Nature-based Interventions and the Environment-Microbiome-Health Axis

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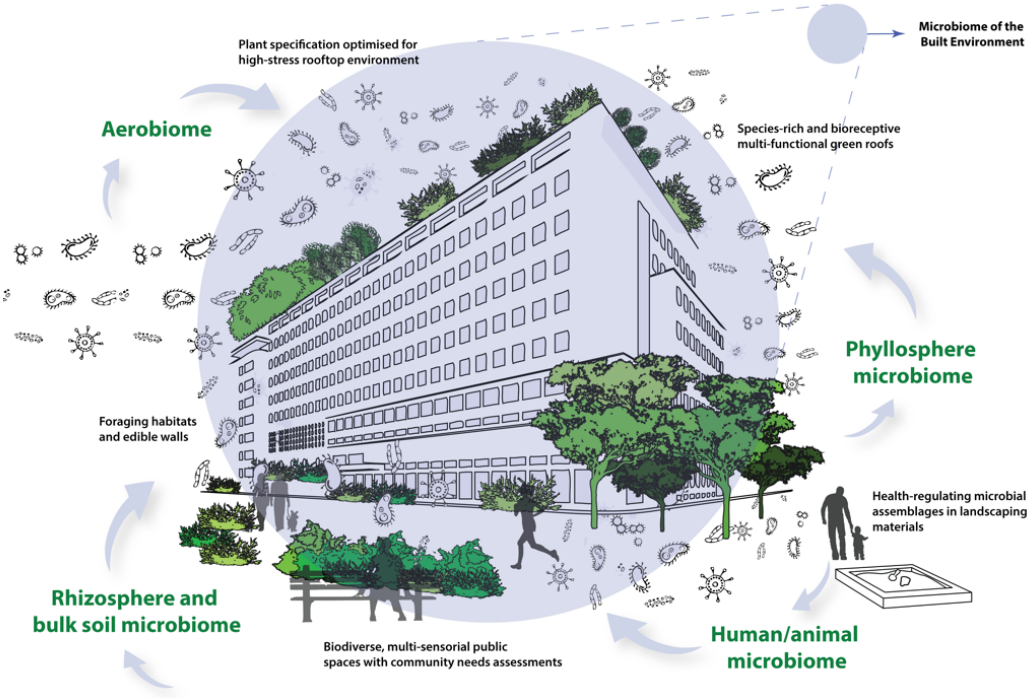

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ABSTRACT

This interdisciplinary PhD is an innovative socioecological investigation into two key phenomena: 1. nature-based interventions, which are structured nature-based activities that aim to facilitate behavioural changes for the benefit of health and wellbeing; and 2. the relationship between the environment, the microbiome and human health. The aims of the PhD study include:

- Determining the distribution of, and socioecological constraints and opportunities associated with nature-based interventions.
- Ascertaining how engagement with nature may have supported population health during the COVID-19 pandemic, and whether the pandemic affected nature engagement.
- Investigating the environment-microbiome-health axis, including a review of potential anthropogenic disruptions to this relationship, and determining the spatio-compositional and ecological factors that affect exposure to the aerobiome (microbiome of the air) in urban green spaces.
- Determining whether relationships with and knowledge of biodiversity affect attitudes towards microbes.
- The development of novel conceptualisations that transcend the boundaries of current knowledge, including Microbiome-Inspired Green Infrastructure (MIG) – multifunctional green infrastructure that aims to enhance ecosystem functionality and human health through considerations for the microbiome; the *Lovebug Effect* – microbially-mediated nature affinity; and, the *Holobiont Blindspot* – a

newly proposed cognitive bias that may result from the failure to recognise the microbiome's role in behaviour and health.

This is a PhD by publication comprising 9 chapters and 12 publications. Chapter 2 presents publications on nature-based interventions (Robinson and Breed, 2019; Robinson et al. 2020a), and the potential health benefits of engaging with nature are presented in Chapter 3 (Robinson et al. 2020b). This is followed by an overview of the environment-microbiome-health axis (Robinson and Jorgensen, 2020) and how nature engagement may affect our attitudes towards microbes in Chapter 4 (Robinson et al. 2021e). This is followed by the aerobiome studies in Chapter 5 (Robinson et al. 2020c; Robinson et al. 2021b) and potential disruptions to the environmental microbiome and human health in Chapter 6 (Robinson et al. 2021c). Chapter 7 presents Microbiome-Inspired Green Infrastructure (Robinson et al. 2018; Watkins and Robinson et al. 2020), and finally in Chapter 8, novel conceptualisations are presented, including the *Lovebug Effect* (Robinson and Breed, 2020) and the *Holobiont Blindspot* (Robinson and Cameron, 2020).

The methods used in the nature-based intervention studies included geospatial analysis using geographic information systems (GIS) and online questionnaires. The methods used in the microbiome studies included the creation of novel sampling methods to collect bacterial DNA in urban green spaces, DNA sequencing, and the application of bioinformatic tools. Although systematic reviews were not included, the reviews in this thesis did follow the preferred reporting system for systematic reviews (PRISMA) method to ensure robust data collection.

This PhD makes several important contributions to knowledge. For example, constraints to nature-based interventions were identified, and these show that enhanced transdisciplinary collaborative pathways and efficient infrastructure are needed. The research also identified that people significantly changed their patterns of nature engagement during the COVID-19 pandemic and many visited nature for important wellbeing and coping benefits. This highlights the immense value of nature to health and wellbeing during unprecedented times. The research also revealed for the first time that attitudes towards microbes might be influenced by nature engagement and biodiversity literacy, and that anthropogenic pollution (light and sound) could affect the microbiome with important ecological and health implications. In the aerobiome studies, the cutting-edge discovery of ecological factors that drive aerobiome assembly and potentially affect exposure types and routes could have important implications for public health and landscape management.

Nature-based interventions have the potential to enhance human and environmental health, and the environmental microbiome will likely play an important role. The novel conceptualisations developed during this PhD add new knowledge to the fields of landscape design, microbiome science, and environmental psychology and have stimulated new research agendas across the world. The work in this thesis is influenced by the emerging planetary health paradigm, the ‘interconnectedness’ and intrinsic value narrative associated with Indigenous cultures, and ‘systems thinking’ (a holistic analytical approach that focuses on the way a system’s constituent parts –– and how systems themselves — interrelate). Fostering deep reciprocity with the natural world to enhance personal and planetary health has never been more important. We have a key opportunity to redefine our relationship with the wider biotic community by establishing a new appreciation of our ‘microbial friends’ and the profound interconnectedness between the environment, the microbiome and human health.

List of publications in this PhD thesis:

1. **Robinson, J.M.,** and Breed, M.F. (2019). Green Prescriptions and Their Co-Benefits: Integrative Strategies for Public and Planetary Health. *Challenges*. 10, pp. 1-14.
   Part of the Special Issue - The Emerging Concept of Planetary Health: Connecting People, Place, Purpose, and Planet.
2. **Robinson, J.M.,** Jorgensen, A., Cameron, R., and Brindley, P. (2020). Let Nature Be Thy Medicine: A Socioecological Exploration of Green Prescribing in the UK. *Int J Environ Public Health*. 17, pp. 1-24.
   Part of the Special Issue "Planetary Health: From Challenges to Opportunities for People, Place, Purpose and Planet”
3. **Robinson, J.M.,** Brindley, P, Cameron, R., MacCarthy, D., and, Jorgensen, A. (2021). Nature’s Role in Supporting Health During the COVID-19 Pandemic: A Geospatial and Socioecological Study. *Int J Environ Res Public Health*. 18, pp. 1-21.

4. **Robinson, J.M.,** and Jorgensen, A. (2019). Rekindling Old Friendships in New Landscapes: The Environment-Microbiome-Health Axis in the Realms of Landscape Research. *People Nat*. 2, pp.339-349.
5. **Robinson, J.M.,** Cameron, R., and Jorgensen, A. (2021). Germaphobia! Does our Relationship with, and Knowledge of Biodiversity, Affect our Attitudes Towards Microbes? *Front Psychol*, 12, pp. 1-10.
6. **Robinson, J.M.,** Cando-Dumancela, C., Liddicoat, C., Weinstein, P., Cameron, R. and Breed, M.F. (2020). Vertical Stratification in Urban Green Space Aerobiomes. *Environ Health Persp*, 128, p.117008.
7. **Robinson, J.M.,** Cando-Dumancela, C., Antwis, R.E., Cameron, R., Liddicoat, C., Poudel, R., Weinstein, P., and Breed, M.F. (2021). Urban Green Space Aerobiomes: Exposure to Airborne Bacteria Depends Upon Vertical Stratification and Vegetation Complexity. *Sci Rep*, 11, pp. 1-17.
8. **Robinson, J.M.,** and Parker, B. (2021). The Effects of Anthropogenic Sound and Artificial Light Exposure on Microbiomes: Ecological and Public Health Implications. *Front Ecol Evol.* 9, pp. 1-7.
9. **Robinson, J.M.,** Mills, J.G., and Breed, M.F. (2018). Walking Ecosystems in Microbiome-Inspired Green Infrastructure: An Ecological Perspective on Enhancing Personal and Planetary Health. *Challenges.* 9, pp.1-15.
10. Watkins, H., **Robinson, J.M.,** Breed, M.F., Parker, B. and Weinstein, P. (2020). Microbiome-Inspired Green Infrastructure: A Toolkit for Multidisciplinary Landscape Design. *Trends in Biotech. 38,* pp.1305-1308.

11. **Robinson, J.M.,** and Breed, M.F. (2020). The *Lovebug Effect*: Is the Human Biophilic Drive Influenced by Interactions Between the Host, The Environment, and the Microbiome? *Sci Tot Environ.* 720, p.137626.
12. **Robinson, J.M.,** and Cameron, R. (2020). The *Holobiont Blindspot*: Relating Host-Microbiome Interactions to Cognitive Biases and the Concept of the "Umwelt". *Front Psychol. 11,* p.591071.

GLOSSARY

Aerobiome:

The network of all microbial communities (and their genetic material) within a given airspace.

ALAN:

Artificial Light at Night (anthropogenic light pollution).

Alpha diversity:

Species richness in a system (the number of species in a population) and species evenness (the abundance of each species in a population).

Archaea:

Single-celled microorganisms with a structure similar to bacteria. They are evolutionarily distinct from bacteria and eukaryotes. They form the third domain of life and often prefer extreme environmental conditions.

Beta diversity:

The extent of change in community composition. Allows for a comparison of diversity between systems.

Biodiversity hypothesis:

Contact with natural environments enriches the human microbiome, promotes immune balance and protects from allergy and inflammatory disorders (Haahtela, 2019).

Bioinformatics:

The science and computational study of biological information. An interdisciplinary field that develops methods and tools to understand often large and complex biological data.

Biophilia:

A hypothesis that proposes humans possess an innate affinity to connect with other forms of life. Edward O. Wilson introduced and popularized the hypothesis in his book, Biophilia (1984).

Biophilic drive:

The process to describe the motivation behind an individual's desire to seek out natural environments and other life forms.

BLAST:

Basic Local Alignment Search Tool – an algorithm for comparing primary biological sequence information such as the nucleotides (building blocks) of DNA.

Bray-Curtis dissimilarity:

A statistic used to quantify the compositional dissimilarity between two different sites, based on the counts at each site (Bray and Curtis, 1957).

Cognitive bias:

A systematic error in judgement or deviation from rationality – primarily to save our brains time/energy.

Decibel (dB):

A measure sound intensity also known as amplitude, and measured on a logarithmic scale.

Deep Ecology:

A term coined by Arne Naess (1973) that describes an ecological philosophy which promotes the intrinsic value of all living beings regardless of their instrumental value to humans, and a restructuring of human societies in accordance with these ideas.

DNA sequencing:

The process of reading and interpreting the nucleic acid sequence i.e., the order of the building blocks of DNA.

Dysbiosis:

A term used to describe an imbalance or maladaptation in a microbiome (collection of microbial communities in a given environment), typically with adverse effects on animal health.

Ecological restoration:

The process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed. Restoration ecology is the corresponding scientific discipline.

Environment-microbiome-health axis:

The relationship between the environment, its microbiome, the human microbiome and human health.

Forest bathing:

A Japanese practice (Shinrin-yoku 森林浴) of immersing oneself in a forest environment – a method of being calm amongst trees for a wellbeing benefit.

Germaphobia:

The pathological fear of, or aversion towards microorganisms and dirt.

GIS:

Geographic Information System – a system for acquiring, processing and interpreting geospatial information.

Green Infrastructure:

‘Strategically planned network of natural and semi-natural areas with other environmental features designed and managed to deliver a wide range of ecosystem services’ in both rural and urban settings,’ (European Commission’s Green Infrastructure Strategy, 2013).

Green prescribing:

A systematic way of prescribing time engaging with nature and meaningful nature-based activities for the benefit of health and wellbeing.

Holobiont:

A term first coined by Margulis (1990) defined as a “biomolecular network composed of the host plus its associated microbes [i.e. the Holobiont], and their collective genomes forge a Hologenome” (Bordenstein and Theis, 2015).

Holobiont blindspot:

Failing to consider the role of host–microbiome (collectively termed a “holobiont”) interactions in a given behavior, may underpin a potentially important cognitive bias – referred to as the *Holobiont Blindspot.*

Hologenome:

Collective genomes of holobionts.

Horizontal gene transfer:

Also known as lateral gene transfer, and is the movement of genetic material between unicellular and multicellular organisms by means others than vertical transmission of DNA from parent to offspring.

Lux:

The SI (International System of Units) derived unit of illuminance, measuring luminous flux per unit area.

Metagenomics:

The study of genetic material recovered directly from environmental samples.

Microbe:

Also known as microorganism. Microscopic organisms that exist as unicellular, multicellular or cell clusters. Examples include bacteria, fungi, viruses, archaea, protozoa, and algae.

Microbiota-gut-brain axis:

A bi-directional communication link between the microbiota, the gut, and the brain.

Microbiome:

The entire collection of microorganisms (and their genetic material) in a given environment, their habitat and conditions.

Microbiomics:

The science and study of microbiomes.

Microbiome-Inspired Green Infrastructure (MIGI):

Natural infrastructure that is restored and/or designed and managed to promote reciprocal (often health-promoting) relations between humans and environmental microbiomes, whilst sustaining microbially-mediated ecosystem functionality and resilience.

Microbioscape:

Microbioscape research is the investigation and application of innovative research methods to characterize and visualize the structure, composition and distribution of environmental microbial communities and their relationships with their hosts. Furthermore, Microbioscape research aims to understand the social implications and functional ecology of these communities, focusing on their importance for people, place and nature (Robinson and Jorgensen, 2019).

Nature-based interventions:

An intervention involving nature-based activities for the benefit of health and wellbeing.

Nature connectedness:

One’s affective, cognitive and experiential connection with the rest of the natural world.

NDVI:

Normalised difference vegetation index – a measure of relative landcover greenness.

Network analysis:

A set of integrated techniques to read and analyse relationships among and between elements and social structures.

Noncommunicable disease:

Chronic, non-infectious diseases such as diabetes and inflammatory bowel disease.

Old Friends hypothesis:

An update by Rook et al. (2003) on the hygiene hypothesis (Strachan, 1989)- suggesting that because of our long evolutionary association with certain microorganisms, they are recognised by the innate immune system as harmless or in some cases, treated as “friends” because they are needed for regulation.

Operational Taxonomic Unit (OTU):

A system to classify groups of closely related individuals, and in the case of microbes, it represents a taxonomic unit based on the similarity of microbial DNA sequences.

Ordination plot:

A plot to summarise community data by producing a low-dimensional ordination space in which similar species are plotted close together, and dissimilar species are plotted further apart.

Perceived Stress Scale (PSS):

A validated psychological instrument for measuring the perception of stress (Cohen et al. 1994).

Phyllosphere:

A term used to refer to the total aboveground portions of plants in terms of them being habitat for microbes.

Protozoa:

Single celled eukaryotes, which include amoebas, flagellates, ciliates, sporozoans and others. Treated as phyla belonging to the kingdom Protista.

Read:

An inferred sequence of base pairs corresponding to all or a single fragment of DNA.

Sequence:

In this thesis, this refers to DNA sequence – a sequence of nucleotides (building blocks of DNA).

Shinrin-yoku (森林浴)**:**

Japanese term for forest bathing.

Short-chain fatty acid (SCFA):

The main metabolites (metabolic by-products) with fewer than six carbon atoms, produced by gut microbes.

Symbiocene:

A philosophical term used to describe a proposed geological epoch that is defined by a mutually advantageous relationship between all living beings (particularly between humans and the rest of nature).

System 1 Thinking:

This refers to a conceptual branch of cognition characterized by “fast and automatic thinking” – popularised by Daniel Kahneman (Kahneman, 2011).

The Lovebug Effect:

An evolutionary model to describe microbially mediated nature affinity.

The Holobiont Blindspot:

A new concept that describes a failure to recognize potential microbiome influences in perception and action (a cognitive bias).

Umwelt:

The world as it is perceived by a particular organism.

Vertical Stratification:

Vertical layering of a particular medium – in this thesis this refers to the layering of the microbiome in a given airspace.

Warwick-Edinburgh Mental Wellbeing Scale (WEMWBS):

A validated psychological instrument used to acquire information on one's perceived level of mental wellbeing (University of Warwick, 2019).

16S rRNA gene:

A gene that is conserved across all bacterial species, but which contains 'hyper variable' regions that allow discrimination between taxa (for identification).

CHAPTER 1

1. INTRODUCTION

This interdisciplinary PhD thesis by publication lies at the interface between ecology, public health and landscape architecture. It is an innovative socioecological investigation into two key phenomena: **(a)** nature-based interventions, which are structured nature-based activities that aim to benefit health and wellbeing; and, **(b)** the relationship between the environment, the microbiome (the network of microbial communities in a given environment) and human health.

1.1. Global public health and biodiversity issues

1.1.1. Problem statement

The global megatrends of rising non-communicable diseases and declining biodiversity are interrelated. A transdisciplinary, planetary health approach could ameliorate the coupled effects on human and ecosystem health.

1.1.2. Public health issues

In recent decades, advances in knowledge and innovation have given rise to improvements in several areas of public health, particularly in affluent countries (Pretty and Barton, 2020). Notable examples include improved infant survival rates, reductions in cases of measles, mumps and rubella as a result of widespread immunisation programmes (Peltola et al. 1994), and an increase in breast cancer survival due to upstream mammographic screening (Vyse et al. 2002). However, with a profuse and ever-increasing global population, deeply intertwined with sociopolitical and cultural complexities, there is still a considerable amount of work to be done to improve personal and planetary health. Indeed, noncommunicable diseases (NCDs) are on the rise in many countries (Bollyky et al. 2017; Pretty and Barton, 2020). NCDs are characterised by chronic, non-infectious conditions such as diabetes, asthma, and cardiovascular disease (Adesina et al. 2020; Eryurt and Menet, 2020). In recent decades the prevalence of asthma (El-Gamal et al. 2017; Borna et al. 2019), diabetes (Holman et al. 2010), allergic rhinoconjunctivitis (Kainu et al. 2013), and autoimmune disorders (Dinse et al. 2020; Paramasivan et al. 2020) has increased worldwide, and many countries have reported current or looming mental health epidemics (Bhagat, 2020; Usher, 2020; Wang et al. 2020). The risks of infectious diseases have also been illuminated in recent times, largely due to the current (as of January 2021) COVID-19 pandemic. A growing body of evidence now connects urbanisation to a disconcerting rise in communicable diseases such as dengue fever and chikungunya (Andrea, 2019; Goryakin et al. 2017; Alirol et al. 2011; Ali and Dasti, 2018). Indeed, pandemics are expected to increase in frequency in the future (Hall et al. 2020), and global urbanisation could potentially increase hazardous (human-wildlife habitat) interfaces for emerging infectious disease exposure and transmission (Gibb et al. 2020).

1.1.3. Biodiversity issues

Our vastly diverse global and local ecosystems provide an abundance of provisioning, supportive, regulatory and cultural benefits to humans, commonly referred to as ‘ecosystem services’. It cannot be overstated that the health and survival of the human race is intimately dependent upon these ecological provisions

(Mtwana et al. 2016; Henderson and Loreau, 2020). Our ecosystems can be considered our ‘life support systems’ (Barnosky et al. 2014). Anthropogenic pressures (e.g., pollution and resource exploitation) have been and continue to be detrimental to the environment (and its intrinsic and instrumental value) to the point where the terms ‘biodiversity crisis’ and ‘climate crisis’ are now commonplace (Albert et al. 2020; Borges et al. 2020; Engström et al. 2020).

Biodiversity loss is now recognised to be a global megatrend, with current species extinction rates estimated to be 1,000 times higher than natural background rates, and future rates likely to increase to 10,000 times higher (Haahtela et al. 2013; De Vos et al., 2015). This is driven, in part, by anthropogenic trends such as urbanisation, population growth, and associated processes including unsustainable resource exploitation, pollution and climate change (Sol et al. 2014; Hughes, 2017; Crenna et al. 2019). Indeed, without immediate and scalable interventions, it is projected that by 2050, 95% of Earth’s land will be affected by degradation (Yu et al. 2020). To reflect these enormous environmental issues, the United Nations heralded 2021-2030 as the UN Decade on Ecosystem Restoration (Waltham et al. 2020).

Microbial communities (comprising bacteria, algae, fungi, viruses, archaea, and protozoa) can be considered the foundations of our ecosystems, influencing the health of all organisms (Cavicchioli et al. 2019). However, ecosystem degradation negatively affects microbial communities, which, along with a reduction in our exposure to biodiversity, can be detrimental to human health e.g., by inhibiting interactions that promote immunoregulation (Liddicoat et al. 2018; Cavicchioli et al.

2019). Studying the relationship between the environment, the microbiome, and human health is an emerging area of research that is central to this thesis.

1.1.4. Policy, practice, and the 'burning platform'

All of the 17 interlinked global Sustainable Development Goals (SGDs) are relevant to these twinned issues of human and environmental health. In relation to this thesis, the three most directly relevant SDGs include Goal 3: Good Health and Wellbeing; Goal 11: Sustainable Cities and Communities; and, Goal 15 Life on Land (protect, restore and promote sustainable use of terrestrial ecosystems) (UN, 2021). In a UK context (the author's home country), the UK Government's Department for Environment, Food & Rural Affairs (Defra) published a 25 Year Plan to Improve the Environment (Defra, 2018). This plan set out goals to restore nature, improve sustainability, and connect people with the environment to improve health and wellbeing over a generational period (i.e., 25 years). The goals include encouraging the exploration of nature-based interventions, which are structured nature-based activities that aim to facilitate behavioural changes for the benefit of health and wellbeing (Shanahan et al. 2019). The 25 year plan states: "these actions will, we hope, ensure that this country is recognised as the leading global champion of a greener, healthier, more sustainable future for the next generation" (Defra, 2018, p.8).

Ensuring a greener, healthier, more sustainable future for the next generations is imperative, and the current trajectory of global ecosystem collapse is the 'burning platform'. There have been recent calls to take an integrative and transdisciplinary approach towards addressing the challenges associated with the intricately connected phenomena of ecosystem resilience and human health (Gabrysch, 2018). For example, the emerging *planetary health* conceptual framework focuses strongly on mutual considerations for human and environmental health at both local and global scales (Prescott and Logan, 2018).

The COVID-19 pandemic has emphasised the importance of unsustainable human actions on global public and ecosystem health. Indeed, anthropogenic land-use change and human encroachment into 'wilder' habitats likely contributed to the outbreak of COVID-19 (Plowright et al. 2021). Despite this, the COVID-19 pandemic also provides a crucial opportunity to 'press the metaphorical reset button', and seek immediate policy and practice transformation (Sinsky and Linzer, 2020). As Albert Einstein reportedly said: "*in the middle of difficulty lies opportunity*" (Riker and Fraser, 2018). COVID-19 (and other diseases) along with ecosystem degradation, present immense difficulties. Applying a transdisciplinary planetary health approach to address global environmental and public health issues is a vital opportunity.

The problems of the world are complex and when faced with questions regarding the biodiversity and public health crises, the traditional scientific paradigm often provides inadequate solutions (Annerstedt, 2010). The planetary health paradigm is inherently transdisciplinary in that it draws together different disciplines to form a holistic strategy and includes non-academic stakeholders in the process of knowledge production (Rigolot, 2020), This approach could potentially remove the obstacle of incommensurability between disciplines (Annerstedt, 2010) and address the coupled phenomena of human and ecosystem health. However, the success of planetary health solutions is predicated on an enhanced understanding of proactive interventions across scales and domains — including the microbial and macro scales and the sociosphere (Prescott and Logan, 2018).

1.2. The disciplinary orientation, and theoretical framework of the thesis

1.2.1. Existing scientific theories

Due to the interdisciplinary nature of this work, several scientific theories constitute the basis for the studies within the thesis. From the environmental psychology discipline, these include the biophilia hypothesis i.e., describing our potential innate affinity for nature (Wilson, 1984), the attention restoration theory e.g., looking at natural features allows the mind to sit in the default mode network and restore attention capacities (Kaplan, 1982), and pro-environmental behaviours — upstream solutions to address human and ecosystem health issues by applying methods of behaviour change that prompt pro-environmentalism, with downstream human health benefits (Annerstedt and Depledge, 2015). In the biological sciences, the core scientific theories include the old friends and biodiversity hypotheses, which suggest that biodiversity loss and our reduced exposure to immunoregulatory microbial biodiversity, is increasing human non-communicable diseases.

1.2.2. Nature and human health

There is now a well-established body of evidence to support the idea that spending time in and engaging with ‘natural environments’ is beneficial for human health and wellbeing. Several mechanistic pathways and health outcomes have been identified by researchers. The nature-health pathways can be divided into 4 categories (excluding broader ‘ecosystem services’). These include biological, psychological, sociocultural, and physical activity pathways (Hartig et al. 2014). Health outcomes range from immunoregulation and reduced blood pressure, to improved physical activity and reductions in stress and anxiety (Ideno et al. 2017; Soga et al. 2017; Roslund et al. 2020).

In terms of biological pathways and health outcomes, the Japanese practice of Shinrin-yoku (森林浴) also known as ‘forest bathing’, in which a person would immerse themselves in a natural, calming woodland environment (pathway), has been shown to reduce diastolic and systolic blood pressure (outcome) (Ideno et al. 2017), enhance innate immunity via lymphocyte cell activity (Li et al. 2007) and reduce stress and anxiety (Chen et al. 2018). There is also evidence to suggest that exposure (pathway) to phytoncides — plant-based chemicals — can enhance sleep via interactions with Gamma-aminobutyric acid (GABA), a potent neurotransmitter (Woo and Lee, 2020). Gardening can improve symptoms of depression (Soga et al. 2017), provide relief from acute stress and anxiety (van den Berg et al. 2011), improve diets and facilitates physical exercise to combat obesity (Watson and Moore, 2011). There is also emerging evidence to suggest that human interactions with the environmental microbiome — the diverse consortium of microorganisms in a given environment — could be essential to the development and regulation of our immune system and potentially our mental health (Liddicoat et al. 2020; Roslund et al. 2020). This will be discussed further in subsequent sections.

The ‘reconnecting with nature’ narrative has gained popularity recently, particularly in western cultures (Ives et al. 2018; Snep and Clergeau, 2020), which reflects an important psychological pathway to nature-associated health benefits. An example is the concept of *nature connectedness,* which is considered to be a validated psychological construct that aims to measure one’s affective, cognitive and experiential connection with the natural world (Richardson et al. 2019; Choe et al. 2020). Higher levels of nature connectedness have been linked to more favourable wellbeing outcomes and pro-ecological behaviours (Pritchard et al. 2020). It is also believed that nature connectedness can be enhanced through nature-based engagement activities (Nisbet et al. 2019).

Simply walking in natural environments and proactively noticing natural features (such as birds and flowers) has been shown to enhance eudemonic wellbeing and psychological restoration — or the ability to recover from stressful events (Ollafsdottir et al. 2020; Richardson et al. 2021). The vast body of quantitative evidence to support the psychological pathways and benefits is strong and it is important to emphasise that vital qualitative evidence has also been gathered in recent years. For example, Birch et al. (2020) explored the lived experiences of young people (aged 17-27 years) who had mental health difficulties, and investigated how nature supported their mental health and wellbeing. They found that urban nature provided a stronger sense of self, and feelings of escape, connection and care, offering a new relational understanding of health and place. Natural environments also provide important places for introspection, cultivating awe, inspiration and feelings of freedom, and can facilitate convivial, cultural, and recreational activities (van den

Bosch and Nieuwenhuijsen, 2017; Bethelmy and Corraliza, 2019). These factors could help to improve mental health and enhance social cohesion (Jennings et al. 2019). Another recent study suggested that the presence of urban trees in areas with lower socioeconomic status was associated with a significantly lower probability of being prescribed anti-depressants (Marselle et al. 2020). Other research has shown that it is not simply the presence of 'green' features that is important for health and wellbeing, but the quality of these features, such as biodiverse, safe, clean, accessible green spaces (Mears et al. 2019).

Neighbourhoods with trees and green spaces provide a setting for social interactions, which likely increases social cohesion (Marselle et al. 2021). Social cohesion or positive friendly relationships and the feeling of being accepted, is linked to favourable health and wellbeing (Hartig et al. 2014). Therefore, the provision of safe and accessible natural environments likely plays an important role in various positive health outcomes via this social pathway. Access to natural environments also contributes towards building capacities and facilitating physical activity, which itself is strongly associated with favourable physical and mental health and wellbeing outcomes (Marselle et al. 2021). Research also suggests that physical activity in natural environments may provide greater health benefits than indoor-based exercise (Bowler et al. 2010).

Despite the supporting evidence, spending time engaging with nature should not automatically be viewed as a panacea. Different nature-based activities, contexts, environments and methods of engagement may have different effects on different individuals. For example, a recent study suggested that perceived social pressure to visit nature was associated with lower visit happiness and higher visit anxiety in people with common mental health disorders (Tester-Jones et al. 2020). Therefore, any efforts to promote nature-engagement as a health intervention should also explicitly consider individual preferences, needs, and life history traits.

1.2.3. Nature-based interventions

Nature-based interventions aim to facilitate behavioural changes that benefit health and wellbeing through the structured promotion of nature-based activities (Shanahan et al. 2019). The fundamental principles of nature-based interventions have been applied for centuries in Western cultures. For example, the ancient Romans and Greeks created health-promoting spa baths at thermal springs (Pérez et al. 2019). Moreover, to treat symptoms of disease in the Hippocratic era (460–370 BC) a "change of habits and environment was advised, which included bathing, perspiration, walking, and massages" (van Tubergen and van der Linden, 2002). In their contemporary form, nature-based interventions, which are also known as green prescriptions, have emerged in response to the relatively recent rise in noncommunicable diseases (e.g., cardiovascular and inflammatory diseases) and social isolation issues (Shanahan et al. 2019). Activities associated with nature-based interventions include biodiversity conservation and ecological restoration volunteering, horticultural therapy, park walks, and other meaningful activities that often involve an important social element (Jepson et al. 2010; Breed et al. 2020). Nature-based interventions have strong parallels with and even intersect and converge with social prescribing interventions. Social prescribing is a system of referral used by primary health care professionals and can be defined as “supporting people via social prescribing link workers to make community connections and discover new opportunities, building on individual strengths and preferences, to improve health and well-being” (Pretty and Barton, 2020). Nature-based interventions follow a similar referral process and are often considered to be social prescriptions themselves, particularly those involving group-based activities. A systematic review by Annerstedt and Währborg (2011) showed that a small but reliable evidence base supports the effectiveness and appropriateness of nature-based interventions, with 6 studies of high evidence grade reporting significant health improvements.

Many examples of nature-based interventions and schemes/providers exist. These include Muddy Fork, a therapeutic horticulture team based in Nottinghamshire, UK, who provide gardening and apiary-centric mental health services for both patients and GPs (Muddy Fork, 2020), and the ParkRx project. ParkRx is currently running across the USA, and offers nature-based activities organised by public land agencies in collaboration with healthcare providers (ParkRx, 2020). The interventions are typically designed for patients with a defined need. They have potential to supplement orthodox medical treatments whilst providing economic benefits via reduced public service use (Pretty and Barton, 2020). Furthermore, nature-based interventions could contribute towards reactive (health care) and proactive (health promoting) solutions to public health issues. In the long-term, NBIs should ideally be viewed as part of an upstream, preventative health care model that includes opportunities for salutogenic contact with nature in everyday urban environments. This holistic mode of health, social, and nature care delivery should not simply be seen as a replacement for other vital public services. Moreover, as mentioned earlier, any efforts to promote nature-based interventions should explicitly consider individual preferences and needs to ensure efficacy and sustainability (Tester-Jones et al. 2020).

There are likely to be important practical challenges to the implementation of nature-based interventions and to realising the full potential of any associated co-benefit. For example, Bloomfield (2019) highlighted an important point. He said that coordinators of NBIs will likely be required to speak two disciplinary ‘languages’: 1. the language of the healthcare profession, and 2. the language of the ecologist. Prospective schemes that fail to do so, may focus on gains for their respective disciplines. This could potentially devalue the co-benefits and lead to counterproductive outcomes for human health and the environment. Another important factor will be to secure the provision of high quality green spaces and the availability of NBI facilitators (such as suitably trained wildlife conservation groups and therapeutic horticulture groups). This will help to create an accessible, well-funded, and equitably distributed network of NBIs. Nature-based interventions should focus on contributing towards a holistic planetary health cultural mind-shift. In order to care for ourselves in the short and long-term, it is imperative that we care for our environments.

1.2.4. The environment-microbiome-health axis

Humans are spending less time in biodiverse environments. This is due in part to habitat loss, urbanisation, and sociocultural barriers that may lead to an ‘extinction of experience’ (loss of human-nature experiential connections) (Snell et al. 2017; Cox et al. 2018). Reduced interactions between humans and a diverse suite of coevolved immunoregulatory microorganisms or ‘old friends’ (e.g., as posited by the old friends and biodiversity hypotheses) is a likely result (Rook et al. 2003; Haahtela, 2019). As mentioned, noncommunicable diseases such as diabetes, asthma, inflammatory bowel disease, and other autoimmune conditions are on the rise. The development and progression of these ‘modern maladies’ has been linked in part to the breakdown of this evolutionary relationship between humans and environmental microbiomes (the collection of microorganisms in a given environment) (Renz and Skevaki, 2020; Donovan et al. 2021). As such, the megatrends of biodiversity loss and rising NCDs are thought to be intricately connected (Von Hertzen et al. 2015; Haahtela, 2019).

Exposure to diverse environmental microbiomes could allow a variety of microorganisms with different functional roles to colonise the gut. This may play an important role in gut health, which in turn affects our overall health and wellbeing (Valdes et al. 2018). Dysbiosis literally translates to ‘*life in distress*’. The term is used to describe an imbalance in the microbial ecosystem via the loss of compositional and/or functional diversity. Dysbiosis of the gut microbiome has been implicated in a plethora of diseases from Alzheimer’s disease to depression (Capuco et al. 2020; Liu et al. 2020), and atherosclerotic cardiovascular disease to coronary artery disease (Jie et al. 2017; Trøseid et al. 2020).

A biodiversity intervention recently demonstrated the induction of immuno-regulation (Roslund et al. 2020). This was the result of exposure to an enhanced diversity of environmental microbes, particularly those belonging to the Proteobacteria phylum.

Indeed, environmental factors are thought to be more important than genomic factors in shaping the gut microbiome composition (Rothschild et al. 2018). Prior research suggests that early life exposure to a diverse range of microbiota is particularly important (i.e., until the weaning age, typically 0-4 years). During this period the human gut microbiome composition is highly dynamic and readily colonised by environmental microbiota (Yang et al. 2016; Moore and Townsend, 2019). However, recent research suggests the adult microbiome is also more plastic than previously thought. For example, Martinson et al (2019) provided evidence to show that certain bacterial families in the adult human gut microbiome such as Enterobacteriaceae exhibit high plasticity. Furthermore Schmidt et al. (2019) recently showed that one in three microbial cells from the oral environment pass through the digestive tract to settle and replenish the gut microbiome of healthy adult humans. Browne et al. (2016) showed that anaerobic spore-forming bacteria dominate the gut, comprising >50% of bacterial genera, and display greater change in abundance and species over time in comparison with non-spore formers. This implies that a range of gut microbes may come and go from natural environments (such as soil, where anaerobic spore-forming microbes thrive). Biodiverse environments could supplement the gut microbiome with functionally important microbes.

Short chain fatty acids (SCFAs) including butyrate are produced by bacteria as metabolic by-products and are known to support human health (Tan et al. 2014; Sanna et al. 2019). Indeed, butyrate is linked to intestinal tumour and atherosclerosis inhibition (Roy et al. 2009; Du et al. 2020), can support bone formation, and promote epithelial integrity (Geirnaert et al. 2020; Li et al. 2020). Such microorganisms may be transferred through aerobiomes (the microbiome of a given airspace). For example, in a randomised controlled mouse study, a putative soil-associated butyrate-producing bacteria was found to supplement the mouse gut microbiome following trace-level airborne soil dust exposures (Liddicoat et al. 2020). The abundance of these bacteria associated with reduced anxiety-like behaviours.

Gut colonization aside, exposure to the aerobiome in biodiverse environments also has implications for the human skin and airways. Several studies have shown that the human nasal microbiome composition is significantly influenced by the surrounding aerobiome (Shukla et al. 2017; Kraemer et al. 2018). A recent study also showed that the diversity of skin and nasal microbiota increased after short-term exposure to urban green spaces (Selway et al. 2020). Furthermore, a recent systematic review highlighted that despite the relative infancy of aerobiome–human health research, two studies have demonstrated shifts in immune function away from allergic (Th2-type) responses due to rural (compared to urban) aerobiome exposure (Flies et al. 2020; Lehtimaki et al. 2020). Indoor studies have also demonstrated a link between microbial composition (and endotoxin levels) in biodiverse house dust and immuno-protection (Gehring et al. 2008; Stein et al. 2016). In these studies, the difference in microbial composition was thought to be due differences in farming practices/lifestyles i.e., traditional farming practices (more immuno-protective) versus modern, highly mechanised and chemical-based practices (less immuno-protective). Other studies have shown that aerobiomes contribute to skin, nasal, and oral microbiomes (Lai et al. 2017; Chen et al. 2019). However, very little is known about the ecological and urban anthropogenic factors that affect aerobiome spatial assembly, community composition, exposure types and routes. Understanding this could allow landscape managers and public health practitioners to optimise human-environmental microbe interactions, whilst promoting ecosystem functionality and resilience. This is also highly relevant in the design of urban areas and living environments more generally.

1.2.5. Theoretical framework

The previous paragraphs underpin the theoretical framework of this thesis, and a graphical model is presented in Fig. 1. Nature-based interventions and quality, equitable green infrastructure could in theory, form an important part of a more holistic and upstream ‘health creation’ model that recognises the interconnectedness between human and ecosystem health. From a knowledge perspective, the emerging contributory pathway to health and wellbeing — via human and environmental microbiota interactions — plays an important role in the theoretical foundations of this thesis.

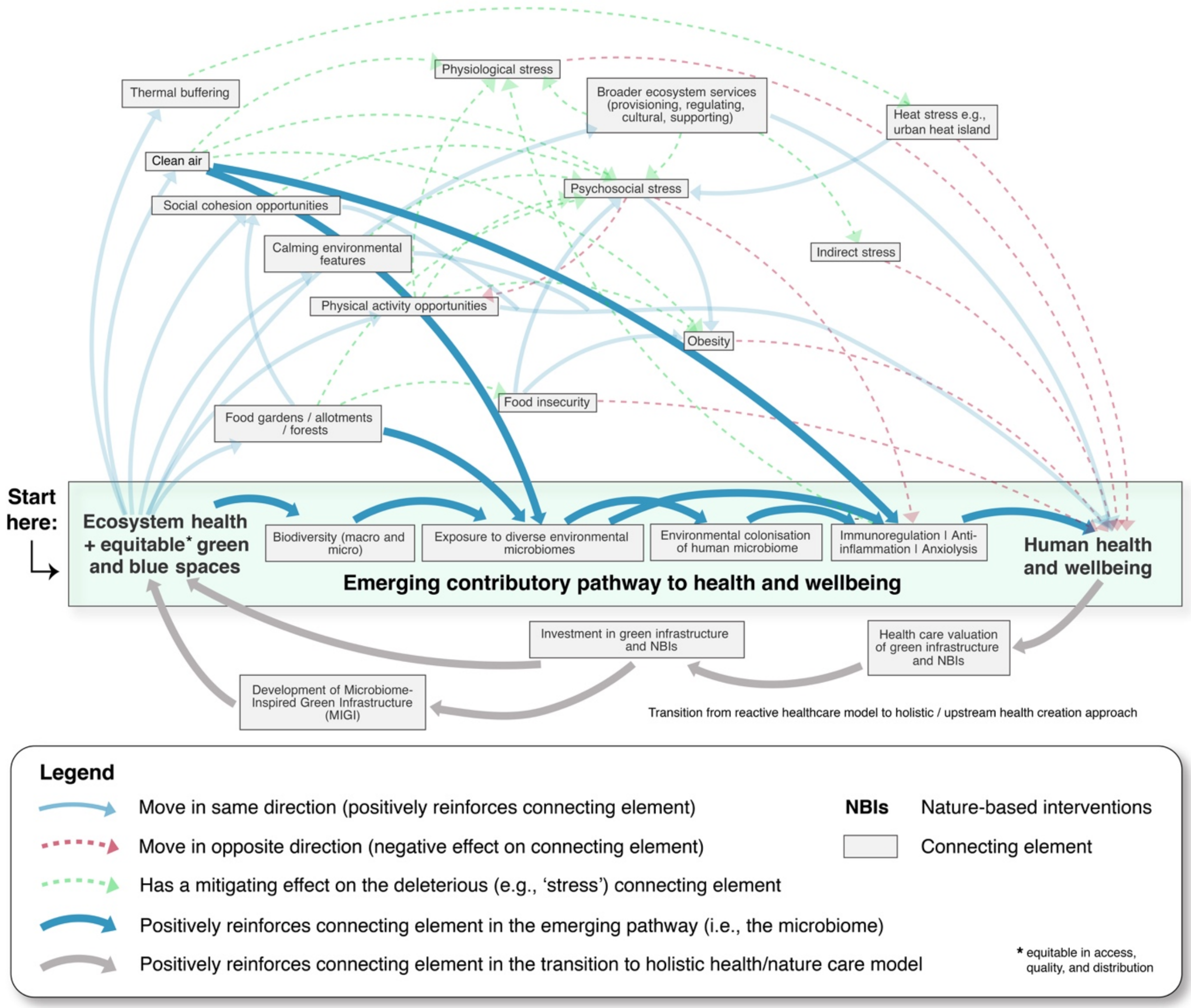

Fig. 1. Schematic model of the theoretical framework that underpins the work in this thesis. This model acknowledges the various pathways linking healthy ecosystems and equitable green/blue spaces to different dimensions of health and wellbeing. This is predominantly indicated by the network of connections and elements in the upper part of the diagram. The emerging (in terms of knowledge) contributory dimension i.e., the environment-microbiome-health axis, is also presented. The lower part of the diagram shows theoretical positive feedback as a result of valuation and investment in a more holistic 'health creation' model which includes quality green infrastructure and nature-based interventions. This is a simplified model (adapted from Flies et al. 2018). The author recognises the additional complexities involved

such as socio-political drivers, and the broader cultural implications associated with transformational change.

1.3. Knowledge gaps and research questions

Several knowledge gaps which are being addressed by this PhD exist in the following areas, and are divided into 1. Research elements, and 2. Conceptual elements. Each knowledge gap is followed by the associated research question/s.

Research element

- Understanding where nature-based interventions are active in the UK and the constraints to (and co-benefits of) implementation.
    - Where in the UK are nature-based interventions active?
    - Does green space presence and deprivation affect nature-based intervention provision?
    - Are there differences in the perceived constraints to nature-based interventions between prescribers (GPs) and providers (nature-based organisations)?
    - What are the co-benefits associated with nature-based interventions?
- Understanding how nature supported health and wellbeing during the COVID-19 pandemic, and how the pandemic may have affected the ability to engage with nature.
    - Did nature help people cope with the COVID-19 pandemic, and what kinds of health benefits were acquired, if any?

    - Did the restrictions associated with COVID-19 affects peoples' patterns of behaviour and engagement with nature?
- Understanding which spatial and ecological factors determine exposure to airborne bacteria in urban green spaces.
    - Does vertical stratification in aerobiome diversity and composition occur in urban green spaces?
    - Does habitat type (e.g., amenity grassland vs. scrub) influence the composition of urban green space aerobiomes?
    - Do the above factors affect the relative abundance of pathogenic or potentially beneficial bacterial taxa?
- Understanding whether anthropogenic stressors (noise and light) affect microbiomes and as a result, the health of humans and ecosystems.
    - Does anthropogenic sound and artificial light affect human and environmental microbiomes?
    - If so, what are the implications for human and ecosystem health?
- Understanding whether nature engagement and microbial literacy affect attitudes towards microbes.
    - Does knowledge of biodiversity and our relationship with nature affect attitudes towards microbes?

Conceptual work

- Understanding how knowledge of microbial ecology can be applied to landscape design and management frameworks to improve human and ecosystem health.

- Understanding how the microbiome may affect human behaviour and exposure to nature.

More broadly, the research aims to contribute towards a transformational change from a simple responsive healthcare model, towards a more systemic health and nature care model; one that embeds the importance of reciprocity between humans and the rest of nature, including microorganisms.

1.4. Overview of methods

To explore the distribution, socioecological constraints and opportunities associated with nature-based interventions, and the potential multifaceted health benefits of engaging with nature, online questionnaires were designed using SmartSurvey (Smart Survey, 2020). Geospatial analysis was also carried out using QGIS 3.14. The geographic information systems (GIS) work involved using buffer analysis, the normalised difference vegetation index (NDVI) as a proxy for greenness, the OS Open Green Space datasets, and deprivation data such as the Index of Multiple Deprivation (IMD). To assess potential relationships, various statistical analyses were applied. These include logistic and generalised linear regression models, bootstrap resampling of Pearson's correlation, chi-squared, and spatial statistics. Questionnaires (Appendix II) were ethically reviewed by the University of Sheffield's Department of Landscape internal review committee and by the National Health Service's (NHS) Health Research Authority (HRA).

The methods used in the microbiome studies included the creation of novel sampling methods to collect bacterial DNA in urban green spaces. This was followed by extracting the DNA in the lab using low biomass techniques. The resulting DNA samples were amplified using polymerase chain reaction (PCR) and sequenced on high-throughput Illumina platforms to provide raw data for the bioinformatics stage. The application of complex next generation bioinformatic tools included Quantitative Insights into Microbial Ecology (QIIME) along with analysis packages in R such as Phyloseq, DeSeq2, Decontam, and Vegan (McMurdie and Holmes, 2013; Davis et al. 2018). Statistical analysis approaches included co-occurrence network analysis, ordinations, bootstrap resampled correlations, and differential abundance analysis. Although systematic reviews were not included in this thesis, the reviews in this thesis did follow the preferred reporting system for systematic reviews (PRISMA) method to ensure robust data collection (Moher et al. 2015; Tricco et al. 2018). An additional research project was designed but abandoned due to COVID-19 restrictions. This project is described below.

Summary of the cancelled Green Prescribing project (due to COVID-19)

A practical green prescribing study was designed to follow the studies in chapter 2. However, this was cancelled due to constraints associated with the COVID-19 pandemic. The study would have involved a randomised controlled trial (RCT) intervention lasting between 3-6 months. The intervention would have involved adult patients with mild-moderate depression, as determined using the well-established PHQ-9 questionnaire. A key aim was to sustain this green prescribing service once the research was completed and to stimulate other trials across Sheffield and the UK, providing opportunities for important meta-analyses.

The intervention would have included the creation of ‘pocket gardens’ (and activities in small, semi-permanent, versatile gardens) and nature-based activities hosted in the premises of GP practices in Sheffield’s Network North region. The idea was to evaluate the green prescribing trial and assess potential changes to patient mental health and wellbeing, and time/cost effectiveness for general practices. Due to the considerable amount of time spent planning this project and acquiring NHS ethics approval during this PhD, the cancelled project will be discussed further in the Discussion section and the designed research protocol can be found in Appendix II. The COVID-19 pandemic was used as a topic for a replacement project. This replacement project is presented in chapter 3.

1.5. Structural overview of the thesis with chapter synopses

This is a thesis by publication. To address the research aims, twelve publications were included. These are presented across seven chapters (Chapters 2-8). The ninth chapter presents an in-depth discussion of the publications, followed by conclusions (Fig. 2).

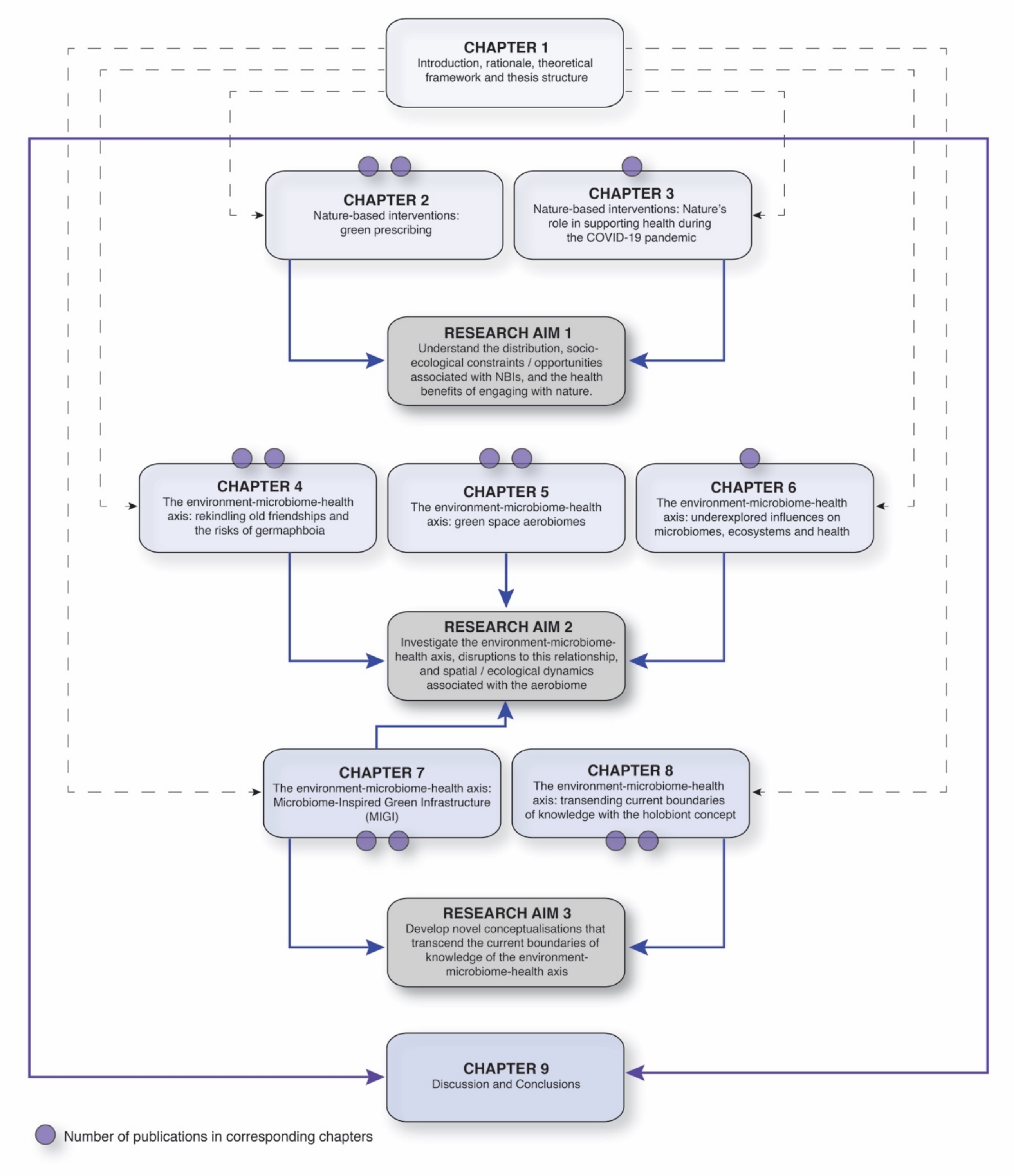

Fig. 2. Diagram showing the structure of the thesis. Arrows indicate how the different chapters combine to address the research aims.

1.5.1. Chapter 2 synopsis

Nature-based interventions may generate several potential co-benefits (e.g., ecological, economic, social). However, these have not been explored and presented in a single publication. This would allow readers to view and understand the multiple dimensions of NBIs. The first publication in chapter 2 addresses this gap. Furthermore, at the start of this PhD, only a limited amount was known about the practice and awareness of, and socioecological constraints and opportunities associated with green prescribing. In the UK, the distribution of green prescribing had yet to be comprehensively mapped. Understanding these factors is important to advance the NBI approach e.g., by helping to identify and address constraints and opportunities at different scales.

The second publication in chapter 2 aimed to address some of these NBI knowledge gaps (Box 1). Views were collected from general practitioners (GP) and nature-based organisations (who typically help to facilitate the interventions). This was followed by an analysis of social (e.g., deprivation) and ecological (e.g., green space distribution) factors. It is hoped that mapping green prescribing resources, acquiring views from GPs and nature-based organisations, and conducting spatial and socioecological analyses provide important insights and spur further research into nature-based interventions.

Box 1. Publications in Chapter 2.

Robinson, J.M., and Breed, M.F. (2019). Green Prescriptions and Their Co-Benefits: Integrative Strategies for Public and Planetary Health. *Challenges*. 10, pp. 1-14.

This was part of the Special Issue - The Emerging Concept of Planetary Health: Connecting People, Place, Purpose, and Planet.

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Acknowledgement of contributions: Jake Robinson was first author on this publication and lead the conceptualisation, methodology and investigation. Jake Robinson lead the full manuscript writing, preparation and editing, and produced the visualisations. Martin Breed contributed to the write-up and the critical review and editing process.

Robinson, J.M., Jorgensen, A., Cameron, R., and Brindley, P. (2020). Let Nature Be Thy Medicine: A Socioecological Exploration of Green Prescribing in the UK. *Int J Environ Public Health*. 17, pp. 1-24.

This was part of the Special Issue "Planetary Health: From Challenges to Opportunities for People, Place, Purpose and Planet"

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Acknowledgement of contributions: Jake Robinson was first author on this publication. Jake Robinson coordinated all aspects of the study, analysis, write-up, visualisations, review and funding acquisition. Prof. Anna Jorgensen also contributed to the conceptualisation, supervision, review and editing, and funding acquisition. Dr Paul Brindley contributed to the methodology (review), and the review and editing process. Dr Ross Cameron contributed towards the review and editing and provided supervision.

1.5.2. Chapter 3 synopsis

Chapter 3 presents research on the multifaceted health benefits of engaging with nature during the COVID-19 pandemic (Box 2). For this study, an online questionnaire was created, and several validated wellbeing instruments were used. This was followed by the application of geospatial methods to analyse the socioecological data.

This research replaced the planned green prescribing project (involving GP surgeries) which was cancelled due to the COVID-19 pandemic (Appendix II). This research project still continued with the theme of nature-based interventions, albeit more in the realms of ‘self-prescribing’, and aimed to reveal novel insights into the value of nature for health and wellbeing, particularly in response to a disease pandemic.

Specific questions included: has nature helped people cope with the COVID-19 pandemic, and has the pandemic changed people’s patterns of visiting nature? Gaining a greater understanding of this could help to promote green infrastructure and NBIs (e.g., highlighting their value). It could also provide insights into which environments are most important during disease pandemics.

Box 2. Publication in Chapter 3.

Robinson, J.M., Brindley, P, Cameron, R., MacCarthy, D., and, Jorgensen, A. (2021). Nature's Role in Supporting Health During the COVID-19 Pandemic: A Geospatial and Socioecological Study. *Int J Environ Res Public Health*. 18, pp 1-21
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Acknowledgement of contributions*:* Jake Robinson was first author on this publication. Jake Robinson coordinated all aspects of the study, analysis, write-up, visualisations, review and funding acquisition. Prof. Anna Jorgensen also contributed to the conceptualisation, supervision, review and editing, and funding acquisition. Dr Paul Brindley contributed to the methodology (review), and the review and editing process. Dr Ross Cameron contributed towards the conceptualisation, review and editing and provided supervision. Danielle MacCarthy contributed to the review and editing process.

1.5.3. Chapter 4 synopsis

Chapter 4 presents an exploration of the relationship (i.e., what is known) between the environment, the microbiome, and human health in the context of landscape research. This helps to set the agenda for subsequent research in this interdisciplinary area.

The loss of biodiversity and our emotional connection with nature, along with poor microbial literacy may be augmenting the negative ecological consequences of germaphobia (the pathological aversion to microorganisms). This could be contributing to an explosion in human immune-related disorders via mass sterilisation of surfaces and reduced exposure to (micro)biodiversity. The publication in the second part of Chapter 4 asks the question of whether our relationship with, and knowledge of biodiversity, affects our attitudes towards microorganisms? (Box 3). Understanding this could be essential, if, for example, an appreciation for, and engagement with the microbial world are to play roles in developing a more holistic approach to health and nature care. For this project, pilot-tested online questionnaire were created and a validated nature connectedness instrument (the Nature Relatedness Scale-6) (Nisbet and Zelenski, 2013) was used.

Box 3. Publications in Chapter 4.

Robinson, J.M., and Jorgensen, A. (2019). Rekindling Old Friendships in New Landscapes: The Environment-Microbiome-Health Axis in the Realms of Landscape Research. *People Nat*. 2, pp.339-349.
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Acknowledgement of contributions: Jake Robinson was the first author on the publication. Jake Robinson conceived the ideas and produced the visualisations. Jake Robinson also led literature collection and the writing of the manuscript. Prof. Anna Jorgensen contributed to the review, editing, and provided vital supervision throughout.

Robinson, J.M., Cameron, R., and Jorgensen, A. (2021). Germaphobia! Does our Relationship with, and Knowledge of Biodiversity, Affect our Attitudes Towards Microbes? *Front Psychol.* 12, pp. 1-10.
This publication will be Open Access and under the Creative Commons 4 (CC 4.0) license.

Acknowledgement of contributions: Jake Robinson was the first author on the publication. Jake Robinson conceived the ideas and produced the visualisations. Jake Robinson also led literature collection, data analysis, interpretation and the writing of the manuscript. Prof. Anna Jorgensen and Dr Ross Cameron contributed to the review, editing, and provided vital supervision throughout.

1.5.4. Chapter 5 synopsis

Chapter 5 presents two novel and cutting-edge experimental studies relating to the aerobiome. In particular, the spatio-compositional and ecological dynamics were investigated. This helped to unravel the complexities of environmental microbiome assembly and potential exposure types and routes (Box 4). The author created the very first urban green space aerobiome vertical stratification measurement station and passively collected environmental bacterial DNA (using petri dishes) in Adelaide Parklands, Australia. Bacterial DNA was sequenced, and bioinformatics, geospatial, and network analysis methods were applied to examine the data.

Specific questions included: how does urban green space habitat type and near-surface altitude affect aerobiome assembly, composition and exposure potential? Understanding these factors could help landscape managers design appropriate vegetation communities to optimise human-microbial interactions that promote health, for example, via immunoregulation.

Box 4. Publication in Chapter 5.

Robinson, J.M., Cando-Dumancela, C., Liddicoat, C., Weinstein, P., Cameron, R. and Breed, M.F. (2020). Vertical Stratification in Urban Green Space Aerobiomes. *Environ Health Persp*, 128, p.117008.
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Acknowledgement of contributions: Jake Robinson was the first author on the publication. Jake Robinson conceived the ideas and produced the visualisations. Jake Robinson also led the fieldwork, lab work, literature collection, bioinformatics, data analysis, the writing of the manuscript, acquisition of funding and multi-stakeholder liaison. Christian Cando-Dumancela provided vital help with the fieldwork and lab work, and review. Dr Craig Liddicoat contributed toward the data analysis and review. Dr Ross Cameron and Prof. Philip Weinstein contributed towards the review and editing, and Dr Ross Cameron provided vital supervision. Dr Martin Breed contributed to the initial concept, review, editing, and provided supervision throughout.

Robinson, J.M., Cando-Dumancela, C., Antwis, R.E., Cameron, R., Liddicoat, C., Poudel, R., Weinstein, P., and Breed, M.F. (2021). Urban Green Space Aerobiomes: Exposure to Airborne Bacteria Depends Upon Vertical Stratification and Vegetation Complexity. *Sci Rep*, 11, pp. 1-17. This publication will be Open Access and under the Creative Commons 4 (CC 4.0) license.

Acknowledgement of contributions: Jake Robinson was the first author on the publication. Jake Robinson conceived the ideas and produced the visualisations. Jake Robinson also led the fieldwork, lab work, literature collection, bioinformatics, data analysis, the writing of the manuscript, acquisition of funding and multi-stakeholder liaison. Christian Cando-Dumancela provided vital help with the fieldwork and lab work, and review. Dr Rachael E. Antwis, Craig Liddicoat and Dr Ravin Poudel contributed toward the data analysis and review. Dr Ross Cameron and Prof. Philip Weinstein contributed towards the review and editing, and Dr Ross Cameron provided vital supervision. Dr Martin Breed contributed to the initial concept, review, editing, and provided supervision throughout.

1.5.5. Chapter 6 synopsis

There are potentially several underexplored anthropogenic influences which could disrupt the environment-microbiome-health axis. Chapter 6 presents a mini-review of the potential impacts of anthropogenic light and sound pollution on microbiomes and the downstream ecological and health implications (Box 5). Many underexplored anthropogenic activities could conceivably alter the assembly and composition of environmental microbiomes. This could have subsequent implications for ecosystem functionality. It could also have indirect ‘downstream’ health impacts, or even direct impacts via the human microbiome. Specific research questions in this chapter include: does anthropogenic sound and artificial light affect microbiomes, and what are the human and ecosystem health implications? Understanding these factors could help in the development of interventions that enhance human and ecosystem health.

Box 5. Publication in Chapter 6.

Robinson, J.M., and Parker, B. (2021). The Effects of Anthropogenic Sound and Artificial Light Exposure on Microbiomes: Ecological and Public Health Implications. *Front Ecol Evol.* 9, pp. 1-7. This publication will be Open Access and under the Creative Commons 4 (CC 4.0) license.

Acknowledgement of contributions: Jake Robinson was the first author on the publication. Jake Robinson conceived the ideas and produced the visualisations. Jake Robinson also led literature collection, review and the writing of the manuscript. Dr Brenda Parker and Dr Ross Cameron contributed to the review and editing process.

1.5.6. Chapter 7 synopsis

Chapter 7 presents work that expands the concept of Microbiome-Inspired Green Infrastructure (MIGI). This concept was first developed by the author during this PhD. MIGI can be defined as multifunctional green infrastructure that is restored and/or designed and managed to optimise reciprocal human-environmental microbiome interactions, whilst supporting essential microbially mediated ecosystem processes, which are fundamental to ecosystem health (Box 6). It essentially calls for stakeholders to apply (eco)systems thinking to landscape management. It calls for explicit considerations for the foundations of our ecosystems (microbes) in relation to wider ecosystem functionality and resilience, and human health. Fig. 4 and Fig. 5 in Appendix I highlight some of the MIGI bioscience and design considerations that have been proposed by the author of this thesis (Robinson et al. 2021a). These include planting considerations (Fig. 4 a, b, and e), socioecological interactions (Fig.

4 c) soil management (Fig. 4 d), and broader habitat considerations (Fig. 4, f). A horizon scan of MIGI considerations (including bioreceptive materials, microbial inoculations, supportive infrastructure and opportunities for engagement) is presented in Fig. 5 (a-f) in Appendix I. The MIGI concept has been used to help establish a new research agenda at the Bio-Integrated Design Lab at University College London (UCL). MIGI has also received interest from urban planners who have integrated the ideas into urban development masterplans. Organisers of international ‘multispecies urbanism workshops’ aimed at transforming our urban ecosystems have also incorporated MIGI ideas. Multispecies urbanism is a framework for urban development that is driven by considerations for reciprocal relationships between humans and non-humans (including microbes) (Rupprecht et al. 2020; Robinson et al. 2021a).

The second publication in this chapter presents an overlay to the Royal Institute of British Architects (RIBA) plan of works. This allows initial considerations for the microbiome at different stages of built environment projects. Multiple stakeholders are developing this concept further.

Box 6. Publications in Chapter 7.

Robinson, J.M., Mills, J.G., and Breed, M.F. (2018). Walking Ecosystems in Microbiome-Inspired Green Infrastructure: An Ecological Perspective on Enhancing Personal and Planetary Health. *Challenges.* 9, pp.1-15.

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Acknowledgement of contributions: Jake Robinson was the first author on the publication. Jake Robinson conceived the ideas and produced the visualisations. Jake Robinson also led literature collection and the writing of the manuscript. Jacob Mils and Dr Martin Breed contributed to the writing, review and editing.

Watkins, H., **Robinson, J.M.,** Breed, M.F., Parker, B. and Weinstein, P. (2020). Microbiome-Inspired Green Infrastructure: A Toolkit for Multidisciplinary Landscape Design. *Trends in Biotech. 38,* pp.1305-1308.
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Acknowledgement of contributions: Jake Robinson was the second author on the publication. Jake Robinson conceived the MIGI ideas and produced the visualisations. Jake Robinson also co-led literature collection and the writing of the manuscript. Harry Watkins co-led the literature collection and writing of the manuscript, and led the plan of works aspects. Prof. Phil Weinstein, Dr Brenda Parker and Martin Breed contributed to the review and editing.

1.5.7. Chapter 8 synopsis

Chapter 8 presents novel conceptualisations developed by the author over the course of the PhD. The concepts draw upon the diverse but interconnected realms of the environment-microbiome-health axis (Box 7). These concepts apply lateral thinking and imagination but are also grounded in scientific evidence and theory. The *Lovebug Effect* is an evolutionary model that describes the possibility of microbially mediated nature affinity, again highlighting profound interconnectedness. The

Holobiont Blindspot describes a newly proposed cognitive bias characterised by a failure to recognise potential microbiome-derived influences in perception and action.

Box 7 . Publications in Chapter 8.

Robinson, J.M., and Breed, M.F. (2020). The *Lovebug Effect*: Is the Human Biophilic Drive Influenced by Interactions Between the Host, The Environment, and the Microbiome? *Sci Tot Environ.* 720, p.137626.

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Acknowledgement of contributions: Jake Robinson was the first author on the publication. Jake Robinson conceived the ideas and produced the visualisations. Jake Robinson also led literature collection and the writing of the manuscript. Dr Martin Breed provided critical input on the conceptual refinement, review and editing.

Robinson, J.M., and Cameron, R. (2020). The *Holobiont Blindspot*: Relating Host-Microbiome Interactions to Cognitive Biases and the Concept of the “Umwelt”. *Front Psychol. 11,* p.591071.

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Acknowledgement of contributions: Jake Robinson was the first author on the publication. Jake Robinson conceived the ideas and produced the visualisations. Jake Robinson also led literature collection and the writing of the manuscript. Dr Ross Cameron contributed to the review, editing, and provided vital supervision throughout.

CHAPTER 2

NATURE-BASED INTERVENTIONS: GREEN PRESCRIBING

“What a marvellous cooperative arrangement - plants and animals each inhaling each other's exhalations, a kind of planet-wide mutual mouth-to-stoma resuscitation”

— Carl Sagan, 1980

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Green Prescriptions and Their Co-Benefits: Integrative Strategies for Public and Environmental Health

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2.1. Abstract

There is a growing recognition of the links between the increasing prevalence of noncommunicable diseases, environmental concerns including biodiversity loss and ecosystem degradation, and socioecological issues such as ecological (in)justice. This has encouraged a number of recent calls for the development of integrative approaches aimed at addressing these issues – also known as nature-based solutions.

An example of an emerging nature-based solution is a ‘green prescription’, broadly defined as a nature-based health intervention. Green prescriptions are typically designed for patients with a defined need and they have the potential to supplement orthodox medical treatments, particularly those aimed at addressing noncommunicable diseases. It is also thought that green prescriptions could bring about significant environmental, economic, and social co-benefits. However, researchers have recently expressed concerns over taking the ‘dose of nature’ approach, in that it may be too reductionistic for the complex social settings in which it is provided. Here we frame a holistic philosophical perspective and discuss green prescribing logic, types, mechanisms and fundamental remaining questions and challenges. We place a significant emphasis on the potential co-benefits of green prescriptions, and the importance of taking a planetary health approach. More research is needed to determine how this potential can be realised, and to further understand the complexities of the nature–human health relationship. However, with additional research and support, there is huge potential for green prescriptions to contribute to both reactive (health care) and proactive (health promoting) public health solutions whilst enhancing the natural environment.

Keywords: green prescriptions; planetary health; urban nature; biodiversity; microbiome; mental health; nature connectedness; greenspace; noncommunicable diseases; nature-based interventions

2.2. Introduction, Issues and Philosophical Perspective

There have been many recent improvements to public health across the planet. Notable examples of these include significant reductions in cases of measles, mumps and rubella (MMR) as a result of widespread immunisation programmes in the mid-late 20th century (Peltola et al. 1994; Vyse et al. 2002), and an increase in breast cancer survival rates following successful upstream mammography screening initiatives (Tabar et al. 2003; Park et al. 2015). However, with such an abundant and ever-increasing global population, deeply intertwined with social and cultural complexities (preventing equity in resource distribution and the associated health benefits), there is still a significant amount of work to be done to improve human health and wellbeing.

Environmental health issues such as biodiversity loss and ecosystem degradation may at first appear to develop independently to those in public health—however, they are often deeply connected (Haahtela et al. 2013; Von Hertzen et al. 2015). There is growing recognition and understanding of these links, particularly between the increasing prevalence of noncommunicable human diseases, environmental concerns, and socioecological issues such as ecological (in)justice. This has encouraged recent calls for the development of integrative approaches that aim to address these issues holistically—also known as nature-based solutions (NbS) (Raymond et al. 2017; Ostfield, 2017). An example of an emerging NbS is green prescribing, broadly defined as a nature-based health intervention. Green prescriptions are typically designed for patients with a defined need, and they have the potential to supplement orthodox medical treatments, particularly those aimed at addressing noncommunicable diseases (Bragg and Leck, 2017). It is also thought that green prescriptions have the potential to bring about significant environmental, economic, and social co-benefits (Bloomfield, 2017; O'Brien et al. 2010).

Our paper aims to explore green prescriptions and their co-benefits in greater detail. We provide an overview of two of the key issues that green prescriptions could potentially help to address—a need for public health innovation, and new strategies that support ecosystem resilience and biodiversity conservation. This is followed by a planetary health-driven philosophical framework that underpins this 'integrative strategy' approach. We then discuss green prescribing logic, types and mechanisms, which are followed by a discussion of their potential co-benefits. Finally, we highlight some of the fundamental remaining questions and concerns regarding their effectiveness.

2.3. Issue 1: A Need for Public Health Innovation

In a number of studies investigating the social determinants of public health, attention has been drawn to the uncomfortable reality of the pressing need for public health and socioeconomic innovation (WHO, 2008; Walsh et al. 2010). These studies highlight significant health inequalities in many countries. Other studies arrive at equally striking conclusions regarding the risk factors for chronic diseases and the associated impacts upon general health, mortality and the economy. For example, Scarborough et al. (2011) estimated that in July 2006, chronic diseases related to poor dietary habits, physical inactivity and obesity cost the UK National Health Service (NHS) £11.8 billion. A similar story unfolds for mental health and wellbeing, with an annual expenditure of up to £13 billion allocated to the management of mental health conditions in England alone (Naylor et al. 2011).

Despite significant improvements in certain areas of public health, recent estimates suggest that 61% of adults in England are considered to be overweight or obese (NHS, 2017)—both of which are risk factors for noncommunicable diseases (NCDs) such as diabetes and cardiovascular-related illnesses (Al-Goblan et al. 2014; Riley et al. 2016). Moreover, around one in four people experience mental health issues in the UK each year (Bridges et al. 2014; McManus et al. 2014). These conditions are diverse, often co-morbid with other NCDs (Patel and Chatterji, 2015; Varghese, 2017) and they are thought to be responsible for 38 million annual deaths across the planet (Allen and Feigl, 2017; Nethan et al. 2017).

These findings highlight the need to develop innovative strategies to:

Improve public health and wellbeing;
Close the health inequality gap; and,
Alleviate the financial burden currently facing health services and the wider economy

2.4. Issue 2: Biodiversity Conservation and Ecosystem Resilience

The natural environment provides a significant amount of provisioning, supportive, regulatory and cultural benefits to humans, and the processes and features that generate these benefits are now commonly referred to as ‘ecosystem services’. It cannot be overstated that the existence of humanity, along with societal health and wellbeing, is highly dependent upon these so-called ‘services’, and the diverse array of organisms they support (Soliveres et al. 2016; Pienkowski et al. 2017). However, our decisions and actions are often detrimental to the environment, and the urban lifestyle is one of the fundamental anthropogenic drivers of biotic restructuring, often to the detriment of biological diversity and ecosystem functionality (Oliveira et al. 2017). For example, two issues associated with biodiversity loss are natural resource exploitation and pollution. The former is a key input in the process of urban development, often resulting in habitat loss and a subsequent reduction in genetic and species diversity (Allentoft and O’Brien, 2010; Pinsky and Palumbi, 2014); whereas pollution is a key output and ‘by-product’ of urbanisation, and it can lead to widespread negative impacts on the global biological systems that support biodiversity (Ceulemans et al. 2014).

There have been recent calls to take an integrative approach towards addressing challenges associated with ecosystem resilience and human health and wellbeing (Figure 1). For example, Raymond et al. (2017) put forward a framework to promote the co-benefits (for environmental and public health) of nature-based solutions. Furthermore, the field of planetary health also focuses strongly on the simultaneous management of human and environmental health (Ostfeld, 2017).

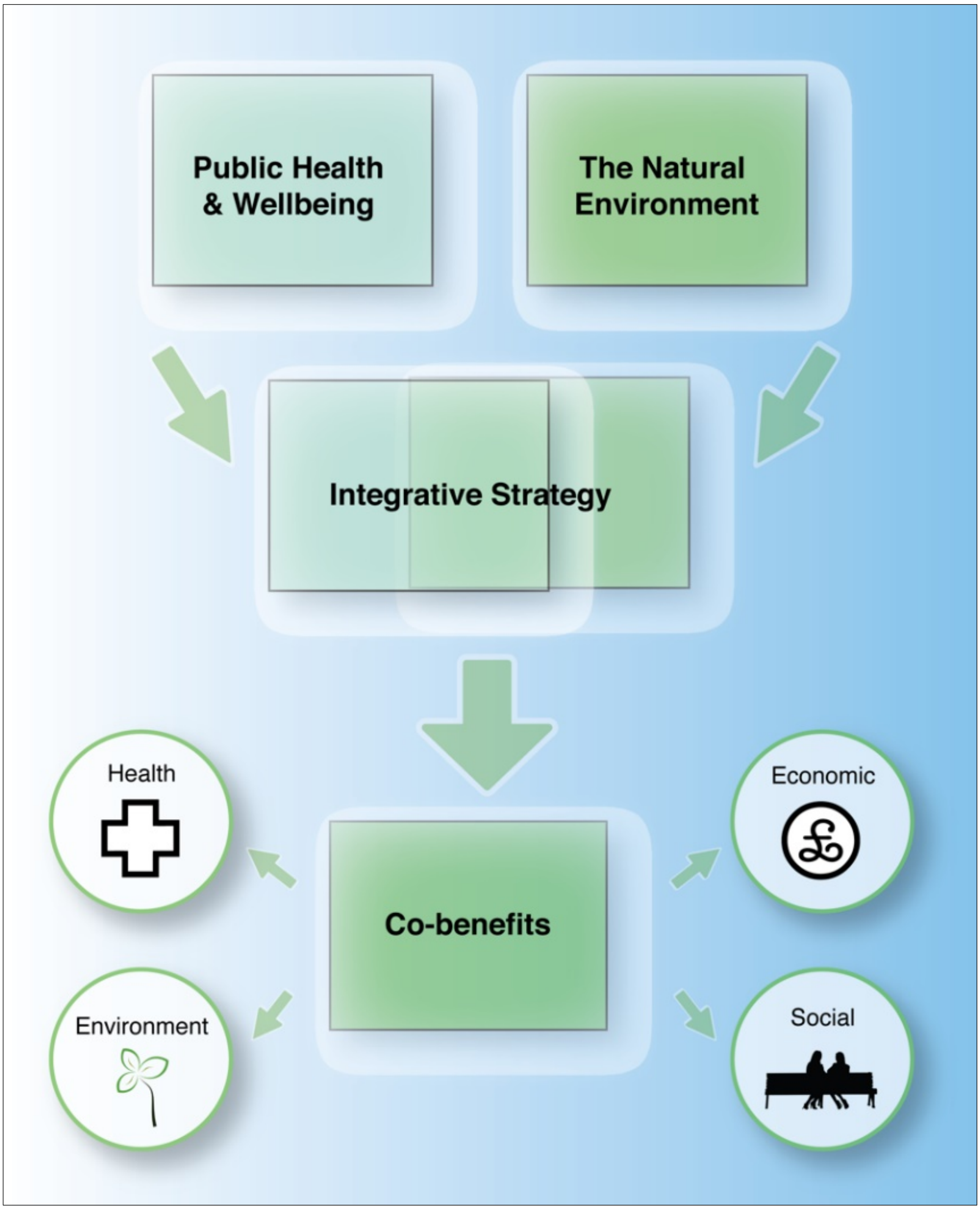

Figure 1. A call for integrative strategies that provide co-benefits for humans and nature (created by the authors).

2.5. Planetary Health, Biophilosophy and the Symbiocene

Issues surrounding public health — particularly noncommunicable diseases — and the growing threats to biodiversity and ecosystem resilience, can be viewed from a single overarching philosophical perspective, that is, from a planetary health perspective. Planetary health is an increasingly popular term that is used to describe the coupling nature of human and environmental health (Prescott and Logan, 2018). The concept was born out of the view that both of these growing issues are inextricably linked. For example, anthropogenic habitat alteration increases the risk of infectious diseases across the planet, and pollution increases the risk of cardiovascular diseases and cancer (Myers, 2017); there are also strong relationships between planetary health and the quality of human nutrition, as well as displacement, conflict and mental health (Myers, 2017; Canavan et al. 2017; Schwartz et al. 2017).

From an anthropocentric perspective, there are social, biological and psychological determinants of public health, and each domain can influence the other in a complex bio-eco-psycho-social network (Borell-Carrió et al. 2004). This phenomenon, along with the notion that all realms of life — both seen and unseen — are interconnected (Prescott and Logan, 2017), is central to the philosophical foundations of this narrative. This philosophy lends itself to emerging calls for a newly recognised epoch, as voiced by several researchers and eco-philosophers [Prescott and Logan, 2017; Albrecht, 2014)]. These calls refer to an epoch dominated by mutually-advantageous relationships between humans and the environment, and not simply a unidirectional and exploitative relationship, as often characterised by the current 'anthropocene'. This proposed epoch has been labelled the 'symbiocene' (Huff, 1977), based upon aspirations for a move towards a more symbiotic, holistic and ecological approach to living. The symbiocene also includes a drive towards a healthcare model dominated by holistic medical and social practices (Prescott and Logan, 2017). However, epochs aside, to contribute to a value system characterised by a human–nature relationship with mutually-beneficial health outcomes, a philosophical approach similar to the ‘biophilosophy’ popularised by Salk appears to be a worthy starting point (Salk, 1962). This approach requires a “coalition of thought from biologists, philosophers and humanists” (Prescott and Logan, 2017), and has parallels with the recent interests in transdisciplinary research and the calls for integrative models, which include considerations for both biodiversity and healthcare (reactive) and health promotion (proactive) (Gehlert et al. 2010; Nyatanyi et al. 2017).

Also central to this narrative is the perceived growing ‘disconnect’ between human beings and natural environments (Kesebir and Kesebir, 2017). Public health and social issues attributed to this disconnect manifest in various forms, including social stress and reduced psychological wellbeing (Lederbogen, 2011; Cox et al. 2017). Potential pathways leading to this disconnect include reduced exposure to natural environments, green space access issues, reduced environmental awareness and time spent in nature (Bertram and Rehdanz, 2015; Soga and Gaston, 2016)). Furthermore, immunodeficiency disorders such as asthma and inflammatory bowel disease (IBD) are on the rise (Kuenzig et al. 2017). This has been partially attributed to reduced exposure to immunoregulatory microorganisms and other health-inducing biological compounds (Earl et al. 2015; Robinson et al. 2018; Robinson and Jorgensen, 2019; Rook, 2013). Obesity has also increased, resulting in part, from lower levels of outdoor-based physical activity and an increasing intake of ultra-processed foods (Wen et al. 2009; Rosiek et al. 2015).

Many ecological issues associated with the human–nature disconnect are multidimensional and include habitat destruction, pollution, biodiversity loss and human–wildlife conflict (Bekoff and Bexell, 2010). This disconnect likely diminishes pro-environmental behaviours and a general sense of environmental stewardship (Bekoff and Bexell, 2010). This may further exacerbate the pressures exerted on the environment, and subsequently exacerbate the human health issues, potentially setting a negative feedback loop in motion. Integrative strategies that exploit the reverse positive nature of the aforementioned feedback loop have huge potential. Green prescribing (Box 1) is one example of an integrative strategy that aims to support the nature–human relationship, and this will now be discussed in greater detail.

Box 1. A definition of a green prescription.

Green Prescription:

A prescription for a monitorable activity that involves spending time in

natural environments for the benefit of human health and wellbeing.

2.6. Green Prescriptions: An Integrative Approach

It is perhaps important to note here that although the use (or at least the popularisation) of the term 'green prescription' has been a recent occurrence, the fundamental principles of nature-based therapies have been applied for centuries. For example, the Romans (influenced by the Greeks) constructed spa baths at thermal springs to enhance health and wellbeing (Pérez et al. 2017). Furthermore, to treat the symptoms of disease in the times of Hippocrates (460–370 BC) a “change of habits and environment was advised, which included bathing, perspiration, walking, and massages” (van Tubergen and van der Linden, 2002, p. 1). In Germany a similar practice called the *Kur* (cure or treatment) was also influenced by the Roman practice of therapeutic bathing, and became mainstream by the 1700s (Maretzki, 1987). The *Kur* is now “part of a complex system of rehabilitation medicine utilizing medical bathing and environmental stimuli” (Maretzki, 1987, p. 1). Recent evidence (including the use of cortisol as a biomarker) points to the use of spas/*Kur* as an effective intervention for managing stress-related disorders (Antonelli et al. 2018). These treatments have also been associated with improvements in a number of conditions, from rheumatic and musculoskeletal diseases, to circulatory and nervous disorders (Antonelli et al. 2018).

A green prescription (in its contemporary form), also known as a nature-based health intervention, is an emerging, innovative strategy that is designed to improve physical and mental health and wellbeing through exposure to, and often multisensorial interaction with natural environments (Bragg and Leck, 2017). A regular walk through a green space, systematic participation in gardening (horticultural therapy), and/or the undertaking of biodiversity conservation activities, such as habitat creation and restoration, are all potential examples of green prescribing activities (Bragg and Leck, 2017; Jepson et al. 2010). They can be viewed as a monitorable activity that involves spending time in natural environments for the benefits of health and wellbeing.

Green prescriptions are typically designed for patients with a defined need, and they have the potential to supplement orthodox medical treatments, particularly those that are aimed at addressing noncommunicable diseases and mental health issues (Bragg and Leck, 2017). Furthermore, it could be argued that green prescriptions could provide a reactive (health care) and a proactive (health promoting) solution to public health issues. Indeed, this dual approach could potentially materialize through contributions to improving the health of patients whilst promoting a healthy lifestyle and supporting the development and maintenance of health-promoting infrastructure.

The term 'green prescription' or 'GRx' was first coined by health professionals in New Zealand in the late 1990s to underscore a range of physical and dietary activities that aim to enhance the health and wellbeing of patients, and reduce NCDs such as Type-2 diabetes (Patel et al. 2011). In this initiative, GPs provide patients with diet plans and strategic physical activities such as 'progressively increasing' steps, monitored with pedometers and follow-up telephone counselling (Kolt et al. 2009). The term has since been broadened to include nature-derived activities (e.g., horticulture, nature walks, biodiversity conservation) as part of a holistic and integrative approach aimed at addressing NCDs, mental health issues and social isolation.

It is acknowledged that the foundations of green prescribing often require the interactions of three main phenomena—natural environments, a social context and meaningful activities (Bragg and Leck, 2017). However, there is still a significant amount of research to be undertaken to answer a number of fundamental questions

(Figure 2), such as what kinds of nature-based interventions (or elements of these) work best for whom, where and when?

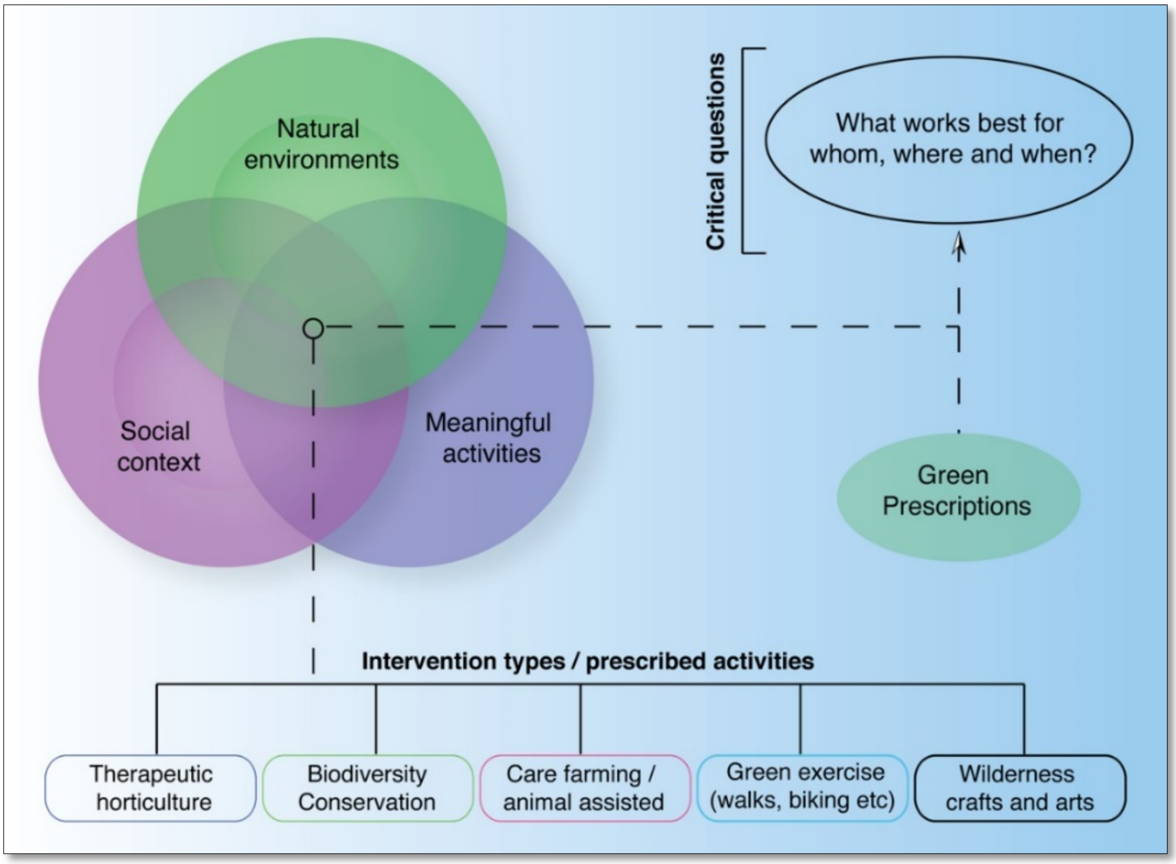

Figure 2. The interaction of the social context, natural environments and meaningful activities, and the potential types of nature-based interventions (created by the authors, partially adapted from Bragg and Leck, 2017).

Jepson, Robertson and Cameron (2010) and Bragg and Leck (2017) highlight the range of potential activities that may constitute a green prescription, and these include:

- therapeutic horticulture;
- biodiversity conservation;

- care farming (the use of farming practices for health, socialising and education);
- green exercise (e.g. nature walks, biking, climbing);
- wilderness arts and crafts

Many of the activities are suitable for a diverse range of users. Taking biodiversity conservation-based green prescriptions as an example, the associated tasks (often group-focused) are typically aimed at restoring, conserving and enhancing the diversity of flora and fauna. Whilst it is recognised that biodiversity conservation activities are not suitable for everybody, due to ability, interest and willingness (e.g., there may be several physical, mental, social, and/or access issues to take into consideration), based on the authors' experience of attending a wide range of conservation groups, the events tend to be highly sociable and attended by a diverse cohort. Furthermore, whilst acknowledging that this may differ between groups, no apparent pressure to participate in the more physical aspects of the activities was witnessed in the groups attended by the authors. Conservation activities are considered to be highly flexible in type and timescale, providing a range of benefits to people with very different abilities and backgrounds (O'Brien et al. 2010). As with gardening equipment (Victoria State Government, 2018), conservation tools and infrastructure can also be adapted for people with disabilities.

2.7. Green Prescriptions: Potential Co-Benefits

Hitherto, variations of green prescribing have been shown to generate health, social and financial gains, but only in a limited number of studies with small sample sizes and an absence of robust control measures (Bloomfield, 2017; Poulsen et al. 2018)—

hence the emphasis on *'potential'* co-benefits. However, a large number of potential co-benefits spanning areas of health, socioeconomics, and the environment have been identified by the authors (Figure 3). Mounting evidence from a wide range of studies investigating the relationship between nature and human health supports this, and in particular, the environmental benefits (Bloomfield, 2017; Millar and Donnelly, 2013; Seymour et al. 2018; Annis et al. 2017].

By developing strategic nature-based activities, there is an important opportunity to help address public and environmental health issues—that is, through the application of a well-defined co-beneficial strategy. An example where this could be realised is the Healthy Urban Microbiome Initiative (HUMI; www.humi.site)—a multidisciplinary initiative that was recently supported by the United Nations Secretariat for the Convention on Biological Diversity (CBD; https://www.cbd.int/health/cop14/default.shtml). This initiative aims to understand and recreate the immune-boosting power of high quality, biodiverse green spaces in our cities to maximise population health benefits, bringing significant savings to health budgets while delivering gains for biodiversity (Flies et al. 2018. Although more evidence of the mechanisms linking nature to health is needed, improving and maintaining green spaces in urban areas, particularly with high levels of biodiversity, could potentially lead to important health and environmental benefits. Enhancing the diversity of the human microbiome by encouraging interactions with natural environments and their microbial inhabitants (microbiome rewilding) is one such potential route (Mills et al. 2017). Building on this theory, microbiome-inspired green infrastructure (MIGI) has recently been proposed as a collective term for the design and management of innovative living urban features that could potentially facilitate the process of microbiome rewilding in towns and cities across the planet (Robinson et al. 2018; Robinson and Jorgensen, 2019).

Green prescriptions potentially fit into this by providing an important social interface—a systematic process to enhance one's connection with biodiverse urban green spaces, which could be designed and monitored to suit the individual needs of the patients. As mentioned, biodiversity conservation volunteering is an activity that is associated with green prescriptions; therefore, the enhancement of green spaces and biodiversity across urban areas could be incorporated into a green prescribing strategy itself. Patients could contribute towards the enhancement and maintenance of biodiverse urban green spaces and MIGI, whilst improving their own health as a result of their participation.

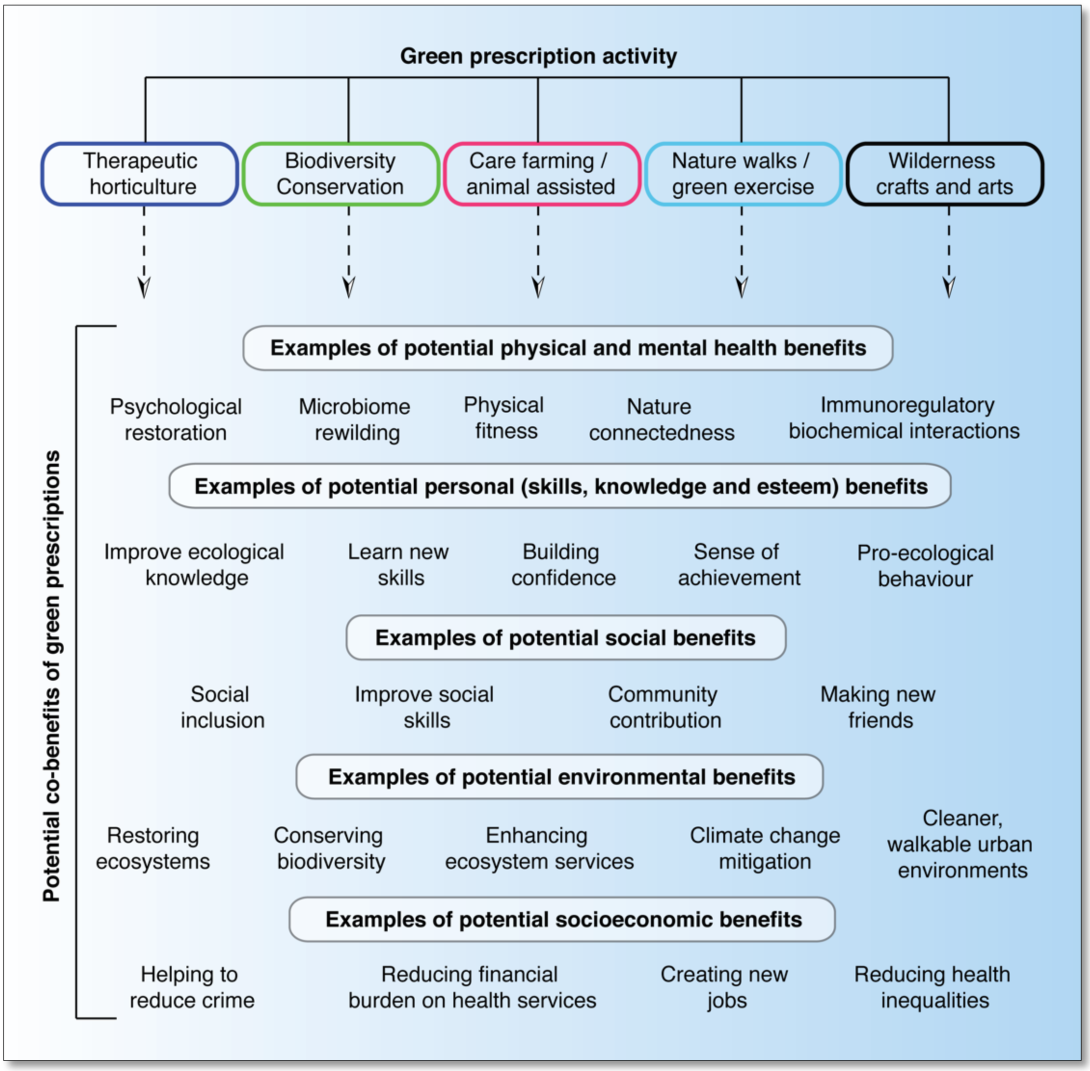

Figure 3. Potential green prescribing activities and co-benefits (created by the authors).

