Quantifying the Soil Community on Green Roofs

A thesis submitted to the University of London for the degree of Doctor of Philosophy

By

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I hereby declare that the thesis, submitted in fulfilment of the requirements for the degree of Doctor of Philosophy and entitled ‘Quantifying the soil community on green roofs’, represents my own work and has not been previously submitted to this or any other institution for any degree, diploma or other qualification.

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Abstract

With the majority of people living in cities, innovative solutions for greening the urban environment are necessary to provide ecosystem services such as urban cooling and remediating habitat loss. Green roofs are one potential solution within green infrastructure.

Few studies have investigated whether green roofs are a good urban habitat, particularly for soil organisms. The soil food web is vital to above-ground ecosystem processes as it regulates nutrients and can alleviate drought stress, so could be an important but overlooked factor in green roof design. This is the first multi-season study to examine green roof soil organisms in detail, whilst tracking abiotic factors and plant cover.

The first part of this thesis characterises the microarthropod and microbial community present on two green roofs in Greater London. It was found that the mite population was dominated by a xerophilic family (Scutoverticidae) and that collembola suffered population crashes in summer. Soil bacteria and fungi were low in abundance, but were more prevalent in dry weather. In general the soil community was impoverished and influenced by drought.

The second part of this thesis explores the use of microbial inoculants to improve the soil community. Bacteria, mycorrhiza and *Trichoderma* were added to a new and mature roof. On the mature roof, plant growth was not affected by treatments, but collembola populations were higher when *Trichoderma* were added. On the new roof, inoculants negatively affected plant growth and mite populations, but benefitted collembola.

Soil organisms on the new roof colonised independently and from the *Sedum* plugs. One species of rarely recorded collembola (*Sminthurinus trinotatus)* colonised early after construction. Planting with *Sedum* was found to improve the soil community compared to leaving the substrate bare.

The results presented here highlight that current green roof designs do not support a functional soil community but that microbial inoculants have the potential to improve them.

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Glossary

Amorphous	Without a clear shape
Arbuscular mycorrhizal fungi	Fungi that forms associations within the roots of plants. Three structures can be defined by microscopy: 1. Hyphae – Branching filaments of the fungus 2. Vesicles – Storage bodies of the fungus 3. Arbuscules – Nutrient exchange sites of the fungus
Axenic	Sterile conditions (except the study organism)
Biodiverse *(green roof)*	Green roof seeded with native wildflowers or left to self-seed
Bioturbation	The mixing of particles *(of soil)* by an organism
Collembola	*(or springtail)* An order of arthropods
Compost tea	Liquid produced by the aerobic digestion of microbe rich soil
Ectomycorrhizal	Fungi that forms an association with the outside of plant roots
Epigeic	Living on the surface
Extraradical	Hyphae of a mycorrhizal fungus growing in soil that is not directly associated with the roots of a plant
Exudate	A substance released by a porous surface such as a root
Femora	The third segment, closest to the body, of insects and arthropods
Furca	Jumping apparatus used by collembola
Gram negative	A bacterium that does not take up violet stain when subject to Gram's method
Gram positive	A bacterium that does take up violet stain when subject to Gram's method
Graminoid	Grasses
Microarthropod	Very small, often microscopic, invertebrates possessing an exoskeleton
Mycophagous	Fungus eating
Oribatid	Superfamily of mites, typically oval in shape
Parthenogenetic	Method of reproduction where an unfertilised egg develops into a new individual
PCA	Principal Components Analysis: transforms data orthogonally, organising data along axes that explain the most variance within a dataset. Axis 1 explains the most variability, with subsequent axes decreasing in the amount they explain.

Phoresy	A non-parasitic mutualism where one organism uses another to travel
Propagule	A structure that can become detached from an individual to form another individual
Refugia	An area that can be utilised by an organism so that it may survive unfavourable conditions
Rhizobacteria	Root colonising bacteria found in legumes
Ruderal	A plant species able to first colonise disturbed lands
Saprophytic	Obtaining food from dissolved organic matter
Selection, K	Organisms with traits related to stable or predictable environments, such as long life span, few offspring and long parental care
Selection, r	Organisms with traits related to unstable or unpredictable environments, such as short life span, many offspring and limited parental care
Senescence	Deterioration of cell function
Shannon-Wiener diversity	An index used to determine the number of different species and their evenness in a population
Sporulating	Produce spores
Succession	Process by which a virgin habitat is colonised by species
Thermotolerant	Able to withstand heat
Xerophilic	Organism adapted to life with little water

Chapter 1

Introduction

1.1 Green roofs

As urbanisation continues at an increasing rate and cities face the challenges posed by increasing population densities and climate change, including extreme climatic events, new approaches to architecture and town planning are emerging (Gill *et al*., 2007). Green infrastructure is a large part of this, promoting the inclusion of natural habitats into urban settings and green roofs contribute to this new technology (Brenneissen, 2006; Gill *et al*., 2007). Green roofs are increasingly becoming of interest to architects, town planners and ecologists as a way of remediating the habitat lost during building construction and as technologies to adapt to climate change (Gill *et al*., 2007). The term 'green roof' is used to describe any kind of vegetated roof, from the traditionally vegetated sod roofs typical of Northern Europe (Plate 1.1a) to ultra-modern habitat reconstructions such as the Vancouver Convention and Exhibition Centre (Plate 1.1b), housing 400 000 native Canadian plants (Green, 2009). It is a term that's also used for less obvious roofs, such as London's Jubilee Park, built over a London underground station (Plate 1.1c) or New York's elevated park, the High Line (Plate 1.1d). Although there are a number of green roof types (Table 1.1), the most common in the UK is known as an extensive green roof (Plate 1.1e) and these are currently being built in large numbers internationally.

Type of roof	Substrate	Substrate depth	Planting regime
Intensive	100% organic matter	>150cm	Not limited – shrubs to trees
Extensive	80-90% crushed brick 10-20% organic matter	<150cm	Drought tolerant plants
Biodiverse/Brown	Usually secondary aggregates such as brick	<150cm	Wildflowers or left unplanted

Table 1.1. Typical building specifications for the most commonly installed green roofs in the UK. Of these, extensive green roofs are the most common.

Extensive green roofs are designed to be low cost and maintenance and are usually built using the standard procedures outlined in the German FLL guidelines (2008) (Plate 1.2a). They typically consist of a substrate of no more than 15cm in depth (though more usually 8cm or less). In the UK crushed brick with 10-20% of rough organic matter is usually used as a substrate. These roofs are then planted with hardy plants, usually of the genus *Sedum* (Grant,

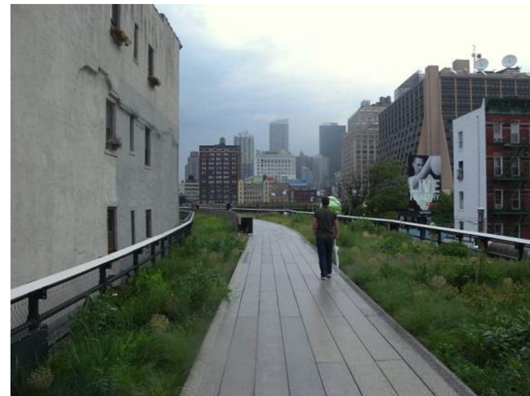
2006), that are either plug planted (Plate 1.2b) or rolled out in mats (Plate 1.2c). Roofs installed with mats may contain no substrate at all.

a.

b.

c.

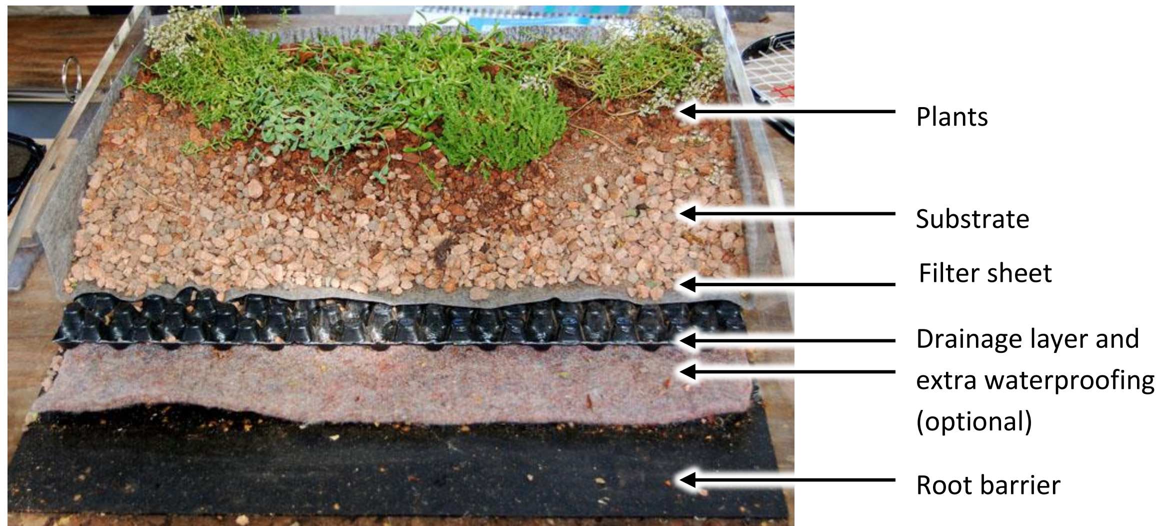
d.



e.

Plate 1.1. Green roof examples: (a) A traditional Norwegian sod covered house on the Faroe Islands (Erik Christensen, 2002), (b) the Vancouver Convention Centre green roof, planted with native species (www.flynn.ca, n.d.), (c) Jubilee Park, Canary Wharf, built over an underground station (Danny Robinson, 2007), (d) The New York High Line, a converted elevated railway line that is now a park (Jim Henderson, 2011), (e) A *Sedum* planted extensive green roof on the Hardley Mill Visitor Centre, Norwich (Evelyn Simak, 2009).

a.



b.

c.

Plate 1.2. (a) A green roof layering system (thingermejig, 2006), (b) *Sedum* plugs ready to be installed on a green roof, (c) A *Sedum* mat growing at a *Sedum* farm before installation (www.sedumgreenroof.co.uk, n.d.).

Green roofs provide a range of economic benefits. Jaffal *et al*., (2012) found that the energy efficiency of buildings installed with a green roof was higher and Getter *et al.,* (2009) determined that these energy savings would offset the carbon used to build a green roof in nine years. Getter *et al.,* (2009) also found that green roof plants may contribute towards carbon sequestration, further reducing the carbon offset of a green roof to seven years. As green roofs have been shown to have at least doubled the life of a conventional flat roof, with some examples over 90 years old (Brenneisen, 2006), this is a significant contribution to remediating some of the carbon used in the construction of a building. Green roofs are also installed for their ability to retain stormwater (van Woert *et al.,* 2005), helping to prevent watercourses being flooded. This is a major driver for green roof installation in London, where it is predicted that surface flooding will become a major issue due to climate change (GLA, 2009). For many years green roofs have also been cited as reducing pollution runoff. However,

a recent review paper by Berndtsson (2010) notes that this varies greatly between roofs and that while green roofs may have the potential to do this, more research is needed in the area. Much research is also being done into whether green roofs can reduce the urban heat island effect (Takebayashi and Moriyama, 2007).

However, aside from the economic benefits of green roofs, they are also of increasing interest to ecologists as a novel urban habitat (Oberndorfer *et al*., 2007) and in the construction industry they can be an important asset to a building's value. For example, Building Research Establishment Environmental Assessment Method points, or BREEAM points (where buildings are scored based on their environmental impact) can be awarded for including a green roof on a building, if it is established that habitat remediation has occurred by doing so. Additionally, in London, Mayoral guidelines state that a green roof must be considered for new flat roof building projects where feasible, contributing to the Mayor, Boris Johnson's, target to have greened 5% of central London by 2030 (GLA, 2011). In 2004 and 2008, audits were carried out on green roofs in London and it was estimated that over 50ha of green roofs had been installed, mostly in Islington where they had been actively encouraged by the planning department (Livingroofs.org, 2013). This is only a small fraction of the estimated flat roof space in London. In 2008, of 29ha of roof area assessed in the Victoria area, 25ha could have been retrofitted with green roofs (Livingroofs.org, 2013).

a.

b.

Plate 1.3. Two examples of green roofs designed as urban habitats: (a) The Checkland Building, Brighton University, is designed to mimic a chalk grassland (©University of Brighton, n.d.), (b) The California Academy of Sciences living roof in San Francisco is planted with only native plants (PerryPlanet, n.d.).

Though some green roofs have been built deliberately to recreate valuable habitats (Plate 1.3), relatively little is known about whether standard extensive green roofs are a valuable habitat for wildlife or not. Often no discrimination is made between a good green roof and a bad one in terms of policy and incentives. It is vital to assess the value of a green roof, both from the standpoint of mitigating habitat loss and to ensure that green roofs do not become 'greenwash' (Velasquez, 2011); devices designed to maintain an air of sustainability, without

actually doing so. Green roofs have been shown to benefit a wide variety of organisms, from spiders (Kadas, 2006) to birds (Gedge, 2003) and could even improve human health (Velarde, 2007). The following discussion summarises what is currently known about green roofs as a habitat and where further work needs to be done.

1.2 Green roofs as an urban habitat – current knowledge

The shallow substrates and exposed aspect of extensive green roofs mean they pose a challenging environment for plants (VanWoert *et al*., 2005). However, few studies have determined the capability of other organisms to survive and benefit from this environment.

1.2.1 Humans

The benefit of greenery in urban landscapes to human health is not a recent discovery. Ulrich (1979) found that individuals suffering from stress improved faster when subject to scenes of nature than exposed to urban scenery. In 2007, a review by Velarde *et al.,* on the effects of greenery on urban health concluded that, despite many papers being too vague in their descriptions of nature and non-nature, several aspects of human health could be improved by exposure to greenery. These included stress reduction, improving attention capacity, facilitating recovery from illness as well as improving physical wellbeing in elderly people. It was also found that green spaces could positively change behaviours, with the result of improving mood and general wellbeing. Green roofs, particularly those overlooked by other buildings, could therefore substantially improve the quality of life for those urbanites viewing them.

1.2.2 Mammals and birds

Green roofs have the potential to provide a habitat for some of our largest urban species, birds and mammals. No evidence of ground-dwelling mammals has yet been found on green roofs, presumably because their height makes them inaccessible. However, bats could benefit from green roofs. Pearce and Walters (2012) investigated this, recording the bat usage of green and biodiverse roofs in London. Biodiverse roofs were shown to attract more bats than conventional roofs, although the value of *Sedum* as a habitat for bats over conventional roofs was questionable. Feeding calls were rare over the roofs but when they did occur they were more prevalent over biodiverse roofs. The surrounding habitat around the roofs was also important for bats, emphasising the importance of green roofs at the landscape, as well as individual level. The insect communities supported by green roofs could be the driving force here and will be studied in subsequent papers (Pearce, pers. comm.). Bats have also been

recorded flying over the roofs used in the current study (Itter, unpublished data), though whether they are feeding or just commuting (i.e. passing over the green roofs) is unknown.

Birds have also been shown to benefit from green roofs. In London, the black redstart (*Phoenicurus ochruros*), a former British Action Plan species, became the flagship species for green roof development in London in the early 2000's, with many biodiverse roofs built with its habitat needs in mind (Gedge, 2003). However, the use made of *Sedum* planted extensive green roofs by other European birds is unclear. Many species, such as blackbirds (*Turdus merula*) and wagtails (*Motacilla alba*), have been reported to have bred on green roofs (Fernandez-Canero and Gonzalez-Redondo, 2010). However, the paper often cited as evidence (Ohlsson, 2003), actually lists species breeding near green roofs, rather than on them. No information regarding the influence of the green roofs themselves or information about how the green roofs are used by these species is provided in this paper, emphasising the caution needed when reviewing the green roof literature. Some reports are, however, quite clear about bird use on green roofs, though little is reported about *Sedum* roofs. A grass roof in Lancashire has attracted breeding mallards (*Anas platyrhynchos*) and has been visited by skylarks (*Alauda arvensis*), finches (*Carduelis spp.*) and thrushes (*Turdus philomelos*) (Johnston and Newton, 2004). Itter (unpublished data) found that the green roofs used for the current study (*Sedum* roofs) were used by magpies (*Pica pica*), jackdaws (*Corvus monedula*), crows (*Corvus corone corone*) and pigeons (*Columba spp.*) and the latter two used the roofs extensively for feeding. The Sussex Express (Sussex Express, 2013) reports that the feeding habits of crows and gulls (*Larus spp.*) on grass green roofs have become a nuisance, and birds of prey have been employed to ward them off. Green roofs planted with grass could, therefore, be an important breeding habitat and some species seem to feed on *Sedum* roofs.

However, for some bird species, such as ground nesting plovers (*Charadrius dubius*) and lapwings (*Vanellus vanellus*) studied in Switzerland, extensive green roofs have been reported as sink habitats. Adult birds laid eggs on green roofs, but their chicks later died due to food stress (Baumann, 2006). Controversially, this paper is often cited as proof that green roofs are a good habitat for ground-nesting birds (Colla *et al*., 2009). However, Francis and Lorimer (2011) note that the short sample period in this study means that we should not rule out this habitat as a breeding site. In agreement with Francis and Lorimer (2011) there are reports of successful breeding attempts of oystercatchers (*Haematopus ostralegus*) on conventional flat roofs (Lynch, n.d.). This is a ground-nesting species that could benefit from green roofs. Unlike lapwings and plovers (Schekkerman and Visser, 2001; Birds in Cheshire and Wirral, 2008), oystercatchers bring food to their young (Tjørve and Tjørve, 2010), perhaps circumventing the food stresses seen by Baumann (2006). Green roofs may not be a suitable habitat for all birds,

but they show potential for some species. With monitoring of breeding activity, including the success of clutches, the green roof industry may be able to tailor designs to prevent them from becoming sink habitats.

1.2.3 Insects and arachnids

The study of insects and arachnids on green roofs is perhaps the most comprehensive area of green roof research to date, with a number of large-scale studies determining their use. Jones (2002) found a variety of unusual insect and arachnid species on green roofs in London, including species of Coleoptera, Hemiptera and Aranae. Beetles, bugs (Hemiptera) and spiders (Aranae) were the most prevalent organisms and mostly consisted of hardy species usually found on brownfield sites, suggesting they were resident on the roof. Jones (2002) designated these three groups to be 'tecticolous', roof dwelling, as they were most probably resident and adapted to the roof conditions. Jones (2002) also noted that Diptera, wasps, aphids, ants, Orthoptera, Isopoda, Chilopoda, Gastropoda and Lepidoptera were in notably low numbers, probably due to the habitat being unsuitable. He also suggested that height may be an issue and that those organisms found on very high roofs, such as the HSBC tower, Canary Wharf, probably arrived with the *Sedum* matting. This applied particularly to Gastropoda and Isopoda.

Jones (2002) suggested that the green roof invertebrate community was similar to a brownfield site. Kadas (2006) tested this theory, performing a similar study on green roofs in London. Where Jones (2002) used suction sampling, Kadas (2006) placed pitfall traps on the roofs. She found that insects and spiders were more prevalent on *Sedum* green roofs than both biodiverse roofs and ground-level brownfield sites. She also found that 10% of the insects present on green roofs were either red data book species, nationally rare, scarce or localised. Again Coleoptera and Aranae were the most common species but in addition, bees and wasps were also found. Hemiptera were less common than found by Jones (2002), but were still one of the most prevalent invertebrates on the green roofs.

Aside from these two papers, invertebrate research on green roofs has also been conducted internationally. Smith and Palmer (2010) found that Hemiptera, Diptera and Hymenoptera were prevalent on *Sedum* planted green roofs in New York, but Aranae and Lepidoptera were few. Coffman and Davis (2005) found similar communities to Kadas (2006) but over five weeks found only a few families. Schindler, Griffith and Jones (2010), sampling in Boston, studied a non-*Sedum* planted roof in the same way and it appears that they found all the orders listed above, but with Diptera, Lepidoptera and Orthoptera better represented. Schindler, Griffith and Jones (2010) also seemed to have a wider range of orders on non-*Sedum* planted extensive green roofs, including ants. This research suggests that those organisms low in

number in Jones (2002) may be more common on green roofs planted with non-*Sedum* species.

Additional research on the suitability of green roofs for bees and wasps has also been conducted. Colla *et al.,* (2009) found that green roofs in Toronto harboured as diverse species assemblages of bees as ground-level urban habitats. As with the bats mentioned previously, it is unknown whether the roofs were used for feeding or just for commuting. However, Tonietto *et al.,* (2011) found that on green roofs in Chicago bees were actually visiting flowers on green roofs, though less frequently than in parks. They also found that the species richness of bees using green roofs was comparable to those using parks, but on green roofs abundance was lower. Green roofs also harboured a different type of community to parks. This means that though both habitats were most popular with ground-dwelling species, cavity nesters were higher in prevalence on green roofs than in parks. It is worth noting, however, that Tonietto *et al.,* (2011) found that bees were correlated overall with the diversity of flowering plants and that this was lowest on *Sedum* green roofs.

It is clear from the research presented that green roofs can harbour unusual species and may even harbour more invertebrates than some ground-level habitats, such as brownfield sites. It seems that *Sedum* roofs may not be a suitable habitat for all groups, as Lepidoptera and Orthoptera are usually not found on them. Grass roofs seem to be better for these species. A comprehensive comparison between green roofs and other ground-level habitats would enable green roof developers to be more explicit about the kind of habitat a green roof represents and provide accurate data about how to encourage more insects and spiders onto them.

Plate 1.4. Spider on a green roof at Royal Holloway (Rumble)

1.2.4 Soil microarthropods

A number of studies have documented the prevalence of soil microarthropods on extensive green roofs. Schrader and Böning (2006) found collembola on green roofs in Hannover in similar abundances to ground-level soils (Fountain and Hopkin, 2004). They hypothesised that an absence of earthworms on green roofs could have allowed greater success of collembolan proliferation. They also noted a slow succession of collembola, with ubiquitous early colonisers, followed by more specialist species later on. Smith and Palmer (2010) also found high abundances of collembola on *Sedum* planted green roofs in New York, along with some species of Thysanoptera.

In contrast, Jones (2002) found very few collembola on green roofs and suggested that they may not be suited to green roof life. However, as suction sampling was used, it is likely that a bias towards epigeic collembola would be prevalent and many species would have been missed.

Schindler, Griffith and Jones (2010) widened their green roof soil sampling beyond collembola but still only found collembola and Chilopoda on *Sedum* planted green roofs in Boston, both in low abundances. On non-*Sedum* roofs they also found Coleoptera larvae and Hymenoptera. They suggest microarthropod species richness was linked to plant cover, although sample sizes in this study were low. Davies, *et al*., (2010), whilst not looking at *Sedum* planted green roofs, but a native planted extensive green roof, found high numbers of both mites and collembola in emergence traps in Waitakere, New Zealand.

This research suggests a very mixed picture for microarthropods on green roofs. It is important to note, however, that all of these studies were conducted either on only one sample date, or over a short time period within the same season. Hence, they only present a snapshot of microarthropod diversity on green roofs. Schindler, *et al.,* (2010) suggest that the time of year they sampled was when collembola were likely to be most active. However, when appraising the suitability of a habitat for an organism, sampling when it is most abundant could bias our view. Schrader and Böning (2006) saw dynamic shifts seen between roofs of different ages, leading us to hypothesise that green roof microarthropod communities change over time. Therefore incorporating seasonal fluctuations into sampling regimes will be vital in determining their value as a good habitat all year round and to confirm that the community shows dynamic population shifts over time.

1.2.5 Vegetation, fungi and bacteria

As previously mentioned, extensive green roofs are generally planted with *Sedum*, either in the form of a mat or plugs (Grant, 2006). Emilsson (2008) found that both establishment methods produced the same level of cover several years after construction, although substrate type greatly affected cover success. As a man-made environment, planting regimes and subsequent development of vegetation on green roofs is driven by industrial practises. Emilsson (2008) noted that extensive green roofs are rarely installed with the long-term development of vegetation in mind. However Köhler (2006) notes that with careful gardening, maintenance can improve species richness on extensive green roofs, demonstrating that a healthy green roof plant community can be achieved if desired. From the customers' point of view, fast establishment of vegetation is paramount to the aesthetic value of a roof, as is the low maintenance nature of green roofs. Despite this, most UK green roof manufacturer's recommend that green roofs are 'weeded' i.e. ruderal plants and other self-colonisers are removed, at least once a year. This recommendation certainly increases the revenue acquired from green roof maintenance contracts, but might not create the best possible habitat. Some authors do note, however, that particularly voracious plant species may damage the waterproofing membrane (Fejes and Gerzson, 2006; Damas *et al.*, 2010), but in most European constructions a root barrier is installed to combat this.

In fact, for a functioning habitat, self-colonising plants could be important for insects (Gedge *et al.,* 2012) and provide an input of carbon into the soil (Zikeli *et al.,* 2002). Emilsson (2008) found that the establishment of self-colonising species on green roofs was low and was affected by both planting method, substrate type and the planted species present. There are few studies on those species that self-colonise *Sedum* planted extensive green roofs in Europe, but a table summarising the major studies is included in Appendix I. In the case of Emilsson (2008), more self-colonising species were found on plug planted roofs than those roofs installed with a *Sedum* mat and Emilsson (2008) suggests that they may have arrived on the roofs in the plugs themselves. However, Dunnett *et al.,* (2008) found that most self-colonising plants were effective dispersers, suggesting that they arrived independently of the roof's construction. They also found that substrate depth significantly affected colonisation and survival rates of self-colonising plants and warn that if establishment of self-colonisers is limited by, for example, substrate depth, some species may end up dominating the community.

Moss is also a dominant feature on extensive green roofs, reaching high levels in the study by Emilsson (2008) where it was suggested that the presence of one species, *Ceratodon*

purpureus, could reduce the self-colonisation of vascular plants due to its propensity to dry out between rain events. This species, along with *Tortula muralis* was also found on the Canary Wharf *Sedum* roof (Grant, 2006), as well as the roofs studied by Köhler (2006) and could be a valuable habitat for species such as tardigrades (Grant, 2006).

Few records exist recording the presence of fungi, including arbuscular mycorrhizal (AM) fungi on green roofs. Emilsson (2008) suggests that substrates (that are fired prior to installation) will likely be lacking AM fungi, unless the organic matter added contains soil microbes. However, green roof manufacturers are increasingly applying mycorrhizal inoculants to green roofs (Drought Smart Plants, 2013) providing a potential avenue of AM fungal input. However, *Sedum* is not reported to be mycorrhizal in the wild (Harley and Harley, 1987). Therefore empirical evidence pertaining to the presence of fungi on green roofs is needed.

To the author's knowledge, there is only one study exploring any other aspect of the green roof microbial community. Molineux (2010) used phospholipid fatty acid analysis (PLFA, see section 2.3) to determine the microbial mass (including fungi and bacteria) present in green roof soils after application with AM fungi and a mixture of other microbes called 'compost tea'. She found that the abundance and community structure of microbes in different substrates varied, but that bacteria were usually the most abundant microbe.

1.3 Problems with green roofs

The research summarised above highlights a number of problems with current extensive green roofs. A lack of long term studies means that an overall picture of the suitability of green roofs as a habitat is absent. The invertebrate species previously found on green roofs (Kadas, 2006; Jones, 2002) and the research done on green roof plants strongly suggests that drought is a problem (Dunnett and Kingsbury, 2004). Additionally, the low input of organic matter into the green roof system means that it could also be a nutrient limited environment. Molineux (2010) suggests that 50% organic matter in a green roof substrate is the best solution for maximum plant diversity but minimal weight. However this much organic matter is rarely added. The potential remediation of roofs by using inorganic fertilisers is not necessarily a viable solution as this can have a negative impact on water run-off quality (Berndtsson *et al.*, 2007) and could require multiple applications. Molineux (2010) suggests that the depth of the substrate and the type of substrate are the main factors in sustaining plant communities on green roofs. However, cost implications could be a barrier to the uptake of deeper and more novel substrates. Moreover, there are already countless green roofs constructed that may need to be improved, where changing the depth or type of substrate is no longer a realistic option.

The evidence also suggests that whilst *Sedum* roofs can support a number of rare invertebrates, roofs planted under different regimes, such as grass, support more taxonomic groups. However, as with substrates, there are already large numbers of *Sedum* roofs in existence and they will undoubtedly remain a popular planting choice due to their drought resistance. Therefore a more robust system of remediation needs to be explored, working with the current status quo. Molineux (2010) reports that the soil microbial community on green roofs could currently be lacking. Improving the soil food web could, therefore, be an alternative to adding additional fertiliser and water. The potential importance of the soil food web to green roof functioning is now discussed.

1.4 The soil food web and green roofs

Though the studies in section 1.2 give an initial idea as to what organisms inhabit green roofs, very little work has been done to determine if these communities are sustainable. The sustainability of communities comes not only from the standpoint of individual success, but whether an ecosystem functions as a whole. This can only be assessed by determining how the organisms present in an ecosystem interact with one another and their environment.

Many previous studies on green roofs focussed on determining above-ground processes (Kadas, 2006; Schindler *et al.*, 2010; Davies *et al.,* 2010), but below-ground interactions have been largely ignored, despite the two being inextricably linked (Wardle *et al.*, 2004). Below-ground processes are key for nutrient cycling, promoting plant productivity, permitting decomposition, buffering environmental changes and improving water retention (Neher, 1999). All of these are whole ecosystem processes, required for a healthy above-ground community.

Much of the nutrient cycling occurring in soils relies on plants being decomposed, exudates produced by living plants and inputs of inorganic nitrogen (Neher, 1999). Decomposition is facilitated by microbes, including bacteria and fungi, microarthropods, such as mites and collembola, and macro-arthropods, such as earthworms, all of which reside in the soil. Bardgett (2005) suggests that bacteria and fungi are responsible for the majority of decomposition taking place in soils, but this varies between habitats. In desert soils, for example, the removal of fungi from soils can cause a decrease in soil decomposition of nearly 30%, whilst the exclusion of microarthropods can reduce decomposition by over 50% (Santos and Whitford, 1981). Thus, in an artificial environment such as a green roof, previously found to be nutrient limited (Emilsson, 2008; Molineux, 2010), it is important to establish which organisms are key for nutrient cycling. Additionally, whether the soil community required can self-assemble or needs to be supplied to the roof, as the plants are, also needs to be

established. Whether the kinds of secondary aggregates and 'rough' compost commonly installed on green roofs can harbour such a community is unknown.

It is also important to establish if the community that is present is balanced. Soils can accumulate antagonists such as herbivores and pathogens that may produce a negative feedback loop, limiting plant growth and diversity (Mills and Bever, 1998; Bever, 2002; Klironomos, 2002; Brown and Gange, 1989). Conversely, some soils produce positive feedback mechanisms (Bever, 2003). For example, some plants form mutualist associations with mycorrhizal fungi, benefitting their success in a community (van der Heijden *et al.,* 1998). Changes in the balance between positive and negative feedbacks can have profound impacts on above-ground biota as they drive above-ground diversity through the processes of growth promotion (van der Heijden *et al.,* 2008) and competition (Moora and Zobel, 1996). This could be particularly important on *Sedum* planted green roofs as plants that are high in abundance can accumulate higher pathogen loads, incurring a negative feedback effect (Klironomos, 2002). However, the fact that green roofs are not the natural habitat of *Sedum,* in addition to its relative rarity in the UK, could mean that pathogens affecting *Sedum* are less prevalent in this environment than would be expected (Klironomos, 2002), at least while *Sedum* green roofs are not overly dense. However, this could become an issue if areas dense in green roofs all harbour the same planting regime, as is conceivable in large cities.

An additional factor to consider is that the complexity of the interactions between above and below-ground organisms is highly species specific. For example, although mycorrhizal fungi associate with most terrestrial plant species (Gerdemann, 1968), many plants are more productive when coupled with a specific mycorrhiza species (Wardle *et al.*, 2004). Some mycorrhiza species can even become parasitic on their host plants (Gange and Ayres, 1999), particularly when phosphorus (P) is not limiting (Smith and Read, 1997). *Sedum* is a rare plant in the UK and not reported as being mycorrhizal in the wild (Harley and Harley, 1987), but whether this is due to a lack of sampling is unknown. I hypothesise that mycorrhiza will be absent from the root system due to its absence in wild *Sedum* and that adding it to the soil would change the dynamic of the roof considerably.

In addition to soil microbes, micro and macro-arthropods also have considerable effects on above ground productivity and diversity. Wardle *et al.,* (2004) noted that in fertile ecosystems, earthworms are a major nutrient cycler, whereas nutrient poor soils tend to be dominated by fungi, collembola, mites and millipedes. There are also differences between the decomposition effects within these species, with earthworms more effective at breaking down rapidly decomposing litter compared to millipedes that are effective at breaking down slowly

decomposing material (Hättenschwiler and Gasser, 2005). Increased soil productivity in the presence of earthworms is a well-studied phenomenon (Zaller and Arnone, 1999; Atiyeh, 2002; Eisenhauer *et al*., 2009), but Partsch *et al.,* (2006) found that grassland plant communities were also more productive in the presence of collembola, in addition to earthworms. However, as with the microbial interactions previously discussed, these effects can be specific to the plant community present. Zaller and Anorne (1999) found that graminoid species in particular were associated with earthworms in complex plant communities and Eisenhauer *et al.,* (2009) found that legumes in particular benefitted from earthworms in grassland. It can therefore be concluded that competition outcomes and therefore plant diversity are affected by the presence of earthworms. This phenomenon has been shown to extend to many invertebrate species including collembola (Scheu *et al.,* 1999) and beetles (Cottam *et al.,* 1986).

The significance of invertebrate species to above-ground processes means that the species assemblage below-ground and its stability on green roofs is likely to greatly impact the plant community. Liiri *et al.,* (2002) hypothesise that species redundancy is high in soils and that they are therefore resistant to perturbations. However, Srivastava (2002) notes that this may only be the case in saturated environments and that in already impoverished environments, species loss and therefore functional loss is difficult to mitigate. In harsh environments like green roofs, this could have an even greater effect, as a change in vegetation structure also means a change in the microhabitats available to above-ground organisms. For example, plant architecture is known to significantly influence which spider species will use it (Hatley and MacMahon, 1980). Aside from the plant community, many of the above-ground insects reported to have been found on green roofs (see section 1.2) can also be affected by below-ground organisms. Scheu *et al.,* (1999) found that the presence of collembola in soils positively affected aphids feeding on *Trifolium repens,* but negatively affected aphids feeding on *Poa annua,* another example of the complex species specific interactions in soil food webs.

Thus far I have assumed that green roofs are controlled by below-ground forces impacting above-ground mechanisms via nutrient supply i.e. a 'bottom-up' controlled food web. However, food webs can also be driven by 'top-down' forces, such as predation. Kadas (2006) found a large number of predatory spiders on green roofs, so a 'top-down' control is plausible. It is important to establish which of the two has a larger influence on green roofs (if any) to enable researchers and green roof manufacturers to target those areas which are most dysfunctional, or have the most beneficial impact. This will maximise the efficiency of delivering green roof habitats. However, as the least is known about below-ground processes, the current study will focus on these, aiming at closing the gap in our current knowledge. This

said, the microarthropod community encompasses many predatory species, such as mites, so although we are primarily exploring the possibility of a 'bottom-up' controlled system, some information will be gleaned about the possibilities of a 'top-down' system, or a combination of the two.

1.5 Overview of research aims and chapters

Currently, green roofs have yet to realise their full potential, due to the economic factors associated with installation (Clark *et al*., 2008) and difficulties in establishing and sustaining vegetation (Cook-Patton and Bauerle, 2012). The latter paper suggests that a fuller exploration of animal-plant interactions needs to be performed on green roofs, combined with studying ways of enhancing diversity. The overall aim of this work is to do exactly that, using a combination of observational studies and manipulative research. The main methods used throughout are outlined in Chapter 2.

Prior to manipulative experiments, it was essential to determine the state of the existing community. Thus, the aim of Chapter 3 is to characterise the green roof soil community and to understand the reasons for the occurrence (or lack) of certain organisms. The work presented constitutes the first multi-season study of microarthropods in extensive green roof soils and will help to determine what organisms inhabit the green roof community and what challenges they face.

Green roofs have been hailed as a valuable habitat for many organisms, but the below-ground fauna has been largely neglected. One of the major research themes in this project, therefore, is to determine the value of green roofs to subterranean organisms. Unquestionably, two of the most important factors affecting plant growth on green roofs are the availability of soil organic matter and water (Nagase and Dunnett, 2011). In other field soils, many invertebrates (collembola in particular) are known to be limited by the availability of moisture (Verhoef and van Selm, 1983). Furthermore, arthropod species richness on roofs can be correlated with vegetation cover (Schindler *et al*., 2011). I therefore hypothesise that soil microarthropod abundance in green roofs will be related to plant cover and moisture availability. Harsh conditions present on the roof and lack of plant diversity will cause communities to be impoverished.

It is well established that there are complex interactions between soil invertebrates and soil microbes, including AM fungi, in plant communities (Gange and Brown, 2002). To date, only one study (in German) has searched for the presence of AM fungi in the roots of green roof plants (Busch and Lelley, 1997). As the plants for green roofs are generally supplied by the

horticultural industry as plugs or modular units, grown either indoors or outdoors, opportunities for mycorrhizal colonization vary. Thus, I hypothesise that arbuscular mycorrhizal presence in green roof substrates will be low, due to a lack of inoculum and invertebrates to disperse it (Gormsen *et al*., 2004) and that based on the findings of Molineux (2010), the microbial community will also be impoverished.

The second part of this study attempts to manipulate the soil food webs present on green roofs to determine if the microbial community could be improved and if subsequent improvements in plant and microarthropod abundance can be seen, as discussed in section 1.4. Prior to large scale manipulative experiments in the field, it was important to run trials *in vitro* to determine if the inoculants would have any effect at all. There were many problems associated with replicating green roof conditions *in vitro* so I present only the most successful of the experiments conducted. This is outlined in Chapter 4. Chapter 4 also includes an introduction to microbial inoculants and the rationale behind their use.

Based on knowledge gained during these assessments, Chapters 5 and 6 are manipulative experiments designed to better understand the interactions between soil organisms by adding soil microbes. The hypothesis is that if the addition of, for example, mycorrhizal fungi, alters populations of soil microbes and microarthropods or produces better plant growth, it could reasonably be assumed that this element of the food web was lacking previously. Additionally, as some of these amendments are already being marketed by green roof manufacturers, this would serve as an appropriate field experiment to determine if they are actually effective and should be used by the industry.

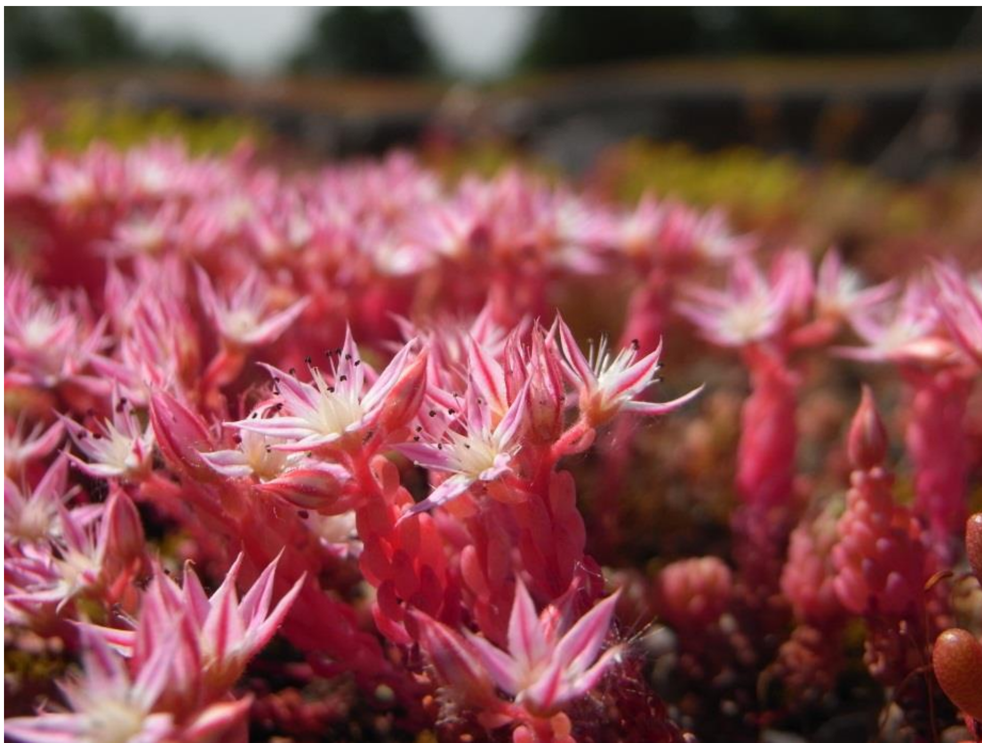
Chapter 5 focuses specifically on adding these microbial inoculants to a newly constructed roof. The hypothesis is that the addition of inoculants will produce a different successional pattern than the natural development of a green roof (Probanza *et al.,* 2002), potentially producing a healthier soil food web. To uphold the low maintenance ethos of extensive green roof design, application of inoculants at construction would be the most convenient and cost effective time to do so. Additionally, I tested the effect of planting on the subterranean community to determine the value of unplanted biodiverse roofs for plants and animals. Plant diversity is often higher on biodiverse roofs than on *Sedum* planted roofs (Molineux, 2010), and as many studies link above and below-ground diversity, I hypothesise that unplanted plots will harbour a more diverse community of both plants and microarthropods than *Sedum* roofs.

Despite the first years of a green roof being the most convenient time to apply inoculants, London alone already harbours a vast number of extensive green roofs (Livingroofs.org, 2013). If microbial inoculants are found to significantly enhance the habitat for biodiversity on a new

roof, it would also be valuable to assess whether this technique can be used to remediate impoverished communities by being applied to already failing roofs. I therefore converted one of the mature roofs used in Chapter 3 into an experimental site and replicated the experimental design of Chapter 5, testing the efficacy of inocula on a mature roof. The hypothesis that the addition of inoculants would enhance the microbial community and thus improve above-ground biodiversity is shared with Chapter 5. However, I hypothesised that because the mature roof is likely to have had some time to establish a microbial community, the results would be different to those seen with application on a new roof. This is the focus of Chapter 6, before a general discussion drawing together the results from previous chapters is presented in Chapter 7.

Chapter 2

General Methods



2.1 Introduction

Three techniques were used frequently throughout this study and methods for these are included here. These are microarthropod extraction, visualisation of mycorrhizas and analysis of the microbial community using phospholipid fatty acid profiling.

2.2 Microarthropod extraction

2.2.1 Introduction

Methods of extraction of microarthropods from soil can be active, relying on the movement of live organisms, or passive where organisms are removed from soil by the researcher (McSorley and Walter, 1991). Both methods have complications and biases.

Passive removal of microarthropods usually relies on differences in specific gravity by, for example, adding sugar or salt solutions to soil to separate particles of different density (McSorley and Walter, 1991), or on the affinity for hydrocarbons such as heptane or propane inherent in microarthropod cuticles (Walter *et al.,* 1987), causing them to float to the surface. Both techniques suffer from a lack of ability to distinguish between live and deceased organisms (McSorley and Walter, 1991).

The most common active technique used to extract microarthropods from soil is by using Berlese Tullgren funnels (Macfadyen, 1953). These devices heat and dry soil samples suspended above funnels, causing microarthropods that are averse to such conditions to move downwards in the soil where they fall through a mesh into a collecting vial. This technique has been shown to vary in its success, with some species being underrepresented due to rapid desiccation or an ability to withstand desiccation (Macfadyen, 1953). However, it does alleviate problems associated with living and dead organisms.

For the purposes of this study extraction by Berlese Tullgren funnel was chosen. However, initially propane flotation was used on soils that had been extracted to determine if some species were missed with Berlese Tullgren extraction and to determine the length of time to leave samples in the extractors. After 7 days of extraction, only 2 individuals in a standard sized sample were detected using propane flotation, one of which was not intact so was presumed deceased at the time of sampling. Including this deceased individual, this equates to less than 1% of the average number of microarthropods found by Berlese Tullgren extraction, suggesting efficient extraction by the funnels over this time period. Thus, extraction by Berlese Tullgren funnel was determined to be an efficient way of sampling microarthropods from green roof soils.

2.2.2 Method

Volumes of soil used in microarthropod extraction varied in each experiment depending on the size of the experimental site and the impact this removal of soil would have on the community. Sample sizes were kept as small as possible and the specific volumes of soil used in each experiment are outlined in the methods sections of each chapter.

In all cases soil samples were weighed to obtain wet weight and then placed in Berlese Tullgren funnels at approximately 18˚C for 7 days (MacFadyen, 1953). Soil organisms were collected in 70% ethanol and stored until further analysis. Microarthropods were sorted to morphospecies using a dissecting microscope at x100 magnification. Species identification, where possible, was then performed at higher magnifications (x200-1000) using a compound microscope. In the case of mites, this was usually restricted to the most prevalent mite, and then species level identifications were rare. Less common mites were identified to the highest level possible or assigned a morphospecies. All collembola were identified to species level. In early field surveys specimens of collembola were sent to an expert in the field (Fountain, M., East Malling, Kent) for verification. Other species present were identified to the highest resolution possible.

Collembola were identified using Hopkin (2007). Mites were identified using Strandtmann (1971), Strandtmann and Davies (1972), Walter and Proctor (2001) and Krantz and Walter (2009). Hemiptera were identified using Southwood and Leston (2005).

2.3 Mycorrhizal analysis

2.3.1 Introduction

Visualisation of AM fungi is commonly achieved by staining of the roots, as described by Vierheilig et al (1998). Identification of individual species can be done by identifying spores (Liberta *et al.,* 1983) or infection morphology (Abbott and Robson, 1979), but not only is this difficult but it is not then directly relatable to hyphal counts obtained with staining methods, making quantification of individual species difficult. However, it is a simple method for assessing whether mycorrhiza are present and in what abundance. Polymerase chain reaction (PCR) techniques looking at ribosomal ribonucleic acids (rRNA) within roots have the potential to overcome these problems (de Souza *et al.,* 2004) but as yet these techniques are in their infancy and were not deemed appropriate for this study.

2.3.2 Method

To obtain counts of mycorrhizal colonisation, a small portion of root mass, around 2g, of root was taken and stored in 70% ethanol until ready to be analysed. Roots were washed with tap water and cleared in 10% potassium hydroxide (KOH) in a water bath at 80˚C for 20-30 minutes, depending on the species. In the case of *Sedum spp.,* which the majority of samples belonged to, roots were cleared for 25 minutes. KOH was then disposed of and roots were thoroughly washed and dried. Visualization of mycorrhizas in the roots was performed using a modified ink staining method of Vierheilig et al. (1998), whereby commercial ink mixed with 1% HCl and water in the ratio 84.4:15:0.6 was added to the samples and heated at 80˚C in a water bath for 15 minutes. Root samples were stored in stain until ready to be analysed.

