The Hidden Half:

Blanket Bog Microbial Communities across a Spectrum of Site and Management Conditions and Impacts on Peatland Carbon Cycling

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Abstract

Monitoring blanket bog condition and functions is critical in ensuring the ecosystem services they provide, especially as their environment becomes increasingly pressured. Microbial DNA based monitoring tools allow assessment of soil communities, but our knowledge of the microbial processes underpinning ecosystem service provision is limited. Specifically, (a) how microbial communities vary with management and climate, (b) how the microbial community is related to environmental variables, and (c) whether microbial community measurements can be used to predict water quality and carbon fluxes, including methane.

Here, microbial community taxonomy and fungal community function was measured on UK blanket bog with different management, habitat or restoration status using sequencing techniques, alongside a novel carbon partitioning mesocosm experiment. Water quality variables were measured alongside this experimental set up, along with a suite of environmental, chemical and soil edaphic variables.

Fungal, bacterial and archaeal microbial communities changed with management and location. Microbial communities were highly variable but showed some categorisation based on climatic conditions. Communities subject to managed grouse moor burning were frequently different to every other habitat category.

Concentrations of zinc, magnesium and calcium were strongly associated with microbial community structure, alongside soil temperature, rainfall and the abundance of *Calluna vulgaris*. Blanket bogs with a legacy of pollution contained specifically adapted microbial taxa.

Grouse moor burning was linked to changes in fungal trophic groups, with potential consequences for water quality. Changes to the fungal and bacterial communities, alongside the abundance of sedges, were linked to changes in methane flux, and fungal community change was linked to soil respiration.

The findings here indicate that ecosystem services can be linked to management and habitat status via microbial processes, but that our knowledge remains incomplete. This thesis provides the basis for further in depth experiments, and recommends focusing on a functional, rather than a taxonomy, based approach

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I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as References.

Chapter 1: Introduction

List of abbreviations

- ARISA fingerprinting - Automated Ribosomal Intergenic Spacer Analysis fingerprinting
- ASSI - Areas of Special Scientific Interest (Irish SSSI)
- C - Carbon
- CSM - Common Standards Monitoring
- DEFRA - Department for Environment Food and Rural Affairs
- DGGE - Denaturing Gradient Gel Electrophoresis
- DOC - Dissolved Organic Carbon
- ERM – Ericoid Mycorrhiza
- ES - Ecosystem Services
- GHG - Greenhouse gase
- JNCC - Joint Nature Conservation Committee
- LTMN - Long Term Monitoring Network (Natural England initiative)
- N - Nitrogen
- NE - Natural England
- NEE - Net Ecosystem Exchange
- NGS - Next Generation Sequencing
- NVC – National Vegetation Classification
- OTU - Operational Taxonomic Unit
- P - Phosphorus
- POC - Particulate Organic Carbon
- PLFA - Phospholipid Fatty Acids
- SAC - Special Area of Conservation
- SPA - Special Protected Area
- SSSI - Site of Special Scientific Interest
- T-RFLP - Terminal Restriction Fragment Length Polymorphism
- TTGGE - Temporal Temperature Gradient Gel Electrophoresis

1.1 Introduction

Peatlands contain approximately 30% of the world's soil organic carbon, despite covering only ~3% of the global land area (Parish *et al*., 2008). In the northern hemisphere circumpolar region, generally low temperatures, high peat moisture, high water-table depth and slow decay rates of net primary production (NPP) allow soil organic matter to accumulate and peat to form, resulting in peatlands being an important habitat for carbon storage.

Blanket bog is a type of peatland, defined by poor nutrient levels (ombotrophy) and being rain fed. It comprises approximately 9.4% (Bain *et al.,* 2011) of the UK land area, which contains about 13% of this globally rare (Littlewood *et al.,* 2010) and important habitat, especially in terms of the ecosystem services (ES) it provides, such as carbon (C) storage and water quality. These bogs blanket large parts of the UK uplands with peat that has a mean depth of around 1-4 m on all but the steepest slopes (Lindsay, 2010). As with other peatlands, blanket bogs are characterised by an acrotelm/catotelm separation in relation to the mean water table depth, which generally shows an annual average depth of around 5-10 cm. Blanket bogs have been intensively managed throughout the history of the UK , particularly over the last two centuries (through agricultural reclamation and grouse shooting, Simmons, 2003) , and have suffered

from pollution, erosion, artificial drainage and agricultural management alongside burn rotation management on heather dominated grouse moors situated on blanket bog. These disturbances resulted, in some peatlands, in a change from an 'active' bog status with a long-term net C sink, to a C source, which has been associated with increases in dissolved organic carbon (DOC) in stream water, and thus, a deterioration in drinking water quality (Whitehead *et al.,* 2009, Yallop *et al.,* 2010, Holden *et al.,* 2012). However, the underlying reasons (e.g. temperature rise versus recovery from N-deposition and acidification or management) for the increased DOC over recent decades are still under debate (Holden *et al.,* 2012, Worrall *et al.,* 2018; Harper et al., 2018). In fact, recent habitat assessments point to only around 18% of the UK blanket bogs being in a natural or near natural 'favourable' condition, where the habitats and features are assessed as being in a healthy state, with appropriate management (Littlewood *et al.,* 2010) and restoration programmes are underway by several partnerships between charities, NGOs and agencies such as Natural England (NE) to halt and reverse this trend of peatland habitat degradation. Moreover, current research (e.g. Heinemeyer et al., 2019) also addresses management alternatives for grouse moors such as mowing, typically used as an alternative for burning as a vegetation fuel load management tool, and its ES impacts compared to burn management on heather dominated blanket bog, which is still widespread and increasing (Douglas *et al.,* 2015) although mostly restricted to grouse moors in the north of England.

A key part of restoration work involves monitoring the habitat condition. A significant component of this is assessment of above ground vegetation composition and structure (JNCC, 2009), and restoring the hydrological functioning of the bog (i.e. ensuring wet conditions with high water tables), which is based on the acrotelm/catotelm concept (Ingram, 1978). Generally, sedges and *Sphagnum* mosses are seen as indicative of an 'active' peat forming condition, whereas species such as heather and non-*Sphagnum* mosses are perceived as indicative of degraded bog with a deep acrotelm, acting as a carbon source (losing carbon and often showing signs of erosion). So far, no common UK habitat assessment method uses belowground biota in their habitat assessments, despite soil biota likely having a large impact upon soil processes (e.g. peat decomposition, DOC export) with impacts on ES provisioning (e.g. C storage and drinking water). Although some work exists on below ground taxa in peatlands worldwide (Andersen *et al.,* 2013), which has identified some changes in peatland soil microbial communities following disturbance, considerable knowledge gaps remain. For example, questions remain regarding the relative importance of diversity, redundancy and function of peatland soil biota, specifically, **is loss of diversity or the loss of individual taxa more important in governing the ecological responses of peatlands to change?**

We have good descriptions of many individual taxa present in peatland soils, such as phenolic degrading bacteria (Fenner *et al.,* 2005), methanogens (Juottonen *et al.,* 2005), N-fixing bacteria (Doroshenko *et al.,* 2007) and increasingly so the fungi – especially ascomycetes and zygomycetes, with basidiomycetes tending to be less common (Thormann, 2006). However, we are likely missing many taxa. Specifically, the role of symbiotic ericoid mycorrhizal fungi in peat decomposition remains unclear (Morton, 2016). Older techniques such as phospholipid fatty acid profiles and culturing have only limited potential to reveal details of biodiversity, and next generation sequencing may provide much-needed deeper ecological insights (Kip *et al.,* 2011), although whether this has been realised is a difficult question. It remains unclear how peatland soil communities relate to spatial and temporal factors (e.g. vegetation, seasons, and climate), how communities might change across a spectrum of active to degraded bog habitat status (Anderson *et al.,* 2010) and management (Anderson *et al.,* 2013). Additionally, there are questions over whether there are plant-soil related tipping points (that is, biophysical thresholds, beyond which ecosystem function is flipped to an alternative state,

from Jassey, *et al.,* 2018). The key research questions are, **how will the soil biota community react to multiple pressures of management and a changing climate, and what impact will this have on carbon storage and water quality?**

Previous Defra-funded work (Heinemeyer et al., 2019) on grouse moors situated on blanket bog covered a relatively narrow spectrum of degraded, heather dominated, habitat condition, defined by vegetation assessment. It revealed higher C accumulation (based on C flux measurements) and water storage (based on precipitation and stream flow rates) on less degraded sites (i.e. wetter and higher *Sphagnum* cover) and under mown management compared to burning and more degraded status (i.e. drier and less *Sphagnum* cover). Moreover, vegetation type was found to be correlated to DOC and water quality indicators. However, burning indicated decreased soil decomposition rates and considerable charcoal input was linked to increased carbon accumulation under higher burn frequencies, which related to peat physical changes (i.e. higher bulk density) and also potential impacts on soil microbes and their activity (Heinemeyer et al., 2019). The overall aim of this PhD is to build on this work and ascertain whether below ground biota can be linked to vegetation, restoration management and habitat status in blanket bogs, and whether this has predictable impacts upon peatlands' ES provisioning such as C storage and water quality. This project will

(i) Test whether previous empirical evidence (Heinemeyer et al., 2019) on C fluxes, hydrological functioning and associated ecosystem services (i.e. carbon storage, water quality) holds true across a wider habitat spectrum that includes more degraded as well as more intact bog.
(ii) Unravel plant-soil mediated changes in C cycling and water quality,
(iii) Contribute to the unpicking of the underpinning soil processes involved in decomposition,
(iv) Identify potential key habitat indicator biota and microbial groups/associations, and
(v) Determine climate and management impacts on the microbial community and its functioning.

Ultimately, this project aims to enable organisations (e.g. NE) to undertake wider and more robust assessments of habitat status, restoration potential and trajectory towards intact and active blanket bog.

This literature review aims to review all the major literature related to microbial populations in peatlands, especially blanket bogs. Much of the research in this area is and has been focused on Canadian and Russia peat bogs, which, whilst having many similarities, are often different in terms of their climatic envelope, vegetation and, frequently, their management (or lack thereof). Consequently, the review's focus was on research specific to the UK or research where the study site was considered sufficiently similar to UK conditions.

This review first defines and elaborates on blanket bogs, the ecosystem services concept, habitat assessments in the UK, and briefly describes the blanket bog carbon cycle, and how it relates to microbial populations. This is then followed by a more detailed examination of microbial communities in peatlands, the methods scientists have used to measure them, and past research on how they are affected by common UK management practises. It concludes with a short section on studies that feature some likely key taxa in relation to ecosystem functioning and ecosystem services.

1.2 Blanket Bogs

For the purposes of this work, blanket bog refers strictly to upland mire which is rain fed (ombrotrophic) and therefore generally nutrient poor - as opposed to peat fen which is stream or groundwater fed and therefore often richer in nutrients, being minerotrophic (Lindsay, 2010). Importantly, whilst the definition here refers to upland blanket bog, it also most likely existed at lowland elevations in the UK, but disappeared due to peat cutting and land use change such as agricultural use and cultivation (Heinemeyer et al., 2019).

The two differ in their ecosystem conditions, dominant vegetation types and could therefore likely differ in their microbial communities. Fen peatlands will not be considered as part of this thesis. Active blanket bog habitat is dominated by NVC (National Vegetation Classification) vegetation types M1, M2, M3, M15, M17, M18, M19, M20 and M25 and their intermediates (inc. common groups like sedges and the *Sphagnum* family), (Maddock, 2011). However, where peatland degradation has occurred and the water table is low, "inactive" blanket bog may be dominated more by dry heath vegetation (Hedley, 2007). This definition of blanket bog is based on the DEFRA JNCC definitions of UK Biodiversity Action Plan priority habitat (Maddock, 2008).

Bogs may be defined by the depth of their peat because this depth is fundamental to their functioning, but it is important to note that there is no formal widely held definition of "peatland" or "peat". Consequently, different soil surveys, and different scientific fields, use different peat depths to define a given area. Ecologists, for example, might use a minimum depth of 30 cm to define bog, whilst geological surveys use a depth of 1 metre. The Soil Survey of Scotland uses a minimum depth of 50 cm whilst the Soil Survey for England and Wales ranges from 40 cm depth (JNCC, 2011). This depth increases with peat accumulation, which is very slow due to the nutrient poverty of bog waters, and can range from 0.5 mm (Lindsay *et al.,* 2014) to 2.5 mm (Clymo, 1984) a year, linked to the ratio of litter input from net primary productivity (NPP) versus litter and peat decomposition (see, for example, Heinemeyer et al., 2010)

Whilst these blanket bog definitions are quite precise, it is important to note that blanket bogs are vulnerable to change, especially in terms of habitat degradation due to management (i.e. drainage), but also under climate change. Given their defining features, blanket bogs are highly sensitive to changes in temperature and precipitation (*Figure 1*), and, under a model using UKCIP02 (Met Office climate projections), the UK bioclimatic envelope, more simply stated as the amount of area where blanket peatlands can form or continue to exist, is predicted to be ~84% smaller under a high emissions scenario (Gallego-Sala *et al.,* 2010).). The potential threats to blanket bog distribution include a reduction in this bioclimatic envelope (Gallego-Sala *et al.,* 2010, Figure 2). However, these predictions by Gallego-Sala *et al.,* 2010 are based on bioclimatic envelope models and do not include plant-soil processes, nor do they consider additional aspects such as sensitivity to pollution and degradation, including from burning and deposition of atmospheric pollutants, particularly nitrogen.

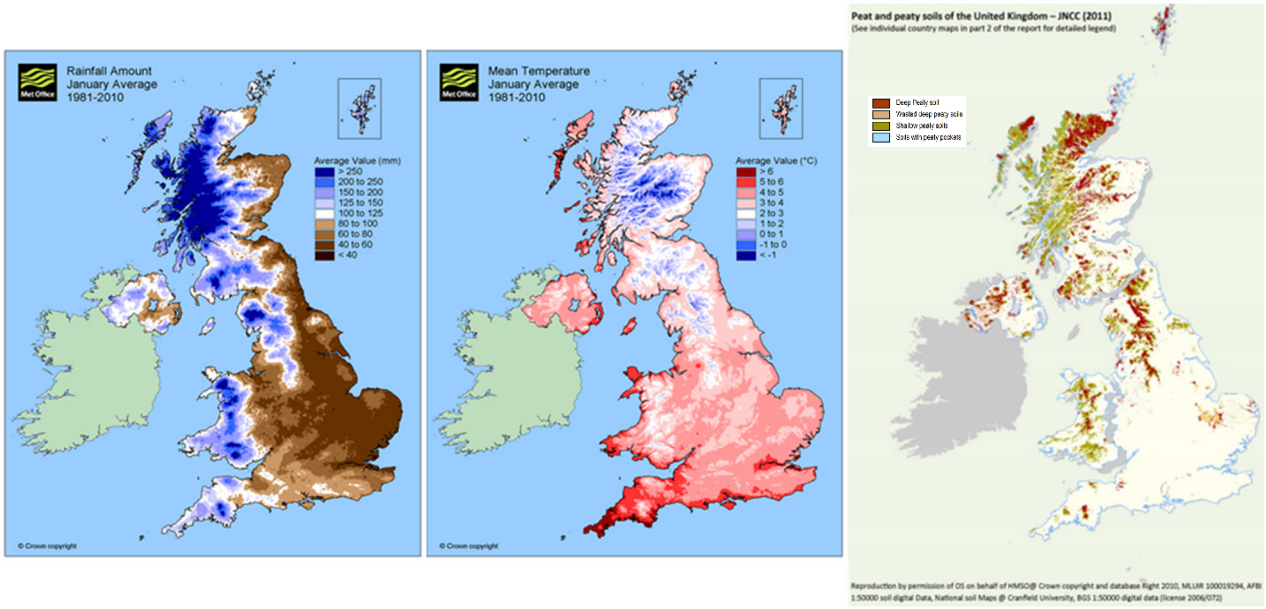


Figure 1.1: Maps of the British Isles showing the spatial correlation between Mean Rainfall, Mean Temperature and Peatland Extent. Rainfall and Temperature maps are January average 1981-2010 (Met Office, 2018). Peatland extent map taken from JNCC (2011).

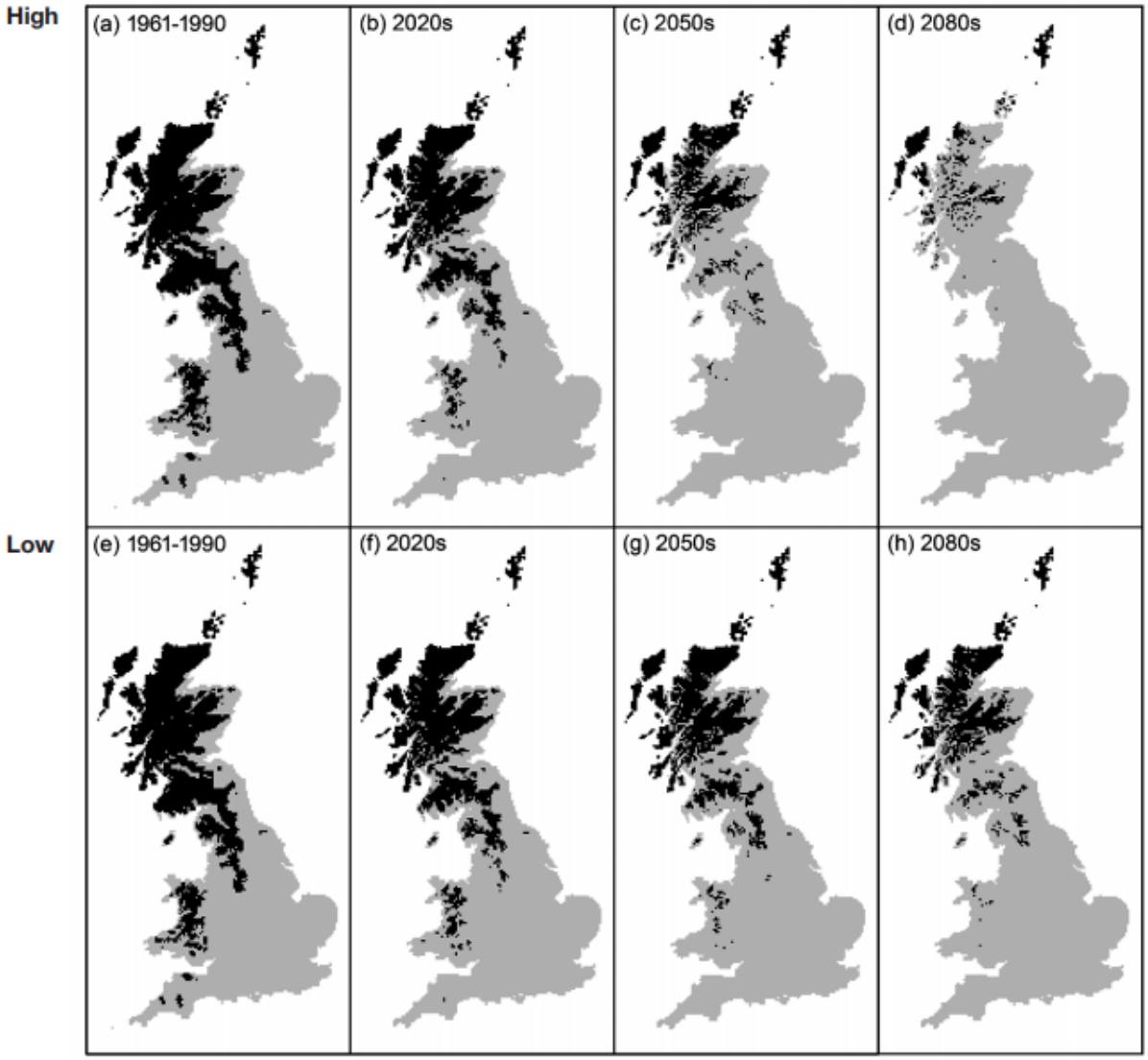


Figure 1.2: The predicted area covered by the bioclimatic envelope of peatlands under “low" and "high" emissions (defined by UKCIP02 scenarios) for times: 2020s, 2050s, and 2080s. Figure taken from Gallego-Sala et al., 2010

1.3 The Carbon Cycle in Peatlands

Bogs are characterised by their large storage of soil carbon in the form of peat (soil type classification as histosol): where net primary productivity (NPP) in an ecosystem exceeds (in an active state) decomposition of soil organic matter (SOM), this will lead or led (in the past) to increasing long-term deposition of largely undecomposed, humified SOM termed peat. This process is how peatlands function as a net carbon sink. This is primarily as a function of the water table, which, when high as it is in active upland bogs, induces anaerobic conditions in some or the entire peat column and thus acts to limit the rate of SOM decomposition. From this key function arises a model of peatland function, which divides, by depth, the peatland profile into two layers: the acrotelm and the catotelm (Ingram, 1978). The acrotelm is the uppermost layer of highly permeable and mostly aerated and vegetated but periodically saturated peat layer, which overlies the catotelm, a layer of low permeability characterised by permanently saturated denser peat with very little or no living plant matter (apart from some deep roots) and thus much reduced or no fresh carbon input from NPP. Whilst this model is a

simplification (Holden & Burt, 2003) of a more complex hydrology that is unlikely to operate over a single discrete boundary (Morris *et al.,* 2011), it is important in the context of carbon accumulation in peatlands being driven by anaerobic zones controlling rates of decomposition, which is frequently used by peatland SOM models (e.g. Bauer et al., 2003; Heinemeyer et al., 2010).

In the UK, upland peatlands evolved during the Holocene after the glacial period allowed new soil formation, leading to considerable peat accumulation in relation to key factors such as climate and vegetation but also aspect and slope (see MILLENNIA model by Heinemeyer *et al.,* 2010). Tallis (1991) provides detailed information on the initiation periods for UK peatlands, which in the Pennines mostly formed around 5-9 ky ago, but also as recently as a 1-2 ky ago. Peat initiation processes (Tallis, 1991) include natural water logging but also management impacts (e.g. deforestation) and resulting changes in vegetation cover and soil conditions (e.g. acidification). In general, there is a clear progression in soil development (pedogenesis) from mineral to peat soils linked to humus accumulation and ultimately peat layer development (see Rhodeghiero et al [in Kutsch, Bahn & Heinemeyer, 2009]).

There is also a clear positive correlation between peat depth and initiation age (Tallis, 1991). Because of these generally long-term carbon inputs, boreal and subarctic (northern) peatlands now represent a global store of some 455 Pg of carbon (Gorham, 1991), although other, more recent estimates are higher with estimates of up to 600 Gt (Yu *et al.,* 2011). However, many biotic and abiotic factors control the C cycle processes and peatlands also efflux carbon in several ways, including as part of SOM decomposition atmospherically and via the water cycle as DOC (Dissolved Organic Carbon) and from erosion as particulate organic carbon (POC).

Carbon dioxide (CO_2) and other gases such as methane (CH_4) are released from bogs when plant biomass is degraded by the belowground biota community (CO_2 is produced in aerobic conditions and CH_4 under anaerobic conditions), which in turn is controlled by various biotic and abiotic factors (Brown, 1998). These factors include, but are not limited to, water table depth, temperature, nutrient availability (in terms of soil composition and litter quality but also nitrogen and phosphorus availability [Lin *et al.,* 2014]), above ground vegetation composition, their roots and activity, and pH. CH_4 is not just important in terms of C export but also because of secondary climate forcing effects; CH_4 is approximately 25 times more potent as a greenhouse gas (GHG) measured over a 100-year period (Boucher *et al.,* 2009) than CO_2.

Another principal means of carbon export from bogs is via DOC in runoff and peat water directly into fluvial systems, which also includes export from erosion as particulate organic carbon (POC). The amount of DOC and POC in blanket bog carbon budgets has been estimated to be around 10% for DOC and 1% for POC (Heinemeyer et al., 2019, Worrall et al., 2014). DOC increases in particular, are associated with changes in water colour, which can range from dark brown to black (mediated by humic acids) to pale brown to yellow (mediated by fulvic acids), which is commonly measured by the Hazen scale (Watts *et al.,* 2001). DOC also contains colourless substances including waxes, fats, proteins and carbohydrates, which are more easily decomposed and thus have a shorter lifespan (Morton, 2016). DOC is removed from the water at water treatment plants, but the fulvic component and non-coloured substances are harder to remove, and during chlorination produce trihalomethanes, which are believed to be carcinogenic (Singer, 1999). Consequently, the release or retention of DOC can be considered as a water quality related ecosystem service, as reducing the fulvic and colourless components of DOC would result in savings to water treatment companies. POC

mostly causes sedimentation in reservoirs or blocks in filters needed to clean drinking water, thus causing additional costs in water treatment works.

Consequently, DOC is very important in the carbon budget of blanket bogs and for water provisioning and water quality (for example, burning has been linked to changes in the aquatic community in watercourses draining peatland catchments with observed lower taxonomic richness and Shannon's diversity [Brown *et al.,* 2013]). Moreover, given that DOC decomposes mostly or at least partly to CO_2 and/or CH_4 further downstream (Worrall & Moody, 2014) it is no surprise that DOC turnover related evasion of GHGs from stream surfaces returned 12% of the total C captured by NEE back to the atmosphere in a study of a Scottish ombrotrophic bog by Dinsmore *et al.,* (2010).

Recently, UK peatland DOC has been increasing with a 65% increase between 1988 and 2000 (Freeman *et al.,* 2001) rising to a 91% average by 2006 (Evans *et al.,* 2006). There are several hypotheses to explain this trend; including soil warming, increased river discharge, shifting rainfall trends and CO_2 mediated stimulation of primary productivity (Freeman *et al.,* 2004) and land management such as burning (Clutterbuck & Yallop, 2010; Yallop et al., 2010). Additional explanations include declining sulphur deposition (Reynolds *et al.,* 2013) and declining sea salt load, (Evans *et al.,* 2006). All of these hypotheses are potential drivers of change in microbial communities and their associated processes, who ultimately are the mediators of the peatland C cycle.

1.4 Ecosystem Services in UK blanket bogs

Ecosystem Services are defined as the benefits society and people obtain from ecosystems. The Millennium Ecosystem Assessment described four types of ecosystem service: *provisioning services* such as water, *regulating services* such as flood regulation and water quality, *supporting services* such as soil formation and carbon sequestration, and *cultural services* such as recreation and other non-material benefits (François *et al.*, 2005). Blanket bogs and indeed all peatlands provide ecosystem services in all four of these categories, and consequently they are important for human wellbeing. For example, in terms of provisioning, approximately 70% of our drinking water comes from upland catchments (RSPB, 2014) and in terms of regulating services, healthy peatlands certainly provide water quality related benefits, although these are hard to quantify (Martin-Ortega *et al.,* 2014). Carbon sequestration as a supporting service can be quantified, and rewetting damaged peatland (via grip blocking) can result in 2.5 t CO_2e savings per ha per year, costing approximately £15/t CO_2. This is effective relative to other CO_2 saving methods such as afforestation which can cost up to £25/t CO_2 (Bonn *et al.,* 2014). There are few papers on the value of cultural ecosystem services derived from peatlands (Kimmel & Mander, 2010).

Scientists have identified between 13 and 18 individual ecosystem services provided by upland peatlands (Bonn *et al.,* 2009, Kimmel & Mander, 2010). However, work by Evans *et al.* in 2014 found the underpinning evidence on management and climate impacts on ecosystem services for blanket bogs is weak, particularly in terms of anthropogenic (e.g. management) factors. This is because quantifying a pressure-response is difficult, particularly due to differing methods across studies, which impedes the integration of data from several areas. In addition, many studies attempt to detect a single and significant experimental effect rather than searching for the continuous relationship (e.g. we not only need to see what DOC concentrations in drainage waters do in relation to climate, but also over different temporal and spatial scales) required to make conclusions about the effects of various interacting pressures on ES provisioning. Such problems with quantifying pressure-response measures are

compounded by spatial and temporal variability in UK blanket bogs - an anthropogenic pressure may lead to decreases in water quality measures in the Peak District but may not have the same effect on the Flows of Caithness. Additionally, temporal variability can confuse the situation, as the pressure response relationship may change by time/season, and ecosystem reactions may lag behind pressures. Clearly, empirical data, which link a pressure to its response, is an important avenue for further study. This work will focus mainly on two ecosystem services: carbon storage and water quality.

Whilst the ecosystem services concept has been criticised for undervaluing ecosystem functions (Peterson *et al.*, 2009) and for ignoring the intrinsic value of natural systems (McCauley, 2006) it is seen as a useful tool for presenting to policy and decision makers the benefits of sustainably managing our natural assets.

1.5 Habitat Assessments of British Blanket Bog

Current habitat surveys and mapping tend to be based mainly either upon the JNCC Phase 1 (structure) and Phase 2 (composition) Habitat Survey (which is designed to quickly survey an area based upon its vegetation characteristics and wildlife habitat, whilst also making note of topographical and substrate features (JNCC, 2010)), or the vegetation communities of the National Vegetation Classification (NVC), (Rodwell *et al.*, 1991 *et seq.*). The JNCC Common Standards Monitoring (CSM) guidance is used to assess the condition of habitat features in designated (SSSI/ASSI, European SAC and SPA, and RAMSAR International Convention sites). CSM is used to assesses the site features for which the site was designated rather than the site in general (Williams, 2006). It has also been used to assess corresponding habitats on non-designated sites (Critchley, 2011).

Both the Phase 1/2 assessments and the CSM mainly assess habitats based on vegetation composition and structure, which excludes measures of function, though physical features and impacts are also recorded - for example, in blanket bog, the amount of bare peat or eroded peat and burning and grazing impacts (JNCC, 2009). However, there is no current habitat assessment used in the UK that includes information on soil microbial communities. Consequently, the soil microbial composition, diversity or functionality is not directly considered when assessing habitat status of UK blanket bog. This is primarily because of technological, logistical and financial issues; when the Phase 2 survey method (aimed at classifying plant communities) was first used in 1979, measuring the microbial community composition was impossible using genetic techniques (sequencing DNA had only been achieved a few years before). Although high throughput sequencing was invented in the year 2000, it is only a decade or so later that the cost has fallen to a reasonable level to allow its application in wider ecological assessments of soil microbial communities. Therefore, tools are now available to conduct Phase 2 survey equivalents for soil microbial community classification.

As key drivers of ecological soil processes and functions in peat bogs, it is important, now that the tools are available, to include the soil microbial community in the assessment of peat bog health. This has been recognised by Natural England, who have started to use PLFA measurements (for microbial biomass and community composition) and TRFLP (for genetic diversity) in the Long Term Monitoring Network of sites (Nisbet *et al.,* 2017, Natural England EIN024, 2017). This PhD intends to expand upon that work by attempting to link microbial community structure and diversity measures to habitat health and function of blanket bog using high throughput next generation sequencing methods. However, one of the inevitable downsides of microbial community studies is their high fine-scale spatial heterogeneity and

temporal (e.g. seasonal) changes. Consequently, there are issues to be considered regarding the relevant spatial and temporal scales of data for guiding future management or monitoring changes over time, for example, due to climate or drainage change drivers.

1.6 Microbial communities in blanket bogs

Blanket bogs host a diverse microbial community that extends across prokaryote, eukaryote, archaeal and fungal flora (Anderson *et al.,* 2013). The microbes are constrained by the rather poor environmental (e.g. low temperatures, nutrient poor), physical (e.g. peat pore spectrum, high water-tables) and chemical (e.g. low pH and often limited oxygen with steep concentration gradients and no mineral component) soil conditions, and therefore many are highly functionally specialised and the populations have a wide metabolic diversity. The microbial community is instrumental not just in carbon turnover, but also in nutrient cycling and uptake and as a result, they are linked with plant productivity and whole ecosystem functioning (Anderson *et al.,* 2013).

The model of depth stratification provided by Ingram (1978) where bogs are divided into acrotelm and catotelm, with a periodically saturated mesotelm between them (Clymo & Bryant, 2008), is a defining feature of blanket bog. It is not surprising that the microbial communities within peat bogs are similarly depth stratified (Lin *et al.,* 2012). This stratification is likely controlled by the hydrological balance, which further controls soil edaphic factors such as litter accumulation, nutrient and oxygen availability and thus decomposition rates. In general terms, there is a decrease in biomass and community diversity with depth (Jaatinen *et al.,* 2007), and the community profile changes with depth (Morales *et al.,* 2006), as decomposition in the deeper layers relies on more specialised species coping with the low oxygen and nutrient levels (Artz *et al.,* 2006) in addition to increased recalcitrant matter (e.g. Heinemeyer et al., 2010). A study of microbial communities in bog and fen at a site in the United States found a P-limitation in the acrotelm and catotelm, but greater soluble P level in the mesotelm, wherein is found a corresponding hotspot of bacterial diversity (Lin *et al.,* 2014).

The microbial community within blanket bogs is likely to be the key mediator of the decomposition processes that govern the provision of ecosystem services such as carbon sequestration and water quality. The very nature of a bog being either a net C sink (growing) or C source (depleting) is the imbalance between production (from vegetation) and decomposition, which is largely a product of the composition and activity of the below ground microbial community driving the humification of soil organic matter (peat) and the resulting decomposition products (e.g. DOC). There is, however, very little work that provides mechanistic understanding of the roles specific microbes or groups play in these processes (Littlewood *et al.,* 2010, Andersen *et al.,* 2013, Elliot *et al.,* 2015).

In the UK, blanket bogs are dominated by several common microbial phyla, most often the Proteobacteria, Acidobacteria, Actinobacteria and the Bacteroidetes. Verrucomicrobia, Elusimicrobia, and bacteria from the AD3,TM-6 and WPS-2, groups of bacteria that have never been cultured but have only been detected via sequencing: taxonomically they are “candidate phyla”, have also been found in UK samples of managed peat bog, from degraded (bare) peat to ‘active’, pre-disturbance vegetation (Elliot *et al.,* 2015). Other studies in the UK have found cyanobacteria and archaea such as Euryarchaeota (Potter *et al.,* 2017). However, hardly anything is known about climatic or habitat differences in these communities nor about variability in space and time. For example, at a phylum level taxonomic resolution, it is difficult to include ecological information about the more ubiquitous phyla. Proteobacteria, for example, contains over 1600 morphologically and physiologically diverse species (this is

reflected in it being named after Proteus, the Greek god who could take many forms!) and without a finer taxonomic resolution, it is hard to tell how members fit into the environment (Kersters *et al.,* 2006). The Acidobacteria are similarly diverse (Thrash & Coates, 2015) although many of them, including the type species, are (unsurprisingly for a group found in bogs) acidophilic (Hiraishi *et al.,* 1995). The Bacteroidetes, one of the main phyla making up the bacteria, is likewise diverse (Gupta & Lorenzini, 2007) and identifying these phyla within peat does not provide us with many clues regarding ecosystem function.

Other identified groups, such as the Elusimicrobia, are more interesting. Originally named "Termite Group 1", as it was originally found in the hindgut of termites, this group is now found in soils and contaminated aquifers (Zheng & Brune, 2015). It is an obscure group, but at least one of the species, *Elusimicrobium minutum*, is obligate anaerobic, being from the gut biome (Herlemann *et al.,* 2009) and another species, *Endomicrobium proavitum* possesses a set of genes (nifHDK) required for nitrogen fixation (Zheng & Brune, 2015). The Verrumicrobia are similarly interesting, as some are known to be acidophilic methane oxidisers, which, due to a lack of some gene groups, can be inferred to use novel methanotrophic pathways (Dunfield *et al.,* 2007). Other species of Verrumicrobia are extreme methanotrophs, using methane as a sole energy source under oxygen limitation in pH conditions as low as pH 0.8 (Pol *et al.,* 2007).

1.7 Microbial methods in peatland ecology

Research into the microbial populations of peatlands has been occurring for some time, primarily with the use of cultures (Dedysh *et al.,* 1998, Bräuer *et al.,* 2006) yet we have known for over a century that the majority of microbial life cannot be cultured in this way (Stewart, 2012). Consequently, it is hard to capture the microbial diversity of peatland soils, and how they are linked to function.

One alternative has been to measure proxies of microbial life, such as the quantity of phospholipid fatty acids (PLFA) in the soil. PLFAs are components of all cellular membranes, and they can be assigned to groups, giving a (very) broad overview of the community composition, likely function and physiology. PLFA analysis has been used successfully in studies in bogs, especially where simple measures have been made, such as changes in total microbial biomass (Bragazza *et al.,* 2012) or distinguishing total amounts of bacteria and fungi (Jaatinen *et al.,* 2007). PLFA does have drawbacks, however, as it can only distinguish macro-taxonomic groups and not smaller taxonomic units such as species (Quideau *et al.,* 2016), giving only an overview of the microbial community. It is for this reason that PLFA cannot, and should not, be used for assessing diversity and results cannot be converted into diversity indices such as Shannon-Wiener index (Frostegård *et al.,* 2011). Other issues affect the accuracy of a PLFA result. For example, the quantity of PLFAs in cells can change under different physical conditions (especially pH). This means that comparisons between two communities with differing physical conditions might not be easily comparable, and, in addition, there are questions posed by some researchers over whether PLFA are preserved in dead organisms existing within deep, saturated sediment - a problem that would affect peat soils in particular (Green & Scow, 2000).

An alternative to these methods is to measure and categorize the community directly through DNA based analyses, for which there are methods that have been developed at a rapid pace over the past few decades. These are culture-independent *in situ* approaches that can elucidate many more members of the community. However, DNA methods sometimes cannot distinguish between DNA from inactive (e.g. old fragments) and active (i.e. living microbes)

organisms. Moreover, methodological issues are likely with different affinity of organisms' DNA during the extraction or amplification processes and different abundance levels of certain DNA material within cells.

A simple method of examining soil community DNA is denaturing gradient gel electrophoresis (DGGE) which has been successfully used in several peatland studies in Britain. DGGE separates PCR products from environmental samples, "clamped" at one end by a high GC sequence into bands along a gel, which contains an increasing concentration of denaturing compound. As DNA moves along the gel, it denatures at different points depending on its sequence composition, and stops because of the GC clamp. Each sequence stops at a different point, creating a "fingerprint". DGGE was used by Linton *et al.* (2007) in peatlands of the Southern Pennines to determine differences in microbial community profiles across peat bogs of varying heavy metal, which included copper, lead, zinc, cadmium, aluminium, iron and manganese. Sites with highly bioavailable metals were found to have more extremophile bacteria. The study found that stress from toxic heavy metals only affected species composition and not diversity - but this would not have been found had Linton *et al.* not employed additional DNA sequencing on each band of the DGGE gel. This demonstrates one of the problems with using DGGE described by Sekiguchi *et al.* in 2001, who found that single bands in the DGGE gel could sometimes represent several different bacterial isolates. Other studies have found that multiple bands can represent single isolates (Neilson *et al.,* 2013). Both of these problems means that whilst DGGE is a cost effective and quick method for assessing change in the community structure of soil microbial communities, it cannot be used singularly to quantitatively assess the diversity of the community. These band problems may affect the results of studies that assumed a single band represents a single species, such as the study by Trinder *et al.* (2008), which examined fungal community differences between different litter types with varying water table depths.

Another method of DNA analysis commonly employed in past peatland studies is terminal restriction fragment length polymorphism analysis (TRFLP). TRFLP investigates polymorphisms in the terminal restriction fragments of conserved molecular markers such as the 16S RNA gene (used because of its slow evolution and therefore ubiquity across groups [Woese *et al.,* 1990]). It is more sensitive than DGGE, and still provides a low-cost option for assessing microbial communities compared to next generation sequencing (Marsh, 1999), which is discussed below. The method has been used successfully in peatland studies, especially by Kim *et al.* (2012), who used TRFLP to investigate the effect of warming on a mesotrophic peatland in Wales. Kim *et al.* used TRFLP to obtain richness, evenness and Shannon diversity index figures for the microbial communities, finding that all of these measures decreased under warming treatments in the methanogen groups. However, because of the weakness of TRFLP (and any molecular technique using marker genes) being that multiple copies of a marker gene can exist in the same cell (Case *et al.,* 2007; Prakash *et al.,* 2014) it is important to note that these measures are an estimate of microbial abundance. Nethertheless, TRFLP can provide a fast and cost-effective way of assessing microbial communities, being slightly easier to quantitate than DGGE because it is detected using fluorescence (Liu *et al.,* 1997).

In the last ten to fifteen years, new techniques that use DNA sequencing have revolutionised microbiology and has given some access to what used to be a largely untapped reservoir of soil DNA (Rappe & Giovannoni, 2003, Wooley *et al.,* 2010). Technological advances gave rise to Next-Generation Sequencing (NGS) technologies, which are able to measure the metagenome - defined as the collective DNA of an environmental sample (Hugenholtz & Tyson, 2008). This NGS can be used to provide information on the microbial diversity and ecology of a given

environment without the need to culture. Whilst the term was first used in 1998, it was in 2005 that the first soil metagenomic study generated more than 100 Mbp of genetic data (Tringe *et al.,* 2005). Probably the first application of metagenomic techniques to peatland soils was by Chen *et al.* (2008b), who used metagenomic high throughput shotgun sequencing combined with DNA stable isotope probing (SIP) to search for microbial life specifically associated with methanogenesis. DNA SIP is a culture independent method of linking the identity of a microbe with its function inside the environment by using stable isotopes to probe individuals in a sample. This method has the advantage of only finding the metabolically active microbes in a sample (Radajewski *et al.,* 2000). The Chen *et al.* (2008b) study used peat samples from the Moor House National Nature Reserve (NNR), a blanket bog site in the North Pennines of England, a historically important study site for moorland studies since the 1930s. The study found previously uncultured but predominant, active methanotrophs in the peat soils there.

Lin et al. (2012), undertook a key study of microbial communities in bogs of the Glacial Lake Agassiz Peatland (in the United States) using NGS technology. They examined specific functional guilds of microbial life, including methanogens, methanotrophs, sulphate reducers, fermenters, and microbes involved in nitrogen cycling. The study compared a bog and a fen as a scenario that is analogous to the vegetation composition transitions that might occur during climate change (a favouring of grasses and sedges over mosses). Lin *et al.* (2012) found a dominance of prokaryotes in the peat soils and using their community composition measures, were able to draw interesting conclusions about the roles certain groups may be playing using already known physiological and ecological information. The community changed with changing depth and environmental variables. The biggest factors that structure microbial community composition were ammonia (which is negatively correlated to oxygen availability in the vertical soil profile) and pH. The study concluded that to be able to understand the role of certain bacterial and fungal clades in the peatlands (required for linking them to ecosystem services) more field experiments and lab ecophysiology experiments are needed.

Since Lin *et al.* (2012) there have been surprisingly few studies that sequenced the microbial community in UK peatlands. Elliot *et al.* (2015) examined the differences in microbial community assemblages across a spectrum of peat bog habitats in the Peak District, using pyrosequencing. This study is the closest in scope to the subject of this PhD. The authors characterised microbial and fungal communities in field samples using high throughput sequencing in the acrotelm and mesotelm of six different peats: bare peat, restored grass (where grass had been planted post restoration to hold the surface together), young heather, 25 year old heather, a gully, and the original vegetation. This is a similar spectrum of habitats that we will sample in the experiment (noted in the conclusion of this chapter). The study found that the peat samples did not significantly differ in pH, which, given the differences in microbial communities, is surprising, as other work in wetlands has found the microbial community to correlate strongly with changes in pH (Hartman *et al.,* 2008, Griffiths *et al.,* 2011, Ye *et al.*, 2012). Further soil physicochemical differences were contrasting mainly between two groups of samples: the unmanaged areas (including bare peat, the gully and the original vegetation) which had significantly higher levels of ammonium and nitrates, and the managed areas (heather and restored habitats). Alongside this, OTU (Operational Taxonomic Unit, roughly equivalent to species) rank abundance data indicated that a low number of OTUs dominated the bare peat and old heather, whereas the other zones had more even populations. Overall, the study found low bacterial diversity in bare peat, and low fungal diversity in 25-year-old heather zones.

When analysing the effect of habitat type on the microbial community, Elliot *et al.* (2015) made several interesting observations in Holme Moss, situated in the Peak District. The study found significant differences in dominant taxonomic groups (Figure 3) between habitat areas, especially between bare peat (degraded) and the original vegetation. Noteworthy is the relative stability throughout the zones of the abundance of Proteobacteria: finer taxonomic resolution may reveal more subtle differences. More apparent is the differences in fungal communities across the habitat areas, especially in terms of Zygomycota abundance. The study included 6 habitat areas, bare peat (featuring no vegetation), restored grass (dominated by *Lolium* and *Festuca* species), young heather (dominated by young heather plants), 25 year old heather (dominated by long growth *Calluna* stands), gully habitat (gullied area featuring generally damp with exposed bedrock, and dominated by *Eriophorum angustifolium* and other acid grasses) and original vegetation (containing the mature moorland vegetation including *Empetrum nigrum*, *E. angustifolium* and *Vaccinium myrtillus*). Whilst the study points out that the most abundant phyla, Proteobacteria, showed the least variation between habitat areas, it must be noted that this is among the most functionally diverse bacterial groups (Fierer *et al.,* 2007). Had the community been viewed with a finer taxonomic resolution (involving a technique that looked at species level taxonomy) then functional or taxonomic differences between habitat areas might have become apparent. Given that the study found phylum level differences across the habitat spectrum, it is likely that any analysis that provides finer resolution will find differences in functional and taxonomic diversity between habitats. The study-interpreted differences in phylum between the bare peat and other habitat areas as being indicative of a microbial copiotroph (rich nutrient environment species)/oligotroph (low nutrient level species) divide, with bare peat sustaining an oligotrophic community. The study did not measure the microbial community in the catotelm, however, which may have given interesting additional insights. The study concluded that the different habitats support unique communities, which is important in the context of this PhD project, because it indicates that certain microbial groups or community patterns might be used to assess habitat condition and function.

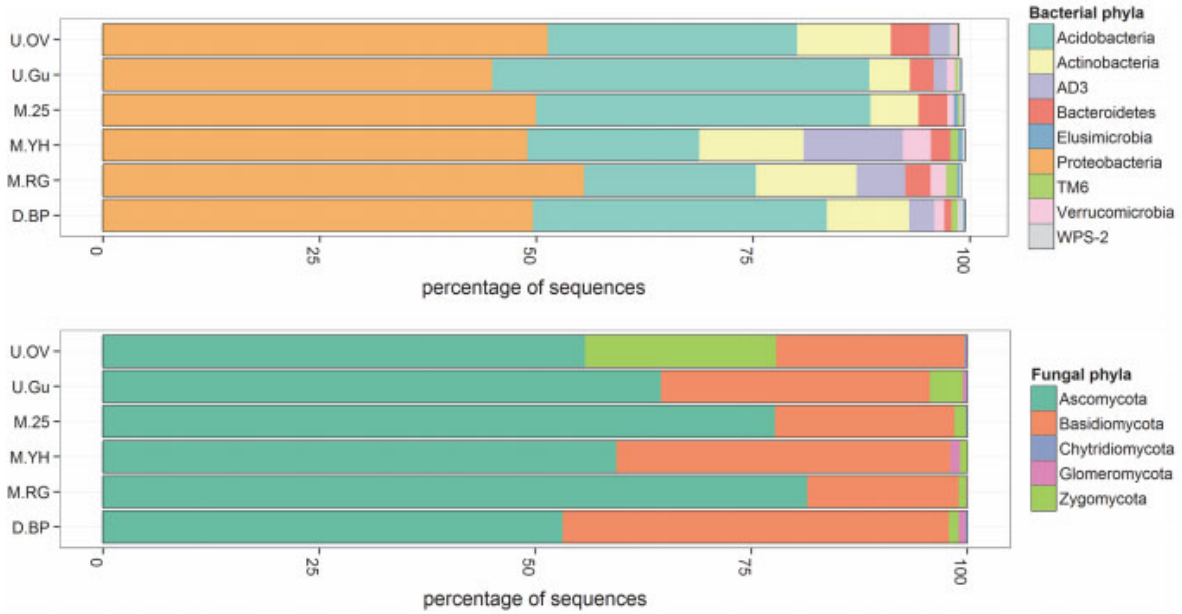


Figure 1.3: Microbial community profiles in the study by Elliot et al., 2015. Represented are the relative abundance of bacterial and fungal phyla from the size zones used within the study (U.OV = Original Vegetation, U.Gu = Gully Vegetation, M.25 = 25 year old stand of Calluna, M.YH = young heather stand, M.RG = restored grassland, D.BP = bare peat). Abundances obtained from the mean of three samples from each zone.

A more recent study into the microbiology of UK peats was undertaken by Potter *et al.* (2017), who examined the change in microbial community under controlled conditions, in peat samples from Wales, in both an upland bog and lowland fen ecosystem. Similar to the study proposed for this PhD, they used peat mesocosm cores and treated them with a drought, and measured the microbial community using high throughput marker gene sequencing which was then analysed using an ARISA (Automated Ribosomal Intergenic Spacer Analysis) community fingerprinting method as well as sequencing to characterise OTUs. This multi method approach is robust, because without additional sequencing, ARISA (indeed community fingerprinting in general) on its own cannot be used to assess diversity or OTU richness (Bent & Forney, 2008), especially in diverse communities where multiple species can share the same intergenic spacer length (Kovacs *et al.,* 2010). Whilst the study found that bacterial and fungal communities were both affected by habitat and depth, the effect sizes (R^2) were small (All were $R^2 < 0.09$). This suggests that differences in the microbial communities within peat bogs is probably controlled by many interacting factors. Contrary to the results of Elliot *et al.* (2015), where Acidobacteria dominated the bog sample, in the Potter *et al.* (2017) study Proteobacteria (making up ~50% of the samples in the Elliot *et al.* (2015) study) only contributed 20% of the sequence reads. This might be a reflection of methodological differences, or it might be a real environmental difference between relatively "pristine" bog with high water tables used in the Potter study, and the particularly degraded/polluted bog at Holme Moss. The study concluded that although carbon dioxide emission levels changed during drought treatment, the soil microbial community composition did not seem to, perhaps indicating that although the microbial community did not change its levels of activity did. Consequently, metatranscriptomic analysis, which provides details of the transcribed genes in a sample (and therefore the genetic activity of the community rather than just its composition), might provide useful insights.

1.8 Interactions of microbial communities with management practices

The microbial community, as a driver of change, is affected by and interacts with multiple levels of management, both current and historical. This PhD aims to unravel the relationship between management practices and the microbial community, and how this affects ecosystem service provision such as carbon sequestration and water quality. The following sections outline literature on measured direct impacts of management (e.g. heat from burning) on the microbial communities within peat bogs. There are also many indirect impacts that management can have on a bog, for instance, mowing leaves brash (litter and therefore soil organic matter – unless it is removed) whereas burning leaves charcoal – these are also considered.

It is noteworthy that whilst there has been some research into the individual effects of various management practices on the microbial community, there has been no multifactorial study that examines any of these management impacts in combination.

Mowing

Mowing has been investigated as a potential alternative to burning on blanket bog because it is safer (i.e. fire risk) and retains biomass (i.e. litter) on the ground as a brash (instead of losing it to the atmosphere during combustion), which should contribute to peat formation under the right conditions (although in between burn periods litter still adds to the peat layer). There is, however, very little research studying its effects on microbial populations and

on ecosystem service provision in blanket bogs. Specifically the question of decomposing brash layers adding microbial habitat and as such carbon compounds affecting water quality has not been adequately addressed for most vegetation types: studies specifically of *Molinia* (Purple Moor Grass) found no negative affect on water quality (Walker, 2016).

One study in Scotland used mowing as a management technique to reduce the cover of heather (*Calluna vulgaris)* in order to favour the production of the bilberry (*Vaccinium myrtillus)* and increase the biomass of spiders, both important food groups for the Capercaillie (*Tetrao urigallus),* a type of large forest grouse. The mowing management did decrease the abundance of heather and increased the biomass of spiders, but the study did not measure any other variables (Hancock *et al.,* 2010) nor did it investigate changes in the soil microbial community.

Another experiment found evidence that under mowing and burning the distance to water table decreased (the peat became wetter) compared to unmanaged control plots, most likely because vegetation loss reduced the water loss from evapotranspiration, and also that management reduced the concentrations of DOC in the soil (Worrall *et al.,* 2013). Other studies have found the opposite of this in regards to burning, e.g. the EMBER project (Brown *et al.,* 2014) which found water tables were deeper in burnt catchments. Water table changes are bound to affect microbial communities in terms of their activity, and, potentially their diversity and taxonomic composition. Consequently, a mowing management that changes the hydrological regime, for example, with higher water tables and higher soil moisture in mown compared to burnt areas (Heinemeyer et al., 2019), will certainly have measurable effects on community composition and function.

Whilst literature on how mowing impacts water quality is scant, there are some effects of grazing recorded: for example, Ward *et al.* (2007) found a small increase in the quantity of DOC produced from shallow soil margins when catchments were grazed. Perhaps mowing might have a similar effect on DOC (albeit, mowing can result in a brash layer if being left, whereas grazing it is digested and excreted).

Burning

Burning is a common management technique in the UK uplands, and is used to promote new growth and vegetation structural diversity for the benefit of the red grouse (*Lagopus lagopus scoticus*) and livestock, and occasionally is proposed as a mitigation strategy to deal with wildfire potential. It is estimated that about 114 km^2 of ericaceous dominated peatland is burnt per year in the UK (Yallop *et al.,* 2006). Burning is likely to dramatically affect the microbial structure of the peatland because it changes the vegetation composition (promoting heather germination) and the carbon cycle (decreasing litter input whilst increasing recalcitrant charcoal input) extensively (Harper *et al.,* 2018), as well as changing the hydrological regime through changes in runoff and infiltration rates (Holden *et al.,* 2015).

A recent review found prescribed burning generally reduced or modified (in terms of changing levels of activity, or changing composition or diversity without affecting biomass) microbial biomass in examples of non-peatland soils (Alcaniz *et al.,* 2018). Some studies have directly examined the effect of fire on peatland microbial communities. An investigation into wildfire in a Russian ombrotrophic peat bog, for example, found fire reduced the abundance of methanotrophic bacteria. In addition it modified the community from acidophilic Type II methanotrophs to Type I, which are less active in the acidic environment (Danilova *et al.,* 2015), even 7 years after a single burn. These findings seem to contradict an older study on UK

blanket bog (undertaken at Moor House), which found Type II methanotrophs were more abundant in frequently burned peat soils than Type I (Chen *et al.,* 2008a). This may be a consequence of sampling and/or burning frequency, whereas the peat in the Danilova *et al.* study was assessed 7 years after a single wildfire, the peat in the Chen *et al.* study was assessed on peatland that is cyclically subjected to prescribed burning. Similar drastic changes to the taxon composition of peatland microbes immediately after fire have been found in testate amoebae occurring in a Chinese bog (Qin *et al.,* 2017). The study found that fire greatly changed the taxon composition from those tests constructed from idiosomes to those constructed of xenosomes, probably because of the direct destruction of idiosome tests by extreme heat. It is important to note that this study examined the testate amoeba community directly after a (hot) wildfire, rather than a prescribed burn.

In assessing the effect of fire on peatland microbial communities, considering the limited peatland studies, it might be informative to look to other biomes. In forest ecosystems, microbial communities serve similar functions to those in peatlands: enhancing soil nutrient cycling, engineering and maintaining soil structures, often by forming (e.g. mycorrhizal) symbioses with plants to improve their nutrient uptake. Experiments have found changes in microbial community and activity up to 12 years after a single fire in a coniferous forest (Fritze *et al.,* 1993), with a higher microbial C corresponding to an increase in C sources that followed post fire vegetation succession. A later review (Hart *et al.,* 2005) also concluded that post fire vegetation dynamics were drivers of microbial change in forests subject to burning in pine-dominated forests. The authors asserted that the short-term immediate effects of fire on microbial communities (heat-mediated mortality) are transient, but that fire alters the vegetation dynamics long term, which then drives microbial community composition and function. Further, another study found that the ectomycorrhizal spore bank survived a California wildfire, with specific fire adapted fungal species increasing in abundance (Glassman *et al.,* 2016).

One might also consider the effect of burning on the microbial community from an outcomes perspective. For instance, where burning (or any other management) results in a change in ecosystem service provision, it is reasonable to assume that some change in the microbial community has occurred. One example is DOC - if burning were affecting DOC levels then most likely there is a change in the peat carbon cycling processes or turnover and this should be mediated by microbial change/activity. However, in the UK, the evidence on burning impacts on water quality is not clear (See Table 2 in Harper *et al.,* 2018, which shows conflicting studies examining the influence of burning on UK peat bog DOC outputs, as an example – however, it must be noted that the table presents research from several papers that come from single studies). Some studies found an increase in DOC from burnt catchments (Yallop *et al.,* 2010, Grayson *et al.,* 2012, Ramchunder *et al.,* 2013). Others reported no effect, such as Ward *et al.,* 2007; however, this was not a catchment scale study (being plot scale) and was at the end of a 10-year burn cycle, which means no effect might have been expected anyway, if all of the change occurred immediately post-fire. Other work found burning was associated with DOC reduction (Worrall *et al.,* 2013, although this is more likely to do with a wetter bog due to loss of vegetation and therefore less evapotranspirative loss). Clearly, the actions of the microbial communities in determining DOC in these studies are also impacted by other environmental factors, and so it is difficult to tell to what extent prescribed burning effects this water quality variable - perhaps the magnitude of effect changes with space (both on a micro and macro scale) and in time (seasonally etc.).

Draining/Drought/Changes to Hydrology

Changes to the hydrological regime via purposeful draining (often and especially done in post war Britain to make land more agriculturally useful for grazing or cultivation) can have effects on the microbial community. This can change the availability of nutrients and oxygen, which ultimately affects the balance between organic material deposition and decomposition (Holden *et al.,* 2004, which discusses the impact of lowering the water table on microbial communities and therefore decomposition rates)

A key theory concerning the effect of hydrological regime on peat microbial communities is the enzyme latch theory proposed by Freeman *et al.,* (2001). This proposes that there is an enzyme 'latch' on global carbon stores in peatlands, wherein anoxic conditions restrain the activity of the extracellular enzyme phenol oxidase, which is able to degrade phenolic compounds deposited as litter. The theory proposes that the accumulation of peat is due to the inhibition of these enzymes, which allow phenolic compounds to accumulate in the anaerobic environment. Other studies have suggested the phenol oxidase activity is controlled by temperature, and pH (Pinsonneault *et al.,* 2016). This potentially validates earlier studies which found a suppression of phenol oxidase was synergistic with DOC increases and phenol compound deposition under treatments of warming and CO_2 addition (Fenner *et al.,* 2007). Recent studies have confirmed that oxygenation increases decomposition and carbon loss in droughted cores, but found no change in phenolic compounds, suggesting it is an oversimplification to propose that a single enzyme is a driver of the system (Brouns *et al.,* 2014).

Under drought, or where the water table is lowered via draining (thus droughting the upper layers) we might reasonably expect that more peat becomes oxygenated, and therefore aerobic decomposition is encouraged, thus causing the microbial community to change. Work on droughting via TTGGE found that a greater diversity and abundance of phenolic catabolising bacteria was found with concurrent increased phenol oxidase activity. Droughting decreased DOC and increased carbon dioxide flux. Conversely, increased rainfall simulations led to an increase in phenolic compounds, an increase in DOC efflux and increased anaerobic gas trace fluxes (Fenner *et al.,* 2005). Whilst this study measured a specific gene (XylE) using TTGGE, and therefore only detected changes in community function, rather than composition, this indicates drastic community change due to managed changes to the hydrological regime.

Perhaps in terms of ecosystem services such as water quality, microbial community function is more important than composition. For example, whilst the study from Fenner *et al.,* (2005) found microbial community function changes in relation to altered DOC outputs, a direct measure of the community composition by Potter *et al.,* (2017) found weak relationships between drought treatments and community composition. Potter *et al.* used ARISA community fingerprinting to assess the differences in taxonomic composition between drought treatments of peat bog mesocosms and found significant but weak relationships between community composition and drought treatments, where differences in community were better explained by habitat and depth between mesocosms.

Whilst the literature indicates that drainage and water levels have a profound effect on proxies for microbial community function such as key enzyme levels or specific genes, it is not clear whether hydrological regimes have an effect on composition.

1.9 Ericoid Mycorrhizas and other Fungi in Blanket Bogs

Whilst hypotheses 1-3 of my experiment (listed at the end of this chapter) will focus on the entire microbial community of the bog soils, hypotheses 4 and 5 focus specifically on the role

of ericoid mycorrhiza (ERM) in peat bogs. ERM are symbiotic associations between a variety of soil fungi and the roots of plants in the family *Ericaceae*, which includes important peatland plants such as those in the genera *Erica*, *Calluna*, and *Vaccinium.* We hypothesise that ERM play a crucial role in soil processes by altering soil communities, decomposition processes, and access to recalcitrant soil C, discussed later in this section. Additionally, soil C cycle and water quality impacts are driven by plant induced changes in the biota community, which affects decomposition. The below section outlines the major pieces of literature relating to ERM, and their potential role in linking soil processes to ecosystem services.

Bragazza et al. (2012) suggested; "the quantity and quality of DOC might be controlled by vascular plants through a greater rhizodeposition of labile C compounds". Ericoid mycorrhizas can form symbioses with ericaceous species, such as *Calluna*, or exist as 'free-living' fungi in the soil. The success of Ericaceous plants in peat bogs could be attributed to these ericoid associations, which can shield the plants from stressful conditions (such as low pH, low temperature, poor drainage, and occasionally in British peat soils, readily available metals [Cairney & Meharg, 2003]) as well as helping the plant acquire nutrients via their saprotrophic properties (Haselwandter *et al.,* 1990), by breaking down SOM or 'priming' SOM turnover. Considering their wide range in enzymatic activity related to decomposing SOM (see below section), the ERM can help their host by mobilising otherwise inaccessible nutrients from the soil (Read, 1991). It would be no surprise, then, that ERM could be a significant driver in the structuring of above and below ground communities, especially given that many peatlands, including peat bogs, are dominated by Ericaceous vegetation and that the majority of those have ERM (Thormann *et al.,* 1999). It is important to note, however, that ERM contain a diverse group of fungi, and may form associations not just with Ericaceous plants, but also with other non-vascular land plants such as liverworts (Kowal *et al.,* 2018). Additionally, whilst many ERM fungal species were presumed to be in the Ascomycetes (Read *et al.,* 2005) recent work has found species of ERM that belong to the Basidiomycota (Kolařík & Vohník, 2018) which complicates microbial work that aims to relate fungal communities to the extent of Ericaceous plant communities and function in blanket bog.