In addition to physical and practical aspects (such as enhancing ecosystem services), gaining ecological knowledge, social confidence and communication skills are all potential co-benefits associated with nature-based interventions. Enhanced pro-ecological behaviours and environmental stewardship are some of the key potential co-benefits that could conceivably generate positive cascading impacts on the natural environment by influencing lifestyle decisions and positive changes that benefit nature. For example, these changes could include deciding to recycle, reducing the consumption of unsustainable products, and simply being more mindful of the wider biotic community.

However, although the potential co-benefits of green prescriptions are promising, there is still a need for further rigorous and evaluative research and interventions to confirm this, and to understand the dynamic complexity of the way in which individuals experience the natural world.

As mentioned earlier, green prescriptions also have a potential financial co-benefit associated with them. Bloomfield (2017) pointed out that Mind's 'Ecominds' (a nature-based intervention programme) (Vardakoulias, 2013), reported savings per participant of £7082 via reduced costs to the NHS (p. 83). This further corroborates studies that report significant financial gains in non-nature-based social prescribing (Kimberlee, 2016; Dayson et al. 2016).

It could be argued that green prescribing is founded on holistic principles (as with social prescribing models), and so it is also important to recognise that in addition to the proposed nature-derived pathways to health, green prescribing activities are often considered 'social events', which could potentially facilitate other indirect health-inducing benefits that have a social and community focus. This, in itself is another potential co-benefit.

2.8. Challenges and Remaining Fundamental Questions

There are copious studies promoting the potential benefits of nature-based health interventions (Seltenrich, 2015; Maier and Jette, 2016) and the evidence-base supporting the broader concept in terms of the nature–health relationship is growing increasingly stronger. However, there are limited empirical studies that scrutinise the functionality and sustainability of green prescribing schemes in practice. This is a key knowledge gap that warrants further in-depth investigation, preferably based on longitudinal study designs with robust evaluation frameworks.

One study conducted recently in Cornwall, UK (Bloomfield, 2017) adopted different nature-based interventions. The different interventions included conservation activities, nature-walking and meditation. The interventions were carried out in areas defined as ‘highly biodiverse’; however, the methods for determining this were not defined, and the habitats in which the interventions took place differed and included woodland and coastal habitats, which could have affected the results. For example, the various biotic and abiotic features of a woodland may (or may not) elicit different psychological, microbial and biochemical-based health-inducing mechanisms and effect sizes when compared to coastal habitats.

In terms of practical challenges associated with green prescriptions, Bloomfield highlighted an important point—that it will be imperative for coordinators of green prescribing schemes to speak two disciplinary ‘languages’: the language of healthcare, and the language of ecology. The author suggested that unless these two languages are acknowledged, coordinators may focus on gains for their respective disciplines, which could potentially devalue the important co-benefits.

Numerous researchers from New Zealand have evaluated the views of general practitioners (GPs) in relation to green prescribing (Swinburn et al. 1997; Gribben er al. 2000). Although these studies focus on the non-nature-based form of green prescribing (physical activity and diet), they do provide some interesting insights into unorthodox prescriptions, and the constraints and barriers associated with early implementation. For example, one study found that GPs were generally very receptive to the concept of green prescribing (Swinburn et al. 1997). Another study assessed GP views following a green prescribing programme (Gribben et al. 2000). The authors identified several implementation-related constraints, namely, lack of time, perceived loss of revenue and the simplistic perception of green prescribing. However, these constraints, the authors suggested, were subsequently assisted in the wide-spread development of green prescriptions in New Zealand.

It could be argued that another key challenge facing the application of green prescribing is the way in which we currently think about public health—both in research and practice. This has recently been highlighted in a publication on biodiversity and urban healthcare initiatives, where the authors argue that we need more “place-based, preventative, wellness-focused healthcare systems that interact with urban planners, environmental managers and politicians to promote healthy urban designs and living” (Flies et al. 2018, p. 5). There is no doubt that forming multi-stakeholder collaborations and taking integrative approaches (such as green prescriptions) to public and environmental health will be challenging, but they are challenges that are worth rising to, as the coupling issues of noncommunicable diseases and environmental degradation increase.

Although green prescriptions have the potential to enhance human and environmental health, important questions are now being asked in terms of what works best for whom, where and when (Lovell et al. 2018). Answering these questions is critical if we are to gain the level of understanding that is needed to develop effective nature-based interventions that can justify the reallocation of limited resources. Rigorous evaluations are needed to identify appropriate interventions, and to understand the mechanisms and the contexts in which they are effective. There have been recent calls to take a more critical view of the 'dose of nature' approach, and to include a focus on social practices to cater for the complexities of how individuals experience and interpret nature (Bell et al. 2018). This exemplifies the importance of diversity and holism in our approaches to public and planetary health.

2.9. Future Studies

It would be prudent for researchers to continue conducting natural experiments to assess the health and social impacts of green prescriptions, and in particular—to grasp research opportunities where green prescriptions are already (or are in the process of being) implemented. For this approach to be effective, systems must be developed to maximise intervention uptake (and engage those who will benefit the most), minimise 'drop out' rates, and ensure that the green prescribing in practice is both sustainable and consistent (Husk et al. 2018). Furthermore, understanding who is currently prescribing nature-based interventions, and gaining insights into how they are funded, as well as what infrastructure/resources are needed, will play an important role in establishing productive collaborations between researchers and practitioners. By working closely with green prescription providers (e.g., environmental conservation charities) and prescribers (e.g., general practitioners), researchers have the opportunity to not only apply existing and to acquire new knowledge of the processes that influence exposure and effect, but importantly, to shape the intervention approach. This will be vital when it comes to gathering the appropriate evidence to inform the choice of intervention, and to maximise their potential co-benefits (e.g., health, environmental, social and economic).

Natural experiments are particularly important for population-based and epidemiological studies. However, studies with a clinical-style design, such as randomized control trials (RCT) will also be important for gaining a greater mechanistic understanding of the factors involved with health gains from green prescriptions. However, RCTs present a distinct set of challenges in natural environments, and they comprise less than 1% of published research in the field of environmental health [80]. As with any health-centric study, there are many potential confounding factors to consider (e.g. genetics, general health, diet, physical exercise, pets, age, and social contact), and the challenges are compounded by the complexity of the natural environment. However, with appropriate control measures and thorough consideration for these factors, RCTs are most certainly feasible.

Additional research should also be carried out to evaluate the potential economic impact of green prescribing on general medical practice. This will undoubtedly influence the sustainability of the interventions, and researchers may ask questions such as:

Can green prescriptions save medical practices money and other resources?
If so, which type of intervention is most effective?
Does this vary between geographic regions or between areas with different socioeconomic statuses?
Which patients will benefit the most from green prescriptions?

It will also be vital to gain a greater understanding of the downstream social and environmental impacts of green prescriptions. To investigate these factors, studies will likely benefit from longitudinal and mixed method designs. This further highlights the importance of establishing systems and collaborations that focus on the sustainability of nature-based (and other social) interventions.

2.10. Conclusions

Green prescriptions have the potential to contribute towards improving human health and wellbeing. As an integrative strategy, green prescriptions also have a wide range of potential co-benefits. These include environmental benefits that are generated through the involvement of patients in activities that are aimed at enhancing biodiversity and by influencing the allocation of resources to maintain green infrastructure. The concept of green prescribing could also add an important dimension to the recent calls to rewild the microbiome by establishing microbiome-inspired green infrastructure (MIGI) in towns and cities—in that it could provide an interface that helps to facilitate health-inducing interactions with biodiverse environments.

Enhancements in pro-ecological behaviours and environmental stewardship could result from participating in nature-based interventions, and this could have important longer-term positive impacts on the environment. There is also evidence to suggest that nature-based interventions can generate a range of important social and financial benefits.

However, more research is needed — particularly longitudinal studies and evaluations of interventions — in order to fully understand the mechanisms behind the interventions, and the contexts in which they are most effective. The shift in thinking towards embracing a mutually advantageous relationship with nature and a move towards place-based health interventions will be challenging, but they are challenges worth rising to in the face of increasing noncommunicable diseases and environmental degradation. The importance of taking a holistic and diverse approach to enhance public and planetary health cannot be overstated. With further research, green prescriptions could make an important contribution to this approach, whilst providing reactive (health care) and proactive (health promoting) solutions to public health.

Let Nature Be Thy Medicine: A Socioecological Exploration of Green Prescribing in the UK

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2.11. Abstract

Prescribing nature-based health interventions (green prescribing) — such as therapeutic horticulture or conservation activities — is an emerging transdisciplinary strategy focussed on reducing noncommunicable diseases. However, little is known about the practice of, and socioecological constraints/opportunities associated with green prescribing in the UK. Furthermore, the distribution of green prescribing has yet to be comprehensively mapped. In this study, we conducted a socioecological exploration of green prescribing. We deployed online questionnaires to collect data from general practitioners (GPs) and nature-based organisations (NBOs) around the UK and conducted spatial analyses. Our results indicate that GPs and NBOs perceive and express some common and distinct constraints to green prescribing. This highlights the need to promote cross-disciplinary communication pathways.

Greenspace presence and abundance within close proximity (100m and 250m) to GP surgeries (but not greenness, as a proxy for vegetation cover) and NBO presence within 5km were associated with higher levels of green prescribing provision. Lower levels of deprivation were associated with higher frequency of NBOs. This suggests that the availability of greenspaces and NBOs could be important for green prescribing provision, but there could be greater opportunities in less deprived areas. Important foci for future research should be to establish transdisciplinary collaborative pathways, efficient infrastructure management and a common vocabulary in green prescribing—with the overall aim of reducing inequalities, and enhancing planetary health.

Keywords: green prescriptions; planetary health; nature-based interventions; urban nature; biodiversity; mental health; nature connectedness; greenspace; noncommunicable diseases; upstream health interventions

2.12.Introduction

It is now widely accepted that spending time in natural or semi-natural environments (e.g., forests, grasslands, gardens and parks) can result in significant positive mental and physical health benefits (McEwan et al. 2019; Sarris et al. 2019; White et al. 2019). For example, the Japanese practice of Shinrin-yoku (森林浴) or ‘forest bathing’ has been shown to enhance innate immunity via lymphocyte cell activity and can reduce diastolic and systolic blood pressure (Ideno et al. 2017; Li, 2010); gardening can provide relief from acute stress and improve symptoms of depression (Soga et al. 2017; Clatworthy et al. 2013); and simply walking in nature can enhance psychological restoration or the ability to recover from stress (Pasanen et al. 2018; Wyles et al. 2019).

Furthermore, there is evidence to suggest that the environmental microbiome — the diverse consortium of microorganisms in a given environment — can have positive developmental and regulatory influences on the immune system and potentially anxiolytic effects (Largo-Wight et al. 2018; Sbihi et al. 2019; Deckers et al. 2019). This latter claim is supported by a recent mouse study where exposure to trace levels of biodiverse soil dust was significantly associated with reduced anxiety-like behaviours (Liddicoat et al. 2020). Natural environments can also provide important places for reflection and introspection, for cultivating feelings of awe, inspiration and freedom, and for facilitating group-based convivial activities—which could help to improve social cohesion and enhance mental health (Liddicoat et al. 2020; Bethelmy and Corraliza, 2019; Barrable, 2019; Jennings et al. 2019).

Interacting with nature for salutogenic effects is by no means a novel concept. From a Western-societal perspective, the fundamental principles of nature-based therapies can be traced back to the Hippocratic era (460-370 BC) when changing environments and lifestyle practices were advised by the physicians of the time (Fuller et al. 2007). Furthermore, the Greeks and Romans established thermal spa baths to improve health and wellbeing (Van Tubergen and van der Linden, 2002; Jackson, 1990; Vladeva et al. 2016). From a traditional ecological knowledge perspective, indigenous Australians recognised the deep connections between mental and physical health and the "land and river", and Canadian aboriginals' holistic view of health highlights the interrelatedness of human wellbeing and the environment (Gianfaldoni et al. 2017; Wheatley and Wyzga, 1997). Nonetheless, it is important to recognise that our complex societies have evolving views, social behaviours and health-related needs, and it is unrealistic to view spending time in nature as a panacea, i.e., it will not be the ‘silver bullet’ for everyone, on every occasion, and for all conditions.

However, there is growing interest in ‘green prescribing’ (GRx) as a contemporary practice of prescribing nature-based health interventions, particularly for noncommunicable diseases (Ganesharajah, 2009; Shanahan et al. 2019; Crnic and Kondo, 2019). Green prescribing builds on the earlier concept of prescribing exercise and diet-based interventions (Margoliers, 2018)—a variant that was pioneered by general practitioners (GPs) in New Zealand in the 1990s (Patel et al. 2011). It also builds on the recent social prescribing movement, which can be defined as: “*a way of linking patients in primary care with sources of support within the community – usually provided by the voluntary and community sector, offering GPs a non-medical referral option that can operate alongside existing treatments to improve health and wellbeing*” (Swinburn et al. 1998, p.7; Bragg and Leck 2017; Aggar et al. 2020).

Green prescriptions are typically administered to patients with a defined need and can be used to complement orthodox medical practices (Gearey et al. 2019; Van den Berg, 2017). Nature-based intervention activities can include therapeutic horticulture, biodiversity conservation activities, care farming (i.e., farming practices for health, socialising and education), nature walks, and social activities in greenspaces (Bragg and Atkins, 2016; Elsey et al. 2016; Husk et al. 2018)—and although the social element is often important, it is not a necessity. To establish effective and sustainable green prescribing schemes, cooperative interactions between primary care professionals and nature-based organisations (NBOs) are typically required, and the ability to speak multiple disciplinary ‘languages’ is considered an essential asset (Hart, 2016).

There is potential for green prescribing to contribute to healthcare (reactive), sustainable health promotion (proactive), while potentially bringing important co-benefits (e.g., social, environmental, and economic benefits) (Bloomfield, 2017). However, it is still an emerging and unorthodox strategy. As such, initial adoption may be sporadic and limited. In the UK, little is known about the status of (distribution and practice), and socioecological constraints and opportunities associated with green prescribing. To our knowledge, no one has explicitly mapped nationwide green prescribing services/infrastructure. To this end, mapping could be a useful policy action (e.g., for informing targeted resource allocation). Moreover, gaining insights into the perceived constraints of green prescribing from the view of primary care professionals and NBOs could help to synchronise knowledge and empathy and identify disciplinary barriers to aid in future management and delivery. Furthermore, exploring ecological, spatial and social factors that may affect green prescribing could also provide important insights for policy makers.

In this study, we conducted a socioecological exploration of the green prescribing health intervention model in the UK. We deployed online questionnaires to collect data on awareness, constraints and opportunities from general practitioners (as potential prescribers) and nature-based organisations (as potential providers) around the UK. A total of *n* = 284 respondents were included in the questionnaire analysis which consisted of general practitioners (*n* = 114) and nature-based organisations (*n* = 170).

N = 714 records were obtained from a manual web-scrape for nature-based organisations.

We also collected spatial data to estimate the general distribution of green prescribing and conducted spatial analyses using a Geographic Information System (GIS). For the spatial element, we specifically aimed to explore whether available services, geography, greenspace, and deprivation influenced green prescribing awareness, provision and constraints. The pre-existing datasets we used included Ordnance Survey's (OS – Great Britain's national mapping agency) Open GreenSpace, Indices of Multiple Deprivation (IMD), NASA Landsat 8 Imagery, and results from the web-scrape. We used a combination of parametric and non-parametric statistical tests and qualitative coding to facilitate the analyses.

2.13. Materials and Methods

2.13.1. Online questionnaire and web-scrape protocol

We formulated two online-based questionnaires; one for GPs (as potential service *prescribers*) and one for nature-based organisations (as potential service *providers*). The questionnaires included 8-10 structured questions, formulated with the aid of a pilot study and a group of GP volunteers prior to commencing the research. The questionnaires were ethically reviewed by the University of Sheffield's Department of Landscape internal review committee and by the National Health Service's (NHS) Health Research Authority (HRA); Integrated-Research Application System (IRAS) reference number: 261514.

The research questionnaires included key questions regarding geolocation, awareness and status of green prescribing, and a question to ascertain what the respondents considered to be the main constraints to green prescribing. The questions are set out in Figures A1, and A2 in Supplementary Materials.

The online questionnaires were distributed to GPs and NBOs across the UK (between March and September 2019) via an introductory email with a detailed participant information sheet, consent form and a secure link to the questionnaire. The questionnaires were hosted by the University of Sheffield's Google Forms account. Contact details for the GPs were obtained via the publicly-available NHS online contact directory (www.nhs.uk/service-search/find-a-gp) and by contacting the Clinical Commissioning Groups (CCG) directly. The protocol for approaching GPs was also ethically reviewed by the HRA.

The contact details for the NBOs obtained via a web-scraping process (web data searched and copied into a central local database) combined with an approach based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) workflow (Moher et al. 2015; Tricco et al. 2018).

To obtain a list of all the relevant organisations either currently facilitating or having the potential to facilitate green prescribing schemes in the UK, a set of relevant search terms were compiled (e.g., "Green prescriptions"; "Green care"; "Nature-based intervention"). These were then tested and refined in the Google search engine and filters were applied to include only UK results. Additionally, green prescribing activity search terms were used for each of the 100 geographic counties (subnational divisions) in the UK (Figure 1). Where possible, email contact details were obtained and geographic coordinates were acquired for subsequent GIS analysis.

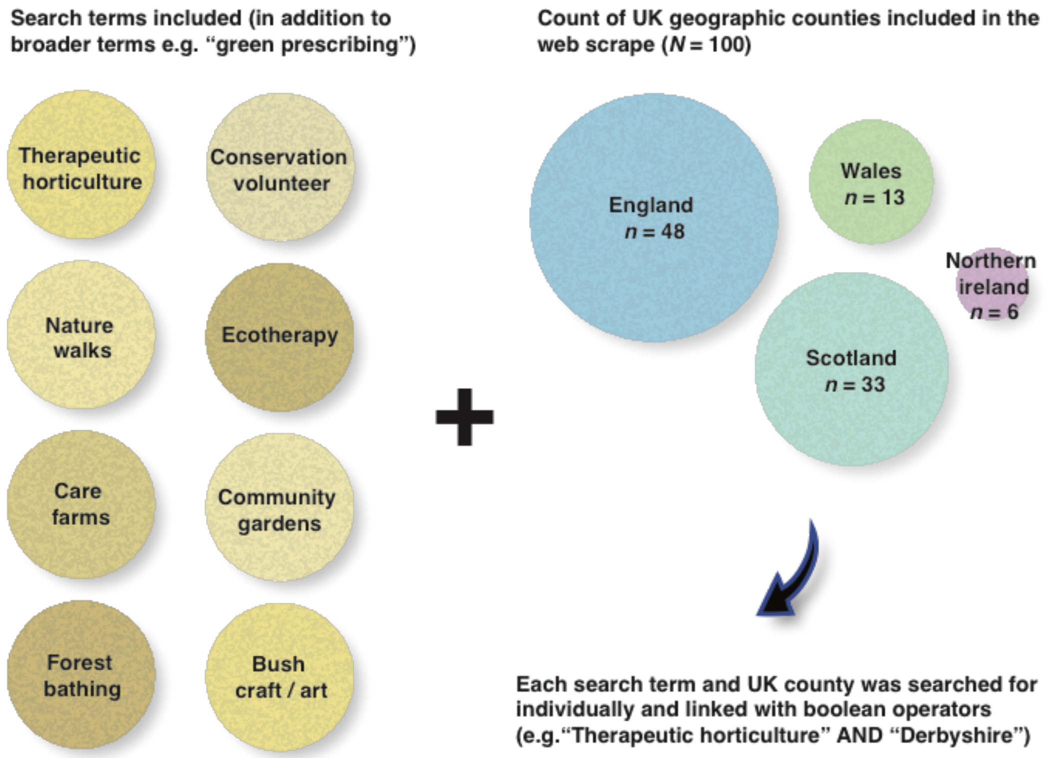


Figure 1. Green prescribing web scrape search method for nature-based organisations. Search terms are shown on the left, and a count breakdown of UK counties per country on the right.

A detailed participant information sheet and informed consent form was also provided to the nature-based organisations. Once the responses were entered and submitted, they were downloaded by the researchers in a comma separated values (.csv) format for subsequent processing and analysis. The questionnaire structure and plan for maximizing response rate was informed by references (Williams, 2003; Brace, 2018; Patten, 2016).

2.13.2. Coding of open-ended responses

For the perceived barriers question (Q.7 Figure A1 in Supplementary Materials), the open-ended response format was chosen to allow respondents to “use their own language and express their own views” (Rowley, 2014, p. 9). To classify and “clean” the data for subsequent analysis, the responses to the questions with the open-ended answer format (descriptive) were coded.

The approach to interpret these textual responses was to read through each answer several times in a spreadsheet, seeking key recurring themes. These themes specifically related to the focal topics and respondent views. A set of theme codes were generated, providing “the basis for surfacing the frequency of occurrence of themes” in preparation for subsequent quantitative analysis(Rowley, 2014, p.29). A short and perfunctory response or more in-depth response could be assigned the same code, for example, “lack of funding” and a detailed response with an obvious focus on the lack of funding would be given the code ‘Funding’ (as a key constraint to green prescribing).

2.13.3. GIS data

Once the spreadsheets containing the responses and geolocations were cleaned, they were saved as .csv files and imported into QGIS 3.4 as vectors layers. These were then converted to ESRI point shapefiles.

2.13.4. Buffer analysis

The point files were separated into four categories, as follows: “Yes” to green prescribing provision (responses from GPs); “No” to green prescribing provision (responses from GPs); “Yes” to green prescription facilitation (responses from NBOs); “No” to green prescription facilitation (responses from nature-based organisations). Using vector geoprocessing tools, circular buffer zones (radii from central coordinate of GP surgery or NBO) of 50m, 100m 250m, 500m, 1km and 5km, were then created around each point to facilitate spatial analyses (Figure 2). These radii have been used in several spatial studies involving the built environment, urban green spaces and human health (Browning and Lee, 2017; Houston, 2014; Hochadel et al. 2006).

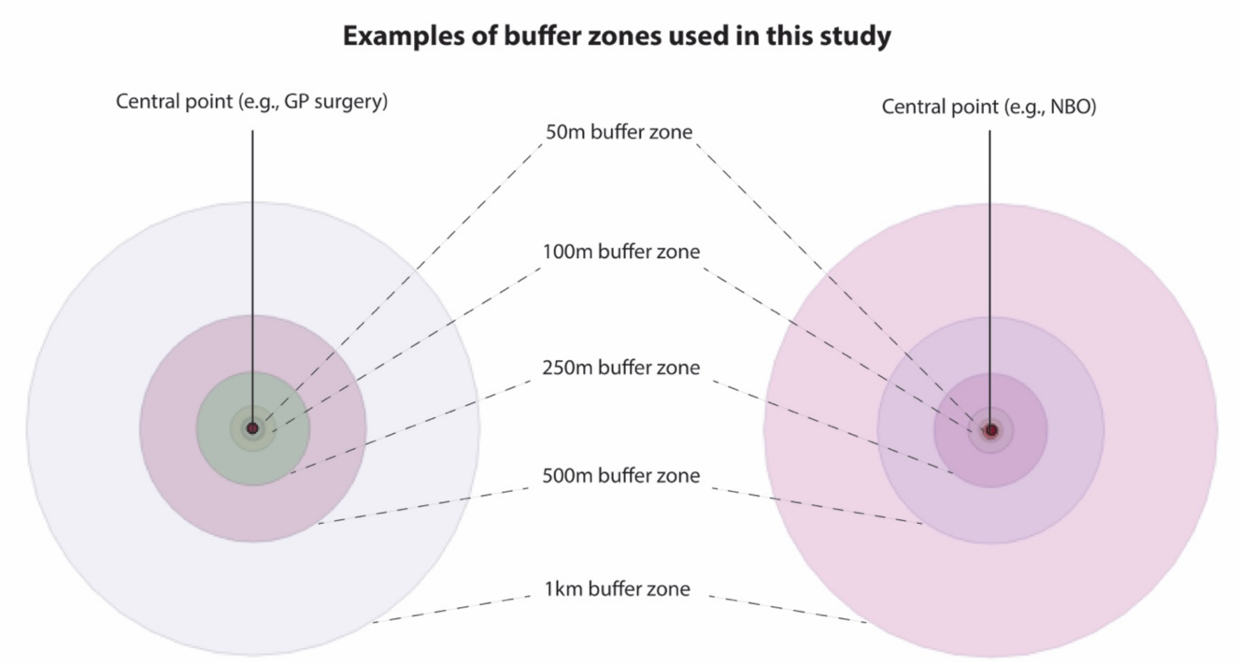


Figure 2 Example of buffer zones created around each point file containing attribute data (spatial information and questionnaire responses) for GPs and nature-based organisations in the UK.

2.13.5. Graduated symbology

To provide map outputs and descriptive statistics of the web-scrape results, UK county boundary datasets were obtained from UK government sources (e.g., https://ckan.publishing.service.gov.uk/dataset and https://opendatani.gov.uk/dataset). Green prescribing activity attributes were then joined 'by location' to the county boundary datasets using vector data management tools. The symbologies were subsequently graduated and classified to provide a visual representation of quantitative differences in values using defined colour ramps.

2.13.6. Landscape / environmental datasets

To analyse aspects of greenspace and infrastructure, the OS Open Greenspace dataset (a comprehensive dataset of publicly accessible urban greenspaces) was imported into QGIS as a polygon vector layer with a point layer for greenspace access locations. These datasets have been used in several urban greenspace studies (Mears et al. 2019; Dennis et al. 2018).

A measure of greenness (mean greenness for each buffer zone) was also calculated using NASA Landsat 8 Imagery (30m resolution), isolating spectral bands 4 (Red) and 5 (Near Infrared) and applying the equation for the Normalised Difference Vegetation Index (NDVI). This process provides a score of estimated landcover greenness, where -1 represents no greenness and 1 represents high levels of greenness—used as a proxy for vegetation cover. The equation to obtain this metric is as follows:

$$\frac{Near\ Infrared\ Light - Red}{Near\ Infrared\ Light + Red}$$

Using the Raster algebraic expression calculator, the above equation was applied to the two spectral band layers i.e., Red and Near Infrared (NIR). The resulting outputs were subsequently rendered into a single band pseudocolour and represented using a RdYlGn (Red-Yellow-Green) colour ramp.

2.13.7. Deprivation data

To explore relationships between green prescribing and deprivation, quintile scores from an Index of Multiple Deprivation (IMD) dataset previously adjusted for each UK country was used (Abel et al. 2016). IMD data have been used in several greenspace epidemiology studies (Southon et al. 2018; De Keijzer et al. 2019; Coldwell and Evans, 2018). The IMD provides multivariate data on relative deprivation in Lower Super Output Areas (LSOAs) for England, Wales and Northern Ireland and Data Zones layers for Scotland (Figure 3). LSOAs are boundary areas containing an average population of approximately 1,500 and up to 1,000 in Data Zones. These geographic boundaries have been used in similar socioecological studies (Brindley et al. 2019; Mears et al. 2019; Houlden et al. 2017).

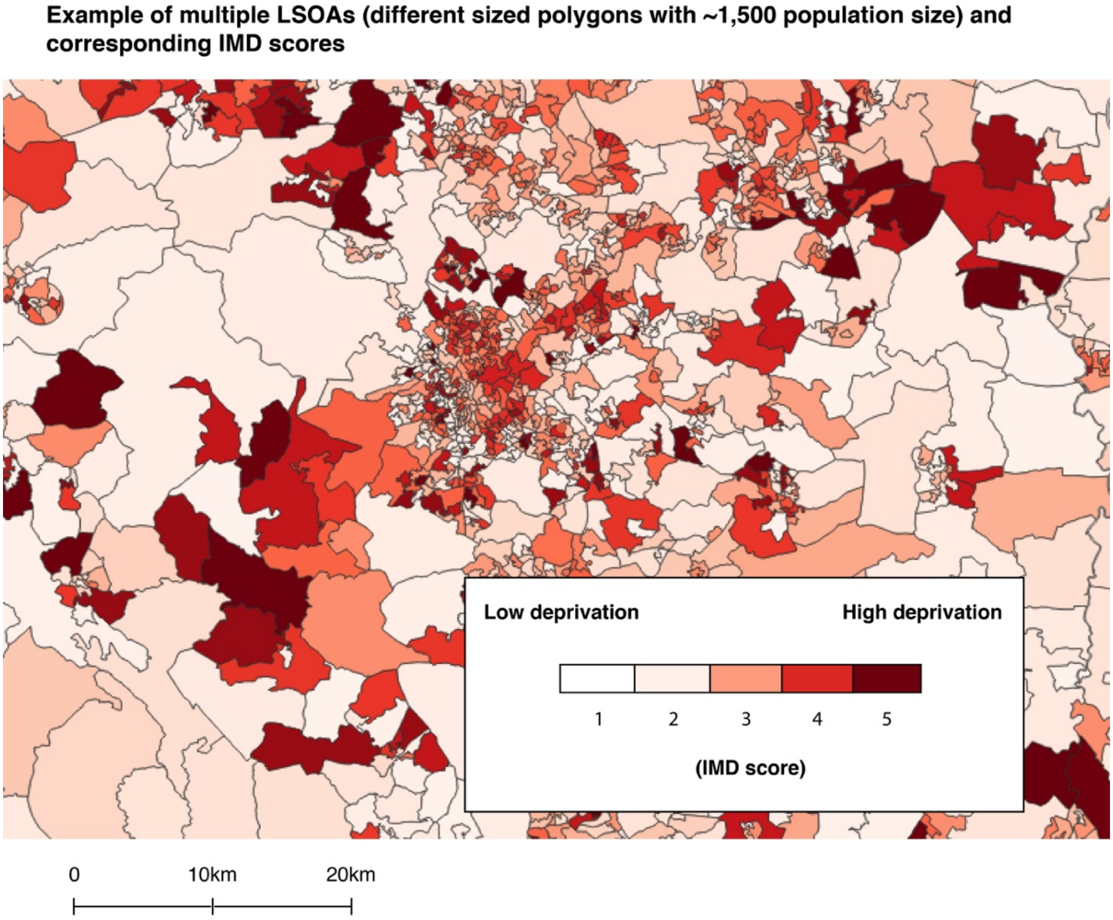


Figure 3. Example of LSOAs (boundaries) with IMD scores using ‘categorised’ symbology in QGIS.

2.13.8. Spatial and statistical analyses

To facilitate quantitative analysis and maximise UK-wide representation, the aim was to acquire *n* = 367 responses from GPs based on an approximate population size (of UK GP practices) of 8,000 (RCGP, 2019) with a 95% Confidence Level and 5% Margin of Error. For NBOs, the aim was to acquire a sample size of *n* = 251. This was based on the *n* = 714 results from the web-scrape with 95% Confidence Level and 5% Margin of Error.

To facilitate quantitative analysis of potential relationships between the presence or absence of green prescriptions and the independent variables (e.g., greenspaces; deprivation etc.), the ‘Yes’/’No’ questionnaire responses for Question 3 (i.e., *“Does your GP practice provide green prescriptions?”*) were extracted and recoded to numerical binary variables, where 1 = Yes/Present; and 0 = No/Absent.

2.13.9. Landscape and environmental metrics

To determine whether the presence (and count) of greenspaces within (and touching i.e., greenspaces partially in the buffer zone were included) a certain radius of GP surgeries was associated with green prescribing provision, the OS Open Greenspace dataset and the georeferenced binary responses for Question 3 were imported into QGIS. The greenspace polygons within each buffer zone (50m, 100m, 250m, 500m, 1km and 5km) were extracted and counted using vector data management tools. The joined data were then exported to a .csv file for subsequent statistical analysis in the R statistical computing environment via the R Studio interface version 1.2.1335.

Due to the non-normal (right skew) distribution of the samples, nonparametric statistical tests were selected. A Mann-Whitney U test was conducted to explore differences between number of greenspaces within 100m and 250m of the GP surgeries that provided green prescribing vs GP surgeries that did not provide them (500m and 1km radii were excluded due to no relationships for these ranges, and the 50m buffer was excluded due to an absence of greenspaces within this radius).

For the NDVI analysis, firstly we reprojected the vector (buffer) layers to match the coordinate reference system (CRS) of the Landsat 8 raster files and then calculated the mean NDVI values for all buffer zones using the zonal statistics raster analysis tool

(Figure 4). The updated attribute table was exported as a .csv file for subsequent statistical analysis.

Once the mean NDVI scores were calculated, a binomial logistic regression model was used to predict whether mean NDVI (a representation of greenness) in each buffer zone had a significant influence over the binary dependent variable (where 1 = “Yes” to represent the GPs that do provide nature-based interventions; and 0 = “No” to represent the GPs that do not provide nature-based interventions).

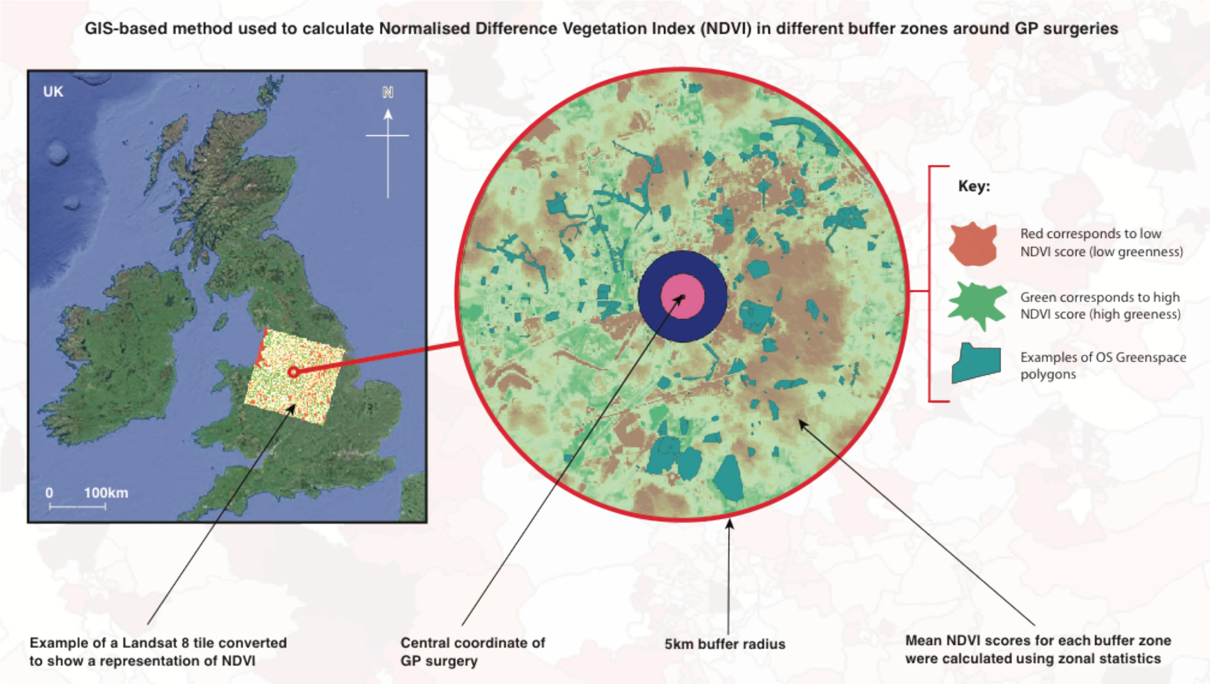


Figure 4. Example of buffer zones around GP surgeries with a visual representation of the NDVI in the background (where red is closer to -1 and green is closer to 1). The mean values within these buffers was calculated and exported for further analysis. The whole of the UK was overlaid with the NASA Landsat 8 tiles to facilitate NDVI calculations.

2.13.10. Deprivation

For the analysis of deprivation, UK quintile scores from 1 (lowest deprivation) to 5 (highest deprivation) were extracted from the adjusted IMD dataset. These scores were joined to each LSOA and Data Zones and used for subsequent analysis. To explore whether deprivation influenced the provision of nature-based interventions, Mann-Whitney U tests were conducted. This approach was suitable for comparing IMD scores between the four variables (GPs that did and did not prescribe GRx; and NBOs who did and did not provide GRx).

To test whether a relationship existed between levels of deprivation and NBO presence, we joined the web-scrape results for NBOs with the UK IMD and boundary datasets. We subsequently conducted Chi Sq (X2) tests to compare expected vs. actual observations. This test provided what the probability was that differences in values (frequency of observations) are by chance under the assumption of independence.

2.13.11. Nature based organisation presence and GRx provision

We also tested whether presence of NBOs was associated with provision of GRx by GP surgeries. For this element we explored the potential incidence of the NBOs confirming GRx facilitation (from the questionnaire responses) and also data from the web-scrape of NBOs (n = 714). We used a Mann-Whitney U test and a 2-sample test for equality of proportions.

2.14. Results

2.14.1. Descriptive statistics

A total of *n* = 284 respondents completed the research questionnaire. The number of GPs participating in the study was *n* = 114. Confidence Level and Margin of Error for this sample size are 95% and 9%, respectively. For NBOs (from *n* = 714 identified by the manual web-scrape), a total of *n* = 170 responded. Confidence Level and Margin of Error for this sample size are 95% and 6.6%, respectively. The majority of responses came from England-based practices and organisations.

2.14.2. Results from the questionnaire (presence/absence of green prescription provision)

Based on the count of questionnaire responses by GPs, *n* = 29 GPs did prescribe nature-based interventions and *n* = 85 GPs did not. In terms of NBO responses, *n* = 131 did provide (i.e., facilitate activities) nature-based interventions and *n* = 39 did not (Figure 5).

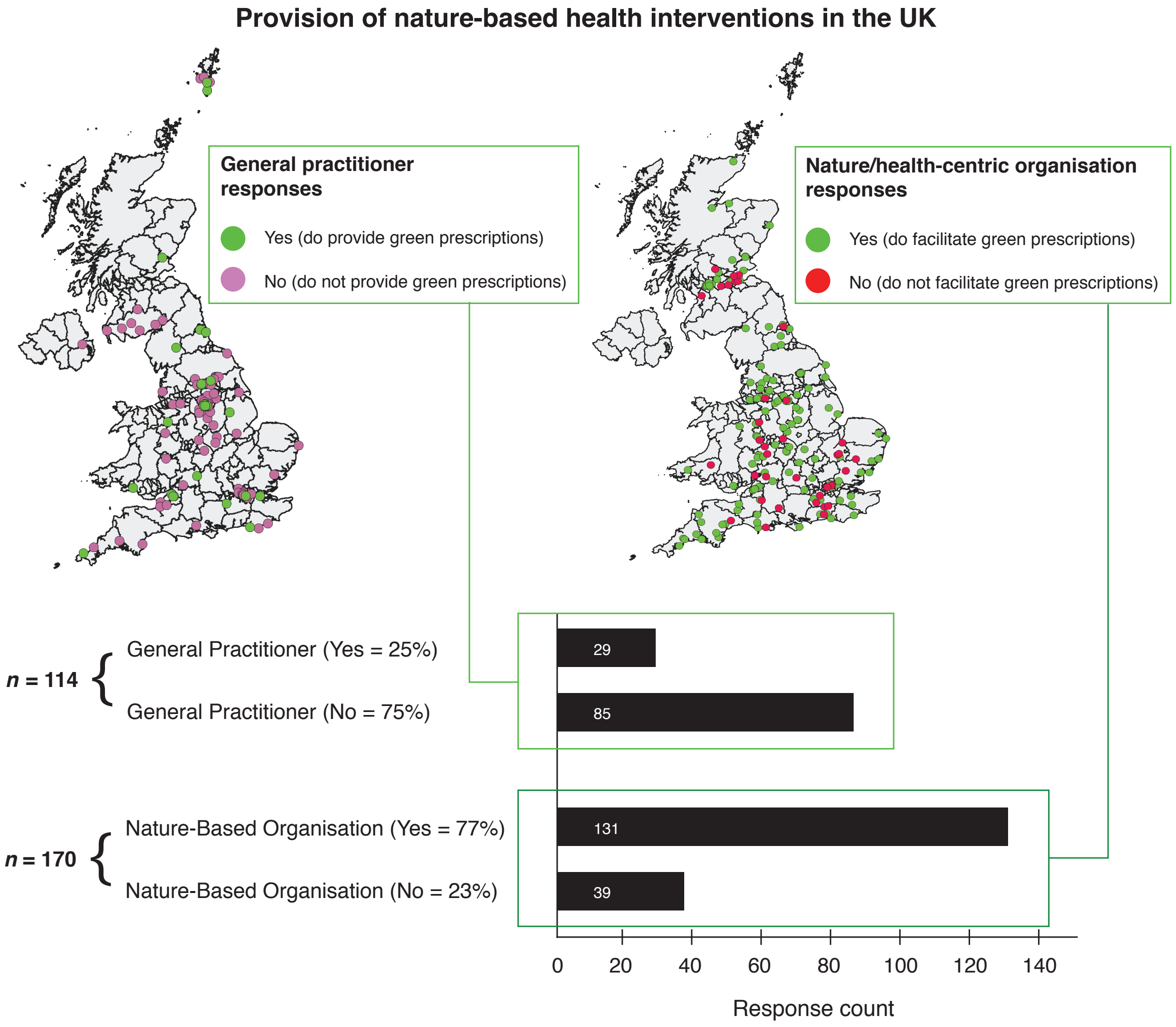


Figure 5 Provision of nature-based health interventions (green prescriptions) in the UK based on the questionnaire responses. This figure shows the location and distribution of responses to the question "*Does your GP surgery provide green prescriptions*" (or similar question for nature-based organisations).

2.14.3. Results from the coding of the perceived constraints question (for GPs)

The results of the analysis of what GPs perceive as key constraints to green prescribing showed that ‘available services’ (organisations and processes that facilitate nature-based interventions) was mentioned the most frequently by GPs (*n* = 33). Funding for the service and awareness of the green prescribing concept were also frequently mentioned (*n* = 31 and *n* = 29, respectively). However, we are unable to confirm whether ‘awareness’ refers to GPs, patients or both.

Time constraints (*n* = 25) (note: there is an assumption here that this refers to GP time), ‘know-how’ (i.e., knowledge of how to set up a green prescribing service) (*n* = 24), patient motivation (and confidence to attend the interventions) (*n* = 20), and having the appropriate resources to provide a green prescribing service (this could overlap somewhat with time and funding) (*n* = 13) were also mentioned by several GPs (Figure 6).

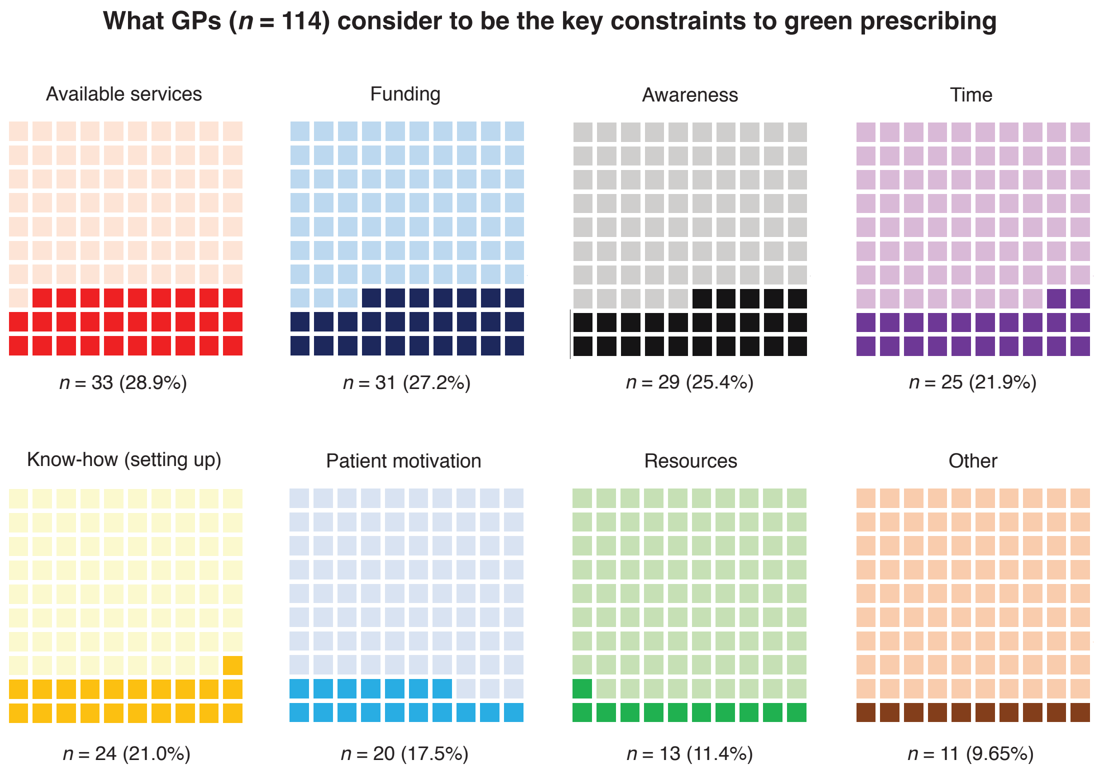


Figure 6. Waffle charts showing what GPs consider to be the key constraints to green prescribing. These charts show proportions with actual response counts and corresponding percentages below. The proportions are presented in descending order (i.e., of response frequency) from top left to bottom right.

2.14.4. Results from the coding of the perceived constraints question (for nature-based organisations)

The results of the analysis of what NBOs perceive as key constraints to green prescribing showed that funding (i.e., the organisations typically have small financial budgets) was the most frequently mentioned constraint (*n* = 86). Awareness and understanding of the benefits of spending time in nature were also conveyed as important constraints several times by NBOs (*n* = 30 and *n* = 38, respectively). It is likely that these constraints are aimed at GPs and potentially also patients as the responses suggest that, in general, NBOs are aware of the potential benefits.

Distinctively NBO-based themes included engaging GPs (*n* = 33) (some respondents suggest it is difficult to "*engage the NHS at all levels, and disseminating information through the NHS can* [also] *be difficult",* and GPs are *"not able or willing to green prescribe"),* greenspace access (*n* = 11) (e.g., landowner permission, transport costs, but also some people are "*house bound*"), green prescribing 'referrals' which could be synonymous with engaging GPs (*n* = 9), and 'evidence' to support benefits of green prescribing (*n* = 11) (some respondents feel there is still not a strong enough evidence base to persuade health professionals to engage in the interventions) (Figure 7).

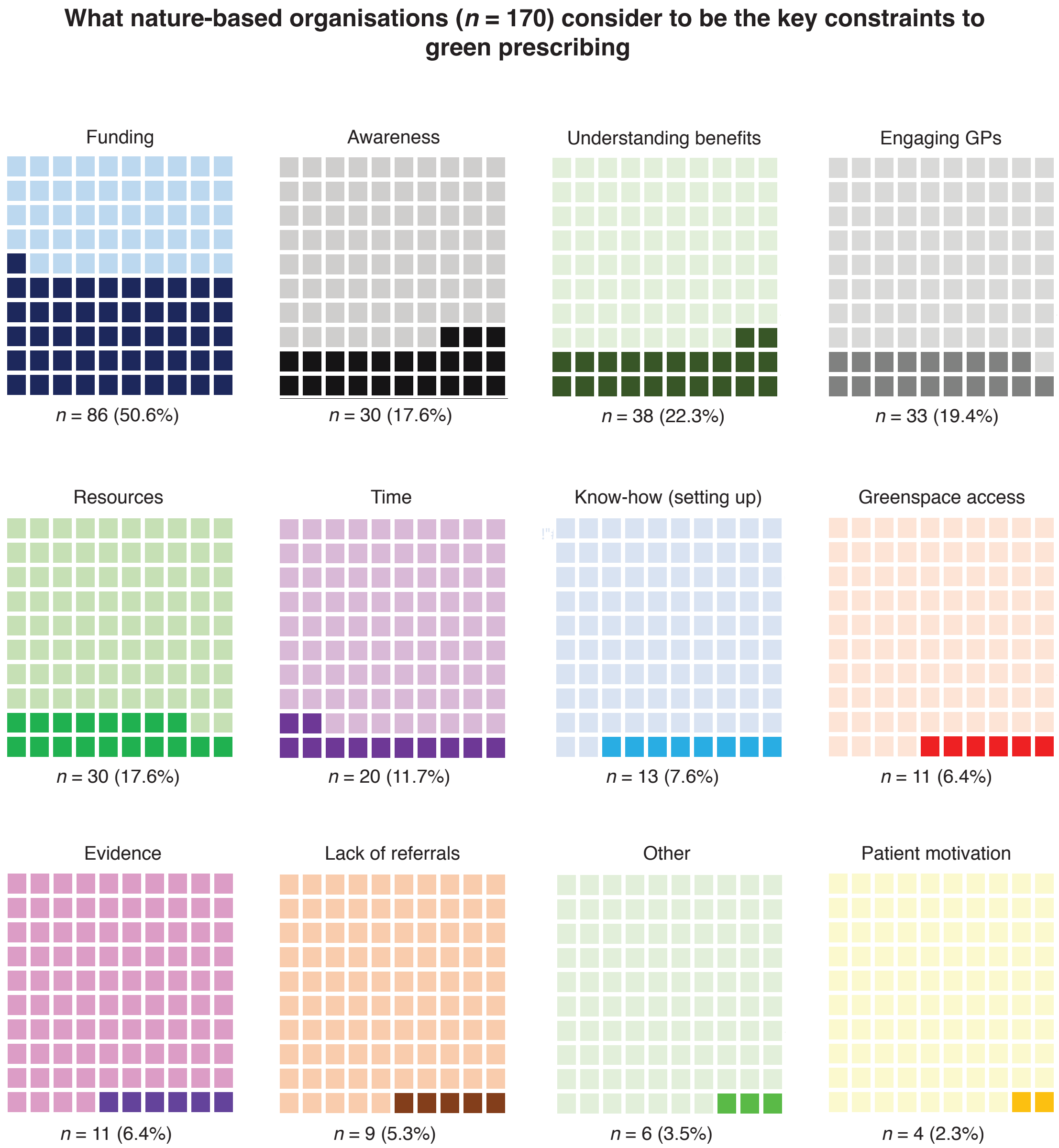
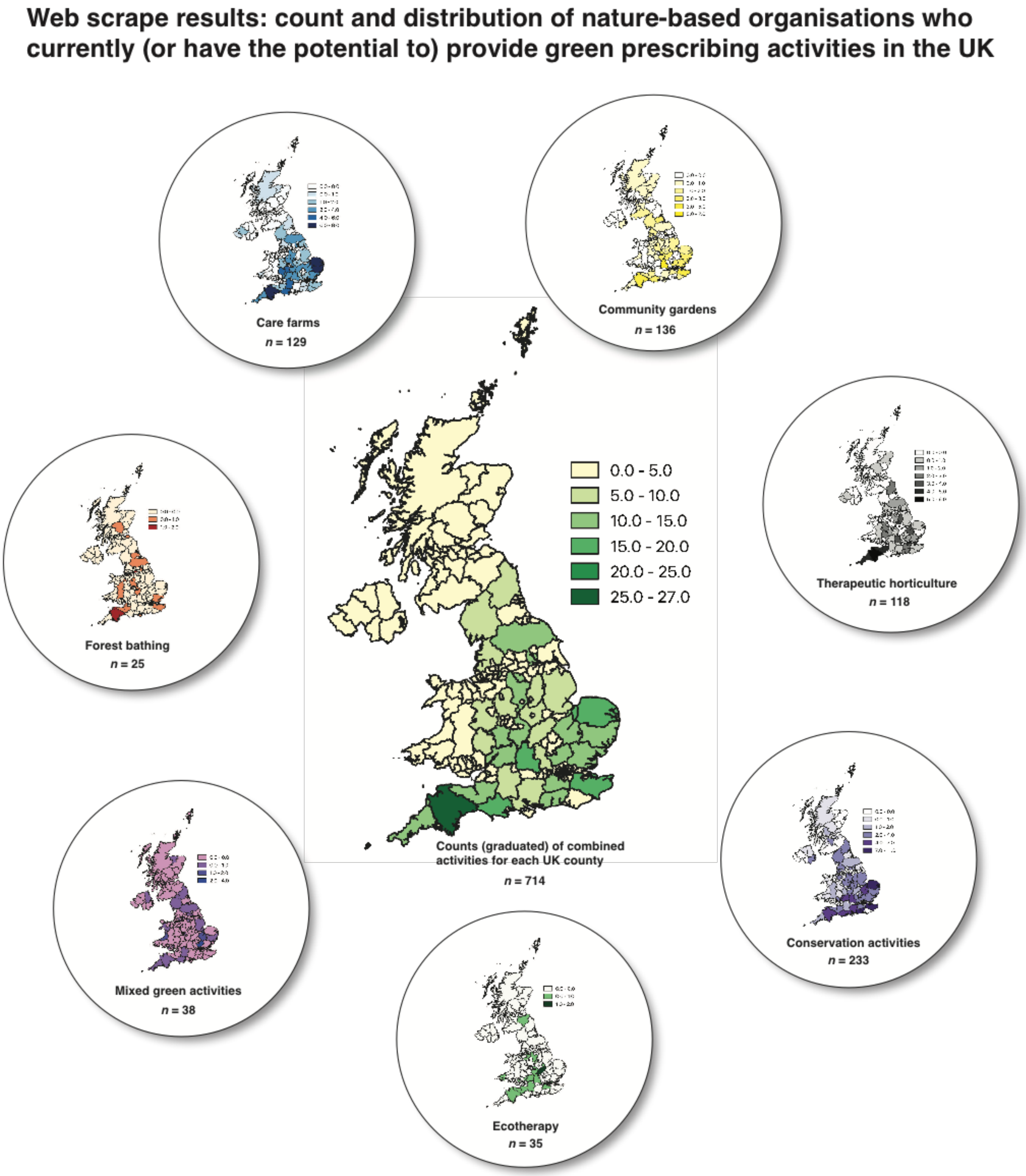


Figure 7. Waffle charts showing what nature-based organisations consider to be the key constraints to green prescribing. These charts show proportions with actual response counts and corresponding percentages below. The proportions are presented in descending order (i.e., of response frequency) from top left to bottom right.

2.14.5. Results from the web-scrape process (for nature-based organisations)

The web-scrape resulted in the acquisition of *n* = 714 NBOs who either provided green prescribing activities or had the potential to do so based primarily on organisation/service type. These fall into seven themes including Care farms (*n* = 129), Community gardens (*n* = 136), Therapeutic horticulture (*n* = 118), Conservation activities (*n* = 233), Ecotherapy (*n* = 35), Mixed green activities (such as bush crafts and walks; *n* = 38), and Forest bathing (*n* = 25) (Figure 8).



Web scrape results: count and distribution of nature-based organisations who currently (or have the potential to) provide green prescribing activities in the UK

Figure 8. UK map of counties showing count (*n* = 714) and distribution of nature-based organisations which currently (or have the potential to) provide green prescribing activities (inlets show counts/distribution for individual activities). The quantitative differences in values are presented using graduated symbology and an appropriate colour ramp. This was processed in QGIS.

Conservation activities/organisations returned the highest number of records (*n* = 233) and forest bathing the lowest (*n* = 25). There are clear differences between the number of advertised NBOs in England (i.e., more abundant) compared to Northern Ireland, Scotland and Wales. Zero records were returned for several UK counties (e.g., Kincardineshire in Scotland), whereas *n* = 27 records (the highest number) were returned for Devon in the southwest of England.

2.14.6. Results from spatial and inferential statistical analyses

The following section presents the results from both the spatial analyses conducted in QGIS using landscape/environmental and sociological (deprivation) datasets, and the statistical analyses carried out primarily in the R statistical computing environment.

2.14.7. Landscape and environmental metrics

The data for greenspace presence within different buffer zones around GP surgeries, were found to have non-normal (right skew) distributions. Therefore, nonparametric tests were used for statistical analysis. We conducted a Wilcoxon rank sum test with continuity correction and found that mean greenspace abundance within 100m of group 1 (GPs prescribing nature-based interventions; $\bar{x} = 1.17$) was significantly different (greater) to the same radius for group 2 (GPs not prescribing nature-based interventions; $\bar{x} = 0.51$) (W = 853, *p* = 0.005) (Figure 9).

A 2-sample test for equality of proportions also confirmed that a greater proportion of GPs who prescribed nature-based interventions had a greenspace present within (including partial intersect) 100m radius (17 out of 29 or 58.68%) compared to those who did not (31 out of 85 or 36.4%) (X-squared = 5.05, df = 1, *p* = 0.047). The same analysis but for greenspaces fully within the 100m radius buffer (6 out of 29 or 20.68%) compared to those who did not (4 out of 85 or 3.4%) also resulted in statistically significant differences (X-squared = 5.05, df = 1, *p* = 0.02).

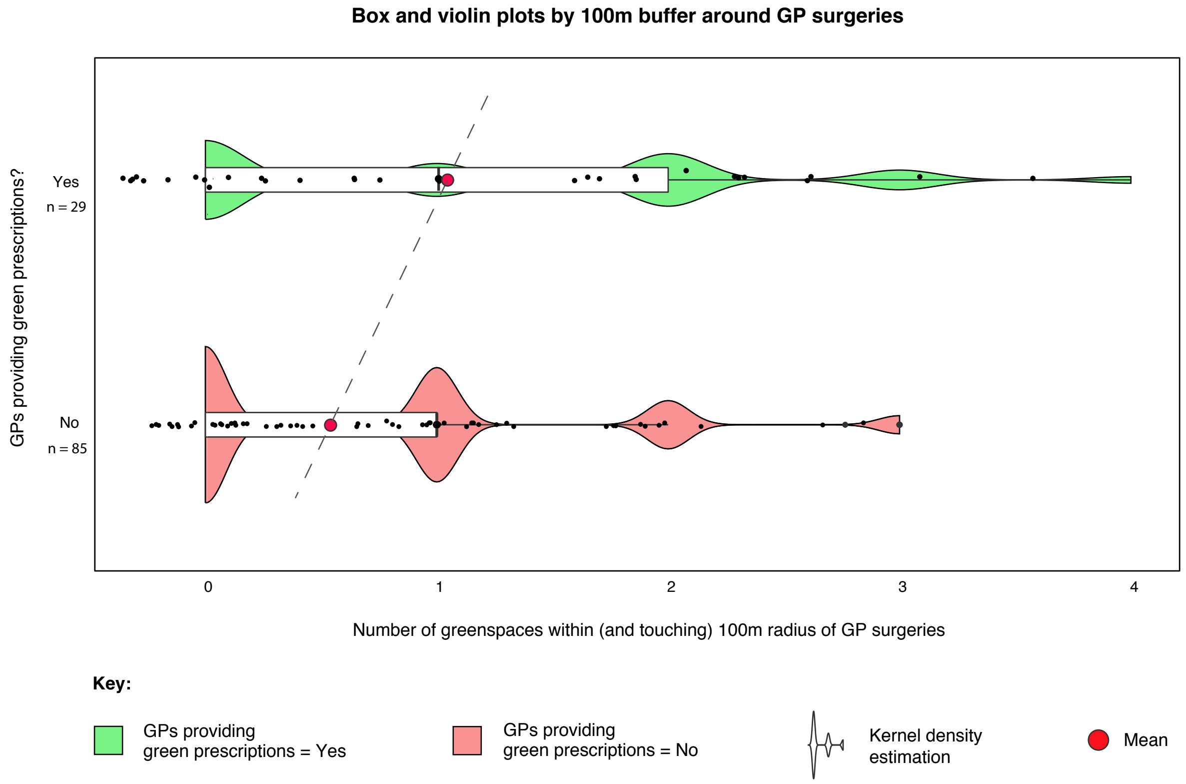


Figure 9. Boxplot showing differences in greenspace abundance within the 100m buffer zone around GP surgeries that did (green) and did not (red) prescribe nature-based interventions. The maximum number within 100m of any practice was *n* = 4. The violin plots show kernel density estimation i.e., representing the distribution shape of the data and the points have a small amount of random variation (jitter) to reduce over-plotting.

The types of greenspace within the 100m buffers are presented below in Table 1. We further explored the 'type' of greenspaces around this 100m radius, and used Google Street View (GSV) as a manual confirmation tool. Following GSV Public park or garden analysis, it was also discovered that in four of the 100m buffers for GPs that did prescribe GRx, there were additional large greenspaces (public parks, *n* = 2; sports field, *n* = 1, and scrub/grassland, *n* = 1) not registered in the OS Open Greenspace dataset, and only one additional greenspace (sports field, *n* = 1) within 100m of GPs that did not prescribe GRx (highlighted with asterisks). These additional greenspaces were included in the aforementioned analysis.

Table 1. A list of greenspace type within 100m buffer radius of GPs that do and do not prescribe GRx.

Type of greenspace	**Number in 100m of GRx = "Yes" *(n* = 29)**	**Number in 100m of GRx = "No" *(n* = 85)**
Playing field	5	6
Other sports facility	5	3
Play space	3	6
Cemetery	1	1
Allotment or community garden	3	5
Religious grounds	7	8
Public park or garden	6	10
Bowling green	1	1

Tennis court	1	1
Golf course	0	1
Public park*	1	0
Sports field*	0	1
Grassland/scrub*	1	0

A 2-sample test for equality of proportions confirmed that in terms of greenspace *presence* within 250m radius of GPs who prescribed nature-based interventions (23 out of 29 or 79.3%) compared to those who did not (69 out of 85 or 81.1%) there was no significant difference (X-squared = 1.78e-30, df = 1, $p = 1$). However, we conducted the Wilcoxon rank sum test with continuity correction on the 250m buffer and found that mean greenspace *abundance* within 250m of group 1 (GPs prescribing nature-based interventions; $\bar{x} = 3.69$) was significantly different (greater) from the same radius for group 2 (GPs not prescribing nature-based interventions; $\bar{x} = 2.74$) (W = 524, $p = 0.013$) (Figure 10).

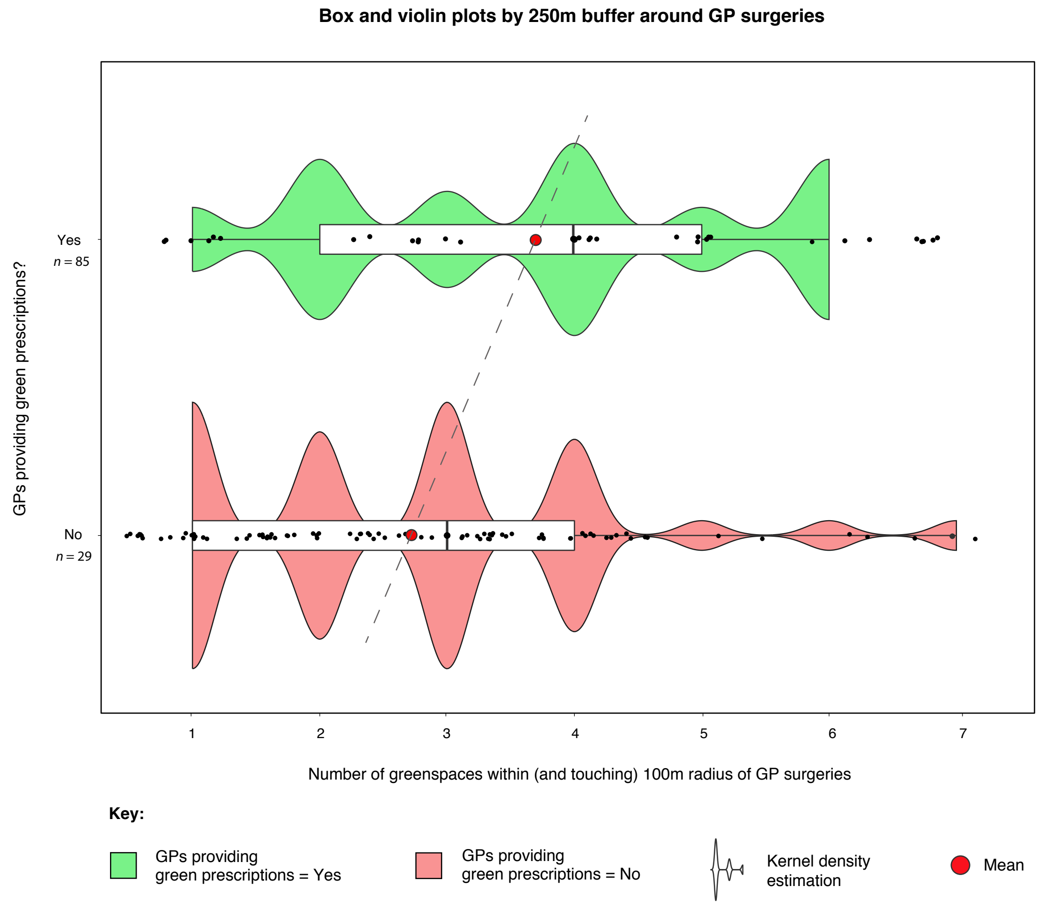


Figure 10. Box and violin plot showing differences in greenspace abundance within the 250m buffer zone around GP surgeries that did (green) and did not (red) prescribe nature-based interventions.

Table 2. Greenspace abundance for all buffer radii between 100m-5km (50m excluded due to data deficiency) around GP surgeries.

Radius	**Total greenspaces**	**Mean**	**Median**	**Standard deviation**
100m GRx Yes	34	1.17	1	1.12
100m GRx No	85	0.51	0	0.81
250m GRx Yes	85	3.69	4	1.66
250m GRx No	188	2.72	3	1.49

500m GRx Yes	239	8.24	8	3.80
500m GRx No	554	6.50	6	3.50
1000m GRx Yes	602	20.70	21	11
1000m GRx No	1669	19.60	19	9
5000m GRx Yes	8120	280.00	297	210
5000m GRx No	19936	234.00	190	209

Initial indications suggested that greenspace abundance was higher for the remaining radii, however, these failed to reach statistical significance. For example, greenspace abundance within 5km of the GP surgeries that prescribed nature-based interventions ($\bar{x}$ = 280) was higher compared to areas (within 5km) where GP surgeries did not prescribe nature-based interventions ($\bar{x}$ = 234). However, following a Wilcoxon rank sum test with continuity correction, these failed to reach statistical significance (W = 1044, $p = 0.22$).

For the NDVI analysis, the mean NDVI values (within 50m and 100m buffer zones) where GPs prescribed nature-based interventions were higher ($\bar{x}$ = 0.095 and $\bar{x}$ = 0.098, respectively) compared to the same radii where GPs did not prescribe nature-based interventions ($\bar{x}$ = 0.085 and $\bar{x}$ = 0.086) (Figure 11). However, we generated a binomial logistic regression model for these parameters, and the differences were shown to be non-significant (GLM, $p = 0.539$ for 50m; $p = 0.497$ for 100m).

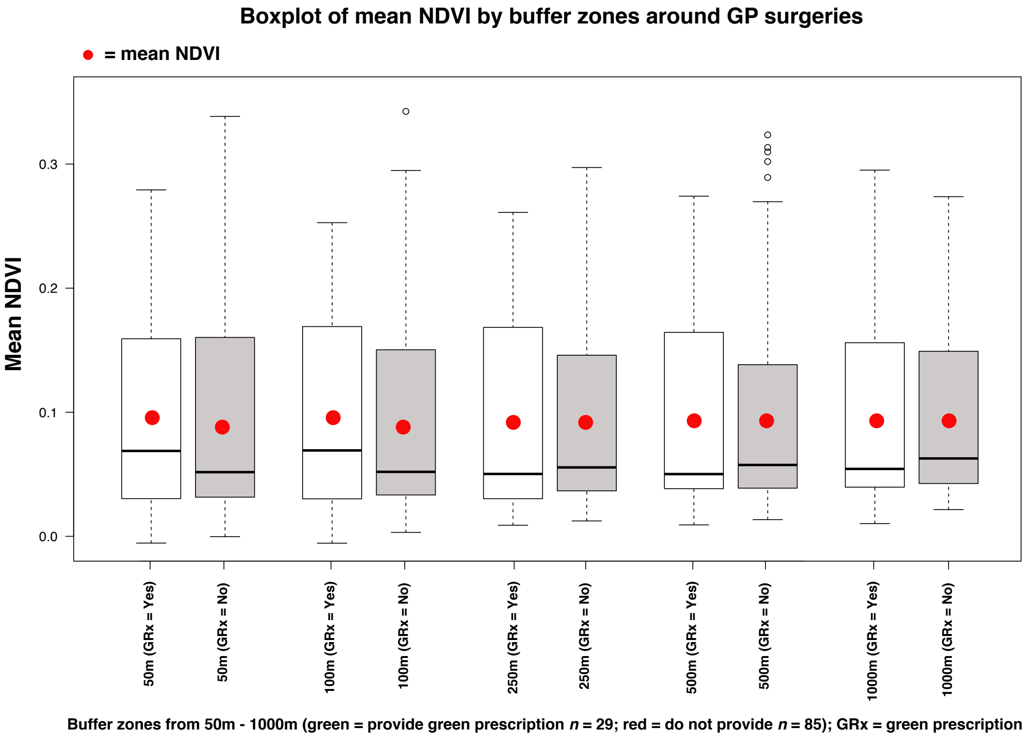


Figure 11. Boxplots showing mean NDVI scores for each buffer zone (50m – 1000m) around GP surgeries that either did prescribe nature-based interventions (GRx = Yes) or did not (GRx = No).

2.14.8. Deprivation analyses

Mean IMD scores for areas (LSOAs) where GPs did prescribe GRx ($\bar{x} = 3.58$) were higher than mean IMD scores for areas where GPs did not prescribe GRx ($\bar{x} = 3.18$). However, based on the results of a Wilcoxon rank sum test with continuity correction in R, these were not statistically significant (W = 1339, $p = 0.1703$).

When analysing NBOs from the web-scrape (a combination of confirmed and unconfirmed GRx providers; $n = 714$) we found significant differences in the frequency of NBOs between areas with different levels of deprivation ($X^2 = 35.36$, df = 4, $p = 3.71966E\text{-}07$) (Figure 12). For sensitivity analysis, we also converted IMD quintile scores 1 and 2 into a "Low" deprivation category, and quintile scores 4 and 5 into a "High" deprivation category, which confirmed statistically significant differences ($X^2 = 4.4$, df = 1, $p = 0.035$) (Figure 13). This test calculated what the probability was that the difference in values (frequency of observations) was by chance under the assumption of independence.

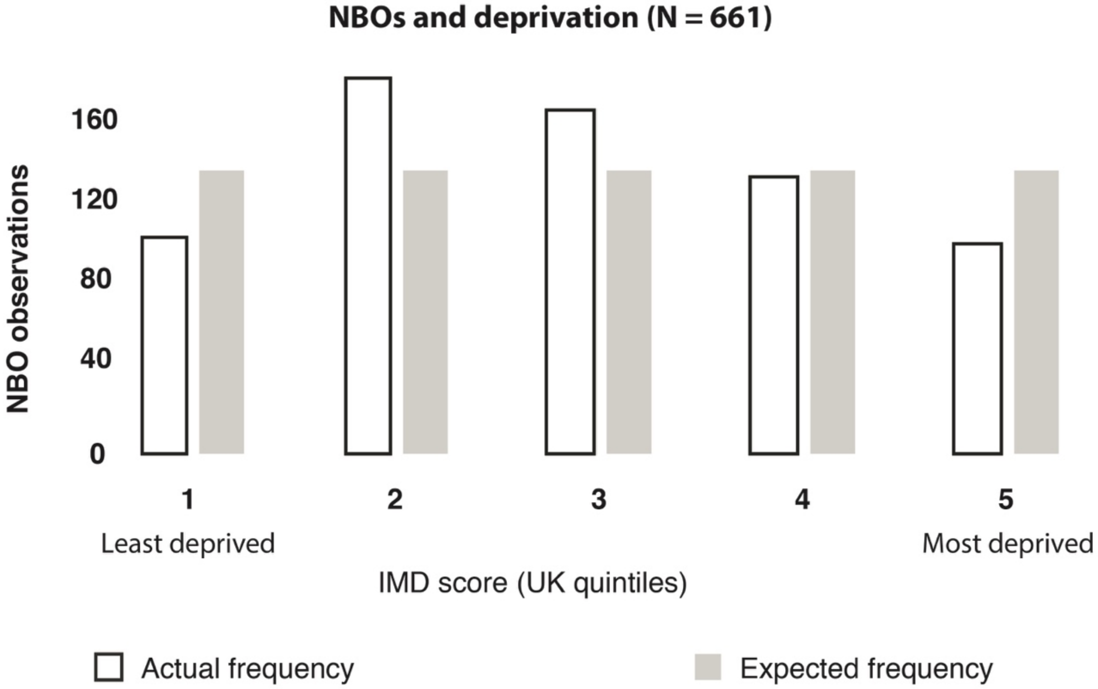


Figure 12. Output of X^2 results: The frequencies of NBOs were significantly different between areas with different levels of deprivation (based on UK IMD quintile scores), where 1 = least deprived and 5 = most deprived. Note, *n* = 53 NBO records contained zero IMD data.

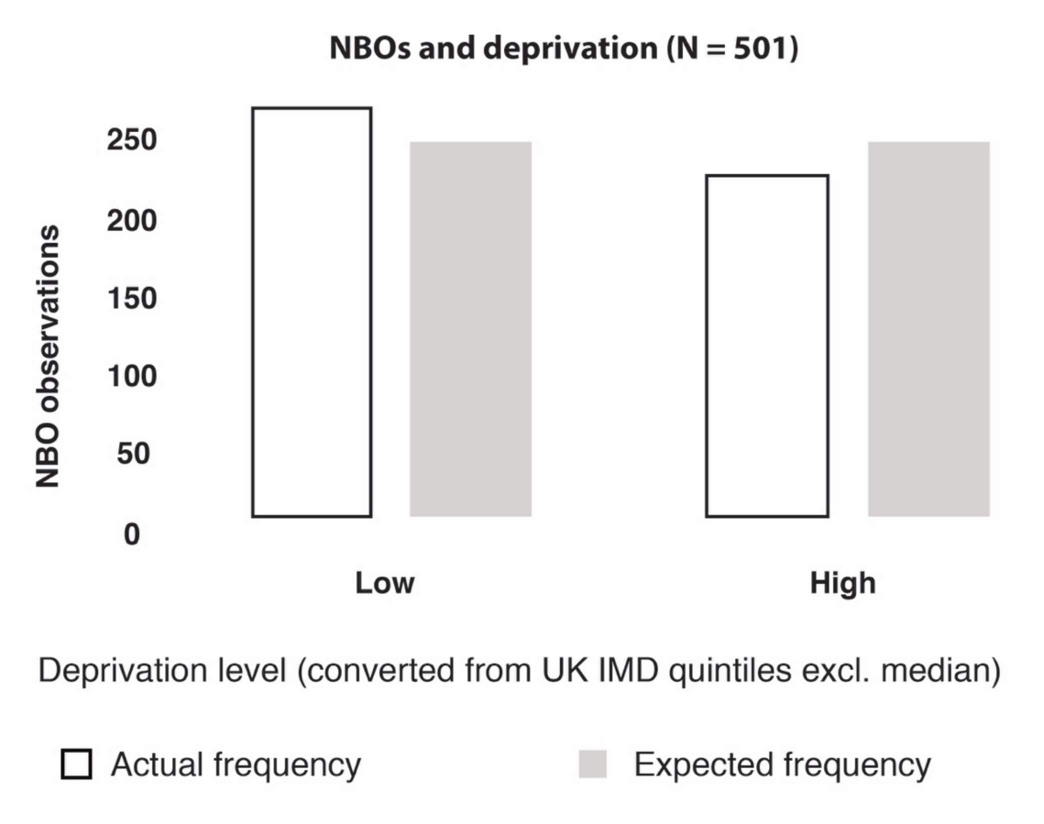


Figure 13. Output of X^2 results: the frequencies of NBOs were significantly different between areas with low and high levels of deprivation (based on converting UK IMD quintile scores into Low and High deprivation categories).

2.14.9. Geographical presence of NBOs (confirmed and unconfirmed GRx providers)

There were more likely to be NBOs who did provide GRx activities present within 5km of GP surgeries that did prescribe nature-based interventions (14 out of 29 or 48.3%) compared to GP surgeries that did not prescribe nature-based interventions (22 out of 85 or 25.8%). This was confirmed by a 2-sample test for equality of proportions (X-squared = 4.0355, df = 1, *p*= 0.04455).

When including all NBO records acquired by the web-scrape (a combination of confirmed and unconfirmed providers; $n = 714$), the mean number of NBOs ($\bar{x} = 2.7$) within 5km of GP surgeries prescribing nature-based interventions ($n = 29$) was greater than the mean number of NBOs within 5km of GP surgeries not prescribing nature-based interventions ($\bar{x} = 1.7$; $n = 85$). However, this difference was not statistically significant (W = 1481, $p = 0.09187$).

2.15. Discussion

In this study, we aimed to contribute to the growing but still limited knowledge base underlying green prescribing (i.e., prescribing nature-based health interventions) as a practical service. To this end, we mapped green prescribing services in the UK, explored spatial and socioecological relationships, and acquired the views from both GPs — as potential *prescribers* — and NBOs — as potential *providers*.

A diverse suite of studies now supports the concept that spending time in nature can improve one's health and wellbeing (Rogerson et al. 2020; Song et al. 2019; Garrett et al. 2019), and calls have been made to integrate nature-based and social prescribing into public health strategies (Hunter et al. 2019; La Puma, 2019; Husk et al. 2019). There is also growing advocacy to support holistic integrative strategies such as green prescribing to enhance planetary health (through co-benefits to humans and the environment) (Nelson et al. 2019; Cook et al. 2019). However, there is limited understanding of the current status of (awareness and distribution), and socioecological relationships and constraints associated with green prescribing as a practical model of healthcare. An improved understanding of this could aid the optimization of management strategies and spur further research to overcome the constraints.

Our study confirms that green prescribing is active in numerous areas of the UK. We mapped some of the potential prescribers (GPs) and providers (NBOs), and acquired a diverse list of nature-based activities across the UK via a comprehensive web-scrape. With additional collaborative input, this latter process could form the basis of an expandable/editable database to allow primary healthcare professionals to search for local nature-based organisations and services that could support their patients.

Our results suggest that GPs and NBOs perceived and expressed some common but also distinct constraints to green prescribing. Some of the common constraints included a shortfall of funding and time, and a lack of awareness of the green prescribing concept. The constraint most frequently expressed by GPs was not funding but the perceived lack of available services (i.e., organisations to support patients in engaging with interventions). Interestingly, a key constraint expressed by NBOs was the inability to engage with GPs and other primary care professionals. This disharmonic perception exemplifies the importance of establishing transdisciplinary collaborative pathways that are time-efficient, and a common vocabulary in the area of green prescribing. Alongside the research that is needed to gain a greater understanding of the interventions themselves (as evidence may be lagging behind practice) (Husk et al. 2019; Leavell et al. 2019), additional action is needed to improve the infrastructure management required to connect the different stakeholders (e.g., primary and social care, NBOs and patients) and to establish effective referral and monitoring processes—with personalised approaches in mind. In the UK, the recent formation of primary care networks (PCNs) (networks of practices that serve 30,000-50,000 patients) — and the provision of funding to employ 'social prescribers' — could provide an important opportunity for early integration of green prescribing and could stimulate support for the additional research that is needed.

It is widely accepted that greenspaces have an important role to play — ecologically and socially — in supporting personal, community and planetary health (Barbosa et al. 2007; Larson et al. 2016; Wood et al. 2017; Flies et al. 2017). Furthermore, greenspaces are a fundamental resource (e.g., the archetypal setting) for GRx activities (Barton et al. 2012; Buckley et al. 2016; Peacock et al. 2007; Razani et al. 2016). The significant association between greenspace presence and abundance within 100m and 250m radius of GP surgeries and the likelihood of providing green prescriptions was an interesting finding. This prompts a suite of additional questions such as: does the presence of local greenspaces influence the decisions by the GPs to prescribe GRx, or the decision by patients to enquire about GRx? Is the presence of greenspaces an indication of potential GRx activities in the area, and as such, does the availability of services equate to increased GRx provision and vice versa i.e., does the lack of available services/infrastructure equate to limited GRx provision? Another of our findings suggests that significantly more NBOs were present within 5km of GP practices that did prescribe GRx. This implies that the presence of available services could indeed affect the provision of GRx, however, further research is needed to verify this. Promisingly, collaborative networks involving medical authorities and nature-based organisations are increasing in presence and activity (e.g., the Centre for

Sustainable Healthcare; www.sustainablehealthcare.org.uk). Providing more support for these kinds of networks on locals scales would likely bring considerable value.

Other future pertinent questions include: does surrounding greenspace influence the decision of eco-centric GPs (who may be more likely to prescribe GRx) to move to a given practice? Does the presence of greenspace reflect the socioeconomic status of an area, and does this increase the likelihood of GRx provision? And what element/s of the greenspace are important (e.g., size, type, quality, greenness, biodiversity)? We have made an initial contribution towards understanding this latter point, i.e., our results suggest that greenness (based on mean NDVI calculations for different buffer radii around GP surgeries) may not be a significant factor. Further research into the quality of greenspaces may be beneficial and there are several dimensions that could be explored, such as: maintenance, biodiversity, aesthetics, accessibility and the presence of facilities (De Vries et al. 2013; Akpinar, 2016; Zhang et al. 2017).

Studies have suggested that less deprived areas have a much higher prevalence of voluntary organisations than more deprived areas (Clifford, 2012; Mohan and Bennett, 2019). Considering that the majority of NBOs fall into the voluntary sector category, our results echo these previous studies and support the calls for governments, local authorities and also the NBOs themselves, to help secure ecological justice and provision of resources in areas of greatest need.

Nonetheless, it is positive to see the initial indication of no significant differences between provision of GRx in areas of low and high deprivation—however, the small sample size calls for a cautionary approach to interpretation. Equitable access to high quality greenspaces is likely to be important for personal and planetary health, and should therefore be a primary goal of health-centric urban policies (Braubach et al. 2017). If green prescribing is to play a key role in future healthcare strategies — alongside research that is needed to personalize these strategies — additional research into infrastructure management is needed to strengthen transdisciplinary collaborations. Further research into how local greenspace accessibility and quality may influence GRx would be beneficial, as would research that further scrutinises the equitable status of GRx resources.

2.16. Limitations

Our study has some important limitations to consider. For example, the relatively small sample size for the questionnaire element means that our findings should be interpreted with caution — particularly in the realm of representativeness (for both the significant and non-significant results). Our questionnaires did not reach all of the GP practices in the UK due to ethical and hierarchical issues, and the lack of a comprehensive list of contacts. Secondly, the results of our study are correlational, and as such, more conclusive evidence is required to infer causation for any of the findings. Thirdly, our list of NBOs from the web-scrape process is highly unlikely to be an exhaustive list of these organisations in practice. The records only represent NBOs that are sufficiently advertised (with appropriate search engine optimization e.g., the inclusion of relevant keywords) and have an active web presence. We were unable to isolate the intended stakeholder for ‘awareness’ category in the questionnaire (i.e., whether this refers to GP, patients or both). There are several categories in the questionnaire results for perceived constraints that may have a degree of overlap – for example, “funding” and “resources” may overlap, as may “engaging GPs” and “lack of referrals”. However, these were considered to not significantly affect the interpretation the results. “Ecotherapy” is also a vague category from the web-scrape that could include several of the other activities.

2.17. Conclusions

We have shown that green prescribing is happening in numerous parts of the UK. We created GIS-outputs to highlight (based on the questionnaire results) the distribution of GPs that did prescribe nature-based interventions and the GPs that did not. We also plotted where NBOs facilitated green prescribing activities and where they did not, and we provided a comprehensive distribution map of NBOs (i.e., those with an online presence) via the web-scrape process. Our results suggest that GPs and NBOs perceive and express some common but also distinct constraints to green prescribing. Greenspace presence (but not greenness) and abundance within close proximity (100m and 250m) to GP surgeries and NBO presence within 5km were associated with higher levels of green prescribing provision. Lower levels of deprivation were associated with a higher frequency of NBOs but not with higher levels of green prescribing provision.

We hope that mapping green prescribing resources, acquiring views from GPs and NBOs, and conducting spatial/socioecological analyses will spur further research in this area. Establishing transdisciplinary collaborative pathways and a common vocabulary in the area of green prescribing would no doubt bring immense value, as would additional research on personalised interventions. Action is needed to improve infrastructure management, particularly strategies that optimise stakeholder connectivity, referral mechanisms and monitoring processes. Further research into how local greenspace accessibility and quality may influence green prescribing could also bring value. Green prescribing has the potential to make an important contribution to personal and planetary health, but more support and research is needed to initiate, optimize and sustain these strategies.

CHAPTER 3

NATURE-BASED INTERVENTIONS: NATURE'S ROLE IN SUPPORTING HEALTH DURING THE COVID-19 PANDEMIC

> *"Acting on behalf of life transforms. Because the relationship between self and the world is reciprocal. As we work to heal the earth, the earth heals us"*
>
> *– Wall-Kimmerer, 2013*

3. PUBLICATION IN CHAPTER 3:

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Nature's role in supporting health during the COVID-19 pandemic: A geospatial and socioecological study

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3.1. Abstract

The COVID-19 pandemic has brought about unprecedented changes to human lifestyles across the world. The virus and associated social restriction measures have been linked to an increase in mental health conditions. A considerable body of evidence shows that spending time in and engaging with nature can improve human health and wellbeing. Our study explores nature's role in supporting health during the COVID-19 pandemic. We created web-based questionnaires with validated health instruments and conducted spatial analyses in a geographic information system (GIS). We collected data (*n* = 1,184) on people's patterns of nature exposure, associated health and wellbeing responses, and potential socioecological drivers such as relative deprivation, access to greenspaces, and land-cover greenness. The majority of responses came from England, UK (*n* = 994). We applied a range of statistical analyses including bootstrap resampled correlations and binomial regression models, adjusting for several potential confounding factors. We found that respondents significantly changed their patterns of visiting nature as a result of the COVID-19 pandemic. People spent more time in nature and visited nature more often during the pandemic. People generally visited nature for a health and wellbeing benefit and felt that nature helped them cope during the pandemic. Greater land-cover greenness within a 250 m radius around a respondent's postcode was important in predicting higher levels of mental wellbeing. There were significantly more food-growing allotments within 100 m and 250 m of respondents with high mental wellbeing scores. The need for a mutually-advantageous relationship between humans and the wider biotic community has never been more important. We must conserve, restore and design nature-centric environments to maintain resilient societies and planetary health.

3.2. Introduction

The 2020 COVID-19 pandemic has brought about unprecedented changes to human lifestyles across the world. This includes considerable disruptions to urban mobility patterns and social interactions (Kleinschroth and Kowarik, 2020; Venter et al. 2020).

In many countries, governments have imposed ‘lockdowns’ and other ‘social distancing’ restrictions to reduce the transmission and spread of the SARS-CoV-2 virus and prevent the collapse of health services (Atalan, 2020; Batlle-Bayer et al. 2020). However, evidence suggests that these social restrictions are associated with higher rates of negative mental health outcomes such as depression, insomnia (Rossi et al. 2020), suicidal ideation (Every-Palmer et al. 2020), and anxiety (Benke et al. 2020).

Although not a panacea, the importance of spending time in and engaging with natural environments such as parks and woodlands for physical and mental health is well documented. For example, Shinrin-yoku (森林浴) or ‘forest bathing’ has been shown to reduce blood pressure and anxiety (Yau and Loke, 2020). Urban nature supports mental health and wellbeing (Birch et al. 2020), and access to a garden is associated with higher levels of wellbeing (de Bell et al. 2020). Furthermore, green spaces can harbour diverse microorganisms (Robinson et al. 2020) that transfer to humans after a short period of time spent in these environments (Selway et al. 2020). Importantly, exposure to a diverse range of microorganisms from the environment can regulate the human immune system (Roslund et al. 2020).

Recent studies have demonstrated that patterns of visiting natural spaces such as urban parks and woodlands have changed as a result of COVID-19 (Heo et al. 2020; Venter et al. 2020). Other studies have called for keeping parks and green spaces accessible during the COVID-19 pandemic due to their health benefits (Slater et al. 2020; Ugolini et al. 2020). A recent study showed that participation in some nature-based activities increased (e.g., foraging, gardening, hiking, jogging, and watching wildlife), while others decreased (e.g., camping) (Morse et al. 2020). Although these studies have commented on the potential health and wellbeing benefits of engaging with nature during the pandemic, to our knowledge, none have specifically explored the multifaceted benefits on mental health and wellbeing using validated research instruments. Furthermore, no studies have explicitly investigated how socioecological factors such as deprivation, access to green spaces, and vegetation cover may influence health and wellbeing outcomes.

In this mixed-method study, we provide a comprehensive analysis of the effects of natural spaces (e.g., parks, woodlands, lakes) on self-reported health and wellbeing. We also investigate aspects of changes to patterns of nature exposure, and potential socioecological drivers of wellbeing outcomes. We use online pilot-tested questionnaires with validated wellbeing instruments including the Warwick-Edinburgh Mental Wellbeing 14-item Scale (WEMWBS) and the Perceived Stress 10-item Scale (PSS). To assess nature connectedness (one's affective, cognitive and experiential connection with the natural world) (Richardson et al. 2019; Cheung et al. 2020) we used the Nature Relatedness 6-item scale (NR-6). We also used a geographic information system (GIS) to study how socioecological factors including deprivation, presence/abundance of green spaces, and relative greenness, may affect wellbeing outcomes.

The primary objectives of this study were to: **(a)** assess whether people's patterns of exposure to nature changed as a result of the COVID-19 pandemic (and to characterise these changes); **(b)** assess whether nature provided a health and wellbeing benefit during the pandemic (and to characterise these benefits); and, **(c)** investigate whether potential health outcomes were significantly affected by socioecological factors such as deprivation, the presence and abundance of green spaces, and relative greenness.

Gaining a better understanding of how socioecological factors affect human health and wellbeing during the COVID-19 pandemic will help to inform environmental management and public health policy. This study also provides important information on how populations respond to emerging infectious disease pandemics and how we can optimise the mitigation of the associated negative impacts. This knowledge will be increasingly valuable as the number and diversity of human infectious diseases outbreaks have increased since 1980 (Smith et al. 2014). Moreover, pandemics are expected to increase in frequency in the future (Hall et al. 2020). Indeed, the projected increase in global urbanisation has the potential to augment hazardous interfaces for zoonotic pathogen exposure (Gibb et al. 2020). Therefore, we believe natural environments should be conserved and restored on a global level, but also maintained and promoted at the urban/community level to support health and wellbeing in the face of emerging pandemics.

3.3. Methods

3.3.1. Study design and participants

3.3.1.1. Digital questionnaire and validated wellbeing instruments

We created a web-based research questionnaire using the Smart Survey online platform (Smart Survey, 2020). The questionnaire included 52 multi-format questions (Supplementary Materials, Link S1) aimed at measuring different aspects of mental wellbeing and nature connectedness. To measure wellbeing, we used the 14-item Warwick-Edinburgh Mental Wellbeing Scale (WEMWBS) (Tennant et al. 2007; Trousselard et al. 2016; Smith et al. 2017). Between April and July 2020, we asked participants to answer questions regarding their wellbeing in recent weeks, as well as in the weeks prior to the COVID-19 pandemic. The WEMWBS includes 14 items, on a 1–5 Likert scale relating to perceived state of mental wellbeing (emotional and cognitive). The continuous scale was scored by summing the responses to each item answered, ranging from 14 (lowest possible wellbeing score) to 70 (highest possible wellbeing score). We measured perceived stress using the 10-item Perceived Stress Scale (PSS) (Mondo et al. 2019; Monk et al. 2020). The PSS measures the degree to which one feels stressed by evaluating coping recourses and feelings of control. We asked participants to answer questions regarding perceived stress in recent weeks, as well as in the weeks prior to the COVID-19 pandemic. The PSS includes 10 items, on a 1–5 Likert scale. The PSS scores range from 0 (lowest possible stress score) to 40 (highest possible stress score), and higher scores indicate higher levels of perceived stress. We also measured nature connectedness using the Nature Relatedness Scale (NR-6) (Nisbet and Zelenski, 2013; Kettner et al. 2019). The NR-6 includes 6 items, on a 1-5 Likert scale, and presents questions such as “I feel very connected to all living things and the earth” and “my relationship to nature is an important part of who I am”. Items were averaged, and higher scores indicated stronger subjective connectedness to nature. All of the validated instruments used in this study have been used in previous green space epidemiology studies (Stigsdotter et al. 2010; Wood et al. 2017; Soga et al. 2018). We also asked several pilot-tested questions regarding nature exposure such as duration and frequency of visits, environment type, and reasons for visits (Supplementary Materials, Link S1).

The questionnaire was ethically reviewed by the University of Sheffield's Department of Landscape Architecture's internal review committee. The questionnaire also requested key demographic information including age, gender, location (postcode), highest level of education, and occupation. The questionnaire was distributed across the world (between April and July 2020) via a secure weblink with a detailed participant information sheet, consent form and the questionnaire. We used a range of non-random sampling approaches to reach potential participants including: emailing volunteer groups, posting on social media, and undertaking a web scrape of publicly available community group directories, and emailing the group leaders. People under the age of 18 years were not included in the study (the only exclusion criterion).

3.3.1.2. Geospatial analysis

We cleaned the spreadsheet containing the responses and geolocations, imported it into QGIS 3.4 as a comma separated value (.csv) vector layer, and converted it to an ESRI point shapefile. Using vector geoprocessing tools, buffer radii of 50 m, 100 m, 250 m, and 500 m were generated around each point (respondent's postcode) to facilitate spatial analyses (Figure 1). Similar buffers have been used in previous geospatial and socioecological studies (Su et al. 2019; Robinson et al. 2020). To explore green space presence and abundance, we imported the OS Open Greenspace dataset (publicly accessible urban green spaces in the UK) into QGIS as a polygon vector layer. These datasets have been used in several urban socio-ecological studies (Mears et al. 2019; Dennis et al. 2020). Figure 1 highlights the concept of buffer and green space analysis used in this study. We also imported UK forest shapefiles (>5 ha) from the National Forest Inventory (Forestry Commission, 2020) using the same methods.

