The Influence of Green Roof Substrate Composition on Plant Growth and Physiological Health

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Summary

Green roofs are becoming increasingly common in urban areas due to the range of benefits they provide to their host building and to wider surrounding areas. Green roofs are formed of a number of engineered layers culminating in substrate into which vegetation is planted. Despite the critical role of green roof substrate for plant growth and physiological health, surprisingly little empirical research has been carried out on the importance of green roof substrate composition.

This thesis aims to further our knowledge of the effect of green roof substrate composition on the growth and physiological health of green roof plants through greenhouse and module experiments. A wider scale sampling approach is also used to document substrate characteristics on established green roofs.

Greenhouse trials presented here show that relatively minor changes to green roof substrate composition can have major effects on plant growth, physiological performance and drought tolerance. Therefore it has been found that green roof substrate can be optimised for specific climates or service provision. Module trials showed that mycorrhizal networks can be successfully introduced to green roof substrate with commercial inoculum, increasing physiological health and flowering performance. Finally the physical, chemical and biological characteristics of three established green roofs were measured in order to assess inter- and intra-roof variability. This data has been used as the basis of a potential future green roof Substrate Health Index which could be used to assess the performance of green roofs substrate and to direct management.

Data from this thesis has been used by Boningale Ltd. the co-sponsoring company, to develop new products and services. Specifically a new range of green roof substrates as well as an award winning online substrate selector tool has been created as a direct consequence of this thesis. In addition, plans exist to commercialise further aspects of this thesis, most notably the development of pre-inoculated mycorrhizal plug plants.

Overall this work has shown that green roof substrate is vital for the performance of green roof vegetation and a combination of greenhouse, module and established roof sampling is needed to fully understand how substrates can be modified for optimal performance.

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Chapter 1

Introduction: The role of green roof substrate in the development of green roof technology

1.1 Development of Green Roofs

Green roofs are roofs with vegetation intentionally planted on them. Also known as living roofs, eco-roofs, planted roofs or vegetated roofs, they have experienced a large surge in popularity in recent years and are now a common feature in most western urban areas (Dunnett and Kingsbury 2010; Snodgrass and McIntyre 2010). Despite being used on buildings throughout the world for hundreds of years, architects and urban planners have only relatively recently started to use green roofs in modern urban developments (Oberndorfer et al. 2007).

Modern green roof technology has its origins in early 20th Century Germany where vegetation was used on buildings to reduce the damage of roof surfaces by sunlight and the risk of fire on tar-paper-gravel roofs (Köhler and Poll 2010). Further research in the 1960/70's by Prof. Hans-Joachim Liesecke and Dr. Walter Kolb provided the majority of the groundwork for the development of the modern commercial green roof (Dunnett and Kingsbury 2010). The creation of a green roof study group within the FLL (Forschungsgesellschaft Landschaftsentwicklung Landschaftsbau: *The Landscaping and Landscape Development Research Society*) in 1977 led to the first definition of green roof

specifications and industrial guidelines which have continued to direct green roof construction standards to the present day, including the present UK GRO standards (FLL 2008; Dunnett and Kingsbury 2010; GRO 2011).

1.2 Green Roof Classification

Modern green roofs can be split into three different categories depending on their substrate depth and composition: extensive, semi-intensive and intensive (Table 1.1) (Fig. 1, 2, 3). By far the most common green roof is the extensive type (Fig. 1) due to its low weight loading and therefore greater suitability for retrofitting onto existing roofs that have not originally been built with the need to support a green roof (Dunnett and Kingsbury 2010; Snodgrass and McIntyre 2010). Semi-extensive roofs have the same type of substrate composition as extensive but generally to a deeper depth, whilst intensive roofs refer to anything with a substrate depth over 150mm and/or with substrate with large amounts of organic matter. As the vast majority of green roofs currently constructed are extensive, the term green roof will be used throughout this thesis to specify extensive green roofs.

1.3 Benefits of Green Roofs

As green roofs have become more common in urban areas the benefits that they can provide have become more understood. These benefits, or green roof services, are provided to the host building as well as the surrounding area and include energy savings from the cooling and insulation of a building, reduced impact of urban heat islands (UHI), storm water attenuation, reduced air and sound pollution, increased urban biodiversity as

well as improved aesthetic properties and psychological benefits (Getter and Rowe 2006; Oberndorfer et al. 2007) (See summary in Table 1.2).

1.4 Green Roof Design

Modern green roofs all follow a similar design pioneered in Germany in the 1970/80's which involves adding various additional layers to an existing or new roof (Fig. 1.4, Table 1.3). These layers have been designed to work in tandem with one another and therefore the removal of some layers may result in reduced green roof performance. As many green roofs are retrofitted to buildings additional layers of structural support are sometimes added to the existing roof in order to support the new load. A waterproof layer is added first and is designed to prevent any moisture leakage into the host building. This is protected from any damage by excessive root growth with a root proof membrane. Specially designed drainage layers are placed between waterproof layers and the substrate in order to aid drainage from the substrate and to also sometimes act as an additional water reservoir (Fig. 1.5) (Dunnett and Kingsbury 2010; Snodgrass and McIntyre 2010). It is also becoming more common for an additional water retention mat to be used below the drainage layer to further increase water holding capacity (Savi et al. 2013). A filter layer is usually placed on top of the drainage layer to prevent substrate loss. Substrate is added to this structure to a depth of usually 80-120mm. Finally plants are added to the substrate in the form of either a) pre grown *Sedum* or wildflower blankets b) *Sedum* cuttings c) plug plants d) direct seeding.

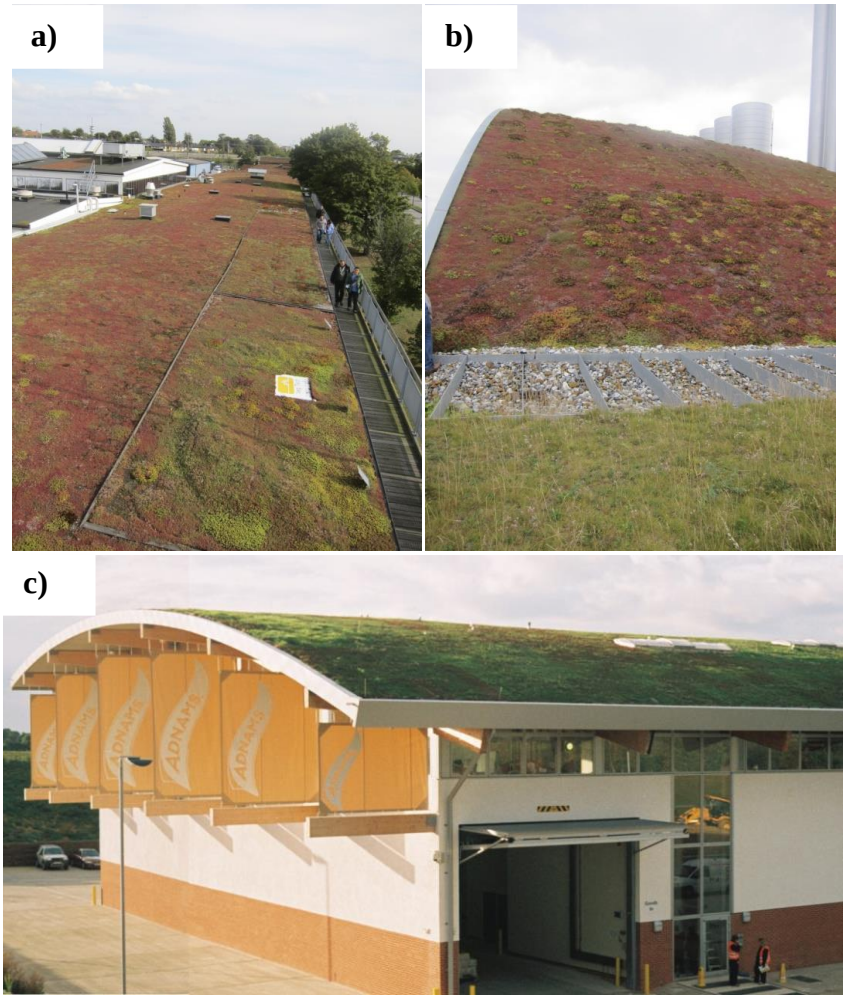


Figure 1.1: Typical extensive *Sedum* spp. green roofs. Going clockwise; a) Augustenborg Urban Development, Malmo, Sweden, b) Kastrup Power Plant, Copenhagen, Denmark, c) Adnams Brewery, Southwold, UK. Photos by Elisa Olivares Esquivel and Jeff Sorrill, University of Sheffield.

Figure 1.2: Typical semi-intensive green roof located on Sharrow School, Sheffield, UK. Substrate depths vary from 60-300mm. Photo by Jeff Sorrill, University of Sheffield.

Figure 1.3: Typical intensive green roof located West One development, Sheffield, UK. Substrate >250mm. Photo by Jeff Sorrill, University of Sheffield.

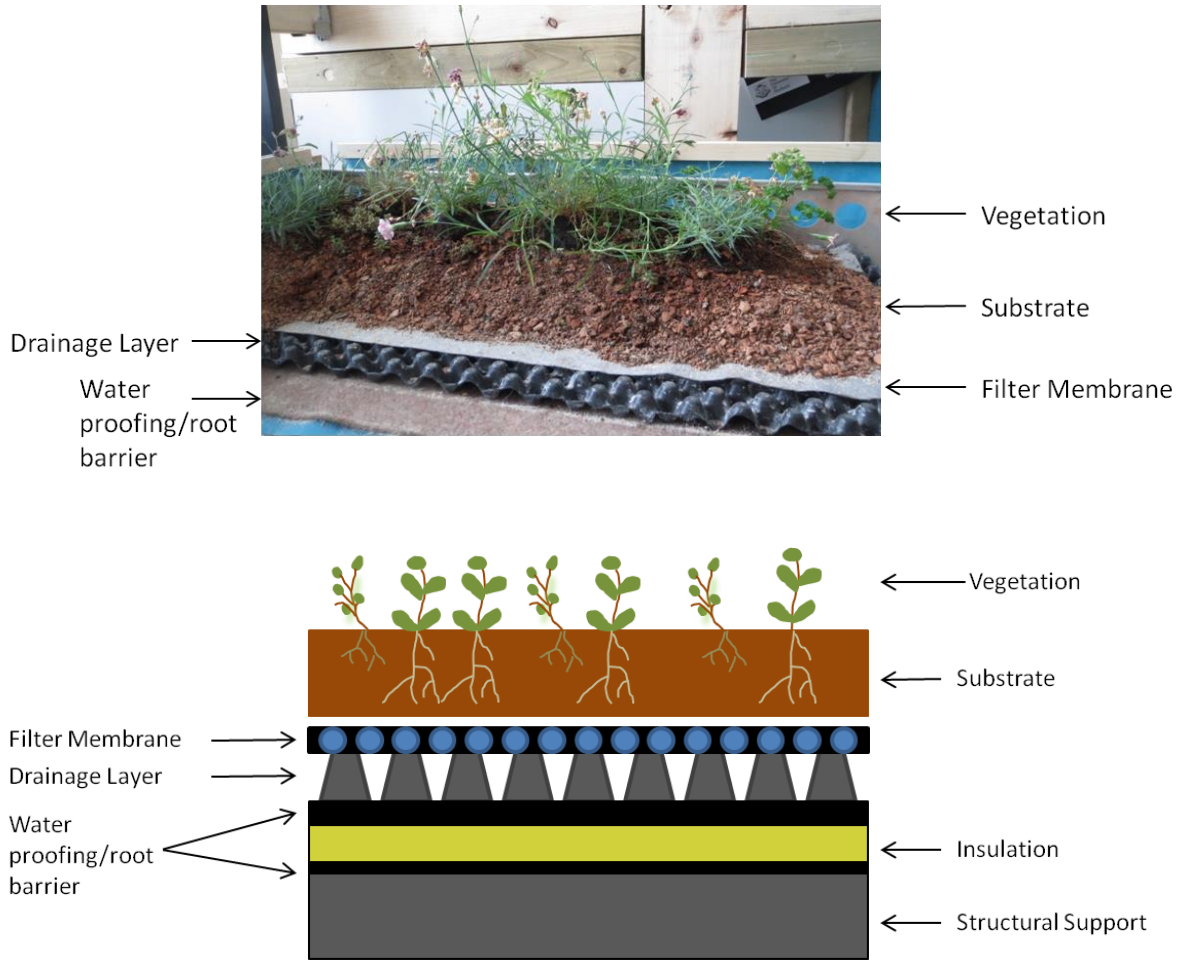


Figure 1.4: a) Small scale model of a standard extensive green roof. b) Cross Section of a Standard Extensive Green Roof (adapted from Oberndorfer *et al.*, 2007). Photo by Thomas Young, University of Sheffield.

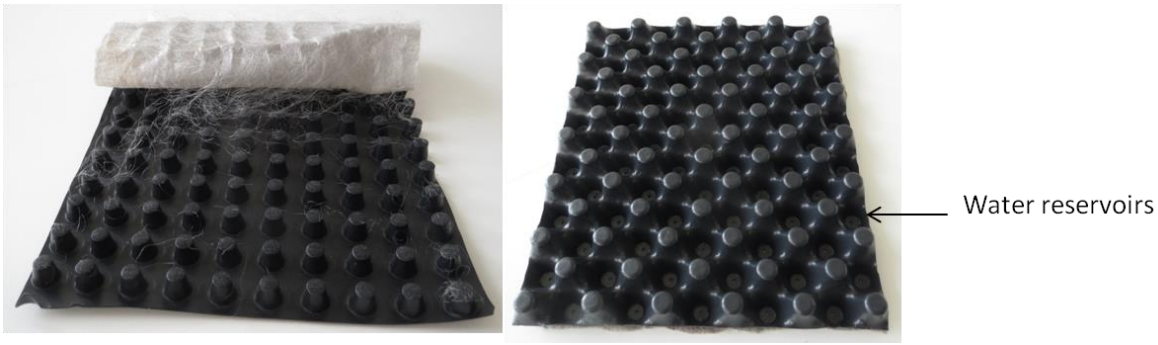


Figure 1.5: a) Common type of drainage board b) Drainage board with water storage capacity. Photo by Thomas Young, University of Sheffield.

Table 1.1- Three main modern green roof classifications (Dunnett and Kingsbury 2010; Snodgrass and McIntyre 2010)

Characteristic	Extensive	Semi-Intensive	Intensive
Substrate Depth	40-100mm	80-120mm	150mm+
Substrate Composition	Lightweight, low organic matter (0-20%)	Lightweight, low organic matter (10-20%)	Higher density, higher organic matter (20-40%)
Maintenance	Low (annual)	Medium (bi-annual with some vegetation clearance)	High (regular weeding and vegetation cutting required)
Weight Loading	Low (60–150 kg/m^2)	Low/Medium	High (>300 kg/m^2)
Irrigation	No irrigation required	Some irrigation may be required	Irrigation required depending on species selection
Planting	Mixture of *Sedum* spp., some drought tolerant perennials and grasses	Mixture of *Sedum* spp., dry meadow spp., drought tolerant perennials, grasses and alpines	Mixture of *Sedum* spp., dry meadow spp., drought tolerant perennials, grasses, alpines, turf grass, sub shrubs, shrubs, edible plants, small trees

Table 1.2- Summary of the main services that green roofs provide to their host building and to the surrounding area.

Green Roof Benefit	Mechanism	Additional Comments
Building Cooling	Green roof reduces heat transfer into building from the roof (Castleton et al. 2010; Jaffal et al. 2012). Vegetation shades the roof and absorbs solar radiation whilst substrate acts as insulation layer (Fioretti et al. 2010; Saadatian et al. 2013). Passive cooling may occur from evaporation of moisture from substrate as well as transpiration from vegetation (Ouldboukhitine et al. 2014).	Can shift internal temperature peak to 1-5 hours later in day i.e. when building is not occupied or when cooling is needed less (Simmons et al. 2008; Spolek 2008). Less heat is emitted to the building throughout the night, reducing the amount of cooling needed the next day (Castleton et al. 2010). Plant form, transpiration rate and biomass structure all strongly determine cooling performance (Jim 2012; Blanusa et al. 2013)
Building Insulation	Green roof acts as an extra insulation layer for the building and therefore can help to maintain higher internal temperatures (Castleton et al. 2010; Berardi et al. 2014). Substrate also acts as thermal mass which retains heat and slowly releases it (Castleton et al. 2010; Saadatian et al. 2013).	A green roof will provide insulation benefits to buildings without modern roof insulation, (Castleton *et al.*, 2010) however when a building possesses modern roof insulation the additional benefits of a green roof are negligible (Niachou et al. 2001).
Reduced Urban Heat Island (UHI)	Cumulatively the individual cooling effects of each roof can reduce the severity of an UHI through increased albedo and evapotranspiration (Bowler et al. 2010b; Susca et al. 2011).	Impact of green roofs on UHI depends on climate of city, with vegetation likely to have a much larger impact in hotter drier cities (Alexandri and Jones 2008). Widespread implementation of green roofs is expected to reduce urban air temperatures by 0.3-3^{o}C (Berardi et al. 2014; Santamouris 2014).
Rainwater Attenuation	Vegetation on green roofs intercepts rainfall and slows the speed of runoff, delaying the time taken to reach peak runoff (Stovin 2010). A proportion of the runoff from rainfall events will be retained in the substrate and vegetation and slowly released through evaporation/transpiration, reducing the total amount of runoff that a roof produces (Berndtsson 2010).	A number of factors influence the ability of a green roof to attenuate rainwater which include substrate type & depth, vegetation type & coverage, roof age, slope of roof, preceding weather conditions and type of rainfall (Berndtsson 2010). Reviews of extensive green roof runoff studies conclude that annual runoff can be reduced by roughly 45-100% depending on rainfall intensity and longevity (Mentens et al. 2006; Berardi et al. 2014).

Biodiversity	Green roofs can act as an important habitat in urban areas for various plant and animal species (Dunnett 2006; Dunnett and Kingsbury 2010).	Vegetation on the roof can attract and benefit pollinators (Tonietto and Fant 2011), whilst the substrate can also support various insects and birds as well as many plant species including rare orchids (Brenneisen 2005; Kadas 2005; Fernandez- and Gonzalez-R 2010; Köhler and Poll 2010; MacIvor and Lundholm 2011a; Madre et al. 2013; Madre et al. 2014). Substrate and type of vegetation present have a large influence on biodiversity, with extensive roofs likely to support a smaller amount of species (Dunnett and Kingsbury 2010).
Carbon Sequestration	Carbon is taken up by plants through photosynthesis and then sequestered in plant biomass and substrate (Getter et al. 2009). Overtime a green roof will reach a carbon equilibrium but could still act as a net sink for carbon (Rowe 2011).	Lack of experimental evidence but extensive Green Roofs in Michigan and Maryland, USA, sequestered 375g $C.m^{-2}$ over a period of two years (Getter et al. 2009). Long term dynamics of carbon balance in green roof substrates is unknown. Imbedded carbon costs of roof construction may outweigh amount of carbon sequestrated by vegetation (Rowe 2011). Depth of substrate and vegetation type can influence amount of carbon sequestered (Rowe 2011; Whittinghill et al. 2014)
Noise Abatement	Vegetation, substrate and air pockets on the roof absorb sound waves to a greater extent than conventional roofs which are generally hard and reflect a much larger amount of sound (Rowe 2011).	Van Renterghem & Botteldooren (2009) observed a linear decrease in sound pressure on opposite side of building to sound source with increasing green roof coverage (Van Renterghem and Botteldooren 2009).
Reduced air & water pollution	Direct uptake of gaseous pollutants by vegetation and interception of particular matter on leaves (Rowe 2011). Reduction of local air temperatures could lead to a decreased amount of photochemical reactions which lead to formation of ozone (Rowe 2011).	Large amount of work on ability of plants to improve air quality but little specific research on green roof plants (Rowe 2011). A number of models have predicted that green roofs can reduce air pollution (NO_3, O_3, PM_{10}, & SO_2) (Currie and Bass 2008; Yang et al. 2008). Intensive roofs are more effective than extensive (Currie and Bass 2008). Runoff from green roofs is generally cleaner than conventional roofs due to the uptake of pollutants by plants (Rowe 2011). However this effect is still poorly understood and green roofs, (in particular newly constructed roofs) can cause increased levels of pollutants in runoff due to fertilizer leaching and decomposition of organic matter (Aitkenhead-Peterson et al. 2011; Rowe 2011; Speak et al. 2014).

Aesthetic and Psychological	In neighbourhoods where ground-level recreational or green space is at a premium, green roofs can provide additional green space. Attractive well planted green roofs can (depending on an individual's discretion) improve the aesthetics of a building (Hartig et al. 1991; Dunnett and Kingsbury 2010; Lee et al. 2014).	Few studies have been conducted on the physiological benefit of green roofs but they are likely to be similar to those of other green spaces in urban areas (Bowler et al. 2010a). These can include increased sense of well being, stress reduction creative thinking and calm well being (Hartig et al. 1991; Loder 2014).
Economic Savings	The energy savings from reduced cooling/heating can be translated into direct savings on energy bills (Niachou et al. 2001; Castleton et al. 2010; Jim 2014). A green roof may increase the lifespan of a conventional roof by 20-45 years by protecting waterproofing membranes from large temperature fluctuations and UV light (Oberndorfer et al. 2007; Clark et al. 2008).	The improvement of air quality, reduction in storm water runoff and enhanced aesthetic value can also have an economic benefit, although this is hard to quantify (Clark et al. 2008). Rent prices have also shown to be 16% higher in New York apartments where a Green Roof is present (Ichihara and Cohen 2011). There is also some evidence to suggest that the cooling effect of an extensive Green Roof can improve PV cells output by 1.3-6% (Köhler et al. 2007; Chemisana and Lamnatou 2014). Urban vegetable production is also possible on green roofs and can provide an additional source of income for buildings (Whittinghill and Rowe 2012; Whittinghill et al. 2013)

Table 1.3 - Description and order of common green roof construction layers. Adapted from (Getter and Rowe 2006; Oberndorfer et al. 2007; Dunnett and Kingsbury 2010; Snodgrass and McIntyre 2010)). Note that some green roof architects design roofs with only three layers (water proof layer, geotextile layer and substrate) due to the increased simplicity of such systems.

Layer	Function	Additional Comments
Structural support	Provides additional support for green roof as many existing roofs are not designed to support the additional weight of a green roof.	Green roofs, although designed to be as lightweight as possible can become extremely dense when wet.
Insulation	Provides additional insulation to the host building.	Insulation is usually standard polystyrene slabs roughly 85mm thick (Kosareo and Ries 2007). Cooling effect of green roofs is higher when additional insulation is used (Jim 2014).
Water proof membrane	Provides an additional water proof layer above the roof and ensures that no leaks occur.	Three main types; 1. Single ply plastic/rubber membranes 2. Fluid applied membranes 3. Bitumen/asphalt roofing felt
Root proof barrier	Protects waterproof layer against root penetration and the activity of micro organisms in substrate.	Usually composed of PVC (0.8-1mm thick) rolls which are adhered together to prevent any weak points that can be exploited by roots.
Drainage layer	Efficient drainage of rainwater is extremely important for green roofs in order to prevent damage to the roof membranes, excessive weight loadings, and poor anaerobic growing conditions for plants.	All green roof drainage should occur below the surface as underflow which should only occur once a substrate is saturated. Many green roofs now use drainage layers that also have the capacity to store water and therefore act as an additional water reservoir for plants as well as further reducing runoff. Sometimes a water absorption mat is also used to increase water retention. Common systems used include; 1. Plastic drainage modules which allow water storage as well as drainage 2. Porous mats made from recycled materials which can absorb large volumes of water 3. Use of granular materials (gravel, clinker, pumice, broken tiles) to provide a well aerated space for excessive water
Filter membrane	Prevents fine particles from the substrate from clogging up drainage layer and drainage outlet.	Using constructed with a semi-permeable polypropylene mat.
Substrate	The substrate acts as an artificial soil and as such must provide plants with physical support, water and nutrients. It must also be free draining, lightweight, well aerated, have low fertility and chemical stability.	Substrate must have low fertility in order to prevent excessive plant growth which increases maintenance and reduces plant drought tolerance and also to prevent undesirable weed species establishment. Substrates are therefore usually composed of around 20% organic matter (bark, green waste compost, coir, peat) and 80% mineral matter (pumice, crushed brick, heat expanded clay, broken tiles, perlite, crushed concrete).

Vegetation	Provides the 'green' aspect of a green roof. Plants provide the majority of green roof services through transpiration which cools the building and recharges storm water holding capacity, by having a large surface area to absorb air and noise pollution and also increasing the roofs aesthetic value.	The depth of substrate is the main driver of green roof plant selection with a general rule that deeper substrates are able to support a larger range of plants. The primary selection criterion for plants is their ability to survive in the inhospitable environment of a green roof where they are exposed to drought, high substrate temperature extremes, low nutrients and high wind shear. The following planting regimes are often found at these depths; 0-50mm: *Sedum* (other succulents are used) and moss 50-100mm: *Sedum*, dry meadow spp, drought tolerant grasses and low growing perennials and alpines 100-200mm: Dry habitat perennials, grasses and annuals, small shrubs, turf grass 200-500mm: Medium shrubs, edible plants, generalist perennials and grasses

1.5 Green Roof Substrate

1.5.1 Substrate Composition

Substrate is critical to the performance of green roofs since plant growth and health directly depends on the substrate (Ampin et al. 2010). Unlike other types of growing media green roof substrate is highly engineered and can contain a wide range of materials (Table 1.4). Therefore substrates can be extremely variable in their composition, which is likely to have significant impacts upon green roof vegetation. For example commonly used mineral components of substrate (e.g. crushed brick, pumice, tiles, heat expanded clay) are supplied at a wide range of particle sizes which affects the physical properties of the substrate and therefore ultimately plant growth and performance (Graceson et al., 2014; Olszewski and Young, 2011). Similarly the type of organic matter used will have a large influence on the amount of nutrients available, substrate biological activity and

therefore also plant growth and performance (Nagase and Dunnett 2011). Despite this there has been a lack of scientific research into how green roof substrate composition affects green roof plant growth and ultimately the performance of a green roof (Graceson et al., 2014).

There are two general types of green roof substrates, commercial (developed by green roof companies) and non-commercial (developed by individuals or research institutions) (Ampin et al. 2010). Non-commercial mixes are usually based on basic guidelines given in the FLL, however commercial mixes are often confidential and therefore not widely known (Emilsson and Rolf 2005). Despite this, most green roof substrates are specified to vary little in their physical characteristics as they all need to be lightweight, stable, well aerated, free draining and able to retain nutrients (Friedrich 2008), however this may not be the case on installed roofs (See Table 1.4 for a summary of common substrate components).

1.5.2 Effect of Substrate Depth on Plant Growth

Substrate depth is nearly always restricted on a green roof since it directly increases weight and volume of substrate needed (and hence cost of supporting the roof and purchasing the substrate). Since substrate depth is typically quite shallow (80-120mm) it is one of the main factors affecting plant growth and diversity (Dunnett and Kingsbury 2010; Madre et al. 2014). Increasing the depth of substrates usually leads to greater plant survival, coverage, biomass production and drought tolerance (Durhman et al. 2007; Getter and Rowe 2008; Thuring et al. 2010; Olly et al. 2011; Rowe et al. 2012), which are

due to increased moisture retention (VanWoert et al. 2005a; VanWoert et al. 2005b), nutrient availability and increased buffering against freezing (Boivin et al. 2001).

Some *Sedum* species can survive in shallow substrates (25-40mm) but nearly all species tested show a preference for deeper substrates of 75-120mm (Durhman et al. 2007; Thuring et al. 2010). However for some species, increasing substrate depth from 70mm to 100mm (Getter and Rowe 2008), or from 40mm to 100mm (Getter and Rowe 2007) makes no difference to their initial growth coverage and survivability. Long-term survival tends to be greater in deeper substrates (Rowe et al. 2012). In some instances the timing of planting may more important than substrate depth for the initial survival of vegetation (Getter and Rowe, 2007). A general rule is that deeper substrates can support a much wider range of plants, although greater diversity of plants may be found in shallower substrates as greater environmental stress prevents a small number of species from dominating (Dunnett et al. 2008b; Rowe et al. 2012). In addition heterogeneity in substrate depth across a green roof can further improve diversity by encouraging species co-existence and providing niches for different plant species (Heim and Lundholm 2014b; Lundholm et al. 2014).

1.5.3 Effect of Substrate Composition on Plant Growth

The amount and type of organic matter in substrates is key to green roof plant growth and survival. Greater amounts of organic matter increase nutrient and water availability which results in greater growth, as seen for Sedum *spp.*(Rowe et al. 2006a; Olszewski et al. 2010; Graceson et al. 2014b) and herbaceous perennials and grasses (Rowe et al. 2006a; Molineux et al. 2009; Nagase and Dunnett 2011; Graceson et al. 2014a). However,

luxuriant growth of plants is not desirable on green roofs as it can lead to reduced drought tolerance as plants have more biomass to maintain and a lower root:shoot ratio (Rowe et al. 2006a; Nagase and Dunnett 2011; Rowe et al. 2012). It is now generally accepted that an optimal amount of organic matter for a sustainable and diverse plant community is between 10-20% (Rowe et al. 2006a; Ampin et al. 2010; Nagase and Dunnett 2011). The type of organic matter will also affect plant growth through the initial amount of plant available nutrients, rate of decomposition and therefore nutrient release, as well as stage of organic matter decomposition (WRAP 2011).

Table 1.4 - Description of commonly used green roof substrate components. Adapted from (Ampin et al. 2010; Dunnett and Kingsbury 2010; Snodgrass and McIntyre 2010).

Substrate Component	**Materials Used**	**Comments**
Natural Minerals	Sand, clay, gravel, lava & pumice	Gravel not often used due to its heavy weight and poor nutrient and moisture retention capabilities. Sand is used sparingly due to its small particle size and high density. Clay has good nutrient and water retention as well as cation exchange but can clog up drainage system if used in large quantities. Lava and pumice are lightweight and have high porosity but can be expensive.
Artificial/Modified Minerals	Expanded shale, clay & slate (ESCS). Perlite, vermiculite & rockwool	ESCS and perlite are porous, lightweight and have good nutrient and water retention, although are too lightweight to make up majority of substrate and have an extremely high embedded carbon value (Peri et al. 2012).
Recycled Waste	Crushed clay bricks/tiles and concrete. Paper ash, quarry fines sewage sludge and sub soil from construction (Molineux et al. 2009)	Generally quite heavy and poor at nutrient/water retention. Often used in brown/biodiverse roofs as they are common components of derelict urban habitats that the roofs are trying to recreate. Waste can contain pollutants e.g. lead from old paint that may contaminate runoff (Alsup et al. 2010).

Plastic Foams	Encapsulated styrofoams & resin foams	Very light but poor nutrient and water retention. Can degrade over time and have low nutrient composition.
Organic Matter	Peat, compost (bark, poultry litter & green waste) & coir	Peat has high nutrient content, water holding capacity and cation exchange capacity although can be expensive and can experience decomposition which leads to media shrinkage and nitrogen depletion. Compost also has high nutrient content but can be of variable quality as well as salinity. Coir has extremely high water holding capacity but can be deficient in calcium and sulphur.
Water retention additives (Super Absorbent Gels)	Cross-linked polyacrylamide gel, cross-linked polyacrylic acid-potassium salt, silicate additive (Farrell et al. 2013; Savi and Marin 2014)	Designed to absorb large amounts of water relative to their own mass (2000g/g). The water stored in gels is slowly released back to the substrate as drying out occurs reducing the likelihood of plants experiencing drought stress (Hüttermann et al. 2009).
Fertilizer	Slow release pellets	Fertilizer must be able to have long lasting effect to reduce maintenance of roof. Amount must also be carefully regulated as too much fertilizer will negatively affect runoff quality as well as make the roof more susceptible to invasions from undesirable plants.

The physical properties of substrates are highly influenced by the type of inorganic materials used in green roof substrate (Graceson et al. 2013). Such materials include crushed brick, pumice, heat expanded clay and slate, and crushed tiles. The characteristics of these materials in turn influences plant growth and health (Olszewski and Young 2011). Increasing the number of finer particles in a substrate increases its water holding capacity which in turn increases the growth of plant species (Molineux et al. 2009; Olszewski and Young 2011; Graceson et al. 2014a). An optimal particle size distribution should include a wide range of particle sizes in order to prevent water logging, increase substrate aeration/porosity and reduce bulk density (Ampin et al. 2010; Graceson et al. 2013).

However it is not widely known how altering the physical properties of a substrate affects plant resistance to environmental extremes e.g. drought. Increasing water holding capacity (WHC) may provide a greater water reservoir for plants, but may also encourage excessive growth which could lead to plants actually being more vulnerable to drought in the long term (Nagase and Dunnett 2011; Bates et al. 2013).

In recent years there has been increased interest in using additional substrate amendments, for example polyacrylamide water absorbent gels, to further increase the WHC of substrate during droughts (Farrell et al. 2013; Savi and Marin 2014). However it is not clear what effect amendments such as these have on plant growth and performance during ambient or extreme climatic periods. In addition it is not known how their performance is affected by the physical and chemical characteristics of the substrate they are placed in.

1.5.4 Substrate Biology

Very little empirical work has been conducted on biological populations within green roof substrate despite the importance of such populations in nutrient cycling and success of plant communities (Wardle et al. 2004; Rumble and Gange 2013). Of the very little work that does exist, it has been shown that beneficial Arbuscular Mycorrhizal Fungi (AMF) can colonise a number of roofs/trial plots, although it is not clear how this may affect plant growth or performance (McGuire et al. 2013; Rumble and Gange 2013). In many other anthropogenically made/altered biological systems AMF is often artificially introduced to the soil in order to sustainably increase plant growth and physiological performance

(Smith and Read 2008). The use of AMF in green roof substrates could potentially be extremely beneficial for green roof plants, however the use of this technology on green roofs has not yet been fully explored (Sutton 2008; Molineux et al. 2014).

In addition, established green roof substrate has been shown to host populations of important soil conditioning *Collembola* species, although these populations are highly variable and often low in density (Schrader and Böning 2006; Rumble and Gange 2013). It is expected that other important soil conditioning invertebrates such as earthworms cannot survive in green roof substrate due to low moisture and organic matter levels (Brenneisen 2005; Schrader and Böning 2006; Molineux et al. 2014). However only one German study has directly looked at earthworm numbers (Steiner and Schrader 2002). There have also not been any studies which have focused on how biological characteristics of green roof substrate are affected by substrate composition and how this impacts on plant growth and performance.

Like soil, green roof substrates are dynamic systems which will change over time. The physical and chemical characteristics of green roof substrate from different aged roofs (20-50 years old) have shown high variance, with older roofs showing greater levels of organic matter, shallower depths and higher pH (Köhler and Poll 2010; Thuring and Dunnett 2014). However it is not known how the biological characteristics of substrate influence this development and whether other types of green roofs experience similar substrate development. There is also a lack of available data on the physical, chemical and biological characteristics of established green roof substrate (Emilsson 2008; Köhler and Poll 2010; Rumble and Gange 2013) which means that an accurate benchmark of successful green roof substrate characteristics is not currently available.

1.5.5. A Health Index for Green Roof Substrate

Soil Health Indexes are used in agriculture as an objective method to assess the relative quality of soils (Karlen et al. 2008). These measure a number of physical, chemical and biological soil characteristics which can then be compared to the past performance of similar systems in order to assess how well or 'healthy' particular soils are (Andrews and Karlen 2004; Karlen et al. 2008). The development of a similar method for established green roof substrate could provide green roof practitioners with more practical advice on how to manage green roofs for optimal performance.

As a dynamic living system green roof substrate will change its physical, chemical and biological characteristics over time. However current FLL guidelines do not specify how green roof substrate is expected to develop over time once installed (FLL 2008). Much more work needs to be done in order to understand how green roof substrate performs as a vital living biological system in its own right and not just as a physical media host for plants (Rumble and Gange 2013).

1.5.6 Effect of Vegetation on Green Roof Service Provision

The presence of a diverse and healthy plant community is vital for the provision of high quality green roof services (Lundholm et al. 2010; Cook-Patton and Bauerle 2012). Increased plant structural and life form diversity can increase canopy interception and evaporation of rainwater, which reduces runoff and helps storm water management (Dunnett et al. 2008a; Wolf and Lundholm 2008; Lundholm et al. 2010). Transpiration from plants recharges the water storage capacity of the substrate and also cools the roof by absorbing heat energy (Castleton et al. 2010; Voyde et al. 2010). However, vegetation

on green roofs is typically dominated by water efficient succulents (*Sedum spp.*) which have extremely low transpiration rates and growth forms (Wolf and Lundholm 2008). This gives a much lower cooling performance, and increases the time taken for the substrate to recharge its water storage capacity compared to other types of vegetation such as broad leaved perennials (Wolf and Lundholm 2008; Lundholm et al. 2010; MacIvor and Lundholm 2011b; Blanusa et al. 2013). Vegetation also cools by directly absorbing or reflecting heat due to its higher albedo compared to bare roofs (Castleton et al. 2010), with structurally diverse vegetation systems showing a greater cooling capacity (Kolb and Schwarz 1986; Cook-Patton and Bauerle 2012). It is possible that despite their low transpiration rates the presence of hardy succulents like *Sedum* can improve other less hardy plant performance during drought stress by reducing substrate temperatures (Butler and Orians 2011). Therefore the selection of plants currently used on green roofs could be expanded by using *Sedum* 'nurse' plants which facilitate the growth of other less hardy plants which have greater cooling ability (Butler and Orians 2011).

Greater plant diversity and complexity of growth forms can also increase noise and air pollution absorption due to the increased leaf surface area (Cook-Patton and Bauerle 2012; Speak et al. 2012; Weber et al. 2014). A wider variety of wildlife can also be supported by a more diverse plant community, for instance the diversity and abundance of bee and arthropod populations. (Brenneisen 2005; Tonietto and Fant 2011; Cook-Patton and Bauerle 2012; Madre et al. 2013).

A green roof that supports a wider range of plant species and structural forms (plant functional groups) should provide a higher level of green roof services in the long term due to the reasons outlined above. In addition greater species diversity in analogous plant

communities has also been shown to increase plant resilience to environmental extremes by compensating for less tolerant plants and also by increasing resistance to disease and weed invasions through maintaining full plant coverage and greater genetic diversity (Cook-Patton and Bauerle 2012).

However the long term establishment of a diverse and healthy green roof plant community is inherently determined by the properties (Emilsson 2008; Graceson et al. 2014a) and depth of substrate (Dunnett et al. 2008b; Madre et al. 2014). Therefore if a poorly designed or too shallower substrate is used on a green roof, plant diversity and health will be adversely affected and therefore also the provision of green roof services (Dvorak and Volder 2010; Cook-Patton and Bauerle 2012).

1.6 Key Gaps in Green Roof Substrate Research

Green roof substrate has been relatively neglected in terms of biological and soil scientific research and a number of significant research gaps exist (Nagase and Dunnett 2011; Farrell et al. 2013). These encompass all areas of a substrate's life cycle;

1. Designing and manufacturing substrate mixes.

Although many green roof companies use standard industrial substrate mixes, there is little available information on how altering substrate materials and individual components can change the physical, chemical and biological characteristics of substrate (Graceson et al. 2013; Graceson et al. 2014b).

2. The effect of substrate composition on plant growth and performance during ambient climatic conditions.

The majority of published substrate research has generally used one substrate mix and altered individual components of the substrate (e.g. depth, amount of organic matter (Nagase and Dunnett 2011; Rowe et al. 2012)). However it is very hard to directly compare these individual trials due to the different substrates, growing conditions and plant selection used. This then makes it hard to make accurate conclusions about which substrate components have the largest effects on plant growth and performance.

3. How substrates can be optimised for extreme climatic conditions.

Due to their location and design green roofs are often extremely harsh environments for plants. The availability of hardy species such as *Sedum*s has allowed the development of thin extensive green roofs. However due to the success of *Sedum* species on green roofs there has been a lack of pressure to develop substrates which can support less hardy species without significantly increasing depth. In order to optimise substrates for extreme climatic conditions, individual components within the substrate will need to be altered or new components added for sustainable plant growth. However it is not known what is the best method for improving plant tolerance to extreme climatic conditions through altering substrate components.

4. The suitability of utilising existing biological interactions to improve plant growth and physiological performance.

In many other anthropogenically made or altered biological systems the positive interactions between AMF and plants are often utilised. However there has been a lack of

research and available knowledge on a) presence of AMF on established green roofs b) the viability of artificially introducing AMF to green roofs to improve plant growth and physiological performance (Molineux et al. 2014).

5. Biological characteristics of green roof substrate a) during installation b) once the roof is established.

FLL guidelines specify recommended ranges for physical and some chemical characteristics of substrate (FLL 2008) but do not mention any recommended biological characteristics. Biological components of any soil/substrate are integral in the cycling and provision of nutrients to plants, and often have a significant influence on the plant community they host (Wardle et al. 2004). Despite this, little research has been done and no guidelines exist for the biological characteristics of green roof substrate (Rumble and Gange 2013).

6. Long term performance of green roof substrate.

Once installed green roof substrate has a dynamic state and will develop over time (Köhler and Poll 2010; Thuring and Dunnett 2014). FLL guidelines exist for physical, some chemical and no biological characteristics of substrate when it is installed. However there are no guidelines as to how biological, chemical and physical characteristics of substrate should develop over time. In addition there is a lack of data of the biological, chemical and physical characteristics of established green roof substrate, which is needed to determine what desirable substrate characteristics of a successful green roof are. These data are needed if practical management tools such as a "Substrate Health Index" tool are to be developed for green roofs.

1.7 Aims of the thesis

Green roof vegetation, substrate characteristics and services are all intrinsically linked with one another and the modification of any of these will impact upon all the others. It is vital that this holistic view of green roof substrates is taken if they are to be optimised for plant growth and ultimately for green roof service provision. This thesis will address the research gaps indentified for green roof substrate through green house pot experiments, modular green roof trials and sampling of established green roofs.

Chapter 2: Importance of different components of green roof substrate on plant growth and physiological performance

In this chapter common green roof substrate components (brick size, compost type and depth) and a component not commonly used in green roof substrate (polyacrylamide water absorbent gel) were altered to create eight different substrates at two depths as part of a balanced factorial experiment. It is relatively well known to what extent brick size, compost type and depth affect green roof plant growth. However, it is not clear how they interact with one another to affect plant growth as well as physiological performance.

The use of water absorbent gels in green roof substrate is also not currently widespread. However due the low moisture levels often experienced in green roof substrate and the gels ability to hold large amounts of water they could potentially aid green roof plant growth and physiological performance. *Lolium perenne* was used as a phytometer species

and was grown in these substrates for three months after which a number of plant growth and physiological health measurements were taken.

The following research questions were asked:

1. Does the use of small as opposed to large crushed brick particles increase the water holding capacity of green roof substrate, improve *L. perenne* shoot growth, physiological performance and pot evapotranspiration?
2. Does the use of green waste compost as opposed to bark compost increase the nutrient availability of the substrate, *L. perenne* nutrient status, shoot growth, physiological performance and pot evapotranspiration?
3. Does the presence of polyacrylamide water absorbent gel (SwellGel) increase the water holding capacity of the substrate, *L. perenne* shoot growth, physiological performance and pot evapotranspiration?
4. Which combination of substrate components is best for *L. perenne* growth and physiological health? Can substrates be optimised for specific climatic areas or green roof services?

Chapter 3: Optimizing green roof plant drought tolerance through the use of substrate amendments and novel planting methods

In this chapter the results from Chapter 2 are built upon. The ability of two substrate components previously investigated (brick size and SwellGel presence) as well as a planting regime (additional *Sedum* "nurse" plants) to alter the drought tolerance of two common green roof species (*Festuca ovina, Linaria vulgaris*) was investigated. Results

from Chapter 2 indicated that SwellGel can promote plant growth and increase substrate water holding capacity (WHC), whilst larger brick particles promote lower, more sustainable growth due to a lower WHC. However it was not clear what combination of components would be better for extreme drought conditions. Research by (Butler and Orians 2011) has also indicated that the presence of *Sedum* can help cool the substrate and assist the growth of less hardy species under drought stress. The *Sedum* planting treatment was designed to assess how much of a beneficial impact a planting regime has on plant drought tolerance in comparison to altering substrate components.

The following research questions were asked:

1. Does SwellGel would increase plant survival and physiological health by providing a slow release water reservoir in the substrate during an extreme drought?
2. Does small as opposed to large crushed brick increase the growth of plants during ambient conditions which would make plants more vulnerable to an extreme drought, despite having a larger water holding capacity?
3. Does the presence of *Sedum spp.* reduce plant growth during ambient conditions, reduce water loss via evaporation and therefore increase plant performance during extreme drought conditions?

Chapter 4: The use of arbuscular mycorrhizal fungi (AMF) inoculum in assisting the initial establishment of Prunella vulgaris plug plant in green roof substrate

This chapter aims to assess the viability of introducing commercial AMF inoculum to green roof substrates planted with *Prunella vulgaris*. Due to the low water availability, nutrient and shallow depth it was expected that the presence of AMF would improve the growth, flowering and physiological health of *P. vulgaris* throughout the trial. A number of AMF application methods were used in order to determine which one was the most efficient at infecting *P. vulgaris* and therefore would be most suited for commercial application.

The following research questions were asked:

1. Does the addition of mycorrhizal inoculum to green roof substrate aid the establishment of *P. vulgaris* plugs?
2. Does the addition of mycorrhizal inoculum to green roof substrate increase the amount and length of *P. vulgaris* flowering?
3. What method of applying mycorrhizal inoculum is the most efficient at infecting and improving the growth and physiological health of *P. vulgaris* plugs?

Chapter 5: Moving towards an integrated substrate health index (SHI) for green roof substrate

This chapter aims to assess the potential of developing a SHI for green roofs. In order to do this a number of common physical, chemical and biological soil measurements were taken on three established green roofs in Sheffield. These were then assessed to see which ones were most appropriate for use on green roof substrate and also which ones showed

the most variability inter-roof and intra-roof. Therefore the basis of a SHI was created, although significant work is still needed in order to determine what substrate characteristics are desirable on green roofs.

The following research questions were asked:

1. What standard soil health measurements are most applicable for analysing green roof substrate?
2. What standard soil measurements are the best for predicting green roof substrate biological health?
3. By what degree does established green roof substrate vary between different green roofs as well as between sites on the same green roof?

Chapter 2

Importance of different components of green roof substrate on plant growth and physiological performance

2.1 Summary

Green roof substrate is arguably the most important element of a green roof, providing water, nutrients and physical support to plants. Despite this there has been a lack of research into the role that different substrate components have on green roof plant growth and physiological performance.

To address this, we assessed the importance of three green roof substrate components (organic matter type, brick particle size and water absorbent additive) for plant growth and plant physiological performance. *Lolium perenne* (Ryegrass) was grown in eight substrates in a controlled greenhouse environment with a factorial design in composition of (i) small or large brick, (ii) conifer bark or green waste compost organic matter, and (iii) presence/absence of polyacrylamide water absorbent gel ('Swellgel™').

We found that large brick substrates had a lower water holding capacity than small brick (-35%), which led to decreased shoot growth (-17%) and increased root:shoot ratio (+16%). Green waste compost increased shoot and root growth (+32% and +13%) shoot nitrogen concentration and chlorophyll content (20% and 57%), and decreased root:shoot

ratio (-15%) compared to bark. The addition of swell gel increased substrate water holding capacity (+24%), which increased shoot growth (+8%). Total evapotranspiration (a proxy for potential cooling) was increased by greater shoot biomass and substrate water holding capacity. Overall, this study provides one of the first quantitative assessments of the relative importance of commonly used green roof substrate components. It is clear that substrate composition should be considered carefully when designing green roofs, and substrate composition can be tailored for green roof service provision.