Percentage root length colonized was obtained with the cross-hair eyepiece method of McGonigle et al. (1990), whereby samples are spread evenly across a slide and observed at x200 magnification. Each root piece crossing the centre of the eyepiece, or the crosshair, is observed for presence or absence of fungi in the form of hyphae, vesicles or arbuscules, and recorded. Approximately 100 counts were obtained from each sample (100 minimum unless there was insufficient root mass to achieve this[1]).

2.4 Phospholipid fatty acid (PLFA) profiling of fungi and bacteria

2.4.1 Introduction

Whilst many analyses of bacteria and fungi are conducted using culturing techniques, it is estimated that only a small fraction of the microbial community is recoverable in this way (Hill *et al.,* 2000). To try to circumvent this problem, indirect observations have also been used to characterize the soil community, using systems such as BIOLOG, which detects differences in carbon utilization by microbes and therefore enables changes between sites or treatments to be evaluated against one another (Garland & Mills, 1991).

However, to achieve higher resolution results, two methods are commonly used: PLFA and PCR based methods, the latter of which looks at ribosomal RNAs (rRNAs) (Zelles, 1999). The latter technique is of a higher resolution and recent advances enable quantification of microbes as well as species identification (Hirsch *et al.,* 2010). However, although in the future this may become the most widely used method for characterizing the soil community, currently PCR based methods are expensive and time consuming. Furthermore, the techniques for

[1] Insufficient root mass was only a problem in seedlings grown in *in vitro* experiments.

performing such analysis as well as the identification databases required are still being developed (Hirsch *et al.,* 2010).

Therefore, for the purposes of this study, PLFA analysis was chosen to characterize the soil community. PLFA analysis has become an extremely common method for characterizing the soil community and produces rapid, sensitive and reproducible results (Frostegård *et al.,* 2011). Phospholipid fatty acids (PLFAs) are membrane components in all living cells and therefore can be used as indicators of living organisms (Zelles, 1999). They have been shown to constitute a relatively stable portion of the biomass of organisms and changes in PLFA patterns

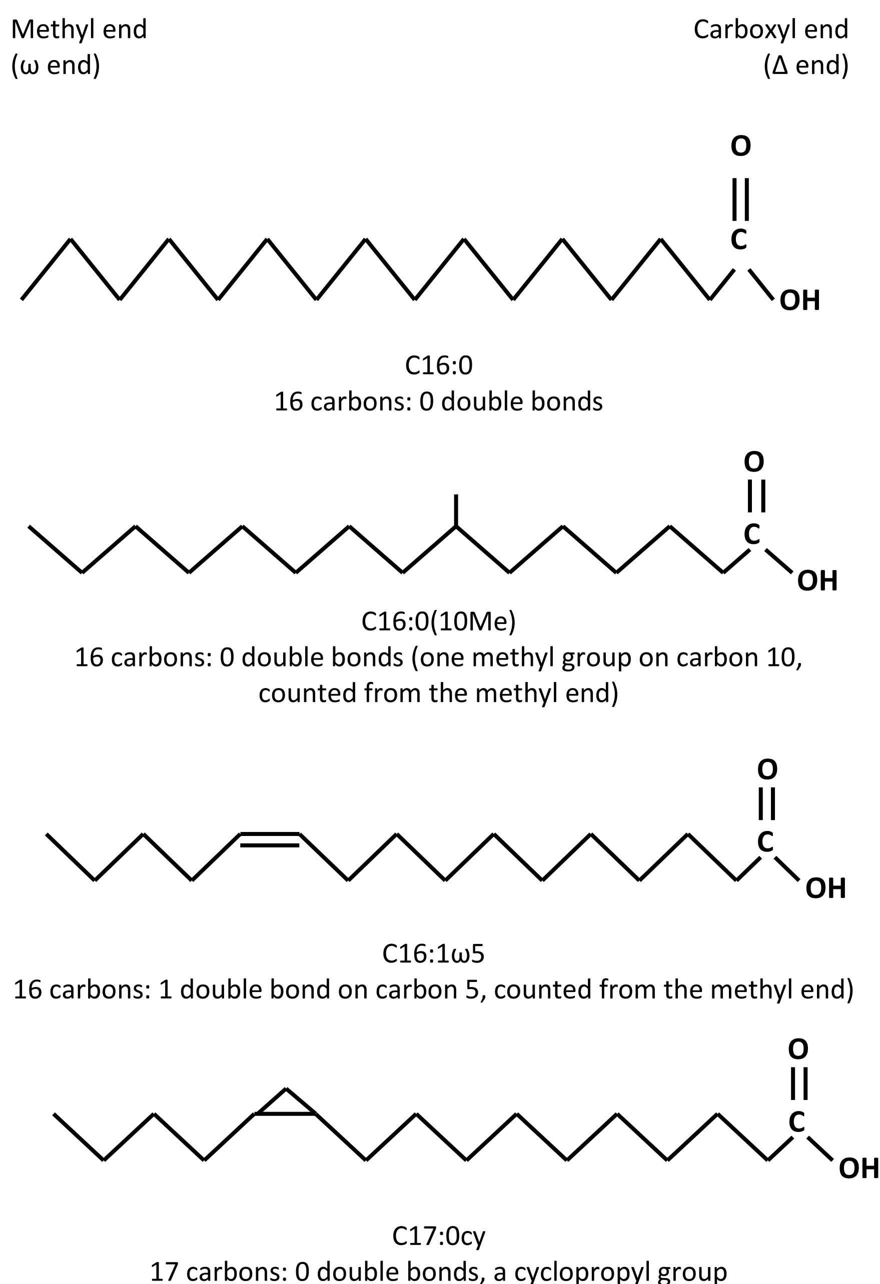


Fig 2.1. Nomenclature and structure of fatty acids in this study is as used by Frostegård et al (1993).

are indicative of changes in the microbial community (Zelles, 1999).

Fatty acids are comprised of chains of carbon that may be unsaturated (containing double bonds) or saturated, determined by their metabolic pathways (Ruess & Chamberlain, 2010) (Fig 2.1). Branched carbon chains, cyclopropyl groups, hydroxyl (OH) groups and saturated and polyunsaturated (>1 double bond) fatty acids can occur and be diagnostic of certain organisms. This technique, therefore, has been commonly used to glean information about specific organisms in the community, as well as general community changes between environments or treatments. However, many PLFAs are shared by multiple organisms due to the uniform methods of fatty acid synthesis between them (Ruess & Chamberlain, 2010) and interpretation of communities using PLFAs as markers should, therefore, be approached with caution. Some fatty acids, such as C16:0 (see Fig 2.1 for nomenclature of fatty acids), are ubiquitous in the soil environment and are, therefore, not suitable biomarkers (Ruess & Chamberlain, 2010).

Specific fungal biomarkers are a particular problem in this respect. For example, a common biomarker used to determine if mycorrhizal fungi are present, C16:1ω5, is also found in bacteria (Nichols *et al.,* 1986). C18:2ω6,9 is also used as an indicator for fungi, and one that will be used in this study. Research has shown it to be correlated with ergosterol, another fungal biomarker (Klamer & Bååth, 2004), and although it is also found in other eukaryotic organisms including plants, most plant material was removed from our samples and a plants contribution to C18:2ω6,9 is, anyhow, thought to be negligible (Kaiser *et al.,* 2010). C18:1ω9 is also common in both fungi and plants and is present in soil when there are no fungi, probably because some bacteria have a small amount of it, but it has been shown to increase in tandem with 18:2ω6,9 if other fungi are present (Frostegård *et al.,* 2011). Therefore, in this study 18:1ω9 has been used as a fungal marker only when 18:2ω6,9 was present. C20:1w9, an indicator of mycorrhizal fungi was also used in the analysis (Table 2.1). Diagnostic PLFAs for bacteria are usually saturated, often with characteristic features, such as branched carbon chains, found in gram-positive bacteria, or cyclopropyl chains, found in gram-negative bacteria (Zelles, 1999), and those specific to bacteria and therefore used in this study can be seen in Table 2.1.

Fatty acid patterns within an individual bacteria or fungi may change in response to environmental variables, such as stress and so have also been commonly used as an indicator for this (Hammesfahr *et al.,* 2008; Calderón *et al.,* 2000). However, Frostegård (2011) states that changes in abundance of these PLFAs could equally be suggestive of changes in the community structure and using PLFAs in this way have not been found to be good long-term

indicators of stress in some environments (Fischer *et al.,* 2010). For the purposes of this study, therefore, stress ratios commonly used in other publications will not be used.

2.4.2 Method

3(±.05) g of soil was taken per PLFA sample and stored at -20˚C until analysis. In those samples that were taken from microarthropod soil samples, fresh weights of 3g samples were taken and dry weights calculated from percentage water loss in the microarthropod samples. In samples taken specifically for PLFA (of which there were few), fresh weight was obtained in the same way but dry weight was obtained by drying soil samples after lipid extraction in an oven at approximately 40˚C for 5 days before reweighing. All PLFA samples were processed in batches of 19, plus a blank run to ensure quality control. All glassware for use in PLFA analysis was soaked in Decon 90 for at least 24 hours, rinsed in tap water and fired in a muffle furnace at 400˚C for a minimum of 4 hours. Glassware was then rinsed in hexane immediately before use. Pipette tips and other plastics were autoclaved at 121˚C for 20 minutes. The PLFA process used is a modified version of the method described by Frostegård *et al* (1993) and involves a five step process, as follows, with dried samples stored in a freezer at -20˚C between steps.

2.4.2.1 Lipid extraction

To each sample, 1.5ml of citrate buffer, 1.9ml of chloroform, 3.75ml of methanol and 2ml of Bligh and Dyer extractant (hereafter referred to as B&D) were added and vortexed for 10 seconds. These were then left in darkness for two hours with half hourly 10 second vortexes to ensure contact between the soil and the solvents. Samples were then centrifuged at 1500rpm for 17 minutes to eliminate debris from the supernatant. The supernatant was removed and placed in a new, clean tube before the soil samples were subjected to a second wash, adding a further 2.5ml of B&D and centrifuging as before. The supernatants were then combined and 3.1ml of chloroform and 3.1ml of citrate buffer were added before being left in a fridge at approximately 0˚C overnight to allow the supernatants to phase into an upper aqueous layer and a lower lipid layer. The following day, the upper layer was disposed of and the lower layer, containing the soil lipids was dried down under nitrogen.

2.4.2.2 Lipid fractionation

Silicone columns (Biotage isolute; Cronus[1]) were washed with 5ml of chloroform to ensure no contaminants were present. Samples were taken up in 200µl of chloroform and transferred to

[1] Two brands of silicone column were used during the study period. To ensure this did not affect the results, a test run was conducted whereby samples were split into two and placed in both types of

the silicone columns. This was repeated a further two times. Columns were then washed with 6ml of chloroform, releasing the neutral fatty acids (NLFA's) from the samples. Columns were then washed with 6ml of acetone, releasing glycolipids. These two fractions were not desired so were disposed of and replaced with new, clean test tubes. 6ml of methanol was then used to wash PLFA's from the columns. This fraction was then dried down under nitrogen.

2.4.2.3 Alkaline methanolysis

200µl of the internal standard, methylnonadecanoate (C19:0), were placed in each sample. Samples were then taken up in 1ml of methanol/toluene (1:1 v/v) and 1ml of methanolic potassium hydroxide (made fresh each day) was added. Samples were then placed in a water bath at 37˚C for 15 minutes before being wiped clean of water and left to cool to room temperature. 2ml of hexane/chloroform (4:1 v/v) were then added along with 0.3ml of acetic acid and 2ml of ultrapure water. Samples were then centrifuged for 2 minutes at 1500rpm. Samples were once again phased and the supernatant was transferred into new, clean tubes whilst the lower, aqueous phase was subject to a second wash of hexane/chloroform (4:1 v/v) before being centrifuged as previously. Both supernatants were then combined and evaporated off under nitrogen.

2.4.2.4 Gas chromatography

Samples were taken up in 100µl of chloroform and transferred to a gas chromatography (GC) vial. This was repeated a further two times. These samples were then evaporated off under nitrogen. Samples were then taken up in 300µl of hexane and placed in a Perkin Elmer autosampler. Analysis was performed by a Hewlett Packard (HP) 5890 Series II GC, equipped with a flame ionization detector and a DB-5 capillary column (30mm x 0.25mm i.d., film thickness 0.25µm). The injection temperature was 250˚C and the detector temperature regime started at 100°C increasing at 20°C min^{-1} before being held at 160°C for 5 minutes. Temperature increased again at 3.5^{o}C min^{-1} to 280°C where it was held for 3 minutes before finally increasing at 20°C min^{-1} to 320°C. Injection was splitless and helium was used as a carrier gas. Fatty acid methyl esters (FAME's) were identified on an HP 5970 mass spectrometer.

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column for a total of 5 replicates. No significant differences were seen in the results of the two column runs, nor were there significant changes in data after the switch.

Fatty acid type	Fatty acid	Lipid fraction	Predominant origin	Reference	**Fatty acids used in this study**
Saturated					
Iso/anteiso methyl-branched	i, a in C14-C18	PLFA	Gram-positive bacteria	Zelles (1997, 1999)	**C14:01, C15:0i, C15:ai, C16:1i, C16:0i, C17:0i, C17:0ai**
10-methyl-branched	10Me in C15-C18	PLFA	Sulphate reducing bacteria	Dowling et al (1986), Kerger et al (1986)	**C16:0(10Me),C17:0(10Me), C18:0(10Me)**
Cyclopropyl ring	C17:0cy, C19:0cy	PLFA	Gram-negative bacteria	Zelles (1997, 1999)	**C17:0cy, C19:0cy**
Unsaturated					
Double bond C9	C18:1ω9	PLFA	Fungi	Bååth (2003), Vestal & White (1989)	**C18:1ω9**
		PLFA	Gram-positive bacteria	Zelles (1999)	
		PLFA, NLFA	Plants	Harwood & Russell (1984), Ruess et al (2007)	
	C20:1ω9	PLFA	AM fungi (*Gigaspora*)	Sakamoto et al (2004)	**C20:1ω9**
ω6 family	C18:2ω6,9	PLFA	Fungi (saprophytic, EM)	Frostegård & Bååth (1996), Zelles (1999)	**C18:2ω6,9**

Table 2.1. Modified from Ruess & Chamberlain (2010). Organisms in which PLFAs are found and are appropriate for use as biomarkers, as cited in the literature. Only PLFAs common to one group were used in the study, with the exception of 18:1ω9, which was only used in tandem with 18:2ω6,9

2.4.2.5 Analysis

FAMEs were identified by chromatographic retention times and bacterial PLFAs verified with a standard bacterial FAMEs mix (Sigma-Aldrich, St Louis, USA). Fatty acid nomenclature followed Frostegård et al (1993) (Fig 2.1). Microbial markers were used to characterize the community, as summarised by Ruess and Chamberlain (2010). The PLFAs 18:2ω6,9, 18:1ω9 (Zelles 1999; Frostegård *et al* 2011) and 20:1ω9 (Sakamoto 2004) were used as indicators of fungi, although 18:1ω9 only when 18:2ω6,9 was present, as described in Frostegård et al (2011). C14:0i, C15:0i, C15:0ai, C16:1i, C16:0i, C16:1ω7c, C16:0(10Me), C17:0i, C17:0ai, C17:0cy, C17:0(10Me), C18:1ω9c, C18:0(10Me) and C19:0cy (Dowling 1986; Zelles 1999) were used to characterize total soil bacteria in the parameters expressed in Table 2.1 (i.e. gram positive bacteria, etc.).

Total microbial mass of identified fatty acids was calculated using the following equation, modified from Hedrick *et al* 2005:

$$\mathrm{FA} = \frac{\left(sum\ {}^{A_{FA}}\!/_{A_{IS}}\right) \times IS \times X}{Y}$$

FA	Total µg of fatty acids per gram of dry mass of sample (µg g^{-1})
sum A_{FA}	Sum of the areas of all the identified PLFAs, excluding the internal standard
A_{IS}	Area of the internal standard
IS	Concentration of the internal standard (µg μl^{-1})
X	Volume of internal standard (µl)
Y	Mass of dry soil (g)

All fatty acids are expressed as equivalent responses to the internal standard, C19:0, in µg g^{-1} dry weight of soil. Fungal fatty acids and bacterial fatty acids were also calculated in µg g^{-1} dry weight of soil using the same equation but replacing the sum of the areas of all the identified PLFAs with the sum of the PLFAs belonging to the group of interest (e.g. the retention times of fatty acids denoting bacteria).

2.5 Statistical analyses

The statistical tests used vary in each chapter depending on the variables tested. Generally, differences between treatments or factors were determined using ANOVA. Relationships

between variables were explored using multiple linear regression. Both these tests were performed in SPSS 19.0. Tests for heteroscedacity were performed in SPSS 19.0 using syntax written by Garcia-Granero (2002), including outputs for the Breusch-Pagan and Koenker tests, in addition to examining residuals plots.

Community analyses, also applied to fatty acid profiles, were conducted using principal component analysis (PCA) using the package FactoMineR (Husson *et al.*, 2013) in R (R Core Team, 2012). These were performed on a covariance matrix, which emphasises the most common species/fatty acid. To decide how many factors to include in the analysis, parallel analysis was performed (O'Connor, 2000), whereby random datasets parallel to the dataset of interest are created using Monte Carlo permutations (1000 in these studies). Eigenvalues are then extracted from these and compared to those produced by the data set of interest. Eigenvalues in the dataset of interest that exceed those within the 95th percentile of the randomly produced dataset are included in the analysis. In addition to parallel analysis, scree plots were also produced and examined for similarities and differences. Both these techniques were performed in SPSS 19.0, the latter using syntax written by O'Connor (2000).

Chapter 3

Characterising the soil community on green roofs

Part of this chapter has been published and is appended to this thesis:

Rumble, H. & Gange, A. C., (2013) Soil microarthropod community dynamics in green roofs. Ecological Engineering, 57, 197-204

3.1 Introduction

Though above and below-ground processes are inextricably linked (Wardle *et al.*, 2005), little is known about the soil community on green roofs. Subterranean microarthropods regulate decomposition of organic matter, aid nutrient cycling and shape soil food webs (Moore, Walter & Hunt 1988). They also significantly affect plant (Ingham *et al*. 1985) and fungal (Finlay 1985) growth and can assist movement of fungal spores through soil (Lilleskov & Bruns 2005). Microarthropods are, therefore, a valuable asset, providing multiple ecosystem services. Despite their importance, they have received remarkably little attention in green roof research and design, nor have soil microbes, which form the basis of many bottom-up soil food webs (van der Heijden *et al*., 1998), as discussed in section 1.4. It is as yet unknown, therefore, if green roof soil organisms provide the valuable ecosystem services, such as nutrient cycling, that they would perform at ground-level.

Those studies of below-ground organisms that have been conducted have either focussed on one group of microarthropods, commented on microarthropods as a sub-catch of their main samples or have been over a short time period (Jones, 2002; Schrader and Böning, 2006; Davies *et al.*, 2010; Schindler *et al.*, 2010; Smith and Palmer, 2010). These studies may not, therefore, accurately represent the suitability of green roofs as a sustainable urban habitat.

To address this knowledge gap, a fourteen month study was undertaken on two green roofs within the Royal Holloway grounds to determine the population trends of as full a range of soil organisms as was possible. Microarthropods, nematodes and soil microbes were examined, as vital components of the soil food web encompassing multiple trophic levels (Wardle *et al.,* 2005). Vegetation and mycorrhizal fungi were also surveyed to determine if there were relationships between them and microarthropods, testing the hypothesis put forward by Schindler *et al.,* (2010) who determined that plant cover and microarthropod abundance are linked on green roofs. In addition, to determine the levels of abiotic stress organisms were subjected to, abiotic factors such as temperature and substrate water content were also measured and related to soil microorganism abundance.

The hypothesis was that soil organisms, including microarthropods and microbes, would be limited by abiotic conditions. In addition, if this hypothesis were proven correct, I hypothesised that AM fungi, vital for the growth of many plants, would also be in limited supply due to a lack of organisms to disperse them. Taking this into account, overall it was hypothesised that a lack of these soil organisms would result in poor nutrient cycling and unsustainable communities of plants and animals.

3.2 Methods

3.2.1 Field sites

Two green roofs in the grounds of Royal Holloway, University of London (Fig. 3.1), were used in this study (Roof A and Roof B) (Plate 3.1). Both were built in April 2004 and at the time of construction were plug planted with *Sedum album, S. acre, S. spurium, S. kamtschaticum* and *S. rupestre*, in proportions of approximately 3.5:3.5:1:1:1 respectively. The substrate is 80% crushed brick and 20% organic matter (commercial compost) and is approximately 75mm deep. The roofs are within 40m of one another and are approximately 12m high. Roof A is approximately 1960m^2 in area and Roof B is approximately 2240m^2. No fertilization, supplementary watering or removal of naturally colonising plants has ever occurred.

3.2.2 Abiotic factors

Daily and monthly average temperature and wind speed readings were obtained from a weather station situated on a roof approximately 300m from our study site (Fig. 3.1), on the Royal Holloway Earth Sciences department. Average rainfall for South-East England was obtained from Met Office records (Met Office 2011).

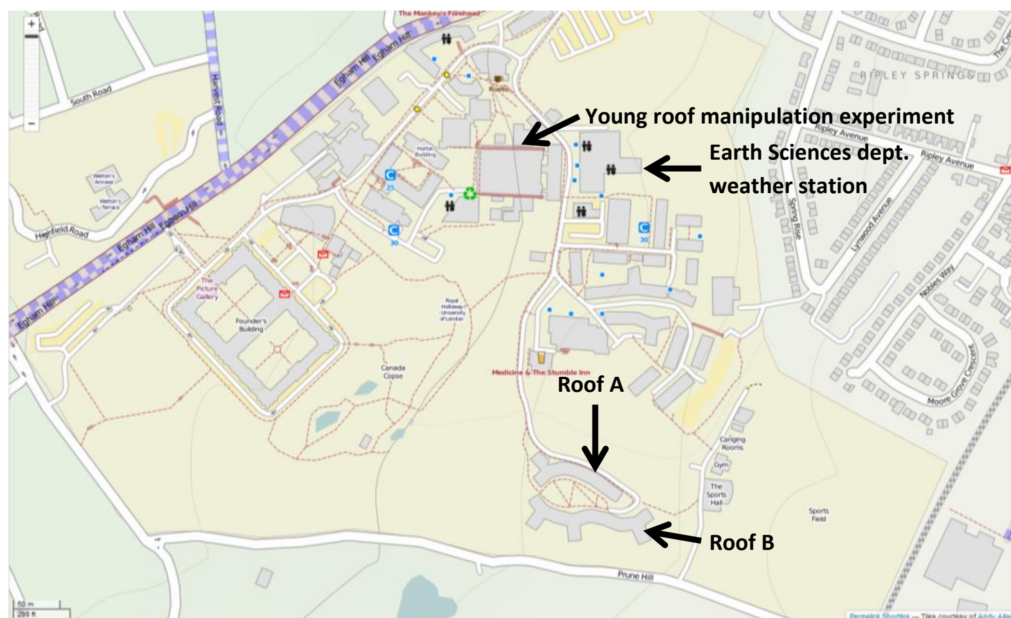

Fig. 3.1. Map of study areas used in relation to one another and to the Geology Department weather station. Roofs A and B were used for roof surveys and the mature roof manipulation experiment (©Open Street Map contributors, 2013)

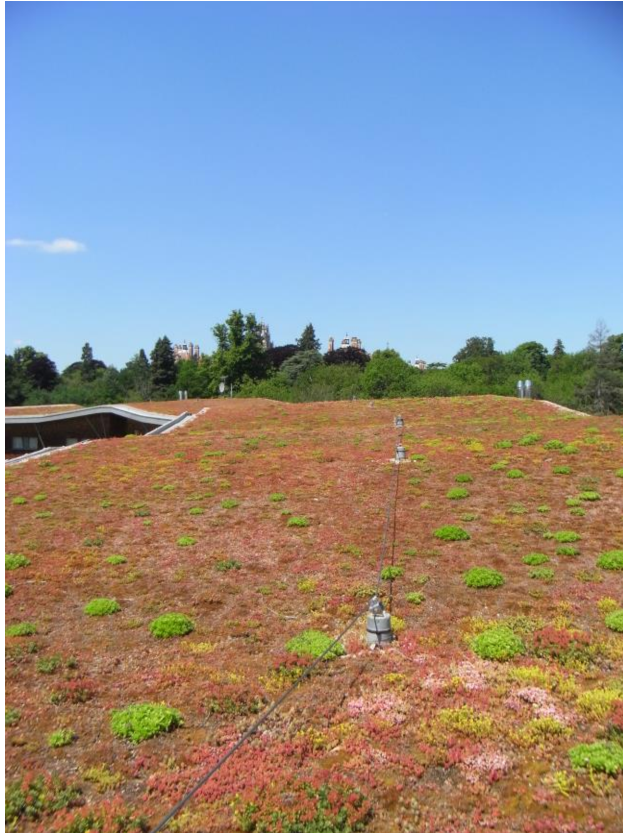

Plate 3.1. Roof A in June 2010. Both roofs were built in April 2004. Planted plugs consist of *Sedum album, S. acre, S. spurium, S. kamtschaticum* and *S. rupestre.* The substrate is 80% crushed brick and 20% organic matter (commercial compost) and is approximately 75mm deep. No fertilization, supplementary watering or removal of naturally colonising plants has ever occurred.

3.2.3 Plants and fungi

Samples to determine percentage root colonisation by AM fungi were obtained alongside invertebrate sampling in October 2010 by removing a portion of root from an individual of *S. kamtschaticum* in each plot. This plant was chosen because it was present in most plots. The procedure was only performed once, so as to limit the impact on the fragile roof community. AM fungi was analysed as per the method detailed in section 2.3.2.

Plant cover and plant diversity estimates were obtained in April, June, July and November 2010 and April 2011 in the same plots used for invertebrate and PLFA analysis. Individuals were counted and identified to species level where possible using Blamey *et al.,* (2003). Additionally, vegetation cover was estimated by eye with the aid of a quadrat split into 1% fractions.

3.2.4 Microarthropod sampling

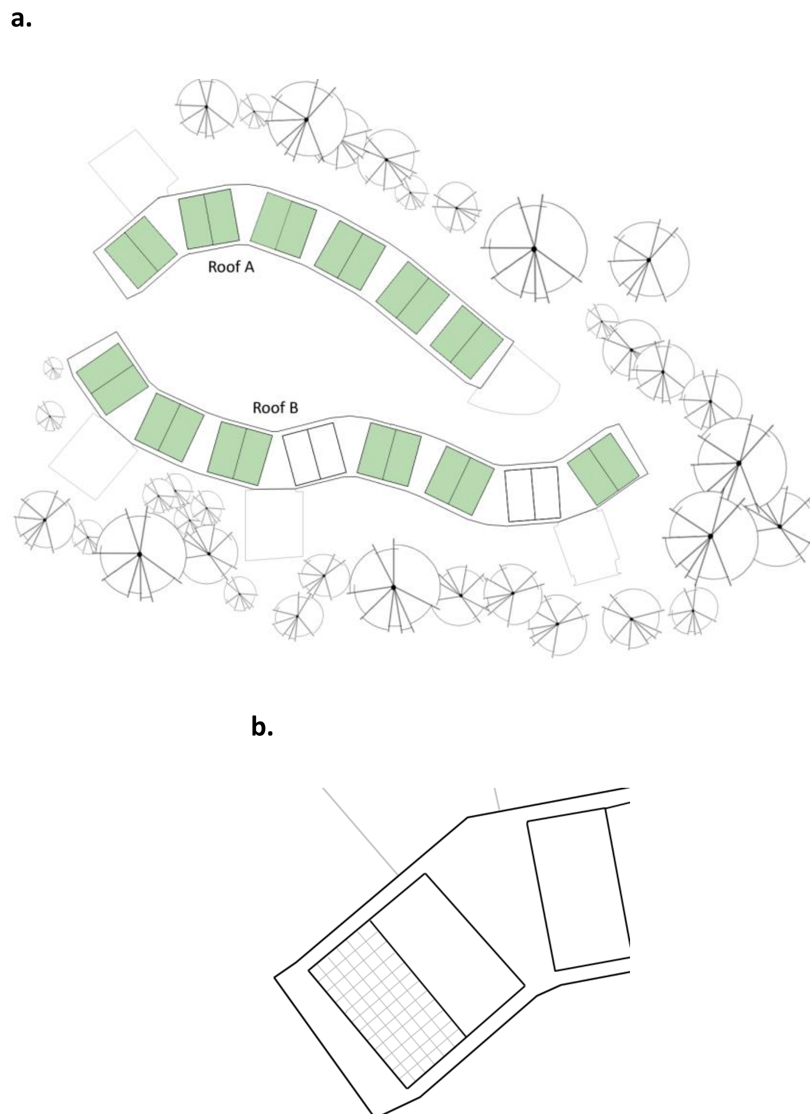

Fig. 3.2. (a) Roofs A and B, split into plots 12 plot each (12mx6m). Shaded plots were used in the study. Circles denote trees, grey outlined structures denote lower roofs. (b) An example of one plot, divided into a 6x12 grid, of which one square was randomly chosen to sample from per month.

Stratified random sampling was employed on each roof; each roof was divided into twelve 6m x 12m strata (Fig. 3.2). On each sampling occasion, in each stratum, a 1m^2 sample area was chosen at random using a random number generator. Two samples were taken from this with an 85mm diameter soil corer, inserted down to the roof lining (75mm). This method was chosen to overcome problems associated with aggregated soil invertebrate distributions (Ettema and Wardle, 2002), and resulted in a sample of 851cm^3 at each sampling point. Larger amounts could not be removed for fear of permanently damaging the roof structure. Samples

were taken at monthly intervals from March 2010 to April 2011 inclusive. Microarthropod extraction was conducted using the methods detailed in section 2.2.2.

Every other month, from April 2010 to February 2011, inclusive, approximately 10g of soil was removed from these samples prior to being weighed. Approximately 3(±.05) g of soil were removed from this, weighed and subject to PLFA analysis as described in section 2.4.2. The remainder of the 10g subsample was stored in a freezer at -20˚C as backup soil, should samples fail during preparation or analysis. If needed, a further 3(±.05) g of soil was once again removed, as needed.

In September 2010, a 50cm^3 subsection of soil was also removed from the microarthropod samples to determine if the roof had a healthy nematode community. This soil was placed in a modified Baermann funnel (Plate 3.2) (S. Edgington, pers. comm.) for 3 days before analysis. Identification of nematodes was completed with the help of an expert in the field (Edgington, CABI, Egham, Surrey) and was conducted using a compound microscope at x1000. Nematodes were categorised using the 'persistence score' of Bongers (1990), where 5 is a long lived species, 1 is a short lived species and numbers in between are intermediate. Dietary group was also attributed, using Bongers & Bongers (1998).

Plate 3.2. Nematode extraction set-up, based on the Baermann funnel technique. The black section comprises of a wide plastic mesh, topped with filter paper. Soil samples are placed on the filter paper and nematodes are collected from the terracotta portion.

3.2.5 Statistical analysis

3.2.5.1 Microarthropods

Analysis of the microarthropod community (m^{-2}) was performed in SPSS 19.0. Normality tests were performed on whole data sets and data were transformed if necessary by *ln*+1 or square root.

Differences between total microarthropod abundance over time were tested using a two-factor, repeated measures ANOVA, employing month of sample and roof as main effects. These were also performed for collembola and mites separately. Months were separated using a Bonferroni post-hoc test.

Relationships between microarthropods and abiotic and biotic factors were examined using linear and curvilinear regressions. Mites, collembola and total microarthropod abundance were the dependent factors and plant cover, plant diversity, mycorrhiza, temperature and substrate water content were the independent factors.

Diversity was measured using the Shannon-Wiener index and was calculated in four variations: all roof organisms, mite morphospecies, collembolan species and all organisms not belonging to mites or collembola. Differences between roofs were examined with a Mann-Whitney U-test.

March 2011 data were examined for spatial separation of mites and collembola between the moss and substrate layers on each roof using a two-factor ANOVA, employing roof and layer as main effects.

3.2.5.2 The microbial community

Principal component analyses (PCA) were performed on individual fatty acids (μg^{-1}) using the FactoMineR package (Husson *et al.*, 2013) in R (R Core Team, 2012). To determine the number of factors to include in the analysis, scree plots were analysed in SPSS 19.0 and parallel analysis was performed using 1000 Monte Carlo permutations, again in SPSS 19.0.

Roof and month were added separately to the PCAs as qualitative supplementary variables to help explain the data, and confidence ellipses are drawn around these data at the 95% confidence level. Average monthly wind speed, average monthly temperature, average daily temperature, average monthly rainfall and substrate water content were also added as quantitative supplementary variables. Correlations between axes and fatty acids were

obtained using the 'dimdesc' algorithm in FactoMineR (Husson *et al.*, 2013) and significant correlations are present in tables where diagrams are unclear.

Fatty acids were also analysed in groups of their respective biomarkers (see section 2.4.1, Table 2.1). These were grouped into total mass of identified fatty acids, mass of bacterial fatty acids and mass of fungal fatty acids. After initial analysis the latter was also analysed by splitting fatty acids into those with 20 carbon atoms and those with 18 carbon atoms, the former representing AM fungi, the latter saprotrophic fungi. Each of these respective groups was tested using a two-factor repeated measures ANOVA with time and roof entered as main effects. Months were separated using a Bonferroni post-hoc test.

To determine if any of the abiotic factors had an influence on the microbial community, these microbial groups were also subject stepwise multiple linear regression, performed in SPSS 19.0. The variables tested were average monthly wind speed, average monthly temperature, average daily temperature, average monthly rainfall and substrate water content.

3.3 Results

3.3.1 Abiotic factors

Temperature for the sample period reached a maximum daily temperature of 30˚C in July 2010 and a minimum daily temperature of -8.3˚C in December 2010, with monthly average temperatures between 18.4(±0.1)˚C in July 2010 and 0.8(±0.1)˚C in December 2010 (Fig. 3.3). Substrate water content was highest over the winter months reaching a maximum of 30% by weight in December 2010. The substrate was driest in April 2011 at 2% water content by weight (Fig. 3.3). Wind speed was low throughout the sample period, between 1-2.5 mph (Fig. 3.3).

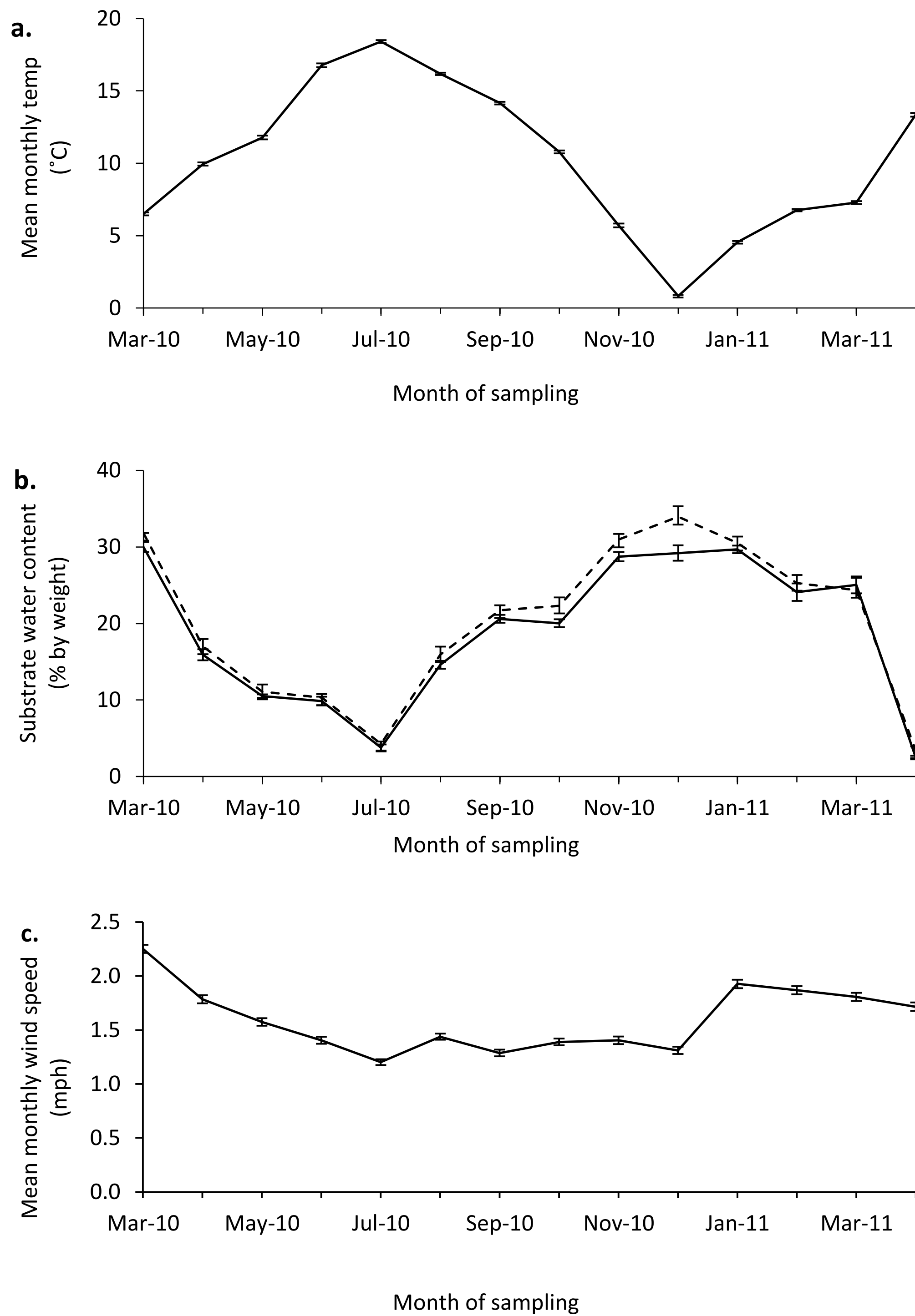

Fig. 3.3. (a) Mean monthly temperature for the local area (˚C) for the same period. (b) Percentage water of green roof substrate (by weight) for Roof A (solid) and Roof B (dashed) for the same period. (c) Mean monthly wind speed between March 2010 and April 2011. Error bars represent SEM.

3.3.2 Plants and fungi

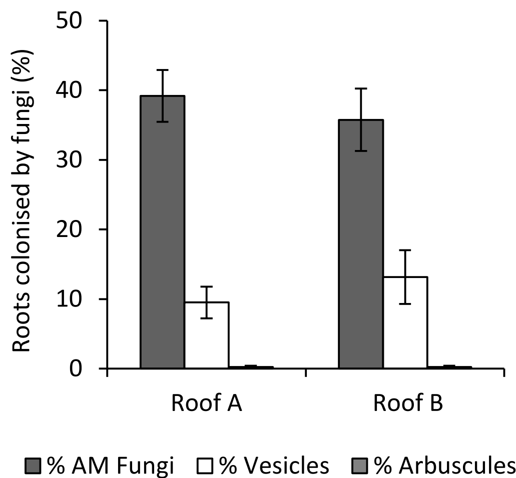

Fig. 3.4. Percentage colonisation of *Sedum* roots by mycorrhizal fungi in October 2010. Error bars represent SEM.

Both roofs had an average of 49(± 4)% root colonisation by mycorrhizal fungi with some individuals as high as 76%. Roots were relatively high in vesicles, averaging 9.5(± 2)% on Roof A and 13(± 4)% on Roof B, but very low in arbuscules, averaging 0.25(± 0.2)% on each roof (Fig. 3.4).

The plant community was dominated by *Sedum spp*. and mosses, with the latter tending to prevail in most plots. Over the five plant surveys, mosses had an average cover of 45(± 2)% and Sedum 28(± 1)%. Some plots had bare areas and these accounted for 20(± 2)% of average plot area. Lichen accounted for 2(± 0.6)% of vegetation cover (Fig. 3.5).

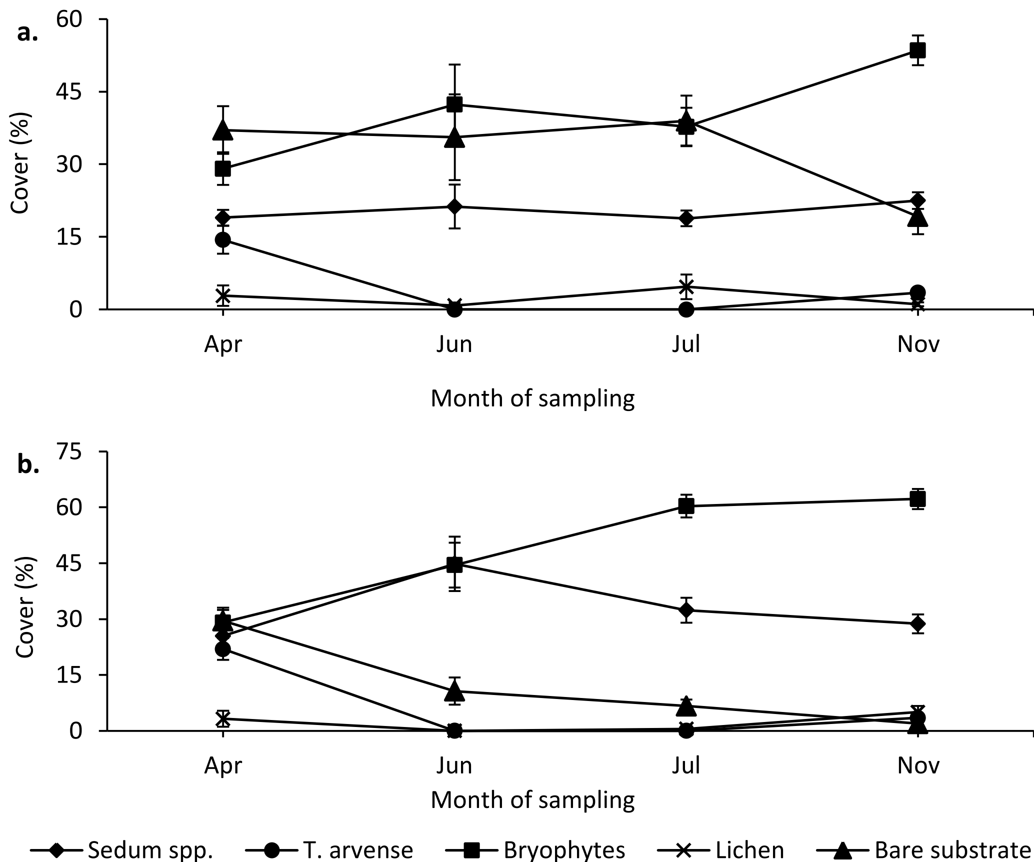

Fig. 3.5. Percentage cover of plants achieving cover of >1%, recorded over four sampling dates on (a) Roof A and (b) Roof B. Error bars represent SEM.

Seasonal colonisers (Table 3.1) were absent in June and July 2010 but abundant in April 2010, 2011 and November 2010. *Trifolium arvense* made up a large proportion of these, particularly in April 2010 where it accounted for an average of 14(± 3)% of plant cover on Roof A and 22(±4)% on Roof B. Mean Shannon-Wiener diversity for non-Sedum and non-moss species for April 2010 for Roof A and B were 0.11(± 0.07) and 0.23(± 0.07) respectively, for April 2011 were 0.08(± 0.04) and 0.09(± 0.04) respectively and November averaged 0.05 (± 0.04) on Roof A and 0.04(± 0.03) on Roof B. Two species of Basidiomycete fungi were observed on the roof, *Melanoleuca polioleuca* and *Omphalina pyxidata*.

Plants			
Sedum			
Sedum album	*Sedum acre*	*Sedum kamtschaticum*	*Sedum rupestre*
Sedum spurium			
Seasonal colonisers			
Arabidopsis thaliana	*Anthyllis vulneraria*	*Cirsium arvense*	*Geranium robertianum*
Jacobaea vulgaris	*Leontodon hispidus*	*Melilotus officinalis*	*Sonchus asper*
Sonchus oleraceus	*Taraxacum officinalis*	*Trifolium arvense*	*Trifolium dubium*
Tree saplings			
Acer pseudoplatanus	*Betula pendula*	*Pinus sylvestris*	
Fungi			
Melanoleuca polioleuca	*Omphalina pyxidata*		

Table 3.1. Plant and fungal species encountered during the sample period. In addition to this lichen and bryophytes were present as well as six unidentifiable plant species[1] and one species of grass, also not identified.

3.3.3 Microarthropods

3.3.3.1 Total microarthropods

Overall, total microarthropod diversity was low, with key soil groups such as Annelida and Diplopoda absent. Only 42 species/morphospecies were found over the 14 month period (Table 3.2). The fauna was dominated by collembola (61%) and mites (38%) but also included

[1] These individuals were either at the seedling stage or were deformed through drought stress.

small numbers of Chilopoda, Coleoptera, Hemiptera, Aranae and larvae, mostly of Diptera, Lepidoptera and Coleoptera. Of these less prevalent groups, larvae were most common but no group represented more than 1% relative abundance. No correlations were found between total abundance and any abiotic or biotic factors.

Order	Mean individuals (m^{-2})	Relative abundance (%)	Number sp./morphospecies
Collembola (ad & juv)	20637.8 (± 1056.7)	62.13	5
Acarina (ad & juv)	12359.7 (± 888.5)	37.21	15[a]
Hemiptera (ad & juv)	54.4 (± 8.7)	0.16	6[a]
Aranae (ad & juv)	9.6 (± 2.3)	0.03	1
Chilopoda (ad & juv)	13.1 (± 3.7)	0.04	1[a]
Coleoptera (ad)	6.4 (± 1.4)	0.02	3
Diptera (ad)	9.9 (± 1.7)	0.03	1[a]
Unidentified insect larvae	89.2 (± 5.1)	0.3	11[a]

[a]morphospecies, as opposed to species

Table 3.2. Number of species and morphospecies of microarthropod found on both roofs A & B (pooled) during the sample period. Ad=adult, juv = juvenile.

3.3.3.2 Collembola

Only six collembola species made up the 72 978 individuals counted. 74% were *Sminthurinus aureus*, 23% *Deuterosminthurus pallipes*, 1% *Parisotoma notabilis* and less than 1% were made up of *Bourletiella hortensis*, *D. bicinctus* and *Isotomurus palustris*. *Sminthurinus aureus* and *D. pallipes* showed almost identical seasonal trends, although *D. pallipes* was always lower in abundance.