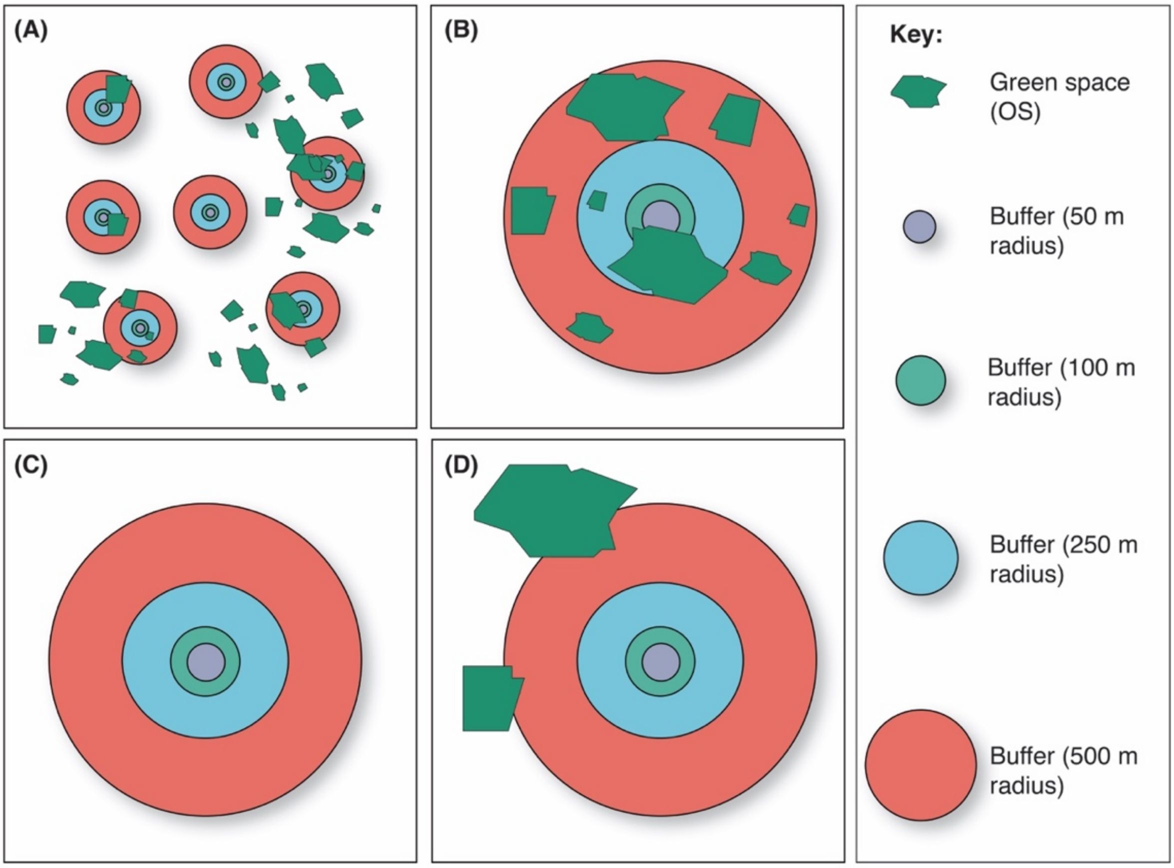

Figure 1. Buffer types and green space polygons used in the study. Green space shapefiles (green polygons) were imported into QGIS and buffer radii of 50 m, 100 m, 250 m, and 500 m were created. (A) shows an example where green space presence/abundance differs between buffer zones; (B) shows buffer zones with several green spaces within; (C) shows a buffer without any green spaces; and (D) provides an example of where green space polygons are touching the 500 m buffer but are not completely encapsulated—these would still be counted as being within this buffer zone.

To acquire a measure of mean greenness for each buffer radius, we used the Copernicus Sentinal-2 satellite imagery (10 m resolution), downloaded from the EDINA Digimap Ordnance Survey Service (Digimap, 2020). We isolated spectral bands 4 (Red) and 8 (Near Infrared) and applied the following equation for the Normalised Difference Vegetation Index (NDVI):

$$\frac{Near\ Infrared\ Light - Red\ Light}{Near\ Infrared\ Light + Red\ Light}$$

This equation provides a score of estimated land-cover greenness, whereby -1 represents a very low level of greenness and 1 represents a very high level of greenness. The greenness score can be used as a proxy for vegetation biomass and cover (Finstad et al. 2016; Tian et al. 2016). We used the algebraic expression calculator in QGIS to process the raster files (the two Sentinel-2 spectral band layers: red and near-infrared). We then calculated the mean NDVI values for all buffer zones using the zonal statistics raster analysis tool. The attribute table was then exported as a .csv file. This enabled downstream analysis in R (version 4.0.2).

3.3.1.3. Deprivation

To explore relative deprivation, we calculated quintile scores from the 2019 Index of Multiple Deprivation (IMD) dataset. The IMD has been used in a range of epidemiology and urban ecology studies (Garrett et al. 2019; Schneider et al. 2020). In England, the IMD provides an output of relative deprivation based on multivariate analysis of demographic data acquired for Lower Super Output Areas (LSOAs) (Yuan and Choudhary, 2020). LSOAs are a geographic hierarchy designed for the reporting of small area statistics. The LSOA boundaries represent an average population of approximately 1,500 and have been used widely in socioecological studies (Flouri et al. 2014; Houlden et al. 2017).

3.3.1.4. Statistical analysis

To assess proportional differences between pre/during COVID-19 patterns of exposure to nature we used 2-sample tests for equality of proportions with continuity corrections in R. We used one sample t-tests to compare differences in mean frequency of visits and duration of time spent in nature before and during the pandemic. We applied the one-way analysis of variance (ANOVA) test to determine whether socioeconomic status (based on IMD) affected the mean frequency of visits and duration of time spent in nature before and during the pandemic. A binomial regression generalised linear model (GLM) was used to explore responses to environmental preferences, and point estimates were used to indicate which environments were associated with the greatest odds for visits.

To analyse self-reported wellbeing and perceived stress, the WEMWBS and PSS scores were recoded into binary variables by division into high and lower scores. For WEMWBS, we used scores of 60+ as an indication of high wellbeing (University of Warwick, 2020). For the PSS, we used scores of 16+ as an indication of high stress (University of Wisconsin-Milwaukee, 2020). We built logistic regression models to investigate relationships between wellbeing, perceived stress and different ecological variables including green space presence and abundance, forest presence and abundance, and vegetation cover/greenness (via NDVI). An odds ratio (OR) of 1 or above means the predictor variable increases the odds of scoring a high level of wellbeing. An OR <1 means the predictor variable decreases the odds of scoring a high level of wellbeing (and the same for perceived stress). We applied model adjustments for gender, age, socioeconomic status, level of education, work/living situation, and nature connectedness. We repeated these models for each buffer area (50 m, 100 m, 250 m, and 500 m).

We also examined associations between nature connectedness and duration of nature visits, frequency of visits to nature per week, and self-reported wellbeing via the WEMWBS. We applied Pearson's product-moment correlation test. Using the psych (Revel, 2020) and boot (Canty and Ripley, 2020) packages in R, we applied bootstrap resampling to assign a measure of accuracy to sample estimates for correlations with a minimum of 1,000 iterations.

3.4. Results

A total of $n = 1184$ respondents completed our research questionnaire. We acquired a broad distribution of responses, predominantly ($n = 993$ or 96% of georeferenced responses) from across England, UK (Figure 2, B). We also acquired complete datasets for green spaces, IMD, and forests (>0.5 ha) for England to conduct the geospatial analysis (Figure 2, A, C, and D). There was a skew towards respondents who identified as being female ($n = 851$ or 72%) compared to male ($n = 331$ or 28%), trans woman ($n = 1$ or 0.1%), and non-binary ($n = 1$ or 0.1%), and towards respondents with a higher level of education (*n* = 847 or 72% with ≥ undergraduate degree). Taking the median age category, the distribution either side was similar (*n* = 624 or 53% were 55 years old or over; and *n* = 560 or 47% were 54 years old or younger).

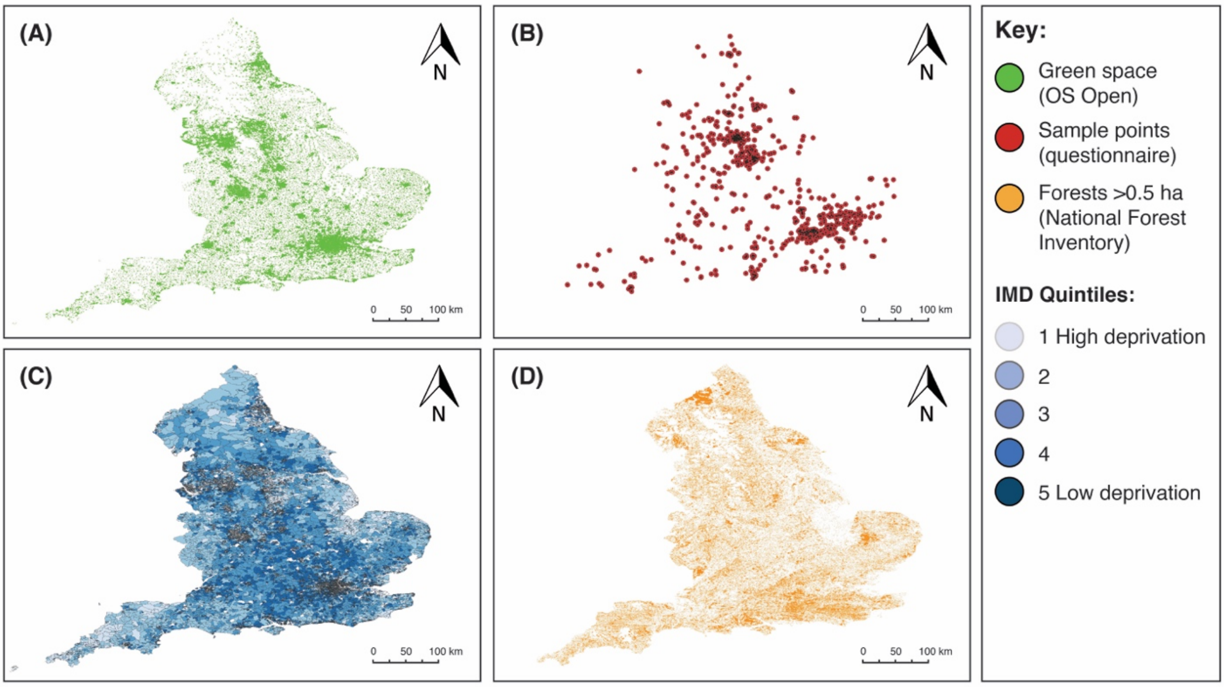

Figure 2. Spatial outputs combined with England boundary datasets. (A) shows the distribution of OS Open Green Space polygons; (B) shows the distribution of georeferenced samples from the survey; (C) shows the Lower Super Output Areas with joined Index of Multiple Deprivation quintile data, whereby 1 corresponds to relatively high deprivation (and lighter blue) and 5 corresponds to relatively low deprivation (and darker blue); and (D) shows distribution of forests >0.5 ha.

3.4.1. Changing patterns of exposure to nature during the COVID-19 pandemic

Our results show that a significantly greater proportion (88%) of participants spent more time in natural environments as a result of the COVID-19 pandemic, confirmed by a 2-sample test for equality of proportions with continuity correction ($X_2 = 1525$, df = 1, $p = < 0.01$). This was in contrast to those who spent less time in nature (7%) and those whose patterns of exposure did not change (5%). Table 1 shows a breakdown of the most popular responses. The most popular environments (based on a duration increase) were private gardens (47.7%), followed by woodlands (13.7%), and urban parks (10.9%). Over 80% of all participants reported they were likely to spend more time in nature once the COVID-19 pandemic is over, which is also a significant proportional difference ($X_2 = 853$, df = 1, $p = < 0.01$).

Table 1. Patterns of change in visits/exposure to natural environments as a result of the COVID-19 pandemic.

Response	**No. of responses**	**% of responses**
Increase in the amount of time spent in private gardens	565	47.7
Increase in the amount of time spent in woodlands	162	13.7
Increase in the amount of time spent in urban parks	129	10.9
Decrease in the amount of time spent in natural environments	71	6.0
Increase in the amount of time spent in natural environments	71	6.0

No change	60	5.1
Increase in the amount of time spent around waterbodies	49	4.1
Increase in the amount of time spent on an allotment	30	2.5
Increase in the amount of time spent at the beach	11	0.9
Decrease in the amount of time spent in urban parks	9	0.8
Increase in the amount of time spent on mountains/hills	9	0.8
Increase in the amount of time spent in meadows	8	0.7
Decrease in the amount of time spent in woodlands	4	0.3
Increase in the amount of time spent in arable land	3	0.3
Decrease in the amount of time spent around waterbodies	2	0.2
Decrease in the amount of time spent on mountains/hills	1	0.1

The average duration that participants spent in natural environments increased during the COVID-19 pandemic ($\bar{x} = 106$ min) compared to before the pandemic ($\bar{x} = 66$ min), and was statistically significant ($t = -15.491$, df = 2310.8, $p = < 0.01$) (Figure 3, A). The average frequency of visits to natural environments per week also increased during the COVID-19 pandemic ($\bar{x} = 5$ visits) compared to before the pandemic ($\bar{x} = 4$ visits), and was also statistically significant ($t = -4.8263$, df = 2336, $p = < 0.01$) (Figure 3, B).

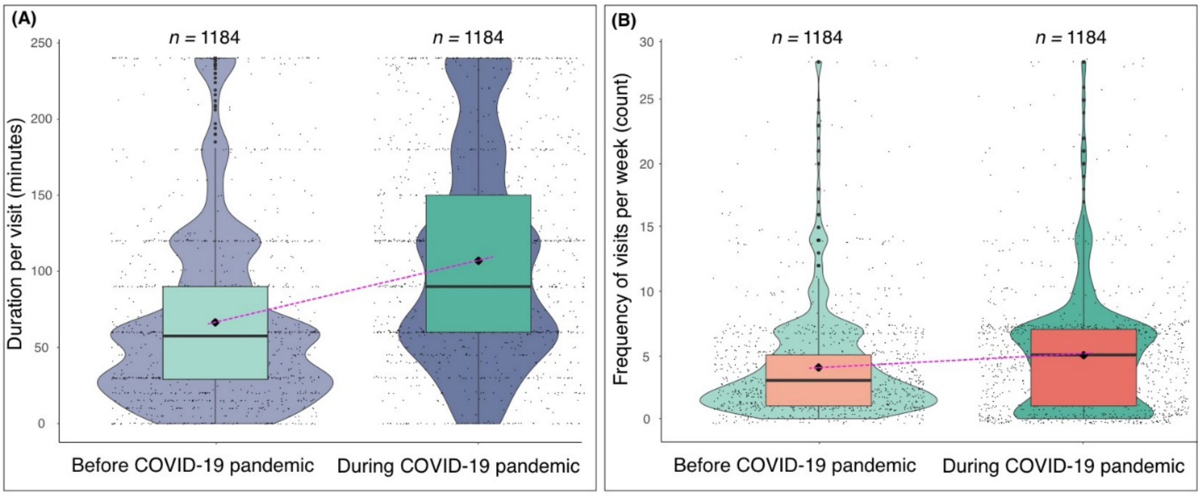

Figure 3. Violin plots (A) typical duration spent in natural environments (e.g., parks, woodland) before (left) and during (right) the COVID-19 pandemic; and (B) typical frequency of visits to natural environments per week before (left) and during (right) the COVID-19 pandemic. The black diamond represents the mean value.

Our results show that IMD did not significantly affect duration spent in nature before or during the pandemic (ANOVA, df = 4, F = 0.74, *p* = 0.6; and df = 4, F = 0.55, *p* = 0.7, respectively). Furthermore, IMD did not significantly affect frequency of visits to nature per week before or during the pandemic (ANOVA, df = 4, F = 1.5, *p* = 0.2; and df = 4, F = 1.1, *p* = 0.3, respectively). Gender did not significantly affect duration or frequency (ANOVA, df = 2, F = 0.5, *p* = 0.5). We confirmed these non-significant relationships for each IMD quintile with a Tukey multiple comparison of means test.

The ANOVA test results showed that duration of nature visits before the pandemic was significantly different depending on age (ANOVA, df = 7, F = 2.3, *p* = 0.02). However, the Tukey multiple comparison of means test showed that differences were only significant between 75-84 year olds and both 45-54 year olds ($\bar{x}$ difference = +26 mins, $p = 0.02$) and 55-64 year olds ($\bar{x}$ difference = +23 mins, $p = 0.04$). In other words, the 75-84 year olds spent more time per visit to nature than 45-64 year olds before the pandemic. However, there were no significant differences in duration between any age group during the pandemic (ANOVA, df = 7, F = 1.375, $p = 0.2$). There were also no significant differences in frequency of visits per week between any age group before the pandemic (ANOVA, df = 7, F = 1.2, $p = 0.3$) or during the pandemic (ANOVA, df = 7, F = 0.4, $p = 0.9$).

There was a statistically significant difference in responses to the question "*Are there any outdoor environments that you would be concerned to visit as a result of COVID-19*?" ($\text{GLM}_{\text{Binomial}}$, $X_2 = 743$, df = 6, $p = <0.01$). Point estimates indicate that beaches and urban parks are associated with the greatest odds for (perceived) concern due to COVID-19 (Figure 4). This implies that concern for contracting SARS-CoV-2 virus may influence people's decision to spend time in certain environments.

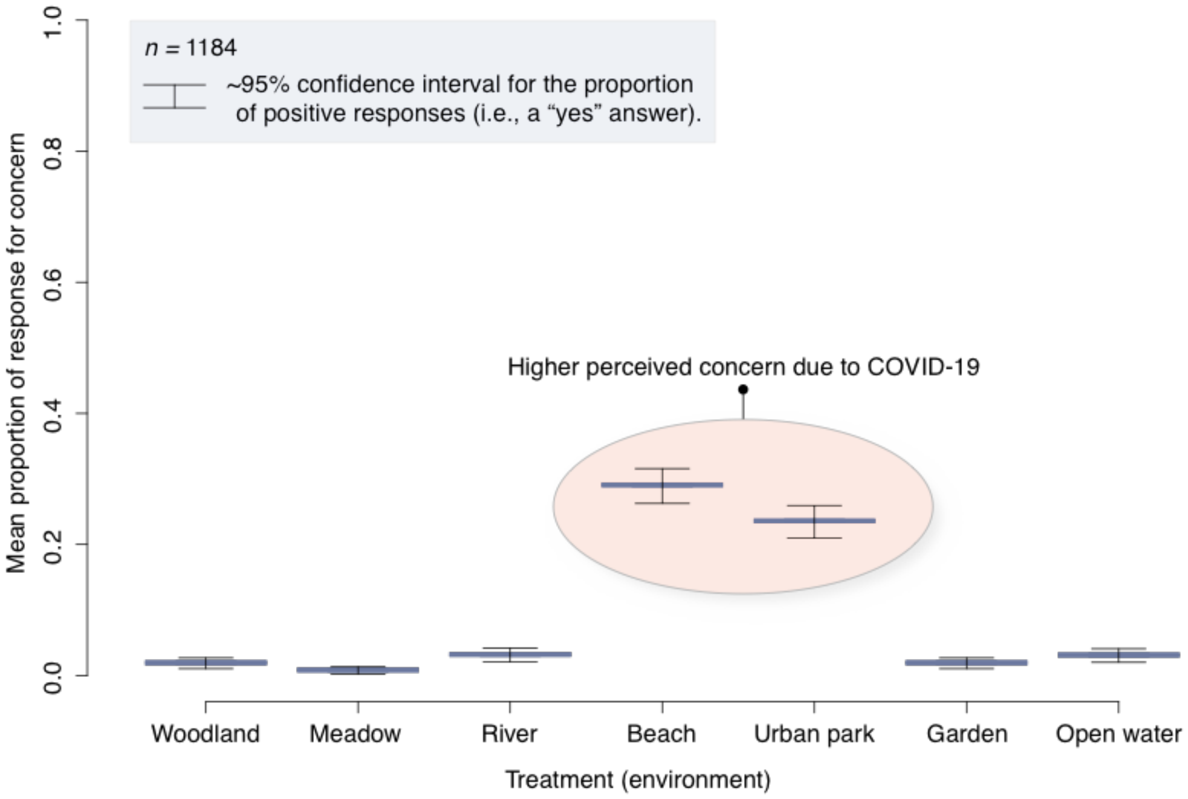

Figure 4. Boxplot for the GLM analysis (regarding environments of concern due to COVID-19), showing means and approximate 95% confidence intervals for the proportion of positive responses, where "yes" was recoded to "1".

We also show that 34% of participants visited natural environments that they would not usually visit as a result of the COVID-19 pandemic. There was a statistically significant difference in responses ($\text{GLM}_{\text{Binomial}}$, $X_2 = 22$, df = 11, $p = 0.02$), and point estimates indicate that woodlands (56% of responses) are associated with the greatest odds for novel visits (Figure 5).

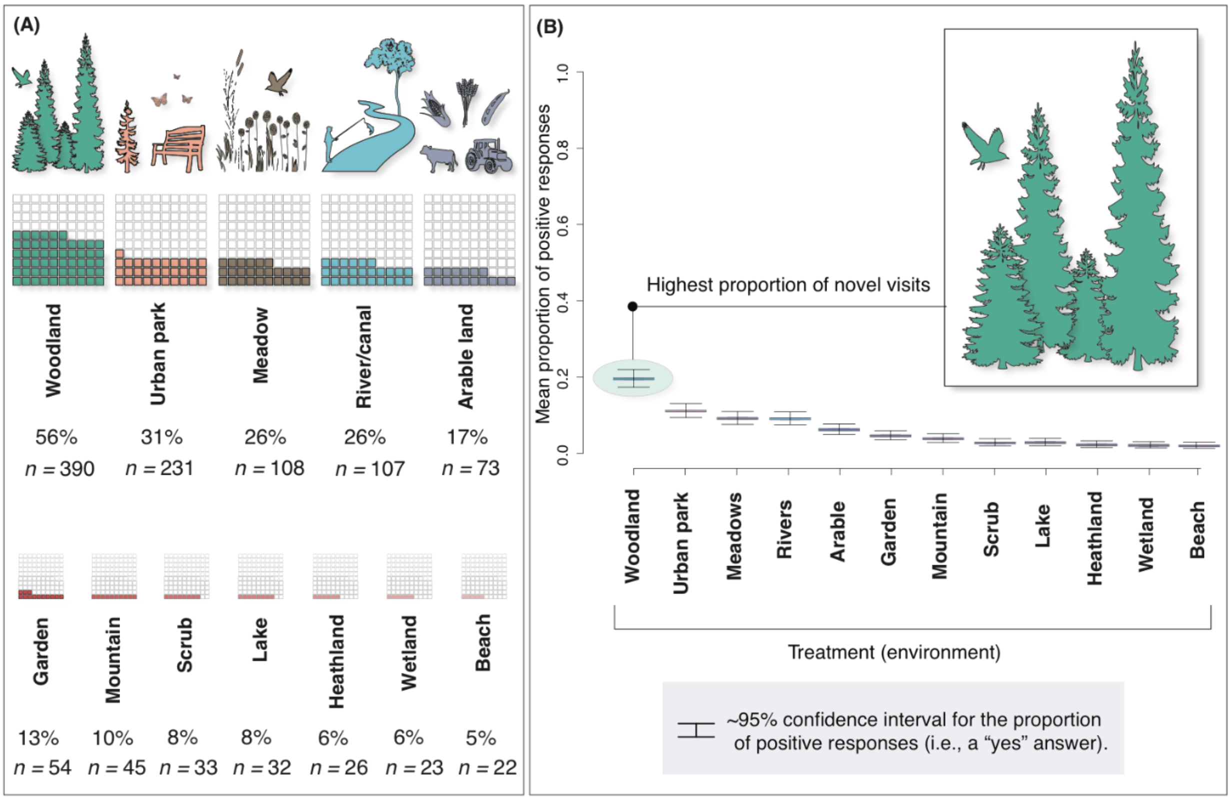

Figure 5. Proportions of participants who visited natural environments they would not usually visit (as a result of the COVID-19 pandemic). The top left (A) waffle plots show the most popular natural environments and, (B) boxplot for the GLM analysis, shows means and approximate 95% confidence intervals for the proportion of positive responses, where "yes" was recoded to "1".

3.4.2. Nature's influence on health and wellbeing during the COVID-19 pandemic

Overall, respondents' self-reported mental wellbeing reduced significantly (t = 19.1, df = 2349, *p* = <0.01) during the pandemic compared to before the pandemic. Interestingly, the mean perceived stress scores were slightly lower during the pandemic compared to before the pandemic (t = 1.9, df = 2305, *p* = 0.05). However, mean perceived stress scores before and during the pandemic were both in the highest

PSS scoring range (Table 2). Of the respondents whose duration in nature increased during the pandemic (*n* = 911), a significantly greater proportion showed decreased perceived stress (X^2 = 8, df = 1, *p* = <0.01). Moreover, of the respondents whose frequency of visits to nature increased during the pandemic (*n* = 632), a significantly greater proportion showed decreased perceived stress (X^2 = 5.5, df = 1, *p* = 0.01). Furthermore, when comparing people's work/living situation, there was only a significant difference in perceived stress levels before and during the pandemic for those who were "*furloughed or unemployed as a result of COVID-19*". Their perceived stress levels were significantly lower during the pandemic (t = 2.4, df = 350, *p* = 0.01).

Table 2. Differences in mean scores (before vs. during COVID-19 pandemic) for the WEMWBS and PSS tests.

Instrument	***n***	**Mean (±SD)**	**t**	**df**	***P*-value**
WEMWBS before	1184	51.5 (8.2)	-	-	-
WEMWBS during	1184	44.7 (8.9)	19.1	2349	<0.01***
PSS before	1184	20.9 (3.3)	-	-	-
PSS during	1184	20.6 (3.8)	1.9	2305	0.05·

'***' <0.01 '**' <0.05 '*' 0.05

Eighty-four percent (*n* = 1,004) of respondents agreed that spending time in nature helped them cope with the COVID-19 pandemic, and 56% (*n* = 569) of these 'strongly agreed'. When comparing the responses for male and female we found a significant difference in the strength of respondents' agreement (W = 17060, *p* = < 0.01). The median female score was 7 (strongly agree), while the median male score was 6 (agree). We also found that the strength of respondents' agreement was significantly different depending on their living situation (H = 14.357, df = 6, *p* = 0.02). For example, the median score for participants "*at home and not working due to being furloughed or unemployed as a result of COVID-19*" (*n* = 211) was 7 (strongly agree), and the median score for those working (either at home or still at their workplace) (*n* = 564) was 6 (agree) (Figure 6).

There were also differences in the perceived ways in which nature helped respondents cope with COVID-19 ($GLM_{Binomial}$, X_2 = 1138, df = 6, *p* = <0.01) (Table 3, A). The most popular response was that nature provided a place to exercise ($\bar{x}$ = 0.7), followed by helping to reduce stress ($\bar{x}$ = 0.6) and providing a calm space to think ($\bar{x}$ = 0.58).

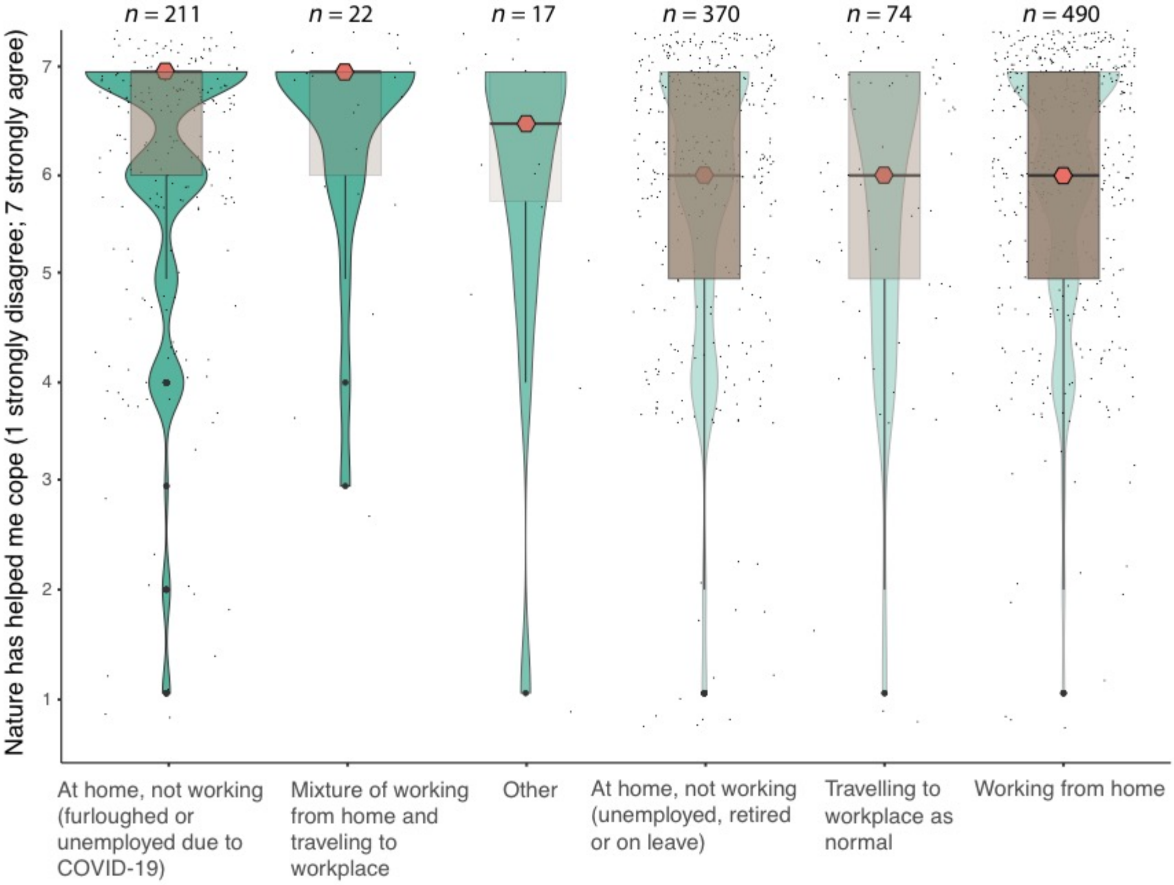

Figure 6. Violin plots of different Likert scores (Y-axis) denoting level of agreement ('nature has helped me cope with COVID-19') analysed by home/work situations (X-axis). Plots display median values (red diamond), interquartile range (brown) and kernel density estimation (green). The strength of the kernel colour corresponds to the median value, and the strength of the boxplot colour corresponds to the sample size.

Ninety-seven percent of participants (*n* = 397) who visited novel (to the respondent) natural environments as a result of COVID-19, reportedly did so for a health and wellbeing benefit. There were significant differences in terms of popularity of responses ($GLM_{Binomial}$, X_2 = 836, df = 8, *p* = <0.01). Physical exercise ($\bar{x}$ = 0.3) and fresh air (x̄ = 0.3) followed by relaxation (x̄ = 0.23) were the top three most popular perceived nature-mediated benefits (Table 3, B).

Table 3. Estimated regression parameters for comparisons of perceived nature-mediated coping benefits (A). Estimated regression parameters for comparisons of perceived nature-mediated benefits of visiting novel environments (B). All coefficients were significantly different from the intercept apart from the fresh air response. Perceived benefits are in descending order based on popularity of response (indicated by the mean).

(A) Perceived benefit (of nature on coping)	**Mean**	**Estimate**	**Std. Error**	**z value**	***p*-value**
Nature provided a place to exercise	0.70	0.46	0.08	5.29	<0.01***
Nature helped reduce stress (Intercept)	0.60	0.40	0.05	6.84	<0.01***
Nature provided a calm space to think	0.58	-0.07	0.08	-0.92	0.38
Nature helped reduce anxiety	0.48	-0.48	0.08	-5.80	<0.01***
Nature helped provide perspective	0.46	-0.56	0.08	-6.73	<0.01***
Nature provided a place to be creative	0.20	-1.78	0.09	-19.04	<0.01***

Nature is a judgement free environment	0.18	-1.91	0.09	-19.91	<0.01***

(B) Perceived benefit (of novel environment)	Mean	Estimate	Std. Error	z value	*p*-value
Physical exercise (Intercept)	0.30	-0.82	0.06	-13.08	<0.01***
Fresh air	0.30	-0.05	0.08	-0.62	0.53
Relaxation	0.23	-0.37	0.09	-4.03	<0.01***
Reduce stress	0.20	-0.62	0.09	-6.43	<0.01***
Reduce anxiety	0.15	-0.91	0.10	-8.83	<0.01***
Space to think	0.15	-0.94	0.1	-9.08	<0.01***
Boost immune system	0.07	-1.77	0.13	-13.54	<0.01***
Beneficial microbes	0.02	-3.00	0.21	-14.20	<0.01***
Bathe in phytoncides (plant based chemicals)	0.01	-3.52	0.26	-13.20	<0.01***

‘***’ <0.01 ‘**’ <0.05 ‘*’ 0.05

There was no significant association between level of nature connectedness and self-reported mental wellbeing before the pandemic, as shown by a bootstrap resampled Pearson's correlation ($r = 0.05$, df = 1179, $\beta = 0.05$ (-0.01 – 0.11), $p = 0.13$). However, level of nature connectedness did show a weak but significant association with self-reported mental wellbeing during the pandemic ($r = 0.07$, df = 1179, $\beta = 0.07$ (0.02 – 0.13), $p = 0.01$). When we compared the scores for females and males, we found that the association between nature connectedness and self-reported mental wellbeing before the pandemic was not significant for females ($r = 0.01$, df = 849, $\beta = 0.01$ (-0.05 – 0.08), $p = <0.74$) and the association during the pandemic was also not significant ($r = 0.04$, df = 849, $\beta = 0.04$ (-0.02 – 0.12), $p = <0.16$). However, the association between nature connectedness and mental wellbeing before the pandemic was significant and stronger for males ($r = 0.12$, df = 328, $\beta = 0.12$ (0.01 – 0.24), $p = 0.02$), and the association during the pandemic was also significant ($r = 0.13$, df = 328, $\beta = 0.13$ (0.02 – 0.24), $p = 0.02$).

The correlation results also show there was a weak but significant positive association between frequency of visits to natural environments and level of nature connectedness ($r = 0.12$, df = 991, $\beta = 0.12$ (0.06 – 0.19), $p = <0.01$). We also show a significant positive association between duration of visits to natural environments and level of nature connectedness ($r = 0.17$, df = 991, $\beta = 0.17$ (0.11 – 0.23), $p = <0.01$). However, when comparing scores for female and males, the association between nature connectedness and duration in nature for females was not significant ($r = 0.00$, df = 708, $\beta = 0.00$ (-0.07 – 0.07), $p = 0.95$). The association between nature connectedness and frequency of nature visits was also not significant ($r = 0.00$, df = 707, $\beta = 0.00$ (-

0.06 – 0.08), *p* = 0.83). The association between nature connectedness and duration in nature for males was not significant (*r* = 0.03, df = 280, *ß* = 0.03 (-0.08 – 0.16), *p* = 0.53). The association between nature connectedness and frequency of nature visits was also not significant for males (*r* = 0.04, df = 280, *ß* = 0.04 (-0.08 – 0.14), *p* = 0.53).

3.4.3. The relationship between health outcomes and spatial / socioecological factors

Our results show that 94% (*n* = 1,118) of the survey responses came from the UK. Of these respondents, 92% (*n* = 1,031) provided georeferenced identifiers (in the form of postal codes). Ninety-six percent (*n* = 993) of these respondents were based in England. Therefore, *n* = 993 responses were included in the logistic regression models built to investigate potential relationships between green space, NDVI, mental wellbeing and perceived stress. This enabled a standardised analysis of socioeconomic status via the IMD (unique to England).

The results from our unadjusted logistic regression models show that there was a significant positive effect of NDVI (greenness) on self-reported mental wellbeing in all of the spatial radii around a respondent's home location (50 m, 100 m, 250 m, 500 m). For the 250 m buffer, the significant positive effect of NDVI on self-reported mental wellbeing remained significant and with a relatively high odds ratio (>8) when adjusting for all of the covariates (OR: 8.04 (1.44, 45.01), *p* = 0.01).

However, in the 50 m, 100 m and 500 m buffer radii (around a respondent's home location), the significant effect remained only when adjusting for gender (OR: 4.92 (1, 24.13), *p* = 0.04; OR: 5.26 (1.03, 26.90), *p* = 0.04; OR: 5.2 (0.95, 29.3), *p* = 0.05, respectively) and not when adjusting for age (apart from the 65-74 year age range), socioeconomic status (IMD), nature connectedness, work/living situation and level of education (Table 4). The positive effect of NDVI on self-reported wellbeing was significant for the 65-74 year age range for both the 100 m buffer (OR: 4.49 (1.05, 19.22), *p* = 0.04) and the 500 m buffer (OR: 4.66 (1.09, 19.95), *p* = 0.03).

Our results also show no significant associations between green space (or forests – Supplementary Materials, Table S1) presence and abundance and self-reported mental wellbeing for any of the spatial buffers (Table 4).

In terms of perceived stress, there was a significant effect of NDVI on reducing stress in the 100 m (OR: 0.38 (0.15, 0.94), *p* = 0.03) and 250 m buffer zones (OR: 0.37 (0.14, 0.96), *p* = 0.04) with the unadjusted models (Table 5). In adjusted models, however, these significant levels tended to be lost; there being no other significant associations for NDVI, and green space presence on stress.

Table 4. Association between NDVI, green space presence and abundance, and self-reported mental wellbeing during the COVID-19 pandemic.

	NDVI 50 m	**NDVI 100 m**	**NDVI 250 m**	**NDVI 500 m**	**Green space Pr 50 m**	**Green space Ab 50 m**	**Green space Pr 100 m**	**Green space Ab 100 m**	**Green space Pr 250 m**	**Green space Ab 250 m**	**Green space Pr 500 m**	**Green space Ab 500 m**
Model 1: Unadjusted†	5.14 (1.05, 25.09)** $p = 0.04$	5.48 (1.07, 27.94)** $p = 0.03$	8.04 (1.44, 45.01)** $p = 0.01$	5.32 (0.95, 29.96)* $p = 0.05$	0.97 (0.58, 1.63) $p = 0.91$	1.05 (0.73, 1.49) $p = 0.86$	1.13 (0.74, 1.73) $p = 0.58$	1.0 (0.81, 1.24) $p = 0.92$	1.13 (0.61, 2.09) $p = 0.68$	0.99 (0.91, 1.08) $p = 0.91$	0.71 (0.27, 1.86) $p = 0.50$	0.96 (0.93, 1.0) $p = 0.06$
Model 2: Adjusted for gender	4.92 (1, 24.13)** $p = 0.04$	5.26 (1.03, 26.90)** $p = 0.04$	7.74 (1.38, 43.37)** $p = 0.01$	5.2 (0.95, 29.3)* $p = 0.05$	0.98 (0.58, 1.67) $p = 0.94$	1.05 (0.73, 1.5) $p = 0.80$	1.16 (0.75, 1.8) $p = 0.49$	1.01 (0.82, 1.25) $p = 0.91$	1.2 (0.64, 2.24) $p = 0.56$	0.99 (0.91, 1.08) $p = 0.96$	0.83 (0.31, 2.22) $p = 0.72$	0.97 (0.93, 1.01) $p = 0.15$

Model 3: As 2 + adjusted for age	2.93 (0.56, 15.38) p = 0.2	3.32 (0.61, 17.93) p = 0.16	6.16 (1.03, 36.89)** p = 0.04	4.83 (0.81, 28.87) p = 0.08	0.97 (0.57, 1.62) p = 0.89	1.04 (0.73, 1.49) p = 0.81	1.12 (0.73, 1.72) p = 0.59	1.0 (0.81, 1.24) p = 0.93	1.12 (0.61, 2.07) p = 0.70)	0.99 (0.91, 1.08) p = 0.91	0.69 (0.26, 1.81) p = 0.47	0.96 (0.93, 1.0) p = 0.06
Model 4: As 3 + adjusted for SES§	2.96 (0.55, 15.88) p = 0.2	3.39 (0.61, 18.88) p = 0.16	6.74 (1.07, 42.48)** p = 0.04	5.42 (0.84, 35.11) p = 0.08	1.0 (0.59, 1.69) p = 0.99	1.08 (0.75, 1.54) p = 0.68	1.15 (0.75, 1.77) p = 0.52	1.02 (0.83, 1.27) p = 0.83	1.17 (0.63, 2.16) p = 0.62	1.0 (0.91, 1.09) p = 0.96	0.71 (0.27, 1.85) p = 0.49	0.97 (0.93, 1.01) p = 0.08
Model 5: As 4 + adjusted for nature connectedness¶	2.76 (0.51, 14.79) p = 0.23	3.15 (0.57 17.49) p = 0.19	6.05 (0.96, 38.11)* p = 0.05	4.84 (0.75, 31.35) p = 0.09	0.97 (0.58, 1.63) p = 0.91	1.04 (0.73, 1.48) p = 0.82	1.15 (0.75, 1.76) p = 0.52	1.0 (.081, 1.24) p = 0.93	1.17 (0.63, 2.16) p = 0.61	0.99 (0.91, 1.08), p = 0.92	0.75 (0.29, 1.97) p = 0.57	0.97 (0.93, 1.0) p = 0.06
Model 6: As 5 + living/work situation	3 (0.55, 16.46) p = 0.2	3.29 (0.58,	6.08 (0.95,	4.56 (0.70,	1.0 (0.59, 1.68) p = 0.98	1.05 (0.74,	1.15 (0.75,	1.01 (0.82,	1.09 (0.58,	0.92 (0.09,	0.72 (0.27,	0.97 (0.93, 1.0) p = 0.08

		18.63) *p* = 0.17	38.98)* *p* = 0.05	29.79) *p* = 0.10		1.49) *p* = 0.78	1.78) *p* = 0.51	1.25) *p* = 0.89	2.02) *p* = 0.79	1.08) *p* = 0.86	1.9) *p* = 0.52	
Model 7: As 6 + level of education	1.1 (096, 1.39) *p* = 0.2	3.33 (0.59, 18.74) *p* = 0.17	5.97 (0.94,37.79)* *p* = 0.05	4.71 (0.73, 30.23) *p* = 0.09	0.96 (0.57, 1.62) *p* = 0.89	1.04 (0.73, 1.49) *p* = 0.81	1.12 (0.73, 1.72) *p* = 0.60	1.0 (0.81, 1.24) *p* = 0.94	1.13 (0.61, 2.09) *p* = 0.69	0.99 (0.91, 1.08) *p* = 0.90	0.71 (0.27, 1.84) *p* = 0.49	0.97 (0.93, 1.0) *p* = 0.06

Pr = presence; Ab = abundance

Odds ratio and 95% CI reported

'***' <0.01 '**' <0.05 '*' 0.05

†*n = 933;* §Adjusted by index of multiple deprivation (IMD) quintiles; ¶Based on nature relatedness-6 scale (NR-6)

Table 5. Association between NDVI, green space presence and abundance, and perceived stress during the COVID-19 pandemic.

	NDVI 50 m	**NDVI 100 m**	**NDVI 250 m**	**NDVI 500 m**	**Green space Pr 50 m**	**Green space Ab 50 m**	**Green space Pr 100 m**	**Green space Ab 100 m**	**Green space Pr 250 m**	**Green space Ab 250 m**	**Green space Pr 500 m**	**Green space Ab 500 m**
Model 1: Unadjusted†	0.45 (0.18, 1.08) p = 0.07	0.38 (0.15, 0.94) ** p = 0.03	0.37 (0.14, 0.96)** p = 0.04	0.43 (0.17) p = 0.08	1.06 (0.78, 1.43) p = 0.71	1.03 (0.84, 1.28) p = 0.76	0.9 (0.7, 1.15) p = 0.4	0.99 (0.88, 1.13) p = 0.98	0.87 (0.62, 1.23) p = 0.4	1.0 (0.95, 1.06) p = 0.74	0.88 (0.47, 1.65) p = 0.6	1.02 (1, 1.04) p = 0.06
Model 2: Adjusted for gender	0.5 (0.2, 1.23) p = 0.13	0.50 (0.17, 1.06) p = 0.06	0.46 (0.16, 1.06) p = 0.06	0.46 (0.17, 1.319 p = 0.10	1.08 (0.79, 1.46) p = 0.6	1.04 (0.84, 1.29) p = 0.8	0.9 (0.7, 1.16) p = 0.4	0.99 (0.88, 1.13) p = 0.9	0.85 (0.59, 1.21) p = 0.4	1.0 (0.95, 1.06) p = 0.76	0.95 (0.5, 1.79) p = 0.8	1.02 (1, 1.04) p = 0.06

Model 3: As 2 + adjusted for age	0.66 (0.26, 1.27) *p* = 0.38	0.54 (0.21, 1.38) *p* = 0.2	0.49 (0.19, 1.3) *p* = 0.15	0.52 (0.2, 1.38) *p* = 0.18	1.03 (0.76 1.41) *p* = 0.8	1.02 (0.82, 1.27) *p* = 0.8	0.86 (0.66, 1.11) *p* = 0.2	0.99 (0.87, 1.12) *p* = 0.86	0.88 (0.68, 1.16) *p* = 0.4	1.0 (0.95, 1.05) *p* = 0.86	0.84 (0.44, 1.61) *p* = 0.6	1.01 (0.99, 1.04) *p* = 0.17
Model 4: As 3 + adjusted for SES§	0.69 (0.27, 1.77) *p* = 0.43	0.55 (0.21, 1.47) *p* = 0.2	0.5 (0.18, 1.39) *p* = 0.18	0.53 (0.19, 1.5) *p* = 0.23	1.02 (0.75, 1.4) *p* = 0.87	1.01 (0.81, 1.26) *p* = 0.9	0.85 (0.66, 1.11) *p* = 0.2	0.98 (0.87, 1.12) *p* = 0.89	0.84 (0.59, 1.2) *p* = 0.4	1.0 (0.95, 1.05) *p* = 0.92	0.85 (0.44, 1.62) *p* = 0.6	1.01 (0.99, 1.04) *p* = 0.2
Model 5: As 4 + adjusted for nature connectedness¶	0.59 (0.23, 1.53) *p* = 0.27	0.47 (0.17, 1.25) *p* = 0.19	0.4 (0.14, 0.14) *p* = 0.08	0.43 (0.15, 1.23) *p* = 0.11	1.02 (0.74, 1.4) *p* = 0.9	0.99 (0.79, 1.24) *p* = 0.9	0.89 (0.68, 1.15) *p* = 0.3	0.99 (0.86, 1.12) *p* = 0.88	0.88 (0.61, 1.26) *p* = 0.5	1.0 (0.95, 1.05) *p* = 0.89	0.94 (0.48, 1.81) *p* = 0.8	1.02 (0.99, 1.04) *p* = 0.14
Model 6: As 5 + living/work situation	0.59 (0.23,	0.38 (0.15,	0.37 (0.14,	0.41 (0.14,	1.02 (0.74,	0.99 (0.79,	0.89 (0.68,	0.99 (0.86,	0.89 (0.62,	1.0 (0.95,	0.96 (0.49,	1.02 (0.99, 1.04) p = 0.12

	1.53) *p* = 0.27	0.94) *p* = 0.11)	0.96) *p* = 0.07	1.2) *p* = 0.10	1.4) *p* = 0.9	1.24) *p* = 0.99	1.16) *p* = 0.4	1.12) *p* = 0.89	1.28) *p* = 0.5	1.06) *p* = 0.85	1.85) *p* = 0.9	
Model 7: As 6 + level of education	0.59 (0.23, 1.53) *p* = 0.29	1.06 (0.95, 1.17), *p* = 0.3	0.39 (0.14, 1.11) *p* = 0.07	0.43 (0.17, 1.12) *p* = 0.10	1.02 (0.74, 1.4) *p* = 0.9	0.99 (0.79, 1.24) *p* = 0.98	0.88 (0.68, 1.16) *p* = 0.4	0.99 (0.86, 1.12) *p* = 0.89	0.89 (0.68, 1.16) *p* = 0.4	0.99 (0.86, 1.12) *p* = 0.89	0.96 (0.49, 1.86) *p* = 0.9	1.02 (0.99, 1.04) p = 0.12

Pr = presence; Ab = abundance

Odds ratio and 95% CI reported

'***' <0.01 '**' <0.05 '*' 0.05

†*n = 933;* §Adjusted by index of multiple deprivation (IMD) quintiles; ¶Based on nature relatedness-6 scale (NR-6)

However, we further explored green space typology and found that within the 100 m and 250 m buffer radii around a respondent's postcode, the mean number of food-growing allotments was higher for those who had higher mental wellbeing scores ($\bar{x}$ = 0.07 and 0.31, respectively) compared to lower ($\bar{x}$ = 0.03 and 0.21, respectively). This was confirmed as a significantly greater proportion of allotments within 100 m and 250 m of respondents with high mental wellbeing scores compared to low (X^2 = 4.3 and 10.8, df = 1, p = 0.03 and <0.01, respectively). See Supplementary Materials (Table S2) for a full breakdown of green space typologies.

3.5. Discussion

Our study shows that respondents reported significant changes in their patterns of visiting nature as a result of the COVID-19 pandemic. People reportedly spent significantly more time in nature and visited nature more often during the pandemic. People generally visited nature for a health and wellbeing benefit and the majority of respondents felt that nature helped them cope during the pandemic. Greater land-cover greenness within a 250 m radius around a respondent's postcode was important in predicting higher levels of mental wellbeing. There were also significantly more food-growing allotments around respondents with higher mental wellbeing scores. This study provides an important contribution towards understanding how populations respond to infectious disease pandemics. It also further highlights the importance of conserving, restoring and designing nature-centric environments for human health and wellbeing.

As a result of the COVID-19 pandemic, over 90% of respondents increased the amount of time they spent in natural environments such as woodlands, parks, and gardens. Forty-eight percent of respondents spent more time in their private gardens. Fourteen percent of respondents spent more time in woodlands, and 11% spent more time in urban parks. People responded differently to the question "*Are there any outdoor environments that you would be concerned to visit as a result of COVID-19*?". Beaches and urban parks were the environments that caused most concern with respect to visitations during the COVID-19 pandemic. This implies that concern for contracting SARS-CoV-2 virus influenced people's decision to spend time in certain environments. Perhaps this is intuitive as beaches and urban parks traditionally attract crowds of people for recreational and social activities (Chen et al. 2016; Dodds and Holmes, 2020). Moreover, there was considerable media coverage in the UK about overcrowding parks and beaches at the time, thus conceivably increasing the perceived risk of viral transmission. This information could be valuable to landscape managers and the public health sector. For example, understanding where additional anthropogenic pressures on the landscape (and upon sensitive ecological receptors) are likely to occur in response to pandemics could help with the formulation of appropriate mitigation measures. From an epidemiological perspective, comprehending patterns of behavioural change is also important for tracking and understanding disease dynamics (Macpherson, 2020; Arthur et al. 2017).

Thirty-four percent of respondents also visited environments that they would not usually visit as a result of COVID-19. Our results indicate that woodlands were the most popular novel environment with 56% of these respondents visiting woodlands when they would not usually. This further highlights the value of conserving and restoring woodlands and provides novel insights into human-environment interactions in the face of infectious disease pandemics.

Overall, respondents' self-reported mental wellbeing reduced significantly during the pandemic. This corroborates other studies highlighting increases in anxiety (Benke et al. 2020), depression and insomnia (Rossi et al. 2020) as a result of COVID-19. Interestingly, the slightly lower stress levels during the pandemic do not corroborate previous work (McKay and Asmundson, 2020). We found that respondents who increased their duration and frequency of visits to nature, a greater proportion had lower perceived stress levels. This suggests that nature may provide a role in perceived stress relief and warrants further research. We also explored whether work/living situation affected the overall reduction in perceived stress and found an intriguing result. Only respondents who were furloughed or unemployed as a result of COVID-19 showed significantly lower stress levels during compared to before the pandemic (although both were still in the highest stress range). This could be due to a reduction in work-related stress, particularly for those who were furloughed and still receiving government-assisted payments. However, to fully understand these psychosocial dynamics, further research is warranted.

The majority of respondents agreed that spending time in nature helped them cope with the COVID-19 pandemic. This again highlights the immense value of conserving and restoring natural environments for human health and wellbeing. Perhaps in terms of our psychological resilience and ability to withstand disease pandemics, this has never been more salient. Indeed, the number and diversity of human infectious diseases outbreaks has increased significantly in the last 40 years (Smith et al. 2014). Furthermore, as urbanisation continues to augment hazardous interfaces for zoonotic pathogen exposure (Gibb et al. 2020), pandemics are expected to increase in frequency in the future (Hall et al. 2020).

Ninety-seven percent of participants who visited novel natural environments – that is, novel to the respondent – as a result of COVID-19, reportedly did so for a health and wellbeing benefit. This suggests that people were actively seeking out new environments as a therapeutic response to COVID-19, and highlights the human appreciation for nature-centric features. The majority of respondents perceived natural environments as being important places for exercise, stress reduction and anxiety reduction. This corroborates results from previous green space and epidemiological studies (Gladwell et al. 2013; Birch et al. 2020; de Bell et a. 2020) and underscores the multifaceted benefits of engaging with nature.

Nature connectedness (one's affective, cognitive and experiential connection with the natural world) (Cheung et al. 2020; Choe et al. 2020), which has previously been shown to associate with enhanced mental wellbeing (Howell et al. 2011; Martin et al. 2020), only associated with higher wellbeing before and during the pandemic for male participants. Further research is warranted to elucidate the reasons (and generalisability) for this gender difference and to ascertain the directionality of the association. Interestingly, our results show there was a significant positive association between frequency of visits and duration of visits to natural environments and level of nature connectedness. This supports the idea that spending time in and engaging with nature can increase one's nature connectedness (Richardson and McEwan, 2018; Nisbet et al. 2019). However, when analysing the results for females and males separately, the results were not statistically significant. This could be due to the p-value being a function of sample size as well as variance, and thus the reduction in sample size when stratifying the analysis may have affected the significance. Therefore, increasing the sample size would likely provide a richer and more accurate picture of the relationship between nature connectedness and duration/frequency of visits to nature.

Our results show that within the 250 m spatial buffer (around each respondent's postcode), there was a significant positive effect of land-cover greenness on self-reported mental wellbeing during the COVID-19 pandemic. The relatively high odds ratio (>8) implies that a higher level of greenness (measured via the NDVI) significantly increases the odds of scoring a high level of wellbeing. This suggests that neighbourhood-scale greenery may be an important factor in the mental wellbeing of members of the community, which corroborates other studies (Brown et al. 2018; Wang et al. 2020). There was no association in the 50 m, 100 m, or 500 m buffers, suggesting that very proximal land-cover greenness (e.g., in private gardens) and landscape greenness beyond the neighbourhood scale are potentially less important in moderating wellbeing. These results provide additional support for calls to augment neighbourhood vegetation cover and highlight the multidimensional benefits associated with urban greening.

When analysing publicly accessible green space as a single typology, there were no associations between these and mental wellbeing or perceived stress. These results could be affected by only analysing the presence and abundance of green spaces and not fully considering their typology and quality (e.g., biodiversity, recreational potential, facilities, safety). For example, some of the OS green spaces include church yards (which many people may not visit), golf courses and bowling greens (often exclusive to members only). We did find that with deeper analysis, there were significantly more food-growing allotments within 100 m and 250 m of respondents with higher mental wellbeing scores compared to lower. This again strengthens the calls for more quality and community-focused neighbourhood green spaces and urban gardens. As discussed, many people may have avoided parks due to overcrowding and the associated risks of contracting SARS-CoV-2. However, allotments have provided an important community space during COVID-19 (Niala, 2020), and may provide a multiplicity of wellbeing benefits (Dobson et al. 2020). Further research focusing on the typology and quality of green spaces and their relationships with mental wellbeing is warranted.

3.6. Limitations

There are several important limitations associated with this study. For example, non-random sampling methods were used, which means robust calculations of error and inferences of representativeness are not possible. It is possible that people who consider green spaces as important, and those who use green spaces, were over-represented in the sample. There was also a deficit of samples from outside of England to include in socioecological analyses and there were age and gender skews. The inclusion of additional wider-scale georeferenced samples would have provided a richer picture of socioecological dynamics. Temporally-objective information on nature exposure and analysis of seasonal influences vs. pandemic influences would also bring value. For example, as mentioned, seasonality (and the one-time sampling point) may have significantly affected our results. People are probably more likely to spend time outdoors engaging with nature during the spring and summer months (in the northern hemisphere, where the majority of samples were acquired) as the conditions are favourable for recreational activities and more flora and fauna are active during this period. We used the term “as a result of the COVID-19 pandemic” in the framing of many of our questions, and the questionnaire information sheet described how the project was a study of the behavioural responses to the COVID-19 pandemic. Future research should aim to control for this factor. The results in this study are also association based. Therefore, inferences of causation and directionality of the relationships are not possible. There are also inherent biases associated with self-reported methods and potential for responder bias, i.e., did the respondents guess what the survey was looking for and respond accordingly? Further in-depth and controlled research is warranted. A re-assessment of the data, or follow-up work could benefit from providing a deeper examination of, for example, the social structure of the sample of individuals who responded to the questionnaire and using the wellbeing instrument scores as continuous variables may provide different results (as information can be lost when recoding variables). Another limitation is that the survey was written in the English language only, and as such, only English-speaking individuals were likely to respond.

3.7. Conclusion

This study provides novel insights into the value of natural environments, particularly in response to an infectious disease pandemic. People need quality natural environments in their neighbourhoods to maintain favourable health and wellbeing. The COVID-19 pandemic has further highlighted the immense value of connecting and engaging with nature. The need for a mutually advantageous relationship between humans and the wider biotic community has never been more important. We must conserve and restore nature to maintain resilient societies and planetary health.

CHAPTER 4

THE ENVIRONMENT-MICROBIOME-HEALTH AXIS: REKINDLING OLD FRIENDSHIPS AND THE RISKS OF GERMAPHOBIA

"Beneath our superficial differences we are, all of us, walking communities of bacteria. The world shimmers, a pointillist landscape made of tiny living beings."

– Margulis, 1989

4. PUBLICATIONS IN CHAPTER 4:

Robinson, J.M., and Jorgensen, A. (2019). Rekindling Old Friendships in New Landscapes: The Environment-Microbiome-Health Axis in the Realms of Landscape Research. *People Nat*. 2, pp.339-349.

Robinson, J.M., Cameron, R., and Jorgensen, A. (2021). Germaphobia! Does our Relationship with, and Knowledge of Biodiversity, Affect our Attitudes Towards Microbes? *Front Psychol. 12, pp. 1-10*.

Rekindling Old Friendships in New Landscapes: The Environment-Microbiome-Health Axis in the Realms of Landscape Research

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4.1. Abstract

Humans are spending less time in biodiverse environments, and according to the Old Friends and Biodiversity hypotheses, this has led to fewer interactions with diverse immunoregulatory microorganisms or “old friends”. Noncommunicable diseases such as asthma and inflammatory bowel disease (IBD) are on the rise, and the development and progression of these ‘modern’ diseases may be attributed in part, to the breakdown of this evolutionary relationship between humans and environmental microbiota. There is a growing interest in the environment-microbiome-health axis as a mechanism to explain some of the health benefits linked to spending time in nature. This may provide a platform for proposing a new, holistic and transdisciplinary approach to public and environmental health. The field of landscape research — which combines social and natural sciences — responds to emerging socio-ecological issues and can make a significant contribution towards this approach. This paper explores innovative, landscape research-based approaches to understanding the complex relationships between the environment, the microbiome and human health. Transdisciplinarity will play an important role moving forward. This forms a major discussion point in this paper, along with future research directions, key research questions and novel concepts supported by recent technological advancements. The development of a new field of study – *Microbioscape Research* as a crossover between microbiome science and landscape research is also discussed.

Keywords: microbiome; biodiversity; microbiome-inspired green infrastructure; landscape research; urban microbiome; environmental microbiome; urban ecology; green space; noncommunicable disease; old friends hypothesis; biodiversity hypothesis

4.2. Introduction

The Old Friends hypothesis (Rook, 2003), a revision of the Hygiene hypothesis (Strachan, 1989), puts forward a mechanism to explain the rise in immunological dysfunction and allergic disorders in highly urbanised populations. The hypothesis is based on the premise that humans have co-evolved with a diversity of microbiota (or 'old friends') in biodiverse environments, and this relationship was essential to the evolution of resilient immune systems (Rook and Brunet, 2005; Rook et al. 2014). The hypothesis supports the relatively recent view that humans are 'holobionts' — that is, a host plus trillions of microorganisms working symbiotically to form a functional ecological unit (Salvucci, 2016; Robinson, Mills and Breed, 2018). There is an increasing body of evidence pointing to the involvement of the microbiome (the collection of microorganisms and their genetic material in a given environment) in the health and wellbeing of humans — for example, in processes such as emotional regulation, nutrient processing, and the modulation of inflammatory diseases (Schirmer et al. 2016; Koppel, Maini Rekdal, and Balskus, 2017; Thomas et al. 2017; Bicknell et al. 2019).

Several authors have suggested that a diverse microbiome plays an important role in the maintenance of favourable health (WHO and CBD, 2015; Heiman and Greenway, 2016; Flies et al. 2017; Gibbons, 2019). This has parallels with broader ecological observations that suggest ecosystems with higher biodiversity can be more stable and resilient (Tilman, Reich and Knops, 2006; Ptacnik et al. 2008; Mori, Furukawa, and Sasaki, 2013; Lohbeck et al. 2016). However, it is important to note that fragile ecosystems can also be attributed to functional relationship failures and other factors (Dobson et al. 2006; Donohue et al. 2017).

It has recently been argued that reduced contact with microorganisms from biodiverse environments (Haahtela et al. 2013), along with increases in stressors associated with urbanised lifestyles (e.g. antibiotic overuse, exposure to pollution and poor nutritional intake), has led to a ‘dysbiotic drift’ (Logan, 2015). Indeed, dysbiosis or ‘life in distress’, is considered by some researchers to manifest as an imbalance in the microbial assemblages in the human body to a state that is detrimental to health

(Logan, Jacka and Prescott, 2016; Schepper et al. 2017; Sokol et al. 2019). However, it is important to note that the complexities of characterising ‘dysbiotic’ patterns are considerable and the concept remains controversial.

Since the advent of Germ theory (c.1860s) a strong focus has been on the negative impacts of pathogenic microorganisms, and the potentially vital role that symbiotic environmental microorganisms play in regulating our health has been neglected. This historic approach to public health (and to microorganisms) may have inadvertently contributed to an epidemiological transition, characterised by the current rise in noncommunicable diseases (NCDs) (Rook and Lowry, 2014; Flandroy et al. 2018). Furthermore, it is suggested that urbanisation perpetuates the spread of emerging pathogens e.g. through antimicrobial resistance, land-use change and overcrowded populations (Ayukekbong, Ntemgwa and Atabe, 2017; Hassell et al. 2017). Alongside these theories, it is important to acknowledge other etiological models that take into account the dynamic complexities of social phenomena (e.g. housing and education) such as the social determinants of health (SDOH), and the developmental origins of health and disease (DOHaD) – which recognises the importance of the microbiome and other exposures across the life-course (Haugen et al. 2015; Taylor et al. 2016).

The renewed interest in the microbiome — and more broadly, the exposome i.e. the measure of all exposures throughout the life-course — provides a platform for proposing a new, more holistic and transdisciplinary approach to public health. Consequently, it is important to work across disciplines with the aim of uncovering the mechanisms at play in the environment-microbiome-health axis (the relationship between the environment, the microbiome and the health of humans). Recent calls have been made to initiate this via concerted, widespread, interdisciplinary research (Flies et al., 2017). For example, Mills et al. (2017) propose the Microbiome Rewilding hypothesis, which calls for researchers to understand whether ‘rewilding’ biodiversity (including environmental microbiota) in urban environments could benefit public health whilst promoting resilient ecosystems. In this paper we extend these broader calls to landscape research.

Landscape is “an area, as perceived by people, whose character is the result of the action and interaction of natural and/or human factors” (European Landscape Convention, 2019). Landscape research is well-established as a transdisciplinary field of study that addresses a range of social and environmental challenges (Swaffield and Deming, 2011; Vicenzotti et al. 2016). In particular, landscape research deals with the cultural, social, ecological, and spatial factors that shape urban areas and promote interactions with green and blue spaces (semi-natural terrestrial or aquatic environments). As an integrative field of study, landscape research offers landscape literacy: the ability to ‘read’ and interpret the cultural, social, spatial, and material aspects of place. This includes a strong understanding of how to plan, design and manage urban places. In this paper we argue that landscape research can make an important contribution towards rekindling the ‘old friendships’ between humans, biodiverse environments and microbiota.

An interdisciplinary framework is used to consider future environmental microbiome research and practice and to propose a new field of study – Microbioscape Research. This proposal reflects a new way of thinking about the characterisation and visualisation of the environmental microbiome and its relationship with people and nature. Although the methodology for this approach stems from a traditional materialist ontology, it could also be applied to incorporate other perspectives such as new materialism (perspectives that re-think subjectivity, question anthropocentrism and emphasise the materiality of both the natural and sociospheres) (Connolly, 2013; Fox and Alldred, 2016).

The discussions within this paper are divided into three themes. The process of selecting these themes was informed by past reviews of landscape research, highlighting the diversity and evolution of this interdisciplinary field (Powers and Walker, 2009; Vicenzotti et al. 2016). This is not an exhaustive list, however, each theme was identified as being highly relevant to the environment-microbiome-health axis.

The three themes are:

1. *Human and Environmental Relationships* (landscape usage and meaning, health and wellbeing);
2. *Landscape Planning and Ecology* (planning, surveys and ecological design); and,
3. *Communication and Visualisations* (mapping, modelling, visualisation).

4.3. Theme 1: Humans and Environmental Relationships

Health intervention discourse is active and growing in landscape research (Ernstson, 2013; Vicenzotti et al. 2016). This reflects an evolving framework that addresses emerging social challenges, including changes in human health and wellbeing. A robust understanding of socio-ecological dynamics is required to discern the complexities of the human-environment-health relationship. These qualities are present in the landscape research discipline and are arguably transferable to environment-microbiome-health axis research. Environmental justice and nature-based interventions (discussed in the following subsections) have strong socio-ecological foci, and could provide useful lenses to study the environmental-microbiome-health axis.

Environmental justice

One aspect of environmental justice is the consideration for the basic needs of communities in terms of equity of natural resources (Schlosberg, 2013). This is an issue with far-reaching implications for the human-environment relationship. It is recognised as playing a central role in the “upstream determinants of health” (Prescott and Logan, 2016). A prime example of environmental injustice is the disparity in the quality and accessibility of urban greenspaces (Rutt and Gulsrud, 2016). Indeed, several studies have revealed that urban greenspace distribution can disproportionately favour particular social groups, for example, those with a higher socioeconomic status and those from white ethnic backgrounds (Wolch, Byrne, and Newell, 2014; Wüstermann, Kalisch, and Kolbe, 2017). Other studies suggest that it is not necessarily greenspace distribution or spatial proximity, but quality, composition and access that differ between areas of higher and lower deprivation (Jones, Hillsdon and Coombes, 2009; Roe, Aspinall and Ward-Thompson, 2016; Mears et al. 2019). Therefore, some urban groups and individuals may also be less exposed to diverse microbiota of natural environments due to distribution, access, composition and/or quality issues. As such, the potential health benefits associated with environmental microbiome exposure may also be unequally distributed.

People with lower socioeconomic status tend to eat higher proportions of ultra-processed foods and may face additional barriers to accessing affordable fruit and vegetables (Moran et al. 2019; Schnabel et al. 2019). Growing evidence suggests that this has detrimental effects on health, and associated changes in the microbiome may be involved (Zinöcker and Lindseth, 2018). Therefore, a lack of access to quality green spaces may further impoverish the human microbiome and increase health inequalities. As the diet can have a substantial and rapid influence on the gut microbiome (David et al. 2014; Zhang et al. 2018), it could be beneficial to increase opportunities for people to get involved in growing healthy foods and harvesting activities that promote contact with diverse microbiota in natural environments e.g. in community gardens.

Furthermore, it is important to consider environmental justice in the context of pathogenic microbiota: for example, do certain environments contain higher proportions of non-beneficial assemblages? Liddicoat et al. (2019) found that disturbed land may favour opportunistic bacteria (including pathogenic strains), albeit in a non-urban setting, and Talamantes et al. (2007) found anthropogenically disturbed land can release pathogenic fungal spores. Moreover, densely urbanised environments can prevent the transfer of diverse microbiota indoors (Patajuli et al. 2018), and indoor environments can harbour higher proportions of human associated pathogens (Kembel et al. 2012). As such, creating socially inclusive, high quality biodiverse greenspaces may also help to reduce contact with pathogens.

It has been suggested that spatial proximity to greenspaces and associated microbiota may play an important role in noncommunicable diseases. For example, Ruokolainen et al. (2015) showed that greenspace proximity was inversely associated with atopic sensitisation in children, and surrounding land-use explained variations in commensal skin microbiota. Similar conclusions were reached by Hanski et al. (2012), who demonstrated significant associations between surrounding biodiversity, residents with allergic dispositions and diversity of gammaproteobacteria. They found residents living with higher surrounding biodiversity supported a higher diversity of immunoregulatory gammaproteobacteria. Therefore, establishing equity in the provision of high quality and biodiverse greenspaces, could play an important role in the process of optimising interactions with beneficial microbiota.

It is important to note that there is still a dearth of evidence to demonstrate microbiome plasticity in later life. Ruggles et al. (2018) provided evidence for stability in the adult human gut microbiome in the face of environmental disturbance (e.g. human translocation to different habitats and dietary changes). This apparent ecological stability in the adult gut microbiome is corroborated in previous studies (Faith et al. 2013; Rodríguez et al. 2015). However, several authors now suggest that the gut microbiome in adults may be more plastic than previously thought. For example, Martinson et al. (2019) recently provided evidence for plasticity of the bacterial family *Enterobacteriaceae* in the adult human gut microbiome, and Schmidt et al. (2019)

challenged the notion of an oral-gut barrier by showing that one in three microbial cells from the oral environment pass through the digestive tract to settle and “constantly replenish” the gut of healthy humans. As such, additional research focusing on the timing, magnitude and stability (and transmission routes) of environmental microbiome effects on post-infant human health is required.

Environmental justice could be a useful lens for landscape researchers and others to study place and inclusion, understand social and ecological trade-offs, and promote equitable distribution of biodiverse urban greenspaces with strategic considerations for the role of the microbiome. Another useful lens could be nature-based interventions.

Nature-based interventions for health and wellbeing

Building on a rich foundation of nature and human health research (Takano, Nakamura, and Watanabe, 2002; De Vries et al. 2003; Groenewegen et al. 2006), improving the health and wellbeing of communities through landscape interventions is another area that has received widespread attention. This is a fundamental topic in the Human-Environment Relationship theme. For example, the ‘social prescribing’ movement, which connects patients in primary care with a range of non-clinical services in the local community, takes a holistic approach to address the complex needs of people, often through landscape and community-focused interventions (Bragg and Leck, 2017; Kings Fund, 2018). Furthermore, there is a continued interest in the role of nature-based health interventions (a subset of social prescribing) as a means of enhancing human health through interactions with natural environments (Maller et al. 2006; Burls et al. 2007; Bloomfield, 2017; Bragg and Leck, 2017). Interactions with natural environments includes interactions with a range of microbial communities, but the potential beneficial impacts on health have received limited attention. However, our growing understanding of the relationship between the microbiome and human health make this topic highly relevant. Furthermore, advances in microbiome science offer opportunities to consider human and environmental microbial interactions as part of nature-based intervention research.

There is also an opportunity to address interconnected human-environment relationship issues such as ecosystem resilience and public health, with explicit considerations for the environment-microbiome-health axis through integrative strategies. Raymond et al. (2017) outline a ‘co-benefits’ framework for promoting nature-based solutions (NbS) with the aim of generating benefits for humans and the environment (Fig. 1). Furthermore, the need for integrative strategies is highlighted by the planetary health conceptual framework, which is a systems thinking approach that applies considerations for the inextricable links between human and environmental health (including at the planetary scale) (Ostfeld, 2017; Prescott and Logan, 2017; Gabrysch, 2018; Prescott and Logan, 2018).

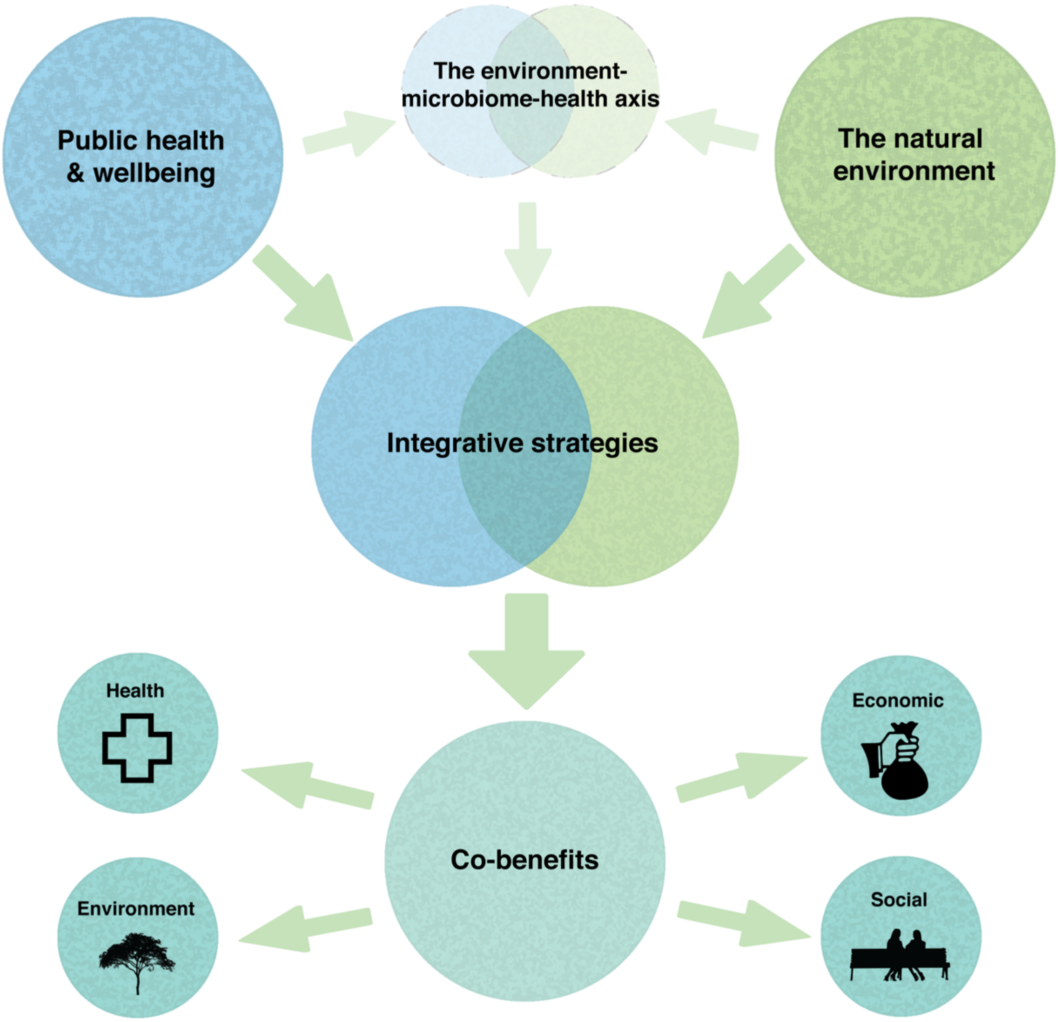

Figure 1. Integrative strategies and their potential co-benefits for humans and the environment. Considering the environment-microbiome-health axis could be important (created by authors, adapted from Robinson and Breed, 2019).

Green prescribing schemes (prescribed nature-based interventions, which build on the 1990's concept of prescribing exercise and dietary-based interventions) have the potential to provide co-benefits for public and environmental health through integrative approaches (Swinburn et al. 1998; Gribben et al. 2000; Robinson and Breed, 2019). Green prescribing schemes can include therapeutic horticulture, biodiversity conservation activities, or simply social activities in greenspaces, which could potentially enhance interactions between humans and environmental microbiota. Further research in this area is needed (see Box 1 for example research questions), but using biological markers could provide valuable objective evidence of the health benefits of interacting with natural environments. Next we will consider the second landscape research theme — Landscape Planning and Ecology — and its relevance to the environment-microbiome-health axis.

Box 1. Examples of theme-specific research questions:

- *Can environmental microbiome research be incorporated into integrative strategies to meet both public and planetary health objectives?*
- *How do the aesthetics of different landscapes entice people to have the social and environmental interactions they need to enhance and regulate their microbiome?*

4.4. Theme 2: Landscape Planning and Ecology

Through planning, design and management, landscape architects can have an important influence on the ecology of urban environments (Rottle and Yocom, 2017). This includes selecting, shaping and managing natural elements based on their functional (proximal and distal) roles in the landscape. Understanding how planning, design and management can influence urban microbial ecology through landscape research is highly relevant to the current conceptual framework.

Relatively recent advances in molecular biology have enable high-throughput sequencing of microbial DNA, revolutionising our ability to understand the diversity and dynamics of microbial communities (Wooley et al. 2010; Zhang et al. 2019). By revealing the unseen but integral components of ecosystems, this technology provides an opportunity to gain greater insights into the composition and functional roles of microbiota, and to investigate how these interface with nature-based features and humans in urban (and other) environments. The next sections will consider how landscape design, planning and ecology could play a role in environment-microbiome-health research and practice.

Innovation in planting schemes and urban design

An emerging objective for those involved in urban ecological design is to understand whether green infrastructure could be designed and managed to generate microbiome-associated health benefits (Robinson, Mills and Breed, 2018; Watkins et al. 2019). This will require a comprehensive understanding of the various physical, spatial and biological factors that affect the composition, function and transmission of environmental microbiota in urban landscapes, and of the social factors that influence interactions (Fig. 2). Fulthorpe et al. (2018) discuss the importance of green roofs as an ecosystem service provider, and the importance of plant-microbe interactions, presenting a list of hypotheses for the positive role of environmental microbiota. These include drought tolerance, pathogen protection and phytohormone production. Here, we present a new addition to this list of hypotheses for green roof scientists to consider:

Green roofs can be designed to promote beneficial interactions between humans and environmental microbiota.

Investigating the functional roles of green infrastructure and choosing planting designs supported by empirical evidence, already plays a fundamental role in landscape research (Cameron and Hitchmough, 2016). For example, Blanusa et al. (2016) investigated different green roof planting schemes to promote urban resilience under various scenarios. The authors suggest that a strong case should be made for the indirect benefits of more complex planting designs, particular those with a greater diversity of morphological characteristics and physiological regulatory factors. Suggested benefits include localised air cooling, greater rainfall and pollutant capture, and thermoregulation. Building on these suggestions, researchers could also investigate whether there are direct and indirect public health benefits to be made through optimising human-environmental microbiome interactions.

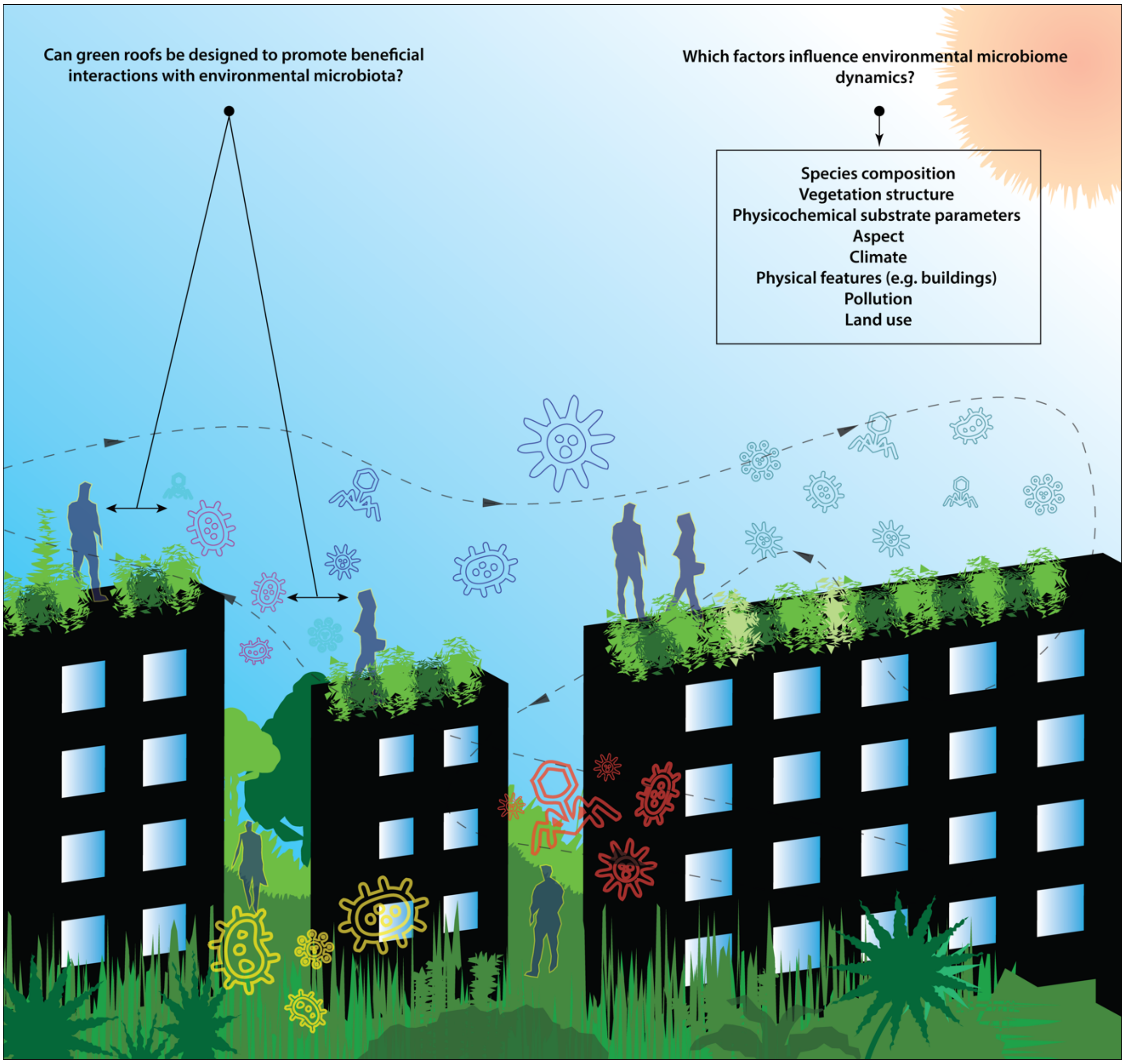

Figure 2. Can green roofs be designed to promote beneficial interactions between humans and diverse microbial assemblages, specific immunoregulatory taxa, or ‘old friends’? (created by authors).

Alternative green infrastructural concepts

There are numerous other types of multifunctional green spaces in urban areas. These range from rain gardens to urban parks; hedgerows to wildflower verges; wildlife overpasses to community allotments. All of these act as natural reservoirs of microorganisms emitting rich clouds of immunoregulatory biochemical compounds

(Rook, 2018, in van den Bosch and Bird, 2018, p. 62). Considering the environment-microbiome-health axis in future green infrastructure designs could potentially have a profound impact on human health. In addition to species composition, spatial and social considerations are likely to play a role in maximising the impact of what we call 'microbiome-inspired green infrastructure' (MIGI) (Robinson, Mills and Breed, 2018; Watkins and Robinson, 2019; Watkins et al. 2019). For example, it will be essential to understand how size, proximity, aspect, and urban physical features affect microbiome dynamics. Community needs assessments could also help inform the design and management of any green features aimed at optimising interactions with environmental microbiota. Moreover, extending beyond the domain of localised impacts, determining whether interconnected systems of MIGI can improve the microbial network fragility of larger urban areas such as 'megacities' (which have been linked to human diseases) (Kim et al. 2018) could also be an important line of enquiry. However, it is also important to recognise that the complexities of microbial ecology and our current limited understanding of microbiome-human health dynamics poses a considerable challenge to this research. Further studies which integrate landscape ecology with fine-scale metagenomics (the study of genetic material from environmental samples) and metatranscriptomics (the study of gene expression in natural environments) such as those in Mehta et al. (2018) would likely bring considerable value to this field of research.

Ecological restoration, microbiome rewilding and "types of nature"

There is evidence to suggest that allowing ecological processes to develop in the absence of anthropogenic pressures, through passive and active restoration processes could potentially ‘rewild’ environmental microbiomes (Gellie et al. 2017; Liddicoat et al. 2019). Mills et al. (2017) propose the Microbiome Rewilding hypothesis, which outlines a case for restoring urban ecosystems and their microbial communities to a state that benefits human health. This has the potential co-benefit of promoting resilient natural ecosystems and could complement the designed greenspaces. The theory behind microbiome rewilding leads to further questions as to whether it can be extended to other “types of nature” in urban environments: from remnant vegetation (“old wilderness”), designed/managed habitats (“functional urban greening”) to extant and/or emerging urban wildscapes (“new wilderness”) (Kowarik and Körner, 2005).

Urban wildscapes are ‘wilderness’ landscapes in urban areas that have naturally established and developed in the absence of human management (Jorgensen and Keenan, 2008). Urban wildscapes include ‘wastelands’, vacant lots, and former industrial sites typically dominated by ruderal vegetation. Several authors have discussed the value of urban wildscapes, highlighting important contributions to climate change adaptation, supporting biodiversity, and promoting social inclusion (Aurora et al. 2009; Kitha and Lyth, 2011; Rupprecht et al. 2015). The process of natural succession in urban wildscapes has ecological parallels with rewilding, which points to the plausibility that they could support an important ‘rewilded’ microbial resource. Urban wildscapes are ubiquitous and provide the potential benefit of enhancing the urban microbiome with limited human input. Interestingly, a recent study showed significant differences in airborne microbiome composition (aerobiome) between non-vegetated parking lots and nearby greenspaces (Mhuireach et al. 2016).

As such, the process of natural succession from a non-vegetated site to a vegetated urban wildscape may alter the composition of the aerobiome. Further research is needed to determine whether these potential changes exist and whether they translate to beneficial outcomes for human health.

Landscape planning can include locating optimal wildscapes in proximity to managed areas, and understanding social needs to optimise interactions between humans and potentially beneficial microbiota. ‘Design’ can include framing wildscapes in a way that makes them acceptable to/usable by a broader range of people. Many researchers in this area have transferable knowledge of landscape, community and functional ecology. Working across disciplines, these skills can be applied to investigate environmental microbiota of urban wildscapes and other “types of nature” - including the ‘designed and managed’ type. This could potentially lead to important public health benefits (see Box 2 for a potential research questions). The final section will consider how the Communication and Visualisation research theme is relevant to the environment-microbiome-health axis.

Box 2. Examples of theme-specific research questions:

- *Can multifunctional green spaces be designed to promote beneficial interactions with diverse environmental microbiota, specific taxa or ‘old friends’?*
- *Can a network of urban wildscapes enhance the aerobiome (airborne microbiota)?*

4.5. Theme 3: Communication and Visualisation

The requirement for innovative modelling, visualisations and geospatial analyses has increased as landscape research has expanded to address societal issues (Lovett et al. 2015). Innovative data integration has the potential to generate new knowledge in environment-microbiome-health axis research, and can play an important role in communicating complex datasets and concepts to broad audiences. This section discusses the crossovers between innovative modelling, visualisation techniques, and microbiome datasets.

4D modelling and microbial cartography

Wissen et al. (2008) suggest that 3D visualisations can help to ensure landscape conditions are communicated in an intelligible manner, using visual and non-visual landscape information. This is pertinent to environment-microbiome-health axis research as both visual (e.g. vegetation, buildings, geomorphological features) and non-visual (e.g. microbial communities, biochemical compounds, meteorological factors) landscape data can produce informative models for the environment and health sectors. Three-dimensional modelling offers benefits to the representation of complex spatial, temporal and compositional data. This is important when collaborating with a diversity of stakeholders (often non-designers) — where clear visual interpretations of current findings and future projections are necessary (Lindquist, Lange and Kiang, 2016).

Kapono et al. (2018) recently conceptualised ‘3D molecular cartography’. The researchers highlighted human-environmental interactions using microbial and metabolic sampling methods and 3D modelling techniques. They were able to map different molecular signatures in indoor environments. Extending this idea to the environment-microbiome-health axis, the nomenclature can be adapted to 4D microbial cartography (4DMC) and the concept adapted to create 4D models (3-dimensions plus a temporal dimension) for mapping and analysing environmental microbiome dynamics. Due to the complexities of microbial ecology, providing a molecular reading of the landscape and explicitly linking these to human health dynamics is currently unrealistic. However, 4D microbial cartography could potentially provide a valuable starting point by generating intelligible outputs of microbial dynamics in the landscape and communicating these to transdisciplinary audiences.

Using either terrestrial scanners or unmanned aerial vehicles (UAVs) with photogrammetry technology (a process also known as Structure from Motion or 'SfM'), 3D models of habitats can be created at different scales. The latter method could be combined with light detection and ranging (LiDAR; i.e. laser-based technology) for detailed outputs. Once the 3D model is created, microbiome sampling is conducted and the sequenced datasets integrated to produce an interactive visualisation of microbial spatiotemporal dynamics (Protsyuk et al. 2018) (Fig. 3). An integrative system for modelling and visualising these data with changeable layers to display the distribution of certain taxonomic groups and heatmaps of diversity, is currently being developed.

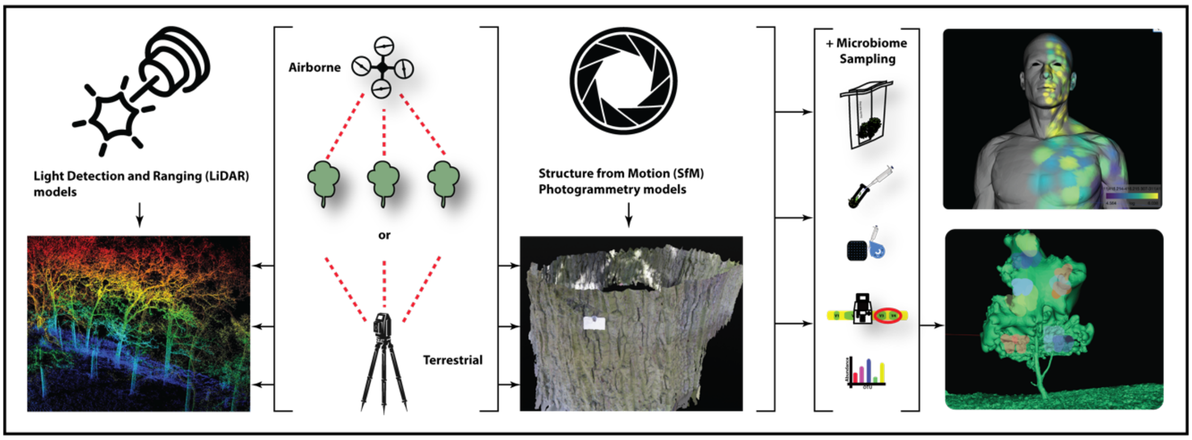

Figure 3. 4-Dimensional Microbial Cartography (4DMC) could contribute to the monitoring of environmental microbial dynamics. The top right image (human) is taken from the open-source ‘ili software, as per Kapono et al. (2018) (created by authors, from Watkins et al. 2019).

Flexible scenarios can be built, compared and analysed by integrating 4D models with other spatial, temporal and compositional datasets. Crucially, the integrated 4D models can help to create context, realistic representations, and enable interactive data exploration. This allows representations of current and future (invisible) elements of the landscape to be visualised, and could be used to help understand exposures/interactions.

The Microbioscape

As alluded to above, technologies and disciplines can now be combined to gain a better understanding of the structure, distribution, and functional roles and relationships of microbial communities within and across different landscapes. Affordable DNA sequencing technology is now widely available to characterise the environmental microbiome on a larger scale than was previously possible. For example, the Earth Microbiome Project, an initiative launched to characterise “global microbial taxonomic and functional diversity” highlights the scale of the potential (Earth Microbiome Project, 2018). Using innovative sequencing technology and working across disciplines, landscape researchers could help to pioneer a new concept, hereby termed the Microbioscape, and with it, a new interdisciplinary field of study — Microbioscape Research. Below is a preliminary definition of this proposed field of study:

> *“Microbioscape research is the investigation and application of innovative research methods to characterise and visualise the structure, composition and distribution of environmental microbial communities and their relationships with their hosts. Furthermore, Microbioscape research aims to understand the social implications and functional ecology of these communities, focusing on their importance for people, place and nature.”*

Microbioscape research can add an important dimension to landscape literacy and the ability to ‘read’ and interpret landscape functions and characteristics. With the availability of advanced technology to characterise microbial communities, the previously unseen constituents of natural environments can now become visible (represented) through modelling and visualisation interfaces. Developing skills in microbial cartography, 4D modelling, GIS, and other spatially-orientated technology will play important roles in Microbioscape research. These are roles that landscape researchers and ecologists are well-placed to develop. Microbioscape research could also incorporate other ontologies such as new materialism, e.g. to explore how “relational networks or assemblages of the animate and inanimate” may produce the world (Fox and Alldred, 2015, p.1; Monforte, 2017). This could lead to additional lines of socioecological enquiry and novel approaches to understanding the environment-microbiome-health axis in the future.

To establish the Microbioscape as a field of research, a strong interdisciplinary (socio-spatio-ecological) approach will be needed. Microbioscape research could make an important contribution towards understanding the environment-microbiome-health axis (see Box 3 for potential research questions).

Box 3. Examples of theme-specific research questions:

- *Can environmental microbiomes be characterised and visualised in a way that more effectively informs landscape planning and design for human/ecosystem health?*
- *Which spatial and design characteristics will provide the optimal conditions for beneficial microbial distribution?*

4.6. Conclusion

A growing body of evidence supports the presence of a health-regulating relationship between humans, biodiverse environments and microbial ‘old friends’. This highlights the importance of a concerted research effort to enhance our understanding of the mechanisms and dynamics at play in this relationship. Emphasis on ‘co-benefits’ is also important, and a transdisciplinary approach is needed to address the interrelated issues of human and environmental health. There is potential to extend the scope of landscape research well beyond the domains of current knowledge to combine microbial ecology and social research. Generating new strategies for human and environment health with explicit considerations for the environmental microbiome and understanding social needs is possible. However, it is important to acknowledge the complexities involved in microbial ecology and in studying the relationships between the environment, the microbiome and human health.

Ultimately, it is hoped this paper stimulates new discourse and lines of enquiry in the area of environment-microbiome-health axis research, and a response of working across disciplines to better understand the relationships involved. In the future, the development of Microbioscape research as a crossover field between microbiome science and landscape research has the potential to inform optimal (health promoting) urban designs, and potentially uncover some of the mechanisms that influence the development and progression of NCDs. Developing Microbioscape research aims to bring together researchers to transcend disciplinary boundaries and help establish integrative strategies for the benefit of people and nature.

Germaphobia! Does our Relationship with, and Knowledge of Biodiversity, Affect our Attitudes Towards Microbes?