2.2 Introduction

Green roofs can have significant beneficial impacts in urban areas including storm water attenuation, urban heat island reduction, passive individual building cooling and provision of urban green space for recreational and aesthetic use (Oberndorfer et al. 2007). Due to these environmental benefits, the green roof industry has experienced a rapid expansion in the last twenty years and green roofs are now a common feature in most western urban areas (Oberndorfer et al. 2007). The amount of empirical green roof research conducted in the last ten years has also expanded, however many aspects of green roof technology and design have still not been fully investigated or optimised, in particular green roof substrate which is arguably the most important component of a green roof. The substrate has to perform the role of an artificial soil for plant growth and therefore must provide moisture, nutrients and physical support to plants, whilst also being lightweight, chemically stable, aeratable, and free draining (Friedrich 2008; Ampin et al. 2010). These characteristics are vital for the long term survival of green roof vegetation and provision

of the benefits (services) that green roofs provide. To date however, there has been little empirical research into the role of substrate on provision of green roof services (Roth-Kleyer 2005; Ampin et al. 2010; Olszewski and Young 2011), into new substrate materials (Molineux et al. 2009; Solano et al. 2012), biological properties of substrate (Kolb et al. 1982; Molineux et al. 2014) or the influence of substrates on green roof vegetation growth (Rowe et al. 2006b; Emilsson 2008; Nagase and Dunnett 2011; Farrell et al. 2012; Kotsiris et al. 2012). There has also been a lack of research into the effect that each individual substrate component (e.g. mineral content, type of organic matter, artificial additives, mixing ratios) has upon the growth and physiological performance of the vegetation it supports and ultimately the services that it provides (Dvorak and Volder 2010; Ouldboukhitine et al. 2012; Graceson et al. 2014a).

Most previous green roof substrate research has focused on the effect that substrate depth has on plant establishment, growth and long term survival (Durhman et al. 2007; Getter and Rowe 2007; Getter and Rowe 2008; Thuring et al. 2010; Rowe et al. 2012). It is generally agreed that plant growth and physiological performance increases with substrate depth, although substrate depth is not always a limiting growth factor for some green roof species, most notably for hardy succulents (Getter and Rowe 2008). Increased depth can protect plants from temperature extremes and also increases the potential reservoir of water available for plants, reducing the chance of plants experiencing drought stress (Dunnett and Kingsbury 2010; Thuring et al. 2010). However increasing substrate depth comes at an economic cost (greater volume of substrate required) and also may not be viable due to inadequate strength in the roof to support the greater substrate weight. An alternative is to design substrates to be more efficient and tailored towards specific or

multiple services by modifying individual components in order to change substrate properties (e.g. increase water holding capacity or nutrient provision). However in order for this to occur, a full understanding of the effect that all components of green roof substrate have on plant growth and performance must first be gained (Dvorak and Volder 2010).

Due to the relatively shallow depth and free draining nature of green roof substrates, water stress is one of the most common limitations for plant growth on green roofs (Thuring et al. 2010; Rowe et al. 2012). The water holding capacity of substrates can be increased by decreasing particle size which increases the amount of inner particle pore space, although this can increase the potential of water logging (Olszewski and Young 2011; Graceson et al. 2013). It has been shown that increased substrate water holding capacity can increase survival of five different succulents during an extreme drought in Australia (Farrell et al. 2012), however it is not fully known how a change in green roof substrate particle size and therefore water holding capacity impacts upon non succulent plant growth and performance during typical growing conditions (Olszewski and Young 2011).

An alternative to increasing the amount of smaller particles in a substrate, which can have negative effectives on drainage and water logging, is the use of artificial water retention gels. These are often used in horticulture and regeneration of degraded land to increase a soil/substrate's water holding capacity and reduce plant exposure to water stress without the need for large amounts of extra growing media (Hüttermann et al. 2009; Agaba et al. 2010; Kabiri et al. 2011; Williamson et al. 2011). Three previous trials have reported that similar benefits may be possible for green roof vegetation by providing longer term storage of water in the substrate (Sutton 2008; Olszewski et al. 2010; Savi and Marin

2014). It has also been shown that water retention gels can increase the water holding capacity of green roof substrate (Farrell et al. 2013). However this does not necessarily always benefit plants during periods of drought as this water may not be available or accessible to plants, and the effectiveness of the gel may be species dependent or vary depending on substrate composition (Farrell et al. 2013).

The type of organic matter used in green roof substrate can also affect water holding capacity due to different absorption properties. However subtle changes to its composition or quantity may have much larger effects on the substrate's moisture dynamics due to its impact upon the establishment and long term survival of green roof vegetation through nutrient availability (Emilsson, 2008; Nagase and Dunnett, 2011). The amount and type of vegetation present alters the rate at which a substrate's water reservoir is depleted via evapotranspiration (Berghage et al. 2007; Wolf and Lundholm 2008). Therefore altering organic matter type and amount in substrate will also alter green roof performance through influencing plant growth, rate of water use and transpiration (Wolf and Lundholm 2008; Nagase and Dunnett 2011; Graceson et al. 2014b).

Despite the potential for substrate composition to heavily influence green roof vegetation and therefore green roof service performance, the extent to which substrate components and their ratios influence green roof vegetation remains unknown. Without this knowledge it is challenging to engineer substrates that are tailored towards providing optimised services e.g. storm water retention at all times of the year.

With these concerns in mind, a pot experiment was established where the growth and physiological performance of the grass *Lolium perenne* (ryegrass) was assessed in controlled environment greenhouse trials. *L. perenne* was grown on green roof substrates composed of factorial combinations of commonly used green roof components of (i) small or large brick, (ii) organic matter as bark or green waste compost, and (iii) presence/absence of a polyacrylamide gel (SwellGel^{TM}). Trials were also undertaken using two substrate depths of 80 and 120mm.

It was hypothesised that;

1. Small brick would increase the water holding capacity of green roof substrate compared to large brick, increasing evapotranspiration and improving *L. perenne* shoot growth and physiological performance.
2. Green waste compost would increase nutrient availability of the substrate, leading to improved *L. perenne* nutrient status, shoot growth, physiological performance and increases in evapotranspiration.
3. Polyacrylamide gel (SwellGel) would increase water holding capacity of the substrate, leading to greater *L. perenne* shoot growth and physiological performance.
4. In light of these hypotheses, the best performing green roof substrate in terms of *L. perenne* shoot biomass production, evapotranspiration and plant physiological condition would contain small brick, green waste compost and SwellGel.

2.3 Methods

2.3.1 Experimental design

The study was undertaken in a temperature controlled greenhouse in a day/night regime of 16 hours 20ºC/ 8hours 15ºC from 28.2.13 to 29.5.13. Where necessary, supplementary lighting was used to ensure the required day length (Helle Lamps, IR 400 HPS, 400W).

The eight substrates had three component variables: (i) brick size (small brick at 2-5mm particle diameter; large brick of 4-15mm diameter), (ii) organic matter type (bark or green waste compost) and (iii) presence or absence of a polyacrylamide gel "SwellGel™" (www.swellgel.co.uk). Brick was crushed waste red brick, sieved to ensure brick fragments were within the size limits set. Green waste compost (Green Estate, Sheffield, UK) was composed of composted garden waste collected in Sheffield, whilst bark was sourced as common garden mixed conifer bark mulch. Due to the high C:N ratio of bark compost it was expected that greater N 'lock up' would occur and lower levels of available N and other nutrients would be present compared to pre decomposed green waste compost (WRAP 2011). SwellGel™ (www.swellgel.co.uk) is a soil additive made of cross linked polyacrylamide which is designed to expand and store water during high moisture levels and release it slowly back to the plant as moisture levels decline.

The substrate was made up of 20% of either organic matter type (no extra fertilisation was added), with the remaining 80% made up from one of the two crushed brick size categories. Dry SwellGel was then added as 1% of the total substrate volume as per manufacturer's instructions. Substrate was added to pots (12cm x11cm x 11cm) with two depths of substrate (80mm and 120mm), both of which are commonly used depths on extensive green roofs. The experiment therefore had a fully factorial design of brick size (2-5mm/4-15mm), organic matter type (green waste compost/bark), SwellGel (presence/absence) and substrate depth (80mm/120mm). Eight replicates of each substrate type and depth were used to give a total of 128 pots.

2.3.2 Plant species and water regime

Although not commonly found on green roofs in the UK, *Lolium perenne* (Hitchcock and Green 1929) was used as a phytometer species due to its lower stress tolerance than hardier green roof grasses, and its relatively high growth rate. This was desirable given the primary aims of this project was to detect effects of substrate composition and differences in plant physiological performance between substrates, which would be more readily quantifiable with *L. perenne* than with slow growing green roof species over the duration of the experiment. 1g of seed (Emorsgate Seeds, Kings Lynn, UK) per pot (approximately 500 seeds) were sown uniformly onto saturated substrate and then watered to saturation every day until two weeks following germination. After this point each pot was subjected to a watering regime of 150ml per week, spilt over two days (with each day being two watering events of 37.5ml) in order to make the watering event less intense and to prevent excessive leaching. As a percentage of total pot water holding capacity the weekly watering total was equivalent to 59-122% at 80mm and 45-95% at 120mm. This

is the equivalent to 50mm month^{-1} which is average for London, UK during winter months (Met Office 2010).

2.3.3 Substrate water holding capacity and evapotranspiration

Unplanted substrates were air dried in the greenhouse for three weeks and weighed to quantify substrate dry weight. They were then saturated (in standing water for two days) and allowed to drain for 15 minutes to reach field capacity, after which they were weighed and the difference in weights given as water holding capacity.

During the experiment, pots were weighed daily as well as 15 minutes after each watering event. Any reduction in pot weight over time or between watering events was attributed to evapotranspiration (following 15 minute draining there was never evidence of further leached losses). Total evapotranspiration of each pot over the duration of the experiment was calculated as the sum of all the weight differences over all time periods. We did not correct for plant biomass in this weight since we did not want to destructively harvest mid-way through the experiment, and plant biomass was less than 1/500th the mass of the evapotranspiration mass.

2.3.4 Plant biomass and shoot nitrogen content

After 16 weeks growth following germination, all above ground biomass was harvested, oven dried at 80^{o}C for two days and weighed to obtain dry weight. To determine root biomass, material was washed in water to remove all traces of brick and compost. After

cleaning, roots with SwellGel still attached were then soaked in water overnight to expand the gel, which was then manually removed using a scalpel. All root material was dried (80^{o}C for two days) before weighing.

Leaf tissue nitrogen (N) content was determined on oven-dried ground samples from the final biomass harvest, following Kjeldahl digestion (Allen et al. 1974). For this approximately 50mg dry plant biomass was digested in 1 ml concentrated sulphuric acid with 1 microspatular of catalyst (1:10 $CuSO_4$:$LiSO_4$) for 7hours at 375^{o}C. After a dilution (N=1:100 dH_2O) total nitrogen was determined by Flow Injection Analysis (Burkard FIA Flo2, Burkard Scientific, Uxbridge, UK).

2.3.5 Chlorophyll content

Biomass production and shoot nitrogen content were supported by physiological indicators of plant health. Mean leaf chlorophyll content for each pot was determined through acetone extraction (Cameron et al. 2009). After the last watering event, five grass shoots (0.25-0.5g fresh weight) from different parts of the pot were harvested and kept on ice in the dark until extraction of chlorophyll (within 1h to prevent degradation). The grass shoots were ground in a pestle and mortar with acid washed sand to form a paste. 5ml of ice cold 80% acetone was added and the mixture further ground then transferred to a 25ml centrifuge tube. The pestle and mortar were rinsed twice with 2ml ice cold 80% acetone and transferred to the same centrifuge tube then diluted to 10ml with ice cold 80% acetone. Samples were centrifuged at 8000 g for 5min and absorbance of the supernatant measured at 645 and 663nm using a Cecil Ce 1020 spectrophotometer (Cecil Instruments Ltd,

Cambridge, UK). Chlorophyll content was calculated using the following equations according to (Arnon 1949)), and re-expressed as mg chlorophyll per dry shoot weight.

Chl*a* (mg l^{-1}) = (12.7 × OD_{663}) – (2.69 × OD_{645})

Chl*b* (mg l^{-1}) = (22.9 × OD_{645}) – (4.68 × OD_{663})

2.3.6 Statistical analyses

To determine the main factorial effects and interactions of the substrate components (brick size, organic matter type, SwellGel and substrate depth), four-way ANOVAs were performed. Tukey HSD tests were used to determine differences between each individual substrate. All statistical analyses were carried out in R Studio version 2.15.1 (22.6.2012), (R Development Core Team, 2011).

2.4. Results

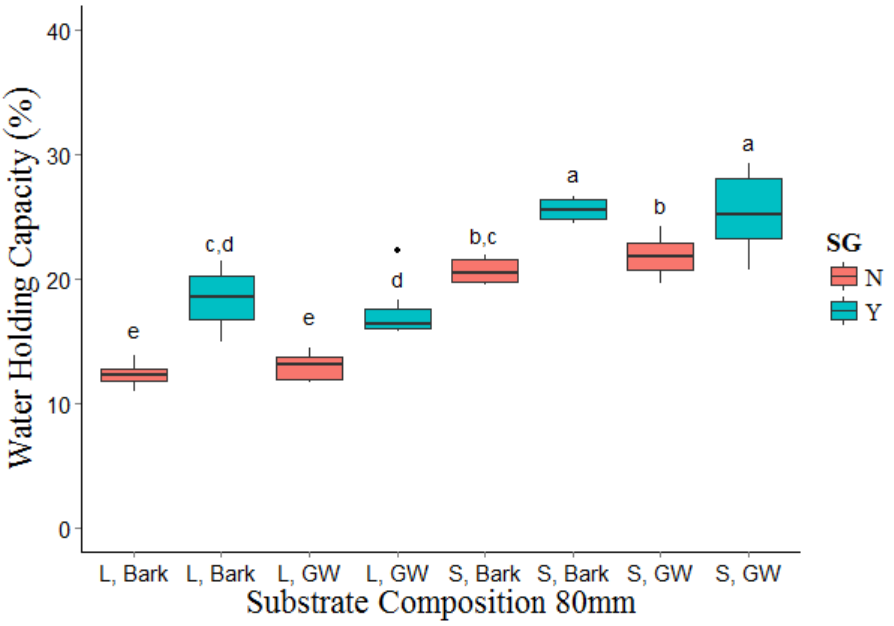
2.4.1 Water Holding Capacity of Substrates

The presence of SwellGel increased water holding capacity by 24% (four way ANOVA, $p<0.05$), whilst large brick reduced water holding capacity by approximately 35% compared to small brick (Fig. 2.1 a,b, Table 2.1a). Organic matter type (bark or green waste) did not significantly affect water holding capacity (Table 2.1a). Increasing the substrate depth from 80mm to 120mm significantly increased water holding capacity by 28% (four way ANOVA, $p<0.05$) (Fig. 2.1a,b, Table 2.1a). Overall substrates containing small brick and SwellGel always had a significantly higher water holding capacity than substrates containing large brick and no SwellGel at both depths regardless of organic matter content (Tukey HSD, $P<0.05$).

2.4.2 Evapotranspiration

SwellGel and green waste organic matter both significantly increased evapotranspiration by 4% and 7% respectively compared to no SwellGel and bark (four way ANOVA, $p<0.05$). Large brick significantly decreased evapotranspiration by 12% compared to small brick (four way ANOVA, $p<0.05$) (Fig.2.2 a,b, Table 2.1b). Substrate depth had a significant effect on total evapotranspiration, with evapotranspiration 11% greater from 120mm depth substrate (four way ANOVA, $p<0.05$) (Fig.2.2 a,b,, Table 2.1b). At both substrate depths, small brick with green waste organic matter had greater evapotranspiration than large brick with bark organic matter (Tukey HSD, $p<0.05$).

a)



b)

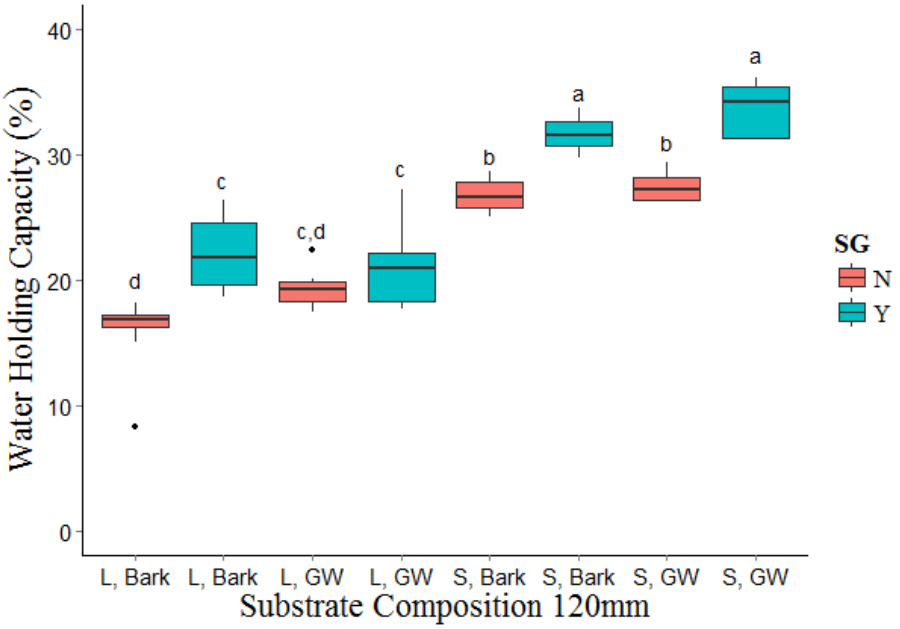
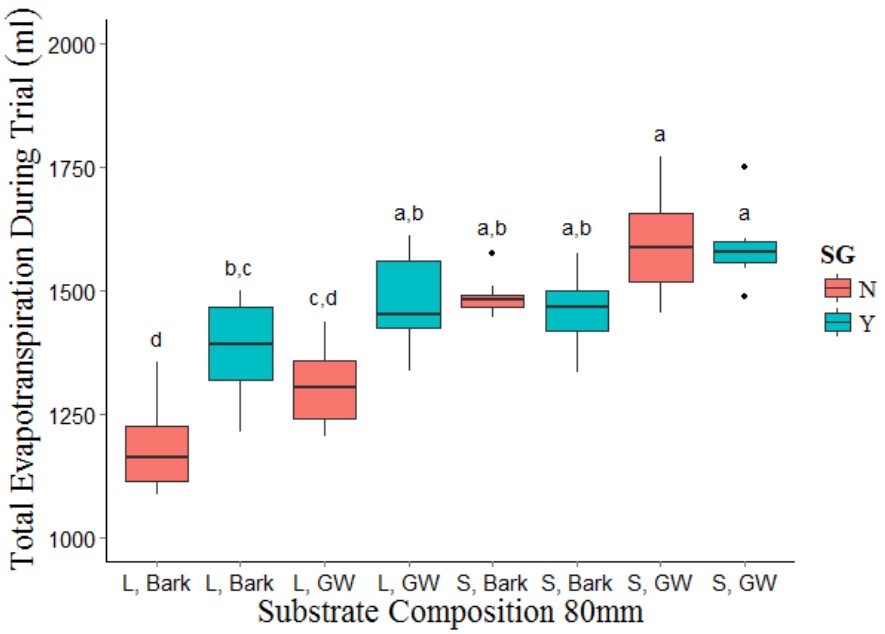


Figure 2.1: Water holding capacity (%) of each substrate at (a) 80mm substrate depth, (b) 120mm substrate depth. Axis and bar label codes are as follows; L= Large Brick, S= Small Brick, Bark= Bark Organic Matter, GW= Green Waste Compost, SG Y= SwellGel Present, SG N= SwellGel Not present. The middle bar represents the treatments mean, the upper and lower box hinges the 1st and 3rd quartiles, the thin black line the complete spread of data and outliers as black dots. Treatments with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$).

a)



b)

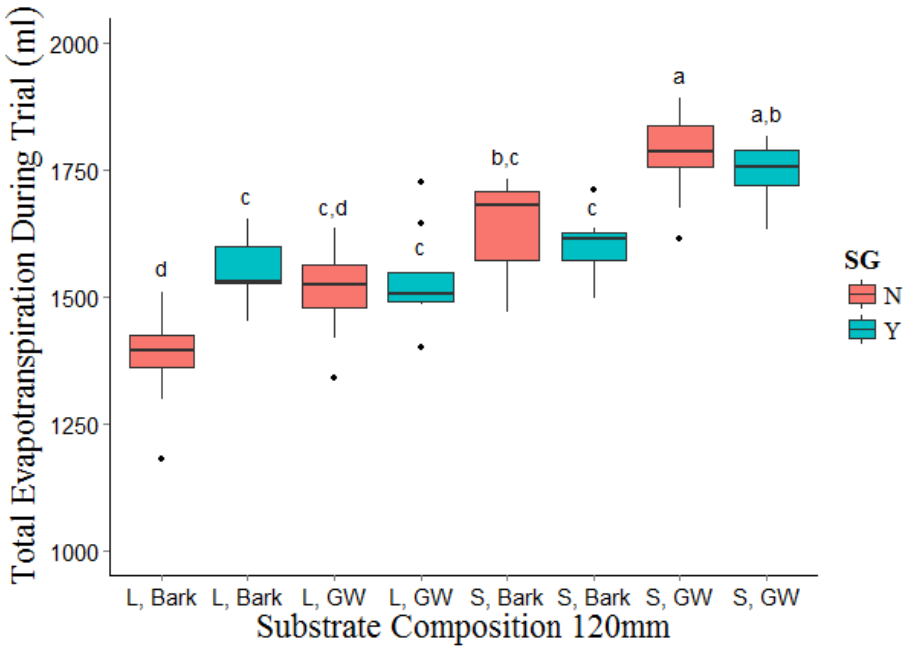


Figure 2.2: Total Evapotranspiration (ml) of *L. perenne* grown at (a) 80mm substrate depth, (b) 120mm substrate depth. Axis and bar label codes are as follows; L= Large Brick, S= Small Brick, Bark= Bark Organic Matter, GW= Green Waste Compost, SG Y= SwellGel Present, SG N= SwellGel Not present. The middle bar represents the treatments mean, the upper and lower box hinges the 1st and 3rd quartiles, the thin black line the complete spread of data and outliers as black dots. Treatments with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$).

Table 2.1: Main factor effects (four-way ANOVA) for (a) substrate water holding capacity (ml per L substrate) and (b) total evapotranspiration of *Lolium perenne* grown for 3.5months in eight different green roof substrates. Main factors are brick size (small vs. large), organic matter (bark vs. green waste compost) and SwellGel (absence vs. presence). Main factor means are shown with the % change also shown between the two levels within that factor (e.g. absence vs. presence of SwellGel). Significant factorial interactions are also shown. Statistical significances of P-values: * p<0.01, **p<0.001, *** p<0.0001. Statistical significances were calculated from four-way ANOVA. Abbreviations for each factor are as follows, Org=Organic matter type, GW=Green Waste organic matter, Sw.G=SwellGel. DF= 1 for each Factor.

(a)

Factor	F-value	P-value	Main factor means of water holding capacity (ml per L substrate)								% Change (±SE, n=64)
			Brick Size		Organic		SwellGel		Depth		
			Small	Large	Bark	GW	No	Yes	80mm	120mm	
Brick	640.6	***	266.2	174.0							-34.6± 1.9
Organic	3.8	0.053			216.5	223.6					+3.3± 3.7
SwellGel	168.6	***					196.4	243.7			+24.1± 3.8
Depth	222.4	***							192.9	247.2	+28.1± 4.0
Sw.G:Org	4.9	*									
Brick:Depth	9.3	**									
Sw.G:Org:Brick	4.6	*									
Sw.G:Org:Brick:Depth	4.0	*									
(b)			Main factor means of total pot evapotranspiration (ml)								
Brick	162.9	***	1612.2	1415.5							-12.2±1.2
Organic	47.0	***			1461.0	1566.7					+7.2±1.4
SwellGel	14.9	***					1484.1	1543.6			+4.0±1.1
Depth	108.2	***							1433.7	1594.0	+11.2±1.3
Sw.G:Brick	7.8	***									

2.4.3 Shoot biomass

Organic matter type had the largest effect on shoot biomass, with this being 32% greater on green waste than bark substrates (four way ANOVA, $p<0.05$) (Fig.2.3 a,b, Table 2.2a). The presence of SwellGel more modestly increased dry shoot biomass by 8%, and large brick size decreased shoot biomass by 17% (four way ANOVA, $p<0.05$) (Fig.2.3 a,b, Table 2.2a). Overall this meant that substrates containing green waste with either brick size or SwellGel presence had significantly greater biomass production than all bark based substrates at both 80 and 120mm depths (Tukey HSD, $p<0.05$). Shoot biomass did not differ significantly between 80 and 120mm substrate depth (Table 2.2a).

2.4.4 Root biomass

Organic matter type had the greatest effect on root biomass production. Overall green waste significantly increased root biomass by 13% compared to bark (four way ANOVA, $p<0.05$). SwellGel had the next greatest effect on root biomass, decreasing this by 7% overall (four way ANOVA, $p<0.05$) (Fig. 2.4 a,b, Table 2.2b). There was a significant interaction between SwellGel and organic matter type (four way ANOVA, $p<0.05$), with bark substrates producing significantly greater levels of root growth when SwellGel was not present. The same interaction occurred between SwellGel and brick size with SwellGel significantly decreasing root biomass on small brick, but not on large brick (Fig. 2.4 a,b, Table 2.2b). Increasing the depth of substrate from 80mm to 120mm significantly

increased root biomass by 22% (four way ANOVA, $p<0.05$) (Fig. 2.4 a,b, Table 2.2b). Brick size did not have a significant effect on root biomass.

2.4.5 Root:shoot ratio

Root:shoot ratios with green waste organic matter was significantly reduced by 15% compared to bark, while large brick significantly increased root:shoot ratios by 16% compared to small brick (four way ANOVA, $p<0.05$) (Fig. 2.5 a,b, Table 2.2c). The presence of SwellGel reduced root:shoot ratios by 15% (four way ANOVA, $p<0.05$) (Fig. 2.5 a,b, Table 2.2c). The same factorial interactions observed for root biomass were also observed for root:shoot ratios, with SwellGel reducing root:shoot ratios more when the organic matter was bark rather than green waste, or small rather than large brick (four way ANOVA, $p<0.05$) (Fig. 2.5 a,b). Root:shoot ratios at 120mm depth were 17% higher than at 80mm depth at 120mm depth (four way ANOVA, $p<0.05$) (Fig. 2.5 a,b, Table 2.2c).

2.4.6 Shoot nitrogen concentration

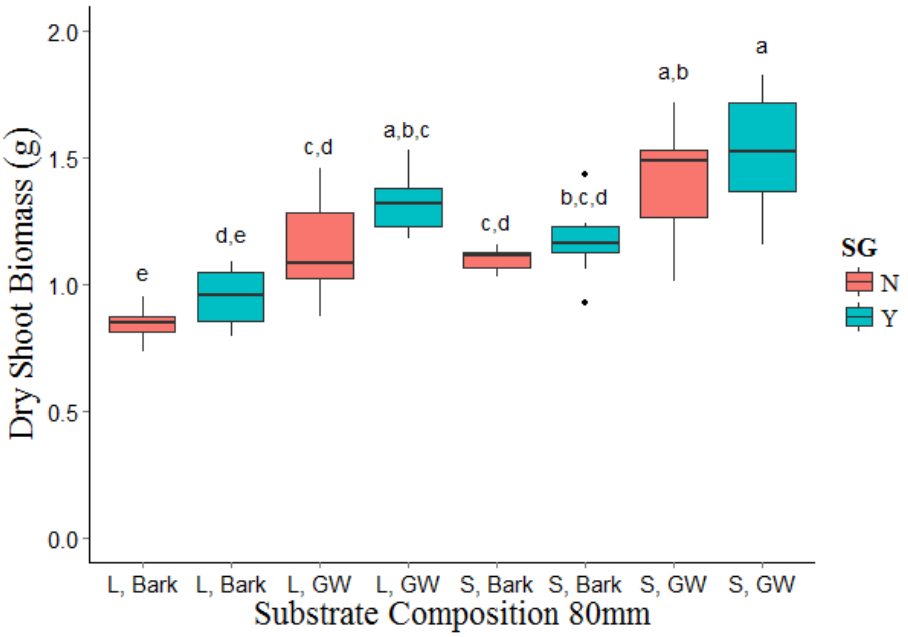
Green waste, SwellGel and large brick had very similar effects on shoot nitrogen concentration, increasing this by 21% , 20% and 22% compared to bark, no SwellGel and small brick respectively (four way ANOVA, $p<0.05$) (Fig. 2.6 a,b, Table 2.3a). A significant interaction showed that the increase in shoot nitrogen concentration due to SwellGel was much larger when it was present with green waste rather than bark, although this effect only occurred in small brick (four way ANOVA, $p<0.05$) (Table 2.3a).

Substrates containing SwellGel and green waste had significantly higher shoot nitrogen concentrations than substrates without SwellGel and bark based at 80mm depth (Tukey HSD, $P<0.05$) and partly at 120mm. Substrate depth did not significantly affect shoot nitrogen concentration (Table 2.3a).

2.4.7 Chlorophyll content

Shoot chlorophyll content was most significantly affected by organic matter type and substrate depth, with green waste increasing chlorophyll content by 57% compared to bark, and 120mm substrate depth increasing chlorophyll content by 40% compared to 80mm (four way ANOVA, $p<0.05$) (Fig. 2.7 a,b, Table 2.3b). Increasing brick size from small to large caused a decrease in chlorophyll (-14%) content (four way ANOVA, $p<0.05$) (Table 2.3b). A significant interaction between SwellGel and organic content occurred with large brick only with SwellGel increasing chlorophyll content in bark based substrates but decreasing chlorophyll content in green waste substrates (Table 2.3b).

a)



b)

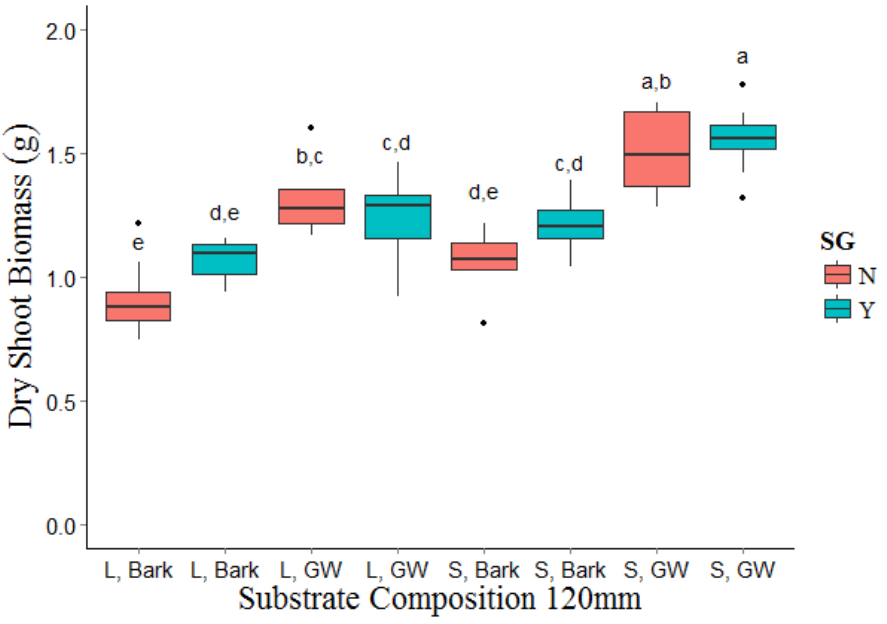
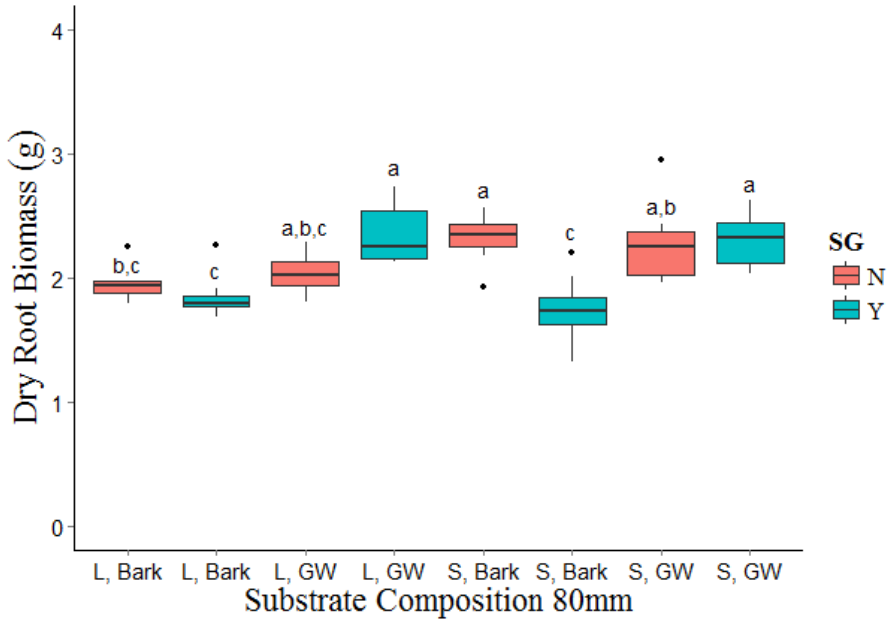


Figure 2.3: Shoot biomass (g) of *L. perenne* grown at (a) 80mm substrate depth, (b) 120mm substrate depth. Axis and bar label codes are as follows; L= Large Brick, S= Small Brick, Bark= Bark Organic Matter, GW= Green Waste Compost, SG Y= SwellGel Present, SG N= SwellGel Not present. The middle bar represents the treatments mean, the upper and lower box hinges the 1st and 3rd quartiles, the thin black line the complete spread of data and outliers as black dots. Treatments with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$).

a)



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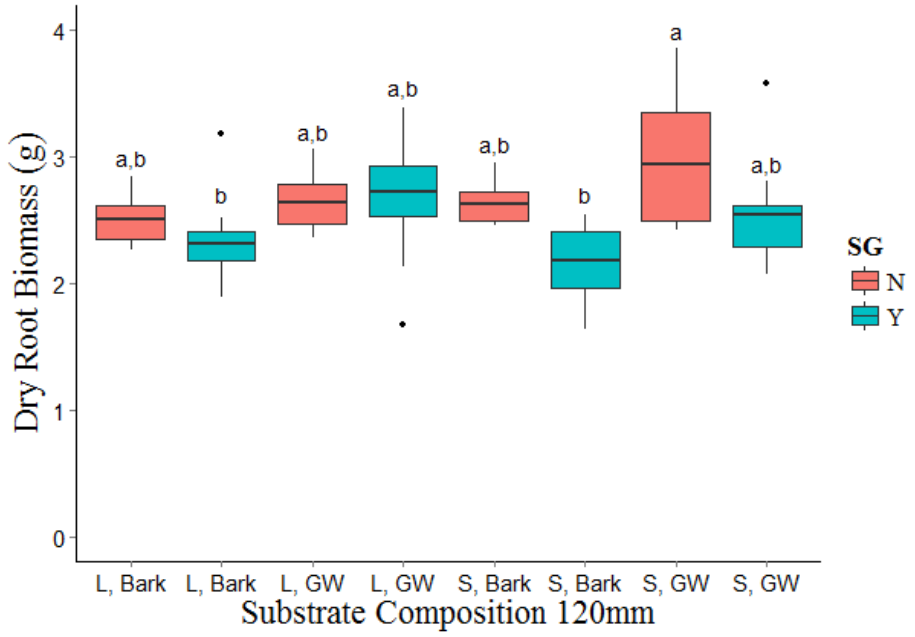
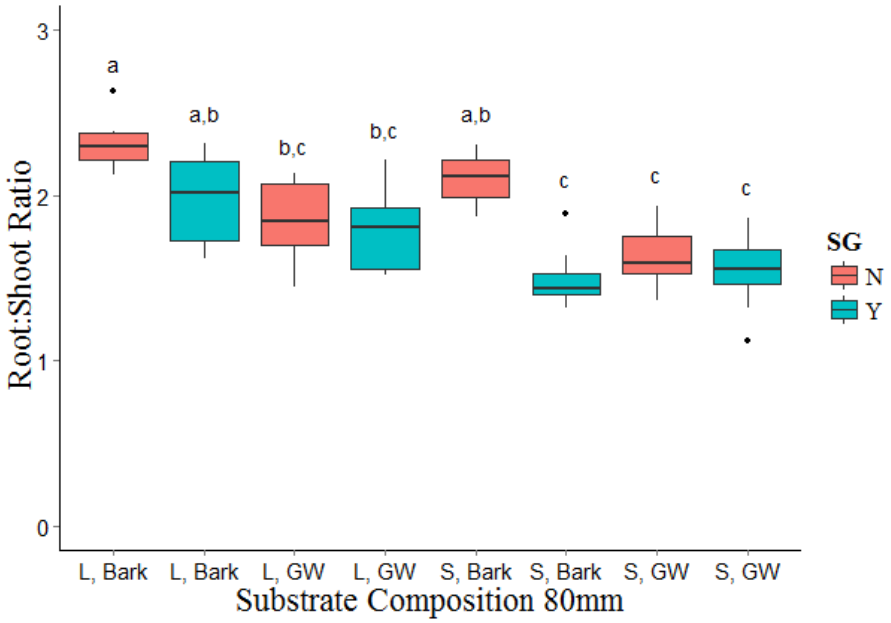


Figure 2.4: Root biomass (g) of *L. perenne* grown at (a) 80mm substrate depth, (b) 120mm substrate depth. Axis and bar label codes are as follows; L= Large Brick, S= Small Brick, Bark= Bark Organic Matter, GW= Green Waste Compost, SG Y= SwellGel Present, SG N= SwellGel Not present. The middle bar represents the treatments mean, the upper and lower box hinges the 1st and 3rd quartiles, the thin black line the complete spread of data and outliers as black dots. Treatments with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$).

a)



b)

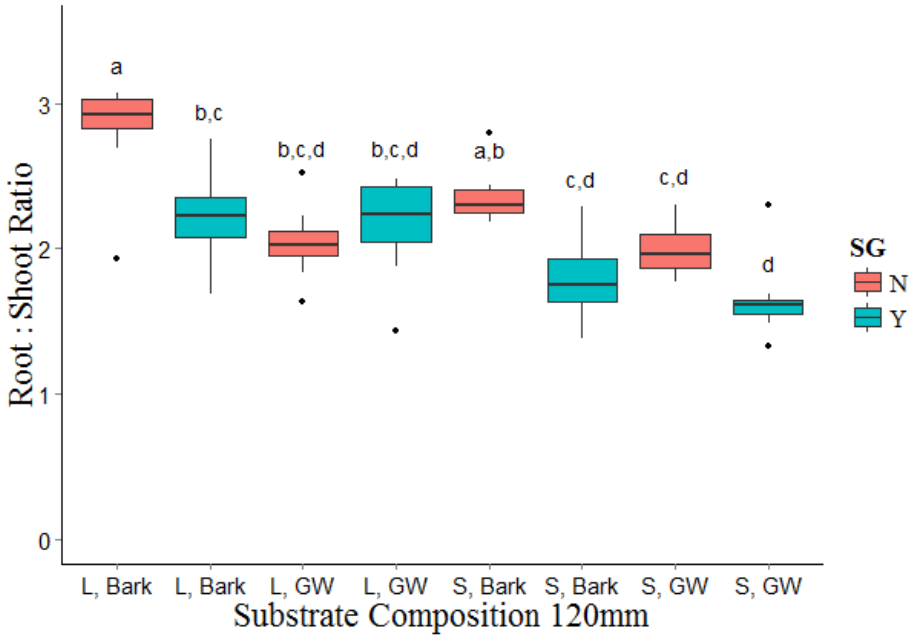


Figure 2.5: Root:Shoot ratio of *L. perenne* grown at (a) 80mm substrate depth, (b) 120mm substrate depth. Axis and bar label codes are as follows; L= Large Brick, S= Small Brick, Bark= Bark Organic Matter, GW= Green Waste Compost, SG Y= SwellGel Present, SG N= SwellGel Not present. The middle bar represents the treatments mean, the upper and lower box hinges the 1st and 3rd quartiles, the thin black line the complete spread of data and outliers as black dots. Treatments with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$).

Table 2.2: Main factor effects (four-way ANOVA) for (a) shoot biomass, (b) root biomass and (c) root:shoot ratios of *Lolium perenne* grown in eight different green roof substrates. Main factors are brick size (small vs. large), organic matter (bark vs. green waste compost) and SwellGel (absence vs. presence). Main factor means are shown with the % change also shown between the two levels within that factor (e.g. absence vs. presence SwellGel). Significant factorial interactions are also shown. Statistical significances of P-value: * p<0.01, **p<0.001, *** p<0.0001. Statistical significances were calculated from four-way ANOVA. Abbreviations for each factor are as follows, Org=Organic matter type, GW=Green Waste organic matter, Sw.G=SwellGel. DF= 1 for each Factor.

(a)

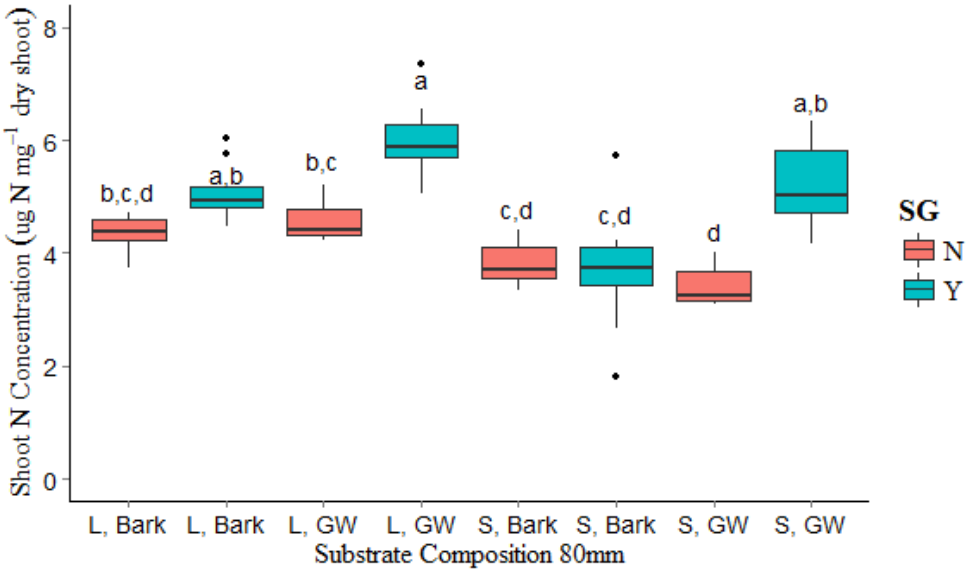
Factor	F-value	P-value	Main factor means of dry shoot biomass (g)								% Change (±SE, n=64)
			Brick Size		Organic		SwellGel		Depth		
			Small	Large	N	Y	No	Yes	80mm	120mm	
Brick	68.5	***	1.31	1.10							-16.7± 2.0
Organic	157.5	***			1.04	1.38					+32.1± 2.7
SwellGel	13.2	***					1.16	1.26			+8.3± 2.6
Depth	3.9	0.05							1.18	1.23	+4.2± 2.6
Sw.G:Org:Depth	4.3	*									
(b)			Main factor means of dry root biomass (g)								
Brick	2.2	0.14	2.38	2.30							-3.4± 2.1
Organic	26.7	***			2.19	2.48					+13.1± 2.6
SwellGel	9.4	**					2.42	2.25			-7.0± 2.3
Depth	70.6	***							2.11	2.57	+22.2± 2.6
Sw.G:Org	7.3	**									
Sw.G:Brick	12.1	***									
(c)			Main factor means of root:shoot ratios								
Brick	35.8	***	1.84	2.14							+16.2± 2.8
Organic	42.6	***			2.16	1.83					-15.1± 1.8
SwellGel	43.6	***					2.16	1.83			-15.3± 2.1
Depth	38.7	***							1.84	2.15	+16.9± 3.2
Sw.G:Org	22.5	***									
Sw.G:Brick	5.0	*									

Table 2.3: Main factor effects (four-way ANOVA) for (a) mean shoot nitrogen concentration (mg^{-1} g shoot biomass), (b) mean chlorophyll content (mg g^{-1} dry shoot biomass) of *Lolium perenne* grown in eight different green roof substrates. Main factors are brick size (small vs. large), organic matter (bark vs. green waste compost) and SwellGel (absence vs. presence). Main factor means are shown with the % change also shown between the two levels within that factor (e.g. absence vs. presence SwellGel). Significant factorial interactions are also shown. Statistical significances of P-value: * p<0.01, **p<0.001, *** p<0.0001. Statistical significances were calculated from four-way ANOVA. Abbreviations for each factor are as follows, Org=Organic matter type, GW=Green Waste organic matter, Sw.G=SwellGel. DF= 1 for each Factor.