Collembolan density varied between 0 – 120 000 individuals m^{-2} (average ≈ 19 000(±1000)m-^{2}, median ≈ 14 000m^{-2}). Total abundance did not vary between roofs but varied greatly over time ($F_{6.4,\ 128.3}$ = 47.8, p <0.001) with peaks in March of each year (Fig. 3.6).

Density decreased with rising average monthly temperature (Roof A: R^2 = 0.175, $F_{1,\ 166}$ = 35.2, p < 0.001; Roof B: R^2 = 0.249, $F_{1,\ 142}$ = 47.1, p < 0.001) with population crashes occurring when water content was low, followed by a recovery time as water content increased. *Deuterosminthurus pallipes* was slower to recover from these than *S. aureus*. Collembolan abundance showed a logarithmic relationship with substrate water content (R^2 = 0.22, $F_{1,\ 331}$ = 93.3, p < 0.001), with a threshold value of approximately 5%, below which numbers decreased dramatically (Fig. 3.6). Of the biotic variables measured, collembolan abundance was positively

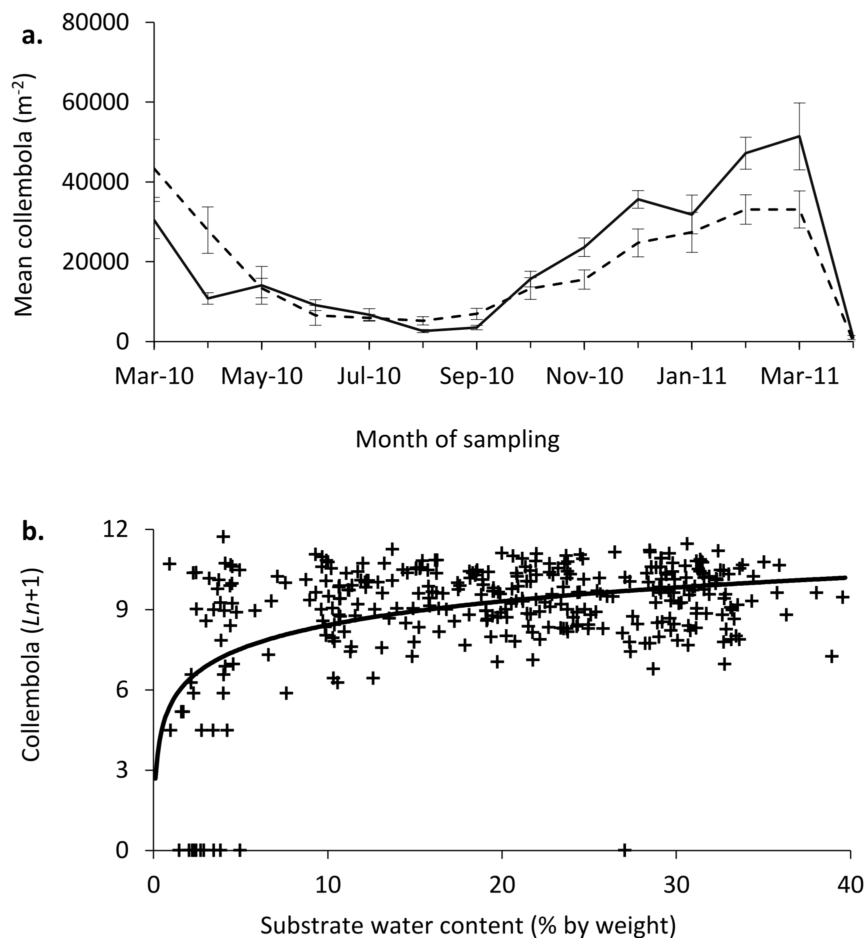

Fig. 3.6. (a) Mean number of collembola between March 2010 and April 2011. Solid line denotes Roof A; dashed line denotes Roof B. Error bars represent SEM. (b) Total collembola on both green roofs, *Ln*+1 transformed, and plotted against percentage substrate water content (% by weight) between March 2010 and April 2011. A logarithmic fit is displayed.

related to moss cover, but only on Roof B (R^2 = 0.102, $F_{1, 56}$ = 6.3, p = 0.05). However, on both roofs collembola were considerably more abundant in the substrate layer than the moss fraction ($F_{1, 44}$ = 59.1, $p < 0.001$) (Fig. 3.7).

Collembolan diversity was poor, reaching only 0.5 at its highest. Diversity was highest in April 2010, March 2011 and over winter (Fig. 3.8). There were no differences between roofs in diversity or seasonal pattern.

Collembolan diversity decreased with increasing daily average temperatures (R^2 = 0.147, $F_{1, 286}$ = 49.3, $p < 0.001$), as well as increasing monthly average temperatures (R^2 = 0.089, $F_{1, 310}$ = 30.177, $p < 0.001$). These were the only abiotic factors measured to affect collembolan diversity.

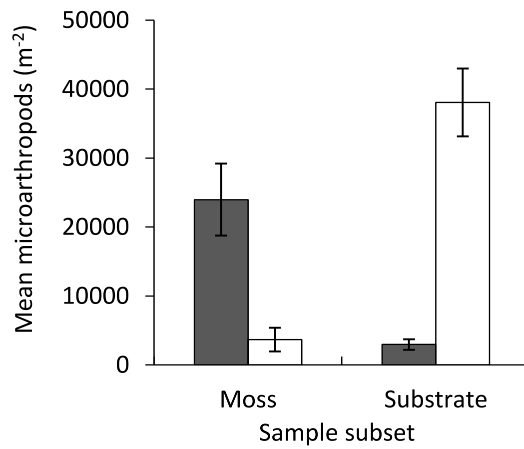

Fig. 3.7. Microhabitat preferences for mites and collembola in June 2010 on both roofs. Dark bars represent mites, white bars represent collembola. Error bars represent SEM.

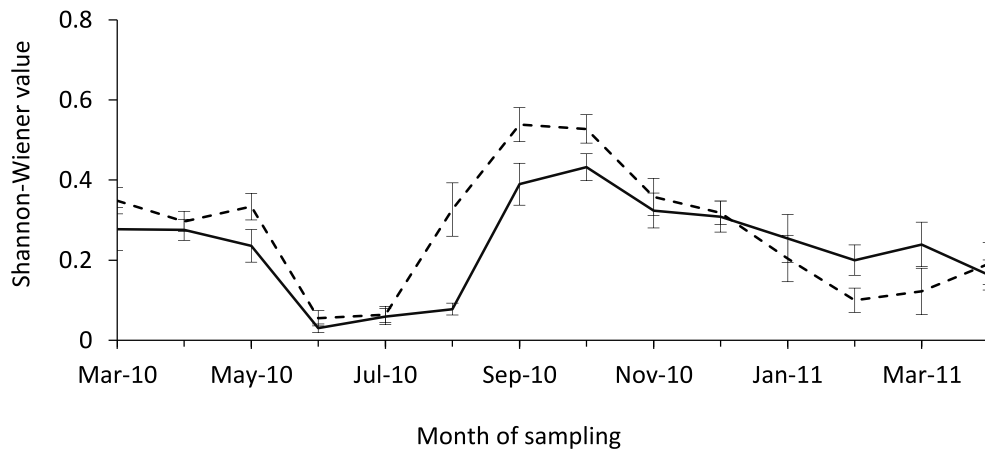

Fig. 3.8. Shannon-Wiener indices for collembolan diversity between March 2010 and April 2011. Solid line denotes Roof A; dashed denotes Roof B. Error bars represent SEM.

3.3.3.3 Mites

Fifteen morphospecies of mite were present on the roofs and density varied between 180 and 109 000 mites m^{-2} (average ≈ 12 000(± 800)m^{-2}, median ≈ 7000 m^{-2}). The two most abundant mites were a prostigmatid, *Eupodes viridis*, which was particularly abundant in summer 2010, and an oribatid mite from the Scutoverticidae family. These represented 23% and 62% of mites respectively. Mite abundance did not differ between roofs (Fig. 3.9) but did change over time ($F_{3.1,\ 61.8}$ = 11.1, p < 0.001) with higher abundances in August/September 2010 (*E. viridis*) and December 2010 and March 2011 (Scutoverticidae) (Fig. 3.9). The Scutoverticid was usually the most dominant mite.

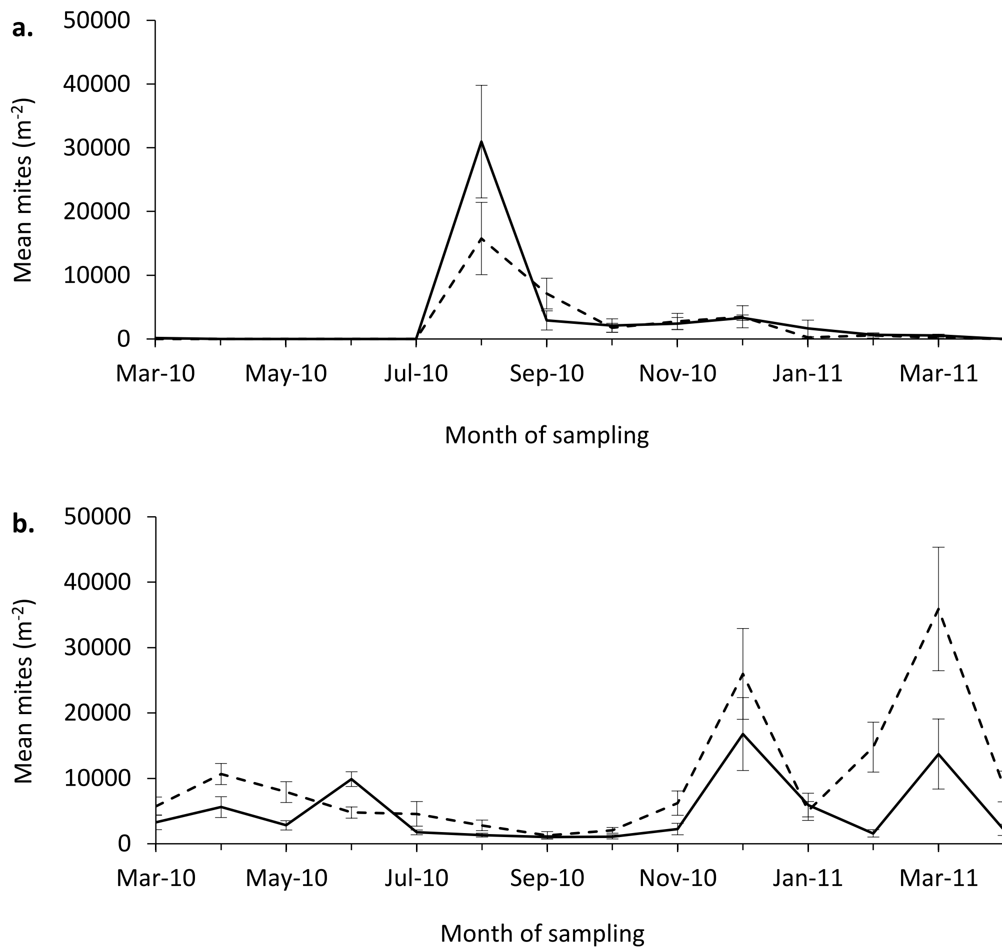

Fig. 3.9. Abundance plots of the two most common mites encountered on the two green roofs between March 2010 and April 2011. Solid line denotes Roof A, dashed denotes Roof B and error bars represent SEM. (a) *E.viridis* (b) Scutoverticidae.

Mite abundance was not affected by any of the variables investigated. No relationship was found between mite abundance and substrate water content or temperature. No association between mites and plant cover, plant diversity or mycorrhizal colonisation of nearby roots was found either. However mites showed a strong preference for the moss fraction of the habitat ($F_{1,\ 44}$ = 34.3, p < 0.001) (Fig. 3.7), creating a clear spatial separation between mites and collembola.

Mites were more diverse than collembola, reaching a maximum of 0.7 in September 2010 but decreasing to 0 in June 2010 (Fig. 3.10). Mite diversity remained high over winter and also peaked in early and late summer. There was no significant difference in diversity or seasonal pattern between roofs.

Mite diversity decreased with increasing daily (R^2 = 0.135, $F_{1, 286}$ = 44.809, p < 0.001) and monthly (R^2 = 0.1, $F_{1, 310}$ = 25.9, p < 0.001) average temperature but was affected by no other factors.

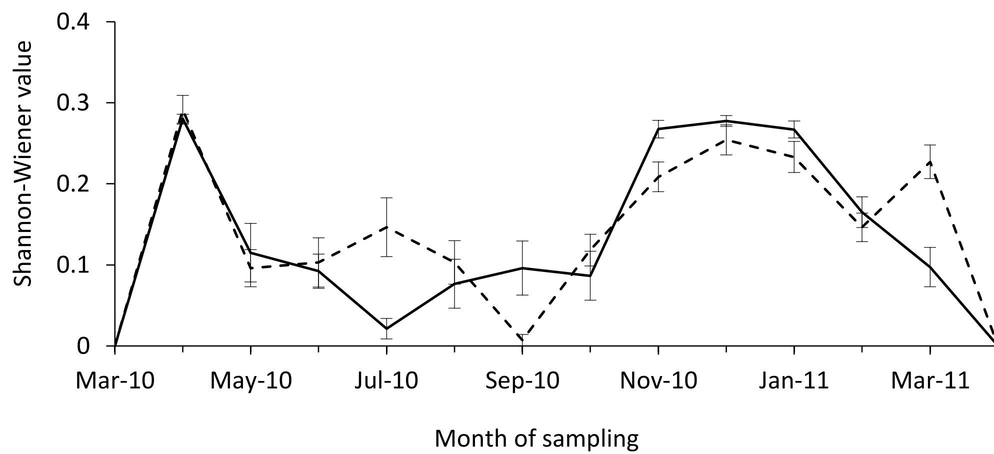

Fig. 3.10. Shannon-Wiener indices for mites between March 2010 and April 2011. Black represents Roof A, grey Roof B and error bars represent SEM.

3.3.4 The microbial community

PCA on individual fatty acids (FA's) attributed their distribution to two axes (Fig. 3.11). Axis 1 accounted for 59.1% of the variation in the data (Fig. 3.11) and axis 2 for 29.6% of the variation. The bacterial fatty acids were tightly grouped, with Axis 1 a good

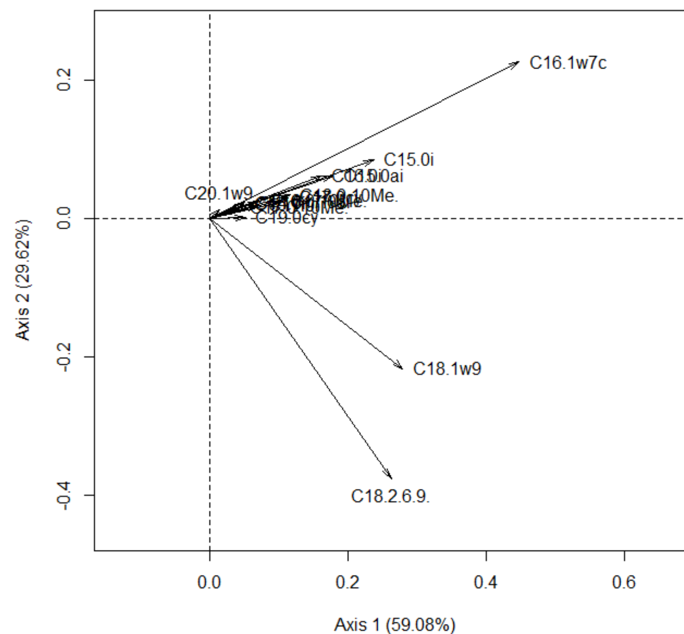

Fig. 3.11. PCA biplot of fatty acids found throughout the entire sample period.

predictor for all except C19:0cy. The fungal fatty acids were not correlated with bacterial fatty acids and were the least well described by axis 1. C20:1w9 in particular was weakly correlated with axis 1 (Table 3.3). However, axis 2 explained the variance in C18:1w9 and C18:2w6,9 better, with both of these saprophytic fungal fatty acids being strongly negatively correlated with axis 2. C20:1w9 was not significantly correlated with axis 2, nor was C19:0cy, suggesting that these fatty acids were not well correlated with bacteria or saprophytic fungi.

Month	Correlation
Axis 1	
June	0.52
April	0.45
August	0.37
October	-0.53
December	-0.69
Axis 2	
June	0.29

Table 3.4 Correlations between PCA axis and months in the analysis.

PCA also showed months to differ in their community composition. December's community had the strongest correlation with axis 1 (though it was negative), followed by October (negative), June, then April, with August having the least correlation and February showing no correlation. June was the only month to be described by axis 2 and this was weak (Table 3.4).

Confidence ellipses around the means of months suggest that in the winter months the community is different at each sampling point, overlapping little. Summer months, however, overlap more, suggesting the community changes little over the summer. Confidence ellipses for all summer months are larger though, suggesting that this summer population is variable (Fig.3.12).

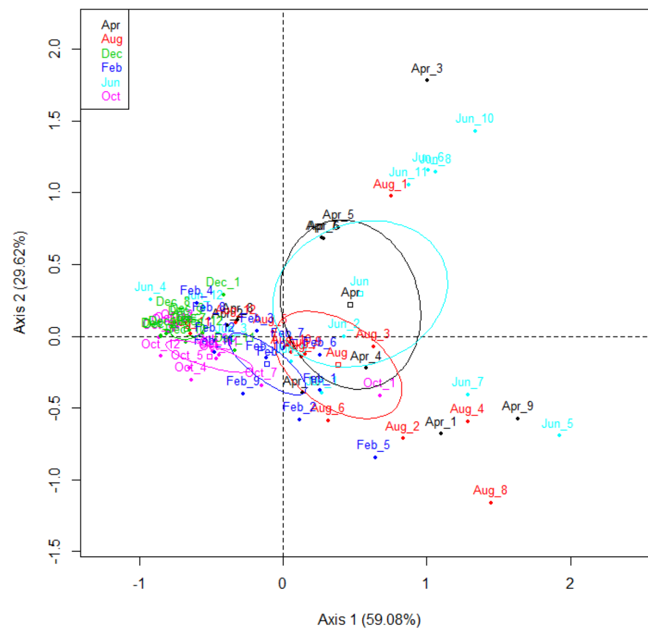

Fig. 3.12. PCA ordination plot of monthly PLFA samples. Confidence ellipses are drawn at the 95% confidence limit.

Fatty acid	Organism	Correlation
Axis 1		
C17:0cy	Bacteria – Gram -	0.92
C15:0i	Bacteria – Gram +	0.91
C17:0ai	Bacteria – Gram +	0.91
C17:0i	Bacteria – Gram +	0.90
C15:ai	Bacteria – Gram +	0.90
C16:0i	Bacteria – Gram +	0.89
C16:1w7c	Bacteria	0.88
C16:0(10Me)	Bacteria – Sulphate reducing	0.84
C18:0(10Me)	Bacteria – Sulphate reducing	0.84
C14:0i	Bacteria – Gram +	0.81
C17:0(10Me)	Bacteria – Sulphate reducing	0.79
C16:1i	Bacteria	0.79
C18:1w9	Fungi	0.72
C18:2w6,9	Fungi	0.55
C20:1w9	Fungi – Mycorrhizal	0.38
C19:0cy	Bacteria – Gram -	0.35
Axis 2		
C16:1w7c	Bacteria	0.44
C20:1w9	Fungi – Mycorrhizal	0.38
C14:0i	Bacteria – Gram +	0.37
C16:1i	Bacteria	0.34
C16:0i	Bacteria – Gram +	0.34
C15:0i	Bacteria – Gram +	0.33
C15:ai	Bacteria – Gram +	0.32
C16:0(10Me)	Bacteria – Sulphate reducing	0.31
C17:0(10Me)	Bacteria – Sulphate reducing	0.29
C17:0i	Bacteria – Gram +	0.26
C18:0(10Me)	Bacteria – Sulphate reducing	0.25
C17:0cy	Bacteria – Gram -	0.24
C18:1w9	Fungi	-0.55
C18:2w6,9	Fungi	-0.79

Table 3.5 Correlations between PCA axes and fatty acids in the analysis. Bold entries signify unique values for that axis.

Confidence ellipses around the means of each roof on the PCA suggest that the community varies little between roofs (Fig.3.13). ANOVA confirmed these observations. The total mass of identified PLFAs changed over the sample period (time: $F_{5, 50}$ = 13.816, p=<0.001), with smaller fatty acid mass in autumn and winter compared to spring and summer. Mass was high in April, June and August 2010, before significantly decreasing in October and December 2010. Two months later, in February 2011 mass once again increased. Both roofs showed the same pattern and did not differ from each other overall (roof: $F_{1, 10}$ = 0.21, p = 0.66), but seasonal changes on Roof B were less pronounced than on Roof A (time * roof: $F_{5, 50}$ = 3.12, p = 0.02) (Fig. 3.13).

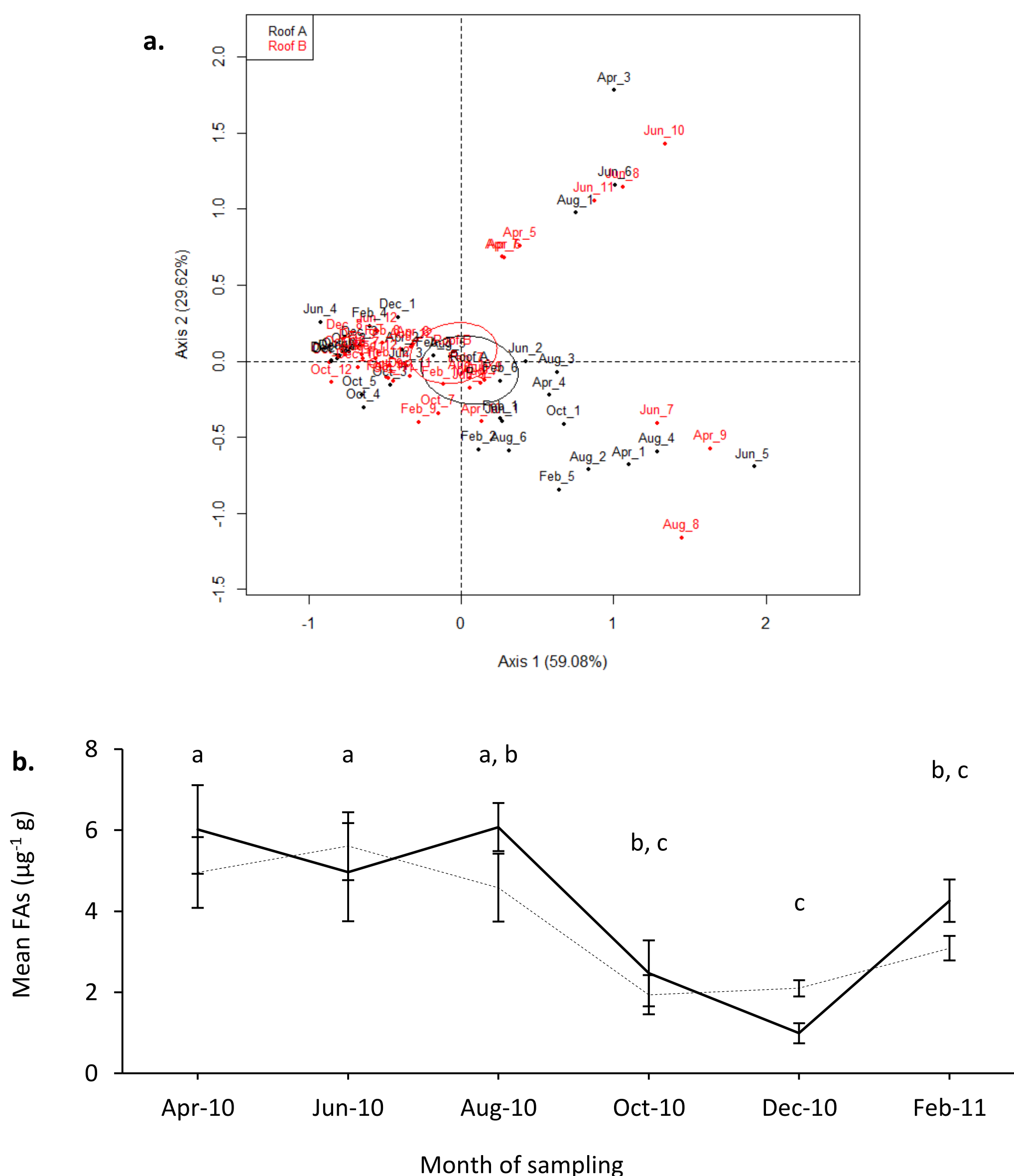

Fig. 3.13. (a) PCA ordination plot of samples on different roofs. Confidence ellipses are drawn at the 95% confidence level, the black ellipse denote Roof A, red ellipse Roof B, (b) total microbial mass over time on each roof. Solid line denotes Roof A, dashed Roof B. Error bars represent SEM.

Fatty acids attributed to bacteria, which were predominantly gram positive bacteria, showed the same pattern (time: $F_{5, 40}$ = 15.34, p = <0.001, time * roof: $F_{5, 40}$ = 3.16, p = 0.02, roof: $F_{1, 10}$ = 0.91, p = 0.36) (Fig. 3.14). The three bacterial parameters, gram positive, gram negative and sulphate reducing also followed the same pattern (data not shown).

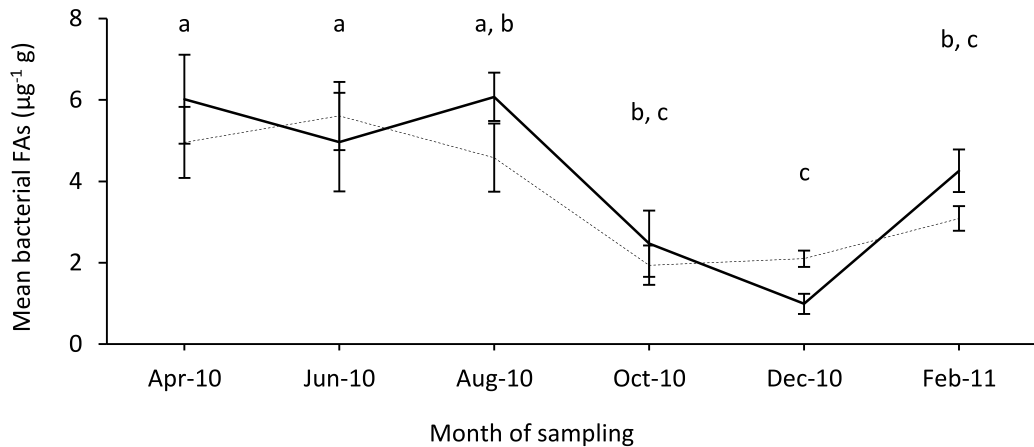

Fig. 3.14. Mass of fatty acids identified as bacterial. Solid lines represent Roof 1, dashed lines Roof 2. Error bars represent SEM. Letters denote statistically similar months according to Tukeys HSD test.

However, fungal fatty acids followed a slightly different pattern, changing over time (time: $F_{5, 50}$ = 2.83, p = 0.03), but with less pronounced differences between roofs over the year (time*roof: $F_{1, 50}$ = 1.37, p= 0.25, roof: $F_{1, 10}$ = 0.56, p = 0.47), but Tukeys HSD test failed to determine where differences between months lay. Variability seemed high in the fungal fatty acids (data not shown), and as C20:1w9 was not correlated with the other two fatty acids in the PCA, it was also plotted and analysed separately to determine if it was masking information about C18:1w9 and C18:2w6,9. Fig. 3.15 shows C20:1w9 drop in abundance in August, October and December 2010, whilst C18:1w9 and C18:2w6,9 increased in August. However, the variance remains high and so analysing these two groups separately did not produce different ANOVA results. Tukeys HSD test was still unable to detect differences between months.

Stepwise multiple regression determined that two factors explained the variance for each fatty acid group, but none very strongly. For total microbial mass, two factors explained 33% of the variance (R^2=0.33, $F_{2, 67}$ = 8.24, p = <0.001). Substrate water content had an effect on total microbial mass (β = -0.54, p = <0.001) as did mean monthly wind speed (β = 0.26, p = 0.009). For bacterial fatty acid mass, two factors explained 36% of the variance (R^2=0.36, $F_{2, 67}$ = 18.91, p = <0.001). Substrate water content had an effect on bacterial microbial mass (β = -0.57, p = <0.001) as did mean monthly wind speed (β = 0.20, p = 0.046). For fungal fatty acid mass, two

factors explained 13% of the variance (R^2=0.13, $F_{2,\ 67}$ = 5.19, p = 0.008). Mean monthly temperature had an effect on fungal microbial mass (β = 0.28, p = 0.016) as did mean monthly wind speed (β = 0.25, p = 0.030). When C20:1w9 and the C18 group were split up, the variables left in the analysis changed. The same two factors affected the C18 group, once again explaining 13% of the variance (R^2 = 0.13, $F_{2,\ 67}$ = 4.85, p = 0.01). These factors were mean monthly temperature (β = 0.28, p = 0.017) and mean monthly wind speed (β = 0.24, p = 0.04). However, 18% of the variance in the C20 group was accounted for by two different variables (R^2 = 0.18, $F_{2, 67}$ = 7.39, p = 0.001), this time mean monthly wind speed (β = -0.28, p = 0.02) and mean monthly rainfall (β = 0.27, p = 0.02). All independent variables met the assumption of homoscedacity.

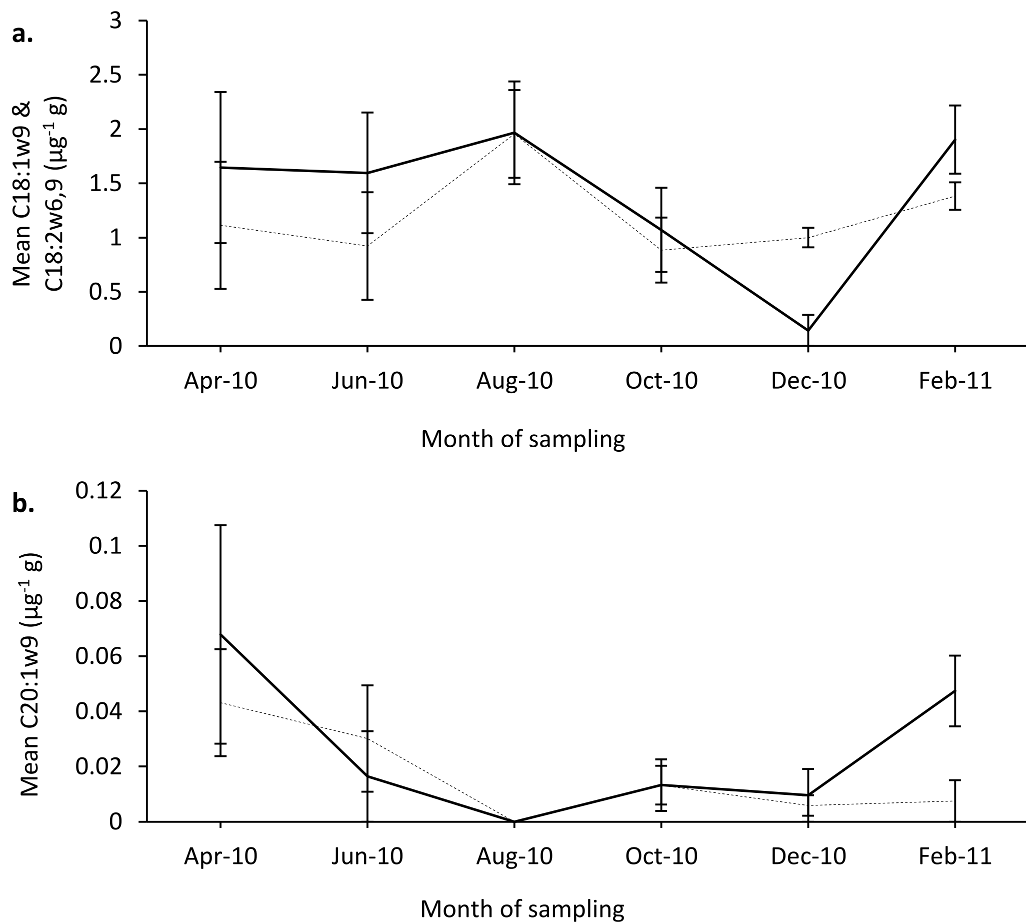

Fig. 3.15. Mass of fungal fatty acids over time. (a) Saprophytic markers, (b) mycorrhizal markers. Solid lines represent Roof A, dashed lines Roof B. Error bars represent SEM. No differences between months could be determined by Tukeys HSD test.

3.3.5 Nematodes

A range of nematodes were identified from the roof samples, belonging to both short and long lived species (Bongers & Bongers, 1990) and representing a number of different trophic groups including omnivores/predators, bacterial feeders and plant parasites (Bongers & Bongers, 1998) (Table 3.5).

Family[1]/genus[2]	Persistence score	Feeding group
Actinolaimus[2]	4	Predator/omnivore
Longoridae[1]	Not listed	Plant ectoparasitic
Bastianiidae[1]	3	Bacterial feeding
Mononchidae[1]	4	Predator

Table 3.6. Nematode families/genera encountered in September 2010 on the roof. Persistence scores are taken from Bongers (1990), whereby 1 denotes a short-lived species, whilst 5 is a long lived species. Feeding groups are assigned according to Bongers & Bongers (1998).

3.4 Discussion

3.4.1 Microarthropods

3.4.1.1 Total microarthropods

Overall, microarthropod diversity on the roofs was lower than would be expected in ground level soils, supporting the hypothesis put forward in section 3.1. Additionally, there were rarely differences between roofs. This suggests that similarly constructed roofs in a given location will likely face the same challenges and harbour similar communities, making this study relevant to a large proportion of roofs in the UK. Key functional groups of the soil biota were missing and the uniform, depauperate communities observed emphasises the importance of providing varying green roof designs within a city, to maximise diversity of communities.

The species assemblage on these roofs is comparable to other early successional environments. Similar communities are found in desert soils (Wallwork, 1972) and glacial foreland soils (Kaufmann *et al.,* 2002). In both, the fauna is dominated by mites and collembola but some other organisms, such as insect larvae, also occur. Soils with lower abundances but a higher diversity of collembola and mites (but no other species) include Antarctic soils (Convey and Smith, 1997; Caruso and Bargagli, 2007) and polluted urban sites such as roadside lawns and roundabouts (Eitminaviciute 2006a, b). In these examples mites tend to dominate over

collembolans, converse to the current study where the collembolan count was higher, if more variable, than mites. Our sites perform poorly compared to reclaimed mining sites (Dunger *et al*., 2001; Wanner and Dunger, 2002) where both abundance and diversity of microarthropods was higher.

Organisms expected in urban soils, such as Diplopoda, Isopoda and Annelida (Hartley *et al*., 2008; Santorufo *et al.,* 2012) were absent. This impoverished soil food web could have serious implications for nutrient cycling, which although mites and collembola contribute to, may be less efficient than ground level soils (Sheehan *et al.,* 2006). Despite spiders having been found in abundance on green roofs previously (Kadas, 2006), the low numbers of spiders, centipedes and predatory mites in this study indicate that the soil food web available to above-ground predators could also be inadequate and highlights that the community is probably driven by bottom-up forces more than top-down. The ecology and diversity of the roof as a whole, therefore, could be vastly improved by enhancing the soil community.

3.4.1.2 Collembola

The six collembola species encountered were cosmopolitan, native UK species (Hopkin, 2007). *S. aureus, I. palustris, B. hortensis* and *P. notabilis* have been previously recorded on green roofs (Schrader and Böning, 2006) but this is the first record of *D. pallipes* and *D. bicinctus* to the authors' knowledge.

Collembolan density was negatively affected by high temperature and low soil moisture, but the latter only below a certain threshold. Petersen (2011) found that the density of Symphypleona (*S. aureus, D. pallipes, B. hortensis, D. bicinctus*) subjected to warm, dry treatments for one month in Britain was unaffected. However, in warm, sparsely vegetated Spanish sites (more like a green roof), drought negatively affected Symphypleona, particularly *S.aureus*, despite its ability to produce drought resistant eggs (Alvarez *et al*, 1999). Contrary to our findings, *D.pallipes* was unaffected in their study. The longer period of drought in the current study, or an unmeasured buffering factor, such as food availability, could cause these disparities. Beyond what was needed to survive, collembolan abundance was driven by an unknown factor, such as competition or diet (Petersen, 2002). It is clear that on our roofs, *S. aureus* and *D. pallipes* share some tolerance to the harsh conditions.

Habitat colonisation by collembola relies on both dispersal ability and favourable conditions for persistence (Auclerc *et al.,* 2009). All six species that dispersed to the roofs were mobile, long-legged species with active furcas (jumping apparatus), yet three did not persist. Conditions on the roof are therefore likely to be unfavourable for them. *Isotomurus palustris*

is vulnerable to drought (Alvarez *et al.,* 1999), being a species found in wet habitats such as lakes (Hopkin, 2007). However, it has been found on green roofs before (Schrader and Böning, 2006) suggesting survival might be possible if drought is alleviated.

Maximum abundance of collembola was comparable to other green roofs in Hannover (Schrader and Böning, 2006) and to urban soils (Fountain and Hopkin, 2004), but neither of these studies report the drought-driven population crashes seen in populations in the current study, emphasising the importance of long-term studies in determining the stability of populations across a range of seasonal changes.

Fewer species were encountered than in Schrader and Böning (2006), whose roofs in Hannover were of a similar age, height and depth but whose substrate consisted of expanded clay or shale pellets, not crushed brick, which may have a different water holding capacity and a different nutrient profile. Molineux (2010), although not having tested expanded shale, found that different substrates do vary in these properties. Hannover also has a different climate to South-East England, though no studies have determined the effect of either climate or substrate type on green roof soil communities as yet. Diversity was lower than that expected in urban UK soils (Fountain and Hopkin, 2004), again, perhaps due to differences in drought tolerance between species.

In general, collembolan abundance was comparable to other urban habitats at certain times of the year but this was unstable and overall diversity was low. Colonisation and extinction occurred throughout the sampling period, with little persistence. A snapshot taken at one point in the year on these roofs, such as that by Schrader and Böning (2006), though valuable for producing well-rounded data sets covering different roofs, would have produced vastly different conclusions regarding the suitability of this habitat for microarthropods.

3.4.1.3 Mites

Mite density was low and consisted mainly of Scutoverticidae. Abundance was slightly lower than that of ploughed soils (Perdue and Crossley, 1989) and was comparable to terrestrial sub-Antarctic habitats (Barendse *et al.,* 2002). However, abundance has not been reported as low as the minima in the current study in either of these habitats. Even in the poorest dry Mediterranean plots, Tsiafouli *et al.,* (2005) found densities of oribatid mites (which formed the majority of our samples) higher than ours. This, with the absence of other functional groups on the roof, supports the hypothesis that harsh conditions on the roof generally have a negative effect on mites (Taylor and Wolters, 2005). It is also plausible that a lack of prey for predatory mites (Koehler, 1999) and low levels/poor quality of organic matter for detritivores

(Taylor and Wolters, 2005) produces unfavourable conditions for specialist mites. Observing the mite community at the family/species level further exemplifies this point. One mite dominated at any one time, with the two most abundant mites being characteristic of stressful environments.

Eupodes viridis has a cosmopolitan range but can be found in environments such as the sub-Antarctic (Strandtmann and Davies, 1972). Diet preference is unknown for this species (Krantz and Walter, 2009), but its physiology, with an enlarged leg IV femora, suggests an active lifestyle, therefore perhaps suggests it is predatory. Little is known about dispersal of the genus, but some are canopy specialists so dispersal from the nearby trees is plausible (Fagan *et al.,* 2006). Generation times of *Eupodes spp* are speculated to be slow, around two to three years (Booth and Usher, 1986), perhaps enabling it to survive the harsh conditions. However, it must be noted that studies into generation times in Booth and Usher (1986) were conducted in the Antarctic where metabolisms and generation times of many organisms may be unusually slow compared to warmer environments.

Plate 3.3 Oribatid mite on a lichen in May 2010 (Rumble, 2010)

The oribatid family Scutoverticidae is also found in extreme environments. Primarily inhabiting moss and lichen, they are also found on exposed rocks and rooftops (Schäffer *et al.,* 2010b) and are primary colonisers of young soils (Lehmitz *et al.,* 2011). DNA analysis has also shown them to be excellent dispersers, probably facilitated by phoresy on birds (Schäffer *et al.,* 2010a) but also capable of wind dispersal (Lehmitz *et al.,* 2011), useful strategies for roof dwellers. Scutoverticidae were unaffected by any factors in this study and are known to be tolerant to desiccation and temperature flux (Schäffer *et al.,* 2010b) as well as possessing anti-

predatory mechanisms such as thick armour (Krantz and Walter, 2009). The family are thought to be generalist feeders (Smrž, 2006). Generation times are suggested to be two to six months (Schäffer *et al.,* 2010b), which would correspond with the peaks in abundance in the current study. The dominance of xerophilic oribatids on the roof mirrors our conclusions regarding collembola; the hot, arid nature of the roof is capable of supporting only a small and unstable community.

Mite diversity was higher than collembolan diversity but also crashed in June 2010 when Scutoverticidae dominated the fauna. Diversity was lower than in reclaimed Mediterranean mining sites (Andrés and Mateos, 2006) but comparable to Swedish agricultural soils (Gormsen *et al.,* 2006).

3.4.1.4 Relationships with plants and fungi

The hypothesis outlined in Chapter 1, that a lack of organisms to disperse AM fungi spores would contribute to low AM fungal presence, was not upheld; AM fungi were extremely prevalent on the roof, reaching colonisation levels typical of highly mycorrhizal plants such as *Plantago lanceolata* (Ayres *et al.,* 2006). Whether this was present in the initial Sedum plugs or has successively colonised is unknown. The limited space available for spread of *Sedum* roots may maximise spore contact without the need for dispersing organisms. Neither collembola, nor mites were found to associate with AM fungi, also contrary to the hypothesis. The two fruiting bodies recorded on the roofs, *M. polioleuca* and *O. pyxidata*, are not mycorrhizal but may contribute to collembola diet, as they are known to preferentially feed on non-AM fungal species if present (Gange, 2000).

The plant communities on roof, aside from *Sedum*, were ephemeral, dying off in summer and winter. I hypothesised that the plant community would be unsustainable if there was found to be a lack of supporting nutrient cyclers, such as microarthropods and fungi. However, it is unclear in the current study whether plants died as a result of limited nutrients or drought. The species present mainly consisted of nitrogen fixers, suggesting at least a lack of N in the soil (Ritchie and Tilman, 1995), but they also died during times of drought. This suggests that both drought and a lack of nutrients hamper the development of a diverse and resilient plant community.

Contrary to the hypothesis that plant cover and microarthropods would be correlated, there was no correlation between total plant cover and collembola, mite or total soil microarthropod density or diversity. Schindler *et al.,* (2011) found that plant cover was correlated with soil microarthropod abundance on green roofs. However, their roofs were younger and do not

mention mosses, which had a large effect in our study. Their roofs also had a more diverse flora than those in the current study, perhaps due to differences in construction, climate or sampling season (cover and diversity of flora changed throughout the year in the current study). What drives these populations when water is not a limiting factor is, therefore, still to be discovered.

3.4.1.5 Habitat preferences

Collembola and mites showed distinct spatial separation, dominating the substrate and moss layers respectively. Scutoverticidae have a well-documented association with mosses (Schäffer *et al.,* 2010b) and the separation of the two could suggest competition avoidance. Despite inhabiting the underlying substrate, collembola were positively affected by moss cover on one of the roofs. Neither dominant species of collembola are known to be moss-associated but the moss crust could provide secondary benefits such as moisture retention (Chamizo *et al.,* 2012) or may support fungi, a collembolan dietary component (Gange, 2000).

Plate 3.4. A depression in the green roof substrate creating a microhabitat for a spider. Emulating micro-structures such as this, as well as providing other types of microhabitats, could improve microarthropod diversity on green roofs. (Rumble, 2009)

The implications for green roof design are great if these spatial separations are temporally consistent. McGeoch *et al.,* (2006) tested microhabitats in Antarctic micro-arthropod communities, finding that mites (including *Eupodes spp*.) avoid shade, whilst collembola avoid warm, dry regions. Spatial separation is therefore likely to be influenced by availability of

suitable microhabitats and emphasising these in green roof designs to ameliorate the effects of warmth and drought could enhance the microarthropod community. It is likely that once suitable habitat is provided on green roofs, further species changes will occur as food availability becomes a limiting factor. This may be where we see effects of plant and fungal diversity on microarthropods, rather than the ability to survive harsh conditions. By enhancing the soil food web, we could directly enhance above-ground biodiversity and enable green roofs to realise their ecological potential (Cook-Patton and Bauerle, 2012).

3.4.2 The microbial community

The two green roofs were bacteria dominated, with nearly twice the bacterial mass on average than fungal mass, as would be expected from an early successional soil (Bardgett *et al.,* 2002; Ohtonen *et al.,* 1999). Within the bacterial community, gram positives dominated while gram negative bacteria and sulphate reducing bacteria were at lower mass and at similar levels to one another. Mass of fatty acids was extremely low compared to ground level soils, at around a quarter of the mass found in grasslands (Bardgett and McAlister, 1999) and a sixth of that found in forests (Myers *et al.,* 2001). However, mass was double that found in Antarctic soils (Malosso *et al.,* 2004) and comparable to other green roof soils (Molineux, 2010). Overall, microbes showed a summer maximum and winter minimum. In grassland soils it is more usual for soil microbes to display a spring maxima and autumn minima (Bardgett *et al.,* 1999; Grayston *et al.,* 2001) but Grayston *et al.,* (2001) note that planting regime has a profound impact on microbial structure. This may, therefore, be a pattern unique to the vegetation present on green roofs. However, the resolution afforded by PLFA allows us to draw some conclusions about the specific dynamics in different microbial groups.

PCA determined that the bacterial community drove the microbial community structure in the winter, whilst in the summer months saprophytic fungi played an equally important role. This suggests that fungi become more active during the summer months, probably due to an input of carbon from decaying ruderal plant plants and increased metabolic activity of bacteria supplying soil nutrients (Lynch and Panting, 1980; Patra et al., 1990; Blume *et al.,* 2002).