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4.7. Abstract

Germaphobia — a pathological aversion to microorganisms — could be contributing to an explosion in human immune-related disorders via mass sterilisation of surfaces and reduced exposure to biodiversity. Loss of biodiversity and people's weaker connection to nature, along with poor microbial literacy may be augmenting the negative consequences of germaphobia on ecosystem health. In this study, we created an online questionnaire to acquire data on attitudes towards, and knowledge of microbes. We collected data on nature connectedness and interactions with

nature and explored the relationships between these variables. Although the study had an international reach (*n* = 1,184), the majority of responses came from England, UK (*n* = 993). We found a significant association between attitudes towards microbes and both duration and frequency of visits to natural environments. A higher frequency of visits to nature per week, and a longer duration spent in nature per visit, were significantly associated with positive attitudes towards microbes. We found no association between nature connectedness and attitudes towards microbes. We found a significant relationship between knowledge of 'lesser known' microbial groups (e.g., identifying that fungi, algae, protozoa, and archaea are microbes) and positive attitudes towards microbes. However, we also found that people who identified viruses as being microbes expressed less positive views of microbes overall — this could potentially be attributed to a 'COVID-19 effect'. Our results suggest that basic microbial literacy and nature engagement may be important in reducing/preventing germaphobia-associated attitudes. The results also suggest that a virus-centric phenomenon (e.g., COVID-19) could increase broader germaphobia-associated attitudes. As the rise of immune-related disorders and mental health conditions have been linked to germaphobia, reduced biodiversity, and non-targeted sterilisation, our findings point to a feasible strategy to potentially help ameliorate these negative consequences. Further research is needed, but greater emphasis on microbial literacy and promoting time spent in nature could potentially be useful in promoting resilience in human health and more positive/constructive attitudes towards the foundations of our ecosystems – the microorganisms.

4.8. Introduction

Germaphobia – also known as 'mysophobia' – is the pathological fear of, and aversion to dirt and microorganisms (henceforth referred to as 'microbes') (Zemke et al. 2015). The rise of germaphobia has likely been influenced by decades of advertising campaigns creating negative perceptions of microbes, and falsely prompting mass (non-targeted) sterilisation of surfaces to achieve 'safe' human environments (Timmis et al. 2019). Symptoms of germaphobia include avoiding certain 'dirty' environments (e.g. soil) due to perceived to fear of microbial exposure, excessively washing hands, over-use of sanitisers and antibiotics (Qadir and Yameen, 2019). However, far less than 1% of the microbes on the planet are human pathogens (Balloux and van Dorp, 2017; Zobell and Rittenberg, 2011). Moreover, germaphobia may have contributed to the current explosion in human immune-related disorders (such as diabetes, asthma, and inflammatory bowel disease) (Jun et al. 2018; Timmis et al. 2019). This is thought to be attributed to the notion that exposure to environmental microbiomes – the diverse network of microbes in a given environment – plays an important role in human health (Rook et al. 2003; Dannemiller et al. 2014; Stein et al. 2016; Arleevskaya et al. 2019; Liddicoat et al. 2019; Selway et al. 2020). Indeed, from a young age, exposure to a diverse range of environmental microbes is considered to be essential for the assembly of our microbiome and the training and regulation of our immune systems (Flies et al. 2020; Renz and Skevaki, 2020; Roslund et al. 2020). A stable and functional human microbiome is colonised following birth. Firstly by the mother's skin and breast milk, and later supplemented from visitors, pets, biodiverse environments, and a 'normal dirty' (not overly cleaned) home environment (DeWeerdt, 2018). Germaphobia and associated overly-clean disposition (whilst recognising targeted hygiene is essential) could conceivably inhibit all of these activities (e.g., avoiding playing in soil or staying away from animals), and if the microbiome assembly process is derailed, the negative health consequences such as immune dysfunction, could be long-term (Gensollen et al. 2016; Renz and Skevaki, 2020). In relation to the current COVID-19 pandemic — a situation that could conceivably increase germaphobia — in addition to being hygienic, we need to promote the concept that the majority of microbes are in fact innocuous and/or beneficial to human health via immunoregulation and other functional roles (Rook, 2013). Indeed, through the modulation of host immune responses, the gut microbiome may even have a direct role in regulating COVID-19 severity (Yeoh et al. 2021).

Microbial communities and their interactions also play essential roles in carbon and nutrient cycling, climate regulation, animal and plant health, and global food security (Cavicchioli et al. 2019; Li et al. 2020; Trivedi et al. 2020). Therefore, microbial biodiversity is of vital importance for the ability of ecosystems to simultaneously provide multiple ecosystem services (Guerra et al. 2020). Consequently, ongoing degradation of microbial communities likely poses an important threat to global macro-level biodiversity and to human societies across the planet (Cavicchioli et al. 2019). Loss of biodiversity and our affective, cognitive and experiential connection with the natural world (also known as 'nature connectedness'), along with poor microbial literacy (such as awareness of the different types of microbes and their importance) and germaphobia, may be detrimental to ecosystem health (Cavicchioli et al. 2019; Robinson and Breed, 2020). Studies have suggested that environmental knowledge (particularly of macro-ecological features) can play a role in fostering pro-ecological attitudes and behaviours (Choe et al. 2020; Sat Gungor et al. 2018), while other suggest knowledge is not an important factor (Qomariah and Prabawani, 2020). A recent study investigated the factors that account for pro-ecological behaviours, and found that nature connectedness, nature experiences (time spent in nature and nature engagement) and nature-based knowledge and attitudes explained 70% of the variation in people's actions for nature (Richardson et al. 2020). Other studies have shown that connectedness to nature and frequency of visits to nature are linked to pro-ecological behaviours (Collado et al. 2015; Duron-Ramos et al. 2020). Recent work suggested that outdoor nature experiences can help overcome fears of 'creepy crawlies' such as insects and snakes and can help develop respectful and positive attitudes towards nature (Chawla, 2020; Hosaka et al. 2017).

Is our diminishing connection with (the rest of) the natural world helping to drive germaphobia-associated attitudes (which may subsequently affect behaviours)? To our knowledge, no studies have investigated the relationship between nature engagement (duration and frequency in nature), nature connectedness and attitudes towards the invisible constituents of nature (i.e., microorganisms). Furthermore, no studies have explored whether there is a relationship between basic knowledge of microorganisms and attitudes towards microorganisms.

In this study, we used an online questionnaire to acquire data on attitudes towards microbes. We collected data on respondents' nature engagement (including typical duration and frequency of visits to nature), and data on nature connectedness using the Nature Relatedness 6 Scale – a validated psychological instrument (Nisbet et al. 2013). To gauge respondents' basic knowledge of microbes, we asked them to select all of the organisms (from a list) that they considered to be microbes. The relationships between these variables (i.e., between nature connectedness, nature engagement and attitudes towards microbes; and between basic microbial literacy and attitudes towards microbes) were then assessed using a range of statistical methods including logistic regression models, Mann Whitney U tests, and 2-sample tests for equality of proportions with continuity correction in R.

The primary objectives of this study were to: **(a)** assess whether people's patterns of exposure to nature associated with their attitudes towards microbes (i.e., a positive or negative view); **(b)** assess whether people's level of subjective connectedness to nature associated with their attitudes towards microbes; and, **(c)** investigate whether basic knowledge of microbial groups (e.g., identifying that fungi, algae, protozoa, and archaea are also microbes) associated with attitudes towards microbes.

Gaining a better understanding of the factors that may aid in reducing/preventing germaphobia-associated attitudes (e.g. negative attitudes that may influence subsequent behaviours) could help to inform environmental and public health policy. For example, improving microbial literacy and promoting campaigns that seek to reconnect humans with the wider biotic community could potentially bring value to both human and environmental health. Microbes are the foundations of our ecosystems and are essential to the survival of all life on Earth (Cavicchioli et al. 2019). Targeted hygiene approaches and continued efforts to control infectious diseases are undoubtedly vital. However, germaphobia (and associated actions such as soil/nature avoidance, and mass sterilisation of the environment) only serves to inhibit a more nuanced awareness of, and mutually-advantageous relationship with these diverse, underappreciated, and indispensable lifeforms.

4.9. Materials and Methods

4.9.1. Online questionnaire

We produced a research questionnaire using the Smart Survey online software (Smart Survey, 2020). The questionnaire included 21 multi-format questions (Supplementary Materials, Appendix I). The questions were devised to gather data on respondents based on four variables: (1) nature engagement (via determining frequency and duration in nature); (2) nature connectedness; (3) attitudes towards microbes; and, (4) basic knowledge of microbes. The online survey was active between April and July 2020.

4.9.2. Nature engagement

As the study was conducted during the COVID-19 pandemic, we asked participants to provide answers by referring to their typical patterns of visiting nature before the pandemic. For example, the following questions were asked: “how many times would you visit any natural environments (e.g., parks, woodlands, the beach) in a typical week before the COVID-19 pandemic?”; and “Approximately how long would you spend in any natural environment per visit before the COVID-19 pandemic?”. For this study ‘natural environments’ and/or ‘nature’ were considered to be less anthropogenic/built-up environments, typically containing a large proportion of vegetation and wildlife such as woodlands, parks, and meadows.

4.9.3. Nature connectedness

We asked participants to answer questions regarding how emotionally and cognitively connected they felt to nature using the Nature Relatedness Scale (NR-6) (Kettner et al. 2019; Nisbet et al. 2013). The NR-6 comprises 6 questions, and answers are recorded using a 1-5 Likert scale. Examples of questions include “My relationship to nature is an important part of who I am”, “My ideal vacation spot would be a remote, wilderness area”, and “I feel very connected to all living things and the earth”. Items were averaged, and higher scores indicated stronger subjective connectedness to nature. This validated instrument has been used in several previous environmental psychology studies (Nisbet et al. 2013; Obery and Bangert, 2017; Whitburn et al. 2020). We also asked several pilot-tested questions regarding typical exposure to nature such as duration and frequency of visits to natural environments.

4.9.4. Attitudes towards microbes

To acquire data on respondents’ attitudes towards microbes, we devised a pilot-tested word-association measure using three categories: positive association, neutral association, and negative association. To reduce potential bias, the categories were not revealed to the respondents and each category contained five randomly-ordered words, displayed as one amalgamated list (Appendix A). In the positive category, respondents could choose from words such as ‘essential’ and/or ‘beneficial’. In the neutral category respondents could choose from words such as ‘nature’ and/or ‘mobile’. In the negative category respondents could choose from words such as ‘disease’ and/or ‘nuisance’. Respondents were asked to select a total of three words that best reflected their view of microbes. We also used the questions "do you consider microbes to be good?; bad?; some are good, some are bad?; or, neither are good or bad?", the resulting positive and negative categories were used in the models to explore the influence of nature connectedness. To gauge respondents' basic knowledge of microbes, we asked them to select all of the organisms that they considered to be microbes. The list included bacteria, viruses, fungi, algae, protozoa, and archaea. Due to the current COVID-19 pandemic, which is of viral origin, we separated out viruses in some of the analyses in case they affected people's overall perception of microbes.

4.9.5. Demographic data, distribution, exclusion and ethics

We also acquired key demographic information including postal code, deprivation (based on the Index of Multiple Deprivation, which takes into account socioeconomic, occupational, housing, and environmental factors to estimate deprivation), age, gender, highest level of education, and occupation. The questionnaire, along with a detailed participant information sheet and consent form was distributed across the world via a secure weblink. We used several non-random sampling methods to reach respondents including: social media posting, emailing volunteer groups, and carrying out an online search of publicly available community group directories. The only exclusion criterion for the study was: people under 18 years of age. The questionnaire was ethically reviewed by the internal review committee in the Department of Landscape Architecture at the University of Sheffield (the authors' academic institution).

4.9.6. Statistical analysis

To test the hypothesis that nature engagement i.e., duration and frequency of visits to nature, may positively influence a person's attitudes towards microbes, we acquired a score from the word-association output by summing the positive, neutral and negative values given by each respondent – this was used as a proxy to indicate positive vs. negative attitude towards microbes. We then assigned the positive and negative scores into two groups and compared the mean duration and frequency of visits to nature of each group using the two-sample Mann-Whitney U test with continuity correction in R.

To test the hypothesis that nature connectedness influences people's attitudes towards microbes, we built logistic regression models. For these models, an odds ratio (OR) of 1 or above equated to the predictor variable (nature connectedness score) increasing the odds of a positive attitude towards microbes. An OR <1 equated to the predictor variable decreasing the odds of a positive attitude towards microbes. Answers from the question "do you consider microbes to be good" were coded into a 'positive' category, and "do you consider microbes to be bad" were coded into a 'negative' category, and these were then used in the regression models as binary dependent variables. We adjusted for several covariates including age, gender, deprivation, and level of education.

To test the hypothesis that basic knowledge of microbes influences people's attitudes towards microbes, we assessed proportional differences between groups, in which respondents either did or did not identify different microbial groups (i.e., bacteria, viruses, fungi, algae, protozoa, and archaea) and their respective word-association scores (summing the negative, neutral and positive scores as a proxy to indicate a positive or negative attitude as a variable) using the 2-sample tests for equality of proportions with continuity correction in R. For example, 3 positive words = net positive score; 2 positive words and one negative or neutral = net positive score, and the reverse formula was used to acquire a net negative score.

4.10. Results

A total of n = 1184 respondents completed the questionnaire. A broad distribution of responses from across the world was acquired (Fig. 1, A); however, the main cluster (n = 993) was from England, UK (Fig. 1, B).

Respondents who identified as being female (n = 851 or 72%) outnumbered those who identified as being male (n = 331 or 28%), trans woman (n = 1 or 0.1%), and non-binary (n = 1 or 0.1%). There was also a skew towards respondents with a higher level of education (n = 847 or 72% with ≥ undergraduate degree). In terms of age, the distribution either side of the median was similar (n = 624 or 53% were ≥55 years old; and n = 560 or 47% were ≤54 years old).

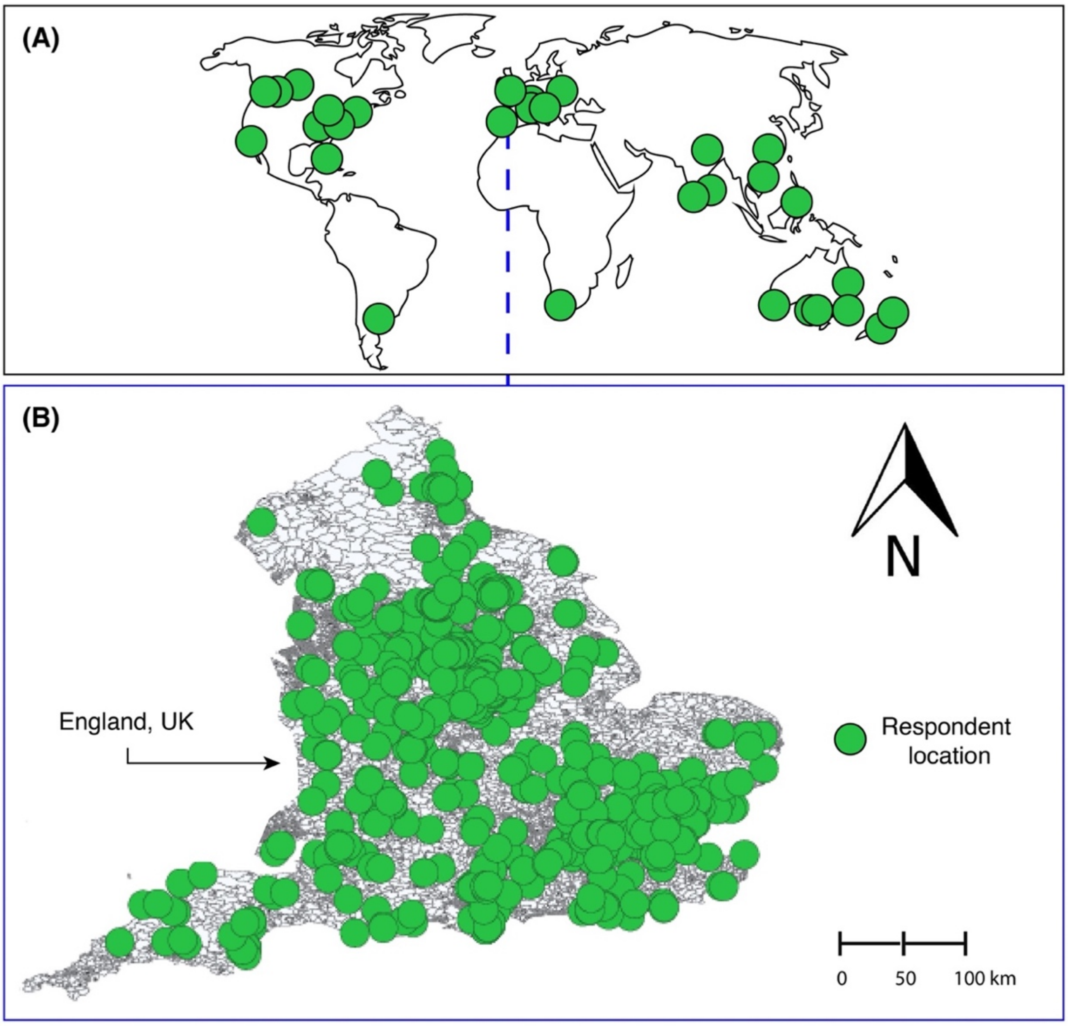


Fig. 1. Distribution of respondents, whereby (A) shows the global distribution, and (B) shows England, UK – the geographical source of the majority of responses (*n* = 993).

4.10.1. Nature engagement, and attitudes towards microbes

Our results show that respondents with a net positive word-association score for microbes (i.e., those who viewed microbes more positively) spent significantly more time per visit ($\bar{x}$ = 87 mins) to natural environments such as woodlands, parks, and meadows compared to respondents with a net negative word-association score for microbes ($\bar{x}$ = 70 mins) (W = 3995, *p* = <0.01) (Fig. 2).

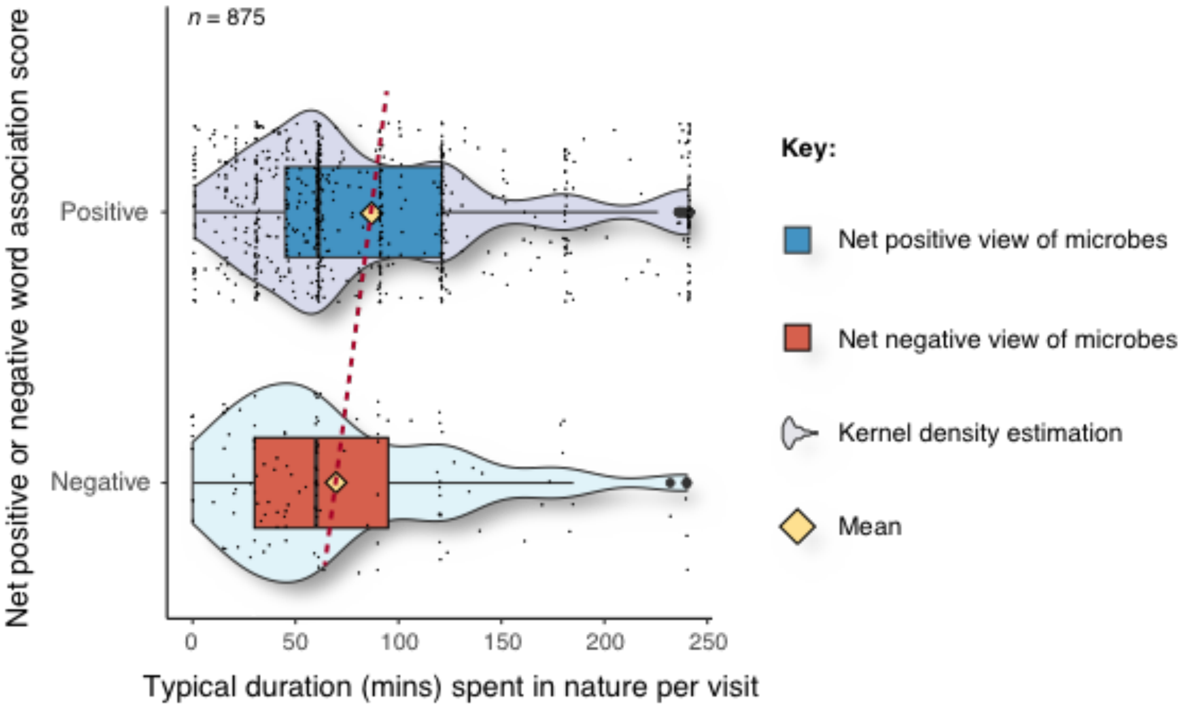


Fig. 2. Typical duration spent in natural environments per visit for respondents with net positive and net negative word-association scores. The yellow diamond represents the mean value. The dashed red line is a visual aid to track the difference in means.

Our results also show that respondents with a net positive word-association score for microbes visited natural environments such as woodlands, parks, and meadows significantly more often ($\bar{x}$ = 4.2 visits in a given week) compared to respondents with a net negative word-association score for microbes ($\bar{x}$ = 3.8 visits in a given week) ($W = 3935$, $p = <0.01$) (Fig. 3).

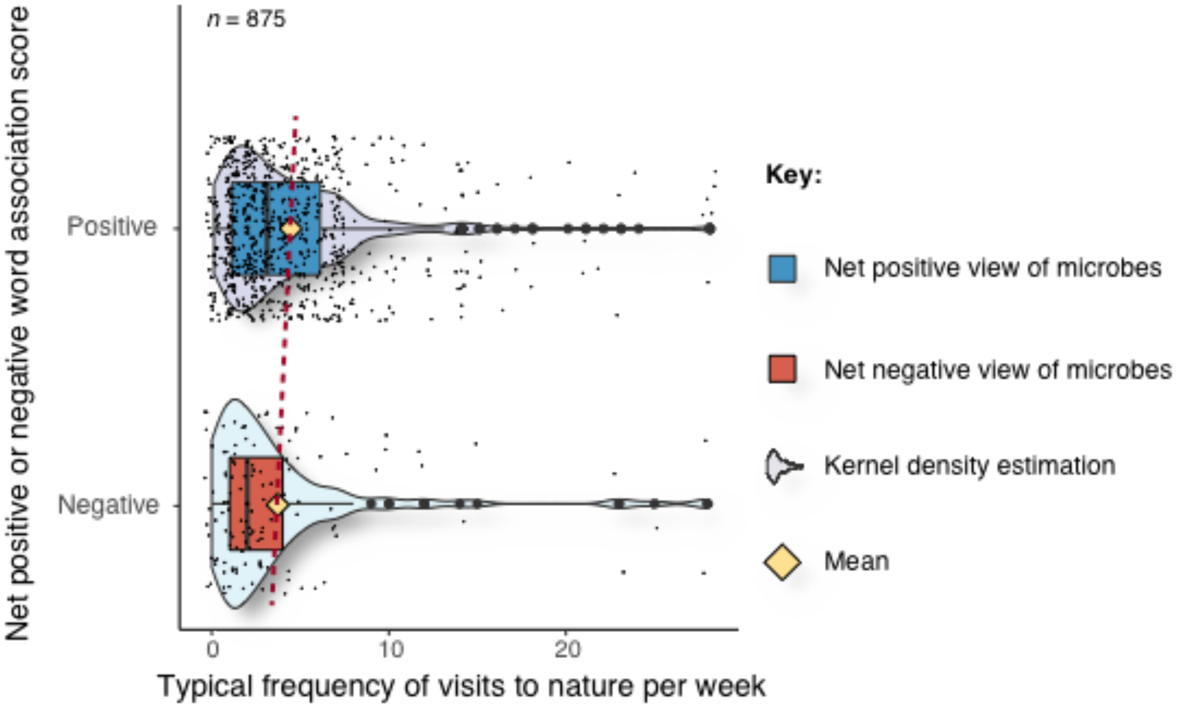


Fig. 3. Typical frequency of visits to natural environments per week for respondents with net positive and net negative word-association scores. The yellow diamond represents the mean value. The dashed red line is a visual aid to track the difference in means.

4.10.2. Nature connectedness and attitudes towards microbes

We found no association between nature connectedness (measured using the NR-6 Scale) and attitudes towards viruses (OR: 0.99 (0.95, 1.02) *p* = 0.54) or all other microbes (OR: 1.01 (0.89, 1.16) *p* = 0.86) (Table 1).

Table 1. Associations between attitudes towards microbes and nature connectedness, adjusting for relative deprivation, education, age and gender.

	Model 1	**Model 2**	**Model 3**	**Model 4**	**Model 5**

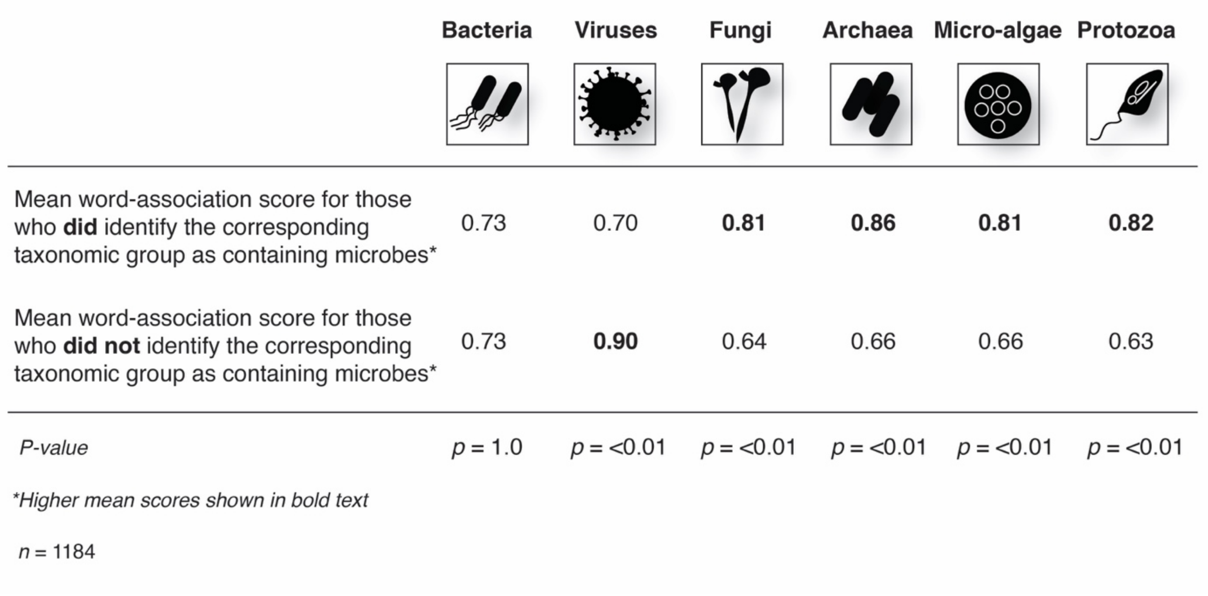
Viruses†					
Nature connectedness unadjusted¶	0.99 (0.95, 1.02) *p* = 0.54 N.S	-	-	-	-
Adjusted for IMD§	-	0.98 (0.89, 1.09) *p* = 0.70 N.S	-	-	-
Adjusted for Education level	-	-	1.07 (0.96, 1.19) *p* = 0.21 N.S	-	-
Adjusted for Age	-	-	-	0.97 (0.90, 1.05) *p* = 0.50 N.S	-
Adjusted for Gender	-	-	-	-	1.13 (0.85, 1.52) *p* = 0.46 N.S
All other microbes†					
Nature connectedness unadjusted¶	1.01 (0.89, 1.16) *p* = 0.86 N.S	-	-	-	-
Adjusted for IMD§	-	0.98 (0.89, 1.09) *p* = 0.70 N.S	-	-	-
Adjusted for Education level	-	-	1.19 (0.75, 1.88) *p* = 0.46 N.S	-	-
Adjusted for Age	-	-	-	1.29 (0.94, 1.79) *p* = 0.12 N.S	-
Adjusted for Gender	-	-	-	-	0.55 (0.17, 1.75) *p* = 0.60 N.S

†Positive vs. negative view
Odds ratio and 95% CI reported
'N.S' not significant
n = 1184; §Adjusted by index of multiple deprivation (IMD) quintiles;

¶Based on nature relatedness-6 scale (NR-6)

4.10.3. Basic microbial literacy and attitudes towards microbes

Mean positive scores (derived from word-association) towards all microbes were significantly higher for those who correctly identified that fungi (X^2 = 42.5, df = 1, p = <0.01) archaea (X^2 = 52, df = 1, p = <0.01) micro-algae (X^2 = 30, df = 1, p = <0.01) and protozoa (X^2 = 51, df = 1, p = <0.01) were microbes compared to those who did not identify these groups as being microbes. Mean positive scores towards all microbes were significantly lower for those who correctly identified that viruses were microbes compared to those who did not identify viruses as being microbes (X^2 = 30.7, df = 1, p = <0.01). There were no significant differences in scores between respondents who correctly identified bacteria as being microbes (n = 1124) compared to those who did not (n = 60) (X^2 = <0.01, df = 1, p = 1.0) (Fig. 4).



	Bacteria	Viruses	Fungi	Archaea	Micro-algae	Protozoa
Mean word-association score for those who **did** identify the corresponding taxonomic group as containing microbes*	0.73	0.70	**0.81**	**0.86**	**0.81**	**0.82**
Mean word-association score for those who **did not** identify the corresponding taxonomic group as containing microbes*	0.73	**0.90**	0.64	0.66	0.66	0.63
P-value	p = 1.0	p = <0.01	p = <0.01	p = <0.01	p = <0.01	p = <0.01

**Higher mean scores shown in bold text*

n = 1184

Fig. 4. Differences in mean microbe word-associated scores for respondents who correctly identified a given taxa as being a microbe compared to those who did not identify the taxa as being a microbe. There were significantly higher (in positivity) word-association scores for respondents who correctly identified that fungi, archaea, micro-algae, and protozoa are microbes compared to those who did not.

4.11. Discussion

Our study shows a significant positive relationship between nature engagement (a respondent's duration and frequency in nature) and the respondents' attitudes towards microbes. However, we found no association between nature connectedness (a person's affective, cognitive and experiential connection with the natural world) (Cheung et al. 2020; Choe et al. 2020) and attitudes towards microbes. Importantly, we found a significant relationship between knowledge of 'lesser known' microbial groups (e.g., identifying that fungi, algae, protozoa, and archaea are microbes) and positive attitudes towards microbes. This study suggests that nature engagement and basic microbial literacy may be important in improving positive attitudes towards microbes. Further confirmatory research is required, with a focus on whether these potential changes to attitudes translate to changes in germaphobia-associated behaviours.

As mentioned, nature engagement significantly associated with positive attitudes towards microbes. This finding supports our first hypothesis, and is corroborated by other (non-microbiological) work that suggests nature engagement may reduce fears of 'creepy crawlies' and help foster respectful and positive attitudes towards nature (Chawla, 2020; Hosaka et al. 2017). It is important to note that the directionality of the relationship is unknown (i.e., whether spending more time in nature helps to establish more positive attitudes towards microbes, or whether other factors related to more positive attitudes increase the likelihood of spending more time in nature). Conceivably, being less averse to microbes could increase one's desire to spend time in environments with natural features such as plants and soil – key sources of dense microbial communities (Liddicoat et al. 2019; Robinson et al. 2020). On the other hand, a greater habituation to these kinds of environments and an affinity for natural environments with its diverse life-forms could conceivably reduce one's aversion to microbes in general (as shown with 'macro' organisms). It is important to acknowledge here that spending time in natural environments exposes us to a diverse suite of microbial communities (Robinson et al. 2020; Selway et al. 2020) that are thought to have important beneficial effects on our health (Haahtela, 2019; Renz and Skevaki, 2020). Therefore, whatever the actual directionality of the proposed relationship is (which requires further research to determine), it is likely to have an important impact on our health and could help to ameliorate the negative consequences of germaphobia (e.g., immune dysfunction) (Rook, 2003). In one direction (i.e., contingent on factors related to more positive attitudes towards microbes increasing the likelihood that we will spend more time in nature), we could potentially gain the many benefits associated with nature engagement. These include improvements in immune health (Li et al. 2010; Rook, 2013), mental health (Birch et al. 2020; Callaghan et al. 2020), and cardiovascular health (Yao et al. 2020; Yeager et al. 2020). In the alternative direction (i.e., spending more time in natural environments which may help to establish more positive attitudes towards microbes), we can hypothesise that our positive attitudes towards microbes could conceivably reduce the likelihood that we carry out mass (non-targeted) sterilisation of our local environments, which could also have important implications for our health (Jun et al. 2018; Parks et al. 2020; Prescott, 2020; Renz and Skevaki, 2020). This hypothesis requires further research and would benefit from the collection of data on people's actions (e.g., related to environmental avoidance and sterilisation). This relationship could also be non-dichotomous (or potentially even a virtuous loop) in the sense that our positive attitudes towards microbes may predispose us to spend more time in nature—an act that may enhance our positive attitudes towards microbes, and the feedback continues. This theoretical relationship warrants further research.

Given that we have shown that nature engagement (duration and frequency in nature) associates with positive attitudes towards microbes, it would perhaps be expected that nature connectedness may also associate with positive attitudes towards microbes (our second hypothesis). Studies have shown that people who exhibit higher levels of nature connectedness are more likely to spend time in and engage with natural environments (Capaldi et al. 2014; Capaldi et al. 2015), and reciprocally, spending time in nature can enhance one's nature connectedness (Chawla, 2020; Nisbet et al. 2019). However, the results of our study show that no significant relationship existed between the nature connectedness of our respondents and their attitudes towards microbes. This could be confounded by other factors, however, age, gender, education and deprivation were controlled for with similar non-significant results. It may simply be that a person's affective, cognitive and experiential connection with nature is not an important factor in predicting one's attitude towards microbes. We can only speculate and say that the invisibility of microbes to the human eye could conceivably negate the affective, cognitive and experiential connection that one may establish with, for example, charismatic fauna or aesthetically-appealing flora. There is a deficit in research on people's emotional and cognitive connection with the invisible constituents of the natural world, and as such, future studies focusing on this relationship would be valuable. It is worthwhile to point out that in contrast to macro-level organisms (e.g. birds and trees), it is only recently – evolutionarily speaking – that humans have been aware of diverse microscopic lifeforms, and only in the past few decades have we been able to comprehensively characterise microbial communities and understand their ecology (Hugenholtz and Tyson, 2008). At this stage, it can only be speculated that this may have an effect on the relationship between nature connectedness and attitudes towards microbes, that is, via a lack of a developed emotional link through sense (e.g. sight, sound, touch)-stimuli interactions over evolutionary timescales. Alternatively, this result could be a facet of the nature connectedness instrument used (the NR-6 Scale). Perhaps a more detailed version of the instrument such as the 17-item Connectedness to Nature Scale (CNS) (Mayer and Frantz, 2004) would reveal alternative findings. This warrants further research.

Finally, our study shows a significant relationship between basic level of microbial literacy and attitudes towards microbes, which supports our third hypothesis. Previous work has suggested that environmental knowledge can positively affect attitudes towards nature (Choe et al. 2020; Sat Gungor et al. 2018), although other research suggests this is not important (Qomariah and Prabawani, 2020). In our study, respondents who correctly identified that lesser publicised (as microbes) organisms — such as algae, fungi, archaea, and protozoa — were microbes, showed higher positivity scores towards microbes. This implies that basic microbial literacy may be an important factor in the formation of a person's attitudes towards microbes, and thus could potentially influence the onset of germaphobia. Determining whether any potential influences on people's attitudes subsequently translates into 'germaphilic' or microbe-appreciative behaviours, requires further research. Interestingly, mean positive scores towards all microbes were significantly lower for those respondents who correctly identified that viruses were microbes compared to those who did not identify viruses as being microbes. Although further research is needed, one explanation could be that the COVID-19 (virus) pandemic had an effect on people's overall view of microbes. This may be unsurprising given the damage the pandemic has caused and the multi-pronged approach taken to try and eliminate the SARS-CoV-2 virus. However, it could conceivably have negative cascading effects on our health by contributing to broader germaphobia.

Microbes are the foundations of our ecosystems and are essential to the survival of all life on Earth (Cavicchioli et al. 2019). We now have the technology to easily characterise and learn about these diverse invisible communities that continuously surround us, providing essential ecosystem services. Although further research is required to build upon our preliminary findings, it is conceivable that in the future, strategies that aim to enhance positive attitudes towards microbes could include the promotion of nature engagement (spending more time and more often in nature), which has several important co-benefits for health and wellbeing (Birch et al. 2020; Rook et al. 2013). Moreover, perhaps in an educational context, greater emphasis can be placed on microbial literacy moving into the future. With a more nuanced awareness of, and mutually-advantageous relationship with these diverse, underappreciated, and indispensable lifeforms, germaphobia-associated attitudes can potentially be reduced, while still maintaining the critically important targeted-hygiene and efforts to control infectious diseases.

Limitations

Our study has some important limitations. Firstly, the results in the study are correlational. Therefore, strict inferences of causation are not possible. Along similar lines, inferences regarding the directionality of the relationships are also not possible. Non-random sampling methods were used in this study. This means accurate calculations of error and representativeness are not possible. Perhaps one of the most important limitations is that self-reported data collection methods come with inherent biases. For example, responder bias — where participants, either intentionally or by accident, choose an untruthful or inaccurate answer, or where people who consider nature important are over-represented in the study. We acknowledge our attitude assessment was limited, and future studies would benefit from investigating behaviours such as environmental avoidance and sterilisation. Further controlled research is required to fully unravel the complexities of the observed relationships. There was also a deficit of samples from outside of England, UK. The study would have benefited from the inclusion of additional international georeferenced samples to be representative on a wider scale. Temporally-objective nature-engagement data that represents scenarios before the COVID-19 pandemic, during the pandemic, and after the pandemic would also bring considerable value.

Conclusions

This study suggests that basic microbial literacy and nature exposure may be important in reducing/preventing germaphobia-associated attitudes. As the rise of immune-related disorders and mental health conditions have been linked to germaphobia, reduced biodiversity, and non-targeted sterilisation, our findings point to a simple strategy to potentially help ameliorate these negative consequences, although further research is required to explore this in greater detail. Indeed, a greater emphasis on microbial literacy and promoting time spent in nature could potentially be useful in promoting resilience in human health and more positive/constructive attitudes towards the foundations of our ecosystems – the microorganisms.

CHAPTER 5

THE ENVIRONMENT-MICROBIOME-HEALTH AXIS: URBAN GREEN SPACE AEROBIOMES

> *"The visible is set in the invisible; and in the end what is unseen decides what happens in the seen; the tangible rests precariously upon the untouched and un-grasped. The contrast and the potential maladjustment of the immediate, the conspicuous and focal phase of things, with those indirect and hidden factors which determine the origin and career of what is present, are indestructible features of any and every experience."*
>
> *– Dewey, 1958*

5. PUBLICATIONS IN CHAPTER 5:

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Vertical Stratification in Urban Green Space Aerobiomes

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5.1. Abstract

5.1.1. Background

Exposure to a diverse environmental microbiome is thought to play an important role in 'educating' the immune system and facilitating competitive exclusion of pathogens to maintain human health. Vegetation and soil are key sources of airborne microbiota — the *aerobiome*. Only a limited number of studies have attempted to characterise

the dynamics of near surface green space aerobiomes, and no studies to date have investigated these dynamics from a vertical perspective. Vertical stratification in the aerobiome could have important implications for public health and for the design, engineering and management of urban green spaces.

5.1.2. Objectives

The primary objectives of this study were to: (a) assess whether significant vertical stratification in bacterial species richness and evenness (alpha diversity) of the aerobiome occurred in a parkland habitat in Adelaide, South Australia; (b) assess whether significant compositional differences (beta diversity) between sampling heights occurred; and (c) to preliminarily assess whether there were significant altitudinal differences in potentially pathogenic and beneficial bacterial taxa.

5.1.3. Methods

We combined an innovative columnar sampling method at soil level, 0.0, 0.5, 1.0, and 2.0 m, using passive petri dish sampling to collect airborne bacteria. We used a Geographic Information System (GIS) to select study sites, and high-throughput sequencing of the bacterial 16S rRNA gene to assess whether significant vertical stratification of the aerobiome occurred.

5.1.4. Results

Our results provide evidence of vertical stratification in both alpha and beta (compositional) diversity of airborne bacterial communities, with diversity decreasing roughly with height. We also found significant vertical stratification in potentially pathogenic and beneficial bacterial taxa.

5.1.5. Discussion

Although additional research is needed, our preliminary findings point to potentially different exposure attributes which may be contingent on human height and activity type. Our results lay the foundations for further research into the vertical characteristics of urban green space aerobiomes and their implications for public health and urban planning.

5.2. Introduction

Vegetation and soil are known to be key sources of airborne microbiota, i.e., the *aerobiome* (Joung et al. 2017; Liu et al. 2018). Exposure to a diverse suite of microbes from the environment (including the aerobiome) is thought to be important for the development and regulation of the human immune system (Rook et al. 2003; Rook et al. 2013; Arleevskaya et al. 2019). Furthermore, studies now link the microbiome to a plethora of maladies from Alzheimer’s disease (Kowalski and Mulak, 2019) and myalgic encephalomyelitis (Hansom and Giloteaux, 2017), through inflammatory bowel (Aschard et al. 2019) and skin diseases (Prescott et al. 2017), to respiratory health (Sokolowska et al. 2018). Environmental factors are thought to be more important than genetic factors in shaping the composition of the gut microbiome (Rothschild et al. 2018). Indeed, Browne et al. (2016) showed that spore-forming bacteria (which survive in aerobic conditions) dominated the human gut, comprising 50-60% of bacterial genera, and displayed greater change in abundance and species over time compared to non-spore formers, suggesting that many gut bacteria may come and go from the environment.

Gut colonisation aside, exposure to airborne microbiota has implications for the human skin and airways. For example, several studies (particularly in agricultural settings) have demonstrated that the composition of the human nasal microbiome is significantly influenced by airborne microbial communities from the surrounding environment (Shukla et al. 2017; Kraemer et al. 2018). A recent study also showed that the diversity of skin and nasal microbiota increased after exposure to urban green spaces (Selway et al. 2020). Furthermore, a recent systematic review highlights that despite the relative infancy of aerobiome-human health research, two studies have shown that rural aerobiomes shifted immune function away from allergic (Th2-type) responses (Flies et al. 2020). In the indoor environment, studies have also drawn the link between microbial composition and endotoxin levels in dust and immuno-protection (e.g., against asthma) (Gehring et al. 2008; Stein et al. 2016). Other indoor-based studies show airborne microbes contribute to nasal, oral and skin microbiomes (Lai et al. 2017; Chen et al. 2019). Studies have also shown that up to 10^6 microbial cells can be found in a cubic meter of air (Šantl-Temkiv et al. 2018; Tignat-Perrier et al. 2019). Therefore, there is considerable potential for aerobiome-respiratory system interactions.

A limited number of studies have attempted to characterise the community structure and spatiotemporal dynamics of near-surface green space aerobiomes. For example, Mhuireach et al. (2016) compared bioaerosol samples in green spaces and parking lots and found compositional distinctions in bacterial communities between the two land cover types. Furthermore, Mhuireach et al. (2019) explored spatiotemporal controls on the aerobiome and suggested that localised site factors were likely to be important in driving bacterial community structure. However, no known studies have investigated the spatial and compositional factors from a vertical perspective. Support for the existence of aerobiome vertical stratification can be drawn from studies of pollution, allergenic pollen and fluid dynamics of particulates where stratification has been shown to occur at various scales. For example, in an indoor agricultural environment and under ventilated conditions, Miles (2008) showed that NH_3 molecule concentrations decreased vertically with increasing distance from source (i.e., the ground). Gao and Nui (2007) found that vertical concentration stratification of particles up to PM10 (10.0 µm) occurred under different ventilation conditions. Particles smaller than 2.5 µm were less affected by gravitational factors, and submicron particles with small relaxation times (i.e., the time required for particles to adjust their velocity to new conditions of forces) behaved more like trace gases following main airstreams. Alcázar et al. (1998) found higher concentration of *Urtica membranacea* pollen at the upper region of their sampling height range of 1.5 m-15 m, and higher concentrations of *U. urens-Parietaria sp.* at lower heights — possibly due to pollen mass and different fluid dynamics. The size range of bacterial cells can vary by eight orders of magnitude (0.013 µm to 750 µm) (Levin and Angert, 2015) and can clump together and adhere to larger suspended particles (Tham and Zuraimi, 2005; Haas et al. 2013; Gong et al. 2020). These factors, along with turbulent mixed flow could conceivably influence aerobiome stratification.

The existence of aerobiome vertical stratification could have important implications for the design, engineering and management of urban green spaces — particularly those aimed at promoting public health via microbial exposure (Watkins et al. 2020). For example, do children receive the same exposure to airborne microbiota as taller adults? Do people who lie down or work close to the ground (e.g., gardeners bending over to dig) have different exposure levels to those who remain upright, and what are the downstream implications for health? Developing a refined understanding of this aerobiome-human interface could also have implications for the design and monitoring of nature-based health interventions, for example via green/nature prescribing (Robinson and Breed, 2019; Shanahan et al. 2019; Robinson et al. 2020).

Furthermore, protocols for sampling the aerobiome to date have often included a reasonable yet arbitrary sampling height of 2 m (Airaudi et al. 1996; Cordeiro, 2010; Mhuireach et al. 2016; Domingue, 2017). Therefore, investigating aerobiome composition at various heights could also provide important methodological insights to fine-tune future study protocols and public health recommendations.

In this proof of concept study, we combine innovative columnar aerobiome sampling methods along with remote sensing techniques and high-throughput sequencing of the bacterial 16S rRNA gene. The primary objectives of this study were to: (a) assess whether significant vertical stratification in bacterial species richness and evenness (alpha diversity) of the aerobiome occurred; (b) assess whether significant compositional differences (beta diversity) between sampling heights occurred; and (c) to preliminarily assess whether there were significant altitudinal differences in putative pathogenic and beneficial bacterial taxa.

5.3. Materials and Methods

5.3.1. Site selection

Our study site comprised three vegetated plots totalling seven ha of the southern section of the Adelaide Parklands (Kaurna Warra Pintyanthi), South Australia. The justification for the selected study site was as follows:

1. Its broadly consistent soil geochemistry, as the southern Parklands generally fall within the Upper Outwash Plain soil boundary (coalescing alluvial soil, draining the Eden Fault Block).

2. This area is managed by a single division of the City of Adelaide, minimising variation in site management and allowing for simpler study logistics.
3. A single study site (i.e., the southern section) in the Parklands provided a degree of control over potential variation in landscape effects on the aerobiome (e.g., dominant vegetation type, distance to coast, elevation, orientation, aspect).

4. Urban Parkland is representative of conditions that both child and adult residents might be exposed to.

Following site selection, boundaries of three plots (as polygons) were defined in QGIS 3 (v3.0.2). These polygons were subsequently converted to shapefiles (.shp) and a random point algorithm was generated. This provided randomly selected sampling points within each vegetated plot to include in our study (Figure 1). The spatial coordinates for each sampling point were recorded and programmed into a handheld global positioning system (GPS) device. This was operated on site to allow us to identify the relevant locations for setting up the sampling stations.

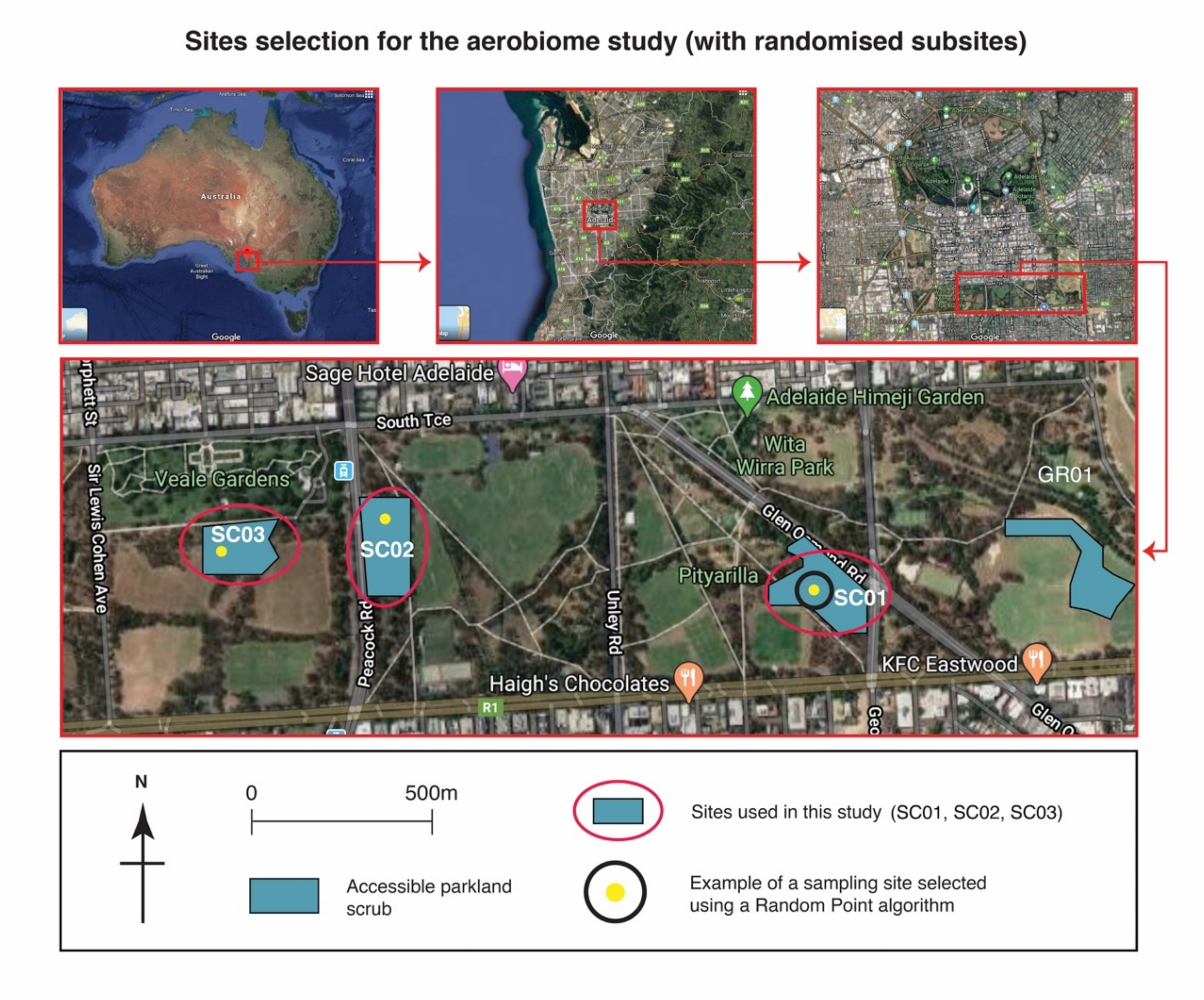

Figure 1. Location of study sites, showing the randomly selected sampling locations (indicated by yellow points). Accessible sites are indicated by the blue polygons and the sites used in this study are surrounded by the red rings. SC01, SC02, and SC03 refer to the three scrub habitat study sites.

5.3.2. Sampling equipment

The sampling stations (Figure 2) were constructed using timber (SpecRite 42 mm x 28 mm x 2.7 m screening Merbau). The sampling stations comprised a timber stand with 45° leg braces. Hooks and guy ropes were also installed, ensuring stability in the field. We used standard lab-grade clear plastic petri dishes (Nest Cell) supported by steel brackets (and attached to the brackets with Velcro tabs) to passively sample the aerobiome as per Mhuireach et al. (2016).

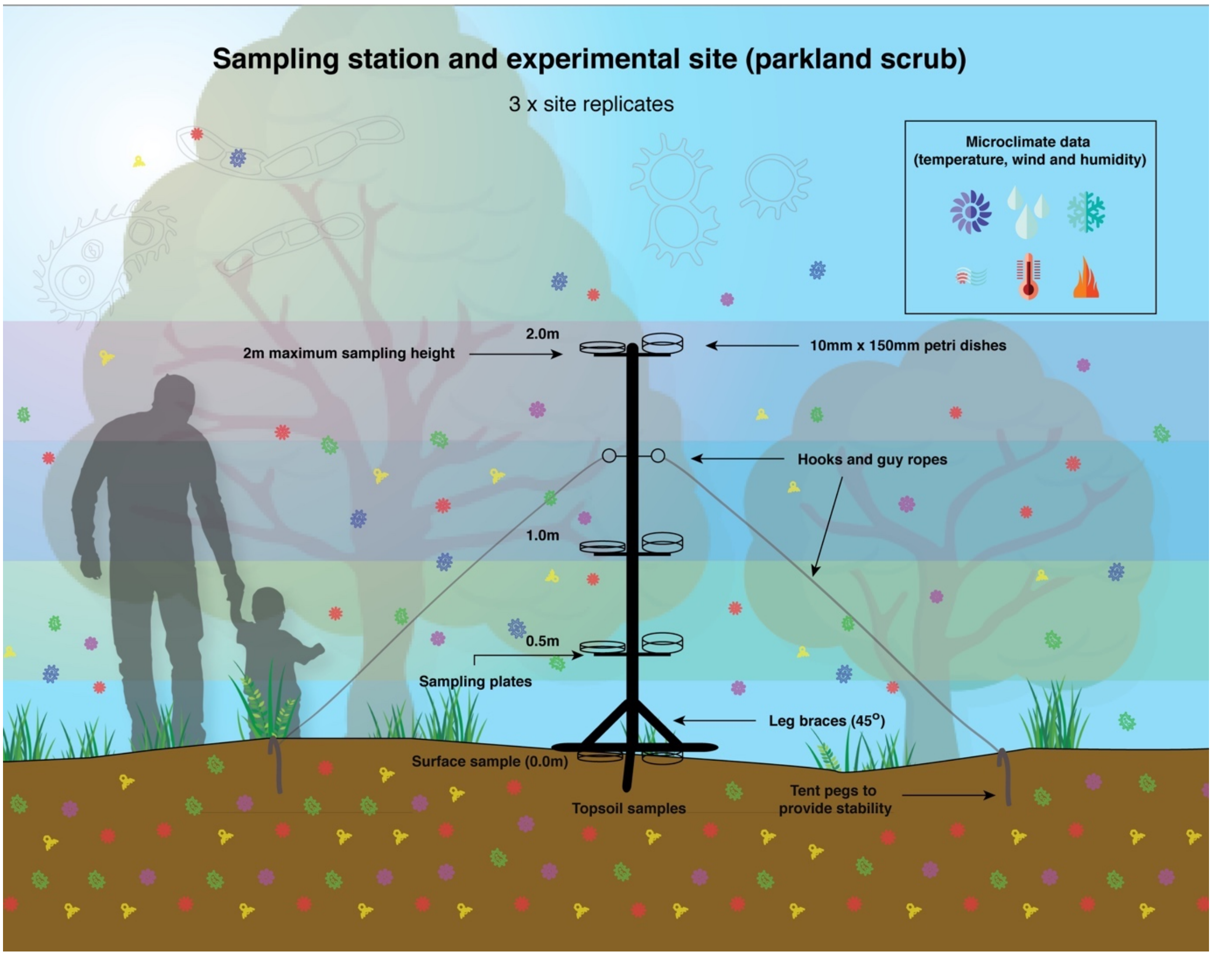

Figure 2. Design of the aerobiome vertical stratification sampling stations. These were deployed in scrub habitat in the Adelaide Parklands. The figure also shows a silhouette of humans to provide perspective.

The level of stability was tested in two phases – *Phase 1*: during windy conditions (~Beaufort scale No. 5) in a yard environment, and *Phase 2*: in situ, prior to the sampling phase.

5.3.3. Data loggers

We installed temperature and relative humidity data loggers (Elitech RC-4HC) at each sampling station. Each logger was programmed to record data at 8-second intervals for the entire sampling period. The dataloggers were calibrated at the start of each sampling day using a mercury thermometer (Gerotherm) and a sling psychrometer (Sper Scientific 736700) taking the range between the two bulbs to determine baseline humidity.

5.3.4. On-site setup procedure

The sampling stations were placed into position between 0600-0800hrs on 4th, 5th and 6th November 2019. This ensured sufficient time was allocated to travel between the sampling locations. From 0800hrs onwards and prior to installing the petri dishes for passive sampling, the sampling stations were decontaminated using a 5% Decon 90 solution. The microclimate data loggers were then decontaminated and installed on the sampling stations. The nearest trees (all <10 m height and 20 cm-50 cm in diameter at breast height) were between 2 m and 5 m from the sampling stations.

5.3.5. Sampling protocol

The sampling procedure involved collecting soil samples (actively) and airborne microbiota (passively). Environmental metadata were also collected (e.g., windspeed, temperature and relative humidity). Soil pH at each site was measured using a digital pH meter (Alotpower). The probe of the pH meter was inserted into the soil and left for a period of 1-minute prior to taking a reading, as per manufacturer's instructions.

Windspeed and direction data for the entire study area were obtained from Adelaide's meteorological weather station at Ngayirdapira (West Terrace): Lat: -34.93, Lon: 138.58, Height: 29.32 m. Windspeed and direction was also recorded at each sampling site on an hourly basis (Mhuireach et al. 2016) using the handheld anemometer (Digitech *QM-1644)*.

5.3.6. Soil samples

Topsoil samples were collected using a small shovel and stored in 50 mL sterile falcon tubes. The shovel was decontaminated using the 5% Decon 90 solution prior to use. Wearing gloves, we sampled five topsoil samples (depth: 5-7cm) at equidistant sampling points, 20-30 cm from the central stem of each sampling station (Zarraonaindia et al. 2015). The soil samples were subsequently pooled and then homogenised, passed through a 1 mm pore sieve, and placed in new sterile 50 mL Falcon tubes. The sample tubes were labelled using a predefined labelling system. We included field controls of soil samples by opening 50 mL sterile falcon tubes for 60 s at each site (Mbareche et al. 2019). All soil and field control samples were immediately chilled by placing in an ice box in the field, and then storing at -80˚C in the lab prior to DNA extraction and sequencing (Zarraonaindia et al. 2015). In total, we collected 15 soil subsamples per sampling day across the three sampling stations for each of the three sampling days. Subsamples were pooled and homogenised by sampling station and day, which gave a total of nine homogenised samples (three per sampling station) plus three field controls.

5.3.7. Aerobiome samples

Passive sampling methods were used to collect low biomass aerobiome samples following established protocols (Mhuireach et al. 2016; Mhuireach et al. 2019). Petri dishes (100 x 15 mm) were attached with decontaminated Velcro tabs on the sampling stations at four sampling heights: ground level, 0.5 m, 1 m, and 2 m. The total height of the sampling stations was 2 m from ground level (in a cohort study across Europe, North America, Australia and East-Asia, 95% of adult human heights fell within 2 SD at 1.93 m for males and 1.78 m for females) (Jelenkovic et al. 2016). One metre is the average height of a 4-year old child (RCPCH, 2020) — typically the maximum weaning age (Mutch, 2004; Clayton et al. 2006) and the time when the gut microbiome is thought to become less plastic (Milani et al. 2017) — and is the approximate height of a pram bassinet (Thule, 2020). Fifty cm is the approximate height of an adult torso from the hip to the mouth (representing the height of an adult sitting on the floor) (Nikolova et al. 2017)—although this will vary depending on size/age. The ground surface is also considered to be an important sampling level, for example, representing the point of contact for a crawling child or an adult lying on the floor. The petri dish sampling plates were also decontaminated using the 5% Decon 90 solution prior to use.

The petri dishes were secured to the sampling stations (Figure 2) and left open for 6-8 hours (Mhuireach et al. 2016). At the end of the sampling period, we closed the petri dishes. A new set of gloves was worn for the handling of petri dishes at each vertical sampling point to reduce potential contamination. The petri dishes were then sealed using Parafilm, labelled, immediately placed on ice, and transported to the laboratory for storage at -80˚C prior to DNA extraction (Mhuireach et al. 2019). Unused petri dishes were left open for 60 s in the equipment box carried on site and then sealed at each site as field controls. Dishes were later swabbed during the DNA extraction process using nylon flocked swabs (FLOQSwabs Cat. No. 501CS01, Copan Diagnostics Inc., CA, USA) (Mhuireach et al. 2019; Bae et al. 2019; Liddicoat et al. 2020).

5.3.8. DNA extraction, amplification and sequencing

We extracted DNA from samples at the Evolutionary Biology Unit (EBU), South Australian Museum. The order of processing samples was randomised using a digital number randomiser, including the soil samples (higher biomass), which were processed after the low biomass, aerobiome samples to minimise cross-contamination.

The petri dishes for each sampling station were swabbed with FLOQSwabs for 30 s (with consistent back and forth strokes) in a laminar flow cabinet type 1 (License No. 926207). The base and lid samples for each height, station and date were then pooled, prior to extraction. The swabs were cut with decontaminated scissors directly into labelled 2 mL Eppendorf tubes. Extraction blank controls were used to demonstrate the absence of sample contamination during extraction and were the last samples in the extraction. Sterile water and reagents were used instead of a sample and all DNA extraction steps were performed as if they were normal samples. We used Qiagen QIAamp DNA Blood Mini Kits to extract DNA from the swabs together with extraction blank controls, and Qiagen DNAeasy PowerLyzer Soil Kits to extract DNA from the soil samples (and extraction blank controls). We followed the manufacturer's instructions throughout the extraction process.

PCR amplification was done in triplicate using the 341F/806R primer targeting the V3-V4 region of the 16S rRNA gene (5' -CCTAYGGGRBGCASCAG- 3'/5' -GGACTACNNGGGTATCTAAT- 3'). The 300 bp paired end run was sequenced on an Illumina MiSeq platform at the Australian Genome Research Facility Ltd (AGRF) using two flowcells (ID 000000000-CW9V6 and 000000000-CVPGT). Image analysis was done in real time by the MiSeq Control Software (MCS) v2.6.2.1 and Real Time Analysis (RTA) v1.18.54. Then the Illumina bcl2fastq 2.20.0.422 pipeline was used to generate the sequence data. A minimum of 0.20 ng/uL of usable PCR product was required in order to generate sequencing output guarantee of 10,000 raw reads and to be included in the analysis.

5.3.9. Bioinformatics and statistical analysis

Paired-end reads were assembled by aligning the forward and reverse reads using PEAR (version 0.9.5). Primers were identified and trimmed. Trimmed reads were processed using Quantitative Insights into Microbial Ecology (QIIME 1.8.4), USEARCH (version 8.0.1623), and UPARSE software. Using USEARCH tools, reads were quality filtered, full length duplicate reads were removed and sorted by abundance. Singletons or unique reads in the data set were discarded. Reads were clustered and chimeric reads were filtered using the "rdp_gold" database as a reference. To obtain the number of reads in each operational taxonomic unit (OTU), reads were mapped back to OTUs with a minimum identity of 97%. Taxonomy was assigned using QIIME.

We used the phyloseq package (McMurdie and Holmes, 2013) in R to import and analyse the sequencing data, and decontam (Davis et al. 2018) to identify and exclude contaminants.

Lower biomass samples (i.e., air, field blanks, and extraction blank controls) were analysed using the isNotContaminant() function, where contaminants were identified by increased prevalence in negative controls. Higher biomass samples (i.e., soil, and corresponding extraction blanks) were analysed using the isContaminant() function. Using isContaminant(), contaminants were identified by the frequency that varies inversely with sample DNA concentration, or by increased prevalence in negative controls. All taxa identified as contaminants were pooled and removed from further analysis. To estimate OTU alpha diversity we derived Shannon Index values based on rarefied abundances (Liddicoat et al. 2020) in phyloseq. The lowest number of reads in a sample was used to rarefy the datasets (Liddicoat et al. 2020). We generated box and violin plots with ggplot2 (Wickham and Wickham, 2007) to visualise the distribution of the alpha diversity scores for each sampling height. Microbial beta diversity was visualised using non-metric multidimensional scaling (NMDS) ordination of Bray-Curtis distances based on rarefied OTU abundances. The ordinations plots show low-dimensional ordination space in which similar samples are plotted close together, and dissimilar samples are plotted far apart.

We used permutational multivariate analysis of variance (PERMANOVA) to test for compositional differences between sampling heights. The Pearson's product-moment and Spearman’s rank correlation tests were used to examine correlations between sampling height and alpha diversity scores. A Mann-Whitney Wilcoxon test was used to examine differences in alpha diversity between merged air sampling heights (0.0 - 0.5 m and 1.0-2.0 m) and a Kruskal Wallace chi-squared test to explore differences in correlations between sites and dates. We also calculated OTU relative abundances using the phyloseq package in R to examine the distribution of taxa that have potential implications for public health. To compare presence and proportions of taxa we used 2-sample tests for equality of proportions with continuity corrections and created radial charts using pivot tables with comma separated value (csv) files. A data point was considered to be an outlier if it was more than 1.5 x above the third quartile or below the first quartile.

5.4. Results

We obtained 3,781,284 raw reads from air samples with an average length of 300 base pairs and 3,278,433 reads after quality control (QC). For soil, we obtained 1,830,395 raw reads and 1,287,303 reads after quality control. The range of reads per samples after QC was 19,966-251,822. Reads were clustered into 10,563 OTUs. Overall, bacterial communities were diverse at each sampling height and bacterial phyla were dominated by:

- Proteobacteria (at 2.0 m: 49.5%, 1.0 m: 43.8%, 0.5m: 28.1%, 0.0m: 27.1% and soil level: 23.12%); and,
- Actinobacteria (at 2.0 m: 19.7%, 1.0 m: 17.5%, 0.5m: 26.6%, 0.0m: 43.5% and soil level: 47.2%).

10 bacterial phyla represented 100% of OTUs over 1% relative abundance including: Proteobacteria, Actinobacteria, Bacteriodetes, Chloroflexi, Cyanobacteria, Firmicutes, Gemmatimondates, GN02, OD1, and TM7. Proteobacteria were dominant at upper sampling heights, and Actinobacteria were dominant at lower sampling heights.

We observed a significant negative correlation between alpha diversity (air and soil for all sites/dates) and sampling height (*r* = -0.58, df = 38, *P* = <0.01; Figure 3A; Table 1).

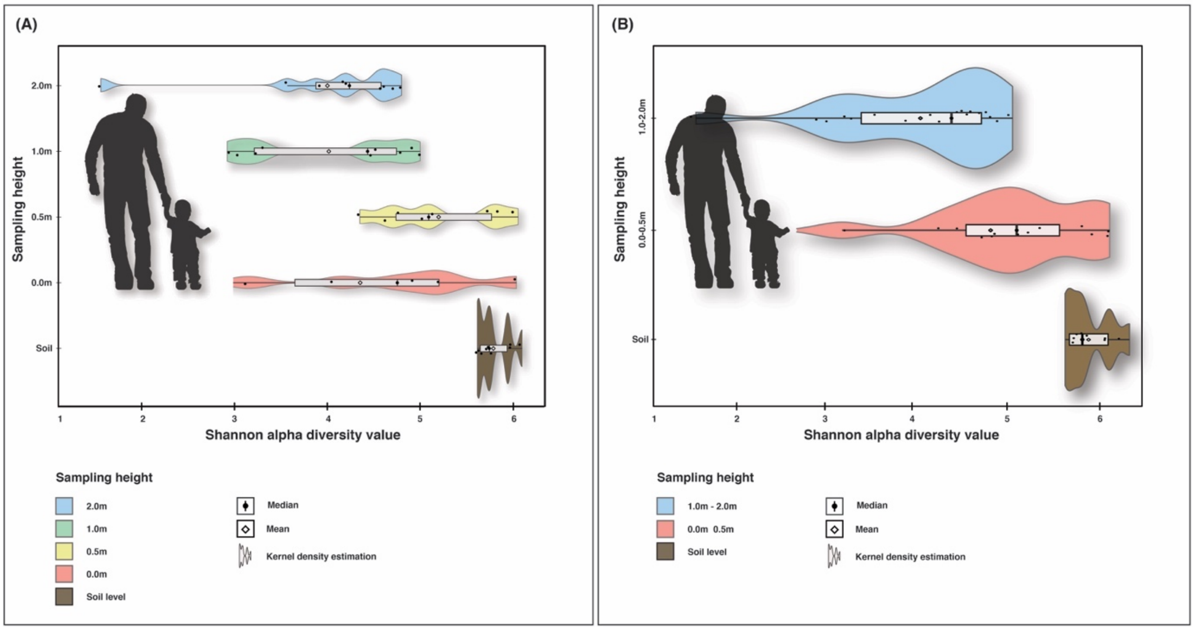

Figure 3. Box/violin plots of Shannon alpha diversity scores for each sampling height including soil (A) and for merged lower heights 0.0-0.5 m and upper heights 1.0-2.0 m, with soil (B). Plots also display mean values, interquartile range and kernel density estimation. Boxplots indicate a vertical stratification trend of airborne bacterial alpha diversity decreasing with increased sampling height.

Table 1. Shannon alpha diversity scores for each spatial and temporal replicate, along with means and standard deviations.

Days/ sampling height	**Scrub 01 (SC01)** **Shannon diversity score**	**Scrub 02 (SC02)** **Shannon diversity score**	**Scrub 03 (SC03)** **Shannon diversity score**	**Mean (± SD)**
Day 1				
Soil	5.73	5.60	5.93	5.75± 0.16
0.0 m	5.26	6.01	4.74	5.34± 0.63
0.5 m	4.63	5.82	5.72	5.39± 0.66
1.0 m	4.43	3.21	4.48	4.04± 0.71

2.0 m	1.54*	3.87	4.53	3.31± 1.57
Day 2				
Soil	5.63	5.60	5.93	5.72± 0.18
0.0 m	-	3.15	4.15	3.65± 0.70
0.5 m	4.35	6.01	5.14	5.16± 0.83
1.0 m	3.01	4.86	2.90	3.59± 1.10
2.0 m	4.67	4.79	4.14	4.53± 0.34
Day 3				
Soil	5.68	5.74	6.00	5.81± 0.17
0.0 m	-	-	-	-
0.5 m	4.77	5.02	-	4.89± 0.17
1.0 m	3.28	4.98	4.74	4.33± 0.92
2.0 m	4.57	3.53	4.23	4.11± 0.53

- = missing data (failed to reach minimum DNA concentrations: 0.20 ng/uL of usable PCR product was required to generate sequencing output of 10,000 raw reads); * = outlier. A data point was considered to be an outlier if it was more than 1.5 x above the third quartile or below the first quartile. Scrub 1, 2 and 3 refer to samples collected from the scrub habitat study sites.

Alpha diversity ranged from 1 to 6 and was highest at soil level followed by the lower air sampling levels (0.0 m-0.5 m) and the upper sampling levels (1.0 m-2.0 m), respectively.

When the lower sampling heights and the upper sampling heights were merged (0.0 with 0.5 m; 1.0 m with 2.0 m), we observed a significant negative correlation between alpha diversity and sampling height (*r* = -0.68, df = 38, *P* = <0.01) (Figure 3B). Following an examination of alpha diversity scores for individual sites and dates, all variants showed negative correlations between alpha diversity and sampling height. Four out of six indicated strong and significant relationships (Day 1: *r* = -0.76, *P* = 0.00; Day 3: *r* = -0.64, *P* = 0.01; SC01: *r* = -0.68, *P* = <0.01; and, SC03: *r* = -0.73, *P* = 0.01; Table 2). It is important to note that we omitted 6 samples from the lower heights due to failure to reach minimum DNA concentrations (as denoted by "-" in Table 1).

With the merged sampling heights, all correlations increased in strength and were all statistically significant (Table 2). A Mann-Whitney Wilcoxson test for differences in alpha diversity between the merged air sampling heights (0.0m-0.5m and 1.0m-2.0m) showed a statistically significant difference (*W* = 188, *P* = <0.01). A Kruskal Wallace chi-squared test indicated no significant difference in correlations between sites or dates (*P* = 0.44).

Table 2. Correlation scores of alpha diversity and sampling height based on all air and soil samples, followed by merged air sampling heights (0.0m-0.5m and 1.0m-2.0m) and soil samples.

Days/sites	***r* score**	***df***	***P-value***
Day 1 (04-11-19)	-0.76	11	<0.01***
Day 2 (05-11-19)	-0.31	12	0.17
Day 3 (06-11-19)	-0.64	11	0.01**
Scrub 01 (SC01)	-0.68	13	<0.01***
Scrub 02 (SC02)	-0.41	12	0.14
Scrub 03 (SC03)	-0.73	9	0.01**
Merged air sampling heights (0.0m-0.5m and 1.0m-2.0m):			
Day 1 (04-11-19)	-0.76	11	<0.01***
Day 2 (05-11-19)	-0.59	12	0.02*
Day 3 (06-11-19)	-0.72	11	<0.01***
Scrub 01 (SC01)	-0.72	13	<0.01***
Scrub 02 (SC02)	-0.54	12	0.04*
Scrub 03 (SC03)	-0.86	9	<0.01***

<0.01 ‘***’ 0.01 ‘**’ 0.05 ‘*’ . The Pearson’s product moment correlation test was used. Correlation scores for each sampling date and site are included. Scrub 1, 2 and 3 refer to samples collected from the scrub habitat study sites.

Using these same merged sampling heights, a 2-sample test for equality of proportions with continuity correction showed a significant difference in proportions of taxa that occurred in lower air sampling heights (compared to upper sampling heights) that also occurred in the soil samples. The positive relationship between the proportion of taxa occurring in the air that also occurred in the soil decreased as vertical distance from the soil increased. For example, at the genus level, 84.4% of taxa in the lower air samples also occurred in the soil samples, whereas only 76.1% of the taxa in the upper air samples occurred in the soil. This difference was statistically significant (Chi-squared = 9.5376, df = 1, *P* = <0.01; Figure 4 shows taxonomic breakdown).

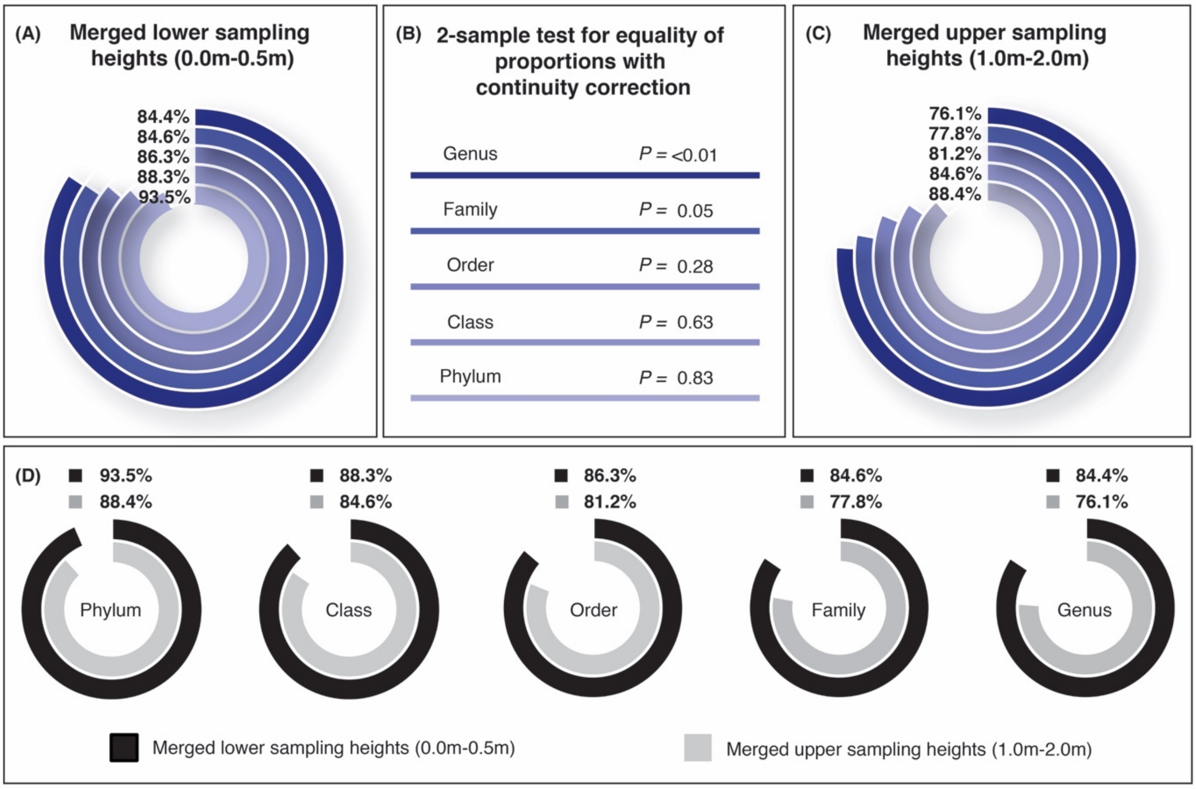

Figure 4. Radial charts showing proportions (as %) of taxa from the air samples that also occurred in the soil samples for each sampling height and across all available taxonomic levels. A 2-sample test for equality of proportions shows significant differences between lower and upper sampling heights for both genus and family taxonomic levels. Merged lower sampling heights are shown on the left (A), with the

radial bar colours corresponding to the taxonomic level shown in (B), and merged upper heights are shown on the right (C). Proportional differences for individual taxonomic levels are compared in (D) with black radial bars indicating lower sampling heights, and grey indicating upper heights.

Sampling heights displayed distinct bacterial signatures (Figure 5, panel A). Sampling height explained 22% of the variation in environmental microbiota when all air sampling heights and the soil level were included, and this was statistically significant (PERMANOVA df = 4, F = 2.50, R^2 = 0.22, P = <0.01, permutations = 999).

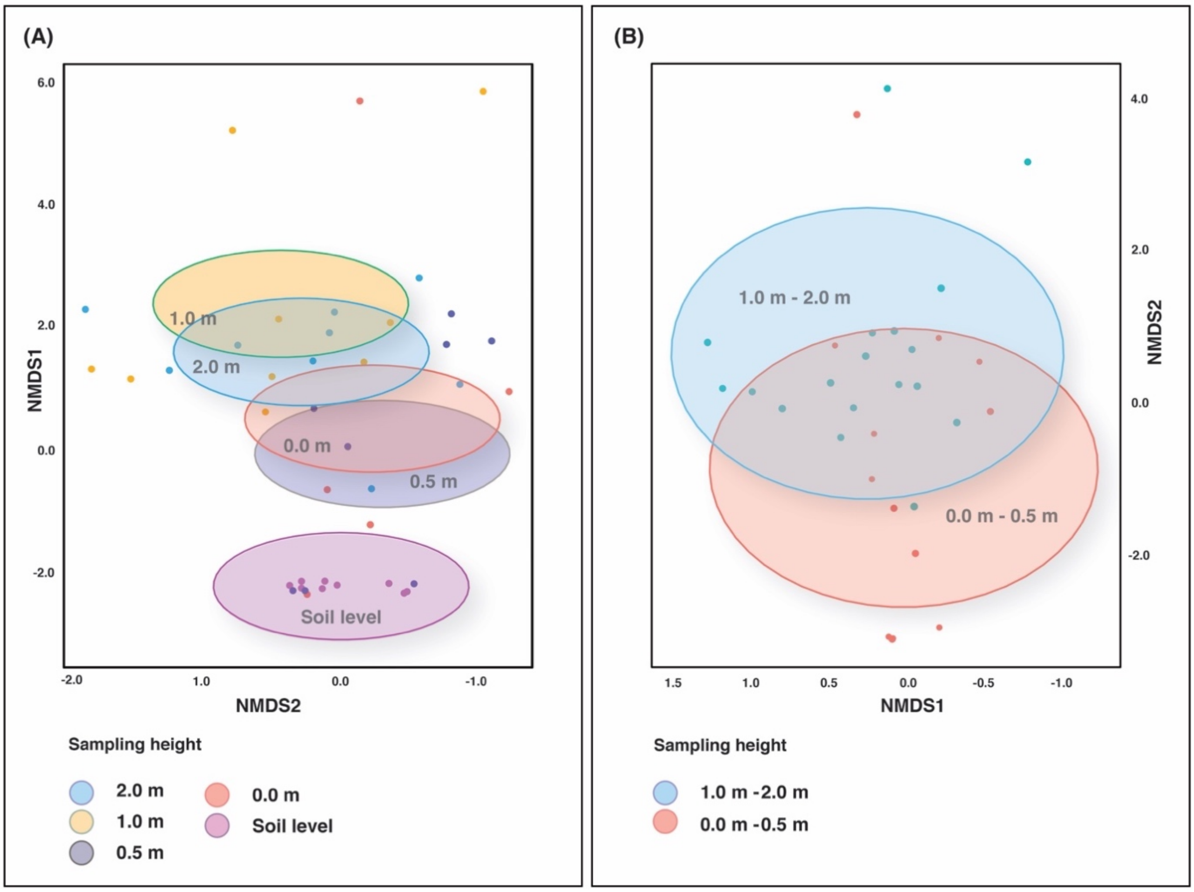

Figure 5. Non-metric multidimensional scaling (NMDS) ordination plots for visualising bacterial beta diversity (community composition) for all sampling heights, including soil (A) (Stress: 0.09, R^2 = 0.22) and for all sampling heights, excluding soil

and merging within lower and upper samples (B) (Stress: 0.10, $R^2 = 0.06$). Ellipses represent Euclidian distance from the centre – with the radius equal to the confidence level (0.95). Clusters suggest clear differences between communities at different sampling heights (indicated by the colours).

When analysing air samples in isolation, sampling height explained 11% of the variation in environmental microbiota, however, this was not significant (df = 3, F = 1.18, $R^2 = 0.11$, $P = 0.15$, permutations = 999). When we merged within lower and upper sampling heights, sampling heights explained 6% of the variation and this was statistically significant (df = 1, F = 1.98, $R^2 = 0.06$, $P = 0.01$, permutations = 999) (Figure 5, panel B).

The dominant taxa in the soil and lower sampling heights were Actinobacteria (based on mean relative abundance >1%), and the dominant taxa in the upper sampling heights were Proteobacteria (Figure 6; segments 1 and 9). A significantly greater proportion of Actinobacteria were present in lower air sampling heights (merged 0.0m-0.5m; 43.52% and 26.61%, respectively; $\bar{x} = 35.07\%$) compared to upper air sampling heights (merged 1.0m-2.0m; 17.52% and 19.67%, respectively; $\bar{x} = 18.59\%$) (Chi-squared = 6.1032, df = 1, $P = 0.01$).

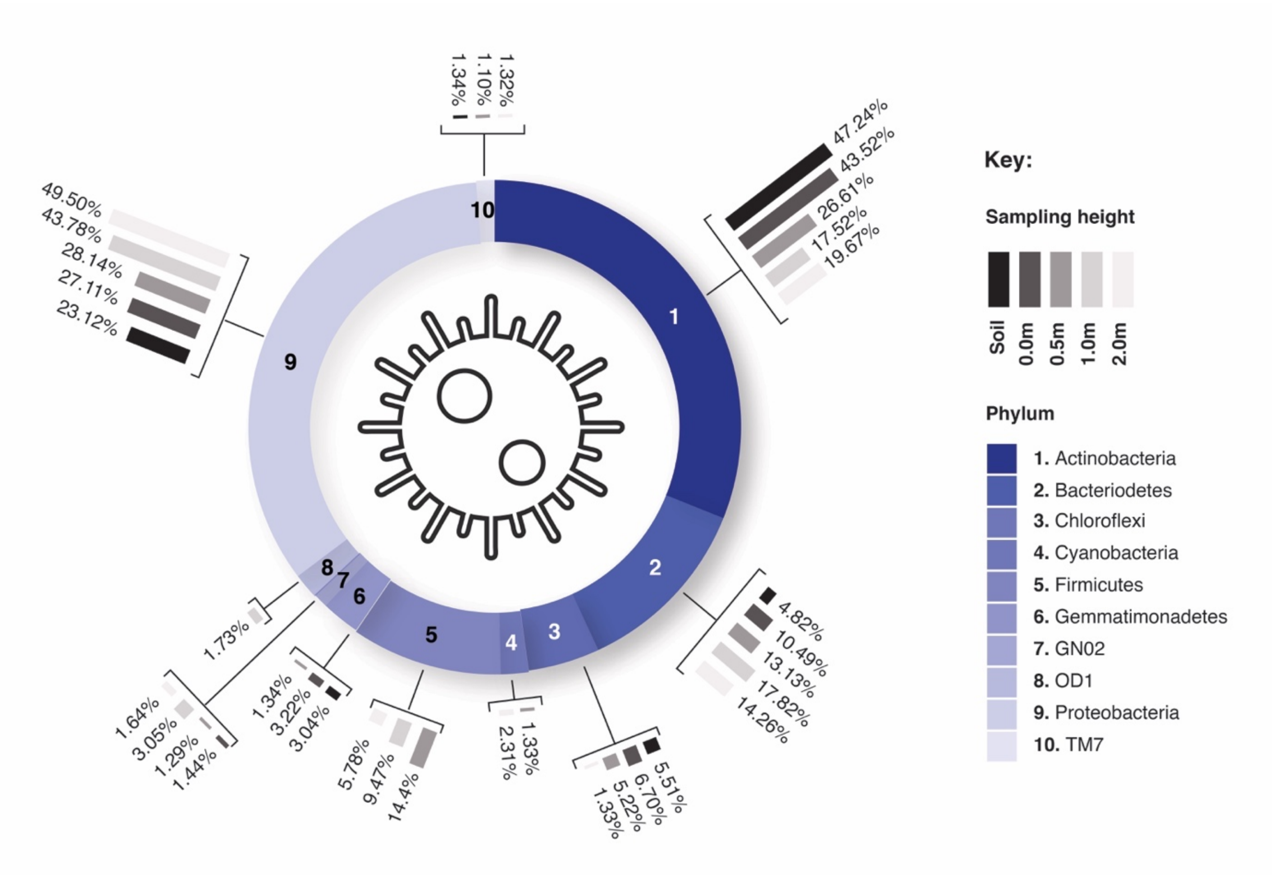

Figure 6. Relative abundance of bacterial OTUs at the phylum taxonomic level (based on mean relative abundance >1% for each sampling height). Ring segments relate to phyla via the number key on the right; segment size corresponds to mean relative abundance across all heights; mini bar charts relate to relative abundance of taxa for individual sampling heights where applicable. Actinobacteria (1) dominate lower sampling heights (indicated by the darker coloured bars), Proteobacteria (9) dominate upper sampling heights (indicated by the lighter coloured bars).

A significantly greater proportion of Proteobacteria was present in the upper air sampling heights (merged 1.0m-2.0m; 43.78% and 49.50% respectively; x̄ = 46.64%) compared to the lower air sampling heights (merged 0.0m-0.5m; 27.11% and 28.14%, respectively; x̄ = 27.63%) (Chi-squared = 6.9471, df = 1, *P* = <0.01).

A number of relatively abundant and notable taxa (contingent primarily on their implications for public health) were identified in the samples (Figure 7). The relative abundance of these taxa differed across sampling heights and all significantly correlated with sampling height, ranging from moderate to strong relationships (Table 3). The relative abundance of these taxa are as follows: *Streptomyces* (3.63% and 3.7% in soil and 0.0 m, respectively), *Kingella* (2% and 4.1% in 1.0 m and 2.0 m, respectively), *Lactobacillus* (5.9% and 3.8% in 1.0 m and 2.0 m, respectively), *Flavobacterium* (4.3% in 0.0 m , 7.5% in 0.5 m, 7.9% in 1.0 m, and 4.8% in 2.0 m), and *Sphingomonas* (4.3% in 0.0 m, 4.8% in 0.5 m, 6.5% 1.0m, and 6.8% in 2.0 m). The potential implications of these taxa for public health are highlighted further in Table 4 in the Discussion.

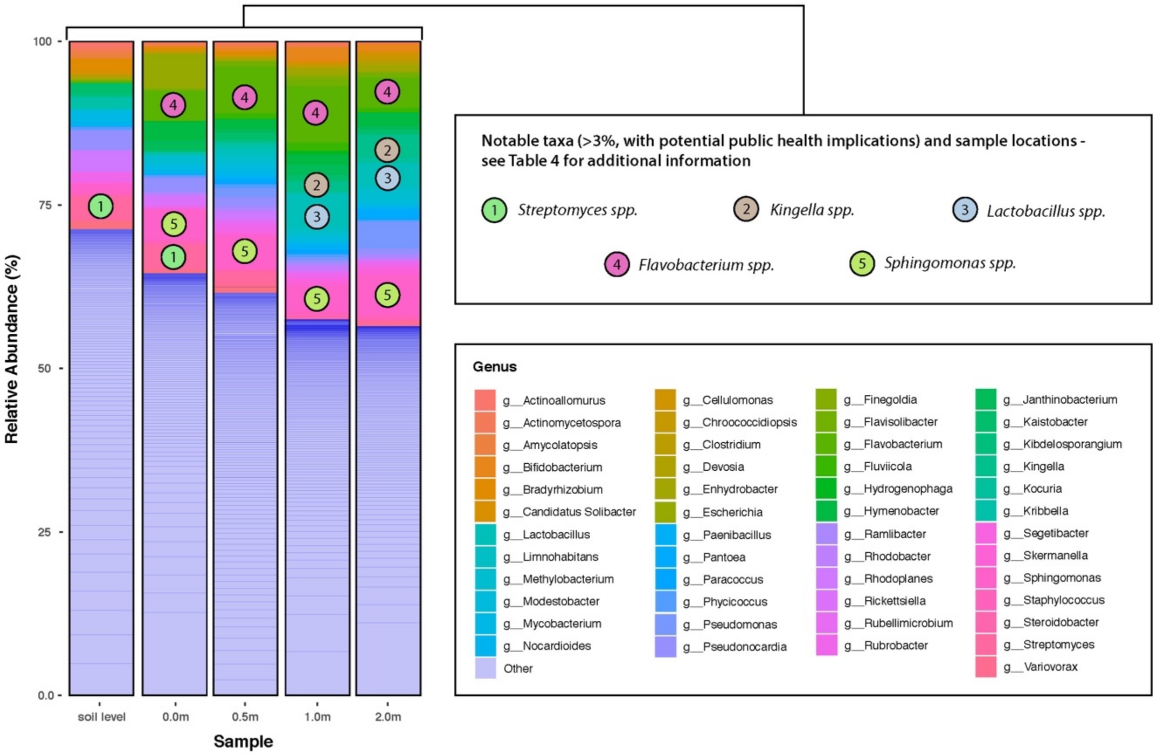

Figure 7. Relative abundance of bacterial OTUs at the genus taxonomic level and identification of notable taxa. Refer to Table 4 for potential public health implications of notable taxa. Reference numbers within the relative abundance bars correspond to the number key and notable taxa displayed in the upper-right pane.

Table 3. Correlations for notable taxa at the genus level across sampling heights, based on mean relative abundance (>1%) for each sampling height.

Ref	Taxa (genus)	r_s score	S	*P-value*
1	*Streptomyces*	-0.66	23596	<0.01***
2	*Kingella*	+0.39	8606	<0.01***
3	*Lactobacillus*	+0.54	6470	<0.01***
4	*Flavobacterium*	+0.53	6639	<0.01***
5	*Sphingomonas*	+0.39	8577	<0.01***

<0.01 '***' 0.01 '**' 0.05 '*'; The Spearman's rank order correlation test was used. S = (n3-n) × (1-*rs*)/6 where n is the number of bivariate observations and *rs* is Spearman's rank correlation coefficient; Reference number refers to the number key and corresponding notable taxa in Figure 7 and Table 4.

5.5. Discussion

5.5.1. Vertical stratification of aerobiome alpha diversity

Here we show that vertical stratification of aerobiome alpha diversity occurred in an urban green space habitat - scrub in Adelaide Parklands, South Australia. This transpired as a significant association in the reduction of bacterial alpha diversity as height increased (i.e., between the ground surface level and two vertical meters of the air column). When considering all sampling heights, alpha diversity reduced with greater height. This vertical stratification in alpha diversity was neither spatially (i.e., site specific) or temporally dependent. The strength of the negative relationship between alpha diversity and height increased when we merged lower sampling heights (0.0 m with 0.5 m) and the upper sampling heights (1.0 m with 2.0 m). This implies that the required spatial frequency to elucidate vertical stratification in alpha diversity — specifically, five sampling heights across a 2 m vertical transect — may have been overestimated. However, several omissions in the lower sampling heights due to failure to reach minimum DNA concentrations could have affected the strength of this association.

The decay in observed alpha diversity as height increased could be the result of increasing distance from the primary source, that is, potentially the soil. It is widely accepted that soil represents one of the most microbially-diverse terrestrial habitats (Briones, 2014; Bender et al. 2016; Dumbrell, 2019; Zhu et al. 2019). Therefore, it seems reasonable to suggest that lower sampling heights may possess a higher level of microbial diversity as they are closer to a potentially greater concentration of microbiota. We observed that a greater proportion of bacteria taxa found in the lower sampling heights (compared to the upper sampling heights) were also present in the soil samples, both at genus and family levels. Together, these results suggest that soil does appear to play a key role in supplementing the local aerobiome, particularly at lower heights.

The presence of vertical stratification of bacterial diversity in the aerobiome could have important implications for human health. Indeed, exposure to environmental microbes is thought to prime and ‘educate’ the immune system (Belkaid and Hand, 2014; Hanski, 2014; Minchim et al. 2020) particularly in early life, and a recent mouse study suggests that exposure to environmental microbes such as the butyrate-producer *Kineothrix alysoides* could also have anxiolytic (anxiety-reducing) effects (Liddicoat et al. 2019). The vertical stratification concept could also be important for exposome researchers, who investigate the types and methods of exposures to both endogenous and exogenous chemical composites (including microbes and their biological compounds across the life-course) (Escher et al. 2017; Daiber et al. 2019; McCall et al. 2019). The presence of vertical stratification implies that the potential for exposure to environmental microbial diversity may differ throughout the human life-course due to age and gender differences in height, activity types, and methods of motion. However, our static experimental conditions fail to capture the dynamics of human movement and activity within and between environments. Further research is required to understand how vertical stratification may impact human colonisation with particular focus on the dynamic nature of human movement through environments. Additional research into aerobiome stratification could lead to improved design and management of three dimensional urban structures and vegetation assemblages which may influence aerobiome dynamics. In the future, this could lead to ways of optimising human-environmental microbe interactions.

Humans are spending more time indoors (Ergan et al. 2019). Therefore, future aerobiome studies should also consider whether vertical stratification occurs indoors, and consider the relative influence of the outdoor environment and the potential health implications of these dynamics. Understanding how patterns of human behaviour influence exposure to airborne microbiota will also be important to understand. For example, ongoing changes to commuting, recreation and living environments may have important implications for aerobiome characteristics and exposure potential.

5.5.2. Vertical stratification of aerobiome beta diversity

We also showed vertical stratification of aerobiome beta diversity, where sampling height explained 22% of the variation in environmental microbiota when all sampling heights were included. This was corroborated by the analysis of equality of taxonomic proportions between the air and the soil samples. As mentioned, the proportion of bacterial taxa from the air samples that were also present in the soil decreased as altitude increased. This provides preliminary evidence that soil has a stronger influence on aerobiome composition at lower heights and allochthonous sources make a key contribution to the aerobiome higher up.