(a)

Factor	F-value	P-value	Main factor means of total nitrogen shoot concentration (µg N g^{-1} shoot biomass)								% Change (±SE, n=64)
			Brick Size		Organic		SwellGel		Depth		
			Small	Large	Bark	GW	No	Yes	80mm	120mm	
Brick	53.897	***	4.14	5.04							+21.9± 2.8
Organic	43.866	***			4.18	5.00					+19.6± 3.1
SwellGel	50.765	***					4.15	5.03			+21.2± 3.4
Depth	1.148	0.29							4.52	4.65	+2.9± 3.0
Sw.G:Org	7.815	**									
Org:Depth	4.707	*									
Sw.G:Org:Brick	8.014	**									
Sw.G:Org:Depth	6.490	*									
(b)			Main factor means mean chlorophyll content (mg g^{-1} dry shoot biomass)								
Brick	5.4	*	0.16	0.14							-14.4±5.7
Organic	44.4	***			0.12	0.18					+56.7±8.9
SwellGel	1.7	0.20					0.15	0.14			-8.2±5.8
Depth	24.3	***							0.12	0.17	+39.5±8.0
Sw.G:Org	11.7	***									
Org:Brick	6.7	*									
Sw.G:Org:Brick	12.9	***									
Sw.G:Org:Brick:Depth	6.1	*									

a)



b)

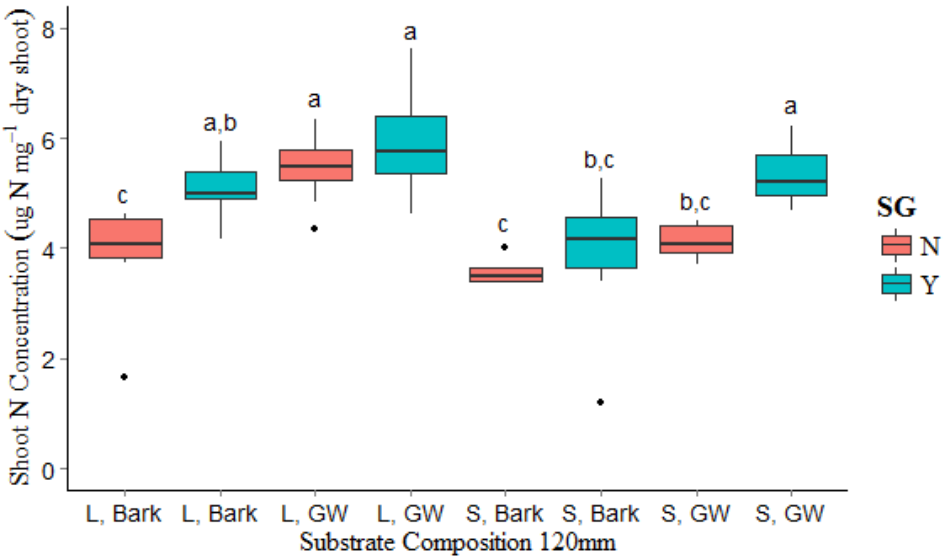
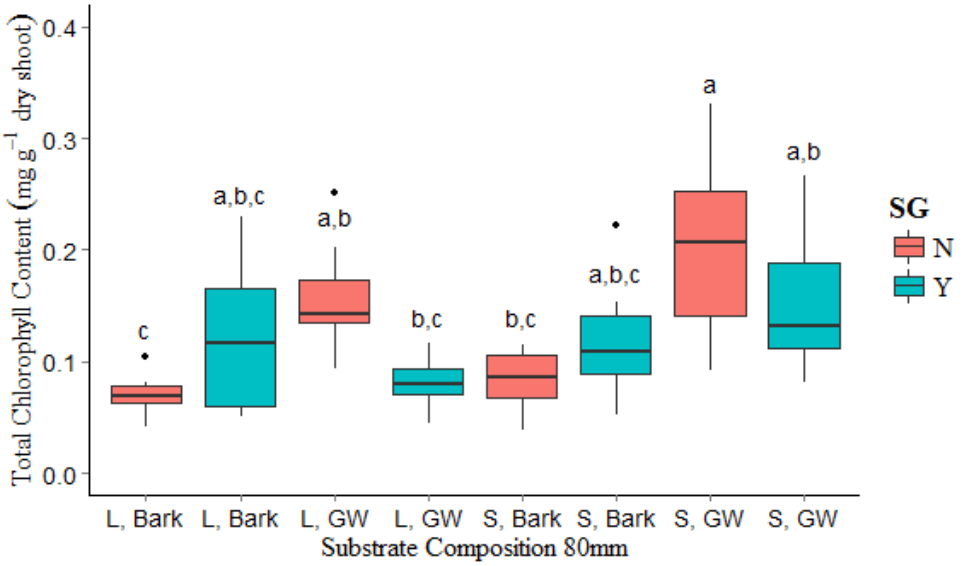


Figure 2.6: Shoot nitrogen concentration (µg N g^{-1} dry shoot) of *L. perenne* grown at (a) 80mm substrate depth, (b) 120mm substrate depth. Axis and bar label codes are as follows; L= Large Brick, S= Small Brick, Bark= Bark Organic Matter, GW= Green Waste Compost, SG Y= SwellGel Present, SG N= SwellGel Not present. The middle bar represents the treatments mean, the upper and lower box hinges the 1st and 3rd quartiles, the thin black line the complete spread of data and outliers as black dots. Treatments with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$).

a)



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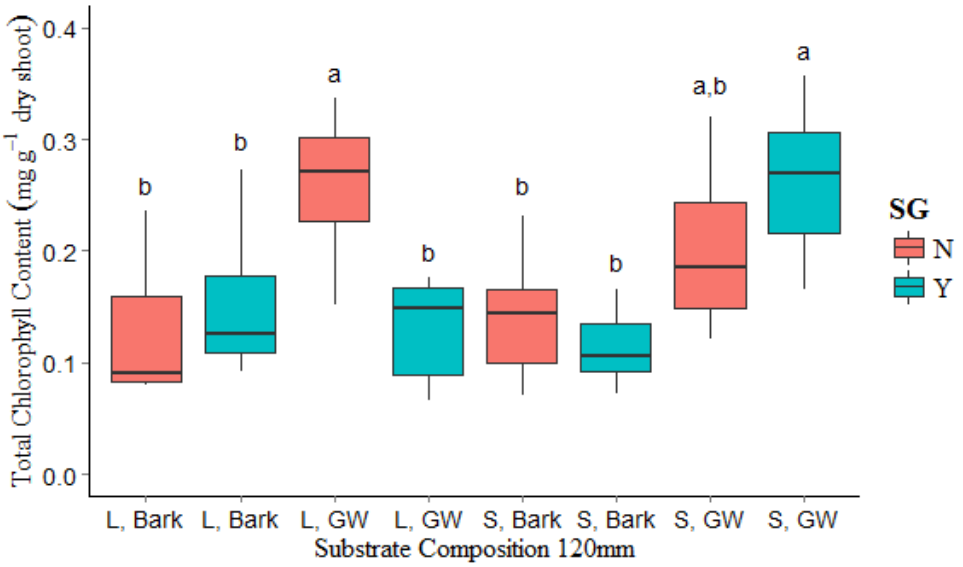


Figure 2.7: Shoot chlorophyll concentration (mg g^{-1} dry shoot) of *L. perenne* grown at (a) 80mm substrate depth, (b) 120mm substrate depth. Axis and bar label codes are as follows; L= Large Brick, S= Small Brick, Bark= Bark Organic Matter, GW= Green Waste Compost, SG Y= SwellGel Present, SG N= SwellGel Not present. The middle bar represents the treatments mean, the upper and lower box hinges the 1st and 3rd quartiles, the thin black line the complete spread of data and outliers as black dots. Treatments with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$).

2.5 Discussion

This study is one of the first systematic investigations to quantify the importance of widely used green roof components for plant growth and physiological performance. It is clear that altering the composition/type of substrate components can have substantial effects on plant physiological performance and water balance. All three substrate composition factors studied (presence of a polyacrylamide gel (SwellGel), organic matter and brick size) had significant effects on *L. perenne*, which were largely consistent across both substrate depths, and indeed often had larger effects than the often previously studied substrate depth. Although this trial only assessed initial plant establishment, these findings can therefore begin to inform substrate composition choice depending on plant growth requirements (fast growing/high maintenance/lower drought tolerance vs. slow growing/low maintenance/higher drought tolerance).

2.5.1 Water holding capacity & evapotranspiration

SwellGel increased the water holding capacity of green roof substrates, which explains its benefit to shoot growth and evapotranspiration. In this trial its effect on water holding capacity is less than that of brick size (small brick increased water holding capacity ~50% more compared to adding SwellGel). This does not mean that SwellGel has a limited impact on water holding capacity since it constituted only 1% volume in our substrates compared to 80% brick. Indeed, greater impact of SwellGel could be achieved by increasing the amount used, although there are limitations in the amount that can added due to substrate disturbance from constant expansion and contraction during wetting and

drying cycles, physical limitations and negative effects on biomass yield (Farrell et al. 2013). In fact, SwellGel may be more important in times of drought as water stored in it may be released much more slowly to plants than water stored in inner particle pore space (Hüttermann et al. 2009; Agaba et al. 2010; Savi and Marin 2014). However it should be noted that this trial did not assess the effect of SwellGel on plant available water which does not always increase with greater substrate water holding capacity and can be species dependent (Farrell et al. 2013). Where substrates are used in regions with prolonged periods of low rainfall, or where a greater frequency of drought events are predicted from climate change (Coumou and Rahmstorf 2012), then SwellGel is likely to be an important and beneficial component of substrates (Savi and Marin 2014). Nonetheless, using small instead of large brick size appears to be the simplest (and likely most cost effective) way of improving substrate water holding capacity (Graceson et al. 2013).

Vegetation plays a major role in increasing evapotranspiration rates from green roofs (Wolf and Lundholm 2008; Voyde et al. 2010; Metselaar 2012), and in this trial the presence of *L. perenne* increased total evapotranspiration by between 13-57% compared to non-vegetated substrate (data not shown). The amount of transpiration that *L. perenne* contributed to the total evapotranspiration amount is dependent on the total amount of biomass produced (evapotranspiration and *L. perenne* biomass were significantly correlated; r^2=0.482, p<0.0001), which in turn is dependent on the nutrient content and water storage capacity of the substrate. Organic matter type did not affect the water holding capacity of the substrate but did indirectly affect the rate at which water was lost from the substrate by influencing biomass production and therefore transpiration. This highlights that water holding capacity should not be the only substrate property that is

considered when selecting a substrate for its influence on water dynamics, as vegetation growth also has a considerable influence on this (Wolf and Lundholm 2008).

Limited evapotranspiration, however, may not always be desirable since this can play an important role in temperature regulation of host buildings (Castleton et al. 2010; Blanusa et al. 2013). Similarly, when designing green roof substrate to promote greater plant growth in order to increase cooling from evapotranspiration, one has to consider the effect that higher evapotranspiration rates may have upon the substrate water reservoir during times of drought. If this is depleted too quickly, leading to water stress and stomatal closure, plants no longer transpire at the same rate, mortality may occur and the net cooling effect of the green roof could be reduced. In addition, by developing a green roof solely for one service, for example building cooling, other green roof services may be compromised, such as biodiversity provision or aesthetic qualities. Therefore such trade-offs must be taken into account when optimising a green roof substrate (Ampin et al. 2010; Lundholm et al. 2010).

2.5.2 Plant Growth

Plant biomass was substantially increased when green waste compost was used as the organic matter component instead of bark. Green waste compost will have more nutrients available to plants due to its preconditioned state (composted) and greater diversity of source material. It has been shown previously that increasing the organic fraction of a green roof substrate increases plant growth (Nagase and Dunnett 2011), although to our

knowledge this is the first time that it has been demonstrated that different organic matter types have a significant effect on green roof plant growth. Again, increased plant growth may not always be desirable since it can be detrimental to long term plant survival as plants with more luxuriant growth can be more susceptible to the drought stresses common to green roofs (Bates et al. 2013), and will also require more maintenance compared to slower growing coverage (Nagase and Dunnett 2011). None-the-less, higher nutrient content (through increased organic fraction or different organic matter type) of green roof substrates increases plant growth (Olszewski et al. 2010; Nagase and Dunnett 2011) and can improve long term substrate development due to a larger build up of dead biomass, which can also help prevent nutrients from being leached out of the system (Emilsson 2008).

The greater fund of nutrients in green waste is also consistent with the lower root:shoot ratios found in green waste compost substrate compared to bark substrates. This indicates less need for plants to allocate resources to nutrient capturing roots in green waste based substrates, and a greater allocation to the photosynthesising shoots (Hermans et al. 2006). The same response in root:shoot ratios was observed for SwellGel and small brick, likely due to the increased availability of water which reduced the need for water capturing root biomass. However substrates that do the opposite and promote a higher root:shoot ratio (i.e. promote resource allocation to roots) may actually be more desirable for green roofs during the establishment phase of plants, especially in areas subject to low precipitation and high temperatures, where greater water capture capacity (roots) and less surface for transpiration (shoots) is desirable (Grossnickle 2005; Nagase and Dunnett 2011).

Plant growth was significantly decreased when brick particle size was increased from 2-5mm to 4-15mm. This may be due to the poorer water holding capacity of the large brick substrates, as larger particle sizes reduces inter-particle pore space and therefore reduces water holding capacity (Farrell et al. 2012; Graceson et al. 2013; Graceson et al. 2014a). This effect may also be due to the higher amounts of nitrogen leached from large brick substrates throughout the trial, which could have depleted nitrogen stocks in the substrate at a faster rate (data not shown).

SwellGel had a relatively small effect on plant growth, although this may be due to the regular watering regime not resulting in great enough water stress for the benefits of SwellGel to be realised. Much larger increases in *Sedum* shoot biomass with polyacrylamide gel amendment has previously been demonstrated, although a higher temperature and less frequent watering regime were used in that study (Olszewski et al. 2010). However different types of water retention amendment seem to differ in their ability to influence green roof plant growth (Farrell et al. 2013).

Depth of substrate had no effect on plant growth, which contrasts with many other studies that have stated this to be a major factor in green roof plant establishment and growth (Durhman et al. 2007; Getter and Rowe 2007; Getter and Rowe 2008; Thuring et al. 2010). Past studies have concluded that increased water availability in deeper substrate is one of the most important factors for plant growth (Rowe et al. 2012), but water availability may not have been a major limiting factor in this trial. Increased depth also protects plants from frost damage (Boivin et al. 2001), as well as reducing extreme temperature fluctuations from solar radiation (Butler and Orians 2011), both of which were not present in the

controlled temperature environment of the greenhouse. These benefits of deeper substrate would therefore not have become fully apparent in our study.

2.5.3 Shoot nitrogen and chlorophyll concentration

Shoot N concentration was increased by SwellGel, however the mechanism behind this is unclear. It may be due to the SwellGel degrading to form acrylamide and then ammonium or nitrogen oxides (Smith et al. 1997; Holliman et al. 2005), or by SwellGel absorbing nitrogen from the substrate. Alternatively it could be due to increased microbial activity around the moisture pockets created by the SwellGel as it has been shown that fungi and bacteria can readily colonise polyacrylamide gel and utilise the nitrogen held within it (Kay-Shoemake et al. 1998; Holliman et al. 2005). As chlorophyll content was not significantly affected by SwellGel but did show significantly higher levels in plants grown in green waste compost substrates, it could indicate that any additional nitrogen supplied through the presence of SwellGel was not a significant factor in chlorophyll production. Green waste compost increased shoot nitrogen concentration, probably by increasing the amount of nitrogen available for plant uptake (supported by KCl plant available nitrogen analysis of substrates, data not shown). This is also the likely reason for significantly higher chlorophyll content on green waste as it has also previously been shown that higher chlorophyll content in temperate grasses is correlated with high shoot nitrogen concentration (Gáborčík 2003). The higher concentration of shoot N in large brick is, in contrast, likely to be caused by a negative growth dilution as brick size did not have a significant effect on total tissue nitrogen stocks (data not shown), but did reduce shoot growth, and so potentially "concentrating" the nitrogen in the smaller shoot biomass.

It must be noted that both shoot N (5 µg N g^{-1}) and chlorophyll (0.15 mg g^{-1}) shoot concentrations were significantly lower than those found in other studies utilising *L. perenne* in controlled greenhouse conditions (32 µg N g^{-1} and 2.8 mg g^{-1} respectively) (Smith et al. 1980; Smith et al. 1985).

2.5.4 Depth of substrate

Increasing the depth of green roof substrate generally improves green roof plant growth and survival by increasing water and nutrient availability, especially during times of drought (Durhman et al. 2007; Getter and Rowe 2007; Getter and Rowe 2008; Thuring et al. 2010). Although this trial did not show such dramatic improvements to plant growth and physiological performance with depth as previous trials, it was conducted under controlled temperature conditions and therefore plants did not experience some of the environmental extremes that roof top trials encounter.

2.6 Conclusions

This study has shown that altering the characteristics of commonly used green roof substrate components can significantly alter the initial growth and physiological performance of the plants grown upon them. This is especially important for green roofs because vegetation plays a core role in provision of green roof services (Oberndorfer et al. 2007).

All four hypothesises were supported by the experimental data. By looking at each substrate component in turn it is clear that organic matter type was found to have the most

influence on plant growth and health. Increasing plant available nutrients by switching from bark to green waste compost significantly increased *L. perenne* shoot N concentration, chlorophyll content and shoot and root biomass, which in turn increased total evapotranspiration. However by also reducing *L. perenne* root:shoot ratio, green waste compost potentially reduced this plant's ability to survive drought stress. The effectiveness of SwellGel to provide water storage during drought was not thoroughly tested in this trial due to the absence of drought conditions. However, SwellGel still improved plant growth and substrate water holding capacity. Brick size had a larger effect than SwellGel on shoot growth and water holding capacity, however SwellGel may be more effective at providing water to plants during a drought stress, although more studies on the plant availability of water stored in SwellGel must be conducted.

Therefore our fourth hypothesis which predicted that substrates containing small brick, green waste compost and SwellGel would be the best performing substrate in terms of shoot biomass production, evapotranspiration and plant physiological condition was correct. However this does not necessary mean that this mixture of substrate components will be the optimum for every green roof, with designers needing to consider the particular environmental stresses at that location and the core reason why that green roof is being built (e.g. high rainfall areas will not need high water retention for plant growth, but may need it for storm water mitigation). Clearly, compositional changes in green roof substrates- even among commonly used substrate materials- can have large influences on the properties and physiological performance of the vegetative component of the roof, and emphasises the fact that substrate composition should be considered carefully when designing green roofs for optimal provision of particular green roof services.

Chapter 3

Optimizing green roof plant drought tolerance through the use of substrate amendments and novel planting methods

3.1 Summary

The provision of green roof services is largely dependent on the health of the vegetation on the green roof. Despite this, there has been a lack of research on improving green roof substrate for drought tolerance.

This chapter assessed the impact of two substrate components (brick particle size and water absorbent gel additive, 'SwellGel™') and a planting technique (companion planting of *Sedum spp.*) on the growth, physiological and visual health of newly established plugs of *Festuca ovina* and *Linaria vulgaris* during droughts of 10, 15 and 25 days.

SwellGel and large brick substrates increased both plant species' drought tolerance. SwellGel increased the reservoir of water that plants could access, whilst large brick promoted slower and therefore more drought resistant plant growth. *Sedum* reduced plant growth but had no effect on drought tolerance.

Overall this study shows that water retention gels and courser particle size substrates can significantly improve the drought tolerance of green roof plants through two different mechanisms.

3.2 Introduction

Green roofs are becoming much more common in urban areas due to the many services that they can provide (Getter and Rowe 2006; Oberndorfer et al. 2007). These services range from storm water attenuation, urban heat island reduction, building cooling and provision of urban green space (Getter and Rowe 2006; Oberndorfer et al. 2007). The vegetation component of a green roof is always key to the successful provision of these services, for example transpiration of plants increases the cooling effect of a roof (Castleton et al. 2010) whilst at the same time recharging the available storm water reservoir (Wolf and Lundholm 2008). However a relatively narrow spectrum of plant species are currently used on green roofs due to the harsh growing environment (Dunnett and Kingsbury 2010). Plants most often used include hardy succulents (e.g. *Sedum spp.*), mosses, and some herbaceous perennials and annuals (Snodgrass and Snodgrass 2006; Rowe et al. 2012). In particular the use of succulent *Sedum spp.* on green roofs (especially on extensive roofs where substrate depth is less than 100mm) is very common due to their low growth, shallow rooting and ability to withstand extremely harsh growing conditions (Getter and Rowe 2006; Snodgrass and Snodgrass 2006). However due to these qualities *Sedum spp.* do not always provide an optimum level of green roof services. For example, due to their water conservation strategies they can severely reduce the amount of water leaving the substrate via evapotranspiration which leads to a reduced cooling service (Lundholm et al. 2010; Blanusa et al. 2013) and a slow recharge of available storm water retention capacity (Voyde et al. 2010).

An alternative to using the small range of plants that are extremely well adapted to the green roof environment is to modify the substrate so it can support a wider range of plants. Increasing the depth of substrate will increase the water holding capacity

(WHC) of a roof (VanWoert et al. 2005a; Mentens et al. 2006) and usually leads to more successful plant establishment, growth and long term survival (Durhman et al. 2007; Getter and Rowe 2008; Getter and Rowe 2009; Thuring et al. 2010; Rowe et al. 2012). However this is not always possible due to economic and structural constraints and therefore a better alternative may be to alter the physical characteristics of the substrate by modifying or adding components. By modifying for example; inter particle pore space (Graceson et al. 2013; Young et al. 2014a), organic content (Rowe et al. 2006b; Nagase and Dunnett 2011), or the addition of water retaining additives (Olszewski et al. 2010; Farrell et al. 2013; Savi and Marin 2014) the WHC can be increased. This increase in WHC in combination with increased organic matter (Nagase and Dunnett 2011) and depth (Dunnett et al. 2008b; Thuring et al. 2010) can lead to greater plant growth. However promoting high levels of luxuriant plant growth may not be desirable on a green roof as plants may then become more vulnerable to drought conditions (Rowe et al. 2006b; Nagase and Dunnett 2011; Savi and Marin 2014). Alternatively, it is possible to increase green roof substrate WHC without causing a large increase in plant growth through the use of water retention gels which are able to absorb large amounts of water (Olszewski et al. 2010; Farrell et al. 2013; Savi and Marin 2014; Young et al. 2014a).

Similarly the use of smaller sized particles in green roof substrate has been shown to increase WHC and also plant growth and physiological health (Graceson et al. 2013; Graceson et al. 2014a; Young et al. 2014a). It is still not clear whether this is will increase plant tolerance to drought conditions or leave plants more vulnerable due to their greater amount of shoot biomass (Rowe et al. 2006b; Nagase and Dunnett 2011; Young et al. 2014a).

An alternative to increasing the WHC of a substrate is to reduce the rate at which water is lost from it. Commonly used *Sedum spp.* mats or the addition of moss *spp.* have previously been shown to reduce green roof substrate temperatures (Butler and Orians 2011; Heim et al. 2014; Heim and Lundholm 2014a), maintain higher levels of water in substrate by impeding evaporation (Wolf and Lundholm 2008) and improve companion plant appearance during a severe drought (Butler and Orians 2011; Heim et al. 2014). However it is not clear how this effect compares to the benefits offered by substrate modification.

To address these questions, a pot experiment was established where the growth and physiological health of two common green roof perennial species (*Festuca ovina* and *Linaria vulgaris*) was assessed during three droughts of 10, 15 and 25 days. Both plant species were grown on green roof substrates composed of factorial combinations of, (i) small or large crushed brick, (ii) presence/absence of a polyacrylamide gel (SwellGel™) and (iii) presence/absence of *Sedum spp.* on the substrate surface.

It was hypothesised that;

1. SwellGel would increase plant survival and physiological health by providing a slow release water reservoir in the substrate.
2. Small brick would increase the growth of plants during ambient conditions which would make plants more vulnerable to drought, despite having a larger WHC.
3. The presence of *Sedum spp.* would reduce plant growth during ambient conditions, reduce water loss via evaporation and therefore increase plant performance during drought conditions.

3.3 Methods

3.3.1 Experimental Design

Substrates with two component variables were used: (i) brick size (small brick at of 2-5 mm particle diameter; large brick of 4-15 mm diameter) and (ii) presence or absence of a water retention polyacrylamide gel "SwellGel™" (www.swellgel.co.uk) (Table 3.1). Brick was crushed waste red brick, sieved to ensure brick fragments were within the size limits set. Green waste compost (Green Estate, Sheffield, UK) was composed of composted garden waste collected in Sheffield. SwellGel™ is a soil additive made of cross linked polyacrylamide which is designed to expand and store water during high moisture levels and release it slowly back to the plant as moisture levels decline.

20% of the substrate volume was made up of green waste compost, with the remaining 80% made up from one of the two crushed brick size categories. Dry SwellGel was then added as 1% of the total substrate volume and substrate added to pots (12 cm x 11 cm x 11 cm) to a level of 120 mm.

3.3.2 Plant species and growth conditions

Festuca ovina and *Linaria vulgaris (*sourced as SkyPlugs™ from Boningale Nurseries Ltd) were planted as plug plants at a density of 4 plugs per pot. These two species were used as they are common green roof species and gave a spectrum of drought tolerance from relatively high (*F. ovina*) to low tolerance (*L. vulgaris*). Plugs were washed before planting to remove any substrate and *F. ovina* plugs split into roughly four separate plugs in order to make up the required number. A treatment of *Sedum* cuttings (mixture of *Sedum album* and *Sedum acre*) was applied to half of the pots at

a density of approximately 100 g m^{-2} (1.21 g per pot) to achieve full coverage of the substrate.

Planted pots were kept in a controlled greenhouse environment for 3.5 months with a temperature regime reflective of a temperate summer climate (16 hours 20 ^{o}C, 8 hours 15 ^{o}C) to allow plants to mature and *Sedum* to achieve high coverage of substrate. Where necessary, supplementary lighting was used to ensure the required day length. A watering regime representing an average month in Sheffield, UK (240 ml per pot $week^{-1}$ = 80 mm $month^{-1}$) was used from planting until drought initiation.

3.3.3 Drought Treatments

Each treatment combination received four drought treatments with six replicates each, to make a total of 384 pots (Table 3.1). Drought treatments were as follows, Short = 10 days, Medium = 15 days, Long = 25 days and Control = 0 days After each drought treatment ended the initial watering regime was restarted for two weeks in order to provide plants with a recovery period.

3.3.4 Biomass Harvest

After the two week recovery period shoot biomass was harvested, dried at 80 ^{o}C for 48 hours and weighed. Root biomass was removed, washed, dried (80 ^{o}C for 48 hours) and weighed from the long drought pots only. Roots from substrates containing SwellGel were soaked overnight in water in order to expand the gel, which was then manually removed from the roots using a scalpel.

The experiment therefore had a fully factorial design of two plant species (*F.ovina* and *L.vulgaris)*, brick size (2-5 mm and 4-15 mm), SwellGel (presence or absence), *Sedum* treatment (presence or absence) and four drought treatments (Table 3.1).

Table 3.1: Factorial design of drought experiment. S= short drought (10days), M=medium drought (15days), L= long drought (25days) and C=control.

Species	Brick Size 80% by volume Small=2-5mm Large=4-15mm	SwellGel 1% by volume	Sedum	Drought Treatments (Six replicates of each)	Total Number of Pots
Festuca ovina	Small	Yes	Yes	S, M, L & C	24
			No	S, M, L & C	24
		No	Yes	S, M, L & C	24
			No	S, M, L & C	24
	Large	Yes	Yes	S, M, L & C	24
			No	S, M, L & C	24
		No	Yes	S, M, L & C	24
			No	S, M, L & C	24
Linaria vulgaris	Small	Yes	Yes	S, M, L & C	24
			No	S, M, L & C	24
		No	Yes	S, M, L & C	24
			No	S, M, L & C	24
	Large	Yes	Yes	S, M, L & C	24
			No	S, M, L & C	24
		No	Yes	S, M, L & C	24
			No	S, M, L & C	24
Total					**384**

3.3.5 F_v/F_m

Physiological health was determined by chlorophyll fluorescence of Photosystem II (PSII). This was quantified using a Walz Mini-PAM photosynthesis yield analyser using the saturation pulse method (Heinz Walz GmbH). Plants were measured every three to four days and at least two hours after sunset in order to ensure full dark adaption. Maximum quantum efficiency of PSII was recorded (F_v/F_m) each time on randomly selected *F.ovina* shoots (enough to fill the leaf clip) and on a randomly selected *L.vulgaris* leaf. This process was repeated once for each plant and therefore

four times for each pot. Measurements began the day that drought commenced and continued until the day before harvesting.

3.3.6 Plant Wilting Index

The visual health of the plants was assessed with a wilting index adapted from Engelbrecht et al., (2007) (Table 3.2). A score between zero and five was assigned to the plants in each pot depending on the visual signs of wilting (Table 3.2). This was carried out every three/four days throughout the trial.

Table 3.2: Wilt Index scoring system for *F. ovina* and *L. vulgaris*. Adapted from Engelbrecht et al., (2007).

Wilting Index Score	*F.ovina*	*L.vulgaris*
0	Dead (no resprouting after watering)	Dead (no resprouting after watering)
1	Fully wilted (horizontal form)	Fully wilted and nearly dead (all leaves dead but stem still alive)
2	Severely wilted (only a few vertical grass blades showing)	Severely wilted (strong change in majority of leaf angle/curling up, stem still alive)
3	Half wilted (50% spilt)	Half wilted (some change in leaf angle, some healthy leaves still)
4	Sporadic/slightly wilted (relatively few grass blades wilted)	Sporadic/slightly wilted (some changes to leaf angle but no folding or rolling of leaf)
5	No wilting	No wilting

3.3.7 Water Holding Capacity/Physical Characteristics of Substrate

Each pot was weighed daily (excluding weekends) throughout the trial. After the plant harvest each pot was soaked overnight in order to fully saturate the substrate and then allowed to freely drain for fifteen minutes. After which the pots were considered to be at field capacity and weighed. They were then oven dried at 80 ºC for four days and weighed again to obtain dry weight. The difference between dry and field capacity weight was taken as the water holding capacity. Thus the rate of water loss via evapotranspiration for each pot (change in weight over time) was calculated as a percentage of total water holding capacity.

Physical properties of the substrate (Table 3.3) were determined according to Forschungsgesellschaft Landschaftsentwicklung Landschaftsbau (FLL) (FLL 2008) methods.

3.3.8 Temperature of substrate

The temperature of the substrate was measured throughout the trial with remote temperature loggers (Maxim Integrated™ iButton®). These were buried 2cm below the surface of the substrate in the centre of each pot and programmed to take the temperature every hour to an accuracy of 0.5 ºC. Each iButton® was wrapped in Clingfilm to prevent moisture damage. Due to the number of treatments only the temperature of the long drought treatment was measured.

Table 3.3: Main factor effects and means (two-way ANOVA) for substrate physical characteristics. Tests performed according to FLL standards. Statistical significances of P-values: * p<0.01, **p<0.001, *** p<0.0001. Statistical significances were calculated from two-way ANOVA.

	FLL physical characteristics of substrate											
	SwellGel						Brick Size					
Physical substrate characteristics	No	Yes	% Change ±SE, n=6)	P-value	F-value	Df	Small	Large	% Change ±SE, n=6)	P-value	F-value	Df
Max Water Holding Capacity (%)	29.8	46.4	+55.7±8.7	***	108.9	1	42.6	33.6	-21.1±4.2	***	31.9	1
Permeability (mm/min)	24.8	17.4	-29.8±4.3	*	10.9	1	12.4	29.8	+140.3±20.5	***	59.1	1
Air Dry Density (g/cm^3)	1.06	1.02	-3.8±2.5	0.12	3.0	1	1.05	1.04	-1.0±2.7	0.66	0.2	1
Oven Dried Density (g/cm^3)	1.12	0.95	-15.2±2.8	***	44.3	1	1.02	1.05	+2.9±3.2	0.27	1.4	1
Saturated Density (g/cm^3)	1.38	1.17	-15.2±2.6	***	46.7	1	1.30	1.25	-3.8±2.2	0.09	3.7	1
Total Pore Volume (%)	31.2	62.5	+100.3±14.1	***	177.2	1	51.9	41.7	-19.7±5.6	**	18.7	1
Air Content at WC Max (%)	1.04	1.35	+29.8±2.8	***	145.4	1	1.19	1.20	+0.8±2.3	0.92	0.01	1

3.3.9 Statistical Analysis

To determine the main factorial effects and interactions of the substrate components on the substrates physical characteristics, shoot biomass, root biomass, root:shoot ratio, and substrate temperature, three-way ANOVAs were performed (with SwellGel presence or absence, brick size and *Sedum spp.* presence or absence as the fixed

factors). Three way repeated measures ANOVAs were performed on the F_v/F_m and wilt index values. F_v/F_m values were Arcsine square root transformed before analysis. *L. vulgaris* data between days 20-37 was analysed with type III ANOVA due to an unbalanced data set caused by an incorrect watering event. A cumulative link model from the R Package 'Ordinal' was used to analyse the wilt index data due to the ordinal nature of the index. The models used to predict water loss were 2^{nd} order polynomial. All statistical analyses were carried out in R Studio version 2.15.1 (22.6.2012), (R Development Core Team, 2011).

3.4 Results

3.4.1 Physical Characteristics of Substrate

SwellGel significantly increased substrate WHC by 56% (two way ANOVA, $p<0.05$) whilst large brick decreased WHC by 21% ($p<0.0001$) relative to small brick (Table 3.3). Substrate permeability was significantly reduced by SwellGel by 30% but significantly increased by large brick by 140% (two way ANOVA, $p<0.05$) (Table 3.3). SwellGel also reduced oven dried and saturated density by 15%, and increased pore volume by 100% (two way ANOVA, $p<0.05$) (Table 3.3).

3.4.2 Shoot Biomass

Festuca ovina shoot biomass was not significantly affected by drought treatment (three way ANOVA $p>0.05$) (Table 3.4a). *Linaria vulgaris* shoot biomass differed significantly between drought treatments and consistently decreased as drought length increased (three way ANOVA, $p<0.05$) (Table 3.4b). SwellGel increased shoot biomass of both species for all four drought treatments by between 10-34% (Table 3.4a,b). The positive effect of SwellGel on *F. ovina* was relatively constant, increasing shoot biomass by around 30% for the short and long drought, although the 10% increase observed for the medium drought was not significant (three way ANOVA, $p=0.099$) (Table 3.4a). SwellGel's relative effect on *L. vulgaris* increased as drought length increased (increasing biomass from 28% to 42%), however even with SwellGel, biomass was smaller as drought length increased (Table 3.4b). Large brick significantly reduced biomass of both species by between 23-40% for all drought treatments (three way ANOVA, $p<0.05$). The presence of *Sedum* significantly reduced

biomass at all drought treatments by between 36-63% for *F. ovina* and 24-43% for *L. vulgaris* with this effect being relatively constant between drought treatments (three way ANOVA, $p<0.05$) (Table 3.4 a,b).

3.4.3 Root Biomass

SwellGel significantly decreased control drought root biomass by 36% for both species (three way ANOVA, $p<0.05$) (Table 3.5 a,b). Large brick also significantly reduced *F. ovina* control root biomass by 26% although no effect of brick size was observed for *L. vulgaris* (three way ANOVA, $p>0.05$). The presence of *Sedum* had no significant effect on either species control drought root biomass (three way ANOVA, $p>0.05$) (Table 3.5 a,b).

3.4.4 Root:Shoot Ratio

SwellGel significantly decreased the control drought root:shoot ratio for both species by 43-44% (three way ANOVA, $p<0.05$) (Table 3.5 c,d). Large brick significantly increased this in *L. vulgaris* by 50%, although it had no significant effect on *F. ovina* (three way ANOVA, $p<0.05$). The presence of *Sedum* significantly decreased root:shoot ratio by 38-39% for both species (three way ANOVA, $p<0.05$) (Table 3.5 c,d).

Table 3.4: Main factor effects and means (three-way ANOVA) for (a) *F. ovina* shoot biomass and (b) *L. vulgaris* biomass after four drought treatments. Main factor means and the % change are shown between the two levels within that factor (e.g. absence vs. presence of SwellGel). Significant factorial interactions are also shown. Statistical significances of P-values: * p<0.01, **p<0.001, *** p<0.0001. Statistical significances were calculated from three-way ANOVA. Abbreviations for factor interactions is SG=SwellGel.

a) Mean factor means of *F. ovina* shoot biomass (g) after four drought treatments

		Factors												Significant factor interactions			
		SwellGel				Brick Size				Sedum				SG:Brick	SG:Sedum	Brick:Sedum	SG:Brick:Sedum
		No	Yes	%	P-value	Small	Large	%	P-value	No	Yes	%	P-Value	P-Value			
Drought	Control	2.41	2.8	+16.2	*	2.98	2.22	-25.5	***	3.21	1.2	-62.6	***	*			
	Short	2.7	3.55	+31.5	**	3.89	2.35	-39.6	***	3.9	2.35	-39.7	***				*
	Medium	2.58	2.83	+9.7	0.099	3.16	2.26	-28.5	***	3.45	1.96	-43.2	***	*			**
	Long	2.25	3.02	+34.2	***	3.16	2.11	-33.2	***	3.22	2.05	-36.3	***			**	*

b) Mean factor means of *L. vulgaris* shoot biomass (g) after four drought treatments

		Factors												Significant factor interactions			
		SwellGel				Brick Size				Sedum				SG:Brick	SG:Sedum	Brick:Sedum	SG:Brick:Sedum
		No	Yes	%	P-value	Small	Large	%	P-value	No	Yes	%	P-Value	P-Value			
Drought	Control	1.33	1.44	+8.3	0.26	1.7	1.07	-37.1	***	1.57	1.2	-23.6	***				
	Short	1.12	1.43	+27.7	**	1.46	1.08	-26.0	***	1.62	0.92	-43.2	***		*		
	Medium	1.06	1.28	+20.8	**	1.32	1.02	-22.7	***	1.35	0.98	-27.4	***			***	
	Long	0.57	0.82	+42.1	***	0.75	0.56	-25.3	**	0.78	0.53	-32.1	***				

Table 3.5: Main factor effects and means (three-way ANOVA) for (a) *F. ovina* control pot root biomass (g), (b) *L. vulgaris* control pot root biomass (g), (c) *F. ovina* root:shoot ratio, (d) *L. vulgaris* root:shoot ratio. Main factor means and the % change are shown between the two levels within that factor (e.g. absence vs. presence of SwellGel). Significant factorial interactions are also shown. Statistical significances of P-values: * p<0.01, **p<0.001, *** p<0.0001. Statistical significances were calculated from three-way ANOVA. Abbreviations for factor interactions is SG=SwellGel.

Factor	F-value	P-value	Factor means of *F. ovina* root biomass (g) from control drought pots						% Change (±SE, n=64)
			SwellGel		Brick Size		Sedum		
a)			No	Yes	Small	Large	No	Yes	
SwellGel	31.6	***	2.40	1.53					-36.3±9.3
Brick Size	12.8	***			2.23	1.66			-25.6±12.6
Sedum	3.2	0.08					2.09	1.81	-13.4±8.0
SG:Brick	8.2	**							
b)			Factor means of *L. vulgaris* root biomass (g) from control drought pots						
SwellGel	31.6	***	1.18	0.75					-36.4±6.8
Brick Size	0.8	0.39			1.00	0.93			-7.0±4.8
Sedum	0.02	0.90					0.96	0.97	+1.0±7.3
c)			Factor means of *F. ovina* root:shoot ratio from control drought pots						
SwellGel	62.0	***	1.04	0.58					-44.2±4.5
Brick Size	1.2	0.27			0.78	0.83			+6.4±12.5
Sedum	21.7	***					0.68	0.94	+38.2±13.2
SG:Brick:Sedum	5.4	*							
d)			Factor means of *L. vulgaris* root:shoot ratio from control drought pots						
SwellGel	49.6	***	0.98	0.56					-42.9±4.7
Brick Size	27.2	***			0.61	0.92			+50.8±9.8
Sedum	18.2	***					0.64	0.89	+39.1±10.3
SG:Brick	5.4	*							
Brick:Sedum	4.9	*							
SG:Brick:Sedum	7.2	*							

3.4.5 Fv/Fm & Wilt Index

Control, Short & Medium Droughts

Festuca ovina and *L. vulgaris* F_v/F_m and wilt index values did not significantly decrease or differ between substrate treatments for the control and short drought

treatments (repeated measures ANOVA, $p>0.05$). *Festuca ovina* F_v/F_m and wilt index values also did not significantly decrease or differ between substrate treatments for the medium drought (repeated measures ANOVA, $p>0.05$). *Linaria vulgaris* F_v/F_m and wilt index values for the medium drought were significantly lower after 13 days for small brick substrates (repeated measures ANOVA, $p<0.05$) although SwellGel and *Sedum* had no effect. Due to these limited effects, for brevity, these data are not shown and we focus on the long drought data.

Long Drought- F. ovina

Festuca ovina showed a decrease in F_v/F_m and wilt index values throughout the trial with a significant decrease in values after 20 days of drought (Fig 3.1a, 3.2a). F_v/F_m and wilt index values after 20 days were significantly higher when SwellGel or large brick were present in the substrate (repeated measures ANOVA, $p<0.05$). This increase in F_v/F_m and wilt index values continued after the drought had finished with substrates containing SwellGel showing a much faster recovery. The presence of *Sedum* did not significantly affect *F. ovina* F_v/F_m values (repeated measures ANOVA, $p>0.05$) but did significantly reduce wilt index values throughout the trial (repeated measures ANOVA, $p<0.05$) (Fig 3.1a, 3.2a).

Long Drought- L. vulgaris

Linaria vulgaris showed a decrease in F_v/F_m and wilt index values throughout the trial with a significant decrease in values after 13 days of drought (Fig 3.1b, 3.2b). F_v/F_m and wilt index values after 13 days of drought were significantly lower for small brick substrates and significantly higher for substrates containing SwellGel (repeated measures ANOVA, $p<0.05$). The increase in F_v/F_m and wilt index values for large brick substrates only occurred between days 13-20, after which no significant effect of large

brick substrates was observed (Fig 3.1b, 3.2b). SwellGel continued to increase F_v/F_m and wilt index values until the end of the drought at day 25 and showed a faster recovery once watering had restarted. The presence of *Sedum* did not significantly affect *L. vulgaris* F_v/F_m and wilt index values (repeated measures ANOVA, $p>0.05$) throughout the trial (Fig 3.1b, 3.2b).

3.4.6 Drying Out Curves

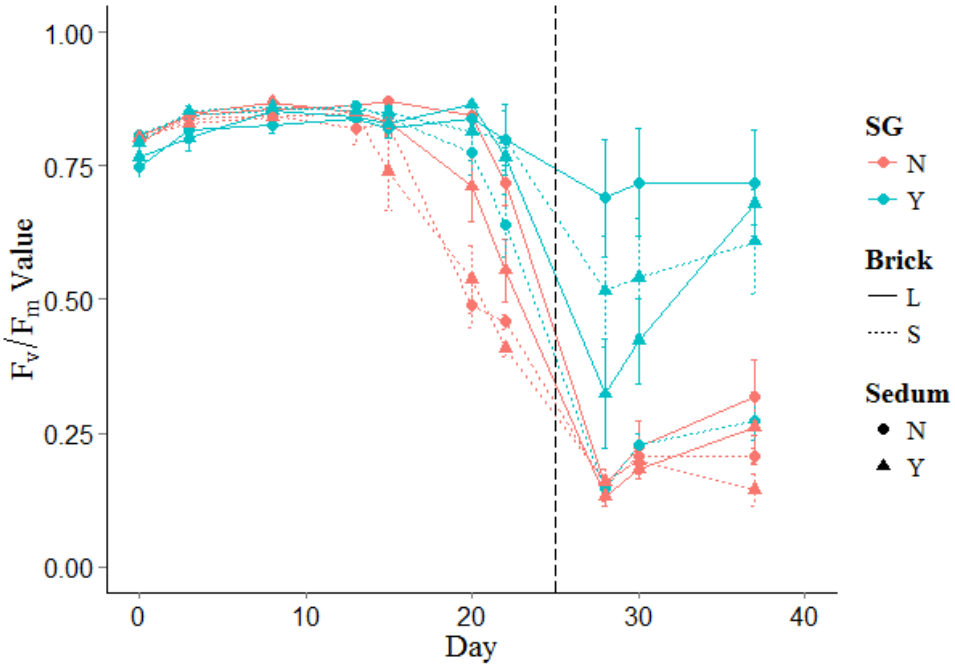
SwellGel and large brick increased the time taken for substrates to move from 70% to 20% WHC by 1.6 days for *F. ovina* and by 1-1.7days for *L. vulgaris* (Table 3.6). The presence of *Sedum* also increased time taken to move from 70% to 20% WHC by 0.49 days and 1.27 days for *F.ovina* and *L. vulgaris* respectively (Table 3.6).

3.4.7 Substrate Temperature

The mean daily, minimum and maximum substrate temperatures for *F. ovina* were all significantly increased by the presence of *Sedum* by 0.28-0.37 ^oC (Table 3.7). *F. ovina* daily mean and maximum substrate temperatures were also significantly increased by large brick by 0.46 ^oC and 0.78 ^oC respectively, whilst SwellGel significantly decreased daily maximum substrate temperature by 0.31 ^oC (Table 3.7).

The mean daily, minimum and maximum substrate temperatures for *L. vulgaris* were not significantly affected by any substrate factor (Table 3.7).

a)



b)

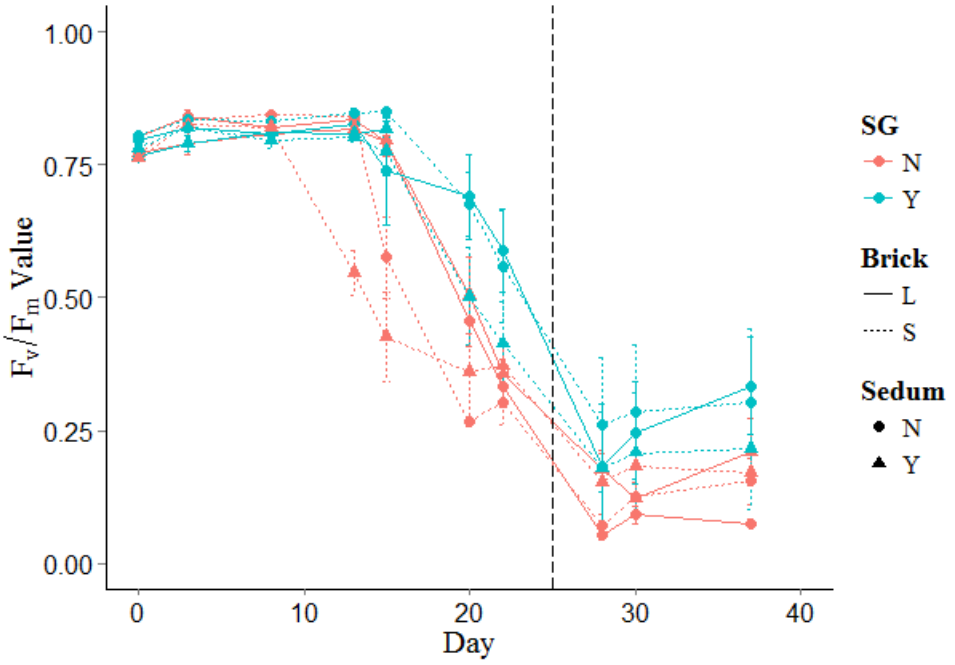
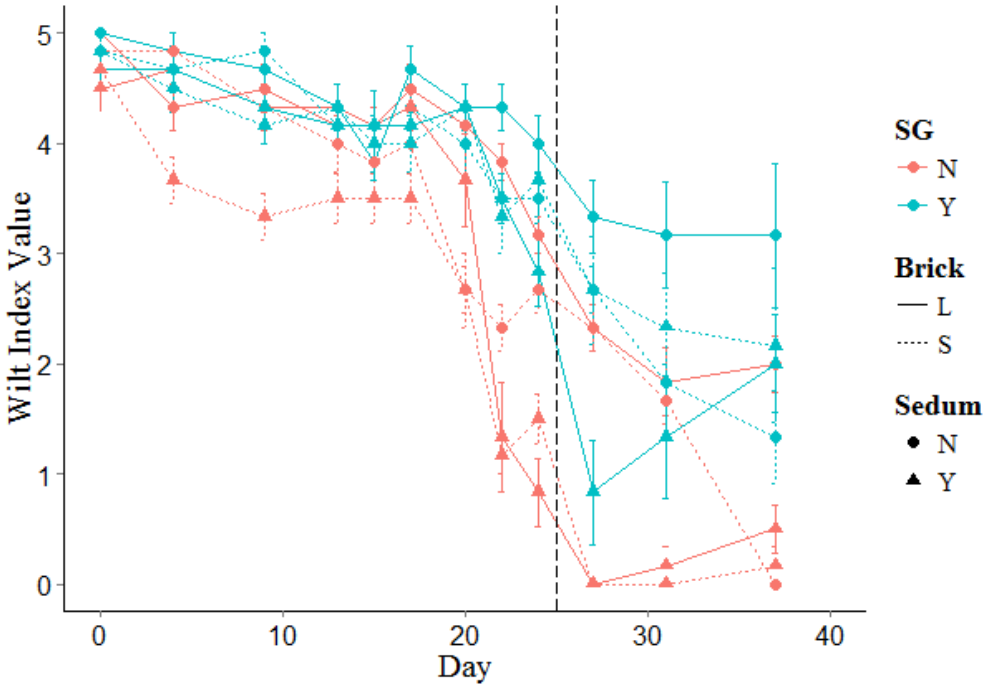


Figure 3.1: (a) Mean F_v/F_m values for *F. ovina* during long drought (25 days), (b) Mean F_v/F_m values for *L. vulgaris* during long drought (25 days). Black dashed line indicates end of drought and start of two week watered recovery period. Error bars are± one standard error.