The dominating bacteria community was highest in summer, with the three bacterial parameters, gram positive, gram negative and sulphate reducing bacteria showing broadly the same pattern as one another. Bacterial PLFAs were also tightly grouped according to PCA, suggesting little variability in community structure patterns between them. The bacterial community was negatively correlated with substrate water content and increased wind. It seems, therefore, that the bacterial community is xerophilic, more successful in dry conditions

than moist. Steinberger *et al.,* (1999) analysed bacterial communities in desert soils using PLFA and found that in the areas with the least precipitation, bacterial biomass was low overall and decreased during times of precipitation. Clark *et al.,* (2009) found similar patterns in arid and semi-arid areas of the Chihuahuan desert and suggested that rainfall events may alter the bacterial community from a community consisting of drought adapted r-selected species to one of k-selected species that need moister conditions. Thus, the frequency of rainfall events leading to drought is an important factor in shaping the microbial communities. This has implications for green roof design, particularly with reference to alleviating drought. Long term changes to the soil moisture holding capacity of the substrate is likely to alter communities as a whole, perhaps increasing biomass, but infrequent watering of the substrate is likely only to destabilise the current microbial community, lowering microbial mass after watering.

In general it seems that, unlike the majority of the microarthropods on the roofs, the microbial community is low in abundance but adapted to the arid conditions. However, it is important to note that some of the microarthropods on the roof also increased during times of higher soil moisture, perhaps increasing grazing pressure on bacteria (Bell *et al.,* 2008). This would significantly affect the interpretation of these results. The community shifts seen in PCA suggest that the structure of the community itself changed over time and once again this is in concordance with Clark *et al.,* (2009) as a result of drought, but also Griffiths and Bardgett (1997) as a response to grazing. Identification of the organisms involved, coupled with *in vitro* experiments would be the only way of uncoupling this interaction.

The saprophytic fungal community broadly followed the same pattern as bacteria, reaching its lowest mass in December. However, the mass of AM fungi remained extremely low throughout the sample period, increasing only in spring of each year. Spring is when many mycorrhizas are reported to be at their maximum abundance (Allen, 1983; Lopez-Sanchez and Honrubia, 1992, Mohammad *et al.*, 1998) and so this is most likely a function of the natural cycle of the AM fungi. Fungi as a whole were little affected by the abiotic conditions measured, suggesting that their populations are driven by other factors. Responses to biotic and abiotic factors by fungi are species specific, with some species more tolerant to certain conditions than others (Bardgett, 2005). However, the lack of an effect of temperature or moisture on the green roof fungi in this instance is encouraging, as it suggests that the fungi present can cope with the harsh conditions of the roof. Zak *et al.,* (1995) have noted that although soil fungal function is water regulated, there is scope for fungal communities to adapt differently in various microclimates, including dry ones. Planting regime could, however, affect the fungal community (Kourtev, 2003; Batten *et al.*, 2006). In the case of mycorrhizas this would be dependent on perennials, self-colonising or otherwise, as according to Read (2002), ruderal

plants do not associate with mycorrhiza and the establishment of a mycorrhizal community is what drives ecosystems past the ruderal plant stage. Substrate type could also influence the fungal community as a whole; Griffin (1963) suggests that aeration is an important contributory factor to soil fungi survival and so pore size of substrates is also likely to have a profound effect on fungal communities.

As with the microarthropod community, the microbial community did not differ between roofs. Again, this suggests that roofs constructed in a similar way and of a similar age are likely to harbour the same communities and, at least in the first decade of their existence, will follow the same successional development. This emphasises the dangers of producing a monoculture at the landscape level, but also suggests that amendments to the soil in the form of microbial inoculants will likely produce similar effects across a vast number of current extensive green roofs, as the majority are constructed in a similar way. It seems that both the bacterial and fungal community are well adapted to the harsh conditions on the roof, but all microbial abundance was low. Microbial inoculants may improve this by enhancing the microbial community present.

3.4.3 Nematodes

Although only a cursory study of the nematode community was carried out, a wide range of feeding types were encountered including plant feeding, fungal feeding, bacterial feeding and predatory nematodes, suggesting that there is a healthy and diverse nematode community present on green roofs. These nematodes are a valuable source of food for many organisms higher in the food chain (Read *et al.,* 2006), and are predators of some of the target organisms in this study (Yeates *et al.,* 1993). They are, therefore, a valuable contributor to the green roof soil food web and should be studied in more detail in the future. Procter (1990) suggests that in favourable conditions, nematodes show moderate diversity and low abundance because their generalist nature means they are outcompeted by more specialist organisms. However, because of this generalist nature, in more extreme environments nematodes adapt well to climatic stress, so are often found in higher abundances and at higher diversities in these ecosystems. Thus, providing there is adequate water available for movement and feeding on green roofs, nematodes may be a more important member of the green roof ecosystem than microarthropods because of their high propensity to adapt to climatic stress. If this hypothesis proves correct, it would be expected that a high proportion of microbe feeding nematodes would be prevalent on green roofs due to their adaptability and because of the decomposer dominated food chain (Procter, 1990). Though no precise quantification of the nematodes found in this study was conducted, bacterial feeding nematodes were extremely prevalent.

However, it must be noted that nematode sampling took place in September, when substrate water content was relatively high. As nematodes are essentially aquatic and need water for movement and feeding, it cannot be assumed that there was a healthy nematode population on the roof throughout the year. Their presence after the summer drought in addition to the fact that some nematode species are r-selected generalists and some are parthenogenetic, suggest that the nematodes present on green roofs may have the adaptations for rapid population recovery necessary in this type of environment (Procter, 1990).

3.5 Conclusions

With regards to microarthropods, extensive green roofs are either in an interrupted or extremely slow successional process capable of supporting only the hardiest of soil organisms. They present a boom and bust community, with key functional groups missing, but support a few ephemeral colonisers, such as beetle and fly larvae. Few species manage to survive in the long-term due to hot, arid conditions, an impoverished soil food web and low plant diversity. Amelioration of these conditions and manipulation of the soil food web to provide a diverse food source could benefit microarthropod and plant communities on these roofs.

Water is a serious limiting factor for collembola and mites on these roofs. The development of superior water retention properties could significantly benefit microarthropod diversity and microbial abundance, but infrequent watering in times of drought is likely to damage resident microbial populations. Alternatives to crushed brick substrates are available and should be seriously considered, not only for their ability to support plant growth (Molineux *et al.,* 2009) but also for soil faunal sustainability.

Temperature was also a key factor and previous research (McGeoch, 2006) demonstrates how refugia can ameliorate unfavourable conditions, a lesson to be learnt for green roof construction. This emphasises the importance of varying green roof habitat designs as the similarities between communities on the current field sites suggest that in high density areas of green roofs of the same design, as is perfectly conceivable in London, a monoculture could develop. Thus, diversity of the green roof landscape could increase its resilience to changes, including the temperature and moisture changes that could be posed by climate change (IPCC, 2007).

Nematodes are capable of surviving in this harsh habitat and it seems that a wide diversity of feeding types existed on the current roofs studied. As an important component in the soil food web, these organisms should be studied in more detail, particularly with regards to discovering how they recover from drought stress.

The microbial community, like the mites, seem to be adapted to the arid conditions on the roof, mirroring patterns of abundance seen in other arid environments. However, microbial abundance was low, potentially having serious implications for bottom up soil food web processes. Improvement of the microbial community could, therefore stabilise higher trophic levels on green roofs.

In conclusion, I suggest that the current standard for extensive green roof design is not adequate to support a biodiverse soil microarthropod community especially in the dry Thames corridor, and that this could have detrimental effects on above-ground communities. Research into the successes and failures of other designs, such as intensive and semi-intensive systems, needs to be conducted to improve the delivery of extensive green roofs, whilst retaining the benefits of having a low cost, low maintenance system.

Increasing rooftop soil biodiversity in our cities may require not only heterogeneous designs at the roof level but also careful planning at the landscape level, rather than accepting a monoculture of industry standards.

Chapter 4

Microbial inoculant trial, *in vitro*

4.1 Introduction

The evidence from having characterised the soil community on two green roofs in Chapter 3 suggests that both the microarthropod and microbial community is low in abundance and is not sustainable. Drought is a limiting factor but alleviating this requires changes to the structural properties of the substrate or roof design, rather than watering in times of drought as there is a risk of diminishing the already low numbers of microbes (section 3.4.2).

Little has been determined so far about how microbes, microarthropods and plants relate to one another and if the low microbial mass on green roofs has a negative impact on soil microarthropods and plant life. Cook-Patton and Bauerle (2012) suggest an exploration of animal-plant interactions on green roofs needs to be performed, combined with studying ways of enhancing diversity. Manipulating the soil food web from the level of a primary consumer may help do this.

As discussed in section 1.4 there is much evidence to suggest that above and below-ground processes are inextricably linked (Wardle *et al.*, 2005). However, relationships between the two are often difficult to determine due to the cryptic nature of soil. The vast majority of soil food web manipulative experiments centre on removing or adding soil components and observing changes in the flora and fauna and much can be gleaned about soil food webs in this way.

Chen and Wise (1999), for example, exploring whether soil food webs are bottom-up controlled, added nutrients to the soil in the form of mushrooms, potatoes and instant *Drosophila* medium (Formula 4-24, Carolina Biological Supply, Burlington, N. Carolina). They then studied soil arthropod communities to determine if changes in populations could be noted. Most groups studied in this experiment increased in number with the additional nutrient input, from grazers of the microbial community, right up to predators. Other studies testing the same nutrient addition principal have found similar results (Kajak, 1981; Davidson and Potter, 1985). Sibi *et al*., (2008) focused on the abundance of *Trichoderma harzianum* in particular, after adding organic matter to soils, finding an increase with certain types of organic matter. This increase in *T. harzianum* not only increased mycophagous mite and saprophytic nematode populations, but later predatory mites and nematodes also increased.

Taking a slightly different approach to answering the same question, Scheu and Schaefer (1998) added carbon (glucose), nitrogen (as NH_4NO_3) and phosphorus (as NaH_2PO_4) directly to soil, so as not to influence soil structure. Additionally, they analysed changes in the microbial community as well as the arthropod community. They found that microbial mass increased

with the addition of carbon and nutrients, with the highest increase occurring with all three amendments. However, they did not see resultant increases in macro and microarthropods. Biomass of earthworms, for example, increased when carbon alone was added, but decreased in the presence of the other two nutrients. Responses within the earthworm community were also species specific. Scheu and Schaefer (1998) hypothesise that earthworms may be in competition with soil microbes for resources in this instance. Millipedes increased with phosphorus application but Isopods barely responded to treatments and centipedes were negatively affected by carbon addition. Maraun *et al.,* (2001) repeated this experiment, adding the analysis of microarthropods, nematodes and protozoa. Oribatid mites declined under additional carbon, both alone and when phosphorus was added. Collembola also suffered under the mixed carbon and phosphorus treatment. However, protozoa and nematodes increased with all three treatments, either in combination or singly. Predatory mites did not respond to any of the treatments. Maraun *et al.,* (2001) suggest that the negative effects on collembola and oribatid mites were due to increased bioturbation and mucus excretion by earthworms under the treatments and that the increased soil moisture caused by earthworms also benefited nematodes and protozoa, although earthworms were not studied in this paper.

Altering nutrient inputs in the soil can, therefore, be a useful tool in determining the links between trophic levels, particularly with regard to competition for nutrients. However, there is evidence that a slight increase in leaching occurs after the addition of fertiliser to green roofs due to the free draining nature of the soil (Berndtsson, 2010). Additionally, fertilisation may need to be applied more than once which is not necessarily a sustainable solution for green roof development.

Both Chen and Wise (1999) and Scheu and Schaefer (1998) found that the microbial community was a vital factor in these soil networks and, as seen in section 3.3.4, that the microbial community on green roofs is less abundant than we would find in ground-level soils. Though a low nutrient content no doubt influences this, it is also possible that the manufacturing process for green roof substrates, where substrates are fired (Emilsson, 2008) eliminates the microbial community to begin with. Therefore adding a microbial community to green roofs could aid nutrient cycling and thus benefit both the plant and animal community. Molineux (2010) found that the addition of microbial inoculants significantly increased soil organic matter in shallow green roof soils. The addition of a multispecies microbial inoculant (compost tea) also increased available phosphates. However, the results were not straightforward with the addition of both compost tea and mycorrhizal fungi causing increased nitrate levels in one substrate but decreasing them in others. Potassium also decreased when

both inoculants were added, regardless of the substrate. This shows that there is the potential for microbial inoculants to alter green roof soil food webs, but whether this is positive or negative is to be determined. The lack of earthworms in the current study also makes the results of inoculant addition unpredictable. Scheu and Schaefer (1998) found that the microbial and macroarthropod communities were not linked, probably because of the presence of earthworms and, as previously mentioned, Maraun *et al.,* (2001) hypothesise that earthworms interact significantly with microarthropods. However, as an earthworm-free zone, it is plausible that direct benefits of a functioning primary consumer group on green roofs could benefit microarthropods, especially those feeding directly on the microbial community. To the authors' knowledge, there have been no soil food web experiments to determine if the addition of soil microbes has an effect on microarthropods.

An additional reason for altering the microbial community is that some commercial inoculants, including mycorrhizas and other microbes, are already used in the green roof industry (Circle Organics, 2010; Motherplants, n.d.) and inoculant manufacturers are keen to determine if a wider range of inoculants would be successful in this environment. To date, no intensive tests of the suitability of commercial inoculants on extensive *Sedum* roofs have been conducted. Molineux (2010) applied a commercial mycorrhiza mix and compost tea to biodiverse roofs in London and used a bait plant, *Plantago lanceolata*, to determine if plant growth was affected. She found that the addition of mycorrhiza increased plant height, but only in the first year of application. She also reported an antagonistic relationship between the two inoculants and an increase in species richness after the addition of mycorrhiza. Interestingly, although increased growth was seen in the mycorrhizal treatments, it was the compost tea that produced the highest level of mycorrhizal colonisation, emphasising the point that root length colonised does not necessarily translate to increased plant growth. Applications of inoculants to other environments have also shown mixed results and some authors have recommended that testing be carried out on each environment before industrial scale application (Corkidi *et al.*, 2004). Golf courses provide a good analogy for green roofs as they too have free-draining soil and much work on commercial inoculants has been done in this area, including under low nutrient input regimes. Butler and Hunter (2007), for example, found that the addition of microbial inoculants increased plant tolerance to stress, although questioned the ability of mycorrhiza to colonise roots in this environment.

In the current study, three commercial inoculants were used: A *Trichoderma* mix, a bacteria mix and a mycorrhizal mix, the constituents of which are outlined in Table 4.1. Mixes of inoculants have been proposed as a way of ensuring that the species specific responses between microbes and resident organisms are catered for, with the likelihood that at least one

combination of microbes will be beneficial to one another (Koomen, 1987). However, Molineux (2010) warns that antagonistic effects due to competition can also be seen. The outcome of this experiment will therefore, determine if these inoculant mixes are appropriate or if single species need to be investigated.

Inoculant	Species	Concentration (propagules g^{-1})
Trichoderma treatment	*Trichoderma harzianum*	$5x10^{-8}$
	T. viride	$5x10^{-8}$
	T. koningii	$5x10^{-8}$
	T. polysporum	$5x10^{-8}$
Bacterial treatment	*Bacillus subtilis*	$2x10^{-9}$
	B. laterosporus	$2x10^{-9}$
	B. licheniformus	$2x10^{-9}$
	B. megaterium	$2x10^{-9}$
	B. pumilis	$2x10^{-9}$
Mycorrhizal treatment	*Gigaspora margarita*	>4.4
	Glomus aggregatum	>48.4
	Glomus brasilianum	>4.4
	Glomus clarum	>4.4
	Glomus deserticola	>4.4
	Glomus etunicatum	>48.4
	Glomus intraradices	>48.4
	Glomus monosporus	>4.4
	Glomus mosseae	>48.4

Table 4.1. Species present in inocula and their concentration according to the suppliers, Symbio Ltd. (Wormley, Surrey).

Trichoderma have been shown to increase tolerance to plant disease (Papavizas, 1985; Mousseaux *et al.,* 1998; Cuevas *et al.,* 2011) and as they are saprophytic fungi, should also benefit fungal feeding microarthropods (Sibi *et al.,* 2008). *Trichoderma* have also been reported to alleviate abiotic stress (Mastouri *et al.,* 2010). *Trichoderma* are hypothesised to have an ability to survive in a wide range of ecological niches due to their metabolic versatility, ability to degrade various substrates and their resistance to microbial inhibitors (Papavizas,

1985), making them a robust choice for green roof application. The successful application of *T. harzianum* to plants in drought stress experiments (Harman *et al.,* 2004) suggests that it is drought tolerant. *T. viride* and *T. koningii* have been found in desert ecosystems (Durrell and Shields, 1960; Guiraud *et al.,* 1995), again suggesting they can cope with the arid conditions on green roofs, although *T. viride* is more commonly found in temperate environments (Klein and Eveleigh, 1998). *T. polysporum* may be restricted to cooler climes (Danielson and Davey, 1973; Klein and Eveleigh, 2002). *T. viride, T. polysporum* and *T. koningii* are capable of growing at temperatures as low as 2˚C (Tronsmo and Dennis, 1978) so may also fare well for most of the winter. Consequently, this mix should be robust to the extreme changes in temperature seen on green roofs.

Bacteria of the genus *Bacillus* have been shown to have various beneficial effects on plant growth depending on species. All five of the species used in this study are associated with the rhizosphere and all are found in field soils (Andrade *et al.,* 1997; McSpadden Gardener, 2004). They could, therefore, introduce a natural bacterial community to the green roof soil. They broadly fall into the group of plant growth-promoting rhizobacterias (PGPR's), groups of bacteria indigenous to soils that are studied for their growth promoting qualities (Siddiqui, 2005).

B. megaterium and *B. thuringiensis* have been shown to be effective phosphate solubilisers, although not always with subsequent increases in plant growth (Freitas *et al.,* 1997). *B. thuringiensis* is an effective biocontrol agent (Raddadi *et al.,* 2012). *B. subtilis* has been found to have both plant growth promoting and disease suppressive effects (Baker *et al.,* 1985). *B. laterosporus* has been patented as an agent for maintaining alkaline pH, nutrient cycling and inhibiting soil pathogens (O'Donnell, 1995). *B. pumilus* and *B. licheniformis* have been shown to produce the plant growth hormones, gibberelins (Gutiérrez-Mañero *et al.,* 2008). All five species are mesophilic, preferring temperate environments (Knight and Proom, 1950; Madigan *et al.,* 2011), though *B. licheniformis* has been shown to be thermotolerant (Raddadi *et al.,* 2012). All these bacteria are able to survive the worst of conditions by sporulating (Holt and Leadbetter, 1969; Pham *et al.,* 1995; Smirnova *et al.,* 1996; Hecker and Völker, 2001).

Mycorrhiza can increase nutrient uptake in plants (Smith and Read, 1997) and give them greater resistance to drought (Davies *et al.,* 1992), pathogens (West, 2002), and herbivores (Koricheva *et al*., 2009), as well as increasing their competitive ability (Davies *et al.,* 1992). As mentioned in section 1.4, mycorrhiza form associations with most terrestrial plant species (Gerdemann, 1968), but plants may benefit most from a particular species (Wardle *et al.,* 2004). However, it is unknown which of these species best associates with the *Sedum spp.*

frequently used on green roofs. *Glomus mosseae* has been found to colonise *S. alfredii* in China, so may be a successful coloniser of green roof *Sedum*. Kowalczyk and Błaszkowski (2011) also found *S. maximum* to be colonised by mycorrhizal fungi in Poland and report the majority of this to be *Glomus spp. G. intraradices, G. deserticola, G. margarita* and *G. mosseae* have all been successfully applied in drought treatments, suggesting some resistance to the green roof conditions (Ruiz-Lozano *et al.,* 1995; Mathur and Vyas, 2000; Vivas *et al.,* 2003). *G. intraradices* and *G. clarum* have also been found in arid areas of China (Tao and Zhiwei, 2005), again suggesting they may be well adapted to the harsh conditions on the roof.

Combinations of these inoculants have also been shown to have positive benefits in some environments. Atef (2008) found that the addition of *T. harzianum* and *B. subtilis* were effective at controlling disease in wheat crops, particularly when used together. This combined effect against pathogens, along with increased plant growth by the two inoculants, was confirmed by de Jensen *et al.,* (2002). Additionally, bacteria, including *Bacillus spp.,* can facilitate mycorrhizal colonisation, acting as 'helper bacteria' (Frey-Klett *et al.,* 2007). They achieve this by altering nutrient mobilisation from soils and protecting plants from pathogens (Frey-Klett *et al.,* 2007) and have been shown to significantly increase the success of mycorrhizal colonisation under arid conditions (Vivas *et al.,* 2003). However, particular combinations of inoculants in specific soil conditions may react badly with one another. *Trichoderma* has, for example, been shown to have negative impacts on mycorrhizal establishment in lettuce (McAllister *et al.,* 1994). However, tested under axenic conditions, Calvet *et al.,* (1992) found that *Trichoderma* enhanced the germination success of mycorrhiza. It is therefore not only important to trial inoculants individually, but also in combination as although in some cases they may act beneficially towards one another, at other times they may not.

Though the potential positive benefits of inoculants have been outlined here, negative effects may occur in some instances, for example if pollution is present (Killham and Firestone, 1983) or if conditions differ from those the inoculants are adapted to (Compant *et al.,* 2010). This emphasises the specific responses of these treatments to their environment. It is, therefore, paramount that inoculants are tested thoroughly before widespread application: this is the aim of the following experiments.

Aside from their composition, the concentration of inoculants also affects their success. The success rate of inoculants on colonisation and improved growth varies widely in the literature (see Appendix II for application rates in the literature). The conditions under which the inoculants have been stored and transported are also a contributory factor (Schenck *et al.,*

1975; Wiseman *et al.,* 2009), emphasising the importance of testing the viability of each new batch used. As the aim of this experiment is to simulate the real-world applications of these inoculants, it was decided that in light of the variable evidence of efficacy in the literature, the manufacturers recommended dose would be used (Table 4.2). Continual monitoring would also be conducted and a reapplication would be initiated if no effect was seen. Bashan (1998) noted that microbial inoculants are more successful in sterile soils where they compete less with other microbes, adding to the hypothesis that the construction of a roof may be the best time to apply inoculants. However, as the level of microbial mass on the mature roof was also impoverished, competition may not be an important factor.

Inoculant	Bacteria	Mycorrhiza	*Trichoderma*
Manufacturers recommended rate	0.96ml in 0.6l water m^{-2}	0.2-0.3g per plant	2.46ml in 0.6l water m^{-2}

Table 4.2. Application rates for inocula, based on rates by the suppliers, Symbio Ltd (Wormley, Surrey).

Before inoculants were applied in the field (Chapters 5 and 6) it was important to obtain some idea as to whether inoculant addition would have an effect either on the microarthropod or plant community so *in vitro* trials were set up. These had limited success, particularly those using sterile substrates, with the majority of plants failing to germinate or dying soon after transplantation. One trial, however, did produce some interesting results and it is this trial that is presented here. The aim was to determine if the presence of microarthropods in green roof substrates affected the growth of *Sedum acre* and an annual coloniser to the roof, *Melilotus officinalis*, and if the addition of microbial inoculants influenced this interaction or influenced plant growth directly. This experiment also served as a pilot to determine if the inoculants to be added to the roof were viable.

4.2 Methods

To determine if the addition of inoculants would affect the growth of *M. officinalis* and *S. acre* under semi-natural conditions, plants were grown in unsterilized substrate obtained from the mature roof analysed in Chapters 3 and 6 (Roof B).

A small subsection (500g) was removed from the collected substrate and subjected to extraction by Berlese Tullgren funnel (see section 2.2.2) to determine the composition of the microarthropod community at the start of the experiment. The rest was placed into 4cm diameter pots for the experiment.

Seeds of *M. officinalis*¸ a plant previously recorded on the roof (see section 3.3.2) were obtained from Herbiseed (Twyford, Berkshire) and planted directly into pots. Previous *in vitro* experiments had found transplantation from a growing media to pots to be variable in its success (data not included). *S. acre* however was germinated in sterilised sand for ten days and transferred to pots as seedlings. Three *M. officinalis* seeds and three *S. acre* seedlings were placed in each pot, after having been dipped in treatments at the concentrations outlined in Table 4.2. Control plants were dipped in deionised water.

There were nine treatments overall: a bacteria treatment, a mycorrhiza treatment, a *Trichoderma* treatment, the three different pairs of these, a mix of all three ('all treatment'), a control with the mature roof substrate and a control with sterilised green roof substrate (substrate that had been autoclaved twice, each time at 121˚C for 20 minutes). There were five replicates of each and these pots were placed in randomised blocks in boxes covered with fine mesh, to prevent the escape of microarthropods. Sterile pots were to be analysed to determine if microarthropods could move between pots and to determine if plants grew better in sterile substrate. Additionally, an extra box was set up with eight sterile pots to compare with those in boxes with unsterilized substrate. In total, there were 98 pots in this experiment.

The boxes were placed in a controlled temperature room set to a 16:8 hour light/day cycle at approximately 23°C and watered every three days. Date of germination and deaths were also recorded every three days. Plants were harvested after 12 weeks. Mean shoot length per pot, tallest shoot and shortest shoot were measured as well as fresh weight. Dry weight was obtained as in the previous experiment and mycorrhizal colonisation was determined as discussed in section 2.3.2. Substrate from each treatment was also pooled together and microarthropods were once again extracted as outlined in section 2.2.2.

Data analysis was performed using SPSS 19.0. Each variable was analysed separately using a multivariate factorial ANOVA with the addition of bacteria, mycorrhiza and *Trichoderma* as main effects. Data was transformed if necessary by square root transformation or *Ln*+1 transformation to meet the assumptions of ANOVA, but if this was not possible main effects of treatment were obtained using a Kruskal-Wallis test. Additionally, the effect of sterilisation was also tested, again using a one-way ANOVA, with type of control as a main effect (those pots that were isolated and sterilised, those pots that were sterilised but mixed with unsterilized pots and the unsterilized controls). If assumptions of normality were not met and data could not be transformed, a Kruskal-Wallis test was used instead.

4.3 Results

Of the 53 seeds of *M. officinalis* planted, only 40 germinated and by the end of the experiment most of these seedlings had died. Growth parameters could not, therefore, be measured and so instead the germination success of *M. officinalis* was analysed. *Sedum acre,* however, grew successfully and so growth parameters were measured.

At the end of the sample period, only the oribatid mite belonging to the family Scutoverticidae and a member of the Tydeidae family were found in pots. The latter was found in abundance in the controlled temperature room but was absent from initial analysis of the substrate, so was considered to be a contaminant species. Scutoverticidae ranged from 0-8 individuals per pot with an average of 1±2 per pot. Scutoverticidae were found in sterile controls within the non-sterile boxes but were not found in the sterile box. They were also found in all treatments. There was a suggestion that numbers were lower in the mycorrhizal treatment and in the sterile controls (in mixed boxes) than in other treated pots but numbers were too low to analyse with confidence (data not shown).

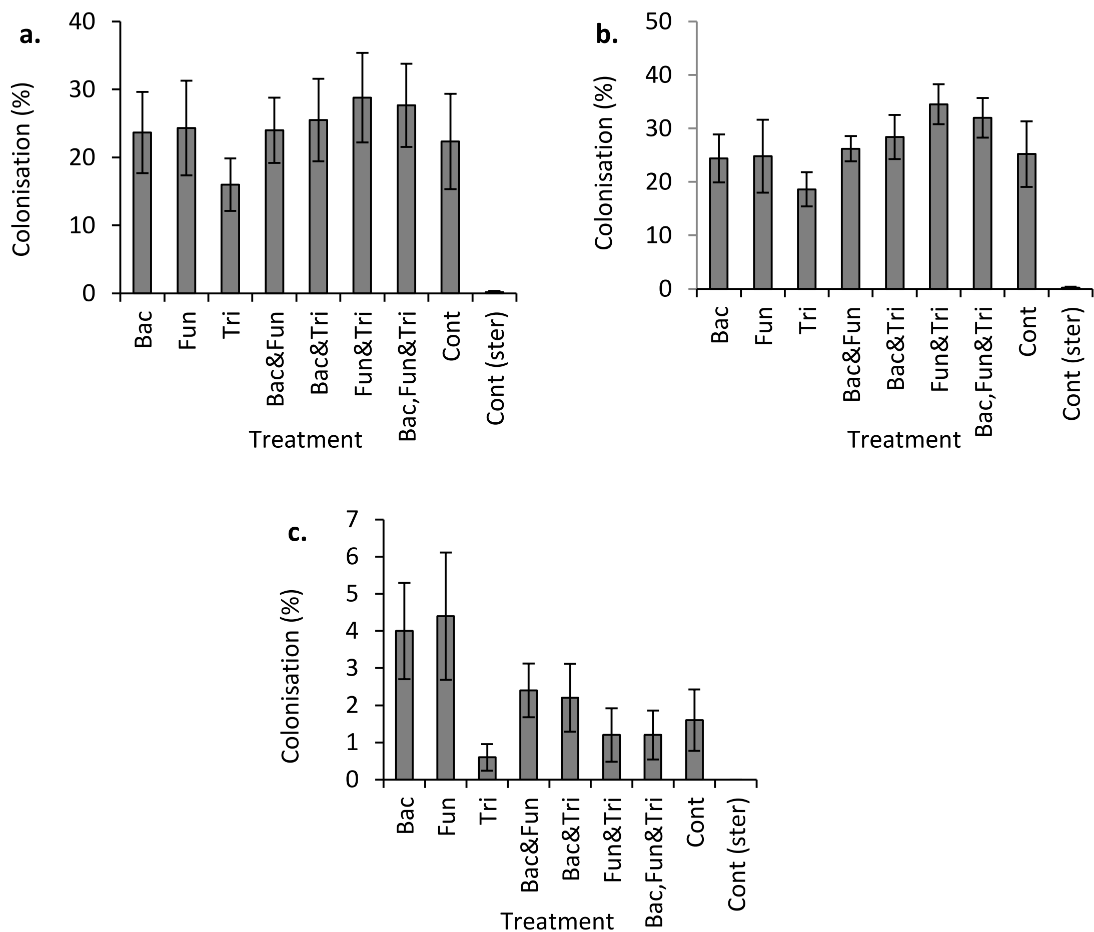

Fig. 4.1. Percentage colonisation per treatment of (a) total mycorrhiza (b) hyphae alone and (c) vesicles. Error bars represent SEM.

The germination success of *M. officinalis* was not affected by any of the inoculants added. For *S. acre*, mean shoot length per pot, fresh weight, dry weight, smallest shoot, longest shoot and shoot range were also unaffected by the inoculants. Mycorrhizal colonisation, however, was affected by the addition of inoculants. The addition of the mycorrhizal treatment produced significantly higher mycorrhizal colonisation (total mycorrhizal structures) ($F_{1, 36}$ = 4.50, p < 0.05) and percentage counts of hyphae alone ($F_{1, 36}$ = 4.49, p < 0.05) (Fig. 4.1). However, the addition of mycorrhizal fungi had no effect on vesicle number produced. Conversely, the addition of *Trichoderma* had a negative effect on vesicle production ($F_{1, 36}$ = 5.06, p < 0.05), as did the combination of bacterial and fungal treatments ($F_{1, 36}$ = 5.72, p < 0.05) (Fig. 4.1).

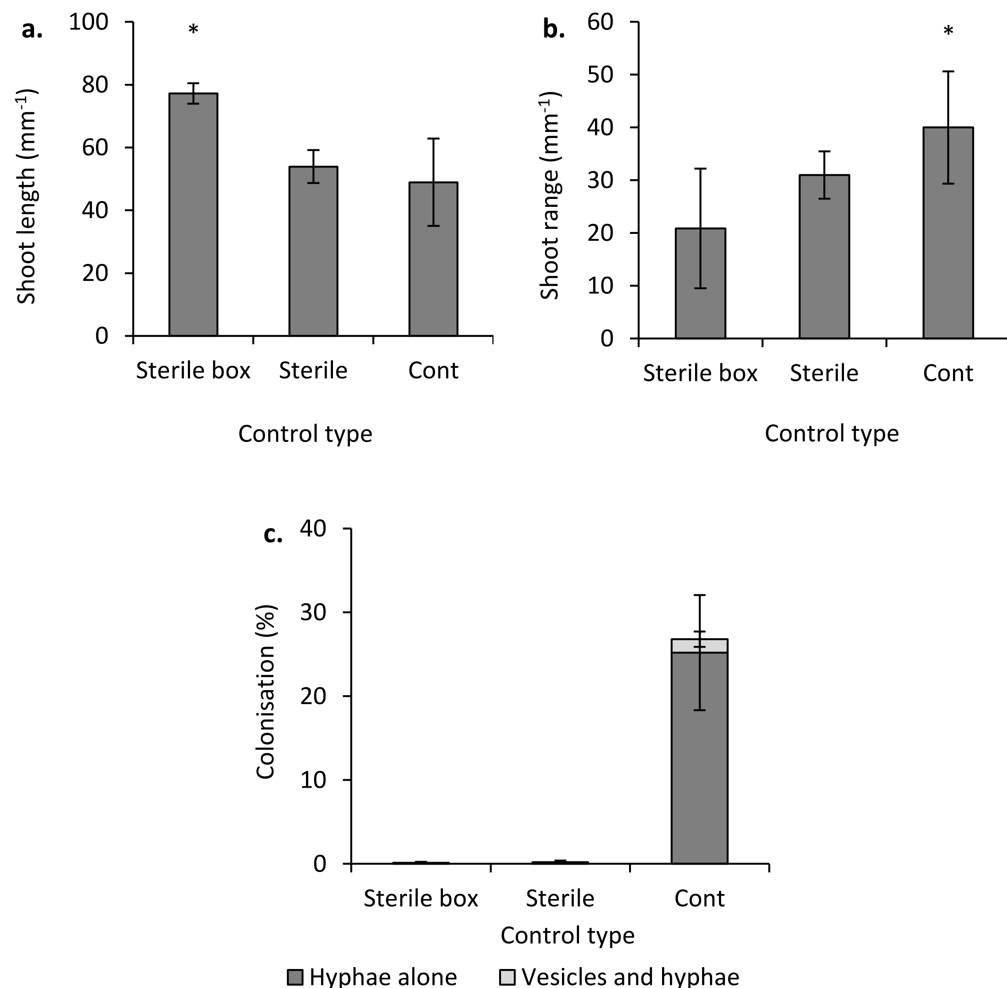

Fig. 4.2. (a) Mean shoot length of *S. acre* per control, (b) Mean shoot range of *S. acre* per control, (c) Percentage colonisation of *S. acre* roots by AM fungi. Error bars represent SEM.

Some differences in growth characteristics and mycorrhizal colonisation were also found between isolated sterile pots, sterile pots that were mixed with unsterile pots and unsterilized controls. Sterilised, isolated pots had a higher mean shoot length than the unsterilized controls ($F_{2, 15}$ = 4.38, p < 0.05) (Fig. 4.2) and those pots that were isolated and sterilised had a lower

range of sizes within each pot than both the sterilised, mixed pots and the unsterilized mixed pots ($F_{2,15}$ = 19.86, p < 0.001) (Fig. 4.2). There were no differences with regards to fresh weight, dry weight, longest shoot or shortest shoot.

Mycorrhizal colonisation was significantly higher in the unsterilized pots (total colonisation: $F_{2,15}$ = 16.46, p < 0.001, hyphae alone: $F_{2,15}$ = 469.15, p < 0.001, vesicles: $F_{2,15}$ = 4.03, p < 0.05) (Fig. 4.2). Mycorrhiza was not totally absent from the sterilised pots, even when isolated but was extremely low in these pots (sterilised mixed pots: $\bar{X}$= 0.02 ±0.02, sterilised isolated pots: $\bar{X}$ = 0.01 ±0.01) and within the limits of experimental error (Fig. 4.2).

4.4 Discussion

4.4.1 Microarthropods

The presence of a member of Tydeidae, a family of mites, in this experiment, along with the knowledge that this genus had not been previously found on the roof but was abundant in the CT room suggests that the mesh used to retain microarthropods in the experimental boxes was insufficient. Few microarthropods were present at the end of the study and, though it is possible that many had died, the primary cause is most probably that they escaped from the boxes. The effect of microarthropods on the plants in this trial is, therefore, likely to be negligible. However, the substrate used was still likely to contain microbes and nutrients comparable to field soils and so some conclusions can still be drawn. That these conclusions should be interpreted with caution, as with any *in vitro* experiment designed to mimic field soils, should be kept in mind.

One microarthropod species had not escaped or died and was found in all pots, that of the oribatid mite of the family Scutoverticidae. Although numbers were extremely low, there was a suggestion that Scutoverticidae were less prevalent in sterile controls and mycorrhiza treated plots. Though little can be concluded based on abundance in pots in this study, the presence of Scutoverticidae in sterile pots (in mixed boxes) suggests that microarthropods did travel between pots and that this species may colonise substrates with little or no microbial life or detritus and may, therefore, be an important primary coloniser. It has been mentioned in section 3.4.1.3 that this species has been found on bare rock surfaces and even bare roofs previously (Schäffer, 2010).

4.4.2 Effect of inoculants

The only effect elicited by the addition of inoculants on *S. acre* in this trial was a change in levels of mycorrhizal colonisation, demonstrating once again that *S. acre* is strongly mycorrhizal, contrary to previous findings (Harley and Harley, 1987). Mycorrhizal colonisation occurred in all pots (excluding the sterile controls), suggesting that a source of inocula is present in the green roof substrate. This is hardly surprising given the high levels of colonisation seen in the *Sedum spp.* present on the roof (see section 3.4.1.4). However, the pots inoculated with mycorrhiza showed higher levels of colonisation suggesting that the added species were also able to associate with *S. acre*.

Hepper *et al.,* (1988) found that mycorrhizas have different competitive abilities with regards to root colonisation, with only one mycorrhiza usually colonising roots where two species had been added. It could be that the added mycorrhiza colonised first and was more vigorous with regard to hyphal production than the native mycorrhiza, thus colonisation was higher in mycorrhizal treatments. However, it could also be the case that there was an additive effect, with both species inhabiting the rootzone of *S. acre*. Daft and Hogarth (1983) found that inoculation with two different species that were equally distributed in the soil meant that both were able to colonise. Cano and Bago (2005) and Alkan (2006) also report that mycorrhizas are able to niche partition within roots by inhabiting different areas and are, therefore, able to co-occur. It is likely that the mechanisms of co-occurrence and competitive exclusion rely heavily on a myriad of factors including the concentration of propagules in the soil, which can later influence the amount a species colonises the roots (Wilson and Trinick, 1983), time of harvest, i.e. age of root (Wilson and Trinick, 1983), soil nutrients (Thomson *et al.,* 1986), and the specific mycorrhizal (Hepper *et al.,* 1988) and plant species (Daft and Hogarth, 1983) involved. To elucidate the true mechanism in this experiment would require these factors to be investigated more thoroughly. Nevertheless, it can be concluded that in the current study, competitive interactions of co-occurrence of mycorrhizal species have little effect on early *S. acre* growth. This phenomenon has been previously reported in the literature (Medina *et al.,* 2003; Johansson *et al.,* 2004).

Vesicle formation was also affected by treatment, with vesicles in fewer numbers in both the dual mycorrhiza and bacteria treatment and the *Trichoderma* treatment alone. Vesicle number can increase under stress (Cabello, 1997) so, assuming that these effects are due to the same mechanism in each treatment, it could be concluded that these inoculants reduced the impact of stress. The source of this stress is unknown but the small pot size in this experiment may mean that nutrients were limited. In addition, the abundance of legumes on green roofs, seen

in section 3.3.2, suggest that the substrate is already nitrogen limited, as legumes are likely to outcompete non-nitrogen fixing plants (Crews, 1999). In the context of this experiment there are few avenues for new nutrient sources to be added to the soil, no doubt exacerbated by the small pot size. Plants and microbes compete for nitrogen in soils (Kaye and Hart, 1997) and carbon losses are also proposed to be higher in nutrient poor soils (Fontaine *et al.,* 2004), so stress caused by a decrease in available nutrients is plausible.

However, a separate mechanism for each treatment could also cause a decrease in vesicle abundance. With regards to the mycorrhiza and bacteria treatment, Abbott and Robson (1981) and Hepper *et al.,* (1988) hypothesise that vesicle formation can be indicative of certain species and thus, changes in frequency could denote species changes within the root. If this was the mechanism, it is plausible that the addition of bacteria to the mycorrhiza treatment competitively favoured a mycorrhizal species which has lower vesicle formation than other pots. The *Bacillus spp.* added have been shown to facilitate the colonisation of roots by mycorrhiza (Toro, 1997; Medina, 2003; Vivas *et al.,* 2003a; Vivas *et al.,* 2003b), but these interactions vary between different fungal, bacterial and plant species (Medina *et al.,* 2003).

Trichoderma could also have a direct effect on vesicle formation in the mycorrhiza as mycorrhiza and *Trichoderma* have also been shown to be antagonistic to one another, with *Trichoderma* shown to reduce colonisation of AM fungi and perforate and damage mycorrhizal extraradical hyphae in some situations (Rousseau *et al.,* 1996; Green *et al.,* 1999). These antagonisms could conceivably cause stress within the mycorrhiza and hence decrease vesicle production.

Though inoculants had no effect on plant growth, different substrates did. Interestingly, those plants that were grown in sterile conditions in sterile boxes grew taller than those that were unsterilized and the growth range was lower in these pots than in both the sterile and unsterile controls in mixed boxes. The lack of nutrients in a sterile soil could have meant that plant growth was less in this trial. However *Sedum spp.* did better in the sterile soil than the non-sterile. It is conceivable that *Sedum spp.*, native to primary successional soils (Houle, 1990), may do better where there are fewer nutrients and, more importantly, fewer soil microbes with which to compete. Though it may be argued that more nutrients are present in the field substrate due to an increase in detritus, increased carbon can sometimes have a negative effect on available C to plants, due to competitive soil microbes (Fontaine *et al.,* 2004) and in this study it seems that *S. acre* does worse in field soils than sterile soils. Boorman and Fuller (1982) found that the frequency of *S. acre* in a sand dune was unresponsive to increased nutrients and in some years was even at lower frequencies when fertiliser was

applied. The authors hypothesised that within a plant community, competitive exclusion was probably the driver, but as no other plants were present in the current study I suggest that competition with microbes is far more likely[1]. Studies on green roofs have hypothesised that the addition of nutrients has no effect on *Sedum spp.* establishment, but that it can have an effect on fresh biomass (Emilsson *et al.,* 2007). Emilsson *et al.,* (2007) hypothesised that an increase in nutrients increases water storage in tissues of *Sedum spp.* This would explain the height increase in this study, caused by increased nutrient uptake due to less competition between the plant and soil microbes.

S. acre's success in sterile soils may also explain why inoculation with mycorrhiza seems to have little effect on growth, as it may be sufficiently good at obtaining nutrients without it. In some instances, this can cause mycorrhiza to become parasitic on its host as the plants net gain associated with mycorrhizal symbiosis decreases (Johnson *et al.,* 1997), again an example of nutrient competition between plants and microbes. This is not to say that natural associations between mycorrhiza and *S. acre* may not be mutually beneficial, but in this case it is unknown if the species inhabiting *Sedum spp.* roots, both *in vitro* and in field soils is the optimum mutualist species for it.

S. acre in sterilised soils was also less variable in height than in non-sterilised soils. It would appear that the effect of competitive interactions are therefore variable, either due to heterogeneity of nutrients and microbes between the pots or due to individuals of *S. acre* having varying competitive abilities. Infection of *Prunella vulgaris* with AM fungi has been shown to increase intraspecific competition (Moora and Zobel, 1996), indicating that in the non-sterile soils the mycorrhiza itself may be the cause of heterogeneous plant growth. The low incidence of mycorrhiza in the sterile pots in mixed boxes suggests that the experimental design was sufficient to prevent transfer of mycorrhiza between pots.

4.5 Conclusions

In summary, the addition of inoculants did not improve the germination success of the wildflower tested (*M. officinalis*) and this plants suitability for *in vitro* experiments is questionable due to its low survival rates. Growth of *S. acre* was also unaffected despite the fact that microbial interactions clearly occurred, and this may be due to the plant's physiology and adaptation to nutrient poor environments. The fact that some plants growth parameters were higher in sterile substrates supports this theory and suggests that *Sedum* may compete

[1] It must be noted that grazing by rabbits also occurred at the sand dune site. The literature is divided on the effect of grazing on *S. acre*, however, with some papers reporting stimulation of growth by grazing (Watt, 1957), others the converse (Pickworth Farrow, 1917).

with soil microbes. Changes in mycorrhizal colonisation are not always indicative of changes in plant growth and it is also conceivable that in a slow growing plant such as *S. acre*, effects of inoculants on growth would not be seen so early, perhaps simply due to the inaccuracy of measurements on small plants. Though this study was designed to be a pilot for field trials, it suggests that green roof conditions are difficult to replicate *in vitro* and that field trials may be more beneficial. Despite the difficulties in replicating field studies in this *in vitro* study, some effect of the addition of inoculants was seen, with added inoculants interacting with the resident microbial populations and plants. They could, therefore, produce measurable effects in the field, though it is unknown if these will be positive or negative.

Chapter 5

Application of microbial inoculants to a new green roof

5.1 Introduction

In section 3.3.3 we saw that on a mature extensive green roof the microarthropod community was impoverished and variable throughout the year. The microbial community too was lower in abundance than we would expect from ground level soils (Chen *et al.,* 2007; see section 3.4.2). Though I surmised that abiotic factors had a large part to play in this, I also hypothesised that an alteration of the microbial community may increase the tolerance of soil organisms to these abiotic factors (Yang *et al.,* 2009; see section 1.3). This could be achieved by the addition of microbial inoculants, and a detailed introduction to the use of these is outlined in section 4.1.

It is important when considering the application of microbial inoculants to green roofs that ideals of extensive green roof design are maintained i.e. to ensure that they require no, or very little, maintenance after construction. This is to ensure that the uptake of constructing these roofs by the green roof industry and by the public remains high. I therefore decided to test whether the addition of inoculants to a newly constructed roof could alter the diversity of green roof biota over the course of a green roof's development. This emulates the most probable entry point for the addition of microbial inoculants in the real construction of a green roof. Aside from testing the commercial reasons for applying inoculants at the point of green roof construction, this experiment also provides an excellent opportunity to determine how a green roof develops after construction and where source populations are found. Additionally, it allows comparisons to be drawn between green and biodiverse roofs by determining the effects of planting on soil community development, as well as enabling us to draw some general conclusions about the soil community dynamics present in green roof soils.

Previous research (Rumble, unpublished) analysed small numbers of soil and plug samples from *Sedum* farms across the UK and found that they contained microarthropods (Appendix III). It was, therefore, hypothesised that this may be a likely source of soil organisms during the roof's construction. However, as the populations in Chapter 3 were impoverished, it would also allow an insight to be gained into whether source populations are also impoverished or whether they become so over time.