It is likely that distance to source makes a key contribution to aerobiome vertical stratification. However, there may be other important biophysical driving factors. For example, the size range of bacterial cells can vary by eight orders of magnitude (from 0.013 µm to 750 µm) (Levin and Angert, 2015). However, many bacteria are thought to occur in the 0.3-5 µm range (Schaechter, 2016). Bacteria can also nucleate and exist as ‘clumps’ or adhere to larger suspended particles (Tham and Zuraimi, 2005; Haas et al. 2013; Gong et al. 2020), thus altering their net particle size that would influence their fluid dynamics. Airborne bacterial concentrations can be influenced by several factors including ambient temperature, humidity, wind dynamics and PM concentrations (Gong et al. 2020), and these factors could also play important roles in vertical stratification, and warrant further research. There also appeared to be some mixing of aerobiome signals within fine vertical resolution strata, while more sensible patterns emerged in larger vertical strata. These findings are consistent with the phenomenon of turbulent mixed (non-laminar) flow, and we might expect some level of vertical mixing in the aerobiome where turbulent flow occurs over and around obstacles and over rough surfaces.

Vertical stratification in bacterial *beta* diversity could also have important implications for public health. For example, our results point to intriguing questions such as: (a) are there significant and consistent differences in potentially beneficial and pathogenic bacterial assemblages at different altitudes in the aerobiome? (b) does this affect exposure and colonisation in humans across the life-course? (c) what are the downstream health implications of this, if any? We provide a preliminary contribution towards answering question (a), as discussed in the following section.

Future research could also consider the potential influence of physicochemical (e.g., anti-microbials, pesticide use) and social (e.g., crowd gathering or isolation/distancing) practices on microbial vertical stratification. For example, efforts to reduce infectious agents such as COVID-19 may disrupt out relationship with environmental microbiomes. Therefore, understanding whether and how these changes affect human-environmental microbial interactions will be essential in the future.

5.5.3. Relative abundances and notable taxa

Following the analyses of relative abundances, the dominant taxa in the soil and lower sampling heights were found to be Actinobacteria, and the dominant taxa in the upper sampling heights were Proteobacteria. This is not surprising given that a large proportion of terrestrial Actinobacteria are soil-dwelling organisms (Barka et al. 2016; Zhang et al. 2019), and both phyla are amongst the largest in the bacterial domain (Verma et al. 2013; Polkade et al. 2016; Rizzatti et al. 2017). Other studies have shown similar dominant roles for these phyla in the aerobiome (Arfken et al. 2015; Maki et al. 2017; Li et al. 2018), but vertical stratification has not, to our knowledge, been explored.

We identified a number of notable dominant taxa at the genus-level, including: *Streptomyces*, *Kingella*, *Lactobacillus*, *Flavobacterium*, and *Sphingomonas*. With the exception of *Flavobacterium*, species in these genera are considered to have potentially beneficial or pathogenic impacts on human health. For example, the Actinobacteria *Streptomyces spp*., is considered to be a microbial 'old friend' and potentially beneficial to human health via production and regulation of anti-proliferative, anti-inflammatory and antibiotic compounds (Bolourian and Mojtahedi, 2018; Nguyen et al. 2020). This genus had higher relative abundance at lower sampling heights. On the other hand, members of the *Kingella* genus such as *K. kingae* are considered to be pathogenic to humans, for example, causing debilitating conditions such as osteomyelitis and septic arthritis, particularly in children (Kiang et al. 2005; Nguyen et al. 2018; Ingersol et al. 2019). These findings warrant further research, because if consistent across time and space, the spatial and compositional differences in microbiota have the potential to be important considerations for public health through the modulation of exposure.

Table 4. Notable taxa (OTUs at the genus level) identified during the examination for bacterial relative abundance – based on mean relative abundance (>1%) for each sampling height.

Ref	**Notable taxa**	**Potential public health implication**
1	*Streptomyces spp.*	These Actinobacteria are relatively more abundant at lower (vertically) sampling levels. They are soil-associated but also considered to be 'old friends' with potential beneficial implications for human health (Bolourian and Mojtahedi, 2018).
2	*Kingella spp.*	Higher relative abundance at upper (vertical) levels. The gram negative *K. kingae* is considered to be pathogenic to humans – causing osteomyelitis and septic arthritis, particularly in children (Kiang et al. 2005; Nguyen et al. 2018).
3	*Lactobacillus spp.*	Gram positive Firmicutes, relatively more abundant at upper levels. Some species are widely considered to be beneficial 'old friends' and probiotics in humans and other ecosystems (Rook et al. 2014) (e.g., *L. acidophilus; L. plantarum; L. rhamnosus).*

4	*Flavobacterium spp.*	Soil and water-dwelling Bacteroidetes bacteria. These are present in all levels but with highest relative abundance at upper levels. Generally not considered to be pathogenic to humans. Spatial distribution suggests potential allochthonous deposition.
5	*Sphingomonas spp.*	These are Proteobacteria, found in a variety of environments. Relatively abundant in all sampling heights but less so in the soil level. These organisms are not considered to be pathogenic to humans and can in fact be highly beneficial via their ability to break down polycyclic aromatic hydrocarbons, which are deleterious to human health (Macchi et al. 2018; Asaf et al. 2020).

The taxa in this table may have important public health implications as highlighted in the third column. Reference number refers to the number key and corresponding notable taxa in Figure 7.

5.6. Limitations

As a proof of concept study, we have demonstrated, for the first time, the presence of vertical stratification of microbial alpha and beta diversity at lower levels of the biosphere (ground level to 2.0 m high). However, data from a larger number of replicates from different environments and geographical areas will be required to establish the generalisability of our findings, i.e., will our results be consistent outside of the Adelaide Parklands environment? We also used OTU picking methods at the bioinformatics stage. We recognise that although this has value for short-read platforms and many studies still use this approach (Dei-Cas et al. 2020; Derilus et al.

2020; Sato et al. 2020), Amplicon Sequence Variant (ASV) analysis would have provided us a more detailed taxonomic picture of vertical stratification. Further, following the DNA extraction process, three samples (each at SC03 0.0 m) failed to reach sufficient DNA concentrations to enable PCR and sequencing, which may have affected the vertical stratification relationship — we can only speculate that the relationship would have been stronger with their inclusion. There are many sensitive variables involved with processing low biomass samples (Eisenhofer et al. 2019; McArdle and Kaforou, 2020) and perhaps even more stringent workflows are required for passive sampling.

5.7. Conclusions

We provide support for the presence of aerobiome vertical stratification in bacterial diversity (alpha and beta), and demonstrate that significant spatial differences in potentially pathogenic and beneficial bacterial taxa may occur. Although the need to promote healthy ecosystems and understand environmental microbial exposures has always been important, in light of the COVID-19 pandemic, it is now justifiably at the forefront of many public health agendas worldwide. As discussed, there is growing evidence to suggest that exposure to the microbiome in biodiverse green spaces contributes towards 'educating' the immune system (Rook et al. 2003; Rook et al. 2013; Arleevskaya et al. 2019; Liddicoat et al. 2020). Furthermore, the microbiome is thought to support the immune system's defensive role against pathogens, and prevent hyper-inflammatory responses and metabolic dysregulation — risk factors for severe COVID-19 (Torres et al. 2019; Guo et al. 2020). Gaining a greater understanding of the transmission routes and physical factors (such as the vertical differential) affecting our exposure to environmental microbiomes — including potentially beneficial and pathogenic species — is likely to play an increasingly important role in the health sciences.

Strategies to explicitly consider the microbiome as part of health-promoting urban green spaces have recently been proposed, such as Microbiome-Inspired Green Infrastructure (MIGI) (Robinson et al. 2018; Watkins et al. 2020). Further exploration of aerobiome vertical stratification could make an important contribution to this approach. For example, there could be value in determining whether different habitats and vegetation management regimes impact vertical stratification in urban green spaces, and elucidating the downstream health effects on urban dwellers. Building on our findings — that vertical stratification did occur in an urban green space aerobiome — has the potential to inform future exposome research, urban biodiversity management, and disease prevention strategies.

Exposure to Airborne Bacteria Depends upon Vertical Stratification and Vegetation Complexity

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5.8. Abstract

Exposure to biodiverse aerobiomes supports human health, but it is unclear which ecological factors influence exposure. Few studies have investigated near-surface

green space aerobiome dynamics, and no studies have investigated aerobiome vertical stratification in different urban green spaces. We used columnar sampling and next generation sequencing of the bacterial 16S rRNA gene, combined with geospatial and network analyses to investigate urban green space aerobiome spatio-compositional dynamics. We show a strong effect of habitat on bacterial diversity and network complexity. We observed aerobiome vertical stratification and network complexity that was contingent on habitat type. Tree density, closer proximity, and canopy coverage associated with greater aerobiome alpha diversity. Grassland aerobiomes exhibited greater proportions of putative pathogens compared to scrub, and also stratified vertically. We provide novel insights into the urban ecosystem with potential importance for public health, whereby the possibility of differential aerobiome exposures appears to depend on habitat type and height in the airspace. This has important implications for managing urban landscapes for the regulation of aerobiome exposure.

5.9. Introduction

Exposure to biodiverse environmental microbiomes – the diverse consortium microorganisms in a given environment – plays an important role in human health (Rook et al. 2003; Dannemiller et al. 2014; Stein et al. 2016; Arleevskaya et al. 2019; Liddicoat et al. 2020). From an early age, a complex network of environmental microorganisms supports the development and regulation of immunity (Rook et al. 2014). Indeed, exposure to a wide range of microorganisms is thought to strengthen our response to noxious stimuli (e.g., pathogens) and reduce the likelihood that our immune systems will be oversensitive to innocuous agents, such as dust particles, pollen, and our own cells — the latter manifesting as autoimmunity (Rook, 2013; Schwinge and Schramm, 2019; Prescott, 2020).

Urbanisation and loss of macro-biodiversity are linked to loss of microbial diversity, which could negatively impact the health-supporting microbial communities residing in and on human bodies – the human microbiome (Prescott et al. 2017; Austvoll et al. 2020). This loss of microbial diversity underpins the *biodiversity hypothesis*, which draws a link between concurrent global megatrends of biodiversity loss (including microorganisms) (Haahtela, 2019) and rapid increases in noncommunicable diseases (NCDs) (Haahtela et al. 2013). A recent study empirically tested this hypothesis and found that exposure to plant diversity and associated microbial communities significantly correlated with reduced risk of acute lymphoblastic leukemia by promoting immune maturation (Donovan et al. 2020).

Furthermore, biodiverse environments could supplement human microbiomes with functionally important microorganisms. Short chain fatty acids (SCFAs) are produced by certain bacteria as metabolic by-products and are known to play important roles in supporting human health. For example, the SCFA *butyrate* is linked to the inhibition of intestinal tumours (Chen et al. 2020) and atherosclerosis (Du et al. 2020), as well as supporting bone formation (Li et al. 2020) and promoting epithelial integrity (Geirnaert et al. 2017). Such microorganisms may be transferred through aerobiomes. For example, in a randomised controlled mouse study, a putative soil-associated butyrate-producing bacteria was found to be supplemented in mice gut microbiota following trace-level airborne soil dust exposures and subsequently linked to reduced anxiety-like behaviour (Liddicoat et al. 2020).

The aerobiome — the collection of microorganisms in a given airspace — is an important source of environmental microorganisms (Uetake et al. 2019; Flies et al. 2020; Selway et al. 2020). Despite this importance, only limited studies have investigated the dynamics of near-surface aerobiomes in urban green spaces. Mhuireach et al. (2016) showed that aerobiomes in urban green and grey spaces had distinct compositions. Subsequent studies have shown vegetation type has a potential modulating effect on aerobiome diversity and composition (Lymperopoulou et al. 2016; Abdelfatttah et al. 2019). Stewart et al. (2020) found that aerobiomes varied in composition and function between urban and suburban sites. Mhuireach et al. (2019) identified localised influences on aerobiomes, including weather and land management (Mhuireach et al. 2016). Our recent work has also demonstrated aerobiome vertical stratification between ground level and 2 m heights in an urban green space (Robinson et al. 2020b). Together, these studies suggest that individuals may be exposed to different aerobiomes depending on the type of habitat visited and human-scale height-based variation in environmental aerobiomes. Consequently, understanding the effects of habitat and height, and their interactions, on aerobiomes could have important implications for public health.

There is growing recognition that urban green spaces are important for human health and wellbeing through provision of psychosocial and biological benefits (Robinson and Breed, 2019; Callaghan et al. 2020; Cameron et al. 2020; Robinson et al. 2020a; Yeh et al. 2020). Gaining a deeper understanding of urban green space aerobiome exposure potential could inform public health and environmental management strategies in the future. In this study, we used an innovative columnar sampling method to sample aerobiome bacterial communities in three urban green space habitat types in the Adelaide Parklands, South Australia. These habitats included amenity grasslands, woodland/scrub (dominated by native *Eucalyptus spp.* trees and shrubs; henceforth referred to as 'scrub'), and bare ground habitat; each is a typical urban green space habitat. We conducted next generation sequencing of the bacterial 16S rRNA gene to characterise the diversity, composition and network complexity of aerobiomes. We also applied geospatial analytical methods to explore the potential influence of trees on the micro-biodiversity of aerobiomes. Our primary objectives were to: **(a)** assess aerobiome composition and micro-biodiversity differences between the three habitats; **(b)** compare aerobiome vertical stratification between the different habitats; **(c)** assess whether tree density, distance to trees, and tree canopy coverage influenced bacterial alpha diversity; and, **(d)** to assess any differences in known pathogenic bacterial taxa between habitats and sampling heights.

5.10. Results

Bacterial communities were dominated by three key phyla in all three habitats: Proteobacteria, Bacteroidetes, and Actinobacteria, however, abundance differed depending on height (**Fig. 1**) (full description of sequencing reads in Supplementary Materials, Appendix B). We now present the results in order of the objectives (a-d) set out in the Introduction.

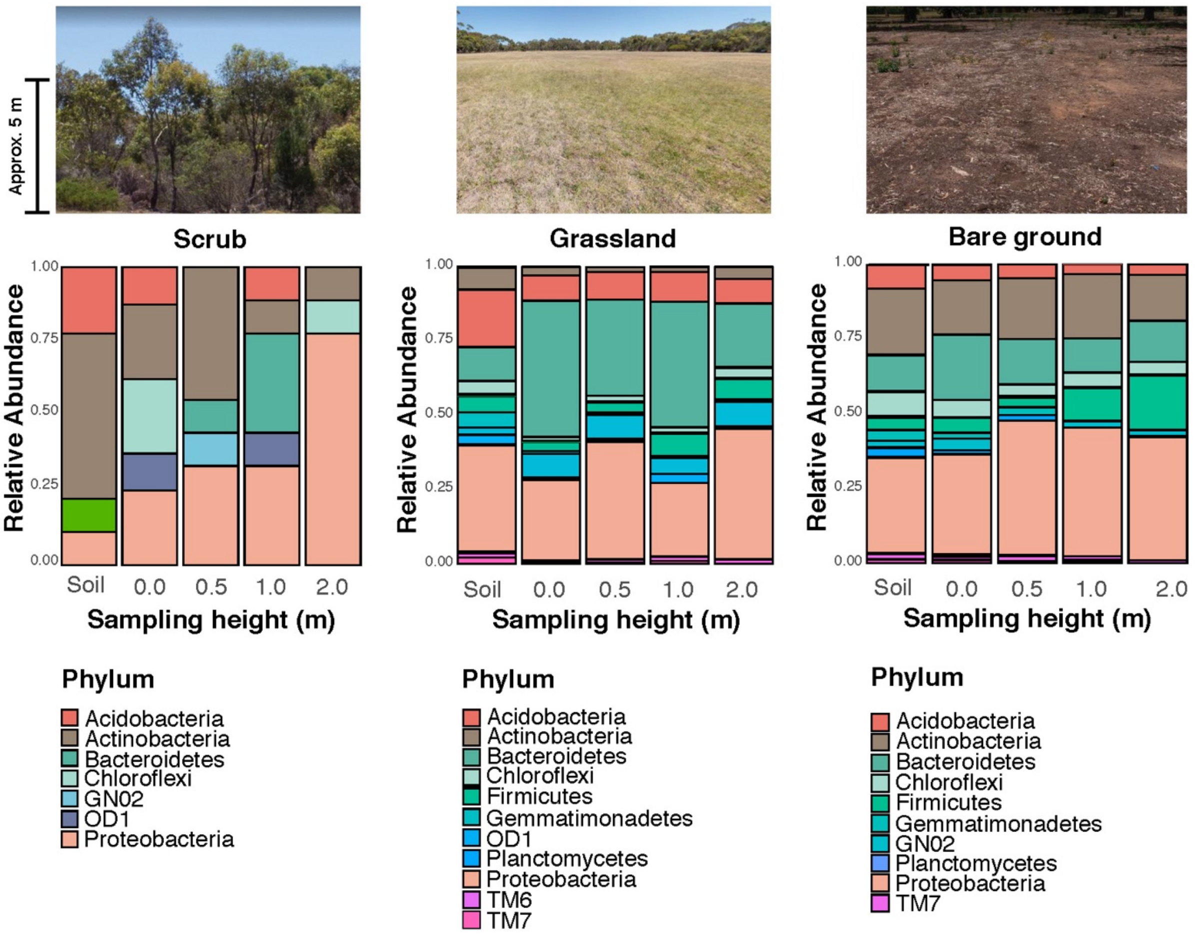

Fig. 1. Profile of bacterial communities from each habitat at the phylum level. The coloured area of each bar represents the relative abundance of the corresponding phylum over 1%. The X-axis displays sampling heights: soil, 0.0 m, 0.5 m, 1.0 m, and 2.0 m (from left to right). The photographs above the plots show examples of each habitat used in the study (photographs by authors).

5.10.1. Comparison of bacterial alpha diversity between habitats

We found that bacterial alpha diversity of the soil differed significantly between habitats (ANOVA F = 3.95, df = 1, *p* = 0.03) (**Fig. 2**). The soil microbiome from the scrub habitat was significantly more biodiverse than the grassland habitat (Tukey multiple comparison of means test; scrub $\bar{x}$ = 5.78; grassland $\bar{x}$ = 5.46; adjusted *p* = 0.02). We also found that bacterial alpha diversity of the air differed significantly between bare ground and scrub habitats (Chi-squared = 11.3, df = 1, *p* =<0.01), with the scrub aerobiome being more biodiverse than the bare ground. Aerobiome alpha diversity of scrub and grassland were also significantly different (Chi-squared = 24.8, df = 1, *p* =<0.01), and the scrub aerobiome was the most biodiverse. No significant difference was observed in alpha diversity between bare ground and grassland habitats (Chi-squared = 0.46, df = 1, *p* =<0.49).

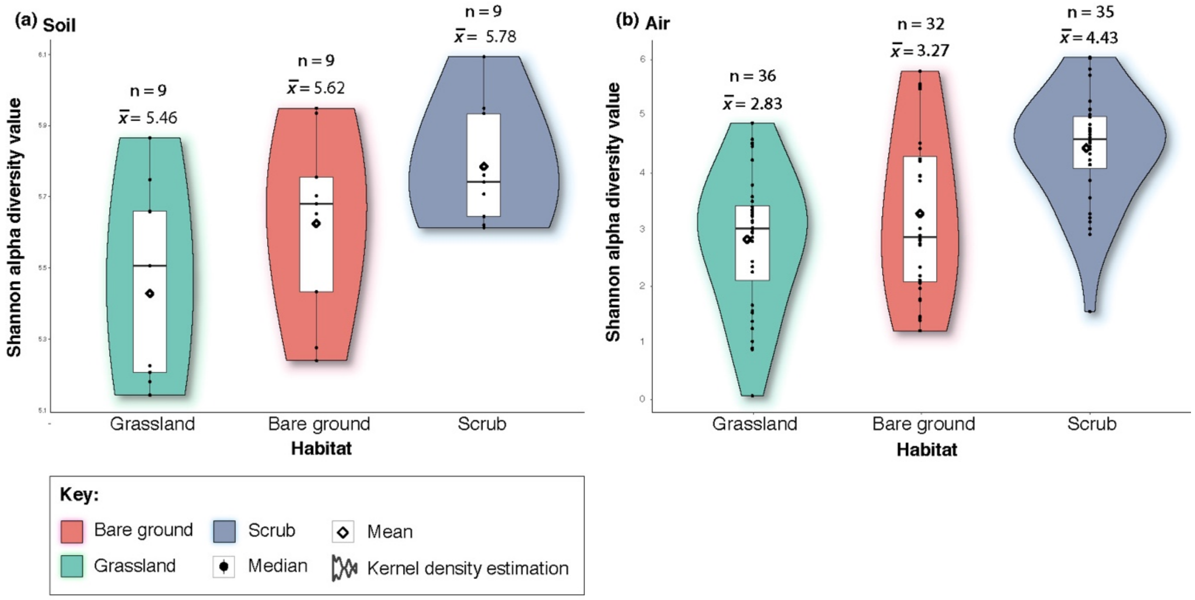

Fig. 2. Box/violin plots of Shannon alpha diversity scores for each habitat; bare ground, grassland, and scrub. Panel (a) shows inter-habitat bacterial alpha diversity for soil samples, and panel (b) shows inter-habitat bacterial alpha diversity for aerobiome samples. Plots also display mean and median values, interquartile range and kernel density estimation (Shannon alpha diversity values for each habitat, divided into days and sites, are in Supplementary Materials, Appendix B). We also tested for mean alpha diversity differences between dates and sites, showing that sampling dates and individual sites were generally not a factor in alpha diversity variation with nearly 90% of comparisons showing non-significant results.

5.10.2. Comparison of bacterial beta diversity between habitats

We observed clear differences in aerobiome compositions (beta diversity) (PERMANOVA, df = 2, F =3.7, R^2 = 0.07, p = <0.01, permutations = 999) and soil samples (PERMANOVA, df = 2, F =6.8, R^2 = 0.36, p = <0.01, permutations = 999 among habitats (**Fig. 3**). For air samples, all habitats displayed significantly distinct bacterial communities, where habitat type explained 7% variation in bacteria community composition. However, there was significant heterogeneity in dispersion (PERMDISP, F = 13, p = <0.01). For soil only, habitat type explained 36% variation in bacteria community composition, however, this increased significantly to 75% and 74% when comparing scrub to grassland and scrub to bare ground, respectively (PERMANOVA, df = 5, F =7, R^2 = 0.75 and 0.74, p = <0.01). There was no significant heterogeneity in dispersion (PERMDISP, F = 2, p = 0.07).

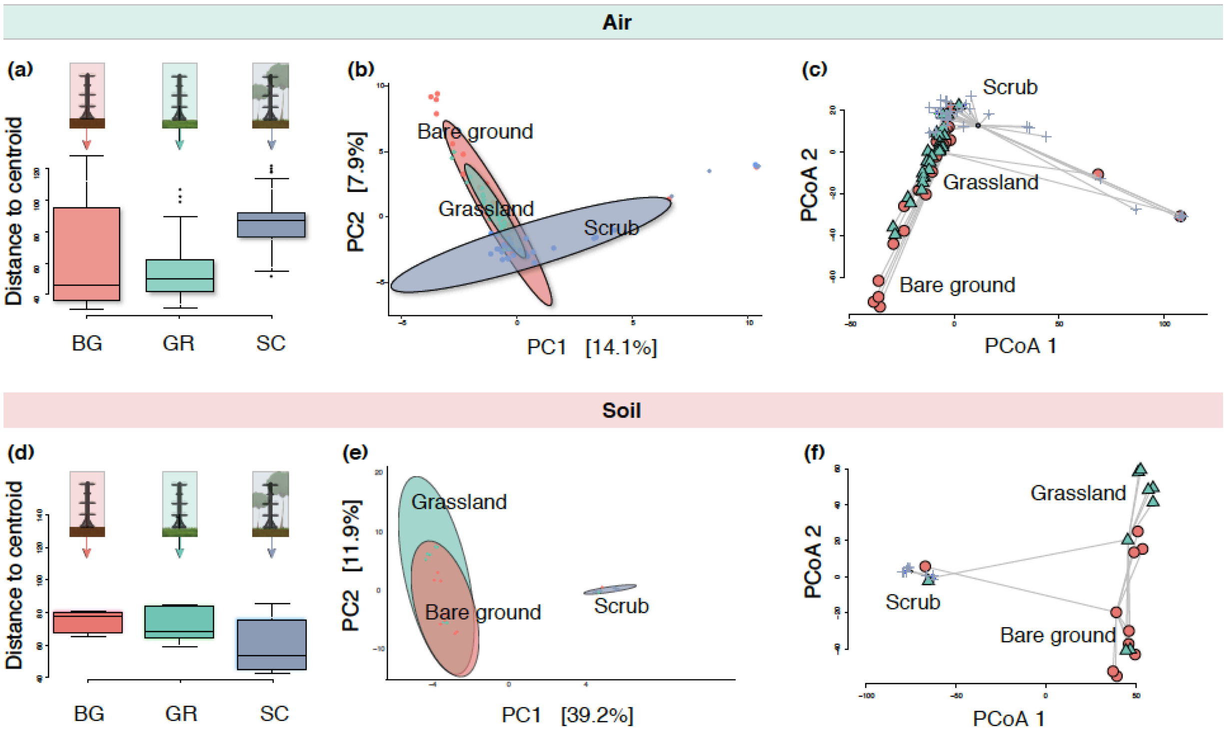

Fig. 3. Upper panels show air samples only, whereby (a) is a boxplot of dispersion (spread); (b) ordination of bacterial communities for all habitats (BG = bare ground; GR = grassland; SC = scrub), ellipses represent Euclidian distance from the centre – with the radius equal to the confidence level (0.95); and (c) ordination of dispersion by Aitchison Distance. Lower panels show soil samples only, whereby (d) is a boxplot

of dispersion; (e) ordination of bacterial communities for all habitats, ellipses represent Euclidian distance from the centre; and (f) shows an ordination of dispersion by Aitchison Distance.

5.10.3. Vertical stratification: alpha diversity

5.10.3.1. Bare ground vertical stratification: alpha diversity

For the bare ground habitat, we observed a strong negative correlation between alpha diversity (air and soil for all sites/dates) and sampling height from ground level to 2 m (Pearson's $r = -0.75$, df = 39, $p = <0.01$) (**Fig. 4, a**). Alpha diversity (Shannon scores) ranged from 1.2 to 5.93 and was highest at soil level, followed by lower air sampling levels (0.0 m-0.5 m) and upper sampling levels (1.0 m-2.0 m), respectively. Analysis of air-only samples also showed a significant negative correlation between height and bacterial alpha diversity, demonstrating vertical stratification in this bare ground habitat (Pearson's $r = -0.60$, df = 30 $p = <0.01$).

5.10.3.2. Grassland vertical stratification: alpha diversity

For the grassland aerobiome, we observed a significant negative correlation between alpha diversity (air and soil for all sites/dates) and sampling height from ground level to 2 m (Pearson's $r = -0.38$, df = 43, $p = 0.01$) (**Fig. 4, b**). Alpha diversity ranged from 1.2 to 5.9 and was highest at soil level. However, once air sample data were isolated from soil sample data and analysed separately, the correlation was weak and not significant, indicating that vertical stratification was not detected in this grassland habitat (Pearson's $r = 0.03$, df = 34, $p = 0.86$; see Supplementary Materials, Appendix B for correlations between individual dates and sites).

5.10.3.3. Scrub vertical stratification: alpha diversity

In the scrub aerobiome, we observed a significant negative correlation between alpha diversity (air and soil for all sites/dates) and sampling height from ground level to 2 m (Pearson's r = -0.59, df = 39, p = <0.01) (**Fig. 4, c**). Bacterial alpha diversity in the scrub habitat ranged from 1 to 6 (Shannon score) and was highest at soil level, followed by lower air sampling levels (0.0 m - 0.5 m) and upper sampling levels (1.0 m - 2.0 m), respectively. Analysis of air-only samples showed a significant negative correlation between height and bacterial alpha diversity, demonstrating vertical stratification in this scrub habitat (Pearson's r = -0.38, df = 30, p = 0.03).

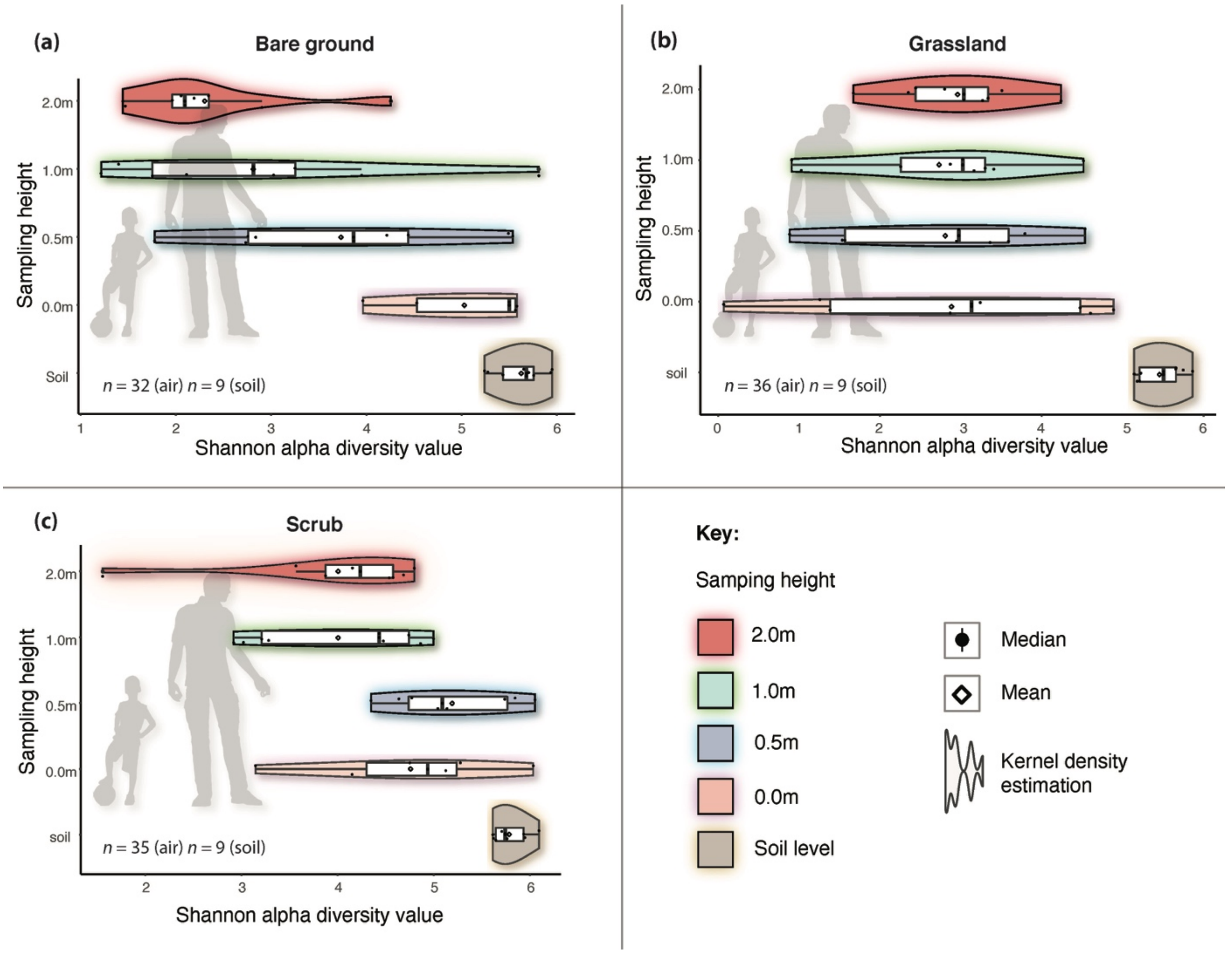

Fig. 4. Box/violin plots of Shannon alpha diversity scores for each sampling height including soil and for each habitat: (a) bare ground; (b) grassland; and (c) scrub. Plots also display mean values, interquartile range and kernel density estimation, and silhouettes of humans for perspective.

5.10.4. Vertical stratification: beta diversity

5.10.4.1. Bare ground vertical stratification: beta diversity

Sampling heights in the bare ground habitat displayed disparate bacterial compositions (**Fig. 5, a**). Sampling height explained 29% variation in bacteria community composition when all air sampling heights were included (PERMANOVA df = 4, F = 3.67, $R^2 = 0.29$, $p = <0.01$, permutations = 999). Analysis of air samples for the bare ground habitat in isolation showed that sampling height still explained 25% variation in bacterial community composition (**Fig. 5, d**) (df = 3, F = 3.06, $R^2 = 0.25$, $p = <0.01$, permutations = 999).

5.10.4.2. Grassland vertical stratification: beta diversity

Air sampling heights in the grassland habitat displayed disparate bacterial communities to the soil (**Fig. 5, b**). Sampling height explained 24% variation in bacterial community composition when all air sampling heights were included (PERMANOVA df = 4, F = 3.17, $R^2 = 0.24$, $p = <0.01$, permutations = 999). However, analysis of grassland air samples in isolation showed that sampling height only explained 9% variation in bacterial community composition (**Fig. 5, e**), and was not statistically significant (df = 3, F = 1.06, $R^2 = 0.09$, $p = 0.24$, permutations = 999).

5.10.4.3. Scrub vertical stratification: beta diversity

Sampling heights in the scrub habitat displayed disparate bacterial communities (**Fig. 5, c**). Sampling height explained 22% variation in bacterial community composition when all air sampling heights and soil were included (PERMANOVA df = 4, F = 2.9, $R^2 = 0.22$, $p = <0.01$, permutations = 999). Analysis of air samples in isolation showed that sampling height still explained 11% variation in bacterial community composition (**Fig. 5, f**) (df = 3, F = 1.30, $R^2 = 0.11$, $p = 0.03$, permutations = 999).

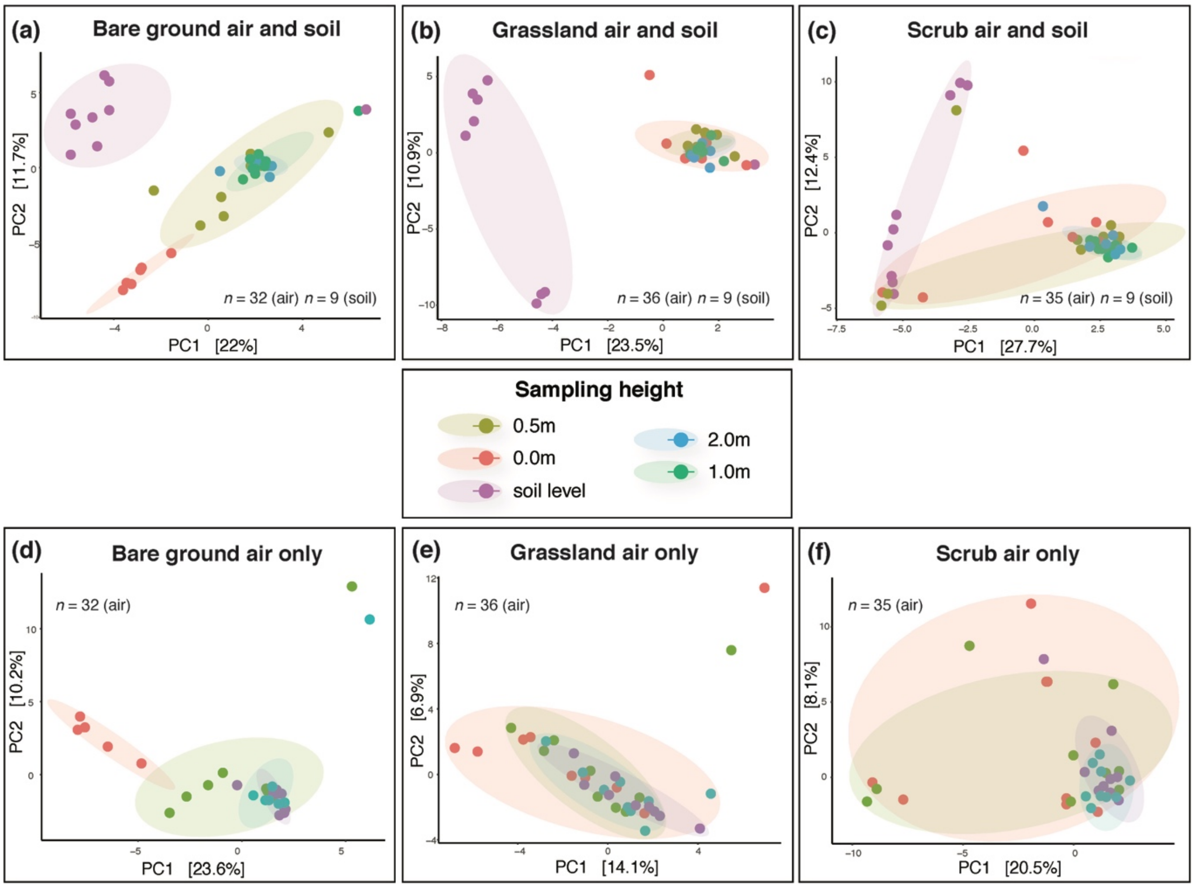

Fig. 5. Visualising bacterial beta diversity with ordination plots of Aitchison distances based on clr-transformations of OTU abundances for each sampling height across the three habitats: (a) Bare ground air and soil, (b) Grassland air and soil, (c) Scrub air and soil, (d) Bare ground air only, (e) Grassland air only, and (f) Scrub air only.

Ellipses represent Euclidian distance from the centre – with the radius equal to the confidence level (0.95). Clusters suggest differences between communities at different sampling heights (indicated by the colours).

5.10.5. Vertical stratification: aerobiome network analysis

In spite of differences in bacterial community composition and alpha diversity among the three study sites, network analyses showed an increase in the community complexity and interactions, defined by node degree and network size, at lower heights as compared to higher heights (**Fig. 6**). Bacterial OTUs in the scrub habitat at 0 to 0.5 m heights had the highest node degree, while the OTUs in the grassland habitat 1 to 2 m had the lowest node degree. At lower heights, the average association of any OTU in the grassland was less (node degree = 2.7) than the average association of OTUs for scrub (node degree= 4.9) and bare ground (node degree= 4.7) habitats. At upper heights, node degree for OTUs was highest for bare ground (2.7) followed by scrub (1.8) and grassland (1.7). Evaluation of link type, either positive or negative links, suggested a positive association among most OTUs, except for scrub 1 to 2 m which only had a small number of negative associations (**Fig. 6**). Comparisons of modularity between heights across the study sites suggested an increase in the network modularity at higher heights, despite the decrease in network connectance and node degree. Percentage of change in the modularity between heights was highest in the grassland (~ 50 %), although there were fewer nodes per module.

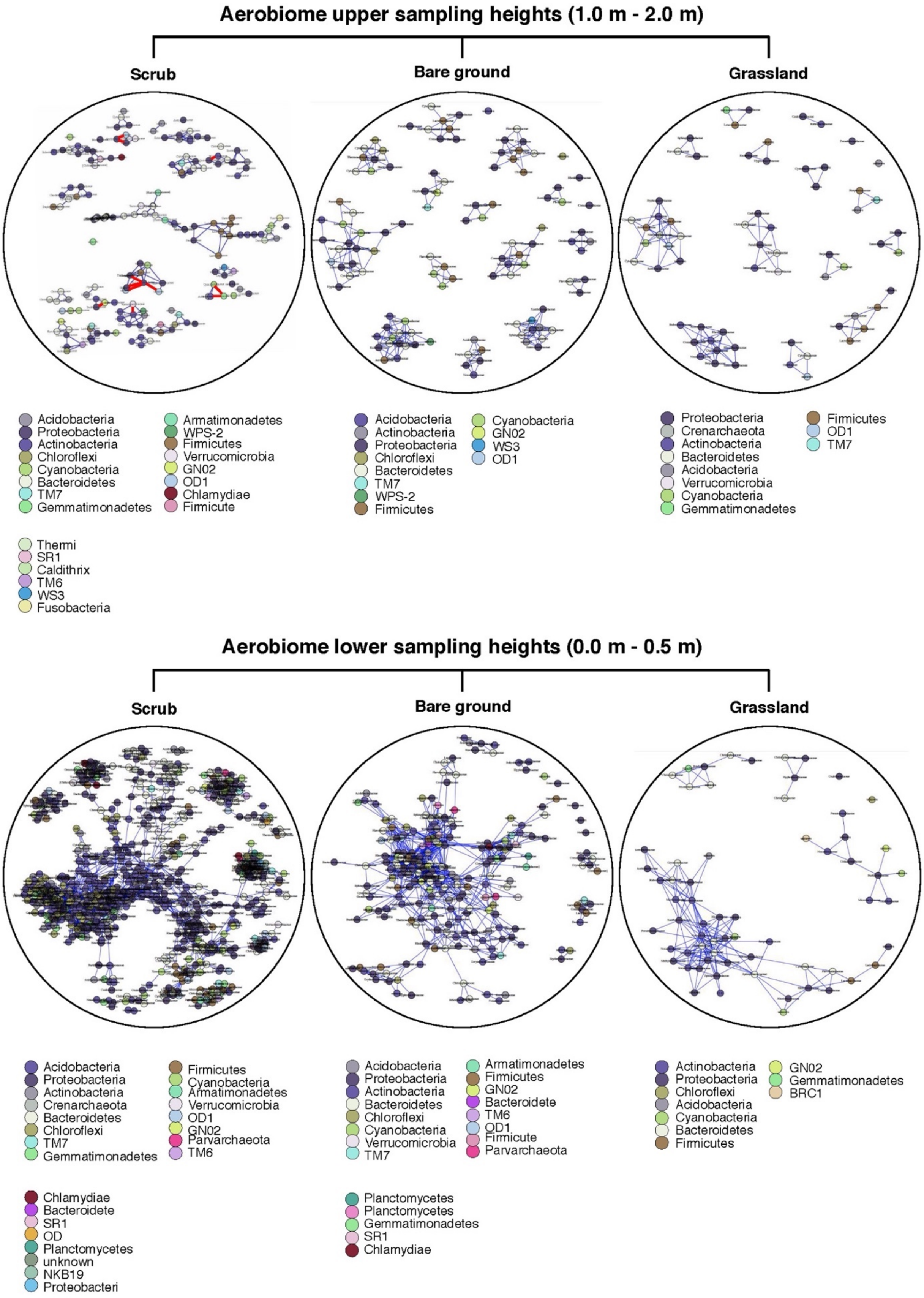
Aerobiome upper sampling heights (1.0 m - 2.0 m)
Scrub
Bare ground
Grassland
Acidobacteria
Proteobacteria
Actinobacteria
Chloroflexi
Cyanobacteria
Bacteroidetes
TM7
Gemmatimonadetes
Armatimonadetes
WPS-2
Firmicutes
Verrucomicrobia
GN02
OD1
Chlamydiae
Firmicute
Thermi
SR1
Caldithrix
TM6
WS3
Fusobacteria
Acidobacteria
Actinobacteria
Proteobacteria
Chloroflexi
Bacteroidetes
TM7
WPS-2
Firmicutes
Cyanobacteria
GN02
WS3
OD1
Proteobacteria
Crenarchaeota
Actinobacteria
Bacteroidetes
Acidobacteria
Verrucomicrobia
Cyanobacteria
Gemmatimonadetes
Firmicutes
OD1
TM7
Aerobiome lower sampling heights (0.0 m - 0.5 m)
Scrub
Bare ground
Grassland
Acidobacteria
Proteobacteria
Actinobacteria
Crenarchaeota
Bacteroidetes
Chloroflexi
TM7
Gemmatimonadetes
Firmicutes
Cyanobacteria
Armatimonadetes
Verrucomicrobia
OD1
GN02
Parvarchaeota
TM6
Chlamydiae
Bacteroidete
SR1
OD
Planctomycetes
unknown
NKB19
Proteobacteri
Acidobacteria
Proteobacteria
Actinobacteria
Bacteroidetes
Chloroflexi
Cyanobacteria
Verrucomicrobia
TM7
Armatimonadetes
Firmicutes
GN02
Bacteroidete
TM6
OD1
Firmicute
Parvarchaeota
Planctomycetes
Planctomycetes
Gemmatimonadetes
SR1
Chlamydiae
Actinobacteria
Proteobacteria
Chloroflexi
Acidobacteria
Cyanobacteria
Bacteroidetes
Firmicutes
GN02
Gemmatimonadetes
BRC1

Fig. 6. Association networks of bacterial OTUs for two vertical stratifications: 1.0-2.0 m (top panel) and 0.0-0.5 m (bottom panel). Node colour represents phylum, and node are labelled at the family level. Blue links represent positive associations, and red links represent negative associations.

5.10.6. The relationship between tree metrics and bacterial alpha diversity

In the air samples, we found strong positive correlations between tree density (based on count of trees in a given radius) and bacterial alpha diversity in the 10 m radius (Spearman's $r_s = 0.67$, $\beta = 0.67$ (0.4 – 0.8), $p = <0.01$) and 25 m radius ($r_s = 0.54$, $\beta = 0.54$ (0.2 – 0.7), $p = <0.01$) (**Fig. 7, a and b**). We also found significant moderate positive correlations between tree density and bacterial alpha diversity in the 50 m (Spearman's $r_s = 0.46$, $\beta = 0.46$ (0.1 – 0.7), $p = 0.00$) and 100 m radii (Spearman's $r_s = 0.50$, $\beta = 0.50$ (0.2 – 0.7), $p = <0.01$) (**Fig. 7, c and d**). Relationships between tree density and bacterial alpha diversity in soil were not statistically significant (Spearman's $r_s = 0.33$, $p = 0.38$).

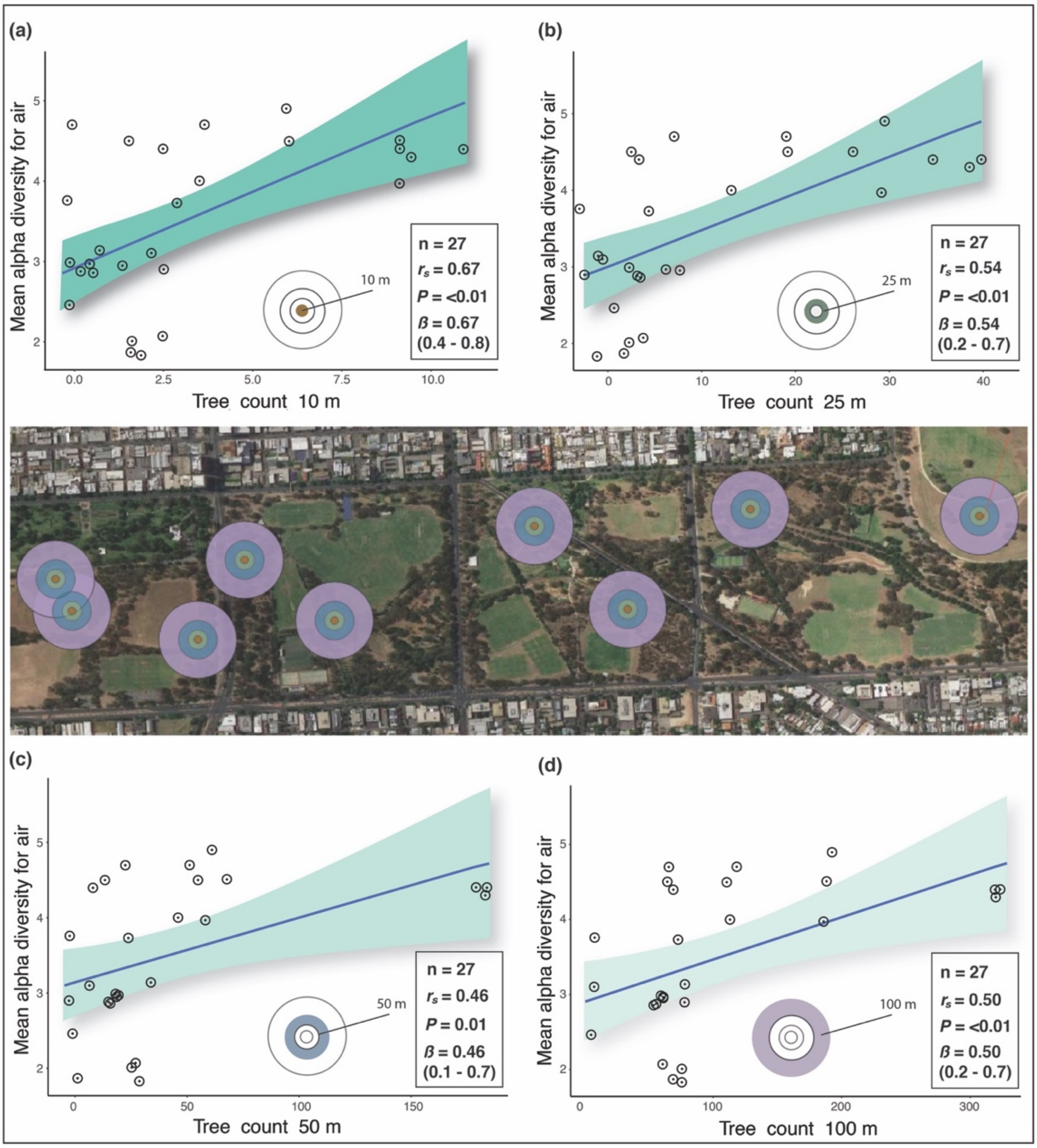

Fig. 7. Scatterplots of Spearman's correlations (r_S) between bacterial alpha diversity (for all habitats and air-only samples) and tree count within each buffer radius: (a) 10 m radius from sampling points, (b) 25 m radius, (c) 50 m radius, and (d) 100 m radius. X-axis shows count of trees within buffer radii. Y-axis shows bacterial alpha diversity of air-only samples using the Shannon diversity index (H). Green shading indicates the 95% confidence intervals for each linear regression. The buffer radii are in the

central aerial image and the corresponding spatial rings are in the plots. Inset also shows bootstrap results (ß) with 2.5% and 97.5% slopes.

We found significant moderate negative correlations between distance to nearest trees (from sampling stations) and aerobiome alpha diversity (Spearman's $r_s = -0.58$, $\beta = -0.58$ (-0.7 – -0.3), $p = <0.01$), and soil bacterial alpha diversity (Spearman's $r_s = -0.40$, $\beta = -0.40$ (-0.6 – -0.1), $p = 0.03$) (**Fig. 8, a and b,** respectively). Moreover, we found significant moderate positive correlations between tree canopy coverage and bacterial alpha diversity of the air in the 10 m (Spearman's $r_s = 0.51$, ß = 0.51 (0.2 – 0.7), $p = <0.01$), 25 m (Spearman's $r_s = 0.66$, ß = 0.66 (0.4 – 0.8), $p = <0.01$), and 100 m radii (Spearman's $r_s = 0.7$, ß = 0.7 (0.5 – 0.8), $p = <0.01$) (**Fig. 8, c, d and f,** respectively). There was a negative correlation between canopy cover and bacterial alpha diversity in the 50 m radius that was not statistically significant (Spearman's $r_s = -0.27$, ß = -0.27 (-0.6 – 0.2), $p = 0.17$) (**Fig. 8, e**).

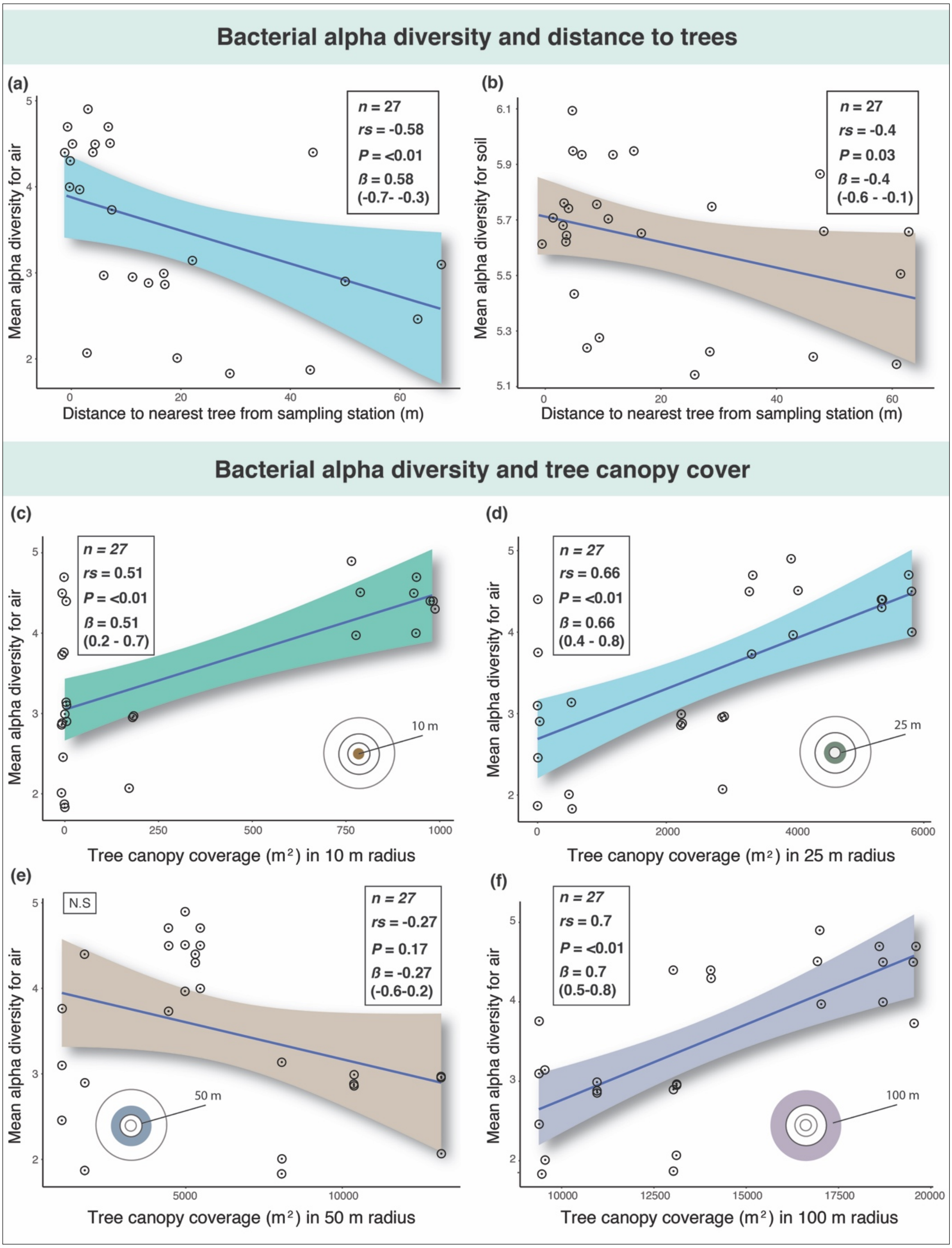

Fig. 8. The upper panels show scatterplots of Spearman's correlations (rs) between bacterial alpha diversity (for all habitats) and distance to nearest trees: (a) air-only samples, and (b) soil-only samples. X-axis shows distance (m) to nearest trees from

sampling points. The lower panels show scatterplots of Spearman's correlations between bacterial alpha diversity (for all habitats) and tree canopy coverage within the sampling point radii: (c) 10 m, (d) 25 m, (e) 50 m, and (f) 100 m. These relate to air-only samples. X-axis shows tree canopy coverage (m^2). Y-axis for both upper and lower panels shows bacterial alpha diversity of samples according to the Shannon diversity index (H). Coloured shading indicates the 95% confidence intervals for each linear regression. Inset also shows bootstrap results (ß) with 2.5% and 97.5% slopes. N.S (not significant).

5.10.7. Differentially abundant and notable taxa

There were 53 differentially abundant genera across habitat types (based on log-2 fold-change with adjusted $p = <0.05$). The top three, for example, in the scrub habitat were: *Gillisia, Sphingobium*, and *Kutzneria*; in grassland: *Parvibaculum, BSV43,* and *Pseudomonas*; and in bare ground: *Rudanella*, *Bacteroides*, and *Actinomyces*. We also observed vertical stratification of differentially abundant taxa. In the bare ground habitat, 77 genera were differentially abundant and significantly increasing in abundance with sampling height, and 97 were significantly decreasing. In the grassland habitat, 137 genera were differentially abundant and significantly increasing with sampling height, and 52 were significantly decreasing. In the scrub habitat, 41 genera were differentially abundant and significantly increasing with sampling height (**Fig. 9, a to c**), and 37 were significantly decreasing.

We also examined differentially abundant taxa at the putative species level. After unclassified taxa were removed, we confirmed identity (100% match) via Basic Local

Alignment Search Tool (BLAST) against the NCBI database (Altschul et al. 1990). In the bare ground habitat, we found 30 differentially abundant taxa assigned at the putative species level. Sixteen of these significantly decreased in relative abundance with sampling height and 14 significantly increased (*p* = <0.01). In the grassland habitat, we found 40 differentially abundant taxa assigned at the putative species level. Thirty-two of these significantly decreased with sampling height and 8 significantly increased (*p* = <0.01). In the scrub habitat, we found 16 differentially abundant taxa assigned at the putative species level. Ten of these significantly increased with sampling height and 6 significantly decreased (*p* = <0.01). Using BLAST and a literature search, we found putative differentially abundant human pathogens in each habitat (**Fig. 9, d**). A 2-sample test for equality of proportions with continuity correction showed a significant difference in proportions of identifiable pathogenic species between grassland and scrub (Chi-squared = 5.57, df = 1, *p* = <0.02) but not between other habitats, where grassland samples exhibited significantly greater proportions of identifiable pathogenic species compared to scrub. Moreover, 87% of these significantly decreased with sampling height based on log-2 fold-change differential analysis (*p* = <0.01). These taxa contain bacteria that have been associated with a number of diseases, including infective endocarditis (*Rothia mucilaginosa*) and gut mucosal damage (*Prevotella copri*). More information on these diseases can be found in Supplementary Materials, Appendix C.

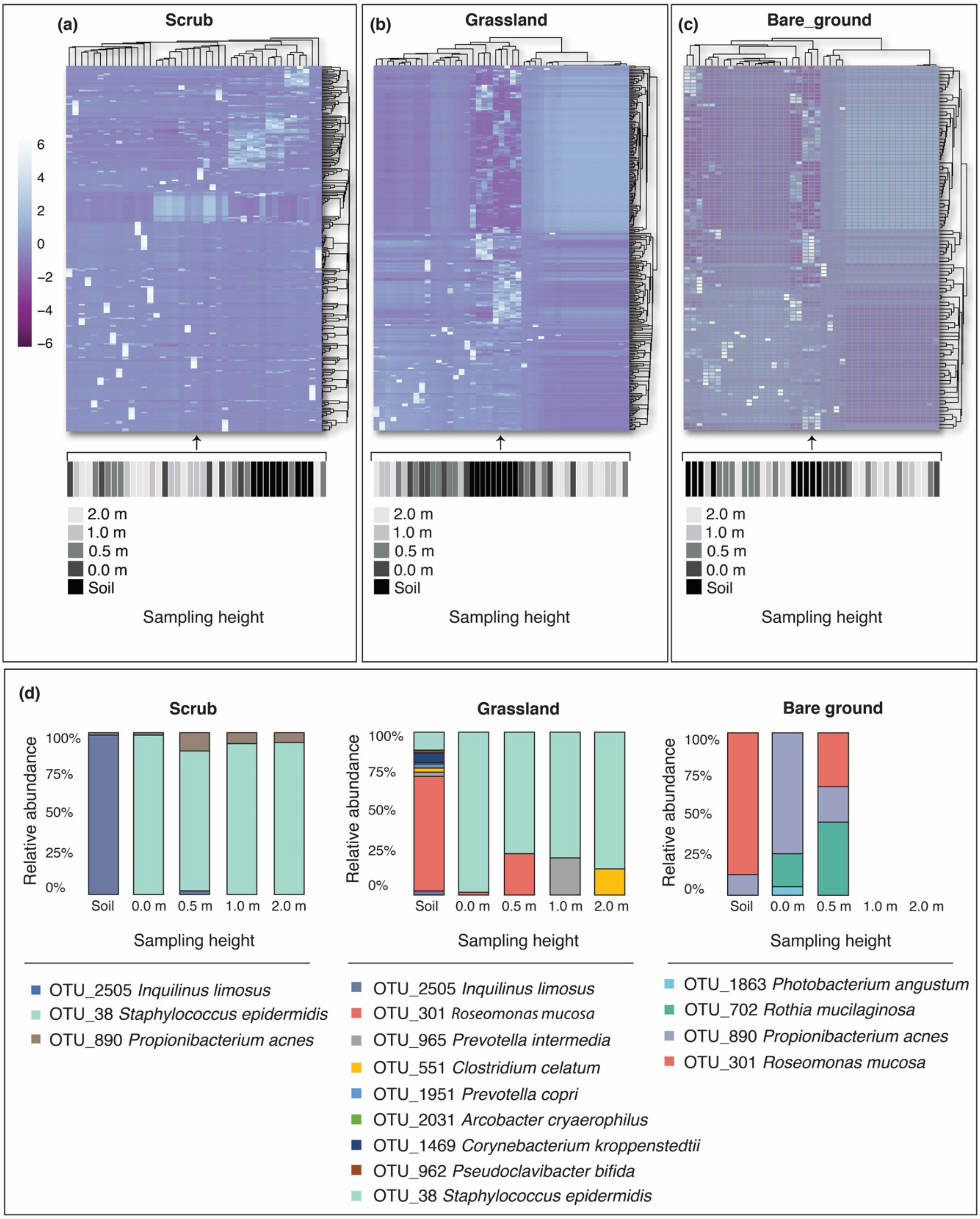

Fig. 9. Top panels show significantly increasing (from soil level to 2 m) differentially abundant *genera* in (a) Scrub habitat, (b) Grassland habitat, and (c) Bare ground habitat measured by log-2 fold-change with a p value < 0.05. Extreme ends of the heat

colour scale represent 6 standard deviations from the mean abundance for each genus across samples. Clustering of genera (rows) is by Manhattan distance. Shaded bars under the heatmap represent sampling heights as indicated by the corresponding colour key (where the lighter the shade, the higher the sampling height). Bottom panel (d) shows potentially pathogenic and differentially abundant *species* for each habitat and their relative abundance for each sampling height. More details on the diseases these pathogens may cause are in Supplementary Materials, Appendix C.

5.10.8. Environmental metadata

In terms of the environmental metadata, there was only one significant association with bacterial alpha diversity; aerobiome alpha diversity decreased significantly in scrub habitat as windspeed increased (Spearman's r_s = -0.88, β = -0.88 (-0.98 – -0.5), p = <0.01) (full details in Supplementary Materials, Appendix D).

5.11. Discussion

Here we show that aerobiome alpha and beta diversity (community composition) differed significantly between urban green space habitat type, and that aerobiome diversity, composition and network complexity also stratified vertically. The level to which this occurred was dependent on habitat type. Therefore, potential bacterial exposure levels and transfer loads to humans will likely differ depending on habitat type as well a person's height and behaviour. Our results confirmed that more trees, closer proximity to trees, and greater canopy coverage associate with higher aerobiome diversity, which could have important implications for landscape management and public health as growing emphasis is placed on designing and managing green spaces for wellbeing (Robinson and Jorgensen, 2019). We also found that grassland samples exhibited significantly greater proportions of identifiable pathogenic bacteria compared to scrub, and their abundance decreased significantly with sampling height. Our study was conducted only in the Adelaide Parklands, South Australia and therefore may not be representative of urban green spaces in other areas. Future work should explore these trends in additional geographical, socioeconomic, cultural areas to understand both generalisability and opportunities to optimise green space exposure for health benefits.

5.11.1. Aerobiome compositional differences between habitats

The scrub habitat exhibited the most biodiverse aerobiome in our study. This corroborates other studies that suggest that environmental microbiomes are more biodiverse in urban habitats with more complex vegetation communities (Baruch et al. 2020; Mills et al. 2020). Growing evidence suggests that exposure to biodiverse environmental microbiomes could have important implications for human health (Liddicoat et al. 2020; Donovan et al. 2020; Honeker et al. 2019). For example, environmental microbiomes are essential in the development and regulation of immunity (Rook et al. 2003; Rook et al. 2014), and soil-derived butyrate-producing bacteria may supplement gut bacteria and have anxiety-reducing effects (Liddicoat et al. 2020). Importantly, urban green space exposure can result in transmission of environmentally-derived bacteria to the skin and airways (Selway et al. 2020). Furthermore, a recent study showed that transfer of bacteria from biodiverse environments enhanced immunoregulatory pathways in children (Roslund et al. 2020). Consequently, environments with different levels of bacterial diversity may affect the potentiality of bacterial exposure levels and transfer loads, warranting further research. We found differentially abundant putative pathogenic taxa and showed significant differences in proportions between grassland and scrub habitat samples. In other words, amenity grassland seemed to exhibit a significantly greater proportion of (identifiable) pathogenic species compared to scrub samples. However, considerably more research is needed to fully explore the validity and generalisability of these results. As with many microbial ecology studies, only identifiable bacterial taxa were used in the differential abundance and analyses that identified the pathogenic taxa (i.e., unclassified taxa were removed). This could result in recording bias with implications for validity.

Our results suggest that tree density, distance to nearest trees, and tree canopy cover could have a considerable influence on aerobiome alpha diversity. This corroborates reports of trees acting as stationary vectors, spreading bacterial cells in the air (Laforest-Lapointe et al. 2017). Complex plant detritus (leaf litter) and organic matter at the base of trees, and corresponding soil-microbe systems, may also contribute to tree-associated aerobiomes. The number of trees and amount of canopy coverage within a given radius correlated strongly with alpha diversity. Furthermore, negative correlations were shown between distance to nearest trees and bacterial alpha diversity for air and soil. This supports the results of the tree density associations and suggests that closeness to trees could be important. These results could have important implications for landscape management and public health. Indeed, there have been widespread calls to improve urban ecosystem services by augmenting tree coverage (e.g., to help reduce urban heat island effects (Chen et al. 2020), support wildlife (Straka et al. 2019; Wood and Esaian, 2020), improve sleep (Astell-Burt and Feng, 2020; Woo and Lee, 2020), and capture precipitation to reduce flood risk (Ross et al. 2020). There is also a need to restore complex vegetation communities and host-microbiota interactions that provide multifunctional roles in urban ecosystems (Honeker et al. 2019; Robinson et al. 2018; Watkins et al. 2020). An important limitation in our study was that tree species and structural diversity metrics were not used. These additional measures could have enriched the quality of analysis and implications of our results and further research that takes these factors into account is needed. However, our findings suggest additional co-benefits from increasing urban tree coverage due to its potential to mediate aerobiome alpha diversity. Our results also corroborate other studies showing microbial alpha diversity increasing along densely-urban to semi-natural environmental gradients (Parajuli et al. 2018; Abrego et al. 2020).

Our results suggest that aerobiome beta diversity (compositional differences) differs between habitats. The results imply that microbial communities in the soil of the scrub habitat are significantly different to bare ground and grassland, which are more compositionally aligned. It is possible that bacterial homogeneity between grassland and bare ground is attributed to homogeneity of vegetation complexity (Socolar et al. 2016). In other words, phyllosphere (total above-ground portion of plants) and rhizosphere (soil root zone) presence and complexity create conditions for different microbial relationships and thus compositional disparity with less botanically-complex or depauperate habitats (Mills et al. 2020; Honeker et al. 2019). Taken together with the alpha diversity results, significantly more bacterial species and unique communities exists in scrub habitat samples compared to grassland and bare ground samples. This could mean that humans are exposed to a greater diversity of bacteria in the scrub habitat. Future studies should focus on the functional relevance of these findings.

5.11.2. Aerobiome vertical stratification

In our study, vertical stratification in bacterial alpha and beta diversity occurred in the bare ground and scrub habitat. However, for the grassland aerobiome, both alpha and beta diversity were relatively stable as height increased. This is the first study to demonstrate that aerobiome vertical stratification is contingent on habitat type, which is important for potential human exposure. As mentioned, urban green space exposure can result in transfer of environmental bacteria to the skin and respiratory tract (Selway et al. 2020), and our study shows that the composition and diversity of aerobiome bacteria may differ between heights (from ground level to 2 m). Consequently, there could be different bacterial exposure levels and transfer loads depending on a person's height and activity (Robinson et al. 2020), however, further confirmatory research is needed. Our results suggest that this may not be the case in amenity grassland where bacterial alpha and beta diversity exhibited high levels of homogeneity among heights. Further research is required to determine the reasons for the lack of vertical stratification in grassland. However, we hypothesise that lower baseline diversity, bacterial resources, openness and airflow in this habitat may be contributing factors. Our study also provides some evidence that different urban green space habitats and heights may not only affect exposure levels and transfer loads of bacterial diversity, but also the presence of notable and potentially pathogenic species for humans. The relative abundance of pathogens identified in the grassland habitat decreased significantly with sampling height. It is possible that a number of these potential pathogens may originate from larger air-sheds (consistent with increasing relative abundance with height), however grasslands may have lesser capacity, compared to scrub or bare ground, to present barriers to this broader airflow or contribute to a more locally distinctive aerobiome. These findings highlight the need for further empirical studies focusing on functional interactions in the environment-aerobiome-health axis.

Our network analyses also provided evidence to support aerobiome vertical stratification. We saw a decrease in bacterial interactions and network complexities with increased network modularity at higher heights compared to lower heights across habitats, which might be attributed to reduced bacterial diversity with sampling height. This pattern might be due to increasing influence, with increasing height, of diluted and somewhat homogenised aerobiomes from larger airsheds, representing the physical mixing of air (and therefore aerobiomes) from multiple different and distant ecological sources. Increased modularity with reduced network size and interactions may also indicate the existence of relatively simplified, yet modular bacterial communities at higher heights. This could be the function of sparse food resources, especially if associations in the networks reflect niche-based interactions. Increased modularity indicates the presence of dense connections between bacteria within modules but sparse connections between bacteria in different modules, whereas reduced connectance means reduced probability of interactions between any pair of bacteria. Increased modularity with reduced connectance often indicates ecological stability (May, 1972). Moreover, presence of mostly positive associations might also suggest cooperation for resources or lack of competition among the interacting OTUs in the community. While association-based networks allow a depiction of potential interactions among OTUs and portray community structure, they do not separate niche-based and biological interactions. Experiments with cultures are recommended to dissociate interaction types and understand the biological and ecological mechanisms behind the observed interactions and network complexity. This action could be important to gain a greater ecological understanding of aerobiome assembly (including vertical stratification), dynamics, and the potentiality of bacterial exposure. Our results provide strong evidence that vertical stratification is a key factor not only in aerobiome diversity and composition, but also in aerobiome interactions, community structure and complexity.

In conclusion, our study provides evidence that bacterial alpha and beta diversity differed significantly between habitats, with scrub habitat providing the most biodiverse aerobiomes. We provide evidence supporting the presence of aerobiome vertical stratification in bacterial community diversity, composition and complexity, which also differed in a habitat-dependent manner. Our results confirmed that more trees, closer proximity to trees, and greater canopy coverage associated with higher alpha diversity of the aerobiome. Finally, we found that grassland samples exhibited significantly greater proportions of identifiable putative pathogenic bacteria compared to scrub, and their richness decreased significantly with sampling height. As discussed, there is growing evidence to suggest that exposure to biodiverse aerobiomes may contribute towards the development and regulation of immunity and support mental health (Rook et al. 2003; Arleevskaya et al. 2019; Liddicoat et al. 2020; Rook et al. 2014). Gaining a greater understanding of bacterial transmission routes, exposure levels, transfer loads, and downstream health implications is required. This aerobiome characterisation study provides novel insights into the urban ecosystem to help encourage further empirical investigations. Future research should focus on the functional interactions between humans and the aerobiome. Although additional research is required, our findings also support calls to increase urban tree cover. Exploring the mediatory roles of trees in aerobiome compositional and functional diversity could have important implications for landscape management and public health.

5.12.Materials and Methods

5.12.1. Site selection

Our study was undertaken in the southern Adelaide Parklands (Kaurna Warra Pintyanthi), South Australia, which comprised nine vegetated plots that spanned approx. 18 ha. The nine plots included three amenity grasslands, three scrub, and three bare ground (exposed soil) habitats.

There were several justifications for selecting this site: (1) the southern Parklands occur within the Upper Outwash Plain soil boundary (coalescing alluvial soil, draining the Eden Fault Block), which provided broad consistency in soil geochemistry; (2) a single section of the Parklands provided control over potential micro-geographic variation effects on the aerobiome (e.g., distance to coast, elevation, orientation, aspect, and dominant vegetation communities); (3) the Parkland habitats are representative of the types of green spaces that urban residents are regularly exposed to when commuting or recreating; and, (4) the City of Adelaide provided guidance in the selection process, identifying accessible (and inaccessible) plots.

We defined the boundaries of the nine plots (as polygons) in QGIS 3 (v3.0.2) in conjunction with the City of Adelaide. Using spatial shapefiles for the plot boundaries, we generated random point algorithms to provide random sampling points within each of the nine study plots (**Fig. 10, a**). We recorded geographic coordinates for each sampling point and programmed them into a handheld global positioning system (GPS) receiver. We operated the GPS receiver in the field, allowing us to pinpoint the locations for the sampling stations.

5.12.2. Sampling equipment

Sampling stations (**Fig. 10, b to e**) were constructed using timber (42 mm x 28 mm x 2.7 m), steel brackets, hooks and guy-lines (Robinson et al. 2020b). We secured lab-grade clear plastic petri dishes (bases and lids) to the sampling stations, which were used to sample the aerobiome via passive sampling (Mhuireach et al. 2016).

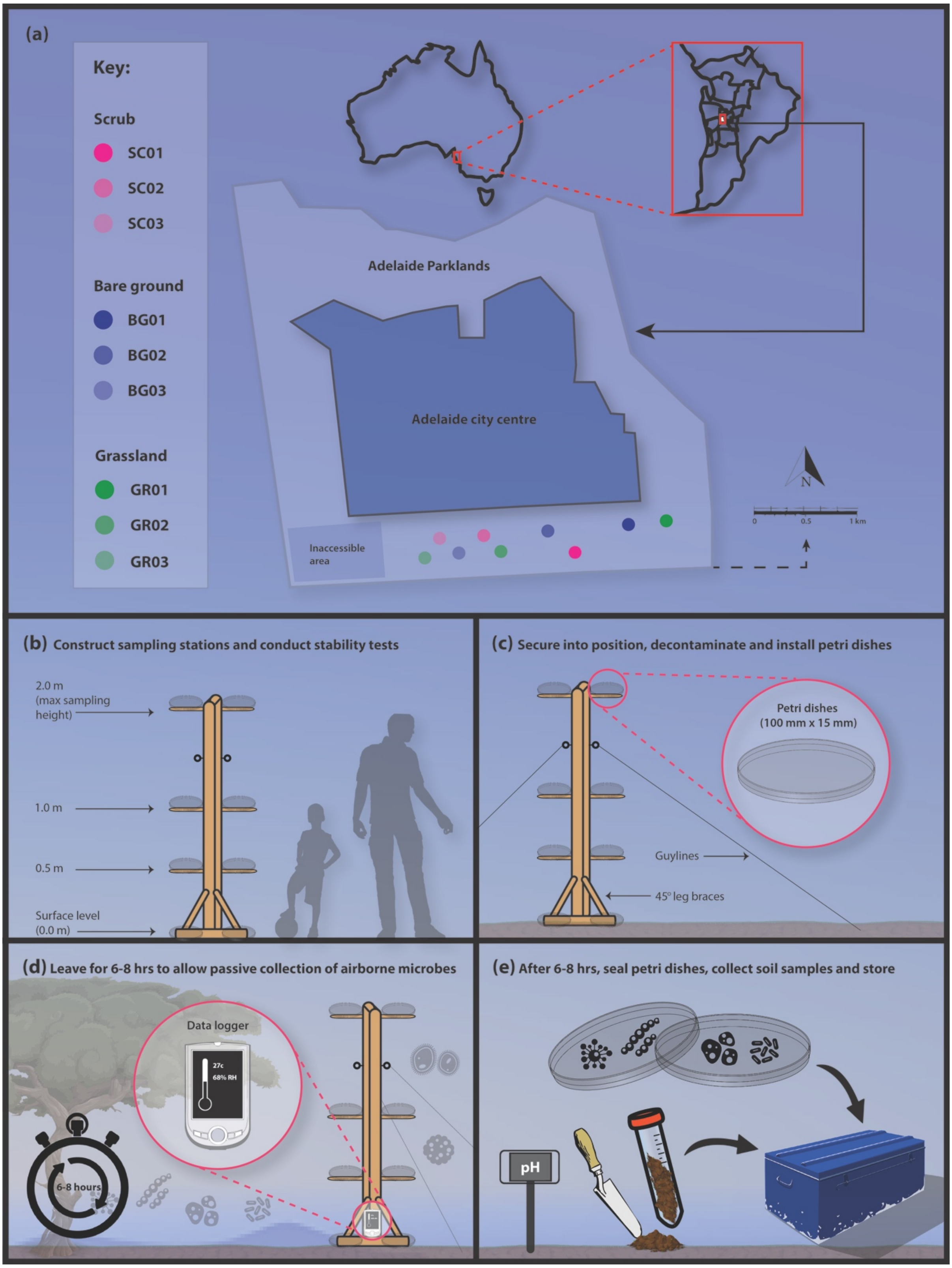

Fig. 10. (a) Location of study sites, showing the different habitat types and randomly selected sampling locations. (b-e) Vertical stratification sampling station and methods

used to collect (passively) air and (actively) soilborne bacteria. We installed the stations in three different habitat types in the Adelaide Parklands.

5.12.3. On-site setup

We installed the sampling stations on site between 0600-0800hrs on the 4th, 5th and 6th November 2019. At 0800hrs, sampling stations were decontaminated using a 5% Decon 90 solution. We then installed the petri dishes for passive sampling. The data loggers were also decontaminated. In the scrub habitat (defined as vegetation dominated by locally native shrubs, usually ≤5 m tall, with scattered trees) (JNCC, 2013), the nearest trees and shrubs were between 2-5 m from the sampling stations, and all trees were <10 m height and 20-50 cm in diameter at breast height (Robinson et al. 2020b).

5.12.4. Sampling protocol

We installed temperature and relative humidity data loggers at each sampling station (Robinson et al. 2020b). We programmed each logger to record data at 8-second intervals for the entire sampling period. At the start of each sampling day, we calibrated the dataloggers using a mercury thermometer (Gerotherm) and a sling psychrometer (Sper Scientific 736700). We collected other metadata including windspeed and soil pH (Alotpower digital meter). We inserted the pH meter into the soil for a period of 1-minute before taking a reading (manufacturer’s instructions). We obtained data for windspeed and direction from Adelaide’s meteorological weather station at Ngayirdapira (West Terrace): Lat: -34.93, Lon: 138.58, Height: 29.32 m. We also used a handheld anemometer (Digitech *QM-1644)* to record these parameters hourly at each sampling site (Mhuireach et al. 2016).

5.12.5. Soil samples

We used a small, decontaminated shovel to collect topsoil samples and stored these in sterile 50 mL falcon tubes. We collected five topsoil samples (approx. 0-5 cm depth) at equidistant sampling points, 20-30 cm from the stem of each sampling station (Zarraonaindia et a. 2015). We pooled and homogenised the soil samples, passed them through a decontaminated 1 mm pore sieve, and placed them in new sterile and labelled 50 mL Falcon tubes. We included field controls for the soil by opening sterile falcon tubes for 60 s in the equipment box at each site (Mbareche et al. 2019). We placed all soil and field control samples immediately into an ice box, and stored the samples in the lab at -80˚C prior to further processing (Dettwyler, 2017). In total, we collected 45 soil subsamples per sampling day across nine sampling stations with three temporal replicates (over three days). We pooled and homogenised subsamples by sampling station and day, which gave a total of 27 homogenised samples (nine per sampling station) plus 9 field controls.

5.12.6. Aerobiome samples

To collect aerobiome samples, we used a passive sampling technique, following established protocols (Mhuireach et al. 2016; Mhuireach et al. 2019). We installed petri dishes (100 mm x 15 mm) with Velcro tabs on the sampling stations at four sampling heights: ground level (i.e., 0.0 m), 0.5 m, 1 m, and 2 m. The total height of the sampling station was 2 m from ground level (95% of typical adult male heights lie within 2 SD at 1.93 m, and 1.78 m for females based on a study across Europe, North America, Australia and East-Asia) (Jelenkovic et al. 2016). Various human characteristics informed the height selection (e.g., representation of adults vs. children, and different activities such as sitting, crawling, walking) (Milani et al. 2017; RCPCH, 2020; Robinson et al. 2020b). We decontaminated the steel plates supporting the petri dishes with 5% Decon 90 solution.

We secured the petri dishes to the sampling stations (**Fig. 10**), leaving them open for 6-8 hours (Mhuireach et al. 2016), and closing them at the end of the sampling period. To reduce contamination, new disposable laboratory gloves were worn for each vertical sampling point. Once sampling was complete, we sealed the petri dishes using Parafilm, labelled and transported them to the laboratory (on ice) for storage at -80˚C (Mhuireach et al. 2019). We collected field control samples by leaving unused petri dishes for 60 s in the equipment box and sealing them at each site.

5.12.7. DNA extraction, amplification and sequencing

We extracted DNA from soil and air samples at the facilities of the Evolutionary Biology Unit, South Australian Museum. Using a digital number randomiser, we processed samples on a randomised basis. We processed the low biomass air samples prior to the higher biomass soil samples to minimise cross-contamination.

To extract DNA, we swabbed the petri dishes in the lab using nylon-flocked swabs (FLOQSwabs Cat. No. 501CS01, Copan Diagnostics Inc.) (Liddicoat et al. 2020; Mhuireach et al. 2019; Robinson et al. 2020b; Bae et al. 2019). All swabbing was carried out in a laminar flow cabinet type 1 (License No. 926207) and each sample was swabbed for 30 s. Samples from the base and lids of each petri dish for each height, station and date were pooled. We cut the swabs directly into Eppendorf tubes (2 mL). We used Qiagen QIAamp DNA Blood Mini Kits to extract DNA from the swabs and extraction blank controls. For extraction blank controls, we used sterile water and reagents instead of a sample and all DNA extraction steps were performed as if they were normal samples. To extract DNA from the soil samples (and extraction blank controls), we used Qiagen DNAeasy PowerLyzer Soil Kits and followed the manufacturer's instructions. PCR amplification was conducted in triplicate using the 341F/806R primer targeting the V3-V4 region of the 16S rRNA gene (5' -CCTAYGGGRBGCASCAG- 3'/5' -GGACTACNNGGGTATCTAAT- 3'). The 300 bp paired end run was sequenced on an Illumina MiSeq platform at the Australian Genome Research Facility using two flowcells (ID 000000000-CW9V6 and 000000000-CVPGT). We conducted image analysis in real-time by the MiSeq Control Software (v2.6.2.1) and Real Time Analysis (v1.18.54). We used the Illumina bcl2fastq (2.20.0.422) pipeline to generate sequence data.

5.12.8. Bioinformatics and statistical analysis

Raw 16S rRNA gene sequences processing, OTU picking, taxonomic assignments, and decontamination were as per Robinson et al. (2020) (described in detail in Supplementary Materials, Appendix A). To estimate OTU alpha diversity we derived Shannon Index values (Liddicoat et al. 2020) in phyloseq (McMurdie et al. 2013) in R. Prior to analysis of compositional data, we used centre log-ratio (clr) transformations (Quinn et al. 2019). Information acquired from this approach is directly relatable to the environment (Gloor et al. 2017). We generated violin plots with ggplot2 (Wickham and Wickham, 2007) to visualise the distribution of the alpha diversity scores for each habitat and height. Bacterial beta diversity was visualised using ordination plots of Aitchison distances based on clr-transformations of OTU abundances. Ordination plots show low-dimensional ordination space in which similar samples are plotted close together, and dissimilar samples are plotted far apart.

We used permutational multivariate analysis of variance (PERMANOVA) to test for compositional differences between different sites, habitats and sampling heights, and permutation tests for homogeneity of multivariate dispersions using vegan (Oksanen et al. 2019) in R. Pearson's product-moment and Spearman's rank correlation tests were used to examine correlations between habitat, sampling height and alpha diversity scores. Using phyloseq, we calculated OTU relative abundances to examine the distribution of taxa that may have potential implications for public health. We used DESeq2 (Love et al. 2014) in R to conduct differential abundance analysis based on log-2 fold-change. To compare presence and proportions of taxa we used 2-sample tests for equality of proportions with continuity corrections. We also applied bootstrap resampling to assign a measure of accuracy to sample estimates for the Spearman's correlations, using a minimum of 1,000 iterations. This was carried out with the psych (Revel, 2020) and boot (Canty and Ripley, 2020) packages in R.

In order to understand the effect of vertical stratification on bacterial interactions and community structures, we evaluated association-networks of bacterial OTUs. We combined the OTU database from 0-0.5 m and 1-2 m for each site, and constructed two networks per site (i.e., lower and upper height), such that in total six networks were evaluated across the three habitats. In the evaluated network, nodes represent OTUs and links exists between a pair of OTUs if their frequencies are significantly associated (absolute abundance > 0.7, $p = < 0.01$). The type of association, whether positive or negative, was represented with blue and red links, respectively. To account for compositional bias associated with OTU data, we used SparCC (Friedman and Alm, 2012) to define associations, and only OTUs with sequence counts >10 were included. Randomly permuted ($n = 100$) data were used to estimate significance of associations, and igraph (Csárdi, 2020) was used to visualize and evaluate the plots. We also ran Spearman's correlation tests with bootstrap resampling to determine whether environmental metadata (pH, temperature, windspeed) associated with bacterial alpha diversity. Outliers were considered as data points more than 1.5 x above the third quartile or below the first quartile.

5.12.9. Geospatial analyses

We investigated possible relationships between aerobiome samples and surrounding vegetation properties using spatial buffer zones. For the buffer analysis, we used vector geoprocessing tools in QGIS 3. Buffer sizes of 10 m, 25 m, 50 m, and 100 m were considered appropriate for the study scale. Similar distances have been used in previous green space and epidemiology studies (Cusak et al. 2017; Klompmaker et al. 2018; Lee et al. 2018; Robinson et al. 2020a). A 100 m maximum buffer radius was chosen; at greater distances, effects would no longer be local to the sampling points (i.e., they would overlap with other sampling points). To determine tree canopy cover within each buffer radii, ESRI shapefiles were imported into i-Tree Canopy (i-

Tree Canopy, 2020). This enabled random sampling points (between 50-250 points per buffer) and selection of land cover classification and associated metrics overlaid with Landsat 8 satellite imagery (Richardson and Moskal, 2014; Soltani and Sharifi, 2017; i-Tree Canopy, 2020). Tree count and distance measures were acquired using geometry tools in QGIS 3.

CHAPTER 6

THE ENVIRONMENT-MICROBIOME-HEALTH AXIS: UNDEREXPLORED INFLUENCES ON MICROBIOMES, ECOSYSTEMS AND HEALTH

> *"Although there is envisaged symmetry between the conceptualizations needed to unify our human community around a set of specific calls-to-action to improve health outcomes from birth onwards for all, there is little evidence to illustrate how our common microbial and molecular communities are being used to inform narratives that unite all life on Earth. The foundation of these developing microbial-based decolonizing narratives are premised on the need for rebalancing our biomolecular functioning within and across species while dampening the supercilious viewpoint that human health should be put ahead of all others on Earth."*
>
> *– Redvers, 2020*

6. PUBLICATION IN CHAPTER 6:

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Mini-review

The Effects of Anthropogenic Sound and Artificial Light Exposure on Microbiomes: Ecological and Public Health Implications

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Keywords: Microbiome; sound exposure; noise pollution; microalgae; artificial light; bioacoustics; ecosystem health; light pollution; photo-sonic restoration hypothesis

6.1. Abstract

Globally, anthropogenic sound and artificial light pollution have increased to alarming levels. Evidence suggests that these can disrupt critical processes that impact ecosystems and human health. However, limited focus has been given to the potential effects of sound and artificial light pollution on microbiomes. Microbial communities are the foundations of our ecosystems. They are essential for human health and provide myriad ecosystem services. Therefore, disruption to microbiomes by anthropogenic sound and artificial light could have important ecological and human health implications. In this mini-review, we provide a critical appraisal of available scientific literature on the effects of anthropogenic sound and light exposure on microorganisms and discuss the potential ecological and human health implications. Our mini-review shows that a limited number of studies have been carried out to investigate the effects of anthropogenic sound and light pollution on microbiomes. However, based on these studies, it is evident that anthropogenic sound and light pollution have the potential to significantly influence ecosystems and human health via microbial interactions. Many of the studies suffered from modest sample sizes, suboptimal experiments designs, and some of the bioinformatics approaches used are now outdated. These factors should be improved in future studies. This is an emerging and severely underexplored area of research that could have important implications for global ecosystems and public health. Finally, we also propose the *photo-sonic restoration hypothesis:* does restoring natural levels of light and sound help to restore microbiomes and ecosystem stability?

6.2. Introduction

Globally, anthropogenic sound pollution (e.g., from traffic, construction, and industrial processes) has increased to alarming levels (Tabraiz et al. 2015; Kumar et al. 2018; Vitkauskaite et al. 2018). Moreover, the rise in artificial light pollution — such as excessive light from buildings and streets, and lighting associated with industry and transportation — is now considered to be a global human health concern (Falci et al. 2019). Anthropogenic sound and artificial light pollution also have a range of impacts on ecosystem processes. For example, it is well documented that anthropogenic sound exposure affects wildlife populations. Indeed, noise-induced reductions in foraging efficiency have been demonstrated in bats (Luo et al. 2015), owls (Mason et al. 2016), flounder larvae *Pseudopleuronectes americanus* (Gendron et al. 2020), and crabs (Wale et al. 2013). Chronic traffic noise was recently shown to alter gene expression in bats, which associated with metabolic dysregulation and stress (Song et al. 2020). Artificial light at night (ALAN) can affect insect movement, foraging, reproduction and predatory behaviour (Owens et al. 2020) and may represent broader disturbances to ecosystems by disrupting mutualistic interactions across trophic levels (Maggi et al. 2020). In terms of direct human health implications, ALAN and sound pollution have been linked to the onset of depression (Min and Min, 2018; Diaz et al. 2020) and insomnia by disrupting circadian rhythms (Hatori et al. 2017; Janson et al. 2020). Research also suggests that sound pollution acts as a biological stressor that can induce cardiovascular and endocrinological disorders (Münzel et al. 2018). For context, hazardous noise to humans is considered to be >85 dB, and a lawn mower or motorcycle emit ~90 dB (Chepesiuk, 2005).

However, limited attention has been given to the potential effects of sound pollution and ALAN on microbiomes. Microbial communities are the foundations of our ecosystems and provide essential ecosystem services. These include carbon and nutrient cycling, climate regulation, global food security, and animal and plant health (Cavicchioli et al. 2019; Guerra et al. 2020; Li et al. 2020; Trivedi et al. 2020). We live in times where anthropogenic ecosystem degradation disturbs many organisms, therefore, understanding how environmental microbiomes may be affected by anthropogenic sound and artificial light could have important ecological and human health implications. The human microbiome (the network of microbial communities in the human body) is also essential for human health (Sharma and Im, 2020). Indeed, a dysfunctional microbiome has been linked to a plethora of diseases from Alzheimer's (Kowalski and Mulak, 2019), skin diseases (Prescott et al. 2017) and mental health conditions (Cryan et al. 2019). Furthermore, exposure to diverse environmental microbiomes is thought to play a role in 'training' and regulating the immune system (Rook, 2013; Robinson and Jorgensen, 2019; Renz and Skevaki, 2020; Roslund et al. 2020).

Therefore, disturbance to environmental and human microbiomes by anthropogenic sound and artificial light pollution, could have important implications for both ecosystem functionality and human health. In this mini-review, we provide a critical appraisal of available scientific literature on the effects of anthropogenic sound and artificial light exposure on microorganisms. We discuss the potential ecological and human health implications of these effects.

6.3. The effects of anthropogenic sound exposure on microorganisms

6.3.1. Bacteria

We found 12 scientific papers pertaining to the exposure of anthropogenic sound on bacteria. A pilot study by Shaobin et al. (2010) investigated the effects of audible sound on *Escherichia coli* growth. The authors placed cultured *E. coli* cells (*n* = 15 plates) into sound chambers and stimulated them using 90 dB sound waves (similar levels to urban traffic). They applied frequencies of 1, 5, and 15 kHz for 1 hour periods with 3 hour intervals over a 24 hour treatment period. They found that *E. coli* growth was significantly promoted and reached colony forming efficiencies of 142%, 130% and 131% after sound stimulation with 1, 5, and 15 kHz, respectively. Although the sample size was modest, this study was later corroborated by Gu et al. (2016) who found that *E. coli* K-12 (*n* = 100 randomly selected cells) exposed to 80 dB sound waves exhibited increased biomass and growth rate at 8 kHz by 1.7 times and 2.5 times (compared to the control), respectively. While variations in the inoculum could impact growth rates, further studies making use of high throughput cell cultivation strategies could be employed to improve robustness. Moreover, the mechanism of sound stimulation on microbial growth is still unknown — therefore further research is required. Similar experiments could conceivably be carried out to investigate microbiome compositional changes and explore different interfaces and media that may affect sound propensity (e.g., water and soil) (Fig. 1). Mechanosensitive channels on bacterial cell membranes might be involved in signal transduction which provides a promising area to focus on. Interestingly, Kim (2016) found that antibiotic resistance to ampicillin increased in soil bacteria (*n* = 10) and *E. coli* (*n* = 10) exposed to low frequency sound (75 dB at 0.1 kHz). The sample size in this study is certainly modest, therefore, caution is needed. However, the indication of increased antibiotic resistance attributed to low frequency anthropogenic noise, warrants further research. The authors conclude that the amount of soil bacteria exposed to noise also increased but chlorophyll optical density (of associated plants) was unaffected. Therefore, it is possible that soil bacteria with mutualistic plant interactions such as nitrogen fixation and denitrification, were outcompeted by less useful bacteria. This also warrants considerably more research due to its potential importance for ecosystem functionality.