It is likely that ERM alter decomposition processes within the soil. Recent genomic and transcriptomic analyses of ERM have found them to be very versatile. Not only can they form mycorrhizal symbioses but they also act as plant endophytes or live independently as saprotrophic fungi (Martino *et al.,* 2018). In addition, ERM, specifically the family Leotiomycetes, contain an exceptional repertoire of genes that code for degradative enzymes. This may explain the success of ericaceous plants in ombrotrophic peat soils; the ERM genome contains genes that code for degradative enzymes involved in the degradation of chitin, hemicellulose and pectin. Crucially, genes coding for lignocellulose degrading enzymes were found in the genome of *O. maius*, providing genetic evidence that these ERM are potentially able to decompose *Sphagnum* moss (Martino *et al.,* 2018). This genetic evidence confirms earlier work, which experimentally observed this ability (Piercey *et al.,* 2002). Therefore, the nature of ERM and their unique decomposition ability warrants further investigation - if ERM, associated with ericaceous species, can efficiently decompose *Sphagnum* litter, then a dominance by vascular ericaceous plants might significantly alter the rates of decomposition in the peatland and consequently the rate of C storage or loss. Additionally, ericoid mycorrhizas, whether in association with *Calluna* roots or not, may break down either peat or charcoal, or both, and therefore be at least partially responsible for the observed increase in DOC production and water colour (Worrall *et al.,* 2003, Worrall *et al.,* 2004, Clutterbuck & Yallop, 2010, Armstrong *et al.,* 2012). As water quality is of great concern to water companies and water decolourisation is a significant process cost, elucidating the causes of colouration and methods of reducing it is of great interest.

In the UK, blanket bog fungal populations are dominated by several fungal phyla namely the Ascomycota, Basidiomycota and Zygomycota with Glomeromycota and Chytridiomycota making up smaller sections of the community. Recent evidence from OTU rank abundance data suggests fungi are much less diverse than bacterial communities are across the UK peat bog habitat spectrum (Elliot *et al.,* 2015). Studies of fungal communities undertaken in North American ombrotrophic *Sphagnum* bog can be considered analogous to UK blanket bog despite containing taxonomically different but functionally equivalent vegetation. Specifically, a study conducted by Lamit *et al.,* (2017), found that fungi in the bog were strongly depth stratified: fungi were four times more abundant in the upper (10-20 cm) layer. Plant functional group was only important in the upper peat layers, with Ericaceous plants resulting in the top layers being dominated by ERM whilst more saprotrophic and endophytic fungi became more dominant with increasing depth.

The findings of Lamit *et al.,* (2017) mirror those of another study that took place in UK peatlands, also finding that depth was a major control on fungal community composition. This study, undertaken by Artz *et al.,* (2007) found microbial biomass carbon decreased with depth across sites of varying habitat quality (including bare peat to sphagnum-dominated bog). The Artz et al. study found statistically significant differences in the quality of the litter in different peat habitats, which all had different vegetation compositions. They linked the changes in fungal community to vegetation succession in the slowly restoring peatlands, which is not surprising given the intimate link between ERM fungi and plants (and other fungi and plant litter). They did point out, however, that the function of many fungi are not known, and that more research into the effects of anthropogenic pressures on the fungal community should be undertaken.

1.10 Conclusion

This introductory chapter has attempted to review the major literature pertaining to the microbial communities of blanket bog in the UK. Blanket bogs are a unique and important habitat, especially in terms of their role as a carbon store. The literature on this topic is scant, but has been increasing in recent years. It is clear that whilst we know some amount of the basic microbiology in blanket bogs, such as the type of lifeforms, their functional genes, and in limited cases, their distributions, there is still much to learn. Many microbial species, including mycorrhizal fungi, are still to be discovered, with their functions remaining elusive (see unassigned reads in Figure 3 of Potter *et al.,* 2017 for a recent example). Recent studies have shown that there are variances in microbial community composition within spectrums of depth, soil edaphic conditions (such as pH) and management, but very little evidence that links these to bog function and, by extension, ecosystem service. The microbial black box, situated in between system C inputs from vegetative matter (as affected by management, vegetation composition etc.) and system C outputs (water quality in terms of DOC, methane emission etc.) is still very much shrouded in mystery. Joined-up field and laboratory studies assessing microbial communities alongside different management and habitat conditions offer a unique potential into enhancing our understanding of the underpinning processes as drivers of key ecosystem services.

In summary;

- Current research into the microbial ecology of peat bogs lacks the evidence necessary for a mechanistic understanding of how system C inputs relate to C outputs (and therefore the associated ecosystem services, specifically carbon storage and water quality).

- Current evidence concerning the influence of management practices upon ecosystem services, which could be used to inform hypotheses concerning the diversity, distribution or function of microbial populations in blanket bogs, is insufficient and frequently contradictory.
- However, microbial methods have advanced sufficiently to allow further work examining the link between microbial communities and habitat status, climate and management in UK blanket bogs.

Consequently, this PhD aims to address the following hypotheses:

1. Habitats across a spectrum of active to degraded peat bog vary in their soil biota with active sites having higher diversity.
2. Across the site spectrum, key indicator species or groups translate to habitat status, which can be used to assess other sites.
3. Biota communities and indicator groups can be linked to C cycle, C accumulation and climatic stress and differ between management, with mowing restoring degraded (burnt) peatland.
4. Ericoid mycorrhizal status plays a crucial role in regulating mycorrhizosphere soil processes (mainly in the acrotelm) by altering soil biota communities, decomposition processes and access to recalcitrant soil C.
5. C cycle and water quality impacts are driven by plant induced changes in the soil biota community affecting decomposition.

Chapter 2: Methods

2.1 Introduction

This chapter outlines the design of the overall study in relation to the research questions and hypotheses discussed in Chapter 1 section 1.10. Whilst there are multiple experiments examining several hypotheses within this thesis, they were all performed on the same experimental setup. The following sections describe the field sampling sites and reasons for their choice, the design of the mesocosms used and how 'intact' mesocosm samples were taken, how the experiment was set up at the University of York and how the microbial sampling was done and samples were processed as part of the laboratory analysis to identify bacterial, fungal and archaeal communities.

2.2 – National Sites

Sites were selected based on the climate and habitat/management categorisations requested from local site managers. The national climate categories were warm/dry, intermediate, wet/cold, and the habitat/management categories were intact, 5-year post restoration, 10-year post restoration, degraded. However, in real terms the latter categories were a hypothesised order of how the sites will be split microbially, as there was no prior awareness of whether this spectrum of habitat would be borne from the data. Nonetheless, this categorisation was helpful as to obtain guidelines to the general site/habitat conditions in terms of vegetation, climate, hydrology *etc.* that testing the hypotheses required.

It was impossible to become an expert in the condition of individual sites and thus identification of sites relied on the help of experienced site managers. For this reason, the site choices can also be seen as a reflection of the opinion of the site manager as to what is "intact" or "degraded", which will of course differ based upon region, organisation, aims for the particular site, etc. For example, the ecologists at RSPB Forsinard may consider a degraded bog to be simply a drained, but ultimately well vegetated one (as there is no muirburn), whereas Moors for the Future in the Peak District may consider degraded sites to be bare peat.

Consequently, we used these categories as a rough guide to site selection, and some flexibility was possible in finding sites to match these criteria: for example, the Peak District restorations were 8 and 4 years ago (at time of collection) rather than 5 and 10. It is impossible for this study to state that a degraded bog has a specific microbial community, and an intact bog has some other, rather, the results were expected to represent which microbial communities correlate overall with which site conditions, in an attempt to reach a more generic understanding of the drivers of microbial community change across a relatively broad categorisation of site condition.

2.3 Site choice

Site choice was based upon a balance of a wide spectrum of conditions, but also financial and logistic constraints. Sites chosen were relatively close to a road (given that the mesocosms

removed from each site amounted to around 60 kg of peat per mesocosm, and this was carried manually) or track that could be accessed by a 4x4 vehicle. The sites also needed to be ombrotrophic blanket bog (not fen, marsh etc.) with a known recent site management history. The sites chosen were clustered based upon latitudinal proximity not only for logistic reasons but also to keep them as similar (climatically) as possible whilst still differing in management.

2.4 Site codes and naming

Sites were coded based upon location and management as well as sample number, with examples in Table 1 below.

Table 2.1: Site codes used in referring to sites throughout this thesis representing the seven site areas

Site codes:	**Management codes:**	**Replicate number:**
Grouse moors M – Mossdale N – Nidderdale W – Whitendale Previous grouse moor MH – Moor House National sites E – Exmoor P – Peak District S – Scotland	Grouse moors F – Burnt M – Mown U – Uncut/not burnt National sites I – Intact 5Y – 5 year post restoration 10Y – 10 year post restoration D - Degraded	1-4

Examples:
Scotland Intact replicate 2 = SI2
Nidderdale burnt replicate 1 = NF1
Peak District 10 year post restoration replicate 2 = P10Y2
Exmoor degraded replicate 3 = ED3

2.5 Site descriptions and site histories

This thesis sampled a wide range of blanket bog under varying climatic conditions, but also variations in management. Whilst peatland restoration now occurs across large areas of the UK, this is often site or area specific, and as such, the actual restoration management/techniques carried out across the sites in this study differ considerably. To provide context, the following sections describe the blanket bog habitat and management/restoration at the three national site areas, as well as the three sites with grouse moors management in Yorkshire and Lancashire and the Moor House (NNR) site.

Peak District (Derbyshire)

The Peak District sites in this thesis are situated on land being restored By Moors for the Future, whom are undertaking restoration activities that are by far the most extreme in terms

of changes to the environment. The peatlands around the areas of Kinder Scout and Bleaklow were extensively grazed, with much bare peat, sometimes down to the regolith, until they were purchased by the National Trust in 1982 (Anderson & Radford, 1984). The Trust began a policy of grazing reduction, involving gathering of trespassing sheep until their owners could collect them. This resulted in some plant re-colonisation but much bare ground – an estimated 8% of all the blanket bog in the Southern Pennines - still remained. The Moors for the Future Partnership was formed in 2002 and began restoration of these areas, which involved the exclusion of grazing and gully blocking, followed by a lime and fertiliser treatment for bare peat, and then seeding of the areas with amenity grasses, dwarf shrubs and heather brash. Two following treatments of lime and fertiliser were applied annually, and plugs of moorland species (inc. *Sphagnum* spp.) were applied (Pilkington, 2015). The sites used in this thesis were those used for the Making Space for Water project, a DEFRA funded study undertaken by Moors for the Future (Walker *et al.,* undated). The intact site in this thesis is the hydrologically active site used for the Making Space for Water study; it has no gullying relative to the other sites here and a high water table. The 5 year post restored site is south of Bleaklow Head (grid reference in Table 2) and was restored from bare, gullied peat in 2012, having had 5 full and two partial growing seasons since the application of fertiliser and seeds. The 10-year post restored and degraded sites are both situated on the northern edge of Kinder Scout. The 10 year post restored area was restored in the summer of 2011, and has had eight full growing seasons since fertiliser and grass seed application. The degraded Peak District site in this thesis represents Moors for the Futures control area, a large area of unrestored heavily degraded peats with no vegetation apart from scarce hags, and extensive, deep gullying, often down to the mineral subsurface (all site information *pers comm.* Michael Pilkington, Moors for the Future, 2019).

Exmoor

The land which was sampled for this thesis in Exmoor was managed by the Exmoor Mires Partnership, for whom the principal restoration focus was ditch blocking. The mires sampled in this thesis were drained for agricultural improvement in the 19th and 20th centuries, but were also peat cut in the past and have been more recently grazed (Grand-Clement et al., 2015, Gatis et al., 2016). Site conditions are described in detail for one of the sites sampled here (Spooners) in a PhD thesis by Luscombe (2014), which is representative of the general history of management and restoration activity in the area, and across our four Exmoor sites. The site at Spooners is ditched approximately every 20 m, with ditches ranging from 0.3 to 0.6m wide. These have been blocked every 7.5 m based upon technical guidance from the Yorkshire Peat Partnership (YPP, 2012) using peat blocks, with occasional wooden supports (Grand-Clement et al., 2015). The same has occurred on all the sites chosen in Exmoor in this thesis, apart from the sites chosen as "degraded" and "intact". The intact sample, Squallacombe, contains deep peat (1.8 – 2.0 m average) with M17/M18 mire and M1/M6 pools which is intact, although there were some ditches to the north dug in the 1970's. The 5-year post restored site, Spooners, contains much shallower peat at 30-40 cm having undergone ditching in the 19th century, and contains impoverished M25 vegetation. The ditches had been blocked 5 years prior to sampling. The 10-year post restoration site, Hangley Cleave, is similar, containing a network of 19th century ditches in an area of peat between 60 cm and 1 m depth. Hangley Cleave, post ditch blocking, underwent positive recovery and at the time of sampling contained an M17/M25 mosaic with *Molinia* limited to 10-20% in most quadrats (although the site sampled

was *Molinia* dominated in this case). Finally, the most degraded site, Halsworthy, is a heavily disturbed site because of extensive 19th century mineral prospecting and drainage efforts (including ploughing). Most of the peat on Halsworthy is 20-50 cm, but this thesis sampled 1 m deep sites presumed to be remnants of past valley mire. The vegetation is impoverished M25 merging into U4 to U5 acid grassland in the worst sections (All site descriptions *pers comm.* Conrad Barrowclough, Southwest Water/Exmoor Mires Partnership, 2018).

Scottish Forsinard Flows

Blanket bog restoration has a complicated and controversial history in the Scottish Forsinard Flows, after conflict in the 1980s over bog afforestation. In the post war period, and moving towards the latter half of the 21st century, afforestation was seen as a sensible and commercially viable use of this large peatland, and this was further encouraged by cheap land, the Thatcherite boom and a favourable tax regime (Warren, 2000). Whilst the work in this thesis actively avoids forest to bog restoration, it is worth mentioning, because the sampled sites in the Flows are surrounded by these formerly afforested bogs. The Forsinard Flows were acquired by the RSPB in 1994 (Wilkie & Mayhew, 2003) when they commenced restoration, which includes felling all the trees, and, in areas with no trees, the primary method of restoration was via drain blocking. It is difficult, however, to find a management history of the parts of Forsinard that were not afforested, but we can infer from the literature on the forestry controversy, which considers local viewpoints (Mather, 1993), that the area was given over to shooting, grazing and stalking, with the consequent possible use of muirburn. It is unlikely, however, that the wettest ("intact") site was burnt, as this was never gripped, and being so wet would never have supported extensive enough *Calluna* to warrant muirburn. The intact Scottish site, the Forsinard bog pools, is considered "near intact" by the RSPB representative but probably represents some of the most "intact" blanket bog in the UK. This thesis did not sample directly in bog pools but sampled close by in a *Sphagnum* dominated lawn/hummock system. The 5-year post restoration site, The Uair, is 6 years post restoration having had gullies blocked in 2012. The 10-year post restoration site, Nam Breac, is 14 years post restoration at the time of sampling, having been gully blocked in 2004. Finally, the most degraded section, Crocach, was deeply gullied. The samples were taken directly adjacent to a ditch on flatter ground. Crocach was gully blocked (restored) after sampling in 2019 (all site descriptions *pers comm.* Daniela Klein, RSPB, 2019)

Yorkshire Dales and Forest of Bowland (Yorkshire & Lancashire)

The grouse moors used in this thesis were Mossdale, Nidderdale and Whitendale, with Mossdale and Nidderdale being located in the Yorkshire Dales, and Whitendale being located in the Forest of Bowland, Lancashire. These are part of the Peatland-ES-UK study and are described in detail in the DEFRA report by Heinemeyer *et al.,* (2019). The three study sites are all in north-west England and represent a controlled experiment, with each site having two sub-catchments (approx. ~10 ha) with each being allocated a burning or mowing management, and the outside of these catchments representing an "uncut" (unmanaged) control. All three sites are classed as blanket bog with a mean peat depth of over 1 m, and were managed as grouse moors. The sites all had a *Calluna* cover of over 50% with some existing bog vegetation

(*Eriophorum* and *Sphagnum).* All three sites are composed of soil that is a poorly draining organic peat from the Winter Hill series (Ewen *et al.,* 2015)

In terms of management, all three sites are managed as grouse moors, including the bird stocking, grit provision, and burning management that this entails (apart from the sampled catchments in the experiment). Nidderdale and Mossdale contain old and sparse grips (now mostly infilled) and the third, Whitendale, is extensively gullied. The sites are grazed, but at a low density of <0.5 ewes ha^{-1}. In Nidderdale and Mossdale, the grips in the study area, dug roughly 40 years ago (late 1970s – 1980s), were naturally infilled by 2010 and no grip blocking took place since that time. Whitendale is extensively gullied, although the mown catchment is less so.

Moor House (NNR)

The Moor House sampling location is located just exterior to the Hard Hill experimental set up. These parts lie within the catchment of Trout Beck, itself within the Moor House National Nature Reserve (Worrall *et al.,* 2007). Blanket peat covers 90% of the catchment, which is dominated by a *Calluna-Eriophorum,* association in terms of vegetation, along with *Sphagnum sp.* (Evans *et al.,* 1999). This area outside of the Hard Hill experimental plots was last subjected to managed burning in 1954, and up until the Worrall study in 2007 was grazed lightly at a density of less than one sheep per hectare (there is no indication this has been increased).

A summary of all sites is located in Table 2, and a national map of sites is in Figure 1.

Table 2.2: Site summary of locations and managements. Managements are brief with a more detailed description in the text. All descriptions are *pers comm.* From site managers at Southwest Water (Exmoor), RSPB (Scotland), Moors for the Future (Peak District) and the Peatland-ES-UK report (Heinemeyer *et al.*, 2019)

Site	National Location	Lat/Long	Site codes	Management
Nidderdale	Yorkshire	54.16619, -1.9154346	NF, NM, NU	Grouse moor: mown, burnt and uncut plots
Mossdale	Yorkshire	54.314726, -2.2940051	MF, MM, MU	Grouse moor: mown, burnt and uncut plots
Whitendale	Lancashire	53.990898, -2.5030333	WF, WM, WU	Grouse moor: mown, burnt and uncut plots
Moor House	Upper Teesdale (Northern Pennines)	54.691597, -2.402717	MH	Previous grouse moor, unburned for 80 years.
Crocach	Scotland	58.376965, -4.0163539	SD	Degraded – an area of deep gripped blanket bog. Possibly previous muirburn although not since early 1990s
The Uair	Scotland	58.411833, -4.0292684	S5Y	5 years post restoration – gully blocked in 2012
Nam Breac	Scotland	58.390931, -3.9936438	S10Y	10 years post restoration – gully blocked in 2004
Forsinard Bog Pools	Scotland	58.369028, -3.9687441	SI	"Intact" area of typical blanket bog, with bog pools nearby.
Halsworthy	Exmoor	51.132925, -3.7561221	ED	Degraded – heavily disturbed by 19th century mineral prospecting and drainage.
Spooners	Exmoor	51.121903, -3.7517172	E5Y	5 years post restoration, which consisted of extensive gully blocking of grips installed in the 19th century for agricultural expansion.
Hangley Cleave	Exmoor	51.164599, -3.6963237	E10Y	10 years post restoration, consisting of gully blocking of 19th century ditches.
Squallacombe	Exmoor	51.16245, -3.8102619	EI	"Intact" blanket bog, has been restored, previous management included ditching.
Kinder Scout	Peak District	53.400729, -1.8664658	PD	Degraded – extensively degraded control area of bare peat (no vegetation)
Bleaklow (south of)	Peak District	53.457536, -1.8583664	P5Y	5 years post restoration – restored from bare peat in April 2012 with lime, fertiliser and grass seed
Kinder Scout	Peak District	53.397933, -1.8656774	P10Y	10 years post restoration – restored from bare peat in early 2011 with lime, fertiliser and grass seed

100m north of Snake Pass (A57 road)	Peak District	53.434574, -1.8692649	PI	"Intact" – a hydrologically intact site used in the University of Manchester "Making Space for Water" project.

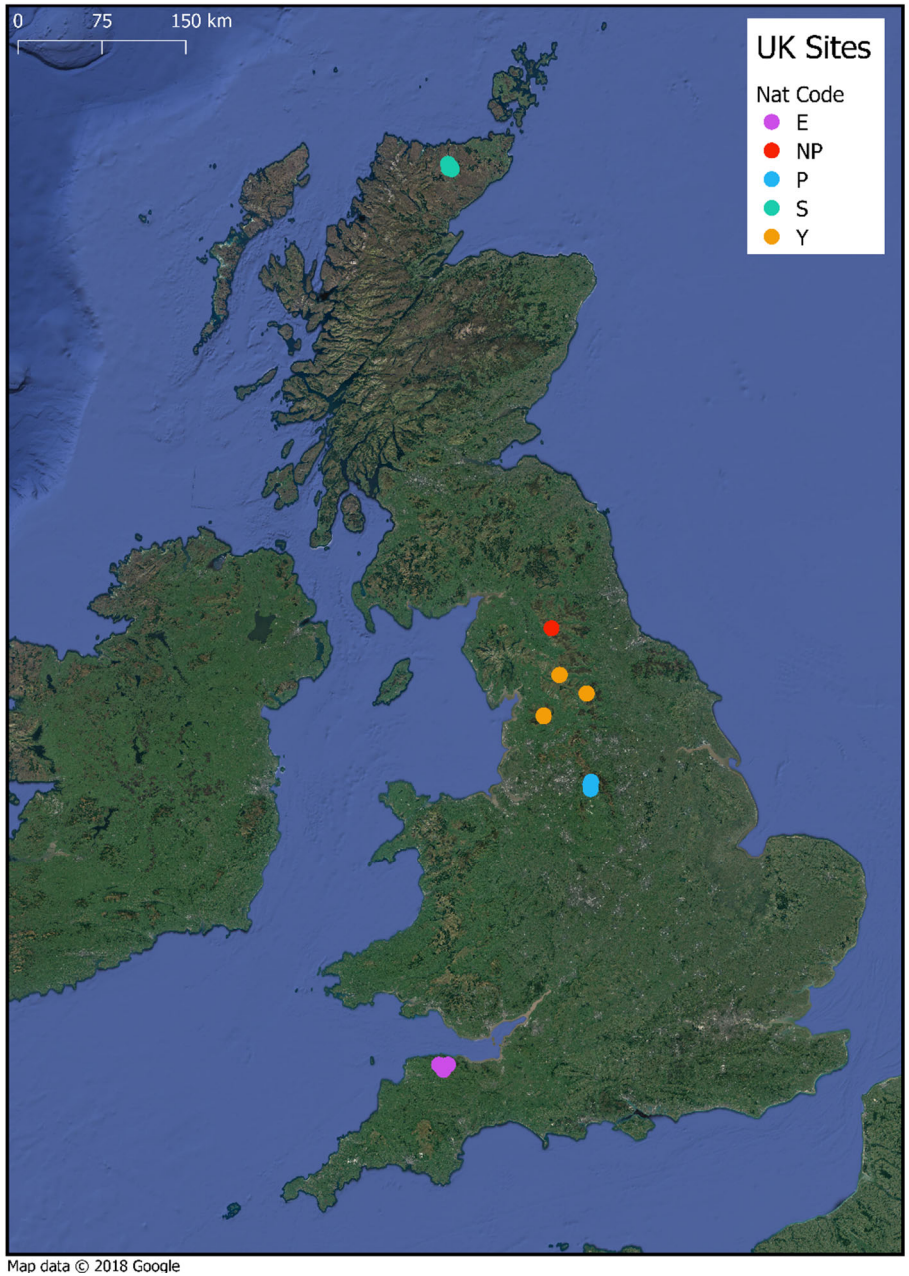
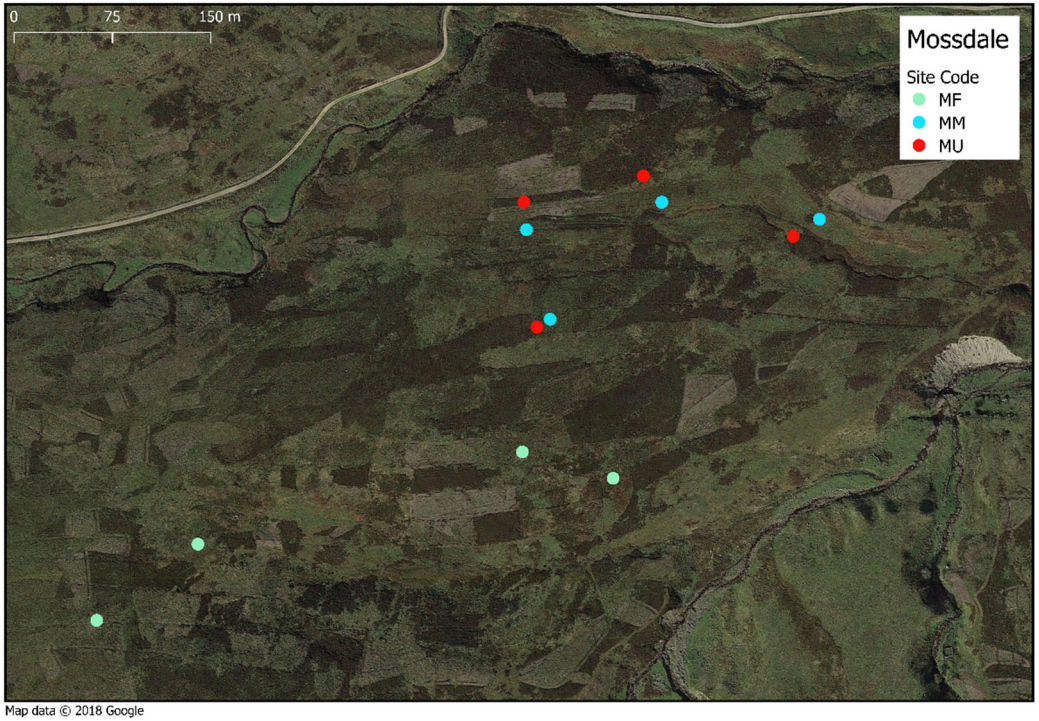
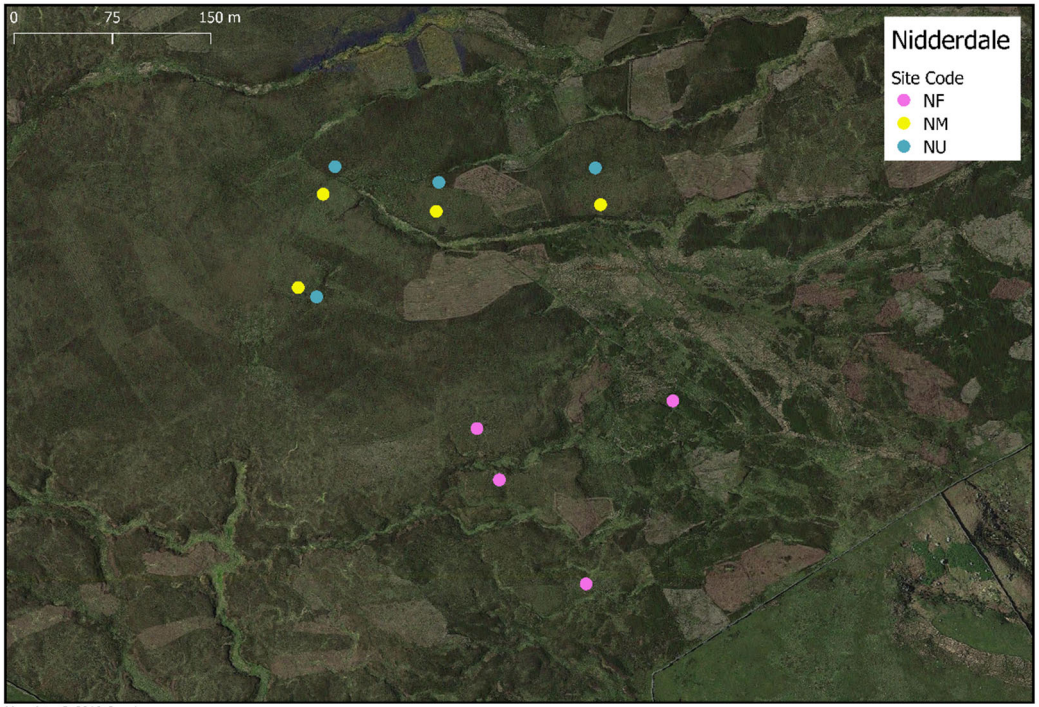
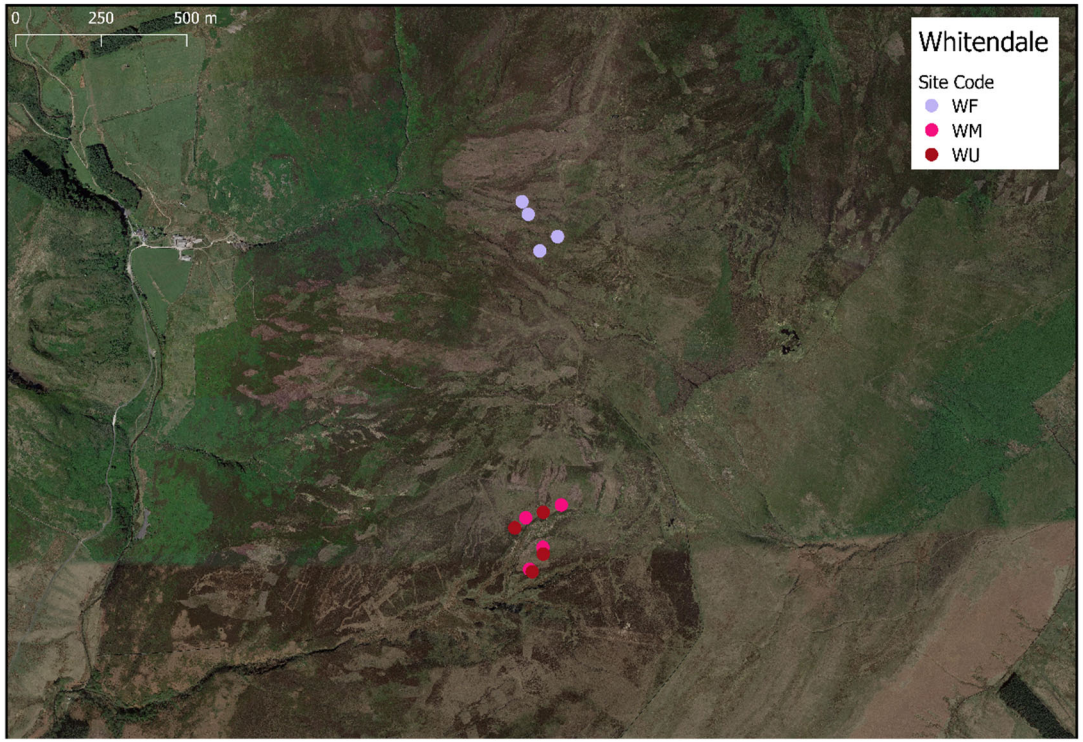
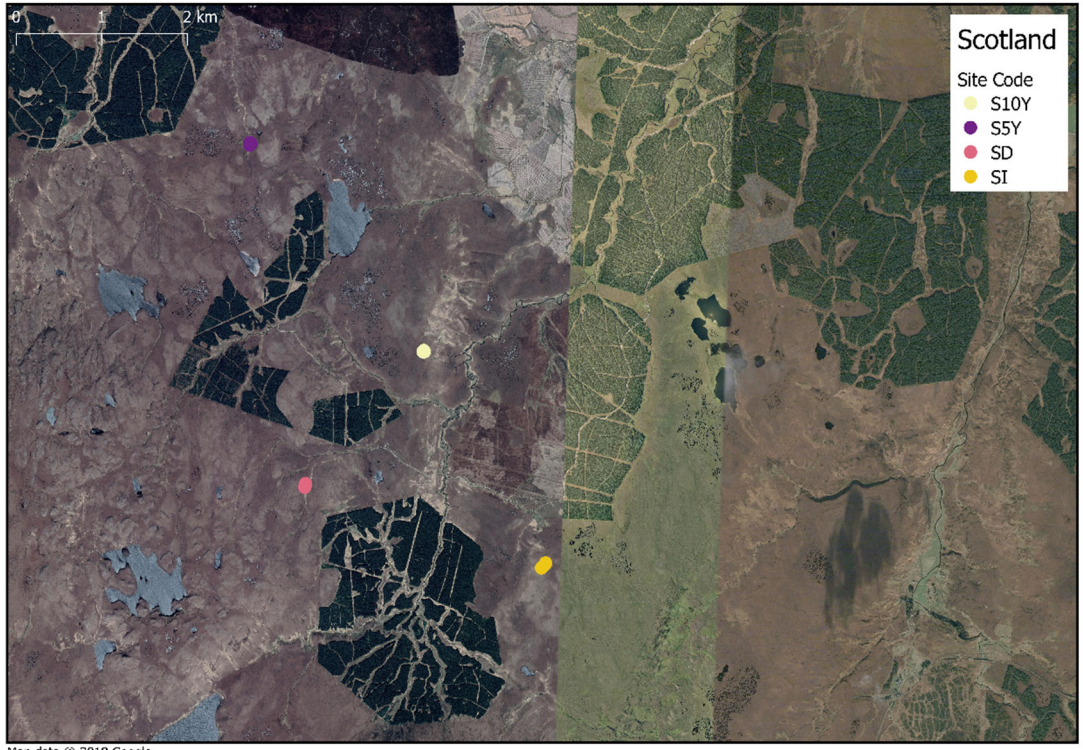


Figure 2.1: Maps of the sites across the UK. For legend details, see section 2.4 of this chapter. It is important to note that these maps are of sites that are disparate in their geographic spread; consequently, the reader should pay close attention to the scale in the upper left hand corner of each map image. Note also, in the Moor House image, the sampling immediately adjacent to the Hard Hill experimental plots.

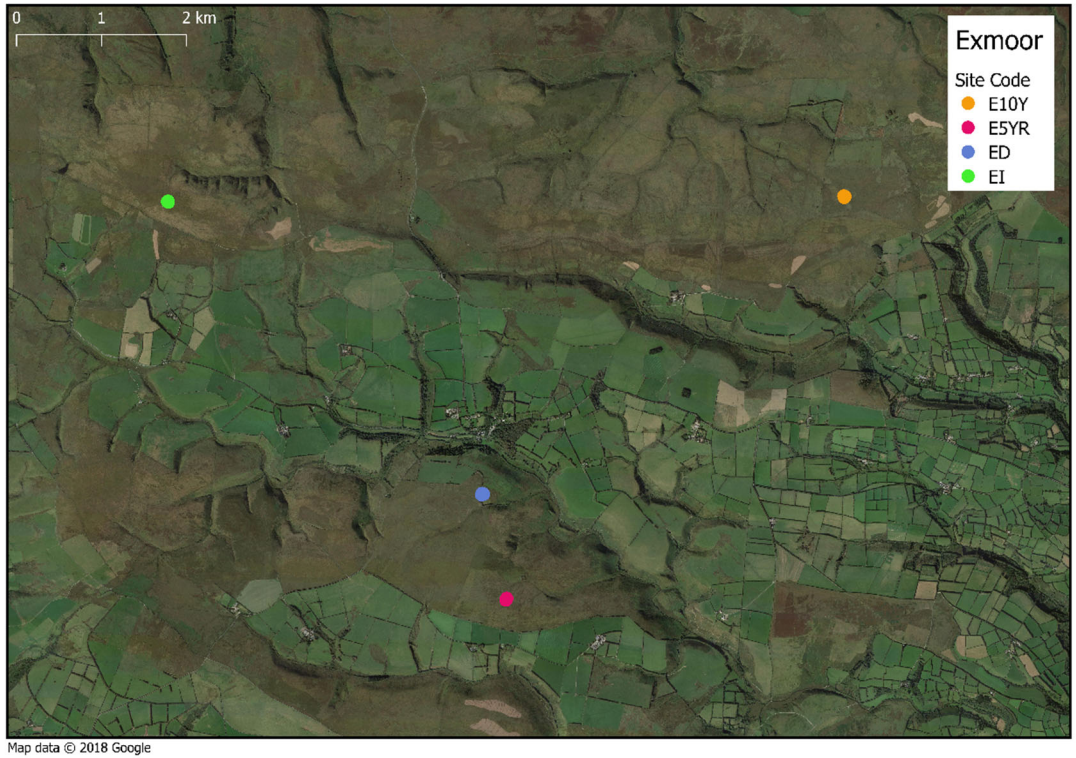








Map data © 2018 Google



Map data © 2018 Google

Figure 1 (Continued)

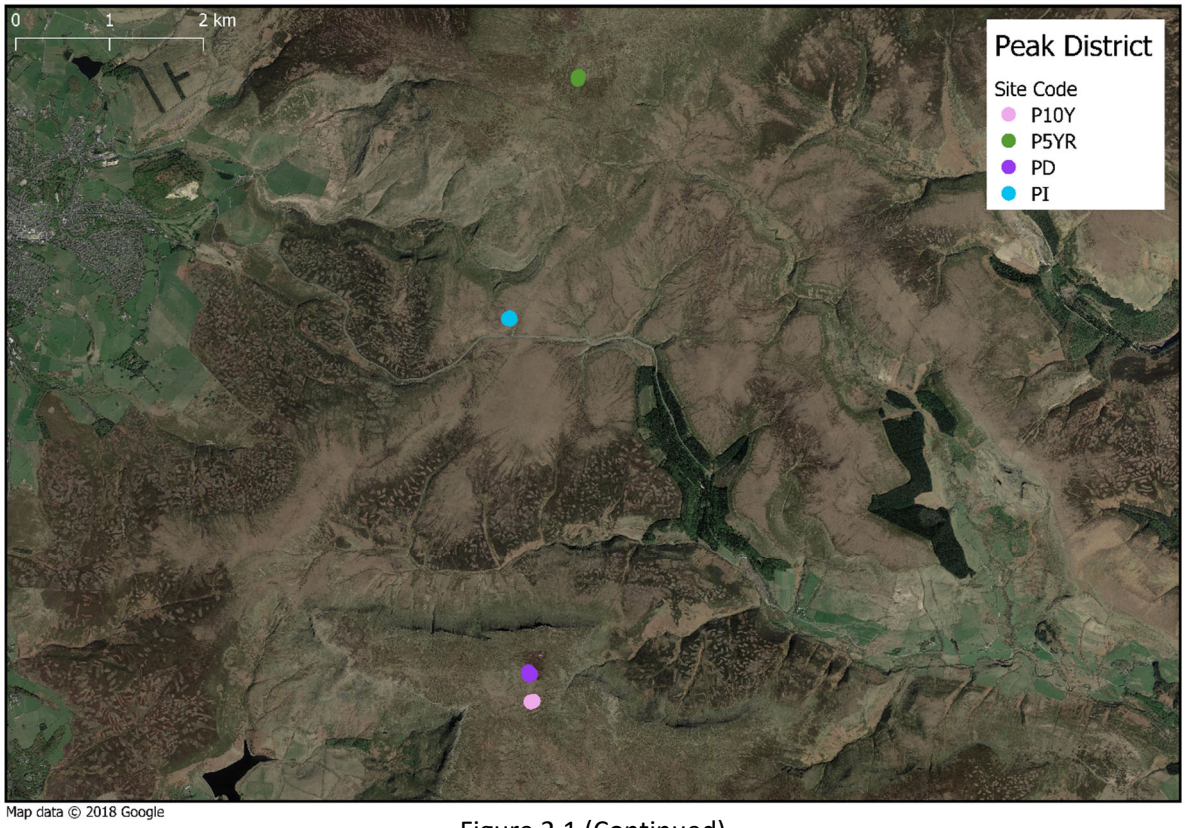


Figure 2.1 (Continued)

2.6 Mesocosm study

For the experiments in this thesis addressing carbon fluxes and water quality under controlled conditions, it was decided a mesocosm study was most appropriate. Mesocosm studies were defined by Odun (1984) as a "bounded and partially enclosed but outdoor experimental setup". They are used with the aim of bridging the gap between smaller, tightly controlled laboratory experiments, which can suffer from a lack of ecological realism (as a "caricature of reality" – Cadotte *et al.,* 2005), and larger "macrocosm" experiments where the complexity of the natural world can obscure the identification of mechanistic relationships (Stewart *et al.,* 2013). Additionally, they can provide a logistically and financially cheaper way of assessing samples that come from disparate areas, allowing the examination of peat bogs over a wide spatial scale but under similar environmental conditions.

Whilst using a mesocosm design aims to take a "best of both worlds" approach, there are still limitations to this method of study. The first of these lies in the isolation of the sample from its parent ecosystem, and, in this case, its movement from one climatic zone to the other. This gives rise to the possibility that over time (and space); the soil and its biota community may diverge from that of its original state (Dzialowski *et al.,* 2014) and that, even worse, this may occur within replicates as well as without (Gamble, 1990). The attempts to address this problem in this study lie in the comparison of climate and mesocosm data to a pre-monitoring phase under known environmental conditions. Data such as carbon flux components and microbial community could be compared for the majority of the cores, using samples taken, in the microbial case, at the time of mesocosm sampling and, in the case of the fluxes, from the years prior to sampling.

2.7 Mesocosm design

The design of the mesocosms used in this experiment employ a plant-soil carbon flux partitioning method whereby the contributions to ecosystem respiration (R_{eco}) from root-derived or rhizosphere (autotrophic), soil microbial or decomposition (heterotrophic) and overall plant-soil (net ecosystem exchange, NEE) components and to the carbon budget were quantified. In addition to calculating NEE, R_{eco} and methane fluxes, soil water was collected for DOC, SUVA and Hazen analysis (see Chapter 5). The central vegetated core was used to derive plant-soil ecosystem fluxes, whilst the two flanking auxiliary vegetation free cores were used to obtain soil fluxes, on the one side soil decomposition with root fluxes, and on the other side soil decomposition only (roots were excluded by repeated cutting – see Figure 2).

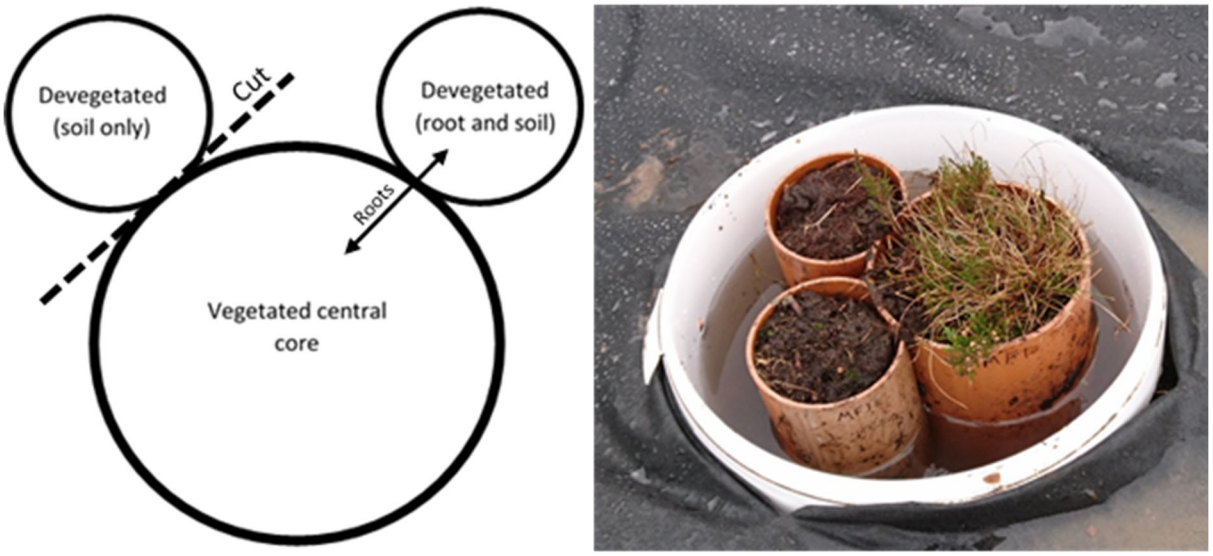


Figure 2.2: Left, Schematic of mesocosm vegetation set up. Right, a picture of a mesocosm in situ.

The mesocosms were located on the field site on Heslington East Campus, University of York. They were all stored in 33.8 cm wide and 41.8 cm deep white plastic buckets (EZ2500-00 Wolf Plastics, Germany) to maintain a constant controlled water table with three 5 mm wide holes at various depth, which were fitted with removable rubber bung. To allow free drainage of water from the buckets into the surrounding soil, and to prevent interaction with mineral soil components, an outside over was placed over the side of the drainage holes). The buckets were sunk into the soil, so that the top of the mesocosm was level with the ground surface – this was intended to ensure that the peat temperatures of the mesocosms' soil component remained comparable to soil temperature experienced under field conditions.

2.8 Mesocosm design rationale

There are several ways of measuring soil CO_2 efflux including root exclusion, physical separation of components, isotopic methods and indirect methods (Subke *et al.,* 2006). For this experiment, the approach included root exclusion by repeated cutting of roots with further overall above- versus belowground flux component separation, whilst maintaining the

connectedness of these components in a novel mesocosm design. The advantage of this design is that "snapshots" of the C cycle can be taken by measuring the various flux components, without damage to the mesocosms' integrity.

It is beyond the scope of this thesis to discuss the advantages and disadvantages of each flux separation method in more detail. However an extensive review conducted by Hanson *et al.,* (2000), identified isotopic methods as the method which least disturbs the sample, however, the high costs of this method prohibited its use in this project. A comparison of three methods, including root exclusion, component integration and isotopic methods by Sapronov & Kuzyakov (2007) found that although the isotopic method was highly accurate and did not disturb the soil, it could not be used to measure the contribution of the rhizosphere (via the soil surface) to the emission of CO_2. It is therefore prudent that we used a combined root exclusion/component integration method.

2.9 Heslington East site preparation and installation

Three trenches were measured, and then dug on the Heslington East site using an excavator. Piles of removed material were sifted by hand to remove stones in order to prevent damage or temperature issues with buckets. Buckets had overflow drains attached on site were situated equidistant from one another as per the map in Figure 3. The trenches were then backfilled by hand around the buckets, which were surrounded by earth, whilst turf and other compostable biomass was left to cover the spaces between the mesocosm trenches to avoid subsidence.

Without removing the bucket lids, black anti-weed matting was placed over the trenches to prevent vegetation growth around and between mesocosms. The anti-weed matting was cut to the shape of the buckets and pegged. After mesocosm installation, chicken wire meshes were placed over the buckets to prevent herbivory.

To aid logistics in keeping water tables at a set level, a rain collection system was built on site. A 3 m by 2 m steel scaffold structure was erected, which collected rainfall over a tilted corrugated plastic roof could fill three connected 200 L water butts. To prevent stagnation the water butts were half drained into the Heslington East Lake regularly. When rainfall was negligible and no water was available, deionised water was taken from the University of York Department of Environment and Geography building to maintain water levels (this was only the case during summer 2019 (twice).

To discourage herbivory, metal meshes (standard chicken wire) covered the mesocosms but allowed easy access at the top. To prevent contamination of mesocosms with mineral soil caused by rain splash, and to prevent the growth of weeds (which may cause shade) the mesocosms were surrounded by a black weed proof membrane soil cover (Figure 3) to about 50 cm width either side (buckets were inserted through slots in the membrane).

A weather station (AWS; MiniMet AWS, Skye Instruments Ltd, Llandrindod Wells, UK) was installed on site for continuous monitoring of air temperature and Photosynthetically Active Radiation at 1.5 m height above ground level alongside soil temperature data loggers (Gemini; Tinytag Plus 2; TGP 4017) used to assess potential differences between surface and buried mesocosm set-ups (one paired set per row) at 5 and 10 cm soil depth.

Figure 2.3: Installed mesocosms were surrounded by black weed proof membrane to limit rain splash from surrounding mineral soil. Note, the picture shows some initial splash as the surrounding soil was still bare before replacing vegetation.

2.10 Experimental design

The mesocosms were transported to the Heslington East site, University of York and sunk into the ground. Sinking the mesocosms up to the rim of the bucket into the ground prevented warming of buckets by the sun and maintained a more constant, realistic soil temperature.

The mesocosms were situated in a random block design throughout three trenches (Figure 3). The three trenches were at different distances from a hedge and tree at the southern border of the site, which shaded the mesocosms to various degrees (seasonal). However, the shade did not affect the mesocosms: in winter, the trees were leafless and in summer, the sun was high enough to illuminate all of the plots for most of the daylight hours.

The mesocosms were arranged within the blocks as per Figure 4, ensuring that a replicate from each management and national site category were present on each trench/block. Because the Peatland-ES-UK sites had four replicates per management category/site combination, the fourth replicate was randomly allocated to a block.

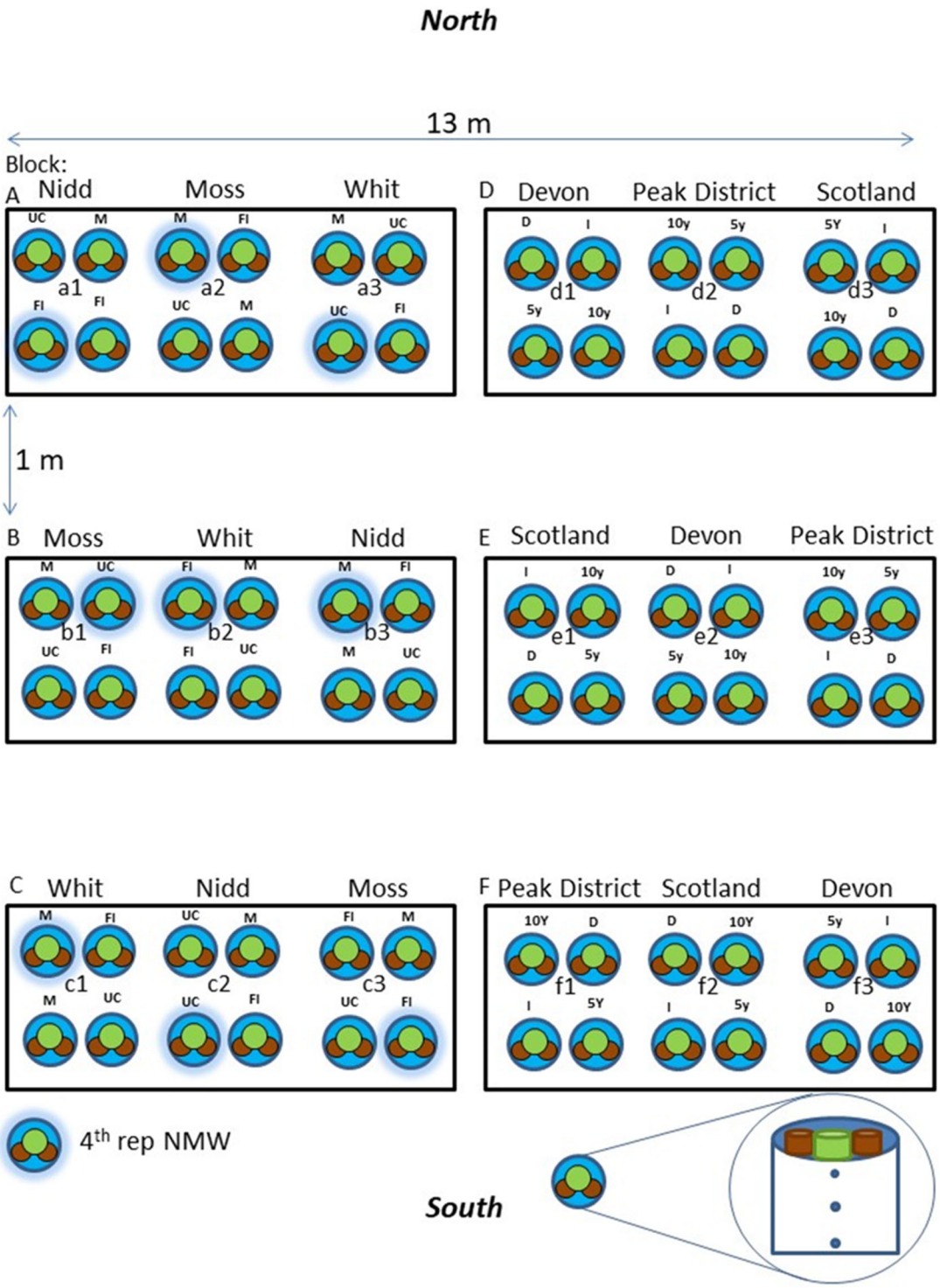


Figure 2.4: Layout of mesocosms on Heslington East site. The side facing South had trees growing nearby, whereas the side to the North was facing towards the University lake.

2.11 Heslington East maintenance

The following tasks were performed on a weekly basis (or when needed):

- - Water level monitoring/top up
- - Site integrity/security including fences, structure, and mesocosm meshes etc.
- - Grass cutting to prevent shading. This was done by hand with a sickle.
- - Removal of weeds/plants within vegetation free soil cores (side cores)

2.12 Mesocosm gas flux and water quality measurements

During the experiment, the mesocosms were monitored for gas fluxes, which included gathering data for NEE, soil respiration, and methane fluxes. The methods used were the same as Heinemeyer *et al.,* (2019), and are described in detail in that report, with these methods reproduced in brief here. Gas flux measurements were taken on the dates described inTable 3.

Table 2.3: dates of flux measurement collections on the Heslington East mesocosms.	
Measurement	Dates taken
NEE, methane flux	1st August 2019, 23rd October 2019, 29th October 2019, 3rd December 2019, 5th February 2020, 21st July 2020, 15th October 2020
Soil respiration	29th July 2019, 15th August 2019, 12th September 2019, 24th October 2019, 2nd December 2019, 6th February 2020, 22nd July 2020, 14th October 2020

Soil respiration

Soil respiration measurements were undertaken on the outer vegetation free (cut and uncut) cores seen in Figure 1. Measurements were undertaken using an Infrared Gas Analysis (IRGA) connected to a 10 cm automated survey chamber (Model 8100-102, LI-COR, Lincoln, NE, USA). The chamber was placed onto the cores, which were designed to accommodate this specific device. The CO_2 concentration was measured every second for 45-60 seconds. LI-COR Viewer software was used to derive CO_2 fluxes from the most linear 30 s portion of the measurement, based on the R^2 value (LI-COR Biosciences, 2007). The software calculated fluxes including the corrections in relation to air temperature and pressure, water vapour as well as chamber volume and surface area. In the mesocosms, soil respiration was partitioned into vegetation free with or without roots, as roots could in-grow from the main (vegetated) mesocosm core.

Net Ecosystem Exchange

Net Ecosystem Exchange (NEE) measurements were undertaken on the central vegetated core of each mesocosm, making sure not to damage any vegetation. Due to different vegetation heights, a number of custom made Perspex® chambers were created by the University of York Biology Workshop with different heights. The flux data were corrected for recorded chamber heights (9, 25, 50 cm). These chambers were connected to an IRGA (Model 8100, LI-COR,

Lincoln, NE, USA). The vegetation within each main mesocosm was bunched together to fit into the chamber, and a Photosynthetically Active Radiation (PAR) sensor (Q55 – PAR Quantum Sensor, Delta-T Devices, Cambridge, UK) was connected to the LI-COR external sensor interface and placed inside the chamber by attaching it to a custom 3D printed widget with a magnetic plate (manufactured by the University of York Biology Workshop), which was fitted to the inner top part of the chamber (without vegetation shadowing the sensor). An air temperature sensor was inserted from the top into the central part of the chamber, together with an in and outflow tube either side of it (at the chamber edge). This temperature was used by the LiCor software to correct gas concentrations (together with air pressure). CO2 fluxes were calculated using the dryCO_2 (i.e. water vapour corrected CO_2). A computer fan, fixed by the University of York Biology Workshop, was glued to the inside of the chamber at the lower part to ensure adequate air mixing in the chamber (seen in visually very stable CO_2 during chamber closure time).

The CO_2 concentration of the chamber was measured every second for between 45 and 90 s per light level, depending on the brightness of the day and temperature affecting vegetation and soil flux activity (winter fluxes need longer measurement times, whilst in summer measurement times can be shorter whilst still producing reliable results). After the first full light measurement (i.e. photosynthesis and respiration), without removing the clear Perspex® chamber, one or two shading mesh layers were placed (in flux measurement sequence) over the chamber, reducing light to 30% to 90% of the available light, respectively. For the final flux measurement, a custom-made dark cover (thick black plastic sheet) was placed over the chamber, blocking all light, to enable an ecosystem respiration (R_{eco}) measurement (i.e. including all plant and soil respiration components).

The LI-COR viewer software was again used to derive the CO_2 fluxes from the most linear 40-60 s portion (LI-COR Biosciences, 2007) of each NEE measurement under each light condition. All fluxes were corrected to account for chamber volume and surface area, temperature and pressure and were expressed in μmol CO_2 m^{-2} s^{-1}.

Using the same methods as Heinemeyer *et al.,* (2019) the combination of NEE and soil respiration measurements allowed a breaking down of the total flux into its main component fluxes for photosynthesis (vegetation) and respiration (shoot, root, microbial) and key carbon balance parameters, enabling the calculation of gross primary productivity (GPP) and net primary productivity (NPP). Very briefly, GPP was derived as the sum of R_{eco} and the additive inverse of NEE. NPP was derived by subtracting plant respiration components from GPP.

Methane Flux

Methane (CH_4) measurements were measured in real time using an Ultraportable Greenhouse Gas Analyser (UGGA; Los Gatos Research, USA) in 2019 or for 2020 a LiCor 7810 GHG analyser (LiCor Biosciences, USA). The methane analysers were connected to the flux chamber with tubing (Bev-a-line IV, Thermoplastic Processes Inc., USA) via a closed loop sub-sampling system from the LiCor analyser (taking gas from the LiCor outflow and circulating it back to the LiCor inflow). A tablet computer was used to view the fluxes in real time by connecting to the analysers via Wi-Fi. Where methane flux spiked, indicating a bubble, the measurement was repeated after venting and replacing the chamber, although this was rare because the chamber was not pressing onto the soil peat surface but rested on the soil core rim. Methane flux was calculated using the LiCor SoilFluxPro software (by combining the LiCor fluxes with

the methane fluxes based on a time stamp) in nmol CH_4 m^{-2} s^{-1}. The flux calculations also included the above corrections and considerations.

Dissolved Organic Carbon, Hazen and SUVA

The dissolved organic carbon, specific UV absorbance and Hazen measurements in this thesis were measured by another PhD student, Abby Mycroft. The method is as per Mycroft (*pers comm.,* thesis to be published 2023). Briefly, water samples were taken from peat samples (at 15 cm depth) taken at the same time when the mesocosm samples were collected (via the mesocosm excavation hole.

To obtain the initial peat sample dissolved organic carbon (DOC) content, 3 ± 0.1 g of peat sample was added to 27 ml ultrapure water and manually homogenised for 60 s. Peat solutions were filtered using 0.45 µm Rhizon filters (Rhizosphere Research Products B.V., The Netherlands) and stored in the dark at 4°C until analysis. 4.5 ml of peat solution was added to 4.5 ml ultrapure water in a VarioTOC vial and sealed with foil to prevent evaporation. DOC was measured as non-purgeable organic carbon (NPOC) using a VarioTOC (Elementar Analysensysteme GmbH, Hanau, Germany). A 5-point calibration of 5, 10, 20, 50, and 100 ppm was used, which was made up from a 500 mg/L stock solution prepared with 4.4121 g sodium carbonate (Na_2CO_3) and 1.0627 g potassium phthalate (KHP) in 1 L ultra-pure deionised water. Prior to calibration standards, a run-in and a drift sample (both 50 ppm stock solution), and two blanks (ultra-pure deionised water) were included. Following calibration standards, a blank and reference standard (stock solution 50 ppm) were run followed by samples with a drift and blank after every 12 samples. After running all samples, a drift, blank and HCl sample were included. All vials were acidified using 100 µl 0.1 M HCl and spared with oxygen for one minute to remove inorganic carbon. Operating temperature and outlet pressure were set to 850°C and 1.0-1.2 bar pressure. The oxygen inlet pressure was set to 950-1000 mbar. Sample volume was 0.250 ml.

The UV absorbency of samples was measured using a 1 cm wide glass cuvette at wavelengths 254, 400, 465, and 665 nm. UV wavelengths were measured using a Lambda ʎ25 UV-vis spectrophotometer (PerkinElmner Ltd, Beaconsfield, UK). Following measurements, the cuvette was rinsed with ultra-pure deionised water and dried using a white Kimtech science paper wipe. Absorbency was blank corrected with an ultra-pure deionised water blank. All samples were measured in triplicate.

SUVA values refer to the specific UV absorbance of the water, normalised by DOC content, which is obtained by dividing the UV absorbance of a sample at 254 nm by the DOC (mg/L) concentration, and is expressed in units of L/mg-m (i.e. the 1 cm readings were multiplied by 100 to convert it to per m).

Hazen was calculated by multiplying the absorbance at 400 nm by 12 based on a previous study (Watts *et al.,* 2001).

2.13 Site mesocosm and microbial peat sampling

Site characteristics were recorded to use as metadata in the analysis of the microbial communities. In the field, we collected the location via GPS, the slope angle and aspect (with compass and clinometer representing the surrounding 50 m area), the peat depth using plastic rods (drain pipe cleaning units; Bailey 19mm width 9 m length black drain rod) pushed into the ground, and the main vegetation cover types (split into Calluna, Moss, Sphagnum, Sedge, Bare and Grass) and percentages thereof. In addition, peat chemical-physical properties were recorded by another PhD student (Mycroft, 2022, in prep), which included bulk density, pH and levels of dissolved organic carbon (DOC) as well as various water quality parameters (the subject of the Mycroft PhD).

The exact mesocosm site choice was based upon the requirement for healthy vegetation (which, in the case of *Calluna vulgaris* did not trail too far over the ground) and that generally represented the character of the area. The mesocosm tubes were first placed over the peat (for the vegetated core over a vegetation area and for the vegetation free side cores over moss/bare peat areas within 50 cm of the vegetation core site) and then the outline was cut using a knife to improve the mesocosm tube insertion through the root mass into the peat. The mesocosms were then hammered into the peat, using a rubber mallet and a wooden plank placed over the top of the PVC tube unit (to distribute the force and prevent breaking). For the vegetated core, another tube was placed on top for the hammering section, so as not to damage the vegetation. The peat-filled tube was then removed using a drain shovel (by undercutting the unit from the side). Care was taken not to lose any material from the base of the core.

Microbial samples were taken from the freshly cut central mesocosm tube excavation area, at -15 cm depth. Two 5 cm diameter metal cookie cutters were pushed into the peat, and then cut out using a knife. This method avoided compaction of the samples, which may have pushed water from the sample, as well as affecting bulk density measurements. Samples were immediately bagged in plastic Ziploc bags, with as much air pushed out as possible to reduce oxygenation of the sample, and then placed into a freezer bag with cooling bags during transport to the research lab.

2.14 Sample storage and preparation

Mesocosms excavated in the field were stored as cold as possible i.e. either in a cold room or outside and were placed in their buckets on Heslington East mostly within 24 hours. Where samples could not be placed within 24 hours (specifically, Scottish sites were placed the next day due to the journey times involved), they were stored in a dark cold store at 4°C in the University of York Environment Department for a couple of days.

Peat soil samples were kept as cool as possible with an insulated bag in the field, and were then frozen at -20°C within 12 hours of sampling using any available freezer. Samples were transported back to York in an insulated sealed icebox.