Another hypothesis was that planting with *Sedum spp.* would increase the abundance of microarthropods, as plant cover could ameliorate the effects of abiotic conditions as well as change the soil structure and provide microhabitats (Sendstad, 1981; Kampichler, 1990; Kay *et al.,* 1999). However, it is generally thought that biodiversity is higher on biodiverse roofs (Molineux, 2010), another hypothesis able to be tested during the course of this experiment.

A third hypothesis was based on the fact that microbial inoculants have been shown to improve plant growth in other environments (see section 4.1) and increase soil nutrients on green roofs (Molineux, 2010). The hypothesis was, therefore, that plant growth would be higher and a resultant increase in microarthropod abundance would occur, both due to the increased plant cover and as additional soil microbes would act as an extra food source for microarthropods. This would also have benefits for the green roof industry as increased speed of *Sedum* cover is desired by customers.

5.2 Methods

5.2.1 Experimental design

A new green roof was constructed in a modular design using trays. 30 trays of dimension 0.52m by 0.42m by 0.10m were installed in June 2011 on a roof within the Royal Holloway grounds at approximately 20m from ground level, with 0.30m between each tray. Holes were drilled in each tray to allow water to drain freely and each tray was lined with a filter sheet (ZinCo SF, ZinCo GmbH, Nürtigen) to prevent leaching of particulate matter. An extensive substrate mix (Shire Green Roof Substrates, Southwater, Kent), consisting of crushed red brick with 10% organic matter (rough compost), was added to each tray to a depth of 0.08m. This depth is within the range commonly used on extensive green roofs (FLL, 2008) and has been used in previous studies (Molineux, 2010), making comparison between studies easier. The bricks that this substrate is made from are obtained from Cambridgeshire, where they are fired during the brickmaking process. Bricks that are not of a suitable standard are crushed and stored outside in 1 tonne bags, creating the potential for seeds and microarthropods to colonise prior to green roof construction. This is standard practise for green roof substrates and as this experiment is designed to replicate what would happen on a real green roof, no modifications (such as autoclaving) were applied to the substrate. The substrate was checked for the presence of microarthropods and a microbial community before being installed (see sections 5.3.1.1 and 5.3.3.1).

Twenty of the trays were planted with nine *Sedum* plugs each, three of *S. album,* three of *S.spurium* and three of *S. reflexum*[1]. These had been grown in a greenhouse by an industry *Sedum* supplier (Sedum Green Roof Ltd, East Knoyle, Wiltshire). After consultation with several green roof manufacturers (Sedum Green Roof Ltd, East Knoyle, Wiltshire; Shire Green Roof Substrates, Southwater, Kent; SkyGarden, Cheltenham, Gloucestershire) about the density at which plugs are normally planted, a distance of 0.1m between each plant was used (the quotes

[1] Except tray 22, which had four *S. spurium* individuals and two *S. album* individuals due to an error in supply number.

given varied between 0.1-0.2m). These plugs were planted uniformly, but the order in which they were planted was random. No attempt was made to remove the soil the plugs arrived in, again to replicate the normal construction of a green roof as closely as possible. Plugs were also checked for presence of microarthropods on arrival using the Berlese Tullgren funnel extraction method described in section 2.1.2 and for microbial communities by the PLFA method, also described in section 2.3.2 (see section 5.2.2 for details of plug analysis).

Planted trays were then inoculated with products manufactured by Symbio Ltd (Wormley, Surrey). Five of these trays were inoculated with a bacterial inoculant, five with a mycorrhizal fungi inoculant, five with both of these treatments and five with no inoculants (planted control) (see section 4.1 for inoculant constituents). These were applied at the suppliers recommended rate (Table 5.1) and deionised water was applied to those trays not receiving inoculants. The *Trichoderma* treatment outlined in section 4.1, was not applied to these plots due to space and time constraints.

Inoculant	Manufacturers recommended rate	Area/plants to cover	Total inoculant	Total water added
Bacteria	0.96ml in 0.6l water per m^2	2.2 m^2	2.09 ml	1.31 l
Mycorrhiza	0.2-0.3g per plant	100 plants	20-30g	1.31 l

Table 5.1. Application rates for inocula per tray. Only the bacterial solution needed to be applied in solution. The same amount of water was added to the mycorrhizal treatment and to controls to ensure consistency.

Ten trays were left unplanted. Initially, half of these were designed to include plants from another *Sedum* farm to determine if the origin of plants had an effect on the microarthropod community. However, the manufacturer was unable to deliver the plants on time and so these plots were left empty, along with another five, to determine how microarthropod communities develop with no plant cover.

All 30 trays were distributed randomly in a 6x5 grid across the roof (Fig. 5.1, Plate 5.1). Dataloggers (EL-USB-2, Lascar Electronics Ltd., Whiteparish, Wiltshire) were placed in trays 9, 15 and 26 to record temperature and humidity at the soil level every 30 minutes.

Sampling of the microarthropod and microbial communities took place every two months from September 2011 (see section 5.2.3), as did plant surveys and estimates of plant cover (see section 5.2.4), until July 2012 when plants in the trays were harvested and weighed (see section 5.2.5).

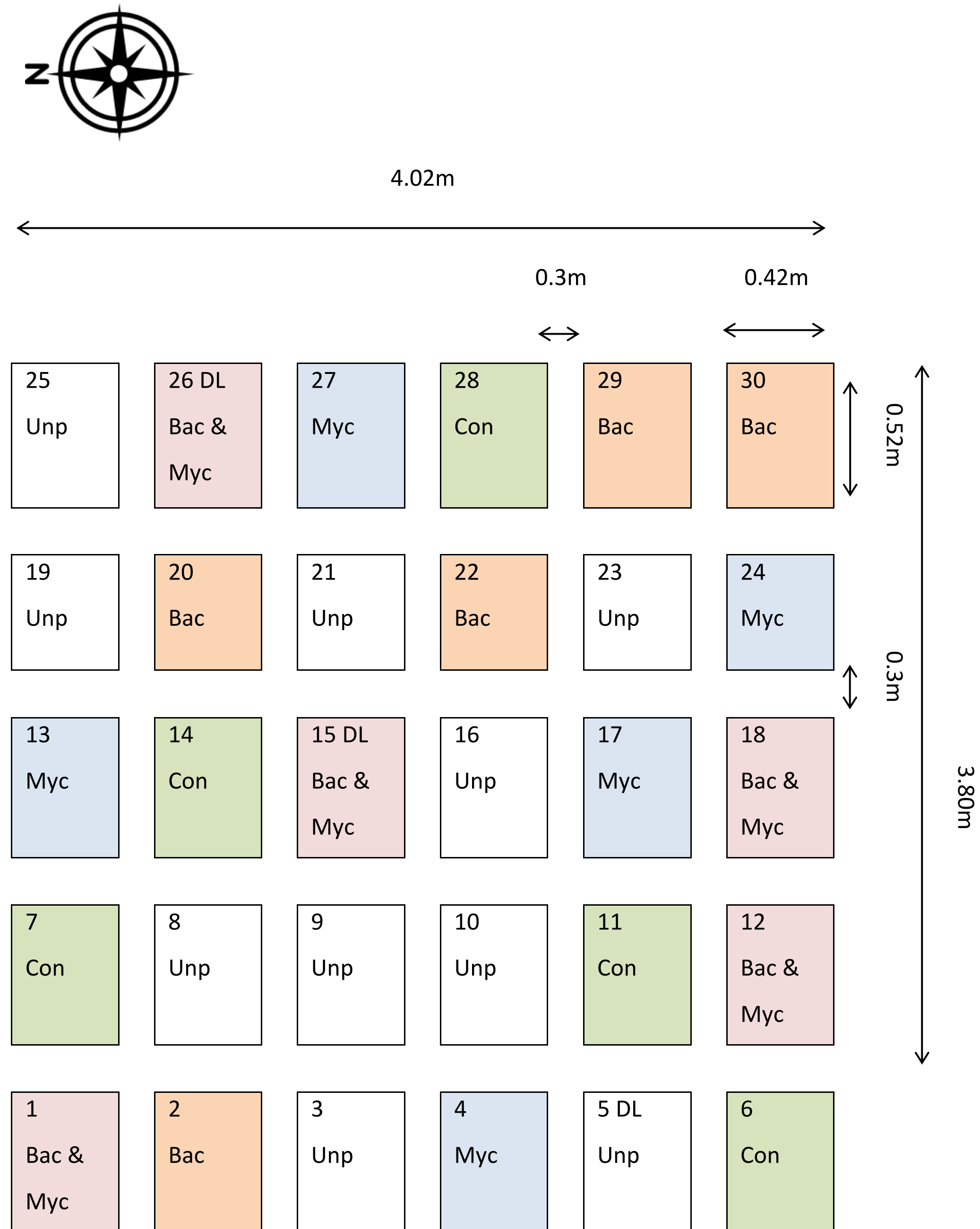

Fig. 5.1. Experimental design for the young roof experiment (not to scale). Trays were 0.52x0.42x0.10m and placed 0.30m apart. Bac denotes bacterial treatment, Myc denotes mycorrhizal treatment, Con denotes the planted controls and Unp denotes trays that were not planted with *Sedum*. DL denotes position of datalogger.

Plate 5.1. New roof at planting. 30 trays were laid out, each with 8cm of crushed red brick substrate.

5.2.2 Microarthropods present in plugs

Before the roof was constructed, samples of the substrate being used and the plugs to be used were taken to determine what microarthropods arrive in them. Five plugs of each species were taken and their vegetation removed to leave 166.38cm^3 of soil. The same volume of substrate was taken from the bags of substrate (again five samples, each from a separate bag) and both plugs and substrate were subject to extraction by Berlese Tullgren funnel and PLFA analysis, both of which are described in sections 2.1.2 and 2.3.2.

Analysis was performed in SPSS 19.0. One-way ANOVAs were performed on total microarthropods, collembola, mites and other microarthropods ('others') with the sample origin (substrate or each *Sedum spp.*) as a main effect. Differences were separated using Tukeys HSD tests. Data were normalised by square root or *Ln*+1 transformation if necessary to satisfy the assumptions of ANOVA. PCA analysis was performed in R (R Core Team, 2012) using the FactoMineR package (Husson *et al.,* 2012), with species of *Sedum* added as supplementary qualitative variables to help explain the data. Confidence ellipses were drawn around these at the 95% confidence level. Correlations between axes and microarthropods were obtained using the 'dimdesc' algorithm in FactoMineR (Husson *et al.*, 2013) and significant correlations are present in tables where diagrams are unclear.

5.2.3 Microarthropod surveys and microbial analysis

Microarthropod samples were taken from each plot every other month between September 2011 and July 2012, inclusive. Soil samples were taken using a 1.5cm corer, driven down to the plot lining (8cm). Two of these were taken from each plot and summed together to overcome problems of clumped microarthropod distributions (Ettema and Wardle, 2002), equating to a total sample area of 3.5cm^2 and volume of 28cm^3. 3(±0.05)g of this soil was removed for PLFA

analysis, as described in section 2.3.2, and after being weighed, the remainder was subject to extraction by Berlese Tullgren funnel for five days at approximately 18˚C (Macfadyen, 1953), also described in section 2.1.2. Soil was then reweighed to determine percentage water loss and microarthropods were stored in 70% ethanol until they were identified, where possible, to species level using a dissecting microscope, or compound microscope for finer detail. Those which could not be identified to species level were sorted into morphospecies. Collembola were identified using Hopkin (2007).

Statistical analysis on the microarthropod community was performed in SPSS 19.0. Repeated measures ANOVAs were used on total microarthropods, collembola, mites, other organisms and total Shannon-Wiener diversity with time, addition of bacteria and addition of mycorrhiza as main effects. Pairwise comparisons between different times were analysed using Bonferroni. Data were normalised by square root transformation if necessary to approximate the normal distributions required by ANOVA. PCAs were conducted in the FactoMineR package (Husson *et al.,* 2012) for R (R Core Team, 2012), where abiotic factors, month and treatment were added as supplementary variables to help explain the data.

Statistical analysis of the PLFA community was performed in SPPS 19.0 on total microbial mass, bacterial mass, fungal mass, gram positive bacterial mass, gram negative bacterial mass, sulphate reducing bacteria mass and AM fungal mass with time, addition of bacterial inoculant and addition of mycorrhizal fungi as main effects. Pairwise comparisons between different times were analysed using Bonferroni. Data were normalised by square root or *Ln*+1 transformation if necessary to satisfy the assumptions of ANOVA. PCAs were conducted in the FactoMineR (Husson *et al.,* 2012) package for R (R Core Team, 2012). Month and treatment were added as supplementary qualitative variables to help explain the data. Confidence ellipses were drawn around these at the 95% confidence level. Correlations between axes and fatty acids were obtained using the 'dimdesc' algorithm in FactoMineR (Husson *et al.*, 2013) and significant correlations are present in tables where diagrams are unclear.

5.2.4 Plant cover estimates

To determine plant cover rates, photographs were taken and analysed using Adobe Photoshop (version 9, Adobe Systems Inc., USA). Each plant was pre-selected using the lasso tool. Colour selection tools were then used to refine this selection to include as much of the plant as possible whilst removing the background (plate 5.2). Uncached histograms were used to count the pixels present in each plant (each plant was then filled to prevent accidental recounting). Total pixels in each tray were also counted and using the known area of the tray in m^2, the area per pixel could then be calculated. These data were then used to calculate the cover, in

square metres and % of the total, of each individual *Sedum* plant initially planted and groups of other plants e.g. All *Stellaria media*, all *Festuca rubra,* all new *S. album* etc. for each plot.

Relative plant cover was determined by expressing plant cover in relation to the plant cover in the plots at the beginning of the experiment in July.

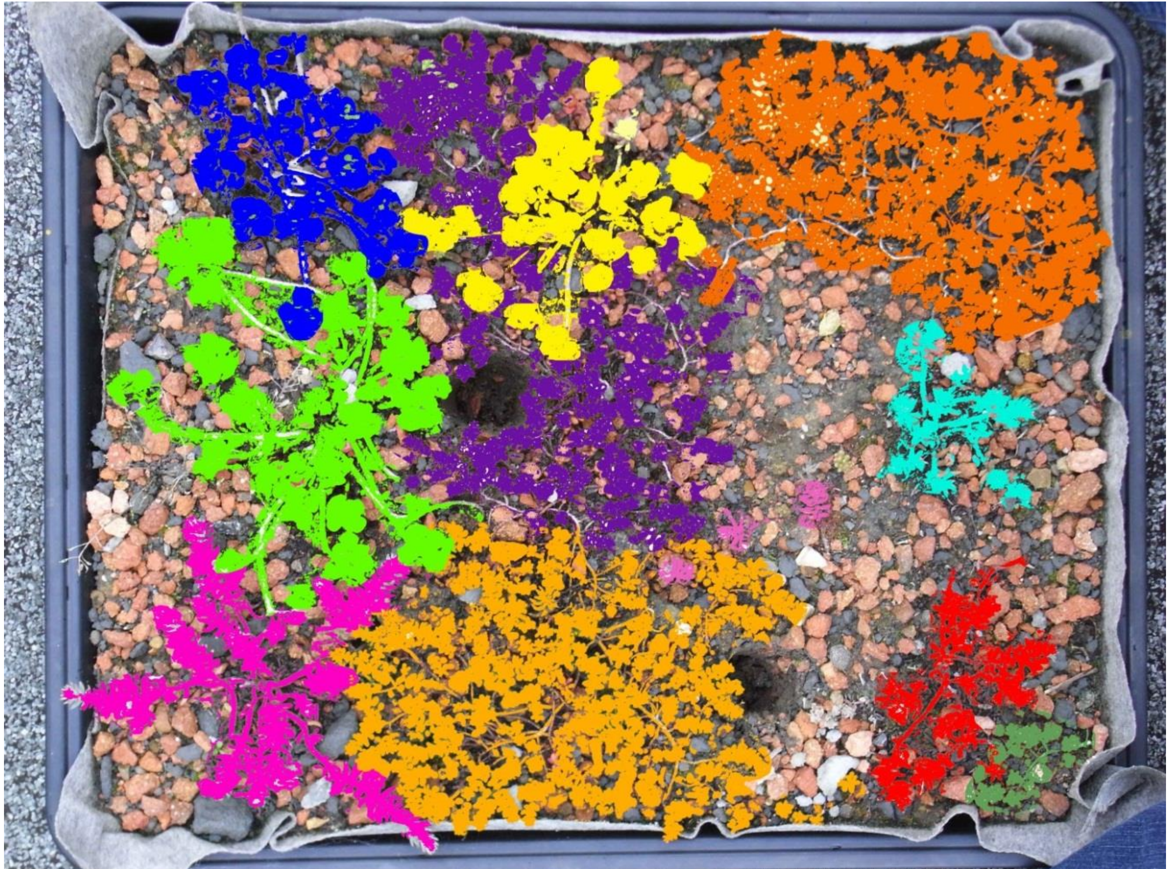

Plate 5.2. Example of an image manipulated in Adobe Photoshop to determine the number of pixels present in each plant. Colour selection tools were used to select each plant, then uncached histograms were used to calculate the number of pixels in each selection. The number of these pixels, compared to the pixels in the whole tray was used to determine the area in square metres each plant occupied.

Analysis was performed using SPSS 19.0. Shannon-Wiener indices were used to assess changes in biodiversity between September 2011 and July 2012 and these were compared using a repeated measures ANOVA with bacterial inoculant, fungal inoculant and time as main effects. The number of different species of plant in each plot was also subject to the same method.

Relative *Sedum spp.* cover per plot was tested to determine if added bacteria or added fungi increased plant cover, by including these as main effects in a univariate ANOVA. This was repeated for each of the three *Sedum* species separately. Absolute, rather than relative, cover

of grasses and forbs was compared between September 2011 and July 2012 using a repeated measures ANOVA, again with added bacteria and added fungi as main effects and time as another main effect. This was due to the high turnover of individual grasses and forbs.

5.2.5 Vegetation survey and fresh and dry weights

365 days after inoculation, vegetation surveys were carried out on the plots. Plants in each plot were identified to species level, where possible, using Blamey *et al*. (2003) and were counted.

The *Sedum spp.* planted initially were then removed from each plot and fresh weight for roots and shoots obtained. Roots were washed to remove soil and excess water was squeezed out. The plugs had not dispersed and roots from these sections proved difficult to extract from the soil. Roots were, therefore, split into two sections, new growth which consisted of all roots outside this 'root ball' and old growth which consisted of the whole 'root ball', including the soil.

A small portion of the new growth roots was removed, weighed and placed in 70% ethanol for later mycorrhizal analysis, as described in section 2.2.2. The remainder of the new growth roots, the old growth roots and the shoots were weighed separately and placed into an oven at 40˚C for two weeks before being reweighed for dry weight. New root dry weight was extrapolated to include the portion removed for mycorrhizal analysis.

SPSS 19.0 was used for all statistical analyses and data were transformed where appropriate, to meet the assumptions of chosen statistical tests using square root or *Ln*+1. To determine if the addition of inoculants or plant type affected the mycorrhizal infection rate of plants, a three-factor univariate ANOVA was used with plant, bacteria added and fungi added as factors. This was performed separately for percentage root length colonised and vesicular formation.

To determine if the addition of bacteria and fungi had an effect on plant growth, these two factors as well as plant species were tested using separate univariate ANOVAs on fresh shoot weight, fresh root weight, dry shoot weight and dry root weight.

Due to the size of harvest, the weighing of some plants was delayed, so a univariate ANOVA with date weighed as a factor was performed to determine if this had affected the results. It was found that fresh shoot weight had been affected, so these individuals, totalling eighteen plants in three trays, were omitted from the analysis of that parameter.

5.3 Results

5.3.1 Microarthropods

5.3.1.1 Pre-planting

The substrate supplied to construct the roofs contained no microarthropods on arrival. However, the plugs of each *Sedum spp.* did, and the microarthropod community differed in both abundance and diversity of microarthropods between plant species (Figs. 5.2-5.4).

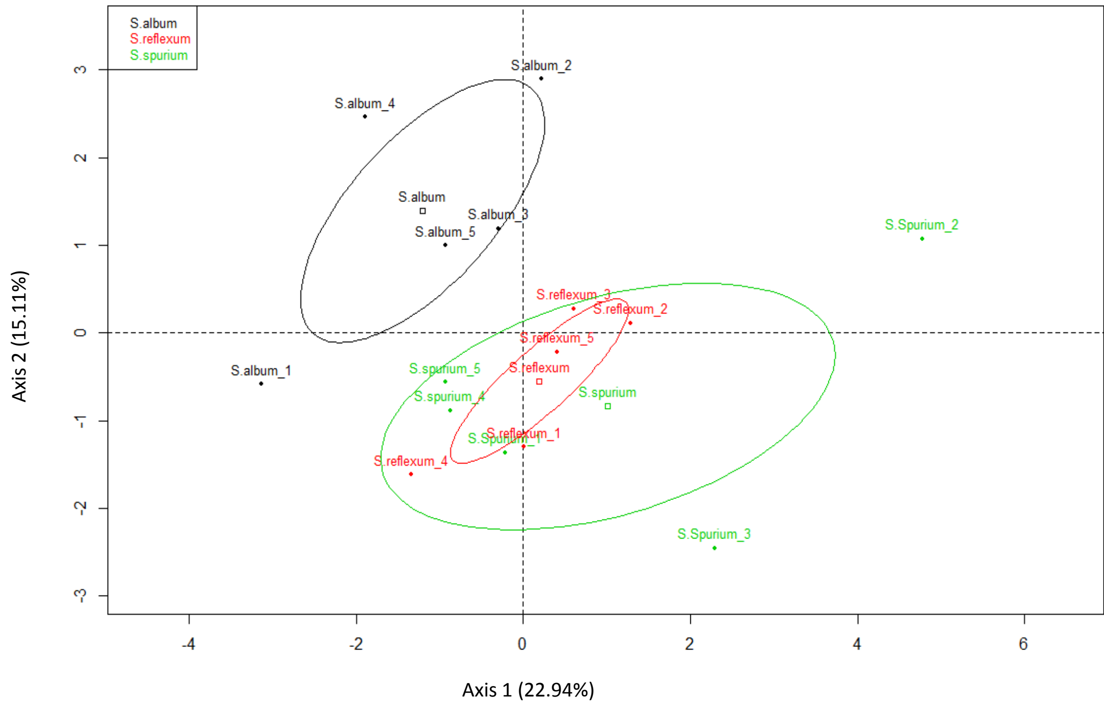

Fig. 5.2. PCA ordination plot of the microarthropod community present in different *Sedum* plugs on arrival. Confidence ellipses are drawn at the 95% confidence level.

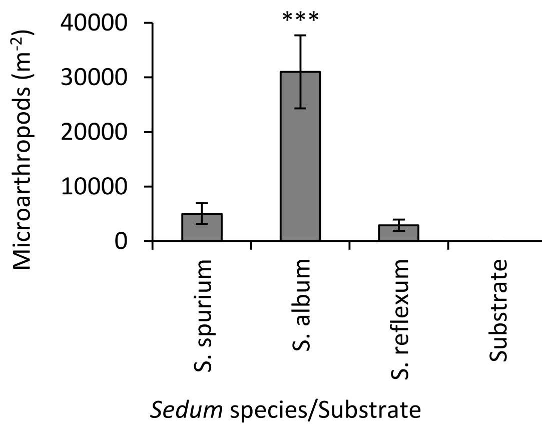

Fig 5.3. Microarthropod abundance in *Sedum* plugs before planting. Error bars represent SEM. Stars denote significance at the 99.9%

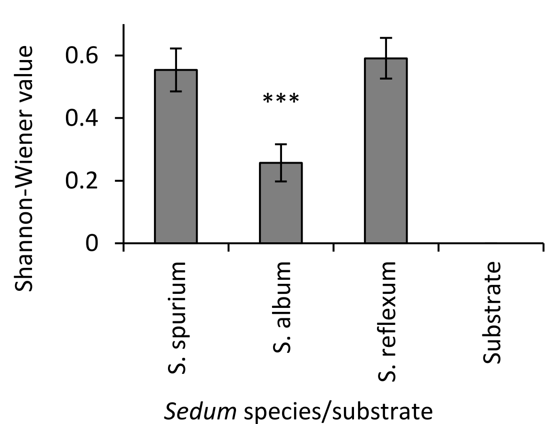

Fig 5.4. Shannon-Wiener diversity indices for *Sedum* plugs before planting. Error bars represent SEM. Stars denote significance at the 99.9% confidence level.

Sedum album supported 10 different species, *Sedum spurium* the same and *Sedum reflexum,* 12 species. In total, these consisted of eight morphospecies of mites including one oribatid and one species in the Bdellidae family, four collembola (*Parisotoma notabilis, Orchesella villosa, Brachystomella parvula* and *Sminthurinus aureus*), one morphospecies of the order Annelida, one aphid morphospecies and one morphospecies of insect larvae (Fig. 5.5). Some species, *Parisotoma notabilis* (Schäffer) for example, were found in all three species, whereas other species, including *Sminthurinus aureus* (Lubbock) was only found in *Sedum spurium* (Fig. 5.6).

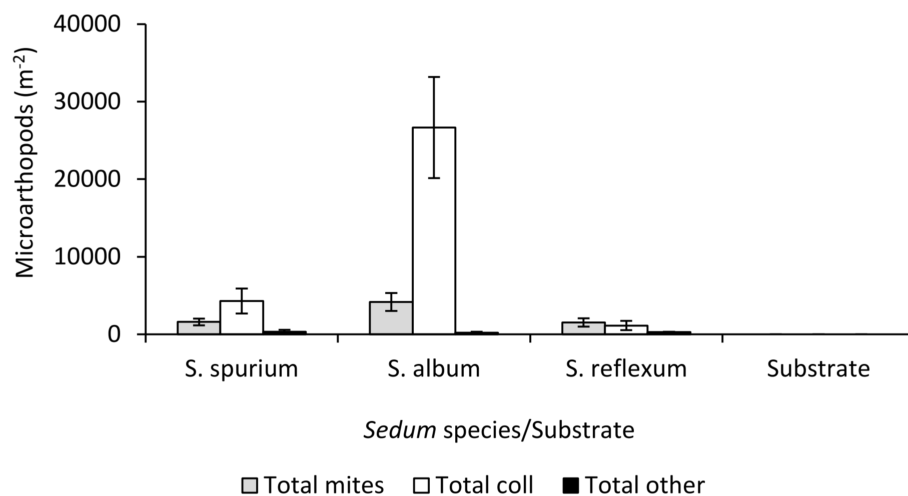

Fig 5.5. Mean microarthropods encountered in each *Sedum* plug before planting. Error bars represent SEM.

Collembola were the most prevalent microarthropod, but more morphospecies of mite were present. *Sedum album* supported a higher abundance of microarthropods than the other two *Sedum spp.* (which supported the same level of abundance as one another) ($F_{3, 16}$ = 16.66, p < 0.001) but lower species diversity ($F_{3, 16}$ = 24.74, p < 0.001) (Figs. 5.4 and 5.5) as it contained the species *P. notabilis* in extremely high abundance (collembola: $F_{3, 16}$ = 13.90, p < 0.001) (Fig. 5.6). Mites did not favour any particular *Sedum spp.* and mite abundance was so low in general that only *S. album* contained significantly higher numbers of mites than the substrate, where no mites were found ($F_{3, 16}$ = 6.64, p < 0.001) (Fig. 5.7). Only three other morphospecies were found in the plugs and these did not differ between plant species (Fig. 5.8).

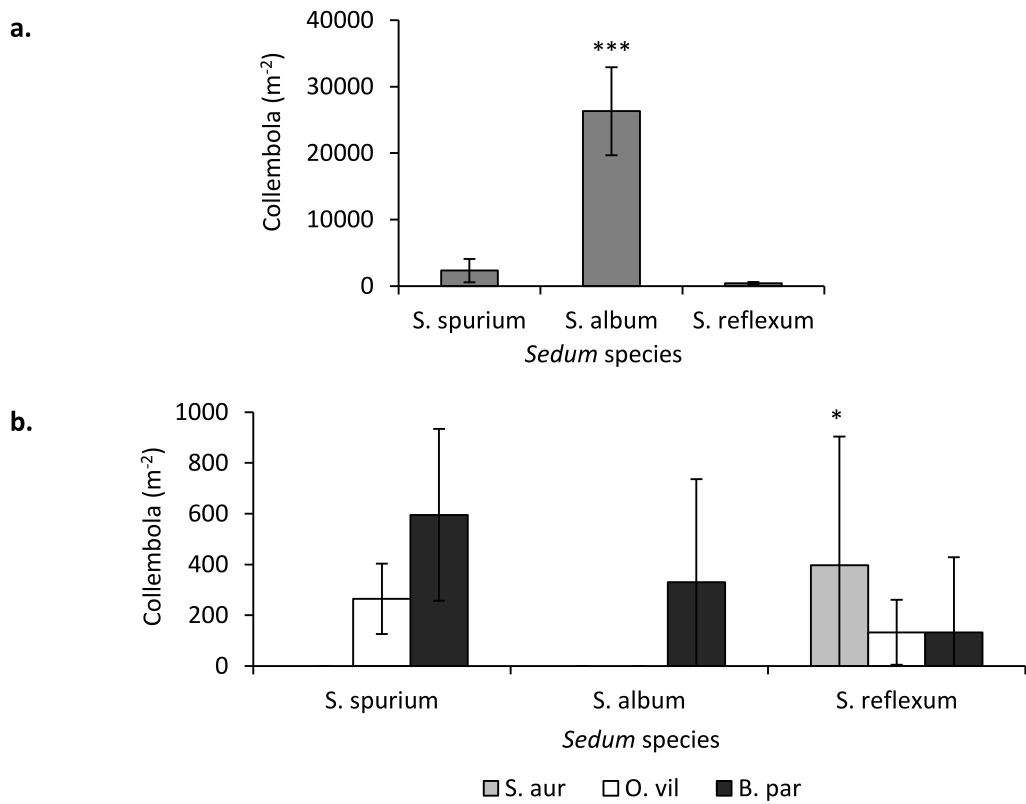

Fig 5.6. Collembola species encountered in *Sedum* plugs before planting (a) *Parisotoma notabilis,* (b) other collembola. O. vil = *Orchesella villosa*, B. par = *Brachystomella parvula,* S. aur = *Sminthurinus aureus*. Error bars represent SEM.

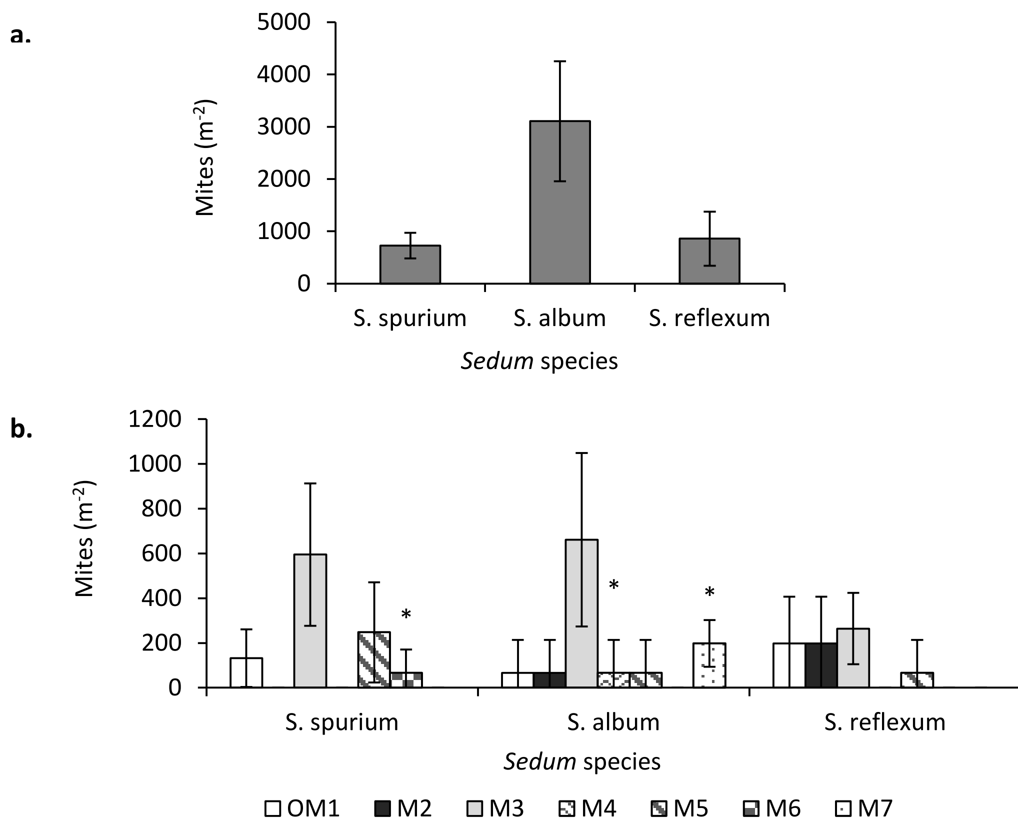

Fig 5.7. Mean mite morphospecies found in *Sedum* plugs (a) Mite 1, (b) other mites. M2, M3 etc. represents Mite 2, Mite 3. OM denotes oribatid mites. * denotes a morphospecies was only found in one *Sedum spp.* Error bars represent SEM.

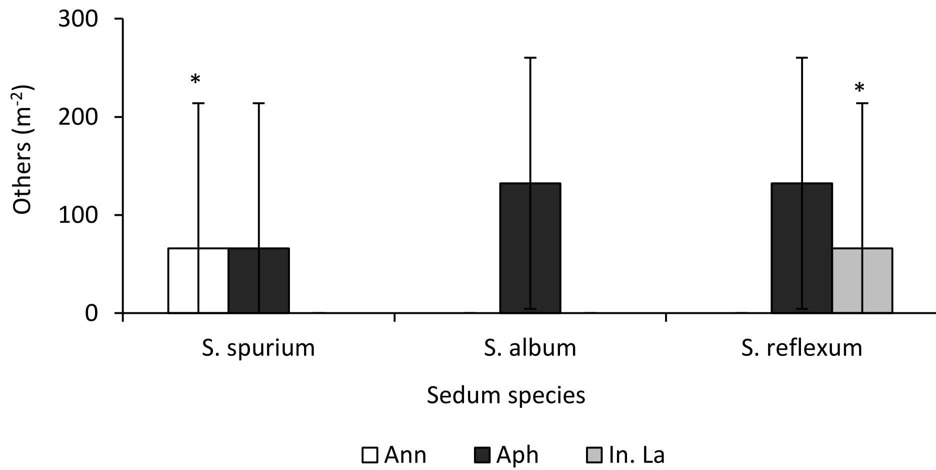

Fig 5.8. Other morphospecies encountered in *Sedum* plugs before planting. Ann = Annelida, Aph = aphid, In. La. = Insect Larvae. * denotes a morphospecies found in only one *Sedum* species. Error bars represent SEM.

5.3.1.2 Post-planting

Changes in the microarthropod community occurred after planting the *Sedum* plugs, with abundance pre-planting higher than that post-planting ($F_{1,\ 163}$ = 27.80, P = <0.001) (Fig. 5.9). Diversity also decreased post-planting ($F_{1,\ 163}$ = 10.95, P = <0.001) (Fig. 5.9) and although new species colonised post-planting, rates were very slow (Fig. 5.10).

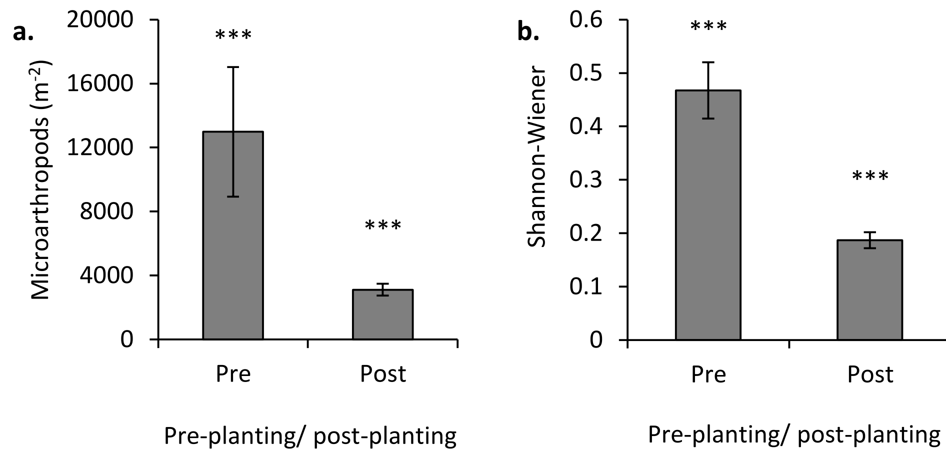

Fig. 5.9. (a) Mean microarthropod abundance and (b) Mean Shannon-Wiener diversity, in initial sedum plugs (Pre) and in plots afterwards (Post, all sample dates). Error bars represent SEM.

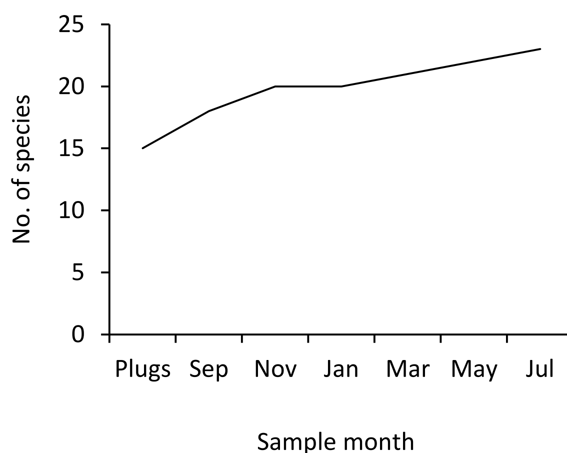

Fig. 5.10. Cumulative species curve of all species for all plots over time, including original plugs.

The mite community changed in species composition, with two mites disappearing post-planting but three morphospecies of mite colonising post-planting. The rate of colonisation varied, with Mite 8 colonising within the first two months, Mite 9 within the first four months and Oribatid Mite 2 colonising in May 2012. Oribatid Mite 1, present both pre and post planting, was extremely successful post-planting, especially in January 2012. Mite 1, which had been abundant pre-planting, became much less common post-planting, as did Mite 3. PCA also showed that the mite community present in *S. album* plugs was dramatically different to later sampling points (Fig. 5.11), whereas the community present in *S. spurium* and *S. reflexum* plugs was not dissimilar to the community found in May 2012. Samples taken from plots only two months after planting (in September), however, were very different to those present in plugs, suggesting a community shift post-planting before seasonal community shifts become apparent.

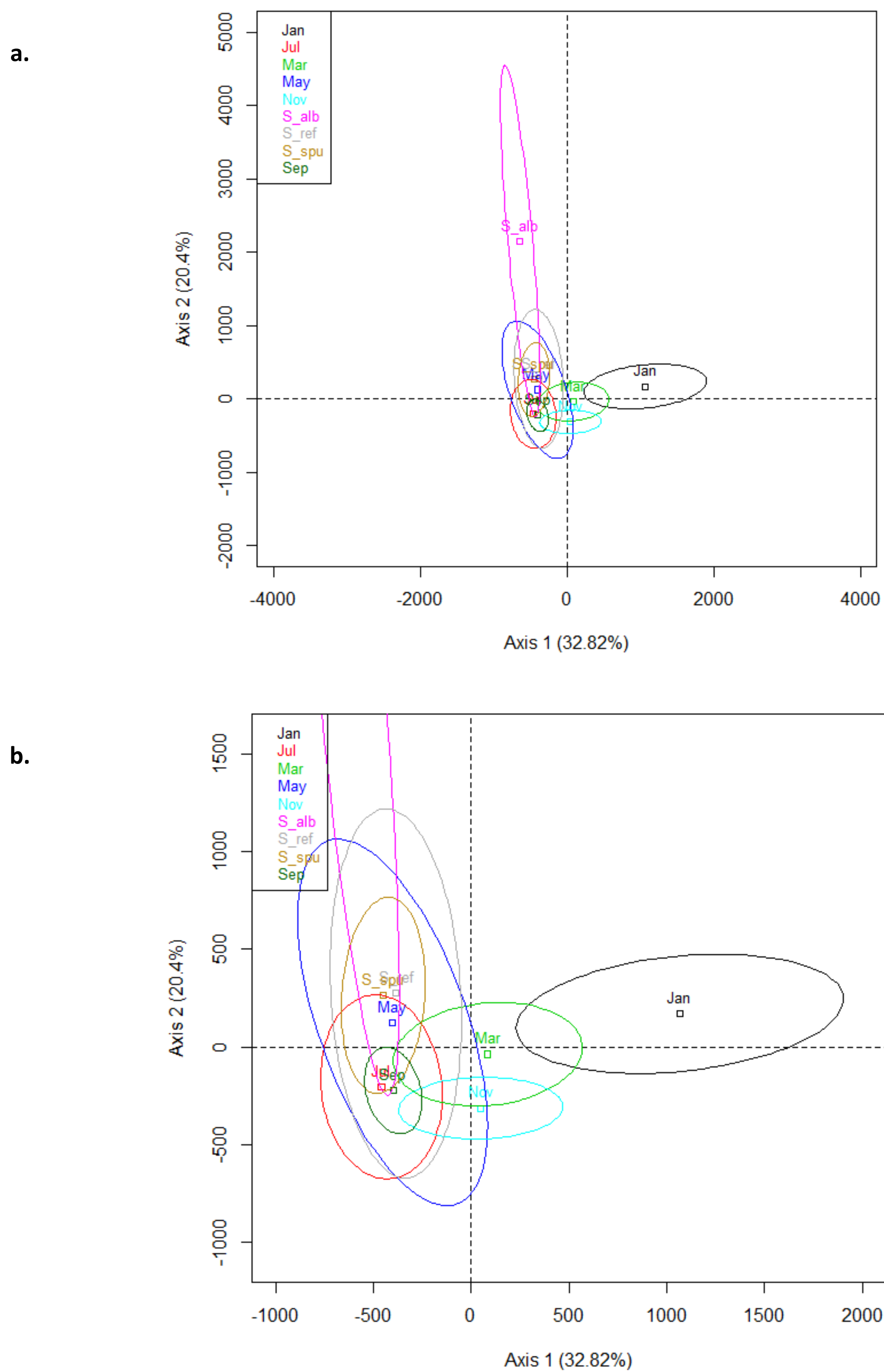

Fig. 5.11. PCA ordination plots of the mite community in plugs (pre planting) and in subsequent sampling months (post planting), (a) entire plot, (b) centre enlarged.

The collembolan community had the most dramatic community shift (Fig. 5.12) with a community present in *S. album* that was very different to other plugs and sample points. Communities in *S. spurium* and *S. reflexum* plugs overlap with some later sampling months, but are distributed differently along PCA axes. *B. parvula* died out post-planting and *S. aureus* disappeared after September 2011 (Fig. 5.12). *O. villosa* was greatly reduced post-planting too, disappearing until May 2012 (Fig. 5.12). *P. notabilis* remained the most common collembola throughout the study period and one collembola colonised post-planting, *S. trinotatus*, which was present in low abundance in most months (Fig. 5.12).

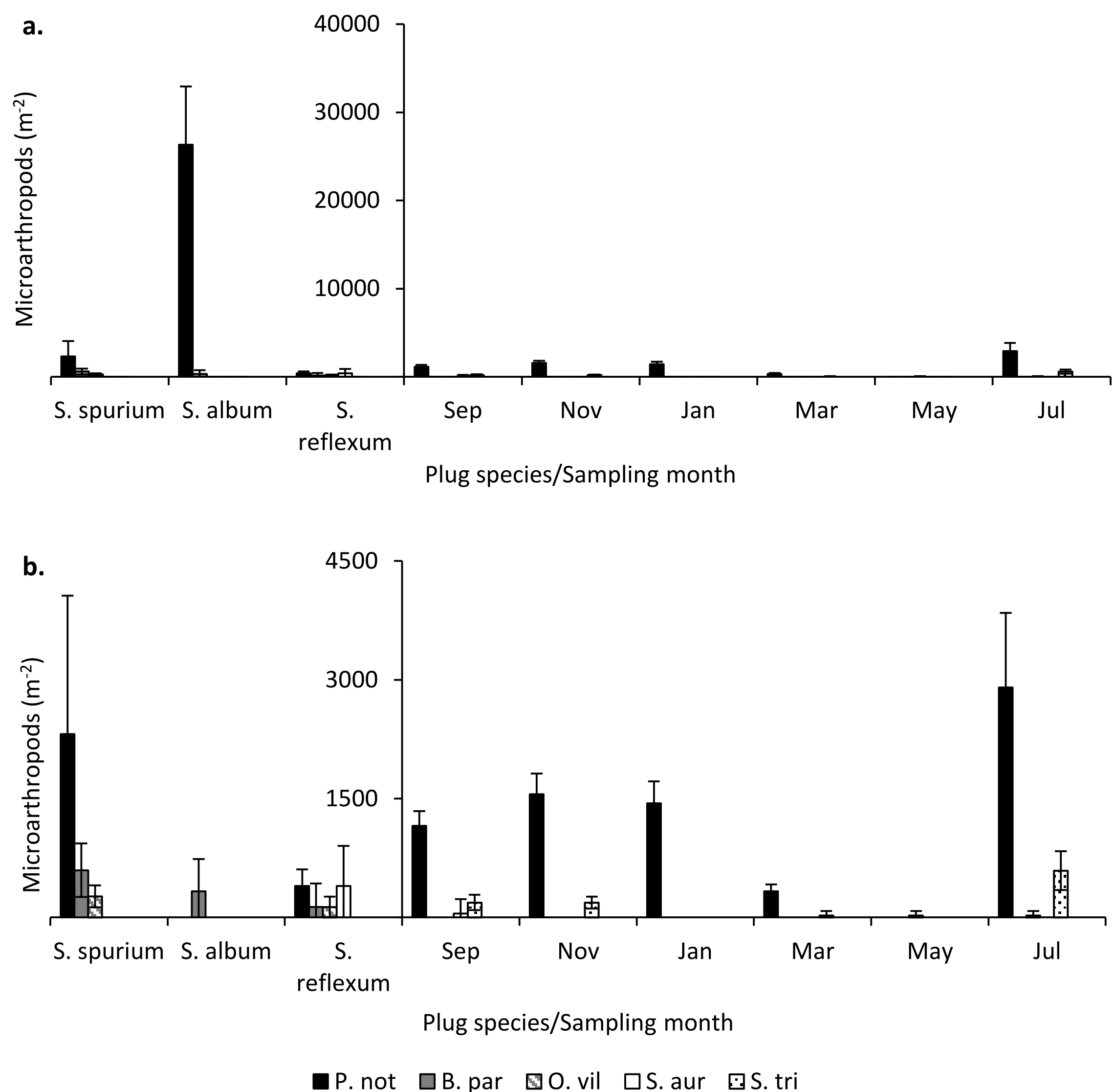

Fig 5.12. Mean collembolan abundance in initial plugs and later sampling months. (a) with *P. notabilis* included, (b) without *P. notabilis* in *S. album*. P. not = *P. notabilis,* B. par = *B. parvulus,* O. vil = *O. villosa,* S. aur = *S. aureus*, S. tri = *S. trinotatus*. Error bars denote SEM.