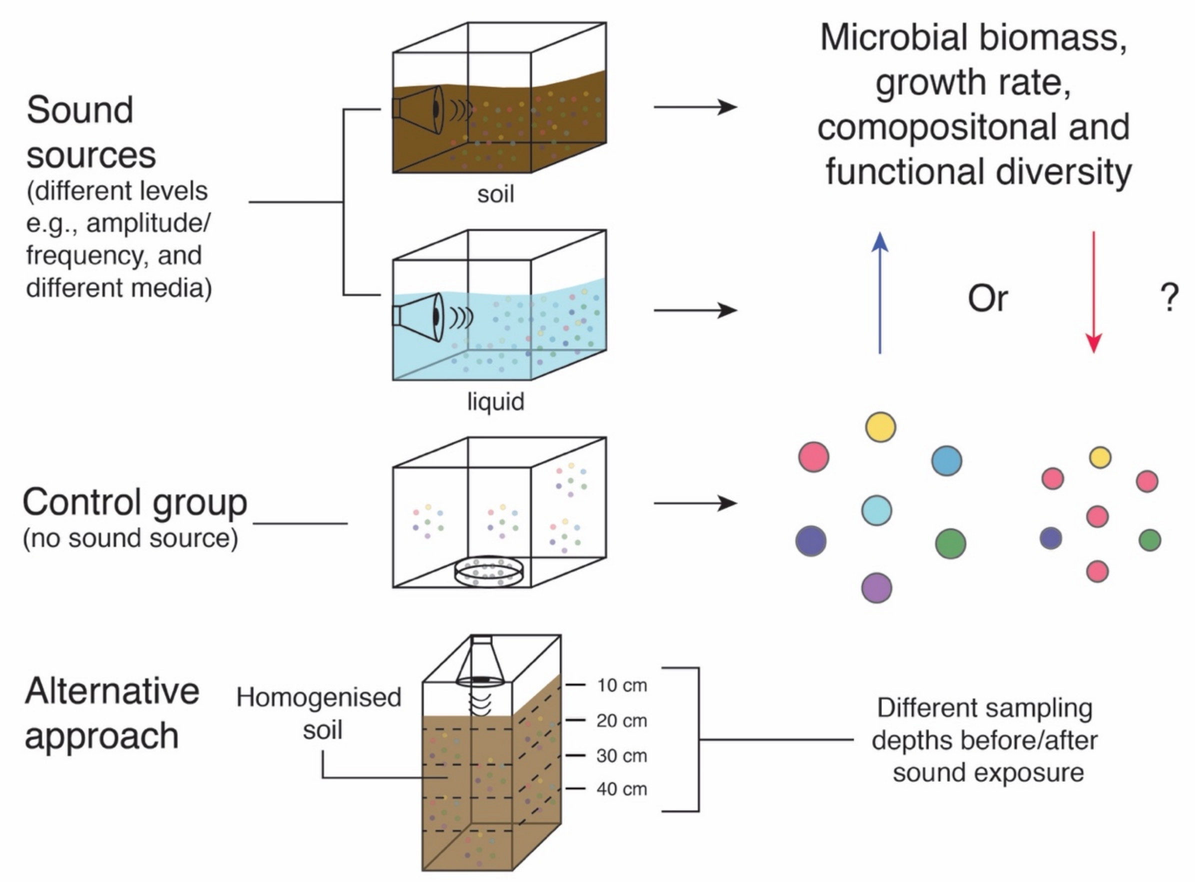

Fig. 1. Schematic of sound exposure experiments. Gu et al. (2016) investigated biomass and growth rate of a single taxa. However, similar experiments could be carried out to investigate potential changes in microbial community compositional and

functional diversity, whilst testing different interfaces/media that may have an important effect on sound propagation.

Murphy et al. (2016) demonstrated that exposing *Pseudomonas aeroginosa* (*n* = 3 plates) and *Staphylococcus aureus* (*n* = 3) to frequencies of 0.1, 0.8, and 1.6 kHz for 48 hours resulted in a significant increase in biofilm formation (compared to the control). Greatest growth for *P. aeruginosa* was recorded at 0.8 kHz, and for *S. aureus* it was 1.6 kHz. This study did not use decibel units in their assessment but the inter-species variation in growth rate was dependent on sound frequency. Again, the sample sizes are low and as such, the results should be interpreted with caution. Inter-species variation in growth was also shown in a study by Sarvaiya and Kothari (2014). The authors exposed *Chromobacterium violaceum*, *Serratia marcescens, Staphylococcus aureus,* and *Streptococcus pyogenes* to music at a frequency range of 38-689 Hz. All bacteria increased in growth (3-40% higher) except for *S. marcescens*, which decreased in growth (-8%) and pigment (prodigiosin) production (-16%). *C. violaceum*'s growth increased by 40% and prodigiosin pigment production increased by 66%. The authors suggest that observed alteration in pigment production is not entirely due to growth, but more likely quorum sensing (i.e., intercellular communication) affected by sound. These studies imply that anthropogenic sound exposure can affect microbial growth, biomass and synthesis of intracellular molecules via a range of pathways, and that certain frequencies and amplitudes may favour some microbial species over others.

A recent mouse-model study demonstrated adverse effects of noise pollution on the gut microbiome (Cui et al. 2020). They used 16S rRNA sequencing to characterise the gut microbiome and the Tax4Fun package in R to predict metagenome content. The authors found that in mice (*n* = 40) exposed to white noise at 98 dB (frequency of 20 kHz for 4 hours per day, for 30 days) but not the control groups, bacterial-encoded functional profiles included an increase in phospholipid and galactose metabolism, oxidative stress, and cell senescence which corresponded with systemic inflammation. The authors suggest this may have implications for early-onset Alzheimer's disease. This study shows interesting results that could have important implications for public health. In subsequent studies, greater value could be added by using whole genome sequencing approaches instead of amplicon-functional profiling approach, and focus on relationship directionality.

Another study investigated glucose metabolism and gut microbiota–host inflammatory homeostasis in rats (*n* = 64) (Cui et al. 2016). The authors found that chronic noise (100 dB, 400Hz-6.3 kHz, 4 hours a day for 30 days) altered the percentage of Proteobacteria and Actinobacteria in the gut and corresponding abnormalities in glucose and insulin regulation relative to controls. They suggest that anthropogenic noise exposure could have cumulative effects on diabetes onset due to microbiome compositional changes and intestinal inflammation. Once again, these results could have important implications for public health by improving our understanding of the factors that may contribute to diabetes. It is worth mentioning that although appropriate in 2016 (time of the study), the approach used to characterise the microbiome — via operational taxonomic units with 97% similarity — is now considered to be outdated. Exact Sequence Variants (ESV) may provide a richer taxonomic picture (Callahan et al., 2017), and whole genome sequencing, although more expensive, would enhance functional profiling.

6.3.2. Algae, Fungi and Zooplankton

We found 2 studies pertaining to the exposure of sound on algae and fungi, and 1 for zooplankton. Cai et al. (2016) exposed the microalga *Picochlorum oklahomensis* to anthropogenic sound at 90 dB and 1.1, 2.2, and 3.3 kHz for 3 hours a day for 30 days. The authors found that all frequencies increased biomass compared to the control, but that 2.2 kHz was the most effective (e.g., oil yield of 40.37 g/L compared to the control of 31.66 g/L). The sample size is not clear, although it appears to be low at only 2 replicates per treatment. As the authors state, an expansion of the study is needed to decipher the mechanism responsible for the increased biomass due to the complexity of interacting variables. Given that lipid accumulation is a stress response to nitrogen limitation, measurements of nutrient uptake would be an interesting complement to growth data. The results of this study align with previous reports Jiang et al. (2012) who cultured *Chlorella pyrenoidosa* for 7 days with sound exposure at 80 dB and frequencies of 0.2, 0.4, 0.7, 1, 2 and 6 kHz. They found that *C. pyrenoidosa* growth due to sound exposure was 30% higher than the control, with an optimal frequency between 0.4-1 kHz. Again, it is not clear what the sample size was for this study, therefore, the results should be interpreted with caution. Kumar (2020) found that the biomass of the yeast *Saccharomyces cerevisiae* increased significantly following sound exposure of 0.1–10 kHz for 8 hours compared to a control. Once again, the sample size is not clearly stated and the study is difficult to interpret. A challenge is that many of these studies rely on optical density (OD) to measure microbial growth. OD measurements are assumed to be proportional to sample concentration (cell numbers) (Stevenson et al. 2016). Taken in tandem with cell counts and dry cell weight, the impact on cell growth could be interpreted with more certainty. Interestingly, Aggio et al. (2012) used metabolomics to compare the physiology of yeast cells (n = 15) exposed to high (10 kHz) and low (0.1 kHz) frequency sonic vibration at 90 dB. All stimuli increased the growth rate of the yeast by 12% but reduced biomass production by 14%, and different frequencies induced different metabolomic responses. Other studies have shown that sound can evoke physiological reactions in plants (e.g., via gene expression in Arabidopsis) (Jung et al. 2018) and potentially enhance growth (Hassanien et al. 2014). Future studies could explore this from a 'holobiont' (collective host and symbiotic organisms) perspective and investigate the directionality of the relationship (e.g., microbe -> host and/or host -> microbe?).

Finally, it is worth noting that anthropogenic noise pollution (e.g., from seismic operations) has been shown to adversely affect zooplankton. McCauley et al. (2017) demonstrated that following seismic air gun exposure, there were 2-3 times more dead zooplankton (n = 78) for all taxa compared with controls, and up to 1.2 km away from the source. All krill larvae found in the exposed samples were dead. This suggests potentially under-acknowledged implications for ocean ecosystem functionality and productivity and warrants further research.

6.4. The effects of artificial light pollution on microorganisms

Artificial light pollution can also have important ecological and public health impacts. We found 8 papers pertaining to the effects of artificial light on microorganisms.

6.4.1. Biofilms and Sediments

Maggi et al. (2020) explored the effects of ALAN (using LED lamps with a mean of 27 lux to mimic coastal urban lighting) on marine biofilms (microphytobenthos). They observed biofilm quadrats (*n* = 24) over a period of 204 days. They showed a significant increase in temporal variance of maximum photosynthetic efficiency under ALAN. This suggests that ALAN may differentially affect certain groups in microbial biofilms due to species-specific sensitivities. The authors conclude that future studies should aim to understand the interactions between ALAN and other anthropogenic disturbances on microbiomes. Hölker et al. (2015) investigated the response of microbial communities in freshwater sediments to artificial light exposure (*n* = 30). They used 70 W high-pressure sodium lamps (2000 K, 96 lm W^{-1}) and nocturnal light levels ranged from 13.3-16.5 lux at the water surface and 6.8–8.5 lux at the sediment surface (50 cm depth). Over a 1-year period they observed an increased abundance of phototrophic taxa (diatoms, Cyanobacteria and green algae) in sediments after five months of ALAN compared to the control. The authors suggest that ALAN over waterbodies could reduce diurnal fluctuations and has the potential to transform freshwater systems to nocturnal carbon sinks. Further studies are needed to ascertain the full ecological impacts (both direct and indirect) of this process.

6.4.2. Coral Microbiome

Baquiran et al. (2020) investigated the effects of ALAN on the coral *Acropora digitifera* and its microbiome. The authors exposed corals (*n* = 45) to ALAN (27-45 lux) for 2 months. They found that microbial diversity remained stable after ALAN exposure, but certain taxa in the families Rhodobacteraceae, Caulobacteraceae, Burkholderiaceae, Lachnospiriaceae, and Ruminococcaceae significantly increased in exposed corals. The observed compositional stability of the coral microbiome in this study may indicate physiological plasticity of different microbes, potentially allowing the community to buffer environmental disturbance with continued provision of important metabolites. Further studies should investigate how longer-term ALAN exposure affects the corals and whether the observed changes in microbial families has positive or negative outcomes for coral ecosystems. Additional research on the potential impacts of ALAN-induced microbiome changes on gene expression of corals would also be beneficial. Rosenberg et al. (2019) found that corals exposed to ALAN have 25 times more differentially expressed genes that regulate cell cycle, proliferation, growth and protein synthesis that may act as a chronic disturbance.

6.4.3. The Gut Microbiome

A recent mouse-model study (*n* = 28) demonstrated that prolonged artificial light exposure can significantly alter the gut microbiome and promote non-alcoholic fatty liver disease (NAFLD) (Wei et al. 2020). The authors used white fluorescent light tubes with a wavelength of 400~560 nm set at 200 lux. They compared normal light-dark ratios with constant light exposure and found that constant light significantly altered gut microbiome composition and promoted functional pathways related to type-2 diabetes in addition to promoting obesity and NAFLD. Future studies would likely benefit from whole genome sequencing as opposed to OTU analysis. However, this study points to important public health implications of artificial light exposure.

Artificial light has also been shown to alter gut microbiome composition in the Eurasian tree sparrow *Passer montanus* (*n* = 40) (Jiang et al. 2020). In this study light (400-1400 lux for 12 hours, followed by 8 lux for 12 hours) reduced bacterial alpha diversity (Shannon 5.70) and significantly affected melatonin synthesis compared to the dark control (Shannon 6.96). As light pollution affects melatonin, which itself helps to regulate the hypothalamus-pituitary-adrenal and microbiota-gut-brain axes (Anderson and Maes, 2015), this could potentially have important cascading impacts on physiological and psychological health. There is a clear deficit in studies exploring the effects of light pollution on the human microbiome, and as such, more research in this area is warranted.

6.5. Discussion

This mini-review shows that a limited number of studies have been carried out to investigate the effects of anthropogenic sound and artificial light pollution on microbiomes. However, the studies do indicate that anthropogenic sound and artificial light may have important influences on microbially-mediated ecosystem processes and human health. Both forms of pollution are considered to be global health issues and have been shown to affect ecosystem composition and functionality. Considerably more research is needed to gain a better understanding of the effects of sound and light pollution on microbiomes. Indeed, ecosystems are under immense pressure from various forms of degradation. By understanding the effects and processes involved, we can start to design appropriate mitigation strategies. Contra to this, we could potentially utilise any positive sound/light-induced microbial effects to improve ecosystem stability and human health outcomes.

The studies mentioned in this paper lay the foundations for important future work in microbial ecology and public health. Understanding that different sound exposures (e.g., amplitude, frequency, durations) induce inter-species variation in growth, biomass and synthesis of intracellular molecules could have important implications for many ecological processes across trophic levels. We also do not yet fully understand the mechanisms by which sound stimulates microbial growth, as suggested by Gu et al. (2016). Mechanosensitive channels on bacterial cell membranes might be involved in signal transduction, but gaining a better understanding will enable optimisation of the processes or mitigation for adverse exposures. The indication that increased bacterial resistance to ampicillin was attributed to low frequency anthropogenic noise certainly warrants further research due to its potential importance in the fight against antibiotic resistance.

Understanding how sound affects plant-microbe (or animal-microbe) interactions as indicated by Kim (2016), could be extremely important given that both anthropogenic sound pollution and ecosystem degradation are increasing globally (Fig. 2.). Plant health is imperative and microbial interactions are essential to the provision of multiple ecosystem services (Guerra et al. 2020). An interesting line of enquiry could be to investigate whether sound pollution influences environmental microbiome assembly and intercellular signalling to the point where it affects plant health and (bioacoustic) communication. The effects of anthropogenic sound on human and non-human animal microbiomes also warrants further research.

Exposure to biodiverse natural environments alters the human microbiome with potential benefits to human health (Roslund et al. 2020; Selway et al. 2020). Exposure routes may differ depending on ecological characteristics such as vegetation complexity and height (Robinson et al. 2020). Another interesting line of enquiry is whether different levels of urban sound pollution affect the composition, assembly and exposure routes of microorganisms.

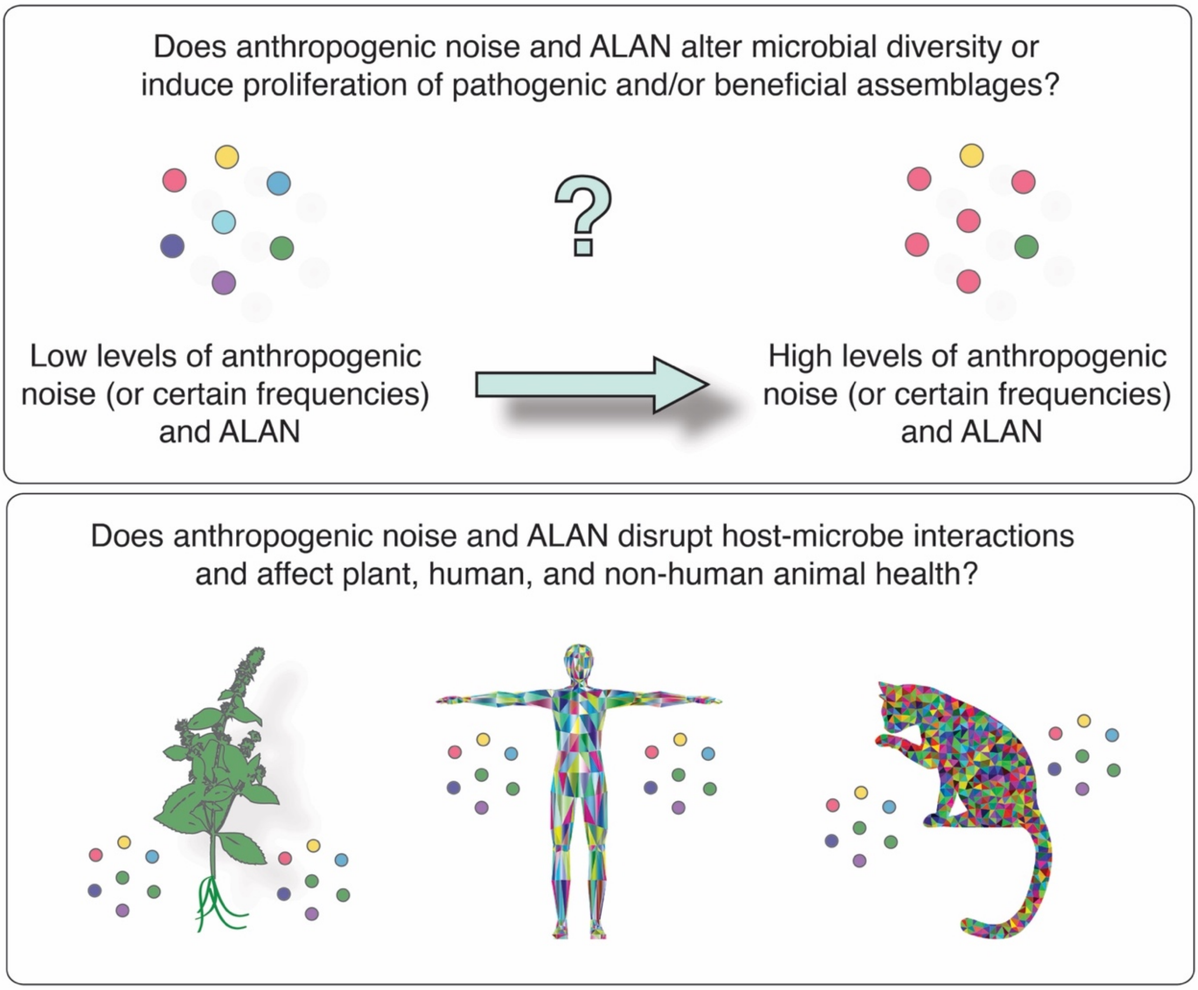

Fig. 2. Future research into the potential effects of anthropogenic sound and ALAN on microbial community composition and host-microbe interactions is an important line of enquiry.

ALAN is also likely to affect human health and ecosystem functionality via impacts on the microbiome. Although initial work suggests that ALAN significantly affects marine and freshwater bodies, it is unclear whether the impacts are negative in the long-term. Indeed, Hölker et al. (2015) suggests ALAN has the potential to transform freshwater systems to nocturnal carbon sinks. Further studies to ascertain the multidimensional ecological impacts of ALAN are needed, because it could potentially have important unforeseen multi-trophic impacts. Indeed, it is a similar story for corals because the studies report variable results. However, as coral reefs are under immense pressure, this is certainly an important area of research.

This mini-review highlights that additional research is needed to unravel the effects of light pollution on the human microbiome. Indications from the studies suggest that artificial light could adversely impact physiological processes via the microbiome, and potentially contribute towards metabolic diseases. If anthropogenic sound and ALAN affect human-environmental microbiome exposure and influence human physiology directly, there could also be important social equity issues to investigate. Social disparities in exposure to anthropogenic sound pollution have been documented (Dregen et al. 2019). Therefore, in some cases, the impacts of exposure will also be unequally distributed across different social groups. This warrants further research.

Many of the studies in this mini-review suffered from modest sample sizes, suboptimal experimental designs (e.g., lack of negative controls, cell counts and particle sizing), and some of the bioinformatics approaches used are now outdated. These factors should be improved in future studies. However, it is clear that anthropogenic sound pollution and ALAN have the potential to influence ecosystems and human health via interactions with microbiomes. This is an emerging and severely underexplored field of research that could have important implications for global ecosystems and public health. There is also an intriguing hypothesis to consider (Box 1).

Box 1. The *photo-sonic restoration hypothesis*

If anthropogenic sound and light disrupt microbiome assembly, potentially favouring certain adaptable species and reducing functional diversity, this could have important ecosystem and health implications. Therefore, does restoring natural levels of light and sound help to restore microbiomes and ecosystem stability? We hereby propose the *photo-sonic restoration hypothesis.*

CHAPTER 7

THE ENVIRONMENT-MICROBIOME-HEALTH AXIS: MICROBIOME-INSPIRED GREEN INFRASTRUCTURE (MIGI)

> *“We are protected by two nested layers of biodiversity: microbiota of the outer layer (soil, natural waters, plants, animals) and inner layer (gut, skin, airways). The latter inhabits our body and is colonized from the outer layer”*
>
> – *Haahtela, 2019*

7. PUBLICATIONS IN CHAPTER 7:

Robinson, J.M., Mills, J.G., and Breed, M.F. (2018). Walking Ecosystems in Microbiome-Inspired Green Infrastructure: An Ecological Perspective on Enhancing Personal and Planetary Health. *Challenges.* 9, pp.1-15.

Watkins, H., **Robinson, J.M.,** Breed, M.F., Parker, B. and Weinstein, P. (2020). Microbiome-Inspired Green Infrastructure: A Toolkit for Multidisciplinary Landscape Design. *Trends in Biotech. 38,* pp.1305-1308.

Walking Ecosystems in Microbiome-Inspired Green Infrastructure: An Ecological Perspective on Enhancing Personal and Planetary Health

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7.1. Abstract

Principles of ecology apply at myriad scales, including within the human body and the intertwined macro and microscopic ecosystems that we depend upon for survival. The conceptual principles of dysbiosis ('*life in distress*') also apply to different realms of life—our microbiome, the macro environment and the socioeconomic domain. Viewing the human body as a holobiont—a host plus billions of microbial organisms working symbiotically to form a functioning ecological unit—has the potential to enhance personal and planetary health. We discuss this ecological perspective in our paper. We also discuss the proposals to rewild the microbiome, innovative microbiome-inspired green infrastructure (MIGI) and the basis of prescribing ‘doses of nature’. Particular emphasis is given to MIGI—a collective term for the design and management of innovative living urban features that could potentially enhance public health via health-inducing microbial interactions. This concept builds upon the microbiome rewilding hypothesis. Mounting evidence points to the importance of microbial diversity in maintaining favorable health. Moreover, connecting with nature—both physically and psychologically–has been shown to enhance our health and wellbeing. However, we still need to understand the underlying mechanisms, and optimal types and levels of exposure. This paper adds to other recent calls for the inclusion of the environment-microbiome-health axis in nature–human health research. Recognizing that all forms of life—both the seen and the unseen—are in some way connected (ecologically, socially, evolutionarily), paves the way to valuing reciprocity in the nature–human relationship. It is with a holistic and symbiotic perspective that we can begin to integrate strategies and address connected issues of human and environmental health. The prospective strategies discussed in our paper focus on enhancing our connections with the natural world, and ultimately aim to help address the global challenge of halting and reversing dysbiosis in all its manifestations.

Keywords: planetary health; microbiome; urban nature; biodiversity; mental health; nature connectedness; green space; Noncommunicable disease; environmental health; health and wellbeing

7.2. Introduction—The Holobiont

Planet Earth's biosphere supports several biomes, each containing many ecosystems. Each ecosystem supports a diversity of abundant macro-organisms, which in turn harbor a multitude of microscopic life forms—the bacteria, viruses, archaea, and microeukaryotes. Each human being can be thought of as a complex and dynamic ecosystem, supporting billions of microbes that provide mutualistic functions (Van de Guchte et al. 2018). Indeed, human beings can be considered *holobionts*, from *holo*—a Greek derivative for the word 'whole', and *biont,* for 'life' and defined as a host plus billions of stable and transient microbial organisms working symbiotically to form a functioning ecological unit (Dheilly, 2014).

Not surprisingly, this concept can be difficult to embrace; consciousness as a biological phenomenon is steeped in intrinsic complexities, and it is perhaps easier to view ourselves as an individual of a species. Even as individuals, a fundamental asymmetry exists between how we view ourselves and how we view others, due in part to deeply complex emotional and cognitive immersion (Pronin, 2008). Although this concept may seem counterintuitive when compared to our default perceptions, taking an ecological approach to viewing humans as holobionts can lead to an influx of fascination and curiosity. Importantly, taking an ecological and holistic view of the human body could also make a significant contribution to both personal and planetary health. This perspective is exemplified by the recent calls for principles of ecology to be included in medical curricula (Smith et al. 2015)—a recognition of the human body as an ecological unit, openly interacting with the environment via complex microscopic processes.

Adopting a holistic philosophical framework, our paper aims to explore these ecological principles in greater detail, whilst highlighting the links between ‘human’, microbial and environmental health. We also provide an overview of prospective landscape and social interventions that have the potential to enhance our connections with the natural world, through health-inducing microbial interactions and psychosocial pathways. These strategies, namely microbiome rewilding, microbiome-inspired green infrastructure (MIGI), and green prescribing, ultimately aim to make an important contribution to both personal and planetary health. Emphasis is given to MIGI—a collective term for the design and management of innovative living urban features that could potentially enhance public health via health-inducing microbial interactions. This concept builds upon the microbiome rewilding hypothesis.

7.3. Interconnectedness and Evolutionary Biology

There is growing recognition that all forms of life are interconnected, ecologically and evolutionarily (Prescott and Logan, 2017; Flandroy et al. 2018). These tangled connections also traverse the boundaries of the sociosphere—the complex realm of dynamic human-centric structures and interactions that weave their way in and out of our social lives and cultural identities. Indeed, it is the interconnectedness of societal health with environmental stability and resilience that are integral to the concept of planetary health (Pongsiri et al. 2017; Seltenrich, 2018).

From an evolutionary perspective, this vast array of connections manifest with great depth and diversity. For example, it has recently been hypothesized that the root-leaf axis in plants may be an evolutionary analogue to the gastrointestinal-brain axis in animals (Tonello et al. 2018). This hypothesis is undoubtedly provocative and draws upon the functional parallels between auxin, a plant hormone, and serotonin, a hormone and neurotransmitter in 'higher animals', and their quantum interactions with photons and nutrients (Tonello et al. 2018).

Following the mid-late 20th century advances in molecular biology, the re-evaluation of evolutionary theory has been a ubiquitous and tantalizing area of interest for evolutionary biologists. It is thought that horizontal gene transfer—that is, the integration of genetic material typically acquired from other species (not vertically transmitted from a parent)—may play a significant role in the evolution of what is traditionally considered to be a species (Daubin and Szöllösi, 2016). Quammen explores this topic in his recent book *The Tangled* Tree (Quammen, 2018). He describes how a substantial proportion of the human genome (roughly 8%) was acquired horizontally through viral infections. This, along with the idea of the holobiont, further challenges our conceptual understanding of a species as a discrete entity, and even human identity and individuality.

A classic example of this interspecies molecular nexus that further strengthens the *tangled* view of evolution, is the existence of mitochondria. These are organelles responsible for synthesising adenosine triphosphate (ATP) via oxidative phosphorylation, producing over 90% of our cellular energy (Sorriento et al. 2014). However, mitochondria are thought to have evolved from a bacterial progenitor in the Alphaproteobacteria phylum (Gray, 2012). In summary, not only can human beings be described as functional ecological units comprised of microbial symbionts, but approx. 8% of our genome is of microbial origin. And not only this, the organelles that contribute significantly to our survival, are also microbial in origin. This overturning of the traditional view of the tree of life, along with the realization that the biosphere and sociosphere are inextricably linked, paves the way for a new holistic philosophy of life and health. It could be argued that the natural step that follows this recognition of interconnectedness, is one that explores mutually-advantageous relationships between the constituents of the whole. The whole being the planet, and the constituents being our environments, our societies, our 'selves', our symbionts, and our genes.

7.4. Forty-Three Percent Human

Recent estimates suggest that human cells (i.e., somatic and germ) constitute only 43% of all the cells that form a human body (Knight, 2018). Due to advances in genomic technologies, this has changed from earlier estimates of 10%, but in any case, it is still an impressively low figure. The other 57% of cells are microbial (bacteria, viruses, archaea, microeukaryotes), and therefore, in terms of cell abundance at least, humans are actually more microbial than human. This statement is also true when considering ratios of gene abundance—microbial genes within the human body are thought to outnumber human genes by between 150 and 1000 times (Qin et al. 2010; Zhang et al. 2017). With a moment of reflection, this can lead to a medley of existential questions such as—what does it mean to be human?

A study has shown that the average palmar surface on the human hand can harbor >150 species-level bacterial phylotypes (Fierer et al. 2008). To put this into perspective, that is more than the total number of all mammal species that inhabit all of the ecosystems in the UK. However, this is dwarfed by a microbial ecosystem less than 1 m away in the oral cavity, where approximately 700 species of bacteria form dense interactive networks of conjugated biofilms (Jordán et al. 2015). This is dwarfed further still by the human gut, which is one of the densest microbial habitats on earth (Mirzaei and Maurice, 2017), with approximately 1,000 species of bacteria (in a 'healthy' gut) and a combined microbial weight of 2 kg (Shreiner et l. 2015; Mazidi et al. 2016) . Like their macro counterparts, microbial ecologists have an important role in studying the relations of microorganisms to one another and to their environments — the network of habitats and biotic processes that support and are supported by a symbiotic conglomerate, a complex system we refer to as the human body.

Viewing the human body as an ecosystem goes far beyond the apt but quirky analogies such as the 'rainforests of our guts', the 'savannahs of our skin' and the 'coral reefs of our mouths'. There are many macro-ecological parallels; for example, just as plants convert energy from the sun, acquire nutrients from the soil and subsequently provide organisms higher up the food web with health-inducing nutrients and bioactive phytochemicals, the microbes living in the human gut consume the food that we ingest and release important metabolites. For example, *Bacteroides* spp. are known to convert carbohydrates into short-chain fatty acids (SCFAs), which are essential in maintaining gut homeostasis (Sivaprakasam et al.

2016; Li et al. 2017). As Cryan said in his 2017 TEDx talk, "*you are what your microbes eat!*" (TEDx, 2017).

7.5. Functional Ecology and Food for Thought

From a health perspective, it may pay dividends to embrace this holobiotic nature; viewing the 'human' as a dynamic ecosystem that requires stability and resilience, just like the macro-ecosystems that humans rely upon for the supportive, provisioning and regulating ecosystem services. When natural habitats are degraded and environments polluted, ecological stability and resilience are often reduced, leading to a loss of trophic and genetic diversity, shifts in ecological communities, and sometimes to a state of ecological collapse (Dobson et al. 2006; Mahoney and Bishop, 2017). The same concept applies to the holobiotic human ecosystem. This is supported by studies reporting positive health outcomes associated with higher microbial diversity such as reduced atopic sensitivity and favorable mental health (Ruokolainen et al. 2015; Clapp et al. 2017). Conversely, reduced microbial diversity has been linked to the onset of inflammatory diseases (Huttenhower et al. 2012). Further to metabolic benefits, it is thought that a diverse assemblage of microbiota in the gut, and on the skin, provides health benefits by outcompeting pathogenic microbiota (Ferreyra et al. 2014). This is analogous to native macroscopic vegetation resisting the potentially deleterious impacts of invasive species in, for example, a forest with high compositional and structural diversity (Guyot et al. 2015).

Extending this argument to a clinical health perspective raises an important objective of microbiome research; that is, to gain a greater understanding of the role of microorganisms in the functioning of the human body. The microbiome is thought to influence various mammalian biotic systems and processes, from appetite and circadian rhythm, to emotional responses and immune regulation (Alcock et al. 214; Thaiss et al. 2016; Bagga et al. 2018; Chhabra et al. 2018). Researchers are discovering complex systems within the human body are partially, if not predominantly, influenced by microbial inhabitants. For example, the gut-brain axis is an area of research currently in the spotlight for this very reason. Researchers now believe that a bidirectional communication network exists between the central and enteric nervous systems and the microbiome within the human gut—that is, human-inhabiting microbiota are communicating with the brain and vice versa (Foster et al. 2017; Bonaz et al. 2018).

A number of radical questions are now being asked, such as: can microbes influence the way we think, and even the dietary choices we make? This notion runs conceptually parallel to the central theorem of the extended phenotype, posited by Dawkins:

“An animal’s behaviour tends to maximize the survival of the genes ‘for’ that behaviour, whether or not those genes happen to be in the body of the particular animal performing it” (Dawkins, 1989, p. 13).

7.6. Dysbiosis—‘Life in Distress’

With a growing understanding of microbial ecology and nature’s complexity, it should be of no surprise that many aspects of human health are now considered to be inextricably linked to processes involving our microbiome. Conversely, several aspects of human ill-health are often a result of an unhealthy balance and reduced diversity of microbiota within and upon the human body (Carding et al. 2015; Ohri et al. 2017). This is also known as dysbiosis or 'life in distress' (Logan, 2015). From a human health perspective, it is important to recognize that there is inter-individual variation in optimal microbial community structure and composition. Diversity of microbial communities is likely to play a major role in health as substantial functional redundancy exists amongst taxonomically distinct microbiota; that is, the microbial constituents of these open ecosystems can share similar functional roles (Louca et al. 2018). In accordance with the redundancy hypothesis, this enables asynchronous responses during perturbation or disturbance, which helps to maintain system stability (Kang et al. 2015).

Dysbiosis exists on various levels, and within different realms—within our bodies, and within the wider environment, and it is the environment and not our genes, that has recently been shown to be the key driver of microbiome composition and health (Rothschild et al. 2018). This notion is also supported by the 'old friends hypothesis', a refinement of the hygiene hypothesis, which proposes that in recent times, humans (particularly in so-called 'industrial' societies) have been exposed to a reduced number of immunoregulatory microorganisms from various sources, such as biodiverse environments and our diet (including breastfeeding), and that these microbes have co-evolved with the human immune system for millennia (Rook and Brunet, 2005). Indeed, it is the downfall of this relationship that has probably made a significant contribution to the etiopathogenesis of noncommunicable diseases such as asthma and inflammatory bowel disease (Haahtela et al. 2013; Bloomfield et al. 2016).

7.7. Microbiome Rewilding and Planetary Health

Humans are spending less time in natural environments, particularly those with higher levels of biodiversity, and less time interacting with natural organisms—physically, psychologically and biochemically. This reduction in exposure to biodiversity has been linked to a wide range of health conditions, from the inflammatory diseases mentioned previously, to depression and anxiety (Pearson and Craig, 2014; Snell et al. 2016). One proposed solution to the health implications of reduced biodiversity exposure is microbiome rewilding (Mills et al. 2017).

The microbiome rewilding hypothesis posits that ecological restoration of urban green spaces can rewild urban spaces to a health-promoting habitat, where urban lifestyles can become interconnected with greater biodiversity (Mills et al. 2017). Importantly, this type of interconnected lifestyle comes with a rich exposure to diverse microbiota and the associated health benefits common to traditional and agricultural communities (Stein et al. 2016). Urban residents surrounded by more green space tend to be healthier, regardless of socioeconomic status (Brindley et al. 2018). As previously discussed, diverse microbiota are fundamental to good human health. Microbiota are involved in the health of most holobionts, including many animals and plants. It is from this co-dependency platform that microbiome rewilding is premised, and we will now explore that further.

Coevolution has produced host species-specific assembly mechanisms for core microbiota (Lundberg et al. 2012; Turnbaugh et al. 2007) that perform various functions, such as mycorrhizal nutrient acquisition in plants and short-chain fatty acid production in mammals. Therefore, a biodiverse community should produce a microbially diverse environment. However, within each holobiont, a portion of the microbiota remains plastic. This plasticity means that microbiota of individual holobionts are to some degree moderated by environmental microbiota. This is evidenced by captive Jamaican fruit bats having skin microbiota more similar to their co-habiting Seba's short-tailed bats than to another population of captive Jamaican fruit bats (Lemieux-Labonté, 2016). Additionally, leaf-surface microbiota of plants have been experimentally shown to be strongly influenced by the richness and functional diversity of their plant community (Laforest-Lapointe, et al. 2017). Furthermore, Finnish children from rural areas have a skin microbiota distinct to those from urban areas (Lehtimäki et al. 2017). Therefore, if the urban habitat is more biodiverse then people living there and using the space should have more diverse microbiota, resulting in better functioning immune systems. However, microbiome rewilding as a health care intervention must be used in concert with healthy dietary and other lifestyle choices that promote diverse symbiotic microbiota and proper immune function.

Microbiome rewilding of human habitats may have important co-benefits for planetary health. Co-benefits include biodiversity conservation (Goddard et al. 2010), urban agriculture benefits (Jansson, 2013), and lower crime rates with increased community cohesiveness (Weinstein et al. 2015). Further, people who are more exposed to natural environments show greater concern for environmental issues beyond the noncommunicable disease epidemic discussed here (Bögeholz, 2006). Such issues can include deforestation, industrial agriculture, single-use plastics, and climate change. These are among the most pressing issues our species has ever faced. These issues require collective consciousness and action at all scales to shape a positive, healthy future for human life on Earth.

7.8. Microbiome-Inspired Green Infrastructure (MIGI)

Building on the microbiome rewilding hypothesis, a term that has recently been proposed is ‘microbiome-inspired green infrastructure’ (MIGI) (Robinson and Jorgensen, 2019). MIGI is a collective term for living, multifunctional green spaces that are designed and manipulated to generate health-inducing microbial interactions (Figure 1). This is based on the premise that biodiverse microbial habitats can be ‘restored’ as per the microbiome rewilding hypothesis.

The ‘inspired’ part of MIGI implies a significant design element. Design considerations include multifunctional roles for social activities and ecosystem services, and both dynamic and static spatial factors within urban settings. For example, understanding how pollution, area, proximity, aspect, and urban physical features such as buildings, roads, and other structures, influence the dynamics of MIGI will be essential. It is also important to extend beyond the domains of localized mechanisms and impacts, and to determine whether interconnected systems of MIGI can “improve the microbial network fragility of larger urban areas such as ‘megacities’, which have been linked to human diseases” (Kim et al. 2018; Robinson and Jorgensen, 2019). Furthermore, as a prospective ecological and public health intervention, MIGI should be designed to maximize ecological justice and reduce health inequalities; for example, by ensuring equity of access where public land is concerned. Gaining a detailed understanding of the dynamics between MIGI availability vs. optimal exposure to, and interaction with, health-inducing microbial community assemblages, will play a key role throughout the MIGI design, implementation and monitoring process.

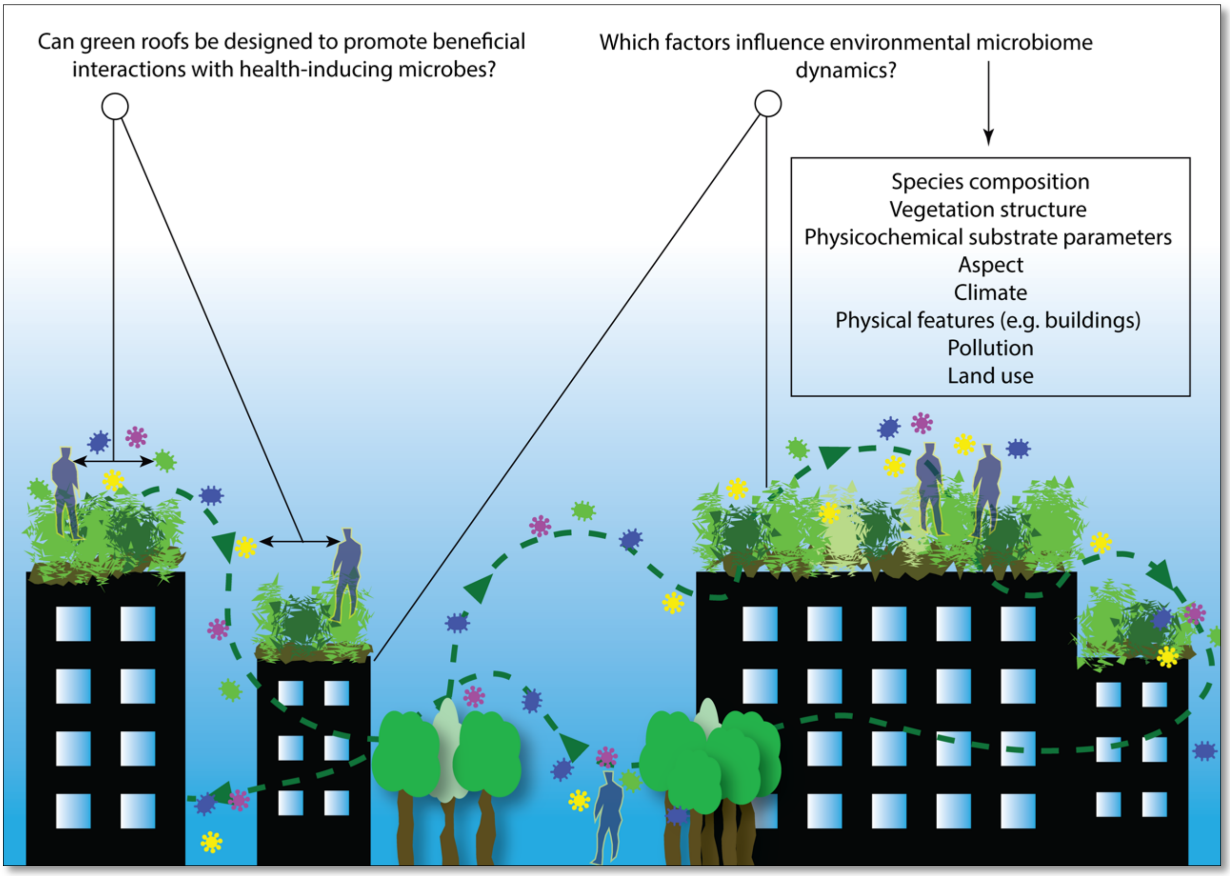


Figure 1. Could green roofs be designed and manipulated to promote beneficial interactions with health-inducing microbial assemblages, i.e., 'old friends'? (created by the author, from Reference Robinson and Jorgensen, 2019).

'Inspired' also implies a detailed understanding of environmental microbiome dynamics, the functional relationships between microbiota and vegetation, the spatiotemporal and compositional dynamics, and the mechanisms and pathways that facilitate human-microbial exchange and associated benefits. These factors are still poorly understood and a concerted effort to establish research and communication methods is needed to rapidly progress our understanding of what is known as “microbial dark matter” (Rook, 2013)—the microbial presence, abundance, composition and functionality in the environment. This continual generation of knowledge will allow for informed applications of MIGI, optimized to benefit humans and the environment.

7.9. Microbiome-Inspired Green Infrastructure—Foraging

From a societal evolution perspective, the human microbiome has gradually decreased in community diversity as human populations have passed along the following trajectory:

Foraging → Rural farming → Urban industrial lifestyles (Gupta et al. 2017).

A number of studies point to the link between high human microbial diversity and the foraging lifestyle (Nasidze et al. 2011; Schnorr et al. 2014; Fragiadakis et al. 2018). Therefore, it is envisaged that the application of MIGI will include foraging-friendly green spaces (Figure 2). This will require a collaborative effort between landscape architects, ecologists, agriculturalists and urban planners to create innovative food planting schemes that replicate (partially, at least) and promote foraging behavior. The ultimate aim of this strategy is to enhance human–environment microbial interactions and increase the diversity of microbiota residing in and on the human body. Foraging also augments the multisensorial experience (i.e., touch, sight, smell), which brings its own intrinsic advantages as nature-derived health benefits arrive through a variety of senses (Franco et al. 2017; van den Bosch and Bird, 2018).

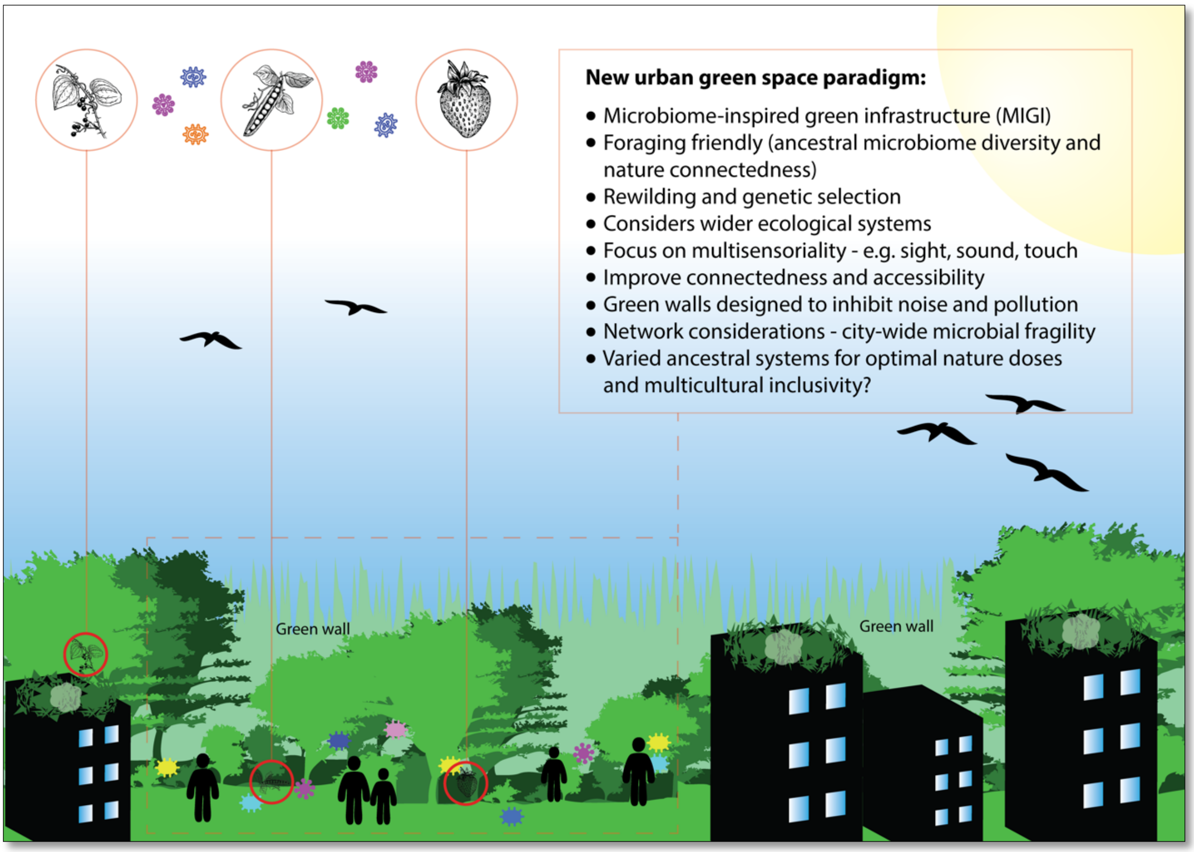
Foraging is already ubiquitous across the globe; however, it is often prohibited or discouraged in urban areas (Shackleton et al. 2017). Formal strategies to draw together the benefits of foraging are needed, and with further research, the potential benefits of health-inducing microbial exchange will likely strengthen this approach. Urban foraging manifests in a variety of forms from harvesting the fruit of street trees to participating in community gardening. It would be prudent for researchers to investigate the differences (in terms of microbial exchange and health outcomes) between these foraging methods, as this would help inform the design and management of MIGI in the future. There are also concerns that need to be addressed, such as urban pollutants and perceived ‘mess’ from fallen fruits (Kowalski and Conway, 2018). The former calls for innovation in planting design plus plant protection and selection, and broader strategies to reduce pollution. The latter would benefit from a shift in perception of the value of these food sources, mobilized perhaps through community-centric groups such as the Grow Sheffield’s Abundance Project (Grow Sheffield, 2018) —an initiative that promotes the harvesting of food plants across the city and redistributes the ‘products’ to food banks and local communities.

Urban foraging schemes may well need to adapt to the intensively dynamic socio-ecological complexities of urbanization; for example, changing ownership of land, building development, and changes to infrastructure. However, recent innovation is helping to address this issue. For example, mobile allotments, such as those created by the arts and environment project Avant Gardening (Avant Gardening, 2018) can be installed on vacant lots to provide communities with a foraging hub, and easily moved if the land status changes. It is also important to note that in addition to the potential of health-inducing microbial exchange, community gardening can generate other health and wellbeing benefits (e.g., through physical exercise, psychological restoration and nature connectedness) and enhance social cohesion (Jang et al. 2017; Veen et al. 2016).

These potentially health-inducing interactions with environmental microbiota may also be enhanced via physical engagement with the substrate that supports the food plants. Cutting-edge research by Lowry on the soil microorganism *Mycobacterium vaccae* has shown that when injected in mice, this non-pathogenic bacterium can activate 5-hydroxytryptamine (serotonin) in the prefrontal cortex, helping to regulate coping responses to "uncontrollable stress" (Lowry et al. 2007). The potential health benefits of *M. vaccae* do not end here; the inoculation of this microorganism has also been shown to protect against neuroinflammation and cognitive dysfunction, and to have immune boosting effects (Fonken et al. 2018; Zheng et al. 2017). This is just the story for a single species of bacterium that can influence cognition, behavior and immunity. This speaks volumes for the potential of the other estimated ~1×10^5 genera of bacteria and archaea on the planet, of which only ~11,000 species have been classified (Yarza et al. 2014). The possibilities here for MIGI are multitudinous—Are there certain natural habitats that can optimize interactions with health-inducing microbiota? Can we isolate different microbial species and manipulate communities to enhance these interactions? The research is in its infancy, but the potential is immense.

7.10. Microbiome-Inspired Green Infrastructure—Green Barriers

Natural green walls such as hedgerows with trees could also be designed as part of MIGI to reduce noise pollution, improve the multisensorial experience, and reduce pollution in green spaces by trapping particulate matter (Van Renterghem et al. 2013; Abhijith et al. 2017). However, these features would also need to be scrupulously curated to allow optimal wind dispersal to prevent localized concentration of pollution (Soyiri and Alcock, 2018). Additional research is needed to understand the impact of green walls on these mechanisms, but green barrier designs could potentially help shield humans and microbial communities in green spaces from industrial pollutants and contribute to the reduction in respiratory illnesses (Soyiri and Alcock, 2018). It is worth noting that despite the benefits, the potential of these features as allergen producers and disease vector habitats should also be considered as part of an assessment of suitability.



New urban green space paradigm:

- Microbiome-inspired green infrastructure (MIGI)
- Foraging friendly (ancestral microbiome diversity and nature connectedness)
- Rewilding and genetic selection
- Considers wider ecological systems
- Focus on multisensoriality - e.g. sight, sound, touch
- Improve connectedness and accessibility
- Green walls designed to inhibit noise and pollution
- Network considerations - city-wide microbial fragility
- Varied ancestral systems for optimal nature doses and multicultural inclusivity?

Figure 2. A vision for the future: microbiome-inspired green infrastructure (MIGI) and multisensorial, multiculturally inclusive, and foraging-friendly green spaces (created by the author).

7.11. Microbiome-Inspired Green Infrastructure—Cultural and Ancestral Microbiomes

Due to increasing cultural diversity of western towns and cities (Crul, 2016), it is essential to emphasize the importance of inclusivity in urban green space designs. It then follows that creating inclusive environments should also be a key consideration for MIGI. Further research into the potential inclusion of inter-ethnic ancestral environments in urban areas to optimize microbial interactions and immune regulation could be important for the development of effective MIGI. Evidence points to ancestry-associated differences in human immune responses, and populations vary in their susceptibility to diseases (Nédélec et al. 2016). Furthermore, human microbiome composition varies significantly across different ethnic groups (Kwok et al. 2014; Gomez et al. 2016), which has only been partially explained by factors such as sociodemographic dynamics and diet (Deschasaux et al. 2018; Gaulke and Sharpton, 2018).

From an immune development perspective, it is possible that specific inter-ethnic interactions with ancestral microbial communities in natural environments are important to the health of these populations. It is also possible that functional redundancy between microbiota and widespread human genetic admixture may neutralize the effectiveness of this theory; however, it has been pointed out that there is evidence to question the "universality of microbiome-based therapeutic strategies" based on ethnic and geographical variation. Ancestry aside, there are also important cultural and generational considerations for MIGI here. For example, in many countries, children are spending less time outdoors and interacting with nature (Soga et al. 2016). This is attributed to a range of factors such as changes in cultural practices and green space accessibility (Soga et al. 2016; Hand et al. 2017). MIGI could also be designed with cultural changes in mind; for example, establishing MIGI in areas where children do spend time, or integrating MIGI strategies with cultural trends. An example of the former could be the establishment of MIGI in and around skate parks, and an example of the latter could be to work with developers of location-based augmented reality games such as Pokémon GO to promote spending time in areas where MIGI has already been established or could be established in the future. After all, this kind of technology is unlikely to disappear, and whilst strong arguments can be made to proactively reduce ‘screen time’ and promote contact with nature, this technology–human–nature interface has also been suggested to provide new links between humans and green space and encouraging physical activity (Berg et al. 2017). More research into the potential salutogenic effects of ‘nature exposure’ whilst using this technology is essential.

The prospect of including different cultural and ethnic ancestral environments to promote health-inducing microbial interactions and multicultural inclusivity is a tantalizing one. However, a significant amount of additional research is needed to further understand the relationships between culture, ancestral environments, microbiota and inter-ethnic health. It will also be essential to consider the potential impacts of including novel environmental features in native ecosystems. A network of closed MIGI systems (i.e., cultural and ancestral biomes) could be an option in the future.

7.12. Microbiome-Inspired Green Infrastructure—Plant Microbiome Selection and Engineering

As with humans, plants and their microbiomes form a holobiont, and the interplay between the plant host and its co-evolving microbial assemblages has a substantial role in maintaining the health of these functional ecological units (Ma et al. 2018). Microbial diversity is a key driving factor in maintaining favorable plant health (Ma et al. 2014; Mallon et al. 2015). Indeed, individual plant genotypes can show distinct microbial diversity, which indicates that some plants have the ability to cultivate a beneficial microbiome and this process may be under natural selection (Urbina et al. 2018). Therefore, strategies to enhance microbial diversity to benefit human health also have the potential to generate important co-benefits for plants, with cascading benefits to the wider ecosystem. This further highlights the importance of the interconnectedness of life. Understanding how plant community composition, independent of diversity, affects the microbiome is also an important factor, particularly in 'designed' urban environments. For example, specific pairwise and synergistic interactions in plant communities can be selected to influence the soil microbiome structure and pathogen suppression (Latz et al. 2016). Plant microbiomes can also be genetically selected to enhance fitness (Mueller and Sachs, 2015); i.e., plant growth promotion, plant health and abiotic stress tolerance (Wallenstein, 2017; Hussain et al. 2018). Genotype-dependent associations between plants and the microbiome could be used to target and establish optimal MIGI dynamics, and careful selection processes may play important roles in the design, implementation and effectiveness of MIGI in the future.

7.13. Nature Connections and Green Prescriptions

Restoring biodiverse urban habitats and designing multifunctional green infrastructure to enhance microbial interactions has the potential to contribute towards planetary health in a number of ways. For example, mounting evidence supports a range of direct health benefits associated with spending time in nature, and there is the obvious enhancement of natural habitats and the contributions this makes towards biodiversity conservation, ecological stability and network connectivity. However, there is also potential to enhance pro-environmental behaviors and environmental stewardship, not only by providing opportunities to promote ecological education, but by providing additional opportunities to access and connect with natural environments. Indeed, it is difficult to overstate the importance of reinstating the intrinsic health-inducing connections between humans and nature; connections that are damaged when we fail to acknowledge the importance of reciprocity in the human–nature relationship (Van Heezik and Brymer, 2018).

From a psychological perspective, 'nature connectedness' is a multidimensional concept that involves one's *"affective, cognitive and physical relationship with the natural world"* (Capaldi et al. 2014). Nature connectedness is an important indicator of pro-environmental behaviors and is associated with psychological wellbeing (Mayer et al. 2009). Furthermore, engagement in nature-based activities can facilitate changes in nature connectedness, and positive impacts derived from exposure to nature could be mediated by an increase in nature connectedness resulting from this very exposure (Mayer et al. 2009; Lumber et al. 2017).

Nature-based health interventions, also known as 'green prescriptions', are an emerging integrative approach, aimed at addressing noncommunicable diseases and social isolation. Green prescriptions work on the premise that exposure to, and interactions with, natural environments bring variable degrees of health benefits, which can be prescribed and monitored over time (Bragg and Atkins, 2018; Van den Berg, 2017). As with microbiome rewilding and ecological restoration, green prescribing has the potential to deliver important co-benefits to humans and the environment. In fact, green prescribing activities can include biodiversity conservation, and as such, patients could potentially help to conserve and restore habitats and rewild the microbiome, whilst simultaneously benefiting from a variety of health-inducing interactions. More research is needed to understand what type of exposure to nature is optimal, and also how much, when and for whom (Lovell et al. 2018). However, for a near-future urban green paradigm that focuses on promoting ecological justice, multicultural inclusivity and widespread foraging-friendly green spaces, green prescribing can play a key role in bridging these objectives and sustaining lifestyles based on underlying holistic principles.

7.14.Conclusions

The human body is a holobiotic organism; a walking ecosystem that intertwines the macro and micro ecosystems in the Earth's biosphere. As Prescott of inVIVO Planetary Health has said "*natural laws of interdependence, mutualism, and interconnectivity underpin life in all forms*"—including the seen and unseen (Prescott and Logan, 2018).

Enhancing the diversity of both the macro and microbiological constituents of the natural world, whilst working towards a greater understanding of microbial functions and dynamics within our bodies and the wider environment, brings a considerable and mutually-advantageous asset to the planetary health paradigm. Furthermore, promoting the advantages of connecting with nature for physical and psychological health and wellbeing (mediated by strategies such as green prescribing, ecological restoration and rewilding, and innovative microbiome-inspired green infrastructure) can only improve this position. This can be enhanced further by a number of potential socioecological benefits such as environmental stewardship, social cohesion and multicultural inclusivity. We believe there are countless possibilities for microbiome-inspired green infrastructure, particular if researchers and those in practice work collaboratively across disciplines to progress this concept. There is an opportunity for a concerted effort to explore the potential of human–environmental microbial exchange and associated health benefits, whilst developing important co-beneficial strategies to maximize the impact of these on humans and the environment.

As our species, or our collective holobionts, moves forward in the Anthropocene, perhaps an epoch that we could aspire to is the 'Symbiocene'. This is a term first coined by Albrecht (2014), the Australian 'eco-philosopher' based on a need to take a more holistic, symbiotic and ecological approach to the way we live. The prospective strategies mentioned in our paper are aimed at addressing the global challenge of halting and reversing dysbiosis in all its manifestations. Collectively, it is hoped these strategies, along with taking an ecological view of the human body, will contribute towards improving personal and planetary health.

Microbiome-Inspired Green Infrastructure: A Toolkit for Multidisciplinary Landscape Design

Harry Watkins, Jake M. Robinson, Martin F. Breed, Brenda Parker, and Philip Weinstein

Keywords

microbiome-inspired green infrastructure; green infrastructure; urban design biodiversity; urban microbiome

7.15. Abstract

Incorporating recent advances in environmental microbiome research and policy is a major challenge for urban design. Here, we set out a framework for managing construction projects so that multidisciplinary teams of researchers and practitioners can explicitly consider environmental microbiota in design and construction contexts, thereby increasing ecosystem functionality and public health.

7.16. The Need to Reverse the Damaging Effects of Urbanisation on Environmental Microbiomes

Organisms across all trophic levels develop complex ecological relationships via their ever-present microbiota. Over evolutionary timescales, these relationships have become fundamental to the maintenance of physiologic processes (e.g., immune regulation or nutrient cycling) in multicellular organisms, such that they scale up to bring considerable health benefits to humans (Cavicchioli et al. 2019), support ecosystem functionality, and deliver regulating and supporting ecosystem services.

The integrity of the relationships between microbes and other organisms, and their associated benefits, are threatened by urbanisation (see Glossary) (Blaser et al. 2016). Furthermore, the loss of interactions between humans and the diversity of environmental microbiota is linked to a wide range of communicable and noncommunicable diseases (Flandroy et al. 2018), including cardiovascular and autoimmune diseases, arterial and non-alcoholic fatty liver diseases, and Alzheimer's disease. As a result, there are potential positive feedbacks between the biodiversity crisis, climate change, public health impacts, and rapid urbanisation (Dutta and Dutta, 2016). To reverse this trend, there is a pressing need for multidisciplinary collaborations between urban designers, public health experts, environmental microbiologists, and microbial ecologists: This will facilitate the development of new ways of enhancing human interactions with beneficial environmental microbiota in urban areas, where pathogenic environmental microbiota could be outcompeted and microbially mediated ecosystem services could be supported (Figure 1).

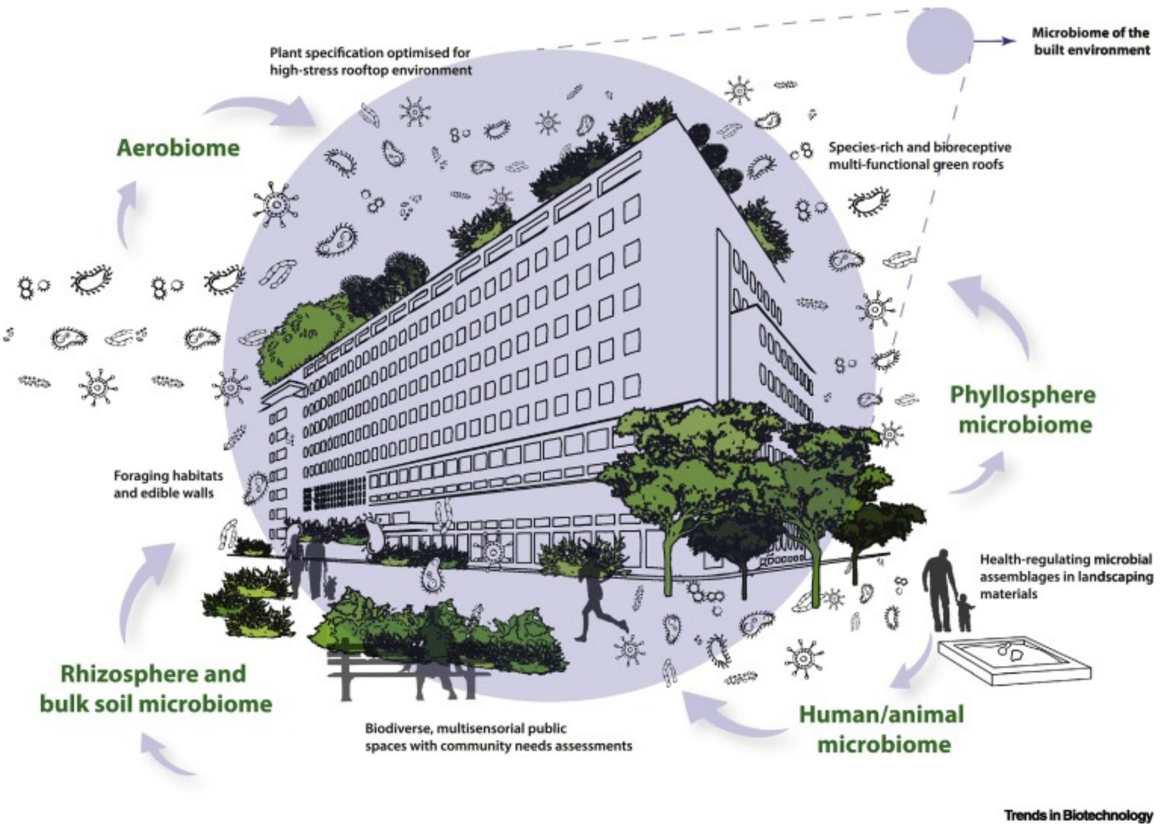


Figure 1. Microbiome-Inspired Green Infrastructure (MIGI). Multifunctional, bioreceptive green features aimed at promoting symbiotic microbial interactions with humans, with several important co-benefits.

7.17. Microbiome-Inspired Green Infrastructure: Increasing the Potential of Green Infrastructure

Microbiome-inspired green infrastructure (MIGI) has been proposed as a means to help mitigate the negative effects of industrial urbanisation on biodiversity and public health (Robinson et al. 2018). Whereas green infrastructure includes strategically designed networks of natural and seminatural features to deliver a wide range of ecosystem services (EEA, 2013), MIGI includes an explicit recognition of the role that microbiota have in urban ecosystem functionality, integrating strategies that optimise the design and management of these multifunctional green systems, and a focus on the sociobiological factors that affect exposure and interactions. Specifically, the MIGI concept is being developed with two primary aims: first, to achieve direct public health gains through improved immunoregulation, and second, to enhance microbially mediated ecosystem services and urban ecosystem functionality.

From an immunoregulation viewpoint, MIGI could take the form of habitat creation (e.g., by explicitly considering plant species and media selection with interspecies interactions in mind to influence the composition of microbiota) (Thompson and Kniffin, 2016) and the inoculation of landscape materials (Hui et al. 2019) with the aim of optimising human–microbial interactions. The opportunities to increase ecosystem functionality (including supporting and regulating ecosystem services) will vary by site, but examples of MIGI in this domain include regulating biochemical cycles (e.g., increasing diversity to optimise C storage and reduce N leaching) (Thompson and Kniffin, 2016) and plant and compost design to prevent N_2O losses from urban soils (Guo et al. 2019). To achieve these aims, environmental microbiologic research is needed to support MIGI through the identification of landscape materials which enhance specific beneficial assemblages (Hui et al. 2019); development of a library of new and existing bioreceptive materials to augment or suppress growth of target species or species assemblages; choice of soil media and strategies to manage soil biodiversity; selection of plants to optimise microbial community structure; and edible (e.g., edible green walls) versus nonedible exposure routes (for immunoregulation) via ecological design that is socially, ecologically, and aesthetically engaging.

However, while researchers and public health experts have attempted to define the benefits of integrating microbe-centric approaches for ecological functionality and public health, few policies have been adopted in design and construction practice (Lõhmus and Balbus, 2015). To overcome this, we identify the key barriers to implementing MIGI and then introduce a framework to demonstrate how environmental microbiologic research could support design and construction projects in the future.

7.18.Identifying the Barriers to Implementing MIGI

There are several conceptual, operational, and technical barriers to implementing MIGI:

(i)

The complexities of characterising microbial assemblages, what functional/ecological roles particular microbiota are playing, and how efficiently they are fulfilling these roles.

(ii)

The lack of an established evidence base for MIGI interventions. Research is needed to monitor the efficacy of microbiota establishment (optimisation), the impacts of pollution and land use on microbial dynamics, and the role of other ecological factors (e.g., competition and biosecurity issues).

(iii)

The cost of developing targeted strategies: Sequencing and bioreactor facilities are expensive, as is research to investigate less technology-centric methods (e.g., planting design and management).

(iv)

Environmental microbiology has not been well integrated into construction industry project frameworks. The lack of access to refined tools for support, implementation, and management of MIGI objectives makes it difficult for clients, designers, and contractors to understand how or why they should try to design MIGI into projects.

(v)

Many elements of MIGI cross project work-stage boundaries or require actions at stages when design team members are not typically engaged. Whereas MIGI embraces systems-based thinking, development projects tend to have goal-orientated processes for delivering discrete objectives. In this environment, benefits that are not perceived to directly result in goal delivery are not prioritised.

7.19. Integrating MIGI Design Principles into Established Construction Workflows

While researchers are actively trying to address the first three barriers, it will be a pyrrhic success to overcome them if the final two barriers remain. As such, it is essential that environmental microbiology researchers understand the context within which their research is applied. In the construction industry, multidisciplinary teams structure projects across several stages, including community consultation, landscape assessment, concept design, detailed design, and contract administration. Due to the complexity of these projects, developers use standardised contracts and frameworks to coordinate objectives with other stakeholders. The Plan of Work published by the Royal Institute of British Architects (RIBA) is one such framework, with clear roles for multiple professional services, providing a ‘common language' for the design and development industries (RIBA, 2020). The RIBA Plan of Work has eight key stages, developing incrementally in scale, complexity, and detail and enabling design teams to work across spatial and temporal scales. The aim of the Plan of Work is to ensure quality and cost efficiency of built work with considerations for behavioural choices and long-term benefits. As the Plan of Work is necessarily interdisciplinary, overlays are sometimes produced to show how specific considerations might be addressed at given project stages, such as building information modelling, biosecurity (Landscape Institute, 2019), or low-carbon building.

The framework for an overlay in Table 1 presents a structure with opportunities for environmental microbiologists to collaborate with landscape architects and other relevant disciplines, notwithstanding the risks and actions associated with each stage of work. Given that successful MIGI requires strategic thinking at the planning stages, this overlay will be essential so that applied biologists are able to strategically introduce their research at the appropriate stage within a construction project and maximise the impact of their proposals. As MIGI aims and objectives develop further and design teams become familiar with them, the research and publication of a MIGI design and intervention guide should be developed (Flies et al. 2018). This should include protocols to deliver MIGI features more effectively and contribute to the design of an optimisation model that realises co-benefits (Pierre et al. 2016; Mills et al. 2019) and integrates ecosystem service considerations.

Table 1. Framework for a Royal Institute of British Architects Plan of Work Overlay, Showing How to Optimise the Timing of Interventions in a Construction Project by Applied Biologists

RIBA work stage	Landscape architect's core tasks	Core tasks for microbiologists and other specialist scientific advisers
Stage 0 Strategic definition	• Horizon scanning • Engage public health experts, environmental microbiologists, and microbial ecologists in design team	• Develop MIGI aims and objectives by identification of priorities for human health and ecosystem services • Prepare an ethics statement to ensure that MIGI prioritises socioecological inclusivity
Stage 1 Preparation and brief	• Landscape assessment • Stakeholder consultation • Agree procurement route • Overcome commercial pressures and value engineering by consulting	• Define brief for biodiversity, bioreceptivity, and interaction • Ecological assessment

	nurseries and materials suppliers at early design stages; consider practices such as contract growing to ensure high biosecurity standards and accurate supply of materials	• Investigate effects of different green infrastructure network configurations and landscape connectivity on environmental microbiota • Identify potential landscape-scale impacts of plant health issues • Identify opportunities to deliver supporting and regulating ecosystem services, including nutrient cycling, soil formation, and primary production • Assess effects of wind, pollution, and land use at various scales on microbial diversity

Stage 2 Concept design	• Strategic landscape planning • Site modelling • Supply chain preparation	• Advise designers on plant selection and growth substrates to manage soil biodiversity and allelopathic factors • Consult with civil engineer to identify opportunities for managing nitrogen cycling in soil water systems • Risk assessment to identify any potentially harmful aspects of MIGI, including carbon sequestration and nitrogen accumulation • Establish MIGI in places where children spend time, such as play areas and skate parks, and integrate MIGI strategies with

		cultural trends • Identify which cultural practices (such as foraging and recreational activities) could maximise cobenefits • Consider microbiome inoculants in landscape materials, depending on results of ecological assessments
Stage 3 Developed design	• Resolve layout design of MIGI features • Consult microbial ecologists to select plant species and design soil structures • Carry out detailed specification of plants • Engage nurseries to begin contract growing	• Consider impacts of aspect, hydrology, and cultural uses on microbial habitats • Maximise macro-biodiversity, such as by using structurally diverse urban meadows instead of amenity grasslands • Evaluate project

		development against aims and objectives • Anticipate future management regimes and create potential for microbiome rewilding
Stage 4 Technical design	• Complete landscape specification • Prepare landscape management plan	• Create biosecurity plan for construction phase • Prepare plan for Stage 7 microbiome monitoring
Stage 5 Construction	• Evaluate contractor's sustainability and biosecurity credentials • Weigh value engineering recommendations against whole-life costs	• Ensure that contractors understand MIGI objectives • Monitor works at critical stages, such as nursery inspection, sourcing of growth media, and inoculation (if a bioaugmentation strategy is used)

Stage 6 Handover and close out	• Record ‘as built’ information to allow future evaluation	• Conduct snagging survey to ensure MIGI features are correctly installed
Stage 7 In use and evaluation	• Record species establishment and sociocultural uses of MIGI features	• Ensure that spirit of MIGI aims is not lost by providing training to management team • Update MIGI management plan as needed • Biogeochemical monitoring of interactome and ecosystem services

7.20. Concluding Remarks

Successful communication between the disciplines of design and microbiology is essential to deliver the benefits associated with MIGI. We suggest that an overlay to the existing industry standards for green infrastructure design will be needed in order to create a means for non-scientists to embrace the importance of environmental microbiota for public health and urban ecosystem functionality. A MIGI design and intervention guide will be foundational and should be created to establish principles and standards for multidisciplinary design, material specifications, and procurement strategies. However, with further research, collaboration, and development, these new integrated approaches could help to deliver the needs of a modern urban environment, based on fundamental considerations for the life-sustaining microbial constituents of the natural world.

CHAPTER 8

THE ENVIRONMENT-MICROBIOME-HEALTH AXIS: TRANSCENDING CURRENT BOUNDARIES OF KNOWLEDGE WITH THE HOLOBIONT CONCEPT

“It is the microbes who will have the last word”

– Pasteur

8. PUBLICATIONS IN CHAPTER 8:

Robinson, J.M., and Breed, M.F. (2020). The *Lovebug Effect*: Is the Human Biophilic Drive Influenced by Interactions Between the Host, The Environment, and the Microbiome? *Sci Tot Environ.* 720, p.137626.

Robinson, J.M., and Cameron, R. (2020). The *Holobiont Blindspot*: Relating Host-Microbiome Interactions to Cognitive Biases and the Concept of the “Umwelt”. *Front Psychol. 11,* p.591071.

The Lovebug Effect: Is the Human Biophilic Drive Influenced by Interactions between the Host, the Environment, and the Microbiome?

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Keywords: Lovebug Effect; Microbiome; Evolution; Holobiont; Nature Connectedness; Hologenome

8.1. Abstract

Psychological frameworks are often used to investigate the mechanisms involved with our affinity towards, and connection with nature, such as the Biophilia Hypothesis and Nature Connectedness. Recent revelations from microbiome science suggest that animal behaviour can be strongly influenced by the host's microbiome, for example, via the bidirectional communication properties of the gut-brain axis. Here, we build on this theory to hypothesise that a microbially-influenced mechanism could also contribute to the human biophilic drive – the tendency for humans to affiliate and connect with nature. Humans may be at an evolutionary advantage through health-regulating exchange of environmental microbiota, which in turn could influence our nature affinity. We present a conceptual model for microbially-influenced nature affinity, calling it the *Lovebug Effect*. This translates to ‘microbio-philia’, from ‘bug’ – a colloquial term for microorganism and ‘philia’– a Greek word for ‘love’ or ‘attraction’. We present an overview of the potential mechanistic pathways involved in the *Lovebug Effect*, and consider its dependence on the hologenome concept of evolution, direct behavioural manipulation, and host-microbiota associated phenotypes independent of these concepts. We also discuss its implications for human health and ecological resilience. Finally, we highlight several possible approaches to scrutinise the hypothesis. The *Lovebug Effect* could have important implications for our understanding of exposure to natural environments for health and wellbeing, and could contribute to an ecologically resilient future.

8.2. Introduction

8.2.1. Biophilia and nature connectedness

The Biophilia Hypothesis (Wilson 1984) proposes that humans have an innate tendency to affiliate with the natural world, and this is suggested to be mediated by a number of evolved survival-based biopsychological responses to environmental stimuli (Kellert, 2016). Indirect support for this hypothesis arrives from research demonstrating links between ‘exposure’ to environmental features (e.g. urban parks, waterbodies, and woodlands) and enhanced physical health and psychological wellbeing (Li et al. 2009; Carrus et al. 2015; Gascon et al. 2017; Berto et al. 2018; Lyu et al. 2019).

Further support for biophilia comes from research into evolutionary predispositions that manifest as ‘phobic’ responses to biotic stimuli. These particular stimuli are considered to be threatening to human survival, such as an aversion to aposematic signals including triangular shapes or body forms associated with predators (Gullone, 2000; Souchet and Aubret, 2016; Prokop, Fančovičová and Kučerová, 2018). The fear responses (referred to as ‘*biophobia*’) are modulated in part by the autonomic nervous system (e.g. the sympathetic ‘fight or flight’ response) and are thought to have evolved in a world where humans were at a heightened threat of predation and/or poisoning by phyto–or–zootoxins. Although biophobic responses are converse to their biophilic counterparts, they represent the same overarching evolutionary framework (Figure 1).

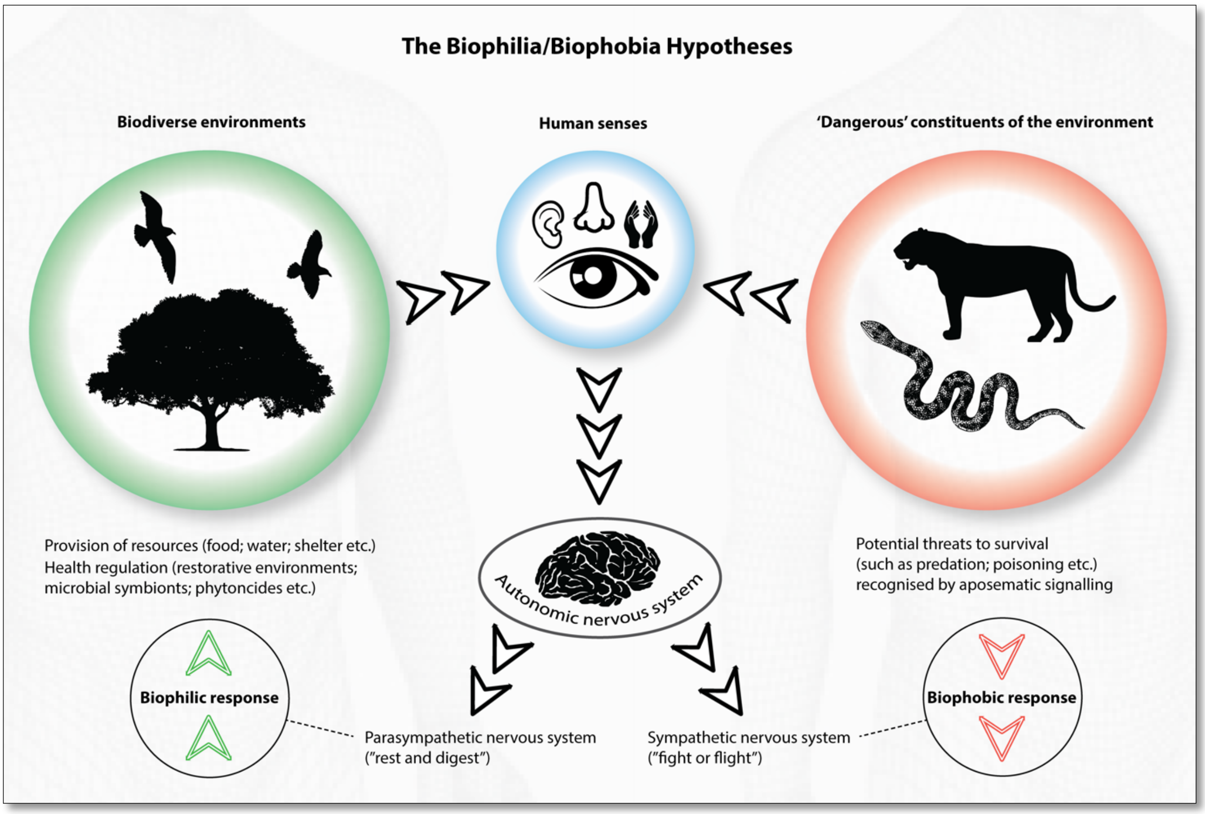

Figure 1. Human Biophilia and Biophobia Hypotheses – showing human behavioural responses to different environmental stimuli, modulated in part by the autonomic nervous system. In general, nature provides health-regulating opportunities and resources for survival. However, some natural features also pose a danger to humans and elicit biophobic responses.

Lumber, Richardson and Sheffield (2017) investigated the mechanisms by which humans connect with nature emotionally — that is, 'nature connectedness' — using the Biophilia Hypothesis as a conceptual framework. The authors point out that nature connectedness and Biophilia are distinct constructs, whereby:

- Biophilia is primarily based on increasing survival opportunities (e.g. via health promoting interactions; resource provision etc.); and,

- Nature connectedness is a recognition that humanity is deeply embedded within nature itself.

The authors noted that nature connectedness is also an "*act of self-realisation of the similarity between other aspects of nature and the individual*" (Schultz et al., 2004 in Lumber, Richardson and Sheffield, 2018, p.15).

Psychological frameworks have been developed to systematically examine how our innate tendencies to affiliate with the natural world are expressed, for example, via the nine values of Biophilia (Box 1), which range from Ecological-Scientific values (e.g. an attraction to learn about nature to meet life's physical and mental requirements, pertinent to evolutionary fitness) to Aesthetic values (e.g. seeking beauty in nature to provide sensory pleasure and the associated wellbeing benefits) (Delavari-Edalat and Abdi, 2010). Furthermore, seven conceptual themes have been identified with significant implications for the "formation and maintenance" of the connection that humans have with the rest of the natural world (Lumber, Richardson and Sheffield, 2018 p.2). Examples of these themes include "investigating nature through scientific enquiry", "noting nature through artistry", and "engaging with wild nature" (Lumber, Richardson and Sheffield, 2018 p.2).

Box 1. The nine values of Biophilia (from Lumber, Richardson and Sheffield, 2017).

1. Utilitarian – Practical use of material nature
2. Naturalistic – Pleasure from contact with nature
3. Ecological-Scientific – Scientific study of the interconnectedness of nature and natural systems
4. Aesthetic – Appeal of nature's physical beauty
5. Symbiotic – Expressing ideas through nature based language and metaphors
6. Humanistic – Emotional bond with, and love for nature
7. Moralistic – Ethical concern/judgements and revering nature
8. Dominionistic – Control and dominance of nature
9. Negativistic – Aversion, removal and fear of nature

However, despite considerable attention being given to the mechanistic pathways involved in biophilia and nature connectedness, some of the potential biological mechanisms that lead to our biophilic drive remain elusive. Furthermore, associations between the microbiome and human biophilic drive have not, to our knowledge, been explored (Figure 1).

8.2.2. Microbially-influenced nature affinity

Here we hypothesise that a microbially-influenced mechanism contributes towards the tendency for humans to affiliate with natural environments (Figure 2; hypothesis detailed below). Our hypothesis partially stems from microbiome research which suggests that microbial interactions via the gut-brain axis and other pathways (e.g. via olfactory dynamics) can have a significant influence on host behaviour (Heijtz et al. 2011; Leitão-Gonçalves et al. 2017; Farzi et al. 2018; Huang et al. 2019). Furthermore, we detail how humans are host to a diversity of symbiotic microorganisms – collectively termed a holobiont or metaorganism – which could potentially form units of selection via effects on host phenotypes. Microbial interactions may influence our affinity towards and connection with nature, thus enhancing our evolutionary fitness through health-regulating microbial exchange. It is important to note that the mechanisms set out in this paper are not intended to replace current perspectives on biophilic tendencies. This is a multidimensional proposition, adopting a predominantly biological framework whilst recognising exogenous social and environmental influences, and is aimed at extending the portfolio of potential pathways to nature affinity.

Building on this newly proposed mechanism to nature affinity, we also hypothesise that an additional pathway to nature connectedness exists – one also mediated by microbial symbionts. As Lumber, Richardson and Sheffield (2018, p.2) state: "*Nature connectedness may result from specific interactions with nature*" via their proposed seven themes. We suggest that a microbially-influenced affinity for natural environments could also form one of the converging pathways to explain nature connectedness. We refer to this collective microbially-influenced mechanism as the *Lovebug Effect* (Figure 2)*.* This translates to 'microbio-philia', from 'philia'– a Greek word for '*love'* or 'attraction' and '*bug*' as a colloquial term for microorganism. This hypothesis builds on the ecological approach to describe humans as dynamic ecosystems, openly interacting with the wider environment (Robinson, Mills, and Breed, 2018; Mills et al. 2019).

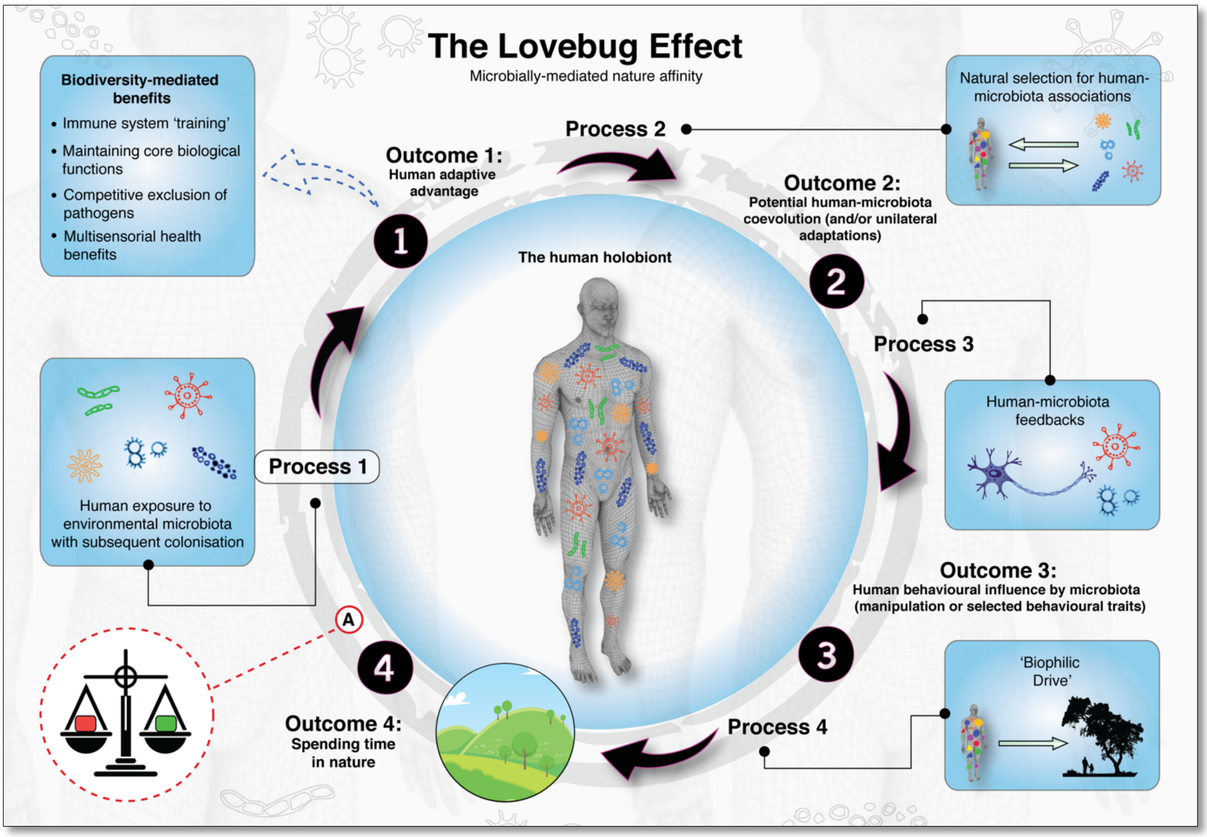

Figure 2. The *Lovebug Effect* - microbially-mediated nature affinity. This hypothesis proposes that our biophilic drive towards natural environments could be influenced by coevolution, biodiversity-mediated benefits and potentially unilateral adaptations. Arrows relate to processes and numbers in circles relate to outcomes. In the absence of anthropogenic impacts, the *Lovebug Effect* continues while subject to a stable pressure-benefits counterbalance. 'A' represents anthropogenic pressures, further defined in Figure 4.

We present a conceptual overview, predominantly of the biological and evolutionary pathways, that could potentially mediate behaviours associated with microbially-influenced nature-affinity. We discuss this concept in relation to broader socioecological implications using two interconnected examples, namely, public health and ecological resilience. We conclude by setting out a number of possible experimental approaches that could be taken to start testing the *Lovebug Effect* hypotheses.

8.3. A mechanistic overview of potential host-symbiont behavioural manipulation and holobiont adaptation

The microbiome — that is, the consortium of microorganisms and their genetic material in a given environment — and in particular, the microbiome of the gastrointestinal (GI) tract, can have a considerable influence on host behaviour, mood, and neurological conditions such as depression (Heijtz et al. 2011; Farzi et al. 2018; Huang et al. 2019). Several mechanisms have been proposed as potential mediators of this process, including the presence of a bi-directional communication system, modulated by the vagus nerve. The vagus nerve is an extensive cranial nerve that links the brain stem to several peripheral organs across the body, and importantly for the current topic, to the GI tract (Ueno and Nakazato, 2016; Breit et al. 2018).

The microbiome of the GI tract has been suggested to 'hijack' this communication infrastructure to relay information to the brain, and thus influence host behaviour (Forsythe, Bienstock and Kunze, 2014; Davidson et al. 2018). Although the mechanisms are not yet fully understood, it is now thought that an array of metabolites produced by microbiota within the gut can initiate the release of peptides and hormones via enteroendocrine cell activation and/or stimulate the vagal afferent fibres that form one of the gut-brain signalling pathways (Lach et al. 2018; Fülling, Dinan and Cryan, 2019). Microbiota within the gut can also produce neurotransmitters such as serotonin (as well as dopamine, noradrenaline and gamma-aminobutyric acid or ‘GABA’), which can directly activate the vagus afferents that connect the gut to the brain (Strandwitz, 2018; Fülling, Dinan and Cryan, 2019).

There are other proposed pathways involved in microbially-influenced host behavioural responses, such as through the synthesis of neuroactive molecules that affect the central nervous system (CNS). These microbially-synthesised molecules include 5-hydroxytryptamine (5-HT), catecholamines, and acetylcholine, and can be transported in the systemic circulatory system to penetrate the blood-brain barrier (Petra et al. 2015). Furthermore, some bacteria are known to release factors that alter peripheral immune cells to stimulate interaction with the blood-brain barrier (Logsdon et al. 2018).

Pasquaretta et al. (2018) suggested that a microbially-mediated pathway to decision-making may also exist, involving active manipulation of host behaviour to select particular food items that favour the nutrient requirements of their microbial symbionts. This is supported by research involving the model fruit fly *Drosophila melanogaster*, which showed that commensal bacteria, and specifically *Acetobacter pomorum* and *Lactobacillus sp.,* work synergistically to become ‘potent modulators of feeding decisions’ – a process that is influenced by the availability of dietary amino acids (Leitão-Gonçalves et al. 2017). Furthermore, Yuval (2017) pointed out that in the invertebrate holobiont, microbial symbionts are known to influence breeding and ultimately speciation.

It has also been suggested that host sociability could be influenced by microbiota, that is, by mediating host behavioural responses to increase inter-host transmission of microbiota, thus increasing dispersal and evolutionary fitness as a consequence (Stilling et al. 2014; Wong et al. 2015). Interestingly, several animal studies support the idea that microbially-influenced behavioural change may be partially governed by olfactory system interactions. For example, both adults and larvae of *D. melanogaster* have been shown to be attracted to volatile compounds of *Saccharomyces cerevisiae* and *Lactobacillus plantarum* but repelled by *Acetobacter malorum* (Qiao et al. 2019). Casadei et al. (2019) showed that microbiota trigger widespread transcriptional responses in the olfactory organs of zebrafish and mice. Studies also suggest that microbiota may influence the structure of the olfactory epithelium, and as Karsas, Lamb and Green (2019) pointed out, human twin studies indicate that the genotype of an olfactory gene (OR6A2) could be related to microbiota (Goodrich et al. 2016; Bienenstock, Kunze, and Forsythe, 2017).

8.4. The extended phenotype

The idea of behavioural manipulation at the metaphorical hand of a mutualistic, commensal or parasitic organism, is by no means a novel concept. Indeed, the central theorem of the extended phenotype (Dawkins, 1989) suggests that the continuity of genes that influence host behaviour tend to be maximised as a result of the behaviour itself, regardless of whether the genes are of host origin (or of the residing symbiont).

Take the classic example of host behavioural manipulation by the protozoan *Toxoplasma gondii*. This organism is a microscopic eukaryote (an obligate intracellular parasite), that, based on current knowledge, can only undergo gametogenesis in the intestines of species in the Felidae family, the definitive hosts (Poirotte et al. 2016). However, *T. gondii* oocysts (zygote-containing sacs) are shed in the felid's faeces where they subsequently sporulate to become infective (Zulpo et al. 2018). Environmental materials contaminated with the infective oocysts are consumed by intermediate hosts, typically rodents and birds (Krücken et al. 2017; Amouei et al. 2018). These intermediate hosts are characteristic prey items of cats, and the maintenance of this virtuous loop is essential for the protozoan's continuity, that is, *T. gondii's* survival is highly dependent on the cat becoming infected by feeding on infected prey (Vyas, 2015). It is this survival pressure that is suggested to have resulted in *T. gondii* evolving mechanisms to acutely manipulate the behaviour of the intermediate host (e.g. rodents). Such behavioural transpositions manifest as reduced innate aversion to the definitive host (the cat), and potentially even a 'fatal attraction' towards the definitive host, thus enhancing the transmission of parasite genes into future generations (Vyas, 2015; Hughes and Libersat, 2019). Although there are still several intermediary manipulation factors to uncover, it is thought that *T.gondii* infection in the intermediate host initiates testosterone production to cause hypomethylation of the medial amygdala, which then leads to loss of innate aversion to their predatory counterparts (Vyas, 2015; Tan and Vyas, 2016; Herbison, Lagrue and Poulin, 2018).

It is important to note that we use the *T. gondii* example to further highlight that a mechanistic pathway for microbially-influenced behavioural manipulation is possible. There are other examples of host manipulation involving viruses (e.g. family

Baculoviridae) and helminths (Hamblin and Tanaka, 2013; Poulin and Maure, 2015). However, we also acknowledge that these examples lack evidence to show that the specific interactions benefit the host in such a way that host behaviour is selected for (although in the *T. gondii* example, the feline is likely to benefit from catching the rodent prey more efficiently). Therefore, more research is needed to identify whether co-evolutionary relationships that benefit the host and their microorganisms exist.

In a recent randomized controlled study, Liddicoat et al. (2019) identified that a soil-derived anaerobic spore-forming butyrate-producer (*Kineothrix alysoides*) was supplemented to a greater extent in the gut microbiomes of mice exposed to trace-levels of higher biodiversity aerobiome treatment (Figure 3). The relative abundance of *K. alysoides* in the gut of these mice was associated with reduced anxiety-like behaviours. These results are relevant to the *Lovebug Effect*, where the authors suggest that their findings point to an intriguing hypothesis that biodiverse soils may supply butyrate-producing microorganisms to the mammalian gut microbiome with potential implications for behavioural regulation.