For SwelGel (SG) and Sedum, “N” and “Y” refer to “yes” and “no”. For Brick, “L” and “S” are “large” and “small” particle size.

a)



b)

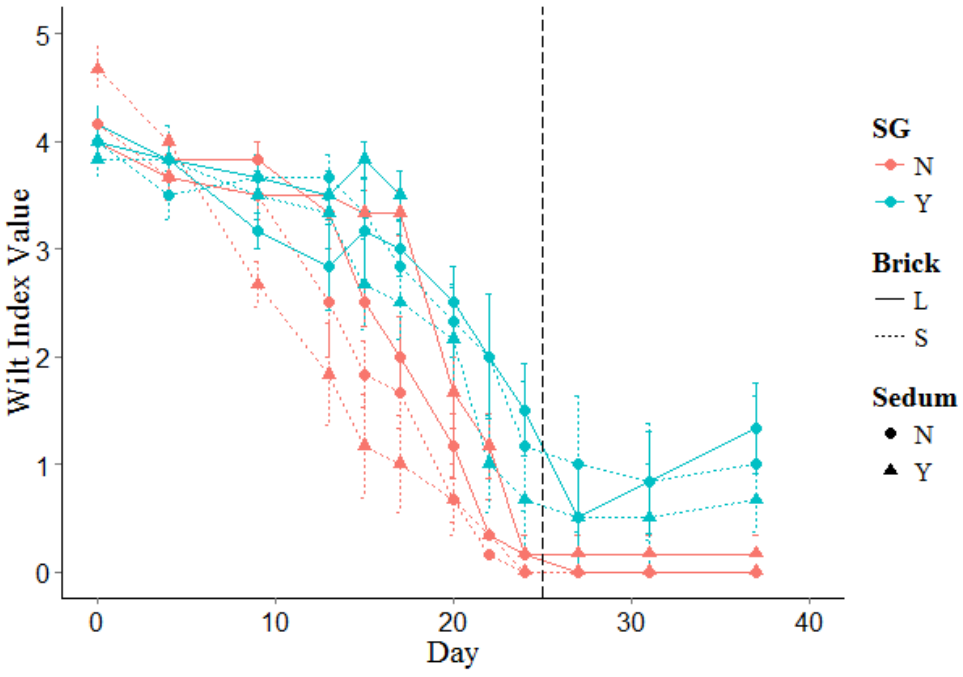


Figure 3.2: (a) Mean wilt index values for *F. ovina* during long drought (25 days), (b) Mean wilt index values for *L. vulgaris* during long drought (25 days). Black dashed line indicates

end of drought and start of two week watered recovery period. Error bars are± one standard error. For SwelGel (SG) and Sedum, “N” and “Y” refer to “yes” and “no”. For Brick, “L” and “S” are “large” and “small” particle size.

Table 3.6: Main factor effects and means (three-way ANOVA) for (a) *F. ovina* drying out curves and (b) *L. vulgaris* drying out curves. Main factor means, the time taken for substrates to dry from 70% WHC to 20% and the difference between each two levels within each factor (e.g. absence vs. presence of SwellGel).

	Mean factor means of substrate drying out curves									
		SwellGel			Brick Size			Sedum		
		No	Yes	Difference	Small	Large	Difference	No	Yes	Difference
a)	Days to 70%	1.38	2.62		1.33	2.88		1.86	1.90	
	Days to 20%	14.60	17.45		14.38	17.57		15.85	16.38	
	Days Between 70-20% (±SE)	13.22±0.38	14.83±0.15	**+1.61±0.23**	13.05±0.49	14.69±0.17	**+1.64±0.32**	13.99±0.23	14.48±0.26	**+0.49±0.03**
b)	Days to 70%	1.14	2.35		1.25	2.44		1.79	1.43	
	Days to 20%	14.61	17.56		14.95	17.17		15.77	16.68	
	Days Between 70-20% (±SE)	13.47±0.46	15.21±0.14	**+1.74±0.32**	13.70±0.52	14.73±0.17	**+1.03±0.35**	13.98±0.24	15.25±0.23	**+1.27±0.01**

Table 3.7: Main factor effects and means (three-way ANOVA) for *F. ovina* and *L. vulgaris* long drought substrate *(*a) Daily mean temperature (b) Daily minimum temperature (c) Daily maximum temperature. Main factor means, the time taken for substrates to dry from 70% WHC to 20% and the difference between each two levels within each factor (e.g. absence vs. presence of SwellGel). Statistical significances of P-values: * p<0.01, **p<0.001, *** p<0.0001. Statistical significances were calculated from three-way ANOVA.

Long drought substrate mean temperature readings

			SwellGel					Brick Size					Sedum				
			No	Yes	Difference ±SE, n=6)	P-value	F-value	Small	Large	Difference ±SE, n=6)	P-value	F-value	No	Yes	Difference ±SE, n=6)	P-value	F-value
a)	Daily Mean (ºC)	*F. ovina*	15.90	15.77	-0.13± 0.07	0.18	1.8	15.61	16.07	+0.46± 0.08	***	26.5	15.70	15.98	+0.28± 0.09	**	10.3
		L. vulgaris	15.75	15.93	+0.18± 0.09	0.22	1.6	15.85	15.81	-0.04± 0.08	0.97	0.001	15.94	15.66	-0.28± 0.09	0.06	3.7
b)	Mean Daily Minimum (ºC)	*F. ovina*	12.79	12.85	+0.06± 0.06	0.48	0.5	12.76	12.89	+0.13± 0.06	0.13	2.4	12.64	13.01	+0.37± 0.07	***	20.7
		L. vulgaris	12.55	12.71	+0.16± 0.07	0.19	1.8	12.62	12.65	+0.03± 0.05	0.57	0.3	12.62	12.65	+0.03± 0.08	0.62	0.2
c)	Mean Daily Maximum (ºC)	*F. ovina*	18.63	18.32	-0.31± 0.12	*	6.7	18.09	18.87	+0.78± 0.12	***	41.1	18.29	18.66	+0.37± 0.14	**	10.5
		L. vulgaris	18.69	18.87	+0.18± 0.14	0.39	0.8	18.81	18.73	-0.08± 0.12	0.86	0.03	18.91	18.55	-0.36± 0.14	0.09	3.1

3.5 Discussion

This study has shown that a polyacrylamide water retention additive (SwellGel) and the use of large crushed brick in green roof substrates can improve the drought tolerance of *F. ovina* and *L. vulgaris*. However the addition of *Sedum* as a companion plant did not improve the drought tolerance of both species despite reducing plant growth. SwellGel and brick size differed in their method of increasing drought tolerance, with SwellGel increasing the WHC of a substrate while only causing a relatively small increase in initial plant growth. The use of large brick particles instead of small brick led to slower and more sustainable plant growth during the ambient watering regime before the drought, leading to greater tolerance during drought.

3.5.1 Physical characteristics of substrate

In this trial SwellGel increased WHC of green roof substrates 50% more than small crushed brick despite only making up 1% of the substrate volume. It has previously been reported that substrate particle size can have a larger impact upon WHC than SwellGel at 1% volume (Young et al. 2014a). However the repeated wetting and drying cycles experienced in the study reported here may have led to a decrease in the ability of SwellGel to retain water (Geesing and Schmidhalter 2004; Bai et al. 2013). The impact of water absorbent gels is also strongly dependent upon the rate of application. This study used a higher application rate of 1% than most other previous studies which have ranged from 0.1-0.6% application (Geesing and Schmidhalter 2004; Olszewski et al. 2010; Farrell et al. 2013; Savi and Marin 2014). Applying water absorbent gels at the higher rate will result in a more expensive substrate, but this extra

cost needs to be balanced out against the clear benefits of 1% addition, compared to lower rates (especially 0.1%) that do not always provide marked benefits (Farrell et al. 2013). More research is needed to find an optimal application rate for green roof substrate.

The observed physical properties of the substrate in this trial supports previous research which shows that a decrease in substrate particle size can increase substrate WHC, pore volume and decrease permeability (Olszewski and Young 2011; Graceson et al. 2013).

3.5.2 Shoot and Root Biomass

All three substrate factors had a significant effect on *F. ovina* and *L. vulgaris* shoot biomass during nearly all of the drought treatments. SwellGel's positive impact upon shoot biomass increased with length of drought but only caused a relatively small increase in biomass during the control treatment. This implies that SwellGel only has a large impact upon shoot growth during times of drought stress and promotes little extra growth during ambient watering conditions. This would help to increase plant drought tolerance as it avoids promoting luxuriant growth that may increase the vulnerability of plants to drought due to the larger amount of shoot biomass to maintain (Rowe et al. 2006a; Nagase and Dunnett 2011; Bates et al. 2013). In addition the substrate water reservoir may be exhausted at a faster rate due to greater transpiration from a larger plant canopy (VanWoert et al. 2005b; Wolf and Lundholm 2008; Young et al. 2014a). Previous studies have shown that water absorbing gels in green roof substrates do not have an effect on the shoot growth of *Triticum aestivum* and *Lupinus albus* at 0.1% volume during drought conditions (Farrell et al. 2013) but

0.05-0.6% volume application can increase *Salvia officinalis* and *Sedum spp.* shoot growth during ambient watering conditions (Olszewski et al. 2010; Savi and Marin 2014). This suggests that plant species differ in their response to water absorbing gels, whilst gel application rate is also important.

The consistent decrease in shoot biomass for both species in our study with large brick substrates was likely to be a function of the lower water holding capacity (Farrell et al. 2012) and high permeability of the substrate, which exposed plants to a degree of water stress before the drought treatments commenced. This may have primed plants to being more drought tolerant before the drought was imposed, and also resulted in greater drought tolerance due to the smaller canopy that transpired less and so caused less water loss (Young et al. 2014a).

Similarly, the presence of *Sedum spp.* reduced shoot biomass for both species on all drought treatments, presumably through a competitive effect for water and nutrition. It has previously been shown that *Sedum spp.* mosses or other mat forming species can reduce the growth of neighbour plants during periods of ambient rainfall, which leads to improve performance during drought conditions through a combination of reduced plant biomass to maintain and cooler substrate temperatures (Butler and Orians 2011; Heim et al. 2014; Heim and Lundholm 2014a).

Root biomass was only measured for the control drought treatment but still showed significant changes in response to substrate factors. Root biomass and root:ratio was reduced by SwellGel for both species which agrees with previous trials (Farrell et al. 2013; Young et al. 2014a), although it is not clear whether this is a direct impact of the SwellGel or an artefact of the process of removing SwellGel from roots prior to weighing which may result in some loss of root biomass. Additionally, care must be

taken in the long-term use of SwellGel if it is truly directly leading to a reduction in root biomass and root:shoot ratio as this may make plants more vulnerable to drought (Grossnickle 2005; Nagase and Dunnett 2011), especially as SwellGel degrades over time and loses its water holding capacity (Al-Harbi et al. 1999; Hüttermann et al. 2009; Savi and Marin 2014). Instead, large brick may provide a more sustainable solution for enhanced drought tolerance.

The decrease in shoot growth caused by *Sedum* and large brick had the result of increasing root:shoot ratios. This indicates that under these conditions, plants experienced a lower amount of available water or nutrients in large brick and increased interspecific competition with *Sedum* and therefore allocated more resources to water and nutrient capturing roots which is likely to make plants more drought tolerant (Poorter and Nagel 2000; Hermans et al. 2006; Robinson et al. 2010), and enhances the benefits of the large brick by also producing a smaller plant canopy with lower rates of transpiration (Young et al. 2014a).

3.5.3 F_v/F_m & Wilt Index

F_v/F_m and wilt index values for both species showed a decline as drought length increased and were also largely correlated with each other in their response to drought.

SwellGel and large brick substrates maintained plant F_v/F_m and wilt index values at significantly higher levels throughout the drought with plants showing faster recovery once watering had commenced again. SwellGel increased the time taken for substrate WHC to decline to 20% once watering was stopped, presumably because the extra water held within SwellGel slowly diffused into the substrate (Savi and Marin 2014), although this can be dependent on the root contact with the gel and conductivity within the substrate (Fonteno and Bilderback 1993). While SwellGel clearly has beneficial

properties for drought tolerance, other water retention additives, such as silicate granules, should not be discounted and may be more effective at providing water to plants as they can increase water availability more homogeneously throughout the substrate (Farrell et al. 2013).

Penetration of SwellGel by roots was observed for both species and therefore it was assumed that the water held within the gels was available to the plants (Woodhouse and Johnson 1991; Farrell et al. 2013; Savi and Marin 2014). However *L. vulgaris* has a much less dense and less finely branching root system than *F. ovina*, which led to less SwellGel contact and penetration and could be a contributory factor to the lower effectiveness of SwellGel (Farrell et al. 2012). As this trial only looked at the short term effect of SwellGel on recently established plants during a single drought, the long term impact upon on green roof plants is unknown. Polyacrylamide gels are known to degrade and rapidly lose their WHC due to UV light exposure, freeze thaw cycles, elevated temperatures and microbial/fungal activity (Smith et al. 1996; Smith et al. 1997; Akhter et al. 2004; Holliman et al. 2005; Savi and Marin 2014). Therefore although SwellGel may initially increase plant drought tolerance, it will lose its ability to store water over time and plants become more vulnerable to drought stress due to their lower root:shoot ratio (Grossnickle 2005). Alternatively plants grown with SwellGel may slowly adapt to the lower amount of available water as the decline in gel WHC is likely to be linear at around 1-9% $year^{-1}$ (Al-Harbi et al. 1999; Hüttermann et al. 2009).

Overall, SwellGel may still be an attractive option to improving the drought tolerance of newly established green roof plants, and given this is likely to be the most vulnerable stage of a green roof's plant coverage; even this early benefit can justify the use of SwellGel. This benefit may be increased further where SwellGel increases

substrate bacterial and fungal populations (Li et al. 2014) as well as plant available nitrogen (Kay-Shoemake et al. 1998).

The ability of large brick to improve F_v/F_m and wilting index values during drought compared to small brick shows that an increase in substrate WHC does not always result in increased drought tolerance. By reducing the amount of water available to plants during ambient watering conditions as well providing a coarse growing media, plants grew at a much more sustainable rate and therefore were more resistant to drought (Bates et al. 2013). The reduced amount of biomass for plants grown in large brick substrates also reduced evapotranspiration rates which increased the length of time for water reservoirs to be exhausted (Savi and Marin 2014; Young et al. 2014a). Therefore, when seeking a substrate suitable for drought tolerance, substrates should not be selected for physical properties alone, but also the impact they have upon plant growth. Although not as effective as SwellGel, the use of coarser substrates with a lower WHC should be considered as a cheaper and potentially longer term method of tailoring green roof substrates for plant drought tolerance.

Despite reducing the growth of both plant species, *Sedum* did not increase their drought resistance and actually reduced *F. ovina* wilt index values. Previous studies have shown that the presence of *Sedum,* mosses or other mat forming species can improve substrate moisture content by reducing evapotranspiration rates (VanWoert et al. 2005a; Durhman et al. 2006; Wolf and Lundholm 2008; Heim et al. 2014; Heim and Lundholm 2014a). However the ability of *Sedum* to reduce water loss appears to have led to an increase in *F. ovina* substrate temperatures throughout this trial. Lower water loss from the substrate is likely to lead to increased substrate temperatures as evapotranspiration accounts for a significant amount of the cooling ability of a green roof (Castleton et al. 2010; Blanusa et al. 2013). Combined with interspecific

competition this could explain reduced growth but also lower drought resistance. Although previous trials have shown that nurse plants can facilitate the appearance of neighbouring plants during drought (Butler and Orians 2011; Heim et al. 2014), this current trial shows that in some cases the negative competitive effects of *Sedum* outweigh any potential benefits of this species as a companion plant.

Although not as accurate or quantitative as chlorophyll fluorescence, the wilt index used in this trial showed good correlation with F_v/F_m values. Chlorophyll fluorescence is an extremely useful tool to quantify the level of stress in plants (Baker and Rosenqvist 2004; Ritchie 2006) but uses specialist equipment that many laboratories/nurseries do not have access to. Our work with Fv/Fm supports the use of the wilt index as a quick, easy and accurate method for quantifying water stress in plants.

3.6 Conclusions

This study confirms the importance of individual substrate components on the drought tolerance of green roof plants. The use of water absorbent gels on green roofs is not common but this trial has shown that SwellGel can increase the drought tolerance of perennial green roof plants by increasing the amount of water available during droughts. The use of large crushed brick can also increase drought tolerance by promoting slower and therefore more sustainable plant growth. However SwellGel increased drought tolerance to a greater extent than large brick. The use of *Sedum* as a nurse plant did not significantly improve plant drought resistance and so cannot be universally considered as a potential method for facilitating support of less drought tolerant plants on green roofs.

Water absorbent gels may be a useful tool for increasing the drought tolerance of green roof plants, expanding the range of suitable green roof plants and increase the cooling potential of a green roof. However the long term effectiveness and optimal application rate of these gels in a green roof environment are not known and more research is required. We propose that a more cost effective and potentially longer term method of increasing green roof plant drought tolerance is to use substrates that promote slower and more sustainable plant growth.

Chapter 4

The use of mycorrhizal inoculum in assisting the initial establishment of *Prunella vulgaris* plug plants in green roof substrate

4.1 Summary

Arbuscular mycorrhizal fungi (AMF) have been shown to improve the growth, health, nutrient uptake, flowering and drought tolerance of many terrestrial plant species. Green roofs are generally deficient in nutrients, organic matter and water and therefore AMF could be extremely beneficial in improving green roof plant performance. Despite this there has been a lack of empirical research into introducing AMF into green roof substrates.

This study applied a commercial AMF inoculum and applied it to *Prunella vulgaris* SkyPlugs™ grown into small green roof modules on a flat roof in Sheffield, UK. The modules were filled with commercial green roof substrate (80% small particle sized crushed brick, 20% green waste compost) to a depth of 100mm. AMF inoculum was applied as four treatments (i) directly with plug, (ii) mixed evenly into surrounding substrate, (iii) split between plug and substrate, (iv) control of no inoculum.

AMF colonisation of *P. vulgaris* roots was detected in all AMF treatments but not in the control. Shoot phosphorus concentration was improved in all AMF treatments, however there was no significant effect on growth rates or biomass production.

Prunella vulgaris flowering time at the end of the 1st growing season was also significantly extended by nearly 100 days in the plug AMF treatment only.

This study has confirmed that commercial AMF inoculum can be used to successfully infect plants and introduce AMF networks into green roof substrate. Since the method of AMF inoculum application did not significantly affect AMF colonisation rates or P concentrations, it is likely that simply inoculating the plug is more efficient and cheaper than inoculating all of the substrate and may even increase the amount of flowering in the 1st growing season.

4.2 Introduction

The majority of plant species have the ability to form symbiotic relationships with mycorrhiza fungi (Smith and Read 2008). Mycorrhizal fungi are able to penetrate and attach onto the roots of a host plant which provides them with direct access to a constant supply of carbohydrates fixed by the plant (Smith and Read 2008). In return the mycorrhizal fungus supplies the plant with nutrients (in particular phosphorus) as well as improving plant health and growth (Brundrett 2009). Nearly 95% of all land plants have the ability to form mycorrhizae relationships (Smith and Read 2008), with 74% of angiosperms forming arbuscular mycorrhizal fungi (AMF) relationships (Brundrett 2009) which is the most common mycorrhizal type, especially among flowering plants and grasses (Smith and Read 2008).

AMF can vastly improve the health of host plants and communities by improving nutrient uptake (van der Heijden et al. 2008), water uptake (Augé 2001), resistance to pathogens and toxicity (Schützendübel and Polle 2002; Jeffries et al. 2003), volume of plant flowering (Garmendia and Mangas 2012; Asrar et al. 2014), surrounding soil structure (Rillig and Mummey 2006) as well as altering plant community structure and increasing biodiversity (Hartnett and Wilson 2002; Bever et al. 2010). Many of these benefits would be extremely valuable to green roof plants as their growing conditions are often very harsh (Getter and Rowe 2009). However to date little empirical research has been conducted into the benefits of artificially introducing AMF inoculum into green roof substrate (Molineux et al. 2014). A few trials have indicated that AMF inoculum can survive in green roof substrate (McGuire et al. 2013; Rumble and Gange 2013; Molineux et al. 2014), however only two conference papers have been published on the effect of artificially introducing AMF inoculum on green roof plant growth,

both of which showed increased plant growth with inoculum (Meyer 2004; Sutton 2008).

AMF spores are naturally present in most soils. However due to the engineered nature of green roof substrates they will probably have very low AMF spore counts (Molineux et al. 2014), although AMF communities have previously been found in commercial green roof substrate planted with a selection of grassland and prairie plants (McGuire et al. 2013). The majority of green roof substrates are composed of 80-90% inert, free draining minerals (pumice, crushed brick/tiles, expanded clay/shale, crushed concrete) and only 10-20% organic matter (green waste compost, bark, coir, worm castings) (Ampin et al. 2010). The mineral content is unlikely to contain significant amounts of AMF inoculum due to its non biological origin. Of the organic matter types only green waste compost is likely to contain significant amounts of inoculum, although green waste compost is sometimes heat treated to denature weed seeds (WRAP 2008) which could also reduce the viability of AMF spores.

AMF increases the potential scavenging area of a plant's root system and increases uptake of phosphorus (van der Heijden et al. 1998) and in some situations nitrogen (Hodge et al. 2001). Green roof substrates are designed to have low nutrient levels and usually only contain between 5-20% organic matter in order to prevent excessive plant growth and weed invasion, although slow release fertilizers are often used to promote extra growth (Dunnett and Kingsbury 2010). Therefore AMF could potentially improve green roof nutrient uptake in what is a nutrient deficient environment, and also help to reduce the need for slow release fertilizer. Plant water uptake can also be increased by AMF, although this is probably due to a combination of higher stomatal conductance, reduced hydraulic resistance in roots, increased root growth and improved soil structure which improves the movement of water through the soil (Augé

2001; Rillig and Mummey 2006). This could potentially increase plant tolerance to drought, which often occurs on green roofs, whilst also increasing plant transpiration, and therefore also increasing the cooling service provided by the green roof. AMF can also improve the soil's total water holding capacity by increasing the amount and stability of soil particle aggregation, which increases the amount water available for plant use (Rillig and Mummey 2006). This can also potentially reduce nutrient leaching from soils, which frequently occurs from green roof substrate (Berndtsson 2010; Aitkenhead-Peterson et al. 2011). AMF can also help reduce plant uptake of non-essential and toxic metals (Pb, Cd, Zn, Fe) by immobilizing and selectively passing on metals to their host (Meharg and Cairney 1999). High concentrations of toxic metals have been found in green roof substrate and leachate, although it is not clear how this can affect green roof plant health (Speak et al. 2014).

AMF can also strongly affect whole plant communities by driving plant diversity and productivity, increasing plant resilience and suppressing non AMF weed species growth (Hartnett and Wilson 2002; van der Heijden et al. 2008; Bever et al. 2010; Cameron 2010). Green roofs with diverse and resilient plant communities are more likely to provide a higher level of green roof services, especially in the long term (Cook-Patton and Bauerle 2012).

A number of green roof substrate companies currently sell substrate and seed mixes with AMF inoculum incorporated into them (Bauder 2012; Mycorrhizal Applications Inc. 2013) and there are numerous case studies in which AMF inoculum has been incorporated into a green roof (Living Roofs 2003; Grothe and Trichie 2006). However there is no empirical evidence that the use of AMF on green roofs has a beneficial impact on green roof plants and service provision. In addition it is also not clear what the most effective and cost efficient method of applying AMF inoculum is.

This chapter aims to explore this major gap in green roof literature by examining the role that AMF can play in increasing green roof plant growth and physiological health. In order to do this a roof top experiment was set up to examine the effect of AMF inoculum on the establishment of *Prunella vulgaris* plugs in green roof substrate over one year. In order to assess the most efficient method of applying AMF inoculum to the plugs four treatments were used, a) inoculum added to plug substrate, b) inoculum added to surrounding substrate, c) inoculum added to plug and substrate, d) no inoculum added.

It was hypothesised that;

1. The addition of mycorrhizal inoculum to a green roof substrate/plug would aid the establishment of *P. vulgaris* plugs.
2. The addition of mycorrhizal inoculum to a green roof substrate/plug would increase the amount as well as length of *P. vulgaris* flowering.
3. Applying AMF inoculum directly to the plugs as opposed to the substrate would result in a much higher rate of AMF infection and greater benefits to the host plant.

4.3 Methods

4.3.1 Location

The roof used for this trial was located in Sheffield, UK (53.23°N, 1.28°W) a city with a temperate seasonal climate. A flat asphalt roof enclosed by a 1.2m high wall and located on the 9th floor of a University of Sheffield building was used as the study site (Fig. 4.1)

4.3.2 Green Roof Modules

Green roof modules were created with plastic trays of 40x30x12cm. Drainage holes were drilled at regular intervals into the base of each tray and a root proof membrane fitted inside the tray to prevent loss of substrates throughout the trial. Each module was filled to a depth of 100mm with green roof substrate which was composed of 80% crushed recycled brick (2-5mm particle size) and 20% green waste compost (Table 4.1). Green waste compost (Green Estate, Sheffield, UK) was composed of composted garden waste collected in Sheffield. The modules were located in a randomised block design (Fig. 4.2) and raised off the roof surface in order to prevent water logging. The outside of each module was painted white in order to reduce the amount of heat absorbed from direct sunlight.

4.3.3 Planting

In June 2013 four *Prunella vulgaris* plug plants (sourced as SkyPlugs^{TM} from Boningale Nurseries Ltd) were planted into each module at equal distances from one another which translates to a planting density of 45 plugs m^{-2}. *Prunella vulgaris* was used as a test species due to its frequent occurrence on green roofs, relatively high

drought tolerance and ability to form mycorrhizal relationships. Due to an especially dry summer each module was given supplementary watering twice a week throughout July 2013 of 4.8L month^{-1} which translates into 40mm rainfall. Additional watering was also given during early August 2014 of 2.5L per module (21mm rainfall) due to a prolonged period of low rainfall. Mycorrhizal inoculum (sourced from rootgrow™ as Rootgrow Professional) was applied to plug plants and substrate as five treatments according to manufacturer's specifications with six replications per treatment (Table 4.2).

Figure 4.1: View of the test site on the 9th storey of the Education Building, University of Sheffield.

Figure 4.2: Installed green roof modules with *Prunella vulgaris* plugs planted

Table 4.1: Physical and chemical characteristics of the commercial substrate used in this trial. Calculated according to FLL 2008 standards. Available from Boningale Ltd.

Measurement	Characteristic	Value
Physical	Organic Matter (%)	11.00
	Permeability (cm/s)	14.81
	Water Holding Capacity (%)	34.99
	Oven Dried Density (g/cm^3)	1.10
	Saturated Density (g/cm^3)	1.39
	Pore Volume (%)	36.78
	Air Content at Water Content Max (%)	1.05
Chemical	Plant Available Phosphorus (µg P g^{-1} Substrate)	11.14
	Plant Available Nitrogen (µg N g^{-1} Substrate)	11.26
	Total Phosphorus (µg P g^{-1} Substrate)	88.26
	Total Nitrogen (µg N g^{-1} Substrate)	208.87

Table 4.2. Mycorrhizal treatments for *Prunella vulgaris* plug plants grown in green roof modules. Plug application rate refers to amount of inoculum placed at the bottom of the plug hole during planting, whilst substrate application rate refers to the amount of inoculum mixed homogenously into the substrate before planting (N=6).

Treatment Number	Mycorrhizal Application Rate		
	Per Plug	Substrate	Total (Module)
1	0 ml	0 ml	0 ml
2	20 ml	0 ml	80 ml
3	0 ml	80 ml	80 ml
4	10 ml	40 ml	80 ml
5 (No Plug Plants)	0 ml	0 ml	0 ml

Three seedlings of *Plantago lanceolata* bait plants were also planted in the middle of each module for two months between August and October 2013 in order to obtain a 'live' update on mycorrhizal colonisation of the substrate. Seeds were surface sterilised with sodium hypochlorite for 3minutes and thoroughly rinsed with

autoclaved water, transplanted to autoclaved sand and grown in a controlled growth cabinet for four weeks prior to planting.

4.3.4. Growth and flowering rates

Prunella vulgaris was measured regularly throughout the growing season to assess maximum plant width and canopy height. A flowering index was used throughout the flowering season where a score of 1 was awarded to the presence of an alive flower bud and a score of 2 to the presence of a flower. Thus the amount of flowers per module was also recorded.

4.3.5 Biomass

Prunella vulgaris was harvested in August 2014 (Day 403) and *P. lancelota* bait plants in October 2013 (Day 70). For both species all above ground biomass was harvested, dried at 80^{o}C for two days and weighed to obtain dry weight. To determine root biomass roots were washed in water to remove all traces of brick and compost. A sample selection of root for mycorrhizal colonisation analysis was removed with a scalpel, dried with a paper towel and weighed. The remaining root material was dried with a paper towel and weighed to obtain fresh weight, and then dried at 80^{o}C for two days and weighed again to obtain dry weight.

4.3.6 Chlorophyll Content

The chlorophyll content of *P. vulgaris* was measured periodically throughout the trial period with a chlorophyll meter (Minolta Chlorophyll Meter SPAD-502). Four leaves from each plant were measured and the mean calculated for each green roof module.

4.3.7 Phosphorus and nitrogen concentrations

Leaf tissue phosphorus (P) and nitrogen (N) content was determined on oven-dried ground samples from the final biomass harvest, following Kjeldahl digestion (Allen et al. 1974). For this approximately 50mg dry plant biomass was digested in 1 ml concentrated sulphuric acid with 1 microspatular of catalyst (1:10 $CuSO_4$:$LiSO_4$) for 7hours at 375^oC. After a dilution (1:50 dH_2O) total phosphorus was determined via colorimetric determination by using a Cecil Ce 1020 spectrophotometer (Leake 1988). After a dilution (N=1:100 dH_2O) total nitrogen was determined by Flow Injection Analysis (Burkard FIA Flo2, Burkard Scientific, Uxbridge, UK).

4.3.8 Root colonisation

After harvesting *P. lanceolata* and *P. vulgaris* roots were carefully washed with dH_2O and a small sample taken for staining. Root staining (according to (Brundrett and Bougher 1996) was used to highlight mycorrhizal colonisation. A sample of root was cleared in KOH (10% w/v) for 120 minutes and then placed in HCl (10% v/v) for 15 minutes. Roots were then stained with Trypan Blue for 15 minutes and stored in 50% glycerol until needed.

Mycorrhizal colonisation rates were quantified using the modified grid line intersection method (Giovannetti and Mosse 1980). Stained roots and a small amount of 50% glycerol were randomly dispersed in a 9cm petri dish with gridlines marked on. Any roots intersecting a gridline were assessed for mycorrhizal colonisation in order to give a % colonisation rate. For each replicate 100 intersections were observed.

4.3.9 Statistical Analysis

To determine the effect of mycorrhizal treatments on *P. vulgaris* shoots, roots, root:shoot ratios, mycorrhizal colonisation and *P. lancelota* mycorrhizal colonisation one way ANOVAs were performed on linear models. Any data not meeting the assumptions of the model were $\log_{10}$ transformed. Any data not meeting the assumptions of the model with values less than 1 were log transformed after the addition of 1 to every value. To determine the effect of mycorrhizal treatments on *P. vulgaris* growth rates, total bud/flower score and number of flowers one way repeated measures ANOVAs were performed.

All analyses were carried out in R Studio version 2.15.1 (22.6.2012) (The R Foundation for Statistical Computing).

4.4 Results

4.4.1 *Prunella vulgaris* mycorrhizal infection

All three mycorrhizal treatments had significantly higher mycorrhizal colonisation rates than the non mycorrhizal treatment (one way ANOVA, $p<0.05$) (Fig 4.2). However when the inoculum was added to just the plug colonisation rate was significantly higher than when the inoculum was added to both plug and substrate (Fig 4.2).

4.4.2 Prunella vulgaris growth

Prunella vulgaris height and width growth throughout both growing seasons did not significantly differ between mycorrhizal treatments (repeated measures ANOVA, $p<0.05$) (Fig 4.4-4.5). All treatments showed little vertical growth in the first growing season with plants spreading laterally (Fig 4.4). Mycorrhizal plants were slightly wider than non mycorrhizal plants at the end of the first growing season but this was not significant (Fig 4.5). All treatments showed large amounts of vertical (Fig 4.4) but little horizontal growth during the second growing season (Fig 4.5).

Prunella vulgaris shoot and root biomass was also not significantly affected by mycorrhizal treatment (one way ANOVA, $p<0.05$) (Table 4.3). However *P. vulgaris* root:shoot ratio was significantly higher in all mycorrhizal treatments (Table 4.3).

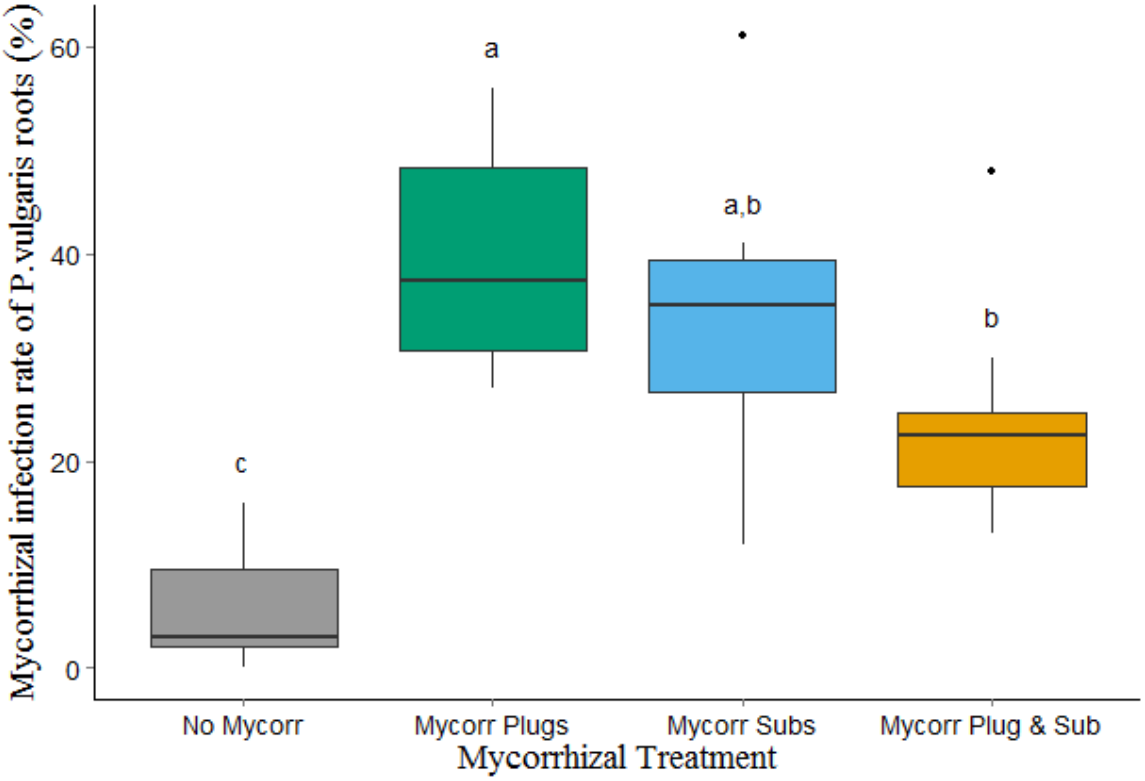


Figure 4.3: Percentage mycorrhizal colonisation of *Prunella vulgaris* roots grown from June 2013 to August 2014. Treatments codes are as follows; No Mycorr= Just plug plants, Mycorr Plugs=Mycorrhizal inoculum added to plugs, Mycorr Subs= Mycorrhizal inoculum added to substrate, Mycorr Plugs & Subs= Mycorrhizal inoculum added to plugs and substrate, No Plugs= No plugs or mycorrhizal inoculum added. The middle bar represents the treatments mean, the upper and lower box hinges the 1st and 3rd quartiles the thin black line the complete spread of data and outliers as black dots. Treatments with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$).

4.4.3 Prunella vulgaris flowering

During the first growing season the addition of mycorrhizal inoculum directly to *P. vulgaris* plugs significantly increased the amount of time that buds and flowers were present (repeated measures ANOVA, $p<0.05$). This increase became noticeable in early October (Day 100) and continued until early January (Day 200) (Fig 4.6-4.7).

During the second growing season the bud/flower scores and total flower numbers for all treatments were not significantly different from one another (repeated measures ANOVA, $p<0.05$) (Fig 4.8-4.9). All treatments showed much higher bud/flower scores and total flower numbers compared to the previous growing season, however all showed a large decline in late July (Day 370) due to a prolonged drought (Fig 4.8-4.9). Values started to increase once additional watering was given.

Table 4.3: Shoot, root and root:shoot ratios of *P. vulgaris* grown from June 2013 to August 2014. Treatments codes are as follows; No Mycorr= Just plug plants, Mycorr Plugs=Mycorrhizal inoculum added to plugs, Mycorr Subs= Mycorrhizal inoculum added to substrate, Mycorr Plugs & Subs= Mycorrhizal inoculum added to plugs and substrate, No Plugs= No plugs or mycorrhizal inoculum added.

	Treatment (±SE)					
	No Mycorr	Mycorr Plugs	Mycorr Subs	Mycorr Plugs & Subs	P-value	F-value
Shoot (g)	46.56 (±3.42)	44.48 (±2.09)	44.1 (±2.37)	39.90 (±1.67)	0.399	1.02
Root (g)	26.41 (±1.75)	28.47 (±0.72)	28.19 (±1.03)	29.39 (±1.22)	0.113	2.67
Root:Shoot	0.57^{b} (±0.01)	$0.65^{a,b}$ (±0.03)	$0.66^{a,b}$ (±0.05)	0.75^{a} (±0.07)	*	2.97

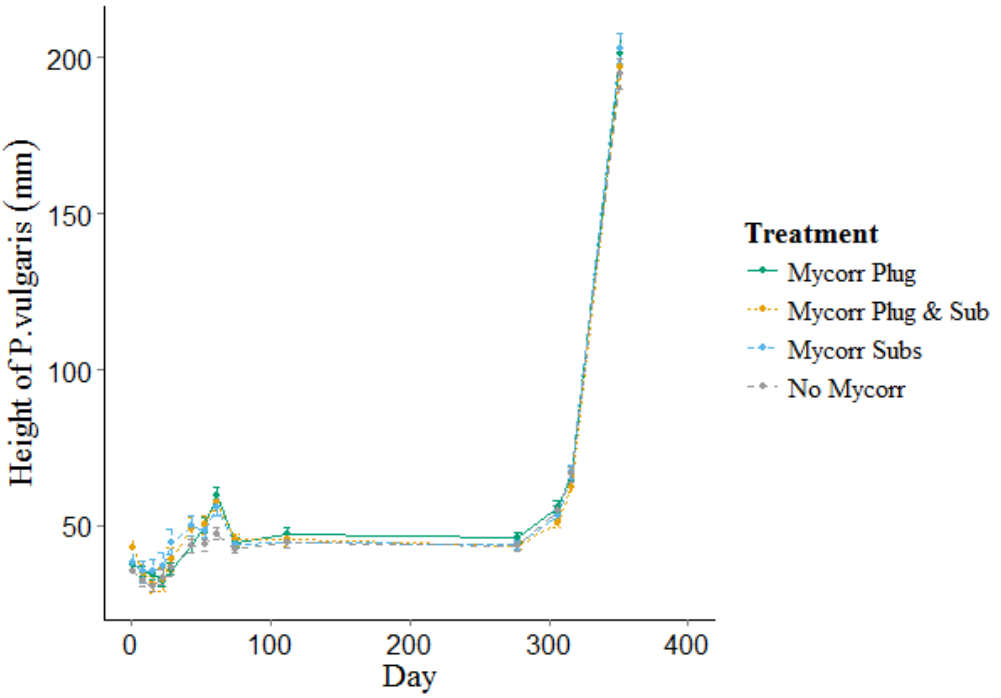


Figure 4.4: Mean height (tallest part of plant) of *Prunella vulgaris* during two growing seasons. Day 1= 26.6.13, Day 400= 2.8.14. Error bars represent one standard error. Treatment codes are as follows; Mycorr Plug= AMF inoculum added to plugs, Mycorr Plug & Sub= AMF inoculum added to plugs and substrate, Mycorr Subs=AMF inoculum added to substrate, No Mycorr= Just Plugs.

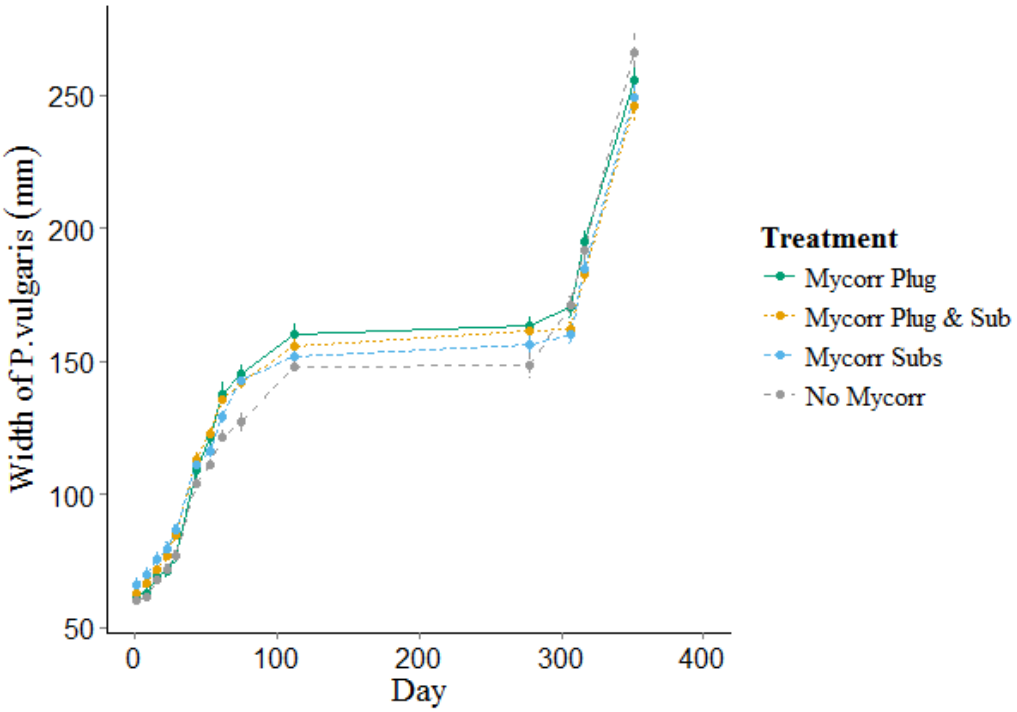


Figure 4.5: Mean width (widest part of plant) of *Prunella vulgaris* during two growing seasons. Day 1= 26.6.13, Day 400= 2.8.14. Error bars represent one standard error. Treatment codes are as follows; Mycorr Plug= AMF inoculum added to plugs, Mycorr Plug & Sub= AMF inoculum added to plugs and substrate, Mycorr Subs=AMF inoculum added to substrate, No Mycorr= Just Plugs.

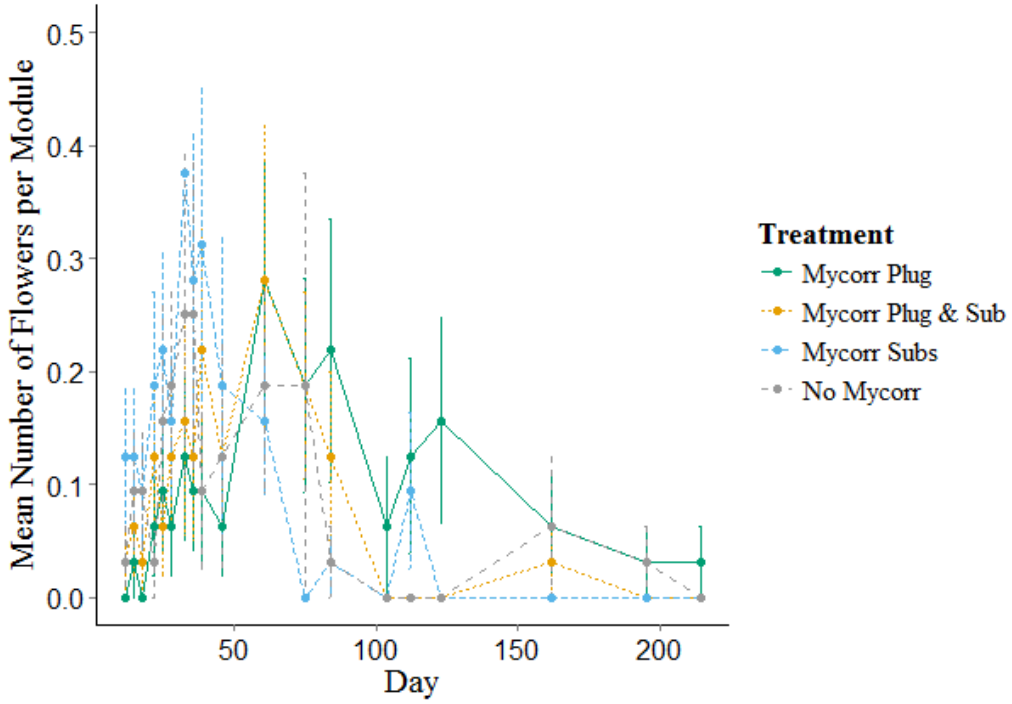


Figure 4.6: Flower production of *P. vulgaris* during first growing season. Day 1= 27.6.13, Day 200= 13.1.14. Error bars represent one standard error. Treatment codes are as follows; Mycorr Plug= AMF inoculum added to plugs, Mycorr Plug & Sub= AMF inoculum added to plugs and substrate, Mycorr Subs=AMF inoculum added to substrate, No Mycorr= Just Plugs.

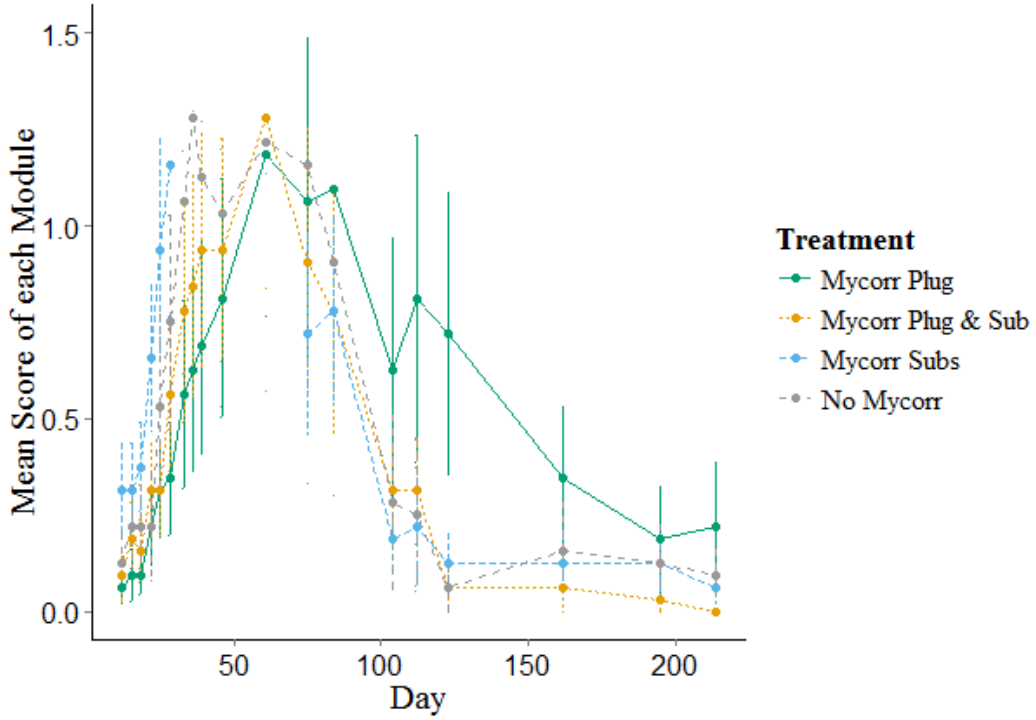


Figure 4.7: Total bud and flower score of *P. vulgaris* during first growing season. Day 1= 27.6.13, Day 200= 13.1.14. Error bars represent one standard error. Treatment codes are as follows; Mycorr Plug= AMF inoculum added to plugs, Mycorr Plug & Sub= AMF inoculum added to plugs and substrate, Mycorr Subs=AMF inoculum added to substrate, No Mycorr= Just Plugs.