Other species also changed in their community composition, with most organisms disappearing post-planting to be replaced with new ones (Fig. 5.13). The only two species present pre-planting to remain post-planting was a species of Aphidae, *Aphis sedi*, and a species of flying insect larvae (a terrestrial chironomid larvae) (Fig. 5.13). The Annelida morphospecies was not to be found post-planting (Fig. 5.13). Diptera, their larvae and Thysanoptera were all colonisers post-planting, although all in low abundance until July 2012 when they reach a peak (Fig. 5.13). In terms of community structure, however, there was little difference between plugs and sample dates post-planting, except in July 2012 (Fig. 5.14).

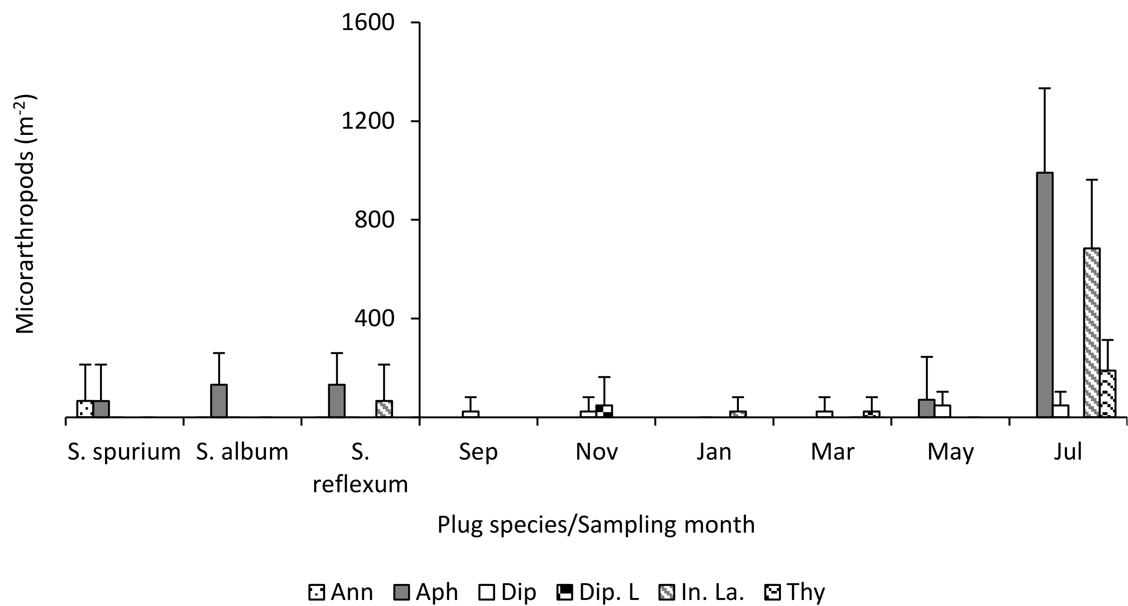

Fig 5.13. Mean abundance of other micorarthropods in initial plugs and later sampling months. Black bars denote species absent post-planting, white bars denote species that colonised post-planting. Ann = Annelida, Aph = Aphidae, In. La = Flying insect larvae, Dip = Diptera, Dip. L = Dipteran larvae, Thy = Thysanoptera. Error bars represent SEM.

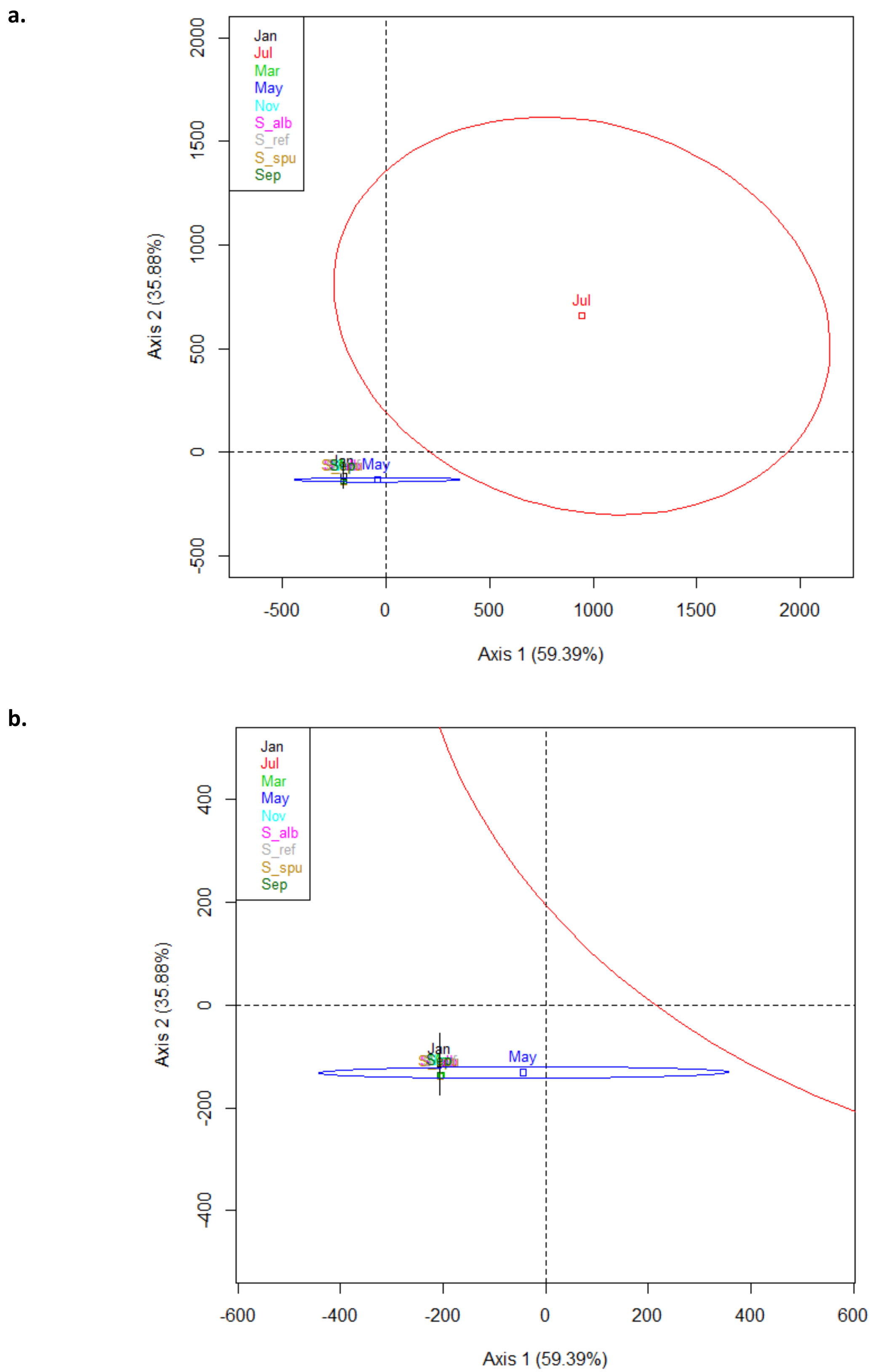

Fig. 5.14. PCA ordination plots of other microarthropods in plugs (pre planting) and in subsequent sampling months (post planting), (a) all months (b) centre enlarged.

5.3.1.3 Seasonal patterns and inoculants

Only 22 species were encountered during the 10 month sample period, all at low abundance. This included 12 morphospecies of mite including two oribatid mites, two Tydeids and one species of the family Bdellidae. Five collembola species were found and four organisms belonging to other groups (Table 5.2). Only eight of these species/morphospecies were not found in the initial plugs: Four mites, including one Oribatid and one Tydeid, *Sminthurinus trinotatus*, Diptera and a species of Thysanoptera.

Collembola
Brachystomella parvula
Orchesella villosa
Parisotoma notabilis
Sminthurinus aureus
Sminthurinus trinotatus
Other organisms
Aphid
Diptera (adults and larvae)
Flying insect larvae (terrestrial chironomid)
Thysanoptera

Table 5.2. Species/morphospecies of collembola and other microarthropods (not including mites) encountered in experimental trays for the whole sample period

Microarthropod abundance on the roof changed over time, with abundance peaking in July 2012 and to a lesser extent January 2012 (Table 5.3, Fig. 5.15). The bacterial treatment supported significantly more microarthropods than other plots (Table 5.3, Fig. 5.15). Seasonal changes in abundance were not affected by the addition of inoculants. Variability compared to the mean (C_V) was less over time in inoculated plots than in control and unplanted plots (Fig. 5.15). However, overall the community varied less in unplanted plots and those inoculated with only fungi than in control plots and plots inoculated with bacteria (Fig. 5.15). PCA determined that treatments containing bacteria overlapped to some extent with the mycorrhizal treatment alone, but that the community was much more variable. The bacteria treatment also produced a different community composition to the unplanted controls (Fig. 5.16).

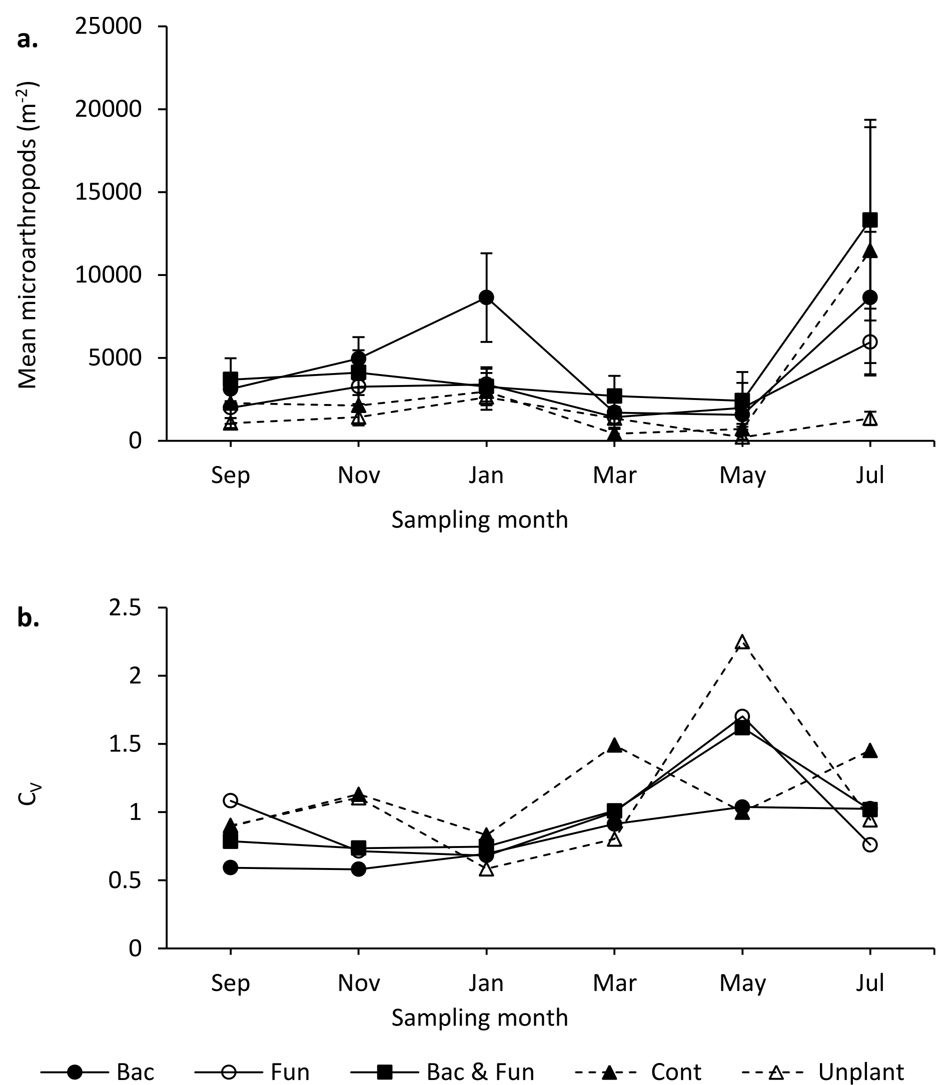

Fig 5.15. (a) Mean micorarthropods per treatment. (b) C_V over time for microarthropod abundance. Error bars denote SEM.

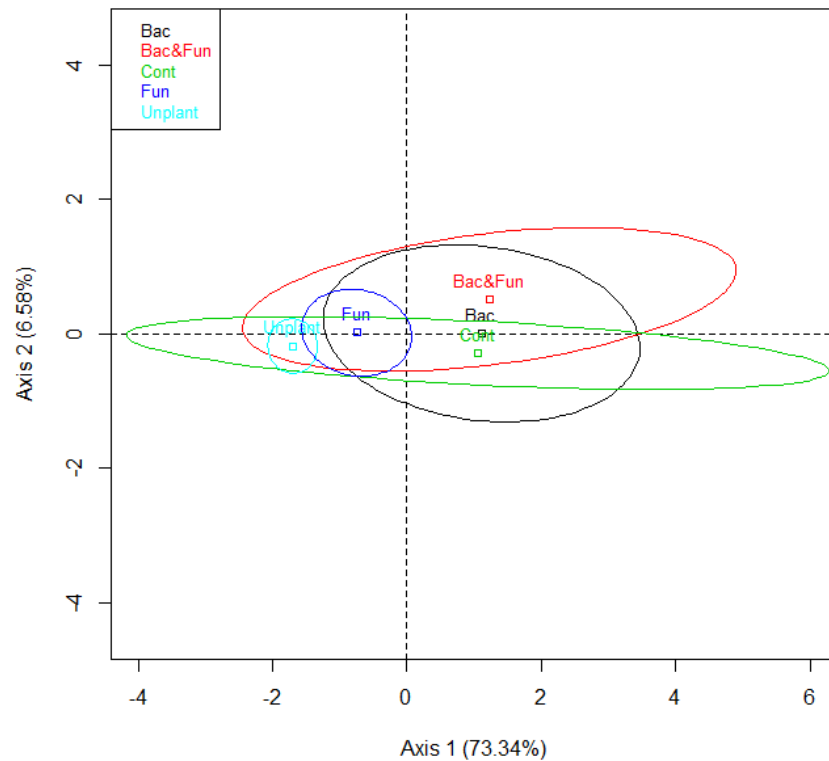

Fig 5.16. PCA ordination plot depicting the total microarthropod community. Confidence ellipses separate treatments and individual samples are omitted for clarity.

The abundance of collembola varied between 0-37 000 individuals (m^{-2}) over the year ($\bar{X}$ = 1400±21) and was higher in all plots that had been treated with the bacterial inoculant (Table 5.3). No treatments changed the pattern of abundance over time (Table 5.3). Plots containing the bacterial inoculant showed similar patterns of variability in abundance over time to those without. In the majority of sampling months, plots treated with inoculants were less variable over time than control or unplanted plots (Fig. 5.17). Again, the community composition in those plots treated with bacteria varied more than other plots. In the case of collembola, analysed with PCA, no plots differed completely in community composition (Fig. 5.18).

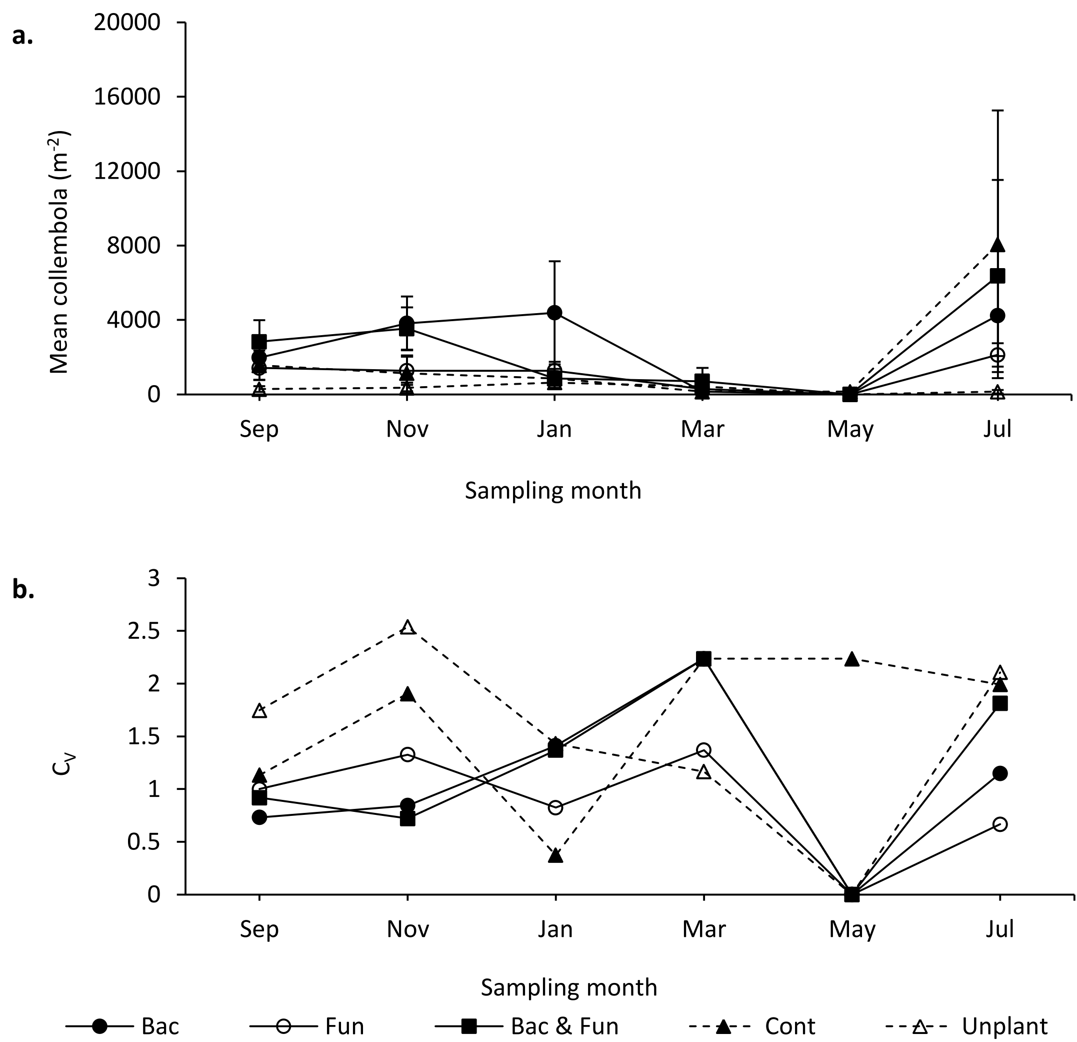

Fig 5.17. (a) Mean collembola per treatment. (b) C_V over time for collembola abundance. Error bars denote SEM.

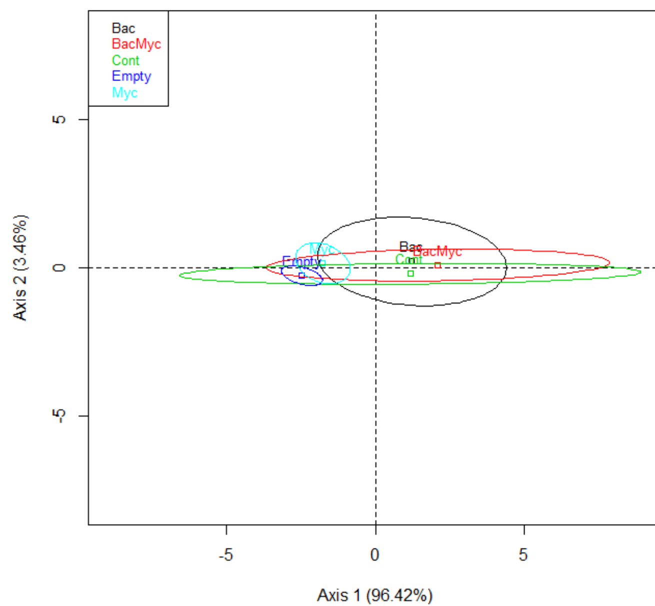

Fig 5.18. PCA ordination plot of the collembolan community in each treatment. Confidence ellipses are at the 95% confidence level. Data points are omitted for clarity.

Total mite abundance was lower than collembola abundance, varying between 0 and 11 000 individuals m^{-2} ($\bar{X}$ = 1000±9) and this also changed over time, peaking in January 2012 (Table 5.3, Fig. 5.19). This corresponded to a peak in Oribatid 1, which was the most prevalent mite over the sample period. In later months other mites such as Mites 5, 3 and 10 also started to increase. Mite 5 was a member of the family Bdellidae, whilst Mites 3 and 10 were Tydeids. No inoculants had a single effect on mite abundance, but the dual treatment reduced mite abundance (Fig. 5.19). This pattern of abundance did not differ over time (Fig. 5.19).

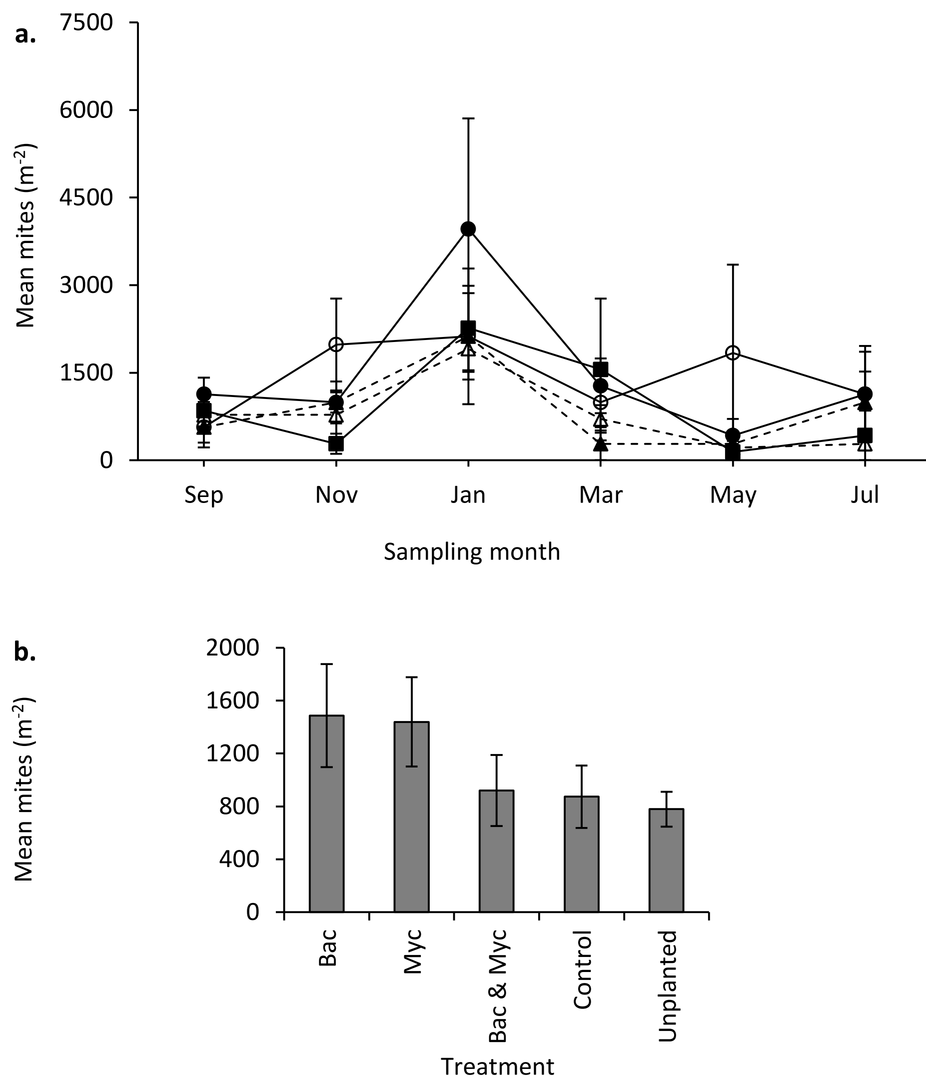

Fig 5.19. Mean mite abundance (a) over time for each treatment and (b) over all time points for each treatment. Error bars represent SEM.

The community was also more variable in inoculated plots than those without inoculants (Fig. 5.20). Oribatid 1 did not vary between plots of different treatments but one mite, Mite 5, was particularly prevalent in plots treated with bacteria ($F_{1,16}$ = 5.31, $p < 0.05$) (Fig. 5.21) and was

the main driver behind axis 2 of the mite community PCA (Fig. 5.22) on which the treatment bacteria and bacteria with mycorrhizae were arranged. However, PCA of the mite community must be interpreted with care as parallel analysis deemed no axes to be significant in explaining variance in the community. Mite 5 was also only in high abundance in May and July.

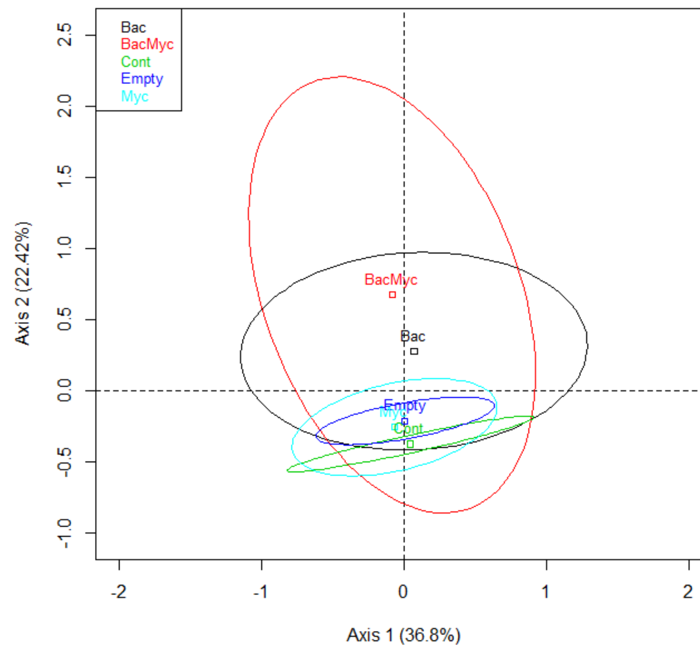

Fig. 5.20. PCA ordination plot of the mite community in each treatment. Confidence ellipses are drawn at the 95% confidence level and data points are omitted for clarity.

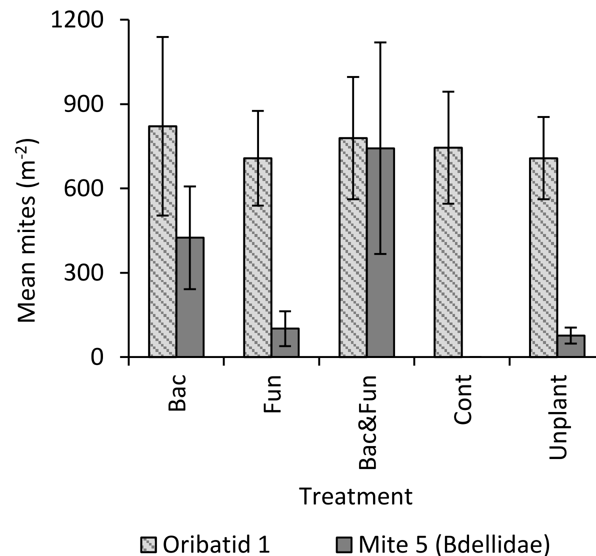

Fig 5.21. The two most prevalent mites in the study per treatment over all time points. Error bars represent SEM.

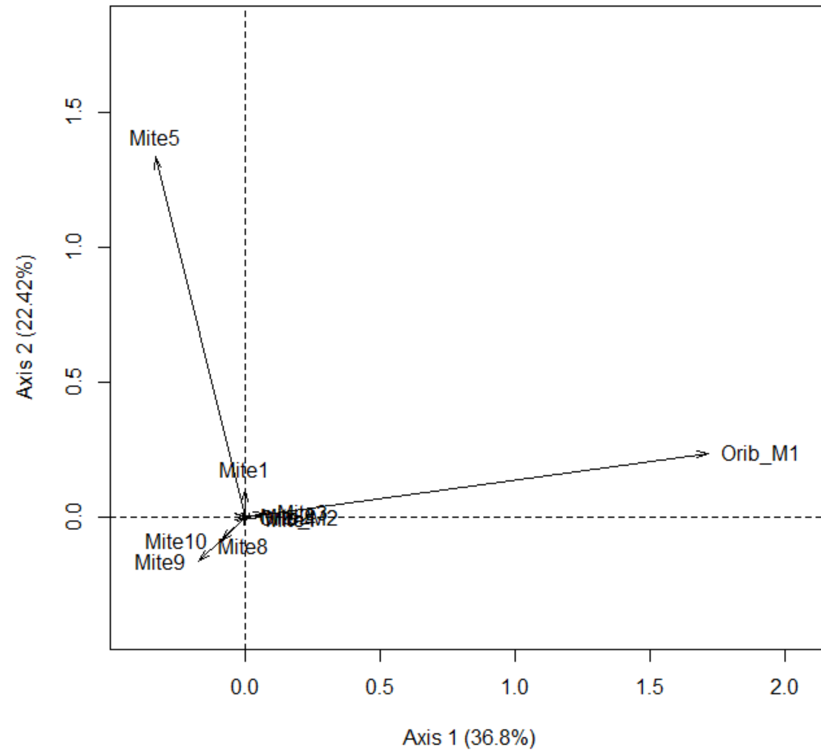

Fig. 5.22. Ordination plot depicting the mite community. Mite 5 is a species of Bdellidae. No axes were deemed significant in explaining mite variance according to parallel analysis.

Other microarthropods (unidentified flying insect larvae, Thysanoptera, aphids and flies) were rare for most of the year, varying between 0 and 12 000 individuals m^{-2} ($\bar{X}$ = 700±10). The population increased over time; Most individuals were counted in May and July (Fig. 5.23).

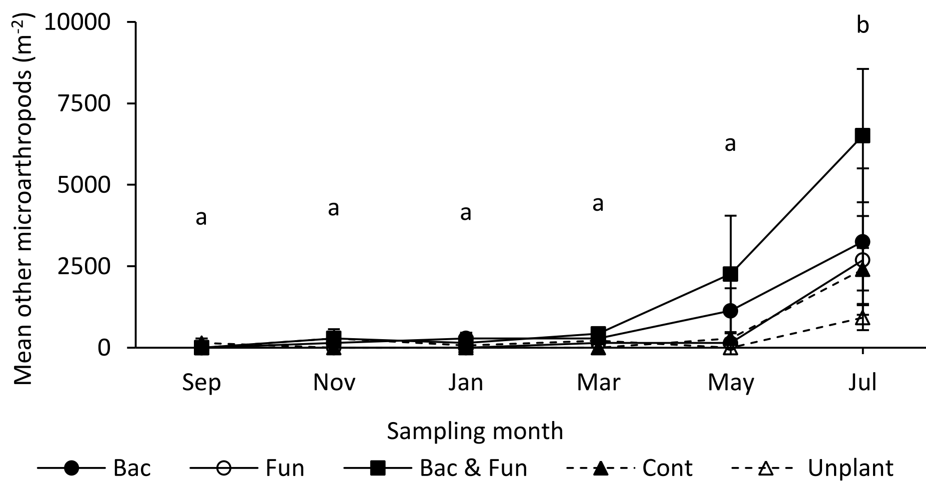

Fig 5.23. Mean non-mite, non-collembola microarthropods for each treatment. Letters denote statistically similar groups. Error bars represent SEM.

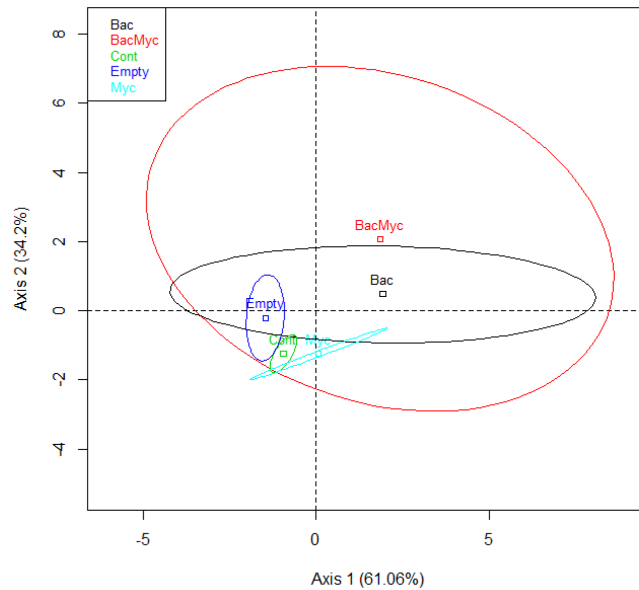

Fig. 5.24. PCA ordination plot depicting the community of microarthropods not belonging to collembola or mites. Confidence ellipses denote separate treatments, drawn at the 95% confidence level. Individual data points are omitted for clarity.

Bacterial inoculated plots supported more of these organisms overall, as did addition of the mycorrhizal inoculant but mixtures of the treatments had no significant effect (Table 5.3). Additionally, the bacterial treatment altered the pattern of abundance over time. Variability in community composition was extremely high in plots treated with bacteria and the bacteria treatment alone produced a different community composition to planted control plots (Fig. 5.24). Both the planted and unplanted controls differed in community structure to the plots treated only with fungi (Fig. 5.24).

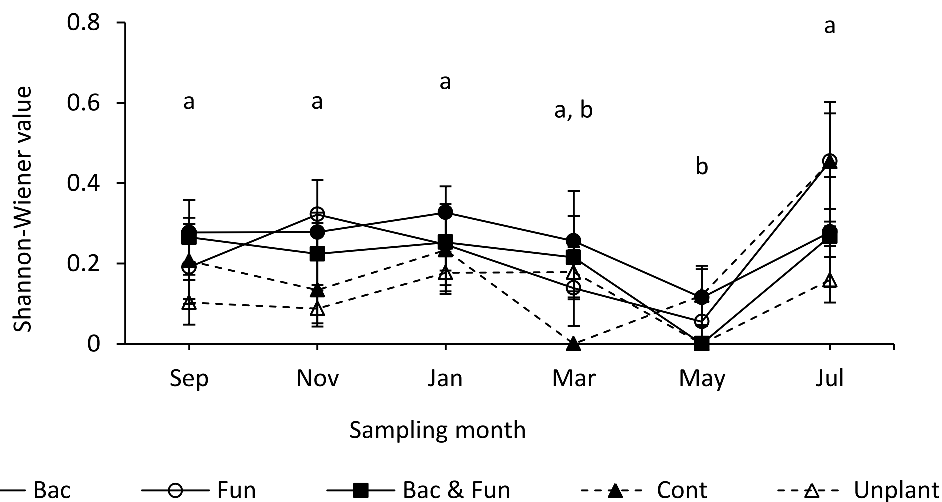

Fig. 5.25. Mean Shannon-Wiener diversity for each treatment. Letters denote statistically similar groups. Error bars represent SEM.

Diversity and the number of species present changed over time but were not affected by the addition of inoculants (Fig. 5.25, Table 5.3).

Parameter measured	Significant factors	Degrees of freedom	*F* value	*p* value
Total microarthropods	Time	1.33, 34.64	7.37	<0.01
	Bacteria	1, 26	7.49	<0.05
Collembola	Time	2.40, 62.50	10.60	<0.001
	Bacteria	1, 26	5.08	<0.05
Mites	Time	5, 130	6.58	<0.001
	Bacteria*Mycorrhiza	1, 26	4.87	<0.05
Other microarthropods	Time	1.55, 40.41	19.19	<0.001
	Bacteria	1, 26	14.95	<0.001
	Mycorrhiza	1, 26	4.81	<0.05
	Bacteria*Time	1.55, 40.41	3.54	<0.05
Diversity	Time	3.16, 82.25	5.78	<0.001
No. of species	Time	2.67, 69.47	8.46	<0.001

Table 5.3. *F* and *p* values for ANOVA results performed on microarthropod parameters with time and treatments as factors. Only significant results are shown.

Collembola were only weakly correlated with any of the abiotic factors over the whole sample period. However, in months where plant surveys were carried out (September and July) they showed a strong correlation with cover of *S. album* and *S. reflexum*, increasing with cover of the former and decreasing with the latter. Mites were weakly correlated with mean daily temperature, decreasing as temperature increased. Other organisms were also weakly correlated with mean daily temperature over the whole sample period, this time increasing with increasing temperature. However, in the two months where plant surveys were carried out, other organisms were explained by different factors, daily humidity and grass cover (Table 5.4).

Community	R^2	*F* value	*p* value	Factor	*β*	*p* value
Collembola (Sep and July only)	0.33	13.74	<0.001	1. *S. album*	1.05	<0.001
				2. *S. reflexum*	-0.73	<0.01
Mites	0.11	22.94	<0.001	Temperature	-0.34	<0.001
Others	0.15	31.64	<0.001	Temperature	0.39	<0.001
Others (Sep and July only)	0.30	12.17	<0.001	1. Humidity	0.60	<0.001
				2. Grass	-0.28	<0.05

Table 5.4. R^2, *F* and *p* values for stepwise multiple linear regressions perfomed on different microarthropod communities in either the whole sample period or in September and July only, when plant surveys were carried out. *β* weights with their respective *p* values for explanatory factors are also included. Degrees of freedom were 1, 178 for total datasets, 2, 57 for Sep/July samples.

5.3.2 Vegetation and fungi

5.3.2.1 Effects of planting

Plots planted with *Sedum sp.* (controls, without inoculants) supported more microarthropods overall than unplanted plots ($F_{1, 13} = 4.90$, $p < 0.05$) (Fig. 5.26). Of the microarthropod groups, only the abundance of collembola was significantly increased by planting ($F_{1, 13} = 6.50$, $p < 0.05$) (Fig. 5.26). However, Fig. 5.26 indicates that, had sample sizes been bigger, the non-mite, non-collembola group and overall diversity may have also benefitted from planting. When inoculated plots were added into the analysis, this was indeed the case (Table 5.5).

Group	*F* value	*p* value
Total microarthropods	11.53	**<0.01**
Mites	2.30	>0.05
Collembola	12.73	**<0.01**
Others	6.26	**<0.05**
Diversity	9.44	**<0.01**

Table 5.5. *F* and *p* values for groups of microarthropods and diversity tested for the effect of planting including those plots that were inoculated. Degrees of freedom were 5, 140 for all groups.

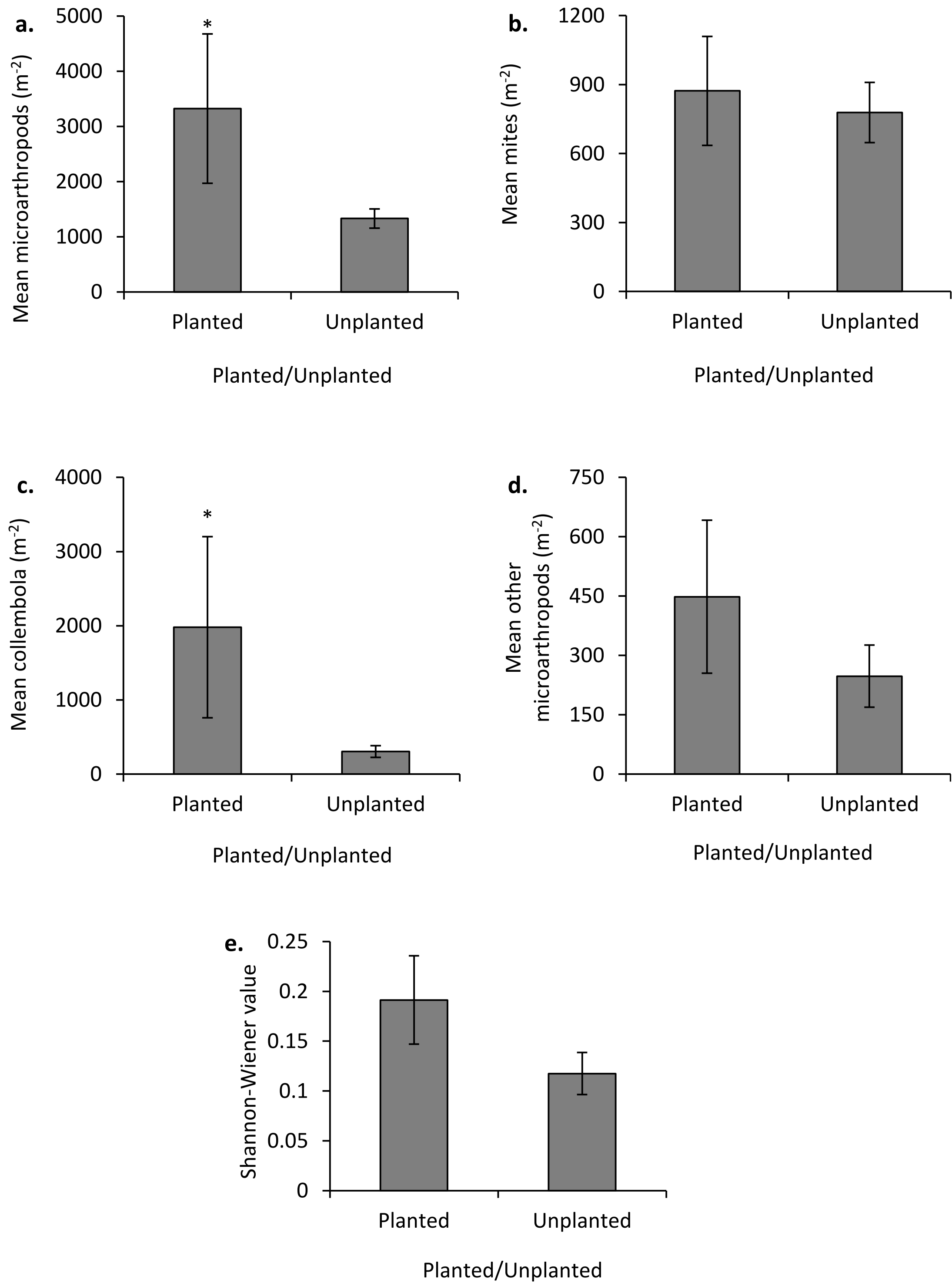

Fig 5.26. Differences in abundance/diversity (e) between planted (without inoculants) and unplanted plots for (a) total microarthropods, (b) mites, (c) collembola, (d) others, (e) Shannon-Wiener diversity. Asterisks denote significance at the 95% (*), 99% (**) and 99.9% (***) level. Error bars represent SEM.

5.3.2.2 Vegetation survey

Twenty-two species of plant were found in the experimental plots at the end of the sampling period, three of which (*S. album, S. reflexum* and *S. spurium*) were initially planted and the rest of which had colonised independently (Table 5.6).

Genus	Species	Notes
Agrostis	*stolonifera*	
Chenopodium	*album*	
Festuca	*rubra*	
Lactuca	*serriola*	
Leucanthemum	*vulgare*	
Matricaria	*discoidea*	
Poa	*annua*	
Polygonum	*aviculare*	
Prunella	*vulgaris*	
Sedum	*album*	(pre-planted)
Sedum	*reflexum*	(pre-planted)
Sedum	*spurium*	(pre-planted)
Stellaria	*media*	
Taraxacum	*officinale*	
Trifolium	*arvense*	
Veronica	*filiformis*	
Unknown 1		
Unknown 2		
Unknown 3		
Unknown4		
Unknown 5		(*Claytonia sibirica?*)
Unknown 6		(*Corinthoides spp?*)

Table 5.6. Species list of plants present on the young roof at the end of the experiment in July 2012

There were new individuals of all three *Sedum* species present in some plots, including unplanted ones. Similarly, grasses and colonisers were found in both unplanted and planted plots.

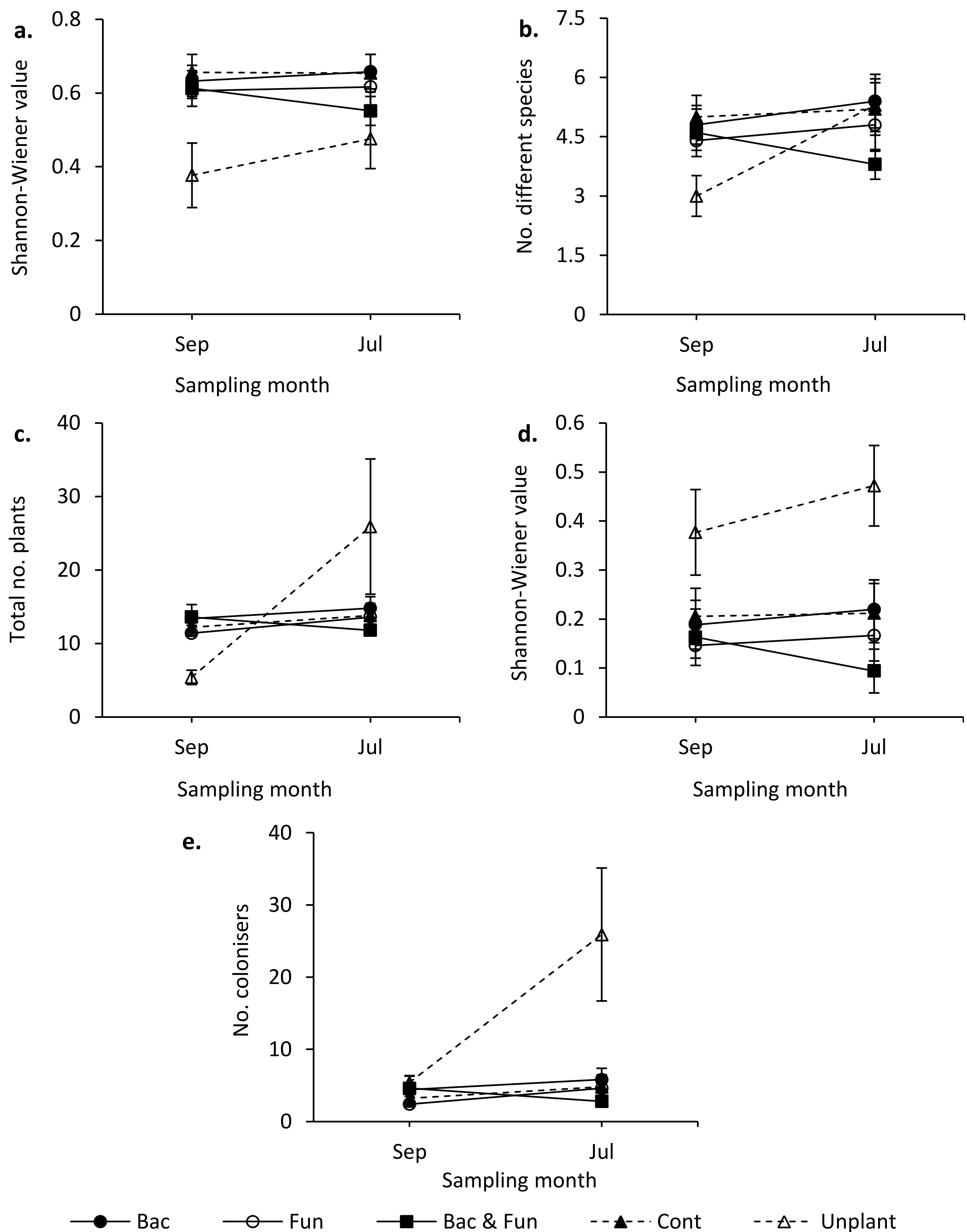

Fig 5.27. Mean values per plot for each treatment. (a) Shannon-Wiener diversity, (b) number of different species present, (c) number of individuals present, (d) Shannon-Wiener diversity of colonisers, (e) number of colonising individuals. Error bars represent SEM.