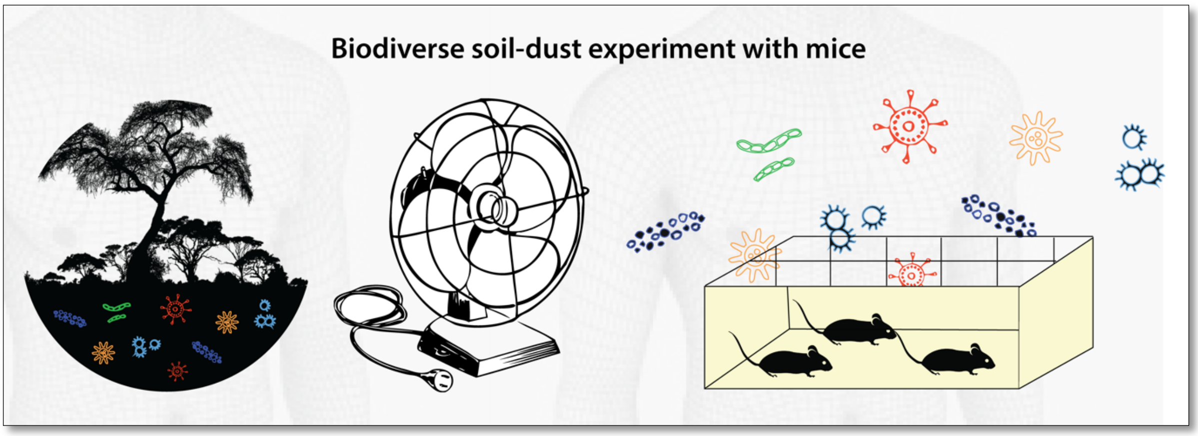

Figure 3. Butyrate-producing *Kineothrix alysoides* was supplemented to a greater extent (with potential anxiolytic effects) in the gut microbiomes of mice that received a higher biodiversity treatment via trace-levels of soil dust exposure in controlled conditions (Liddicoat et al. 2019).

8.5. (Co)evolution and the hologenome concept of evolution

Direct host-manipulation is one potential, albeit controversial, mechanism for microbially-mediated behavioural change. However, Johnson and Foster (2018) suggested that behavioural effects may arise more often as a result of selection on the microorganisms to proliferate in the host, and on the host to depend on their microbial symbionts. The authors suggested that microbial symbionts may preferentially benefit from local manipulation (i.e. changes to the immediate environment) rather than global manipulation (i.e. direct neurological manipulation). This is due to the higher energy investment required to set the neurochemically-intensive global manipulation process in motion, which would potentially leave these organisms vulnerable to competitive exclusion by other species with lower levels of investment. However, this local manipulation by the agency of microbiota could still have considerable downstream effects on host behaviour via the central nervous system.

Human physiology may have adapted to utilise microbiota, thus detecting and responding to certain strains and species assemblages (Johnson and Foster, 2018). Conceptually, this idea has parallels with the Old Friends Hypothesis, which posits that humans are dependent on a diversity of microbiota for immune system ‘training’, development and function (Rook et al., 2014) – factors which may affect brain function, and thus, behaviour (Rook and Lowry, 2008). Indeed, humans may have evolved a dependency on microbiota for ‘normal’ brain function, such that disturbance to the gut microbiome could impact human behaviour. Johnson and Foster (2018) suggested that evolved dependencies could be a simple indirect driver of microbially-influenced behaviour change. Disrupting this relationship through the loss of microbial species or change to microbial communities in the host may translate to cognitive perturbation. Furthermore, the apparent existence of functional redundancy in the gut microbiome (i.e. phylogenetically differentiated microbiota that share similar functional roles and may modulate host dependence) (Louca et al. 2018) may mean that the loss of, or impairment to, important functional *traits* resulting from functionally-important core microbial assemblages (as opposed to specific microbial *species*) may also be important drivers of impairment in host behaviour (Johnson and Foster, 2018).

This coevolution narrative could be explicitly linked to the hologenome concept of evolution (Rosenberg and Zilber-Rosenberg, 2016). Although some aspects of this concept are controversial, it is suggested that the holobiont could operate as a functional system, interacting with the environment as a unique biological entity through its collective traits (Roughgarden et al. 2018). Furthermore, it has been argued that the genome of the microbiome can be altered rapidly via environmental microbial exchange, horizontal gene transfer and DNA mutations (Rosenberg and Zilber-Rosenburg, 2018), leading to changes in the holobiont that could potentially be reproduced in future generations (Roughgarden et al. 2018; Collens, Kelley, and Katz, 2019). Selection at the level of the holobiont may be physiological and developmental (Roughgarden et al. 2018), and thus microbially-influenced regulation and development of behaviour could also be viewed from this multidimensional perspective.

A key criticism of the hologenome concept of evolution is the apparent lack of evidence to support vertical transmission of the gut microbiome. Indeed, with the exception of births delivered through caesarean section, it is thought that the main initial colonization of microbiota in humans arrives through contact with the mother's vaginal microbiome (Houghteling, Pearl, and Walker, 2015; Dreyer and Leibl, 2018). As such, it would seem that multiple temporally-distinct microbiomes coevolving with the host to produce a given behaviour would be required for the transmission of microbially-mediated traits. However, Rosenberg and Zilber-Rosenberg (2019) suggested that there is some evidence to support vertical transmission. For example, supporting studies provided by the authors demonstrate that individuals can maintain the same *Helicobacter pylori* strains as their ancestors, even when they have migrated to different geographical locations (Achtman et al. 1999; Falush et al. 2003), and subsequent supporting studies were also provided (e.g. Ochman et al. 2010; Goodrich et al. 2016; Moeller et al. 2016). However, the authors do indicate that more robust quantitative data are still needed.

Collens, Kelley, and Katz (2019) argued that the hologenome concept of evolution could be an epigenetic phenomenon due to the influence that symbionts can exert on gene expression and patterns of inheritance in host genomes. The authors suggested that the influence of the symbiont on the host genome is outside the Mendelian view of gene transmission and that hologenome interactions can lead to changes in host gene expression without host DNA sequence modification. Examples to support this view are reported for humans, where the gut microbiome can influence epigenetic patterns via the modulation of DNA methylation (Cureau et al. 2016). Furthermore, evidence also supports reciprocal miRNA-mediated epigenetic interactions between the host and the microbiome. This mechanism is supported by studies that report on the interactions between host miRNA secretion and bacterial gene expression in mice (Williams et al. 2017).

It is also worth considering the effect of non-microbially mediated host physical and mental health factors as additional ecological pressures that may influence the functional and compositional dynamics of the microbiome (Alverdy et al. 2017; Karl et al. 2018). Any changes to the host microbiome resulting from health-related impacts could have cascading effects on host–microbiome behaviour. As such, there may be additional complex feedback systems to consider.

8.6. The Lovebug Effect: other potential evolutionary pathways and the natural environment as a restorative domain

Hitherto, we have discussed some of the mechanistic pathways, and hologenome-centric and coevolutionary frameworks, that could potentially be involved in host behavioural adaptation and manipulation by the agency of microbial symbionts (see Process and Outcome 3, Figure 2). However, there are other microbially-influenced processes that could contribute to nature affinity without the need to meet the criteria of vertical transmission of symbiont genomes and direct manipulation.

Indeed, to explain the *Lovebug Effect*, it is important to discuss the fundamental ecological factors associated with microbially-mediated nature affinity in humans. As mentioned above, the portfolio of pathways that influence a human's desire to affiliate with nature must be recognised – some of which include complex psychosociocultural factors. However, from a microbially-mediated perspective, we propose that a biophilic drive towards natural environments (Process 4 in Figure 2) is not only influenced by (co)evolutionary processes between the host and symbionts, but also by interactions with biodiversity that could influence heritable human phenotypes. For example, via regulatory mechanisms that improve human health and do not require vertical transmission of microbial genomes or direct manipulation (Outcome 1 and Process 2 in Figure 2). Such microbially-influenced pathways are also relevant to the biophilia and nature connectedness conceptual frameworks.

For example, life-course exposures that could potentially disrupt the human holobiont ecosystem if left unchecked could include factors that influence immune dysfunction and homeostatic imbalance, human-specialised pathogens, and other health-related disorders (both physical and mental phenomena). These 'normal' pressures could be counterbalanced, in part, by interactions between the host and the wider biotic community – i.e. natural environments (as conceptualised in Figure 2). These environments are potentially rich reservoirs of macro and microbial diversity and other biogenic compounds, such as phytoncides, which are linked to human health (Li et al. 2009; Moore, 2015). Exposure to a diversity of environmental microbiota is critical for immune system ‘training’ to protect against known and novel infectious agents, and to potentially remove pathogens through competitive exclusion whilst maintaining core biological functions (Rook et al. 2014; Mills et al. 2019). As such, these interactions form part of an important survival mechanism, and one that relates strongly to the Biophilia Hypothesis. It is also plausible that these complex interactions contribute to a person’s nature connectedness, that is, the individual’s sense of their relationship with nature (McMahan et al. 2018; Richardson et al. 2018). This could transpire indirectly through the immersive psychological effects and multisensorial experiences of being in nature, experiences that could potentially be influenced by a microbially-mediated biophilic drive – i.e. the process we term the *Lovebug Effect*.

Alternatively, aspects of nature connectedness could be influenced by the transfer of microbiota from the environment to the human body, which in theory, could influence regulatory pathways in both cognitive and affective domains.

It is also important to mention that stress could have a negative impact on the composition and metabolic activity of gut microbiota (Dantzer et al. 2018; Karl et al. 2018). Indeed, several studies have elucidated the negative impacts of host-related stressors on microbiota. For example, stress-induced reductions of the non-spore forming *Lactobacilli* has been highlighted in humans and non-human primates (Bailey and Coe, 1999; Knowles et al. 2007). To this end, there could be potential fitness costs to certain individuals and/or communities of microbiota in the gut and other body sites. It is essential to acknowledge here that gut microbiota have emerged as important mediators of stress responses in humans (Dinan and Cryan, 2012; Foster, Rinaman and Cryan, 2017; Hantsoo et al. 2019). Moreover, stress could have negative (and positive) consequences for reproductive fitness and success in humans and other animals through a range of primary and secondary pathways (e.g. downstream lifestyle choices) that could, for example, elicit immune-endocrine disequilibria (Nakamura, Sheps and Arck, 2008; Mumby et al. 2015; MacLeod et al. 2018; Roychoudhury et al. 2019; Zhou, Cai and Dong, 2019).

Consequently, we argue that spending time in stress-ameliorating environments — for example, in calming natural surroundings that facilitate psychological restoration or eudemonia — could potentially confer positive indirect effects on the human microbiome. To this end, natural environments may provide additional salutogenic stimuli that drive the adaptive evolution of behaviours that benefit the host and its microbial symbionts via stress reduction pathways.

8.7. Discussion

8.7.1. The Lovebug Effect: 'big picture' implications and interventions

Unravelling the mechanisms of the *Lovebug Effect* could have far-reaching implications for researchers, practitioners, the general public, and from a biocentric perspective, the wider environment. This is relevant to nature-based health interventions and nature-based solutions, whereby the management of public health and ecosystems are often considered concurrently, giving rise to important co-benefits (Robinson and Breed, 2019). Augmenting our understanding of the factors that shape the human tendency to affiliate with nature could also help to strengthen our appreciation for planetary health, a relatively recent philosophical framework that describes the inextricable and multiscale links between human and environmental health (Prescott and Logan, 2017; Gabrysch, 2018; Prescott and Logan, 2019).

Mental health conditions such as depression and anxiety, and noncommunicable diseases (NCDs) such as asthma, diabetes, and inflammatory bowel disease are on the rise, which coincides with a global megatrend in biodiversity loss (Haahtela et al, 2013; Haahtela, 2019). It is thought that the key factors driving these megatrends include industrialisation, population growth and the ongoing increase in urbanisation (Pathway A in Figure 4) (Rodriguez et al. 2011; von Hertzen et al. 2011; Rook, 2014; Sartorius et al. 2015; Den Braver et al. 2018). These additional anthropogenic pressures could perturb the cycle of the *Lovebug Effect* by exacerbating 'normal' ecological pressures, and thus contribute to dysbiotic drift, that is, a non-random, industrial urban lifestyle-driven, push towards 'life in distress', microbial imbalance, and socioeconomic disadvantage (Prescott et al. 2018). Furthermore, a ratcheting down effect or the 'extinction of nature experience' (Soga and Gaston, 2016; Lin et al. 2018), along with reduced availability of, and access to biodiverse environments could theoretically compound this effect. This in turn could lead to a degeneration of the *Lovebug Effect*.

As the *Lovebug Effect* could be a potent mechanistic pathway to the survival benefits associated with the Biophilia Hypothesis and the psychological wellbeing benefits of nature connectedness (and associated pro-environmental behaviours), the implications of its degeneration for public health and ecological resilience could be considerable. Nevertheless, there is a range of anthropogenic interventions that could be implemented to help alleviate these pressures, thus allowing the *Lovebug Effect* to be restored (Pathway B in Figure 4).

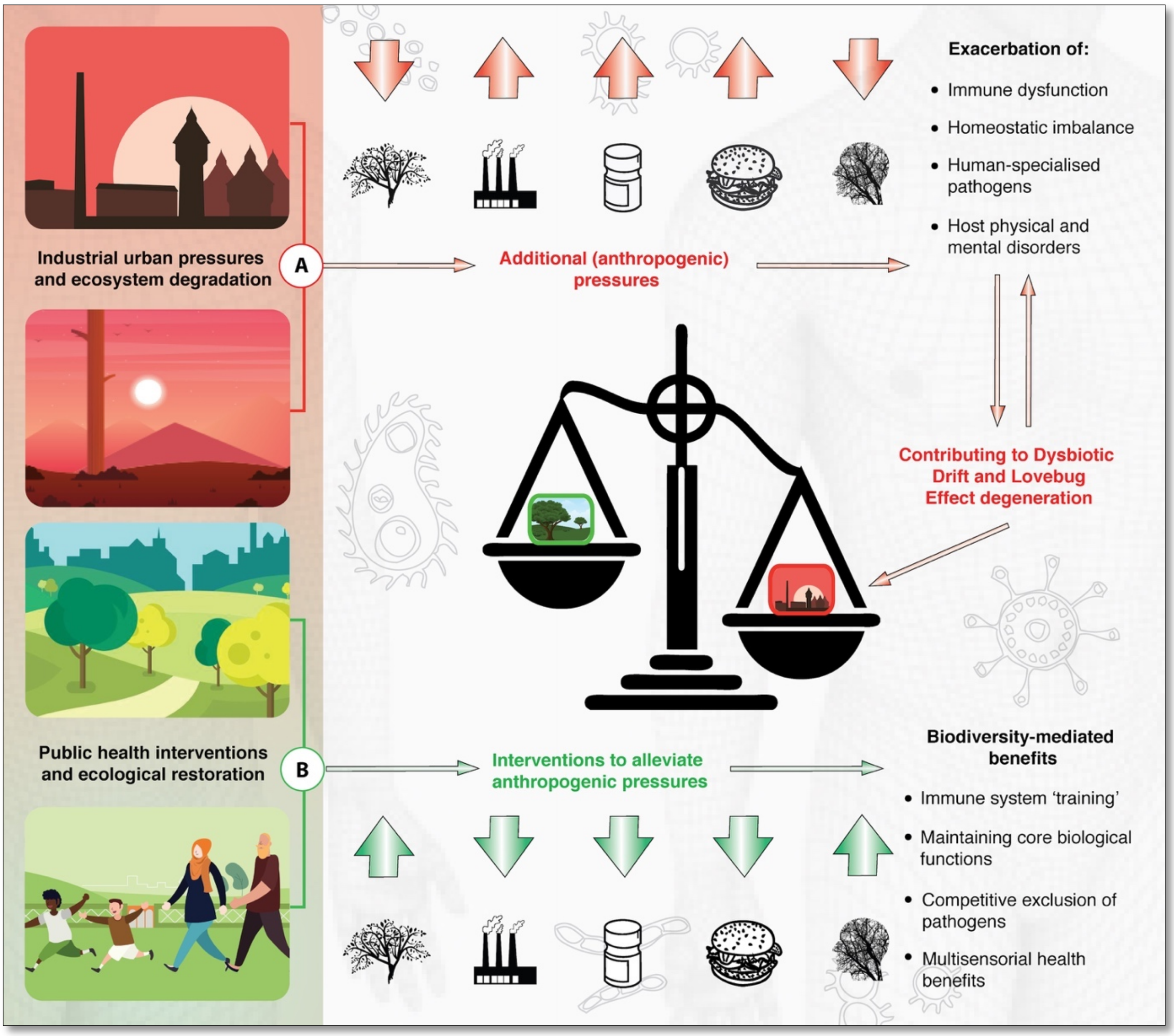

Figure 4. Pathway **(A)**: Anthropogenic pressures and ecosystem degradation could lead to a 'dysbiotic drift' and degeneration of the *Lovebug Effect*. This contributes to an increase in noncommunicable diseases and to a 'ratcheting down effect' (risk of extinction of nature experience and reduced exposure to biodiversity). Pathway **(B)**: Holistic public health and ecological restoration interventions could potentially

alleviate these pressures, allowing the restoration of the *Lovebug Effect* in areas of nature deficit or to continue at a stable level in areas with sufficient supply of biodiversity.

8.7.2. Holistic approaches for public health and ecological restoration

Anthropogenic pressures that could disturb the *Lovebug Effect* are deeply ingrained in complex sociopolitical structures, and are therefore systemic by nature, that is, there are unlikely to be specific isolated factors that would alleviate these issues. Holistic approaches are needed to address social inequalities, loss of biodiversity (including diverse microbial communities), inaccessibility to good quality natural environments, pollution, inappropriate use of antibiotics, ultra-processed diets and extinction of nature experience (as represented in Pathway A in Figure 4). From this perspective, initiatives that explicitly consider multidimensional co-benefits could be valuable. Examples of these integrated approaches include:

- Ecological restoration initiatives (Pathway B in Figure 4), i.e. restoring degraded ecosystems along with their ecosystem services, typically through active management methods (Vaughan et al. 2010; Matzek et al. 2019) with integrated public health evaluations;

- Schemes that aim to empower communities, improve sustainable development, and provide ecological education and opportunities at the ‘grass roots' level - such as community gardening projects (Kim, 2017; Othman et al. 2018);

- Green prescribing (prescribed nature-based activities such as biodiversity conservation, therapeutic horticulture and nature walks), which has potential to enhance human and environmental health (Robinson and Breed, 2019; Shanahan et al. 2019).

Including a microbial model with the psychological frameworks associated with the Biophilia Hypothesis and nature connectedness has the potential to contribute towards a new appreciation for the microbial world, which could ultimately benefit human health. Indeed, it has recently been argued that access to beneficial microorganisms is a facet of public health, and inequitable microbial exposure may compound health inequalities (Ishaq et al. 2019). Developing and integrating a microbe-centric view (Cavicchioli, 2019) is crucial in the face of existential risks such as global biodiversity loss and the climate crisis which ultimately affect human health through the vast array of health-supporting ecosystem services, many of which are microbially-supported (Rashid et al. 2016; Cavicchioli et al. 2019).

At this stage, the *Lovebug Effect* is a hypothesis that requires robust scrutiny. The following section aims to provide an alternative view, counter-arguments, and a starting point for researchers to test the hypothesis.

8.7.3. Challenges and next steps for the Lovebug Effect

As with any newly proposed hypothesis, it is imperative to take a critical view of the conceptual merits and potential pitfalls of the *Lovebug Effect*. To this end, one could easily question why in certain circumstances, some people appear to exhibit a disinclination towards biodiverse environments (Qiu et al. 2013; Hand et al. 2017) – a notion that could be used more broadly to challenge the Biophilia Hypothesis. Furthermore, it is important to also remember there is always a risk of false-consensus cognitive biases.

To counterbalance this perspective, one could point to the importance of anthropogenically-driven changes in life history traits and sociocultural norms in reducing the multiplexity of interactions and connections between humans and the rest of nature (Soga and Gaston, 2016; Colléony et al. 2017; Cox and Gaston, 2018). In other words, could the addition of recent pressures be overriding one's innate and adaptive desire to affiliate with nature? Fattorini et al. (2017) pointed out that some children's preference for less natural and biodiverse environments are likely driven by cultural conditioning, and their innate nature-affinity will fail to flourish if inadequately stimulated.

If the *Lovebug Effect* is fundamentally driven by natural selection, then a degree of natural variation would be expected. Perhaps affinity to nature is beneficial only under certain circumstances (e.g. in certain ecological contexts or life history stages, but not others). If the associated benefit varies spatially and/or temporally, it would lead to variation in selection for this effect, resulting in variation in the trait itself. This has parallels with the concept of adaptive evolution in natural ecosystems, where, for example, adaptive variation in flowering times of plants varies spatially (e.g. later bud-burst in higher latitudes) and through time (e.g. optimal flowering can vary season-to-season). There is no single universally optimal flowering time. On an individual level, the optimisation of this process will depend considerably on location and prevailing environmental conditions. Therefore, the *Lovebug Effect* could fit this evolutionary framework even with high degrees of inter-individual variation in the levels of biophilic drive.

An important line of enquiry, which from a correlative perspective could be investigated with relative ease, is whether an individual's *nature connectedness* is influenced by microbiota (or vice versa). A first step could be to associate the human microbiome with people's Nature Connectedness Index scores via the validated, six-item survey with a seven-point response scale (Richardson et al. 2019). Questions that may arise include: is low or high microbial diversity associated with low or high nature connectedness, and do particular — dominant or diminutive levels of — microbial taxa associate with nature connectedness?

To start testing the *Lovebug Effect* in general, we suggest that researchers explore our eight-step model (see Figure 2) in pairs of process-outcomes, using observational and experimental models for each stage, as follows (summarized in Table 1):

8.7.4. Stage 1. Human exposure to environmental microbiota with subsequent colonisation

For Stage 1, experiments should build on several recent and active studies that investigate human–environmental microbial exchange. For example, Grönroos et al. (2018) demonstrated that short-term direct contact with soil and plants leads to increases in skin microbial diversity. Nurminen et al. (2018) suggested that exposure to nature-derived microbiota associates with gut microbial diversity in the short-term. Ottman et al. (2019) showed that direct soil exposure modifies the gut microbiota in a mouse model. Liddicoat et al. (2019) observed the presence of aerobiome-mediated gut microbiota modulation via exposure to trace-levels of soil dust.

It should be noted that examples of long-term colonisation by environmental microbiota during the adult life stage are limited. Several studies on probiotics show varied results for allochthonous bacterial persistence in the gut (Maldonado-Gómez et al. 2016; Zmora et al. 2018; Xiao et al. 2019). A recent study demonstrated that bile-resistant *Lactobacillus johnsonii* 456 (LBJ 456) can persist in the gut for at least a month following a week-long course (Davoren et al. 2019). Determining the colonisation potential for different body sites and across different life stages will be an important focus point for researchers investigating the *Lovebug Effect*.

Due to the dynamism of the gut microbiome during the human weaning phase, approximately 0-3 years of age (Yang et al. 2016; Moore and Townsend, 2019), it is likely that there will be enhanced opportunities for colonisation by environmental microbiota during this period. Therefore, understanding the microbial influences during this key phase of gut microbiome colonisation should be of early interest in these Stage 1 studies.

Additional randomized controlled trials such as those conducted by Liddicoat et al. (2019) would be a useful framework for testing the *Lovebug Effect*. Detailed experiments to investigate the exposures of different types of microbiomes are needed (e.g. aerobiomes, rhizospheres, phyllospheres), while also studying dose-responses patterns (e.g. compositional changes, durations of effects, longitudinal changes to gut microbiota) and downstream impacts on host phenotypes (e.g. physiology and immune responses).

8.7.5. Stage 2. Selection for human–environmental microbiota associations (does colonization result in health outcomes?)

Experiments for this stage would build on the Old Friends Hypothesis (Rook, 2014). Researchers should aim to identify whether human associations and subsequent colonization (covered in Stage 1) with environmental microbiota can result in improved health outcomes in humans (e.g. via immunoregulation). This idea fits with the hologenome concept of evolution, and perhaps neurological manipulation, but also with more traditional theories of evolution. For example, associations could potentially benefit both the host and the symbionts, and although it is controversial, vertical transmission of microbial genomes could, in theory, contribute to this process. However, interactions with environmental microbiota could also improve health outcomes in humans in a way that adaptively leads to selection for the behavioural traits in humans that maximise exposure to natural environments. As such, these adaptive phenotypes could subsequently be inherited in future generations without vertical transmission of microbial genomes.

Initial studies could include exposing mice to environmental microbiota (as per Stage 1), determining colonization, and examining metabolite production and markers of immunomodulation. Genome-wide association studies combined with microbiome and metabolite characterisation (e.g. short chain fatty acids) could be used to determine the genetic basis of microbiome interactions and metabolic diseases. For example, Sanna et al. (2019) provided evidence of a causal effect of the gut microbiome on metabolic traits (and Type II diabetes) using bidirectional Mendelian randomization analyses.

Karsas, Lamb and Green (2019) pointed out that microbiota may modulate physiology. This is supported by a study that presented evidence for microbial modulation of olfactory epithelium physiology (François et al. 2016). As alluded to earlier, microbially-influenced behavioural changes may be partially governed by olfactory system interactions. Therefore, further investigations into host and bacterial gene associations (e.g. genes related to olfaction such as OR6A2) (Goodrich et al. 2016; Chang and Kao, 2019) could also offer insight into the Lovebug Effect.

8.7.6. Stage 3. Human-microbiota feedbacks

Unravelling the complexities involved in the microbiota-gut-brain axis is an active area of research (Cryan et al. 2019). To explore the *Lovebug Effect*, researchers should conduct environmental microbiome exposure studies (initially using germ free mouse models), followed by fine-scale investigations into the transfer and influence of different microbial taxa with a focus on cognitive and behavioural changes. There is a wide range of validated tests available for the behavioural phenotyping of mice, including protocols for testing basic motor and sensory function, learning and memory, social behaviour, anxiety and depression, impulsivity and personality (Carola et al. 2002; Bailey and Crawley, 2009; Kaidanovich-Beilin et al. 2011; Wolf et al. 2016).

These studies should also integrate functional molecular biology approaches to elucidate the potential biological mechanisms involved in microbially-mediated behavioural change. For example, researchers could focus on immune system responses, tryptophan metabolism, vagal and enteric nervous system activity, while analysing the activity of the microbial metabolites involved in the microbiota-gut-brain axis, such as peptidoglycans, short-chain fatty acids, and branched chain amino acids (BCAAs).

8.7.7. Stage 4. Biophilic Drive

To begin investigating the potential existence of microbial influences on the biophilic drive, researchers could extend the tests in Step 3 with a focus on the response variable being an increased desire for time spent in biodiverse or natural environments. Using randomized controlled trials and mouse models, choice chamber experiments could be designed, whereby two or more microhabitats (initially soil-based) are created with different levels of biodiversity. The experimental mice can then be exposed to and thereby inoculated with different individual strains and assemblages of microbiota (testing a range of diverse microbial communities, pathogens, and microbially-derived metabolites). This should be followed by behavioural tests to determine whether the treatments influence decision making in the mice. There are various other approaches that could be taken, for example, exposing mice to different habitats over varying periods of time, and assessing microbial and molecular effects with subsequent behavioural phenotyping.

Ideally, these types of studies should eventually be modified and scaled up to humans. However, there will be important challenges associated with this process. For example, controlled environments are difficult to create in human studies and there are many potential confounding factors to consider. Some noteworthy, potential confounders of microbiome studies include lifestyle, health, exposures, and psychosocial biases. Overcoming such confounders requires large sample sizes and carefully selected groups.

Other approaches that could be useful for the *Lovebug Effect* include studying the human microbiome composition, structure and dynamics alongside tests for nature connectedness, such as the Nature Connectedness Index (Richardson et al. 2019) and other validated psychosocial instruments. Determining whether spending time in natural environments influences the human microbiome and whether this subsequently correlates to levels of nature connectedness could be an important study for the *Lovebug Effect.* A starting point could be to simply investigate relationships between nature connectedness scores and human microbiome composition (e.g. diversity, individual strains, relative abundances) across different body sites. This could raise questions such as: does a higher level of nature connectedness result in a more diverse human microbiome? Is this a result of a desire to spend time in nature that subsequently increases microbial diversity?

This line of enquiry could be enhanced by longitudinal cohort studies investigating microbiome dynamics from birth with subsequent assessments of nature connectedness and pro-environmental behaviours. Perhaps a study investigating potential relationships between these behaviours throughout the life course with explicit consideration for birth mode (i.e. caesarean section vs. vaginal delivery) could also bring important insights. Furthermore, it is plausible that a parent who is more connected to nature is more likely to expose their children to natural environments during the critical window of microbiome development (0-3 years). As such, studying potential associations between a person's microbiome and their parents' nature connectedness could also be a valuable approach.

8.8. Conclusions

Here we propose the *Lovebug Effect* as a microbially-mediated pathway to help explain the human biophilic drive – the tendency to affiliate and connect with nature. The *Lovebug Effect* is supported by the controversial hologenome concept of evolution. However, the effect would still be relevant in the absence of this evolutionary framework. There are evolutionary processes related to nature affinity that could be microbially-influenced that do not need to meet the criteria of vertical transmission of microbial genomes or direct host manipulation. The pathways discussed in this paper tie together the presence of evolutionary pressures and the mechanisms to microbially-mediated behavioural change (direct or indirect). The foundations have been set to start testing the *Lovebug Effect*, which could extend the portfolio of pathways to nature affiliation. Investigating the *Lovebug Effect* could have implications for the way the Biophilia Hypothesis and nature connectedness are studied in the future. Finally, from a broader perspective, the *Lovebug Effect* could also have implications for the way public health and ecological restoration is approached.

The *Holobiont Blindspot:* Relating Host-Microbiome Interactions to Cognitive Biases and the Concept of the '*Umwelt*'

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8.9. Abstract

Cognitive biases can lead to misinterpretations of human and non-human biology and behaviour. The concept of the *Umwelt* describes phylogenetic contrasts in the sensory realms of different species and has important implications for evolutionary studies of cognition (including biases) and social behaviour. It has recently been suggested that the microbiome (the diverse network of microorganisms in a given environment, including those within a host organism such as humans) has an influential role in host behaviour and health. In this paper, we discuss the host's microbiome in relation to cognitive biases and the concept of the *Umwelt*. Failing to consider the role of host–microbiome (collectively termed a '*holobiont*') interactions in

a given behaviour, may underpin a potentially important cognitive bias, which we refer to as the *Holobiont Blindspot*. We also suggest that microbially-mediated behavioural responses could augment our understanding of the *Umwelt*. For example, the potential role of the microbiome in perception and action could be an important component of the system that gives rise to the *Umwelt*. We also discuss whether microbial symbionts could be considered in System 1 thinking, that is, decisions driven by perception, intuition and associative memory. Recognising *Holobiont Blindspots* and considering the microbiome as a key factor in the *Umwelt* and System 1 thinking has the potential to advance studies of cognition. Furthermore, investigating *Holobiont Blindspots* could have important implications for our understanding of social behaviours and mental health. Indeed, the way we think about *how* we think may need to be revisited.

8.10.Introduction

It is well established that humans are prone to making systematic cognitive errors or 'biases', for example, the susceptibility to overestimate how much one understands about the world (Kahneman et al. 1998; Barton et al. 2016). Some authors (particularly those working within western scientific frameworks) have suggested that anthropomorphism — the heuristic act of attributing human-centric phenotypes to both non-human animals and inanimate phenomena — can lead to misunderstandings of non-human biological processes and behaviours (Burghardt, 2004; Farina, 2012; Bueno-Guerra, 2018). Furthermore, the hierarchical view of nature that positions humans as the pinnacle of species is yet another cognitive bias that may inhibit our understanding and appreciation of the complex interrelated ecologies of biology and behaviour. It should, however, be acknowledged that many Indigenous societies view humans and the rest of nature as a complex web of interconnected subjects (and not discrete, hierarchical objects) (Gratani et al. 2016; De Castro, 2019; Robinson et al. 2020).

The concept of the *Umwelt* was first coined by Jakob Von Uexküll in the early 20th century to describe phylogenetic contrasts in the sensory realms of different species, and the species-specific interactions that occur between the brain, the body and the environment (Von Uexküll et al. 1899; Von Uexküll, 1934/1957; Partan and Marler, 2002). Historically, the *Umwelt* was divided into the *Merkwelt* (perceptual world) and the *Wirkwelt* (effector/action world) to define an animal's sensory unit, from perception to behaviour. However, Bueno-Guerra (2018) recently proposed a broadening of the *Umwelt* concept to include the social sphere or the *Sozialwelt*. An important justification for this proposal was that social dynamics can profoundly influence perception and action. Moreover, transferring the human phenotype of 'cooperative bonding' to their chimpanzee *Pan troglodytes* subjects, led to delusive generalisations in social behaviours (including inconsistent results in task solving with cooperative set-ups) i.e., evolutionary behavioural pathways may not be identical in other species.

In recent years, microbial ecology has seen a rapid expansion in knowledge, attributed in part, to technological advances such as high-throughput DNA sequencing and streamlined bioinformatics (the science of collecting and analysing complex biological data) (Wooley and Ye, 2010; Stres and Kronegger, 2019). It has recently been suggested that the microbiome — the diverse network of microorganisms in a given environment — has an influential role in the behaviour and health of humans and non-human organisms (Rook, 2013; Cryan et al. 2019; Sherwin et al. 2019). Indeed, microorganisms have recently been implicated in host behavioural manipulation through the olfactory system, the microbiota-gut-brain axis, and other biochemical pathways (Davidson et al. 2020; O'Donnell et al. 2020; Robinson and Breed, 2020). Furthermore, it is thought that exposure to the environmental microbiome plays an essential role in 'educating' and regulating innate and adaptive immunity (e.g., via modulation of regulatory T cells), and microorganisms are known to provide a range of functional, physiological roles (Rook, 2013; Rook et al. 2014; Prescott et al. 2017; Chen et al. 2020).

In this perspective article, we discuss host-microbiome interactions in relation to cognitive biases and the concept of the *Umwelt*. We suggest that microbially-mediated host behavioural phenotypes could provide the basis for another conceptual augmentation of the *Umwelt*, that is, to include explicit considerations for the microbiome in the realms of perception and action. Failing to consider the role of interactions between the host and their microbiome (collectively termed a '*holobiont*') in a given behaviour could underpin a potentially important cognitive bias which we refer to as the *Holobiont Blindspot*. This bias could lead to misinterpretations and delusive generalisations in animal (including humans) and non-animal behavioural studies. This is important from a third-person perspective (e.g., the researcher studying another organism or population). However, we also discuss whether microbial symbionts could have an influence from a first-person perspective (integral to the concept of the *Umwelt*) and in the dimension of System 1 thinking, that is, decisions driven by perception, intuition and associative memory, as popularised by

Daniel Kahneman (Kahneman, 2001). If this is the case, there could be important social ramifications, and the concepts of perception and intuition may need to be revisited.

Recognising the *Holobiont Blindspot*, and considering the microbiome as a key component of system that gives rise to the *Umwelt* and Systems 1 thinking, has the potential to advance studies of cognition and social behaviour. Moreover, investigating these concepts could have important social ramifications by restructuring the way we interpret and empathise with social behaviours, and potentially how we understand mental health conditions.

8.11.The Holobiont Blindspot and the Umwelt

Growing evidence suggests that the microbiome can have a considerable influence on the behaviour of humans and non-human organisms (Farzi et al. 2019; Huang et al. 2019; Ezra-Nevo et al. 2020). Although the mechanisms of microbially-mediated host behavioural responses are not fully understood, several biochemical pathways have been proposed. One pathway that has received considerable attention is the microbiota-gut-brain axis (Cryan et al. 2019; Lyte et al. 2020). This refers to the bidirectional communication system linking the central and enteric nervous system to the microorganisms in the gut via the vagus nerve (Ueno and Nakazato, 2016; Breit et al. 2018). Microorganisms in the gut produce an array of metabolic by-products that can stimulate peptide hormone secretion and directly activate the vagus afferents connecting the gut to the brain (Lach et al. 2018; Fülling et al. 2019). Consequently, it has been suggested that microorganisms can metaphorically ‘hijack’ the gut-brain communication highway and influence a range of neuronal processes that result in behavioural responses (Vuong et al. 2017; Davidson et al. 2018). Gut microorganisms can also synthesise compounds such as serotonin (5-hydroxytryptamine), acetylcholine, and peptidoglycan which can penetrate the blood-brain barrier via the systemic circulatory system (Petra et al. 2015; Logsdon et al. 2018; Cryan et al. 2019).

A recent animal study demonstrated that gut bacteria can mimic the functions of cognate host receptor molecules to override host sensory decisions (O'Donnell et al. 2020). In this study, a commensal gut bacterium *Providencia sp*., produced a neuromodulator called tyramine. This compound is thought to act upon the host's olfactory system, modulating aversive responses to certain odours. This process potentially drives mutually-beneficial food decisions, i.e., the host is manipulated into choosing a food source that benefits both the animal host and the commensal bacteria.

This study is only one of several recent animal studies demonstrating modulation of host behaviour by commensal bacteria. For example, the bacteria *Acetobacter pomorum* and *Lactobacillus sp.,* have been shown to work synergistically to manipulate host feeding decisions in *Drosophila melanogaster* (Leitão-Gonçalves et al. 2017; Pasquaretta et al. 2018). Other *D. melanogaster* studies support the notion of behavioural manipulation via olfactory pathways, e.g., individuals can be attracted to compounds secreted by *Saccharomyces cerevisiae* and *Lactobacillus plantarum* but repelled by those from *Acetobacter malorum* (Qiao et al. 2019). Moreover, microorganisms are thought to trigger transcriptional olfactory responses in mice *Mus* *sp.*, and zebrafish *Danio rerio* (Casadei et al. 2019; Cryan et al. 2019). Host sociability and breeding can also be influenced by the microbiome through the mediation of behavioural responses that influence inter-host transmission (Stilling et al. 2014; Wong et al. 2015; Shropshire and Bordenstein, 2016; Sherwin et al. 2019; Simon et al. 2019).

The intricate relationships between host and commensal microorganisms can be framed from a 'hologenomic' perspective. A holobiont, a term first coined by Margulis (1990) is defined as a "*biomolecular network composed of the host plus its associated microbes [...], and their collective genomes forge a hologenome"* (Bordenstein and Theis, 2015).

It is important to acknowledge that the debate is ongoing as to how the hologenome concept of evolution may unfold. For example, an important criticism of this concept is that more evidence is needed to support the notion of vertical transmission of microbiota (from generation to generation) (Robinson and Breed, 2020). However, as Rosenberg and Zilber-Rosenberg (2019) point out, there is some evidence to support this concept. For example, human individuals can retain the same ancestral *Helicobacter pylori* strains, even after migrating to different localities (Achtman et al. 1999; Falush et al. 2003), and other corroborating studies were put forward by Rosenberg and Zilber-Rosenberg (2019) (e.g., Ochman et al. 2010; Goodrich et al. 2016; Moeller et al. 2016). Nonetheless, perhaps a more compelling argument for the hologenomic evolutionary process and its associated behavioural implications, arrives from the notion of *functional associations*. For example, it is likely that evolution has favoured host-microbiome functional associations that precisely reproduce the biochemical networks that give rise to host behaviours across generations (Doolittle and Booth, 2017). Indeed, Suárez (2020) and Suárez and Triviño (2020) argue that in terms of defining the holobiont as an evolutionary unit, less emphasis should be placed on the microbiome's lineages or taxa, and more on its functional traits (encoded by the organisms' genes), referred to as the *stability of traits* concept.

Whilst the precise evolutionary mechanisms still need to be unravelled, one element is clear: the microbiome's functional traits can have a considerable influence on host perception of stimuli (*Merkwelt*) via sensory influences (e.g., olfactory processes), and subsequent behavioural responses or decision-making (*Wirkwelt*). Therefore, this concept could have important implications for evolutionary studies of cognition and may potentially present a cognitive bias if not considered. Here, we propose the *Holobiont Blindspot* to describe this potential cognitive bias. This cognitive bias — also known as a 'blindspot' — could conceivably lead to misinterpretations and delusive generalisations as demonstrated by Bueno-Guerra's (2018) *Sozialwelt*. Indeed, understanding the full sensory spectrum that an animal can perceive (e.g., one element being microbially-derived odours), along with the unique drivers of perception and response (e.g., those functionally-mimicked by commensal microorganisms) could aid in the selection of appropriate controls and relevant stimuli in behavioural studies. Just as a cognitive bias can manifest through the attribution of human-centric phenotypes to non-human animals, treating holobionts as individual subjects divorced from any cognitive influence via symbiotic interactions could also be viewed in this manner. It is also important to note here that plants and even microbes can themselves be holobionts. For example, this was articulated in a recent book, the Entangled Life (Sheldrake, 2020), with the following paraphrased passage:

> *"I attended a conference in Panama on tropical microbes. Someone got up to talk about a group of plants that produced a certain group of chemicals in their leaves. Until recently, the chemicals had been thought of as a defining characteristic of that group of plants. However, it transpired that the chemicals were actually made by fungi that lived in the leaves of the plants. Our idea of the plants had to be redrawn. Another researcher interjected, suggesting that it may not be the fungi living inside the leaf that produced these chemicals, but the bacteria living inside the fungi. The notion of the individual had deepened and expanded beyond recognition. To talk about individuals made no sense anymore" (Sheldrake, 2020, p.18).*

Whilst the idea of a *Holobiont Blindspot* was initially conceived with the third-person view in mind (e.g., the researcher studying the holobiont), we can also consider the *Holobiont Blindspot* from the first-person perspective, which is considered integral to the concept of the *Umwelt* (Baggs and Chemero, 2019). Indeed, in the human dimension the *Holobiont Blindspot* can be positioned in the realm of System 1 thinking. This refers to a conceptual branch of cognition characterised by 'fast and automatic thinking', popularised by Daniel Kahneman (Kahneman, 2001; Moran, 2012). It is important to note that running contra to System 1 thinking is System 2 thinking, a term used to describe the controlled and deliberate mode of thought

(Rottenstreich et al. 2007). However, we find the former to be more relevant to the concepts and scope of this work. Indeed, potential cognitive biases could occur if we assume a System 1-based response in the perception-action cycle (a central principle of the *Umwelt*, also known as the ‘functional loop’) as being purely the result of human intuition and/or associative memory, when it could conceivably be a microbially-mediated behavioural response (Figure 1). For example, via olfactory receptors, leading to an aversive behaviour.

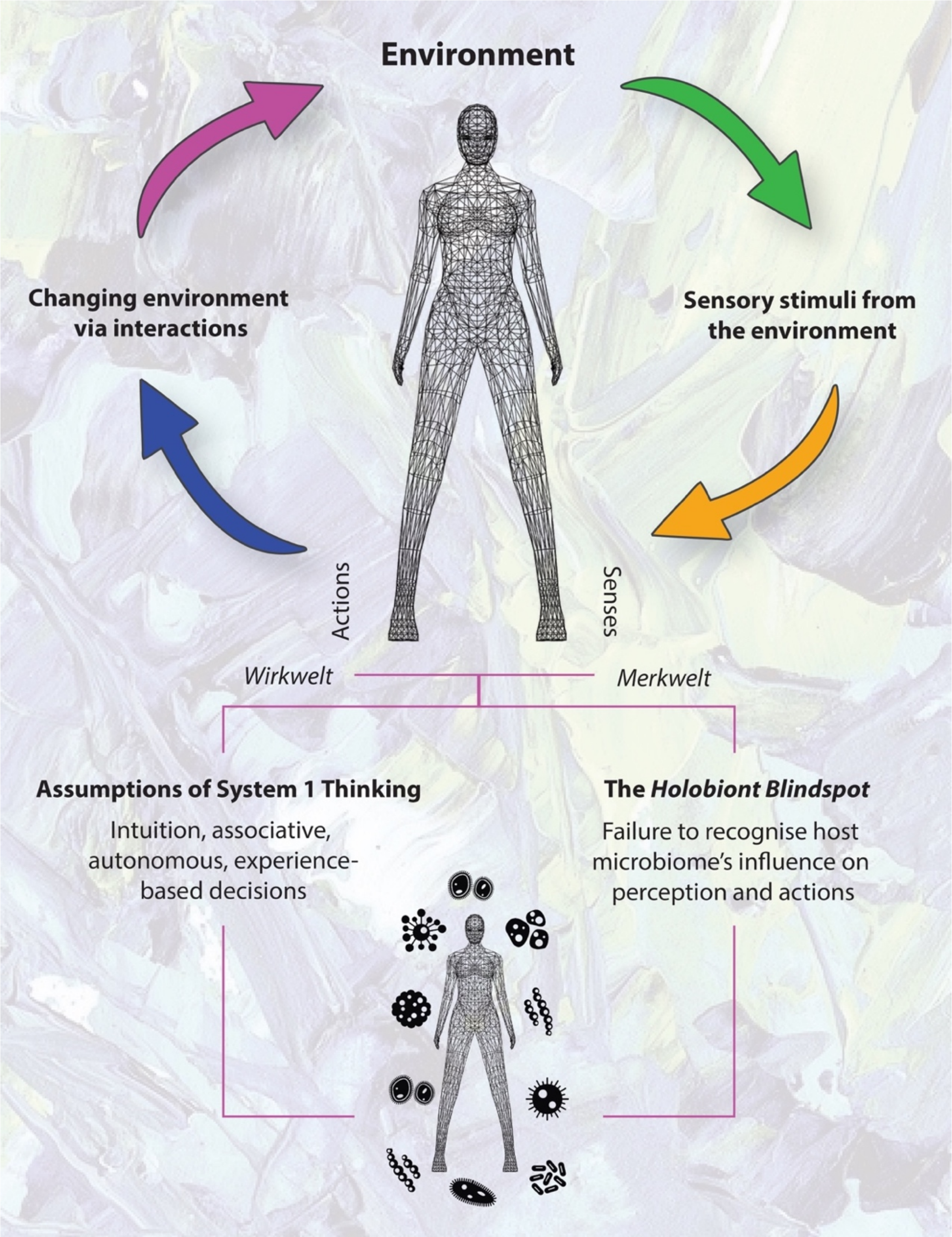

Figure 1. The perception-action cycle and assumptions of System 1 Thinking. Failure to recognise potential microbiome influences in perception and action is the *Holobiont Blindspot*.

As discussed, microorganisms have been shown to influence decision-making in animals via olfactory processes (Qiao et al., 2019; O'Donnell et al. 2020). In humans, the olfactory system plays a major role in social behaviour. For example, olfactory cues can significantly influence memory recall, purchasing behaviour, appetite and sexual arousal (Borg et al. 2019; Jacobson et al. 2019; Sandell, 2019). As such, the *Holobiont Blindspot* could potentially have important social ramifications. To illustrate this, we present a brief thought experiment below:

Changes to (or inter-individual differences in) the human microbiome via environmental disturbances (e.g., pollution exposure; dietary change; antibiotics) → 2. Changes to odour perception in the human host → 3. Changes to preferences (e.g., human odours as 'attractants') → 4. Could the hypothetical individual become less attracted to another individual as a result of this microbially-mediated driver? → 5. Theoretically, this could have important social implications (e.g., leading to relationship issues).

Indeed, it has previously been demonstrated that the microbiome can influence mating preferences in the *D. melanogaster* model. For example, Sharon et al. (2010) divided a population of *D. melanogaster* and reared one half of the population on a molasses-based medium and the other on a starch-based medium. When the populations were mixed together, the flies reared on molasses preferred to mate with other 'molasses flies' and the 'starch flies' preferred to mate with other starch flies. However, subsequent treatment with antibiotics abolished mating preference in the flies suggesting the microbiome was responsible for the preferences. When the flies were inoculated with microbiota from the media, this phenomena was confirmed. It is thought that the microbiome has a role in changing the levels of sex hormones, thus influencing mating behaviour.

Other examples could have important health implications – such as potential effects on food selection or influencing our choices to spend time in certain environments (salutogenic or otherwise). Indeed, the *Lovebug Effect* (Robinson and Breed, 2020) was recently proposed as a microbially-mediated mechanism to help explain our affinity for nature, i.e., could a deficiency in the diversity and functional potential of gut microbiota influenced our decision, via the microbiota-gut-brain axis, to spend time in natural environments where immune supporting microorganisms are abundant? The *Umwelt* of an individual is shaped by the environments the individual resides in, and by the interactions they engage in (Baggs and Chemero, 2019). Therefore, microbial drivers of behaviour could profoundly influence the *Umwelt* of the individual.

Our microbiome is also thought to affect our mood (Bastiaanssen et al. 2020; Talbot et al. 2020). Could this have implications for our relationships and motivations, with downstream effects, for example, on work performance and mental health? Afterall, in the case of depression, oftentimes people do not know (and so cannot articulate) why they feel depressed (Cheng et al. 2020) — could this also be a *Holobiont Blindspot*? If there is a microbial link to depression as suggested by researchers (Foster and Neufeld, 2013), investigating interventions (e.g., through microbial therapeutics) to address this could play an important role in managing mental health in the future (Long-Smith et al. 2020). Several studies have also shown that faecal microbiota transplants can result in the transfer of behavioural phenotypes such as anxiety-like behaviours and anhedonia (inability to feel pleasure) (Bercik et al. 2011; Kelly et al. 2016; Cryan et al. 2019). One study found that altering microbiota in germ-free mice led to changes in hippocampal brain-derived neurotropic factor (a protein involved in brain development and regulation) and subsequent differences in exhibited anxiety-like behaviours (Bercik et al. 2011). Therefore, the *Holobiont Blindspot* could conceivably lead to an inadequate explanation of anxiety-like and anhedonic behaviours, whereas taking host-microbiome interactions into account could provide a much richer and more accurate explanation. Indeed, microbially-mediated anhedonic behaviours is another potential pathway to which microbial drivers could affect one's *Umwelt* i.e., through altering the perception of pleasure.

Our microbial symbionts also affect cognitive traits such as memory, which could affect host memory of food location, as recently shown in wild vertebrates (Davidson et al. 2020). This could have important dietary and health implications, and in humans could conceivably cause relationship issues, e.g., if one partner regularly forgets an important date or forgets to express affection. Further investigations into these theoretical *Holobiont Blindspots* could change the way we understand and empathise with certain social behaviours. As System 1 thinking plays a role in systematic errors through reasoning (Kannengiesser and Gero, 2019; Preisz, 2019), studies aimed at ascertaining the potential effects (deleterious or otherwise) of a host's microbiome in this process could be extremely valuable. If part of our perception and intuition is influenced by 'other' agents (i.e., microorganisms) considered to be constituents of the holobiont, could this change the way we view perception and intuition? Or even the way we view each other – e.g., procuring empathy for decisions ‘out of our control’, or mitigating intuitions/impulses that lead to unfavourable actions? The *Holobiont Blindspot* could also be related to the psychological model of ‘free will’, which has implications for the notions of responsibility and punishment. Indeed, alterations to certain regions of the brain such as the prefrontal cortex can “produce an individual capable of differentiating right from wrong but who, nonetheless, is organically incapable of appropriately regulating their behaviour” (Zeki et al. 2004, p.1). Could our microbiome affect our perception-action cycle and System 1 responses via the modulation of irresistible impulses, and should this be taken into account when considering responsibility and the notions of ‘free will’ and determinism?

Following a similar logic to the recently proposed *Sozialwelt*, we argue that more attention should be given to the hidden components of the system that could influence an organism’s *Umwelt* (e.g., the microbiome). As suggested, microorganisms could have an important role to play in the *Umwelt* through perception (*Merkwelt*) and action (*Wirkwelt*). We should be alert to the possibility of a *Holobiont Blindspot* and consider that ‘thinking’ is not simply a brain-centric process, as microorganisms may play a role in a complex suite of interactions between the brain, body and environment. Indeed, the *Holobiont Blindspot* and the *Umwelt* are also relevant through the lens of biological individuality. If the *Umwelt* refers to an organism’s perceptual world, and the individuality of an organism is in question — particularly given that holobionts can be considered to be individuals and ecosystems simultaneously (Suárez and Stencel, 2020) — then is the *Umwelt* the perceptual world of an organism or an ecosystem?

The *Holobiont Blindspot* questions the very mechanisms and boundaries of the *Umwelt* and even the notions of free will and determinism. It will hopefully generate discussion about how far the microbiome can go in terms of explaining 'our' behaviour and evolution.

8.12. Conclusions

In this perspective article, we have discussed the importance of considering microbial influences on what is traditionally considered to be an organism's perceptual world (*Merkwelt*) and action world (*Wirkwelt*), and in the absence of doing so, there is potential for the *Holobiont Blindspot* (a cognitive bias) which could have important social ramifications. Indeed, it could be important to study the *Holobiont Blindspot* from both the third-person perspective (e.g., a researcher studying animal populations) and from the first-person view (e.g., comprehending the microbiome's influence on our own intuition/behavioural responses and even our mental health). Recognising the *Holobiont Blindspot* and investigating how the microbiome may influence the *Umwelt* and cognition*,* could also provide new and important insights in evolutionary studies of cognition and social behaviour. The *Holobiont Blindspot* may inhibit our understanding and appreciation of the complex interrelated ecologies of biology and behaviour. The way we think about *how* we think may need to be revisited.

CHAPTER 9

9.1. DISCUSSION AND CONCLUSIONS

The objectives outlined in the introductory synopses set out to improve our understanding of the practice and awareness of, and socioecological constraints and opportunities associated with nature-based interventions, and explore their potential co-benefits. Another objective was to gain an understanding of how nature may have supported human health and wellbeing (in the realm of self-prescribing) during the COVID-19 pandemic. Following this, an investigation was carried out into several aspects of the environment-microbiome-health axis, including studies of the complex ecological influences on environmental microbiome assembly, human-airborne microbe exposure types/routes, potential disruptions to this relationship, and factors that may affect attitudes towards microbes. Finally, several novel interdisciplinary phenomena were conceptualised with the aim of transcending current boundaries of knowledge, highlighting the deep interconnectedness of the environment-microbiome-health axis, and setting new research agendas. The multiple aims of this PhD will henceforth be discussed individually, and where appropriate, a degree of cross-referencing between aims will demonstrate how they interconnect.

9.1.1. Chapter 2

Nature-based interventions: Green prescribing

The first part of this chapter explored green prescriptions and their potential co-benefits. This involved an extensive literature review on green prescribing logic, types and mechanistic pathways. The article was framed with a holistic philosophical perspective and aimed to discover some of the fundamental remaining questions and challenges associated with green prescribing. Taking a systems thinking and transdisciplinary approach could potentially procure several other socioecological ‘co-benefits’.

Many types of green prescribing activities are available and active, and often facilitated by the voluntary sector. These include, but are not limited to: therapeutic horticulture (the process by which individuals can achieve enhanced wellbeing through interactions with plants and horticultural practices) (Aldridge and Sempik, 2002); biodiversity conservation or restoration activities (e.g., creating and restoring habitats, often in a group-based setting); care farming (the use of farming practices for health, socialising and education) (Elsey et al. 2016); nature walks/other ‘green’ exercise (such as the cardiovascular-centric activities in parks); and wilderness arts and crafts (such as woodwork using resources from the local environment). By liaising with other researchers and practitioners, the author spent considerable time thinking about the potential co-benefits associated with these activities. Five broad categories of potentially distinct but also interconnected co-benefits were identified. These include:

- physical and mental health benefits (the primary aim of these interventions) which in an ideal situation could include psychological restoration, microbiome enhancements, physical fitness, nature connectedness and others;

- personal (skills, knowledge, and self-esteem) benefits, which could include improved ecological knowledge, learning new skills, building confidence, and a sense of achievement;
- social benefits, which could include social inclusion, improving social skills, community contribution, making new friends;
- environmental benefits, which could include restoring ecosystems, enhancing pro-ecological behaviours, enhancing ecosystem services and improving the quality of the environment; and,
- socioeconomic benefits, which could include helping to reduce crime, reducing the financial burden on public health services, creating new jobs and reducing health inequalities.

More research is needed to determine how these potential co-benefits can be actualised. However, with additional support, there is considerable potential for green prescriptions to contribute to both reactive (health care) and proactive (health promoting) public health solutions, whilst supporting the environment. Any efforts to promote green prescriptions should explicitly consider individual preferences and needs to ensure efficacy and sustainability. Indeed, examples have emerged to show that inappropriately prescribed nature activities can be detrimental to mental health (Tester-Jones et al. 2020).

There are also important practical challenges such as the inability to align disciplinary languages, which could potentially devalue the co-benefits and lead to counterproductive outcomes for health and the environment. It is also essential to ensure that high quality green spaces and nature-based facilitators (typically the community and voluntary sectors) are accessible, are well-funded, and equitably distributed. The UK government recently announced >£4m in funding for green prescribing pilot projects across the country (Marques da Costa and Kállay, 2020). This could be a highly positive step if green prescribing is viewed as part of a more holistic mode of health and social (and nature) care. The intervention designs and evaluations will need to take into account the potential co-benefits and aim to address the multifaceted challenges that face green prescribing sustainability.

Green prescribing is also linked to the environment-microbiome-health axis, which is discussed in future sections. Improving the quality of urban and rural natural environments (e.g., enhancing the biodiversity, safety and accessibility) will likely be important in optimising peoples' exposure to diverse microbial communities. It could also help to reduce pollution exposure, which itself can affect the human microbiome and physiological systems (Abdelsalam et al. 2020; Chiu et al. 2020). Green prescribing could provide an important opportunity to help facilitate health-promoting and disease preventing interactions with biodiverse environments.

This article established the foundations for the subsequent study: '*Let Nature Be Thy Medicine: A Socioecological Study of Green Prescriptions in the UK*'. In this study, the aims were to explore the practice and awareness of, and socioecological constraints and opportunities associated with nature-based interventions, along with mapping green prescription services in the UK. Moreover, building on Bloomfield's (2017) point about the importance of reducing the obstacle of incommensurability, thus speaking two disciplinary languages (i.e., healthcare and ecology), the author investigated the views from two key green prescribing stakeholders: general practitioners and nature-based organisations (NBOs).

The results indicated that green prescribing was active across the UK. GPs and NBOs perceived and expressed some common and distinct constraints to green prescribing. For example, funding and time constraints were generally the most frequently reported concerns This highlights the need for green prescribing services to be properly funded. Many of the NBOs are running other time-consuming activities and working with very small budgets, and it is well understood that GPs can be extremely limited by time. One key constraint for GPs was awareness of available green prescribing services. This is an interesting finding, as a key constraint for NBOs was the inability to engage with GPs and the primary healthcare sector. This highlights the need to promote cross-disciplinary communication pathways which are time and cost efficient, and the need to establish a common language so that all stakeholders can properly engage.

It was found that greenspace presence and abundance within close proximity (100 and 250 m) to GP surgeries (but not greenness—as a proxy for vegetation cover) and NBO presence within 5 km were associated with higher levels of green prescribing provision. This prompts a suite of additional questions such as: does the presence of local greenspaces influence the decisions by the GPs to provide green prescriptions, or the decision by patients to enquire about green prescribing? Is the presence of greenspaces an indication of potential green prescribing activities in the area, and as such, does the availability of services equate to increased green prescription provision and vice versa? Does the lack of available services/infrastructure equate to limited green prescription provision?

Lower levels of deprivation were associated with higher frequency of NBOs. This suggests that the availability of greenspaces and NBOs could be important for green prescribing provision, but there could be greater opportunities in less deprived areas. Arguably, the more deprived areas are where green prescriptions, higher quality green infrastructure, and social activities are needed the most. Other studies have suggested that less deprived areas have a higher presence of voluntary organisations than more deprived areas (Clifford, 2012; Mohan and Bennett, 2019). Considering that the majority of NBOs fell into the voluntary sector category, our results echo these previous studies and support the calls for governments, local authorities and the NBOs themselves, to help secure ecological justice and provision of resources in areas of greatest need.

The questionnaire in this study did not reach all of the GP practices in the UK. The sample size was also relatively small. Therefore, the results should be interpreted with caution. However, this study does contribute to our understanding of green prescribing distribution, awareness, stakeholder perceptions, and socioecological constraints. Establishing transdisciplinary collaborative pathways, efficient and well-funded and equitably distributed infrastructure, a common vocabulary in green prescribing, along with understanding what works for whom, and in what context, are some of the key factors to consider in future research.

To build upon this study, an ‘on-the-ground’ green prescribing study was designed by the author. Unfortunately, this was abandoned due to constraints associated with the COVID-19 pandemic. This study would have comprised a 3-6 month randomised controlled trial (RCT) intervention involving adult patients with mild-moderate depression as determined by the well-established PHQ-9 questionnaire (Kroenke et al. 2001). A key aim was to sustain this green prescribing service once the research was completed and to stimulate other trials across Sheffield and the UK (providing opportunities for important meta-analyses). The intervention would have included the creation of ‘pocket gardens’ (and activities in small, semi-permanent, versatile gardens) and nature-based activities hosted in the premises of GP practices in Sheffield’s Network North region. The idea was to evaluate the green prescribing trial and assess potential changes to patient mental health and wellbeing, and time/cost effectiveness for general practices. One aim was to determine whether the green prescription could procure significant benefits to patient health, wellbeing (including stress levels), and nature connectedness. Another aim was to determine whether there would have been significant reductions in patient attendances as a result of the green prescription. This study took approximately 6 months in planning and the protocol (Appendix II) was rigorously reviewed and approved by the NHS Health Research Authority (HRA) before it had to be abandoned due to the onset of the COVID-19 pandemic.

Nevertheless the planning of the project still provided valuable learning in the context of this PhD. Several multi-stakeholder (e.g., GPs, clinical commissioning groups, nature-based organisations, potential funders) meetings and presentations were attended/given. The support and motivation for the green prescribing project was inspiring. It was also reassuring to observe GPs engage in ecology and planetary health issues alongside primary healthcare issues. This indicated that members of the primary healthcare sector in Sheffield recognised the interconnectedness between human health and environmental health (the key theme of this thesis). To replace this cancelled project, another online study was designed and carried out to find out whether and how nature helped people to cope with the COVID-19 pandemic. It also aimed to determine whether their patterns of nature engagement changed; this will be discussed in the following section.

9.1.2. Chapter 3

Nature-based interventions: Nature's role in supporting health during the COVID-19 pandemic

In this study, we found that respondents significantly changed their patterns of visiting nature as a result of the COVID19 pandemic. People reportedly spent more time in nature and visited nature more often during the pandemic. People generally visited nature for a health and wellbeing benefit and felt that nature helped them cope during the pandemic. This implies that some people were self-prescribing a form of nature engagement in recognition of its health and wellbeing benefits. This further highlights the importance of good quality and equitably distributed green and blue spaces across all neighbourhoods. Referring back to the diagram in Fig. 1, it can be seen that these factors are also an essential part of developing a holistic, upstream health and nature care (i.e., planetary health) model.

People also spent time in unfamiliar natural environments as a result of COVID-19. These included woodlands, rivers, meadows and others. This finding highlights the importance of sustaining a variety of accessible landscapes containing diverse habitats within easy reach of where people live, for health and wellbeing (in addition to biodiversity conservation).

Greater land-cover greenness within a 250 m radius around a respondent's postcode was important in predicting higher levels of mental wellbeing. There were also significantly more food-growing allotments within 100 m and 250 m of respondents with high mental wellbeing scores. Although these results are correlative, they do support other studies that provide evidence for the importance of quality local/neighbourhood green infrastructure for health and wellbeing (Brindley et al. 2019; Marselle et al. 2020). This study provided novel insights into the value of natural environments, particularly in response to an infectious disease pandemic.

There were, however, several important limitations associated with the study. For example, non-random sampling methods were used, which means robust calculations of error and inferences of representativeness were not possible. There are also inherent biases associated with self-reported methods such as responder bias. Nonetheless, taken together with other studies on the nature-human wellbeing relationship, this study supports the need to transition away from a reactive healthcare model towards a holistic model that promotes health creation and stronger human-environment relationships via access to quality and diverse landscapes. These relationships should also include considerations for the network of microscopic life forms that play an integral role in ecosystem functionality and have important influences on human health. This environment-microbiome-health axis will be discussed in subsequent sections.

9.1.3. Chapter 4

The environment-microbiome-health axis: rekindling old friendships and the risks of germaphobia

In the first publication of this chapter, the author of this thesis set out to explore the relationship between the environment, the microbiome, and human health in the context of landscape research. This involved a literature review on what was known about the environmental microbiome and the human microbiome. It also involved an exploration of the interconnectedness of microbiomes, human health, landscape planning, design and management. Another aim was to question current knowledge, and set a research agenda in this area for landscape researchers. This publication was a perspective article and hence drew more upon independent critical and creative thinking as opposed to experimental methodologies.

The discussions within the article were divided into three themes. The process of selecting these themes was informed by past reviews of landscape research, highlighting the diversity and evolution of this interdisciplinary field (Powers & Walker, 2009; Vicenzotti et al. 2016). All of the themes were considered highly relevant to the environment-microbiome-health axis lens.

Human interactions with natural environments include interactions with a diverse range of microbial communities (Roslund et al. 2020; Selway et al. 2020). Advances in microbiome science and reductions in the cost of sampling and processing microbial data, offer opportunities to consider human and environmental microbial interactions as part of nature-based intervention research. For example, future research could investigate whether significant differences exist in exposure and downstream health outcomes between different nature-based intervention activities e.g., therapeutic horticulture vs. nature walks. Roslund et al. (2020) recently demonstrated that a biodiversity intervention in children schoolyards (by adding forest floor material and plants, which children then played in and handled) led to human microbiome compositional changes and stimulated immunoregulation. Selway et al. (2020) showed that simply spending time in an urban green space such as a park could significantly alter your skin and nasal microbiome. Understanding the relative influence of different environments and land management regimes on the human microbiome and downstream health will likely be important factors in future research.

Understanding how landscape planning, design and management can influence urban microbial ecology through landscape research is also highly relevant to the conceptual framework of this thesis. For example, could green infrastructure be restored and designed to promote human health and ecosystem functionality and resilience? This idea is what stimulated the microbiome-inspired green infrastructure ‘MIGI’ concept. We know that vegetation complexity, proximity to trees, native species revegetation (whilst recognising non-natives have value in a resilience and aesthetic sense), wind direction, localised site characteristics, land cover, and potentially even anthropogenic sound and light exposure could influence the assembly, composition and functional profile of the environmental microbiome (Figure 4, Appendix I) (Mhuireach et al. 2019; Mills et al. 2020; Robinson et al. 2020b; Robinson et al. 2021b; Lymperopoulou et al. 2016). Therefore, with this information, could we design our urban landscapes to enhance beneficial human-microbial interactions whilst promoting ecosystem functionality and resilience? These kinds of questions have set a new research agenda in this area, and several stakeholders are now considering MIGI (Fig. 4 and 5, Appendix I). It could be that these factors are too complex to manage effectively or there are too many confounding factors to achieve significant health gains, but it also emphasises the interconnectedness of our complex ecosystems, our decisions and actions, and our health. If this agenda merely stimulates greater consideration in landscape management for microbial communities and their importance in ecosystem functionality and long-term resilience, then this conceptual thinking can still make an important contribution to the planetary health paradigm.

Innovative data integration has the potential to generate new knowledge and can play an important role in communicating complex datasets and concepts to broad audiences. For example, modelling techniques can now be used to map different molecular and microbial signatures in the environment. Microbial cartography (sampling the microbiome and recording its location, then creating dynamic models in software to visualise its composition and distribution) could be used to create 4D models (three dimensions plus a temporal dimension) to map and analyse environmental microbiome dynamics. Generating intelligible outputs of microbial dynamics in the landscape and communicating these to transdisciplinary audiences could pave the way to gaining a better understanding of how land management and environmental factors drive the assembly of the environmental microbiome and how it interacts with humans. A new term called Microbioscape research was put forward by the thesis author, which can be defined as:

> *"the investigation and application of innovative research methods to characterize and visualize the structure, composition and distribution of environmental microbial communities and their relationships with their hosts. Furthermore, Microbioscape research aims to understand the social implications and functional ecology of these communities, focusing on their importance for people, place and nature."*

The technology is now available to develop methods of mapping and visualising the environmental microbiome, particularly at the broader community level and to start understanding how the environment, the microbiome and human health interact. This area of research is highly interdisciplinary. As such, it will require a high degree of collaboration. Generating new strategies for human and environment health with explicit considerations for the environmental microbiome and social needs is possible. However, it is also important to acknowledge the complexities involved in microbial ecology. Ultimately, this paper articulates a new interdisciplinary discourse and adds novel and potentially impactful lines of enquiry in the area of environment–microbiome–health axis research.

This article also highlighted the growing body of evidence supporting the presence of a health-promoting relationship between humans and biodiverse environments. The ongoing loss of biodiversity and loss of our affective, cognitive and experiential connection with nature may be detrimental to planetary health. Furthermore, these factors, along with poor microbial literacy, may be augmenting the negative ecological consequences of what is termed ‘germaphobia’ (the pathological aversion to microorganisms). This could be contributing to an explosion in human immune-related disorders via mass sterilisation of surfaces and reduced exposure to (micro)biodiversity.

The second article in Chapter 4 investigated whether our relationship with, and knowledge of biodiversity, affects our attitudes towards microorganisms. It was found that attitudes towards microbes and both duration and frequency of visits to natural environments were significantly associated. A higher frequency of visits to nature per week, and a longer duration spent in nature per visit, were significantly associated with positive attitudes towards microbes. An important limitation is that the directionality of the relationship is unknown. For example, it was not possible to decipher whether spending more time in nature helps to establish attitudes that are more positive towards microbes, or whether other factors related to attitudes that are more positive increase the likelihood of spending more time in nature. Theoretically, being less averse to microbes could increase one’s desire to spend time in environments with natural features such as plants and soil – key sources of dense microbial communities (Liddicoat et al. 2019; Robinson et al. 2020). On the other hand, a greater habituation to these kinds of environments and an affinity for diverse life forms could conceivably reduce one's aversion to microbes in general. Therefore, whatever the directionality of the proposed relationship is, if confirmed, it may have an important impact on our health and could help to ameliorate the negative consequences of germaphobia.

Interestingly, we found no association between nature connectedness and attitudes towards microbes. This was an unexpected finding given that in this study, nature engagement (spending regular and lengthy times in nature) associated with positive attitudes towards microbes. As mentioned, studies have shown that people who exhibit higher levels of nature connectedness are more likely to spend time in and engaging with natural environments (Capaldi et al. 2014; Capaldi et al. 2015). Reciprocally, spending time in nature can enhance one's nature connectedness (Nisbet et al. 2019; Chawla, 2020). The absence of a significant relationship between nature connectedness and attitudes towards microbes, could be confounded by other factors. However, age, gender, education and deprivation were controlled for with similar non-significant results. It may simply be that one's affective, cognitive and experiential connection with nature is not an important factor in predicting one's attitude towards microbes, or even an issue with the psychological instrument itself. To maximise survey engagement, a basic version (6-items scale) of a nature connectedness instrument was used. It can only be speculated that a more comprehensive instrument may reveal alternative findings. This warrants further research.

Nonetheless, the association between positive attitudes towards microbes and duration and frequency of visits to nature is an important finding that also warrants further research due to its possible implications for public health (e.g., via the potential to enhance immunoregulation) and nature engagement campaigns. It was also found that knowledge of 'lesser known' microbial groups (e.g., identifying that fungi, algae, protozoa, and archaea are microbes) significantly associated with positive attitudes towards microbes. These results suggest that basic microbial literacy may also be important in reducing germaphobia-associated attitudes. This supports the notion that having a greater understanding of a particular phenomenon may increase one's appreciation for the phenomenon, which leads to preservation behaviour (Gribble et al. 2009; Scott-Ireton and Gaimster, 2011).

As concluded in the article, the rise of immune-related disorders and mental health conditions have been linked to germaphobia, reduced biodiversity, and non-targeted sterilisation of human environments. The findings in this paper (if confirmed with further research) point to a feasible strategy to potentially help ameliorate these negative consequences. A greater emphasis on microbial literacy and nature engagement could help to foster enhanced human health and resilience. It could also encourage more positive and constructive attitudes towards the foundations of our ecosystems – the microorganisms.

Despite the evidence that points to human-environmental microbial interactions and benefits (Liddicoat et al. 2020; Roslund et al. 2020; Selway et al. 2020), there is a lack of knowledge in terms of understanding how environmental microbiomes assemble. There is also a knowledge deficit regarding the factors that influence microbiome composition and three dimensional exposure routes. Furthermore, what extent the composition and dynamics may differ between different habitat types (e.g. grassland vs. wooded habitats) is also unknown. In chapter 5 these elements are investigated, and in particular, focus is given to the aerobiome in urban green spaces.

9.1.4. Chapter 5

The environment-microbiome-health axis: urban green space aerobiomes

The first study (a proof-of-concept) in chapter 5 investigated the three-dimensional dynamics of urban green space (scrubby parkland) aerobiomes in the Parklands of Adelaide, South Australia. The publication was titled '*Vertical stratification in urban green space aerobiomes'*.

Standard sampling heights in previous aerobiome studies were 2 m from the ground (Mhuireach et al. 2016; Mhuireach et al. 2019). Microbes will migrate into a given airspace from other areas, driven largely by airflow (Lymperopoulou et al. 2016; Mhuireach et al. 2019). Moreover, the soil is one of the most biodiverse habitats on the planet (Briones, 2014; Bender et al. 2016; Dumbrell, 2019; Zhu et al. 2019) and is therefore a likely key source of environmental microbes in the air. The landscape features (such as the ground, plants and other structures) will also influence the fluid dynamics of particles in the air (Lymperopoulou et al. 2016).

An investigation into some of these ecological factors was carried out to add important knowledge to the environment-microbiome-health axis field. The author of the thesis built the very first instrument to measure urban aerobiome vertical stratification (potential layering of microbes in the air) at the lowest above-ground level of the biosphere using a stand with shelves and petri dishes. This provided a gauge of whether and to what degree the local soil or the airflow from other environments contributed to the aerobiome and to determine whether an altitudinal decay in bacterial diversity occurred from the ground up. This vertical stratification approach also provided an understanding of whether the standard 2 m sampling height used in other studies was capturing a taxonomic picture that was representative of the local airspace from the ground to the approximate maximum human height. The importance of this was that it provided more information on potential exposure types (e.g., what kinds of microbes, how diverse and abundant they were) and inter-height exposure routes (e.g., are smaller adults or children likely to be exposed to different types/levels of microbes compared with taller adults?). This could have important public health implications and adds to the field of nature-based interventions by helping to understand what we could be exposed to (beneficial or otherwise) when spending time in natural environments.

It was found that aerobiome vertical stratification did occur in our sites. Indeed, bacterial alpha diversity (species richness, evenness) decreased from the ground up to 2 m and community composition also varied, depending on height in the airspace. It was found that sampling height explained 22% of the variation in aerobiome community composition and that microbes from the local soil were more dominant at the lower sampling heights, whereas microbes from allochthonous sources (e.g., either from surrounding plants or migrating in with the wind) contributed more to the upper sampling heights. It was also found that significant vertical stratification in potentially pathogenic and beneficial bacterial taxa. For example, *Streptomyces spp*. (potentially beneficial 'old friends' bacteria) were more abundant at lower heights, and *Kingella spp.* (potentially pathogenic – e.g., causing osteomyelitis) were more abundant at higher sampling heights. These results suggest that adults and children or taller vs. smaller adults, and people participating in different activities (e.g., lying down, kneeling when gardening, walking, crawling) may be exposed to different kinds and abundance of microbes. It also suggests that only using a single sampling height – as has previously been done – is unlikely to be sufficient in providing a comprehensive characterisation of the proximal aerobiome.

Further research is required to understand how vertical stratification may affect human colonisation. With this information, we could conceivably improve the design and management of urban vegetation assemblages and structures that may influence aerobiome dynamics, and optimise human–environmental microbe interactions. Finally, it is important to acknowledge the limitations associated with this study. For example, a larger number of replicates from different environments and geographical areas will be required to establish the generalisability of our findings, i.e., will our results be consistent outside of the Adelaide Parklands environment? This warrants further research. Moreover, three samples in the lower sampling heights failed to reach sufficient DNA concentrations to enable PCR and sequencing, which may have affected the vertical stratification relationship; it can only be speculated that the relationship would have been stronger with their inclusion.

As mentioned, this study is relevant to nature-based interventions by adding to our understanding of potentially health-promoting (but also demoting) microbial interactions in natural environments. It is also directly relevant to the microbiome-inspired green infrastructure (MIGI) concept first posited in chapter 4 and discussed further in relation to chapter 7. This aerobiome study is also the precursor to the following study in chapter 5 "*Urban green space aerobiomes: exposure to airborne bacteria depends upon vertical stratification and vegetation complexity*".

The second aerobiome study was also carried out in the Adelaide Parklands, South Australia, using the same sampling techniques but comparing three different habitat types (amenity grassland, parkland scrub, and bare ground) and using additional analytical methods, for example, GIS and co-occurrence network analysis. The objectives were to **(a)** assess aerobiome composition and micro-biodiversity differences between the three habitats; **(b)** compare aerobiome vertical stratification between the different habitats; **(c)** assess whether tree density, distance to trees, and tree canopy coverage influenced bacterial alpha diversity; and, **(d)** to assess any differences in known pathogenic bacterial taxa between habitats and sampling heights.

It was found that habitat type significantly affected bacterial alpha diversity, community composition and network complexity (the number of interactions between microbial species). Bacterial alpha diversity was significantly more diverse is sites with greater vegetation complexity. Indeed, tree density, closer proximity, and canopy coverage associated with greater aerobiome alpha diversity. This suggests that vegetation complexity likely has an important mediatory role on aerobiome diversity. This could provide another example of a co-benefit to having a greater abundance of trees in our urban areas.

Vertical stratification also occurred in the scrub habitat and the bare ground habitat (alpha diversity decreased from the ground to 2 m) but was vertically stable and significantly less diverse in the amenity grassland habitat. The amenity grassland aerobiomes also exhibited greater proportions of putative pathogens compared to scrub. This implies that increasing the vegetation complexity in our urban areas (for example, planting more trees) could potentially improve human health by increasing bacterial diversity. This is thought to be important in immune training and other functional processes (Rook et al. 2003; Rook et al. 2014; Liddicoat et al. 2020). It could also decrease the abundance of pathogens in the airspace.

Further research should investigate the functional relevance of these findings e.g., is vegetation abundance or diversity more important in increasing aerobiome alpha diversity, and does aerobiome alpha diversity increase the number of potential benefits to human health? If so, what are these benefits, and how long do they remain? We know that spending time passively engaging in urban green spaces can significantly change the composition of our skin and respiratory microbiome (Selway et al. 2020), but what are the health implications of this? The research is in its infancy, but the importance will potentially be highly impactful. Finally, there are similar limitations in this study as in the previous aerobiome study, i.e., sample size, number of replications, and the inability to make inferences in terms of generalisability outside of the study location. This study does, however, provide novel insights into the urban ecosystem with potentially important implications for public health and a stimulus for further research.

There are many other interconnected factors to consider in the environment-microbiome-health axis. One is the potential influences of anthropogenic pollution on the microbiome, which could have important ecological and human health implications. For example, chemical pollutants such as nitrogen dioxide (NO_2) and Ozone (O_3) are thought to alter the diversity of the tree pollen microbiome and could increase its allergenicity (Obersteiner et al. 2016). Ozone is associated with lower gut microbial alpha diversity (Fouladi et al. 2020), and traffic-related air pollution may adversely affect metabolic processes via the microbiome (Alderete et al. 2018). A nascent and underexplored area of influence is the potential effects of anthropogenic sound and artificial light pollution on microbiomes. The next publication (a mini-review) in Chapter 6 explores these phenomena in detail.

9.1.5. Chapter 6

The environment-microbiome-health axis: underexplored influences on microbiomes, ecosystems and health

The publication in chapter 6 is titled: “*The effects of anthropogenic sound and artificial light exposure on microbiomes: ecological and public health implications*”. This mini-review set out to provide a critical appraisal of available scientific literature on the effects of anthropogenic sound and light exposure on microorganisms and to discuss the potential ecological and human health implications. It was found that only a limited number of studies (*n* = 23) have been carried out to investigate the effects of anthropogenic sound and light pollution on microbiomes.

However, the studies do suggest anthropogenic sound and light pollution have the potential to significantly influence ecosystems and human health via microbial interactions. Different sound exposures (e.g., amplitude, frequency, and durations) induce inter-species variation in growth, biomass and synthesis of intracellular molecules that could have important implications for many ecological processes across trophic levels. We do not yet fully understand the mechanisms by which sound stimulates microbial growth, as suggested by Gu et al. (2016). However, mechanosensitive channels on bacterial cell membranes might be involved in signal transduction. There was an indication that increased bacterial resistance to ampicillin (an antibiotic) was attributed to low frequency anthropogenic noise. This certainly warrants further research due to its potential importance in the fight against antibiotic resistance. Another study by Kim (2016) suggested that anthropogenic sound could affect plant-microbe interactions and influence plant health, which could conceivably affect ecosystem functionality and downstream ecosystem services.

Another interesting line of enquiry is whether different levels of urban sound and light pollution affect the composition, assembly and exposure routes between humans and microorganisms. Indeed, as mentioned previously, the environmental microbiome could be an important mediator of various health outcomes via human exposure to the aerobiome. Anthropogenic sound and light are increasing in urban areas around the globe (Titulaer et al. 2012; Tabraiz et al. 2015; Kumar et al. 2018; Vitkauskaite et al. 2018). Therefore, we need to understand if these factors disrupt the dynamics of the environmental microbiomes, and if so, what the implications are for ecosystem functionality and human health.

This mini-review suggests that anthropogenic sound and light could affect the growth rate and composition of environmental microbiomes. It also indicates that anthropogenic sound pollution could more directly affect the human microbiome with implications for noncommunicable diseases. Further larger scale research is needed to explore this area in greater detail, and experimental designs (including sample size, bioinformatics, and media comparisons) should be improved in future studies.

In this article, a new hypothesis was proposed: the *photo-sonic restoration hypothesis.*

For example, if anthropogenic sound and light disrupt microbiome assembly, potentially favouring certain adaptable species and reducing functional diversity, this could have important ecosystem and health implications. Therefore, can restoring natural levels of light and sound help to restore microbiomes and ecosystem functionality?

This is an emerging and severely underexplored area of research that could have important implications for global ecosystems and public health. It could also have implications for microbiome-inspired green infrastructure. For example, if certain environments are restored or designed with the microbiome in mind, anthropogenic sound and light could be an important confounding factor if not taken into consideration. The following section will discuss the concept of microbiome-inspired green infrastructure. Two publications relating to MIGI were presented in Chapter 7.

9.1.6. Chapter 7

The environment-microbiome-health axis: microbiome-inspired green infrastructure (MIGI)

In the '*Rekindling old friendships in new landscapes: the environment-microbiome-health axis in the realms of landscape research'* publication in chapter 4, microbiome-inspired green infrastructure was first proposed. MIGI can be defined as multifunctional green infrastructure that is restored and/or designed and managed to optimise human-environmental microbiome interactions, whilst supporting other essential microbially mediated ecosystem processes. MIGI was also discussed in the first paper of chapter 7. This was titled '*Walking ecosystems in microbiome-inspired green infrastructure: an ecological perspective on enhancing personal and planetary health'*.

This article presented a narrative to describe how ecological principles apply at different scales. This includes within the human body and the intertwined macro and microscopic ecosystems that we depend upon for survival. Viewing the human body as a holobiont, that is, a host plus billions of microbial organisms working symbiotically to form a functioning ecological unit, has the potential to enhance personal and planetary health. MIGI provides a means of explicitly considering the microbiome in the landscape and the microbial interactions between the human body and the wider ecosystem (Fig. 3). Considering these interactions and recognising that all forms of life—both the seen and the unseen—are in some way connected (ecologically, socially, and evolutionarily), paves the way to valuing reciprocity in the nature–human relationship.

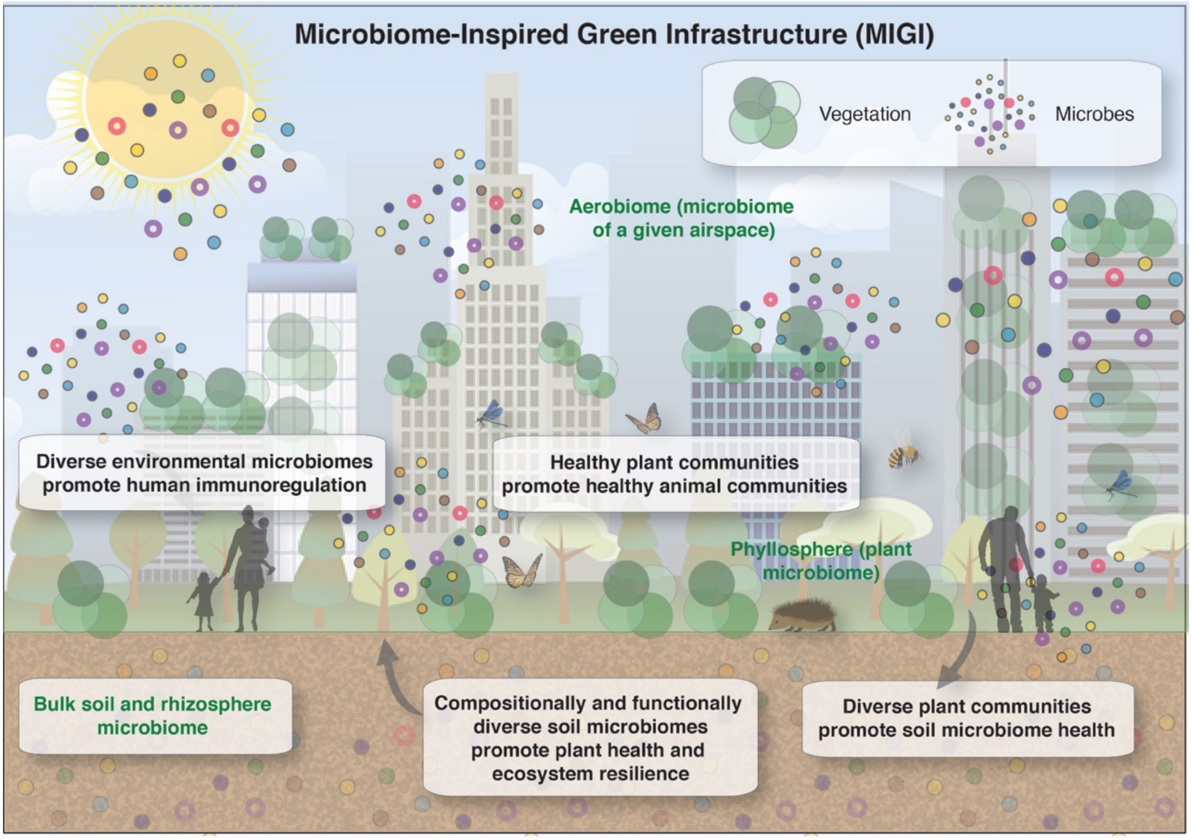

Fig. 3. 'Multispecies health. Environmental microbiomes are the foundations of our ecosystems—promoting plant and animal health (including humans)' (Robinson et al. 2021a).

The general thought-process behind MIGI is that typical landscape (design, creation, management) projects are unlikely to explicitly consider the environmental microbiome. Moreover, some aspects of landscape development projects such as soil replacement practices may even be detrimental. The microbiome is essential for ecosystem functionality, complexity and resilience (Cavicchioli et al. 2019). Therefore, microbial ecology should ideally be integrated in these projects. Furthermore, mounting evidence implies that human-environmental microbiome interactions could be vital for our health and wellbeing (Liddicoat et al. 2020; Roslund et al. 2020). Therefore, could we design and manage green infrastructure with explicit considerations for the microbiome and its role in both human health and ecosystem functionality?

In a human health context, this paper argues that we could potentially enhance human-environmental microbiome interactions through different green infrastructure design/management considerations. These could include: the facilitation of foraging through carefully designed and managed food habitats; natural green walls designed to reduce pollution (chemical, light, sound); plant selection to promote diverse and functionally important microbes; selection and management of appropriate plant-symbiotic microbe relationships (considering this conglomerate as a holobiont). There is also potential to design bioreceptive materials to promote the ability of a landscape material to be bio-colonised (Watkins et al. 2020), thus potentially supplementing the local environmental microbiome. Part of the MIGI concept is to understand the factors that may influence these desired interactions.

For example, understanding how pollution, airflow, area, proximity, aspect, and urban physical features such as buildings, roads, and other structures, influence the dynamics of MIGI will be essential.

MIGI can also be viewed through the nature-based intervention lens. For example, in addition to the social pathways, nature-based health interventions work on the premise that exposure to, and interactions with natural environments bring variable degrees of health benefits (Birch et al. 2020; Cameron et al. 2020; Pretty and Barton, 2020). Nature-based intervention activities can include biodiversity conservation and ecological restoration. As such, service-users could potentially help to conserve and restore habitats (including the environmental microbiome), whilst simultaneously benefiting from a variety of health-promoting microbial interactions. More research is needed to understand what type of exposure to nature is optimal, and also how much, when and for whom (understanding individual needs is essential). However, for a near-future holistic planetary health paradigm that focuses on promoting ecological justice and quality multifunctional green spaces, nature-based interventions could play a role in bridging these objectives.

Incorporating the MIGI concept in landscape design poses considerable multidisciplinary challenges. In the second paper of chapter 7, a framework was set out for managing landscape/construction projects so that multidisciplinary teams of researchers and practitioners can start to explicitly consider the environmental microbiome, thereby improving human health and ecosystem functionality. It is recognised that this area of work is in its infancy. However, this publication provides an initial platform to stimulate more comprehensive considerations as this research area progresses. This publication was titled ‘*Microbiome-Inspired Green Infrastructure: A Toolkit for Multidisciplinary Landscape Design’.*

From an immunoregulation viewpoint, MIGI could take the form of habitat creation (e.g., by explicitly considering plant species and media selection with interspecies interactions in mind to influence the composition of microbiota) and the inoculation of landscape materials (Hui et al. 2019) with the aim of optimising human–microbial interactions. MIGI approaches could also optimise supporting and regulating ecosystem services such as biochemical cycles (e.g., by increasing diversity to optimise carbon storage and reduce nitrogen leaching) (Thompson and Niffin, 2016) and plant and compost design to prevent N_2O losses from urban soils (Guo et al. 2019). There are however, several important challenges to implementing MIGI. These include the complexities of characterising microbial assemblages, determining which functional/ecological roles particular microbiota are playing, and how efficiently they are fulfilling these roles. Nonetheless, advances in technology are enabling rapid and comprehensive assessments of environmental microbiomes, that is, via metagenomics and associated functional profiling.

Due to the complexity of landscape/construction projects (e.g., projects structured across stages, including community consultation, landscape assessment, concept design, detailed design, and contract administration) developers use standardised frameworks to coordinate objectives with other stakeholders. The Plan of Work published by the Royal Institute of British Architects (RIBA) is one such framework. This provides a ‘common language’ for the design and development industries (RIBA, 2019). An overlay to this Plan of Work shows how specific microbiome considerations might be addressed at given project stages. For example, at Stage 2 (concept and design), it will be important to advise designers on plant selection, bioreceptive materials, and growth substrates to manage soil biodiversity and allelopathic factors. Whereas in Stage 7 (In use and Evaluation) it would be beneficial to carry out biogeochemical monitoring of the interactome (where humans, the environment, and microbiomes interact) and an assessment of microbially mediated ecosystem services. An overlay to the existing industry standards for green infrastructure design will be needed in order to create a means for non-scientists to embrace the importance of environmental microbiomes for public health and ecosystem functionality.

MIGI aims and objectives are being developed further. Protocols to deliver MIGI features will need to be intelligible and efficient. However, this Plan of Work overlay provides an initial step towards progressing this agenda and allows multiple stakeholders to see how considerations for the microbiome could, in the future, be integrated into landscape/construction projects. It is also worth reflecting on how environmental microbiome research relates to current biological diversity policy drivers, which are typically focused on the ‘visual spectrum’ of biodiversity, rather than the invisible. Conserving and restoring macro-biodiversity will be imperative to ensure microbial niches and functional diversity are also conserved. However, given the advances in sequencing technology and our understanding of this invisible biodiversity, the question arises as to whether we should place more emphasis on the way our actions affect the environmental microbiome in its own right. In terms of landscape architecture, the 'right tree for the right place' is a term that is currently widely used, and aims to highlight the importance of selecting and planting the trees in a landscape to ensure they survive and provide certain ecosystem services. However, perhaps our growing understanding of microbial ecology and plant-animal-microbe interactions, means we should now be moving towards considering 'the right system (tree-soil-microbiome complex) for the right system (local ecosystem)'. From a human exposure perspective, fostering a deeper fascination for the invisible biodiversity may be important in promoting health and wellbeing in the long-term as demonstrated by the Germaphobia publication. One reason to consider microorganisms in a broader biodiversity conservation framework, is that we depend on them for survival, that is, they have instrumental value. Perhaps a more controversial and philosophical question, is whether microbes should be conserved for their intrinsic value (Cockell and Jones, 2009)?

The MIGI concept pushes the boundaries of current knowledge, yet is supported by emerging evidence on the presence of complex interactions between the human body, mind, the environment, and a plethora of microbial communities (Prescott et al. 2018; Cryan et al. 2019; Liddicoat et al. 2020). Other emerging evidence suggests that we need to re-envisage what it means to be human. For example, as discussed previously, we can be viewed as 'walking ecosystems' or holobionts (Robinson et al. 2018). This deep ecological line of thinking is central to the final chapter of this PhD thesis.

9.1.7. Chapter 8

The environment-microbiome-health axis: novel conceptualisations, transcending current boundaries of knowledge with the holobiont concept

The first paper in chapter 8 is titled '*The Lovebug Effect: is the human biophilic drive influenced by interactions between the host, the environment, and the microbiome?'.* This conceptual paper drew together the fields of microbiome science and environmental psychology to explore the potential role of the microbiome in the human tendency to affiliate with natural environments.

Recent evidence shows that animal behaviour (including mood type and decision-making) can be strongly influenced by the host's microbiome (Cryan et al. 2019). This is due to reasons pertaining to the health of the host's ecological system or 'holobiont' (Robinson et al. 2018). Therefore, a microbially influenced mechanism could potentially contribute to the human biophilic drive. This is the affinity for natural environments – particularly biodiverse types where a health benefit could be gained through multisensorial exposure to ecological features including diverse environmental microbiomes. This conceptual model was called the *Lovebug Effect* (from philia - Greek for affinity, and 'bug' - a colloquial term for a microbe).

The *Lovebug Effect* could add to our understanding of psychological frameworks that are often used to investigate the mechanisms involved with our affinity towards, and connection with nature such as the Biophilia Hypothesis and nature connectedness. It is proposed that by spending time in biodiverse environments, humans may be at an evolutionary advantage through the health-regulating exchange of environmental microbiota. This in turn could influence our nature affinity. The potential mechanistic pathways involved in the *Lovebug Effect* could include direct behavioural manipulation by certain microbes via the microbiota-gut-brain axis (Cryan et al. 2018). Indeed, microbes have been shown to influence feeding decisions and sexual preferences in their animal hosts (Sharon et al. 2010; Leitão-Gonçalves et al. 2017; Pasquaretta et al. 2018). A recent study demonstrated that a neurotransmitter produced by commensal *Providencia* bacteria manipulates host sensory decisions to modulate an aversive olfactory response that favours the fitness of both the host and the microbe (O'Donnell et al. 2020). It is also worth noting that it is not just animal host behaviour that symbiotic microbes can influence. For example, a recent study has demonstrated for the first time that a genus of nectar-dwelling bacteria, *Acinetobacter*, procured pollen nutrition by inducing pollen germination and bursting, i.e., by essentially 'hijacking' the ordinarily rigidly controlled germination mechanisms of pollen for the benefit of the bacterium's fitness and survival (Christensen et al. 2021).