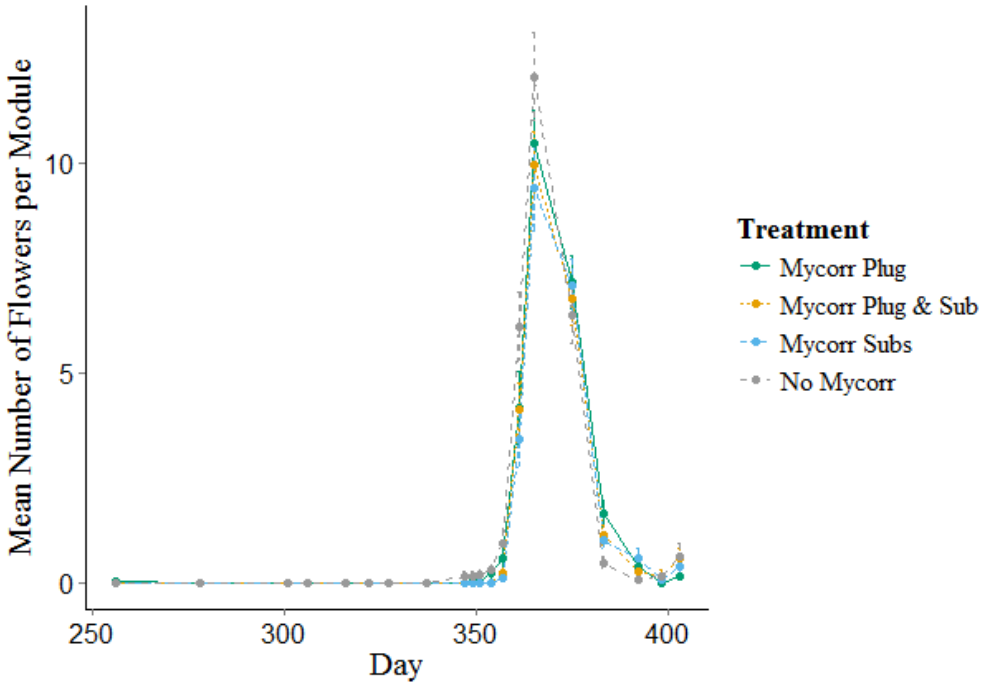


Figure 4.8: Flower production of *P. vulgaris* during second growing season. Day 250= 4.3.14, Day 400= 2.8.14. Error bars represent one standard error. Treatment codes are as follows; Mycorr Plug= AMF inoculum added to plugs, Mycorr Plug & Sub= AMF inoculum added to plugs and substrate, Mycorr Subs=AMF inoculum added to substrate, No Mycorr= Just Plugs.

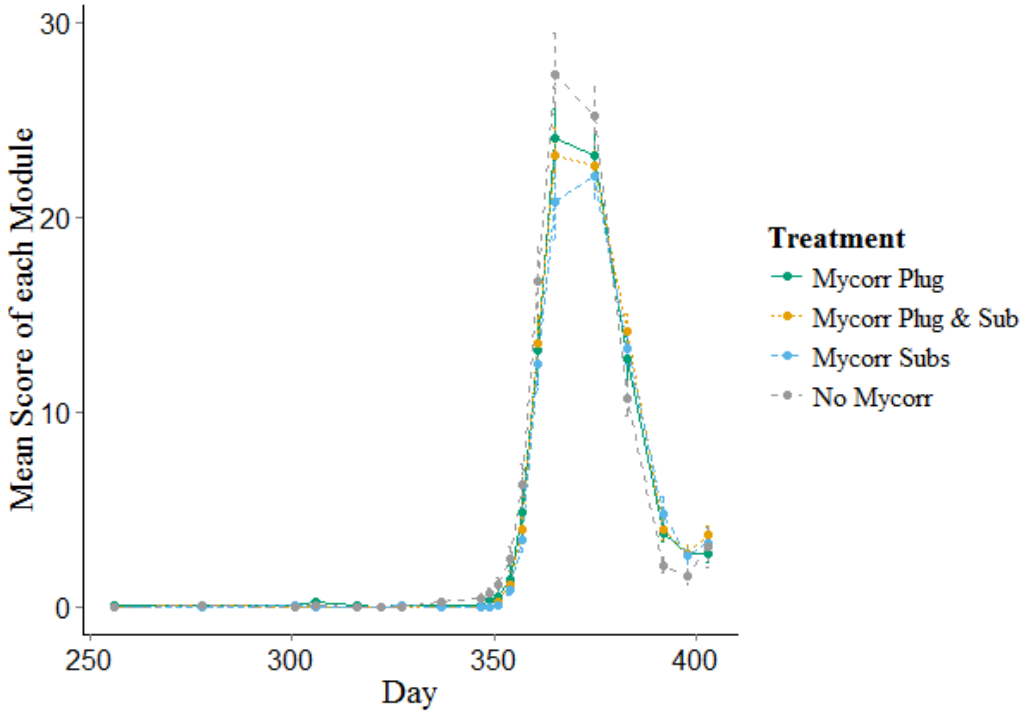


Figure 4.9: Total bud and flower score of *P. vulgaris* during second growing season. Day 250= 4.3.14, Day 400= 2.8.14. Error bars represent one standard error. Treatment codes are as follows; Mycorr Plug= AMF inoculum added to plugs, Mycorr Plug & Sub= AMF inoculum added to plugs and substrate, Mycorr Subs=AMF inoculum added to substrate, No Mycorr= Just Plugs.

4.4.4 *Prunella vulgaris* nutrient status/chlorophyll

Living *P. vulgaris* leaves grown in all three mycorrhizal treatments had significantly higher concentrations of phosphorus (one way ANOVA, $p<0.05$) (Fig 4.10). Of the mycorrhizal treatments, the plug only application had the highest phosphorus concentration, although this was not significant (one way ANOVA, $p<0.05$) (Fig 4.10). However none of the mycorrhizal treatments had any significant effect on the nitrogen concentration of alive *P. vulgaris* leaves (one way ANOVA, $p<0.05$) (Fig 4.11).

P. vulgaris leaf chlorophyll concentration was also not significantly affected by any of the mycorrhizal treatments (one way ANOVA, $p<0.05$), (data not shown).

4.4.5 *Plantago lanceolata* mycorrhizal infection

All three mycorrhizal treatments had significantly higher mycorrhizal colonisation rates of 20-30% than the two non mycorrhizal treatments which was between 0-2% (one way ANOVA, $p<0.05$) (Fig 4.12).

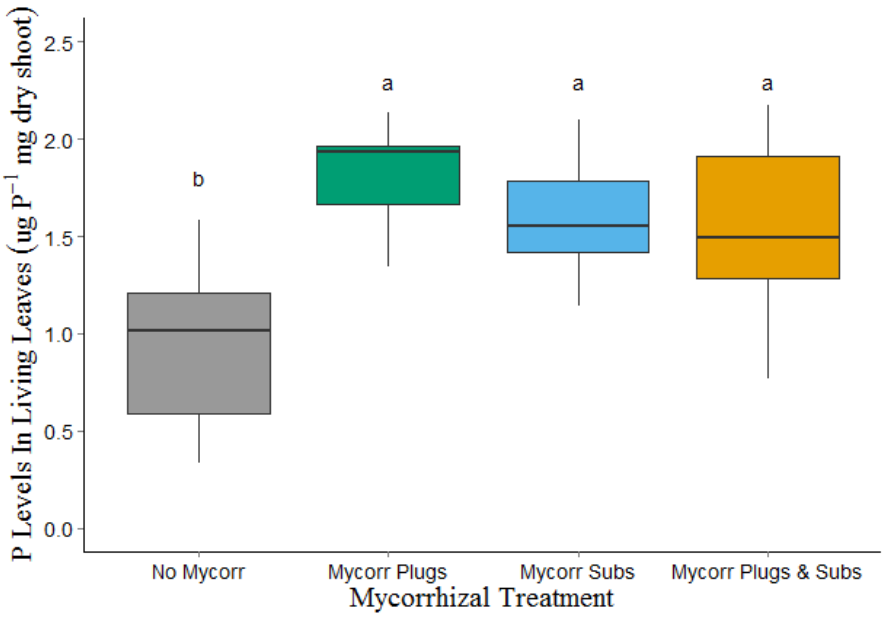


Figure 4.10: Phosphorus concentrations in *Prunella vulgaris* alive leaves after two growing seasons (400 days). Treatments codes are as follows; No Mycorr= Just plug plants, Mycorr Plugs=AMF inoculum added to plugs, Mycorr Subs= AMF inoculum added to substrate, Mycorr Plugs & Subs= AMF inoculum added to plugs and substrate. The middle bar represents the treatments mean, the upper and lower box hinges the 1st and 3rd quartiles the thin black line the complete spread of data and outliers as black dots. Treatments with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$).

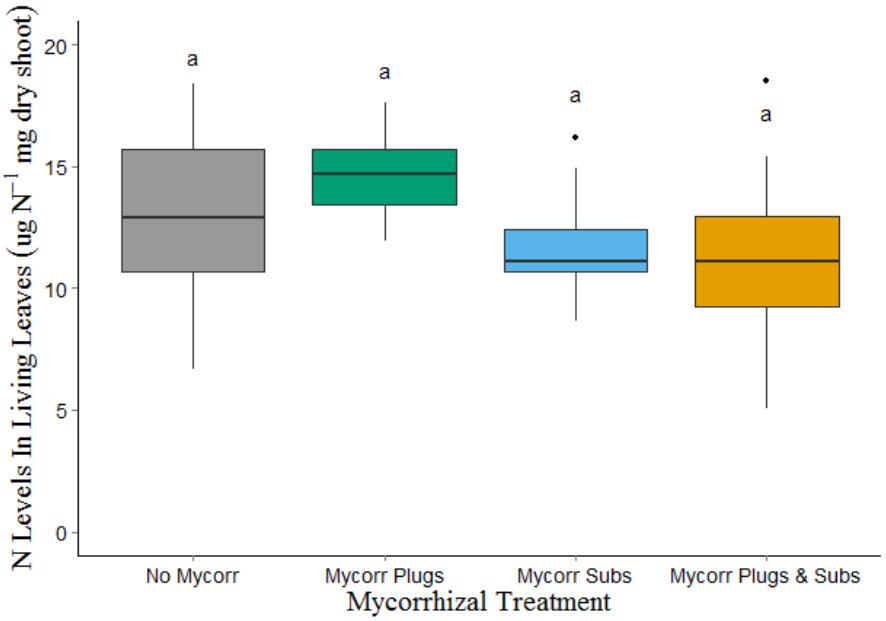


Figure 4.11: Nitrogen concentrations in *Prunella vulgaris* alive leaves after two growing seasons (400 days). Treatments codes are as follows; No Mycorr= Just plug plants, Mycorr Plugs=AMF inoculum added to plugs, Mycorr Subs= AMF inoculum added to substrate, Mycorr Plugs & Subs= AMF inoculum added to plugs and substrate. The middle bar represents the treatments mean, the upper and lower box hinges the 1st and 3rd quartiles the thin black line the complete spread of data and outliers as black dots. Treatments with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$).

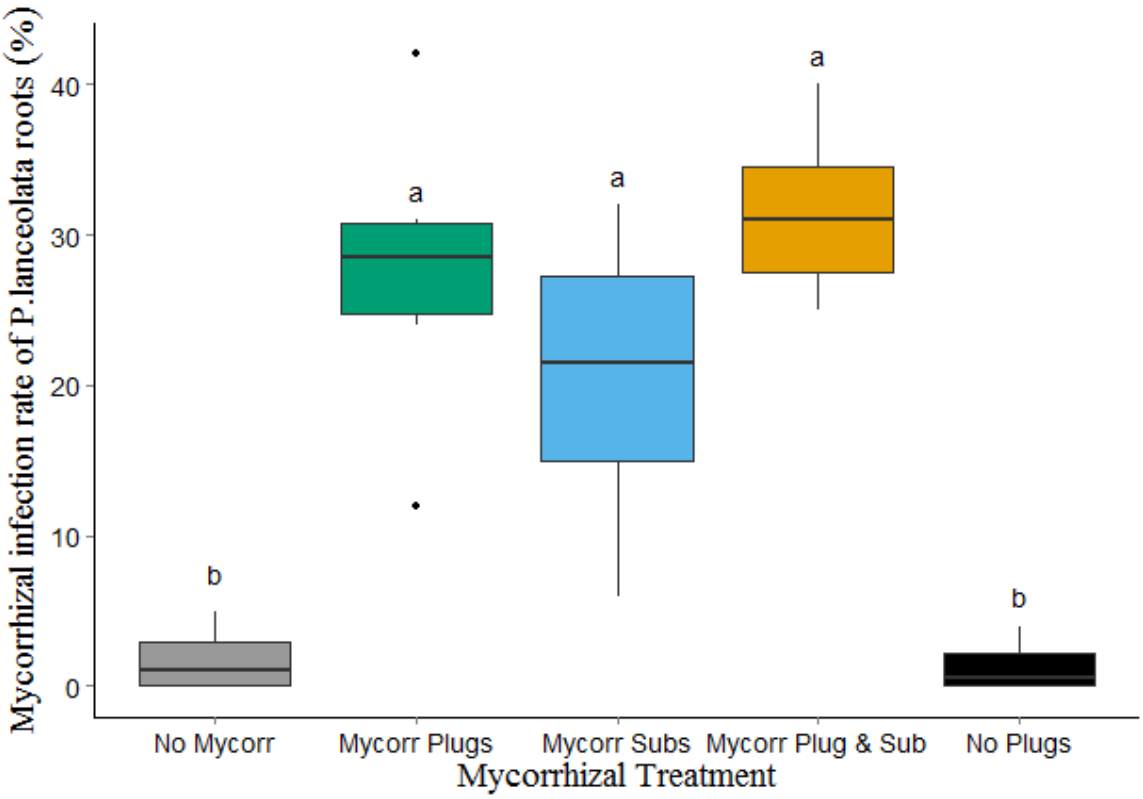


Figure 4.12: Percentage mycorrhizal colonisation of *Plantago lanceolata* roots used as bait plants grown from August 2013 to October 2013. Treatments codes are as follows; No Mycorr= Just plug plants, Mycorr Plugs=AMF inoculum added to plugs, Mycorr Subs= AMF inoculum added to substrate, Mycorr Plugs & Subs= AMF inoculum added to plugs and substrate, No Plugs= No plugs or AMF inoculum added. The middle bar represents th treatments mean, the upper and lower box hinges the 1st and 3rd quartiles and the thin black line the complete spread of data. Treatments with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$).

4.5 Discussion

This trial is one of the first empirical studies to show that AMF networks can successfully be introduced into a green roof system through the use of commercial inoculum. *Prunella vulgaris* plants infected with AMF showed significantly higher amounts of phosphorus in leaves at the end of the trial, although no increase in growth or leaf nitrogen amounts was observed. The application method of the inoculum (either directly with the plug, throughout the surrounding substrate or a mixture of both) significantly altered the degree of AMF root colonisation but not leaf phosphorus amounts. Applying inoculum directly to plug plants also significantly extend the length of flowering and bud production of *P. vulgaris* at the end of the first growing season. Bait plants (*P. lanceolata*) also showed that a viable AMF network was established by the end of the first growing season, approximately four months after inoculation.

4.5.1 Green Roof Mycorrhizal Studies

The use of commercial AMF inoculum on green roofs is still not common despite the many advantages that it could provide to green roof vegetation. This study supports the conclusions of three previous studies that AMF can be successfully introduced to green roof systems (Meyer 2004; Sutton 2008; Molineux et al. 2014). However the effect of AMF on the growth rate of green roof plants is still unclear as this study showed no effect whilst previous studies have (Meyer 2004; Sutton 2008). It has previously been reported that AMF inoculum in the form of prairie top soil can increase the growth of prairie grasses grown in 9 cm depth of substrate (95 %

inorganic, 5 % compost) when added with a polyacrylamide water absorbent gel (Sutton 2008). However when added by itself the inoculum had no significant effect on plant growth, suggesting that the water absorbent gel is needed to facilitate mycorrhizal benefits (Sutton 2008). Similarly the biomass production of alpine grasses and herbs germinated in substrate (95 % inorganic, 5 % compost) from seed was initially increased by the use of AMF inoculum after 10 weeks, although this effect was no longer significant after 25 weeks of growth (Meyer 2004). Molineux et al. 2014 also successfully introduced AMF populations into green roof substrate over two years with commercial inoculum although the effect on plant performance was not measured.

The higher amounts of organic matter in our trial (20%) compared to Meyer 2004 and Sutton 2008 (5%) may have reduced the beneficial role that AMF could play in promoting extra plant growth as a relatively high amount of nutrition was already available. AMF has been previously found on an established green roof with a 80% crushed brick, 20% commercial compost substrate, although its effect on plant performance was not measured (Rumble and Gange 2013). AMF inoculum has also previously been found in commercial green roof substrates (McGuire et al. 2013), although this study suggests that AMF will not always be present in commercial substrates.

It is also debatable whether a large increase in the growth rate of recently established green roof plants is actually desirable. Due to the harsh growing environment of green roofs, rapid luxuriant plant growth can often lead to increased vulnerability to drought and other climatic extremes (Rowe et al. 2006a; Nagase and Dunnett 2011; Young et al. 2014b). Clearly increased plant growth due to AMF is much more sustainable than from high levels of compost or fertilization but could still leave newly established

plants vulnerable. Rapid vegetation coverage is desirable if a green roof has been planted with plugs or seeded, however a trade-off must be made between coverage and long term vegetation survival.

Overall the research in this chapter and the limited previous work suggests artificial introduction of AMF to green roofs via substrate can be successful and lead to benefits for plant growth and physiological performance.

4.5.2 Prunella vulgaris survival

This trial did not detect any differences in *P. vulgaris* survival between AMF treatments, despite the prolonged drought during the second growing season. However there are many examples where AMF has significantly increased newly established plant survival in the restoration of old quarries or mine workings (Khan et al. 2000; Chen et al. 2008). In terms of growing media these sites are similar to green roofs, with low levels of organic matter, drought conditions, wide temperature fluctuations, low nutrient levels and shallow soil depth (Wong 2003). In addition AMF can help plants tolerate high levels of heavy metals which are often present on restoration sites. High levels of heavy metals can also occur on a green roof although to a much lesser degree (Alsup et al. 2010; Speak et al. 2014).

4.5.3 Effect of AMF on *P. vulgaris* flowering

A promising effect of one AMF treatment (adding inoculum direct to plugs) was the extension of the *P. vulgaris* flowering period and bud production in the first flowering season by over 100 days into early January. This was approximately 2/3months longer than expected and could potentially provide an extra justification for the use of AMF

by increasing the aesthetic value of a green roof for a longer period of time. This effect may have also occurred at the end of the second growing season, however due to time constraints the harvest had to be carried out in August. The extension of flowering and budding may be due to the increased phosphorus available in AMF inoculated plants, of which the plug inoculum treatment showed the highest leaf P concentrations. Phosphorus is vital for plant flowering, with allocation of phosphorus for reproduction sometimes reaching 50-60% of total plant amounts (Fenner 1986).

Increased phosphorus content in AMF plants is very common due to the greater foraging ability of AMF mycelium and ability to access immobile forms of phosphorus (Smith and Read 2008). It has been shown that higher levels of available phosphorus to arctic and alpine plants can lead to increased flowering and budding as well as an advancement in flowering time (Heer and Körner 2002; Petraglia et al. 2014; Petraglia et al. 2013; Soudzilovskaia and Onipchenko 2005). AMF colonisation has also been shown to increase plant phosphorus amounts and the number of bud and flowers in ornamental plants (Perner et al. 2007; Garmendia and Mangas 2012) and also plants grown in saline conditions (Asrar et al. 2014). However it must be noted that increased phosphorus availability does not always lead to increased flowering/budding and artificially high levels can impede flowering (Wang 2000; Zhang et al. 2004).

4.5.4 Different methods of AMF inoculation

The highest mycorrhizal colonisation rates of *P. vulgaris* were observed when inoculum was applied directly to the plugs, whilst the lowest was when the inoculum was applied to both the plugs and substrate. This suggests that although applying inoculum directly to plugs is the most effective way to gain high root colonisation, the

amount of inoculum applied is also important as the plug and inoculum treatment had a lower amount of inoculum applied directly to the plugs (Table 4.2). Applying inoculum directly to each plug as it is planted is clearly much more labour intensive than mixing large amounts inoculum off site into substrate, but the improved colonisation rates may justify the added labour. This method may also be cheaper due to the smaller amount of inoculum used. Commercial inoculum is available at around £25 kg^{-1}, although may be available at cheaper prices when brought in bulk. However this is still likely to lead to a significant cost for the installer, especially if applied to all of the substrate. For example a standard extensive green roof of 1000m^2 with a substrate depth of 100mm (Total volume of substrate = 100m^3, £5000-6000) would cost roughly;

a) £5400-6750 to apply inoculum at specified manufactures rates to all of the substrate
b) £1440-1800 to apply inoculum directly to each plug at manufactures rates (assuming a planting density of 40 plugs m^{-2})

An even more efficient method of applying the inoculate would be to pre-inoculate the plugs during germination, which would not increase the amount of labour needed to install the plugs and would also ensure that the plugs were inoculated from the date of installation assisting plant establishment (Kapoor et al. 2008). It is likely that the earlier a plant is inoculated with AMF the greater the benefits the plant will receive from the relationship (Csima et al. 2012). Pre-inoculation with AMF has been shown to improve plant growth in degraded soils (Giri and Kapoor 2004), improve growth in nursery plants (Csima et al. 2012), improve crop tolerance and yield to saline soils (Cantrell and Linderman 2001) and improve the yield of crops in normal field soils (Sorensen et al. 2008). The development of a pre-inoculated green roof plug would be

relatively simple and cost effective as only a small amount of inoculum would be needed per plug.

4.5.5 Establishment of AMF network

The use of *P. lancelota* bait plants showed that a viable mycorrhizal network was present throughout the whole substrate within 4 months of planting. Importantly the method of applying the inoculum did not significantly affect *P. lancelota* infection rates, proving that all of the substrate does not have to be inoculated in order to infect other plants growing on the roof. This could further increase the efficiency of applying inoculum as it would not be necessary to inoculate every single plant in order to introduce a large AMF network.

AMF networks have been shown to modify the structure and function of host plant communities (Cameron 2010). It is generally accepted that AMF can increase the species diversity of a community through a range of mechanisms, most notably through the transfer of resources through Common Mycorrhizal Networks (CMN) and reducing inter-specific whilst increasing intra-specific competition (Moora and Zobel 1996; Hartnett and Wilson 2002; Hart et al. 2003; Bever et al. 2010). In addition the presence of an AMF network may also help to reduce the establishment of invasive weed species (Cameron 2010), which are common management problems on green roofs (Nagase et al. 2013).

Generally the presence of an AMF network will improve green roof service provision due to the presence of healthier plants, which are also more likely to survive environmental extremes, pest damage and disease outbreaks (Jeffries et al. 2003; van

der Heijden et al. 2008). In addition to facilitating better plant growth improved substrate quality and structure from the effects of AMF (Rillig and Mummey 2006) will also act to store more rainwater and prevent runoff.

4.6 Conclusions

This study has confirmed that commercial AMF inoculum can be used to successfully infect plants and introduce AMF networks into green roof substrate. Although this study did not detect any effect on plant growth or survival, leaf phosphorus concentrations were higher in all AMF treatments which was associated with a longer period of flowering/budding at the end of the first growing season.

Significantly higher infection rates were found when the inoculum was applied directly to the plug plants with or without inoculum applied to the rest of the substrate. This suggests that this method of application, despite being more labour intensive, is more effective at infecting plants with AMF. In addition it should also be significantly cheaper as a much smaller amount of inoculum is needed. An even more efficient method of introducing AMF networks onto green roofs would be to pre-inoculate plant plugs in the nursery in order to ensure that plants are infected from the day of planting.

However care should be taken in the use of AMF on green roofs, with the majority of the most commonly used green roof plants (*Sedum* spp.) not generally known for readily forming AMF relationships. In addition the benefits of AMF should not be expected to compensate for poor green roof design or plant choice but should complement existing green roof species as well as increasing the palette of hardy plants used on green roofs.

Chapter 5

Moving towards an integrated substrate health index for green roof substrate

5.1 Summary

Soil Health Indexes have been used in agriculture to assess the overall quality of soil by assessing physical, chemical and biological soil characteristics. In order to create a single 'health' value to indicate soil quality which can be used to inform future management, a number of variables are measured and scored against a desired management goal (e.g. ecosystem service provision).

Green roof substrate is an integral part of any green roof, however there has been a lack of integrated research into the physical, chemical and biological characteristics of established substrate. Therefore the development of an equivalent Substrate Health Index (SHI) for green roofs is proposed in this chapter. Nine roof sites on three extensive/semi-intensive green roofs (allowing intra- and inter-roof comparisons) were sampled in order to assess which commonly used soil variables in Soil Health Indexes would be appropriate for green roof substrate.

Physical variables were mainly determined by 'roof' and therefore original substrate type. However chemical and biological variables were significantly different within as well as between roofs. Higher amounts of organic matter resulted in greater levels of plant available nitrogen and phosphorus and plant coverage, whilst greatest plant diversity was observed at 100mm depth. Substrate depth over 100mm reduced species

diversity but increased total plant coverage, whilst depth of 40mm hosted very low plant diversity.

Variables were chosen for a SHI on account of variance shown as well as appropriateness of measurement. Less frequently used variables such as substrate nitrogen and phosphorus availability, moisture levels and earthworm and *Collembola* density were chosen in addition to common physical measurements (organic matter, water holding capacity) on account of their influence on substrate quality and relative ease of measurement. Therefore the basis of a minimum data set for a future SHI has been established.

This chapter provides the first attempt to holistically study the health of green roof substrate. Widespread sampling of green roofs is needed to establish a baseline data set of established substrate variables and to assess how green roof substrate develops over time. However the proposed SHI method could provide the green roof industry with a cost effective tool for improving the quality and health of green roof substrate.

5.2 Introduction

All green roof plants depend upon their substrate for water, physical support and nutrients (Dunnett and Kingsbury 2010); however there has been a lack of research into the biological components of green roof substrate and its performance as a living system once it is on a roof.

Despite the engineered nature of green roof substrate there is limited biological guidance for substrates, limited knowledge of how substrates develop over time as well as no recognised method for assessing the biological quality of established green roof substrate (Ondoño et al. 2014). This is partly due to the strong engineering influence in green roof design which places a large amount of emphasis on the physical properties of a substrate (e.g. water holding capacity, permeability and granulometric distribution of substrates) and little on the biological quality of the substrate (e.g. presence of microorganisms) (Molineux et al. 2009; Rumble and Gange 2013). It is also partly due to a current over reliance by the green roof industry on a small select range of plants (*Sedum spp.*) which are extremely hardy and therefore do not require substrates with high biological quality (Dunnett and Kingsbury 2010).

Microorganisms play a vital role in nutrient flow and cycling within soil and are now recognised as the main drivers of plant diversity and productivity in terrestrial ecosystems (van der Heijden et al. 2008). It is clear that the success of plant communities is intrinsically linked to the success of soil microorganisms and a combined aboveground-belowground approach to managing ecological systems is needed for all terrestrial ecosystems (Wardle et al. 2004). There has been a lack of empirical research into the microorganism community present in established green roof substrates (Rumble and Gange 2013; Molineux et al. 2014; Ondoño et al. 2014).

However it is probable that the majority of extensive green roofs have microorganism communities of relatively low diversity which are very similar to other early successional environments (e.g. glacial and polluted urban soils) due to the homogeneous design and harsh conditions present on most extensive green roofs (Rumble and Gange 2013).

If an unsuitable or biologically poor substrate is used on a green roof then plant growth and physiological health is likely to be adversely affected (Ondoño et al. 2014). The services that a green roof provides are heavily reliant on the growth and physiological health of its vegetation (Lundholm et al. 2010; Cook-Patton and Bauerle 2012). Therefore if the vegetation experiences poor growth or physiological health then the green roof will only provide a sub optimal level of services (Graceson et al. 2014a; Young et al. 2014a).

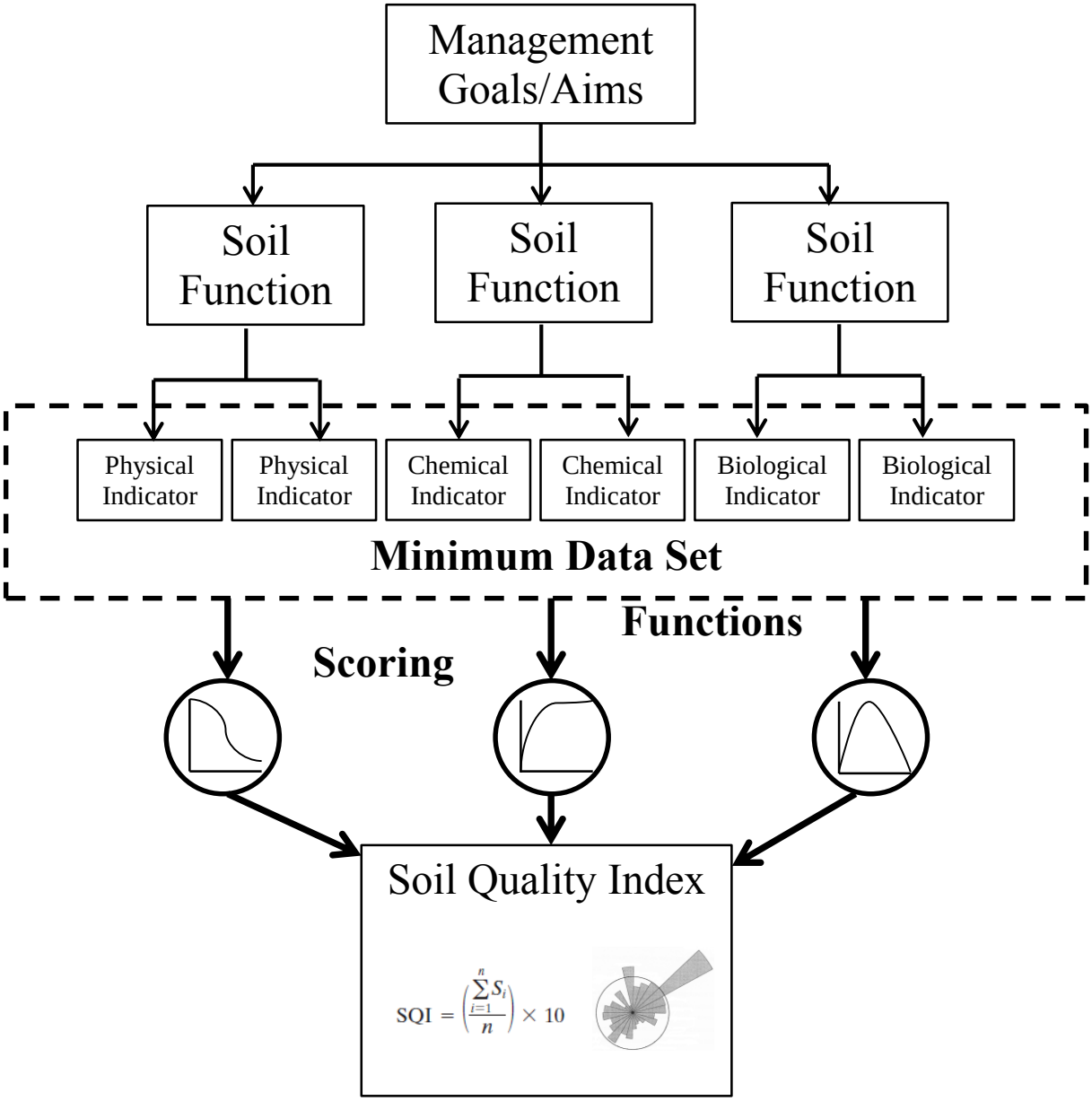


Figure 5.1: Schematic of decision process in constructing a soil health index adapted from (Karlen et al. 2003; Andrews and Karlen 2004)

A similar situation used to be true for agricultural soils, however the vital role that soil plays in agriculture has received increased recognition in the last 20 years with the realisation that current management practices may irreversibly damage the soil (Karlen et al. 2003; Karlen et al. 2008). It has been recognised that productivity should not be the only factor that is measured when assessing the quality of agricultural soils, but also a range of factors that indicate its ability to grow crops in the long term (Doran

and Zeiss 2000). Since Haberern (1992) led the calls for more emphasis to be placed on soil quality, a series of indexes have been created that can easily summarise the current quality or health of a soil and therefore indicate its capacity to function as a vital living system (Doran and Zeiss 2000; Karlen et al. 2008). A common soil health index approach is to measure a selected range of physical (e.g. water holding capacity, particle size distribution), chemical (e.g. pH, plant available nutrients) and biological (e.g. microorganism and mycorrhizae abundance) soil factors (Fig. 5.1). These are then compared to a past or predicted performance of a management goal (e.g. plant growth or ecosystem service provision) (Doran and Zeiss 2000; Karlen et al. 2003; Velasquez et al. 2007) in order to assess how its value will affect the long term provision of that particular management goal (Fig. 5.1). These factors can then be combined together in order to obtain a meaningful, but crucially, simple output that can be used to assess soil quality/health and to inform future management (Fig. 5.1).

It is proposed in this chapter that a substrate health index system similar to previous soil health indexes (Andrews and Karlen 2004; Karlen et al. 2008) should be developed for use on green roof substrates. It is envisaged that it could be used to monitor the biological quality of established green roof substrate in order to influence their management as well as to inform future green roof substrate design. Many established green roofs have poor aesthetic appearance and green roof service provision due to poor substrate quality and a lack of suitable management. However due to cost constraints it is often not feasible to remove such green roofs and install new ones. In addition many green roof managers do not have the resources or expertise to make significant changes to the condition of the roof themselves. A substrate health index will help inform green roof managers of the reasons why their roof may not be performing as they expected and what management can be done to improve its

performance. By establishing clear guidelines for the biological, chemical and physical characteristics of established green roof substrate, management to improve the quality of existing green roofs should become much more achievable.

In order to develop such a substrate health index tool, baseline data from established green roof substrate is needed (Karlen et al. 2008). No such data currently exists for established green roofs with only a few studies available that have been conducted on substrate pH, C:N ratio and porosity (Köhler and Poll 2010), substrate pH, organic matter, depth and nutrient availability (Thuring and Dunnett 2014), vegetation in relation to substrate and general management (Bates et al. 2013; Madre et al. 2014) and the microarthropod community (Rumble and Gange 2013; Madre et al. 2014).

To address the lack of detailed and standardised substrate measurements in the literature three established green roofs in Sheffield, UK were sampled in September 2013. Common Soil Health Index biological, chemical and physical soil measurements were used due to their previous success at predicting soil health in addition to the availability of reference data. The aim of the study was to establish;

1. Which standard Soil Health Index measurements are most applicable for analysing green roof substrate.

2. Which standard soil measurements are the best for predicting green roof substrate health.

3. How established green roof substrate varies within a roof (intra-roof) as well as between roofs (inter-roof) on three similar aged green roofs in the same city.

5.3 Methods

5.3.1 Sites

Three modern (4-6 years old) green roofs (Sharrow, AWEC & SITraN) located within Sheffield (53.23°N, 1.28°W) were selected for field sampling. Each roof differed in its construction, green roof type and appearance (Table 5.1).

5.3.2 Measurements

A number of sites were selected on each roof in order to highlight the variability in conditions within a single roof (Table 5.2). On Sharrow the sites were Wet, Dry, Deep and Shallow, on AWEC Upper and Lower and on SITraN Seeded, Dry and Mid. A number of physical, chemical and biological measurements were taken on each roof (Table 5.3) and in addition substrate samples were transported back to a research laboratory based at the University of Sheffield.

On the AWEC roof due to site access constraints, each site was measured out horizontally from the roof edge at an interval of 1m between replicates (Fig. 5.2). At the SITraN and Sharrow sites a 2x2m square was measured out and six 0.5x0.5m quadrats placed inside (Fig. 5.3, 5.4). These quadrats designated the area from which substrate was removed, field measurements were taken in and the area surveyed for vegetation for each site replicate.

5.3.3 Physical Variables

5.3.3.1 Depth & Moisture

Depth of each site replicate was taken with a metal ruler or wooden meter stick pushed into the substrate until it reached the drainage board layer. Moisture readings were

taken with a Delta-T SM300 Soil Moisture Sensor attached to a HH2 Moisture Meter readout unit.

5.3.3.2 FLL

Physical characteristics of the substrate was determined according to FLL specifications (FLL 2008), which is the approved green roof industry substrate testing method. FLL allows the measurement of substrate water holding capacity, permeability, dry and saturated density, organic content and pore volume.

5.3.3.3 Particle size distribution

Approximately 2-3kg of oven dried substrate (70 ^{o}C) from each site was passed through a number of sieves in order to obtain a particle size distribution curve. Material that did not pass through a sieve was weighed and size was determined to be greater than the sieve mesh size. Sieve mesh sizes were 16mm, 9.5mm, 4mm, 2mm, 1mm, 425µm and 250µm.

5.3.3.4 Temperature regime

The substrate and air temperature of each site was measured throughout the litter decomposition trial (November 2013) with remote temperature loggers (Maxim Integrated™ iButton®). Each iButton® was placed in a small sealed zip lock bag to prevent moisture damage and to assist recovery. Substrate loggers were buried 2cm below the surface of the substrate next to each litter bag and air temperature loggers attached to a tent peg driven securely into the substrate. All loggers were recovered with the litter bags.

Table 5.1: Details of the three green roof study sites all located in Sheffield, UK.

Green roof	Building Description	Year built & age	Type	Substrate depth (mm)	Area (m^2)	Substrate type	Vegetation
Arthur Willis Environment Centre (AWEC)	Single storey university research centre with slight roof pitch (5-10^o). Minimal maintenance which includes drain clearance and sky light cleaning.	2009 4 years	Extensive	30-40	500	Small amount of fine grade substrate particles used in *Sedum* matting	Pre grown *Sedum* matting
Sheffield Institute for Translational Neuroscience (SITraN)	Single storey university research centre. No observable roof pitch. No maintenance in last 3 years.	2010 3 years	Semi-intensive	100-160	750	Bauder Intensive mix consisting of recycled crushed brick, expanded clay shale and composted pine bark	Mix of *Sedum* plugs, wildflower seeds and shrubs
Sharrow Primary School, Sheffield	Two storey local authority school building. No observable roof pitch. Maintenance includes twice yearly strimming and drainage clearance.	2007 6 years	Extensive to intensive	80-300	2000	Recycled crushed brick 80%, green waste compost 20% (roughly mixed on site)	Mix of *Sedum* matting, wildflower seeds and drought tolerant grasses and perennial plugs

Table 5.2: Description of each study site on AWEC, SITraN and Sharrow green roofs.

Roof	Site	Description
AWEC	Upper	Top end of pitched roof
AWEC	Lower	Bottom end of pitched roof (visible pooling of water)
SITraN	Wet	North East facing with wall of building plant room preventing direct sunlight for majority of day.
SITraN	Middle	Little shelter from building plant room leaves site more exposed.
SITraN	Seeded	South West facing. Wall of plant building has little effect on sunlight. Area was reseeded in 2011 with Green Roof Centre, Sheffield wildflower mix.
Sharrow	Wet	Located on the drainage side of a large raised roof in the centre of the green roof.
Sharrow	Dry	Located on the sheltered side of a large raised roof in the centre of the green roof.
Sharrow	Deep	Located around a banked up area of the green roof.
Sharrow	Shallow	Located at site furthest from initial substrate delivery area (substrate was hand spread and therefore this area received less substrate).

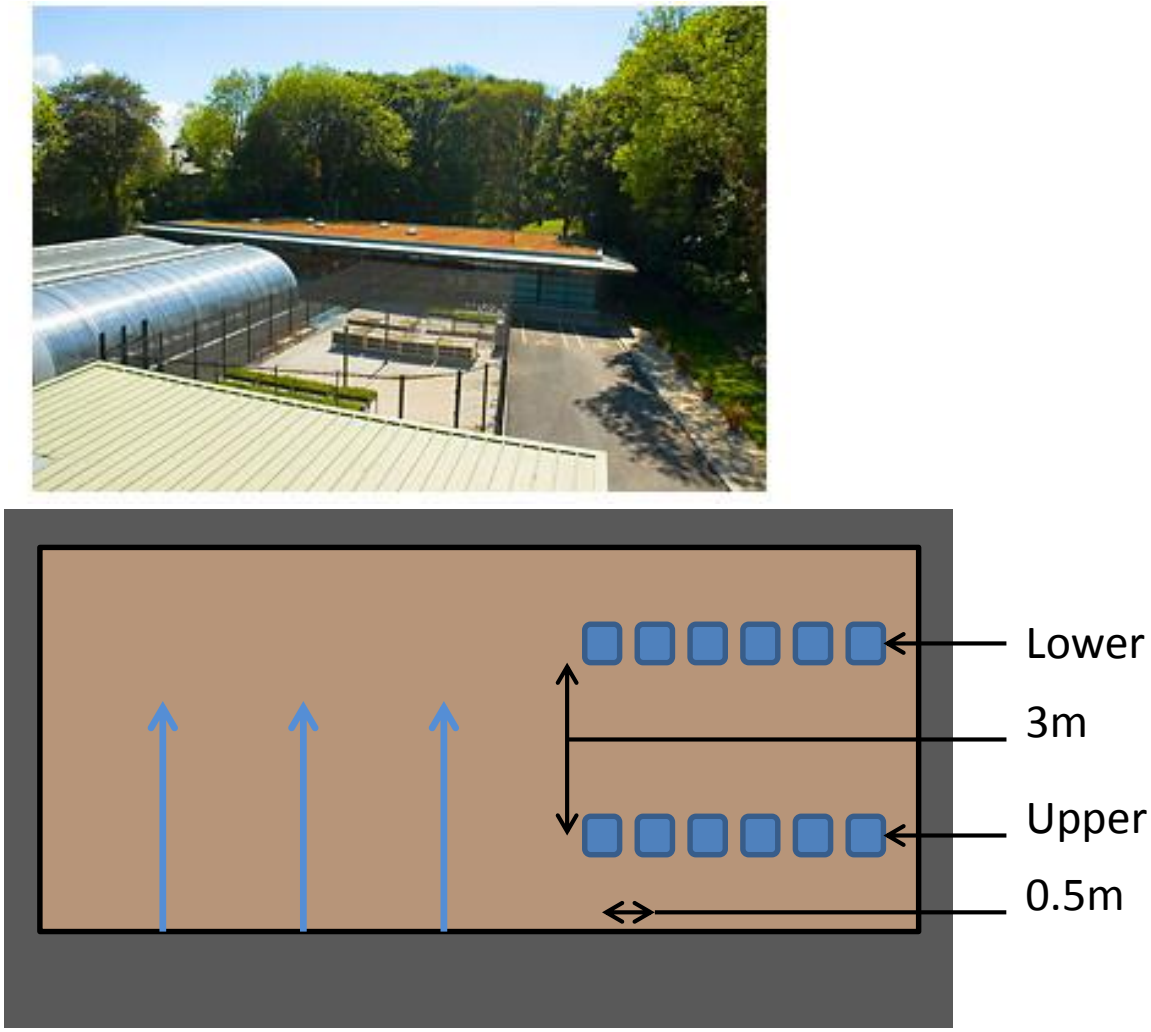


Figure 5.2: a) Aerial view of Arthur Willis Environment Centre (AWEC), b) Blue squares indicate location of sample sites on roof and blue lines indicate direction of roof drainage.

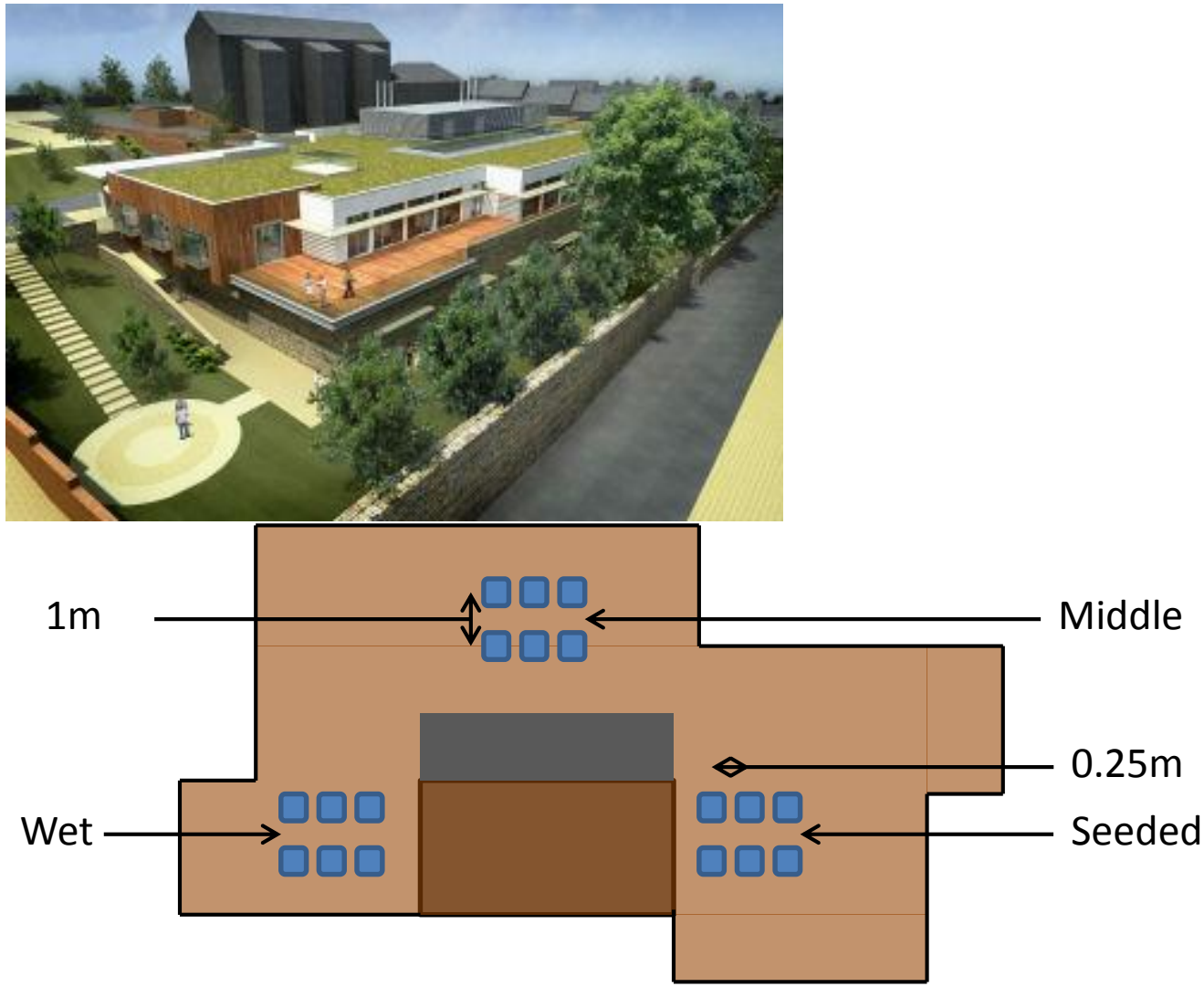


Figure 5.3: a) Aerial view of Sheffield Institute for Translational Neuroscience (SITraN), b) Blue squares indicate location of sample sites on roof.