Peat soil microbial samples were removed from their metal collars gently (by hand) defrosting the outer rim. To obtain a microbial sample, the frozen peat samples were sub-sampled with a hammer and chisel, sterilised between each use. Approximately 1-2g of subsample was taken from the interior of each peat sample.

Peat sub-samples were weighed and then freeze-dried at -55°C using a Labogene Coolsafe freeze drier. Peat subsamples were weighed post drying to obtain a percentage moisture value for each peat sample.

2.15 DNA extraction

DNA was extracted using a PowerSoil DNA Isolation kit (Qiagen). Original extractions, following the manufacturer's protocol, did not result in adequate DNA extraction. The amount of soil put into the initial lysis tube was therefore decreased by half (to approximately 0.125g dry soil) and the lysing time was increased on the vortexer to 15 mins, which resulted in improved DNA extractions.

The extracted DNA was then quantified using a Nanodrop spectrophotometer (Thermo Fisher Scientific). All samples were then diluted to 5 ng/ µl unless the concentration of DNA was already under 5 ng/µl where it was not diluted (it was not concentrated).

Whilst the initial DNA extractions were quantified using a Nanodrop, this was found to give inaccurate readings for the quantity and quality of extracted DNA used in these experiments, and consequently all quantification was undertaken using an Invitrogen Qubit 1 fluorimeter following standard protocols with a Qubit dsDNA HS Assay Kit (both Thermo Fisher Scientific).

2.16 PCR for community profiles

All samples underwent multiple polymerase chain reactions (PCR) with primers from four groups: the fungi, bacteria, archaea and eukaryota. Each group required a different reaction as well as different troubleshooting steps, and therefore the following section is subdivided into PCR details specific to each group/step.

Blotto blocking buffer was made using 1 mg/ml of skimmed milk powder in deionised water. The working concentration was 0.02 mg/ml.

All electrophoresis gels were made using 1 % agarose in Tris/Borate/EDTA (TBE) buffer with 1 µl SYBR Safe DNA gel stain.

2.17 Fungi

All samples underwent PCR using ITS4 and ITS7 primers (Schoch *et al.,* 2012 – see the sequences provided in Table 4 below) followed by gITS7-ill and ITS4-ill in the secondary reaction. Per reaction, the master mix contained 11 µl of nuclease-free water, 4 µl buffer (Flexi Reaction Buffer, Promega), 1.2 µl Magnesium Chloride, 0.4 µl dNTP (10 mM), 0.4 µl forward

primer (ITS4), 0.4 µl reverse primer (ITS7), 0.5 µl Blotto (standard mixture), 0.1 µl GoTaq hot start DNA polymerase (Promega) and 2 µl extracted DNA for a total reaction volume of 20 µl.

Initial tests found only small amounts of DNA replicated in each sample, and so a second nested PCR was undertaken. Each reaction consisted of 15.875 µl of nuclease-free water, 5 µl buffer, 1.5 µl Magnesium Chloride, 0.5 µl dNTP (10mM), 0.5 µl forward primer, 0.5 µl reverse primer, 0.5 µl blotto, 0.125 µl GoTaq and 1 µl of the first PCR product, for a total reaction volume of 25 µl.

In both reaction sets, reactions were cycled at 95°C for 2 min, cycled 30 times at 94°C for 30 s, 55°C for 45 s, and 72°C for 1 min 30s, with a 10 min final extension at 72°C.

2.18 Bacteria

All samples underwent PCR using 27F and 806R primers, followed by 515Y-ill and 806med-ill primers (Caporaso *et al.,* 2011 – see sequences provided in Table 4 below). Per reaction, the master mix contained 10.5 µl of nuclease-free water, 4 µl buffer, 1.2 µl magnesium chloride, 0.4 µl dNTP (10mM), 0.4 µl forward primer (515Y-ill), 0.4 µl reverse primer (806med-ill), 1.0 µl blotto, 0.1 µl GoTaq and 2 µl extracted DNA for a total reaction volume of 20 µl.

Initial sets employed 25 cycles during the reaction but results were poor so this amplification was increased to 30 cycles. Finally, reactions were cycled at 95°C for 2 min, cycled 30 times at 94°C for 30 s, 57°C for 45 s, and 72°C for 1 min 30s, with a 10 min final extension at 72°C. In general, amplification was more difficult in peat compared to soil, possibly because of the degradation of DNA due to acidity, among other potential reasons such as polluting substances.

2.19 Archaea

Initially, all samples underwent PCR using Arch349F-ill/Arch806R-ill (Takai & Horikoshi, 2000), but these primers were later changed (due to their failure), and the original reactions and experiments are described here as a record of null results. Per reaction, the master mix contained 10.5 µl of nuclease-free water, 4 µl buffer, 1.2 µl magnesium chloride, 0.4 µl dNTP (10 mM), 0.4 µl forward primer (Arch349-ill), 0.4 µl reverse primer (Arch806-ill), 1.0 µl blotto, 0.1 µl GoTaq and 2 µl extracted DNA for a total reaction volume of 20 µl.

Reactions were cycled at 94°C for 3 min, cycled 30 times at 94°C for 30 s, 50°C for 30 s, and 72°C for 30s, with a 5 min final extension at 72°C. This amplification again produced poor results and a second nested PCR was used with the same programme and master mix, but using 2 µl of the initial product.

Despite this improvement, some specific sites still had very poor replication with only faint bands present. These sites were limited to the Peak District, but spanned the categories from “intact” to “degraded”. Potentially, this meant that some sort of inhibitor was preventing proper replication or, alternatively that there simply was not enough Archaeal DNA in the samples to be able to obtain a reasonable quantity via replication. It was hypothesised that control samples with a known DNA quantity would fail to properly undergo PCR when spiked

with DNA samples from the Peak District. Therefore, to test this, a spiking experiment was undertaken. Six control samples were used plus a blank as per the diagram in Figure 5.

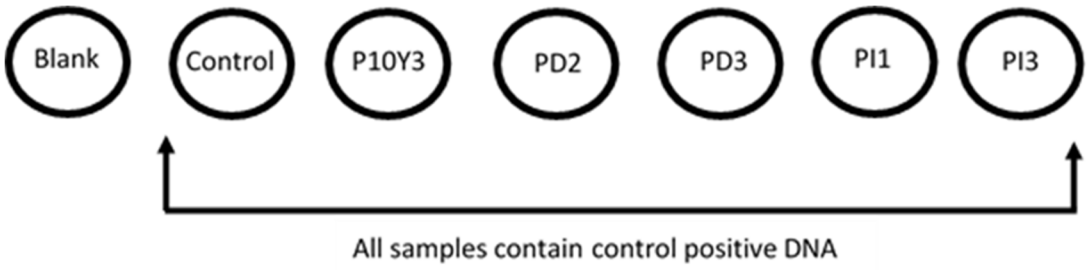


Figure 2.5: Tube labelling in Archaeal spiking experiment. All samples apart from the control are from the Peak District; refer to Table 2 for naming conventions

Each sample contained control DNA, but samples were then spiked with 1 µl DNA sample from the Peak District sites.

All samples underwent PCR using Arch349F-ill/Arch806R-ill primers. Per reaction, the master mix contained 9.5 µl of nuclease-free water, 4 µl buffer, 1.2 µl magnesium chloride, 0.4 µl dNTP (10mM), 0.4 µl forward primer (Arch349-ill), 0.4 µl reverse primer (Arch806-ill), 1.0 µl blotto, 0.1 µl GoTaq, 2 µl of "control" known DNA and 1 µl Peak District sample for a total reaction volume of 20 µl. The control and the blank did not contain the Peak District spike and so the amount of nuclease-free water was increased to 10.5 µl per reaction.

The PCR programme used in the original experiment was kept intact and consisted of a primary PCR followed by a secondary PCR containing 2 µl of the primary PCR product.

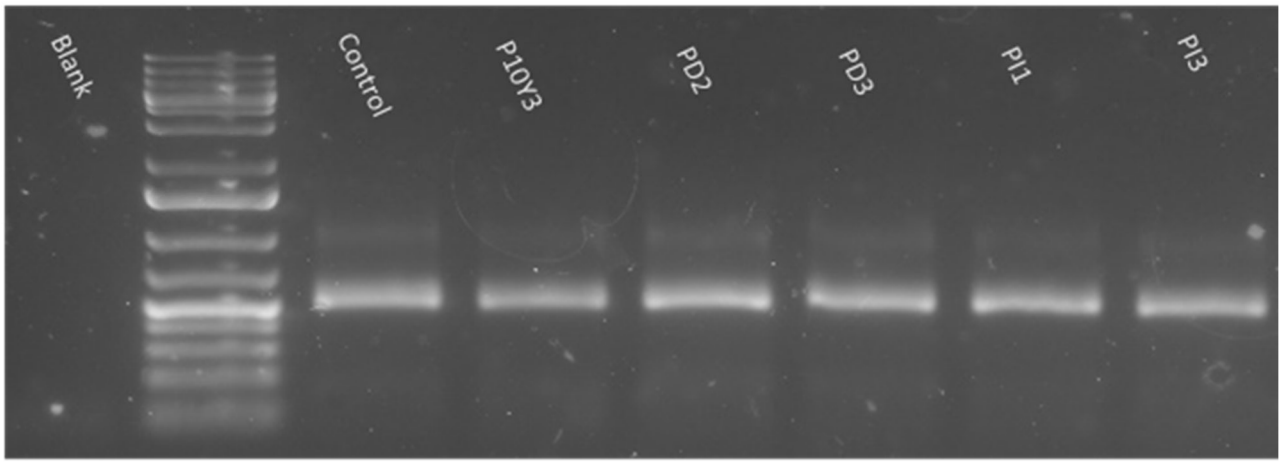


Figure 2.6: Gel showing the presence of Archaeal DNA across all samples. All samples are the same brightness. The codes used are in Table 1, and only Peak District samples were used for this experiment.

The secondary PCR product was loaded on to a gel (for gel details see start of this section) and can be seen in Figure 6. The brightness of a gel band is an indicator of the amount of material at that point in the gel. Because the bands are all the same, it can be justifiably deduce that the spiking with Peak District samples has had no effect on the ability of the reaction mixture to undergo PCR.

This indicates that there are no inhibitors in the DNA extract. Rather, it is more likely that there are simply very low levels of archaeal DNA abundance in these particular Peak District sites.

Therefore, as the above primers produced poor results, a new set of primers was used. These were 344F and 1041R in the initial reaction, followed by 519F-ill and 806R-ill in the second reaction, based on Pausan *et al.,* 2019. The sequences for these primers can be found in Table 4. For these new primers, the master mix consisted of 11.5 µl of nuclease-free water, 4 µl buffer, 1.2 µl magnesium chloride, 0.4 µl dNTP (10mM), 0.4 µl forward primer, 0.4 µl reverse primer, 0.1 µl GoTaq and 2 µl extracted DNA for a total reaction volume of 20 µl. No blotto was used in this reaction.

The initial reaction was cycled at 95°C for 5 min, cycled 25 times at 94°C for 30 s, 56°C for 45 s, and 72°C for 1 min, with a 10 min final extension at 72°C. The secondary nested PCR (to increase yield) was cycled at 95°C for 5 min, cycled 25 times at 95°C for 40 s, 63°C for 2 min, and 72°C for 1 min, with a 10 min final extension at 72°C. These new primers and new reaction programmes produced excellent yields even in the most degraded sites.

2.20 Eukaryotes

The study attempted to extract Eukaryotic community profiles from the soil samples, but this ultimately failed and the attempt to include Eukaryotic profiles was abandoned due to insufficient PCR product yield. Nonetheless, the methods are described below as a record of a failed result.

All samples underwent PCR with the primers TAReuk454F-ill and TAReukREV3-ill (Stoeck *et al.,* 2010). Per reaction, the master mix contained 15.75 µl of nuclease-free water, 6 µl buffer, 1.8 µl magnesium chloride, 0.6 µl dNTP (10 mM), 0.6 µl forward primer (TAReuk454F-illl), 0.6 µl reverse primer (TAReukREV3-ill), 1.5 µl blotto, 0.15 µl GoTaq and 3 µl extracted DNA for a total reaction volume of 30 µl.

Reactions were cycled at 95°C for 5 min, cycled 30 times at 95°C for 1 min, 55°C for 30 s, and 72°C for 30s, and received a 5 min final extension at 72°C. A secondary nested PCR was undertaken using 3µl of the primary PCR product with the same PCR mix and programme.

The initial gel showed low levels of Eukaryotes at the specified band and a contaminated blank at the lower end of the gel between 75-200bp. Whilst it was possible that this was a primer dimer, experiments were conducted to elucidate the source.

First, all of the reagents aside from the GoTaq were replaced (due to cost), and then tested with bacterial primers. Bacterial primers resulted in a gel that did not show a clear indication of a contaminated blank, with only a very faint band possible after a 30-second camera exposure (not visible to the naked eye). However, as it was impossible to rule out contamination during the preparation process, the trial was repeated with fungal ITS (on the basis that it should be very unlikely to contaminate a fungal master mix with human breath). The fungal ITS gel showed a negative blank. The PCR was then repeated with Eukaryotic primers (Figure 6), but this again showed a distinctive and bright band between 75-200bp.

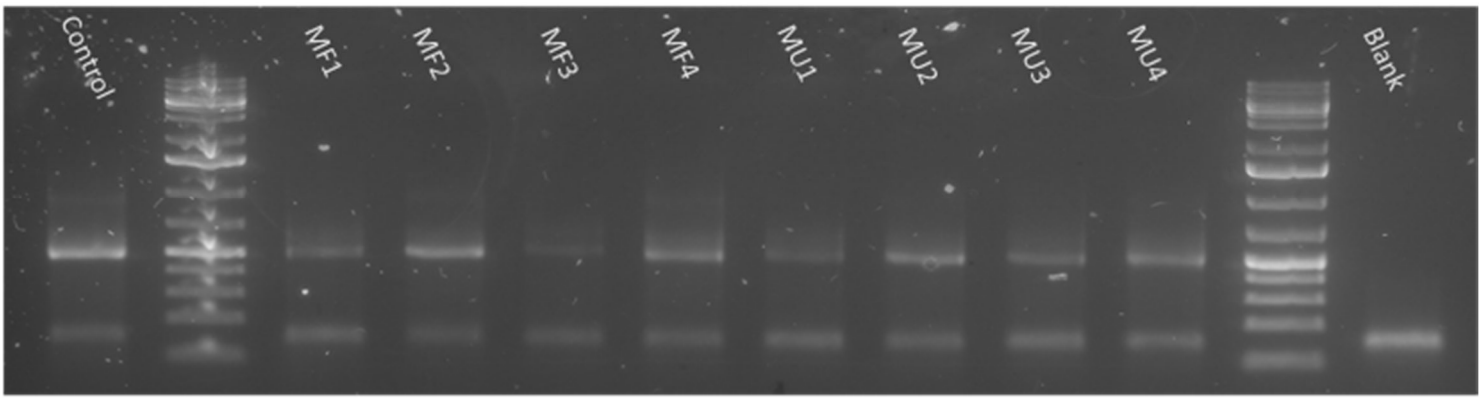


Figure 2.7: PCR products from strip 1 samples

Whilst this lower band contamination was an issue, it is also noteworthy that the PCR products did not produce significant DNA amplification (see the 500bp band in Figure 7). These faint bands are the result of a secondary nested PCR with a programme on 30 cycles. The fact that these were the "best" samples (the ones that repeatedly produced the best amplification of DNA with other primers compared to the much-degraded sites) led to the concern that no sequenceable Eukaryotic DNA could be obtained from the degraded sites at all.

To attempt to discern any problems with GoTaq contamination and to test whether Eukaryotic DNA was amplifiable from the degraded sites, the following trial was designed and tested (Figure 8).

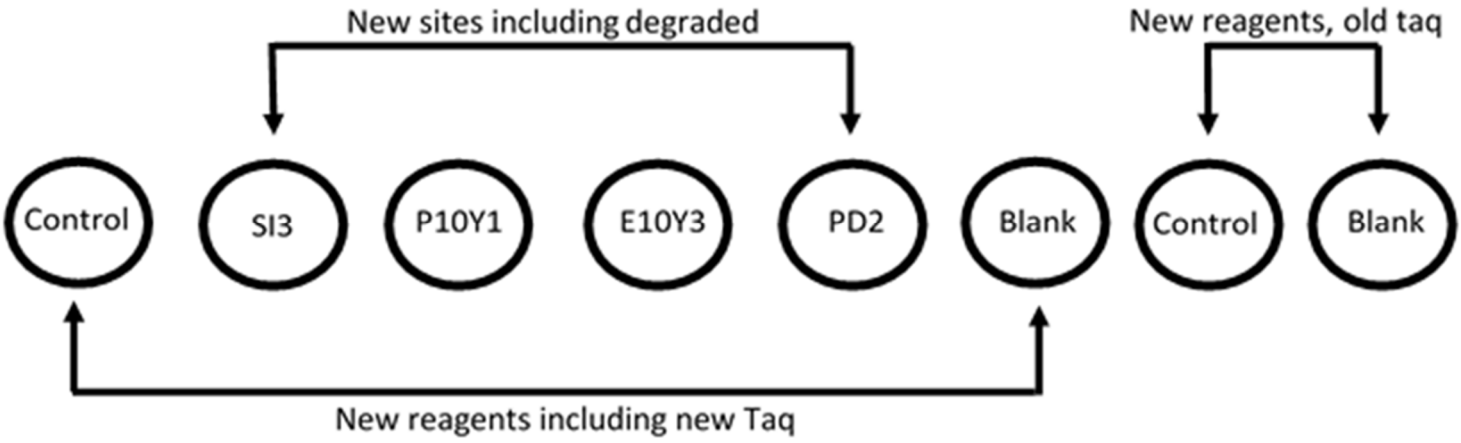


Figure 2.8: Test to determine possible contamination and amplification issues in Eukaryotic PCR

The design was as outlined in Figure 8: the first six samples used entirely new reagents, including new Taq polymerase, whilst the last two samples used new reagents but old Taq polymerase, thus giving the ability to discern whether Taq contamination was an issue. Samples two to four were DNA extractions from a spectrum of sites, including SI3 (Scotland Intact) which had always been successful in amplification, to PD2 (Peak District Degraded) which usually returned blank. The reactions were mixed on an entirely new lab bench with different pipettes after a thorough cleaning using RNAaway. Reaction mixtures and PCR programmes were the same as above, apart from an increase in cycles from 30 x to 35 x in the secondary PCR.

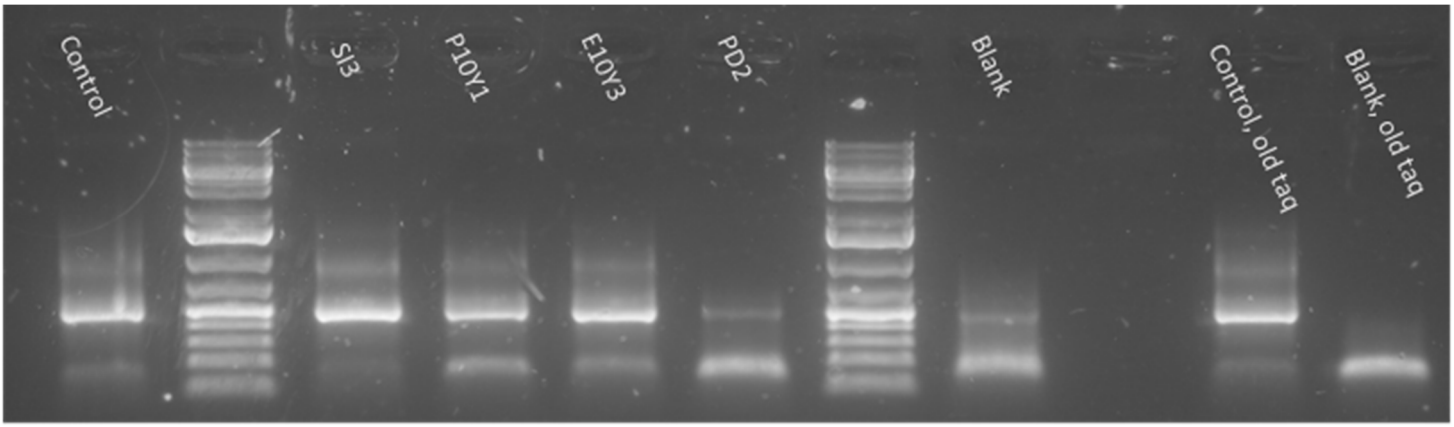


Figure 2.9: Gel of PCR products obtained from the experiment in Figure 8

As can be observed in Figure 9, after thorough cleaning and replacement of all reagents the band between 75 and 200bp was still present, although it was much reduced in the samples where (presumably) plenty of template at 500bp is available (the control and the Scottish Intact site). The gel revealed a contamination in the new set of reagents and that this contamination was almost as bright as the band in the Peak District Degraded (PD2) sample. This contamination of the new blank meant it could not be discerned whether the PD2 sample really had adequate DNA to form a band or whether this was a contamination artefact. The other bands at 500bp were significantly brighter than the contaminated blank and thus indicated that Eukaryote amplification is possible, but was very low in these samples.

To discern the identity of the 75-200bp band, sample 8 (the blank with old Taq, which contained only the new band) was purified with a GenElute PCR clean up kit (Sigma Aldrich) and then sent for direct Sanger sequencing (Source Bioscience). This failed due to insufficient DNA quality and the process was abandoned due to time constraints.

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Table 2.4: Primer names and sequences used in this thesis.		
Group	Primer name	Primer sequence (5'->3') including Illumina tags where indicated in primer name
Bacteria, 1st reaction	27F	AGAGTTTGATCMTGGCTCAG
	806R	GGACTACHVGGGTWTCTAAT
Bacteria 2nd reaction	515F-ill	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGANNNHNNNWNNNHGTGCCAGCMGCCGCGGTAA
	806R-ill	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGGACTACHVGGGTWTCTAAT
Archaea, 1st reaction	344F	ACGGGGYGCAGCAGGCGCGA
	1041R	GGCCATGCACCWCCTCTC
Archaea, 2nd reaction	519F-ill	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-A–NNNHNNNWNNNHCAGCMGCCGCGGTA
	806R-ill	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGGACTACHVGGGTWTCTAAT
Fungi, 1st reaction	ITS1	TCCGTAGGTGAACCTGCGG
	ITS4	TCCTCCGCTTATTGATATGC
Fungi, 2nd reaction	gITS7-ill	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGANNNHNNNWNNNHGTGARTCATCGARTCTTTG
	ITS4-ill	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCTCCGCTTATTGATATGC

2.21 Microbial data processing

All Illumina sequence data was processed via the *DADA2* (Callaghan *et al.,* 2016) pipeline in R with the use of 'cutadapt' (Martin, 2011) specifically for primer removal in the ITS pipeline. Sequences containing ambiguous bases were removed, as were primer sequences. Sequences were then filtered and truncated based on manual inspection of plotted read quality profiles (i.e. the read numbers at each step in Table 5). Amplicon Sequence Variants (ASVs – an alternative to OTUs see Callahan *et al.,* 2017) were then identified, chimeras removed and taxa identified using the UNITE database fungi file version 8.2 (Abarenkov *et al.,* 2020). The data was then combined with environmental data into a *Phyloseq* object (McMurdie & Holmes, 2013) for further processing. After manually checking sequence taxonomic identity using BLAST, amplicon sequence variants were clustered to OTUs at a 97% sequence similarity level, on the basis that some species were split into several ASVs, artificially raising estimates of species richness. Further analysis, including the specific R packages used, are described in their respective data chapters (i.e. chapters 3, 4, 5).

Table 2.5: Number of reads remaining at each step in the sequence data processing process.

Community	Total reads	Post filtering	Post de-noise and merge	Post chimera removal	Percentage loss (%)
Fungi	Unavailable	Unavailable	Unavailable	Unavailable	Unavailable
Bacteria	5,337,285	4,270,788	3,803,713	3,647,505	31.66
Archaea	6,339,805	5,560,857	5,260,728	4,279645	32.50

Chapter 3: The effects of management upon microbial communities in UK blanket bog

3.1 Introduction

The management of UK upland blanket bogs has become a 'hot topic' within both the academic world and the public arena. A wide range of management practices are undertaken, depending largely on site ownership, but also on site history such as past drainage and whether the site is designated, as most English blanket bogs are (Natural England, 2015) and which income streams are the focus.

This chapter will examine the effect of the management and site conditions upon the fungal, bacterial and archaeal soil communities. Broadly, the management categories can be split into two major site uses: grouse moors, managed predominantly for shooting and reserves that are mainly managed for the provision of positive ecosystem service outcomes. All of the Yorkshire sites in this thesis (See Chapter 2 for site descriptions) are managed as grouse moors, and form part of the Peatland-ES-UK project, a long term monitoring experiment that examines the impact of heather burning, alternative mowing or leaving heather to age (no vegetation management). In addition, another National Nature Reserve (NNR) site (Moor House; previously under burn management 80 years ago) is included in the Northern Pennines. Finally, further sites are sampled in Exmoor, the Peak District and Scotland, which are managed by organisations who aim to maintain, or restore land to hydrologically 'active' blanket bog with habitat condition based on vegetation assessments as a focus. The mechanisms by which these sites are managed are described below.

3.2 Grouse moor management

Many moors are rotationally burnt, which is used to maintain a habitat mosaic beneficial to red grouse (*Lagopus lagopus scoticus*) with potentially negative impacts on many bog functions (Brown *et al.,* 2016) although the impacts are uncertain and controversial (Harper *et al.,* 2018, Davies *et al.,* 2016,) for reasons discussed in Chapter 1, section 1.8. Because vegetation burning modifies the vegetation (Noble *et al.,* 2019), the hydrological regime (Holden *et al.,* 2015) and the carbon cycle (Clay *et al.,* 2010, Harper *et al.,* 2018), it is likely to affect the microbial community. Whilst some impacts from burning have been observed in blanket bog microbial communities, such as changes in expression of methanotrophic genes under different bog vegetation types (Chen *et al.,* 2008) and changes in methanotrophic communities after wildfire (Danilova *et al.,* 2015), the long term effects of managed burning on the microbial community relative to other management techniques, especially in terms of diversity, taxonomic composition, and function, is unknown.

An alternative vegetation management is mowing, which would result in the same habitat mosaic (i.e. age structure) and reduction of live vegetation biomass, but without the reported potential negative impacts of burning. However, the effects of mowing on blanket bog are understudied, including impacts on the microbial community. A recent DEFRA report (Heinemeyer *et al.,* 2019) found mowing slightly increased water tables and peat surface moisture, but was associated with a reduction in micro-topography and an increased cover of sedges, which may alter C cycle processes via root exudation and specific microbial associations. Sedge abundance is linked to net methane (CH_4) emissions (Strack *et al.,* 2006)

with impacts on the bog's net GHG balance, and to deep peat layer oxidation via deep rooting and aerenchyma tissue likely facilitating microbial priming and a microbial competition (for oxygen) mediated decrease in oxidation of CH_4 (Waldo *et al.,* 2019). The mowing undertaken as part of Peatland-ES-UK left cut vegetation brash *in situ*, which not only alters peat moisture but also constitutes a nutrient input from decomposition, both of which might affect microbial community composition and functions.

The Peatland-ES-UK sites (see Chapter 2 Section 2.11) also contain a plot-level control that consists of uncut (unmanaged) *Calluna vulgaris* areas. The *Calluna* in these plots (5 x 5 m) has reached >30 years and become tall and rank, passing its mature phase and becoming degenerate (Barclay-Estrup & Gimmingham, 1969), and is starting to become layered (after falling over) with adventitious roots that are associated with wetter stands (MacDonald *et al.,* 1995), although they are generally drier than neighbouring mown areas (Heinemeyer *et al.,* 2019). This lack of management is essentially abandonment but is proposed by conservationists and Natural England (where management is prevented) and the consequences for microbial ecology and related functions are unknown. Yet it would not be surprising to find changes in the microbial rhizosphere composition given the dominance of *Calluna*, a highly ericoid mycorrhizal plant where the fungal partner facilitates (with special enzymatic capabilities) mineralisation and nutrient access (Pearson & Read, 1975). It is possible that mosses forming under this undisturbed and increasingly opening up *Calluna* canopy will alter environmental conditions, also more suitable for *Sphagnum* moss, and that this might also have consequences for the microbial community.

Finally, three samples were also taken from the Hard Hill heather burning experiment on Moor House NNR, in the Northern Pennines. The samples were taken from outside the experimental blocks; consequently, they represent a *Calluna* dominated blanket bog that was previously a grouse moor but has not been burnt since 1954 (Clutterbuck *et al.,* 2020). Whilst not an unmodified blanket bog, it represents a long-term fire recovery plot and is of consequent interest in comparisons to uncut, mown and burnt plots from the Peatland-ES-UK study and in relation to assessing a path of recovery towards a 'natural' bog.

3.3 National Sites and restoration

The other half of the experiment comprises samples taken from Scotland, Exmoor, and the Peak District. At each of these sites, the samples are split into 'intact' plots, degraded plots, plots five years post restoration, and finally, plots that are ten years post restoration. These categories do not follow standardised definitions (see Chapter 2 section 2.9 for site selection criteria) – however, briefly, the sites were selected by asking whoever managed the land to identify their intact and degraded sites, etc. This means that there is within-treatment heterogeneity by design, which is influenced by site-specific organisational objectives. A fuller description of site choice criteria is given in Chapter 2: Methods.

The Peak District contains the most severely degraded peatland in this study; a description of the site history is given in Chapter 2 section 2.11. It has been restored from what might be considered a wasteland of bare and eroding peat, wherein Moors for the Future and the National Trust sought to revegetate the area in order to provide carbon sequestration and water quality benefits. Consequently, the degraded plots contain no vegetation at all, whilst the restored plots contain young heather and a more typical degraded bog vegetation community (NVC M20, moving towards M19, although extensively gullied [Linton, 2007]). The

intact samples in the Peak District are hydrologically intact (i.e. consisting of an acrotelm with a water table depth near the surface) and are the only peat samples that might reasonably be called blanket bog, because they are the only one considered to be hydrologically intact (Walker *et al.,* undated).

The Exmoor sites are also distinct, due to their location in a warmer and wetter area of the UK and the dominance of *Molinia* grasses in the restored plots. These conditions have largely unknown consequences for carbon cycling, including gas fluxes and water quality. Whilst the intact site was a wet *Sphagnum* and sedge dominated community, the degraded samples were in grazed farmland, with an abundance of sedge (*Eriophorum vaginatum* and *E. angustifolium)* and scant *Calluna*. The restored plots, rewetted by the Exmoor Mires Partnership, are *Molinia* dominated blanket bog.

Finally, the Scottish sites are located in the Forsinard Flow Country, Caithness, and managed by the RSPB. These sites were previously managed for shooting and underwent some rotational burning until the 1990's, but have since been restored via drain blocking and grazing cessation. The intact samples come from lawn areas surrounded by typical bog pools, whilst the degraded samples were taken immediately adjacent to a gullied area previously part of the shooting management. None of the samples was taken from sites that were previously forested in the latter half of the last century, although they are surrounded by such afforested areas, which are increasingly under forest-to-bog restoration.

3.4 Microbial communities under differing managements

We might predict that microbial communities will change with management practice (see Chapter 1 section 1.8), but the results from previous studies are inconsistent. Elliot *et al.* (2015) in their study of microbial communities in the Peak District, UK, found distinct microbial communities between managements, and linked these to natural and human induced soil parameters. It is notable however, that when classifying their communities to taxonomic order, around 50% of the communities in all plots were comprised of *Proteobacteria*. This is a hugely taxonomically diverse group, so a finer resolution community analysis may reveal wider diversity, or it may be that a large amount of the community is taxonomically similar regardless of management or habitat state. Importantly in terms of the study in this thesis, the Elliot *et al.,* (2015) management treatments were very distinct, especially in terms of vegetation. The differences in vegetation between mowing and burning are far more subtle in the Peatland-ES-UK plots and increasingly so the further post management it is measured as vegetation recovery is similar. Therefore, the effect of management might be even less pronounced. Burning, for example, is reasonably well studied in terms of its effects on hydrology, but less so in reference to microbial communities. What is often missing is an examination of the range of fire conditions, especially in the UK. Examples from Russia (Belova *et al.,* 2014) have found significant shifts in microbial populations after wildfire on boreal peats, which are hotter and therefore likely burn into the peat, whereas contrasting studies from burnt (and drained) tropical peatlands have found no effect on methanotrophic communities (Arai *et al.,* 2014). It is important to note however, that boreal and tropical peats exist under differing climate regimes than Atlantic UK blanket bog.

Another study by Potter *et al.,* (2017) found contrasting microbial communities in mesocosms undergoing a drought treatment, which were taken from a blanket bog in Wales. The effects of the drought treatment were weak but measurable, with stronger differences between different

mesocosms than between treatments (the habitat only accounted for a small proportion of overall variation). This study potentially indicates that the drivers of microbial community change in blanket bog are diverse and interactive, making their study difficult.

In contrast to the above, a study by Bragina *et al.,* (2015), which compared the "core microbiome" within the plants of an alpine ombrotrophic bog in Austria, found 95 operational taxonomic units (roughly corresponding to species) were shared between the core microbiomes of graminoids and dwarf shrubs. This means that almost 66% of the graminoid (represented by *Carex nigra* and *Eriophorum vaginatum*) core microbiome and ~41% of the dwarf shrub (represented by *Calluna vulgaris*) microbiome is shared in this alpine community. If this is replicated in British ombrotrophic bogs, then we can reasonably expect that the microbiome will be dominated by few taxa (as they all are in the Elliot *et al.,* [2015] paper) and, where vegetation differences are subtle, there may be little discernible taxonomic difference at all. In the study by Bragina *et al.,* samples were taken from the rhizosphere of the plants themselves, and so it is hard to interpret what this means for non-rhizosphere soil. If mowing is designed to have the same impact on the vegetation as burning, but without the negative impacts, then it holds that the microbial community may not change. This is the ideal however, and the reality is that evidence suggests mowing favours a shift towards increasing sedge dominance and methane emissions (Heinemeyer *et al.,* 2019) with impacts on the GHG budget.

3.5 Composition vs. function: does taxonomy matter?

An important question concerns the link between microbial community composition and function. Whilst composition and function may be linked in peatland microbial communities, it remains to be seen how much functional redundancy exists in peat soil communities and therefore how much taxonomic composition will be a predictor of any function. Recent studies using functional community profiling indicate that metabolic community functions can be linked to environmental factors rather than coupled to specific species assemblages (for examples see the recent review by Louca *et al.,* 2018). Indeed, function can remain stable whilst taxonomic composition can be highly variable (Louca *et al.,* 2017). There are relevant examples of this from peatlands: a study by Peltoniemi *et al.,* (2012) on the Finnish Lakkasuo mire complex found that the site type (bog or fen) and water level regime had minor effects on litter-inhabiting microbial community composition, finding instead that the water level modified the community indirectly via changes in litter quality.

This raises the prospect of taxonomically distinct microbial communities that are functionally unchanged by management. However, given the similarities in the vegetation core microbiome, the inverse might also be true: a microbial community unaffected taxonomically but metabolically modified by changes in an environmental factor (e.g. degree of wetness).

Insights into the function of blanket bog can sometimes be examined via the transcriptome. Examinations of transcriptional activities of methanogens and methanotrophs were investigated by Freitag *et al.,* (2010), although they did not characterise the microbial community. They demonstrated a linear relationship between surface mcrA (methanogenesis gene marker) and pmoA (particulate methane monooxygenase) transcript dynamics and the surface flux rates of methane, finding both of these may be related to small variations in soil water content. Clearly, functional genes are related to carbon sequestration potential here, but to what extent this is related to the community taxonomic profile is unclear.

Consequently, for the fungi, this chapter has predicted the functional profile of the community and attempted to analyse this using FUNguild (Nguyen *et al.,* 2015). Bacterial and archaeal communities were not assessed for their functional types.

3.6 Summary and hypotheses

Across sites and management categories, a wide range of conditions occur, especially where sites are in distinct climates or have undergone managements that alter the hydrology, or the make-up of vegetation. Consequently, we expect these communities to be distinct between categories and climate types, and for the variation in environmental variables to have an effect on diversity and taxonomic composition.

This chapter can be distilled into the fundamental question: are soil microbial communities different under different management and habitat condition, and if so, why?

This can be represented by the testable hypotheses:

1. Microbial communities are taxonomically different across management/site categories
2. Microbial communities are taxonomically different based upon national location
3. Diversity varies among managements, with "intact" sites having the highest measures of diversity
4. Diversity and taxonomic composition can be related to environmental variables

3.7 Method

The methods used for this chapter are as per those described in Chapter 2.

Site samples were collected using an incision made during the collection of the mesocosms described in Chapter 2 section 2.12. A sample was taken from -15 cm below soil surface and this was immediately sealed in a freezer bag, with all samples frozen at -20°C within 12 hours. These samples were then subsampled and approximately 1.5 g of soil was freeze-dried to measure soil moisture.

DNA was extracted from the soil samples using a DNEasy PowerSoil kit (Qiagen) with modifications to the standard operating procedure as described in Chapter 2 section 2.14.

Extracted DNA underwent PCR focusing on the 16S (archaea and bacteria) and ITS (fungi) regions, with full PCR, sequencing and processing methods, in Chapter 2 sections 2.15 (onwards).

Data processing was undertaken as per Chapter 2 section 2.20 and tracked reads (including how many were lost to the filtering and chimera process were lost), are described in Chapter 2 section 2.20, in Table 5.

3.8 Results

3.8 Taxonomic variation across managements: Fungi

DADA2 processing outputted 2375 ASVs. Sequences were then clustered to a similarity threshold of 97% resulting in 1418 OTUs (Operational Taxonomic Units). Taxa not identified as Phylum Fungi were removed, and the samples were rarefied to an even depth based on the

sample with the lowest number of observed individuals. This process resulted in a final dataset containing 1029 taxa across 74 samples. The Whitendale burn replicate 4 was removed due to a low read count (<40 reads; perhaps related is the fact that this plot has a near constant deep water table depth of around 45 cm due to peat pipe drainage).

A PERMANOVA was undertaken on the abundance data, implemented using the R package 'vegan' (Oksanen *et al.,* 2019). This used a distance matrix based on Bray-Curtis dissimilarities to assess whether a significant difference was apparent between management and national site categories. Prior to the PERMANOVA, beta-dispersal between factors was tested for both management ($p = 0.017$) and national site groupings ($p = 0.006$). Because both tests for beta-dispersal are significant, it must be acknowledged that differences in samples within-treatment might be contributing to differences observed by PERMANOVA analysis. Fungal communities were significantly different between managements, but also between national sites (Table 1).

Table 3.1: Table of PERMANOVA results from analysis of fungal ITS community data. Df = degrees of freedom, Sum Sq = Sum of Squares, Pseudo-F = pseudo F-statistic, P = p value (significance level: *** p<0.0001).					
Factor	Df	Sum Sq	Pseudo-F	R^2	P
ITS					
management	7	5.69	2.13	0.18	0.0001 ***
residuals	66	25.17		0.82	
total	73	30.86		1	
ITS					
national sites	4	6.74	4.82	0.22	0.0001 ***
residuals	69	24.13		0.78	
total	73	30.86		1	
ITS – 2-way PERMANOVA					
management	7	5.69	2.41	0.19	0.0001 ***
National sites	2	3.28	4.86	0.11	0.0001 ***
Management x National sites	6	2.29	1.13	0.07	0.11
residuals	58	19.59		0.64	
total	73	30.86		1	

A post-hoc pairwise comparison tests were undertaken using the "mctoolsr" package (Leff, 2017) which are presented in Tables 2 and 3.

Table 3.2: Post-hoc comparisons of management effects on fungal communities. Presented in this table are the False Discovery Rate (Benjamini-Hochberg) adjusted *p*-values.							
Management	Burnt	Mown	Uncut	Moor House	10 Year post restoration	5 year post restoration	Degraded
Burnt	X						
Mown	0.04 *	X					
Uncut	0.12	0.34	X				
Moor House	0.006 **	0.04 *	0.04 *	X			
10 year post restoration	0.004 **	0.004 **	0.004 **	0.04 *	X		
5 year post restoration	0.004 **	0.004 **	0.004 **	0.02 *	0.65	X	
Degraded	0.004 **	0.004 **	0.004 **	0.02 *	0.39	0.66	X
Intact	0.004 **	0.004 **	0.004 **	0.03 *	0.7	0.91	0.67

Table 3.3: Post-hoc comparisons of national site effects on fungal communities. Presented in this table are the False Discovery Rate (Benjamini-Hochberg) adjusted *p*-values.				
National Site	Yorkshire	Exmoor	Peak District	Scotland
Yorkshire	X			
Exmoor	0.003 **	X		
Peak District	0.003 **	0.003 **	X	
Scotland	0.003 **	0.003 **	0.003 **	X
Moor House	0.008 **	0.005 **	0.006 **	0.004 **

3.9 Fungal Taxonomic Composition

Across all samples, in all managements, the fungal community is dominated by those species in the phylum Ascomycota comprising a mean relative abundance of 63.3% (range 8.9 – 92.1 %) Basidiomycota (mean 19.4%, range 0.6 - 90.8%) and Mortierellomycota (mean 13.3%, range 0.1 -49.9%) make up the majority of the rest of the community at the Phylum level. The rest of the average fungal community is made up of rare taxa in the Phyla Mucoromycota, Zoopagomycota (formerly Zygomycota, (Spatafora *et al.,* 2014)), Monoblepharomycota, Basidiobolomycota, Rozellomycota, and the rarest phyla including the Olpidiomycota, Chytridiomycota and Kickxellomycota. The arbuscular mycorrhizal Phylum Glomeromycota is notable, having a low mean relative abundance of 2.3%, but comprising as much as 17.9% of the community in one case (full data in Table 4). None of the major phyla differed between managements, apart from the Basidiobolomycota, which was restricted to the Moor House plot samples. The overall lack of difference may be because although phyla-level communities are variable, they are not consistently so between treatments (Figure 1). A fuller graph of Phylum level abundance is represented by Figure 2.

Table 3.4: Mean relative abundance data from all samples across all groups and management types.

Phylum	Mean Relative Abundance	Minimum relative abundance	Maximum relative abundance
Ascomycota	0.63	0.09	0.92
Basidiomycota	0.19	0.006	0.91
Mortierellomycota	0.13	0.001	0.5
Mucoromycota	0.04	0.0002	0.21
Zoopagomycota	0.03	0.001	0.13
Glomeromycota	0.02	0.001	0.18
Monoblepharomycota	0.02	0.001	0.04
Basidiobolomycota	0.02	0.01	0.02
Rozellomycota	0.004	0.0001	0.03
Olpidiomycota	0.001	0.001	0.002
Chytridiomycota	0.001	0.0003	0.001
Kickxellomycota	0.001	0.0006	0.001

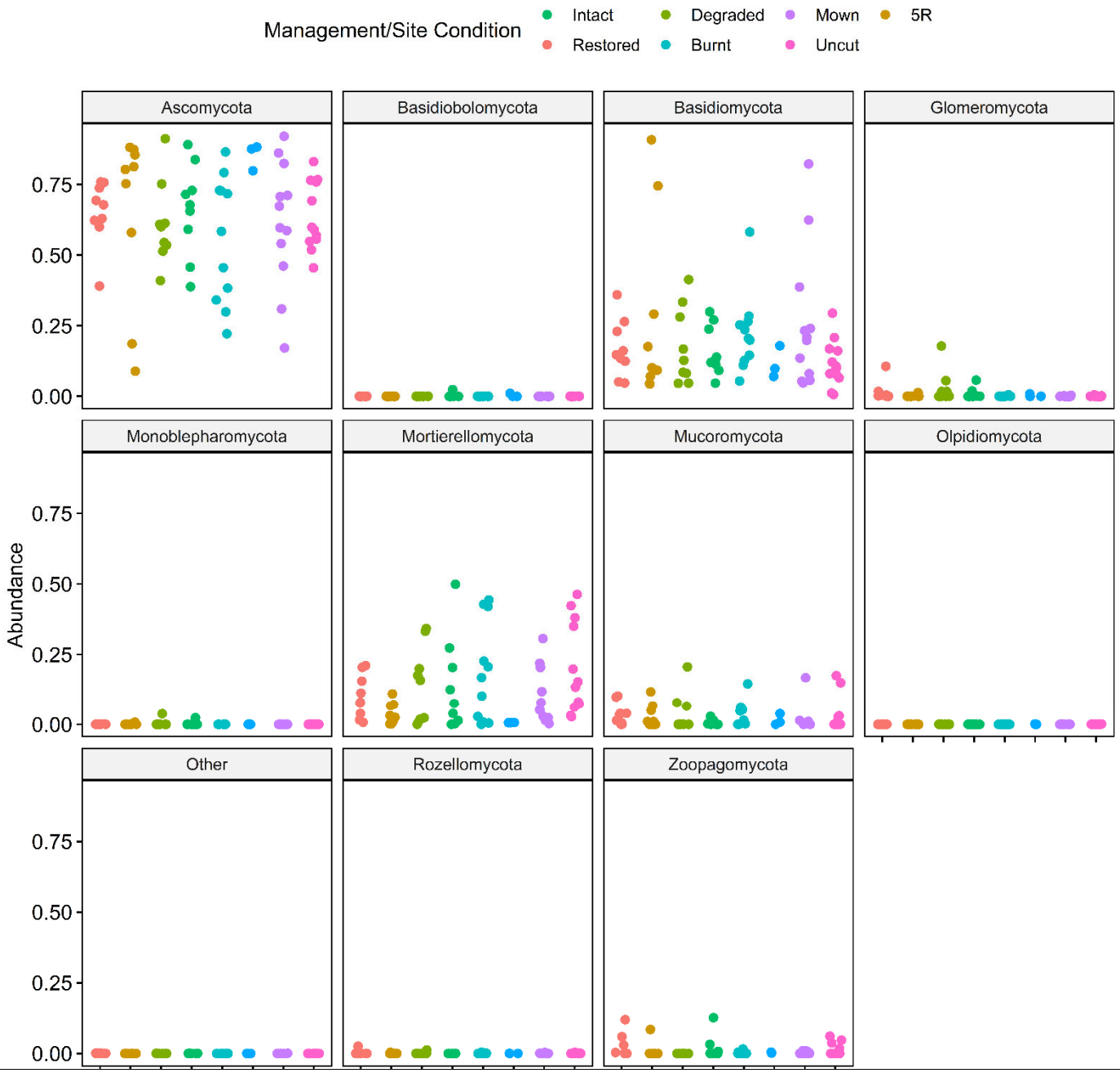

Figure 3.1: Relative abundances of major fungal Phyla, seen in Table 4, split by management.

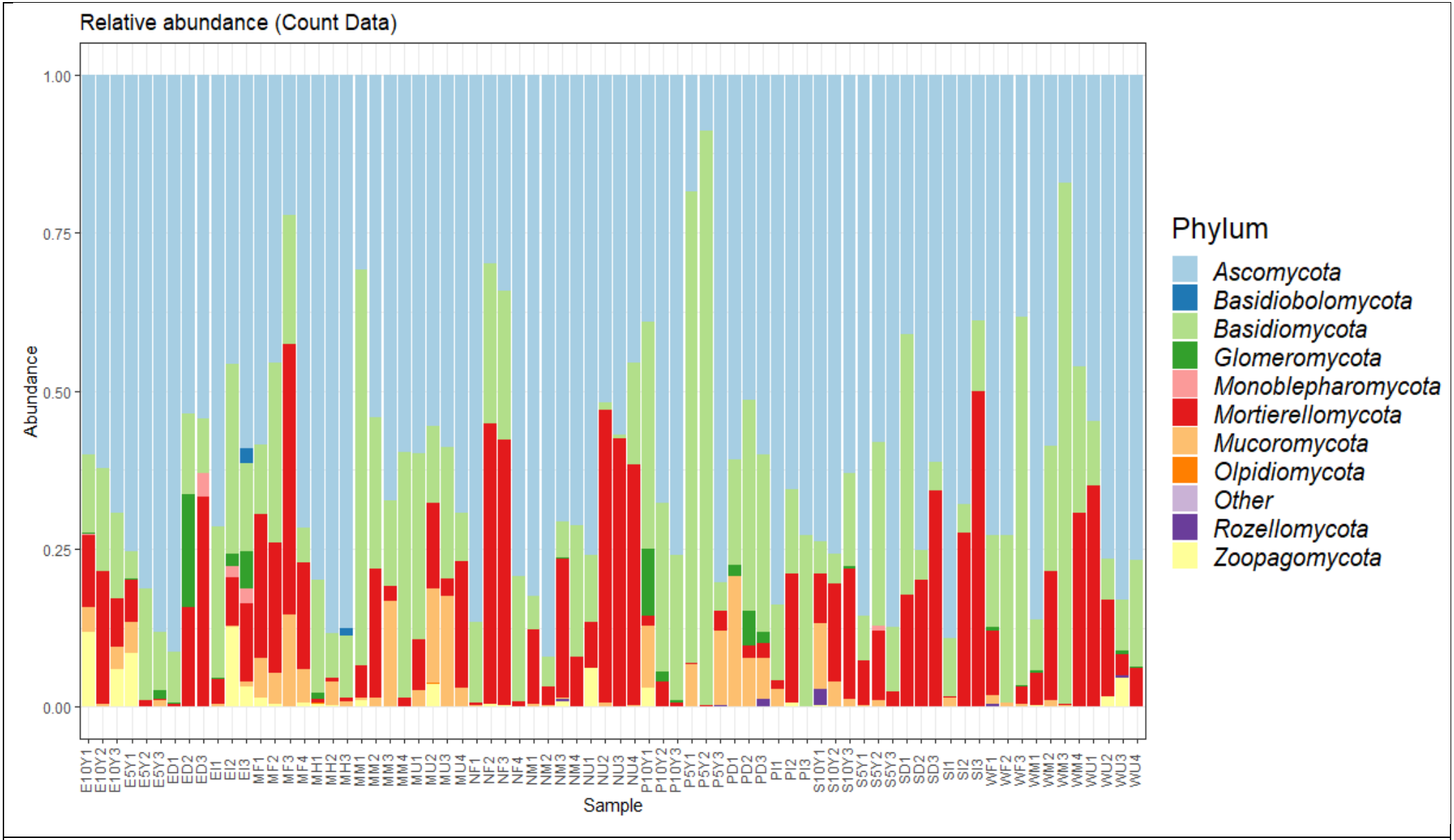

Figure 3.2: Relative abundance of fungal phyla per sample. For site codes, refer to Chapter 2 section 2.10.

3.10 Fungal Diversity

This study assessed both Shannon and Simpson diversity. These indices are mathematical measures of the diversity of the microbial communities, based on the number of species present (richness) and the number of individuals per species (species abundance). The Simpson index gives more weight to common or dominant species, and species evenness, whereas the Shannon index gives more weight to species richness. Because the Shannon index gives weight to less common species, it is more sensitive to diversity changes outside of the core microbiome, and consequently it has been the focus of analysis in this thesis, although Simpsons index has also been used. There is some evidence that Shannon's diversity is better at detecting relationships between community variables (Morris *et al.,* 2014).

Mean Shannon diversity was highest in the 80 year post burn plots (Moor House, n = 3) and Intact plots (n = 9), with the Exmoor Intact plots (n = 3) having the highest mean Shannon diversity of all samples (Figure 3). Shannon diversity values were not significantly different between managements (ANOVA: $F = 1.738$, $df = 7(66)$, $p = 0.115$) or national site categories (ANOVA: $F = 1.386$, $df = 4(69)$, $p = 0.248$). In the DEFRA Peatland-ES-UK sites (Yorkshire), a set of samples with better replication and control over extraneous variables (such as climate), Shannon diversity values did not differ between burnt, mown and uncut treatments (ANOVA: $F = 0.945$, $df = 2(32)$, $p = 0.399$).

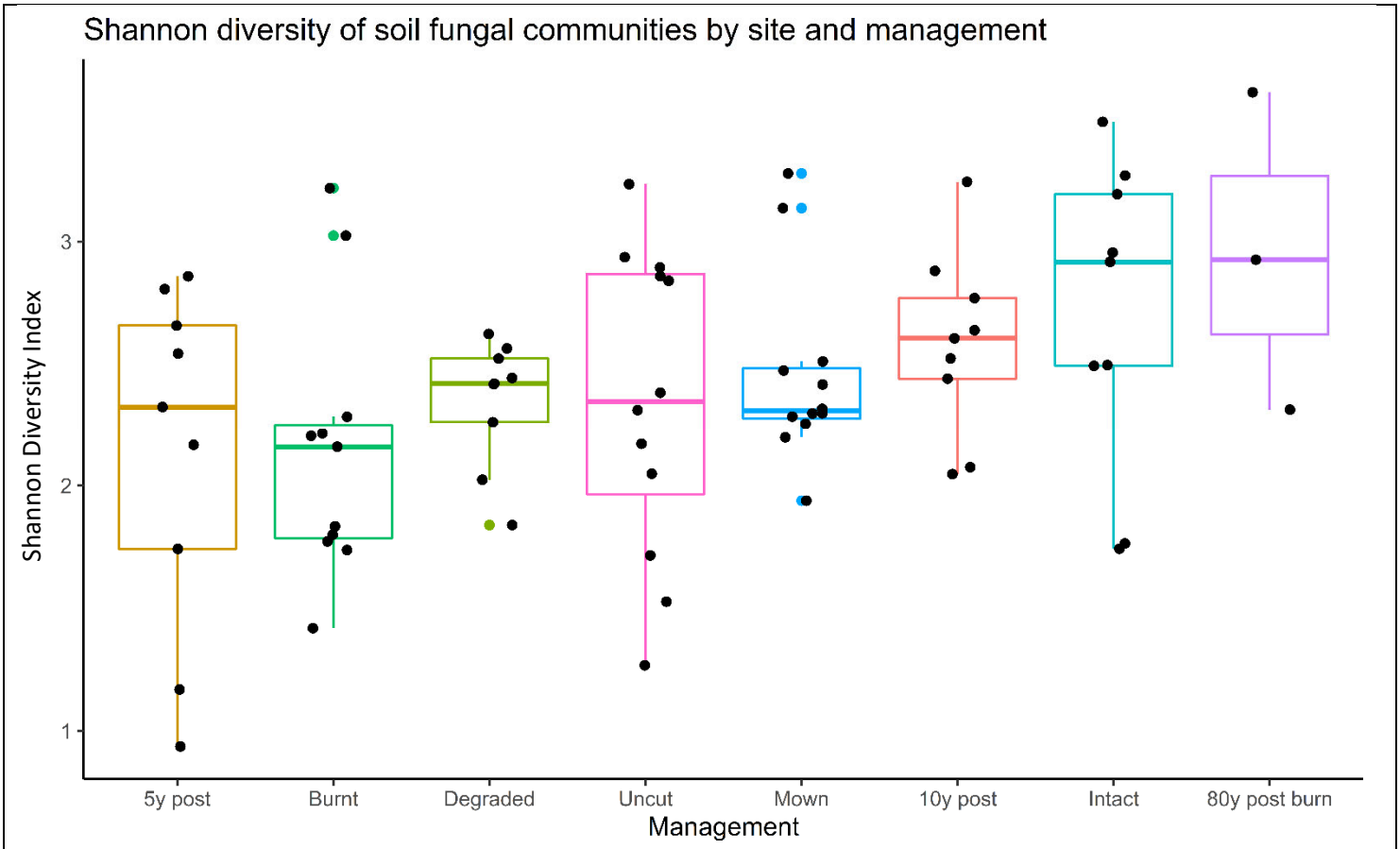

Figure 3.3: Median Shannon diversity values by management/site condition category.

Simpson's diversity did not differ between managements (Kruskal-Wallis: $X^2 = 9.226$, df = 7, p = 0.2368) or national site groupings (Kruskal-Wallis: $X^2 = 3.9853$, df = 4, p = 0.408). Simpson's diversity also did not differ between burnt, mown and uncut treatments on the Peatland-ES-UK sites (ANOVA: F = 0.988, df = 2(32), p = 0.383).

3.11 Environmental drivers of fungal diversity

Environmental variables were investigated to ascertain any effect on Shannon and Simpson's diversity of soil fungi. We hypothesised that Shannon diversity would be affected by soil moisture, pH, and interactions between the abundances of major vegetation groups: *Calluna*, total sedges, *Sphagnum*, *Molinia*, other moss and bare ground. A GLM with a Gaussian distribution was used to model the impacts of these environmental parameters on Shannon index values. Model assumptions were verified by plotting residuals versus fitted values. Models were run with differing combinations of these variables and the model with the lowest Akaike Information Criterion (AIC) was chosen (Table 5). The best model did not include any vegetation indices except *Sphagnum*.

Table 3.5: Estimated regression parameters, standard errors, t-values and P-values for the Gaussian GLM modelling the effect of environmental variables on soil fungal Shannon diversity index values.				
	Estimate	Std. Error	t value	*p*-value
Intercept	-1.69	1.51	-1.12	0.27
Soil moisture	0.06	0.02	3.51	0.0008 ***
pH	-0.26	0.22	-1.18	0.24
Sphagnum %	0.01	0.005	2.49	0.016 *

A second GLM was undertaken on Simpson's diversity index values in the same manner, with the best model (again) including soil moisture, pH and *Sphagnum* cover (See Table 6)

Table 3.6: Estimated regression parameters, standard errors, t-values and P-values for the Gaussian GLM modelling the effect of environmental variables on soil fungal Simpson's diversity index values.				
	Estimate	Std. Error	t value	P-value
Intercept	0.12	0.32	0.36	0.71
Soil moisture	0.01	0.004	2.75	0.008 **
pH	-0.04	0.05	-0.83	0.41
Sphagnum %	0.002	0.001	1.89	0.064

3.12 Environmental drivers of fungal taxonomic composition

The environmental drivers of fungal taxonomic composition were investigated using a distance-based redundancy analysis (dbRDA: Legendre & Anderson, 1999) which is reliable for analysing species-environment relationships (Jupke & Schäfer, 2020). The dbRDA is a means of conducting a redundancy analysis on dissimilarities, which may be non-linear. A matrix based on Bray-Curtis dissimilarities of square root transformed relative abundance at the OTU level was used. Response variables were standardised using z scoring to account for dimensional heterogeneity. Briefly, after the distance matrix is calculated, a principle coordinate's analysis (PCoA) is undertaken on the matrix, and the eigenvalues obtained in the PCoA are analysed using a standard RDA. For our analysis, the "capscale" function in the R package "vegan" was used (Oksanen *et al.,* 2019). The model included 68 observations and 21 of the variables collected (refer to Chapter 2 section 2.12 for details). Variables for inclusion were selected based on their hypothesised relevance and were excluded based on their potential for high degrees of autocorrelation (for example, the dataset variable "abbymoisture" was excluded because it was a repeat soil moisture variable using a slightly different method). The model was significant, with environmental variables explaining variation in the community (dbRDA: df, 20(48), $F = 1.8042$, $p < 0.001$). Testing was undertaken on which variables were significant drivers of community variation (Table 7). Plotted dbRDA ordinations are Figures 4 and 5.

Table 3.7: F-statistics and *p*-values for variables listed as significant drivers of fungal taxonomic variation in the output of a distance-based redundancy analysis.

Variable	F-statistic	*p*-value
Latitude	4.46	0.001 ***
Longitude	6.92	0.001 ***
Slope	1.79	0.01 *
Aspect	2.42	0.002 ***
Elevation	3.51	0.001 ***
Calluna cover %	1.36	0.09
Molinia cover %	1.38	0.08
Dissolved Organic Carbon	1.67	0.03 *
Soil Moisture	1.95	0.004 **

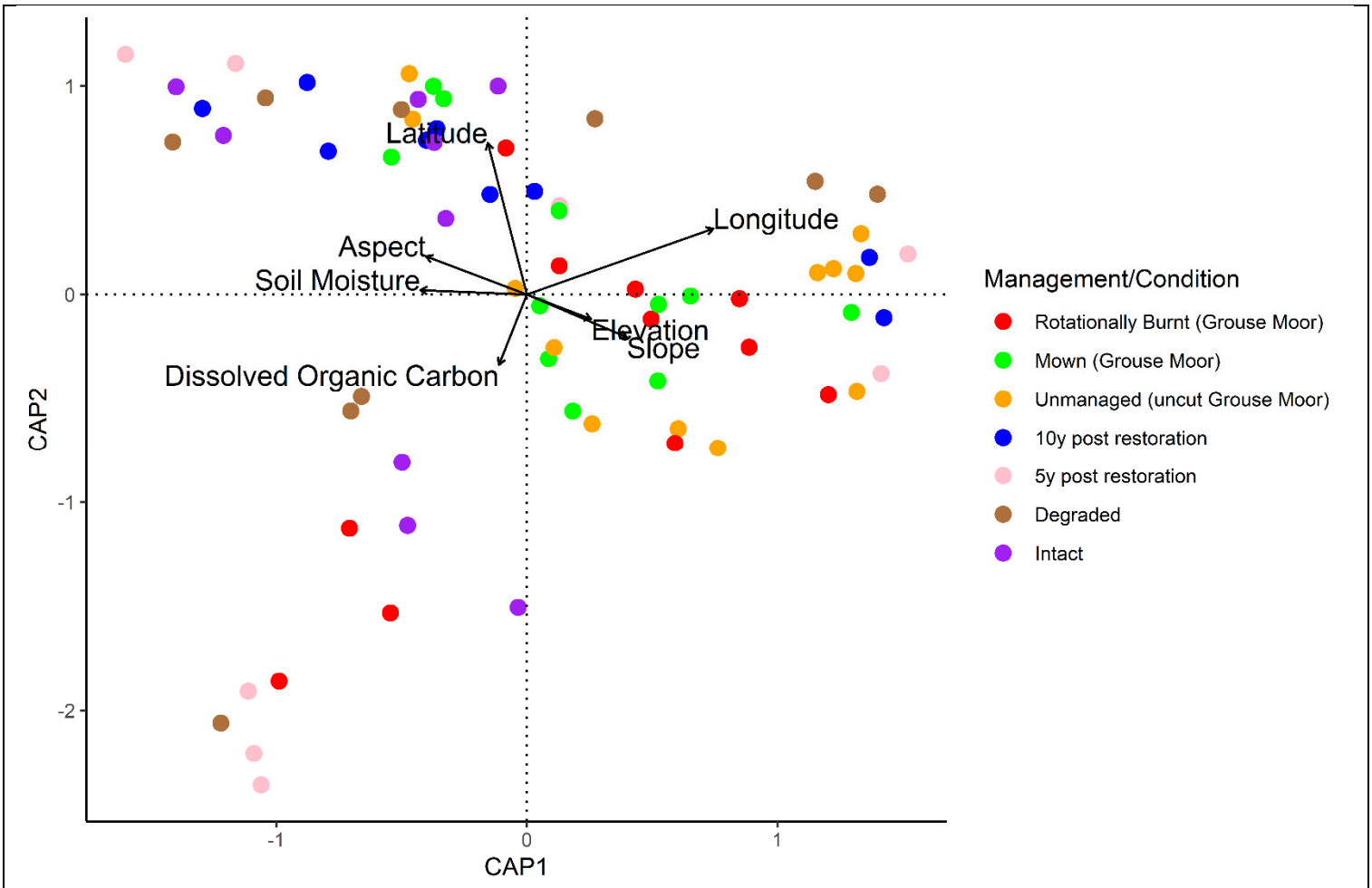

Figure 3.4: Distance based RDA: this model is significant ($p < 0.001$), 42.91% of total variance is explained by constrained variables: pH, Calluna cover and soil moisture drive variation in Axis 1 (CAP1) whilst Latitude, *Molinia* cover ("grass") and Nitrogen ("N) drive variation in Axis 2 (CAP2). Graph coloured by management type: see legend.