Diversity, the number of different species present, the total number of plants, the diversity of colonising plants and the number of colonising plants did not differ significantly between September 2011 and July 2012, nor were they affected by either of the treatments (Fig. 5.27).

5.3.2.3 Plant cover

Relative cover of all *Sedum* species differed between species ($F_{2, 48}$ = 112.86, p < 0.001), with *S. album* achieving a faster rate of cover than *S. spurium* or *S. reflexum,* which obtained cover at the same rate as one another (Fig. 5.28). The addition of single inoculants had no effect on relative cover but there was an interaction between the bacteria and mycorrhiza treatments, negatively affecting relative plant cover ($F_{1, 48}$ = 7.37, p < 0.01) (Fig. 5.28).

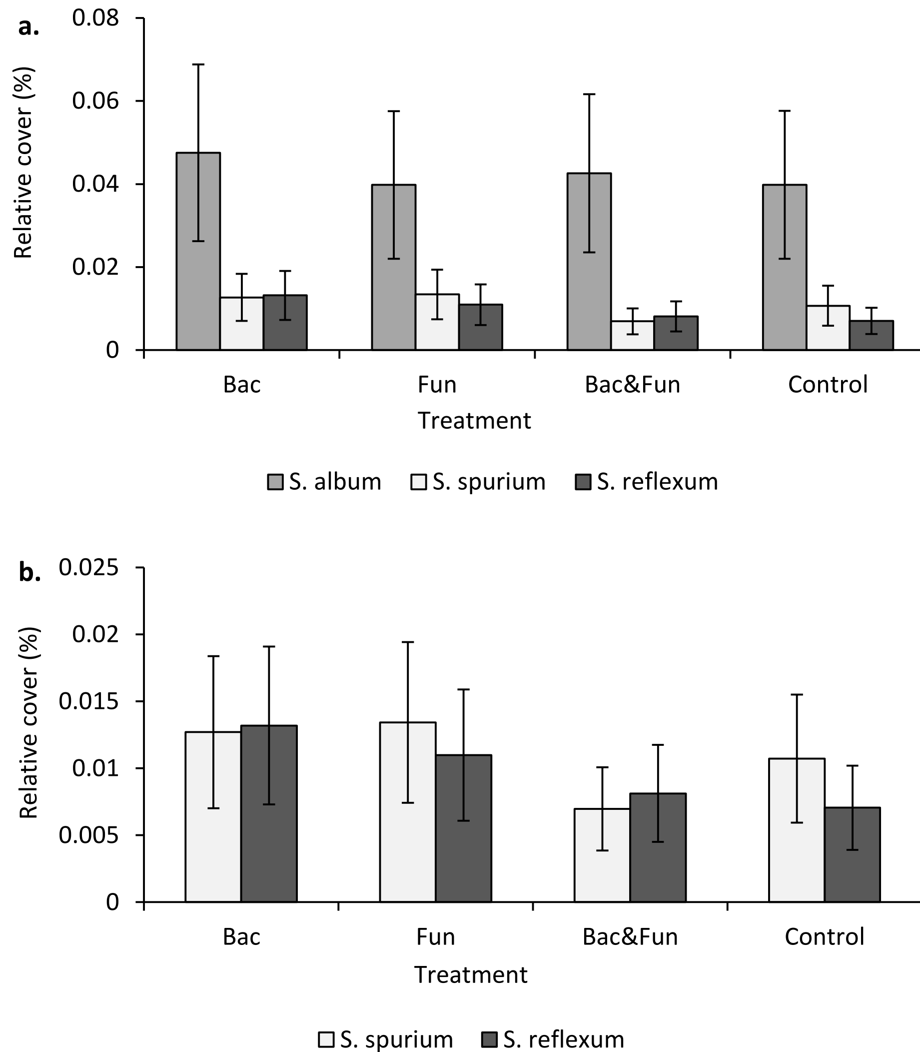

Fig 5.28. Mean relative cover afforded in July 2012 by (a) all three *Sedum spp.* and (b) by *S. spurium* and *S. reflexum* only. Error bars represent SEM.

Cover of grass was higher in July 2012 than in September 2011 in planted plots ($F_{1, 16}$ = 22.29, p < 0.001) and although grass cover was not affected by the addition of any single inoculants, the addition of both together changed the pattern of grass cover over time ($F_{1, 16}$ = 7.98, p < 0.05) (Fig. 5.29). Cover of forbs did not differ between time points, nor between treatments (Fig. 5.29).

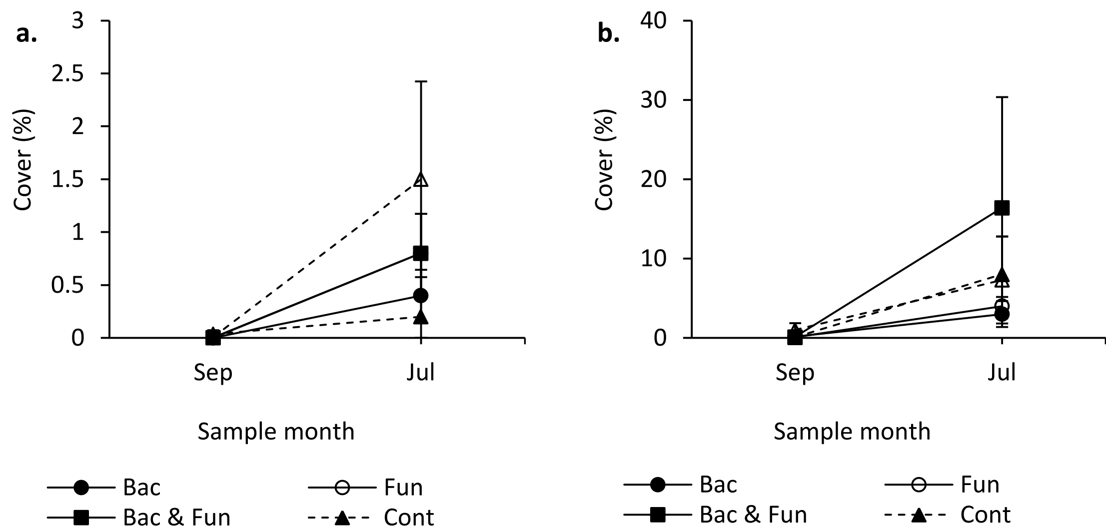

Fig. 5.29. Percentage cover in each plot for each treatment of a.) grasses and b.) forbs. Error bars represent SEM.

The number of different species present in control and unplanted plots changed between the two sampling dates ($F_{1,\ 13}$ = 6.03, p < 0.05), with more species in the planted plots. Shannon-Wiener diversity was the same at both sampling points, but higher in planted than unplanted plots ($F_{1,\ 16}$ = 10.00, p < 0.01). However, diversity of colonising plants was the same in both planted and unplanted plots so this difference was due to the addition of *Sedum*. Diversity of colonisers was also the same at both time points. The total number of colonising plants was lower in planted than unplanted plots ($F_{1,\ 16}$ = 11.21, p < 0.01) and higher in July 2012 than September 2011 ($F_{1,\ 16}$ = 9.27, p < 0.01) and the effect of the planting regime changed over time ($F_{1,\ 13}$ = 5.29, p < 0.05). Including *Sedum* into the total plant count, both planted and unplanted plots harboured the same number of individual plants overall and this increased in July 2012 compared to September 2011 ($F_{1,16}$ = 10.91, p < 0.01).

Cover of grass and forbs was also affected by planting regime. Grass obtained lower levels of cover in planted plots than unplanted plots ($F_{1,\ 16}$ = 7.43, p < 0.05). Grass cover changed over time ($F_{1,16}$ = 17.87, p < 0.001) and was affected by planting regime differently over time ($F_{1,16}$ = 9.75, p < 0.01) with unplanted plots achieving a higher percentage cover between the two sampling points than planted controls. Forb cover was also lower in the planted plots ($F_{1,16}$ = 6.93, p < 0.05) but did not change between the two sampling dates.

5.3.2.4 Plant weight

The addition of bacterial and fungal inoculants had no effect on fresh or dry shoot and root weight (Figs. 5.30 and 5.31). There were differences between plant species, with *S. album*

producing heavier fresh and dry shoots and roots than the other two species and *S. spurium* producing heavier dry roots than *S. reflexum* (Table 5.7).

Plant variable	*F* value	*p* value
Fresh shoot	26.56	<0.001
Fresh root	47.67	<0.001
Dry shoot	27.53	<0.001
Dry root	61.92	<0.001

Table 5.7. *F* and *p* values for the effect of plant type on different plant variables. Degrees of freedom for all variables were 2, 41.

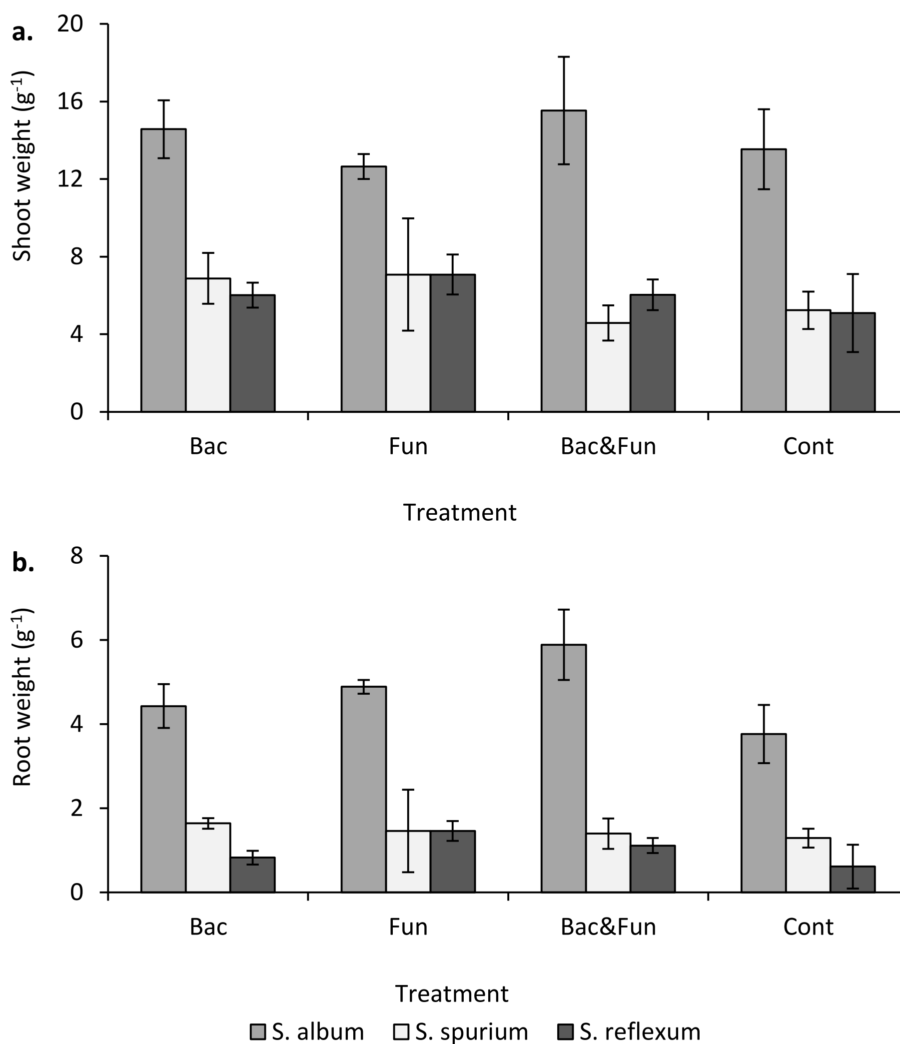

Fig. 5.30. Mean plot dry weights per treatment in July 2012 for (a) shoots, (b) roots. Error bars represent SEM.

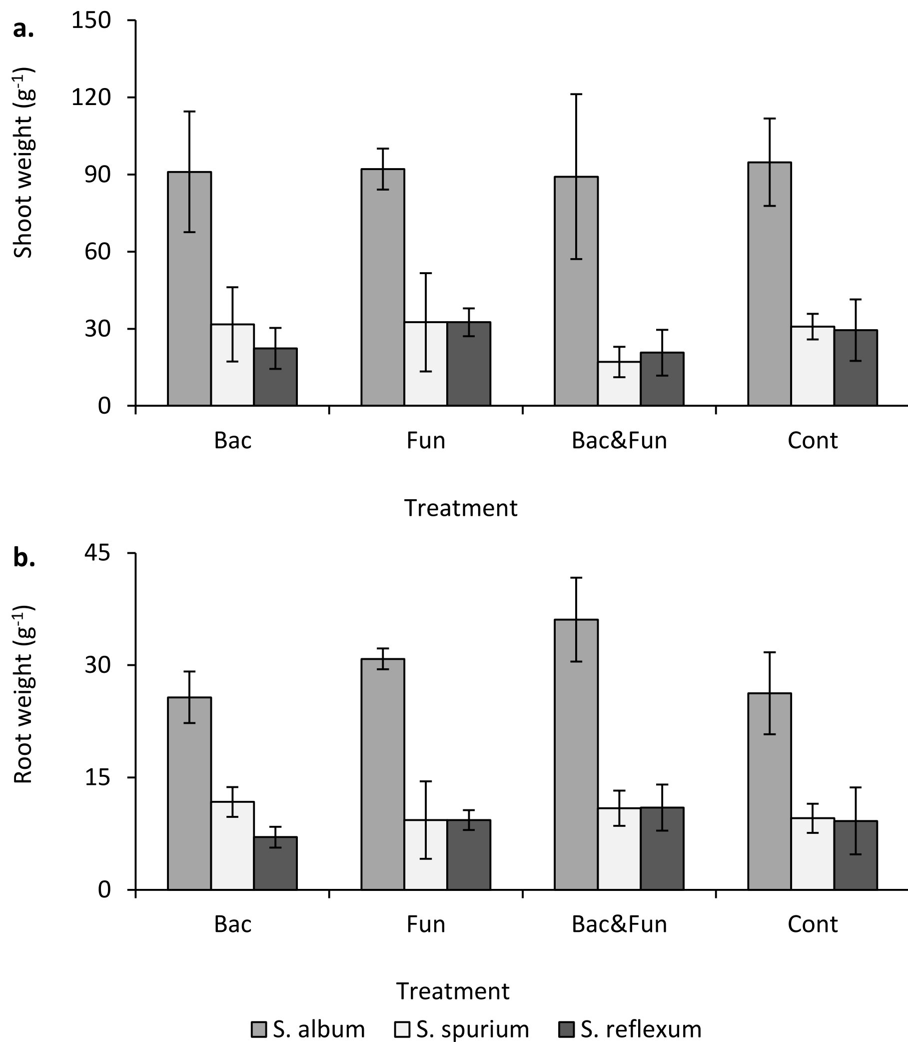

Fig 5.31. Mean plot fresh weights per treatment in July 2012 for (a) shoots, (b) roots. Error bars represent SEM.

5.3.2.5 Colonisation by mycorrhizal fungi

Hyphal counts varied between 0-63% root length colonised in the total sample area. Most plants (95.2%) had colonisation levels below 50%, whilst 3.6% of plants had no mycorrhiza present at all (Table 5.8). Plants that contained vesicles ranged from 0% to 24%, with most (98%) plants containing fewer than 50% of vesicles (Table 5.8). Arbuscules were extremely rare, ranging between 0 and 2%, with 95% of plants containing no arbuscular counts. No arbuscules or vesicles were present in the absence of hyphae.

There were no significant effects of the addition of either bacterial or fungal inoculants on mycorrhizal colonisation overall, nor on the percentage of colonisation consisting of hyphae alone (Fig. 5.32). The plant species did not alter colonisation rates of mycorrhiza overall or hyphae alone either (Fig. 5.33). Inoculant addition singly did not affect the number of vesicles found, but the addition of both mycorrhiza and bacteria together increased vesicle numbers ($F_{1, 48}$ = 4.42, $p < 0.05$) (Fig. 5.32). Plant type also influenced vesicle numbers ($F_{2, 48}$ = 3.25, p <

0.05) although Bonferroni failed to determine where significant differences lay, but Fig. 5.33 suggests that *S. album* had higher levels of vesicular colonisation than the other species.

% colonisation	% plants with hyphal counts	% plants with vesicles counted	% plants with arbuscules counted
0	3.6	22.6	95.2
<1	3.6	31.0	95.2
<5	16.7	76.8	100
<10	35.7	93.5	100
<25	75.6	97.6	100
<50	95.2	97.6	100

Table 5.8. Percentage infection rates of hyphae and vesicles in individual plants across all treatments

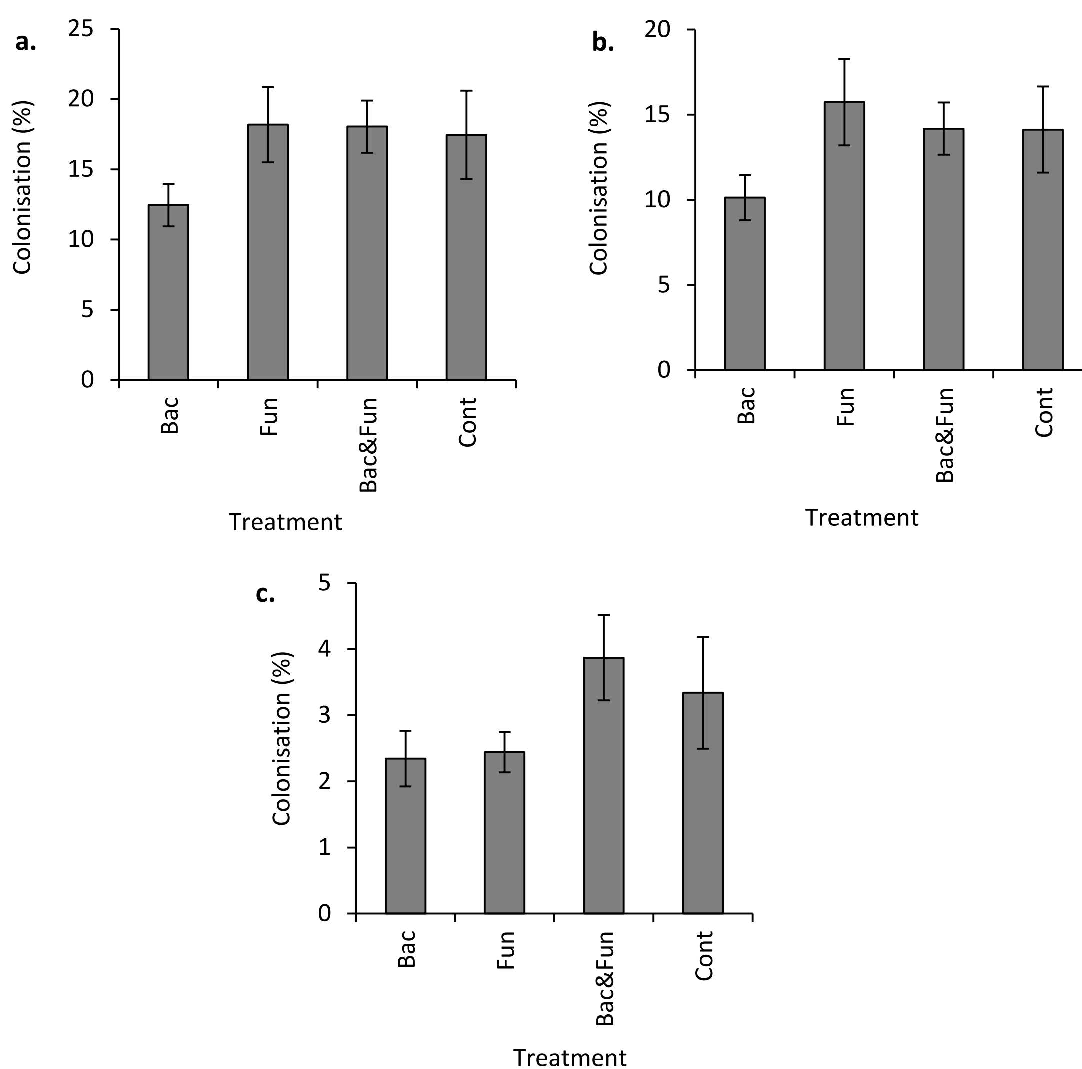

Fig. 5.32. Percentage colonisation by (a) hyhae and vesicles, (b) hyphae alone, (c) vesicles. Error bars represent SEM.

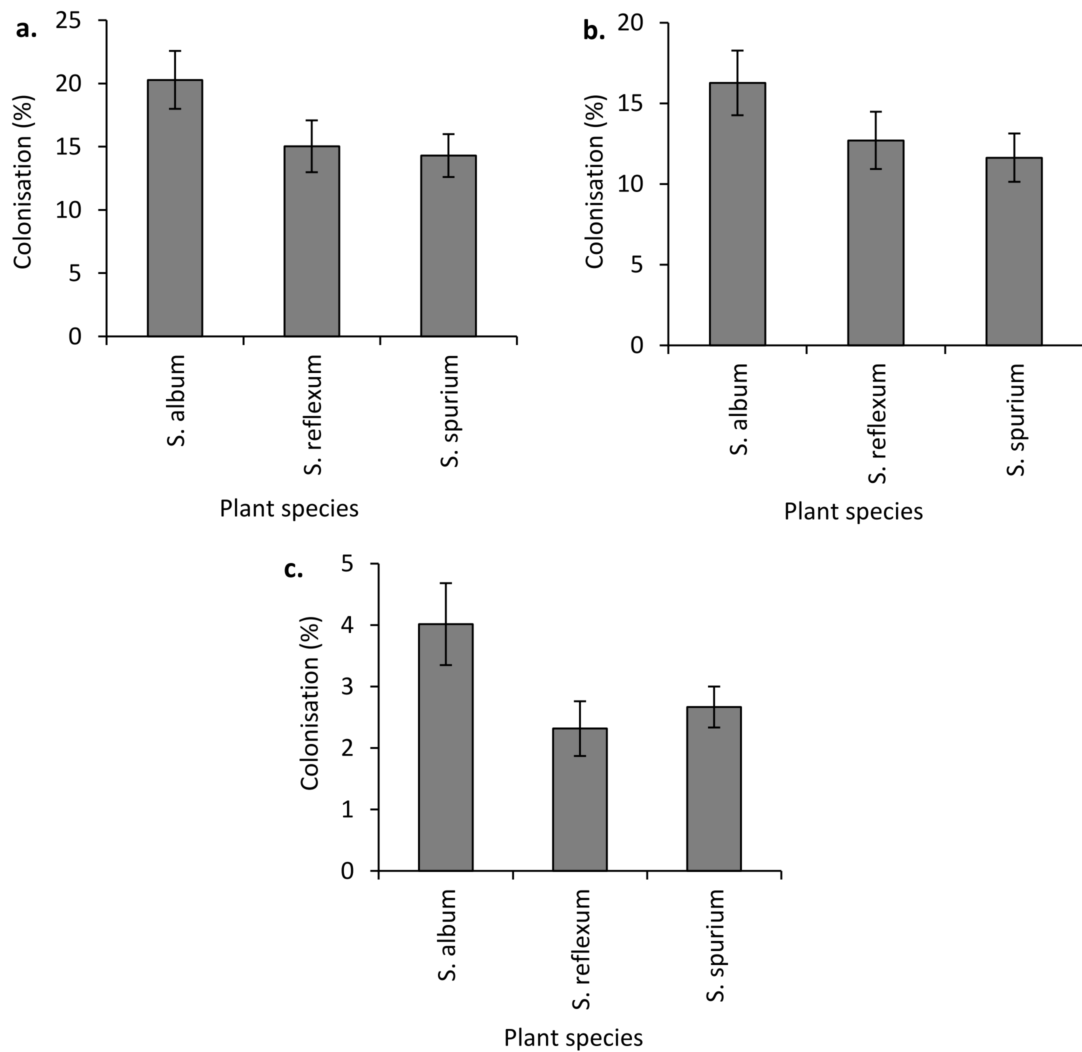

Fig. 5.33. Percentage colonisation by (a) hyphae and vesicles, (b) hyphae alone and (c) vesicles. Error bars represent SEM.

5.3.3 The microbial community

5.3.3.1 Pre-planting

The microbial mass present in the initial plugs was high, with *S. spurium* supporting the most microbial abundance overall (Fig. 5.34). This mainly consisted of bacteria (Fig. 5.34). The substrate supported very little microbial mass, with total microbial mass ranging between 0.3 and 2.2µg g^{-1}(Fig. 5.34).

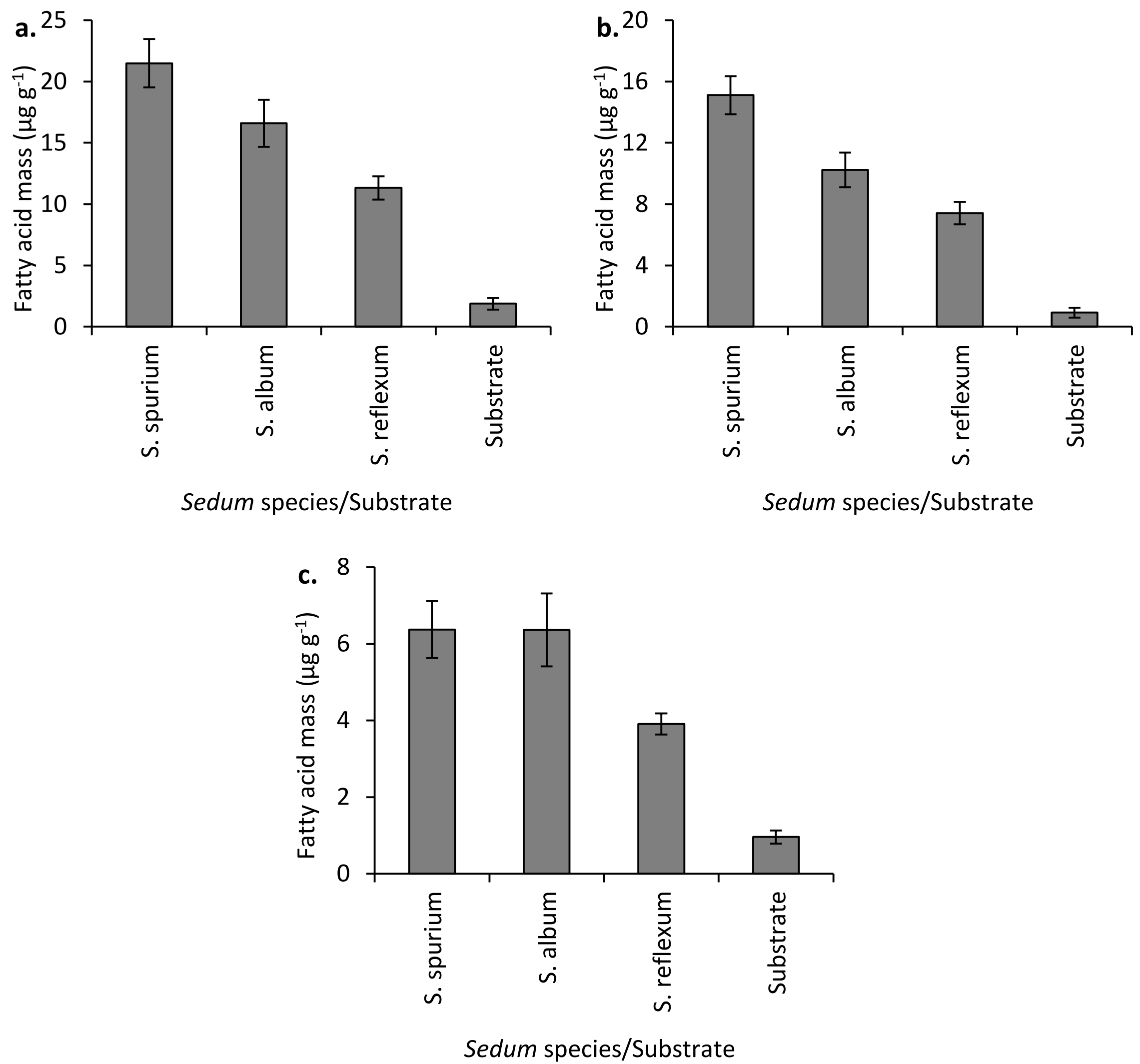

Fig. 5.34. Mean mass of fatty acids in each of the initial plugs, as well as the substrate. (a) Total microbial PLFAs, (b) bacterial PLFAs, (c) fungal PLFAs. Error bars represent SEM.

PCA highlighted that the community structure of fatty acids also varied between plug species, particularly between *S. spurium* and the other two species (Fig. 5.35). *S. album* had the most variable community structure in terms of fatty acids (Fig. 5.35). The substrate was markedly different in community structure to plugs (Fig. 5.35).

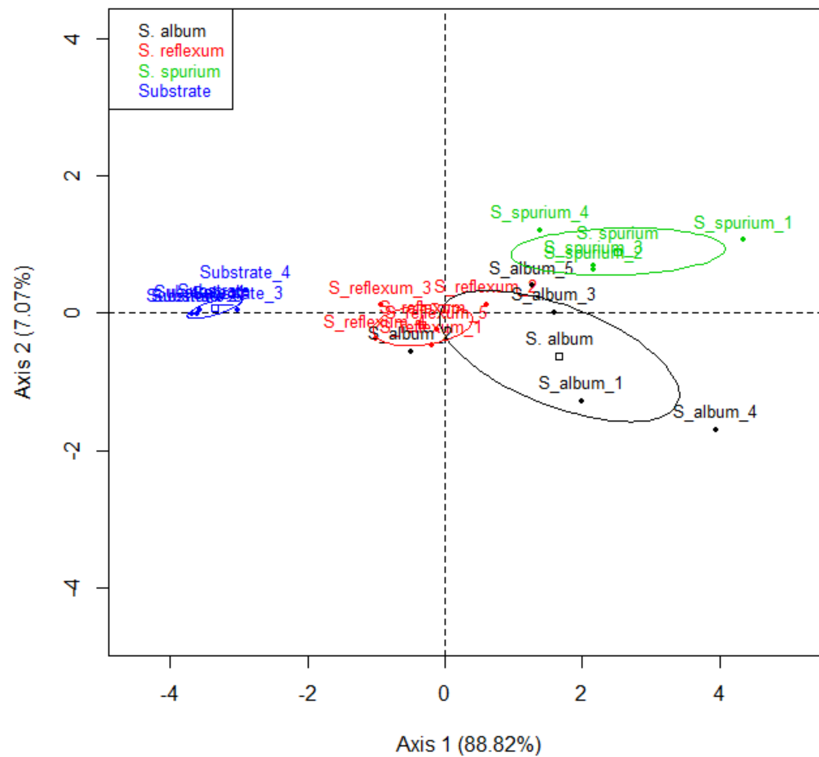

Fig. 5.35. PCA ordination plot of the microbial community present in the plugs and substrate used to construct the green roof.

5.3.3.2 Post-planting

Though fatty acids were in high abundance in the initial plugs, two months after planting these had reduced down to levels more similar to the low mass present in the initial substrate (Figs. 5.36 & 5.37).

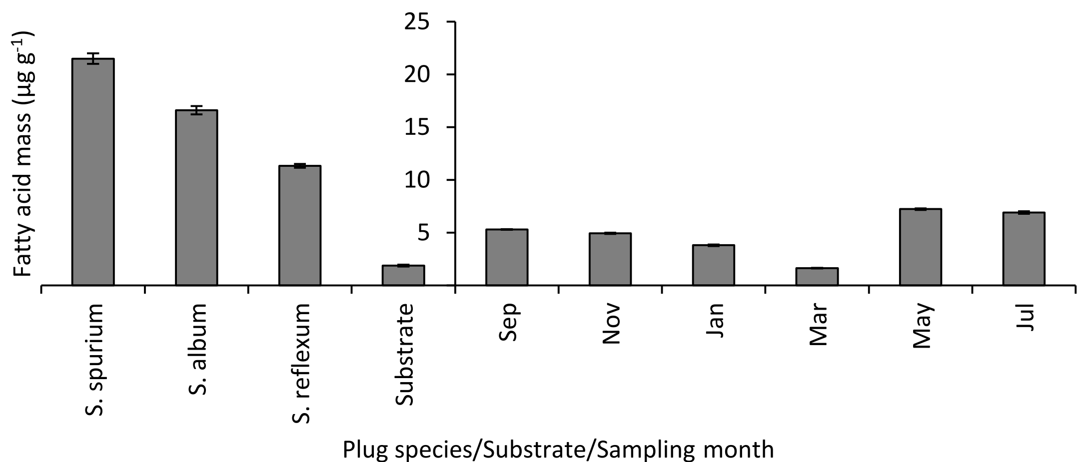

Fig. 5.36. Mean mass of total fatty acids in each of the initial plugs, as well as the substrate and in sampling months subsequent to planting. Error bars represent SEM.

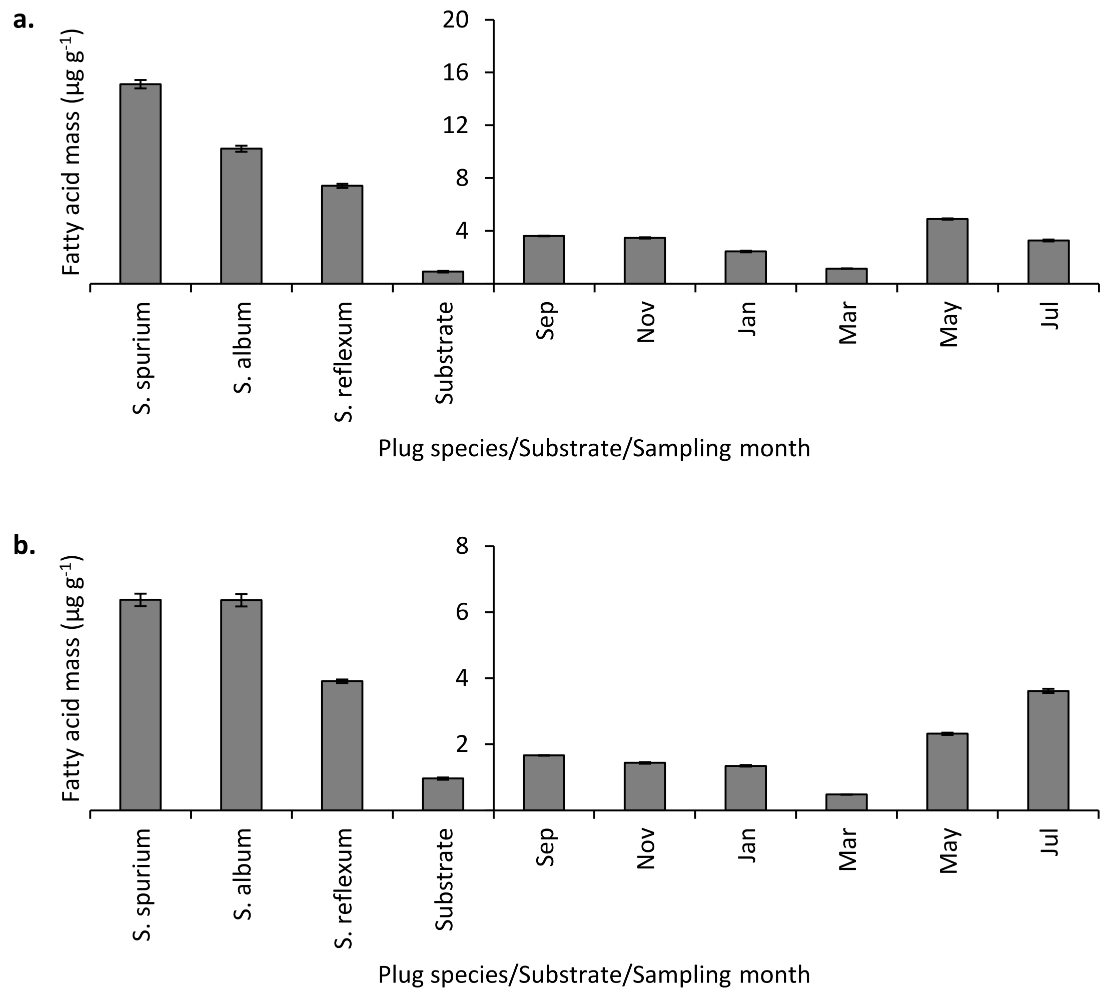

Fig. 5.37. Mean mass of fatty acids in each of the initial plugs, as well as the substrate and in sampling months subsequent to planting. (a) Bacterial PLFAs, (b) fungal PLFAs. Error bars represent SEM.

PCA of the microbial community present in the plugs and substrate used for the green roof construction and the subsequent months of sampling determined that post-planting the microbial community was more like the substrate used than the initial plugs (Fig. 5.38) with regards to community structure.

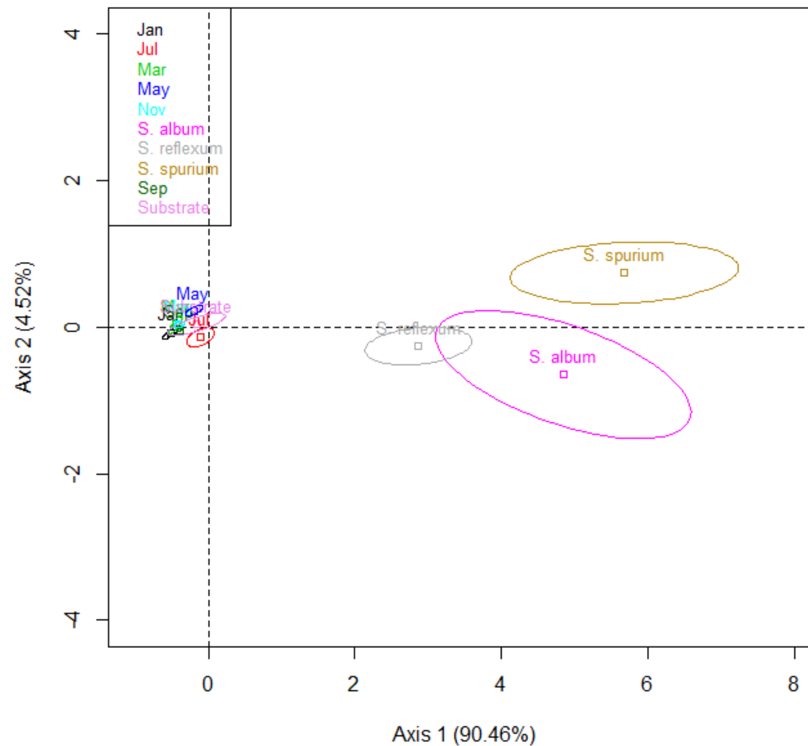

Fig. 5.38. PCA ordination plot of the microbial community in initial plugs and substrate before construction and the subsequent months of soil sampling after planting. Individual sample points have been removed for clarity.

5.3.3.3 Effect of inoculants

Microbial mass was not affected by any of the treatments, although there was a suggestion that it was increased by the mycorrhizal treatment ($F_{1,26}$ = 4.16, p = 0.052) (Fig. 5.39). None of the treatments altered the pattern of microbial abundance over time (Fig. 5.39). Bacteria and fungi were unaffected by treatment, although again there was an indication that the mycorrhizal treatment increased the abundance of fungi ($F_{1, 26}$ = 4.04, p = 0.055) (Fig. 5.39). Gram positive, gram negative and sulphate reducing bacteria were unaffected by any of the treatments (Fig. 5.40). AM fungi was increased by addition of the mycorrhiza treatment ($F_{1, 26}$ = 6.66, $p < 0.05$) (Fig. 5.41). There were no differences in the abundance of microbial fatty acids between planted control plots and unplanted plots for any of the microbial parameters (Figs. 5.39-5.41).

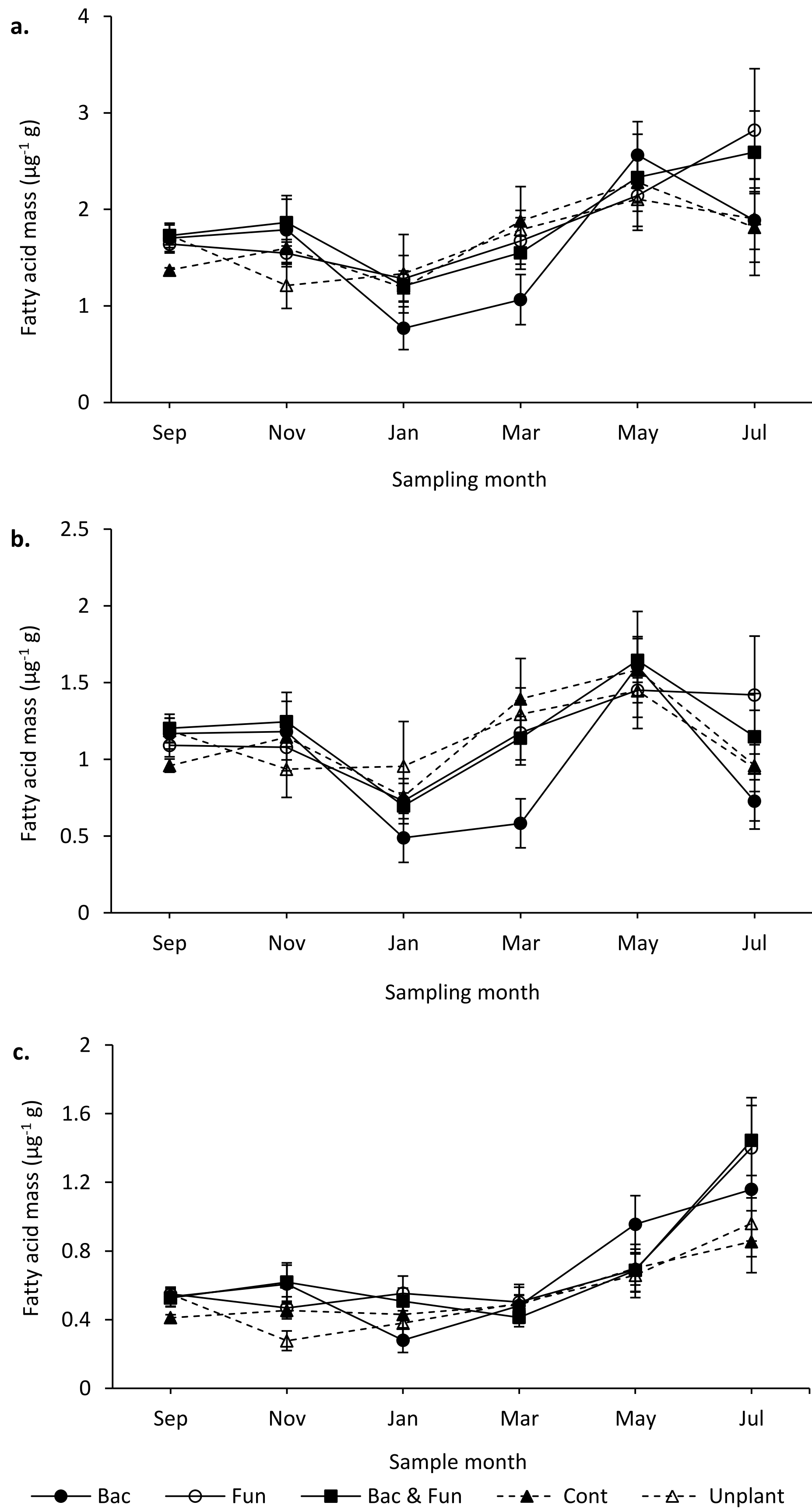

Fig. 5.39. Mean monthly mass of fatty acids for each treatment; (a) total microbial PLFAs, (b) bacterial PLFAs, (c) fungal PLFAs. Error bars represent SEM.