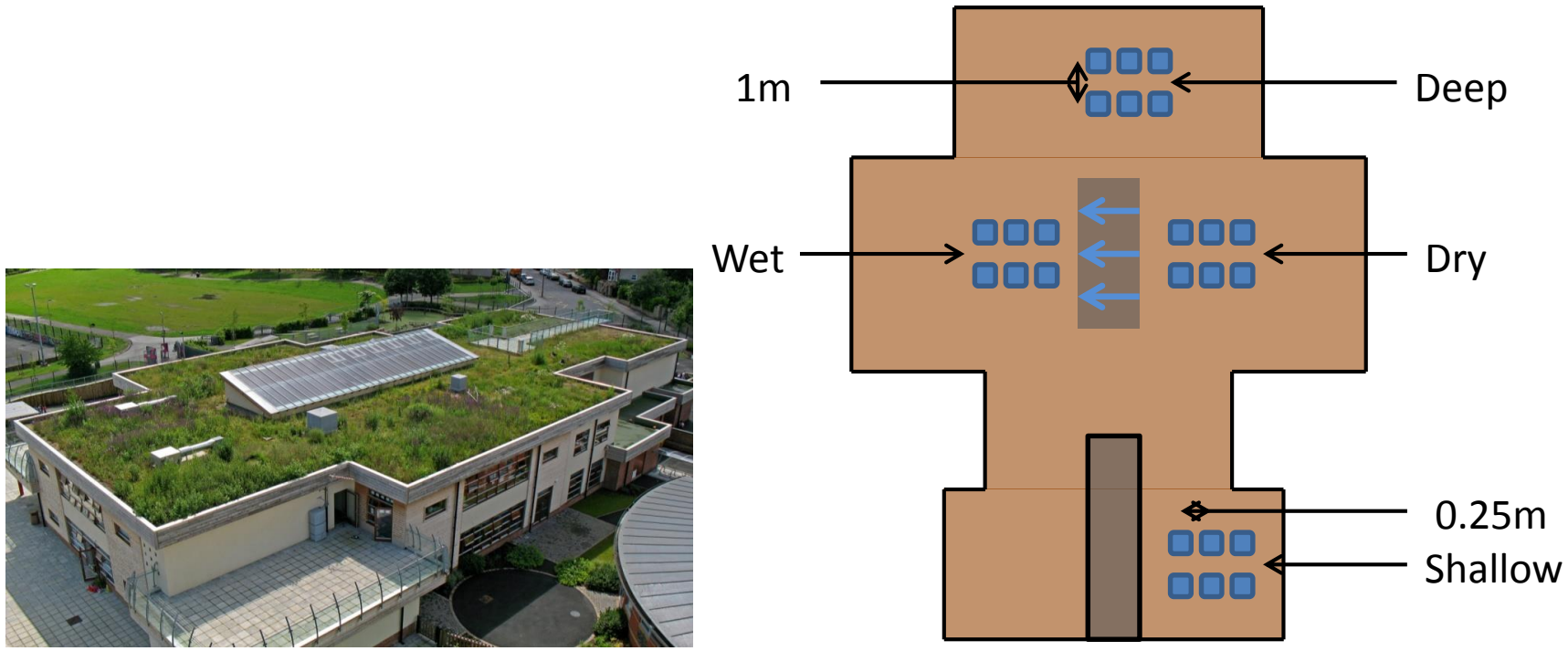


Figure 5.4: a) Aerial view of Sharrow School, b) Blue squares indicate location of sample sites on roof and blue lines indicate direction of roof drainage.

Measurement	Location	State of substrate	Date
Depth	Roof	Fresh	September 2013
Moisture	Roof	Fresh	September 2013
FLL	Laboratory	Dry	January 2014
Particle size distribution	Laboratory	Dry	February 2014
Temperature	Roof	Fresh	October-November 2013
pH	Laboratory	Fresh	September-October 2013
Plant available nutrients	Laboratory	Fresh	September-October 2013
N mineralisation	Laboratory	Fresh	October 2013
Earthworms	Roof	Fresh	September 2013
Collembola	Laboratory	Fresh	September-October 2013
Vegetation	Roof	Fresh	September 2013
Substrate respiration	Laboratory	Fresh	May 2014
Litter decomposition	Roof	Fresh	October-November 2013
Mycorrhizal colonisation	Laboratory	Fresh	November 2013

Table 5.3: Commonly used Soil Health Index physical, chemical and biological measurements taken at each site.

5.3.4 Biological Variables

5.3.4.1 Earthworms

The non toxic 'hot' mustard powder method (Lawrence and Bowers 2002) was used to estimate earthworm density on the SITraN and Sharrow green roofs. Due to access problems as well as the unlikely chance that earthworms would be present on such a thin roof the AWEC roof was not sampled. Following Lawrence and Bowers (2002) 57g of mustard powder (Coleman's Mustard, Norwich, UK) was added to 100ml of water and allowed to stand for 4hours. This was then added to a watering can fitted with a rosette and made up to 7L. Immediately before application the top layer of vegetation in the 0.5x0.5m quadrat was removed in order to expose the substrate and packed up at the edges. The 7L of mustard powder solution was then evenly applied to this square with the watering can. Any worms that emerged in the next 20 minutes were collected, rinsed in water and counted.

5.3.4.2 Collembola

Collembola (springtail) density in the substrate was estimated using the Berlese Tullgren funnel method (Macfadyen 1953). Approximately 200g of fresh substrate was placed in a Berlese Tullgren funnel at room temperature for 5 days with a conical flask containing 70% ethanol below the funnel opening. Identification was carried out with a compound microscope at x80 and samples sorted to subclass level.

5.3.4.3 Plant Diversity

Plant surveys were conducted for each site replicate. Plant species within the 0.5x0.5m quadrat were indentified to species level and the amount of quadrat squares they appeared in counted. The amount percentage cover of bare ground/moss was also recorded.

The Shannon-Wiener Diversity Index was used to assess the diversity and evenness of the plant community at each site using the following equation;

$$Index = \sum (Pi \log[Pi])$$

Where *Pi* is the proportion of total quadrat squares that each species is present in.

5.3.4.4 Microbial Biomass (SIR)

Microbial biomass was determined with the substrate induced respiration method which involves the addition of a glucose solution to samples and the resulting CO_2 response measured (Anderson and Domsch 1978). Fresh substrate was removed from the field in May 2014 and incubated in open bags in the dark at 4^{o}C for 7 days. Approximately 10g of fresh substrate was weighed, oven dried at 70^{o}C for 48 hours and weighed again in order to obtain fresh:dry ratios. Approximately 30g of fresh

substrate was weighed into plastic cylinders previously used as air tight containers for the Respicond VII™ a 96 unit respironmeter (Nordgren 1988). These were incubated in a water bath at 22^{o}C for 36 hours. Glucose optimisation curves for each substrate type were calculated by adding glucose solutions of increasing strength to substrate samples and calculating the point at which increased glucose concentration had no more effect on CO_2. Optimal glucose concentrations were AWEC 1200mg glucose g^{-1} fresh substrate, SITraN and Sharrow 800 mg glucose g^{-1} fresh substrate. After 60 minutes rate of CO_2 evolution was measured using a EGM-4 Environmental Gas Monitor (PP Systems, Amesbury, USA). Microbial biomass was calculated according to (Anderson and Domsch 1978)) using the following equation;

mg biomass C $100g^{-1}$ substrate= 40.04 x (ml CO_2 h^{-1} $100g^{-1}$ substrate) + 0.37

5.3.4.5 Litter decomposition

Fallen oak leaves were collected from Weston Park, Sheffield during October 2013. These were oven dried for 48 hours at 65^{o}C, crushed and sieved to ensure a particle size range between 3-6.7mm. Approximately 0.3g of dried litter was weighed to 4 decimal places and placed in mesh bags (mesh size 1mm) and sewn together with cotton. Litter bags was then planted on each roof at a depth of 1-2cm below the surface in November 2013 and left for 5 weeks. Each bag was recovered, brushed down to remove substrate, oven dried for 48 hours at 65^{o}C and then weighed to 4 decimal places.

5.3.4.6 AMF abundance

AMF abundance in the substrate was quantified by the use of bait plants grown in substrate samples. Seeds of *Plantago lanceolata* were surface sterilised using 70% ethanol, rinsed thoroughly with dH_2O and planted in round pots (diameter 9cm, height 6.9cm) of fresh substrate. These were watered twice a week (75ml $week^{-1}$) for four months in a controlled temperature greenhouse with a day/night regime of 16 hours 20°C/8 hours 15°C. Where necessary, supplementary lighting was used to ensure the required day length (Helle Lamps, IR 400 HPS, 400W).

After four months of growth *P. lanceolata* seedlings were harvested and the roots carefully washed with dH_2O. Root staining was used to highlight mycorrhizal colonisation (Brundrett and Bougher 1996). A sample of root was cleared in KOH (10% w/v) at room temperature for 120 minutes and then placed in HCl (10% v/v) at room temperature for 15 minutes. Roots were then stained with Trypan Blue (Brundrett and Bougher 1996) for 15 minutes and then stored in 50% glycerol until analysed.

AMF colonisation rates were quantified using the modified grid line intersection method (Giovannetti and Mosse 1980). Stained roots and a small amount of 50% glycerol were randomly dispersed in a 9cm petri dish with gridlines marked on. Any roots intersecting a gridline were assessed for mycorrhizal colonisation in order to give a % colonisation rate. For each replicate 200 intersections were observed.

5.3.5 Chemical

5.3.5.1 pH

Approximately 3g of fresh substrate was added to 30ml of dH_2O, stirred thoroughly and allowed to rest for 1 hour. pH readings were taken with a Jenway 3540 pH and conductivity Meter. The probe was rested in the solution until a constant readout was given.

5.3.5.2 Plant Available Nitrogen and Phosphorus

Plant available inorganic nitrogen (N) was determined by calcium chloride ($CaCl_2$) extractions. Substrates were sieved with a mesh size of 6.7mm in order to remove large porous fragments of brick and vegetation and weighed separately. Approximately 5-8g of fresh sieved substrate was weighed and placed in an envelope at 80^oC for 48 hours and weighed again in order to obtain a fresh:dry ratio for the substrate. Approximately 10-15g of fresh sieved substrate was added to 100ml of 0.0125M $CaCl_2$ and then placed in an orbital shaker (250rpm) for 60mins. The $CaCl_2$ solution was then filtered using Whatman Ashless No. 42 filter paper and analysed for ammonium (NH_4^+) and total oxidised nitrogen (TON) by Flow Injection Analysis (Burkard Scientific FIA Flo2). These figures were corrected to account for material sieved out of the substrate earlier and to express N levels per unit of dry substrate.

Plant available phosphorus (P) was determined via Olsen P extractions (Olsen 1954). Approximately 10-15g of fresh sieved (6.7mm mesh) substrate were added to 100ml of 0.5M sodium bicarbonate ($NaHCO_3$) and the solution adjusted to pH 8.5 through the addition of 4M NaOH. 0.8ml of a charcoal solution (15g charcoal in 90ml dH_2O) was added to remove excess organic matter. These were placed in an orbital shaker

(250rpm) for 30mins and then filtered through Whatman Ashless No. 42 filter paper. Total phosphorus (P) was determined via colorimetric determination by using a Cecil Ce 1020 spectrophotometer (Leake 1988). These figures were then corrected in order to account for material sieved out of the substrate earlier and to express P levels per unit of dry substrate.

5.3.5.3 Nitrogen Mineralisation

The rate of N mineralisation was calculated by placing 20-25g unsieved fresh substrate adjusted to 60% WHC in dark plastic tubes sealed with parafilm. These were placed in a tray of water which was in a controlled temperature greenhouse (16 hours 20^{o}C, 8 hours 15^{o}C) for 30 days, after which the substrate was removed, sieved (6.7mm mesh) and inorganic N levels determined using the previous method.

5.3.6 Statistics

To determine the effect of Roof and Site on all variables, nested two way ANOVAs were performed (with Site nested within Roof). Data was $\log_{10}$ transformed if it did not meet the assumptions of the model. In some instances due to low values an additional score of one or three was added to all data before to allow log transformation.

Agglomerative Hierarchical cluster analysis was performed according to (Ward 1963) using the hclust function in R. Data was standardised before analysis by subtracting the median and dividing by the mean average deviation. Due to gaps in the data set *Collembola,* Worm, AMF and Sites with no Microbial Biomass data were excluded from the analysis. All statistical analyses were carried out in R Studio version 2.15.1 (22.6.2012) (R Development Core Team, 2011).

5.4 Results

5.4.1 Physical Variables

5.4.1.1 Depth

Roof and Site both had a significant effect on substrate depth (two-way nested ANOVA $p<0.05$). The two AWEC sites were the shallowest at 35mm. Sharrow Deep had the largest depth at 220mm, although had large variation and therefore was not significantly different from SITraN Mid and Seed (160mm) (two-way nested ANOVA $p<0.05$) (Fig. 5.5).

5.4.1.2 Organic Matter

Roof and Site both had a significant effect on organic matter content (two-way nested ANOVA $p<0.05$). Sharrow Deep contained the highest amount of organic matter at 14%, followed by Sharrow Dry at 8.5%, whilst SITraN sites contained the lowest amounts of organic matter between 1-3% (Fig. 5.6).

5.4.1.3 Particle Size Distribution

Roof had a significant effect on particle size distribution although Site had no effect (two-way nested ANOVA $p<0.05$). AWEC substrates were composed of a much higher percentage of smaller particles than SITraN and Sharrow with 50% of its particles smaller than 1mm. The majority of Sharrow substrate particles were between 4-9.5mm (Fig. 5.7).

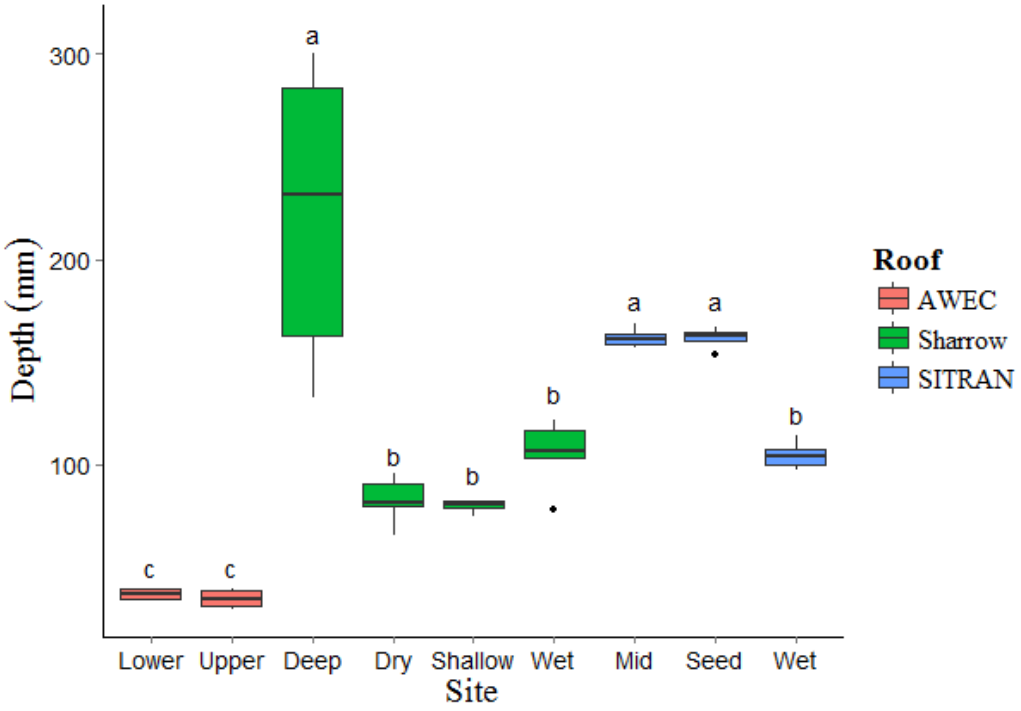


Figure 5.5: Depth of each Site measured in September 2013. The middle bar represents the treatments mean, the upper and lower box edges indicate the 1st and 3rd quartiles, the thin black line indicates the complete spread of data and outliers are black dots. Sites with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$).

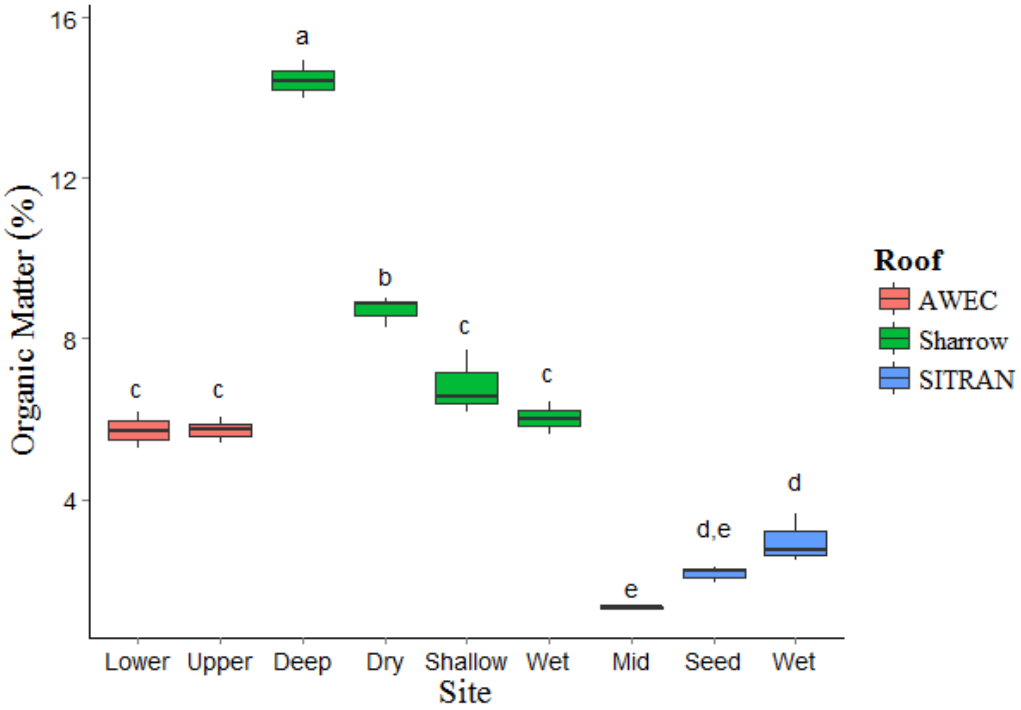


Figure 5.6: Organic matter % of substrate samples taken from each Site measured by loss on ignition. The middle bar represents the treatments mean, the upper and lower box edges indicate the 1st and 3rd quartiles, the thin black line indicates the complete spread of data and outliers are black dots. Sites with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$).

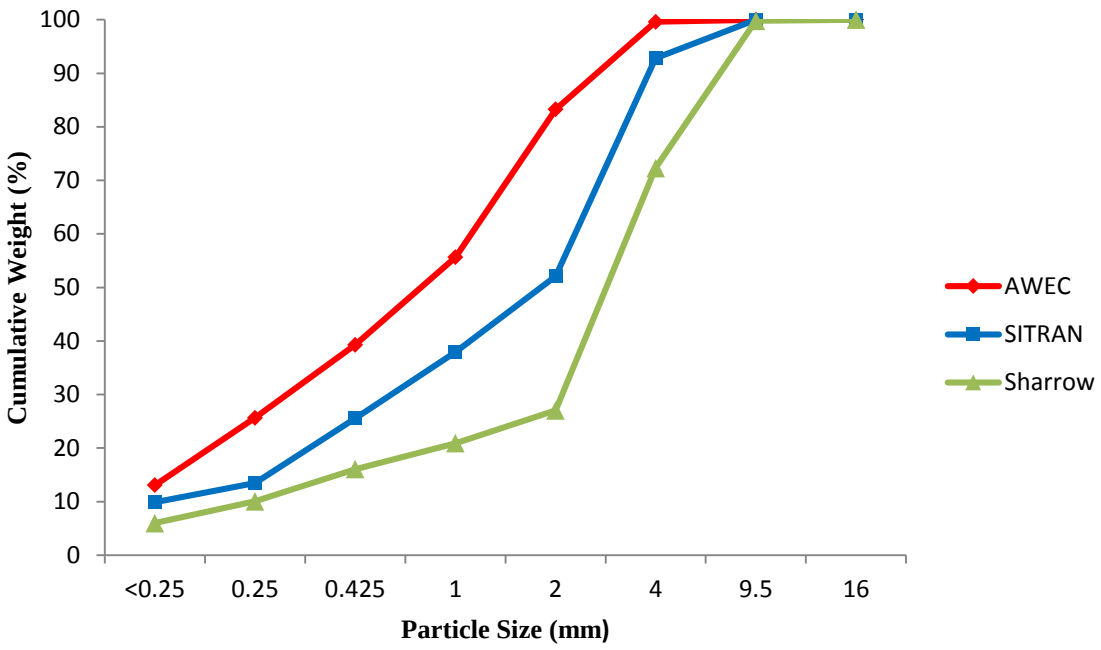


Figure 5.7: Mean Roof Particle size distribution where cumulative weight has been calculated.

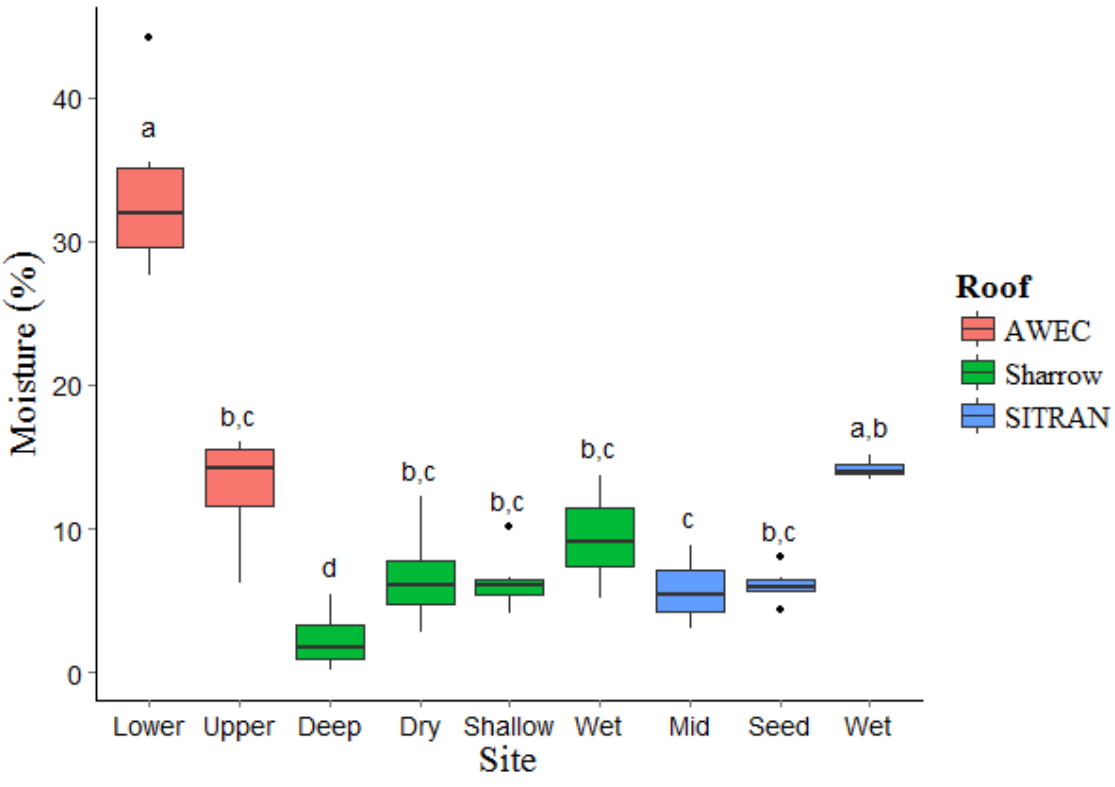


Figure 5.8: Moisture levels (%) at each Site measured in September 2013. The middle bar represents the treatments mean, the upper and lower box edges indicate the 1st and 3rd quartiles, the thin black line indicates the complete spread of data and outliers are black dots. Sites with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$).

5.4.1.4 Moisture

Roof and Site both had a significant effect on moisture levels (two-way nested ANOVA $p<0.05$). Moisture readings were highest at AWEC Lower (33%) and lowest at Sharrow Deep (2%). All other roofs showed intermediate levels of moisture (6-14%) that were not significantly different from one another (Fig. 5.8).

5.4.1.5 FLL Results

Roof had a much larger effect than Site on FLL measured physical characteristics (Table 5.4). AWEC substrates had a much lower permeability but higher porosity and density. Sharrow substrates showed the lowest density and highest permeability with SITraN substrates showing values in between AWEC and Sharrow. AWEC substrates showed the highest water holding capacity (WHC) at 60%, whilst all three SITraN substrates showed the lowest WHC at 32-38% (Table 5.4).

5.4.1.6 Temperature Regime

Daily mean substrate temperature was significantly affected by Roof and Site (two-way nested ANOVA $p<0.05$) with Sharrow showing the highest temperatures and Sharrow Deep the highest daily mean of 6.3^{o}C. SITraN and AWEC showed similar daily mean substrate temperatures of around 4.4^{o}C (Table 5.5a). The range in daily temperatures was also significantly affected Roof and Site (two-way nested ANOVA $p<0.05$) with Sharrow Deep showing the smallest range and AWEC the biggest (Table 5.5a).

Daily minimum substrate temperature was significantly affected by Roof and Site (two-way nested ANOVA $p<0.05$) with Sharrow Deep showing the highest minimum temperature of 5.3^{o}C and AWEC the lowest at 1.5-2^{o}C (Table 5.5a). Daily maximum

substrate temperature was only significantly affected by Roof with Sharrow showing slightly higher maximum temperatures than SITraN and AWEC (Table 5.5a).

Daily mean air temperature was only significantly affected by Roof (two-way nested ANOVA $p<0.05$) with Sharrow and SITraN showing higher temperatures than AWEC (Table 5.5b). Daily temperature range or maximum air temperature was not significantly affected by Roof or Site (two-way nested ANOVA $p<0.05$) (Table 5.5b).

5.4.1.7 Cluster Analysis

Five main clusters of Sites were observed for physical characteristics (Fig. 5.9). Clusters 1-3 were all AWEC Sites and were defined by shallow depth, high WHC (60%) and organic matter levels of around 5%. The largest cluster (No. 4) contained 28 Sites which were predominantly SITraN Sites (Fig. 5.9) and was defined by high permeability, low organic matter (3%), low WHC (37%) and depth of 120mm. Cluster No. 5 was predominantly made up of Sharrow Deep and Dry Sites and was defined by relatively deep substrate (140mm), very low moisture levels (3%) and high organic matter (10%) (Fig. 5.9).

Table 5.4: Physical characteristics of each Site measured according to FLL guidelines (FLL 2008). Statistical differences between Roof and Site are calculated with a 2-way nested ANOVA. Sites with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$). Statistical significances of P-values: * $p<0.01$, **$p<0.001$, *** $p<0.0001$.

Roof	Site	Permeability (cm/s) (±SE)	Air Dried Density (g/cm^3) (±SE)	Oven Dried Density (g/cm^3) (±SE)	Saturated Density (g/cm^3) (±SE)	Porosity (%) (±SE)	Air Content at Water Content Max (%) (±SE)	Water Holding Capacity (%) (±SE)
AWEC	Lower	1.26 ^{CD} (0.9)	1.53 ^{A} (0.1)	1.28 ^{A} (0.1)	1.79 ^{A} (0.2)	63.92 ^{A} (9.9)	4.45 ^{A} (0.4)	59.5^{A} (9.5)
AWEC	Upper	0.50 ^{D} (0.02)	1.57 ^{A} (0.02)	1.28 ^{A} (0.02)	1.77 ^{A} (0.02)	63.32 ^{A} (6.2)	4.80 ^{AB} (2.3)	58.5^{A} (3.9)
SITraN	Mid	9.81 ^{AB} (0.8)	1.34 ^{B} (0.03)	1.20 ^{AB} (0.01)	1.49 ^{B} (0.03)	34.07 ^{C} (3.1)	1.25 ^{ABC} (1.4)	32.8^{D} (1.9)
SITraN	Seed	7.25 ^{AB} (1.3)	1.22 ^{BCD} (0.009)	1.08 ^{BC} (0.01)	1.47 ^{B} (0.02)	38.12 ^{C} (0.8)	0.00 ^{C} (0.0)	38.3^{CD} (0.9)
SITraN	Wet	8.97 ^{AB} (1.7)	1.21 ^{BCD} (0.007)	1.11 ^{BC} (0.01)	1.47 ^{B} (0.01)	36.57 ^{C} (2.0)	0.00 ^{C} (0.0)	36.6^{CD} (2.0)
Sharrow	Deep	4.34 ^{ABC} (1.4)	1.31 ^{BC} (0.02)	1.07 ^{C} (0.01)	1.61 ^{AB} (0.03)	48.98 ^{ABC} (2.8)	0.00 ^{C} (0.0)	50.4^{ABC} (3.1)
Sharrow	Dry	2.42 ^{BC} (0.4)	1.34 ^{B} (0.02)	1.05 ^{C} (0.02)	1.55 ^{AB} (0.04)	58.35 ^{AB} (1.5)	2.83 ^{ABC} (0.9)	55.5^{AB} (1.5)
Sharrow	Shallow	10.91^{AB} (3.1)	1.12 ^{D} (0.007)	1.03 ^{C} (0.007)	1.45 ^{B} (0.006)	40.07 ^{C} (1.9)	0.00 ^{C} (0.0)	40.8^{BCD} (1.4)
Sharrow	Wet	14.74 ^{A} (0.6)	1.16 ^{BCD} (0.03)	1.07 ^{C} (0.03)	1.50 ^{B} (0.04)	42.68 ^{BC} (1.0)	0.00 ^{C} (0.0)	42.7^{BCD} (1.0)
Site		*	***	0.05	0.33	*	*	*
Roof		***	***	***	***	***	***	***
AWEC		0.88^{B} (0.4)	1.55^{B} (0.05)	1.28^{A} (0.04)	1.78^{A} (0.08)	63.62^{A} (4.8)	4.62^{A} (1.0)	59.0^{A} (4.2)
SITraN		8.68^{A} (0.8)	1.26^{A} (0.02)	1.13^{B} (0.02)	1.48^{B} (0.01)	36.26^{C} (1.2)	0.36^{B} (0.5)	35.9^{C} (1.2)
Sharrow		7.50^{A} (1.7)	1.24^{A} (0.03)	1.05^{C} (0.01)	1.53^{B} (0.02)	47.96^{B} (2.4)	0.20^{B} (0.6)	47.8^{B} (2.1)

Table 5.5: (a) Substrate temperatures of each Site and (b) Air temperatures of each Site measured during November 2013.

(a)

Roof	Site	Daily Mean Temperature (°C)	Daily Minimum Temperature (°C)	Daily Maximum Temperature (°C)	Daily Temperature Range (°C)
AWEC	Lower	4.4 ^{DE} (0.1)	2.0 ^{EF} (0.2)	6.7 ^{AB} (0.4)	4.7^{ABC} (0.5)
AWEC	Upper	4.0 ^{E} (0.1)	1.2 ^{F} (0.2)	7.0 ^{AB} (0.3)	5.8^{A} (0.4)
SITraN	Mid	4.3 ^{E} (0.03)	2.4 ^{DE} (0.09)	6.4 ^{AB} (0.09)	3.9^{ABCD} (0.2)
SITraN	Seed	4.9 ^{CD} (0.2)	3.2 ^{BCD} (0.3)	6.8 ^{AB} (0.1)	3.6^{BCD} (0.3)
SITraN	Wet	4.2 ^{E} (0.2)	2.6 ^{CDE} (0.2)	5.7 ^{B} (0.1)	3.1^{BCD} (0.1)
Sharrow	Deep	6.3 ^{A} (0.2)	5.3 ^{A} (0.2)	7.2 ^{A} (0.1)	1.9^{D} (0.1)
Sharrow	Dry	5.2 ^{BC} (0.1)	2.8 ^{CDE} (0.2)	7.8 ^{A} (0.7)	5.0^{AB} (0.9)
Sharrow	Shallow	5.2 ^{BC} (0.1)	3.5 ^{BC} (0.07)	6.8 ^{AB} (0.2)	3.4^{BCD} (0.2)
Sharrow	Wet	5.6 ^{B} (0.06)	4.1 ^{B} (0.08)	6.9 ^{AB} (0.08)	2.8^{CD} (0.1)
Site		***	***	0.11	***
Roof		***	***	**	***
AWEC		4.2^{C} (0.1)	1.5^{C} (0.2)	6.9^{AB} (0.2)	5.3^{A} (0.3)
SITraN		4.5^{B} (0.1)	2.7^{B} (0.1)	6.3^{B} (0.1)	3.5^{B} (0.2)
Sharrow		5.6^{A} (0.1)	3.9^{A} (0.2)	7.2^{A} (0.2)	3.3^{B} (0.4)

(b)

Roof	Site	Daily Mean Temperature (°C)	Daily Minimum Temperature (°C)	Daily Maximum Temperature (°C)	Daily Temperature Range (°C)
AWEC	Lower	4.2^{B} (0.2)	1.2 ^{BCD} (0.2)	7.3^{A} (0.9)	6.1 ^{A} (1.1)
AWEC	Upper	4.0 ^{B} (0.4)	0.2 ^{D} (0.3)	8.1^{A} (0.5)	7.9 ^{A} (0.2)
SITraN	Mid	4.7 ^{AB} (0.2)	0.6 ^{CD} (0.2)	10.0^{A} (0.7)	9.3 ^{A} (0.6)
SITraN	Seed	4.7 ^{AB} (0.2)	1.6 ^{ABCD} (0.4)	8.7^{A} (0.7)	7.2 ^{A} (0.8)
SITraN	Wet	4.7 ^{AB} (0.05)	1.2 ^{BCD} (0.2)	10.1^{A} (0.8)	9.0 ^{A} (0.9)
Sharrow	Deep	5.7 ^{A} (0.06)	3.1 ^{A} (0.3)	8.4^{A} (0.2)	5.3 ^{A} (0.5)
Sharrow	Dry	5.5 ^{A} (0.4)	1.8 ^{ABCD} (0.3)	10.8^{A} (1.4)	9.1 ^{A} (1.3)
Sharrow	Shallow	5.8 ^{A} (0.2)	2.1 ^{ABC} (0.03)	9.7^{A} (0.6)	7.6 ^{A} (0.6)
Sharrow	Wet	5.2 ^{AB} (0.3)	2.3 ^{AB} (0.5)	10.1^{A} (0.4)	6.1 ^{A} (0.6)
Site		0.77	*	0.19	0.05
Roof		***	***	0.07	0.05
AWEC		4.1^{C} (0.2)	0.7^{B} (0.3)	7.7^{B} (0.5)	7.0^{A} (0.7)
SITraN		4.7^{B} (0.1)	1.1^{B} (0.2)	9.6^{A} (0.4)	8.5^{A} (0.5)
Sharrow		5.5^{A} (0.1)	2.3^{A} (0.2)	9.3^{AB} (0.5)	7.0^{A} (0.6)

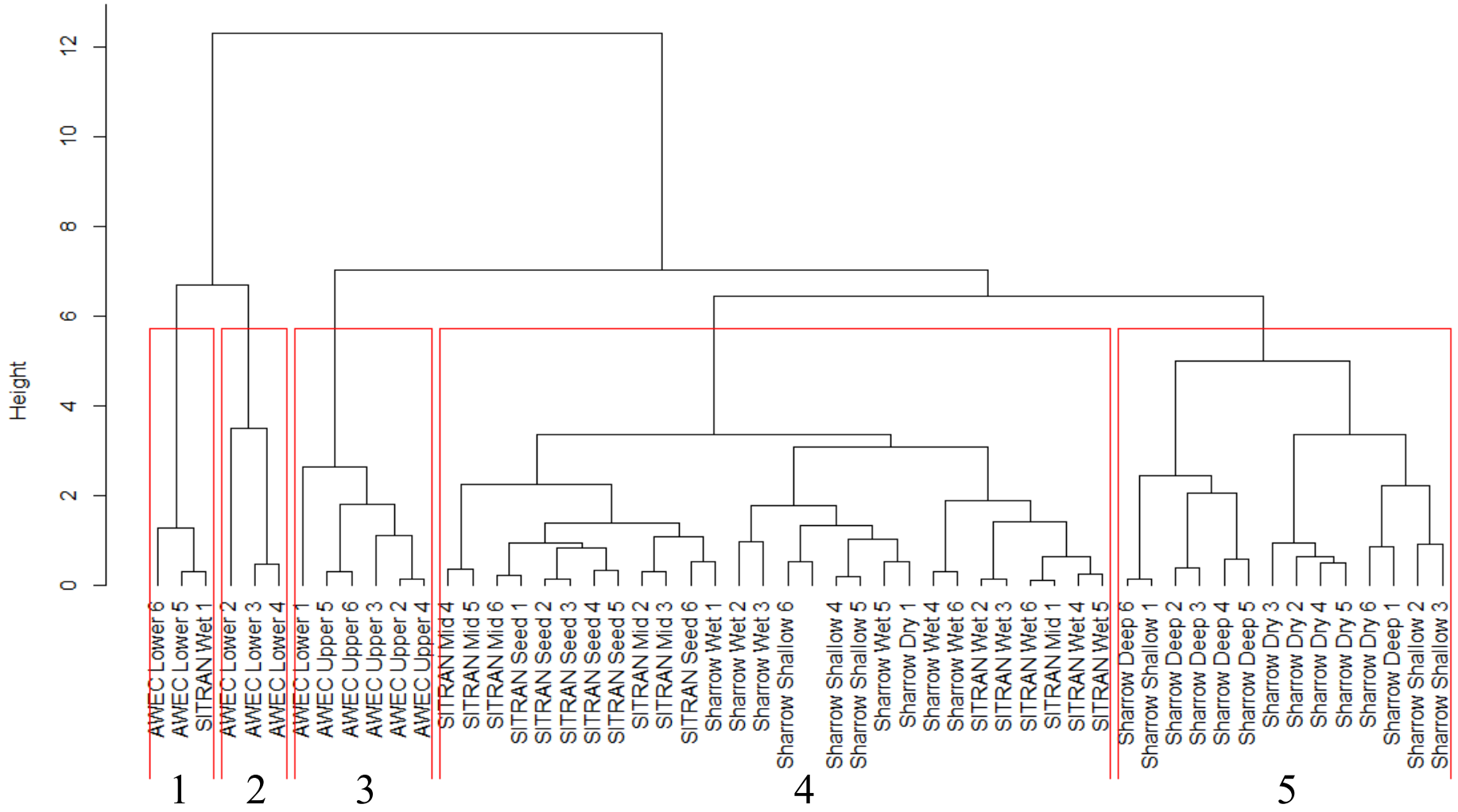


Figure 5.9: Dendrogram of physical substrate characteristics obtained by hierarchical clustering of standardised data.

5.4.2 Biological Variables

5.4.2.1 Worm Density

The density of worms was significantly affected by Site but not Roof (two-way nested ANOVA $p<0.05$). The highest density of worms were both recorded on Sharrow sites (Deep and Wet) although both sites had high variability (Fig. 5.10). No worms were found on Sharrow Dry and Shallow whilst worms were found on all three SITraN sites, although in much lower numbers than Sharrow Wet and Deep (Fig. 5.10). Due to issues with site accessibility sampling could not be conducted on AWEC however worms were visually observed at the Lower AWEC site only.

5.4.2.2 Collembola Density

Density of *Collembola* was not significantly affected by Roof or Site (two-way nested ANOVA $p<0.05$). Despite high density of *Collembola* in some Sharrow Deep substrate samples, high variability within Sharrow Deep samples meant that this site was not significantly different from the others (Table 5.6).

5.4.2.3 AMF Infection Rates

All sites showed AMF infection of *P. lanceolata*, with the amount of infection significantly affected by Roof but not Site (two-way nested ANOVA $p<0.05$). Sharrow (30%) sites had significantly higher infection rates than SITraN (23%) and AWEC (19%) (Table 5.6)

5.4.2.4 Litter Decomposition

The rate of oak litter decomposition was not significantly affected by Roof or Site (two-way nested ANOVA $p<0.05$) (Table 5.6)

5.4.2.5 Plant Diversity

Shannon-Weiner Diversity Index was only significantly affected by Roof (two-way nested ANOVA $p<0.05$) (Table 5.6). AWEC sites showed much lower diversity indexes values of 0.66 respectively than Sharrow and SITraN sites which were between 0.78-0.83 (Table 5.6). The number of plant species present at each site as well as coverage of bare ground/moss was significantly affected by Roof and Site (two-way nested ANOVA $p<0.05$). The highest number of species were observed at Sharrow Shallow and SITraN Seed, and the lowest number at both AWEC sites (Table 5.6), whilst the highest amounts of bare ground/moss were found on AWEC (60%) and the lowest on Sharrow sites (12%) (Table 5.6).

5.4.2.6 Microbial Biomass (SIR)

Microbial biomass was significantly affected by Roof and Site (two-way nested ANOVA $p<0.05$). SITraN sites showed the smallest microbial biomass levels (6mg $100g^{-1}$ dry substrate) with AWEC Lower sites the largest (85mg $100g^{-1}$ dry substrate). Sharrow Deep, Dry and Shallow had similar levels as AWEC Upper (35mg $100g^{-1}$ dry substrate) whilst Sharrow Wet had low levels similar to SITraN sites (Fig. 5.11).

5.4.2.7 Cluster Analysis

Three relatively even clusters of Sites were observed for biological characteristics (Fig. 5.12). Cluster 1 was made up entirely of AWEC sites and was defined by high microbial biomass, low species diversity (4) and high levels of bare ground/moss (60%). Cluster 2 was predominately a mixture of SITran Seed and Sharrow Dry and Shallow and defined by low microbial biomass, low levels of bare ground/moss (14%) and medium levels of species diversity (6.5). Cluster 3 was made up of the remaining

SITraN Wet and Sharrow Deep and Wet Sites and was defined by high levels of species diversity (9).

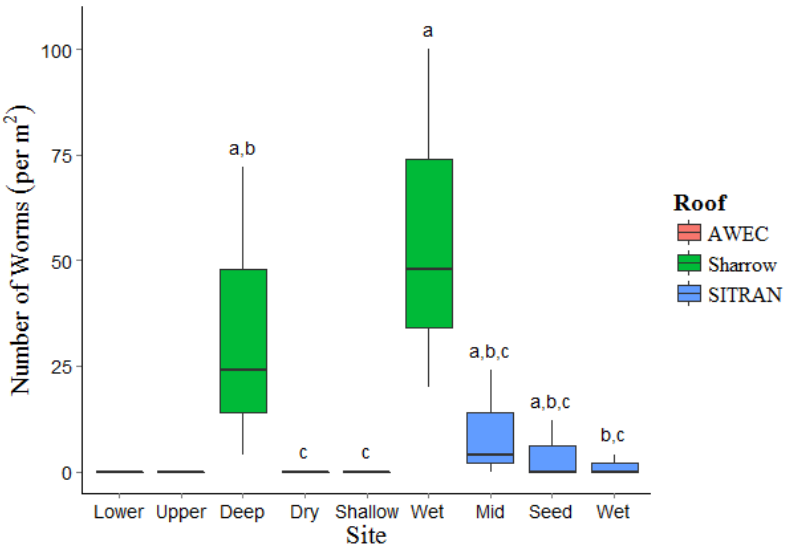


Figure 5.10: Number of earthworms sampled at each Site per m^2. The middle bar represents the treatments mean, the upper and lower box edges indicate the 1st and 3rd quartiles, the thin black line indicates the complete spread of data and outliers are black dots. Sites with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$).

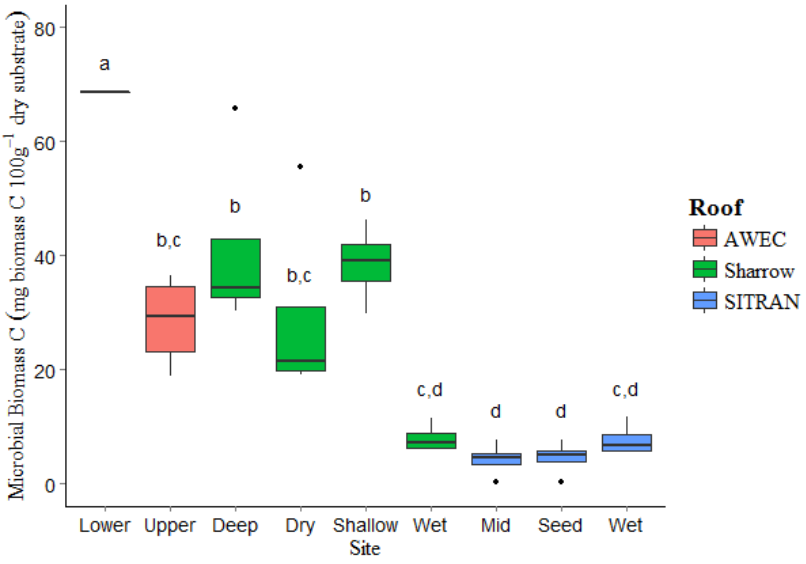


Figure 5.11: Microbial biomass (mg biomass C $100g^{-1}$ dry substrate) measured by substrate induced respiration. The middle bar represents the treatments mean, the upper and lower box edges indicate the 1st and 3rd quartiles, the thin black line indicates the complete spread of data and outliers are black dots. Sites with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$).

Table 5.6: Biological characteristics of each Site. Statistical differences between Roof and Site are calculated with a 2-way nested ANOVA. Sites with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$). Statistical significances of P-values: * $p<0.01$, ** $p<0.001$, *** $p<0.0001$.