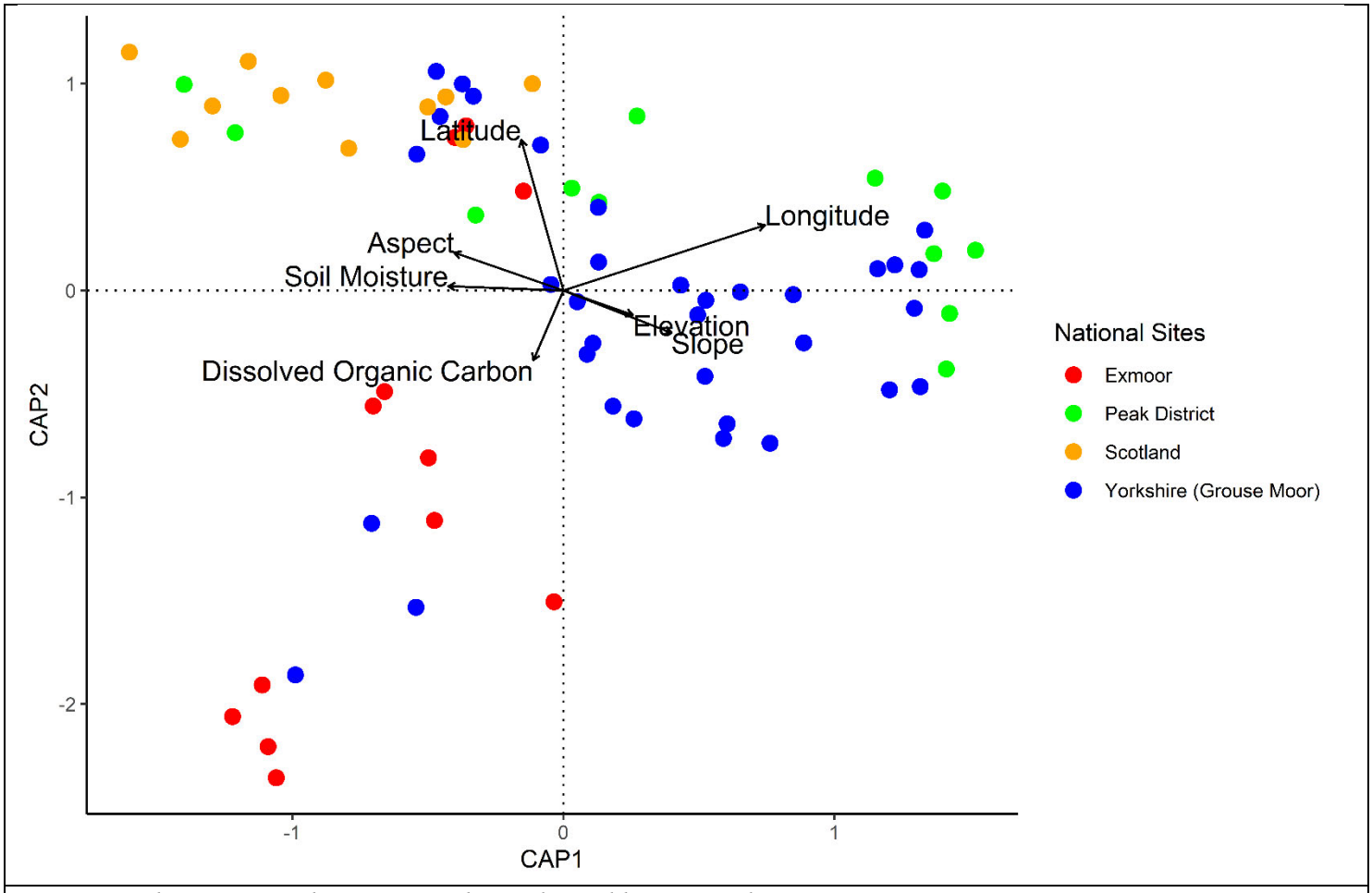

Figure 3.5: the same graph as Figure 5 but coloured by national site category.

3.13 Fungal functional community profiles

To assess broader scale changes in fungal communities, functional categories were assigned to species using FUNguild, an annotation tool for parsing fungal communities by ecological guilds (Nguyen *et al.,* 2015). Each OTU in the fungal community data was assigned a trophic guild and counts were aggregated at the level of trophic guild before being transformed to relative abundance. The fungal community was split into Pathotrophs, Saprotrophs, and Symbiotrophs, an additional "Unassigned" category for unknown species, and additionally, those species that fall into intermediate or functionally flexible groups: Saprotroph-Symbiotrophs, Pathotroph-Symbiotrophs, Pathotroph-Saprotrophs, and Pathotroph-Saprotroph-Symbiotrophs. Relative abundances of taxa fitting into these trophic modes are in Table 8. The most abundant taxa (mean of 64%) in most samples were those taxa not assigned to a trophic mode – likely because of a lack of information that persists regarding fungi in blanket bogs.

Table 3.8: Mean relative abundance, maximum and minimum relative abundance data from all samples across all groups and management types.

Trophic mode	Mean Relative Abundance	Minimum relative Abundance	Maximum Relative Abundance
Undefined	0.64	0.06	0.95
Pathotroph	0.05	0.0004	0.31
Saprotroph	0.14	0.007	0.74
Symbiotroph	0.03	0.0003	0.18
Pathotroph-Saprotroph-Symbiotroph	0.02	0.0007	0.08
Saprotroph-Symbiotroph	0.11	0.0004	0.76
Pathotroph-Symbiotroph	0.004	0.00007	0.01
Pathotroph-Saprotroph	0.06	0.0002	0.6

Differences between managements of the major trophic guilds were tested using ANOVA or a non-parametric equivalent (Kruskal-Wallis) when the data did not meet the assumptions of ANOVA (Table 9).

Table 3.9: Table of Kruskal-Wallis results from analysis of fungal trophic guilds between management/condition classes. Df = degrees of freedom, X2 = chi-squared, P = *p*-value (significance level)

Trophic mode	Factor	Df	X^2	*p*-value
Saprotroph	Management	7	6.61	0.42
Pathotroph	Management	7	6.9	0.44
Symbiotroph	Management	7	19.73	0.0061 **
Saprotroph-Symbiotroph	Management	7	32.78	0.00003 ***
Pathotroph-Saprotroph	Management	7	9.79	0.2
Pathotroph-Symbiotroph	Management	7	8.52	0.29
Pathotroph-Saprotroph-Symbiotroph	Management	7	19.98	0.006 ***
Unassigned	Management	7	6.65	0.47

Based on the result of the Kruskal-Wallis tests in Table 9, post-hoc multiple comparisons were undertaken on Symbiotrophs and Saprotroph-Symbiotroph abundances using pairwise Wilcox tests, presented in Tables 10 and 11.

Table 3.10: Dunn's test post-hoc comparisons of management effects on Symbiotroph relative abundance. Presented in this table are the Benjamini-Hochberg adjusted *p*-values.

Management	Burnt	Mown	Uncut	Moor House	10 year post restoration	5 year post restoration	Degraded
Burnt	X						
Mown	0.03 *	X					
Uncut	0.16	0.38	X				
Moor House	0.07	0.76	0.41	X			
10 year post restoration	0.03 *	0.97	0.4	0.73	X		
5 year post restoration	0.8	0.09	0.41	0.11	0.08	X	
Degraded	0.09	0.65	0.73	0.5	0.63	0.22	X
Intact	0.17	0.42	0.98	0.39	0.42	0.4	0.75

Table 3.11: Dunns test post-hoc comparisons of management effects on Saprotroph-Symbiotroph relative abundance. Presented in this table are the Benjamini-Hochberg adjusted *p*-values.

Management	Burnt	Mown	Uncut	Moor House	10 year post restoration	5 year post restoration	Degraded
Burnt	X						
Mown	0.033 *	X					
Uncut	0.31	0.32	X				
Moor House	0.37	0.56	0.91	X			
10 year post restoration	0.002 **	0.33	0.047 *	0.28	X		
5 year post restoration	0.05 *	0.97	0.35	0.57	0.34	X	
Degraded	0.0001 ***	0.08	0.003 **	0.08	0.46	0.08	X
Intact	0.003 **	0.39	0.06	0.33	0.92	0.4	0.38

Relative abundances are shown for comparison in Figure 6. Note that the mean abundance of unassigned taxa was not significantly different.

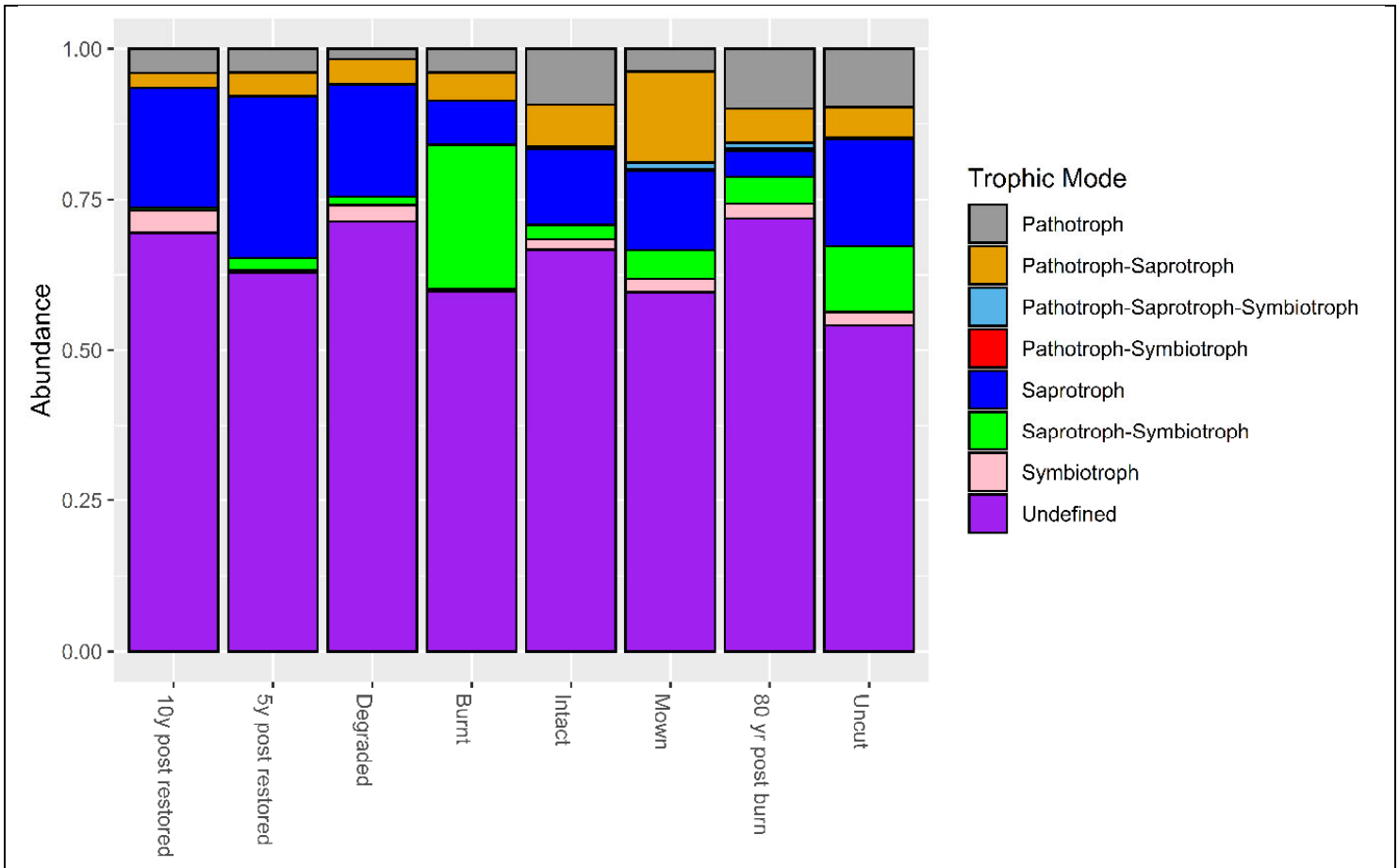

Figure 3.6: Relative abundance of trophic mode groups under different management/site condition categories, including taxa that were "undefined" (not assigned) to a trophic mode

3.14 Bacteria

DADA2 processing outputted 7158 ASVs. Sequences were then clustered to a similarity threshold of 97% resulting in 2366 OTUs. Taxa not identified as being bacteria at the phylum level were removed, and the samples were rarefied to an even depth based on the sample with the lowest number of observed individuals. This resulted in the removal of 178 OTUs, resulting in a final dataset containing 2188 taxa across 74 samples. Like the fungi, the Whitendale plot 4 (-burnt) plot was excluded because it had a low read count.

A PERMANOVA was run on the abundance data, using a distance matrix based on Bray-Curtis dissimilarities to assess whether a significant difference was apparent between management and national site categories. Prior to the PERMANOVA, beta-dispersal between factors was tested for both management ($p = 0.045$) and national site groupings ($p = 0.045$). Because the beta-dispersal test for national sites and management are significant, it must be acknowledged that differences in samples within-national sites and management categories might be contributing to differences observed by PERMANOVA analysis, for this test. Results of these tests are presented in Tables 12, 13 and 14.

Table 3.12: Table of PERMANOVA results from analysis of bacterial 16S community data. Df = degrees of freedom, Sum Sq = Sum of Squares, Pseudo-F = pseudo F-statistic					
Factor	Df	Sum Sq	Pseudo-F	R^2	*p*-value
16S Bacteria					
management	7	4.08	2.88	0.23	0.0001 ***
residuals	66	13.37		0.77	
total	73	17.44		1	
16S Bacteria					
national sites	4	6.47	10.16	0.37	0.0001 ***
residuals	69	10.98		0.63	
total	73	17.44		1	
16S Bacteria – 2 way PERMANOVA					
management	7	4.16	4.57	0.24	0.0001 ***
National sites	2	3.78	14.54	0.22	0.0001 ***
National sites x management	6	1.79	2.29	0.10	0.0001 ***
residuals	58	7.54		0.44	
total	73	17.26		1	

Table 3.13: Post-hoc comparisons of management effects on bacterial communities. Presented in this table are the False Discovery Rate (Benjamini-Hochberg) adjusted *p*-values.							
Management	Burnt	Mown	Uncut	Moor House	10 Year post restoration	5 year post restoration	Degraded
Burnt	X						
Mown	0.47	X					
Uncut	0.45	0.46	X				
Moor House	0.009 **	0.009 **	0.03 *	X			
10 year post restoration	0.007 **	0.007 **	0.03 *	0.03 *	X		
5 year post restoration	0.007 **	0.007 **	0.007 **	0.03 *	0.22	X	
Degraded	0.007 **	0.007 **	0.007 **	0.03 *	0.58	0.42	X
Intact	0.007 **	0.008 **	0.008 **	0.02 *	0.12	0.02 *	0.11

Table 3.14: Post-hoc comparisons of national site effects on bacterial communities. Presented in this table are the False Discovery Rate (Benjamini-Hochberg) adjusted *p*-values.				
National Site	Yorkshire	Exmoor	Peak District	Scotland
Yorkshire	X			
Exmoor	0.002 **	X		
Peak District	0.002 **	0.002 **	X	
Scotland	0.002 **	0.002 **	0.002 **	X
Moor House	0.002 **	0.002 **	0.009 **	0.007 **

3.15 Bacterial Taxonomic Composition

The microbial community across all samples is dominated by the Proteobacteria (mean relative abundance 24.5%), Planctomycetes (21.7%), Acidobacteria (19.2%) and Actinobacteria (19.1%), but between managements the communities are much more variable than the fungi. As seen in Table 15, whilst these phyla dominate most samples on average, they also have very low abundances in some samples where they may be almost absent. Of the eleven largest phyla, the Proteobacteria, Verrucomicrobia, Acidobacteria and Bacteroidetes differ between managements, whereas the others do not (results in Table 16). For a graphical display of the differential abundance of specific taxa, see Figure 7, and a further detailed looked at per-sample relative abundances in Figure 8.

Table 3.15: Mean relative abundance, Maximum relative abundance and minimum relative abundance of bacterial phyla identified from 16s amplicon sequencing.			
Phylum	Mean Relative Abundance	Minimum Relative Abundance	Maximum Relative Abundance
Proteobacteria	0.25	0.1	0.47
Planctomycetes	0.22	0.03	0.49
Acidobacteria	0.19	0.04	0.3
Actinobacteria	0.19	0.04	0.42
Chloroflexi	0.07	0.001	0.33
WPS-2	0.03	0.0009	0.28
Firmicutes	0.02	0.0001	0.26
Verrucomicrobia	0.01	0.002	0.05
Bacteroidetes	0.01	0.0001	0.04
Dependentiae	0.01	0.0005	0.06
Nitrospirae	0.01	0.0001	0.04
Armatimonadetes	0.002	0.0002	0.02
Omnitrophicaeota	0.002	0.0001	0.006
Crenarchaeota	0.002	0.0005	0.006
Spirochaetes	0.002	0.0001	0.01
GAL15	0.002	0.0001	0.007
Patescibacteria	0.002	0.0001	0.01
FCPU426	0.002	0.0001	0.01
Cyanobacteria	0.001	0.0001	0.009

WS4	0.001	0.0001	0.007
Rokubacteria	0.001	0.0001	0.003
Fusobacteria	0.001	0.0003	0.002
Euryarchaeota	0.0008	0.0001	0.002
Elusimicrobia	0.0007	0.0002	0.002
Thaumarchaeota	0.0005	0.0001	0.001
BRC1	0.0005	0.0001	0.002
Fibrobacteres	0.0005	0.0001	0.003
WOR-1	0.0005	0.0003	0.0007
Gemmatimonadetes	0.0004	0.0001	0.001
Latescibacteria	0.0003	0.0002	0.0005
WS1	0.0003	0.0001	0.0006
FBP	0.0002	0.0002	0.0002
Chlamydiae	0.0001	0.0001	0.0003
Nanoarchaeaeota	0.0001	0.0001	0.0002

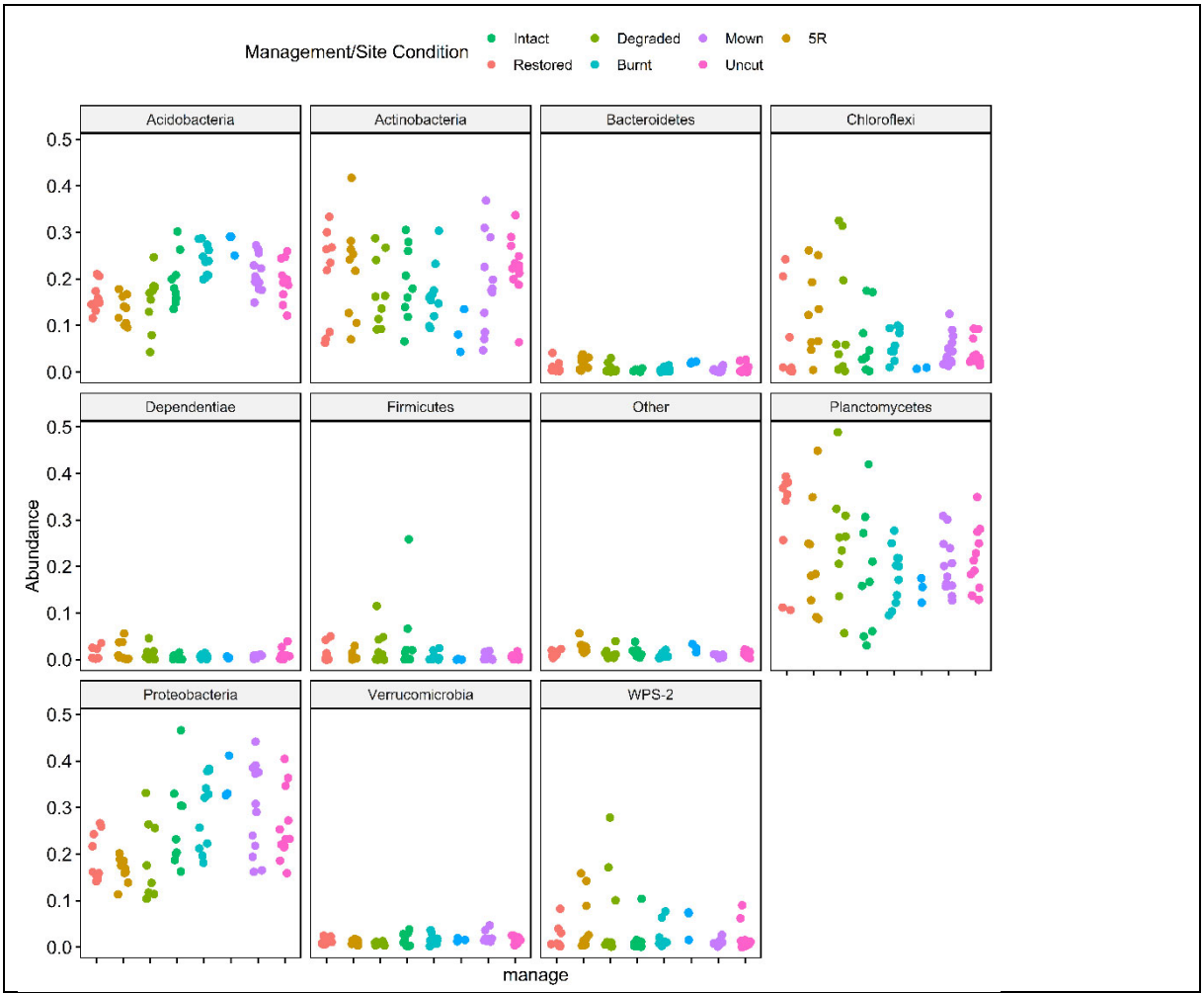

Figure 3.7: Abundances of major phyla, seen in Table 15, split by management.

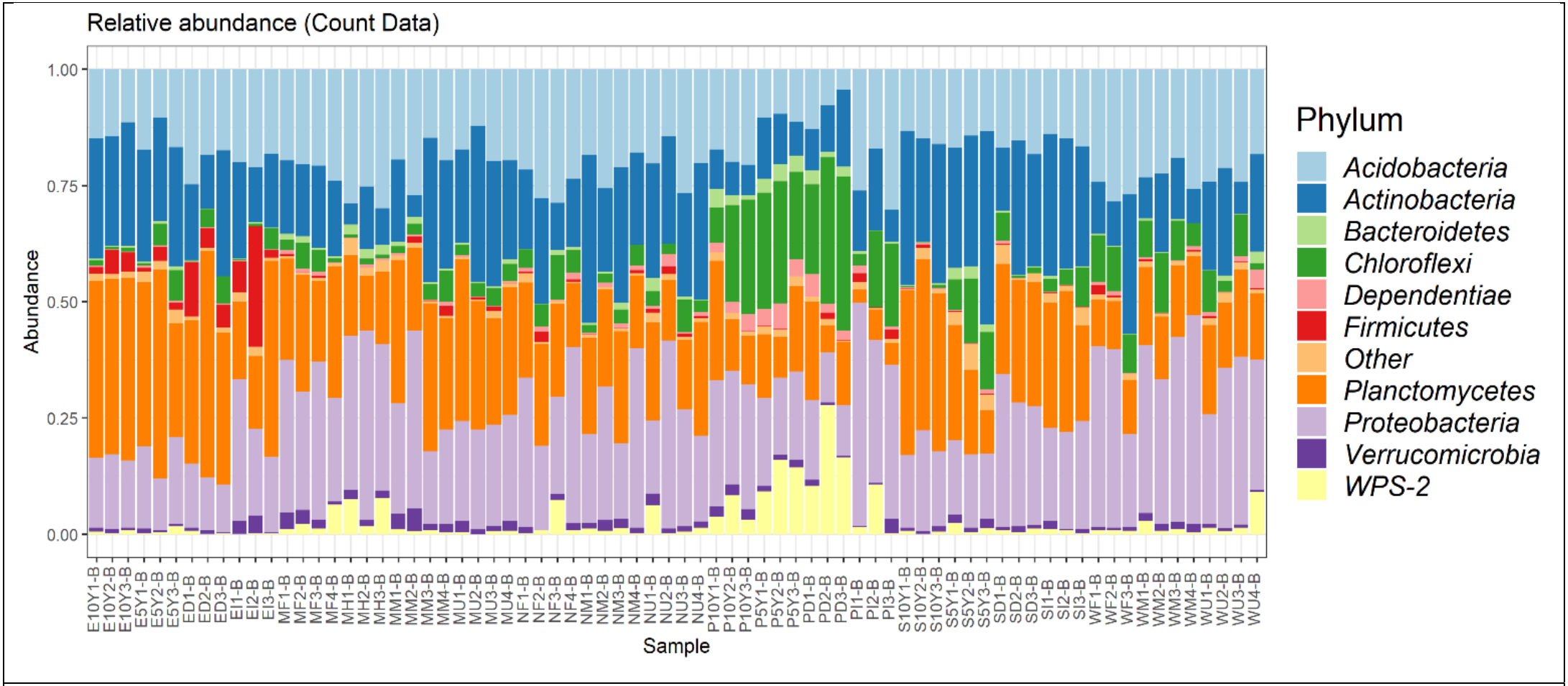

Figure 3.8: Relative abundance of the major phyla of the bacterial community – for site codes see Chapter 2 section 2.10.

Table 3.16: ANOVA and non-parametric Kruskal-Wallis tests of whether phylum relative abundance differs between management. Kruskal-Wallis tests were used where the assumptions for ANOVA (normality and homogeneity of variance) were not met, and the data could not be transformed to meet it.			
Phyla (Kruskal-Wallis)	X^2	Degrees of Freedom	*p*-value
Proteobacteria	27.55	7	0.0003 ***
Planctomycetes	9.75	7	0.2
Chloroflexi	13.55	7	0.06
WPS-2	8.44	7	0.3
Firmicutes	7.01	7	0.43
Verrucomicrobia	14.44	7	0.044 *
Bacteroidetes	17.09	7	0.017 *
Dependentiae	7.74	7	0.36
Phyla (ANOVA)	F-value	Degrees of Freedom	P value
Acidobacteria	8.73	7	0.0000002 ***
Actinobacteria	1.36	7	0.24

3.16 Bacterial Diversity

Like the fungi, mean Shannon diversity for bacteria was highest in the 80-year post burn (Moor House NNR, Hard Hill Plots, n=3) and 5 year restored plots (n=3), and the Degraded plots (n=9) had the lowest mean Shannon diversity of all samples. Shannon diversity values differed significantly between managements (Kruskal-Wallis, $X^2 = 17.522$, df = 7, $p < 0.0143$). Post-hoc tests were undertaken on this data and results are presented in Table 15. Simpsons diversity also differed between managements (Kruskal-Wallis, $X^2 = 22.765$, df = 7, $p < 0.0002$). Results of a post-hoc Dunns' test are in Table 17 and 18. Differences in Shannon diversity are shown in Figure 9.

Table 3.17: Dunn's post-hoc test comparisons of management effects on bacterial Shannon diversity indices for each management category.							
Management	Burnt	Mown	Uncut	Moor House	10 Year post restoration	5 year post restoration	Degraded
Burnt	X						
Mown	0.92	X					
Uncut	0.95	0.97	X				
Moor House	0.13	0.13	0.13	X			
10 year post restoration	0.54	0.45	0.45	0.06	X		
5 year post restoration	0.39	0.45	0.45	0.3	0.18	X	
Degraded	0.28	0.24	0.24	0.045 *	0.64	0.08	X
Intact	0.29	0.25	0.24	0.03*	0.65	0.07	0.97

Table 3.18: Post-hoc Dunn's test on comparisons of management effects on bacterial Simpson diversity indices for each management category. Shown are *p*-values adjusted for multiple tests using the Benjamini-Hochberg method.							
Management	Burnt	Mown	Uncut	Moor House	10 Year post restoration	5 year post restoration	Degraded
Burnt	X						
Mown	0.7	X					
Uncut	0.86	0.78	X				
Moor House	0.18	0.12	0.14	X			
10 year post restoration	0.06	0.12	0.07	0.03 *	X		
5 year post restoration	0.73	0.99	0.79	0.11	0.12	X	
Degraded	0.051	0.11	0.06	0.01 *	0.98	0.11	X
Intact	0.11	0.18	0.11	0.03 *	0.82	0.22	0.81

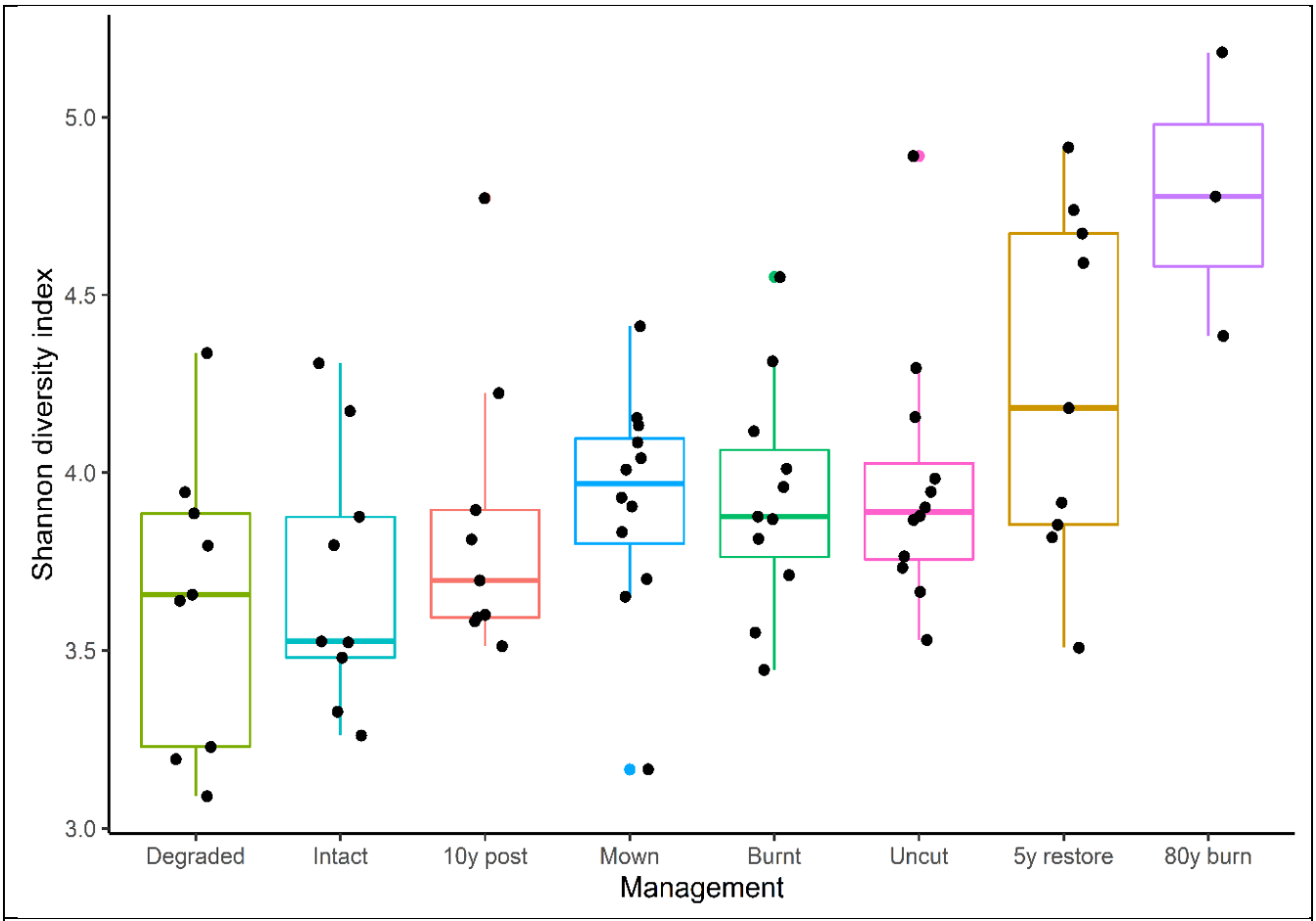

Figure 3.9: Bacterial Shannon diversity of managements and site conditions, ordered by mean diversity.

3.17 Environmental drivers of bacterial diversity

As for the previous taxa, environmental variables were investigated to ascertain any effect on Shannon and Simpson diversity of soil bacteria. Again, it was hypothesised that Shannon diversity would be affected by soil moisture, pH, total organic nitrogen, and interactions between the major vegetation groups' abundances: *Calluna*, total sedge, *Sphagnum*, *Molinia*, other moss and bare ground. A GLM with a Gaussian distribution was undertaken to model the impacts of these environmental parameters on Shannon index values. Model assumptions were verified by plotting residuals versus fitted values. Models were run with differing combinations of these variables and the model with the lowest Akaike Information Criteria (AIC) was chosen, with results presented in Table 19. The best model for Shannon diversity included only soil moisture whereas the best model for Simpson's diversity included soil moisture and *Molinia* percentage cover as explanatory variables.

Table 3.19: Estimated regression parameters, standard errors, t-values and P-values for the Gaussian GLM modelling the effect of environmental variables on soil bacterial Shannon diversity index values.

Variables	Estimate	Std. Error	t value	*p*-value
Shannon				
Intercept	-0.59	1.15	-0.51	0.61
Moisture %	0.05	0.01	3.89	0.0003 ***
Simpson				
Intercept	0.78	0.08	9.17	< 0.00001 ***
Moisture %	0.002	0.001	2.02	0.048 *
Molinia %	-0.0006	0.0001	-4.69	< 0.00001 ***

3.18 Environmental drivers of bacterial taxonomic composition

A dbRDA was applied to the bacterial community in the same way as for the fungi. Variables for inclusion were selected based on their hypothesised relevance and were excluded based on their potential for high degrees of autocorrelation (for example, the dataset variable "abbymoisture" was excluded because it was a repeat soil moisture variable using a slightly different method). The model was significant, with environmental variables explaining variation in the community (dbRDA: df,21(47), F = 3.22, p < 0.001). We tested which variables were significant drivers of community variation, the results of which are listed in Table 20. Plotted dbRDA ordinations are Figures 10 and 11.

Table 3.20: List of F-statistics and *p*-values for variables listed as significant drivers of bacterial taxonomic variation in the output of a distance-based redundancy analysis.		
Variable	F-statistic	*p*-value
Latitude	4.42	0.001 ***
Longitude	24.06	0.001 ***
Slope	2.21	0.02 *
Aspect	2.65	0.02 *
Elevation	10.13	0.001 ***
Calluna cover %	1.79	0.072
Sphagnum cover %	2.34	0.03 *
Molinia cover %	2.85	0.006 **
Sedge cover %	1.96	0.044 *
Soil Moisture	2.34	0.02 *

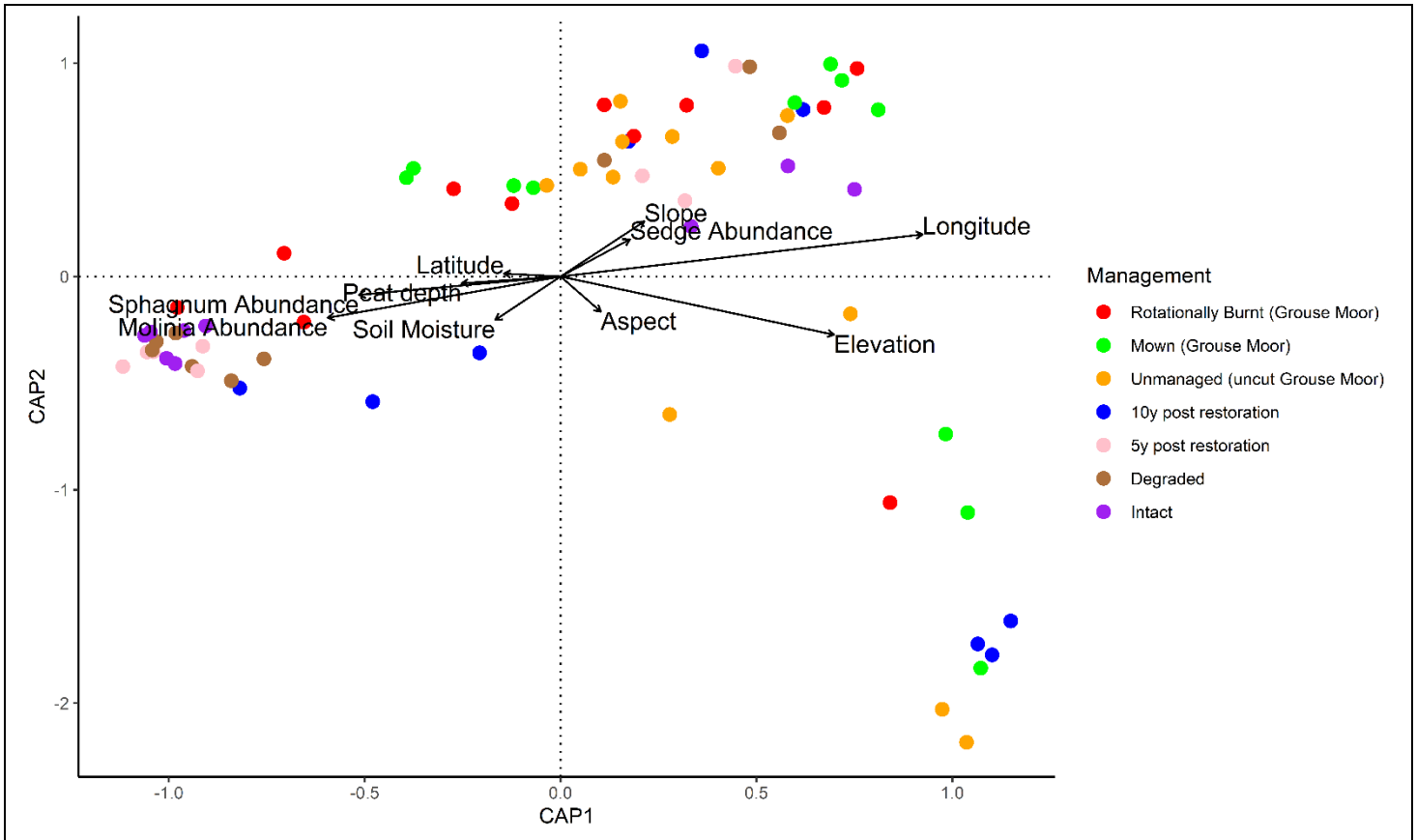

Figure 3.10: Distance based RDA: this model is significant, 59.01% of total variance is explained by constrained variables. Longitude, elevation, pH and *Molinia* % cover (grass) drive variation in Axis 1 (CAP1) whilst aspect and Sedge % cover (sedge) drive variation in Axis 2 (CAP2). Graph coloured by management: see legend.

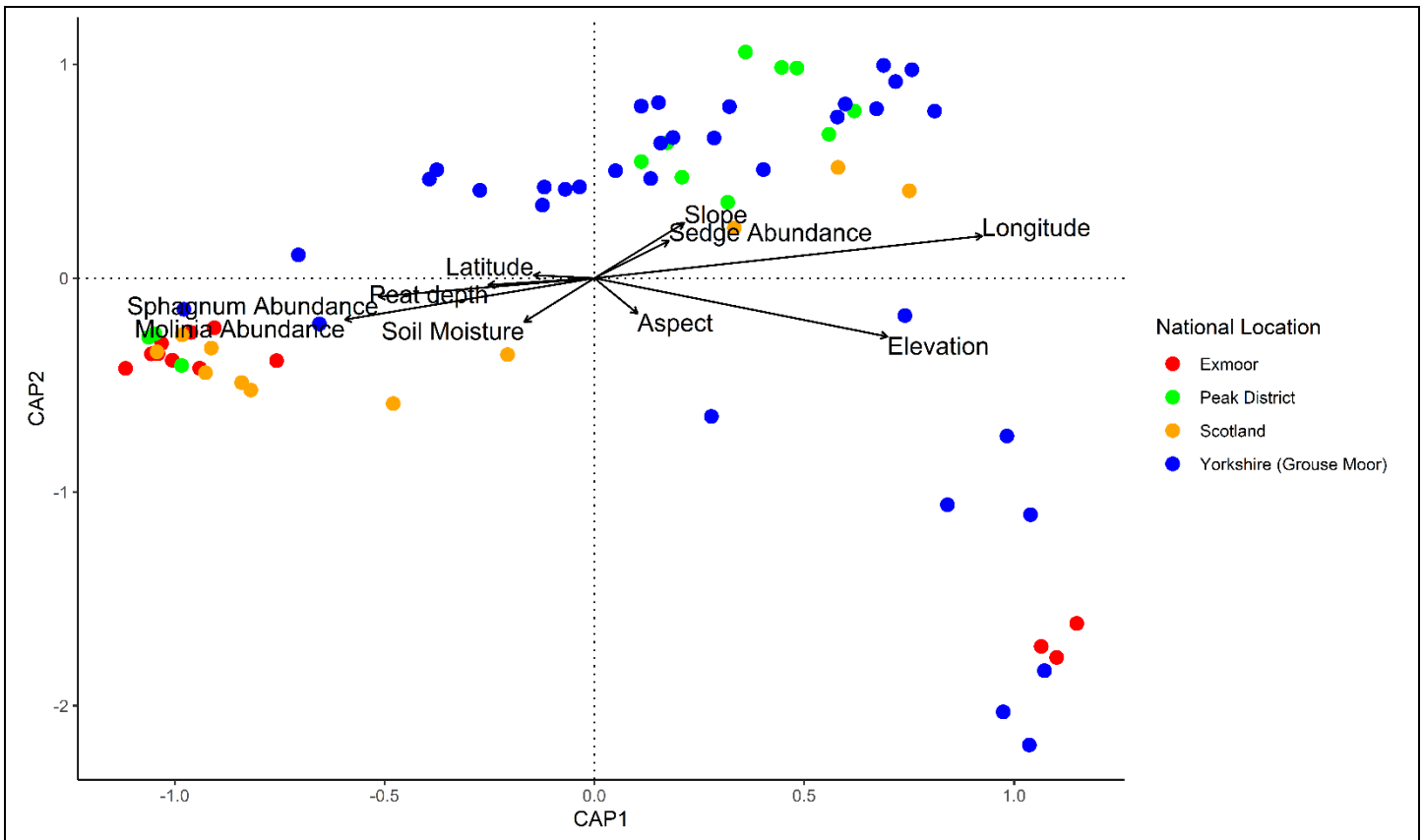

Figure 3.11: The same figure as Figure 11 but coloured by national sites (see legend)

3.19 Archaea

The archaea are much less diverse in the samples, allowing analysis at a lower taxonomic level. DADA2 processing outputted 621 ASVs. Sequences were then clustered to a similarity threshold of 97% resulting in 255 OTUs. Taxa not identified as being archaea at the phylum level were removed, along with any bacterial species in this dataset, and the samples were rarefied to an even depth based on the sample with the lowest number of observed individuals. This resulted in the removal of 30 OTUs. This process resulted in a final dataset containing 71 taxa across 70 samples. Extraction of archaeal DNA from the samples had mixed results – Mossdale Burnt sample 1, Mossdale Uncut sample 1, Peak District Degraded sample 2, and Whitendale Burnt samples 1 and 4 all resulted in amplicon libraries that did not have sufficient DNA to be sequenced.

A PERMANOVA was run on the abundance data, using a distance matrix based on Bray-Curtis dissimilarities to assess whether a significant difference was apparent between management and national site categories. Prior to the PERMANOVA, beta-dispersal between factors was tested for both management ($p = 0.004$) and national site groupings ($p = 0.262$). Because the beta-dispersal test for management is significant, it must be acknowledged that differences in samples within-management conditions might be contributing to differences observed by PERMANOVA analysis, for this test. The result for beta-dispersal between national sites gives

more confidence in the PERMANOVA result. Results of these tests are presented in Tables 21, 22 and 23. A 2-way PERMANOVA has also been included.

Table 3.21: Table of PERMANOVA results from analysis of 16S archaea community data. Df = degrees of freedom, Sum Sq = Sum of Squares, Pseudo-F = pseudo F-statistic

Factor	Df	Sum Sq	Pseudo-F	R^2	*p*-value
Arch					
management	7	4.27	4.05	0.31	0.0001 ***
residuals	62	9.33		0.69	
total	69	13.6		1	
Arch					
national sites	4	5.2	10.05	0.38	0.0001 ***
residuals	65	8.4		0.62	
total	69	13.6		1	
Arch – 2-way PERMANOVA					
national sites	4	5.2	14.95	0.38	0.0001 ***
management	5	2.03	4.66	0.15	0.0001 ***
national sites x management	6	1.68	3.23	0.12	0.0001 ***
residuals	54	4.69		0.35	
total	69	13.6		1	

Table 3.22: Post-hoc comparisons of management effects on archaeal communities. Presented in this table are the False Discovery Rate (Benjamini-Hochberg) adjusted *p*-values.

Management	Burnt	Mown	Uncut	Moor House	10 Year post restoration	5 year post restoration	Degraded
Burnt	X						
Mown	0.03 *	X					
Uncut	0.03 *	0.62	X				
Moor House	0.68	0.23	0.24	X			
10 year post restoration	0.11	0.007 **	0.007 **	0.24	X		
5 year post restoration	0.24	0.007 **	0.007 **	0.3	0.6	X	
Degraded	0.05 *	0.007 **	0.007 **	0.1	0.37	0.48	X
Intact	0.05 *	0.11	0.08	0.52	0.03 *	0.044 *	0.007 **

Table 3.23: Post-hoc comparisons of national site effects on archaeal communities. Presented in this table are the False Discovery Rate (Benjamini-Hochberg) adjusted *p*-values.				
National Site	Yorkshire	Exmoor	Peak District	Scotland
Yorkshire	X			
Exmoor	0.003 **	X		
Peak District	0.003 **	0.003 **	X	
Scotland	0.003 **	0.02 *	0.003 **	X
Moor House	0.53	0.02 *	0.05 *	0.05 *

3.20 Archaeal Taxonomic Composition

The archaeal community is much less taxonomically diverse than the bacteria or fungi and consequently it can be described at a finer taxonomic level than Phylum. There are five archaeal phyla identified in the dataset, with three phyla being dominant in terms of relative abundance, the Thaumarchaeota (mean 24.1%, max 97.0%, but absent from some samples), Crenarchaeota (mean 15.7%, max = 43.2%, absent from some samples) and the Euryarchaeota (mean = 13.1%, max = 84.9%) (Table 24). The phylum Diapherotrites is also present, with a maximum relative abundance of <0.001%, but due to mismatches between most archaeal primers and the Diapherotrites 16S rRNA genes, their rarity may be an artefact of the PCR method used (Youssef et al., 2015). The second rare phylum is the Nanoarchaeota, with a maximum relative abundance of 0.002%. The Nanoarchaeota are nano-sized symbiotic archaea that normally cannot survive without a host (Huber et al., 2003). The typical Nanoarchaeota type species host is not present in our dataset. The archaeal classes are graphed in Figures 12 and 13. Differences in the abundance of species in different classes was assessed, with the results shown in Table 25. Methanobacteria, Group 1.1c, Methanomicrobia and Nitrosphaeria all differ between managements.

Table 3.24: Mean relative abundance, Maximum relative abundance and minimum relative abundance of archaeal phyla and classes identified from 16S amplicon sequencing.				
Phylum	Class	Mean Relative Abundance	Minimum Relative Abundance	Maximum Relative Abundance
Thaumarchaeota	*Group_1.1c*	0.37	0.006	0.98
Euryarchaeota	*Methanobacteria*	0.32	0.0001	0.9
Crenarchaeota	*Bathyarchaeia*	0.19	0.002	0.4
Thaumarchaeota	*Nitrososphaeria*	0.11	0.0002	0.6
Euryarchaeota	*Methanomicrobia*	0.07	0.0001	0.4
Euryarchaeota	*Thermoplasmata*	0.004	0.00003	0.04
Crenarchaeota	*Verstraetearchaeia*	0.003	0.0005	0.008
Nanoarchaeaeota	*Woesearchaeia*	0.002	0.002	0.002
Diapherotrites	*Micrarchaeia*	0.001	0.0009	0.001

Figure 3.12: Abundances of major Archaeal classes, seen in Table 24, split by management.

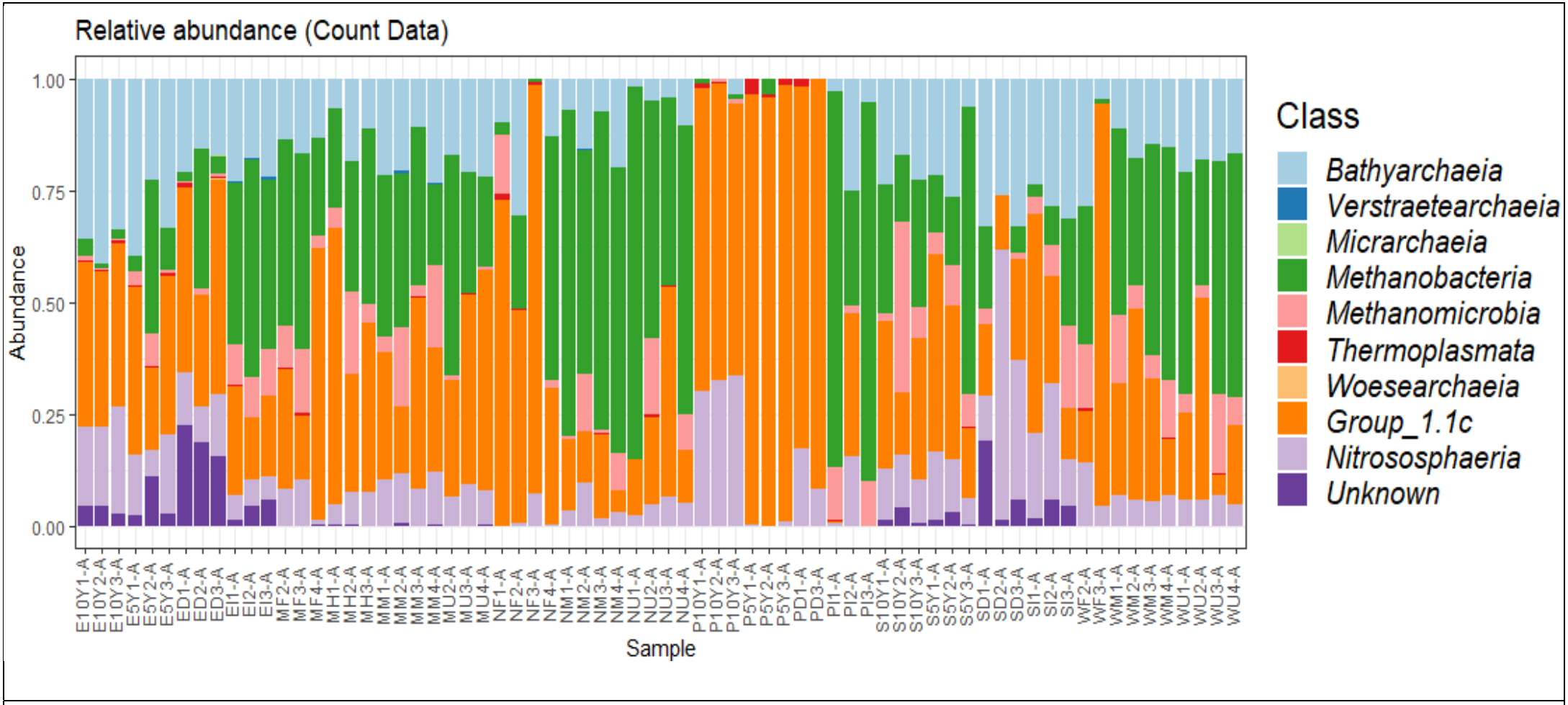

Figure 3.13: Per sample relative abundance of Archaeal classes. For details of sample codes, see Chapter 2 section 2.10.

Table 3.25: Non-parametric Kruskal-Wallis tests of whether phylum relative abundance differs between management. Kruskal-Wallis tests were used where the assumptions for ANOVA (normality and homogeneity of variance) were not met, and the data could not be transformed to meet it. Note: whilst Woesearchaeia and Micrarchaeia are not significantly different between management, they are only present in one sample on one site.

Class (Kruskal-Wallis)	X^2	Degrees of Freedom	*p*-value
Methanobacteria	32.48	7	0.00003 ***
Group 1.1c	15.68	7	0.03 **
Methanomicrobia	18.99	7	0.008 ***
Bathyarchaeia	4.2	7	0.76
Nitrosphaeria	24.52	7	0.0009 ***
Thermoplasmata	13.31	7	0.07
Verstraetearchaeia	9.55	7	0.22
Woesearchaeia	7.75	7	0.36
Micrarchaeia	7.75	7	0.36

3.21 Archaeal Diversity

Unlike the fungi and bacteria, mean Shannon diversity was highest in the 10-year post restoration plots (Mean Shannon = 2.1, N=9) and degraded plots (Mean Shannon = 2.06, n = 8), with the Yorkshire Uncut plots (Mean Shannon = 1.5, n=11) having the lowest mean Shannon diversity of all samples. Shannon diversity values did not significantly differ between managements (Kruskal-Wallis, $X^2 = 12.967$, df = 7, p = 0.07292). Simpson's diversity also did not differ between managements (Kruskal-Wallis, $X^2 = 13.55$, df = 7, p = 0.05978). The observed number of archaeal species did significantly differ between management categories (Kruskal-Wallis, $X^2 = 17.026$, df = 7, *p* = 0.01723), however post-hoc Dunn's test indicates the only significantly different management categories were the Yorkshire Uncut and 10-year post restoration plots. These differences in management are likely to be confounded by the large variation in diversity among management groups due to latitude. Figure 14 demonstrates this: all of the Exmoor sites, whichever management category they are, have high archaeal diversity, whilst all of the Peak District sites show lowest diversity overall.

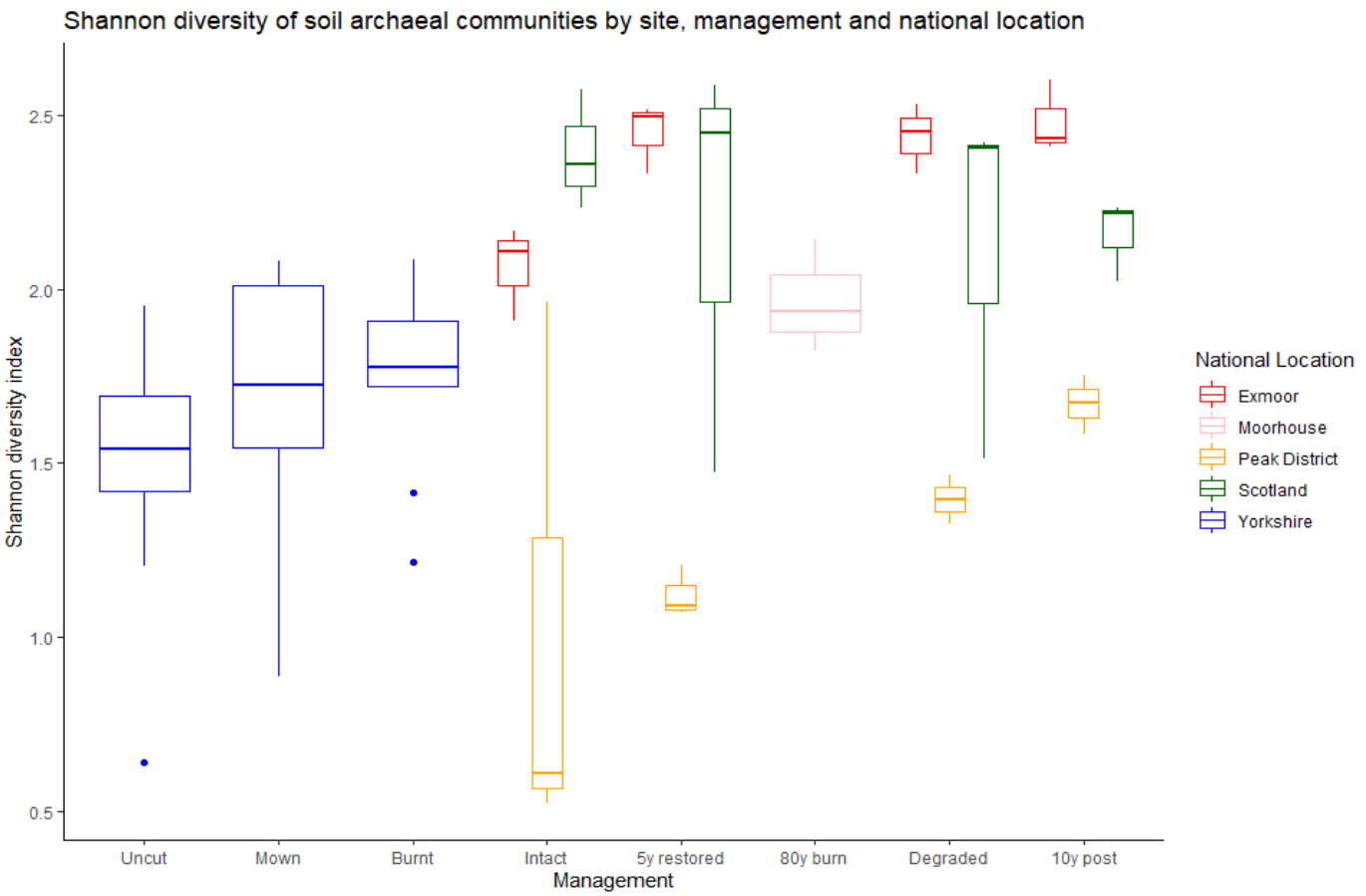

Figure 3.14: Archaeal Shannon diversity values split by management and national location.

3.22 Environmental drivers of archaeal diversity

As for the previous taxa, environmental variables were investigated to ascertain any effect on Shannon and Simpson's diversity of soil archaea. Again, it was hypothesised that Shannon diversity would be affected by soil moisture, pH, total organic nitrogen, and interactions between the major vegetation groups' abundances: *Calluna*, total sedge, *Sphagnum*, *Molinia*, other moss and bare ground. A GLM with a Gaussian distribution was used to model the impacts of these environmental parameters on Shannon index values. Model assumptions were verified by plotting residuals versus fitted values. Models were run with differing combinations of these variables and the model with the lowest Akaike Information Criterion (AIC) was chosen, with results presented in Table 26. The best model for Shannon diversity only included the percentage cover of *Molinia* from the vegetation variables, whereas the best model for Simpson's diversity only included pH as an explanatory variable (Table 27).

Table 3.26: Estimated regression parameters, standard errors, t-values and *p*-values for the Gaussian GLM modelling the effect of environmental variables on soil archaeal Shannon diversity index values.				
	Estimate	Std. Error	t value	*p*-value
Intercept	-1.51	0.77	-1.95	0.06
pH	0.64	0.18	3.61	0.00006 ***
nitrogen	0.25	0.17	2.12	0.04 *
Molinia %	0.004	0.002	2.14	0.04 *

Table 3.27: Estimated regression parameters, standard errors, t-values and *p*-values for the Gaussian GLM modelling the effect of environmental variables on soil archaeal Simpson's diversity index values.				
	Estimate	Std. Error	t value	*p*-value
Intercept	-0.29	0.23	-1.26	0.21
pH	0.22	0.05	4.5	0.00003 ***

3.23 Environmental drivers of Archaeal taxonomic composition

A dbRDA was applied in the same manner as previous. The model for archaea was significant, with environmental variables explaining variation in the community (dbRDA: df,21(43), F = 2.7018, p < 0.001). The significant drivers of community variation were tested, the results of which are listed in Table 28. Plotted dbRDA ordinations are Figures 15 and 16.

Table 3.28: List of F-statistics and *p*-values for variables listed as significant drivers of archaeal taxonomic variation in the output of a distance-based redundancy analysis.		
Variable	F-statistic	*p*-value
Latitude	3.49	0.01 **
Longitude	16.64	0.001 ***
Aspect	2.11	0.06
Elevation	11.44	0.001 ***
Sphagnum cover %	3.62	0.007 ***
Molinia cover %	4.37	0.004 ***

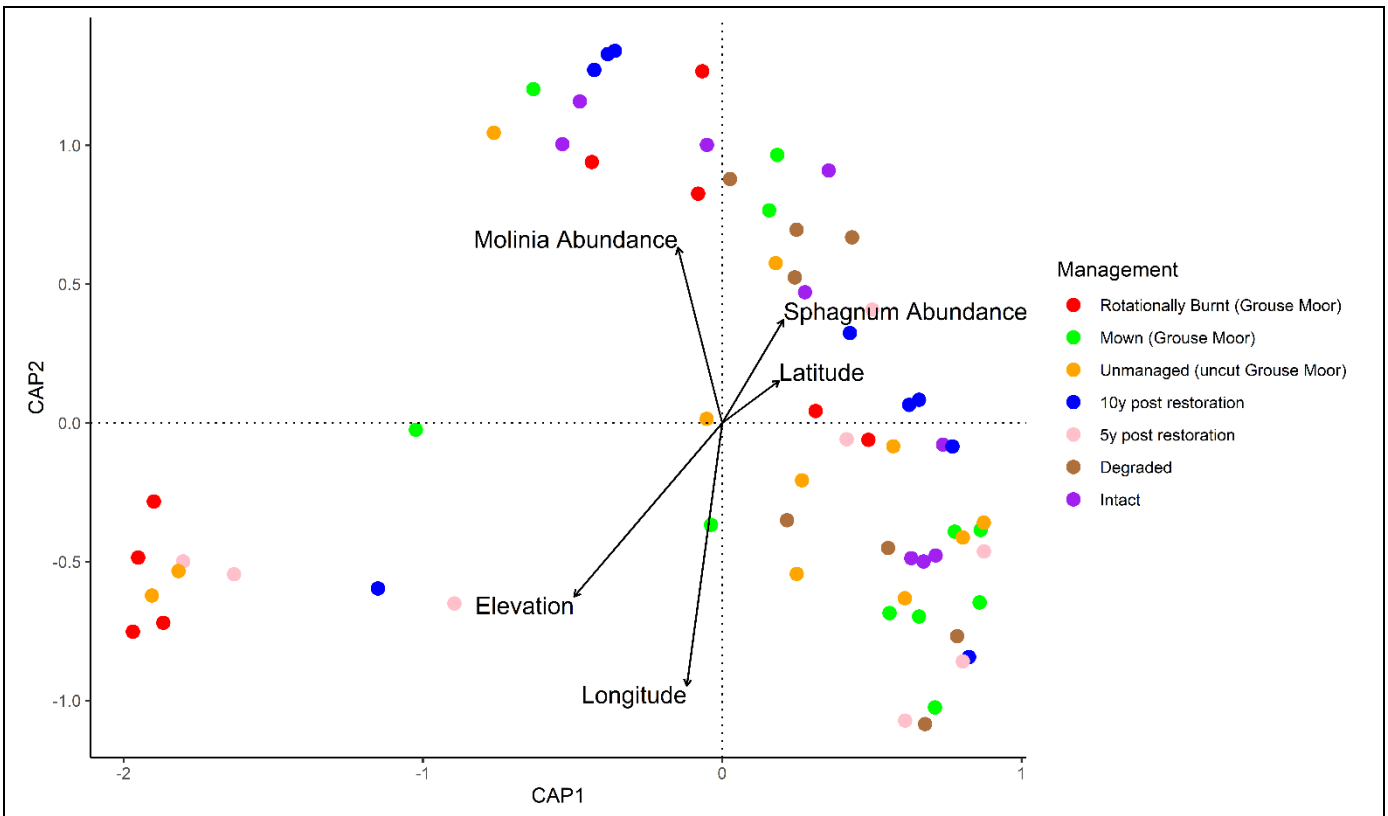

Figure 3.15: dbRDA of Archaeal communities. 61.45% of variance is explained by constrained variables. Latitude and elevation drive variation in Axis 1 (CAP1) whilst *Molinia* % cover, longitude and *Sphagnum* % cover drive variation in Axis 2 (CAP2). Graph coloured by management: see legend.