The process of microbial evolution can be extremely rapid compared to many multicellular organisms (Chevrette et al. 2020) via rapid replication and even horizontal gene transfer: the process by which one organism incorporates genetic material from another organism without mating (Rohner, 2016). Therefore, even if the selection pressure associated with the proposed *Lovebug Effect* developed following the era of industrial revolution, rapid global biodiversity loss, mass microbial sterilisation, and loss of nature connectedness, then microbial mutations to meet the demand of human-microbe ecosystem stability and sustainability of microbial gene proliferation could conceivably still occur in this relatively short (macro-evolutionarily speaking) timeframe.

Microbially mediated behavioural change could also occur through the evolutionary development of host-microbiota associated phenotypes independent of direct manipulation. For example, a selection pressure to interact with environmental microbiota to ensure optimal immune regulation, general homeostasis or a stable microbial ecosystem could conceivably lead to the development of human genes that play key roles in human biophilic behaviours; thus, leading to a greater propensity to interact with biodiverse environments.

At this stage, the *Lovebug Effect* is a hypothesis that requires robust scrutiny. By investigating the *Lovebug Effect* we could gain a greater understanding of how our continued disconnect from biodiverse environments effects our internal microbial ecosystem and our behaviour.

A potentially important line of enquiry would be to compare the microbiomes of humans who exhibit low levels of nature connectedness (one’s emotional, cognitive and experiential connection with the natural world) with humans who exhibit high levels of nature connectedness. Indeed, a dysbiotic human microbiome (analogous to a degraded macro ecosystem) could conceivably perturb the *Lovebug Effect* cycle and lead to continued physiological or behavioural effects that reduce nature connectedness. For example, people with higher levels of nature connectedness are more likely to exhibit higher eudemonic wellbeing (Pritchard et al. 2020). Eudemonic wellbeing is inversely associated with depression (Ruini and Cesetti, 2019). Depression has been linked to a dysbiotic microbiome (Chen et al. 2020). As such, this is an interesting relationship to investigate.

Indeed, it is important to find out whether nature connectedness is affected by the *Lovebug Effect* (i.e., is our perceived subjective connection with nature influenced by our microbial symbionts)? Nonetheless, perhaps equally important and arguably an easier aspect to investigate, is whether the establishment of a ‘healthy’ human gut microbiome during childhood can be influenced by the parents’ level of nature connectedness. For example, it has been suggested that people with a higher level of nature connectedness are more likely to engage in nature-based activities or visit natural environments (Capaldi et al. 2014). Consequently, does this additional nature-engagement and associated exposure to (micro)biodiversity (of parents with their children) have a positive effect on the child’s microbiome assembly with long-term health implications? This warrants a deeper investigation. If this proves to be true, then it has important implications for campaigns aimed at enhancing nature itself, and engagement with nature for health and wellbeing. This is highly relevant to the nature-based intervention elements of this PhD.

It has recently been argued that access to beneficial microorganisms is a facet of public health, and inequitable microbial exposure may compound health inequalities (Ishaq et al. 2019; Robinson and Jorgensen, 2020). Developing and integrating a microbe-centric view (Cavicchioli, 2019) is crucial in the face of existential risks such as global biodiversity loss and the climate crisis which ultimately affect human health through the vast array of health-supporting ecosystem services, many of which are microbially-supported (Rashid et al. 2016; Cavicchioli et al. 2019). To achieve this, it will be imperative to address the rise of germaphobia (Timmis et al. 2019) as discussed in chapter 4 of this thesis.

Failing to consider the microbiome in health and behaviour underpins a potentially newly identified cognitive bias (systematic error). This concept forms the basis of the final paper in chapter 8 and in this thesis, titled: '*The Holobiont Blindspot: relating host-microbiome interactions with cognitive biases and the concept of the umwelt'*. For the *Holobiont Blindspot* publication, a literature review was carried out to find out what was known about the microbiome's influence on human and non-human organisms' behaviour. This publication also involved substantial reading around cognitive biases and the concept of the 'Umwelt', which describes the phylogenetic contrasts in the sensory realms of different species, that is, each species has a unique perception based on the sensory mechanisms it has evolved.

As discussed, we can view most multi-cellular organisms as holobionts (Skillings, 2016). Therefore, it was considered that perhaps we are missing a thorough explanation for different behaviours if we fail to recognise the potential role of the host's microbiome in a given behaviour. This systematic error in thinking is what we called the *Holobiont Blindspot* (the term 'blindspot' is a colloquial term for a cognitive bias). Indeed, cognitive biases can lead to misinterpretations of human and non-human biology and behaviour. Microbially mediated behavioural responses could augment our understanding of our perceptual world (the Umwelt). For example, the potential role of the microbiome in perception and action could be an important component of the system that gives rise to an organism's perceptual world. Our microbiome is also thought to affect our mood (Bastiaanssen et al. 2020; Talbott et al. 2020). Therefore, this could conceivably have implications for our relationships and motivations, with downstream effects, for example, on work performance and mental health. After all, in the case of depression, oftentimes people do not know (and so cannot articulate) why they feel depressed (Cheng et al. 2020) – could this also be a *Holobiont Blindspot*?

If there is a microbial link to depression as suggested by researchers (Foster and Neufeld, 2013), investigating interventions (e.g., through microbial therapeutics) to address this could play an important role in managing mental health in the future (Long-Smith et al. 2020). Several studies have also shown that faecal microbiota transplants can result in the transfer of behavioural phenotypes such as anxiety-like behaviours and anhedonia (inability to feel pleasure) (Bercik et al. 2011; Kelly et al. 2016; Cryan et al. 2019). One study found that altering microbiota in germ-free mice led to changes in hippocampal brain-derived neurotropic factor (a protein involved in brain development and regulation) and subsequent differences in exhibited anxiety-like behaviours (Bercik et al. 2011). Therefore, a *Holobiont Blindspot* could conceivably lead to an inadequate explanation of anxiety-like and anhedonic behaviours. Whereas considering host-microbiome interactions could provide a much richer and more accurate explanation (Robinson and Cameron, 2020).

The Holobiont Blindspot publication also illustrated that this systematic error from a third person perspective is not only relevant animals. Indeed, this is supported by a recent study showing that nectar-dwelling bacteria can manipulate pollination time in order to acquire specific nutrients that facilitate the bacterium's survival (Christensen et al. 2021). Failing to recognise this microbial-relationship could lead us to false assumptions about a plant's behavioural ecology.

Recognising the *Holobiont Blindspot* and considering the microbiome as a key factor in an organism's perception and behaviour has the potential to advance studies of cognition. Furthermore, investigating the *Holobiont Blindspot* could have important implications for our understanding of social behaviours and mental health. Indeed, the *way* we think about *how* we think may need to be revisited.

Once again, this publication highlights the deep interconnectedness in the relationship between the environment, the microbiome and health. It adds a new perspective to science and asks us to recognise that the microbiome likely has an underappreciated role in our complex and nested ecosystems: the biodiversity within us, and the biodiversity around us.

9.1.8. CONCLUSIONS

This interdisciplinary PhD adds to several fields including: landscape architecture, nature-based interventions, microbiome science, urban ecology, public health, and environmental psychology/philosophy. The studies provide novel insights into the value of natural environments, particularly in relation to human health and wellbeing, and also in response to an infectious disease pandemic. The first study provided a map of active nature-based interventions in the UK, and showed that green space presence and deprivation associated with nature-based intervention provision, thus addressing the first set of research questions. Complexities associated with nature-based interventions were highlighted, particularly in relation to stakeholder communication, awareness, and equitable distribution of quality green infrastructure and services. The study confirmed that there are differences in perceived constraints to nature-based interventions. Despite these complexities, it is promising that many primary healthcare practitioners are actively engaging with the planetary health narrative and are recognising the interconnectedness between the environment and human health across scales. Our studies show that actions are required to improve communication between primary healthcare professionals and other stakeholders (e.g., wildlife charities). A greater sample size, particularly for GPs would have been beneficial to gain a broader picture of primary care perspectives, and conducting interviews with the different stakeholders would likely have provided richer qualitative data.

To address the second set of research questions, the second study showed that engagement with nature did help people cope with the COVID-19 pandemic, and that restriction measures changed behavioural patterns. It highlighted the importance of sustaining a diversity of habitats across neighbourhoods for health and wellbeing. Future work in nature-based interventions should focus on promoting quality natural environments, whilst understanding individual preferences and needs.

As discussed, growing evidence suggests that exposure to biodiverse aerobiomes contributes towards the development and regulation of immunity and supports favourable mental health. To address the third set of research questions, the novel aerobiome studies in this thesis provided a greater understanding of aerobiome dynamics and potential bacterial transmission routes, exposure levels, and transfer loads, and confirmed that vertical stratification and habitat differences in aerobiome composition and diversity do occur. These studies provide new insights into urban ecosystems and will hopefully help encourage further empirical investigations in this field. Future research should focus on the functional interactions between humans and the aerobiome. The findings also support calls to increase urban tree cover. Exploring the mediatory roles of trees in aerobiome compositional and functional diversity could have important implications for landscape management and public health.

To address the fourth set of research questions, the review publication in Chapter 6 showed that artificial light and anthropogenic sound could significantly affect microbial dynamics and as a result human and ecosystem health. Additional research is needed to confirm the extent of the impacts, and to determine whether restoring natural levels of sound and light positively affect the human and environmental microbiomes.

To address the final research questions, the work in this PhD showed that peoples' knowledge of biodiversity and relationships with nature may affect their attitudes towards microbes. These findings could have important implications for addressing Germaphobia. However, in hindsight, the findings could have been strengthened through the inclusion of a questionnaire that determined people's usage of microbial detergents/sterilisation behaviours, and acquiring more qualitative data to gain comprehensive multidimensional perspectives.

During this PhD, the author pioneered several novel concepts such as *microbiome-inspired green infrastructure or 'MIGI'*, the *Lovebug Effect*, and the *Holobiont Blindspot*. These concepts have stimulated new research agendas and make an important contribution to the fields of landscape design, microbiome science, and environmental psychology. A deeper recognition of the interconnectedness of these phenomena could pave the way to valuing reciprocity in the nature–human relationship. The concepts could also stimulate specific lines of enquiry that lead to improved management of our landscapes and a better understanding of our health and behaviour (e.g., via the microbiota-gut-brain axis).

It is also important to mention that when we talk about establishing a 'new relationship' with nature, we recognise that this is by no means a pan-cultural revolution. Many Indigenous Peoples already view the human-nature relationship as deeply reciprocal. For example, as Robin Wall-Kimmerer (2013) said: "*In some Native languages the term for plants translates to 'those who take care of us*". The author of this thesis recently published an article which discusses the need to embrace other ontologies in ecology, entitled '*Traditional Ecological Knowledge in Restoration Ecology: A Call to Listen Deeply, to Engage with, and Respect Indigenous Voices'* (Robinson et al. 2021d).

Nature-based interventions could contribute to a transdisciplinary, planetary health framework that promotes flourishing ecosystems and healthy societies. As part of this process, it will be important to recognise the profound interconnectedness between the environment, the microbiome, and human health.

Let Nature Be Thy Medicine…

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Appendix I.

Additional MIGI information

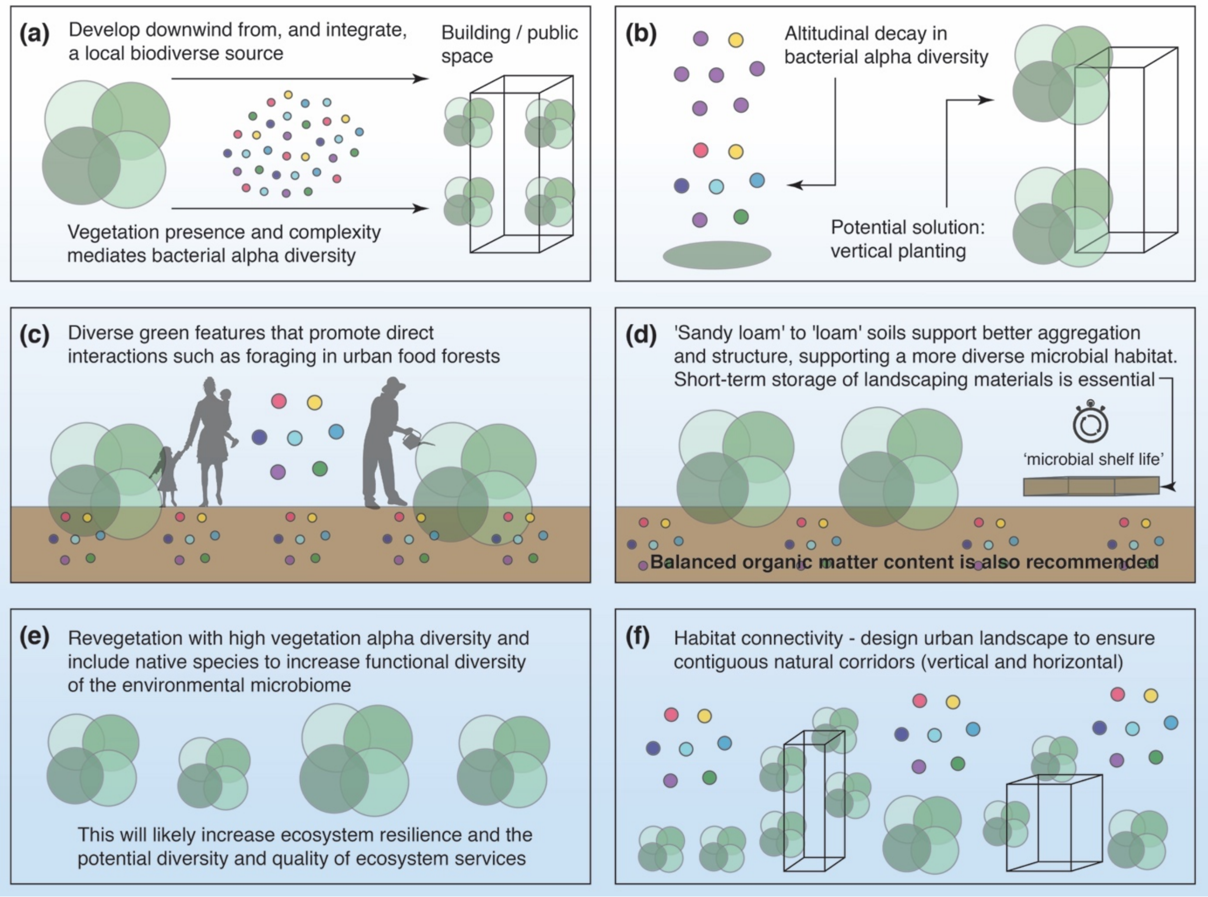


Fig. 4. "Actionable insights for MIGI, including vegetation complexity, downwind development and local integration of biodiverse source (a); a solution to the concept of vertical stratification (b); hands-on engagement with natural features to promote immunoregulation (c); recommended soil types to promote diverse microbial habitat and short-term storage of landscaping materials (d); revegetation with diverse native plants to promote functional diversity (e); the concept of habitat connectivity via contiguous natural corridors to promote long-term multispecies health (f)" (Robinson et al. 2021a).

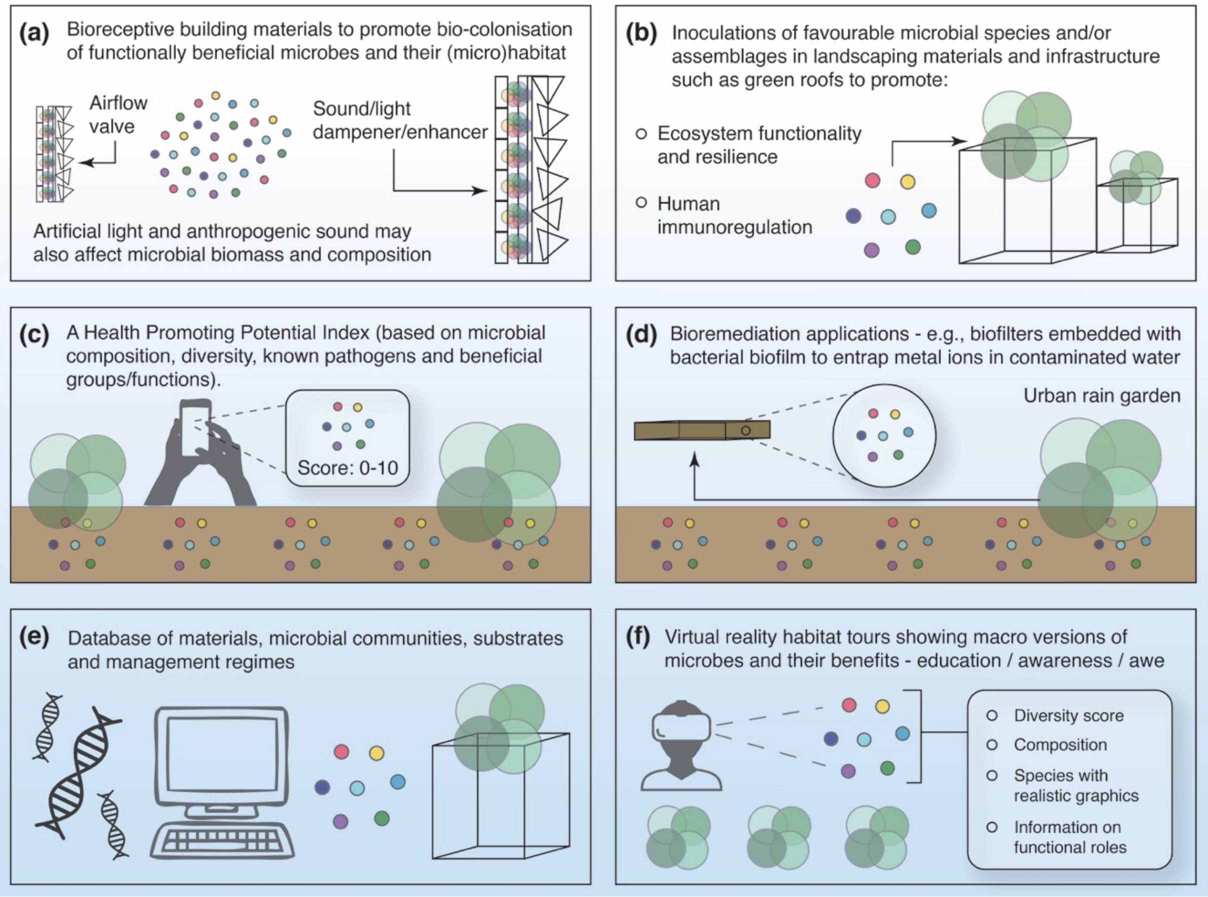


Fig. 5. "Horizon scan of developmental considerations for MIGI, including interventions (b and d), design and supportive features (a and e), and applications for engagement and to acquire useful urban ecosystem health information (c and f)" (Robinson et al. 2021a).

Appendix II.

Green Prescribing NHS-approved Research Protocol

The University Of Sheffield.

Research Protocol / Project Proposal

A Dose of Nature: An Interdisciplinary Study of Green Prescriptions and the Environment-Microbiome-Health Axis

PROTOCOL VERSION NUMBER 3.0 100120

IRAS Number: 263091

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:

Signature: Date:

....../....../......

Name (please print): Deborah McClean (sponsor representative)

Position: Director of Research Services

Chief Investigator:

Signature:

Date:

10/01/2020

Name: (please print):

LIST OF CONTENTS

SECTION

KEY STUDY CONTACTS

Chief Investigator	Professor Anna Jorgensen, 01142 220621,
Study Co-ordinator	Jake Robinson (lead researcher), 07791 944448,
Sponsor	University of Sheffield; Deborah McClean (representative): d.mcclean@sheffield.ac.uk; 0114 222
Joint-sponsor(s)/co-sponsor(s)	N/A
Funder(s)	Economic and Social Research Council (ESRC) –
Key Protocol Contributors	Jake Robinson, 07791 944448,
Committees	N/A

STUDY SUMMARY

Study Title	A Dose of Nature: An Interdisciplinary Study of Green Prescriptions and the Environment-
Internal ref. no. (or short title)	N/A
Study Design	Various – including RCT-style intervention study with
Study Participants	For the project covered in this HRA application, patients
Planned Size of Sample (if applicable)	240 x participants (120 x participants in each arm i.e. intervention vs control group)
Follow up duration (if applicable)	3 months
Planned Study Period	Recruitment, introductory session and a 12-week intervention programme between March

Research Question/Aim(s)	For the project covered in this protocol, the main research question is: - Can the green prescription (pocket gardening activities in GP surgery premises) improve mental health and wellbeing in patients with mild- moderate depression?

STUDY SPONSOR

The study sponsor is the University of Sheffield – the main representative is Deborah McClean who is the Director of Research Services (d.mcclean@sheffield.ac.uk; 0114 222 1449).

PROTOCOL CONTRIBUTORS

Jake Robinson (lead researcher) has designed this research protocol. The research proposals covered by this protocol are part of Jake's PhD doctoral research programme.

Professor Anna Jorgensen (Chief Investigator) has scientifically reviewed this protocol.

KEY WORDS: Green prescriptions; social prescribing; nature; health and wellbeing; microbiome; public health

Allocation

green prescription

Intervention Group

STUDY PROTOCOL

A Dose of Nature: An Interdisciplinary Study of Green Prescriptions and the Environment-Microbiome-Health Axis

1. BACKGROUND

Public health context

In several studies investigating the social determinants of health, attention has been drawn to the need for innovation in public health (Walsh et al. 2010; Marmot and Bell, 2012). These studies highlight significant health inequalities in many countries including the UK. Other studies highlight increasing risk factors for chronic diseases and the associated impacts upon general health, mortality, and the economy. For example, Scarborough et al. (2011) estimated that in 2006-07, chronic diseases related to poor dietary habits, physical inactivity, obesity and social structures cost the UK National Health Service (NHS) £11.8 billion. A similar story unfolds for mental health and wellbeing, with an annual expenditure of up to £13 billion allocated to the management of mental health conditions in England alone (Naylor et al. 2012).

Despite significant improvements in certain areas of public health, recent estimates suggest that 61% of adults in England are considered to be overweight or obese (NHS, 2013) – both of which are risk factors for noncommunicable diseases (NCDs) such as diabetes and cardiovascular-related illnesses. Moreover, around one in four people experience mental health issues in the UK each year (Bridges, 2014; McManus et al. 2014). These conditions are diverse, often co-morbid with other NCDs (Patel and Chatterji, 2015; Varghese, 2017), and are thought to be responsible for 38 million annual deaths across the globe (Prescott and Logan, 2017).

These findings highlight the need to develop innovative strategies to:

- Improve public health and wellbeing;
- Close the health inequality gap; and,
- Alleviate the financial burden currently facing health services and economies

Environmental context

The natural environment provides a significant amount of provisioning, supportive, regulatory and cultural benefits to humans, and the processes and features that generate these benefits are now commonly referred to as ‘ecosystem services’. The health and wellbeing of humanity is highly dependent upon these so-called ‘services’ and the diverse array of organisms they support (Soliveres et al. 2016; Pienkowski et al. 2017). However, our decisions and actions are often detrimental to the environment, and the urban lifestyle is one of the fundamental anthropogenic drivers of biotic restructuring, often to the detriment of biological diversity and ecosystem functionality (Hagen et al. 2017). For example, two issues associated with biodiversity loss are natural resource exploitation and pollution. The former is a key input in the process of urban development, often resulting in habitat loss and a subsequent reduction in genetic and species diversity (Allentoft and O'brien, 2010; Pinsky and Palumbi, 2014); whereas pollution is a key output and 'by-product' of the urbanisation process, and can lead to widespread negative impacts on the global biological systems that support biodiversity (Ceulemans et al. 2014).

There have been recent calls to take an integrative approach to address the challenges associated with ecosystem resilience and human health and wellbeing (Figure 1). For example, Raymond et al. (2017) put forward a framework to promote the co-benefits (for the environment and public health) of nature-based solutions. Furthermore, the emerging 'planetary health' philosophical framework also focuses strongly on the interrelated management of human and environmental health (Ostfeld, 2017).

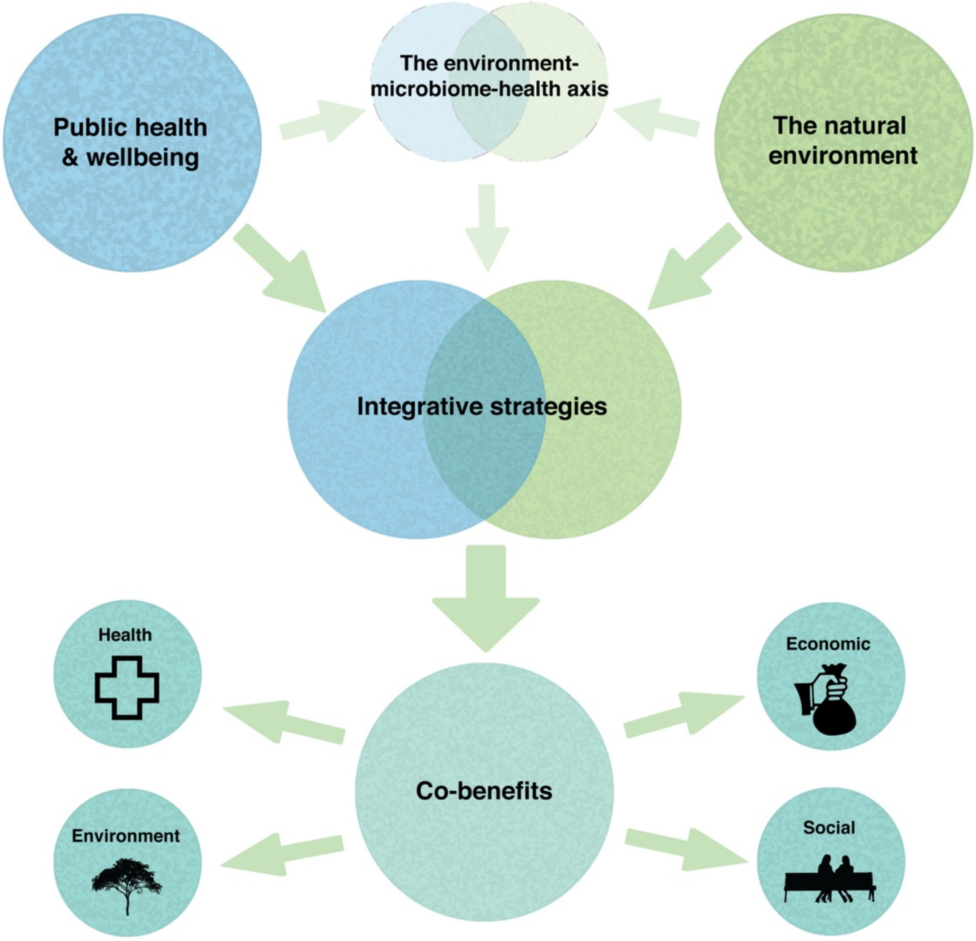


Figure 1. Integrative strategies that result in co-benefits for humans the environment (created by the author).

The nature-human health nexus

It is now widely acknowledged that spending time in natural environments enhances mental health and wellbeing. Although there is an inevitable degree of inter-individual variability in results influenced in part by complex socioecological dynamics, mounting evidence suggest the benefits can be multifaceted and include: psychological restoration, reduction in depression and anxiety, improvements in self-esteem, attentional capacity, happiness, and a sense of peace and security (Bragg and Leck, 2017). A range of studies has linked proximity and access to green space, with a lower incidence rate of depression and anxiety i.e. depressive symptoms decrease as proximity and access increase (Cohen-Cline, Turkheimer and Duncan, 2015; Tomita et al. 2017).

Interacting with natural environments can also stimulate a range of immunoregulatory and health enhancing biochemical processes. Moore (2015) recently put forward the Biogenics hypothesis, suggesting that spending time in natural environments results in airborne exposure to several beneficial phytochemicals through the inhalation and ingestion of airborne particles. Moore’s hypothesis is supported by a study by Li et al. (2008) and Li (2010) who report that forest bathing (Shinrin Yoku) enhances intracellular anticarcinogens in lymphocytes (white blood cells) and natural killer cells (NK) that protect against viral infections and tumour growth. The objective measures and the relatively long duration of the positive immunological impact (lasting 30 days) are promising and would benefit from further studies. There are several other studies linking time spent in ‘nature’ to improved cardiovascular and metabolic systems, DNA protection, enhanced nature connectedness and social integration, and other important phenomena that have potential co-benefits for human and environmental health.

An emerging intervention – green prescribing

A green prescription, also known as a prescribed nature-based health intervention (Robinson and Breed, 2019), is an emerging, innovative strategy designed to improve physical and mental health and wellbeing through exposure to and often multisensorial interaction with natural environments (Bragg and Leck, 2017). A regular walk through a green space, systematic participation in gardening (horticultural therapy), and/or the undertaking of biodiversity conservation activities such as habitat creation and restoration are all potential examples of green prescriptions (Jepson, Robertson and Cameron, 2010; Bloomfield, 2017). It can be viewed as a prescription-led and monitorable activity that involves spending time in natural environments for the benefit of health and wellbeing (Robinson and Breed, 2019). Green prescriptions are typically designed for patients with a defined need and have the potential to supplement orthodox medical treatments, particularly those aimed at addressing noncommunicable diseases and mental health issues (Bragg and Leck, 2017). Furthermore, it could be argued that green prescriptions could provide both reactive (health care) and a proactive (health promoting) solutions to some public health issues. Indeed, this dual approach could potentially improve the health of patients whilst promoting a healthy lifestyle and supporting the development and maintenance of health-promoting infrastructure.

The term ‘green prescription’ or ‘GRx’ was first coined by health professionals in New Zealand in the late 1990s to outline a range of physical and dietary activities with the aim of enhancing health and wellbeing and reducing NCDs such as Type-2 diabetes (Patel et al. 2011). Under this initiative, GPs provide patients with diet plans and strategic physical activities such as ‘progressively increasing’ steps, monitored with pedometers and follow-up telephone counselling (Kolt et al. 2009). The term has since been broadened to include nature-derived activities (e.g. horticulture, nature walks, biodiversity conservation) as part of a holistic and integrative approach aimed at addressing NCDs, mental health issues and social isolation.

It is acknowledged that the foundations of green prescribing typically require the interactions of three main phenomena – natural environments, a social context, and meaningful activities (Bragg and Leck, 2017). However, there is still a significant amount of research to be undertaken to answer a number of fundamental questions such as what kinds of nature-based interventions (or elements of these) work best for whom, where and when (and whether the dose-response approach is too simplistic)? (Figure 2); and also what are the mechanisms, constraints and opportunities, and current status of green prescribing?

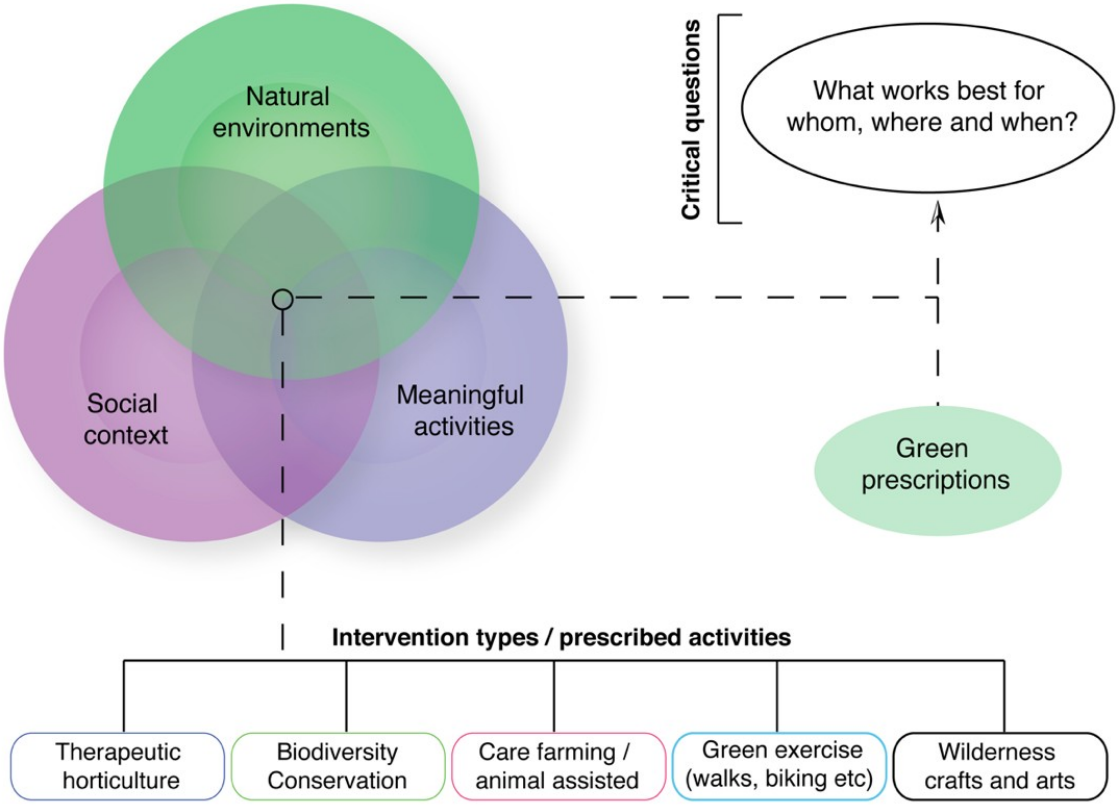


Figure 2. The three interacting phenomena typically required for green prescribing, along with the associated activities and some remaining critical questions (created by author, partially adapted from Bragg and Leck, 2017).

2. RATIONALE and THEORETICAL FRAMEWORK

The idea that spending time in natural environments can enhance one's health has been researched for several years. However, the concept of green prescribing — or prescribing a systematic nature-based intervention that can be monitored over time — has only recently generated traction in practice (although the principles can be traced back to several hundred years ago). Green prescriptions could potentially help to reduce the costs of mainstream healthcare and could have important 'co-benefits', for example, by simultaneously enhancing the environment. There are still a number of critical questions that need answering, such as what works best for whom, where and when, and a number of key constraints need to be addressed. The conceptual framework for this proposed trial has been informed by the results of a UK-wide green prescribing questionnaire (Stage 1 of PhD) and recommendations from the Improving Wellbeing through Urban Nature (IWUN) research project (www.iwun.uk). The main (perceived) constraints to green prescribing acquired from the questionnaire include:

- A lack of funding for all stakeholders
    - The research project aims to be cost-effective and not strictly reliant on other organisations

- A lack of knowledge of how to start a green prescribing service
    - The project aims to demonstrate how to start a green prescribing service

- A lack of opportunities and awareness of service availability

    - As above

- Patient motivation and ease of access/travel etc.
    - The novel situational aspect of the project (within the premises of GP practices) aims to maximise access/minimise travel for patients

- A lack of knowledge of the evidence and mechanisms
    - The research project aims to collect evidence on mental health, wellbeing and nature connectedness via an RCT-style experimental design

- Referral and set-up time
    - As per funding objective - see top. The research project will also evaluate patient appointments/attendances (number and frequency) via the RCT approach

The researcher is proposing to conduct a 3-6 month randomised controlled trial (RCT) interventional study involving adult patients with mild-moderate depression as determined using the well-established PHQ-9 questionnaire. A key aim is to sustain this green prescribing service once the research is complete and to hopefully stimulate other trials across Sheffield and the UK (providing opportunities for important meta-analyses)

The intervention will include pocket gardening (activities in small, semi-permanent, versatile gardens) and nature-based activities hosted in the premises of GP surgeries in Sheffield's Network North region.

A green prescription is a prescribed nature-based health intervention designed for individuals with a defined need (or to complement orthodox medical treatments). Common examples of green prescribing activities include: therapeutic horticulture, biodiversity conservation volunteering, care farming, wild crafts and nature walks (Robinson and Breed, 2019). Green prescriptions are typically provided for a set length of time (e.g. 12 weeks) but can be open-ended.

As a green prescribing activity, therapeutic horticulture has several potential benefits including physical and mental health improvements, facilitating social co-mingling, educational and reward-based e.g. harvesting natural produce (Sempik, Rickhuss and Beeston, 2014; Soga et al. 2017). It has already proven successful as a health promoting activity in projects such as SAGE Greenfingers (sagesheffield.org.uk), which was established following a community needs assessment commissioned by Pitsmoor surgery in Sheffield.

This kind of green prescription can provide natural environmental features, social context and meaningful activities – three important interacting phenomena in nature-based interventions (Bragg and Leck, 2017). By integrating other nature-based activities such as simply noticing the local wildlife, this project also aims to open pathways (via senses, compassion, emotion meaning and beauty) to nature connectedness – one's emotional relationship with the natural world (Richardson, 2019). The novel situational element of this project (i.e. hosted in GP surgery premises) aims to maximise accessibility and minimise travel for the patients, and to minimise multi-stakeholder logistics.

Furthermore, several policy statements—informed by empirical evidence—were recently published by the IWUN research project (www.iwun.uk). Amongst many other recommendations, these call for GP practices to enhance the biodiversity in their premises. Importantly, the question of how GP practices can provide their own greenspaces to improve the mental health of their patients is also raised. The relevant policy document can be found here.

Re-designing the outdoor spaces surrounding GP practices and/or integrating new nature-based features and activities within these spaces could help to enhance patient (and staff) wellbeing. Creating pocket gardens (small multifunctional gardens typically installed in the pockets of empty urban spaces) and biodiverse spaces for green prescribing activities is one potential route, and an evaluation of this concept forms the basis of the research project.

There is also a severe lack of RCT experiments in green prescribing research – further strengthening the rationale for the chosen experimental approach.

3, RESEARCH QUESTION/AIM(S)

Research Hypotheses:

1. The green prescription trial (pocket gardening service) will significantly improve patient wellbeing, nature connectedness and reduce symptoms in adult patients (18+ years old) with mild-moderate depression.

2. The green prescription will significantly reduce patient perceived stress levels (as measured using the Perceived Stress Scale).

3. The green prescription trial will reduce the number and frequency of patient appointments/attendances (over the course of the intervention; reassessed 3 months post-intervention).

3.1. Objectives

To evaluate a green prescribing trial in Sheffield, UK. Assess potential changes to patient mental health and wellbeing, and time/cost effectiveness for general practice, taking a novel approach through 'pocket gardens' and nature-based activities in GP surgery premises.

3.2. Outcomes

- Determine whether there are significant benefits to patient health, wellbeing (including stress levels), and nature connectedness as a result of the green prescription.

- Determine whether there are significant reductions in patient attendances as a result of the green prescription.
- Stimulate future studies and community initiatives (not formally assessed as part of the research).

4. STUDY DESIGN and METHODS of DATA COLLECTION AND DATA ANALYSIS

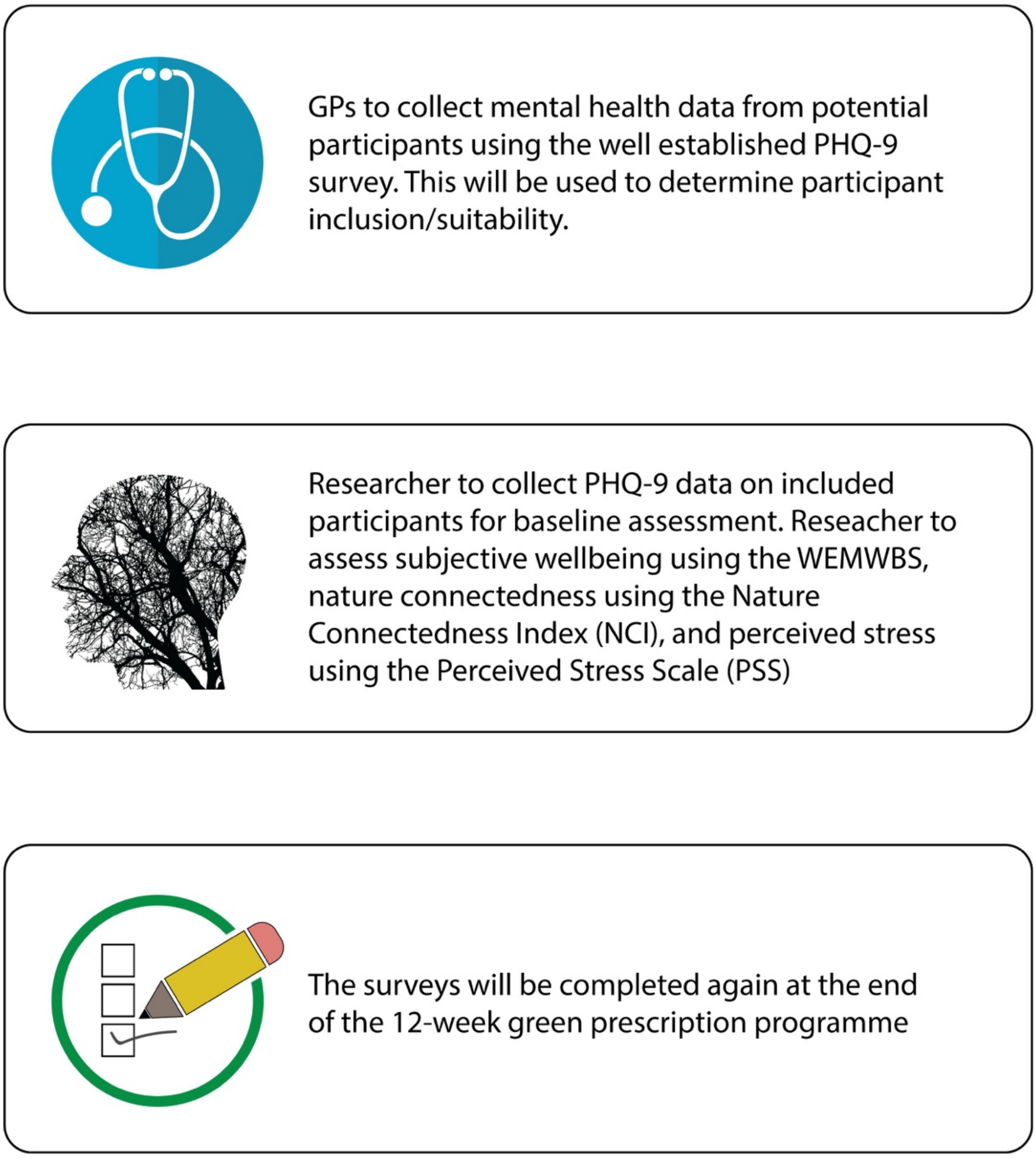
Experimental Design

Summary of Study:

- Run a 12-week Green Prescribing ‘Pocket Garden’ programme based on an RCT interventional study design
- When? Between March-September 2020
- Where? Within the grounds of GP surgeries
- Who will install and run sessions? The researcher (Jake Robinson) and an experienced co-facilitator (Corrine Moss)
- Who will attend? Adult patients (18+ years) with mild-moderate depression
- 5-6 x sites (GP practices in the Network North PCN)
- Each green prescription session will last for 1 hour
- 1 x session at each site per day (5 hours in total)
- 10 x patients per session (aim for 12 assuming 20% drop-out)
- 50 x patients per day
- 2 x days per week e.g. on a Tuesday and Thursday
- Flexibility for patients to cross-over between sites will be enabled
- Green prescription interventions given to 100 x patients (Intervention/treatment group)
- Identify 240 x patients in total (200 + 40 dropout buffer) = 100 x randomly selected patients to participate in the GRx study and 100 x randomly selected non-participating patients (control group).

- Randomisation process will occur following post-introductory session (and post-receipt of informed consent).
- Each patient in the Intervention Group will attend 1 x session per week for 12 x weeks
- Control Group participants will not attend the green prescription sessions but will receive 'usual' care.
- Health and wellbeing data will be collected at baseline i.e. prior to intervention (Fig. 9), and at the end of the intervention). Additional follow-up data collection (3 months post-intervention) will also be conducted to assess patient attendance records. Both the Intervention Group participants and Control Group participants will complete the research questionnaires.

NB: An additional aim will be to ensure this service is available to all patients (i.e. including those from the control group) following the research trial. Legacy planning is underway to maximise the likelihood of sustaining the initiative post-research.



GPs to collect mental health data from potential participants using the well established PHQ-9 survey. This will be used to determine participant inclusion/suitability.

Researcher to collect PHQ-9 data on included participants for baseline assessment. Reseacher to assess subjective wellbeing using the WEMWBS, nature connectedness using the Nature Connectedness Index (NCI), and perceived stress using the Perceived Stress Scale (PSS)

The surveys will be completed again at the end of the 12-week green prescription programme

Figure 9. Health data will be collected by the GPs (for inclusion/suitability) and the researcher (for baseline assessment) using standard questionnaires such as the PHQ-9 (for depression), NCI (for nature connectedness), WEMWBS (for subjective wellbeing), and the Perceived Stress Scale (PSS) for perceived stress levels.

Independent/Explanatory Variables

- The green prescription intervention (pocket gardening)

Dependent/Response Variables:

- Symptoms of depression (severity)
- Level of perceived wellbeing and nature connectedness
- Level of perceived stress
- Patient appointment/attendance number and frequency

Treatment/Intervention Group:

n = 100 randomly selected patients with mild-moderate depression (participating in the intervention).

Control Group:

n = 100 randomly selected patients with mild-moderate depression (not participating in the intervention).

Limitation:

- Intervention blinding is not possible; however, analytical blinding is possible.

Sample Controls to Consider:

- Age
- Gender
- Lifestyle factors

4.2. Data Collection Methods

Patient Health Questionnaire – 9 (PHQ-9)

The PHQ-9 is a validated instrument for criteria-based diagnoses of depression severity (Kroenke et al. 2001). This will be used in the study (before/during/after the intervention). Where possible, the PHQ-9 (or other method for diagnosing depression) will be conducted by GPs prior to the introduction as part of the inclusion/suitability process. This will also be carried out (distributed by the researcher) at the introductory session for temporal standardisation – supervised by a GP. As per Cameron et al. (2008), the PHQ-9 severity cut-off for this study will be as follows:

- Mild = 5-9
- Moderate = 10-14

PATIENT HEALTH QUESTIONNAIRE-9

Over the <u>last 2 weeks</u>, how often have you been bothered by any of the following problems?	**Not at all**	**Several days**	**More than half the days**	**Nearly every day**
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself — or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed? Or the opposite — being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead or of hurting yourself in some way	0	1	2	3

FOR OFFICE CODING

0 + ______ + ______ + ______

=Total Score: ______

If you checked off <u>any</u> problems, how <u>difficult</u> have these problems made it for you to do your work, take care of things at home, or get along with other people?

Not difficult at all	**Somewhat difficult**	**Very difficult**	**Extremely difficult**
☐	☐	☐	☐

Nature Connectedness Index (NCI)

The Nature Connectedness Index (NCI) is a validated measure of nature connectedness – a psychological construct to explain one's attitude towards and emotional connection with nature. This could have implications for pro-environmental behaviours and as such, one's likelihood to participate in nature-based activities.

As per Richardson et al. (2019), this index comprises 6 questions and a 7-point response scale from "completely agree" to "completely disagree".

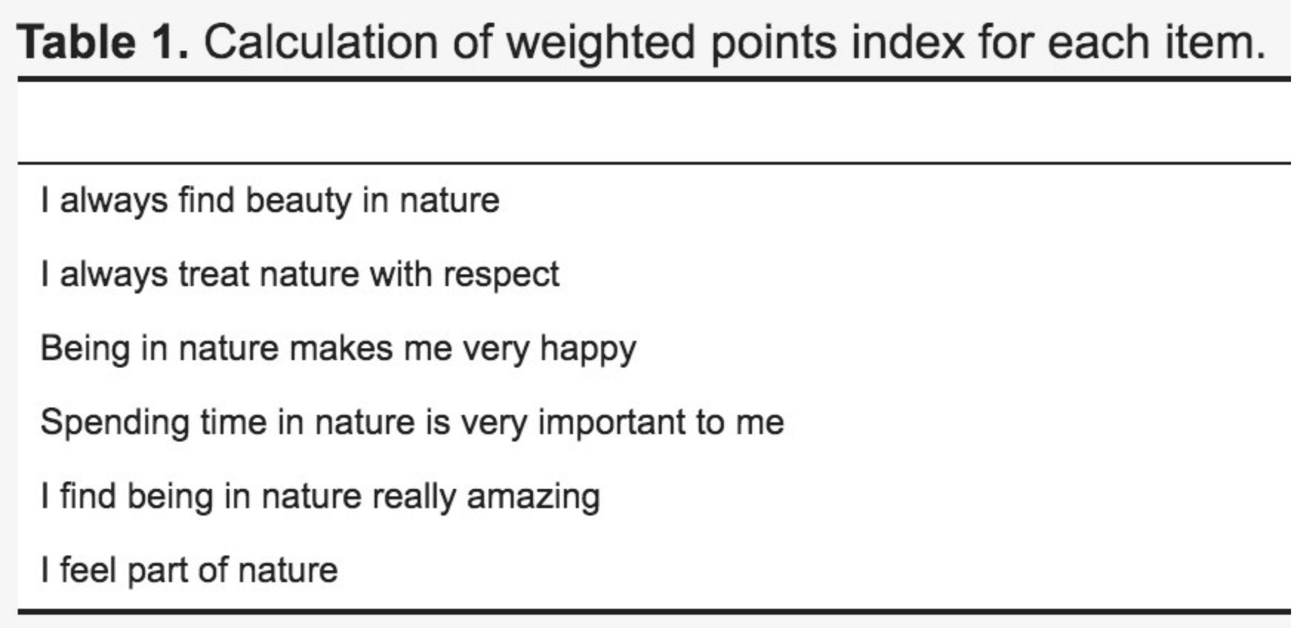


Table 1. Calculation of weighted points index for each item.

I always find beauty in nature
I always treat nature with respect
Being in nature makes me very happy
Spending time in nature is very important to me
I find being in nature really amazing
I feel part of nature

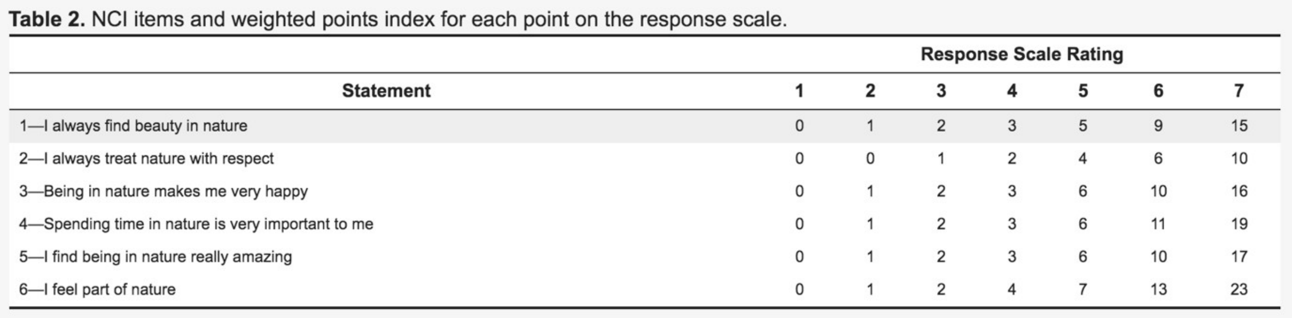


Table 2. NCI items and weighted points index for each point on the response scale.

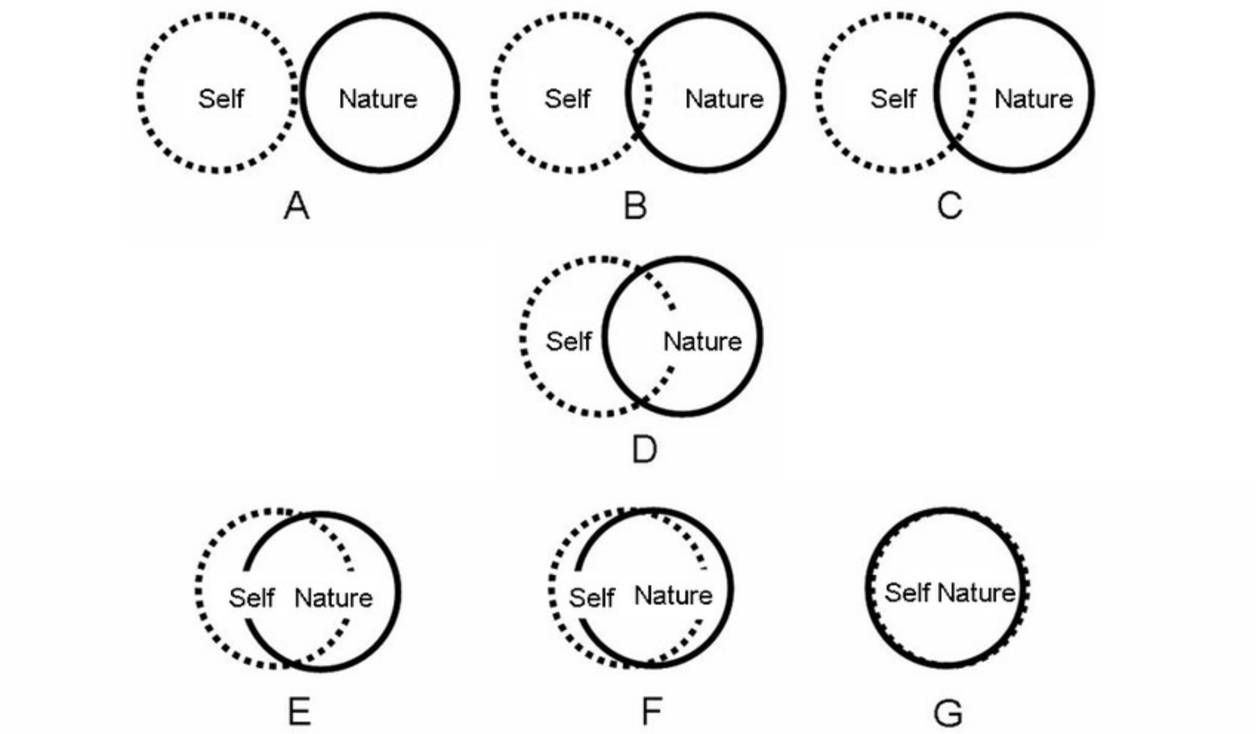
	Response Scale Rating						
Statement	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1—I always find beauty in nature	0	1	2	3	5	9	15
2—I always treat nature with respect	0	0	1	2	4	6	10
3—Being in nature makes me very happy	0	1	2	3	6	10	16
4—Spending time in nature is very important to me	0	1	2	3	6	11	19
5—I find being in nature really amazing	0	1	2	3	6	10	17
6—I feel part of nature	0	1	2	4	7	13	23

From Richardson et al. (2019)

The NCI will be used in this study and the questionnaire will be integrated within the health and wellbeing questionnaires. Data will be collected from patients before, during (mid-point) and after the intervention (and from the control group).

As part of the nature connectedness assessment, the Inclusion of Nature in Self (INS) will also be used as a single item (Schultz, 2002). This is a concise measure and is able to provide important information on one’s subjective connection with the natural world whilst breaking up the formality of standard research questionnaires.

The Inclusion of Nature in Self (INS)



To complete this research item, the participants are asked to rate their connectedness to nature by marking one of the pictures in the above – where A is completely disconnected, and G is completely connected and ‘at one’ with the rest of the natural world.

The Warwick-Edinburgh Mental Wellbeing Scale (WEMWBS):

The researcher will also collect wellbeing data from the patients (this will be integrated with the NCI questionnaire) to determine whether the intervention has an effect on overall wellbeing. This will occur before, during (mid-point) and after the intervention.

The researcher will use the WEMWBS to collect wellbeing data. The WEMWBS has 14 x statements and 5 x response categories, summed to provide a single score.

“The items are all worded positively and cover both feeling and functioning aspects of mental wellbeing, thereby making the concept more accessible. The scale has been widely used nationally and internationally for monitoring, evaluating projects and programmes and investigating the determinants of mental wellbeing” (Stewart-Brown, 2019).

The Warwick-Edinburgh Mental Well-being Scale (WEMWBS)

Below are some statements about feelings and thoughts.

Please tick the box that best describes your experience of each over the last 2 weeks

STATEMENTS	None of the time	Rarely	Some of the time	Often	All of the time
I've been feeling optimistic about the future	1	2	3	4	5
I've been feeling useful	1	2	3	4	5
I've been feeling relaxed	1	2	3	4	5
I've been feeling interested in other people	1	2	3	4	5
I've had energy to spare	1	2	3	4	5
I've been dealing with problems well	1	2	3	4	5
I've been thinking clearly	1	2	3	4	5
I've been feeling good about myself	1	2	3	4	5
I've been feeling close to other people	1	2	3	4	5
I've been feeling confident	1	2	3	4	5
I've been able to make up my own mind about things	1	2	3	4	5
I've been feeling loved	1	2	3	4	5
I've been interested in new things	1	2	3	4	5
I've been feeling cheerful	1	2	3	4	5

Perceived Stress Scale (PSS)

The Perceived Stress Scale (PSS) will be used to help measure individual stress levels. This tool was developed by Cohen et al. (1983) and remains a popular choice for determining how different situations affect perceived stress levels. This is a 10-point measure using a 0-4 Likert style response system.

Individual scores on the PSS can range from 0 to 40 with higher scores indicating higher perceived stress. Scores ranging from 0-13 would be considered low stress. Scores ranging from 14-26 would be considered moderate stress. Scores ranging from 27-40 would be considered high perceived stress.

For each question choose from the following alternatives:
0 - never 1 - almost never 2 - sometimes 3 - fairly often 4 - very often

________ 1. In the last month, how often have you been upset because of something that happened unexpectedly?

________ 2. In the last month, how often have you felt that you were unable to control the important things in your life?

________ 3. In the last month, how often have you felt nervous and stressed?

________ 4. In the last month, how often have you felt confident about your ability to handle your personal problems?

________ 5. In the last month, how often have you felt that things were going your way?

________ 6. In the last month, how often have you found that you could not cope with all the things that you had to do?

________ 7. In the last month, how often have you been able to control irritations in your life?

________ 8. In the last month, how often have you felt that you were on top of things?

________ 9. In the last month, how often have you been angered because of things that happened that were outside of your control?

________ 10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

4.3. Patient Appointment Attendance and Frequency Assessment:

Patient appointment visits (total number of consultations over a given period) and frequency will be recorded throughout the course of the intervention and up to 3-months following the end of the trial. These will be analysed on an inter-treatment level, and if possible, an intra-treatment level. For existing patients, GPs will query patient attendance records from the preceding 6 months to provide additional longitudinal data. This will only occur if the participants give consent (via the consent form).

The researcher will also invite Intervention Group participants to take part in a post-intervention focus group to explore qualitative experiences, and receive feedback – e.g. to find out the most beneficial parts of the intervention (the social, the nature, the physical etc.). The focus group will also be used to find out whether patients feel the green prescription could help to reduce the number of standard GP appointments they attend (qualitative element). These sessions will be used to encourage open discussion amongst participants (Krueger and Casey, 2008).

This deeper, qualitative analysis can be used to inform future improvements. A semi-structured focus group guide will be developed to help explore the experiences of the participants.

The focus group will take place in a relaxing environment and will last for approximately 1 hour. Participants in the intervention will be asked (face-to-face) during the final quarter of the intervention (final 3 weeks) if they would be willing to participate in a focus group and the researcher will record the names of those attending. The aim will be to be have eight participants from each practice (this will be stated at the sessions) – larger than 10 are difficult to control and limit opportunities to share insights and observations. Group dynamics also change when participants are not able to share their experiences (but want to do so) ((Krueger and Casey, 2008). Focus group sessions will be audio recorded and the data will subsequently be transcribed by the researcher for analysis.

4.4. Quantitative Analysis

Core statistical approaches for the primary and secondary outcomes will include:

Two-sample t-Test (or non-parametric counterpart e.g. Mann-Whitney U test) for comparing mean scores of treatment (intervention group) vs. the control group (differences between groups).

Two sample special (i.e. one sample with two measurements) for comparing before, during and after within treatment/control.

As per Johnson et al. (2017), to assess change over time, we will aim to fit a linear mixed model, with time as a categorical variable, and compare baseline scores to mid-point (6 weeks) and 12 weeks, and potentially a 3-month follow-up if time/resources permit.

Multivariate analysis may be conducted to investigate the relationships amongst several different variables (such as gender, nature connectedness etc.) and to see if the outcomes of interest are affected by more than one variable. See Wennmacker et al. (2018) for more information.

Statistical analysis (cleaning, managing, analysing, visualisation) will be conducted by the researcher using various packages in the R statistical computing environment.
During data collection the research will adhere to a double-checking principle to ensure all data points are complete. On the off-chance that there are missing values, data will be removed via listwise deletion i.e. data from any participant with missing values will be removed. The sample size includes a 20% dropout buffer to account for complete withdrawals.

Adjustment for baseline covariates is often advised, firstly to correct for any chance imbalances in important baseline variables following randomisation, and secondly, because adjusting for highly important baseline variables in an RCT can improve the precision of treatment effect estimates even when the outcome measure is binary.

In line with Gilbert et al. (2015):

“key covariates should be selected prior to analysis based on the likely magnitude of the association with the outcome measure (European Agency for the Evaluation of Medicinal Products, 2003). We will therefore also perform a multivariable logistic regression to take into account any imbalance that may occur in important baseline characteristics known to predict outcomes between the groups”. Odds ratios will be quoted together with their 95% confidence intervals and exact P-values.

4.4.1. Sample Size Determination

The sample of participants (N=240) was chosen for several reasons; this was considered a manageable sample size with the available resources, the effect size of the health and wellbeing instruments was also assessed (see next page) and was informed by other studies using the same or similar instruments for RCT interventions, for example:

Potential sample size for RCT – based on other studies investigating health interventions for depression, stress, wellbeing and nature connectedness.

n = 126 (Steardo et al. 2019) – Psychoeducational Intervention for Perinatal Depression (RCT). Using the HAM-D scale.

n = 126 (McClay et al. 2015) – A Community-Based Pilot RCT of Life Skills Classes for Individuals with Low Mood and Depression. Using the PHQ-9 scale.

n = 88 (Karyotaki et al. 2019) – Examining the effectiveness of a web-based intervention for symptoms of depression and anxiety (RCT). Using the PHQ-9 scale.

n = 106 (Eklund, Bäckström and Hansson, 2018) – Psychometric evaluation of the Swedish version of Rosenberg’s Self-Esteem Scale. Using RSES.

n = 173 (Huppert and Johnson, 2010) – RCT of mindfulness training – importance of practice for an impact on well-being. Using WEMWBS and CAMS-R.

n = 200 (Sobko, Tse and Kaplan, 2016) – A RCT promoting healthy eating and active playtime by connecting with nature. Using NRS.

n = 204 (Morledge et al. 2013) – Feasibility of Mindfulness on Stress Management (RCT). Using MAAS and PSS.

n = 90 (Querstret, Cropley an Fife-Schaw, 2018) – Effects of Mindfulness on Perceived Stress, Depression and Anxiety (RCT). Using PHQ-9 and PSS.

n = 96 (Valosek et al. 2018) – Effect of Meditation on Perceived Stress (RCT). Using PSS.

n = 120 (Quach et al. 2016) – Effect of Mindfulness on Working Memory (RCT). Using PSS.

n = 134 (Colgan et al. 2019) – Perceived stress and mindfulness (RCT). Using PSS.

An effect size was searched for in the scientific literature. Soga, Gaston and Yamaura (2017) conducted a systematic review of health studies in the context of gardening and pooled their effect sizes to provide a mean of 0.48. Whilst Nyström et al. (2017) show a much larger effect size of 1.3 using PHQ-9 on physical activity (with rationale), and 1.39 (without rationale).

Taking the former pooled effect size (d=0.48) a sample size of 138 was obtain (for 69 participants in each arm i.e. the intervention and the control). This was then rounded up to 200 (excluding the 20% dropout buffer) which was considered a reasonable target with appropriate power.

Sample size calculation:

Comparing the mean of a continuous measurement in two samples

α (two-tailed) = 0.05 (Threshold probability for rejecting the null hypothesis. Type I error rate)

β = 0.2 (Probability of failing to reject the null hypothesis under the alternative hypothesis. Type II error rate)

q1 = 0.5 Proportion of subjects that are in Group 1 (exposed)

q0 = 0.5 Proportion of subjects that are in Group 0 (unexposed); 1-q1

E = 0.48 Effect size (If $\mu 1$ = mean in Group 1 and $\mu 0$ = mean in Group 0, then E = $\mu 1$ - $\mu 0$.). S = 1 Standard deviation of the outcome in the population

The standard normal deviate for $\alpha = Z\alpha = 1.95996$. The standard normal deviate for $\beta = Z\beta = 0.84162$

Standardized Effect Size = (E/S) = 0.480

Calculation using the T statistic and non-centrality parameter:

N1: 69

N0: 69

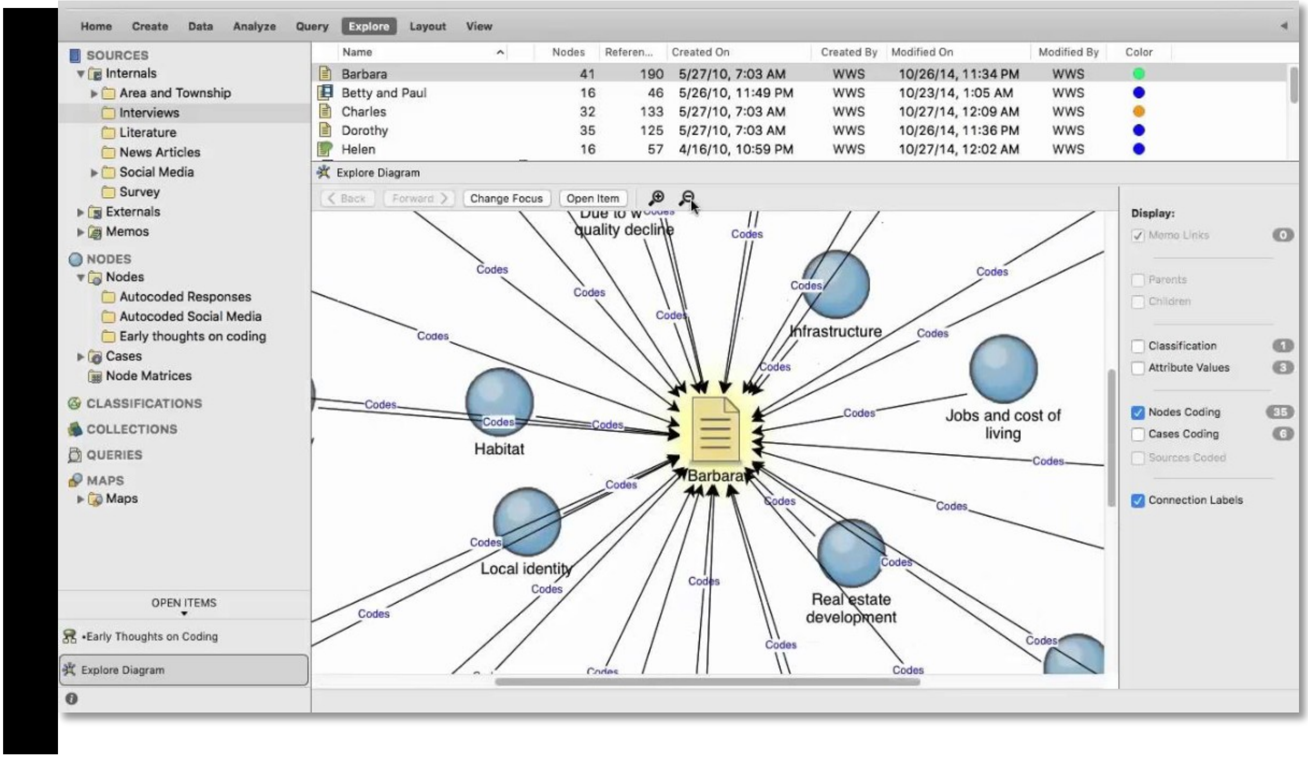
Total: 138

4.5. Qualitative Analysis

For the qualitative feedback research element, the analysis process will involve both inductive and deductive approaches, that is, categorising data according to what emerges from the data themselves (bottom up) and exploring whether the data are consistent with prior assumptions, theories, and hypotheses outlined by the researcher (top down) (Krueger and Casey, 2008).

The former approach will be used to explore participant experiences in the intervention in general (e.g. what worked, what was beneficial, what didn’t work), and the latter approach will be used to explore whether the intervention could have any bearing on a patient’s decision to attend ‘standard’ appointments. Experiences, opinions, feelings, knowledge that emerge from this will be analysed.

NVivo (data storage and analysis software) (Fig. 10) will be used by the researcher to code data and organise the data. Some content analysis may be carried out in addition to the main overall narrative analysis. Codes will be assigned to the data (labels attached to information, with a view of identifying similar or related information).



Name	Nodes	Referen...	Created On	Created By	Modified On	Modified By	Color
Barbara	41	190	5/27/10, 7:03 AM	WWS	10/26/14, 11:34 PM	WWS	🟩
Betty and Paul	16	46	5/26/10, 11:49 PM	WWS	10/23/14, 1:05 AM	WWS	●
Charles	32	133	5/27/10, 7:03 AM	WWS	10/27/14, 12:09 AM	WWS	●
Dorothy	35	125	5/27/10, 7:03 AM	WWS	10/26/14, 11:36 PM	WWS	●
Helen	16	57	4/16/10, 10:59 PM	WWS	10/27/14, 12:02 AM	WWS	●

Display:
- ☑ Memo Links 0
- ☐ Parents
- ☐ Children
- ☐ Classification 1
- ☐ Attribute Values 3
- ☑ Nodes Coding 35
- ☐ Cases Coding 6
- ☐ Sources Coded
- ☑ Connection Labels

Figure 10. NVivo is a qualitative data analysis package - useful for rich text-based and/or multimedia information and deep levels of analysis (QSR, 2016).

4.6. Software used to assist the analysis of data collected will include

- R (statistical computing environment) + various add-on packages
- Microsoft Excel
- NVivo
- Adobe creative suites

The only person to access the raw primary data will be the lead researcher (Jake Robinson) and the Chief Investigator (Prof. Anna Jorgensen). The aggregated data will be archived with the current ESRC Data Service Provider - the UK Data Service.

All data will be kept in digital format and will be stored and encrypted on the university's secure server. No confidential information will be stored on personal devices or portable storage systems such as USB memory sticks or external hard disk drives.

Although no hard / paper copies of the primary data are expected to be produced, in the unlikely event that this is required, the documents will be stored in a locked cabinet and disposed of appropriately after use (i.e. shredded).

5. THE INTERVENTION

- Participant information sheets (and a link to a video) will be provided by the GPs and the patients will be invited to attend an introductory session (ran by the researcher and co-facilitator).
- During the introductory session the research project will be described in full and the patients will have chance to ask questions and shape the green prescribing activities and setting. The research consent form will be given to the prospective participants at these sessions and the PHQ-9 will be given to the patients to complete in their own time (prior to the start of the intervention). The patients can then return the completed survey to the GP or bring to the researcher in a sealed envelope (provided).

- During this session, attendees will be informed that, following the introductory session if they consent to participate in the study, they will be randomised into either an ‘Intervention Group’ or a ‘Control Group’.

The Intervention Group

- Participants who have been randomly selected to participate in the Intervention Group, will attend a green prescribing session at their local GP surgery (once per week for 12 x weeks in total). Each session will last for approximately 1 hour. During the sessions the participants can take part in gardening, activities, creative and nature-based activities, learn about wildlife, and help to make the area more wildlife friendly. They do not need to participate in any given activity but should feel comfortable in the outdoor environment and being around other people as there will be other participants in the study.

- These sessions will be flexible and participants will be able to cross over into sessions held at the other local participating practices.
- The researcher will ask the participant to complete a set of short mental health and wellbeing-related questionnaires at the start, middle and end of the 12 weeks. They will also be invited to attend a focus group at the end to provide feedback.
- An important aim will be to create a welcoming environment and provide refreshments (drinks/snacks) for the participants. Creative input from the participants will also be sought throughout the intervention.
- The researcher and patients will carry out various gardening, creative and wildlife-related activities. For example, seedlings (easy crops e.g. lettuce, radish, spinach, and spring flower bedding) will be planted, using staggered planting methods. Bird feeders will be installed/maintained and wildlife identification skills and guides will be provided

by the researcher. ‘Noticing nature’ prompts will be given and creative/artistic activities will also be considered.

- As the initiative progresses, patients will learn how to maintain crops, learn about wildlife, ecology, nutritious recipes etc. Participants can also help to enhance local biodiversity through strategic planting and maintenance (e.g. biodiverse wildflower meadows).
- Patients will eventually be able to harvest their own crops for free.
- For future coordination of these activities, there is also the potential to provide patient training.

NB: A full (flexible) schedule will be produced prior to intervention, and the researcher has sought advice from experienced social and therapeutic horticulture practitioners, and mental health service users and facilitators.

The Control Group

- If the participants are randomly selected to participate in the Control Group, they will not attend a green prescribing session for the 12 week study but will continue receiving ‘usual’ care. However, they will have the opportunity to use the garden once the 12 week study is complete. The researcher will still ask participants in the Control Group to complete a set of short mental health and wellbeing-related questionnaires at the start, middle and end of the 12 weeks.

6. STUDY SETTING

The proposed research trial will take place across five-six GP practices in the Network North Primary Care Network (PCN) region of Sheffield, UK (area within the red circle in Fig. 1).

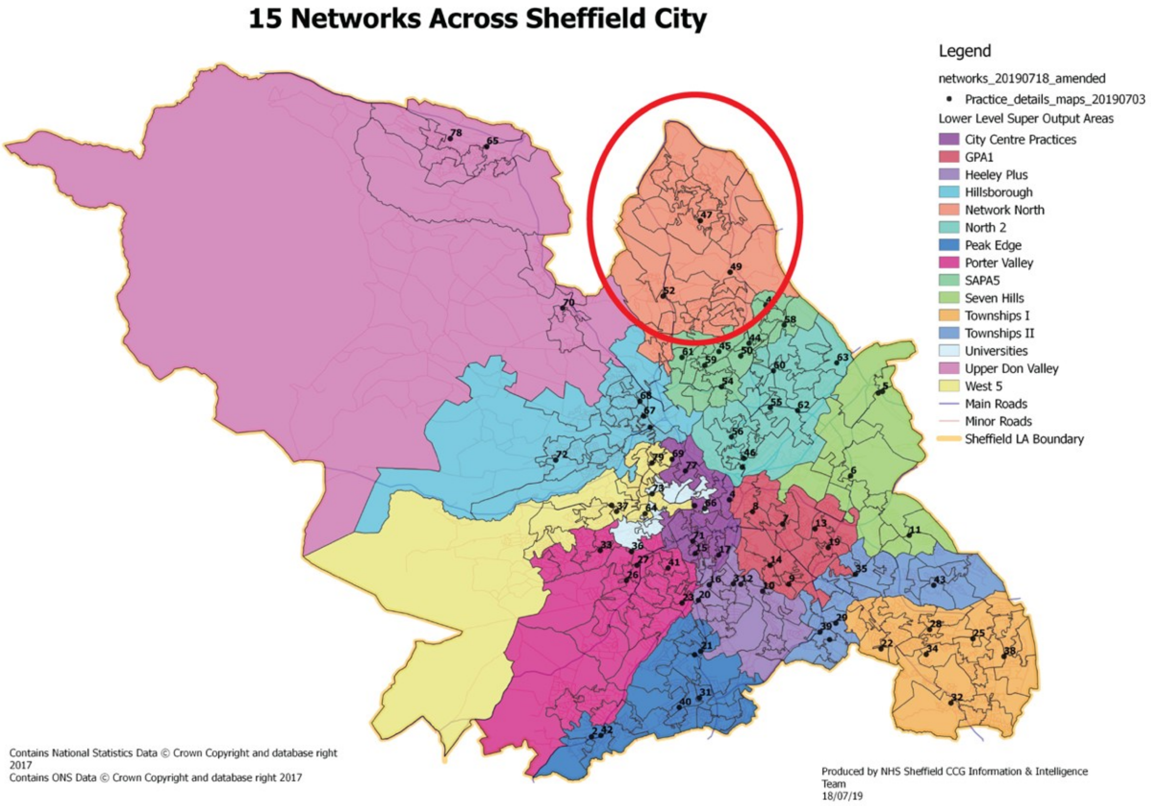


Figure 1. Map showing the fifteen PCN boundaries across the Sheffield City Region. Network North is indicated by the red circle

6.1. Potential Research Sites

6.1.1. High Green Health Centre (Fig. 3)

Vacant area: 1,127m^2 (mostly greenspace)

Figure 3. High Green Health Centre has two potential areas for the pocket gardens. High Green is situated in the far-north of the Network North PCN (inset

6.1.2. Burncross Surgery (Chapelgreen Practice) (Fig. 4)

Vacant area: 880m^2 (60% greenspace)

Figure 4. Burncross Surgery has two potential areas for the pocket gardens. Burncross is situated in the central-north of the Network North PCN in Chapeltown (inset).

6.1.3. Ecclesfield Group Practice (Mill Road) (Fig. 5)

Vacant area: 983m^2 (90% greenspace)

Figure 5. Ecclesfield Group Practice (Mill Road) has three potential areas for the pocket gardens.

Ecclesfield is situated in the central-east of the Network North PCN, to the north of Ecclesfield (inset).

6.1.4. Grenoside Practice (Fig. 6)

Vacant area: 600m^2 (90% greenspace)

Figure 6. Grenoside Practice has two potential areas for the pocket gardens. Grenoside Practice is situated in the central-west of the Network North PCN, in Grenoside (inset).

6.1.5. Ecclesfield Group Practice (Remington Avenue) (Fig. 7)

Vacant area: 200m^2 (90% greenspace)

Figure 7. Ecclesfield Group Practice (Remington Ave) has two potential areas for the pocket gardens. This Ecclesfield Group Practice is situated in the central-east of the Network North PCN, in Ecclesfield (inset

7. SAMPLE AND RECRUITMENT

7.1 Eligibility criteria

Eligibility Criteria is as follows:

Only adults - over 18 years of age with mild-moderate depression. Patients will be residents of North Sheffield and part of the Network North Primary Care Network (PCN), and will preferably be comfortable with outdoors and social settings.

7.2. Size of sample

Potential participants will be identified by the GPs in the Network North PCN (i.e. the GPs will determine initial suitability). The study aims to recruit 240 participants across five practices (120 for the intervention and 120 for the control). This includes a 20% dropout buffer.

7.1.2 Sampling technique

Adhering to the predetermined inclusion criteria, a sample population will be established by the GPs and the aim is to secure x240 potential participants (x120 in the intervention arm, and x120 in the control arm).

To reduce the risk of dysbalanced groups due to confounders, block randomisation/a priori stratified sampling will be conducted (Spieth et al. 2016), whereby gender and age will be blocked and participants from each block will be randomly assigned to the treatment or the control group.

The randomisation process will be explained fully during the introductory sessions, and will occur post-introductory session (and post-receipt of signed consent forms).

7.3 Recruitment

Potential participants will be identified by the GPs in the Network North PCN. The study aims to recruit x240 participants across five practices. The participants will be adults over 18 and will have been clinically diagnosed with mild-moderate depression – i.e. diagnosed by their GP who will use the PHQ-9 depression severity instrument (a method included in the study) or a similar method. There will be a combination of identification processes involved – new patients and existing patients (computerised search to be conducted by GPs).

All aspects of patient record identification will be conducted directly by the healthcare team (GPs).

The researcher will hold an introductory session to discuss the project with the potential participants face-to-face and to allow the participants to shape the activities and ask questions about the study.

The researcher will also collect the informed consent via a simple signature-based document and will collect baseline and follow-up research data via questionnaires.

GPs will take a leading role in the patients’ first contact/introduction to the study during standard appointments for related conditions – i.e. if patients are being treated for symptoms of mild-moderate depression, this study/intervention trial will be suggested by the GPs.

7.4 Consent

For this stage of the research, the participants will be fully informed of all aspects of the research project by providing a comprehensive Participant Information Sheet, summary video, and an invitation to an introductory session where the participants can find out more prior to consenting.

After reading the Participant Information Sheet (provided by the researcher and distributed by the GPs), the reader can choose to either ignore and decline, or agree to attend an introductory session. The consent form will be given to the potential participants at the introductory session and the randomisation process will be fully explained. The potential participants will then be able to take the Consent form away with them and return within 2 weeks. The Consent form will contain the following structure/information:

Taking Part in the Project	**Please**
I have read and understand the Participant Information Sheet dated 100120 and the project has been fully explained to me.	
I have been given the opportunity to ask questions about the project	
I agree to take part in the project and I understand that taking part in the project will include either: 1. Attending 12 x green prescribing sessions (nature-based activities) and potentially a focus group meeting at the end of the trial (not compulsory). This option is only if you are randomly selected to participate in the <u>Intervention Group.</u> 2. Carry on receiving 'usual' treatment/advice from your GP for the same 12 weeks *without* attending the 12 x green prescribing sessions. This is only if you are randomly selected to participate in the <u>Control Group</u>.	

I understand and give permission for the researcher to provide a set of research questionnaires for me to complete at the beginning, mid-point, and the end of the study with the potential for follow-up questionnaires 3 months later.	
I understand that my taking part is voluntary and that I can withdraw from the study at any time; I do not have to give any reason for why I no longer want to take part and there will be no adverse consequences if I choose to withdraw.	

How my information will be used during and after the project	**Please initial**
I understand my personal details such as name, phone number,	
I understand and agree that the anonymised responses I provide on the research questionnaires and/or focus group and the analysed data can be used in the research and published in journal publications, reports, web pages, conferences and other research outputs.	
I agree for medical staff to share with the researcher, how many times I have attended a GP appointment in the six months preceding the study.	

I understand and agree that other authorised researchers may use the anonymised research data in publications, reports, web pages and other research outputs.	
I give permission for the questionnaire and focus group data (if applicable)	
If I choose to take part in a focus group at the end of the study, I give permission to the researcher to record the session and use the transcribed audio recordings in the study.	
So that the information you provide can be used legally by the	**Please initial**
I agree to assign the copyright I hold in any materials generated as part of this project to the University of Sheffield	

See attached Participant Consent form (IRAS 263091 Participant Consent Form 100120) for more information

8. ETHICAL AND REGULATORY CONSIDERATIONS

The participants will be informed that they can withdraw at any stage of the research without providing a reason.

Participating in this research project is not anticipated to cause any significant disadvantage or discomfort, physical or psychological harm to the potential participants. There is a possibility that a minor injury may be incurred through the use of small handheld gardening equipment (i.e. trowel).

Based on the outdoor nature of the intervention, there is potential for harm to participants through typical environmental hazards – in the absence of mitigation these could include sunburn, dehydration, vector-borne pathogens, slips, trips and falls.

There is potential for the research questions (via validated questionnaires) to evoke difficult memories or experiences that may cause upset to some participants who recollect distress or trauma. Besides from the PHQ-9 depression severity questionnaire (carried out by the GPs at inclusion stage and provided during the introductory session for baseline assessment), and PSS, the wellbeing and nature-connectedness questions do not focus on negative mental health experiences – they are deliberately worded in a positive way. The social and outdoor setting may evoke uncomfortable feelings in some of the participants, which is one of the key reasons for fully explaining the nature of the activities and for purposively selecting participants who are more comfortable with this setting. Participants will be informed that they may stop at any time or take a break from the session (and they do not have to participate in any given activity and can opt out at any point).

Participants will receive guidance from the researcher throughout the 12 weeks to highlight the importance of confidentiality among the participants, and to this end, ‘ground rules’ will be shared at the intervention outset.

Information about the study, data collection, storage and who to contact for further information will always be available to the participants through the study via the participant information sheet. The study does not deny any participants access to care / clinical appointments. The researcher will create a welcoming environment in each of the study sites to help the participants feel at ease. The researcher will obtain views of participants during the introductory session as well as from other mental health service users and facilitators to help inform the setting and activities. Participants could potentially feel a sense of loss as the research trial draws to a close. Throughout the sessions the researcher will aim to keep the interventions sustainable following the research trial by liaising with the GPs, the CCG, mental health service providers and the participants. Volunteers could be recruited from the groups to help sustain the initiative once the research part is complete. The researcher also aims to stimulate other pocket gardening projects across Sheffield and potentially the UK, generating interest from various stakeholders to sustain the momentum.

Prior to participating, the potential participant will be informed by the researcher, of how to contact the lead researcher or Head of Department if they have any concerns about the research or are experiencing any stress or harm.

The legal basis for processing the data will be: 'A task in the public interest' (6(1)(e)) under the EU General Data Protection Regulations (GDPR), is considered to be an appropriate legal basis for processing personal data in this research project.

8.0 Indemnity

The insurance for this research is covered by the sponsor (University of Sheffield). The sponsor insurance policy number is: NHE-03CA06-0013 - see the sponsor’s insurance certificate (IRAS 263091 Sponsor Insurance Certificate 160819) for more information.

8.1 Assessment and management of risk

To minimise the risk of injury, each participant will be given their own pair of gardening gloves – providing protection and grip. A ‘toolbox’ talk will also be given by the researcher at the start of each session (to explain how to participate safely) and a dynamic risk assessment will be conducted. The participants will also be informed that they do not need to participate in any activity if they feel uncomfortable in doing so, and will be given assistance where needed.

A detailed Risk Assessment will be produced for general use and a dynamic risk assessment will be produced at the start of each session to ensure the local environment and associated risks remain the same. These hazards will be highlighted in the toolbox talk. A site recce will be conducted during each dynamic risk assessment to make sure the site is free of significant hazards. Refreshments, sun-cream and first aid kits will all be present on site and a named first aider will be established.

There is a risk of tetanus due to presence of Clostridium tetani in soil. The researcher will liaise with the PCN clinical Director Dr Nicola Moody to ensure participants included in the study have up to date tetanus vaccinations. The researcher has up to date vaccinations.

8.2 Research Ethics Committee (REC) and other Regulatory review & reports

This study has been ethically reviewed and approved by the internal ethics review committee in the Department of Landscape, University of Sheffield.

Regulatory Review & Compliance

- The researcher will comply with all conditions set out by the internal research ethics committee and the NHS REC.
- For any amendment to the study, the Chief Investigator or designee, in agreement with the sponsor will submit information to the appropriate body in order for them to issue approval for the amendment

- The Chief Investigator or designee will work with sites (R&D departments at NHS sites as well as the study delivery team) so they can put the necessary arrangements in place to implement the amendment to confirm their support for the study as amended.

Amendments

Amendments will be handled in line with the sponsors and site management organisations policies. See above for more information.

8.3 Patient & Public Involvement

The patients will be involved in the design of the study by providing feedback at the introductory sessions. This will subsequently inform the final activities/environmental design.

The researcher has also involved other members of the public in the design of the intervention. For example, the recruitment strategy and activity selection for this research has been informed by Andy Whitehouse, a psychotherapist and an experienced Resilience Team Leader with the Royal Voluntary Service (and gardening intervention coordinator); Dr Jo Birch, an academic at the University of Sheffield, with experience in mental health interventions; Corrine Moss, an experience community and mental health professional; and Dominic Schad, an experienced mental health and social and therapeutic horticulture service provider. The researchers have also drawn upon advice from Mike Tomson, a retired GP with experience of treating mental health service users, and the Greener Practice group – a group of GPs with interests in the environment, from across the Sheffield area.

The researcher has also visited Sheffield's Flourish Oasis mental health garden (and previously Muddy Fork Social and Therapeutic Horticulture group in Retford) to acquire the views and opinions of the service users and facilitators - e.g. what they thought of the design, methods and activities involved in the proposed study.

8.5 Protocol compliance

Accidental protocol deviations can happen at any time. They will be adequately documented on relevant forms and reported to the Chief Investigator and Sponsor immediately.

It is acknowledged that deviations from the protocol which are found to frequently recur are not acceptable, and will require immediate action and could potentially be classified as a serious breach.

No significant deviations from the protocol are anticipated.

8.6 Data protection and patient confidentiality

The only person/s to access the raw primary data will be the lead researcher (Jake Robinson) and Chief Investigator (Prof. Anna Jorgensen). The questionnaire and focus group responses will be locked in a secure cupboard/room prior to digitisation and data analysis.

Digitisation will occur within 3 weeks following any field data collection and the hard copies/documents will subsequently be destroyed. The aggregated data will be archived with the current ESRC Data Service Provider - the UK Data Service.

All data will be kept in digital format and will be stored and encrypted on the university's secure server. No confidential information will be stored on personal devices or portable storage systems such as USB memory sticks or external hard disk drives.

8.7 Indemnity

Insurance for this research is covered by the sponsor (University of Sheffield).

8.8 Access to the final study dataset

See section 8.6.

9. DISSEMINIATION POLICY

9.1 Dissemination policy

Consent will be secured for rights to disseminate the study data by way of the Participant Consent Form (IRAS 263091 Participant Consent Form 100120). Data from this study may be used in publications, conferences and as part of the lead researcher’s PhD Thesis. Participants will be informed of the results by email notification when the study is published.

In accordance with the ESRC Research Data Policy, all data will be archived with the current ESRC Data Service Provider - the UK Data Service. I will seek to secure consent for data sharing at the start of the project in order to do this. If consent is not secured, the data from these particular individuals will be appropriately anonymised prior to being archived.

In accordance with the ESRC Research Data Policy, all data included in the research will be archived within 3 months of the end of the ESRC grant.

Aggregated data will be archived on the University of Sheffield's Online Research Data Catalogue and Repository (ORDA).

9.2 Authorship eligibility guidelines and any intended use of professional writers

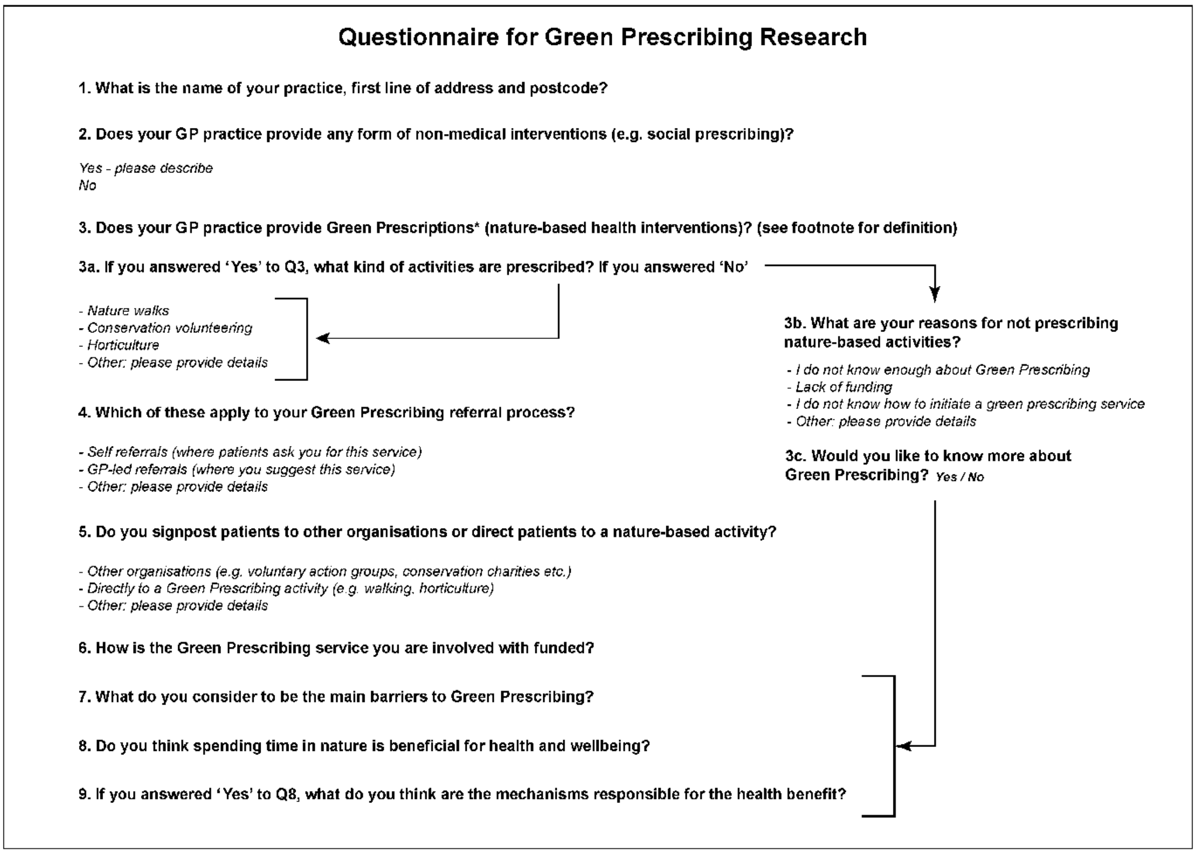
For the PhD thesis, the study author will be Jake Robinson (lead researcher). For journal publications, there may be multiple authors who contribute to the publication. There will be authorship criteria specific to the journals, however, a similar authorship framework to that used by the International Committee of Medical Journal Editors (ICMJE) will be used:

The ICMJE recommends that authorship be based on the following 4 criteria:

- Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
- Drafting the work or revising it critically for important intellectual content; AND
- Final approval of the version to be published; AND
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Appendix III.

Example of the questionnaire used in the Let Nature Be Thy Medicine study



Questionnaire for Green Prescribing Research

1. What is the name of your practice, first line of address and postcode?

2. Does your GP practice provide any form of non-medical interventions (e.g. social prescribing)?

Yes - please describe
No

3. Does your GP practice provide Green Prescriptions* (nature-based health interventions)? (see footnote for definition)

3a. If you answered 'Yes' to Q3, what kind of activities are prescribed? If you answered 'No'

- *Nature walks*
- *Conservation volunteering*
- *Horticulture*
- *Other: please provide details*

3b. What are your reasons for not prescribing nature-based activities?

- *I do not know enough about Green Prescribing*
- *Lack of funding*
- *I do not know how to initiate a green prescribing service*
- *Other: please provide details*

3c. Would you like to know more about Green Prescribing? *Yes / No*

4. Which of these apply to your Green Prescribing referral process?

- *Self referrals (where patients ask you for this service)*
- *GP-led referrals (where you suggest this service)*
- *Other: please provide details*

5. Do you signpost patients to other organisations or direct patients to a nature-based activity?

- *Other organisations (e.g. voluntary action groups, conservation charities etc.)*
- *Directly to a Green Prescribing activity (e.g. walking, horticulture)*
- *Other: please provide details*

6. How is the Green Prescribing service you are involved with funded?

7. What do you consider to be the main barriers to Green Prescribing?

8. Do you think spending time in nature is beneficial for health and wellbeing?

9. If you answered 'Yes' to Q8, what do you think are the mechanisms responsible for the health benefit?