Roof	Site	Litter Decomposition (% weight loss of initial dry weight day^{-1}) (±SE)	Mycorrhizal Infection of Bait Plants (%) (±SE)	*Collembola* Density (no. kg^{-1} dry substrate) (±SE)	Number of Species Present (±SE)	Shannon-Weiner Diversity Index (±SE)	Bare Ground/Moss Present (%) (±SE)
AWEC	Lower	0.23^{A} (0.09)	19.3^{A} (3.2)	98.1^{A} (82.6)	4.0^{E} (0.0)	1.19^{B} (0.03)	54.2^{A} (5.7)
AWEC	Upper	0.45^{A} (0.12)	18.5^{A} (2.2)	82.6^{A} (29.0)	4.3^{DE} (0.2)	1.23^{B} (0.05)	69.2^{A} (5.7)
SITraN	Mid	0.32^{A} (0.01)	18.1^{A} (1.2)	25.8^{A} (15.8)	7.0^{BC} (0.5)	1.67^{A} (0.06)	40^{AB} (5.6)
SITraN	Seed	0.26^{A} (0.07)	27.9^{A} (3.0)	30.2^{A} (12.7)	8.8^{AB} (0.5)	1.86^{A} (0.06)	29.2^{ABC} (9.1)
SITraN	Wet	0.31^{A} (0.01)	22.9^{A} (3.2)	47.7^{A} (14.3)	7.3^{BC} (0.2)	1.76^{A} (0.05)	17.5^{ABC} (2.5)
Sharrow	Deep	0.39^{A} (0.02)	32.8^{A} (4.8)	235.5^{A} (102.6)	7.7^{BC} (0.8)	1.66^{A} (0.1)	13.3^{C} (7.6)
Sharrow	Dry	0.41^{A} (0.01)	24.8^{A} (2.8)	38.7^{A} (16.6)	8.0^{BC} (0.7)	1.82^{A} (0.07)	16.7^{BC} (7.8)
Sharrow	Shallow	0.36^{A} (0.02)	25^{A} (3.0)	20.7^{A} (8.5)	10.3^{A} (0.5)	1.90^{A} (0.08)	19.2^{ABC} (2.4)
Sharrow	Wet	0.31^{A} (0.03)	33.2^{A} (8.5)	66.2^{A} (38.3)	6.5^{CD} (0.6)	1.64^{A} (0.1)	0.8^{D} (0.8)
Site		0.28	0.51	0.24	***	0.07	***
Roof		0.45	**	0.77	***	***	***
AWEC		0.34^{A} (0.08)	18.9^{B} (1.9)	90.3^{A} (39.3)	4.2^{B} (0.1)	1.21^{B} (0.03)	61.7^{A} (4.5)
SITraN		0.30^{A} (0.02)	22.7^{AB} (1.7)	34.6^{A} (8.0)	7.7^{A} (0.3)	1.76^{A} (0.05)	28.9^{B} (4.1)
Sharrow		0.36^{A} (0.01)	30.5^{A} (3.5)	90.3^{A} (32.2)	8.1^{A} (0.4)	1.76^{A} (0.03)	12.5^{C} (3.0)

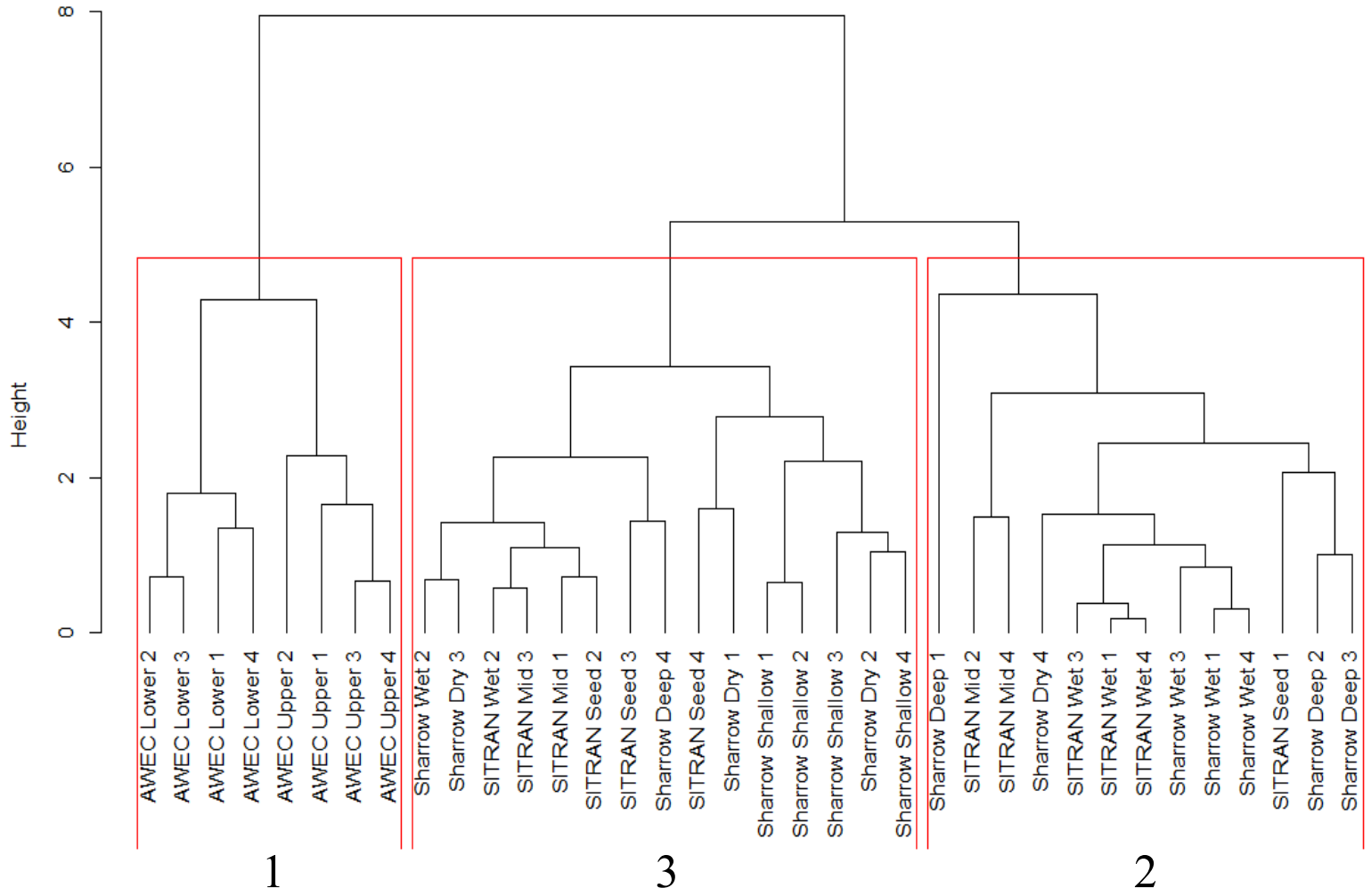


Figure 5.12: Dendrogram of biological substrate characteristics obtained by hierarchical clustering of standardised data.

5.4.3 Chemical Variables

5.4.3.1 pH

Substrate pH was only significantly affected by Roof (two-way nested ANOVA $p<0.05$). Sharrow pH was the highest at 8.8, followed by AWEC at 8.2 with SITraN the lowest at 8 (Fig. 5.13).

5.4.3.2 Plant Available Phosphorus

Plant available P was significantly affected by Roof and Site (two-way nested ANOVA $p<0.05$) with Sharrow substrates (especially Sharrow Deep) containing the highest amounts of P (Fig. 5.14). SITraN and AWEC substrates were not significantly different from one another (Fig. 5.14).

5.4.3.3 Plant Available Nitrogen

Plant available N was significantly affected by Roof and Site (two-way nested ANOVA $p<0.05$). The highest values of N were found in Sharrow Deep, Shallow and SITraN Wet, whilst the remaining sites were not significantly different from one another (Fig. 5.15).

5.4.3.4 Nitrogen Mineralisation Rates

Nitrification (change in TON), ammonification (change in NH_4^+) and mineralisation (net production of plant available N) were all significantly affected by Roof and Site (two-way nested ANOVA $p<0.05$). The highest rates of nitrification were found in Sharrow substrates, although there was a high amount of variation within Sites (Fig. 5.16a). Ammonification was generally negative for all Sites which indicates immobilisation of NH_4^+, with this again being greatest in Sharrow substrates (Fig.

5.16b). Net mineralisation was negative for most sites with Sharrow Sites showing the greatest values and AWEC and SITraN Sites the lowest at around zero (Fig. 5.17).

5.4.3.5 Cluster Analysis

Four main clusters were observed for chemical characteristics (Fig. 4.18). The largest cluster was made up of all the AWEC and SITraN Sites and was defined by low pH (8.1) and low levels of N & P as well as low nitrification and ammonification rates. Cluster 2 was a mixture of Sharrow Sites and was defined by intermediate levels of N & P, but low levels of nitrification. Cluster 3 only contained three Sites which all had high levels of N & P as well as high nitrification and ammonification rates. Finally cluster 4 also contained a mixture of Sharrow Sites and was defined by high P but relatively low N levels.

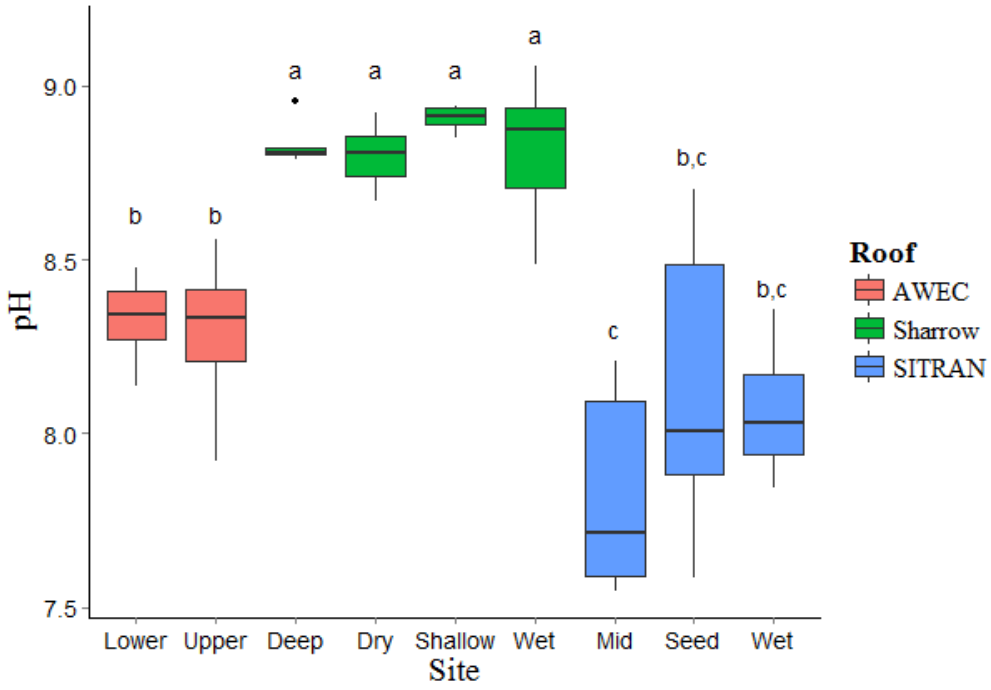


Figure 5.13: pH levels at each Site measured in October 2013. The middle bar represents the treatments mean, the upper and lower box edges indicate the 1st and 3rd quartiles, the thin black line indicates the complete spread of data and outliers are black dots. Sites with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$).

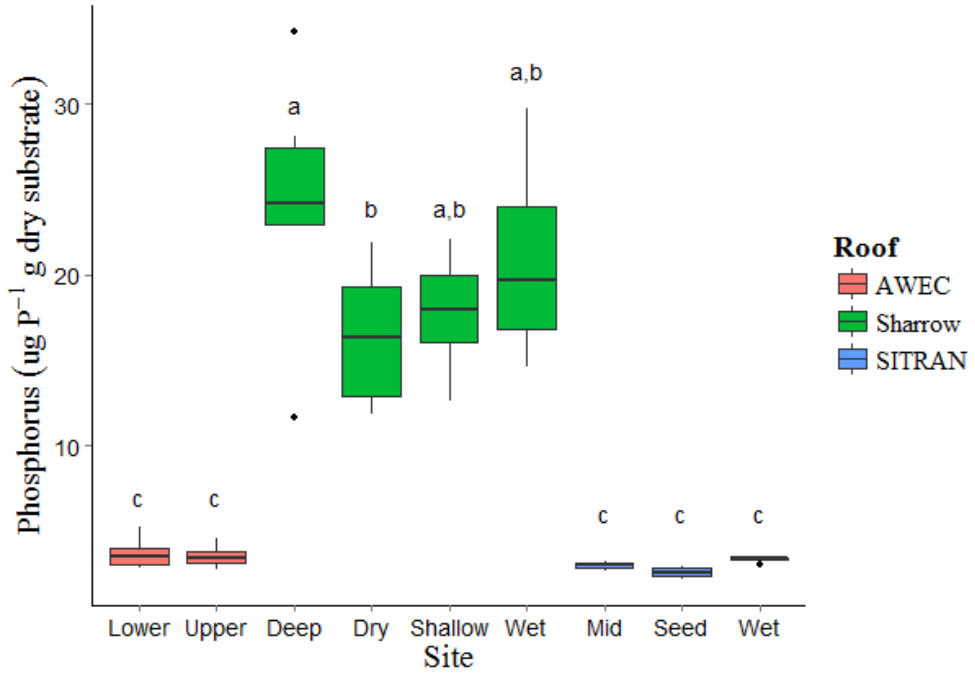


Figure 5.14: Plant available P (µg P g^{-1} dry substrate) in substrate samples from each Site in September 2013. The middle bar represents the treatments mean, the upper and lower box edges indicate the 1st and 3rd quartiles, the thin black line indicates the complete spread of data and outliers are black dots. Sites with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$).

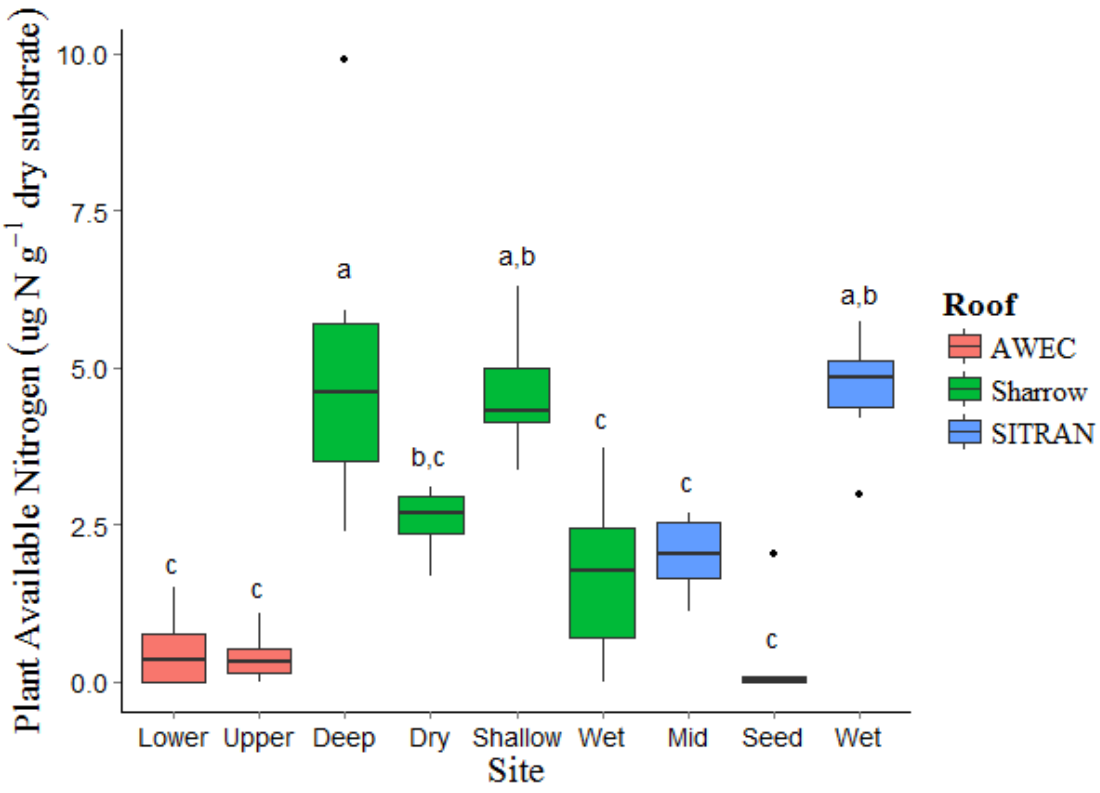


Figure 5.15: Plant available N (µg N g^{-1} dry substrate) in substrate samples from each Site in September 2013. The middle bar represents the treatments mean, the upper and lower box edges indicate the 1st and 3rd quartiles, the thin black line indicates the complete spread of data and outliers are black dots. Sites with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$).

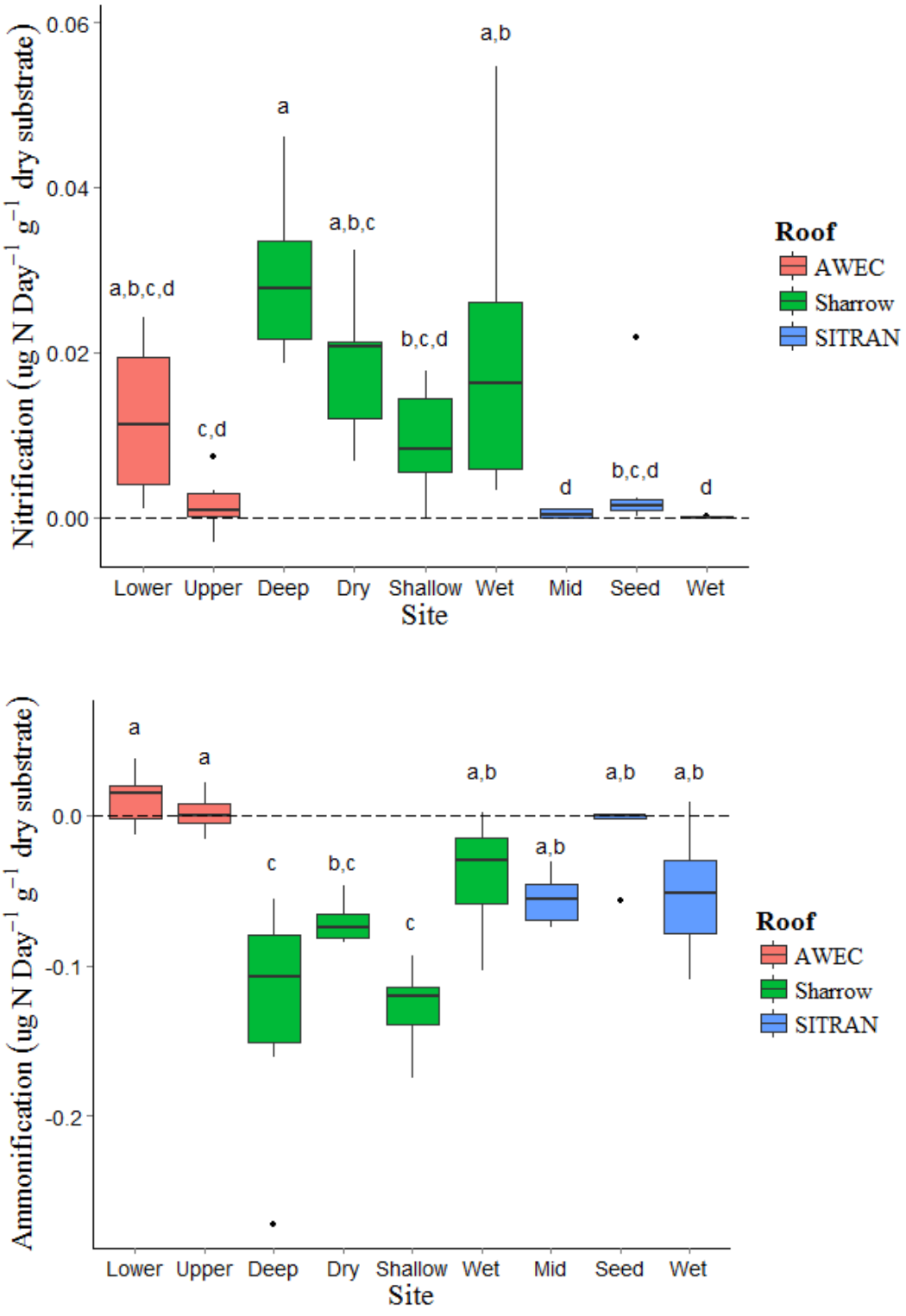


Figure 5.16: (a) Nitrification rates (µg N day^{-1} g^{-1} dry substrate) and (b) Ammonification rates **(**µg N day^{-1} g^{-1} dry substrate) of substrate samples located in Sites for 30days during September-October 2013. Negative values indicate net N immobilisation. The middle bar represents the treatments mean, the upper and lower box edges indicate the 1st and 3rd quartiles, the thin black line indicates the complete spread of data and outliers are black dots. Sites with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$).

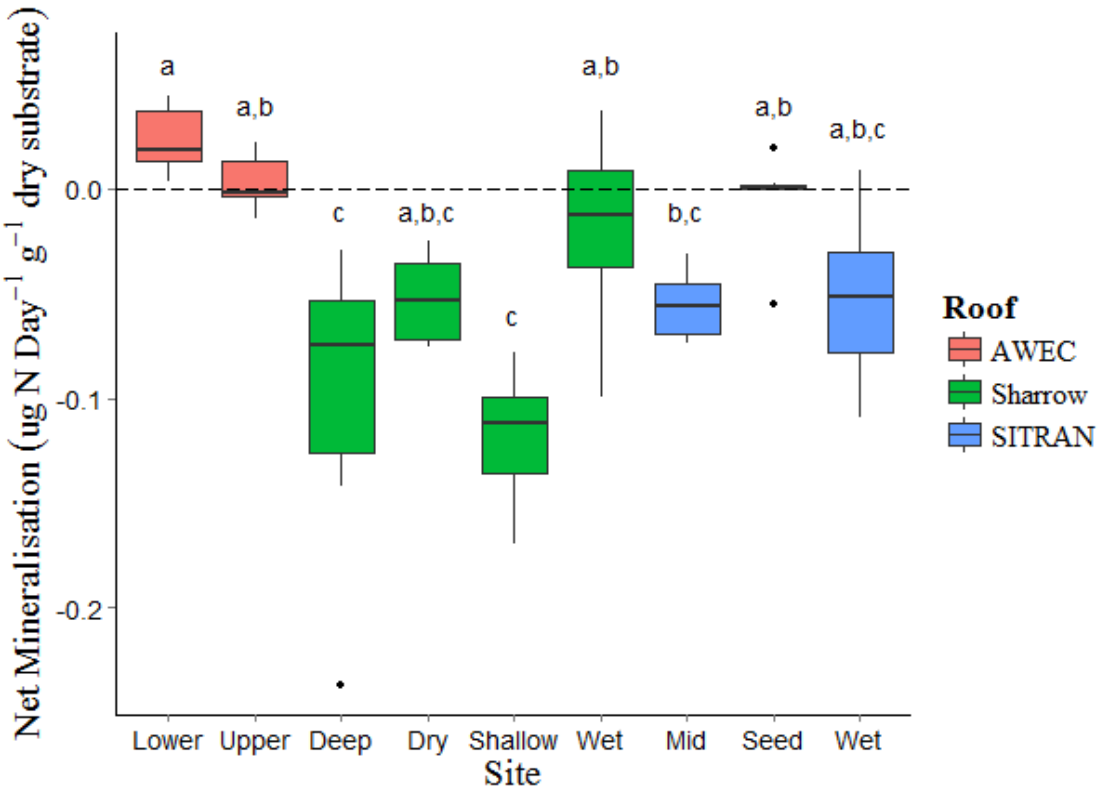


Figure 5.17: Net mineralisation (µg N day^{-1} g^{-1} dry substrate) of substrate samples located in Sites for 30days during September-October 2013. Negative values indicate net N immobilisation. The middle bar represents the treatments mean, the upper and lower box edges indicate the 1st and 3rd quartiles, the thin black line indicates the complete spread of data and outliers are black dots. Sites with the same letter do not differ significantly from one another (Tukey HSD, $p<0.05$).

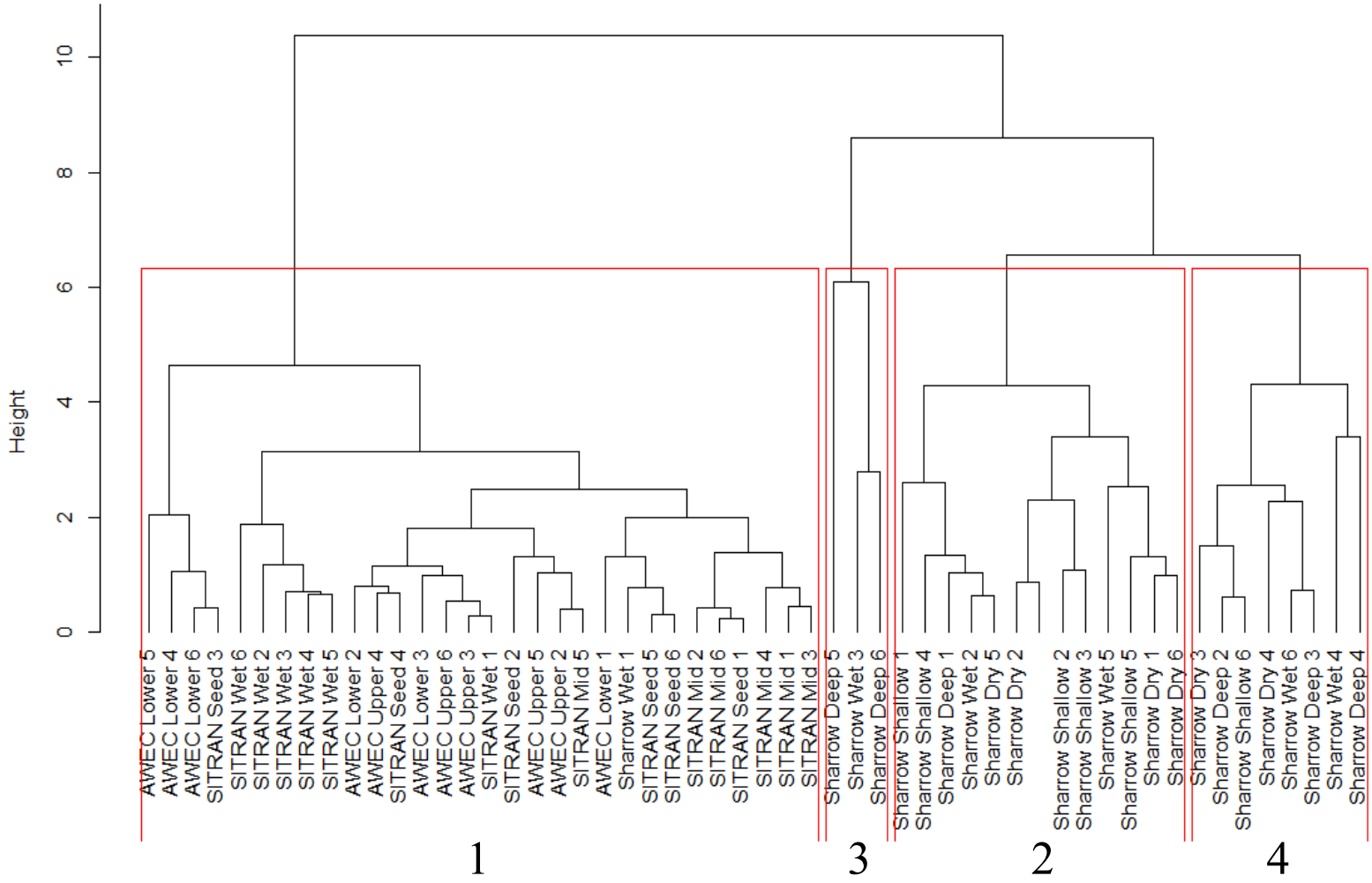


Figure 5.18: Dendrogram of chemical substrate characteristics obtained by hierarchical clustering of standardised data.

5.4.4 Cluster Analysis for All Variables

For all factors five main clusters were found (Fig. 5.19). Cluster 1 contained all AWEC Upper Sites, half the AWEC Lower Sites and one SITraN Wet Site. These were defined by shallow depth (35mm), low N, low species diversity (4) and very high levels of bare ground/moss (70%). Cluster 2 contained the remaining AWEC Lower Sites and had shallow depth (35mm, very high moisture (35%), very high WHC (70%), low species diversity (4), and very low N & P levels. Cluster 3 contained the remaining SITraN Sites and was defined by low pH, relatively deep substrate (140mm) but very low organic levels (2%). Cluster 4 was a mixture of Sharrow Shallow, Wet and Dry and contained relatively shallow depth (90mm), high levels of N & P and high species diversity (9). Cluster 5 was dominated by Sharrow Deep as well as four Sharrow Dry and the remaining Sharrow Sites. This cluster was defined by deep substrate (160mm), high N & P, high nitrification, very high organic matter (11%) and low bare ground/moss coverage (10%).

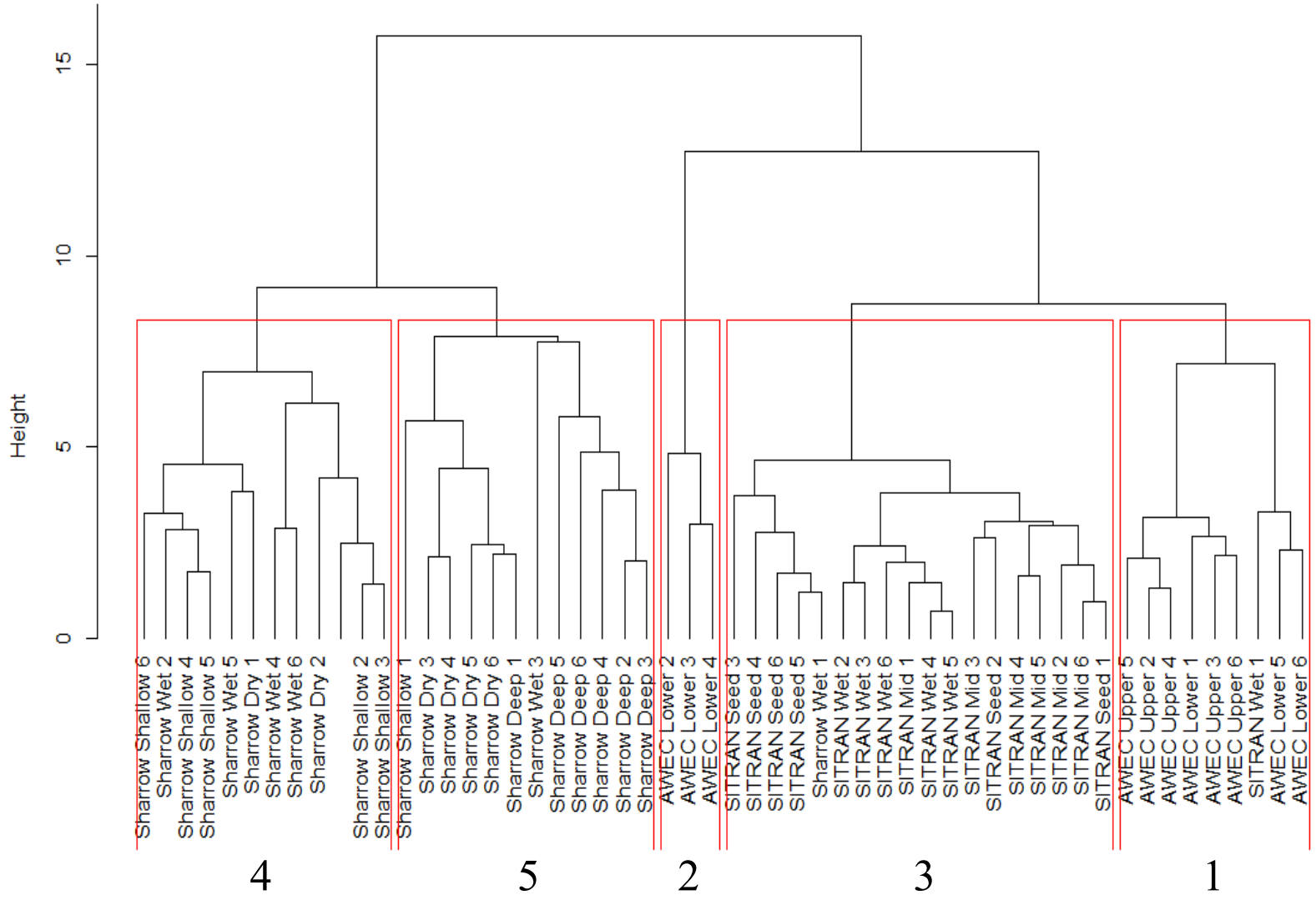


Figure 5.19: Dendrogram of all substrate characteristics obtained by hierarchical clustering of standardised data.

5.5 Discussion

Although this study only sampled three green roofs in a single city, it has shown that similar aged roofs have significant variability in biological, chemical and physical substrate characteristics inter and also intra-roof. Variables that showed the greatest variability intra-roof were biological and chemical indicators not normally measured on green roofs, whilst more commonly measured physical factors showed less variability. Possible reasons for the intra and inter-roof variability will be discussed below as well as the potential to use a selection of the variables to develop a SHI for green roofs.

5.5.1 Physical Characteristics

The substrate depths described on these roofs falls within the general specified range of extensive/semi-intensive green roofs (40-200mm, (FLL 2008). The only exception to this was some of the Sharrow Deep Sites which were located within an artificial mound (up 300mm) of substrate which is unusually deep for an extensive/semi-intensive green roof. AWEC substrates were particularly shallow as the installation technique involved the addition of a pre-grown *Sedum* mat with a fine layer of substrate embedded into it. A much wider study of 115 green roofs in France showed a similar substrate depth range of 20-600mm with a mean of 120mm (Madre et al. 2014), whilst age has been shown to decrease substrate depth on established extensive roofs in Germany with a range of 50-90mm.

Studies on established German green roofs (up to 50years old) has shown that older green roof substrate generally contains higher amounts of organic matter (Schrader

and Böning 2006; Getter et al. 2007; Thuring and Dunnett 2014), however organic matter builds up at a very slow rate (increase from 2 to 3% over 25years) due to slow decomposition of biomass (Köhler and Poll 2010). The slow accumulation of organic matter on established German roofs does not explain the large amount of organic matter at Sharrow Deep (14.5%). However due to the depth of this particular site biomass growth was exceptionally luxuriant and thus could build up relatively rapidly due to infrequent management (1-2 times $year^{-1}$) (Madre et al. 2014). The decomposition and subsequent formation of relatively rich humus was most likely aided by the large amount of earthworms and *Collembola* found in the centre of the mound. However a German study of 4 extensive green roofs (Liesecke 2006) cited in (Thuring and Dunnett 2014)) recorded that organic matter was greatest on the shallowest roofs, although this was perceived to be due to inhibition of microbial activity and therefore organic matter build up was due to un-decomposed plant litter.

The main driver for WHC appears to be substrate particle size distribution with AWEC substrates showing the highest amount of small particles, porosity as well as WHC. Previous trials have reported a similar trend that supports FLL particle size guidelines which are designed to prevent substrates from containing too many small particles which can lead to substrates becoming waterlogged and anaerobic (Molineux et al. 2009; Olszewski and Young 2011; Graceson et al. 2013). Chapters 2 & 3 have shown that reducing the particle size of crushed brick increases the WHC of substrate, which in turn has significant impact of plant growth, health and drought tolerance (Young et al. 2014a; Young et al. 2014b). Age has also been shown to affect WHC, with a sand based substrate (2% organic) increasing its WHC from 17 to 67% over 5 years, most likely due to a increase in micro-pores throughout the substrate which increased total porosity from 40 to 80% (Getter et al. 2007). Organic matter has some impact on WHC

as it has a higher retention ability than mineral components (Graceson et al. 2014a), explaining why Sharrow Deep and Dry showed significantly higher WHC than Sharrow Shallow and Wet.

Although the moisture measurements in this study only provide a snapshot of the water dynamics over the course of a year, they still confirm that contrasting areas of a roof can receive significantly different volumes of water due to slope runoff or interception by other objects. AWEC was the only roof to have a visible roof slope and showed significantly greater moisture levels as well as visible pooling of water at its lower end. Previous research on the effect of slope on water retention has shown that increased slope angle decreases water retention and increases runoff during normal rainfall events (Getter et al. 2007). This alteration of water flow through a green roof will have significant effects on water availability and therefore also vegetation growth and survival. The two Wet sites on Sharrow (receives runoff from another roof) and SITraN (remains in shadow for most of the day) also showed higher moisture levels which suggests that external objects on a green roof can also significantly affect water flow and availability which in turn can affect plant diversity (Köhler 2005; Köhler and Poll 2010)

Despite the temperature measurement of this trial being fairly crude, results suggest that the substrate temperature varies significantly intra-roof. It appears that substrate moisture has the biggest influence on temperature range with higher levels of moisture buffering against temperature extremes. More detailed studies on substrate/air temperature regimes have also shown a similar insulation effect of green roof substrate with substrates experiencing lower temperature extremes than normal roofs (Teemusk and Mander 2010). Depth also played a role in regulating substrate temperature although when substrates were deeper than AWEC's 40mm this effect seemed to be

secondary to moisture. Vegetation is likely to play large role in regulating air and substrate temperatures during the summer, although is less likely to play a major role in autumn/winter (Lundholm et al. 2010; Jim 2012).

The inter-roof results are hard to interpret due to a lack of detailed site information (e.g. solar angle, shading, building insulation, internal temperatures). However there does seem to be a tendency for Sharrow air temperatures to be higher than AWEC or SITraN, and this could due to the denser vegetation on Sharrow sheltering localised pockets of air (Jim 2012).

5.5.2 Biological Characteristics

This is the first empirical English study to demonstrate the existence of earthworms in green roof substrate. One previous study on earthworms has been published although a translated version is not accessible (Steiner and Schrader 2002). It is generally assumed that due to their location as well as shallow depth, low organic matter, coarseness, fluctuating moisture levels and extreme temperature regime that earth worms cannot survive in an extensive green roof, especially one with less than 120mm substrate depth (Brenneisen 2005; Schrader and Böning 2006; Molineux et al. 2014). However this study has shown that earthworms can survive on three different green roofs with different substrate depths and moisture levels (earthworms were seen but not counted at AWEC Lower). The body mass of most earthworms is made up of 75-90% water and therefore moisture retention is critical (Grant 1955; Scharenbroch and Johnston 2011). This is reflected in the presence of earthworms only at the more moist

Sharrow Wet sites. The presence of earthworms on the SITraN was especially surprising due to general poor quality of the substrate and low organic content. SITraN earthworms did appear to be smaller and looked less healthy than Sharrow worms. None-the-less the presence of earthworms on SITraN shows that they can survive on relatively shallow (150mm) substrate with low organic matter content.

Collembola are generally hardier species than earthworms and have previously been sampled on green roofs (Buttschardt 2001; Schrader and Böning 2006; Rumble and Gange 2013). Therefore the presence of *Collembola* spp. at all sites was not surprising. However there was not a significant effect of Site, despite Sharrow Deep having a much larger mean than any other site. Sharrow Deep experienced high variability due to the sampling method which also sampled shallower areas containing lower amounts of *Collembola* around the mound. Of the three previous studies that have focused on green roof *Collembola* populations, two had a single sample point and found relatively low populations of *Collembola* (Schrader and Böning 2006); (Buttschardt 2001) cited in (Schrader and Böning 2006). (Rumble and Gange 2013)) however observed highly variable populations of *Collembola* and mites which they attributed to fluctuating moisture levels in the substrate which often reached very low levels (Rumble and Gange 2013). The authors of that study stress the need for longer term observations of green roof substrate invertebrate populations as they can be highly variable and a single sampling point can give misleading conclusions (Rumble and Gange 2013). The development of green roof substrate has many analogies with the successional development of former open cast mine sites due to the low organic matter levels and shallow soil depth. The appearance of *Collembola* occurs fairly early on in mine sites and helps to develop humus by decomposing plant litter (Dunger et al. 2004). The presence of *Collembola* seems to prelude the arrival of earthworms which requires the

presence of humus and therefore takes longer, but once present can significantly influence the soil (Dunger et al. 2004). If this is also the case with green roof substrate then it is expected that once earthworms are present on a green roof, substrate condition is therefore relatively good, and good plant coverage should be widespread/relatively diverse.

This seems to be the case with plant diversity data with no significant differences between Sharrow and SITraN despite the significant differences in organic matter and available P. The largest number of plant species were found at Sharrow Shallow and Dry which had relatively harsh growing conditions compared to the other Sharrow Sites. Higher plant diversity is often found when moderate levels of stress are present as no single plant species can outcompete the others (Grime 2006). Thus in substrates that are shallow (but still >40mm) or have low organic matter and therefore are likely to have low moisture levels or a smaller pool of available nutrients, higher plant diversity is expected. However once stress levels become too great then only a relatively small number of plant species can survive and diversity decreases (Grime 2006). This is the case on AWEC where only *Sedum* species have survived and large amounts of bare ground/moss have appeared. Clearly the initial diversity of plants planted has a large bearing on future plant communities, but self-colonising species are much less likely to colonise the very harsh conditions and uniform vegetation coverage present on AWEC (Emilsson and Rolf 2005; Dunnett et al. 2008b). However, a spectrum exists where plants are also less likely to colonise green roofs with high plant coverage or deeper substrate (e.g. Sharrow) as there is less opportunity to colonise and more competition (Dunnett et al. 2008b; Nagase et al. 2013). All SITraN sites had high diversity, likely due to relatively high levels of stress on the roof, but also showed high amounts of bare ground/moss indicating that the stress on the roof

is too high for full plant coverage. The high species diversity on SITraN Seed was expected due to being hand seeded with a wild flower mix approximately 2 years before sampling (Sorrill 2013).

A large scale study of 115 green roofs in France has shown that substrate depth is the main driver of plant species diversity, although it is not clear at what depth the greatest diversity was found (Madre et al. 2014). Similarly a study of established German roofs suggests that with increased depth vegetation quality (diversity as well as coverage) also increased, although sun exposure also had an influence (Köhler and Poll 2010). Substrate depth heterogeneity across green roofs has been proposed as a method to increase long-term plant diversity as it has been shown to increase plant co existence (Heim and Lundholm 2014b). Similarly a greater range of substrate fertility and coarseness (lower WHC) has also been proposed as a method to increase plant diversity by creating a series of microhabitats across a roof which a range of species can colonise (Bates et al. 2013). It has also been shown in Chapter 3 that plant drought tolerance can be increased through the use courser substrates which promote slower and more sustainable growth during ambient periods of rainfall (Young et al. 2014b). In terms of green roof service provision, plant coverage is more important than plant diversity, however a greater range of plant species and structural forms has been shown to provide greater service provision as well as being more resilient and sustainable in the long term (Dunnett et al. 2008a; Lundholm et al. 2010; Nagase and Dunnett 2010; Cook-Patton and Bauerle 2012). Therefore future green roof design should try to vary the factors (depth, moisture, nutrition) that influence plant diversity in order to create more resilient as well as functionally diverse green roofs (Dunnett et al. 2008a; Bates et al. 2013; Rumble and Gange 2013).

This is one of the first empirical studies to look at natural AMF inoculation of established green roofs and showed the presence of AMF of all three roofs. Despite not showing any significant Site variability, AMF infection rates were significantly higher on Sharrow and lower on AWEC. Rumble and Gange (2013) detected the presence of AMF in *Sedum kamtschaticum* with infection rates of 49% on two extensive green roofs of depth 75mm (80% crushed brick, 20% commercial compost). It is surprising to find the presence of AMF on AWEC due to the shallow depth of the substrate, however the ground cultivation process of *Sedum* mats should give plenty of opportunity for AMF to colonise the substrate. A study on the natural AMF levels in green roof substrate has shown that AMF can be present in commercial substrate from the day of installation (McGuire et al. 2013). The only likely way that AMF would arrive on a roof after installation would be via spores which can be transported by the wind, water and animals, although this requires local sources of AMF inoculum (McGuire et al. 2013). Chapter 4 has shown that AMF can also be introduced via commercial inoculum, which in turn can improve plant P uptake and potentially flowering length and establishment success (Young et al. 2014c).

Leaf litter decomposition rates are strongly determined by moisture, temperature, and the presence of soil invertebrates (Facelli and Pickett 1991). In this study mean substrate temperatures generally did not differ by a large amount between sites (1-1.5^{o}C), whilst moisture levels on the day measured differed slightly more (5-10% not accounting for AWEC Lower), but without data for a longer time period it is hard to conclude whether moisture levels were constant throughout the study. Similarly *Collembola* and possibly AMF numbers, which both contribute to litter decomposition (Moore et al. 1988; Hodge et al. 2001), were consistent across all sites. However

worm numbers which did significantly differ between sites may not have contributed to any decomposition as it was likely that they could not access litter through the mesh.

Greater amounts of microbial biomass were detected in Sharrow and AWEC than SITran, most likely due to a greater availability of organic matter. Soil respiration is heavily influenced by moisture changes (Sparling 1997). This may explain the very high values in AWEC Lower as these samples had very high moisture levels and therefore were much moister than other Sites when measured. Microbial biomass has previously been measured on green roof trial plots with commercial substrate amended with a microbial 'compost tea' (Molineux et al. 2014). Plots amended with the compost tea showed significantly greater microbial biomass after one year (0.8mg $100g^{-1}$ dry substrate) than those not amended (0.4mg $100g^{-1}$ dry substrate) (Molineux et al. 2014). These values were significantly lower than those measured in this trial (5-40mg $100g^{-1}$ dry substrate), although this may be due to the different measurement technique (Phospholipid Fatty Acid Analysis) used in the (Molineux et al. 2014)) trial or due to lower levels of organic matter. In addition freshly mixed green roof substrate (crushed brick and compost) has showed some microbial activity, although substrates that contained soil instead of crushed brick showed much greater activity (Ondoño et al. 2014).

5.5.3 Chemical

Substrate pH was fairly consistent intra-roof but significantly differed inter-roof suggesting that for relatively young roofs composition of the initial substrate is the biggest driver of pH variability. However the values found on each roof were all quite alkaline, especially Sharrow at around 8.7. Previous studies have found much lower pH values on 20 year old extensive German roofs (5.2-7.2) (Thuring and Dunnett

2014), 3 year old Swedish extensive demo plots (6.9-7.1) (Emilsson 2008), 6-12 year old extensive German roofs (5.3-6.7) (Schrader and Böning 2006) and 3-8 year old extensive German roofs (5.8-7.6) (Buttschardt 2001) cited in Schrader and Böning (2006). Only one study has shown similar high substrate pH of 7-9 in crushed brick substrate, however these values declined over 7 years to 5.8-7.7 (Jauch and Fischer 2000 cited in Thuring and Dunnett 2014). Other studies have also shown that the older a green roof, generally the lower it's substrate pH in relation to similar roofs (Schrader and Böning 2006; Thuring and Dunnett 2014). One possible explanation for the high pH experienced on Sharrow is the use of crushed waste brick which has previously been found to have high pH values of 9.7 (Molineux et al. 2009).

The availability of P was clearly linked to the amount and quality of organic matter in the substrate. The substrate installed on Sharrow was hand mixed on site and contained roughly 20% green waste compost and therefore was likely to be highly nutritious (Sorrill 2013). The amount of P found in Sharrow substrates was similar to that found by (Emilsson 2008)) on 3 year old trial plots of different substrates (2-10% organic matter) which had been given supplementary fertilization throughout the trial (Emilsson 2008). AWEC and SITraN have both not received any additional fertilization since installation which in addition to their low initial amounts of organic matter may account for their low P values.

The availability of N was much more varied intra-roof, however was similar to previous studies (Emilsson 2008). Higher levels of available N on Sharrow is probably due to higher rates of nitrification, although Sharrow also experienced relatively high immobilisation of N which resulted in low/negative mineralisation. Similarly all SITraN sites experienced negative mineralisation which you would expect to reduce the amount of available N but SITraN Mid and Wet also had relatively high amounts

of available N. Temperature strongly influences N cycling (Rustad et al. 2001) and seems to have had some influence with warmer sites showing greater plant available N as well as nitrification rates. The negative mineralisation rates shown by most sites does not necessary result in a lack of available N for plants as shown by the plant available N amounts, especially in Sharrow sites. Modern views on the N cycle stress the importance of not just focusing on mineralisation as a source of plant available N as the amount of depolymerisation of N containing compounds is likely to also be important (Schimel and Bennett 2004).

It must also be remembered that these N concentrations and mineralisation rates only refer to the roofs during autumn 2013 and a much longer sampling regime is needed in order to from more concrete conclusions about N cycling on these green roofs.

5.5.4 Cluster Analysis

Cluster analysis confirmed strong similarities between sites located on the same roof. AWEC roofs were always strongly clustered due to their shallow depth, high moisture and WHC, low nutrient levels, low species diversity and high amounts of bare ground/moss. The type of green roof on AWEC is designed to have low levels of nutrients and species diversity in order to reduce maintenance and prevent colonisation by undesirable species (Dunnett and Kingsbury 2010). However despite being installed with 100% plant coverage (pre-grown *Sedum* mats), roofs like this generally show patchiness relatively quickly and can become dominated by bare ground/moss (Sorrill 2013). A likely explanation for this is the extremely shallow substrate depth (40mm) on which even *Sedum spp.* struggle to survive long term. With large areas of bare substrate/moss and poor plant growth, the level of green roof service provision

by AWEC will be minimal (Cook-Patton and Bauerle 2012). In addition the lack of heterogeneity in substrate depth/ planting regime means that the roof is very vulnerable to extreme climatic conditions, pests and disease (Cook-Patton and Bauerle 2012; Bates et al. 2013; Heim and Lundholm 2014b).