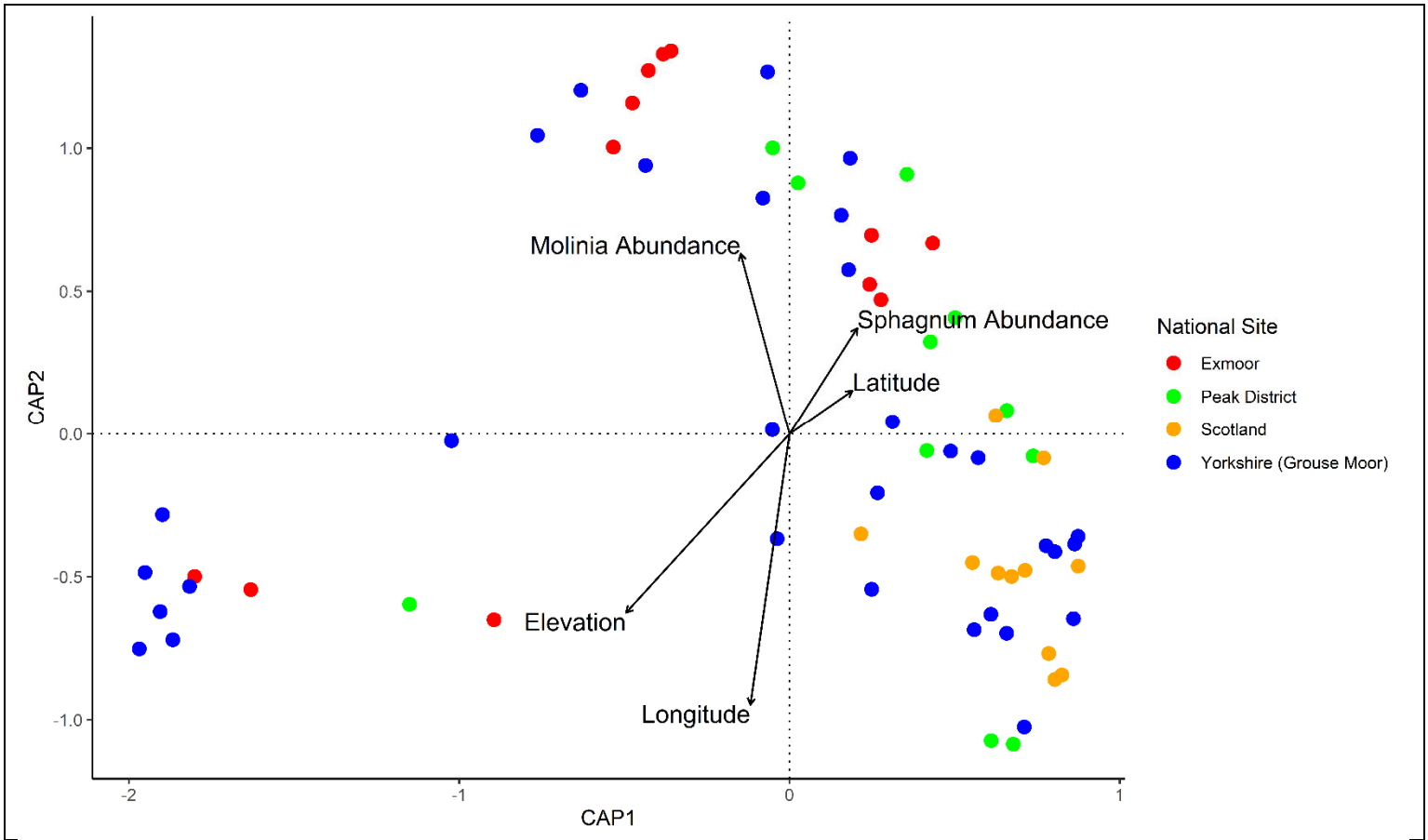

Figure 3.16: the same graph as Figure 15, coloured by national site code (see legend)

3.24 Discussion

National site condition and management differences in taxonomic composition

It is clear from the results that management is related to changes in the microbial community in blanket bogs. This is apparent from the differences detected by PERMANOVA across all three microbial communities (fungi, bacteria, archaea). However, management and national site locations did differ in their strength as explanatory variables for taxonomic composition across the three groups: the archaea were best explained by national site location, management, and the interaction between the two, with an R^2 of 0.66. The bacteria (R^2 = 0.56) were less well explained, and most of the variation in fungi could not be explained by national site location and management category (R^2 = 0.365). The hypothesis that microbial communities vary by management regime can be accepted, although not between states of restoration, but rather as a broad-scale difference between grouse moors and restoration sites.

Potential distinguishing characteristics of North Pennine blanket bogs

In the post-hoc tests undertaken on the site condition and management PERMANOVA tests (Tables 2, 3, 13, 14, 19 and 20) a clear distinction arose between grouse moors (Burnt, Uncut, Mown and Moor House as previous grouse moor) and all other managements. This may represent a genuine impact from different management objectives: grouse moors are principally managed for raising grouse by encouraging heather, often with sheep grazing and past deep drainage, whilst the other sites had contrasting historic management, including over grazing and nutrient deposition. Currently the RSPB, Exmoor Mires Partnership, and Moors for the Future manage their peatlands for ecosystem services objectives such as carbon sequestration, biodiversity and flood management.

Additionally, a number of factors may also cause the major divide between national sites and grouse moors in terms of their microbial taxonomic composition. It is important to note that this is a space-for-time study, where managements have been examined at different sites at the same time (i.e. sampled in the winter of 2019), rather than examining the effect of a particular management as a treatment, as would be the case in a before after control impact study. Consequently, there are confounding factors that make a causal link between grouse moor management and a difference in microbial community challenging.

One explanatory factor for a difference in microbial community could be climate – variables that represent climate, such as latitude and longitude, as well as elevation, were significant drivers of the microbial communities in dbRDA models. Annual rainfall, as well as air and soil temperature do vary between the regions sampled here. However, the Yorkshire sites, with a mean annual rainfall of 1806 mm and a February average soil temperature of 4.16 °C (Heinemeyer *et al.,* 2019), are no different to the Scottish sites with an annual rainfall of 1870 mm and a monthly February average soil temperature of 4.18 °C (Hargreaves *et al.,* 2003, Met Office, 2019). However, seasonal differences in climatic conditions can be expected to be different.

Some further variation may be explained by soil edaphic variables – generally, the Yorkshire grouse moors are the most acidic of the samples, apart from the Peak District. However, there may be an effect from variables that were not measured. For example, atmospheric deposition

of sulphur plays a determining role in the peatland carbon cycle (Boothroyd *et al.,* 2021) and this deposition, although declining is still highest in the Northern Pennines (DEFRA, 2021). Additionally, this study did not measure water table depths due to the logistical constraints of doing so (i.e. need to prepare dip wells), but this is a strong driver of microbial community variation (Lamit *et al.,* 2021). However, soil moisture was measured as a proxy of generic moisture conditions.

Additionally, site histories may play a part in distinguishing between the grouse moors in Yorkshire and in Moor House, and the national sites in Scotland, Exmoor and the Peak District. The Yorkshire sites as well as Moor House have a history of moorland management including burning, as well as light grazing (Heinemeyer *et al.,* 2019) whereas the national sites, in many cases, do not, having been previously grazed but not burnt, or having been used for agriculture where the grouse moors have not (see site histories Chapter 2 section 2.11)

A likely explanatory factor for the major difference in microbial communities is based on vegetation. All three communities were driven by vegetation factors including *Calluna* abundance (for fungi and bacteria) and *Molinia* (for all three communities). The communities where the differences between grouse moor and non-grouse moor are most pronounced, the fungi and bacteria, are also the communities that contain *Calluna* abundance as a statistically significant driver of microbial community difference. Grouse moors are managed to encourage *Calluna*, improving the habitat and providing a main food source for red grouse (*Lagopus lagopus scoticus),* where rotational burning or mowing is used to regenerate *Calluna* stands (Davies *et al.,* 2010). Where land is managed in this way, *Calluna* abundance is higher and consequently this explains a difference in the fungal and bacterial communities measured here because the rhizosphere will be mainly be affected by ericoid *Calluna* roots and their mycorrhizal fungi.

Taxonomic composition of blanket bog microbial communities

In terms of taxonomic composition, there are common themes. Within each community, there are dominant phyla that typically do not vary among sites. Most bogs are dominated by the fungal Ascomycota, the bacteria Proteobacteria and Acidobacteria, and the archaeal classes Methanobacteria alongside the archaeal Group 1.1c. This is somewhat unsurprising: Ascomycota is the largest fungal phylum and contains the type species for the ericoid mycorrhizas, *Rhizoscyphus ericae*, among others in the Helotiales. The bacterial phylum Acidobacteria is also an unsurprisingly abundant group, comprising many acidophilic species, which are common in acidic soils (Kielak et al., 2016). The dominant Archaea, the Methanobacteria, are responsible for methanogenesis and well recorded in anaerobic peatland soils (Bräuer *et al.,* 2020).

The dominance of the Archaeal Group 1.1c across the samples, where it averages 37% abundance, is more difficult to explain. This class of Archaea is related to others in the Thaumarchaeota that require ammonia oxidation for growth, but experimental work by Weber *et al.,* (2015) has found this not to be the case for Group 1.1c. Other studies indicated some species in Group 1.1c might have an aerobic growth habit (Biggs-Weber *et al.,* 2020). Group 1.1c is very dominant in the Peak District sites, at the expense of most other classes (Figure 14), but in the "Intact" sites of the Peak District, a more typical balance, including the Methanobacteria, is present. The degraded, and 5-year and 10-year post restoration sites in the Peak District have a very low pH, with a history of heavy metal deposition including lead,

copper, cadmium and zinc (Linton *et al.,* 2007, measured a site 120 meters from the Peak District 5-year post restoration samples. It is reasonable to hypothesise that the Group 1.1c contains Archaea that are tolerant to such pollution, or a very low pH (or both) given their dominance in these very acidic and polluted peats. However, it must be acknowledged that this may not be a full explanation: as seen in the heavy metals data in Chapter 4, the Peak District sites are not particularly heavily polluted relative to our other national sites. Whilst an thorough explanation for the dominance of Archaeal Group 1.1c is not immediately obvious in the data from this thesis, it does warrant further investigation.

Diversity across management and site categories

As discussed in section 3.10 this study principally relies on Shannon diversity indices because it takes into account both species richness and evenness. Simpson's index is a measure of the dominance of single species within a community. Both indices have been reported here for completeness.

In this study, both Shannon and Simpson diversity indices did not significantly vary across peatland management types in the fungi and archaea. In the bacteria, where the managements do significantly differ in Shannon and Simpson's diversity, post-hoc tests indicated that the only significant differences were for Moor House versus the degraded and intact management categories at the other sites. It is important to note that Moor House was sampled in the summer, rather than with the rest of the samples in February 2019. The number of observed species differed between managements in the archaea but post-hoc tests gave a confusing picture with only the Yorkshire uncut and 10-year post restoration sites being significantly different.

Whilst there are no differences in mean diversity between the management and site condition categories, there are environmental drivers of diversity, but these differ between the major microbial groups. Importantly, this study does not show cause and effect, but rather suggests linkages between environmental drivers and community composition and diversity. Archaeal taxonomic community compositions appears to be driven principally by soil pH (as pH increases, so does diversity), but also total organic nitrogen and the percentage cover of *Molinia,* which is only present in the Exmoor sites. The bacteria and fungi are not impacted by pH changes, but instead variation is principally driven by changes in soil moisture, which was measured at the time of sampling in February 2019. As moisture increases, so does diversity, alongside changes in *Sphagnum* (in the fungi) and again *Molinia* in the bacteria. The reasons for this may be in the split between archaea, which principally live an anaerobic existence in peats, and the bacteria and fungi, which are both limited by soil available oxygen. As previously mentioned, *Molinia* and the sedges may supply the soil with oxygen via their for shunt species characteristic aerenchyma (Waldo *et al.,* 2019), explaining somewhat why some variation in the communities are explained by *Molinia* abundance. These conclusions must be treated with scepticism – *Molinia* is only present in Exmoor and its relationship may represent a climate gradient (very wet) and/or historic management (over burning increases *Molinia* dominance (Brys *et al.,* 2005), but also grazing and ploughing) instead of truly being down to the environmental consequences of its presence.

Environmental drivers of taxonomic composition

The environmental drivers of taxonomic composition are complicated. The principal axes by which fungal taxonomic composition is driven contain pH, soil moisture and *Calluna* percentage cover (Figures 5 & 6) alongside total organic nitrogen, *Molinia* percentage cover and latitude, but of these only soil moisture and latitude were significant and the key drivers of variation. In the bacteria, longitude, elevation, pH and the percentage cover of *Molinia* drive variation in the first and the second, whilst aspect and percentage cover of sedges drive variation along the second axis: all of these variables were significant, and this indicates the bacterial community is structured with much more complexity, and with many more driving variables. The archaea related to much fewer variables, namely latitude, longitude, elevation, *Sphagnum* and *Molinia* percentage cover.

There are common themes in how all three groups are structured. Bacterial, fungal and archaeal composition are all driven by latitude, longitude, elevation or aspect. These are all proxies for the local climate within which the particular peatland is situated and relate to soil environmental conditions such as temperature and soil moisture: these climatic aspects are analysed in detail in the next chapter.

The functional data from the fungi paint an interesting picture. Burning appears to favour functionally flexible Saprotroph-Symbiotrophs at the expense of both Saprotrophs and Symbiotrophs (Figure 7). It is possible that the disturbance, where vegetation, upon which the symbiotic fungi rely, is removed, results in conditions favouring fungi that, in terms of their trophic mode, are functionally flexible generalists (i.e. allowing them to cope with periods of limited vegetation-root C inputs). The consequences of this occurrence for ecosystem services is analysed in detail in Chapter 5.

3.25 Conclusion

Using microbial community data sampled in the field, this study has shown the broad scale differences in soil fungal, bacterial and archaeal communities between blanket bog managements and site conditions in the UK. Hypotheses 1, 3 and 4, discussed in the introduction to this chapter, were accepted: microbial communities are taxonomically different across management/site categories and national locations, and they can be related to environmental variables. However, Hypothesis 2 cannot be accepted, as no variation in diversity across these categories were observed apart from in the bacteria, where Moor House was singularly different from the other sites' degraded and intact categories, with this site having been sampled at a different time of year.

The first overall hypothesis 1 in this thesis is that "Habitats across a spectrum of active to degraded peat bog vary in their soil biota with active sites having higher diversity", as described in Chapter 1 section 1.10. This can be rejected based on the study's data: there is no suggestion that any sites vary in their diversity of microbial communities in a consistent way that relates to habitat condition categorisation.

Chapter 4 – Effects of national location and climate on soil microbial communities and water quality

4.1 Introduction

In the last chapter, the relationship between blanket bog management and soil microbial communities was assessed, and it was found that explaining variability is complicated by site condition (and therefore national location) differences. These differences principally represent differences in climate as all sites contain deep peat.

In this chapter, the impact of measured climate variables on the microbial community within our sampled peatland areas was investigated, and these variables were used to explore improving the models used in chapter 3. The impact of climate variables upon specific microbial groups and some variables related to water quality was then assessed.

It is hypothesised here that microbial communities vary across national sites and that this is linked to climatic gradients of rainfall and temperature and their interaction as outlined below.

4.2 Rainfall influences on peatland microbial communities

Rainfall is linked to peatland hydrology in an intimate fashion; the very definition of the blanket bog ecosystems studied in this thesis is that it is ombrotrophic (rain fed). Whilst the water table in an intact peatland is near the surface and somewhat stable around the mesotelm, it does fluctuate in relation to precipitation events and dry periods. Especially in degraded peatlands, precipitation is likely to be a key factor in determining water table fluctuations (Daniels *et al.,* 2008, Robroek *et al.,* 2009, Ahmad *et al.,* 2020). Degraded locations with higher annual rainfall are likely to undergo more extreme fluctuations in water tables in relation to rewetting and drying than those that are intact, with lower annual totals, with consequences for the microbial community. The microbial habitat conditions therefore change considerably in relation to water table depth changes throughout the peat profile as well as in space and time.

Overall, the measured effects on the microbial community of changes to water levels are mixed, as elucidated by a recent peatland focused review (Kitson & Bell, 2020). Some studies suggest reductions in gene diversity under drought, whereas others have found an increase – whilst some further studies have reported no effect of drainage on species diversity and composition (Kitson & Bell, 2020). Further studies, for example Peacock *et al.,* (2014) found very little effect of ditch blocking (rewetting) on extracellular enzyme activity, indicating that the microbial community remained somewhat unchanged, and is perhaps resilient to changes in hydrology. However, an important aspect in most studies is the short-term nature of monitoring change; for example, studies might not be long enough to detect a trajectory shift and real long-term changes versus variability. Alternatively, this lack of any clear response to water levels could indicate that changes via modifications to the function (i.e. gene expression) of the microbial community can occur without significantly affecting the overall taxonomy, or, effects may be limited to a few individual species or groups who are key functional actors, the effects of which might be obscured in any study focusing on the community as a whole.

The peat hydrology, which is mediated by rainfall, as well as the microbial community and their functions are also potentially linked to the quality of the water flowing out of these peatlands, which in the UK comprises a significant portion of domestic water supply (Xu *et al.,* 2018). However, the relationship between precipitation and dissolved organic carbon (DOC) export from blanket bogs is complicated: in a study on an Irish Atlantic blanket bog, DOC increased or decreased with storm events that entailed increased precipitation, but this was contingent on temperature (Koehler *et al.,* 2009). These differences could likely be because of flow volume dilution effects versus DOC related plant and microbial activity. For example, Ritson *et al.,* (2017) found that post drought riverine DOC increases in UK peatlands were due to soil microbial processes – inferred from a lack of change in DOC production from vegetation litters. Studies that have attempted to link this to the microbial community however, such as Potter *et al.,* (2017), found only weak effects on the microbial community composition, apart from a subset of drought-responsive OTUs (Operational Taxonomic Units – roughly equivalent to species) that changed in relative abundance.

Finally, peatlands produce both CO_2 and CH_4, with the net emissions and the ratio of those two fluxes depending on peat hydrological conditions. Whereas water saturation in the catotelm results in anoxic methane production, drier conditions in the acrotelm result in mainly oxic decomposition and methane oxidation to CO2 and thus high emission of CO_2. Therefore, a very high or rising water table results in increased methane emission, whilst a low or lowering one causes increased carbon dioxide emissions (Moore & Knowles, 1989, Dinsmore *et al.,* 2009). However, any hydrological impact is also mediated by temperature conditions, especially in relation to plant and microbial activity. Consequently, as differences in precipitation are related to differences in soil moisture and water tables, it is likely that there is also a strong effect on the microbial community with resultant consequences for water quality (e.g. DOC) and GHG emissions. In one study (Whitaker *et al.,* 2020), peat mesocosm cores taken from a blanket bog in Scotland under three different plant functional types (PFTs: bryophyte, graminoid, and ericoid shrubs) had their vegetation removed (to exclude direct plant mediated effects) and were found to have their GHG emissions (of both CO_2 and CH_4) increase with temperature as well as increasing water tables. Peat abiotic and biotic properties did not differ with PFTs, except where the removed graminoids left a legacy effect of increasing methane, but not CO_2, emissions. Clearly, changes in GHG emissions in that study were related to changes in microbial respiration, itself a response to water table fluctuation.

4.3 Temperature and peatland microbial communities

At the core of peatland function is soil organic matter (SOM) peat decomposition – its rate is a major determination of the carbon balance. There is debate regarding the effects of temperature on carbon storage in peat soils (e.g. Thornley & Cannell, 2001, Bellamy *et al.,* 2005) but this thesis focuses on microbial decomposition, which, due to enzyme kinetics, generally increases with temperature, unless some physico-chemical process limits it doing so (Wallenstein *et al.,* 2012).

Soil respiration is a an indirect proxy by which the activity of the soil microbial community can be measured, and the temperature sensitivity of soil respiration is often expressed as the Q10 value; that is the factor by which soil respiration increases by over a 10°C increase in soil temperature (Kirschbaum, 1995). Generally, the Q10 is inversely correlated with mean annual temperatures (i.e. highest in coldest regions) and consequently as warming occurs the temperature sensitivity of soil respiration declines (Peng *et al.,* 2009). Because of the

relationship between soil temperature and respiration, it is expected that soil temperatures directly affect soil microbial activity and possibly community composition. The interactions between moisture and temperature in terms of soil respiration is complicated however, and the Q10 value can be quite variable in this regard (Meyer *et al.,* 2018), especially because the Q10 value is only applicable to a constant soil moisture.

Similarly, methane production is linked to temperature in peatlands where generally increased temperatures are linked to increased methane production. A study by Moore & Dalva (1993) which examined CH_4 production and consumption examined the complicated relationship between temperature, water levels and the ability of the gas to be transported to the surface via ebullition, diffusion and via root/shoot systems by examining laboratory mesocosms of peat columns. The experiments found a temperature increase from 10°C to 22.6°C produced a 2.2 times increase in CH_4 emissions in the bog samples, with a decrease in methane production when the water table was lowered from surface to 40 cm. This experiment did not examine vegetation, but more recent studies, such as that by Li *et al.,* (2021) found no effect of moderate warming on CH_4 emissions in a peatland when vegetation was included, although they did observe changes to gross primary productivity due to the warming mediated increase in graminoid biomass.

The direct impacts of temperature changes upon microbial communities in peatlands can be inferred from experiments, which examine litter decomposition, for example, or gaseous and aquatic C export. Bell *et al.* (2018) found drier (lower soil moisture) and warmer treatments lost more carbon via the fluvial pathway than their control, whilst examining the effects of temperature and rainfall on litter decomposition in the Exmoor blanket bog site. They indicated that this was possibly due the effects of microbial enzymatic activity or physical leaching on DOC production. Temperature mediated changes to decomposition must have direct and measurable effects on the microbial community, and this has been observed in boreal fens, which are different to bogs but comparable in many respects (Peltoniemi *et al.,* 2015), where warming decreased microbial biomass alongside changing observed community composition.

Other studies, for example that undertaken by Briones *et al.,* (2021) have found increased ecosystem respiration under a 13-year climate warming experiment at Moor House NNR, with consequent CO_2 emission increases alongside increased leaching of DOC. Their ^{14}C analyses also suggested that warming caused a shift towards increased mineralisation of recent plant-derived C inputs, suggesting a more active microbial community under increased temperatures.

4.4 Methods

Climatic data was collected for each set of site locations to obtain the most accurate information possible. Rainfall data included the annual rainfall (from 2019) and monthly rainfall total for the month the samples were collected in, and temperature data included the daily air and soil temperature average, as well as the monthly air and soil temperature average.

For the Peatland-ES-UK sites (i.e. Mossdale, Nidderdale, and Whitendale, labelled “Yorkshire” in the national data), data was obtained via automated weather stations (AWS; MiniMet AWS, Skye Instruments Ltd, Llandrindod Wells, UK) at each site. These were placed directly adjacent (within <300 m and at similar elevation) to the plots studied, and so represent the most accurate climate data available.

Likewise, in Moor House, the data was collected by an automatic weather station approximately 1.1 km NEE from the site of sampling (but at the same altitude). Data is collected as part of the Environmental Change Network (ECN, 2021) and made available via a licence via its website portal.

Scottish weather data was derived from a flux tower/automatic weather station at Cross Lochs South, Forsinard Flows, which is part of the UK Centre for Ecology and Hydrology's (CEH) long term monitoring as part of the CEH Carbon Catchments project (e.g. Hargreaves *et al.,* 2003). The flux tower is approximately 50-100 m from the Scottish "Intact" sample sites. For annual rainfall, the data was derived from the Kinbrace Hatchery Met Office automated weather station (Met Office, 2019).

In the Peak District, the climate data was derived from two datasets provided by Moors for the Future. Sites on Bleaklow and near to the Snake Pass road (Peak "Intact" and Peak "5 years post restoration") took their climate from the Birchinlee weather station at approx. SK1392, which is approximately 4 km south-east of the east of the sites, and approximately 30 m lower in terms of elevation. For the sites on Kinder Scout (Peak "Degraded" and Peak "10 years post restoration") a different dataset was used; the plateau is approximately 60 m higher in elevation than the other sites. This was derived from the Community Science Environmental Monitoring site which is approximately 2 km south of the sample locations (but still on the Kinder Plateau). Because soil temperature is not measured on the Community Science sites, all the samples' soil temperatures were derived from the dataset at Birchinlee.

Finally, all of the Exmoor climate data was obtained from the Met Office automated weather station located at Liscombe, which is approximately 11.6 km SEE from the sampling sites, albeit at a similar elevation (Met Office, 2019). It is important to note that the Exmoor rainfall data appears high, but that 2019 was an untypically wet year. This data was double-checked not only in the Liscombe weather station data but also on other Met Office weather stations around Exmoor, which showed the same pattern. Whilst it would be possible to repeat this analysis with 2018 weather data for all sites, there is no evidence that rainfall from the period one year prior to sampling would have any more relevance to the microbial community than the period immediately surrounding it.

4.5 Climatic variation across national locations

Climate variables principally represent a latitudinal (temperature) and longitudinal (rainfall) gradient with elevation additionally affecting both. The sites in Exmoor (furthest West) are the wettest, whilst the Scottish sites, which are furthest east, is among the driest, although this is complicated by rain shadow effects in the Peak District and Yorkshire, which are behind the Welsh mountains and the Pennines respectively.

The Exmoor sites, which are furthest south in our data, are the warmest in terms of air temperature. The Peak District was the coldest site in the month of February 2019 (when we collected samples) although it is also the highest in elevation, with all sites being 600 m AOD (Above Ordnance Datum). A sites summary is shown in Table 1.

Table 1: Average (mean) climatic conditions for national site groupings. The highest value in each column is coloured green. All measures are the averages for 2019.						
Site	Annual Rainfall (mm)	Air temp (day of collection) Celsius	Soil temp (day of collection) Celsius	Rainfall (month of collection)	Air temp (month of collection)	Soil Temp (month of collection)
Yorkshire	1806	7.59	7.99	103.43	5.06	4.16
Moor House	2048	6.15	7.01	NA	NA	NA
Scotland	1870	8.40	5.13	60.06	5.90	4.18
Peak District	1472	7.87	7.08	59.70	4.55	6.33
Exmoor	3334	11.10	8.00	178.40	8.21	5.96

4.6 Climate as a driver of habitat variables

The variation in site variables due to climate was investigated. A PERMANOVA undertaken on a Bray-Curtis dissimilarity matrix of vegetation found annual rainfall, alongside management and soil temperature, explained 75.9% of the variation within the vegetation community (Table 2). Temperature was also tested, and this was a significant, although weak, driver of vegetation community dissimilarities.

Table 2: PERMANOVA results comparing the effects of management/habitat condition and annual rainfall combined on the vegetation community (percent cover of *Calluna*, sedge, *Molinia*, bare ground, *Sphagnum* and other moss). Significance is indicated with the symbols * where extremely significant is *** Very significant = ** and Significant = *

PERMANOVA	DF	Sum of Sq.	F Model	R^2	*p*-number
Annual rainfall	1	5.36	82.98	0.34	0.0001 ***
Management	7	4.40	9.74	0.28	0.0001 ***
Annual rainfall and management interaction	6	1.49	3.85	0.094	0.0001 ***
Soil temperature (day of collection)	1	0.78	12.1	0.049	0.0001 ***
Residuals	59	3.81		0.24	
Total	74	15.83		1.000	

The effect of annual rainfall and temperature (day of collection) on measured water quality variables, as well as soil parameters like total available nitrogen and bulk density, was assessed using general linear models with Gaussian distributions. These variables were selected because annual rainfall is the main driver for a bogs water balance, ignoring drainage from management, steep slopes and extreme weather related evapotranspiration losses. The temperature effects are expected to be more short-term in nature and therefore the soil temperature on the day of collection was used – the monthly average air temperature was not significantly related to any variables. As can be seen in Table 3, only DOC (dissolved organic carbon) is affected directly by weather variables and even so, this is only a weak effect. In contrast, SUVA (specific UV absorbance) and Hazen (colour) do not seem to be affected, but these variables are based on the chemical make-up of DOC and consequently they probably more closely represent microbial processes than weather based ones.

Table 3: GLMs undertaken on water quality variables to examine the effects of climatic variables. Significance is indicated with the symbols * where extremely significant is *** Very significant = ** and Significant = *

Variable	Estimate	T value	*p*-value
DOC			
Intercept	-135.84	-2.08	0.04 *
Annual rainfall	0.09	2.58	0.01 *
Soil temperature (day of collection)	18.39	2.20	0.03 *
Annual rainfall and soil temperature interaction	-0.011	-2.41	0.02 *
SUVA			
Intercept	0.72	0.04	0.97
Annual rainfall	0.015	1.43	0.16
Soil temperature (day of collection)	0.24	0.1	0.92
Annual rainfall and soil temperature interaction	-0.002	-1.44	0.16
Hazen			
Intercept	-1.10	-0.14	0.89
Annual rainfall	0.001	0.24	0.81
Soil temperature (day of collection)	0.33	0.32	0.75
Annual rainfall and soil temperature interaction	-0.0002	-0.27	0.79

4.7 Fungi and climate: environmental drivers of taxonomy

After collation of climatic data for the sites, climate-associated variables from the initial environmental analysis in Chapter 3 were replaced with actual climatic data. For example, latitude, longitude and elevation, all significant variables driving microbial communities, are proxies for temperature and rainfall gradients. The dbRDA models on microbial communities with climate variables included on the fungal, bacterial and archaeal communities were therefore re-run. To deal with autocorrelation between different climatic and also vegetation variables, forward selection of explanatory variables was permutationally undertaken (Blanchet *et al.,* 2008) using the "vegan" R package function "ordiR2step" (Oksanen *et al.,* 2019)

dbRDA: Fungal communities

The dbRDA model for the fungal community was still significant when latitude, longitude and elevation were replaced by climatic variables (dbRDA: df, 7(62), F = 2.88, $p < 0.001$). The fewer variables involved after forward model selection decreased the explanatory power to 24.9% of the variance being explained by constrained variables, as opposed to 42.9% in Chapter 3. The significance of some variables changed which are listed in Table 4. To comply with the model requirements of data completeness, the following samples were removed due to missing climate data. Those samples removed included Moor House samples (MH1, MH2 and MH3) and one Scottish 5-year post restoration sample (S5Y3).

Table 4: List of F-statistics and *p*-values for variables listed as significant drivers of fungal taxonomic variation in the output of a distance-based redundancy analysis. Significance is indicated with the symbols * where extremely significant =*** Very significant = ** and Significant = *		
Variable	F-statistic	*p*-number
Annual rainfall	5.52	0.001 ***
Average soil temperature (day of collection)	5.55	0.001 ***
Calluna cover %	2.40	0.001 *
Soil Moisture	2.09	0.002 **
Average air temperature (day of collection)	1.79	0.009 **
Sedge cover %	1.46	0.041 *
Total Nitrogen	1.37	0.07 .

The inclusion of climatic variables meant that some other variables became non-significant compared to the previous analysis in Chapter 3, namely latitude and longitude, slope, aspect, and elevation (all proxies for climate) as well as *Molinia* percentage cover and levels of dissolved organic carbon. In terms of vegetation, *Molinia* abundance may itself be a proxy for climate given it is only located at the most southern of sites. Similar to Chapter 3 results, the abundance of *Calluna* was a significant driver of fungal community assembly, even when differences in climate are accounted for (Figure 1) in addition to sedge abundance.

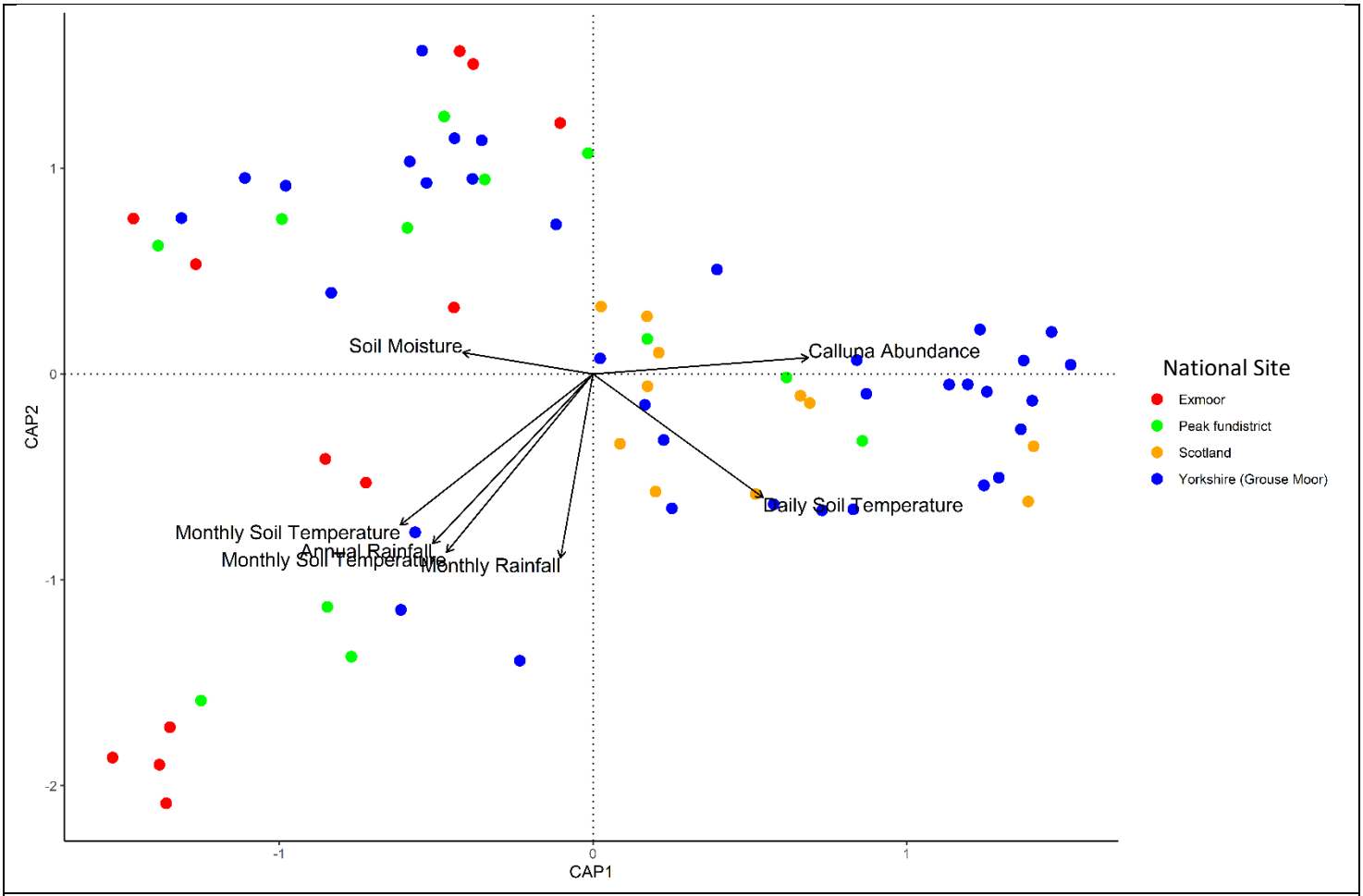

Figure 1: dbRDA of fungal communities split by national site location. 24.9% of the variation is explained by the available variables. All variables were measured in February 2019.

4.8 Environmental drivers of fungal trophic groups

Environmental drivers of the fungal functional groups (trophic groups) first described in Chapter 3 were assessed by employing generalised linear models. Initial data exploration was undertaken using a correlation plot, where correlating variables were highlighted. Only the abundance of saprotrophs and Pathotrophs correlated with environmental and climate related variables, with the saprotrophs being correlated with rainfall (annual and monthly) and monthly air and soil temperature averages, and the Pathotrophs being correlated with monthly and time-of-collection soil temperature, as well as monthly and annual rainfall totals. The resultant general linear models are presented below in Table 5.

Table 5: Generalised Linear Models assessing the impacts of annual rainfall and average monthly soil temperature (for the month of sample collection, February 2019) on fungal trophic groups. Significance is indicated with the symbols * where extremely significant =*** Very significant = ** and Significant = *				
Saprotroph	**Estimate**	**Std. Error**	**t value**	***p*-value**
Intercept	-0.08	0.37	-0.22	0.83
Annual rainfall	0.00001	0.0002	0.06	0.96
Average February soil temperature	0.05	0.18	0.25	0.80
Pathotroph	**Estimate**	**Std. Error**	**t value**	***p*-value**
Intercept	-0.13	0.13	-0.99	0.33
Annual rainfall	-0.00004	0.00008	-0.56	0.58
Average February soil temperature	0.06	0.06	0.93	0.35
Symbiotroph	**Estimate**	**Std. Error**	**t value**	***p*-value**
Intercept	0.18	0.08	2.32	**0.02** *
Annual rainfall	0.0001	0.0001	1.99	0.051
Average February soil temperature	-0.08	0.04	-2.05	**0.044** *
Saprotroph-Symbiotroph	**Estimate**	**Std. Error**	**t value**	***p*-value**
Intercept	-0.46	0.31	-1.50	0.14
Annual rainfall	-0.0004	0.0002	-2.28	0.03 *
Average February soil temperature	0.32	0.15	2.08	0.041 *

4.9 Bacteria and climate

The redundancy model for bacteria was significant (dbRDA: df, 9(61), F = 4.942, p < 0.001) and, as for the fungi, less variation was explained than in the previous chapter, although the constrained variables did account for 42.7% of the variation in bacterial species composition (see Table 6, below). The plotted dbRDA is shown in Figure 2.

Table 6: List of F-statistics and *p*-values for variables listed as significant drivers of bacterial taxonomic variation in the output of a distance-based redundancy analysis, based on samples taken in February 2019. Significance is indicated with the symbols * where extremely significant =*** Very significant = ** and Significant = *		
Variable	F-statistic	*p*-value
Annual rainfall	14.32	0.001 ***
Average soil temperature (day of collection)	10.13	0.001 ***
Bare ground abundance %	6.4	0.001 ***
Average air temperature (day of collection)	3.28	0.003 **
Peat depth	2.67	0.005 **
Calluna abundance %	2.15	0.022 *
Total nitrogen	1.97	0.033 *
Soil moisture	1.81	0.048 *
Sphagnum abundance %	1.77	0.055 .

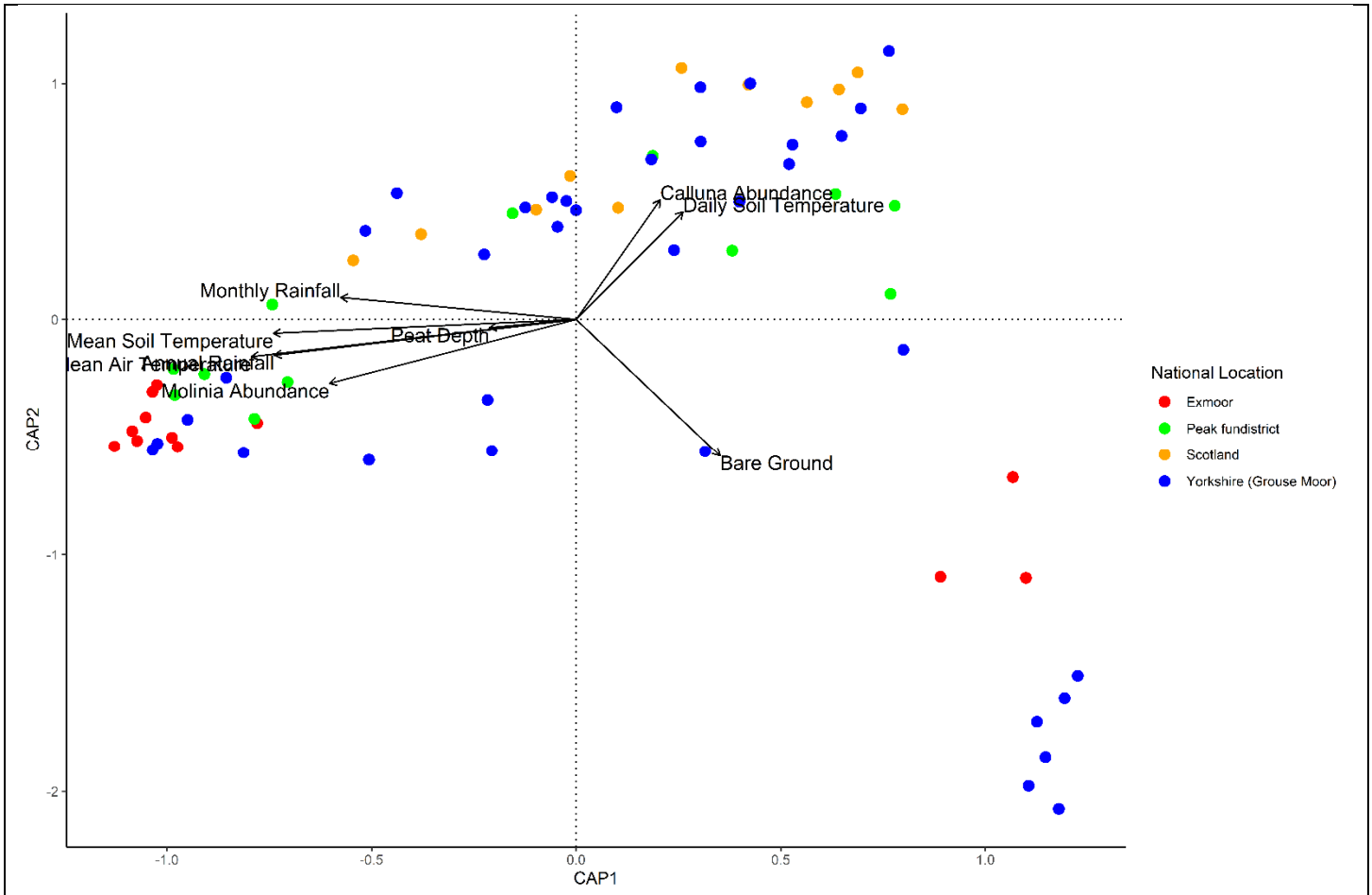

Figure 2: dbRDA graph of the bacterial community, using forward selection to select the best explanatory variables. Where two variables are colliding on the top right of the graph, these two variables are *Calluna* abundance and soil temperature on the day of sample collection.

4.10 Archaea and climate

The model forward selection process for the archaea also produced a model with less variance explained by constrained variables than in the previous chapter 3 – indicating that the explained variance in previous dbRDA models may be overestimated. The model did, however have fewer explanatory variables retained, resulting in a more parsimonious model of environmental effects, which explained 41% of the variance (Table 7). The model was significant (dbRDA: df, 6(59), F = 5.79, p < 0.001) and because of the forward selection, the model contains only significant variables.

Table 7: List of F-statistics and *p*-values for variables listed as significant drivers of archaeal taxonomic variation in the output of a distance-based redundancy analysis based on samples taken in February 2019. Significance is indicated with the symbols * where extremely significant =*** Very significant = ** and Significant = *		
Variable	F-statistic	*p*-value
Annual rainfall	9.92	0.001 ***
Average air temperature (day of collection)	7.8	0.001 ***
Average soil temperature (day of collection)	4.25	0.006 **
Soil pH	5.73	0.001 ***
Peat depth	3.4	0.013 *
Sphagnum abundance %	3.62	0.01 **

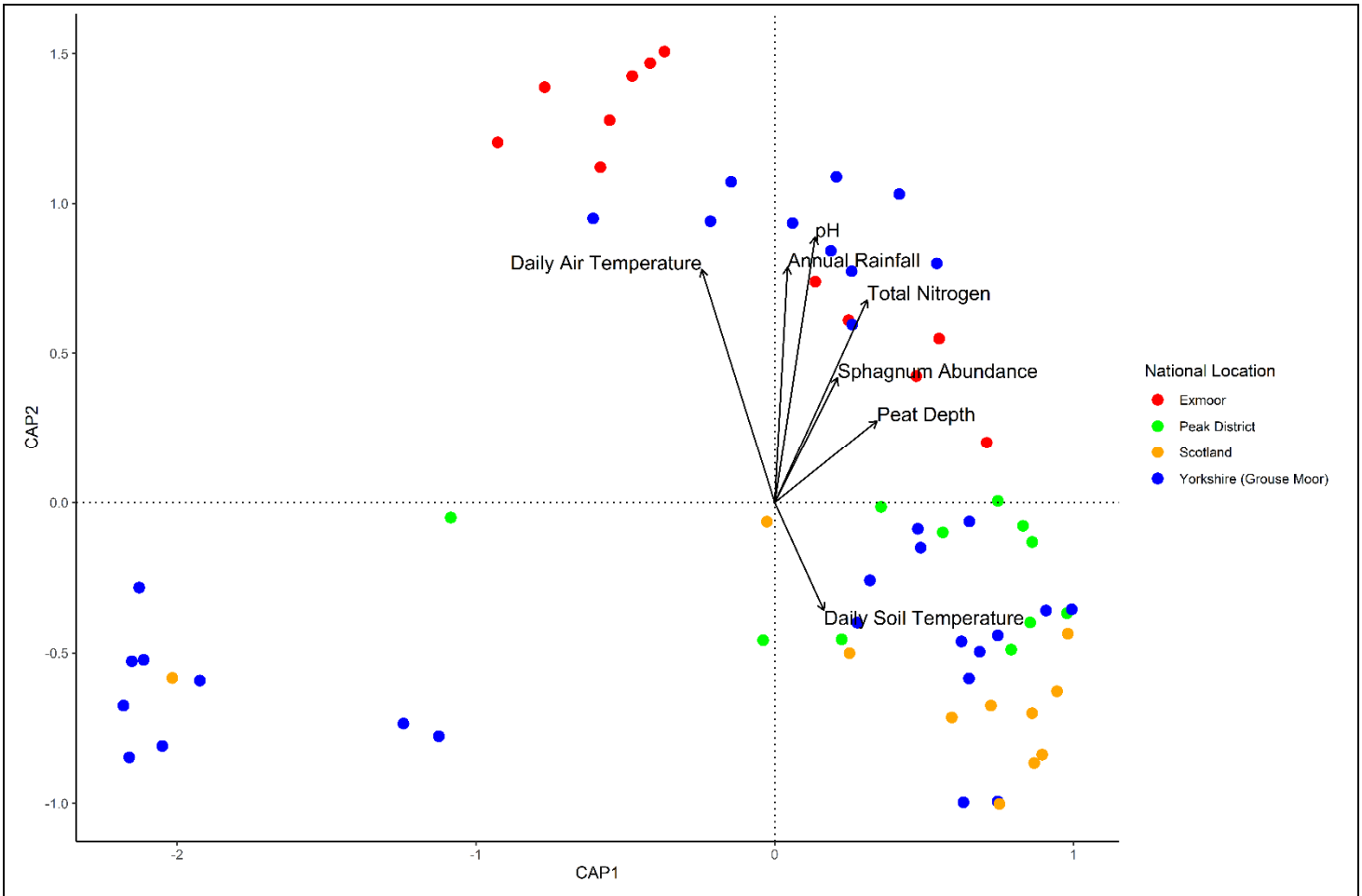

Figure 3: dbRDA graph of the archaeal community, using forward selection to select the best explanatory variables.

4.11 Indicator species analysis: weather variables

Species that were unique to specific climatic conditions were examined by using indicator species analysis (ISA) with the 'indicspecies' R package (Caceres & Legendre, 2009). Because many species in the dataset were not identified, the data was agglomerated to genus level before undertaking the analysis. Consequently, many OTUs not identified to species level are considered equivalent to other OTUs in the same genus.

4.12 Rainfall

To undertake ISA on rainfall, this variable was factorised. The minimum rainfall total was subtracted from the maximum rainfall total to obtain the range (758 mm), which was then divided into three categories: driest, midpoint, and wettest. Exmoor (with an annual rainfall of 3333 mm in 2019), being so far outside the rainfall of the other sites, was excluded and simply classified as "wettest". If Exmoor were included, and the range equally divided, all sites except Exmoor and Moor House would be in the "driest" category, which is not representative of meaningful ecological differences. Which sites are fitted into which category, and what these categories are, is enumerated in Table 8.

Table 8: Site categories of factorised rainfall amounts. Categories represent rainfall of driest (1290 – 1543mm per year), midpoint (1543-1796mm per year) and wet (1796-2049mm per year) as well as wettest, Exmoor.

Site	Annual rainfall	Category	Number and name of samples
Exmoor	3333 mm	Wettest	E samples (n=12)
Mossdale (Yorkshire)	1917 mm	Wet	M samples (n=12)
Nidderdale (Yorkshire)	1637 mm	Midpoint	N samples (n=12)
Whitendale (Yorkshire)	1872 mm	Wet	W samples (n=12)
Moor House	2048 mm	Wet	MH samples (n=3)
Kinder Scout (Peak District)	1653 mm	Midpoint	P10 and PD samples (n=6)
Bleaklow (Peak District)	1290 mm	Driest	PI and P5 samples (n=6)
Forsinard Flows (Scotland)	1869 mm	Wet	S samples (n=12)

Indicator species analysis on the fungal community found 24 OTUs were associated with the driest and 46 OTUs were associated with the wettest categories, but importantly, these were categories with only one site. In the driest category, only Bleaklow (representing the Peak Intact and 5-year restored samples) was included, whilst the wettest category only included samples from the Exmoor sites (all management categories). A full list of species indicators for each annual rainfall level is shown in Table 9. Other than in the "midpoint" category, there were no significant species indicators for rainfall and most of the species in the table are representatives of a specific site.

The same method was repeated on the bacteria, which again was agglomerated to genus, resulting in 253 taxa across 74 samples (as for previous analyses, Whitendale burnt plot 4 was

excluded because of a very low read count and consistently and atypically low water tables). Here, more species were chosen as indicators of weather conditions, with 36 species in total. Of these 36 indicators, 19 were indicators of the "driest" category, which represents only Bleaklow in the Peak District, and 11 represented the four sites in across Exmoor. One OTU represented the midpoint (Kinder Scout in the Peak District and Nidderdale in Yorkshire) and five represented the "wet" category, comprising five diverse sites indicated in Table 10. The full list of species, with associated notes is provided in table 10.

Whilst the same method was repeated for Archaea, the taxa were not agglomerated to genus, as only six genera were present in the dataset, with 77 species. However, due to the lack of previous work in peat bog archaea, the vast majority of OTUs were not identified to species. Results of the analysis for archaea are presented in table 11.

Table 9: Results of indicator species analysis on the fungal community split by rainfall conditions. The "wet" category is not listed because it had no indicator species associated with it. Significance is indicated with the symbols * where extremely significant =*** Very significant = ** and Significant = *				
Annual rainfall	Taxonomy	Correlation	*p*-value	Species notes
Driest	*Galerina calyptrata*	0.51	0.003 **	Common name "Tiny Bog Galerina", moss associated fungal species. This species is reported as being pollution tolerant (Høiland & Dybdahl 1993). Species associates specifically with *Dicranum sp*. mosses (Davy *et al.,* 2013)
	Pseudogymnoascus appendiculatus	0.48	0.004 **	Ink caps in the *Basidiomycetes*
	Xenochalara sp.	0.44	0.01 *	
	Chrysosporium merdarium	0.44	0.005 **	This fungi can degrade Zinc and can be found in polluted environments (Lugauskas *et al.,* 2009)
	Zopfiella pleuropora	0.41	0.007 **	
	Xylodon nespori	0.41	0.0001 ***	
	Plectosphaerella sp.	0.40	0.006 **	
	Neoascochyta graminicola	0.38	0.03 *	A grass pathogen (Golzar *et al.,* 2019)
	Clavaria sp.	0.37	0.002 **	Basidiomycete saprotroph
	Pleurophoma sp.	0.36	0.048 *	Fungal pathogen
	Aureobasidium sp.	0.36	0.03 *	A genus containing pollution resistant black yeasts (Liu *et al.,* 2017)
	Coprinellus radicellus	0.35	0.044 *	A coprophilous basidiomycete with northern distribution (Házi *et al.,* 2011)
Midpoint	*Meliniomyces sp.*	0.38	0.04 *	An ericoid fungal genus phylogenetically related to *Rhizoscyphus ericae* (Hambleton & Sigler, 2005)
Wettest	*Leohumicola minima*	0.60	0.0006 ***	An ericoid fungi of *Vaccinium sp.* (Baba & Hirose, 2020)
	Syncephalis sp.	0.50	0.003 **	Zygomycete mycoparasite (Benny *et al.,* 2016)
	Stagonospora sp.	0.43	0.01 **	Plant pathogen
	Monoblepharis hypogyna	0.41	0.01 *	
	Phaeotremella fimbriata	0.39	0.01 *	Parasite of crust fungi (Spirin *et al.,* 2018)
	Entoloma sp.	0.35	0.04 *	Possibly ectomycorrhizal or parasite fungi (Agerer & Waller, 1993)

Table 10: Bacterial indicators of rainfall conditions. Significance is indicated with the symbols * where extremely significant =*** Very significant = ** and Significant = *				
Annual rainfall	Taxonomy	Correlation	*p*-value	Species notes
Driest	*Acidipila sp.*	0.59	0.002 **	A genus of acidophilic, strict aerobic organotrophic bacteria isolated from acid soils and mine tailings (Okamura *et al.,* 2011)
	Methylovirgula sp.	0.55	0.001 ***	Obligately acidophilic aerobic methanotroph (Voro'ev *et al.,* 2009)
	Paracoccus sp.	0.55	0.001 ***	The type species for this genus, *Paracoccus denitrificans*, is a non-motile denitrifying extremophile (Urakami *et al.,* 1990)
	Novosphingobium sp.	0.53	0.002 **	Members of this genus are involved in degrading compounds in polluted environments including those contaminated by oil and plastic (Segura *et al.,* 2017)
	Candidatus Endonucleariobacter sp.	0.53	0.001 ***	An endosymbiont to amoeba in the *Nuclearia* (Dirren & Posch, 2016)
	Chthoniobacter sp.	0.50	0.01 **	
	Arsenicibacter sp.	0.50	0.003 **	An Arsenic methylating and volatilizing bacterium (Huang *et al.,* 2017)
	Occallatibacter sp.	0.50	0.01 **	Acidobacteria
	Legionella sp.	0.48	0.01 **	
	Candidatus Paracaedibacter sp.	0.47	0.01 **	Amoeba endosymbiont (Midha *et al.,* 2021)
	Massilia sp.	0.46	0.01 *	Copiotrophic root coloniser (Ofek *et al.,* 2012)
	Candidatus Finniella sp.	0.45	0.01 **	Endosymbiont of phagotrophic amoeba (Hess *et al.,* 2015)
	Desulfosporosinus sp.	0.40	0.04 *	Sulfate reducing bacteria sometimes found in contaminated soils but also in pristine soils (Robertson *et al.,* 2001)
	Sporolactobacillus sp.	0.39	0.01 **	Lactic acid bacteria
	Devosia sp.	0.39	0.01 **	
	Bdellovibrio sp.	0.37	0.02 *	A genus of predatory (on other bacteria) highly mobile bacteria (Sockett, 2009)
	Clostridium sensu stricto 9	0.35	0.05 *	
	Roseimicrobium sp.	0.35	0.04 *	Oligotroph often found associated with plant roots (Podar *et al.,* 2020)

	Methanoregula sp.	0.34	0.05 *	A methanogen genus operating at very low pH, isolated from peat bogs (Brauer *et al.,* 2006)
Midpoint	*Candidatus Nucleicultrix*	0.35	0.03 *	A symbiont that infects the nucleus of amoebae (Schulz *et al.,* 2014)
Wet	*Candidatus Solibacter*	0.53	0.01 **	Acidobacteria
	Roseiarcus sp.	0.45	0.01 *	*Roseiarcus sp.* are found in the rhizosphere of *Vaccinium sp.* (Morvan *et al.,* 2020)
	Syntrophobacter sp.	0.41	0.02 *	Sulfate reducing bacterium
	Smithella sp.	0.39	0.03 *	n-alkane degraders that associate with methanogens (Ji *et al.,* 2020)
Annual rainfall	*Taxonomy*	Correlation	*p*-value	Species notes
Wet	*Nevskia sp.*	0.34	0.05 *	A presumably oligocarbophilic neuston bacteria which has been isolated from bog-lakes (Stürmeyer *et al.,* 1998)
Wettest	*Aquisphaera sp.*	0.67	0.001 ***	Common *Planctomycetes* found in *Sphagnum* rich wetlands (Dedysh & Ivanova, 2019)
	Clostridium sensu stricto 12	0.64	0.002 **	
	Candidatus Koribacter	0.54	0.002 **	Acidobacteria
	Conexibacter sp.	0.49	0.005 **	
	Anaerobacterium sp.	0.41	0.02 *	An obligately anaerobic bacterium in the *Clostridium* cluster. Some species have the ability to degrade cellulose (Horino *et al.,* 2014)
	Ktedonobacteraceae (family) 1959-1 group sp.	0.34	0.03 *	
	Tumebacillus sp.	0.40	0.02 *	
	Rhodoplanes sp.	0.39	0.03 *	A genus of phototrophic bacteria (Haraishi & Ueda, 1994)
	Romboutsia sp.	0.39	0.02 *	*Clostridium* group, see above
	Unknown genus in the order Rhodospirillales	0.37	0.02 *	
	Fonticella sp.	0.36	0.04 *	Strict anaerobic halotolerant bacterial genus with one known species, isolated from a hot spring (Fraj *et al.,* 2013)

Table 11: Archaeal indicators of rainfall conditions. Significance is indicated with the symbols * where extremely significant =*** Very significant = ** and Significant = *				
Annual rainfall	Taxonomy	Correlation	*p*-value	Species notes
Driest	Class *Group_1.1c*, unknown Order	0.50	0.01 **	A group of Thaumarchaea with unknown function, whom are highly pH influenced (Lehtovirta *et al.,* 2009). They are potentially symbiotic with methanogens (Lin *et al.,* 2014). They are found in acidic soils with higher moisture and organic content. Unlike their close relatives in the Thaumarchaea they are not ammonia oxidising (Weber *et al.,* 2015)
	Class *Thermoplasmata*, unknown Order	0.49	0.01 **	A group known from very acidic environments (Golyshina *et al.,* 2016)
	Class *Group_1.1c*, unknown Order	0.46	0.02 *	
	Class *Thermoplasmata*, unknown Order	0.44	0.003 **	
	Class *Group_1.1c*, unknown Order	0.43	0.02 *	
	Class *Thermoplasmata*, unknown Order	0.41	0.03 *	
Midpoint	Family *Nitrosotaleaceae* unknown Genus	0.42	0.02 *	Ammonia oxidizing archaea
	Class *Bathyarchaeia*, unknown Order	0.73	0.001 ***	
	Class Group_1.1c, unknown Order	0.67	0.001 ***	
	Phylum Crenarchaeota unknown Class	0.56	0.002 **	
	Class Bathyarchaeia, unknown Order	0.55	0.002 **	
	Class Group_1.1c, unknown Order	0.55	0.01 **	
	Class Group_1.1c, unknown Order	0.49	0.01 **	
	Class Group_1.1c, unknown Order	0.49	0.01 **	
	Class Group_1.1c, unknown Order	0.46	0.01 **	
	Class Bathyarchaeia, unknown Order	0.45	0.01 **	
	Candidatus Nitrosotalea, unknown species	0.44	0.01 **	
	Class *Thermoplasmata*, unknown Order	0.43	0.02 *	
	Candidatus Methanomethylicus, unknown species	0.39	0.05 *	
	Class Thermoplasmata, unknown Order	0.38	0.04 *	

4.13 Temperature

The same process of variable factorisation as done for rainfall was also applied to monthly soil temperatures during the month of sampling in 2019. The minimum soil temperature (4.06°C) was subtracted from the maximum soil temperature (8.21°C) to obtain the range (4.15°C), which was then divided into three categories: coldest (4.06 -5.4°C), midpoint (5.4 – 6.8°C), and warmest (6.8°C +). Which sites are fitted into which category, and these categories are enumerated in Table 12. Monthly average soil temperature was selected because it is more of a stable representative of microbial habitat than the daily temperature, which would be confounded by local weather.

Table 12: Site categories of factorised monthly (February 2019) temperature.

Site	Monthly Average Temperature, February 2019 (°C)	Category	Number and name of samples
Exmoor	8.21	Warmest	E samples (n=12)
Mossdale (Yorkshire)	4.2	Coldest	M samples (n=12)
Nidderdale (Yorkshire)	5.1	Coldest	N samples (n=12)
Whitendale (Yorkshire)	5.2	Coldest	W samples (n=12)
Moor House	NA	NA	MH samples (n=3)
Kinder Scout (Peak District)	4.1	Coldest	P10 and PD samples (n=6)
Bleaklow (Peak District)	5.04	Coldest	PI and P5 samples (n=6)
Forsinard Flows (Scotland)	5.9	Midpoint	S samples (n=12)

The results of indicator species analysis on temperature categories on the fungal, bacterial and archaeal communities are given in Tables 13, 14 and 15, respectively. Very few fungal indicators were found, perhaps exhibiting no temperature specificity in the fungi (two indicators), as opposed to the bacteria where 25 species were found outside of the warmest category, which contains the same species as in the wettest category in the rainfall indicator species analysis. Eight indicator species were found in the archaea, but because none of these was identified to species, and some were not even identified to Class, this makes drawing conclusions about these very difficult.

Table 13: Fungal indicators of soil temperature conditions in the month of sampling (February 2019). Because very few fungal indicators were found (possibly due to variation within groups), categories other than "Warmest" are absent. Significance is indicated with the symbols * where extremely significant =*** Very significant = ** and Significant = *

Average temp (February 2019)	Taxonomy	Correlation	*p*-Value	Species notes
Warmest	*Leohumicola minima*	0.60	0.009 **	Ericoid fungi of *Vaccinium* sp. (Baba & Hirose, 2020). The genus was originally described as a heat (burning) resistant species (Hambleton *et al.,* 2005)
	Syncephalis sp.	0.51	0.02 *	A mycoparasite infecting other fungi.

Table 14: Bacterial indicators of climatic conditions. Significance is indicated with the symbols * where extremely significant =*** Very significant = ** and Significant = *				
Average temp (February 2019)	Taxonomy	Correlation	*p*-value	Species notes
Coldest	*Occallatibacter sp.*	0.69	0.001 ***	An acidophile in the acidobacteria.
	Acidicaldus sp.	0.64	0.001 ***	Thermophilic acidophile (Johnson *et al.,* 2006)
	Granulicella sp.	0.54	0.001 ***	Acidophilic psychrotolerant bacteria isolated from *Sphagnum* peat bogs (Pankratov & Dedysh, 2010)
	Gemmata sp.	0.54	0.002 **	An aerobic acidophilic psychrotolerant species isolated from *Sphagnum* bog (Kulichevskaya *et al.,* 2017)
	Rhodoblastus sp.	0.51	0.001 ***	Peat bog acidophile (Imhoff, 2001)
	Acidibacter sp.	0.47	0.001 ***	Acidophile
	Methylocystis sp.	0.47	0.002 **	Methanotroph
	Singulisphaera sp.	0.43	0.011 *	Acidophile
	Coxiella sp.	0.42	0.003 **	Bacterial pathogen isolated from ticks (Voth & Heinzen 2007)
	Acidipila sp.	0.40	0.01 **	Acidophile
	Methylovirgula sp.	0.38	0.01 **	Acidophilic methylotroph
	Aquicella sp.	0.32	0.03 *	Probably a pathogen (Santos *et al.,* 2003)
	Chthoniobacter sp.	0.31	0.05 *	Verrumicrobia isolated from soil
Midpoint	Family *Enterobacteriaceae* unknown Genus	0.72	0.001 ***	Unidentified members of the *Enterobacteriaceae*, which has over 30 genera and over 100 species.
	Roseiarcus sp.	0.72	0.001 ***	*Roseiarcus sp.* are found in the rhizosphere of *Vaccinium sp.* (Morvan *et al.,* 2020)
	Paludibaculum sp.	0.60	0.001 ***	Anaerobic bacterium in the Acidobacteria capable of reducing iron (Kulichevskaya 2014)
	Candidatus Xiphinematobacter	0.59	0.001 ***	Associated with nematode hosts (Vandekerckhove *et al.,* 2015)
	Mucilaginibacter sp.	0.54	0.001 ***	A Sphingobacteria
	Smithella sp.	0.44	0.004 **	n-alkane degraders that associate with methanogens (Ji et al., 2020)
	Dehalogenimonas sp.	0.43	0.001 ***	
	GOUTA6	0.42	0.003 **	An uncultured species in the family *Nitrosomonadaceae*. All of them are lithoautotrophic ammonia oxidisers (Prosser *et al.,* 2014)
	Inquilinus sp.	0.38	0.02 *	

	Geobacter sp.	0.34	0.02 *	Anaerobic bacteria involved in oxidising organic compounds and metals
	Nitrospirillum sp.	0.34	0.05 *	Nitrogen fixing bacteria
	Class *Acidimicrobiia* unknown Order	0.33	0.03 *	
Warmest	Because the 'warmest' category only contains Exmoor, the species indicators here are the same as for the "Wettest" category (cf. Table 11) for the rainfall indicator species analysis, which also only contained species from Exmoor.			

Table 15: Archaeal indicators of climate conditions.

Average temp (February 2019)	Taxonomy	Correlation	*p*-value	Species notes
Midpoint	Class *Bathyarchaeia* unknown Order	0.60	0.01 **	
	Class *Bathyarchaeia* unknown Order	0.50	0.02 *	
	Family *Nitrososphaeraceae* unknown Genus	0.42	0.05 *	Ammonia oxidising archaea
Warmest	Group 1.1c	0.61	0.02 *	
	Group 1.1c	0.55	0.02 *	
	Phylum *Crenarchaeota* unknown Class	0.50	0.02 *	
	Group 1.1c	0.49	0.02 *	
	Class *Bathyarchaeia* unknown Order	0.26	0.05 *	

4.14 Heavy Metals pollution and microbial communities

On the basis of the above results where species were identified as Peak District specific indicators that were also heavy metal tolerant species, and on the basis of previous experiments finding high levels of heavy metals in Peak District peats (Linton *et al.,* 2007), all samples were run in an inductively coupled plasma mass spectrometer (ICP-MS; iCAP 7000 series ICP spectrometer, Thermo Scientific, Waltham, MA, USA) to ascertain their heavy metal content. The protocol was as per a PhD thesis by Morton (2016) and is described here in brief. Due to the constraints of coronavirus-related health and safety rules on lab occupancy, the laboratory work was undertaken by two technicians, Anthony Jones and Dr. Tom Holmes at the University of York Environment Department.

Samples of approximately 0.5 g were taken from the freeze-dried samples previously used in the microbial work, which were all sampled in February 2019. These were ground in a ball mill and placed in a Kjeldahl tube with 10 ml of 70% nitric acid (AnalaR NORMAPUR grade, VWR International LLC, Radnor, PA, USA). A glass marble was placed on top of each tube, with the tubes then being left overnight. Several blanks, consisting of only nitric acid, were included, and mean blank values were subtracted from measured sample element values.

The following morning the tubes were heated in increasing increments of 10°C until the heating block reached 60°C. Tubes were then left at 60°C for 3 hours then to 110°C using the incremental method. After a further 6 hours, the tubes were removed from the heating blocks and left to cool overnight. A small quantity (5-10 ml) of ultra-pure deionised water was added to each tube and the samples were separately filtered with ashless filter paper into a centrifuge tube. The marble, tube and filter paper were rinsed with ultra-pure water and then the samples were re-filtered through clean filter paper into volumetric flasks, which were then made up to 100 ml with ultra-pure water.

All samples were diluted by half to be run on the ICP-MS, with 10 ml of this sample mixed with 10 ml of ultra-pure water in clean sample tubes. Blanks and washes, where the equipment is washed to avoid contamination, both consisted of nitric acid, which was diluted to the same concentration of that in the samples, with two washes being run every 12 samples. The standards used were the Certipur ICP multi-element standard solution IV (Merck KGaA, Germany), with the addition of Trace-Cert Arsenic Standard for ICP (Sigma Aldrich LLC, USA).

Summary tables of metal values obtained, averages by site (where n = 3), management (where n = 9 – 12) and national location (n = 12 for all sites except Yorkshire where n = 36) are contained in tables 16, 17 and 18. Note that the Moor House samples were not included due to a lack of sample.

Table 16: Average (mean) chemical values per combination of site and management category. All values are in µg/g and are rounded to one decimal place. Metal variables are ordered left to right in decreasing mean abundance, with the most abundant metal on the left.

Site/Management	Iron	Calcium	Aluminium	Magnesium	Phosphorous	Sodium	Potassium	Lead	Zinc	Copper	Manganese	Arsenic	Cadmium
Exmoor 10 year restored	2893.7	839.5	2129.8	681.8	1582.8	305	650.5	178	43.2	29.3	30.8	8.2	2.8
Exmoor 5 year restored	6591.8	1063.6	1993.7	659.4	1682.5	351.6	703.7	61.6	28.9	64.9	13	12.8	3.1
Exmoor Degraded	4734.3	392.4	2295.8	484.4	1632.4	267	534.3	39.9	7.6	13.6	9.3	9.6	1.5
Exmoor Intact	1333.9	517.7	785.1	574.1	1003.5	360.7	265.6	102.5	27.3	24.5	5.2	8.8	2.8
Peak District 10 year restored	358.2	3521.6	386.9	1467.7	273.7	275.2	98.1	1.4	64.8	19.9	5.2	0	3.2
Peak District 5 year restored	682.9	1552.9	195.5	1793.8	225	281.9	90.2	0.3	34.1	14.8	12.6	0	2.9
Peak District Degraded	457	1825.5	399.6	1614.6	202.8	285.4	23.1	0.2	49.8	18.2	22.8	1.2	2.3
Peak District Intact	2163.3	1817.5	1604.3	559.4	412.6	289.7	95.3	513.6	68.8	53.4	6.5	10.4	5.3
Scotland 10 year restored	1231	1117.9	802	1225.6	446.8	460.3	219.1	13.6	11.2	16.5	3.9	0.3	2.7
Scotland 5 year restored	1017.4	3820.7	273.2	1997.5	572.6	542.7	194.6	31.7	18.1	19.4	7.7	1.8	3.1
Scotland Degraded	592	976	690.6	1008.6	629.6	431.5	368.2	7.3	15	17.2	2.7	3.8	2
Scotland Intact	1325.5	1206.6	916.5	1178.6	596.2	420	396.2	22	16.4	12.8	3.7	0.5	1.8
Mossdale Burnt	1929.8	1416.7	666.5	762.2	1107.7	304.3	249	331.6	50.1	44.5	18.3	3.1	4.5
Mossdale Mown	2619.4	1482.6	810.6	980.5	761.1	398.6	317	353	87.8	41.1	11.8	2.8	5.8
Mossdale Uncut	2964.8	1748.2	884.8	746.3	1102.2	271.6	361.5	247.7	46.6	33.6	8.8	6.3	3.5
Nidderdale Burnt	1655.6	1136.6	1379.7	784.4	718.2	243.8	259.9	245.2	71.1	31.1	14.2	2.3	2.8
Nidderdale Mown	1674	1274.1	1150.4	797.3	955.8	302.9	395.4	376.2	74.2	44.3	15.7	6	3.8
Nidderdale Uncut	2060.4	1173.5	1117	617.4	1067.5	216.2	354.2	286.1	52.4	64.1	10.6	7.9	2.5
Whitendale Burnt	1901.9	995	1383.1	782.1	667.6	231.5	230.4	613	70	45.4	10.9	4.2	3.3
Whitendale Mown	1277	1072.2	1356.3	765.8	624.3	266	182.9	364.1	57.7	53	13.1	4.7	3.2
Whitendale Uncut	2095.8	1015.3	1571.8	781.4	722.5	250.6	218.4	548.8	60.3	58.9	8.9	9	2.8

Table 17: Average (median) chemical values per management category. All values are in µg/g and are rounded to one decimal place. All Yorkshire sites (Burnt, Mown, Uncut) are n = 12, whilst national sites (Intact, 10 year and 5 year post, and Degraded) have n = 9. Columns are not ordered.

Management	Copper	Zinc	Lead	Aluminium	Potassium	Magnesium	Calcium	Iron	Arsenic	Sodium	Manganese	Cadmium	Phosphorous
Intact	15.16	27.63	102.53	886.22	265.57	652.74	985.57	1362.82	4.05	355.01	3.75	2.67	637.42
10Y post restored	22.95	29.36	13.84	481.75	238.37	1162.93	1258.1	1047.35	0	295.11	5.88	2.68	452.65
5R post restored	21.51	24.68	25.81	282.38	190.21	1627.81	1399.24	1159.98	0	338.11	8.52	2.59	568.26
Degraded	16.06	11.68	7.34	690.64	318.78	1008.63	975.95	619.94	4.89	294.47	7.79	1.61	629.58
Burnt	33.59	66.97	362.76	1031.19	190.78	752.59	1225.09	1417.47	2.55	249.71	11.62	3.24	798.9
Mown	38.79	72.32	317.27	1075.15	303.12	841.4	1320.7	1415.37	2.07	287.46	13.92	3.76	772.28
Uncut	43.59	58.84	293.04	1141.14	347.23	762.04	1261.28	1697.8	9.23	252.28	8.35	2.92	962.02

Table 18: Average (median) chemical values per national site location. All values are in µg/g and are rounded to one decimal place. All national sites have n= 12, except for Yorkshire sites which have n = 36. Columns are ordered the same as Table 16.

National Location	Copper	Zinc	Lead	Aluminium	Potassium	Magnesium	Calcium	Iron	Arsenic	Sodium	Manganese	Cadmium	Phosphorous
Exmoor	24.71	28.85	69.29	1937.55	660.60	601.0	800.79	3360.79	7.33	302.2	10.97	2.06	1442.82
Peak District	18.80	39.32	0.73	391.5	55.87	1424.63	1770.03	522.71	0	284.38	7.11	3.12	252.17
Scotland	14.17	13.55	13.84	481.75	306.08	1475.88	1258.1	1047.35	0	473.014	3.71	2.01	568.26
Yorkshire	36.66	62.85	320.65	1124.28	247.98	764.12	1248.89	1477.49	4.56	260.57	11.41	3.2	816.83

Kruskal-Wallis tests were employed to test for differences in the means between national locations and the results of these, with notes on *post-hoc* Dunn's tests, are in Table 19. Frequently, the Yorkshire grouse moor locations have the highest levels of metals, including copper, zinc, lead, and cadmium, although it is not established that this can be attributed to management, and the fact they are grouse moors, rather than some difference in historical or geographic context.

Table 19: Results of Kruskal-Wallis tests on the differences between mean values across national categories, with notes on the results of *post-hoc* Dunn's tests.

Metal	X^2	*p*-value	*Post-hoc* Dunn's test notes
Copper	30.07	< 0.00001	**Yorkshire** sites higher than all other sites, other sites no difference
Zinc	31.37	< 0.00001	**Yorkshire** sites higher than Exmoor and Scotland but not Peak District, Peak District higher than Scotland
Lead	40.59	< 0.00001	**Yorkshire** sites higher than all other sites, other sites no difference
Aluminium	26.26	< 0.00001	**Yorkshire** sites different from all other sites, but lower than Exmoor. Exmoor different to Peak District and Scotland.
Calcium	22.29	< 0.00006	**Yorkshire** sites higher values than Exmoor and lower than the Peak District, but no different to Scotland.
Potassium	30.92	< 0.00001	All sites different from one another, except Scotland and Yorkshire.
Magnesium	26.218	< 0.00001	All sites different apart from Scotland and the Peak District
Iron	28.83	< 0.00001	All sites different apart from Scotland and the Peak District
Arsenic	14.99	< 0.003	**Exmoor** different to the Peak District and Scotland (much higher). No difference in other sites.
Sodium	25.127	< 0.00002	**Scotland** significantly higher than other sites, no difference in other sites.
Manganese	20.51	< 0.0002	**Scotland** lowest values and lower than Exmoor and Yorkshire but not the Peak District, no difference among other sites.
Cadmium	11.16	< 0.011	**Yorkshire** significantly higher than Exmoor and Scotland, no differences among other sites.
Phosphorous	45.45	< 0.00001	All sites different apart from Scotland and the Peak District

4.15 db-RDA with heavy metals integration

With new heavy metal data, all 13 metal variables were integrated into the microbial metadata and forward selection of explanatory variables for dbRDA was recalculated. The fungal dbRDA required the subtraction of samples MH1, MH2, MH3, S5Y3, EI2, MM3, and SD1 due to missing samples. The changes to the model, including added heavy metals variables (Table 16), resulted in a slight decrease in the explanatory power of the dbRDA (dbRDA: df, 9(59), F = 2.41, $p < 0.001$, constrained variables account for 27.9% of variance). The bacterial model, where MH1, MH2, MH3, S5Y3, EI2 and SD1 were removed, was improved with the addition of chemical variables (dbRDA: df, 13(55), F = 4.59, $p < 0.001$, constrained variables account for 52.7% of variance). The archaeal model (dbRDA: df, 7(55), F = 6.96, $p < 0.001$, constrained variables account for 52.1% of variance) also offered an improvement. Updated dbRDA biplots are displayed in Figure 4. The Figure 4 dbRDA plots show distinct microbial communities between national sites, but microbial communities in Yorkshire grouse moors being split by environmental variables. In the fungi, this is based on *Calluna* abundance, soil moisture and zinc, where communities change as the peat becomes drier, higher in zinc concentration, and higher in *Calluna* abundance.

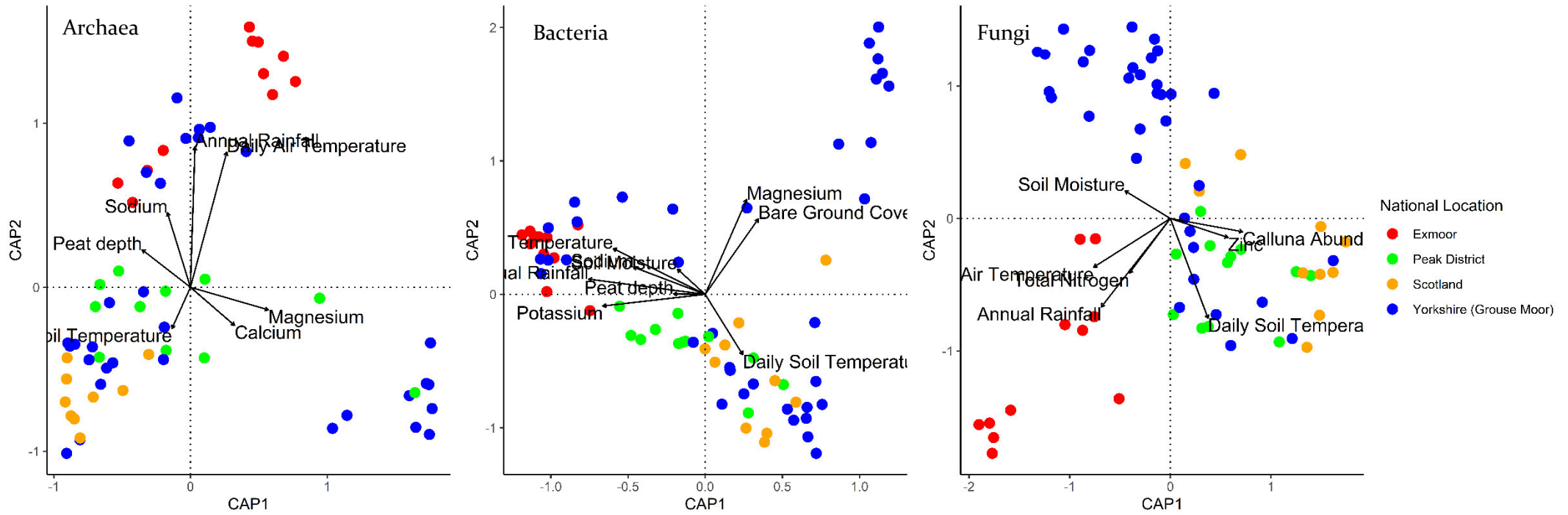

Figure 4: dbRDA biplots from Figures 1, 2 and 3, where the model was updated by adding in heavy metals data. Sites are coloured by their national location as per the legend.

4.16 Discussion

The climatic envelope that a given blanket bog is located in is clearly a key potential driver of its microbial community. In this chapter, the dbRDA models from Chapter 3 were improved using forward selection, and found that climate variables are indeed key drivers of all three microbial communities. Whilst this chapter focused on climatic aspects (in addition to some peat chemical properties) the next chapter will explore the question of how that microbial community affects its function in terms of water quality and carbon cycling.

Climate drivers of microbial communities

For the fungi, temperature and rainfall were found to be key drivers of community differences. Soil moisture, which is presumed to be a function of rainfall but also a function of vegetation (and its evapotranspiration and root carbon inputs) and litter deposition (being highly correlated with soil bulk density and thus water holding capacity), is also a significant driver. The coverage of *Calluna vulgaris* was also shown to be a significant driver of the fungal community, and is potentially a cause of the grouse moor/non-grouse moor split in communities found in Chapter 3. The sites not managed as grouse moors in this study had an average (mean) *Calluna* coverage of 6.6%, whereas on the land managed for grouse it was 34.5%. The bacteria also varied strongly with climatic variables, but also with the cover of bare ground, soil moisture (Table 6, Figure 2, and Figure 4). Bare ground means a lack of vegetation, is probably linked to a lack of root carbon and litter inputs for decomposition, and was identified as a clear driver of separation in the dbRDA (Figure 2). The archaea varied with climate (Table 7), but were also strongly affected by pH, *Sphagnum* abundance, and available soil nitrogen (Figure 3), which is possibly reflective of changes in the archaeal *Nitrosphaerae*.

There are weaknesses in this approach – the inclusion of climatic variables, some of which are auto-correlated, could be obscuring other variables that may also affect the microbial/fungal community at a local level, where our sample size (three samples per site) had a low statistical power (See Chapter 3). Nevertheless, the Yorkshire plots, with 12 samples per treatment, across three sites (within a paired catchment design), allowed an examination of the effect of microbial communities on carbon cycling and water quality, which was undertaken and is elaborated on in Chapter 5.

Indicator Species Analysis does not generally match specific species to climates

An indicator species analysis that examined whether specific species are representative of a given climatic category was conducted. Categorisation of the climatic condition was difficult, with some categories (e.g. “wettest”) having only a single site leading to obfuscation: are the species indicators of climate type or simply species that are confined to a specific site?

For temperature, there are some obvious answers: that known psychrotolerant species are generally more abundant in the colder sites is obvious, as is ericoid fungi in the more *Calluna* (ericoid mycorrhizal) dominated sites. However, the rainfall based indicator species analysis, having only Bleaklow in its driest site revealed a selection of site specific microbial species that are adapted to polluted environments, including acidophilic extremophiles and those with extremophile metabolisms (such as sulphate reducers and those that metabolize arsenic) as

well as pollution tolerant species isolated from mine tailings. This is not surprising: this area has a long history of heavy metal pollution (Linton *et al.,* 2007). The degree to which our samples were contaminated with heavy metals was investigated with further ICP-MS sampling.

Overall, however, it is more likely that species identified here as being climate related are also related to some other management, environmental or edaphic factor. Answering this question, using climatically diverse sites with the same management and general environmental conditions, would potentially confirm whether climatic variation does result in specific microbial community indicators, or whether indicators are more likely to be associated with specific environmental conditions. This further work would prove fruitful for the use of microbial measures as indicators of habitat condition.

Heavy metals and other elements in UK blanket bogs

High levels of all metals are ubiquitous in many blanket bogs around the UK. Peatlands effectively retain heavy metals because of biogeochemical processes (Martinez *et al.,* 2002) and ombrotrophic peat deposits have been shown to accumulate arsenic, cadmium, mercury, lead, sulfur and zinc (Yoon *et al.,* 2012). Additionally, many blanket bog sites in the UK are in proximity to sources of deposition, such as the industrial revolution-era conurbations of Manchester or Leeds in the case of the Peak District and Yorkshire sites. The Exmoor site was subject to mineral prospecting and smelting, and the Wheal Eliza mine is situated within 300m of the site.

Consequently, the high levels observed even in the "intact" sites of this study should not be surprising. Indeed, the presence of some metals otherwise considered to be pollutants is not always negative, and has been shown in synergy with other factors to confer some benefits, for example, to *Sphagnum* (Baxter *et al.,* 1989). However, high levels of metals such as aluminium and heavy metals such as zinc, lead and cadmium are generally considered to exert a negative effect (Smith *et al.,* 2004, Linton *et al.,* 2007).

The complex biogeochemical relationships between metals, and between metals and microbial communities, are beyond the scope of this thesis, but some conclusions and interesting observations can be drawn from the results of the ICP-MS analysis, especially regarding metals concentration and their relationships to other site characteristics and environmental variables.

Potential influences of restoration on soil metals concentration

One of the sites in the study, Bleaklow (Peak District), represented by the P5Y and PI samples, was sampled by Linton *et al.* in their 2007 study on heavy metals concentration in ombrotrophic peats. This presents a unique opportunity for comparing restoration effects on metal concentrations, because the Linton study was undertaken on bare peat before restoration activities on Bleaklow occurred. The soil in this study was not sampled in the same location, but the sampling points of P5Y are only 127 m away from the GPS co-ordinates given in the Linton study.

Table 20: Comparison table of values from the Linton *et al.* 2007 study (Bleaklow pre-restored) and values from this thesis sampled in 2019. Bleaklow 5 year post restored is ~127 m from the site of the Linton study, Bleaklow intact is a hydrologically intact area several hundred metres to the south. DOC values may differ because of the methods used: Linton *et al.* used 20 g of soil in 20 ml of water, but this study used 3 g of soil in 27 ml of water because of sampling constraints (this is discussed in the Linton study). All measures were rounded to two decimal places.

Site	Bleaklow pre-restored 2007	Bleaklow 5 year post restored 2019	Bleaklow intact 2019
pH	3.14	4.47	4.25
DOC (mg/l^{-1})	171	13.23	16.31
Copper ($\mu g\ g^{-1}$)	89.1	19.5	50.9
Zinc ($\mu g\ g^{-1}$)	61.9	31.2	62.5
Cadmium ($\mu g\ g^{-1}$)	0.82	3.7	6.6
Lead ($\mu g\ g^{-1}$)	1066	0.6	458.9
Calcium ($mg\ g^{-1}$)	0.77	1.99	2.02
Magnesium ($mg\ g^{-1}$)	0.34	2.72	0.89
Sodium ($mg\ g^{-1}$)	0.12	0.4	0.45
Potassium ($mg\ g^{-1}$)	0.12	0.13	0.13
Aluminium ($mg\ g^{-1}$)	2.21	0.32	1.46
Iron ($mg\ g^{-1}$)	8.02	0.8	1.83
Manganese ($\mu g\ g^{-1}$)	10.5	19.4	4.3

The comparison between measured metal values is in Table 20. The pH is much higher in both the post-restored samples and the original (unmanaged) intact samples, from 2019, which is presumed to be a consequence of the lime fertiliser applied by Moors for the Future as part of the restoration (revegetation) work. However, this has likely "normalised" the pH, which is now similar to other sites sampled in this thesis, rather than the highly acidic values of 3.14 measured by Linton *et al.* in 2007 (i.e. on bare and eroding peat). Notably, pH is important in determining the partitioning between particulate related metals and peat pore water dissolved metals, which are more likely to end up being exported fluvially.

The values of lead, copper, aluminium and iron have all decreased over time in the hydrologically intact site, but they have also been reduced to lower levels in the post-restored sites. In the case of lead, in the restored site (and almost in the intact) values have now fallen below the (now withdrawn by the Environment Agency) Soil Guideline Value for protecting human health, which was set at 450 $\mu g\ g^{-1}$ (YAHPAC, 2015). Cadmium however was exceeding the same guidelines (1 $\mu g\ g^{-1}$) in 2007 and in both intact and restored sites has accumulated to higher values in 2019.

Generally, chemical species vital for ecological growth, such as calcium, magnesium, and manganese have increased in both restored and intact sites, sometimes more so in the restored sites, which is probably a consequence of the lime and fertiliser treatment undertaken by Moors for the Future. Potassium did not change, either between managements or over time, possibly remaining stable and being underutilised by vegetation due to overall nitrogen and phosphorous limitation in blanket bogs (Table 15).

This study showed low levels of sample replication: Bleaklow 5-year post restored and intact plots, as well as the samples from Linton *et al.* consisted of three samples. In addition, comparisons might be difficult due to a difference in sampling depth; all samples in this thesis were sampled at 15 cm depth whereas the Linton study sampled at 8-10 cm depth where lead levels peak at 5-15 cm depth in the South Pennines (Rothwell *et al.,* 2007). Because of these limitations, there could not be a causal link established between restoration with a lowering of heavy metal concentrations and an increase in vegetation beneficial nutrients, because these reductions may have occurred over time in congruence with a reduction in pollution deposition. It is possible that the reductions in pollutants appear more drastic in the restored bog because this land was bare, and contaminated soil may have eroded away. However, it is also possible that the reintroduction of vegetation to these areas, and the application of lime and fertiliser, has resulted in beneficial reductions in these heavy metals by other means, perhaps principally by changing the pH (e.g. Liang *et al.,* 2017), and this is worthy of further investigation.

Influence of metals on microbial communities

Some metals were found to be of significant influence on community dissimilarity when they were added to the forward selection of distance based redundancy analyses. These were zinc and iron for the fungi, zinc, magnesium and potassium for the bacteria, and calcium, magnesium and sodium for the archaea.

Generally, the abundance of *Calluna vulgaris* increases with the total amount of zinc, and both of these variables increased with decreasing soil moisture, and this exerted an effect on the taxonomic composition of the fungi. This is potentially because, whilst zinc can be phytotoxic in high concentrations, ericoid mycorrhiza are known to confer a major degree of resistance to zinc and copper toxicity (Bradley *et al.,* 1981), possibly by concentrating such metals in the mycorrhiza, thus preventing them from accumulating in the plant. Therefore, it should not be surprising that these variables control fungal community variation, as well as those climatic variables that account for variation in soil moisture. All of the sites fall on this axis (Figure 4) apart from a separated cluster consisting of the Exmoor sites, distinguished by their lack of ericaceous (e.g. heather) vegetation and *Molinia* dominance, alongside nutrient enrichment, specifically of iron and total nitrogen. The iron levels in Exmoor are more than twice as high as the next highest site category (Table 17: Exmoor median 4801 μg g^{-1} versus second highest Yorkshire sites median of 2110 μg g^{-1}). Iron may exacerbate carbon decomposition via precipitation of decomposition inhibiting phenolic compounds, potentially removing the inhibitory effect on microbial activity (Wang *et al.,* 2019), and this might explain why Exmoor generally has the highest levels of pore water dissolved organic carbon (DOC) in this study.

4.17 Conclusion

Here, we have demonstrated that blanket bog microbial communities across the UK are structured by climate variables and their effect on edaphic properties, as well as regional variations in levels of heavy metals pollution. In some sites such as the Peak District, this has led to an adapted microbial community that contains specific species that are tolerant of these pollutants, and these species are not present anywhere else in the same abundances.

Indicator species analysis did not reliably find taxa that differ under environmental conditions; whilst some species are clearly cold tolerant, and some are pollution tolerant, these species all appeared elsewhere. The amount of rain a site receives does not appear to exert overall taxonomic changes, and it is likely that rain is a driver of communities only insofar as it exerts a control on the blanket bog water table seasonally.

It is clear that on a national scale climate is a key driver of the microbial community, which is in turn (as we have seen in the previous chapter), affected by a range of local historical (management) and environmental (peat chemical) conditions. In the next chapter, the effects these microbial community changes have on the water quality and carbon cycling of peatlands are investigate

Chapter 5: Effects of management and microbial community changes on water quality and carbon sequestration.

In the previous chapters, the effects of managements, climate, and environmental parameters on the microbial community were examined. This chapter assesses how that microbial community is related to ecosystem services such as water quality and carbon sequestration.

5.1 Water quality in British blanket bogs

UK upland blanket bogs are principally located in high rainfall and low temperature locations and consequently they comprise a large percentage of the catchments from which we derive our drinking water, especially in the north of England, Wales, and Scotland (Xu *et al.,* 2018). Because of this, water companies especially are interested in the quality of this drinking water and how catchment management may affect it, especially where catchment processes result in downstream capital costs due to water treatment needs (Martin-Ortega *et al.,* 2014). For this study, water quality parameters including DOC, SUVA, and Hazen were measured, which are explained below. Specific methods for obtaining the DOC, SUVA and Hazen values are described in Chapter 2 section 2.8.

DOC

Dissolved organic carbon (DOC) is a measure of the amount of organic carbon contained in water that then flows off the bog (although the soil water DOC is what was measured in this thesis). This is a conduit for carbon (C) loss in peatlands – especially where degradation and associated de-vegetation results in bare surface erosion, such as the degraded Peak District sites in this thesis. This C loss via drainage is an important component in the C balance of peatland ecosystems (Billet *et al.,* 2004) both in terms of organic C carried in the water but also subsequent outgassing from stream surfaces. DOC is a problem for water companies who process this water for eventual drinking: it can promote biofilm formation, act as a complexing agent for micro-pollutants, form disinfection by-products and give the water an undesirable taste and colour (Vik & Eikebrokk, 1988). The formation of disinfection by-products is a significant issue – some trihalomethanes are carcinogens and need to be removed from drinking water, with associated costs (Bond *et al.,* 2014). DOC is usually defined as the fraction which can pass through a specific size of filter, the remaining larger size being referred to as particulate organic carbon, POC. In this study, the filter size was 0.6 micrometres.

SUVA

SUVA refers to the specific UV absorbance of the water, normalised by DOC content. It is obtained by dividing the UV absorbance of a sample at 254 nm by the DOC (mg/L), and is expressed in units of L/mg-m. SUVA is essentially a measure of the reactivity of DOC in relation to its aromaticity. It is consequently typically performed to assess the formation potential of disinfection by-products. A high SUVA value indicates a large proportion of humic matter is present, which is easily removable by coagulants but has a great potential for disinfection by-product formation (a study evaluating SUVA, from Weishaar *et al.,* 2003, introduces its use and utility); low SUVA (about <4) will indicate higher proportion of hydrophilic, uncharged and no light absorbing DOC with small molecular weight, which is not well removed by coagulants (*pers. comm.* Jenny Banks, Yorkshire Water). Some studies have found an association between specific microbial fatty acids and increased SUVA in wetlands

(Bossio *et al.,* 2006) and SUVA has been associated with microbial decomposition in stream sediments (Straus & Lamberti, 2002) but the effect of the microbial community on SUVA export in British blanket bogs is still unclear. In an experimental warming treatment, no change to SUVA was found despite a change in microbial structure (Delarue *et al.,* 2015), but another study did find changes in the bacterial community associated with changes in SUVA, although this was a comparison between bog and fen (Lin *et al.,* 2012).

Hazen

Hazen is a measure of water colour, measured in Au/m^{-1}, obtained by multiplying the absorbance of the water at 400 nm by 12 (Watts *et al.,* 2001 as used by Heinemeyer *et al.,* 2019). Water colour is mainly an aesthetic issue for water treatment companies – especially in peat catchments where humic substances and tannins may make drinking water very dark brown, and the colour may make some industrial processes difficult (Pattinson *et al.,* 1994). Some studies have associated peatland restoration with increasing water colour, but this was in afforested catchments (none of which were studied in this thesis) and was presumed to be due to nutrient leaching, and this is difficult to link to microbial effects (Shah *et al.,* 2019).

5.2 DOC, SUVA and water colour across the study sites

DOC

DOC varied significantly across different national locations (Kruskal-Wallis X^2= 28.82, df=3, p < 0.0003) but did not vary between management conditions (Kruskal-Wallis X^2=5.84, df=6, p = 0.44). DOC did not vary between managements on a per-national site basis, except on Exmoor (Kruskal-Wallis, X^2 = 7.92, df =3, p = 0.048) but on a per-national site level, it is worth noting that each management condition had three samples, with consequently poor statistical power. The three Yorkshire locations did not differ in their DOC values (Kruskal-Wallis, X^2= 0.42, df = 2, p = 0.81).

Table 5.1: Table of summarised DOC values across national site locations. Measurements given are in mg/L

National Location	Mean	Standard Deviation	Median	Max	Min	Range
Exmoor	26.57	6.92	26.24	39.65	16.02	23.63
Peak District	13.21	2.94	13.02	19.7	9.21	10.48
Scotland	24.91	8.22	23.62	40.41	14.19	26.22
Yorkshire	19.66	5.71	18.38	34.29	12.15	22.14

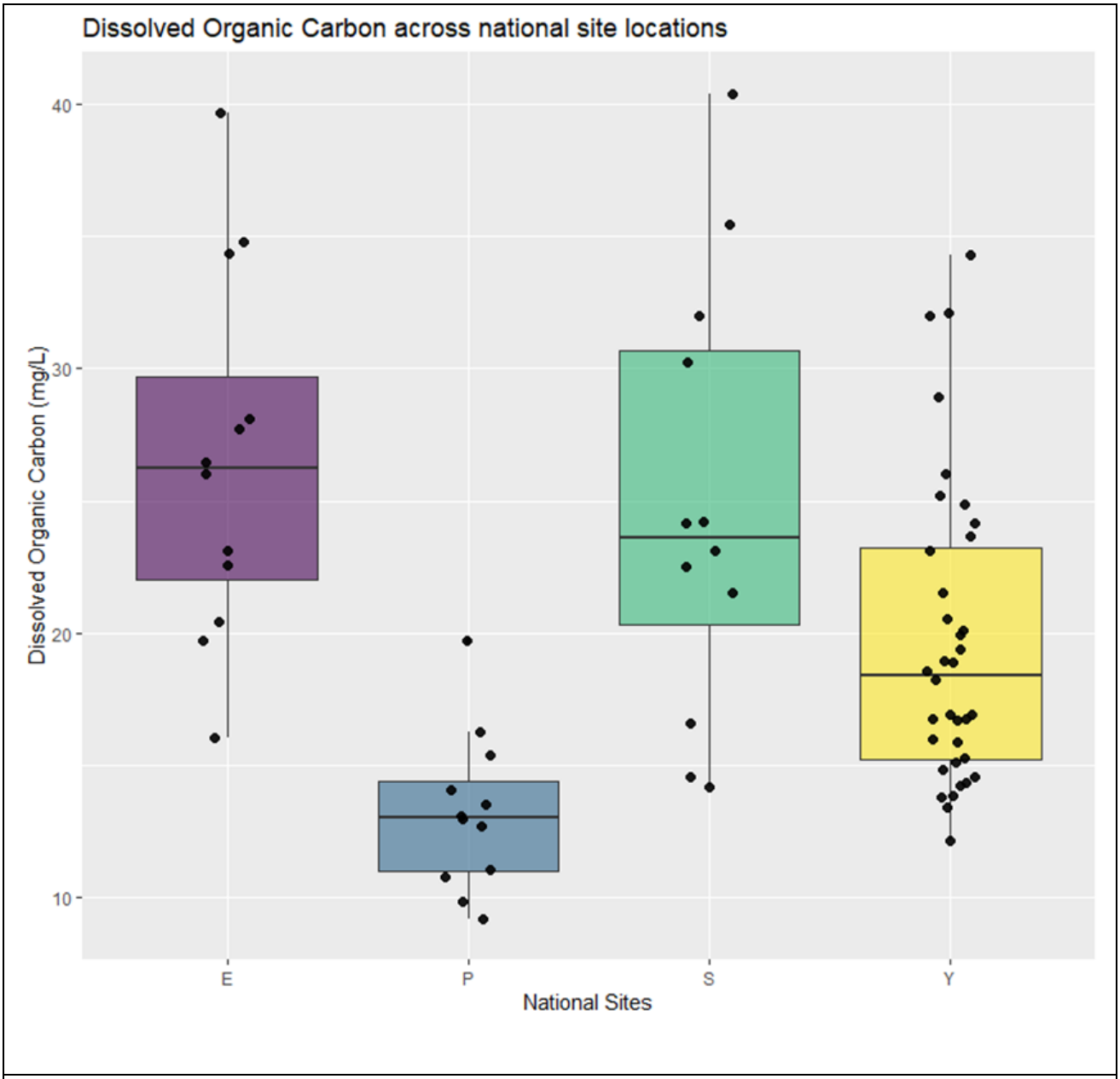

Figure 5.1: DOC values in mg/L across four national site categories: E (Exmoor), P (Peak District), S (Scotland) and Y (Yorkshire Peatland-ES-UK sites).

SUVA

SUVA was highly variable across national sites and showed significant differences (Kruskal-Wallis, $X^2 = 46.11$, df = 3, $p < 0.00000001$); in a post-hoc Dunns test, all national sites differed significantly in their SUVA values except for Scotland and the Peak District. SUVA did not differ between managements (Kruskal-Wallis, $X^2 = 7.95$, df = 6, p = 0.24) on a national scale, nor did it differ on a per-site comparison of management categories. For summary values for SUVA across national sites, see Table 2. SUVA values did differ between the three Yorkshire sites; Whitendale, Nidderdale and Mossdale (Kruskal-Wallis, $X^2 = 14.15$, df = 2, $p < 0.001$), where Mossdale had the lowest values (mean of 1.5 with a standard deviation of ± 0.75) versus the higher values of Nidderdale (2.1 ± 0.83) and Whitendale (2.32 ± 0.67).

Table 5.2: Table of summarised SUVA values across national site locations. Measurements given are in L/mg-m						
National Location	Mean	Standard Deviation	Median	Max	Min	Range
Exmoor	1.45	0.14	1.44	1.71	1.23	0.47
Peak District	3.9	0.79	3.73	5.57	2.84	2.73
Scotland	12.31	3.97	13.59	18.0	2.73	15.27
Yorkshire	2.18	0.89	1.97	4.04	0.5	3.54

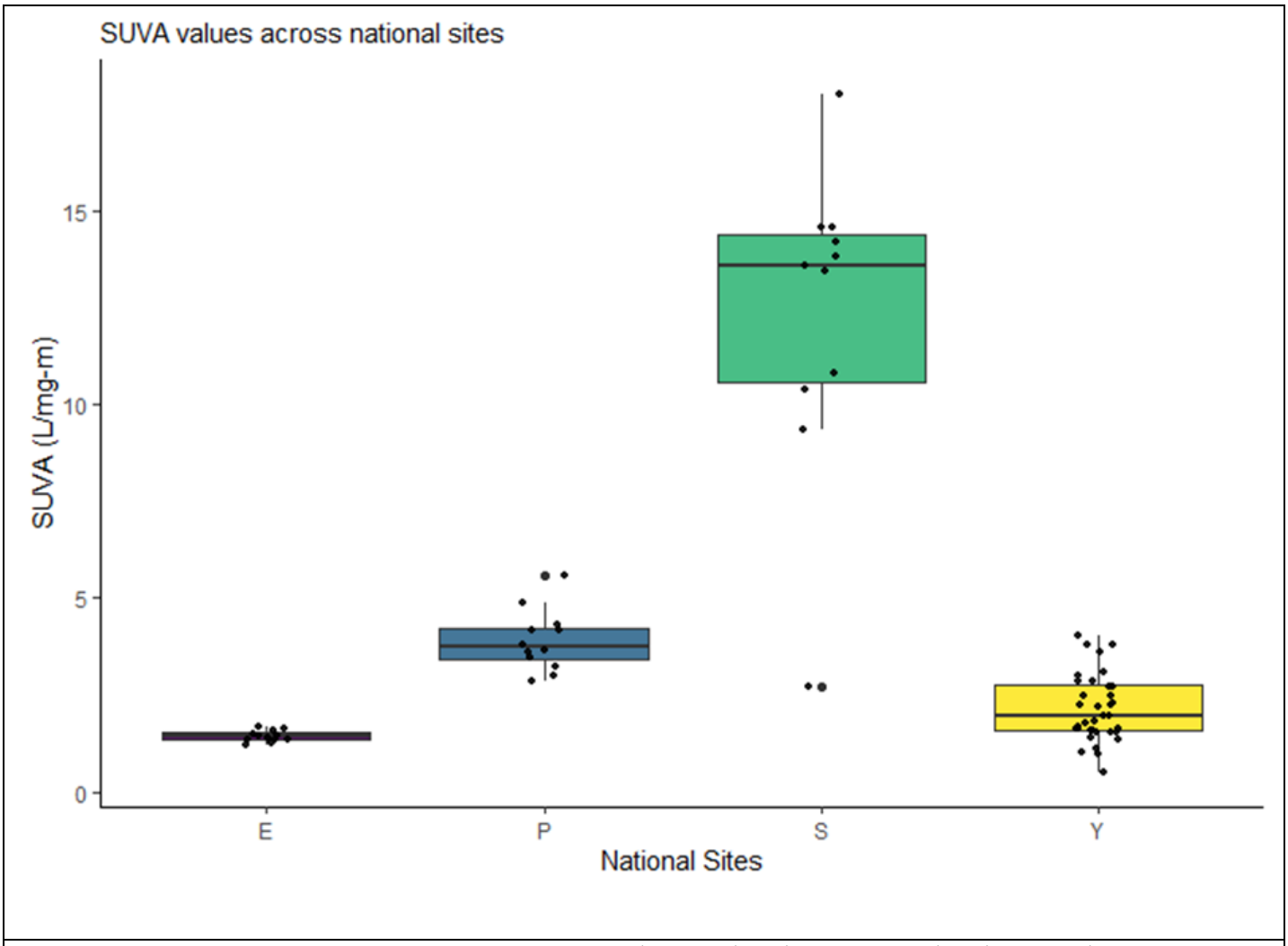

Figure 5.2: SUVA values across national sites where: E (Exmoor), P (Peak District), S (Scotland) and Y (Yorkshire Peatland-ES-UK sites).

Hazen

Two samples, Exmoor Intact 1 and Exmoor Intact 2, were removed from the analysis because they had anomalous Hazen (water colour) values (-162 and 588 respectively). Hazen was significantly different between national sites (Kruskal-Wallis, X^2= 9.95, df = 3, p < 0.02) but only between Exmoor and the Peak District (p < 0.03). Hazen did not differ significantly between management categories on either national (Kruskal-Wallis, X^2= 3.64, df = 6, p = 0.73) or local level (Figure 3). Hazen did significantly differ between the Yorkshire Peatland-ES-UK sites (Kruskal-Wallis, X^2= 7.42, df = 2, p < 0.03) where Mossdale has the lowest Hazen levels (mean of 104 with a standard deviation of ± 53) compared to Nidderdale (138 ± 35) and Whitendale (119 ± 37). Summary values for national sites are located in Table 3.

Table 5.3: Table of summarised Hazen values across national site locations. Measurements given are in Au/m^{-1}.

National Location	Mean	Standard Deviation	Median	Max	Min	Range
Exmoor	92	16	89	118	74	43
Peak District	136	35	131	192	85	107
Scotland	098	21	104	128	58	71
Yorkshire	120	44	112	228	64	164

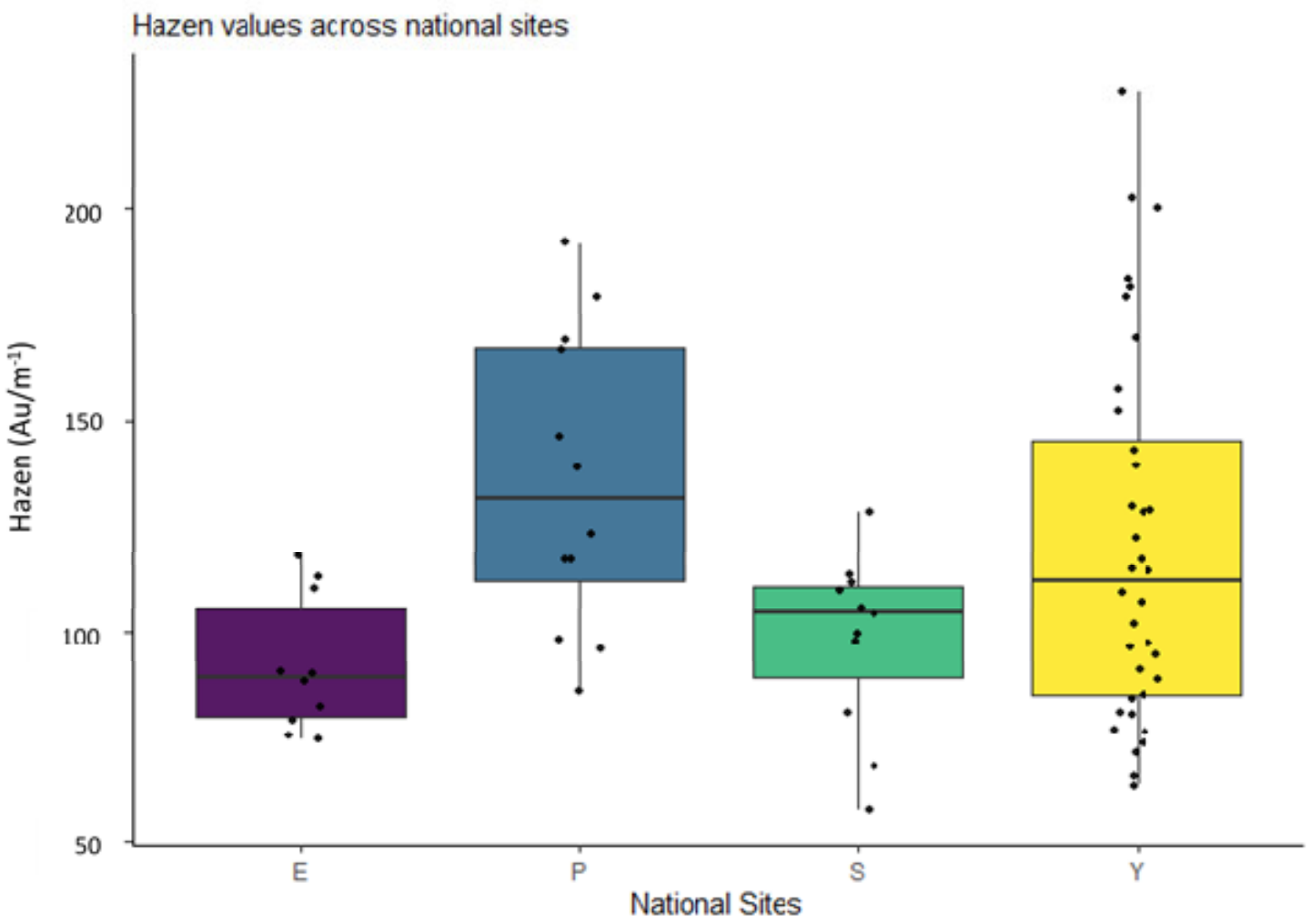

Figure 5.3: Hazen values, in Au/m^{-1} by national site locations, where E = Exmoor, P = Peak District, S = Scotland and Y = Yorkshire.

5.3 The relationship between environmental and water quality variables

As a preliminary step in identifying environmental factors that may be driving water quality parameters, environmental variables were centred and z-scored before creating and plotting a correlation matrix using the R package "corrplot" (Wei & Simko, 2017). A list of significantly correlating variables for DOC, SUVA and Hazen are listed in Table 4.

Table 5.4: lists of environmental variables correlated (with significance p<0.05) with measures of water quality.	
Water quality	List of correlating variables
DOC	Longitude, elevation, pH, covers of *Sphagnum*, *Molinia*, and bare ground, annual rainfall, air temperature (day of collection), monthly rainfall, monthly soil temperature (February 2019) and monthly air temperature (February 2019)
SUVA	Latitude, longitude, slope, elevation, peat depth, pH, the covers of sedge and *Molinia*, total extracted DNA, soil temperature (day of collection) and monthly rainfall (February 2019)
Hazen	Longitude, elevation, peat depth, pH, *Molinia* cover, soil moisture, annual rainfall, air temperature (day of collection), and monthly (February 2019) measures for air and soil temperature and rainfall.

5.4 Dissolved Organic Carbon (DOC)

In order to assess the impact of environmental parameters on DOC, we used a general linear model (GLM) with a Gaussian distribution, on the basis that the water quality variables were continuous and unbounded and approaching a normal distribution.

To deal with issues of multi-collinearity, variables were removed from the dataset based on their hypothesised relevance to water quality variables and their relationship to other data. Latitude and longitude were both removed from the analysis, on the basis that these are measures of climate, which are better represented by the actual climate variables, as was elevation for the same reason. Nitrogen was removed because of its very close correlation with DOC and consequently the other water quality variables. All water quality variables except the one being assessed were removed for each analysis: In the case of DOC, this was SUVA, Hazen, UV254 (Ultraviolet absorption at 254 nm), UV400, UV465 and UV665. Annual rainfall and monthly soil and air temperatures were removed in favour of monthly rainfall and daily soil and air temperatures, because these are more likely to have effects on the snapshot sample of soil water DOC, SUVA and Hazen. The list of environmental variables assessed in the GLM is outlined below.

For DOC, the following variables were used in the initial model: slope, aspect, peat depth, pH, *Calluna,* other moss, Sedge, *Sphagnum, Molinia,* bare ground, soil moisture, total extracted DNA, bulk density, day of collection air temperature, day of collection soil temperature, month of collection rainfall totals.

Further variables of interest were narrowed down by plotting variables against water quality parameters and removing variables that had no discernible relationship with DOC. These variables are plotted in Figure 4: the removed variables include aspect, peat depth, total extracted DNA, and sedge abundance.

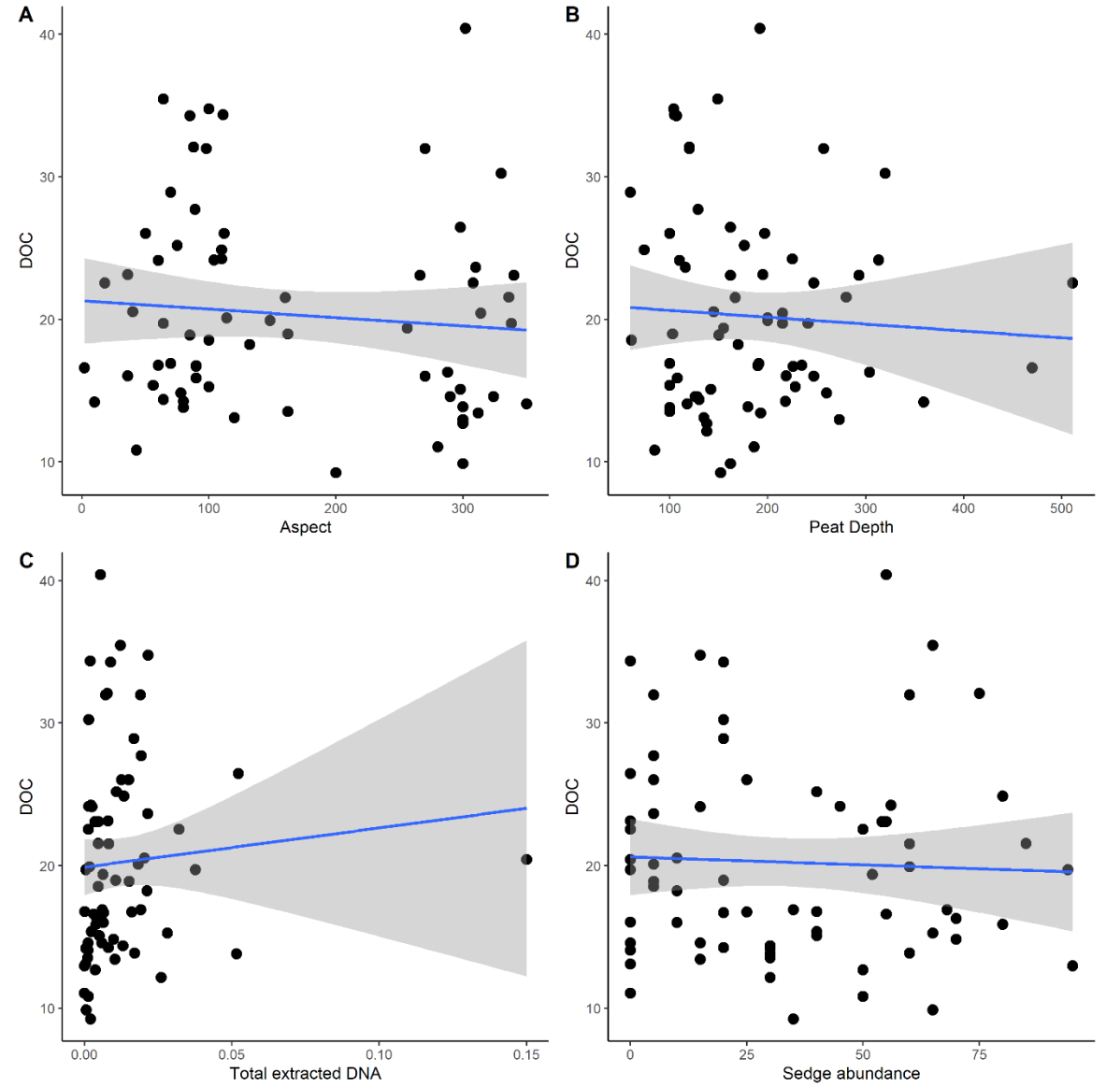

Figure 5.4: DOC plotted against centred and Z-scored variables where A is the aspect, B is peat depth, C is the total extracted DNA and D is sedge abundance.

This model was assessed for multi-collinearity by obtaining Variance Inflation Factors (VIF) using the "car" R package (Fox & Weisberg, 2019). The VIF values obtained indicated a very high incidence of multi-collinearity, especially among the vegetation values but also in the weather values. Sequentially, high VIF value variables were removed from the model until the VIF for all variables was less than five. This resulted in the further removal of *Molinia* cover, air temperature, and soil temperature.

A model was then obtained containing ten variables: slope, pH, *Calluna*, other moss, *Sphagnum*, bare ground, soil moisture, bulk density and monthly rainfall (month of collection). None of these had high VIF values and the model was used.

To test this model, the data were randomly partitioned, where 80% was used for training the model and 20% was retained for testing. Because of the low sample count, this random sub-setting might have resulted in changes to the data structure that may have over-fitted the model, and therefore this random partition (and consequent model training and testing) was repeated ten times. In each of these ten repeats, the model remained unchanged, leaving us confident that the model was correct as applied across all data. In the model containing ten variables above, only two variables were significant predictors of DOC: soil moisture and pH. This was tested by comparing the model trained on the training data against the testing data, and had a Root Mean Square Error (RMSE) of 5.86415 and an R^2 of 0.434.

In an attempt to improve the model, the least performing variables (those with low estimate values, non-significant P value and t values closest to zero) were sequentially removed in order to assess whether a better model could be obtained. The final model did not change when the data was trained and tested on random subsets of the dataset, which gives confidence that it is robust. The final model is given in Table 5.

Table 5.5: Estimates, error, T value, P value and VIF values of a GLM assessing the impact of environmental variables on DOC values.

Coefficients	Estimate	Std. Error	T value	*p*-value	VIF	AIC	Model RMSE	R^2
Intercept	29.42	19.45	1.51	0.136	-	376.51	5.94	0.45
pH	**10.34**	**2.78**	**3.72**	**< 0.001** ***	**1.3**			
Other moss abundance	-0.05	0.04	-1.19	0.24	1.19			
Bare ground cover	-0.06	0.04	-1.54	0.13	1.19			
Soil moisture	**-0.64**	**0.22**	**-2.95**	**< 0.01** **	**1.13**			

5.5 Specific UV Absorbance (SUVA)

For SUVA the same procedure was followed as for DOC. When variables were plotted, aspect, *Sphagnum* abundance, *Molinia* abundance, bare ground, bulk density and air temperature (day of collection) were removed from the model (Figure 5). The consequent initial model contained the variables slope, peat depth, pH, *Calluna,* other moss, sedge, soil moisture, total extracted DNA, soil temperature (time of collection) and monthly rainfall. Terms were then sequentially removed in an attempt to improve the model. The final model only included sedge cover and soil temperature, with a RMSE of 0.350 and an R^2 of 0.905, and is presented in Table 6.

Table 5.6: Estimates, error, T value, P value and VIF values of a GLM assessing the impact of environmental variables on SUVA values. The R2 of this model is 0.461.

Coefficients	Estimate	Std. Error	T value	P value	VIF	AIC	Model RMSE	R^2
Intercept	**29.86**	**2.19**	**13.67**	**< 0 .00001** ***	**-**	**246.73**	**1.43**	**0.91**
Sedge cover	0.003	0.01	0.26	0.8	1.12			
Soil temperature	**-3.51**	**0.27**	**-12.8**	**< 0.000001** ***	**1.12**			

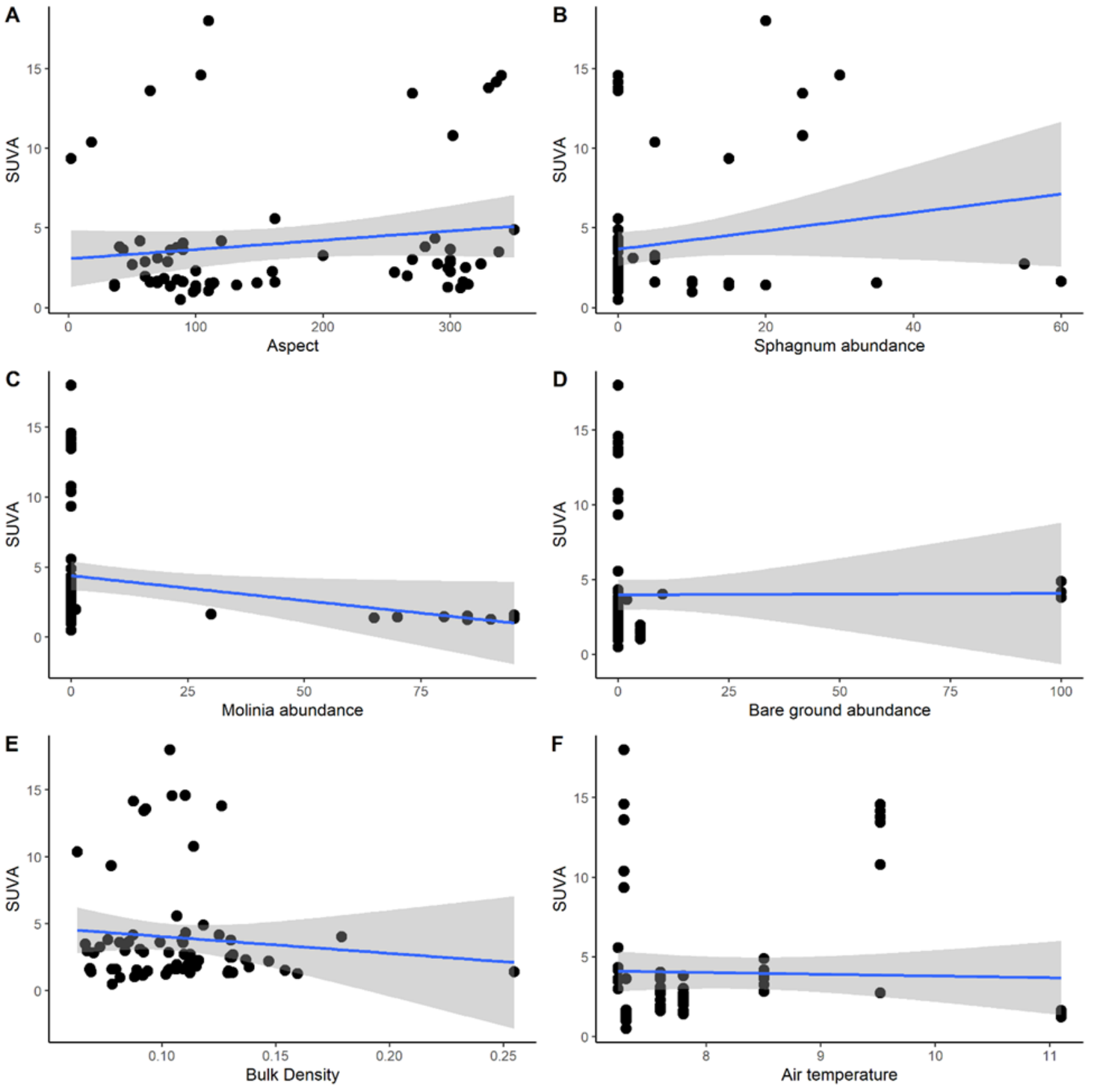

Figure 5.5: SUVA plotted against potentially explanatory variables where A is the aspect, B is *Sphagnum* abundance, C is *Molinia* abundance, D is bare ground abundance, E is bulk density and F is the air temperature on the day of sample collection. All of these variables were removed from the statistical model.