Despite their greater depth and species diversity SITraN substrates were more similar to AWEC due their low nutrient levels, organic matter and high amounts of bare ground/moss. Substrate depth was similar to Sharrow and thus it seems that substrate quality is the main driver for this green roof. The main influencing factor influencing substrate quality seems to be the low levels of organic matter which has never allowed significant plant growth, litter build up and therefore substrate development. This roof also experienced initial installation problems with plug plants which experienced high levels of mortality, most likely due to the poor substrate quality and the use of inappropriate plugs (Sorrill 2013). Despite this SITraN still showed good species diversity, but also had relatively high levels of bare ground/moss indicating that although the harsh conditions encourages species diversity, it also prevents high plant coverage.

Sharrow sites were split into two clusters, with Sharrow Wet and Shallow being much shallower and having lower nutrient and organic matter levels, but greater species diversity than Sharrow Deep and Dry. The range in depths and organic matter/nutrient levels across Sharrow seem to have encouraged greater species diversity but critically also reduced the amount of bare ground/moss. This indicates that Sharrow as well as being productive is also diverse and therefore potentially more resilient and able to provide a greater level of green roofs services (Dunnett et al. 2008a; Lundholm et al. 2010; Cook-Patton and Bauerle 2012; Blanusa et al. 2013).

5.5.5 SHI

The ultimate aim of this study was to measure an extensive list of commonly used soil health index variables and determine (a) if they varied significantly between relatively similar green roofs, (b) which ones are most suited for the development of a green roof SHI.

When choosing variables for use in a SHI a balance must be struck between the variance of the substrate explained by each variable as well as the appropriateness of taking such a measurement on a green roof (Andrews and Karlen 2004). It must also be taken into consideration that the measurements may be taken by an individual who does not have access to high quality laboratory facilities. The variability explained, appropriateness, necessity and overall suitability of the variables used in this study are summarised in Table 5.7. Using the formula in the Total Appropriateness for SHI Score column (Appropriateness*Necessity) a number of variables have been highlighted as particularly suitable to form the basis of a minimum data set of a future SHI due to the relative ease of performing them as well as their importance at influencing green roof substrate. These are summarised in Table 5.8.

As this study only sampled three extensive/semi-intensive green roofs in a single UK city (Sheffield during one month) it would be inappropriate to discuss potential guideline levels of the variables in Table 5.8. Large scale sampling of green roofs is needed in order to build up a baseline data set of these variables. Only one such large scale data set for green roofs has previously been created, with 115 roofs in France surveyed for plant diversity, arthropods, substrate depth, roof management and surrounding habitat (Madre et al. 2013; Madre et al. 2014).

For a SHI to be effective, the expected final management goal of the index must be clearly defined. For the majority of green roofs it is expected that most management goals will be to increase the provision of green roof services (for example; increased cooling, storm water retention, biodiversity, aesthetic appeal). Increased green roof provision usually happens as a result of increased plant growth and diversity of plant species and growth forms (Dunnett et al. 2008a; Lundholm et al. 2010; Cook-Patton and Bauerle 2012; Blanusa et al. 2013). However this must be done sustainably, with plants able to survive in the long term and not made more vulnerable to climatic extremes through excessive growth. Clearly some extreme climatic events will result in plant mortality, but substrates can be designed/modified to increase plant resilience (Bates et al. 2013; Farrell et al. 2013; Cao et al. 2014; Savi and Marin 2014) .

Table 5.7: Overview of all the variables measured in this trial. Statistical significances of P-values: * p<0.01, **p<0.001, *** p<0.0001. Statistical significances were calculated from 2-way nested ANOVA. Ease of Measurement Score values are as follows; 1=Hard, advanced laboratory equipment needed, 2= Difficult without some advanced laboratory equipment, 3= Some laboratory equipment needed, 4= Can done in the field with suitable equipment, 5= Extremely easy, can be done in the field with minimal equipment. Necessity Score values are as follows; 1= Not really needed, 2= Could be useful, 3= Should be obtained if possible, 4= Extra effort should be made to obtain, 5= Vital. Total Appropriateness for SHI Score values are as follows; 1-4= Not needed for SHI, 5-8= Not appropriate in most circumstances, 9-15=Should be used in SHI, 16-25= Vital for SHI.

Type of Variable	Variable	Roof Variability	Site Variability	Notes on Measurement Technique	Ease of Measurement Score (E) (1-5)	Necessity Score (N) (1-5)	Total Appropriateness for SHI Score (=E*N) (1-25)	Additional Notes
Physical-FLL	Permeability	***	*	Only very basic lab equipment required. All part of FLL process.	4	2	8	Can affect water dynamics.
	Air Dried Density	***	***	Only very basic lab equipment required. All part of FLL process.	4	1	4	Only useful for initial design of roof.
	Oven Dried Density	***	0.05	Only very basic lab equipment required. All part of FLL process.	4	1	4	Only useful for initial design of roof.
	Saturated Density	***	0.33	Only very basic lab equipment required. All part of FLL process.	4	1	4	Only useful for initial design of roof.
	Porosity	***	*	Only very basic lab equipment required. All part of FLL process.	4	2	8	Can affect water dynamics and plant growth. Likely to change over time.
	Air Content at Water Content Max	***	*	Only very basic lab equipment required.	4	1	4	

				All part of FLL process.				
	Water Holding Capacity	***	*	Only very basic lab equipment required. All part of FLL process.	4	3	12	Can affect water dynamics and storm water retention service provision.
	Organic Matter	***	***	Advanced lab equipment needed (550^{o}C furnace).	3	5	15	Vital for understanding substrate development and health.
Physical	Depth	***	***	Extremely easy to measure in field.	5	5	25	Vital for understanding intra-roof substrate development and health.
	Moisture Levels	***	***	Extremely easy to measure in field with correct kit.	4	4	16	Vital for understanding intra-roof variation in substrate development.
	Particle Size Distribution	***	0.91	Easy to measure in lab with basic equipment.	4	3	12	Can affect water dynamics.
	Temperature Regime (Below Ground)	Mean:*** Max:** Min:*** Range:***	Mean:*** Max:0.11 Min:*** Range:***	Relatively easy and cheap to measure crudely in field with remote loggers.	4	3	12	Likely to affect biological variables in substrate. Will be highly seasonal.
	Temperature Regime (Above Ground)	Mean:*** Max:0.07 Min:*** Range:0.05	Mean:0.77 Max:0.19 Min:* Range:0.05	Relatively easy and cheap to measure crudely in field with remote loggers.	4	1	4	May be more important in Summer months to assess cooling ability. Hard and expensive to measure accurately.
Chemical	pH	***	0.32	Easy to measure in laboratory.	4	2	8	Most roofs will be consistent but extreme values sometime occur.
	Plant Available P	***	*	Relatively time consuming and laboratory intensive. Relatively expensive to process externally.	2	5	10	Will need multiple measurements over the year to understand seasonal dynamics.

	Plant Available N	***	***	Relatively time consuming and laboratory intensive. Relatively expensive to process externally.	2	5	10	Will need multiple measurements over the year to understand seasonal dynamics.
	Nitrification Rates	***	*	Relatively time consuming and laboratory intensive. Relatively expensive to process externally.	2	3	6	Will need multiple measurements over the year to understand seasonal dynamics. Results can be misleading.
	Ammonification Rates	***	***	Relatively time consuming and laboratory intensive. Relatively expensive to process externally.	2	3	6	Will need multiple measurements over the year to understand seasonal dynamics. Results can be misleading.
	Net Mineralisation	***	***	Relatively time consuming and laboratory intensive. Relatively expensive to process externally.	2	3	6	Will need multiple measurements over the year to understand seasonal dynamics. Results can be misleading.
Biological	Worm Density	0.15	**	Extremely easy to measure in field.	5	4	20	Additional measurements ie size/species could be incorporated. The presence /absence of worms is an extremely easy method of quickly establishing the health of a roof.
	Collembola Density	0.78	0.24	Relatively easy to measure in field.	4	4	16	*Collembola* are probably present on most green roofs (Buttschardt 2001;

							Schrader and Böning 2006; Rumble and Gange 2013), but it less clear how substrate characteristics (other than moisture) affect their density.
AMF Presence	**	0.51	Some fairly advanced lab equipment and chemicals needed.	2	4	8	Presence of AMF is too expected on established green roofs. Benefits of AMF depend on plant species present on roof (i.e. most *Sedum* do not form AMF relationships).
Litter Decomposition	0.45	0.28	Basic lab equipment needed.	4	2	8	Will need multiple measurements over the year to understand seasonal dynamics.
Microbial Biomass	***	**	Some fairly advanced lab equipment needed.	2	3	6	More work needs to be done in order to determine what are acceptable values.
Plant Diversity-Diversity Index	***	0.07	Some knowledge of plant ID needed.	4	4	16	Low diversity isn't necessary a sign of a poor substrate e.g. Sharrow Deep.
Plant Diversity-No. of Species	***	***	Some knowledge of plant ID needed.	4	5	20	Low diversity isn't necessary a sign of a poor substrate e.g. Sharrow Deep.
Bare Ground/Moss	***	***	Extremely easy to measure in field.	5	5	25	Excellent sign of poor substrate health.

Table 5.8: Most appropriate variables (Minimum Data Set) for use in a potential SHI for green roofs.

Type of Variable	Variable	Location of Measurement	Notes;
Physical	Depth	Roof	Could be used as basis to judge the other variable values. For example with very low substrate depth it is not appropriate to expect high nutrient levels or plant diversity/coverage.
	Organic Matter Content	Laboratory	Can be done externally if a 550^{o}C oven is not available.
	WHC	Laboratory	Can be done as part of a wider FLL test.
	Moisture	Roof	Should be measured on a number of occasions to fully understand roof water dynamics.
	Particle Size Distribution	Laboratory	Can be done with sieves.
	Temperature Regime	Roof	Should be measured over a significant time period and in different seasons if possible. Relatively low accuracy temperature loggers can be used (±0.5^{o}C).
Chemical	pH	Roof/Laboratory	One off measurement needed.
	Plant Available P	Laboratory	Should be measured a number of times over the course of a year to understand seasonal dynamics.
	Plant Available N	Laboratory	Should be measured a number of times over the course of a year to understand seasonal dynamics.
Biological	Worm Density	Roof	Should be measured a number of times over the course of a year to understand seasonal dynamics. Size and species data could also be recorded.
	Collembola Density	Laboratory	Should be measured a number of times over the course of a year to understand seasonal dynamics.
	Plant Diversity (a) Diversity Index (b) Number of Species	Roof	Management of roof and initial planting diversity should be taken into account as well.
	Bare Ground/Moss Coverage	Roof	

This study has also highlighted the need for green roof substrate variables to be measured accurately over a period of time including data from the date of installation. Green roof substrates are dynamic and should be expected to change over time, Previous studies have demonstrated that older substrates can show increased porosity (Getter et al. 2007; Köhler and Poll 2010), increased organic matter (Köhler and Poll 2010; Thuring and Dunnett 2014), decreased depth/increased compaction (Thuring and Dunnett 2014), decreased pH (Liesecke 2006; Schrader and Böning 2006; Thuring and Dunnett 2014), variable plant communities (Dunnett et al. 2008b; Rowe et al. 2012; Bates et al. 2013; Nagase et al. 2013) and variable *Collembola* populations (Rumble and Gange 2013). Expecting green roof substrate to maintain its initial characteristics (e.g. FLL guidelines) is clearly unrealistic and a SHI must either take this dynamic nature of substrate into account or be measured over time as a tool for monitoring the changing health of the substrate.

5.6 Conclusions

This study has highlighted the need to take measurements of a range of green roof substrate variables.

Initial results suggest that, (a) initial substrate composition and characteristics can have long term effects on the development of the substrate as well the vegetation, (b) topographical or structural variations can alter substrate characteristics intra-roof and (c) subtle differences in substrate between sites on the same roof can have large impacts on vegetation.

Clearly much more work is needed in order to establish a baseline data set for established green roof substrate that can then be referred to when making future management decisions as part of a SHI. Regular sampling of the same green roofs is also needed to fully understand the seasonal as well as long term dynamics of green roof substrate, how it is expected to change over time and how management can influence this. However the variables listened in Table 5.8 are good candidates to provide the basis of a minimum data set of measured variables for a future green roof SHI.

Chapter 6

General Discussion, Applications of Research and Future Directions

6.1 General Overview

Whilst the amount of green roof research has increased significantly in the last 5 years (Table 6.1), the critical role that substrate plays in determining plant performance and physiological health has still been relatively understudied. In addition the biological characteristics of established substrate have also been relatively ignored, despite the vital role that they play in influencing substrate physical and chemical characteristics and ultimately the green roof plant community (Wardle et al. 2004; Rumble and Gange 2013). This chapter synthesises what is currently known about the critical role that green role substrate plays in influencing green roof plants and incorporates advances made in this thesis.

6.2 Green Roof Substrate-Plant Research

Early green roof substrate research in Germany in the 1970's focused on defining optimal physical characteristics of substrate in regards to weight loading, water retention and physical stability. This led to the creation of the FLL guidelines (FLL 2008) and subsequently UK guidelines (GRO 2011). Both of these documents act as guidelines for practitioners/green roof installers and focus on optimal physical characteristics (including organic/nutrient levels of substrate) of substrate when it is installed. However they provide very little information on

substrate development over time, effect of substrate on plant growth and performance as well as optimal biological characteristics of substrate.

In order to develop products many green roof companies perform their own research into substrate design, and effect on plant growth and performance (Sorrill 2013). Often this research is not publically available as companies have invested significant resources into it.

Of published material, the majority of recent research has focused on the role of substrate depth on plant growth and performance (Table 6.1). Increased substrate depth generally improves plant growth and physiological performance due to the greater amount of water, nutrients and temperature buffering capacity of the substrate (Boivin et al. 2001; Dunnett et al. 2008b; Getter and Rowe 2009). As substrate depth on extensive green roofs is shallow, only a relatively small increase in depth is needed to have a significant impact on plants (VanWoert et al. 2005b; Rowe et al. 2012).

The study in Chapter 2 supports this by showing that increasing substrate depth from 80mm to 120mm increased *Lolium perenne* root biomass, root:shoot ratio, chlorophyll content and substrate WHC and evapotranspiration (Young et al. 2014a). However, shoot biomass was not increased by depth in this study, therefore opposing previous studies, highlighting the point that additional plant measurements other than shoot biomass must be taken if the full effect of substrate composition on plants is to be fully understood.

Chapter 2 also focused on the effect of modifying individual substrate components on plant growth and physiological health. Although the number of studies on this topic has increased in the last 3 years, they often have only compared contrasting substrates (Emilsson 2008; MacIvor et al. 2013; Razzaghmanesh et al. 2014b) or altered one substrate component in order to find optimal levels (Nagase and Dunnett 2011; Olszewski and Young 2011; Graceson et al. 2014a). Whilst these studies have been extremely useful in determining recommended levels of certain

components e.g. organic matter (Nagase and Dunnett 2011) or physical characteristics e.g. WHC (Graceson et al. 2014a) they do not clarify how substrate components interact with one another. In addition many of these studies only used shoot biomass/plant coverage as indicators of plant performance, while other measurements such as root biomass and physiological performance have been neglected. Although shoot growth/plant coverage is vital for green roof service provision, luxuriant shoot growth can be unsustainable and reduce plant tolerance to drought (Nagase and Dunnett 2011). Chapter 2 is one of the first studies to investigate the effect of different substrate components on a range of plant growth variables (shoot, root, root:shoot) as well as physiological health variables (chlorophyll concentration, shoot nitrogen concentration) (Young et al. 2014a). By showing that substrates components can affect a wide range of plant growth and physiological variables, this study will hopefully encourage future studies to utilise a similar range of measurements. In addition this study has advanced the field by looking at substrate components in combination with one another in order to determine optimal substrate mixes for specific growth patterns e.g. optimal plant growth in different climatic regions (Young et al. 2014a).

Chapter 3 expanded on this topic by investigating how the substrate components used in Chapter 2 affected plant drought tolerance (Young et al. 2014b). It was shown that plant drought tolerance can be increased in two ways:

a) Use of courser substrates (larger brick particles) which promote slower and more drought resistant plant growth during ambient watering conditions.

b) Use of substrate amendments (water absorbent gel) to increase substrate WHC without increasing plant growth during ambient watering conditions (as opposed to smaller brick particles).

Water absorbent gels (e.g. hydrogel, SwellGel) have previously been shown to increase the WHC of substrates, available plant water and therefore plant growth or tolerance to drought (Sutton 2008; Olszewski et al. 2010; Savi and Marin 2014) (Table 6.1). However the life span and effectiveness of water absorbent gels in comparison with other water retention additives (e.g. silicate powder) has been questioned (Farrell et al. 2013; Savi and Marin 2014). Therefore much more work is needed in this area, in particular long term studies, before the widespread use of water absorbent gels is recommended on green roofs.

Chapter 3 disagreed with the results of previous trials which have shown that increasing substrate WHC through the use of smaller sized mineral components leads to greater plant drought tolerance (Farrell et al. 2012) (Table 6.1). However plants in this trial generally did not experience significantly greater growth in substrates with high WHC before the drought (Farrell et al. 2012), which was not the case in Chapter 3. Therefore if the WHC of a substrate can be increased without promoting excessive plant growth during ambient watering conditions, plant drought tolerance should also increase.

Chapter 3 also came to a different conclusion regarding the positive effect of 'nurse' plants on drought tolerance and substrate temperature than previous studies on roofs by showing that the presence of *Sedum spp.* did not improve companion plant drought tolerance (Butler and Orians 2011; Heim et al. 2014; Heim and Lundholm 2014a; Young et al. 2014b). However a much lower temperature range and solar intensity was experienced in the trial in Chapter 3 due to its greenhouse location, and therefore complementary planting designs may still provide a cost effective method of increasing green roof species diversity/resilience and should be investigated further (Heim and Lundholm 2014a).

Chapter 4 investigated the viability of artificially introducing AMF to green roof substrate and any effect this may have upon plant growth and physiological health (Young et al. 2014c). This

area of research has previously received very little attention despite the large range of benefits that AMF could bring to green roof vegetation (Molineux et al. 2014). Chapter 4 has shown that it is possible to introduce AMF networks into green roof substrate though the use of commercial inoculum. This can improve plant phosphorus uptake and potentially flowering performance (Young et al. 2014c). However no effect upon plant growth was observed, in contradiction to the few other studies which have used AMF inoculum in green roof substrate (Meyer 2004; Sutton 2008). AMF inoculum is currently added by a number of green roof substrate providers to substrate mixes. Chapter 4 has shown that this method of applying inoculum to the substrate is inefficient and a much more effective method will be to add the inoculum directly to plug plants, or to grow pre-inoculated plugs (Young et al. 2014c).

Chapter 5 proposes a new method for evaluating the 'health' of green roof substrate. As opposed to traditional FLL recommendations that focus on a narrow range of physical attributes, this new method measures a number of chemical, biological and physical substrate variables in order to holistically assess the health/performance of a green roof. It is envisaged that this 'Substrate Health Index' (SHI) tool will predominately be used on existing roofs in order to direct roof management since the time needed for biological health to build up means it is less useful for new installations.

There has been a lack of large scale sampling of established green roofs and therefore only a small data set exists (Table 6.2). A larger body of German data on established green roof exists, however this is mostly un-translated or unavailable (Schrader and Böning 2006; Köhler and Poll 2010; Thuring and Dunnett 2014). The largest study of established green roofs to date looked at the effect of substrate depth and roof management on plant composition and proposed a new plant community classification system for green roofs but did not assess substrate qualities (Madre et al. 2014). Studies on old established German green roofs has highlighted the dynamic nature of substrate and it's tendency to show higher organic matter levels, lower

pH, shallower depth and lower species diversity on older roofs (Schrader and Böning 2006; Köhler and Poll 2010; Thuring and Dunnett 2014). In addition smaller scale studies have documented population fluctuations of *Collembola* spp. in response to moisture availability (Rumble and Gange 2013) and the presence of AMF and microbial populations in green roof substrate (McGuire et al. 2013; Rumble and Gange 2013; Ondoño et al. 2014). However no study has attempted to link physical, chemical and biological characteristics of substrates together in order to gain a holistic overview of green roof substrate which Chapter 5 attempts to do. Clearly much more work is needed in order to fully develop a usable SHI. However Chapter 5 has outlined the need for and determined the basis of a future SHI for green roof substrate.

Table 6.1: Summary of all the known studies that have looked at the effect of green roof substrate on plant growth and physiological health. Relevant work from this thesis has been included.

Study	Duration	Substrate Composition	Substrate Depth	Other Details	Plant/Substrate Response
Substrate Characteristics-Plant Growth					
(Rowe et al. 2006a) Module experiment	3 years	1. 50-100% heat expanded slate, 0-25% sand, 0-10% peat, 0-5% compost. 2. 60 % heated expanded slate. 0-150g m^{-2} slow release fertilizer.	100mm	Natural rainfall + additional irrigation.	1. Higher levels of slate= lower plant (2 *Sedum* spp. 6 non succulents) growth and visual rating. 2. Lower fertilization=lower growth but greater drought tolerance of non succulent plants.
(Emilsson 2008) Newly installed roof /plot sampling	3 years	1. Commercial substrate (contains soil, lava, organic matter) 2. 60% Crushed roof tiles, 37% sand, 3% organic matter 3. 53% Crushed roof tiles, 37% sand, 10% organic matter Slow release fertilizer 15g m^{-2} added.	40mm		Greater amounts of nutrients available in commercial substrate increased succulent spp. biomass and growth.
(Olszewski et al. 2010) Module experiment	9weeks	30% heated expanded fine slate, 50-70% heat expanded course slate, 0-20% compost. Hydrogel added at 0, 0.75, 1.5 & 3.75lb $yard^{-3}$ and slow release fertilizer at 6lb $yard^{-3}$.	'Shallow'	Watered every 10days	Hydrogel increased porosity and WHC. Higher hydrogel and compost increased shoot biomass and coverage of two *Sedum* spp.
(Olszewski and Young 2011) Plot experiment	12 weeks	Heat expanded clay at 10-60% fine grade, 10-60% medium grade, 10% course grade, 20% compost. Slow release fertilizer at 3.56kg m^{-3}	64mm	Natural rainfall + additional irrigation.	Fine grade particles= higher bulk density, WHC and lower porosity. *Sedum* spp.=greater growth & biomass at intermediate levels of particle sizes *Dianthus* spp. =greater growth & biomass at high fine particle levels.
(Nagase and Dunnett 2011) Module experiment	14weeks	Commercial mix (crushed brick base). Organic matter added at 0%, 10%, 25% & 50%.	80mm	Two watering regimes (every 5 or 15 days)	4 contrasting green roof plant species. Optimal level for growth was 10% 5day watering + high organic=excessive growth.
(Bates et al. 2013) Newly installed roof	4 years	97-100% broken brick, concrete & sand (at a variety of coarseness), 0-3% organic matter. Compost mulch added to some areas.	40-120mm		Plants growing in courser and less fertile substrates showed less growth but greater drought tolerance.
(MacIvor et al. 2013) Module experiment	2 years	Organic media= 25% organic matter FLL media= 70% mineral, 25% organic, 5% sand.	100-150mm	Some modules received additional watering	Grass/forb mix of 16 grasses/forbs. *Sedum* mats contained 28 *Sedum* spp. Plant cover & biomass lower for all species in FLL substrate. Irrigated modules had greater plant diversity.

(Zheng and Clarke 2013) Greenhouse experiment	6 weeks	80% Sphagnum peat, 20% perlite. 4.5-7.5 pH range 0.67g N L^{-1} slow release fertilizer.	Unknown		Species specific response of biomass production to pH levels by *Sedum* spp. Optimum levels varied between 5.91-6.43.
(Graceson et al. 2014a) Module experiment	2 years	Factorial design of 6 substrates composed of 70-80% mineral (crushed brick, tile or Lytag) and 20-30% green waste compost.	150mm		Increased WHC and compost amount increased shoot biomass.
(Razzaghmanesh et al. 2014b) Module experiment	12 months	A= crushed brick, scoria, coir & compost B= scoria, pine bark & Hydrocell® flakes	100mm & 300mm	Additional watering given.	Substrate type had little effect on growth and survival of 4 Australian species.
(Razzaghmanesh et al. 2014a) Module experiment	12 months	A= crushed brick, scoria, coir & compost B= scoria, pine bark & Hydrocell® flakes C= 50% of substrate B, 50% compost	100mm & 300mm	Additional watering given.	Poor plant growth in substrate A but good plant growth in substrates B & C.
(Young et al. 2014a) Young Thesis 2014, Chapter 2 Greenhouse experiment		80% mineral, 20% organic. Factorial design of a) brick size (small vs. large), b) organic matter (green waste vs. bark), c) hydrogel (presence vs. absence).	80 & 120mm	Watering regime given.	*Lolim perenne* used as phytometer species. Large brick=lower WHC& shoot but higher root growth Green waste=greater shoot growth, chlorophyll and N content but lower Root:Shoot ratio Hydrogel=greater WHC, shoot growth and N content
Substrate Components & Amendments- Drought Tolerance					
(Sutton 2008) Plot experiment	4 months	95% mineral, 5% compost. Factorial design of just substrate, AMF inoculum & Hydrogel addition (1.2g l^{-1})	90mm		6 grasses, 1 sedge, 5 forbs. Greater plant growth with hydrogel and AMF. AMF only increased plant growth when present with hydrogel.
(Nektarios et al. 2011) Plot experiment	6 months	1. Pumice 50%, perlite 20%, compost 20%, zeolite 10%. 2. Pumice 40%, perlite 20%, compost 20%, zeolite 50%, soil 15%. Slow release fertilizer 6g m^{-2}	75mm & 150mm	2 x watering regimes (high vs low).	*Dianthus fruticosus* planted. Presence of soil in substrate increased WHC and available water throughout trial. Greater growth and chlorophyll content in 150mm substrate.
(Farrell et al. 2012) Greenhouse experiment	113 days	80% mineral components (scoria, crushed roof tiles, bottom ash from power plants) & 20% coir. Slow release fertiliser added.	160mm	Drought treatment vs. Watered once a week	5 succulent species planted. Substrates with greater WHC showed greater plant survival to drought. Lower biomass production increased drought survival.
(Farrell et al. 2013) Greenhouse experiment	2 months	1. 80% scoria, 20% coir. 2. 80% crushed roof tiles, 20% coir Factorial design with a) hydrogel b) silicon based water retention additive. 53g L^{-1} slow release fertilizer.	120mm	45 days watering then drought	Both additives improved substrate WHC. Silicate additive increased drought tolerance of two plant species whilst hydrogel had no effect. Some effect of substrate type on effectiveness of additive.

(Savi et al. 2013) Module experiment	6 months	96.2% mineral, 3.8% organic matter. A=Substrate, B=A + drainage layer, C=B+ water retention mat, D=C+ number of drainage holes doubled	140mm	Additional watering given.	Water retention mat improved growth, water status and drought survival of *Salvia officinalis.* Increasing number of drainage holes improved water movement back into substrate.
(Savi and Marin 2014) Module experiment	6 months	97.1% mineral, 2.9% organic matter. Hydrogel (0, 0.3 & 0.6%)	80-120mm	Additional watering given.	Hydrogel increased WHC, available water and water status of *Salvia officinalis*. Greater impact of hydrogel at 80mm.
(Young et al. 2014b) Young Thesis 2014, Chapter 3 Greenhouse experiment	4 months	80% crushed brick (small or large particles), 20% green waste compost. 2 x hydrogel treatments (0 vs. 1%). 2 x *Sedum* treatments (no coverage vs. substrate coverage)	120mm	Control, 10, 15, 25 day droughts. Plant grown for 3.5months before drought.	*Linaria vulgaris* & *Festuca ovina* planted. Hydrogel and large brick increased drought tolerance of both species. hydrogel increased available water without affecting plant growth whilst large brick reduced growth befor e drought.
(Young et al. 2014c) Thesis 2014, Chapter 4 Module experiment	14 months	80% crushed brick (small particle size), 20% green waste compost AMF inoculum treatments a) none, b) with plugs, c) in substrate, d) in plugs & substrate	100mm	Some additional watering given.	All AMF treatments infected *Prunella vulgaris* and increased shoot phosphorus concentrations. Plug only treatment increased flowering length at end of first growing season. No significant effect of AMF on plant growth or biomass.
Substrate Depth					
(Boivin et al. 2001) Module experiment	3 years	60% mineral components, 40% organic matter	50, 100 & 150mm		6 herbaceous perennials. Greater plant damage at 50mm from low temperatures.
(VanWoert et al. 2005b) Module experiment	88 days	40% expanded slate, 40% sand, 10 % peat, 5% dolomite, 3.33% composted yard waste, 1.67% composted poultry litter.	20 & 60mm	Watering regime every 2,7,14,28 & 88 days.	Larger amounts of biomass (*Sedum* spp.) and also transpiration at 60mm. Optimal watering regime at 20mm was every 14 days and at 60mm was every 28 days.
(Getter and Rowe 2008; Getter and Rowe 2009) Module experiment	20 weeks-4 years	86% sand, 10% silt, 4% clay. 100g m^{-2} slow release fertilizer.	40-100mm	Water retention layer used	Greater growth and coverage of *Sedum* spp. at 70 & 100mm.
(Dunnett et al. 2008b) Plot experiment	6 years	50% expanded clay, 15% medium load, 35% green waste compost. 75g m^{-2} slow release fertilizer.	100 & 200mm	Some additional watering given.	15 species initially planted. Greater survival, diversity, size and flowering performance observed at 200m. Greater amounts of bare ground/moss and colonising species at 100mm.
(Thuring et al. 2010) Module experiment	11 weeks	1. 85% expanded shale, 15% organic matter 2. 85% expanded clay, 15% organic matter	30, 60, 120mm	None, early & late drought	3 succulents & 2 herbaceous perennials. Better plant growth and survival in deeper substrates.
(Olly et al. 2011) Module experiment	20 weeks	66% expanded clay, 33% sand. 1cm topsoil (10% organic, 90% mineral).	100-150mm some with access to bare ground		Herbaceous seed mix used. Greater growth, flowering, ground cover and species richness at 150mm, especially in substrates with access to ground

(Rowe et al. 2012) Module experiment	7 years	40% expanded clay, 40% sand, 5% dolomite, 3.33% composted yard waste, 1.67% composted poultry litter.	25, 50 & 75mm		25 succulent species initially planted. Number of species present declined at all depths over time. Rate of decline was faster in shallower substrates. However stable communities still existed at 25mm depth after 7 years.
(Heim and Lundholm 2014b) Module experiment	1.5 years	Commercial mix. 7% organic matter.	50, 100, 150mm.50/150mm mixed depth.		*Sedum acre* and *Festuca rubra.* Mixed depth showed greater overall coverage and less competition.
Novel Substrate Materials					
(Molineux et al. 2009) Greenhouse experiment	2 months	75-85% mineral (crushed brick, clay pellets, paper ash pellets, carbonated quarry waste pellets). 15-25% top dressing compost	80mm	Watering regime given.	Compost amounts had different effects on *Plantago lanceolata* growth depending on mineral type. All mineral types suitable for use in green roof substrate.
(Mickovski et al. 2013) Module experiment	5 weeks	65% loam, 20% demolition waste, 15% compost	75mm	Watering regime given.	Grass mix and *Sedum* spp, planted. Demolition waste can be used as part in green roof substrate.
(Cao et al. 2014) Greenhouse experiment	2 months	1. 80% scoria, 20% coir. 2. 100% scoria. Biochar added at 0, 10, 20, 30, 40% v/v.	100mm	Watered for 50 days then drought	Biochar increased WHC, plant available water and time until permanent wilting. No effect on biomass.

Table 6.2: Summary of all the known available studies that have sampled established green roofs. Relevant work from this thesis has been included.

Study	Duration	Substrate Composition	Substrate Depth	Other Details	Plant/Substrate Response
Established Green Roof Sampling					
(Schrader and Böning 2006)	3-12 year old green roofs	Heated expanded clay and shale pellets (2-100mm diameter)	80mm	Survey of 10 established roofs in Germany	Older substrates had greater substrate carbon and nitrogen content, slightly higher *Collembola* levels and dehydrogenase activity.
(Köhler and Poll 2010)	20-100 year old green roofs		Mixture of modern extensive and old Tar Paper green roofs.	Survey of 21 established roofs in Germany.	70 colonising vascular plants indentified. Quality of vegetation affected by substrate depth and grade of sunlight exposition.
(Madre et al. 2013; Madre et al. 2014)			Mixture of extensive *Sedum*, semi-intensive & intensive roofs.	Survey of 115 established roofs in France.	176 colonising vascular plants identified. Plant community composition significantly affected by substrate depth, management, age and roof area.
(Thuring and Dunnett 2014)	20-33 year old green roofs	Typical extensive substrate. All adhered to FLL guidelines	60-80mm		Lower species diversity on roofs with higher organic and phosphorus content. These roofs were also generally the older roofs.
Young Thesis 2014, Chapter 5	7-8 year old roofs	Commercial mixes consisting of crushed brick, compost, heat expanded clay and sand	35-300mm	Survey of 3 established roofs in Sheffield, UK.	Lowest species diversity and plant coverage at 35-40mm. Highest species diversity at 80mm, although greater plant coverage at 250-300mm. Substrates varied in their physical, chemical and biological characteristics inter as well as intra-roof.

6.3 Application of Research

This PhD has been co-funded by Boningale Ltd. a plant nursery company which in conjunction with the University of Sheffield has launched a new green roof substrate and plant product line in the last 4 years. Therefore one aspect of this thesis has been to develop new products for Boningale and provide data to support their scientific basis.

There is a current lack of choice for customers when choosing green roof substrate. Companies typically have the choice of a few substrate blends that are designed for either extensive, semi-intensive or intensive green roofs. However there is no choice of substrate depending on a roofs climate, levels of expected management and desired function of the roof. The results from Chapters 2 & 3 were used to develop a new line of green roof substrates known as SkySuperstrates™ and to also inform the creation of a novel online substrate selector tool for Boningale (Fig. 6.1). This tool is designed to recommend substrate mixes and planting densities depending on the location and requirements of each individual customer. It also encourages customers to think about the reasons why they want a green roof and how much management on the roof they are willing to undertake. For example if a customer is located in a area with low rainfall then a substrate that encourages slow sustainable growth for drought tolerance and an appropriate planting density will be recommended (Fig. 6.2).

Despite strong evidence showing that SwellGel can improve the drought tolerance and physiological health of plants (Young et al. 2014b), SwellGel has yet to be incorporated into Boningale's SkySuperstrates™ line. This is due to logistical problems of mixing SwellGel into the substrate which must be done in dry conditions in order to prevent the gel from swelling and sticking together. Most green roof

substrate suppliers are large-scale aggregate suppliers who do not currently have the facilities to mix green roof substrate undercover and therefore cannot guarantee dry mixing (Sorrill 2013). An alternative would be to mix the SwellGel at the roof site, but this would be labour intensive and inefficient. This problem highlights the importance of collaborating with industry in applied ecological research. Substrate amendments can be tested and recommended by the research community, however until a relevant business investigates the logistics of using such an amendment on an industrial scale the research is likely to remain academic.

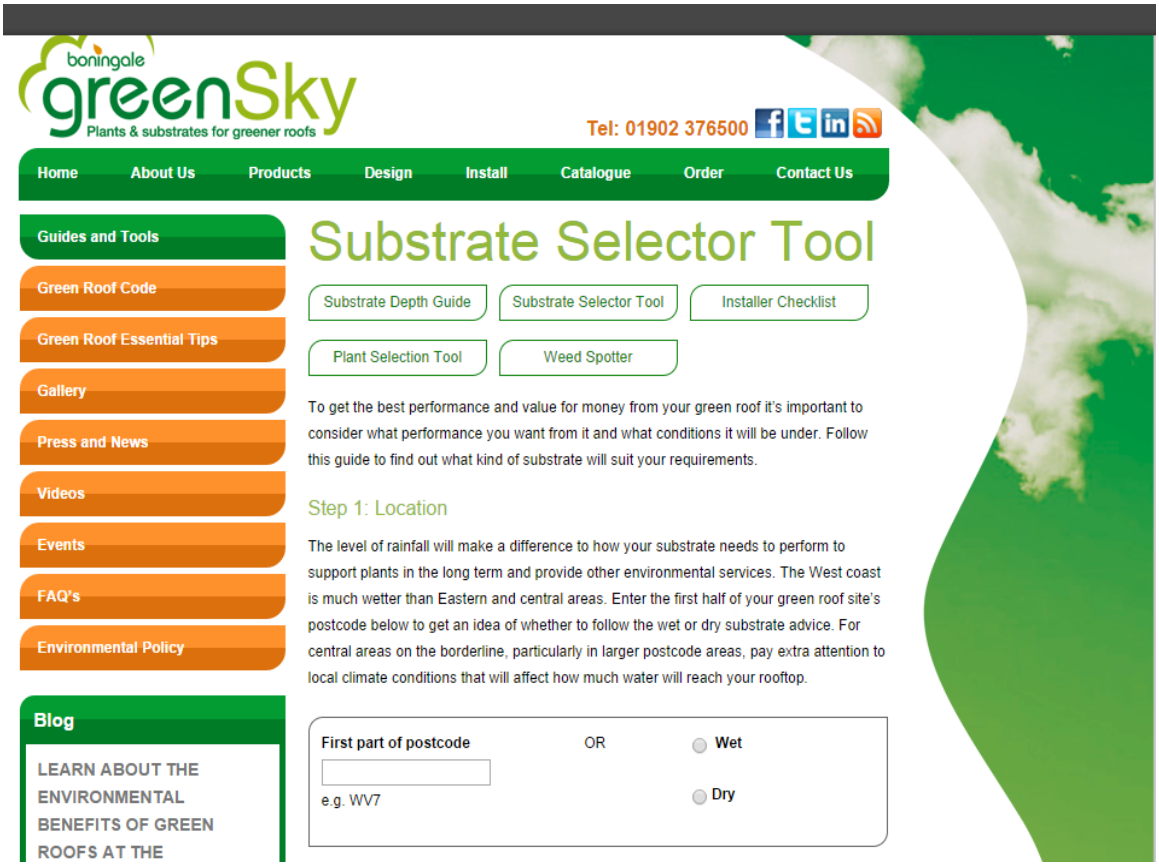


Figure 6.1: Screenshot of the Boningale GreenSky Substrate Selector Tool. Available at http://www.boningale-greensky.co.uk/guides-and-tools/substrate-selector-tool/

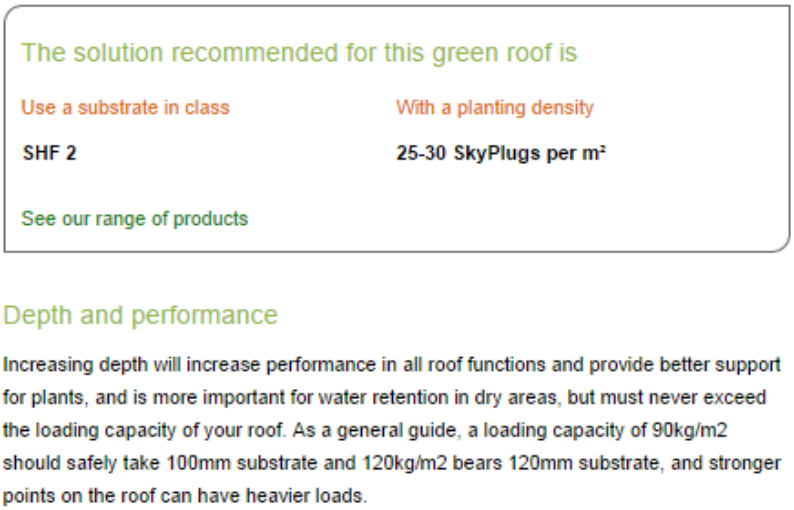


Figure 6.2: Screenshot of the recommended substrate mix and planting density provided by the Boningale GreenSky Substrate Selector Tool.
http://www.boningale-greensky.co.uk/guides-and-tools/substrate-selector-tool/

The novelty and success of the Boningale GreenSky Substrate Selector Tool has been recognised by the industry and has been awarded the title of 'Best Business Innovation' at the UK Growers Awards 2014.

The work in Chapter 4 is also currently being developed at Boningale to supplement their existing SkyPlugs™ line of green roof plug plants. SkyPlugs™ are designed to have improved establishment rates in green roof substrate as opposed to traditional peat plugs. The development of AMF pre-inoculated plugs would allow the efficient transfer of AMF inoculum onto green roofs which in turn would be much more cost effective than current methods of mixing AMF inoculum directly into substrate.

Finally the development of a green roof SHI as discussed in Chapter 5 could potentially provide a useful management tool for the industry. By setting recommended physical, chemical and biological substrate characteristics the quality of new green roof installations could be improved. In addition the tool could be used

to improve existing green roofs through changes in management practices. This solution is clearly much more desirable than replacing poor performing green roofs as it is much more cost effective and sustainable.

6.4 Future Research Directions

6.4.1 Substrate Components

Chapters 2 & 3 have shown that relatively minor changes in substrate composition (e.g. changing crushed brick particle size) can have major effects on plant growth, physiological health and drought tolerance. However a wide range of materials are currently used in green roof substrate (Ampin et al. 2010) and additional recycled materials are being proposed (Molineux et al. 2009; Mickovski et al. 2013; Cao et al. 2014). Due to this wide range of potential substrate components, additional testing is needed to understand how alterations to these components can affect plant growth, performance and green roof service provision.

In addition the effect of substrate components on a wider range of plant species is needed as Chapters 2 & 3 only used three different species. A wide range of plant functional and growth forms are used on green roofs (Dvorak and Volder 2010; MacIvor and Lundholm 2011b; Benvenuti 2014) and they will vary in their performance depending on substrate type and environmental conditions. It is therefore unwise to always make generalised conclusions from single species trials without realising that species will differ in their response to growing conditions.

Additional trials that look at the effect of a wider range of components on a selection of indicator species (i.e. range of growth forms/functional) should therefore be conducted to fully understand how altering substrate components can affect a range of green roof plants.

6.4.2 Water Retention Gels (SwellGel/Hydrogel)

Although Chapters 2 & 3 showed beneficial impacts of SwellGel on the growth and health of *L. perenne* and drought tolerance of *F. ovina* & *L. vulgaris*, much more work is needed to assess the viability of using such amendments in green roof substrate.

The longer term effectiveness of water retention gels has been questioned, with their WHC decreasing by between 25-50% after 5 months in green roof substrate (Savi and Marin 2014), between 5-40% after three wetting cycles in agricultural soils (Akhter et al. 2004) and nearly 90% in the biological restoration of slate waste after 18 months (Holliman et al. 2005). The degradation and subsequent loss of WHC is accelerated by UV light, freeze thaw cycles, elevated temperatures, microbial/fungal activity and repeated wetting/drying cycles (Smith et al. 1996; Smith et al. 1997; Holliman et al. 2005), all of which are present on green roofs (Oberndorfer et al. 2007). It may be possible that this decline in WHC will not significantly affect green roof plants as the gel will have been at its most effective when the plant is at its most vulnerable during establishment (Young et al. 2014b). Alternatively this decline in gel WHC may lead to greater plant vulnerability to drought in the long term if they have not developed sufficient root systems to deal with drought due to the presence of the gels (Young et al. 2014b). In addition, other substrate amendments may actually be more effective at improving plant drought tolerance (Farrell et al. 2013)

Clearly more research needed to asses long-term effectiveness of water retention gels, other substrate amendments as well as optimal application rates (Farrell et al. 2013; Savi and Marin 2014; Young et al. 2014b).

6.4.3 Long-Term Studies

This thesis has only conducted relatively short-term growth trials, with the longest at 14 months. The few long-term green roof trials that exist have highlighted the need for studies that last for longer than 2-12 months (Dunnett et al. 2008b; Rowe et al. 2012). Green roofs are deigned to have a life span of at least 50 years, with some German roofs now reaching 100 years old without need for replacement (Oberndorfer et al. 2007; Köhler and Poll 2010). Studies that show the initial effect of substrate on recently established plants are needed in order to develop optimal substrate mixes and design (Molineux et al. 2009; Graceson et al. 2014a; Young et al. 2014a). However without long-term studies the development of plant communities and substrate is unknown. The few long-term studies suggest that plant diversity will declines over time, however increased substrate depth can be used to slow this decline down and prevent invasive species from establishing (Dunnett et al. 2008b; Rowe et al. 2012). However the long-term effect of altering substrate components on plants is not known and more research is needed to fully optimise substrate design for long-term green roof performance.

6.4.4 Substrate Health Index

Chapter 5 has described the basis of a new SHI tool for green roofs. However due to the small number of roofs sampled and lack of baseline data this tool still requires a large amount of development before it can be used by industry. There is a lack of detailed data on established green roof substrate and therefore it is not known what optimal levels of various physical, chemical and biological variables are. In addition the development of green roofs over time is not fully appreciated. This viewpoint of green roofs as dynamic and changing systems is needed if they are to be managed in order to provide optimal plant growth and therefore optimal green roof service provision.

Therefore, large scale sampling of established green roofs, long term sampled of newly installed roofs and greater knowledge of the influence of plant diversity on green roof service provision is needed if a useful SHI tool is to be fully developed.

6.5 Conclusions

The overall aim of this thesis was to investigate the effect of green roof substrate composition on plant growth and physiological health. This was achieved through two greenhouse trials (Chapters 2 & 3), an outdoor green roof module trial (Chapter 4) and detailed sampling of established green roofs (Chapter 5).

It has been shown that altering green roof substrate components can have significant effects on plant growth, physiological performance and drought tolerance. Altering substrates to reduce plant growth during ambient watering periods is vital in order to increase drought tolerance. These findings suggest that the success of green roof

substrates should not be viewed solely in terms of the shoot growth of plants grown in ambient conditions, and additional measurements such as root biomass, root:shoot ratio, shoot nitrogen, chlorophyll content and fluorescence should also be used. This research has subsequently been used to develop a new line of substrates and an online substrate selector tool for the co-sponsor company Boningale.

It has been demonstrated that AMF networks can successfully be introduced into green roof substrate though the use of commercial inoculum. AMF infection did not affect plant growth but did increase leaf phosphorus levels and potentially the length of flowering time. This research is currently being applied by Boningale to develop pre-inoculated AMF plugs which could be used to as a method of introducing AMF to newly installed green roofs whilst ensuring newly planted plugs plants are also inoculated.

The basis of a new SHI tool for green roofs has also been described. This will take the form of a number of physical, chemical and biological substrate variables which are relatively easy to measure. Depending on the management goal of the roof, these variables are compared against other green roofs in order to assess how 'healthy' the substrate is. This tool could be used to tailor management for particular green roofs in order to improve the health of substrates and ultimately green roof vegetation.

Overall this research has demonstrated the need for applied ecological research into green roof substrates. In such an applied research area partnerships between University researchers and companies are vital if research is to be successfully implemented by industry.

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Appendix A

Papers and manuscripts arising from this thesis

The work in Chapter 2 has been published in Urban Forestry and Urban Greening.

Young, T., D. D. Cameron, J. Sorrill, T. Edwards, and G. K. Phoenix. 2014a. Importance of different components of green roof substrate on plant growth and physiological performance. *Urban Forestry & Urban Greening* 13: 507–516.

The work in Chapter 3 has been accepted by Urban Water for the special edition: Ecosystem Services Through Rooftop Runoff Management on 28th April 2014.

Young, T., D. D. Cameron, and G. K. Phoenix. 2014b. Optimizing green roof plant drought tolerance through the use of substrate amendments and novel planting methods. *Urban Water* In Press.

The work in Chapter 4 has been submitted for review to Urban Forestry and Urban Greening on 19th January 2014.

Young, T., D. D. Cameron, and G. K. Phoenix. 2014c. The use of mycorrhizal inoculum in assisting the initial establishment of Prunella vulgaris plug plants in green roof substrate. *Urban Forestry and Urban Greening* In Review.

The work is Chapter 5 is currently being prepared as a manuscript for Landscape and Urban Planning.

Young, T., D. D. Cameron, and G. K. Phoenix. 2014d. Moving towards an integrated substrate health index for green roof substrate. *Landscape and Urban Planning* In Preparation.