5.6 Hazen

The same procedure was followed as for DOC and SUVA. When variables were plotted, slope, aspect, sedge abundance, *Sphagnum* abundance, and total extracted DNA were removed (Figure 6). We then sequentially removed the least performing variables. The final model, trained and tested on separated data, is located in Table 7.

Table 5.7: Estimates, error, T value, *p*-value and VIF values of a GLM assessing the impact of environmental variables on Hazen values. The R^2 of this model is 0.461.

Coefficients	Estimate	Std. Error	T value	*p*-value	VIF	AIC	Model RMSE	R^2
Intercept	**5.01**	**1.16**	**4.32**	**< 0.0001 *** **	**-**	**50.19**	**0.26**	**0.42**
Bare ground cover	0.003	0.002	1.33	0.19	1.01			
Soil moisture	**-0.04**	**0.13**	**-3.16**	**< 0.003 ** **	**1.0**			
Monthly rainfall	**-0.002**	**0.001**	**-2.01**	**0.049 ***	**1.01**			

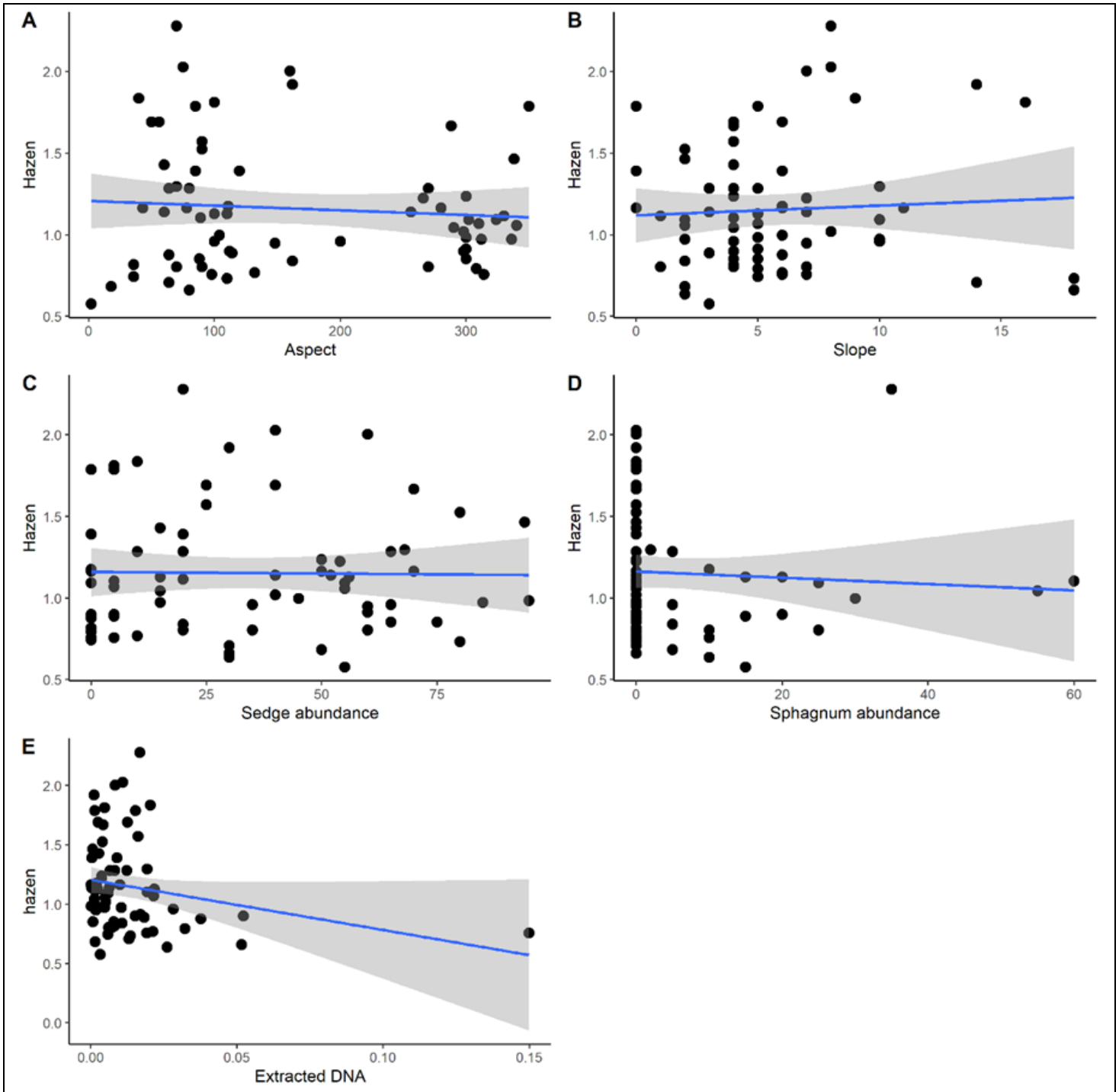

Figure 5.6: Hazen plotted against potentially explanatory variables where A is the aspect (in degrees), B is the slope (in degrees), C is sedge abundance (%), D is *Sphagnum* abundance, and E is total extracted DNA. All of these variables were removed from the model.

5.7 Water quality and environmental variables on the Peatland-ES-UK sites

The same procedures as above were repeated, but only for the Peatland-ES-UK (Yorkshire) sites. The Peatland-ES-UK sites are located on a well-replicated study consisting of paired catchments (comparing heather burning to cutting with no management [uncut] control plots) that controls well for national scale differences such as rainfall and temperature. Additionally, because the sites were chosen for a heather-dominated (grouse moor) vegetation management study, they have similar vegetation (relative to the national sites: e.g., there is a lack of the bare ground seen in the Peak District, and no *Molinia* cover). Consequently, they may be better placed to unpick local scale (soil and vegetation related) drivers of water quality change. This resulted in new models for DOC (Table 8), SUVA (Table 9), and Hazen (Table 10). The differences in DOC, SUVA and Hazen across sites and managements is elucidated in Figure 7. DOC values were not significantly different between the Yorkshire sites (Kruskal-Wallis, X^2= 0.42, df = 2, p = 0.81) or between the managements (Kruskal-Wallis, X^2= 1.34, df =2, p = 0.51). SUVA did significantly vary between locations (ANOVA, df = 2(33), F = 7.75, p < 0.002) where Mossdale, having the lowest SUVA values (1.52 ± 0.75) was significantly different to Whitendale (2.32 ± 0.67) and Nidderdale (2.7 ± 0.83), which were not different from one another. SUVA was not significantly different between managements (ANOVA, df = 2(33), F = 0.24, p = 0.79). Values of Hazen were significantly different between the three Yorkshire sites (Kruskal-Wallis, X^2= 7.42, df = 2, p < 0.03) but did not significantly differ between management treatments (Kruskal-Wallis, X^2= 0.24, df = 2, p = 0.89).

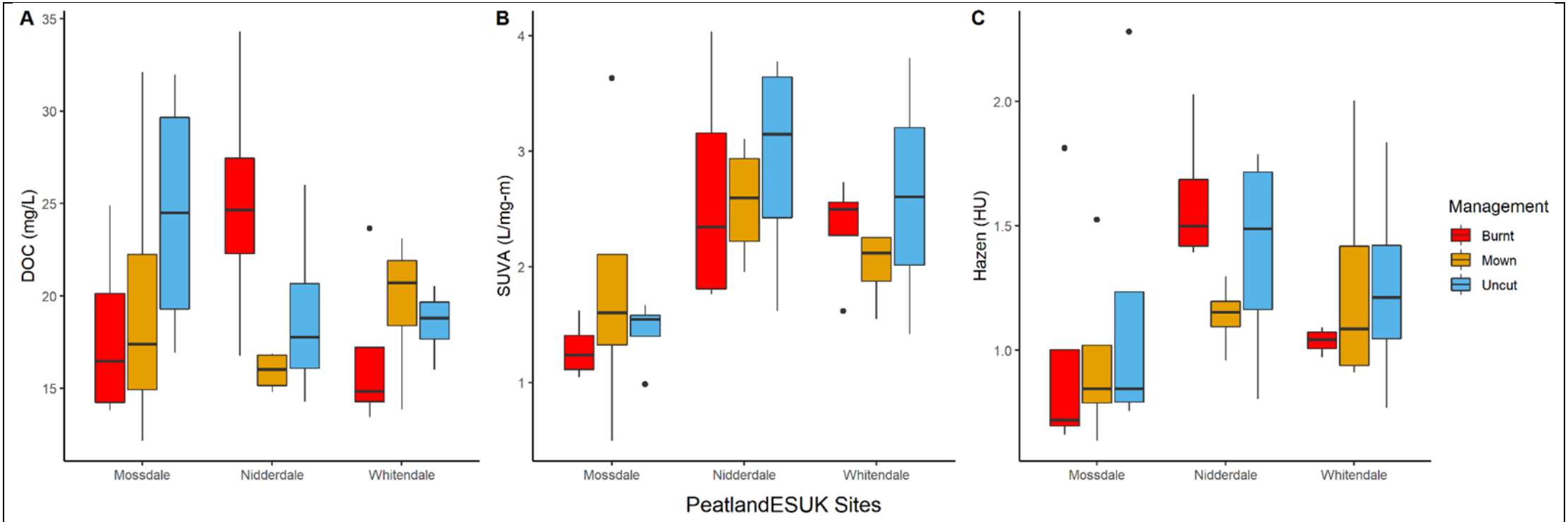

Figure 5.7: Water quality across the Peatland-ES-UK Sites where graph A is DOC, graph B is SUVA, graph C is Hazen.

Table 5.8: GLM model with selected variables for the Yorkshire sites only (Nidderdale, Whitendale, Mossdale) relating environmental variables to water quality variables. Significant variables have been highlighted in bold.

	Variable	Estimate	Std. Error	T value	P value	VIF	AIC	Model RMSE	Model R^2
DOC	Intercept	30.79	27.27	1.13	0.27	-	227.27	5.09	0.18
	pH	10.03	5.27	1.90	0.07	1.12			
	Soil moisture	**-0.65**	**0.27**	**-2.45**	**0.02** *	**1.12**			
SUVA	**Intercept**	**1.83**	**0.25**	**7.28**	**0.00001** ***	-	93.39	0.79	0.18
	***Calluna* cover**	**0.02**	**0.01**	**2.15**	**0.04** *	1.03			
	Sphagnum cover	-0.04	0.02	-1.94	0.06	1.03			
Hazen	**Intercept**	**5.27**	**1.68**	**3.14**	**0.004** **	-	41.67	0.4	0.15
	Soil moisture	**-0.05**	**0.02**	**-2.43**	**0.02** *	-			

5.8 Microbial effects on water quality variables

The previous section of this chapter dealt with the relationship between environmental variables and water quality; often this relationship is assumed to be direct, whereas this is not necessarily the case. In this section, we will investigate whether the microbial community is another (indirect) factor mediating these effects on water quality. The data was deliberately added after the likely environmental variables had been narrowed down in order to avoid model overfitting and multi-collinearity between environmental and microbial variables.

5.9 Fungal diversity and function impacts on water quality variables

To evaluate the effects of microbial populations on water quality variables, fungal diversity and fungal functional group relative abundances were added to the data set to re-evaluate likely predictors of water variables with the same method as used above. This resulted in a dataset of 74 samples with 51 predictors, which is far too many for a reliable model and translates into a high chance of overfitting. Consequently, initial removal of predictors that were not relevant (such as time of collection) or likely to be correlated with other variables (such as latitude) was undertaken, resulting in a dataset with 74 samples and 32 predictor variables. We then removed variables that were not present in the original linear models above, ending with the pre-model possible predictors for each water quality variable as listed in Table 9. Because this method has limitations (outlined in section 5.11 of this chapter) the analysis was not undertaken on bacterial and archaeal communities, but the results for the fungi are presented here as a record.

Table 5.9: Possible predictors for each water quality variable	
Water quality variable	Possible predictors (pre-model)
DOC	Observed species, Shannon, Fisher diversity, pH, other moss abundance, bare ground cover, Soil moisture, undefined fungal trophic group, saprotroph-symbiotroph, saprotrophs, pathotroph-saprotroph, pathotrophs, symbiotrophs, pathotroph-saprotroph-symbiotrophs, and pathotroph-symbiotrophs.
SUVA	Observed species, Shannon, Fisher, Sedge, sedge cover, soil temperature (time of collection), saprotroph-symbiotroph, saprotrophs, pathotrophs, symbiotrophs, pathotroph-symbiotrophs.
Hazen	Observed species, Shannon, Fisher, soil temperature (time of collection), bare ground, monthly rainfall, saprotroph-symbiotroph, saprotrophs, pathotrophs, symbiotrophs, pathotroph-symbiotrophs

The initial dataset contained the 19 predictors listed in Table 9. Further to this, values were plotted against DOC and three variables (the abundance of undefined fungi, pathotroph-saprotroph fungi, and Pathotroph-Saprotroph-Symbiotroph fungi) were excluded (Figure 8).

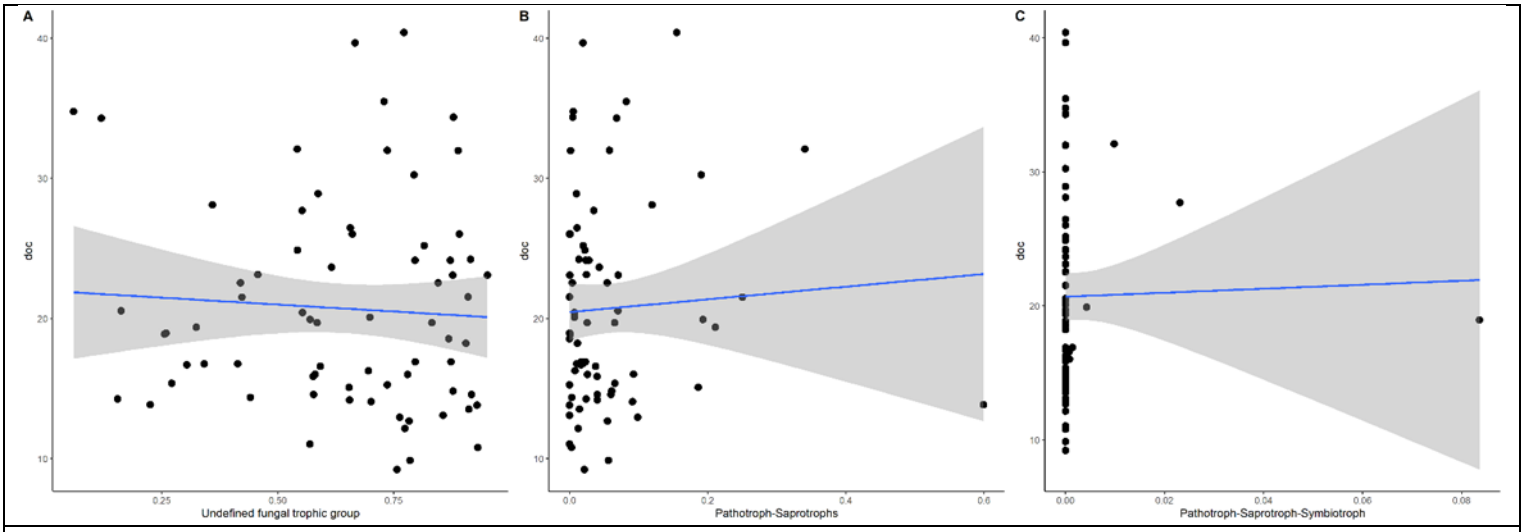

Figure 5.8: Variables excluded from analysis where no discernible relationship was visible, or in the case of the Pathotroph-Saprotroph-Symbiotrophs, very few samples had any species present preventing any meaningful interpretation.

Water quality data was then modelled based on 16 potential predictors. The initial model of DOC versus all 16 predictors contained significant variables, with an AIC of 461.98. The Variance Inflation Factors were investigated and the number of observed species (upon which the Fishers alpha diversity metric is based) was consequently removed. The weakest variables (based on the estimate and t value) were then sequentially removed before retesting the model. The final model, described in Table 10, had an RMSE of 5.233 and an R^2 of 0.731. This method was repeated for SUVA. The initial model for SUVA with all 13 variables had a single significant explanatory variable (soil temperature), an AIC of 260.15, an RMSE of 1.959, and an

R^2 of 0.694. With these added variables, the best model included soil temperature, sedge abundance and pathotroph abundance as variables, and the results are in Table 10. Despite the addition of these variables, soil temperature was still the most robust predictor of SUVA.

The same method was repeated with Hazen nationally with the results in Table 10.

Table 5.10: general linear models describing the effect of environmental predictors on soil water quality parameters. Significant variables have been highlighted in bold.

	Variable	Estimate	Std. Error	T value	P value	VIF	AIC	Model RMSE	Model R^2
DOC	Intercept	38.85	19.48	1.2	0.05	-	383.95	5.23	0.73
	Fungal fisher diversity	**0.60**	**0.2**	**3.0**	**0.004** **	1.07			
	pH	**10.71**	**2.49**	**4.31**	**0.0001** ***	1.1			
	Soil moisture	**-0.83**	**0.22**	**-3.74**	**0.001** ***	1.14			
SUVA	**Intercept**	**28.08**	**2.05**	**13.68**	**0.000001** ***	-	249.9	1.55	0.84
	Sedge abundance	0.004	0.01	0.35	0.73	1.14			
	Soil temperature	**-3.28**	**0.27**	**-12.36**	**0.000002** ***	1.25			
	Pathotroph relative abundance	-1.72	4.12	-0.42	0.68	1.11			
Hazen	**Intercept**	**1.72**	**0.5**	**3.45**	**0.001** **	-	143.26	0.5	0.31
	Shannon diversity	-0.46	0.27	-1.72	0.09	2.04			
	Fisher diversity	0.06	0.04	1.61	0.11	2.17			
	Pathotroph	3.58	1.99	1.8	0.08	1.64			
	Pathotroph-Symbiotroph	**-99.07**	**46.12**	**-2.15**	**0.04** *	1.19			

5.10 Impact of Fungal Microbial Community Data on Water Quality in the Yorkshire Sites

The Yorkshire (Peatland-ES-UK) sites were assessed separately with the fungal data, because it is a well-replicated experiment that controls for national effects such as weather and climate. The Yorkshire sites contain fewer samples (36 in total) and these relationships do not apply to the wider national sites, because of the specific management and climate context within which these sites sit. The final models for SUVA, DOC and Hazen are summarised in Table 13. Having an inadequate sample size for splitting, these data were not split. Models were compared based on AIC, RMSE and R^2 values. Data was not transformed.

Table 5.11: general linear models undertaken in the Yorkshire data describing potential predictors of water quality variables. Significant variables are highlighted in bold.

Water Quality Variable	Variable	Estimate	Std. Error	T value	P value	VIF	AIC	Model RMSE	Model R^2
DOC	Intercept	27.51	25.91	1.06	0.3	-	206.45	3.76	0.54
	pH	7.31	4.85	1.51	0.15	1.35			
	Sphagnum	**0.3**	**0.12**	**2.43**	**0.02** *	**1.18**			
	Bare ground	-0.49	0.39	-1.26	0.22	1.35			
	Soil moisture	-0.47	0.28	-1.68	0.11	1.72			
	Saprotroph-Symbiotroph	6.48	5.46	1.19	0.25	1.77			
	Saprotroph	-7.39	4.68	-1.58	0.13	1.03			
	Pathotroph	-20.7	15.71	-1.32	0.12	1.77			
	Pathotroph-Symbiotroph	**955.08**	**335.02**	**2.85**	**0.01** **	**1.6**			
SUVA	Intercept	3.35	3.85	0.87	0.39	-	90.13	0.66	0.45
	Observed fungal species	**-0.03**	**0.01**	**-2.08**	**0.05** *	**2.88**			
	Shannon diversity	0.72	0.5	1.44	0.16	3.61			
	pH	-0.79	0.8	-1.0	0.33	1.14			
	Calluna abundance	0.01	0.007	1.79	0.09	1.41			
	Sphagnum abundance	-0.03	0.02	-1.2	0.24	1.35			
	Sedge abundance	0.007	0.007	0.98	0.34	1.48			
	Bulk density	8.44	6.53	1.29	0.36	1.43			
	Saprotroph – Symbiotroph	0.89	0.95	0.94	0.36	1.67			
	Saprotroph	1.71	0.91	1.87	0.07	1.22			
Hazen	**Intercept**	**1.69**	**0.19**	**8.78**	**0.0001** ***		29.66	0.3	0.53
	Observed fungal species	**-0.01**	**0.004**	**-3.61**	**0.001** **				
	***Sphagnum* abundance**	**0.02**	**0.009**	**2.23**	**0.03** *				
	Total extracted DNA	-10.58	6.07	-1.75	0.09				
	Saprotroph – Symbiotroph	0.59	0.32	1.83	0.08				
	Saprotrophs	-0.37	0.36	-1.02	0.32				
	Symbiotrophs	2.82	1.51	1.86	0.07				

5.11 Limitations on this use of General Linear Models

This use of GLM has limitations when applied to this dataset. Whilst the relationships applied nationally are robust, the regression models applied to the Yorkshire sites are far less so. This is principally because a low number of samples, combined with the variation among samples (compare the Standard Error to the Estimate in Tables 8 and 10), means these models are likely to be over-fitted and have poor statistical power. The Root Mean Squared Error (RMSE) in these models means they lack predictive power, for example, the RMSE of the national DOC model n Table 10 is 5.233, but DOC only varies between 0 and ~40, the error of 5.233 is therefore quite large. Further, adding in the data and regressing it in this way excludes or might hide environmental variables effecting water quality via microbial community variables – especially where multi-collinear variables are removed from models, but actually represent underlying mechanistic or "latent" variable: for example, it is possible that soil temperature affects SUVA via changes to the microbial community. To attempt to elucidate these relationships, a piecewise structural equation model was undertaken on the Yorkshire site data.

5.12 Piecewise structural equation modelling

In an attempt to assess the impacts of environmental variables via the microbial community, we undertook piecewise structural equation modelling, also called confirmatory path analysis. The aim is to unite predictor and response variables in the same network, using *a priori* hypothesised causal relationships. We used the R package "piecewiseSEM" (Lefcheck, 2016). Because variables can appear as both predictors and responses, this statistical methodology aims to resolve complex causal webs between many correlating environmental predictors that might have cascading or indirect effects, which addresses the problem left unaddressed by previous GLMs. Traditional structural equation models assume independence of variables and that they are normally distributed, which is not appropriate for these data. Consequently a piecewise SEM was used, which calculates each path locally rather than globally, allowing for the inclusion of a wide range of distributions and sampling designs. Piecewise SEMs were created for two sets of data: the national set (n = 74), to evaluate how variables are relayed across climatic and management gradients, and the Peatland-ES-UK set which aims to investigate relationships between vegetation burning, mowing and control. Because of the reduced number of samples (n = 36) in the Peatland-ES-UK set, pSEM is simpler (containing fewer variables), in order to prevent an erroneously good model fit and a lack of statistical power (Grace *et al.,* 2015).

5.13 Piecewise SEM for the national sites

An a priori hypothesised structural equation model as created based upon the findings from previous chapters concerning the microbial communities on a national scale. As a measure of microbial community variation, an NMDS was undertaken on each community (fungi: stress = 0.193 RMSE = 0.0002, bacteria: stress: 0.1, RMSE = 0.0035, and archaea: stress = 0.09, RMSE = 0.00014, all three NMDS with 3 K dimensions) and NMDS Axis 1 scores were used inside the piecewise SEM (as per Jassey *et al.,* 2017). A GLM, undertaken with the aim of ascertaining the vegetation communities' impact on water quality variables, found that vegetation variables

were only significant when included as interactions with other vegetation variables, and based upon this it was hypothesised that it was the difference between communities, rather than any one particular group, which was the principle driver of change. Consequently an NMDS was undertaken on the vegetation community (K dimensions = 2, stress = 0.11, RMSE = 0.0001) and the NMDS axis 1 was used as a measure of vegetation community dissimilarity. The *a priori* model is described in the scheme in Figure 9. As displayed in Figure 9, the *a priori* hypotheses were that the change in vegetation communities as a whole, pH, soil moisture, soil temperature and rainfall would have effects on the three microbial communities as well as on the fungal trophic groups. Further, it was hypothesised that these variables have a direct influence on water quality variables outside the influence of the microbial communities. It was hypothesised that changes in the microbial groups and the fungal trophic groups' relative abundance would directly link to the water quality parameters.

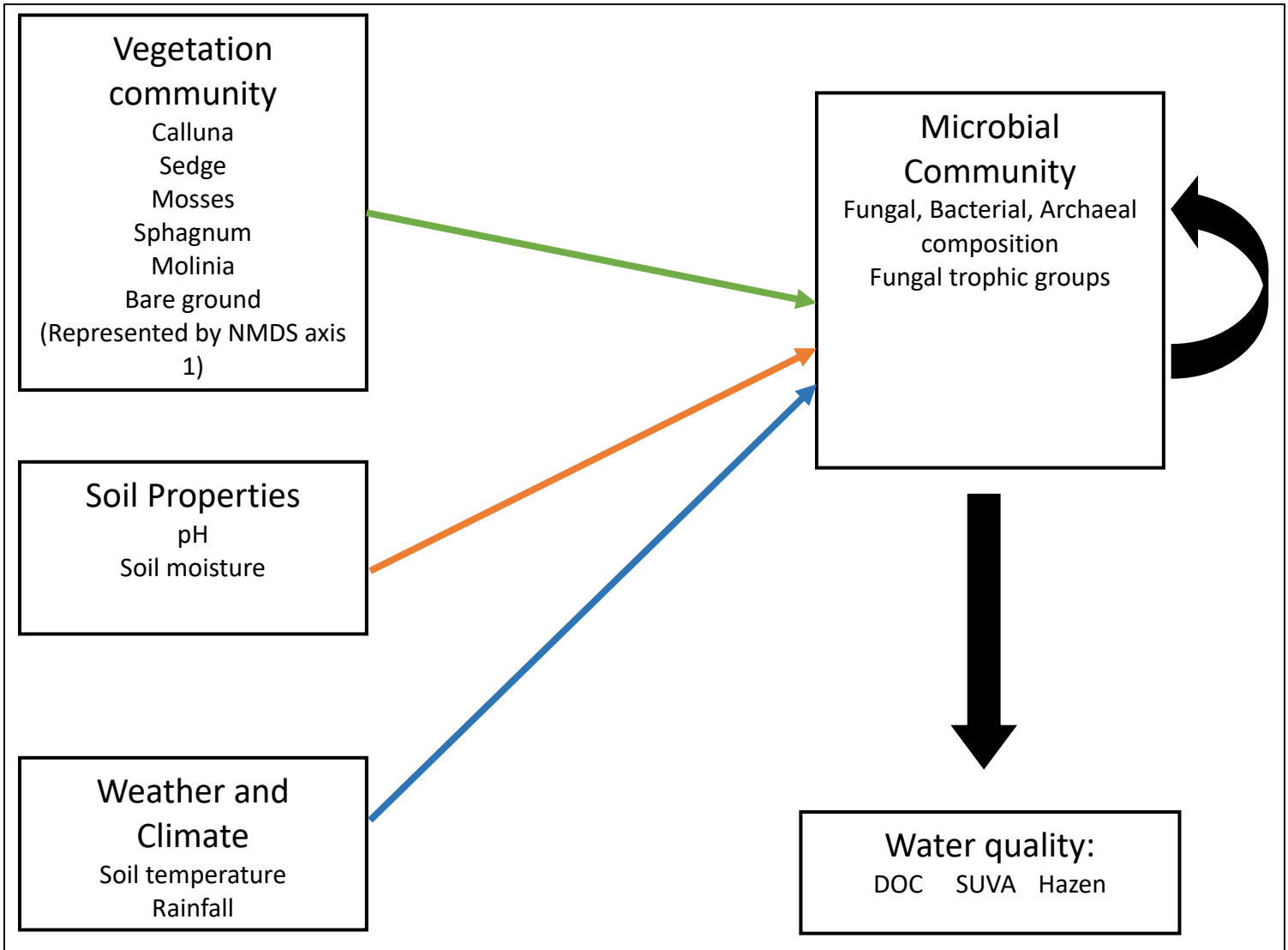

Figure 5.9: Simplified scheme of a piecewise SEM based on national data. Each individual component was included, totalling 14 model parameters: Vegetation NMDS1 (vNMDS1), pH, Soil moisture, Soil temperature (time of collection) monthly rainfall (month of collection), bacterial, archaeal and fungal NMDS axis 1 (bNMDS1, aNMDS1, and fNMDS1 respectively), saprotroph, saprotroph-symbiotroph and symbiotroph relative abundances, DOC, SUVA and hazen.

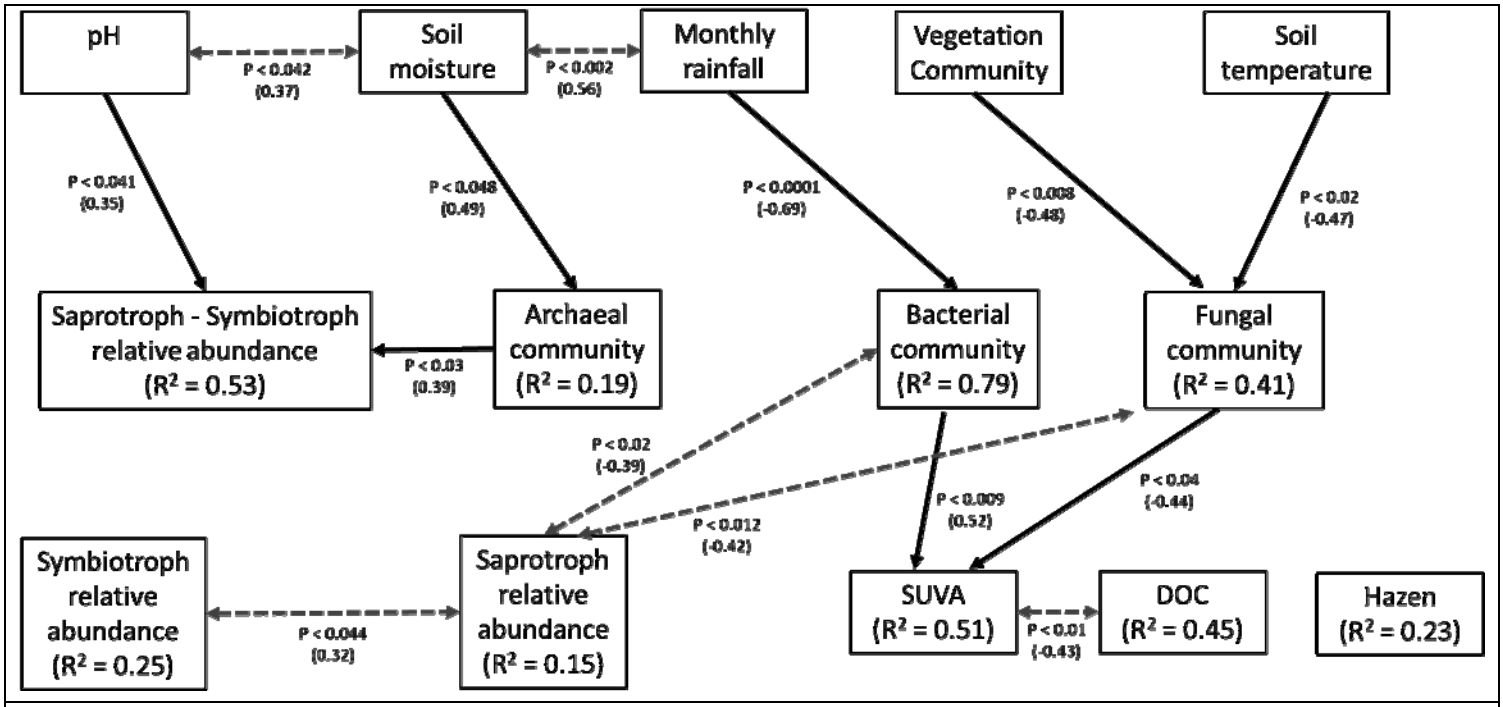

Figure 5.10: A piecewise structural equation model describing the relationships between environmental variables, the microbial community and water quality outcomes on a national scale. This model with a Fisher's C = 13.528, P = 0.957, AIC = 161.53. Numerals placed next to each path indicate the weight of the path relationship, with the level of significance (*p* number). Numerals within boxes (R^2) indicate the percentage of variance explained by the model. For clarity, only significant variables have been included. Dashed arrows represent (significant) correlated errors.

5.14 Piecewise SEM for the Yorkshire sites

The same modelling was undertaken on a subset of the sites in Yorkshire, representing the Peatland-ES-UK experiment, which is a well-replicated paired catchment design. This was undertaken to examine potential relationships without the confounding effects of national variation in weather and climate, and on sites with similar vegetation profiles that principally differ only due to vegetation management. Due to the risk of model overfitting, fewer variables were used in these structural equation models, and two models were undertaken with the *a priori* hypothesised paths conceptualised in Figure 11. For the same reason, and because no effects were shown in the national model, Hazen was excluded as a response variable from these models. Again, as a measure of microbial community variation, an NMDS was undertaken* on each community (fungi: stress = 0.162 RMSE = 0.0005, bacteria: stress: 0.094, RMSE = 0.0002, and archaea: stress = 0.175, RMSE = 0.0002, all three NMDS with 3 K dimensions) and NMDS Axis 1 scores were used inside the pSEM. An NMDS was also undertaken on the vegetation community (stress = 0.042, RMSE = 0.000006 with 2 K dimensions) and used as a representation of vegetation community change.

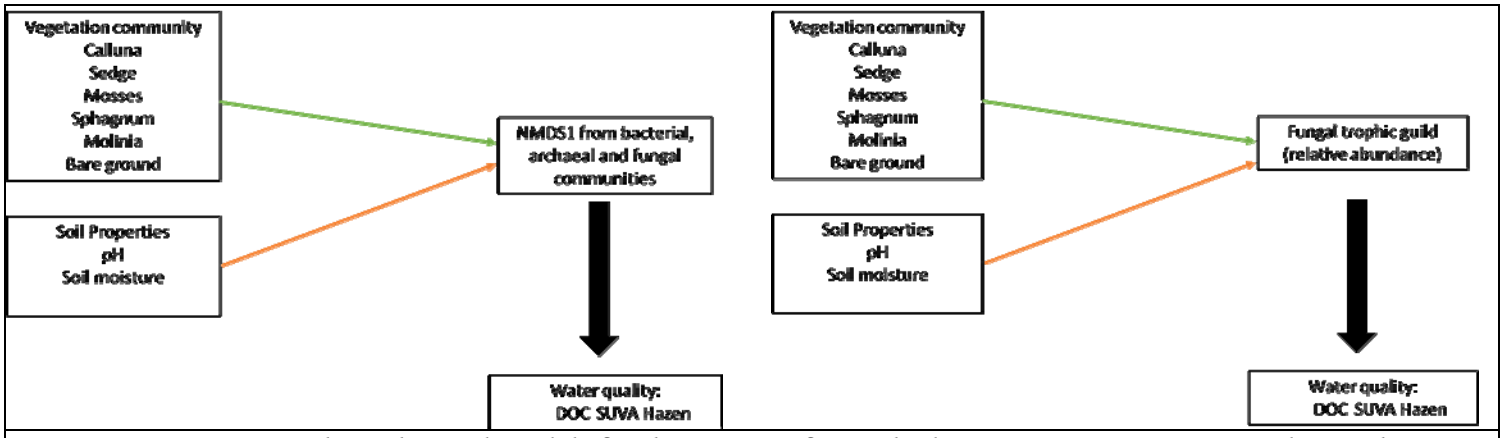

Figure 5.11: Two *a priori* hypothesised models for the impact of microbial communities on water quality in the Yorkshire Peatland-ES-UK sites.

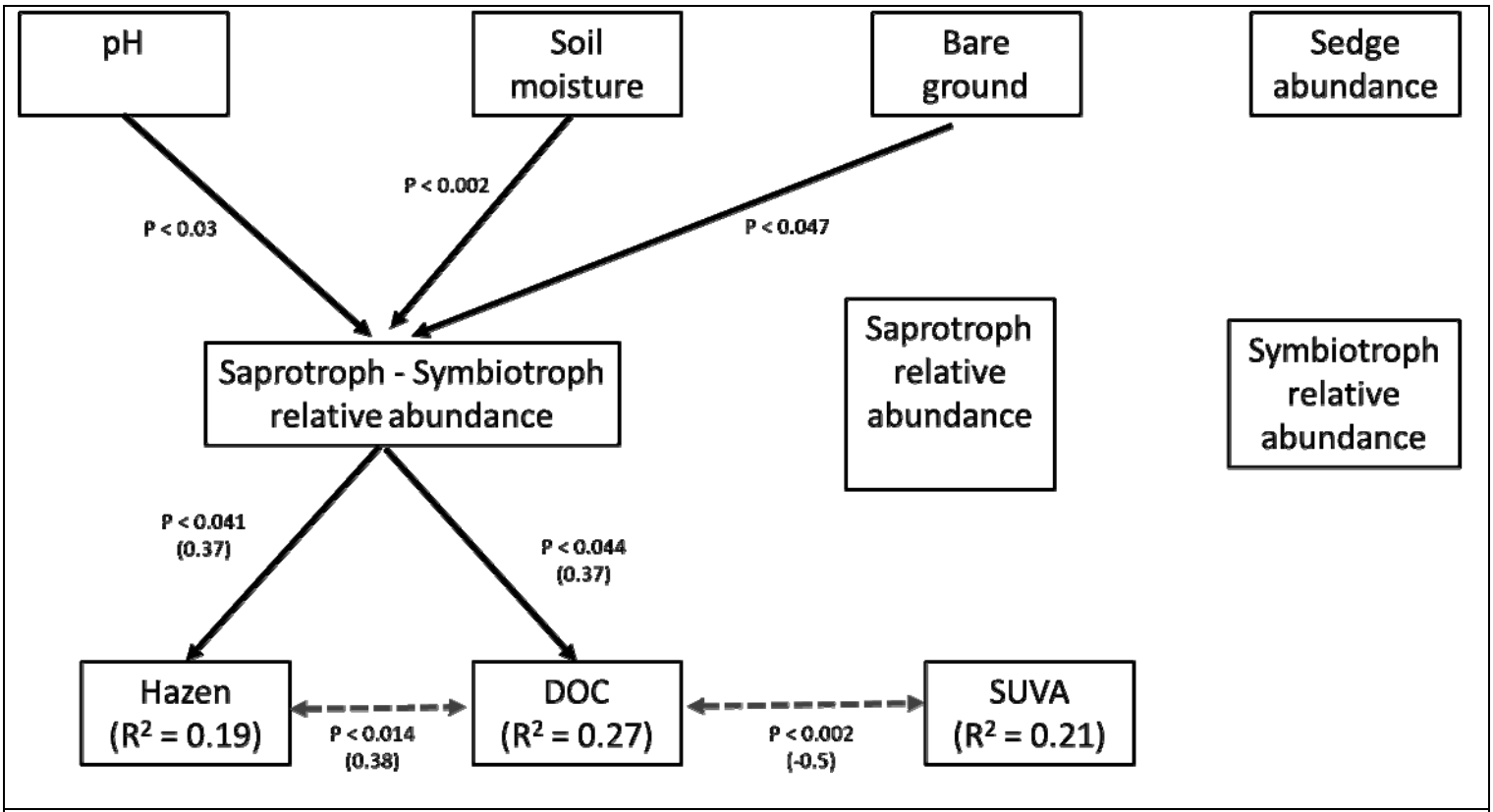

Figure 5.12: A piecewise SEM assessing the impact of fungal trophic groups on water quality variables in the Peatland-ES-UK sites. Boxes containing variables lacking arrows had no significant predictors: non-significant relationships are excluded for clarity. As Figure 10, numerals placed next to each path indicate the weight of the path relationship, with the level of significance (*p* number). Where numerals indicating the path weight are missing, this is due to the use of quasi-Poisson distributions in the general linear models used to assess those paths. Numerals within boxes (R^2) indicate the percentage of variance explained by the model.

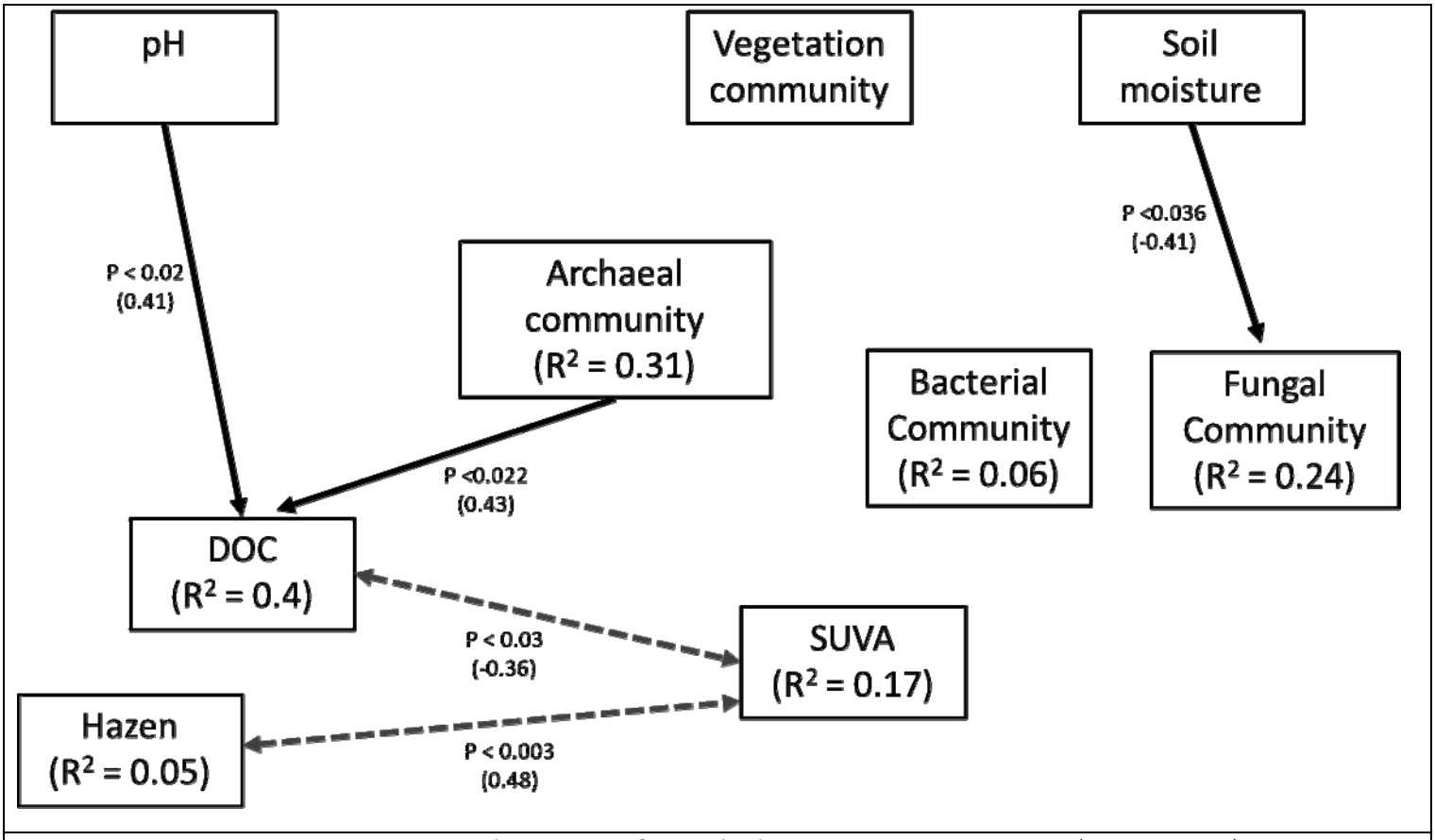

Figure 5.13: A piecewise SEM assessing the impact of microbial community composition (NMDS axis 1) on water quality variables in the Peatland-ES-UK sites. Boxes containing variables lacking arrows had no significant predictors: non-significant relationships are excluded for clarity. As Figure 10, numerals placed next to each path indicate the weight of the path relationship, with the level of significance (*p* number). Where numerals indicating the path weight are missing, this is due to the use of quasi-Poisson distributions in the general linear models used to assess those paths. Numerals within boxes (R^2) indicate the percentage of variance explained by the model.

5.15 Greenhouse gas fluxes

The impact of microbial communities upon gas fluxes, including CO_2 and methane was assessed. The analysis here is based upon gas fluxes measured from the mesocosm set ups described in the methods chapter of this thesis. There are limitations to the comparisons between flux and microbial communities: whilst the microbial samples were taken in the field in February of 2019, the first gas flux measurements from the mesocosms were undertaken in July 2019. It was originally planned that the mesocosms would be sampled directly for their microbial community profiles, but due to the pandemic and associated lockdown (and associated time, logistic, legal and financial constraints) this could not be completed. Consequently, a comparison between the microbial communities sampled in field and the flux measurements taken in July is the best data this thesis has available to address the hypothesis that microbial communities can be linked to the gaseous carbon cycle (see Chapter 1 section 1.10)

The first question that arose during analysis was to what extent the mesocosms and field sites would be different in terms of their gas flux measures. The move from field site to York meant a change of climate: sometimes being warmer (moving from Scotland to York) and sometimes being colder (moving from Exmoor to York). This also meant changes in precipitation,

sunlight hours, and, because the mesocosms were kept at a static water table of -5 cm (during the first year – to enable a direct comparison between mesocosms before an anticipated fluctuating water table depth in the second year), a difference in water table depth. Whilst this made comparing field microbial community samples and mesocosm fluxes difficult, it does have the advantage of controlling for the confounding effects of climate and water table depth in terms of assessing management related effects on carbon sequestration and water quality.

To assess whether there were any difference between field sites and mesocosms, the soil respiration, NEE, and R_{eco} means from the Peatland-ES-UK sites were compared with their corresponding mesocosm samples, from data that was collected between July 2019 and October 2020 (the times at which data is available for both sites). This is a valid comparison as the mesocosms were taken from within 10 m of the Peatland-ES-UK plots, they matched in vegetation and previous hydrological conditions. Annual means were used to avoid the confounding effect of season and the potential for outliers.

5.16 Soil respiration

In the Peatland-ES-UK plots and the mesocosms, two sets of soil respiration were measured: the cut core, where the roots from surrounding vegetation were excluded, and the uncut core, where the roots were allowed to grow into the core. Both cores were bare of vegetation on the surface. Mean soil respiration between the Peatland-ES-UK sites and their corresponding York-based mesocosms did not differ in the cut (Paired t-test, $t = 0.4$, $df = 35$, $p = 0.69$) but was different in the uncut (Paired t-test, $t = -11.47$, $df = 35$, $p < 0.0001$) plots. The uncut soil respiration was much lower in the mesocosms (1.01 ± 0.26) than in the field sites (1.99 ± 0.53). This could potentially be caused by a difference in average soil temperature between the sites which was significantly different (Paired t-test, $t = -2.36$, $df = 35$, $p = 0.024$) where the mesocosms were generally cooler (9.5 ± 0.1), than the field sites (9.93 ± 1.09). However, this temperature difference is not large and might be an artefact of the method of mesocosm temperature collection (mesocosm fluxes did not have exact soil temperature measurements, rather a mean was obtained for between 09:00 and 15:00 on the day of collection). It is important to note that the cut plots represent microbial only respiration whereas the uncut plots represent those still affected by the roots and their attendant rhizosphere (and therefore the main surrounding vegetation).

5.17 Net Ecosystem Exchange

Net ecosystem exchange (NEE) is a measure of the net exchange of carbon between an ecosystem and the atmosphere and thus describes the strength of the carbon sink; it is the sum of gross primary production and ecosystem respiration. NEE was measured using the central vegetated mesocosm core in the mesocosms, and on regular plots close to the mesocosm sampling point in the Peatland-ES-UK field locations. Again, NEE in the field sites and mesocosms was averaged between July 2019 and October 2020. The Peatland-ES-UK NEE values were not normally distributed and so both sets of NEE values were square root transformed. Post transformation both variables met the normality assumption and a t-test was conducted. The NEE values between the Peatland-ES-UK and mesocosm plots square root means were not significantly different (Paired T-test, $t = -1.21$, $df = 34$, $p = 0.24$ – the non-transformed means were also not significantly different in a non-parametric Wilcoxon signed rank exact test, $V = 209$, $p = 0.052$).

5.18 R_{eco} and Gross Primary Productivity

$R_{eco,}$ or ecosystem respiration, represents the combined CO_2 release from the soil and plants within a plot. The calculated mesocosm R_{eco} was compared to the Peatland-ES-UK uncut R_{eco} values. Both variables were square root transformed to meet the assumptions of normality. The square root transformed means were significantly different (t-test, t = 10.055, df = 35, p < 0.00001). R_{eco} was higher in the mesocosms (3.97 ± 1.6) than in the Peatland-ES-UK sites (1.76 ± 0.51).

Gross Primary Productivity is a measure of the amount of carbon fixed by photosynthesis by all producers in the ecosystem. GPP was compared between the Peatland-ES-UK plots and their associated mesocosms and met the assumptions of normality, requiring no transformation. The means were significantly different in their GPP values (t-test, t = 4.84, df = 35, p < 0.00003) where the mesocosms had the highest values (8.5 ± 3.13) versus the fieldwork sites (5.72 ± 1.67).

5.19 Can field microbial communities be compared to mesocosm fluxes?

Above, the difference between the field fluxes and mesocosm fluxes has been assessed. The picture is complicated: whilst overall NEE is not different between field locations and mesocosms, this is because both the Reco and GPP have changed. Certainly, whilst the overall strength of the carbon sink seems not to have changed, mesocosms have become more active in terms of carbon turnover (input and output). The fact that the uncut soil core (devoid of vegetation but with roots allowed to ingress into the core) has changed but the cut core (devoid of vegetation with no roots allowed to enter) has not indicates that this increase in activity is focused on the plants (root). If it were microbial (non-rhizosphere) respiration involved in this change, then the cut core would likely also be different in its levels of respiration.

It would appear then that changes in respiration between field and mesocosm are plant-derived, possibly due to changes in temperature, PAR or seasonality in York. Importantly, this is a hypothesis: the microbial community in the mesocosms was not measured and until it is, it will not be known to what extent the belowground community has changed during mesocosm sampling and incubation.

There is scant literature that examines the impact, in peatland mesocosm studies, of what has been termed the “bottle effect” (from aquatic ecology; the idea that phenomena observed in closed systems [the sampling bottle – or in the case of this study, a bucket] are consequences of confinement rather than being inherent to the ecology of that system [Pernthaler & Amann, 2005]). Other peatland mesocosm studies such as Potter *et al.,* (2019) or PEATcosm (Lamit *et al.,* 2017, and Lilleskov, *pers comm.* 2021) did not take measurements in the field sites from where mesocosms were sampled. One study, by Wilson *et al.,* (2021) examined the microbial community pre- and post- mesocosm collection. Importantly, the study was conducted on lab based (closed system) mesocosms which where it may be expected that they suffer more from the “bottle” effect than larger vegetated cores. The study found that at the broad scale, microbial communities were driven by the same environmental parameters (habitat type of soil depth) in the mesocosms as they were in the field. However, they did find the microbial communities themselves had changed (although not their richness or diversity), demonstrating the possibility of a high degree of functional redundancy in these systems.

Wilson *et al.,* concluded that mesocosms/incubations do reasonably approximate field conditions.

For the reasons above, relationships between the field microbial measurements and the mesocosm fluxes were analysed, with the above caveat that they were sampled at a different time and place.

5.20 Mesocosm fluxes between managements and national locations

The mean carbon flux values on all of the mesocosm sites were calculated for the period July 2019 until October 2020. The mean was used to account for the confounding effects weather, along with (pandemic mediated) irregular sampling periods. The mesocosms were sampled for fluxes once in August 2019, twice in October 2019 (on the 23rd and the 29th), once in December 2019 and once in February, July and October 2020, leaving a 6 month settling period after they were sampled in February 2019.

The Net Ecosystem Exchange (NEE) was tested for differences between national site and management with a two-way analysis of variance (ANOVA). NEE was assessed for normality using a Shapiro-Wilk test, and, not meeting this assumption, was transformed to absolute square root values. A statistically significant difference in average NEE by both management ($f(6) = 2.66$, $p < 0.025$) and national location ($f(2) = 32.64$, $p < 0.000001$) was found, with a significant interaction between these terms ($f(6) = 5.13$, $p < 0.0003$).

A post-hoc Tukey test revealed significant pairwise differences between the Peak District and Exmoor samples (-1.35 NEE real [untransformed] value in the Peak District – a smaller carbon sink), between Scotland and Exmoor (-1.69 NEE in Scotland) and Yorkshire and Exmoor (-0.67 NEE in Yorkshire) and Yorkshire and Scotland (+0.23 NEE in Yorkshire). There were also significant pairwise differences in managements: principally differences between degraded and 10 year post restored plots (-0.48 in degraded), 5 year restored plots (-0.44 in degraded) and Yorkshire uncut plots (+0.35 in degraded). The management differences are primarily due to a difference between the Peak District degraded plots (which have no vegetation at all) which are significantly different from every other combination of national site and management apart from the Scottish degraded and intact sites. Figure 14 shows the plotted NEE values by site and management.

Uncut side (soil) cores, which represent root and soil microbial derived fluxes, were assessed between management and national site categories. A two-way ANOVA indicated a significant effect of management ($f(6) = 3.06$, $p < 0.012$) but not national site ($f(2) = 2.37$, $p = 0.1$). However, a post-hoc Tukey test indicate these differences were only because the Peak District bare cores, which produce almost no flux, were significantly different from other sites: when the Peak District bare sites were removed, there was no longer any significant effect of management ($f(6) = 1.46$, $p = 0.21$). Uncut soil respiration is graphed in Figure 15.

In the cut side (soil) cores, which represent only microbial flux, were not significantly different across management categories (Figure 16) and national sites in a two way ANOVA assessing both management ($f(6) = 2.22$, $p = 0.054$) and national site location ($f(2) = 1.18$, $p = 0.17$). Cut core soil respiration is graphed in Figure 16. Uncut and cut soil cores had significantly different soil respiration values, as assessed by a paired t-test ($df = 71$, $t = -8.78$, $p < 0.001$).

Methane was also measured in the central vegetated mesocosms, and this was compared to the field fluxes (for Peatland-ES-UK). Because methane is highly variable based on seasonal changes, analysis was conducted on both the mean and the median fluxes from between July 2019 and October 2020. Average methane fluxes did not fit the assumptions of normality for a t-test and could not be transformed to normal, either square root values or removing outliers. Consequently they were compared with a paired samples Wilcoxon test and the means ($V = 130$, $p < 0.001$) and median values ($V = 132$, $p < 0.002$) were significantly different between mesocosms and Peatland-ES-UK sites. Generally, mesocosm methane fluxes were much higher. A paired boxplot comparing mesocosm and Peatland-ES-UK mean and median values is in Figure 17.

To assess whether the change in methane flux was related to management or (Peatland-ES-UK) site, a change factor was calculated by dividing the Peatland-ES-UK mean and median flux values by the mesocosm respective mean and median values, on a per-mesocosm pair basis. Whitendale Uncut plot 4 was excluded as an outlier, as its methane flux had decreased by a factor of approximately 418. The change factor of mean methane flux did not significantly differ between management (Kruskal Wallis test [K-W], $X^2 = 2.5$, df = 2, $p = 0.29$) or Peatland-ES-UK site (K-W, $X^2 = 1.87$, df = 2, $p = 0.39$), and the change factor of median methane flux also did not differ between management (K-W, $X^2 = 1.21$, df = 2, $p = 0.55$) or site (K-W, $X^2 = 2.79$, df = 2, $p = 0.25$). Because some of the mesocosms had, essentially, been rewetted, due to the mesocosms being kept at a constant -5 cm water table level, the relationship between the change factor and the soil moisture content was assessed via linear regression, but there was no relationship (linear regression, $F(33) = 0.33$, adj. $R^2 = -0.02$, $p = 0.57$). This indicates that any change in methane fluxes between field and mesocosm is not related to that samples management or site, nor was it related to the water table levels in which the mesocosms were kept.

In the mesocosms, the median was used as a better representation of the average flux, taking into account large variation between sampling time points (as per Heinemeyer *et al.,* 2019). Methane flux was significantly different between managements (K-W, $X^2 = 16.91$, df = 6, $p < 0.01$) and between national locations (K-W, $X^2 = 19.78$, df = 3, $p < 0.0002$). A post-hoc Dunns test indicates from the managements, only the degraded and mown sites were different ($p < 0.004$). In the national site categories, Exmoor and the Peak District ($p < 0.02$), the Peak District and Scotland ($p < 0.002$) and the Peak District and Yorkshire sites ($p < 0.0002$) were different, whilst the Peak District and Yorkshire, and Exmoor and Scotland, were not significantly different (Figure 18)

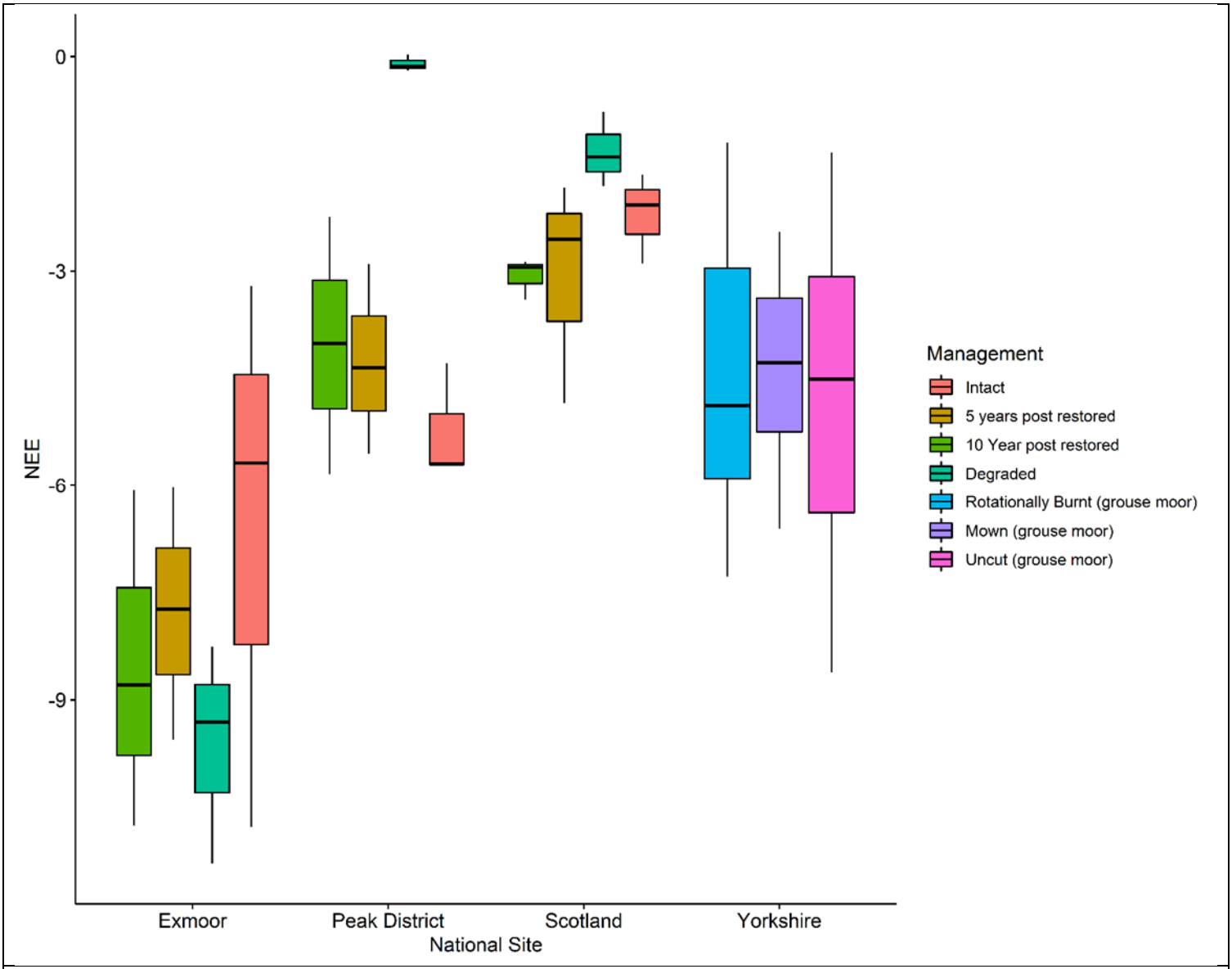

Figure 5.14: NEE values at national sites coloured by management.

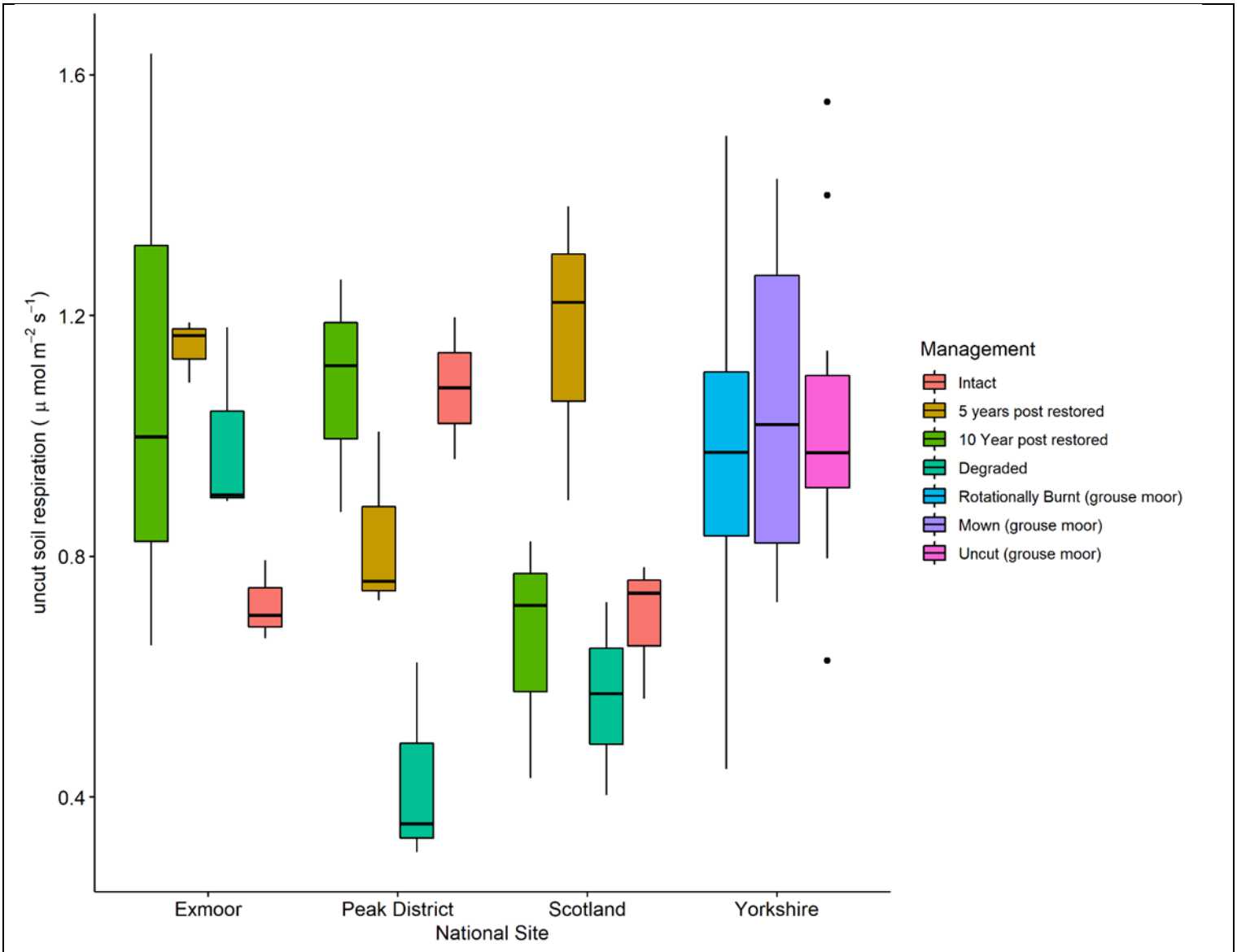

Figure 5.15: Uncut soil respiration values (root and microbial) at national site locations, coloured by management.

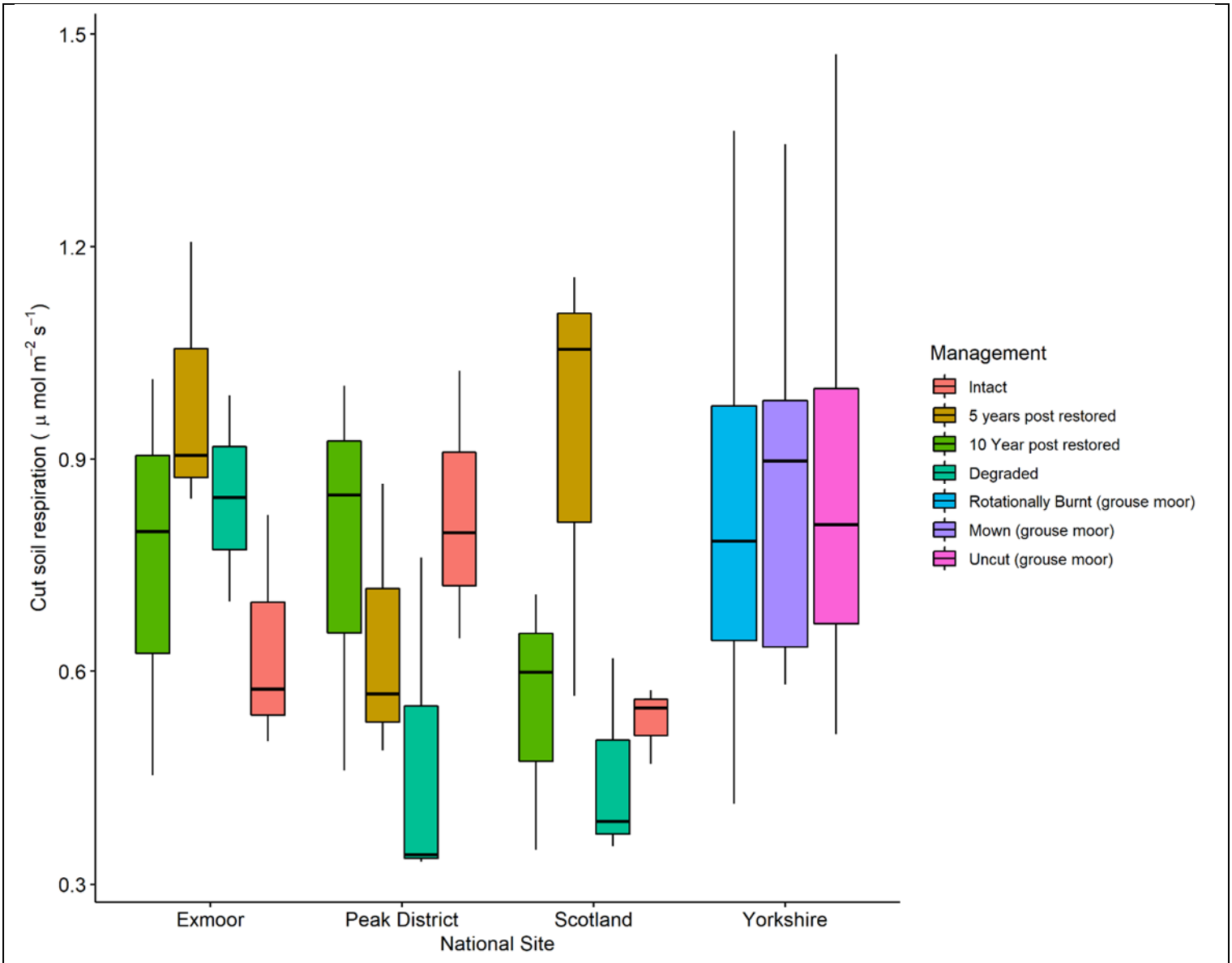

Figure 5.16: Average soil respiration in the cut soil cores (soil microbial only; roots excluded) between national sites, coloured by management category.

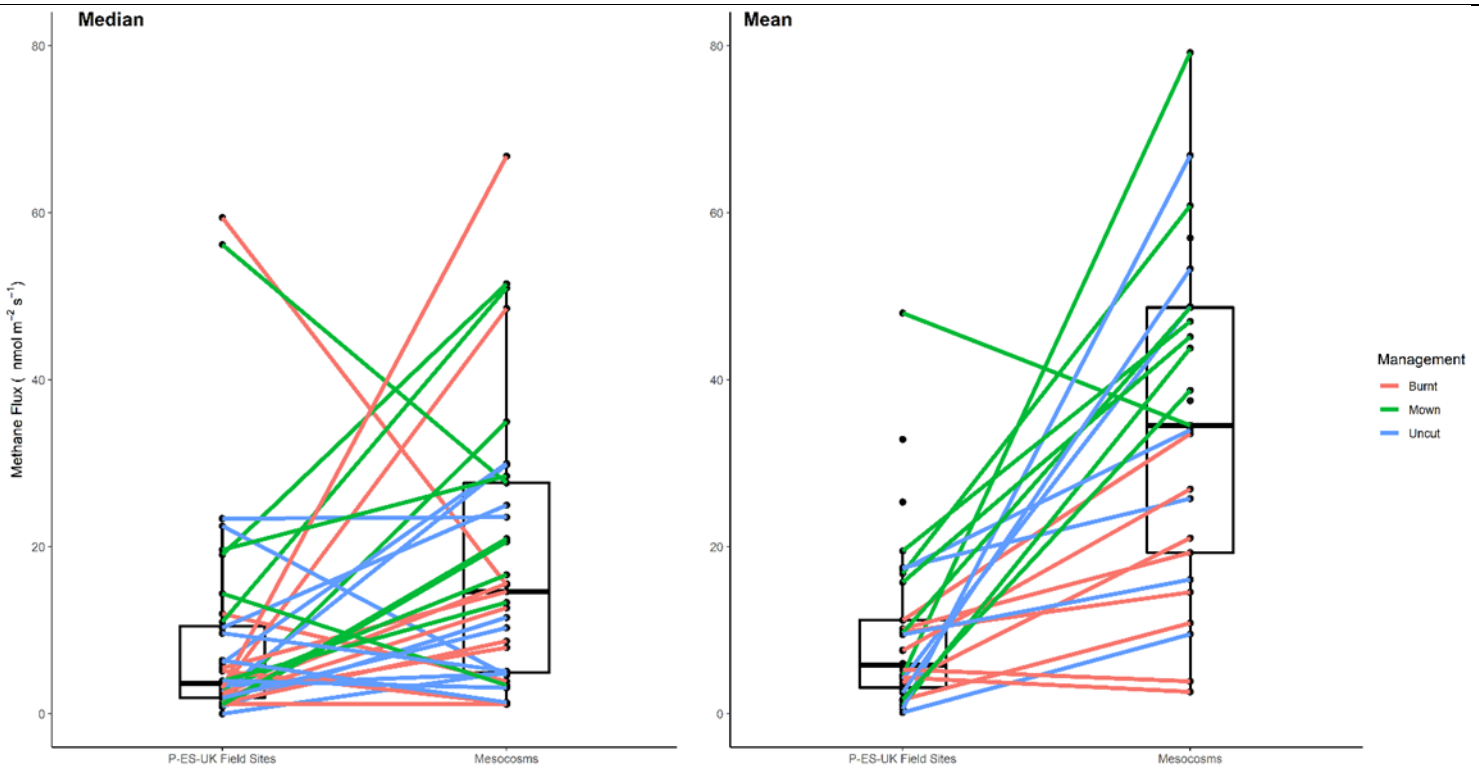

Figure 5.17: Comparisons between mean and median Peatland-ES-UK and mesocosm methane flux values. Samples are linked by lines, which are coloured by management category.

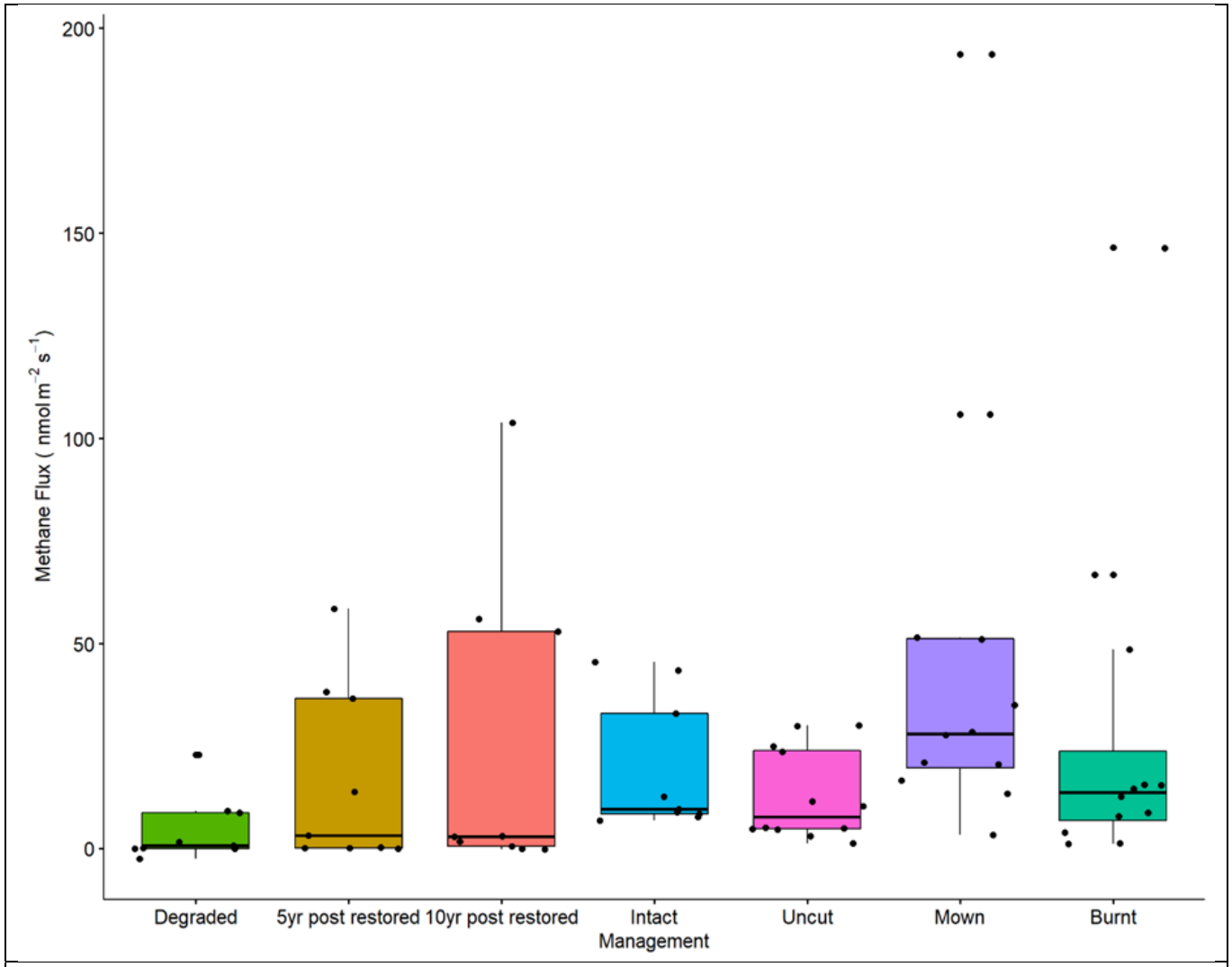

Figure 5.18: Median annual methane flux values in the mesocosms separated by management/site condition category.

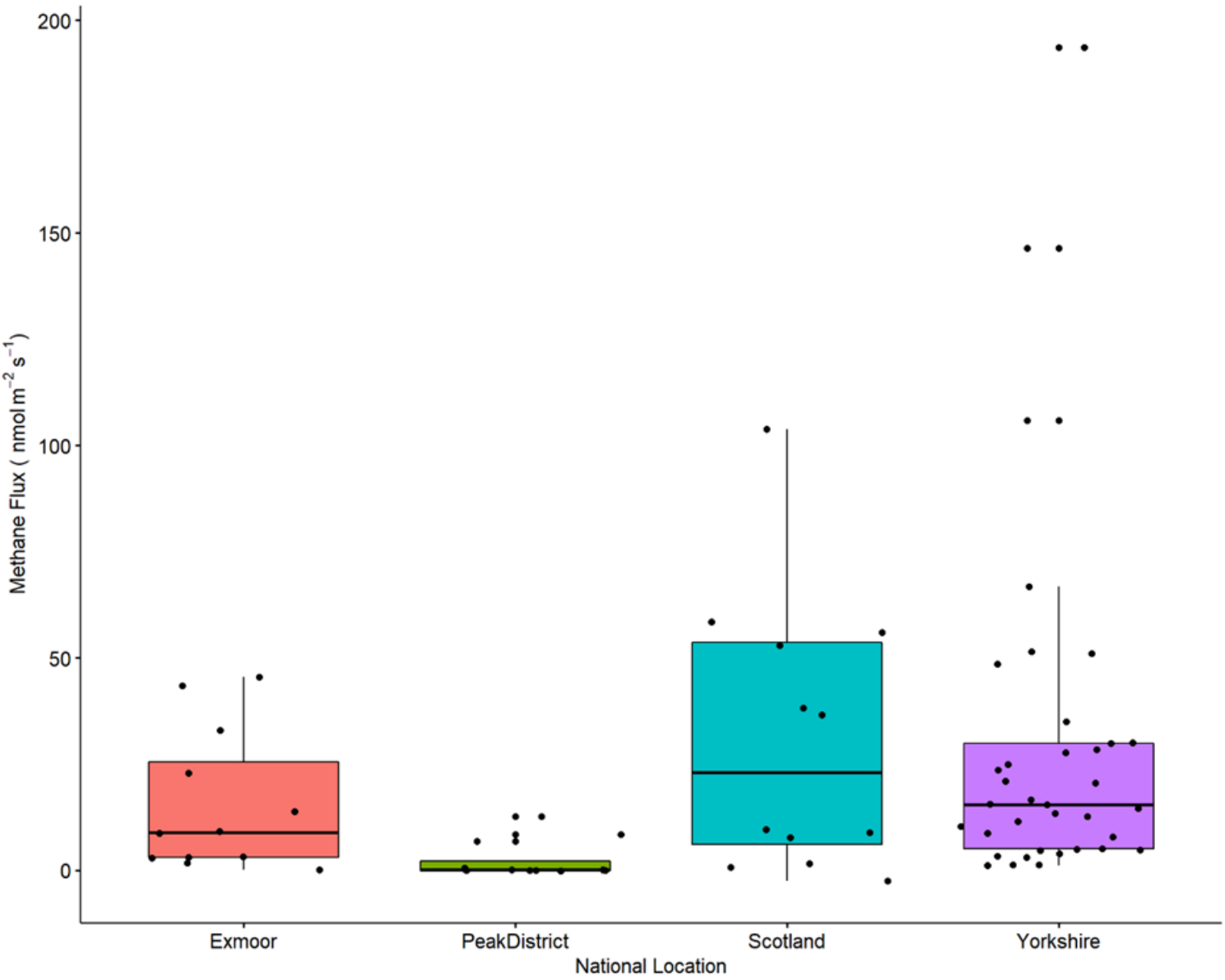

Figure 5.19: Annual median methane flux values in the mesocosms separated by national site location.

5.21 Environmental drivers of GHG fluxes

The environmental drivers of NEE, cut and uncut soil respirations, and methane were assessed using general linear models. The same method as for water quality was used: variables were chosen based upon their hypothesised relevance, and excluded based upon Variance Inflation Factors and manual inspection of relationships to the target variable. The model was created with all variables, and tested by randomly subsampling 20% of the data as a testing set. Variables were then sequentially removed based upon estimate and t value, until the model with the lowest AIC and RMSE, and highest R^2 was found. These models, indicating the biggest drivers of their target flux variable, are described in Table 12.

Table 5.12: general linear models undertaken in the mesocosms data describing potential predictors of GHG flux variables. Significant variables have been highlighted in bold.									
Flux Variable	Variable	Estimate	Std. Error	T value	P value	VIF	AIC	Model RMSE	Model R^2
NEE	**Intercept**	**-2.13**	**0.66**	**-3.37**	**0.002** **	-	306.93	1.9	0.43
	Calluna % cover	**-0.03**	**0.01**	**-2.30**	**0.03** *	**1.38**			
	Sedge % cover	-0.02	0.01	-1.78	0.08	1.49			
	Molinia % cover	**-0.06**	**0.01**	**-4.94**	**0.0001** ***	**2.13**			
	Extracted DNA (mg/g)	-24.78	13.54	-1.83	0.07	1.29			
	Sedge : Calluna	-0.004	0.002	-1.91	0.06	1.15			
Cut core soil respiration	**Intercept**	**0.77**	**0.04**	**17.85**	**0.0001** ***	-	11.66	0.27	0.12
	Fungi NMDS1	**0.14**	**0.05**	**2.74**	**0.009** **	1.07			
	Bacteria NMDS1	-0.09	0.05	-1.74	0.09	1.05			
	Saprotroph % relative abundance	0.23	0.21	1.13	0.27	1.03			
Uncut core soil respiration	**Intercept**	**0.82**	**0.06**	**13.04**	**0.0001** ***	-	19.08	0.26	0.17
	Sedge abundance	0.003	0.001	1.89	0.06	1.34			
	Molinia abundance	**0.004**	**0.001**	**2.856**	**0.006** **	**1.45**			
	Fungi NMDS1	**0.19**	**0.05**	**3.732**	**0.0004** ***	**1.12**			
Methane Flux	Intercept	-7.26	22.01	-0.33	0.74	-	697.74	29.43	0.18
	Peat Depth (field)	-0.07	0.05	-1.28	0.21	1.46			
	Sedge % abundance (field)	**0.35**	**0.15**	**2.4**	**0.02** *	**1.17**			
	Fungal NMDS1	**11.85**	**5.87**	**2.02**	**0.048** *	**1.12**			
	Bacterial NMDS1	**-15.20**	**7.13**	**-2.13**	**0.04** *	**1.54**			
	Fungal Shannon Diversity	**16.52**	**7.20**	**2.30**	**0.03** *	**1.09**			
	Average annual NEE	1.76	1.63	1.08	0.28	1.33			

The results in Table 12 demonstrate the difference in driving factors between cores. In the central mesocosm, which is vegetated, the NEE is driven by (field based) vegetation factors:

Calluna and *Molinia*. The mesocosms have the same vegetation as the field site from which they were sampled. In the bare cores, the root-included (uncut) soil respiration is driven by a combination of vegetation (sedge and *Molinia*) and fungal factors (fungal NMDS axis 1). However, in the root-excluded (cut) core, soil respiration is driven only by microbial elements, including the fungal and bacterial NMDS Axis 1 (a measure of difference between samples; *i.e.* the difference between fungal and bacterial communities between samples is significantly related to the difference in soil respiration here) and the relative abundance of saprotrophic fungi.

5.22 Discussion

In this chapter, the factors most associated with water quality variables and gas flux variables were investigated across all the sites.

Water quality in blanket bogs

All three common water quality parameters used were different among national site locations, with SUVA being the most variable nationally and Hazen only being different across two national locations. Contrary to this, the water quality parameters did not differ between management groupings in any water quality variable. This is potentially because the overall driver of water quality in a blanket bog (relative to others) is the climate in which it is located: in the GLMs undertaken on our data, soil moisture and soil temperature appear as the best explanatory variables for all three water quality variables, with the addition of rainfall as a significant variable for hazen. These are important variables even when direct measures of the microbial community are also included. This is congruent with previous work, that found temperature and precipitation controls DOC export from blanket bog (Koehler *et al.,* 2009, de Wit *et al.,* 2016). Further to climate related controls, fungal richness was found to play a role on a national scale, exerting an effect on DOC and Hazen, whilst pH was also relevant to DOC.

This chapter then assessed the drivers of water quality variables in the Peatland-ES-UK sites, a subset (half) of the overall experiment, that, being a long term, catchment scale and well replicated study, controls for the confounding effects of national level climate differences. The Peatland-ES-UK sites are part of a paired catchment experiment designed to test the differences in ecology between contrasting grouse moor management regimes. DOC, SUVA and Hazen did not differ between burnt, mown and uncut managements, in agreement with other studies examining the effects of burning on DOC concentrations in stream runoff (Clay et al., 2009) but not with others investigating water colour (Clay et al., 2012). Contradictory results regarding the effect of these managements on DOC export and water colour are rife in the literature, possibly due to the short-term nature of some studies and, at the catchment scale, because of confounding environmental factors (Holden et al., 2012, Ashby & Heinemeyer, 2020).

Microbial drivers of water quality

In the Peatland-ES-UK sites, general linear models indicated a positive (where *Calluna* increases as does SUVA) influence of *Calluna*, and when microbial data was included, an influence of fungal diversity, on SUVA. This is similar to previous work on the PEATcosm mesocosms undertaken by Lamit *et al.,* (2017), which found SUVA highest at shallower depths (< 30cm) which varied with plant functional types (Ericaceae *versus* sedges), seemingly dependent on water table depth. Although this experiment and the PEATcosm experiment were undertaken on mesocosms of different sizes, and, importantly, from continental North

American and British blanket bogs, these results indicate that fungal communities, alongside their respective vegetation (e.g. ericoid mycorrhiza in Ericaceae dominated plots) have a direct role in moderating DOC and SUVA values.

These GLMs highlight the difficulty with comparing sites on a national scale, where sites may be influenced by a range of environmental, climate, management or historically related edaphic and biological variables.

Whilst linear regressions provide some insight into the driving factors behind water quality (in a structure *a* -> *b*) they do not assess structural relationships where a variable may affect another *via* one (or more) intermediaries (*e.g.* in a structure *a* -> *b* -> *c*). Doing so with many linear regressions might miss latent effects of variables upon one another (*e.g.* where *a* directly influences *c*). Consequently, structural equation models (SEM: accurately described as a confirmatory path analysis) were undertaken on *a priori* hypothesised relationships driving water quality.

The piecewise SEM incorporating national sites explained 51% of the variance in SUVA, but did not explain DOC or Hazen, in line with the literature discussed above. What is novel is that the model linked changes to the bacterial and fungal communities (NMDS Axis 1) with changes in SUVA, and that these communities were explained by rainfall, changes to vegetation and soil temperature. It has therefore been demonstrated that the 'direct' effects on SUVA by soil temperature and soil moisture espoused by the GLMs above are in fact mediated by the changes that occur in the bacterial and fungal communities. The national SEM also incorporated relationships between microbial community members, including relationships between the bacteria, fungi and archaea, but these are complex and confounded by the separate PCR programmes used to obtain this data. Further analysis of these community interactions in peatlands would be a worthwhile undertaking.

For the Peatland-ES-UK sites, the microbial community variables were split to avoid model overfitting. The first Peatland-ES-UK SEM was undertaken on hypothesised changes to fungal trophic guilds based upon the results in chapter 3, where the burnt plots had a significantly higher relative abundance of saprotroph-symbiotrophs. This indicated a role for soil moisture, the amount of bare ground, and pH in directing the change in symbiotroph-saprotroph relative abundance, which then has impacts on DOC (explaining 27% of its variation) and less so on Hazen (19% variation explained). Changes that occur during a burn, which principally includes the removal of vegetation (and increases in bare ground), may disadvantage symbiotrophs, which rely on said vegetation, and benefit functionally flexible saprotroph-symbiotrophs that can switch between trophic lifestyles. Importantly, we detected this effect in the Peatland-ES-UK plots, which are 7-years post burn. It may be that measuring these variables closer to the burn would reveal an even larger effect, and measuring further in time after a burn may establish whether this effect is long-lived (permanent) or a temporary change.

The second SEM focused on changes to the communities themselves, but the results were far less clear. Only archaeal community change was associated with changes in DOC, which explained 40% of the variation along with pH. No other water quality variables were explained by this model. This indicates that climate variables (excluded from this model) are the principal drivers of bacterial, fungal and archaeal change. The non-significance of the vegetation community in this model indicates that (relatively) lower levels of vegetation change (relative to the national picture, the managements change the abundance and growth

of, but not presence of, functional groups of the vegetation) are not driving microbial community differences at this scale. It would be better to combine all of the microbial community variables into a model examining the effects of contrasting management (burnt, mown and uncut) but this would require far more spatial replicates to be collected, for this study design.

Environmental and microbial drivers of gas fluxes

This chapter also examined environmental and microbial drivers of gas flux measured from mesocosms taken from the sites. The measurements were taken six months after sampling the microbial community and for better comparisons, the microbial community within these mesocosms should be measured again. NEE did not significantly differ between the mesocosms and the field, although the GPP and R_{eco} did, indicating that the mesocosms have become much more active in terms of their soil and plant respiration. In addition, methane fluxes in the mesocosms were generally higher, although this was consistent over all mesocosms, and did not change based on site category or management. These differences were potentially due to changes in temperature, PAR or climate seasonality in York, where the mesocosms were moved to. Ideally, a model would be constructed, utilising a light response curve, to compare NEE, GPP and R_{eco} under similar conditions, as per Heinemeyer *et al.,* (2019), however, the pandemic prevented the completion of this work.

The NEE differed significantly based on management and national location, with the Exmoor sites representing the strongest carbon sinks. The Peak District degraded sites, having no vegetation, had negligible flux (only microbial decomposition but even this was low), and coupled with the very low quantities of DNA that were extracted from these soils, it is reasonable to conclude they are approaching an inert medium. Perhaps unsurprisingly, significant drivers of NEE included vegetation, principally *Calluna* and *Molinia*. These two species probably represent opposite ends of a spectrum of productivity with *Calluna* being least productive, represented in the difference between the *Molinia* dominated sites (Exmoor) and the *Calluna* dominated grouse moors (Yorkshire) represented in Figure 14. Notably, however, there were differences in vegetation survival in the mesocosms – whilst *Molinia* dominated ecosystems maintained their productivity and growth, the uncut rank heather mesocosms lost a significant amount of stems and leaves. These large *Calluna* plants suffered because they are a surface rather than deep rooting species, and the mesocosms restricted rooting to a small area – along with the sampling cutting through the root mass.

There is an important caveat to our NEE figures: NEE is comprised of a calculation involving ecosystem respiration and gross primary productivity. Both of these variables were increased significantly in the mesocosms versus field measurements: it is only because both measures increased that the NEE (the net exchange) has not differed from the field. Our results, finding *Molinia* dominated mesocosms to be the greatest carbon sink, echoes the results of other work such as Leroy *et al.,* 2019. The study found a shift from *Sphagnum* to *Molinia* domination in mesocosms to be beneficial for C sequestration, although the authors cautioned that roots and litter effects (which were not measured in the Leroy study) could provide substrates for emissions that were not taken into account. What is really needed in this instance is the net ecosystem carbon budget, including fluvial and CH_4 carbon losses.

The cut (soil only flux) and uncut (root and soil flux) cores present a complicated picture in Figures 15 and 16. Both were unaffected by management or national site (apart from the degraded "inert" aforementioned Peak District cores) indicating potentially subtle or secondary effects of management (e.g. on root type, abundance of function). As seen in the figures, the national sites (Exmoor, Peak District and Scotland) are similar within-groups; whereas the grouse moor (Yorkshire), side cores are more highly variable in their fluxes. That the cut and uncut cores were significantly different indicates that the mesocosms were functioning as expected, partitioning fluxes into the relevant component, but also points towards the role of root (potentially rhizosphere) effects on soil respiration.

The methane flux in the mesocosms is higher than that in the field for the same time period, although it is important to point out that the higher values in the mesocosms are not outside of the range of values given for the field plots over the past seven years. The cause of the difference in flux values is most likely to be related to the constant high water tables that the mesocosms were kept at; some have essentially undergone rewetting, which does increase methane emissions. In addition, the change of average annual climate conditions (including seasonality) between the field sites and York may have had an impact. Figure 17 would appear to suggest that mown and uncut plots increased in their fluxes more when mesocosms were taken relative to burnt plots, specifically in the mean, but when examining median values the change of flux was more complicated.

Because methane fluxes were taken from mesocosms, and because they are higher than in the field, comparisons between environmental and microbial values recorded and the mesocosm methane flux must be treated with some caution. Nevertheless, methane flux was associated/correlated with some environmental and microbial variables in a multiple linear regression. The abundance of sedge (a methane shunt species) being related to methane flux is reliable, because the vegetation was sampled and has remained similar inside the mesocosms. The results of the models in Table 12 indicates that as sedge abundance increases, so does methane flux. This echoes other research in Canadian drained and natural peatlands (Strack *et al.,* 2006) and by mesocosm studies undertaken on UK peats (Green & Baird 2012). The latter study proposed that vascular plants reduced pore water methane by transporting it via the aerenchyma (shunt) directly to the atmosphere, but that this might be countered by direct stimulation of methanogens by root exudates. Whilst they did find methane fluxes to be higher with sedges (a result mirrored by our results), they found this difference could not be explained by root exudate utilisation. Instead, it could be hypothesised that the change comes about as a result of rhizosphere and soil community changes that arise due to the difference between Ericoid and Sedge plant functional types: where sedges replace ericaceous plants, this results in changes to the below ground community where rhizosphere bacteria and archaea begin to compete with Ericoid mycorrhiza (or other fungi) who are missing a host plant. This is potentially hinted at in our results in Table 12, where we see that the fungal and bacterial NMDS axis 1 (a measure of the overall change in dissimilarity of the communities) is significantly correlated with the annual median methane flux. Might plant-related belowground competitive interactions be driving fluxes? Our study, which measured microbial communities in the field, and compared them with methane fluxes from the mesocosms, cannot establish the causality needed to answer this question, because it cannot be established whether the methane fluxes are associated with changes in the microbial communities themselves, or whether the changes in flux result from the variables driving the variation in NMDS axis 1.

5.23 Conclusion

Using microbial community and environmental data from sites across the UK, and mesocosms to enable long term monitoring, this study has successfully linked environmental conditions across a spectrum of management and site conditions to microbial communities and thence to water quality and carbon sequestration. The piecewise structural equation models linked changes in environmental condition to changes in the microbial community, which was then linked to changes in water quality, but this was not always detectable in a consistent way. Overall, the vegetation community and climate conditions (rainfall) effect changes in the composition of the soil microbial community, which affects SUVA, but not DOC. On a local level in the Yorkshire mesocosms, management-associated environmental changes are linked to changes in specific fungal groups with consequences for DOC and Hazen. The gas flux measurements linked environmental parameters to gas flux outcomes and found differences in the strength of the carbon sink and other flux measurements between different managements/site conditions, albeit with less confidence than the water quality relationships.

The mechanisms underlying these relationships are still unknown. Future work should focus on the chemical changes that occur with changes in fungal trophic groups and bacterial and fungal communities, which then change water quality values, perhaps focusing on the enzyme degrading capabilities of specific taxa, and differences in substrate availability and litter quality between peatland management and site condition. To gain a mechanistic understanding of the sequence of events that lead to water quality change would enable the prediction of water quality variables based on microbial community changes.

Further, microbial taxonomic identification should be undertaken on (outside) mesocosms at the same time as measuring fluxes (or under similar variable environmental / water table conditions), for a range of peatland site and management conditions. This study originally intended to do this, but during the pandemic, this was made impossible. This work is vitally important in understanding the microbial community changes associated with changes to the C sink potential of a peatland under contrasting management regimes. Future work should include the changes in microbial community under different management regimes especially relating this to plant functional types. This has been studied using a plant removal experiment before (Robroek *et al.,* 2015) but should be repeated taking into account the more subtle differences between the management and site conditions found in this study.

6. General Discussion

6.1 Introductory remarks

The overarching question addressed by this thesis asks whether, and how, microbial groups differ across a spectrum of habitat conditions and managements examined on blanket bogs in the UK. Further, the thesis aimed to assess the impact of these microbial differences on key ecosystem services, specifically drinking water quality, carbon storage and climate regulation. In the UK, upland blanket bogs are important for peat accumulation and thus carbon storage (Billett *et al.,* 2010), carbon fluxes including methane emissions and thus climate regulation (Bonn *et al*., 2016) and, water supply given that a large amount of British drinking water is supplied from upland catchments (Xu *et al.,* 2018). Whilst the impacts of specific management/site conditions on these ecosystem services have been examined to a certain extent (e.g. Harper *et al*., 2018; Evans *et al*., 2016), a mechanistic understanding of how the microbial community mediates these impacts remains elusive (Ritson *et al*., 2021).

The literature review given in Chapter 1 found that whilst there is a growing body of evidence examining microbial communities in peat bogs, the specific differences in composition and function among habitat conditions has been paid little attention. Indeed, most of the present literature has focused on the differences between distinct habitats, such as fen and bog (Lin *et al.,* 2012) or large climate gradients (Seward *et al.,* 2020) but only a few (Elliot *et al.,* 2015, Potter *et al.,* 2017) have examined the more subtle differences between habitats in the oceanic temperate (rather than American or European continental) climate of the UK. Further, the rapidly evolving sequencing technologies used in the analysis of microbial communities mean that further information can now be gleaned where only a decade earlier such insights were impossible to uncover.

The specific objectives of this thesis were to **(a)** investigate how the microbial community composition changes across a spectrum of site habitat condition and management including restoration treatments and **(b)** explore how these microbial communities, and functional groups within them, are related to water quality and carbon fluxes, including methane emissions.

Via a combination of exploratory sampling and mesocosm based experimental approaches, the following main hypotheses were investigated:

- H1. Microbial communities (diversity, taxonomic composition, relative abundance of functional groups) are associated with management/site condition:
    - H1a. with "intact"/"active" or "favourable" sites having highest diversity (*Chapter 3)*;
    - H1b. and are nationally controlled by climate variables *(Chapter 4)*.
- H2. Microbial communities can be related to environmental and edaphic variables *(Chapter 3, 4 and 5)*.
- H3. Microbial community measurements (diversity, taxonomic composition, relative abundance of functional groups) can be used to predict water quality and carbon fluxes including methane *(Chapter 5)*.

An overview of the specific chapter aims, hypotheses and the main findings is detailed in *Table 1.*

Table 6.1: A summary table of objectives, hypotheses and main findings for the chapters in this thesis. Hypotheses are related to H1, H2 and H3 above.			
Chapter	Aims and Objectives	Hypothesis	Main findings
Three	Assessing differences in microbial community taxonomic composition between management/habitat condition status categories. In addition, the differences between fungal trophic groups.	H1. Habitats across a spectrum of active to degraded blanket bog vary in their soil biota.	The fungal, bacterial and archaeal communities were significantly different across management and national site categories, including as an interaction between management and national sites. Communities are highly variable across managements and across the UK (national sites).
		H1a and H1b, Biota communities and indicator groups differ between management and site categories.	Fungal trophic guilds, specifically the Symbiotrophs and Saprotroph-Symbiotrophs, differ in burnt plots only versus other categories.
Four	Assessing differences in microbial community taxonomic composition across national site locations and examining principal environmental drivers of composition.	H2, Climate and other plant-soil environmental variables relate to microbial community composition.	Rainfall, air temperature, soil temperature and soil moisture were all significant drivers of fungi, bacteria and archaeal communities, along with vegetation community change, the amount of bare ground, and in the case of the archaea, pH, *Sphagnum* and available soil nitrogen.
	Assessing whether key microbial taxa are climate specific.	H1a and H1b, Some microbial taxa are specific to climate categories e.g. dry sites.	Psychrotolerant species were found to be indicators in the coldest sites, and ericoid fungi indicative of sites higher in *Calluna* abundance, but climate categories were confounded by non-climate site differences: species indicators actually appeared to be related to specific sites/areas with heavy metal pollution tolerant species being indicators of the Peak District sites.
	Examining the impact of heavy metal pollution on microbial communities.	Heavy metal pollution is linked to differences in microbial community composition that is observed in the Peak District samples.	High levels of heavy metals were found in several sites without being restricted to the Peak District. Restoration in the Peak District was associated with generally lower levels of lead, copper, aluminium and iron than was found in previous work. Zinc and iron were significant variables associated with fungal community dissimilarity, with zinc, iron and

		This hypothesis was added to the analysis as a consequence of the results earlier in Chapter 4, addressing H1a and H1b.	magnesium showing the same relationships for the bacteria and calcium, magnesium and sodium affecting the archaea.
Five	Examining the impact of microbial measurements e.g. diversity and taxonomic change, on water quality, carbon sequestration and methane emissions.	Pore water quality differs between peatland management/site condition types with "intact" sites having lowest values. This was a preliminary hypothesis investigated before microbial relationships were investigated.	Water quality variables were variable nationally but did not differ between management/site condition categories. The principle driver of water quality variables is climate, as well as the fungal community and soil pH. DOC was highly variable across national sites with the Peak District having the lowest. Scottish sites had the highest SUVA values.
		H3. Microbial community composition and functional groups are linked to changes in water quality.	Piecewise SEM undertaken on national sites incorporating microbial communities explained 51% of the variance in SUVA, but did not explain DOC or Hazen. On the grouse moor sites, a pSEM incorporating fungal trophic groups explained 27% of the variation in DOC and 19% of the variation in Hazen.
		H3. Microbial community composition is related to Net Ecosystem Exchange and methane emissions.	Fungal community change was associated with changes in soil respiration but not NEE. Fungal and bacterial community change, as well as fungal Shannon diversity, were associated with methane emissions. All respiration indices were also affected by vegetation cover, except for the cut core soil respiration (from which plant roots were excluded).

6.2 Synthesis of Results from Empirical Chapters

Overview of the Studied Systems in this Thesis

This thesis drew its conclusions from two datasets: that obtained from the field mesocosm sample sites, and that obtained from the assembled mesocosms. Field samples were obtained in February 2019, along with environmental and topographic location measurements. Mesocosms were obtained at the same time, and then transported to York, United Kingdom, where they were assembled and kept, buried in the ground, in buckets with a consistent water level where they were continuously monitored for both water quality variables and gaseous fluxes. Due to the Coronavirus pandemic preventing the microbial sampling of the mesocosms, all of the microbial data could only be obtained in the field, as well as the environmental parameters such as bulk density, soil moisture and topographic information such as slope and elevation. However, gas fluxes were obtained from the mesocosms in York, with the monitoring beginning 6 months after mesocosm collection, and could be related to additional long-term flux monitoring data for only the Peatland-ES-UK grouse moor sites.

Here, we address how the results relate to the specific hypotheses outlined in Chapter 1 section 1.10

H1a. Microbial communities vary with management, with "active" or "intact" sites having higher diversity.

The microbial community significantly varied with management across the fungi, bacteria and archaea, and the principle difference was between land managed as grouse moor (the burnt, uncut and mown plots in the Peatland-ES-UK sites), the land previously managed as grouse moor (Moor House) and every other management/site category. The other managements (intact, degraded, 5 and 10 year post restoration in Scotland, Exmoor and the Peak District) did not differ between one another. Fungal trophic groups varied between managements, but only between burnt plots and everything else in the case of the symbiotrophs, and the burnt and uncut plots and everything else in the case of the saprotroph-symbiotrophs (although notably the uncut plots were burnt about 25 years prior to sampling as part of previous grouse moor burn rotation management before the Peatland-ES-UK experiment started).

Shannon diversity did not differ between managements in the fungi or the archaea, but did differ in the bacteria. In the bacteria, only Moor House was different, having much higher Shannon diversity, but notably these samples were collected a few months later in summer, possibly explaining this difference. The hypothesis that "active" sites (represented by the "intact" management categories) show higher diversity was not confirmed.

H1b. Microbial communities vary with national site location, controlled by climate variables.

Microbial communities significantly varied with national site location and every national site category had a different community across the fungi, bacteria, and archaea, except for Moor House (Northern Pennines) and the Yorkshire sites, which had no difference in their archaeal communities. Climate variables played a significant part in explaining this difference; for all three communities, rainfall and soil temperature were significant variables driving community variation in a distance-based redundancy analysis. This hypothesis was accepted.

H2. Measurements of the microbial community relate to environmental and edaphic variables.

The drivers of microbial community variables were assessed in chapters 3 and 4. Some soil edaphic variables were drivers of variation: soil moisture for the bacteria and fungi, peat depth (presumably related to hydrology) in the bacteria and archaea and in the archaea specifically, soil nitrogen and pH were significant. Vegetation appeared to play a lesser role: *Calluna* cover was related to fungal variation and *Sphagnum* being involved in the archaea. Potentially, this may be a "chicken and egg" situation: do we see few effects of vegetation outright because the vegetation community is also driven by climatic and edaphic factors anyway? This might well be borne out in the data: when climatic variables were excluded (or otherwise collapsed into proxies such as elevation, latitude and longitude) in Chapter 3, *Sphagnum*, *Molinia* and sedges were all drivers of bacterial taxonomic change, and in the archaea *Sphagnum* and *Molinia* were significant.

H3. Microbial community measures can be used to predict measures of carbon flux and water quality.

Chapter 5 found significant effects of microbial measures in relation to pore water quality variables, alongside environmental and edaphic variables. DOC was principally related to fungal diversity, pH and soil moisture ($R^2 = 0.73$) whilst SUVA was related to soil temperature ($R^2 = 0.84$) and Hazen was significantly related to the abundance of fungal pathotroph-symbiotrophs, although that model had much lower explanatory power ($R^2 = 0.31$). Piecewise structural equation models (pSEM), based on *a priori* hypotheses, found that SUVA is influenced by the bacterial and fungal communities, which are in turn affected by rainfall, soil temperature and the vegetation community. It is important to note that a structural equation model assumes causative relationships, but these cannot be proven by this analysis. A similar structural equation model was undertaken specifically in the Yorkshire sites, which investigated the difference between mown, burnt and uncut plots. This indicated that, as changes in the levels of saprotroph-symbiotrophs (which, along with the symbiotrophs, are significantly different in their relative abundance in the burnt plots) are associated with changes in DOC and Hazen, and that change in their abundance is linked to the amount of bare ground, soil moisture, and pH. In a separate pSEM, undertaken separately due to a low number of samples, the archaeal community change and pH were linked to variations in DOC.

6.3 The use of microbial communities in habitat monitoring

In the UK, blanket bog is recognised for its importance in terms of biodiversity, landscape value and ecosystem services provision, and consequently much of it is designated within National Parks, National Nature Reserves (NNR), Sites of Special Scientific Interest (SSSI), Special Protection Areas (SPA) and Special Areas of Conservation (SAC). Many of these have been adopted into protected areas known as *Natura 2000,* recognising the value of these sites in a European context: however, the author does not know how this will be affected by Brexit, although future changes will require legislation, and some designations such as the Natura sites are protected under international, rather than EU conventions (e.g. Bonn, RAMSAR). These blanket bogs are assessed for their condition using the upland Common Standards Monitoring (CSM) (JNCC, 2009). The CSM principally assesses blanket bog habitat based upon vegetation composition, as well as erosion (disturbance to the substrate) and management, including drainage features. The CSM does not include information on the carbon sink potential of a blanket bog, or its outflow water quality (although these are partly assessed indirectly by checking vegetation and drainage features), but these are potentially

important measures when assessing bog habitat quality, specifically within the context of an ecosystem services approach. In the future, habitats monitoring is likely to include measures of function, such as the amount of bare ground (linked to microbial community changes in this work – see Chapter 5 figure 12) and there are plans to introduce functional as well as structure/composition attributes, including basic microbial measures such as the ratio of fungi to bacteria, under a draft new Favourable Conservation Status Statement for blanket bog (Crowle *et al.,* In Prep.).

Soil and management features are used in the CSM for upland habitats (JNCC, 2009) as an assessment of disturbance to the substrate, and this includes the effect of drainage (drying) from drainage ditches, where a lack of surface wetness and pool formation in blanket mires is an indicator of poor feature condition. In addition, Natural England have been supporting the development of DNA techniques for a number of years for monitoring biodiversity (individual species and species assemblages) including as part of site condition of SSSIs (e.g. Tang *et al*. 2018, 2020; Price *et al*. 2020), but as far as the author could determine, this has not yet been applied to blanket bog species or sites. This thesis, overall, aimed to address whether microbial community data could be added to such an assessment and to what extent this would be useful.

One caveat to the work in this thesis is that national site and habitat condition categories were not based upon the same categories used in UK CSM habitat assessments. The split into intact, 5-year post restoration, 10-year post restoration and degraded was chosen for the sake of logistical simplicity, allowing local land managers to use their expertise to choose sites based on these criteria. Sites could have been assessed based upon whether they were favourable, unfavourable-recovering, unfavourable-declining etc. (See Annex 1, Natural England, 2019), but these do not take account of many soil related factors, and in some cases are assessments that have not been undertaken in some time – for example, the Peak District 5-year post restoration site (Unit 1015005 within the Dark Peak SSSI) was last assessed in 2015, and the Exmoor degraded sites in 2010 (Unit 1018511 within the North Exmoor SSSI). Whilst this does not discount their usefulness, it does mean that any changes since this last assessment of condition would not be included in the analysis.

One monitoring scheme that does incorporate microbial data successful is Natural England's Long Term Monitoring Network (LTMN). The LTMN continually monitors sites, mostly National Nature Reserves, over time, having started in 2011, and uses TRFLP profiles (discussed in Chapter 1 section 1.7). Whilst full results have not yet been reported, because only baseline surveys have been completed, these measures of genetic diversity will be useful for measuring disturbance from management and climate change, among other drivers (Nisbet *et al.,* 2017). Preliminary results suggest, similar to the data in this thesis, that microbial communities are different between many sites, even where environmental similarities exist (although there is clear overlap).

This thesis has indicated the extent to which environmental factors in blanket bogs influence microbial communities, but this is not consistent across a climate gradient, and so essentially every bog's microbial community is based upon the climate within which it sits, alongside a host of (historic) management related factors. This is similar to other research which found communities shifting along an (albeit larger, American continental) climate gradient (Seward *et al.,* 2020). As a consequence, microbial data cannot, with our current level of understanding, be used to compare sites spatially, in disparate national locations, but the use of TRFLP in the LTMN, and the linking of microbial communities to management related

environmental variables in this thesis, indicates that it can potentially be used for assessing condition over time.

Consequently, the results in this thesis can be used to conclude that the inclusion of microbial data in habitat assessments is not useful with our current level of understanding, unless it is used as part of long-term monitoring. Even with such continuous monitoring, from a cost-benefit point of view, regular microbial community sequencing would be costlier than making simpler, but potentially equally informative measurements such as temperature, pH and vegetation surveys. However, this does not mean that the results advise against microbial community composition sampling: it is highly likely that as our knowledge on the relationships between peatland microbial communities and ecosystem processes develops, re-analysis of previously collected data will prove fruitful. Indeed, this thesis is an example of where this might be the case: the lack of annotated fungi present in the data resulted in problems for analysis and will be improved in future. In that sense, the data collected here will have retrospective value. In the near future meta-analyses (combining a wider range of sites and managements) will likely achieve greater ecological insights, and sampling the range of Natural England CSM habitat conditions (e.g. 'Favourable', 'Near-favourable' etc.) would give an insight into how microbial community taxonomy and function maps on to our current understanding of peatland condition.

6.4 Areas for future work

Vegetation Burning and Water Quality

This thesis has not empirically linked rotational grouse moor burning with increases in DOC, SUVA, or Hazen. Neither of these variables were significantly different across the site categories (Chapter 5 section 5.2, also see Chapter 5 figure 7). However, an effect on water quality via the fungal community can be inferred. Saprotroph-symbiotroph fungi were significantly different between burnt plots and mown plots, as well as between burnt plots and all national sites, (although not different to uncut plots - Chapter 3 section 3.13). The burnt plots had the highest relative abundance of saprotroph-symbiotrophs of any management (approx. 25% - see Chapter 3 figure 6). In a piecewise SEM examining the relationships between microbial variables and pore water quality variables, saprotroph-symbiotrophs were found to be significant in their effect on DOC and Hazen, where as their abundance increased so did the respective water quality measures (Chapter 5 figure 12).

Consequently, the results presented here can only infer an effect of burning on water quality via saprotroph-symbiotrophs, and has not proved a causal link. It is difficult to interpret why this might be so – if higher levels of saprotroph-symbiotrophs are related to higher levels of DOC, then burnt plots with the highest levels of saprotroph-symbiotrophs should have the highest levels of DOC. The relatively low levels of sample replicates and high plot heterogeneity potentially explain this. The principal variables associated with an increase in saprotroph-symbiotrophs are pH, soil moisture and the amount of bare ground. In burnt plots where these three variables have not changed permanently, it is unlikely that saprotroph-symbiotroph relative abundance will have changed. The Peatland-ES-UK plots are more geographically disparate than the rest of the samples examined in this thesis (although they are all still a part of the same or immediately adjacent catchment – see Chapter 2 Figure 1)

and the three sites, which do have significantly different levels of DOC and Hazen, were all included in the analysis. Another explanatory factor may be time since burn – the samples in the Peatland-ES-UK study were examined 7 years after the initial burn, and legacy effects in pore water may not still be present. This latter explanation would fit with the literature, where pore water effects have been found immediately post burn (Clay *et al.,* 2009) but not after 10 years (Worrall *et al.,* 2007).

Whilst the observations made here do not casually link burning and fungi-mediated water quality change, they are interesting. It could be hypothesised that the removal of the photosynthetically active layer of vegetation by burning suddenly deprives symbiotic fungi of the carbon that arrives from the host plant. In such a situation, it is fungi that can switch trophic mode like the saprotroph-symbiotrophs, rather than the obligate symbiotrophs, that would be at an advantage being able to source alternative sources of food. It is obvious why bare ground might be associated with an increase in saprotroph-symbiotrophs, then, but the relationship with soil moisture and pH would make an interesting topic of further investigation. In addition to this, a more thorough understanding of exactly which species have been categorised into which trophic category by FUNguild, and their respective enzymatic abilities, would be of interest. Why are saprotroph-symbiotrophs associated with higher DOC? Do they have enhanced enzymatic capabilities that are able to mobilise (and make soluble) carbon that wouldn't ordinarily be so?

Calluna vulgaris as an ecosystem engineer

A significant proportion of the samples examined in this thesis were *Calluna* dominated. *Calluna* tends to climax towards dominance in grouse moor peatlands and this is aided by a rotational management regime where otherwise the community could likely change due to succession (Marrs *et al.,* 1986). In this thesis, *Calluna* abundance was driven by climate and management, and it was a driving factor in both the fungal and bacterial communities. *Calluna* abundance was in turn related to increases in SUVA values in the grouse moor sites (an effect that disappeared when fungal data was added to the model) as well as being a driver of Net Ecosystem Exchange across all samples.

Calluna may achieve such dominance by virtue of the environmental conditions within which it grows. It is a slow growing dwarf shrub (Gimingham, 1972) generally considered to be a plant that employs a stress tolerating, rather than competitive strategy (Grime *et al.,* 1988; Woodin *et al.,* 1992). It consequently grows well in the nutrient limited, colder, wetter and more acidic conditions of British upland blanket bogs, and changes to these conditions, by, for example, nutrient deposition or changes to grazing regimes, readily results in competition from grasses such as *Nardus stricta* (Hartley & Amos, 1999), *Deschampsia flexuosa* (Hofland-Zijlstra & Berendse, 2010) and *Molinia caerulea* (Aerts, 1993). It is likely that management benefits *Calluna* in terms of this competitive balance: reductions in grazing, for example, and the consequent reduction in nutrients from dung and urine will be of benefit in maintaining a nutrient poor soil. Managed burning may have mixed results: regenerating *Calluna* that is adapted to it and increasing germination (Måren *et al.,* 2010) – although, on the other hand, too frequent and severe burning is advantaging competitors such as *Molinia* (Brys *et al.,* 2005).

It is the fungal symbionts of *Calluna*, the ericoid mycorrhizas, which confer many of the adaptions required to survive in these peat environments, including resistance to low pH and

metallic soil constituents (Leake *et al.,* 1990). The mycorrhizas improve access to nutrients, and confer resistance to some toxic metals such as zinc (Martino *et al.,* 2000). These ericoid mycorrhizas allow *Calluna* to tolerate such, for other species, stressful environments, but it could be possible that in order to provide a competitive advantage, they also have a role in actively maintaining such for other species stressful conditions.

This could be achieved via extracellular controls on soil pH. It is well documented that roots can induce pH changes in the rhizosphere (Dehérain, 1902; see also Hinsinger *et al.,* 2002) principally via H^+ and OH^- release. This has also been observed in the arbuscular mycorrhiza, where mycorrhizal fungi change the pH of a medium up or down depending on nitrogen addition, decreasing the pH when no nitrate were added (Bago *et al.,* 1996, Bago & Azcón-Aguilar, 1997) and also where alkalinisation reduced the bioavailability of Cadmium (Janoušková, & Pavlíková, 2010). Such controls on pH might occur only very close to roots and fungal hyphae, but *Calluna* has an extensive root system of lateral adventitious roots extending into the upper layer, and by virtue of their fine structure (hair roots) and density (>30 km m^{-2} peat over the top 20 cm peat depth; see Heinemeyer *et al.*, 2011) outcompete the roots of *Nardus stricta*, as reported by Genney *et al.,* (2000). Importantly, whilst the authors found that ericoid mycorrhizal infected *Calluna* outcompeted *Nardus*, this was not due to changes in pH, but potentially some form of allelopathy (although, this experiment was completed with a single ericoid fungal isolate, which may not represent the full rhizospheric associations of *Calluna*).

Altering, or maintaining a specific rhizosphere pH might provide *Calluna* with a competitive advantage in some instances. In this thesis, *Calluna* abundance co-varied with the availability of Zinc (Zn), where, as the abundance of *Calluna* increased so did the levels of Zn in the soil (importantly this occurred across several different sites). Mycorrhizal infection provides a major degree of resistance to Zn toxicity (Bradley *et al.,* 1981). Zn availability, and therefore plant uptake, increases with decreasing soil pH (Sims, 1986). Consequently, maintenance of a low rhizosphere pH creates a soil environment high in Zn, tolerable to mycorrhizal *Calluna* but potentially harmful to other non-ericoid plants due to Zn phytotoxicity. This is borne out in the data here: in the *Calluna* dominated managed grouse moors, soil zinc levels were twice as high as in any of the other managements/national site categories (Chapter 4, Tables 16 and 17). This relationship also applies to cadmium and lead (Xian & In Shokohifard, 1989); two metals that were also much higher in the Yorkshire grouse moor sites. This ability, specifically in reference to Zn, may be flexible in terms of application; a study by Martino *et al.,* (2003) found strains of ericoid mycorrhiza behaved differently, mobilising insoluble zinc compounds depending on whether they were located in polluted or unpolluted soil, which they concluded represents specific strategies aimed at maintaining homeostasis of metals under different soil conditions.

Additionally, maintaining a specific rhizosphere pH may allow for efficient nutrient uptake balanced with the competitive advantage of tolerating heavy metals. For example, ericoid mycorrhizas do not confer any particular advantage in terms of phosphorous uptake (Read & Stribley, 1973), but maintaining a pH of around 4.5 would maximise the amount of free phosphorous available (in acidic soils) coinciding with the lowest degree of phosphorous fixation by iron, at pH <4.0, or aluminium, at pH > 5.0 (Penn & Camberato, 2019). This, together with a high density of fine and highly absorbing hair roots, should enable very efficient nutrient capture by *Calluna* plants.

Calluna may simply be a stress tolerant species coincidentally well adapted to a specific niche. However, an investigation to ascertain whether it can adjust its rhizosphere pH would be simple, and worthy. If it can, further questions arise concerning the applicability of this information to ecological restoration of formally bare peats; are there specific pH values or ranges, which can be targeted in order to improve restoration success? Moreover, whether *Calluna* is reactive or proactive in its pH requirements, and bearing in mind the aforementioned results of Martino *et al.,* (2003), are there specific species of ericoid mycorrhiza suited to specific soil conditions, which may be introduced in a form of "fungal inoculum" for the same aim? To the authors' dismay, whilst examining in detail the role of ericoid mycorrhiza was one of the aims of this thesis, this could not be undertaken due to the restrictions during the 2019/2020 coronavirus pandemic.

Unpacking the peatland "Black box"

Microbes are key to peatland functioning, but the underlying processes are still unclear. Whilst it is often known how aspects such as management and climate effect outputs such as carbon fluxes and DOC export, the processes that drive these relationships in the soil exists in somewhat of a "black box" (Ritson *et al.,* 2021). Unpacking this black box and understanding these processes would lead to an ability to not only understand but predict the effects of, or resilience to, management or climate related changes to below ground relationships, and consequently such knowledge would be useful in habitat monitoring.

This thesis has not unpacked this black box, but it offers a peek inside it. Environmental conditions have been linked to changes in water quality mediated by the bacterial and fungal communities, and changes in fluxes have been linked to changes in the fungal community. However, deriving insights only from taxonomy based community composition is difficult, because of community variations across latitudinal gradients found in this work, but also because of the potential for functional redundancy in peatland microbial species (Robroek *et al,* 2017, Kox *et al.,* 2020).

Our more interesting results – finding differences in fungal trophic groups related to environmental changes, were the result of functional data collection. This is probably the more informative approach versus taxonomy based analyses, because specific functional groups are more obviously linked to specific bog functions, for example, examination of the changes in expression of genes responsible for methane oxidation is much more likely to be linked to methane flux (e.g. Freitag *et al.,* 2010). Functional approaches can take many forms; from functional prediction of sequencing data using FUNguild or PICRUST (Jassey *et al.,* 2017) to trait based analyses (Malik *et al.,* 2020) as well as direct measurements of functional genes and gene expression (Steinberg & Regan, 2009, Freitag *et al.,* 2010). Because blanket bog processes are likely to be more closely related to gene abundances than species identity, focusing on the measurement of phenotypic characteristics like trophic mode, metabolism pathways, carbon use efficiency and growth rate is more likely to provide for a mechanistic understanding of the links between microbial community and C cycle processes such as gaseous carbon fluxes and water quality. Ultimately, an integrated approach is probably needed, empirically linking microbial community identity, and microbial processes with environmental parameters (Hall *et al.,* 2018).

6.5 Concluding remarks

Peatlands are a significant carbon store in the UK and the source of much of its drinking water. Managing them in a way that maximises these positive benefits whilst also including the needs of various stakeholders is clearly challenging. Continuous (long-term) monitoring of blanket bog condition seems crucial in understanding the impacts of various management or restoration strategies. It is desirable to expand our assessment of habitats: sampling faster, more often, and geographically more broadly. However, this comes with financial and logistical constraints. Already, sequencing technology allows us to take a single gram of soil and ascertain its microbial community (alongside a suite of environmental variables) but what is missing is not just the what and where (what microbial communities are associated with this habitat, and where are they) but also the why (underpinning conditions and processes).

This thesis has presented data that demonstrate the links between microbial community changes and differences in climate and management, and in relation to consequences for water quality and gas fluxes, especially in the heather-dominated grouse moor sites in Yorkshire. The major drivers of microbial communities in blanket bogs were principally driven by climate related variables such as rainfall and soil temperature, but also local management related variables such as pH and changes or differences in vegetation cover as well as variables effected by both management and climate, such as soil moisture. Fungal symbiotrophs were significantly lower in abundance in the burnt grouse moor plots, but showed no other differences between managements, and this has potential negative consequences for water quality via increased DOC and Hazen levels, suggesting burning has a modifying effect on peat microbial communities. It has also demonstrated that blanket bog microbial communities are different under even subtly different climate regimes, potentially stymying any notion that geographically disparate blanket bogs can be compared in terms of their microbial taxonomy.

The microbial community profiles here will greatly contribute to our understanding of how microbial communities vary across British upland blanket bogs. As ‘snapshots’ of community composition, they also provide the basis for future work assessing how these communities change not just spatially but also temporally. Further, the models presented in this thesis provide evidence for linkages between microbial communities and C-cycling, as well as water quality, which could form the basis for future focused study, possibly again making use of the here presented mesocosm design.

This study has contributed some key insights, focusing on UK blanket bog sites under contrasting habitat condition and management. In the future, a thorough understanding of microbial communities in peatlands may allow us not just to monitor but also to predict a functional output, a trajectory of change, or the effects of a particular management and possibilities of restoration options (Ritson *et al.,* 2021). However, first, a much deeper, mechanistic understanding of peat soil microbial processes is required, that incorporates taxonomic composition, function and biochemical understanding.

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