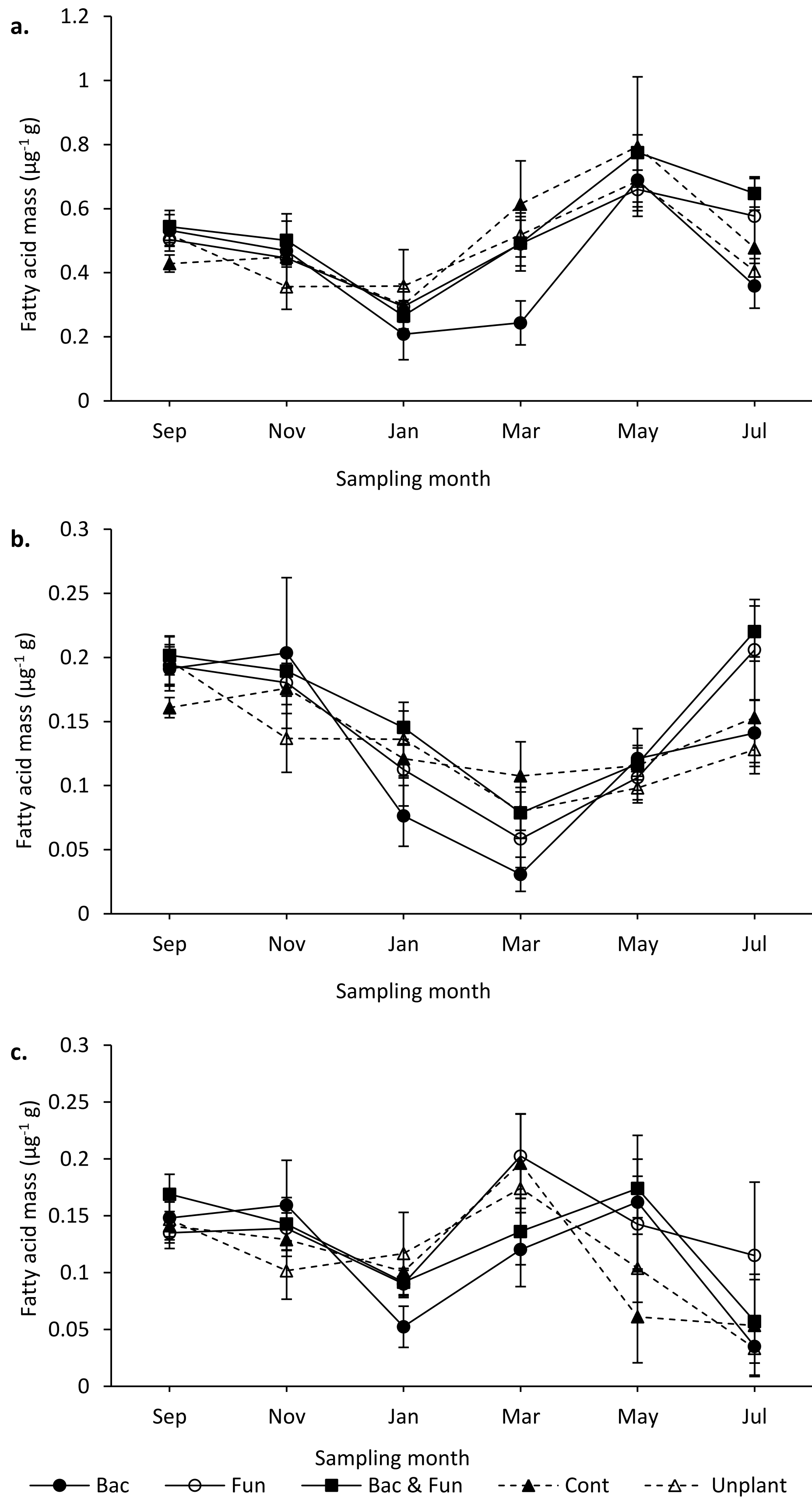

Fig. 5.40. Mean monthly mass of (a) gram positive bacterial fatty acids (b) gram negative bacterial fatty acids and (c) sulphate reducing bacterial fatty acids for each treatment. Error bars represent SEM.

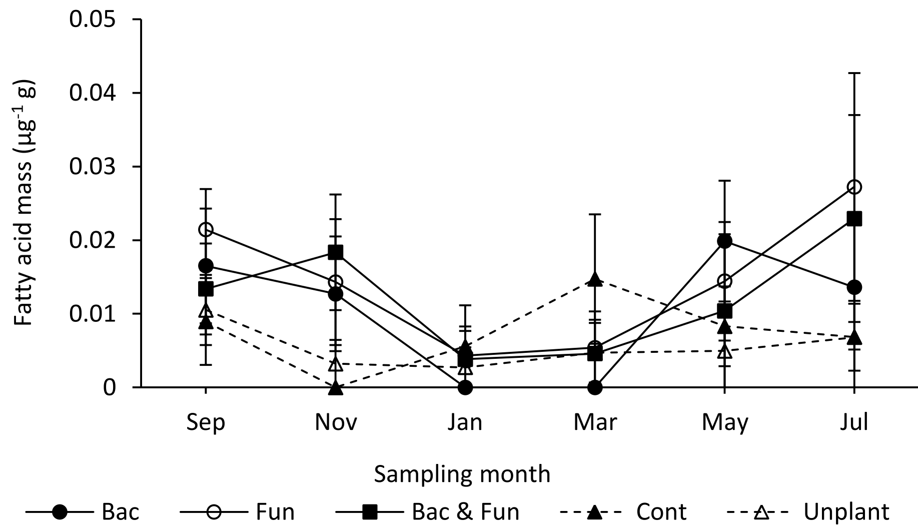

Fig. 5.41. Mean monthly mass of AM fungal fatty acids for each treatment. Error bars represent SEM.

PCA of the fatty acids identified also showed little difference between treatments, although the community present in bacteria treated plots coincided only slightly with the community in unplanted plots (Fig. 5.42).

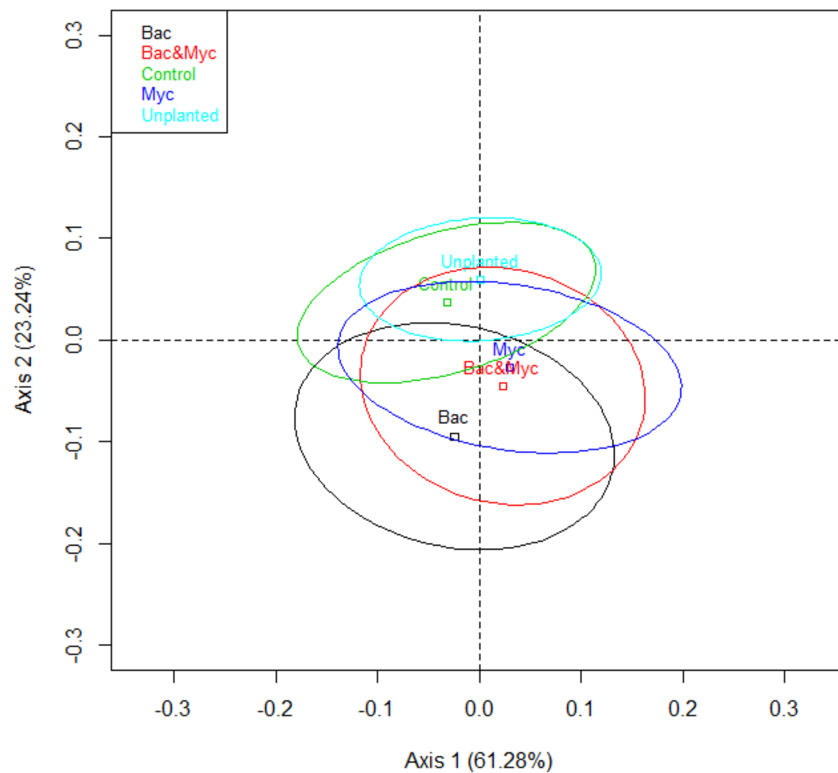

Fig. 5.42. PCA ordination plot for all microbial fatty acids. Confidences ellipses separate treatments and individual samples are omitted for clarity.

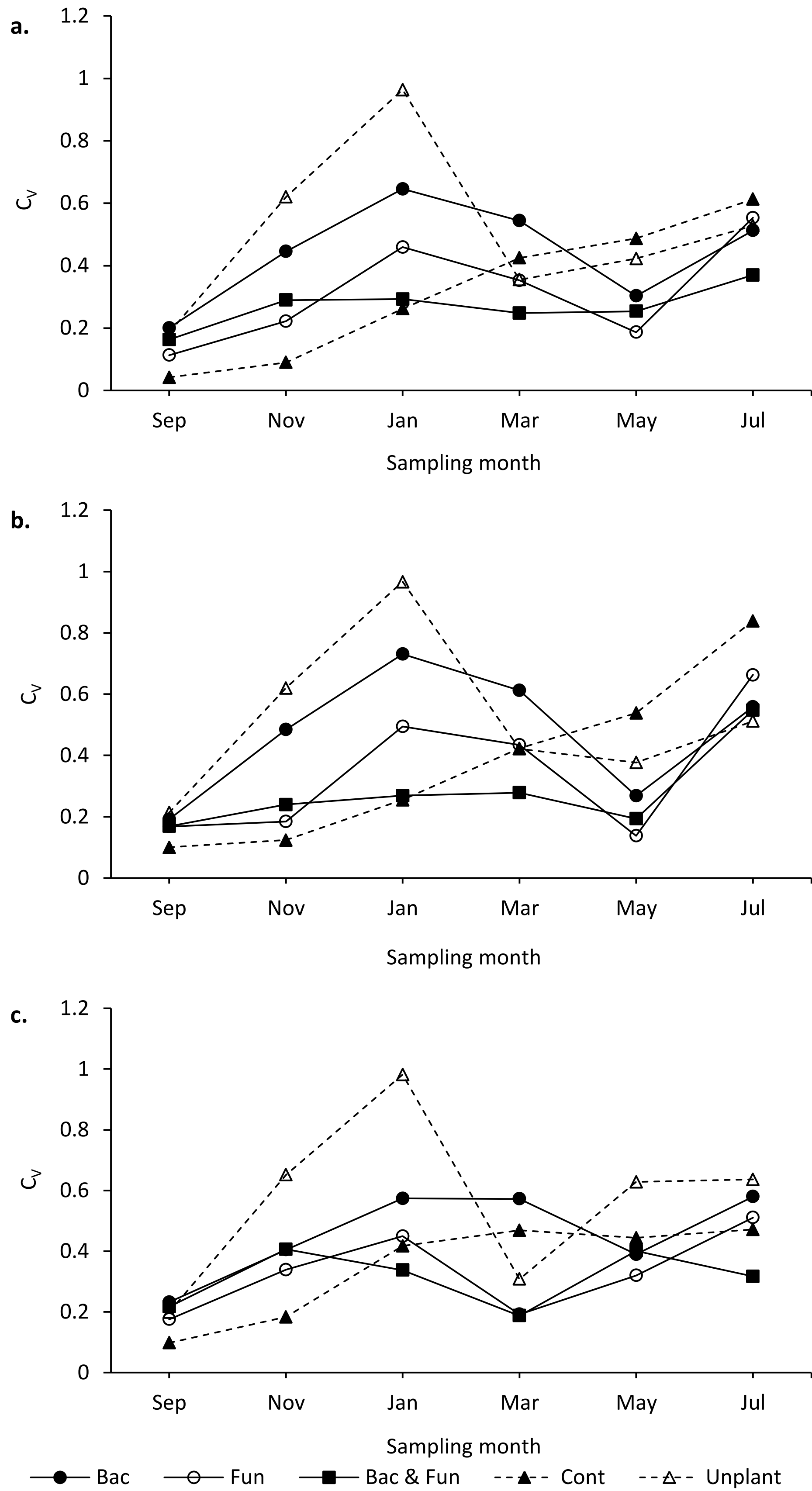

Fig. 5.43. C_V of a.) total microbial mass of PLFAs, b.) mass of bacterial PLFAs and c.) mass of fungal PLFAs.

Treated plots shared similar temporal patterns of variability (Fig. 5.43), particularly within the bacterial community. In most sample months, the bacterial treatment showed the highest variability of the three treatments, with the dual treatment varying least (Fig. 5.43). Control plots became more variable in microbial mass over time and bacterial mass and fungal mass showed the same pattern, although variation of fungal mass plateaued from March onwards where the others did not (Fig. 5.43). The unplanted plots were extremely variable in the winter months, but stabilised to levels of variability nearer to the other treatments in the summer months, except with regards to fungal variability where it was once again higher than in other treatments.

5.3.3.4 Seasonal patterns

Microbial mass changed over time (Table 5.9), with lowest mass occurring in January 2012 and mass increasing thereafter towards May 2012. For total mass in the control, unplanted and bacterial treatments, May 2012 was a high point before a decrease in microbial mass occurred in July 2012 (Fig. 5.39). For the mycorrhizal and dual treatment, July 2012 had the highest total microbial mass of fatty acids (Fig. 5.39). Bacteria showed a similar pattern to total mass, with lowest mass occurring in January 2012 before a rise towards May 2012 and a second decrease in July 2012 (Fig. 5.39). Fungi were lower in abundance overall than bacteria and showed less variation over time, remaining steady throughout September 2011 to March 2012 and then rising towards July 2012 (Fig. 5.39).

PLFA community	*F* value	Degrees of freedom	*p* value
Total microbial mass	8.36	3.76, 97.68	<0.001
Bacterial PLFAs	6.59	3.78, 98.34	<0.001
Fungal PLFAs	20.57	2.60, 67.65	<0.001
AM fungal PLFAs	3.08	2.98, 77.46	<0.050
Gram positive PLFAs	11.10	5.00, 130.00	<0.001
Gram negative PLFAs	20.49	5.00, 130.00	<0.001
Sulphate reducing PLFAs	5.99	3.61, 93.97	<0.001

Table 5.9. Effect of time on the microbial communities.

Gram positive bacteria showed a similar trend to the overall bacteria pattern (Fig. 5.40). However, gram negative bacteria were quite different, decreasing every month from

September 2011, until reaching a low point in March 2012. Gram negative bacteria then increased to September 2011 levels from March to July 2012 (Fig. 5.40). Sulphate reducing bacteria showed less marked peaks and troughs but also followed a general trend of low mass in January 2012, followed by a slight recovery in March and May 2012 before decreasing again in July 2012 (Fig. 5.40).

AM fungal fatty acids alone followed the same general pattern as total fungal fatty acids, though variability in mass was less pronounced (Fig. 5.43).

Variability was higher in the unplanted plots in November 2011 and January 2012 for all microbial groups (Fig. 5.43) whereas the control plots had the least variability in these months. From March to July 2012, the dual treatment was less variable than both the control and unplanted plots for all PLFA groups, suggesting this treatment could stabilise PLFA communities as roofs mature or in summer months (Fig. 5.43).

Parallel analysis determined that three axes explained the majority of the variance in the PLFA community, explaining 61.3%, 23.2% and 5.4%. As in section 3.3.4, the two fungal PLFAs C18:1ω9 and C18:2ω6, 9 and the bacterial PLFA C16:1ω7c were most associated with the two axes, with other fatty acids correlating more weakly with the axes (Table 5.10). Bacteria were less correlated with one another than in section 3.3.4 (Fig. 5.44).

PCA ordination (Fig. 5.44) separated May 2012 and July 2012 from each other and other months. July was also the most variable month according to PCA (Fig.5.45). September and November 2011 also differed from one another, although September, November, January and March were all similar in their community structure.

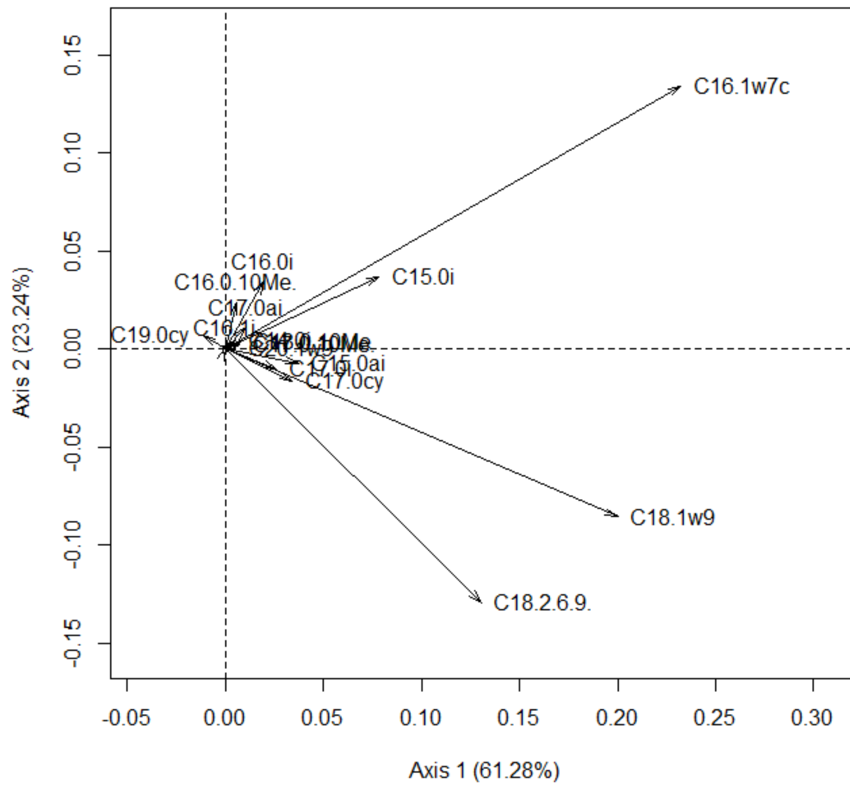

Fig. 5.44. PCA biplot depicting all PLFAs on the new green roof over the sample period.

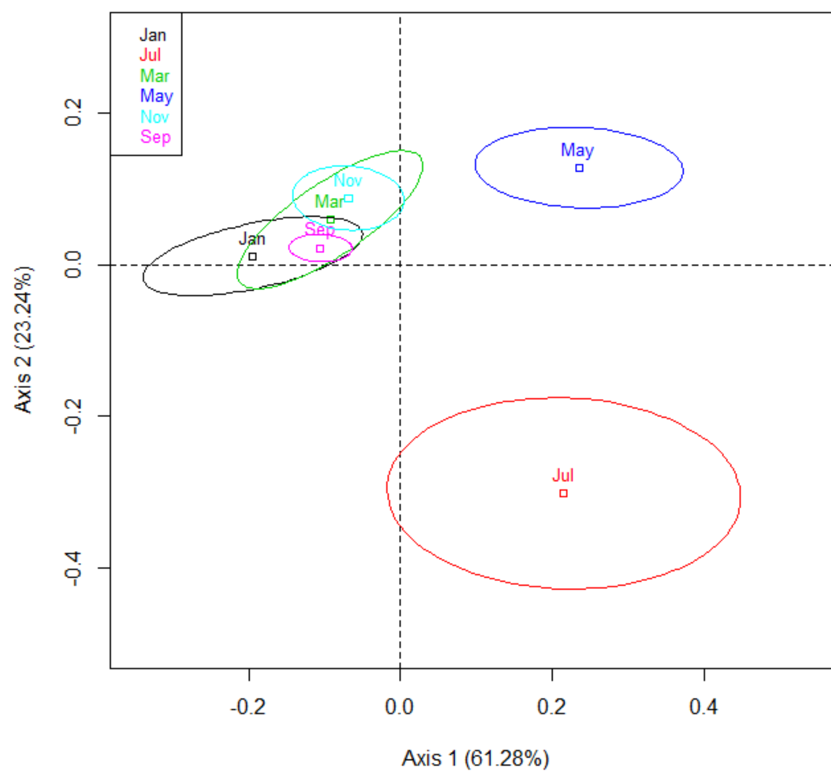

Fig. 5.45. PCA ordination plot depicting all microbial PLFAs, separated by month with confidence ellipses. Individual samples are omitted for clarity.

Fatty Acid		Correlation	*p* value
Axis 1			
C18:1ω9	Fungi	0.90	<0.001
C16:1ω7c	Bacteria	0.86	<0.001
C17:0i	Bacteria – Gram +	0.74	<0.001
C17:0cy	Bacteria – Gram -	0.68	<0.001
C18:2ω6,9	Fungi	0.68	<0.001
C15:0i	Bacteria – Gram +	0.67	<0.001
C17:0(10Me)	Bacteria – Sulphate reducing	0.43	<0.001
C15:0ai	Bacteria – Gram +	0.38	<0.001
C14:0i	Bacteria – Gram +	0.38	<0.001
C16:0i	Bacteria – Gram +	0.37	<0.001
Temp		*0.37*	*<0.001*
C17:0ai	Bacteria – Gram +	0.32	<0.001
C20:1ω9	Fungi – Mycorrhizal	0.30	<0.001
C18:0(10Me)	Bacteria – Sulphate reducing	0.21	<0.010
C19:0cy	Bacteria – Gram -	-0.23	<0.010
Axis 2			
C16:0i	Bacteria – Gram +	0.65	<0.001
Wind		*0.58*	*<0.001*
C16:1ω7c	Bacteria	0.50	<0.001
C16:0(10Me)	Bacteria – Sulphate reducing	0.46	<0.001
C17:0ai	Bacteria – Gram -	0.34	<0.001
C14:0i	Bacteria – Gram +	0.34	<0.001
C15:0i	Bacteria – Gram +	0.32	<0.001
C16:1i	Bacteria	0.19	<0.050
C17:0i	Bacteria – Gram +	-0.28	<0.001
C17:0cy	Bacteria – Gram -	-0.33	<0.001
Humidity		*-0.34*	*<0.001*
C18:1ω9	Fungi	-0.38	<0.001
Water content		*-0.44*	*<0.001*
C18:2ω6,9	Fungi	-0.68	<0.001

Table 5.10. Correlations between PCA axes and fatty acids in the analysis. Bold entries signify unique values for that axis, italic figures denote abiotic factors added as supplementary variables. Gram + denotes gram positive bacterial fatty acids, Gram - gram negative

5.3.3.5 Interactions with abiotic factors

Stepwise multivariate regression determined that three factors were correlated with total microbial mass: daily humidity, average wind speed and substrate water content. Bacteria were less well described by abiotic factors but daily humidity and average wind speed showed some correlation. Fungi were weakly correlated with daily temperature, as did the gram positive bacteria. Gram negative bacteria were correlated with three factors: average wind speed, daily humidity and average daily temperature. Sulphate reducing bacteria showed a positive correlation with wind speed and AM fungi were correlated with three factors: wind speed, daily humidity and daily temperature (Table 5.11).

Community parameter	R^2	*F* value	*p* value	Factor	*β*	*p* value
Total microbes	0.30	25.20	<0.001	1. Humidity	-0.50	<0.001
				2. Wind speed	0.39	<0.001
				3. Water content	0.21	<0.05
Bacteria	0.21	22.84	<0.001	1. Humidity	-0.35	<0.001
				2. Wind speed	0.23	<0.01
Fungi	0.05	8.65	<0.01	Temperature	0.22	<0.02
Gram positive bacteria	0.08	16.03	<0.001	Temperature	0.29	<0.001
Gram negative bacteria	0.22	16.77	<0.001	1. Wind speed	-0.29	<0.001
				2. Humidty	0.42	<0.001
				3. Temperature	0.34	<0.01
Sulphate reducing bacteria	0.19	42.72	<0.001	Wind speed	-0.44	<0.001
AM fungi	0.29	23.90	<0.001	1. Wind speed	-0.37	<0.001
				2. Humidity	0.42	<0.001
				3. Temperature	0.35	<0.001

Table 5.11. R^2, *F* and *p* values for stepwise multiple linear regressions perfomed on different PLFA community parameters (left) and *β* weights with their respective *p* values for explanatory factors. Degrees of freedom were 3, 176 in all cases.

PCA determined that average daily temperature was significantly correlated to axis 1, whilst wind speed, humidity and substrate water content were aligned with axis 2 (Table 5.10, Fig. 5.40).

5.4 Discussion

5.4.1 Pre-planting

A question unanswered so far in green roof ecology is; "How do microarthropods arrive on a green roof?" The hypothesis in section 3.4.1 was that aerial colonisation, phoresy on birds and arrival at construction are all possible sources. The latter has now been shown to be true, as plugs of the *Sedum spp.* supplied to construct this roof had a source of microarthropods residing within them, although the substrate did not. This may be due to the long growth time of *Sedum spp.* thus the length of time soil is exposed to coloniser sources compared to the substrate. On the whole, this finding is encouraging as it means that a source of microarthropods is present at the construction of the roof. It also highlights that any manipulation of this community should focus on plugs, rather than the substrate.

We have also seen that different species of plant harbour different communities, with *S. acre* bringing a more homogeneous community with it than the other two *Sedum spp.* This is important for the later development of the roof as *P. notabilis*, an abundant collembola in *S. acre* plugs, later went on to become a common springtail on the roof at later sampling dates. However, we have also seen that the majority of these organisms do not survive post-planting, presumably due to the harsh conditions on the roof. Therefore, if this source of colonisers is to be exploited to the benefit of the roof, the harsh soil conditions on the roof must be ameliorated to enable the survival of these species. However, there would also be value in experimenting with different growing systems at the farm level. For example, it is still unknown what impact the colonisation ability of less mobile species has on the development of a soil microarthropod community on a green roof. There could be value in growing *Sedum spp.* plugs in green roof media from the onset to ensure that those organisms colonising are suitable for the roof conditions, including less mobile species that would find it easy to colonise at the ground level but would rarely self-colonise a roof.

The microbial community showed a similar pattern to the microarthropod community. Plugs of *Sedum* harboured a high mass of microbial PLFAs, whereas the substrate microbial mass was extremely low. This suggests that the hypothesis proposed by Emilsson (2008), that firing of the substrate before application on a green roof diminishes soil microbes, is extremely likely.

Though the plugs harboured much higher abundance of fatty acid mass, this mass quickly reduced down to masses closer to that of the substrate. As with the microarthropods, there is a source of inoculant within the plugs, but it is unable to survive post-planting.

5.4.2 The microbial community

As expected for a virgin soil, the young roof was characterised by a bacterially dominated microbial community, with PLFAs in low abundance at the start of the experiment. Fungal PLFAs increased over time, suggesting microbial succession occurred (Ohtonen *et al* 1999; Bardgett *et al.,* 2002). Total microbial mass increased over time, with a spring maxima and winter minima. Grayston *et al.* (2001) and Bardgett *et al.* (1999) found that total PLFA mass in grassland soils also had a spring maxima, but was lowest in the autumn months rather than the winter, suggesting some differences in seasonal responses between green roofs and ground-level systems. This could be due to differences in planting regime, as Grayston (2001) notes that in grassland communities, vegetation structure can have a profound impact on PLFAs, even outweighing seasonal effects. This, alongside the fact that studies using PLFAs to determine seasonal changes in microbial structure in field soils are few, makes these findings difficult to compare with others. However, total microbial mass is within the range found on other young green roofs by Molineux (2010).

There was a pronounced community shift in July 2012, when gram positive and sulphate reducing bacteria began to decrease but fungi, including AM fungi, and gram negative bacteria remained high. Gram positive bacteria have been shown to be more tolerant to drought stress than gram negative bacteria due to their thicker cell wall (Fernandez *et al.,* 2012) so another physiological process aside from water stress may have been responsible for this decline. Gram negative bacteria are thought to adapt quicker to environmental change than gram positive bacteria (Fernandez *et al.,* 2012) and tend to increase as organic carbon (C) is added to soil. Fungi (Griffiths *et al.,* 1998; Fierer *et al.,* 2003), including AM fungi (Collins Johnson *et al.,* 1991), also follow this trend, increasing with C, and they too increased in mass throughout the summer. Meanwhile, gram positive bacteria and sulphate reducing bacteria are known to decrease in mass as organic C is added to the soil (Griffiths *et al.,* 1998; Fierer *et al.,* 2003), fitting the pattern on the roof. Studies measuring the activity of soil microbes by looking at microbially produced C or respiration rates have also shown summer peaks in many environments, due to increased temperature and therefore increased metabolic rate of decomposer bacteria (Lynch and Panting, 1980; Patra 1990; Blume *et al* 2002). It is highly probable, therefore, that organic C regulates the seasonal changes in microbial PLFA communities observed on the roof and that these peak in summer when bacterial activity is highest.

The only treatment to have a significant effect on microbial mass was the mycorrhizal treatment, which increased the abundance of the fatty acid marker 20:1ω9, indicating that AM

fungal abundance (*Gigaspora spp.* specifically) had increased (Sakamoto, 2004). However, there was no effect of this treatment alone on either mycorrhizal colonisation of *Sedum spp.* roots, or on plant growth. The colonisation of plant roots by mycorrhizas has been shown often to be unrelated to changes in plant growth parameters, even when improvements in the latter are seen (Medina *et al.,* 2003; Johansson *et al.,* 2004) and so the lack of an effect on root colonisation alone does not explain the lack of plant growth enhancement by the inocula. It may be that *Gigaspora spp.* are not beneficial to *Sedum spp*. The low resolution of PLFA for separating fungal groups makes it difficult to determine if only *Gigaspora spp.* spores proliferated from our inocula or if it was the only fungi detected by PLFA, so the effect of *Glomus spp.* in this context is unknown.

A second hypothesis explaining a rise in soil AM fungi without seeing enhanced growth parameters in plants would be that there is a resident mycorrhizal population in the *Sedum spp.* and that the addition of the inoculant either does not benefit the plant further, because it is the same species or one that is less beneficial to *Sedum spp.,* or that the added mycorrhiza is excluded from colonising the plant roots by the resident mycorrhiza. We saw in section 4.3 that even when added mycorrhiza were able to establish in *Sedum* roots, no benefit to the plant was seen, making either of these hypotheses possible. No changes in vesicle number or arbuscule number were detected in the fungal treatment, perhaps alluding to no change in mycorrhizal species colonised (Abbot and Robson, 1979; Hepper *et al.,* 1988).

Support for a resident population comes from the fact that mycorrhizas were present in the control plants, suggesting that the *Sedum spp.* already had a source of inocula when they arrived as plugs. The three *Sedum spp.* used are not reported as being mycorrhizal in the wild (Harley and Harley, 1987), yet have been found to be consistently highly mycorrhizal in all our studies (other species of *Sedum* such as *S. albertii, S. maximum* and *S. album* are naturally mycorrhizal according to Harley and Harley (1987), Wu *et al.,* (2007) and Kowalczyk and Błaszkowski (2011)), suggesting that *Sedum spp.* supplied for green roofs must have a source of inocula somewhere during their initial growth. As *Sedum spp.* used for green roofs are not grown in their natural environment (greenhouses and fields as opposed to harsh, rocky outcrops), discrepancies between these cultivated plants vs. their wild types may be explained by additional sources of inocula in cultivated *Sedum spp.* Additionally, studies on mycorrhizal associations with *Sedum spp.* seem to be rare and so it may simply be that records of wild type colonisation by mycorrhizas are incomplete. However, as the species colonising these roots are as yet unidentified, it could be that this is not a common species, perhaps one that may exclude additional mycorrhizas. However, the increase of the PLFA C20:1ω9 without an

increase in root colonisation would suggest that added inocula may be able to produce extraradical hyphae in the soil, even if they are unable to colonise plant roots.

Although there were no main effects of the inoculants on the PLFA community structure according to PCA, there were some contrasts in variability. The bacterial inoculant caused higher variability in both the bacterial and fungal PLFAs in most months, perhaps by facilitating organic C to be used as a resource (Buchanan and King, 1992). Control plots rose in variability over time, perhaps due to increasing *Sedum spp.* rootzone and therefore patchier resources due to the addition of root exudates.

No differences were found between planted and unplanted plots, suggesting that the microbial community at least in these early stages is independent of planting regime. However, there were stark differences between planted and unplanted plots with regards to variability of abundance. Unplanted plots were more variable than treated plots and even more variable than planted controls in most months, suggesting that the plant community may act to stabilise microbial abundance, perhaps by buffering the substrate from abiotic conditions or by releasing root exudates. Another theory put forward by Buchanan and King (1992) states that soils with an input of crop residues fluctuate more in microbial mass (measured by measuring microbial C and P) than bare soils. In our study, seasonal ruderal plants better able to colonise bare plots act as a 'crop' input to the soil when they die off in winter and summer. Thus, in these plots one would expect a more variable microbial community than in the relatively more stable *Sedum spp.* planted plots.

5.4.3 Vegetation

No single treatments had an effect on cover of *Sedum spp.* However, increases in cover of *Sedum spp.* were slower in plots inoculated with the dual treatment than in other plots. One hypothesis explaining this is based on the theory that the *Sedum spp.* present were already colonised by a resident population of mycorrhizas. Whereas in the single treatments, additional AM fungi had no effect on the resident AM fungi due to competitive exclusion, in the dual treatment the addition of bacteria may have increased the competitive ability of the added mycorrhiza, placing the resident mycorrhiza under competitive pressure. This would have two outcomes for AM fungal dynamics. The first would be that the added mycorrhiza caused a diversion of resources by the resident mycorrhiza in order to compete, in turn causing a reduction in nutrients available to the plant. Vierheilig *et al* (2000) propose that resident mycorrhizas exclude invading fungi by means that could include production of mycotoxins. These, presumably, would be produced at a cost to the resident mycorrhizas and thus the host plant. The second effect of competition would be that the added mycorrhiza was

able to displace the resident species, lowering plant growth due to its lower specificity with *Sedum spp*. There is evidence to suggest that mycorrhizas better at competing for root space may be less beneficial to plants (Bennett and Bever, 2009) and that one species of mycorrhiza may completely displace another over relatively short time scales (Hepper, 1988). Whether this theory extends to fungi/bacteria mutualisms is unknown however. Displacement of the resident AM fungi population is also supported by the fact that plants treated with the dual treatment had increased vesicle numbers than those without, which could denote a different mycorrhizal species (Abbot and Robson, 1979; Hepper *et al.,* 1988). However, it cannot be overlooked that vesicle production can also be a response to stress, with increased vesicle formation in some fungal species associated with cation (Cooke *et al.,* 1993), nutrient (Davies *et al.,* 2000) and drought (Davies *et al.,* 2002) stress.

Bacterial inoculants have been shown to be effective helper species for mycorrhizal colonisation, with the addition of both together shown to increase plant yield (Toro, 1997; Medina, 2003; Vivas *et al.,* 2003a; Vivas *et al.,* 2003b). However, this effect is extremely species specific in the literature, both for the bacterial and fungal species involved. For example, Medina *et al.* (2003) found that whilst plant yield in alfalfa was increased by the addition of the mycorrhiza *G. mosseae* and either of the two bacteria *B. pumillus* and *B. licheniformis*, neither of these bacteria increased plant yield when co-inoculated with *G. intraradices*. This theory is the driver behind many inoculant companies producing mixes of inoculant species, as used in this study, supposing that at least one successful combination will be present and that mixes will be less susceptible to changes in environment (Koomen, 1987). Additionally, although the literature is divided about whether the mycorrhizas present in commercial mixes compete against one another, there is little evidence to suggest that this competition between mycorrhizas depresses plant growth (see Taylor, 2000 for a rare example), only that the effectiveness of the inocula is less pronounced when mycorrhizas in the mix compete (Daft and Hogarth, 1983; Lopez-Aguillon, 1987; Hepper, 1988; Talukdar, 1994). More recent papers suggest mycorrhizas do not compete at all but in fact exploit different root areas and often co-occur (Cano and Bago, 2005; Alkan, 2006). However, without studies based on a wide range of fungal, bacterial and plant species, it is difficult to draw conclusions about whether AM fungi compete or do not. This study would suggest that, at least in green roofs, they do compete and that in certain plant species, the effect of inoculants may be reduced as a result.

It is clear that the addition of commercial multi-species inoculants into green roof substrates does not increase the cover of *Sedum spp.* when added at construction, and may in fact be detrimental. To establish whether the *Sedum spp.* in this study and on green roofs in general

do have a specific, or at least less common, mycorrhizal association it is recommended that future studies incorporate identification of mycorrhizas and that from this platform, the development of more species specific inoculants be established.

The population of colonising forbs present on the roof consisted mainly of immature seedlings. Forb cover and number was unaffected by any of the inoculants but was affected by planting regime, with numbers severely decreased in planted plots and cover higher in unplanted plots. This means that differences between treated plots were difficult to ascertain due to low numbers, but does exemplify the point that plug planting with *Sedum spp.* may exclude ruderal plants, presumably due to competition for space to germinate and available light. The same pattern was seen in grasses, and although the pattern of grass cover over time was altered by the dual treatment, only three grass individuals (of *P. annua*) were present in the September sample with which to compare with July so this result is most probably confounded by a lack of replicates. Despite the low numbers of ruderal plants though, it is clear that planting effects their colonisation. This could have knock-on effects for predators such as spiders, as plant architecture is known to affect their populations (Hatley and MacMahon, 1980) and it is recommended that this is included in future studies of above-ground organisms.

The grasses present, *F. rubra, A. stolonifera* and *P. annua* are all known to be mycorrhizal (Harley and Harley, 1987; Olsson *et al.,* 1998) and interactions between mycorrhizal fungi and these grass species are widely reported (Sanders and Fitter, 1992; Skalova and Vosatka, 1998). Gange *et al.* (1999) found that in sports turf, areas with mycorrhizas increased the abundance of *A. stolonifera,* whilst populations of *P. annua* decreased. There was also a suggestion that this effect could be replicated with commercial inoculants, including three species present in the inoculant: *G. mosseae, G. intraradices* and *G. caledonium.* In concurrence with this study, mycorrhizal colonisation was not necessarily linked to changes in plant growth. However, Veiga *et al.* (2011) found the opposite effect, with inoculation of *G. intraradices* alone increasing growth of *P. annua* and found that some plant/mycorrhiza interactions were affected by the presence of other plants. This exemplifies the difficulties in comparing different experimental environments and demonstrates the importance of conducting inoculant experiments in the environment intended for the eventual inoculant use.

The increased numbers of forbs and grasses in unplanted plots compared to *Sedum* planted plots supports the opinion that *Sedum* planted green roofs could create urban monocultures. However, exclusion was not complete and ruderal plant populations were ephemeral in all plots, dying out in winter. *Sedum* is important then for supporting organisms requiring year round cover. Perhaps a system that is able to achieve year round cover but high biodiversity

should involve a mixture of the two planting regimes and future studies should focus on the interactions between the two as source and sink habitats for microarthropods.

Overall, numbers of both grass and forbs were very low, making it difficult to ascertain if inoculant addition would improve the colonisation of ruderal plants into *Sedum* planted plots. Planting ruderal species amongst *Sedum spp.* and monitoring their growth would help determine if colonisation ability is a limiting factor for these plants. If this were found to be true and seedlings were able to establish better under this design, further experiments conducted to determine the suitability of inoculants would be more successful.

5.4.4 Microarthropods

The density of collembola was extremely low throughout the sample period, and it was highly variable. This high variability was due to numbers of *Parisotoma notabilis* that were extremely abundant in some plots, but extremely low in others. *P. notabilis* was found in all but one of the 10 *S. acre* and *S. spurium* plugs and all but two of the *S. reflexum* plugs so should have had a source population in all plots. However, collembola form highly aggregated distributions (Bardgett *et al.,* 1993; Chernova *et al.,* 2010) and so the possibility that our method of pseudo-sampling, implemented to try to overcome this, was insufficient cannot be ruled out. However, it could be that varying source numbers and conditions meant that some plots harboured more species than others as aggregation in collembola is usually due to a driving factor, such as water availability (Benhamou, 1992) or microbial activity (Hassall *et al.,* 1986).

The abundance of collembola significantly increased in those plots treated with bacterial treatment. Though most collembola are predominantly fungal feeders, there is evidence to suggest that their diet varies depending on the availability of food and that *P. notabilis,* the dominant collembola in this study, preferentially feeds on amorphous organic material and faecal matter in food choice experiments (Ponge, 1991; Gillet and Ponge, 2003).

It is plausible that the bacteria added in the inoculants produced more, or more palatable, organic matter available to *P. notabilis*, or that an intermediary organism was affected by bacteria altering the palatability/volume of their faecal pellets. However, no other organism's abundance was affected by the bacterial treatment alone, nor could any associations between collembola and another species be found (data not shown), suggesting a qualitative rather than quantitative effect of bacteria on faecal pellet palatability. Bacterial PLFAs did not increase in the bacteria treatment either, suggesting that it was not an abundance of bacteria that had an effect on *P. notabilis,* but rather the community changes present within the bacterial community (as seen in the PCA). The high abundance of *P. notabilis* and its

preference for bacterial based food webs could have had an impact on our PLFA results, explaining why the addition of bacterial inoculants did not produce a corresponding increase in bacterial PLFAs. It may be that grazing by collembola cancelled out the additive effect of the bacteria (Chamberlain *et al.,* 2006).

Another species present, *O. villosa,* is also capable of dietary shifts depending on food availability, including consuming a bacterially dominated diet in some situations (Haubert *et al.,* 2009). *S. aureus* is also capable of diet switching and has a wide range of dietary components, including bacteria and amorphous organic matter, but preferentially feeds on fungi (Gillet and Ponge, 2005). *S. aureus* was too low in abundance to determine if the fungal treatment had an effect on it specifically. *S. trinotatus* is a relatively rare species and so little is known about its dietary preferences and ecology. All four of these species were in such low abundances that it is highly likely that the bacterial effect was mostly due to an effect on *P. notabilis*. However, the generalist nature of these collembola species explain why they are the most common microarthropod in this environment, able to exploit the resources that are available to them in an early successional environment.

The addition of inoculants, regardless of what they were, decreased variability in the collembolan community. This alongside the ability of the collembola found to switch diets suggests that inoculated plots provided a more stable food source than control and unplanted plots. Alternatively, the treatments made the conditions more favourable in another way, for example by changing the pH. Future studies should include analysis of the soil organic matter in plots and pH to determine their effects on collembola numbers.

Planting increased the abundance of collembola, and collembola were found to be correlated to *Sedum* cover, most probably because plants provided a nutrient input to the soil. Hågvar and Klanderud (2009) found that addition of nutrients to soil increased the abundance of *P. notabilis*, although they also noted that plant cover changed with addition of nutrients. *Sedum spp.* did provide more cover than non-*Sedum* species, so this too could be a factor influencing collembolan abundance. There is evidence to suggest that vegetation cover can benefit microarthropod communities by ameliorating harsh environmental conditions (Nyakatya and McGeoch, 2008). pH could also be an influencing factor as Molineux (2010) found that the pH of red brick green roofs were at approximately pH 9.8 at construction but had reduced to around pH 7.4 after one year due to the addition of organics and planting. *P. notabilis* is known to occur at varying soil pH, helping to explain the prevalence of *P. notabilis* over other collembola species in this environment.

The abundance of *P. notabilis* suggests that although young green roofs do not support a diverse range of organisms, they do support secondary consumers, supported by the coprophagous nature of *P. notabilis.* However, the abundance of collembola in bacterially dominated plots also suggests that the fungal community is limited in its ability to support collembolan communities and that only those collembola able to shift their diet to a bacterially dominated one will thrive, at least in the early stages of green roof succession. Planting is a vital green roof component, with significantly reduced collembola numbers in unplanted plots. The presence of *S. trinotatus,* as a collembola rarely recorded in the UK, suggests that although abundance of collembola is low, green roofs are capable of supporting rare species. This supports the findings of Kadas (2006) who found that rare spiders and beetles could also be supported by green roofs.

Mites were present in lower abundance than collembola, perhaps due to the more specialist nature of oribatid mite populations (Ponge, 1991) and their k-selected survival strategies (Norton, 1994). Oribatid mites were the most prevalent mite on the roof and, as in section 3.3.3.3 belonged to the family Scutoverticidae.

The only effect of inoculant addition on the mite community was seen when the dual treatment was added, reducing mite abundance. However, planting regime (which was also lowered by the dual treatment) did not affect mite abundance, suggesting that mite populations are independent of planting and that it is therefore unlikely that corresponding reductions in *Sedum spp.* cover seen in the dual treatment are the cause of this decline. One explanation could be the prevalence of Mite 5, a species of the superfamily Bdellidae, in these plots as this group of predatory mites is known to prey on other mites (Krantz and Walter, 2009). However, this species was only prevalent from May onwards and cannot, therefore, explain why the dual treatment supported lower numbers of mites in earlier months. Nor does it explain why Bdellidae were found in such high abundance in bacterially treated plots, particularly the dual treatment, although the increased numbers of collembola in these plots could be acting as a dietary source. A drop in collembola was also observed when Bdellidae were at their highest abundance, but no association could be found between the two microarthropods (data not shown).

The Scutoverticid mite was unaffected by treatment but did vary seasonally, peaking in January. In section 3.3.3.3, Scutoverticidae also peaked in winter, suggesting that this mite may have a preference for winter months. The predatory mites, Mite 5, the Bdellida and Mite 10, a Tydeid mite, peaked in May, presumably because microarthropod abundance was at its highest at this point, providing an increased abundance of prey.

The presence once more of the drought tolerant Scutoverticid mite again explains why mites were relatively unaffected by abiotic conditions, as these organisms are specialists of harsh conditions (Schäffer *et al.,* 2010b). However, the presence of predatory mites a year after construction denotes that some succession is taking place on the roof and that prey species must be abundant enough to support them. The diversity of mites present on the roof also suggests that there are a range of ecological niches available, despite the relatively few microhabitats available. The addition of more microhabitats, therefore, has the potential to support even higher mite diversity on green roofs. The negative effect of the dual treatment on mite abundance demonstrates the level of trophic niches that can be affected by microbial manipulations and the intimate relationship between microarthropods and the microbial community, demonstrating the importance of incorporating microarthropod surveys into assessments of ecological changes brought about by inoculant addition.

Other organisms were extremely low in abundance throughout most of the sample period and peaked in July, when most individuals were counted. This group were found to be higher in bacterial and mycorrhizal plots (including combinations of these) than in other plots, but as they were found in only 26 out of 180 samples, and in only three plots did they number more than 10 individuals, results must be interpreted with care.

One of these organisms, a larvae of a chironomid midge, was found in only one plot at high abundance but was also found in high abundance on the mature roofs studied. Frouz (1997) found that terrestrial chironomids showed a preference for oviposition in areas of low growing plant cover with open patches and that their larvae were aggregated in these types of habitat. Chironomid numbers also decreased as plant cover increased in this study. Despite the low numbers in these samples, it is plausible that the lowered plant cover in the dual treatment was attractive to ovipositing females. Interestingly, chironomid midge larvae, like *P. notabilis*, are also known to be coprophagous (Ponge, 1991), suggesting some niche overlap must be present on the roof. The second abundant species, found in two plots in high abundance, was a member of the family Thysanoptera, an order with wide dietary preferences (Gillott, 2005).

The presence of chironomid midge larvae is indicative of this type of open habitat and could provide a vital food source for larger organisms such as birds (Delettre, 2000). As this species was consistently found in young and mature roofs, it will be discussed at length in section 7.1.

5.5 Conclusions

It is clear from this experiment that *Sedum* plugs are a potentially valuable source of soil organisms to the green roof environment at construction, but that those species present are

not adapted to green roof conditions. In order to maximise the use of this as a source, ways must be found of either ensuring that drought adapted species are present in plugs or that the conditions on green roofs are ameliorated for their later survival.

For generalist species such as collembola and midge larvae, microbial inoculants did contribute to ameliorating these conditions, providing an additional food source. However, more specialised species, such as mites, were negatively affected by the addition of inoculants. In addition, both the microbial and plant community did worse under inoculated conditions, particularly when mycorrhizae and bacteria were added together, most probably as a result of competition.

These results suggest that a resident microbial population forms rapidly on a green roof with the plugs acting as a source. The addition of inoculants at construction is already too late to prevent this competition from having negative effects on the community. Furthermore, one application of inoculants has long-term effects on soil communities, with effects seen even one year after application. Though these specific inoculants have not been overly successful in this trial, the longevity of their effects and the positive responses by some species suggest that more tailored inoculants would have a positive effect on green roofs and would only need to be applied once.

Furthermore, this experiment has allowed conclusions to be drawn about the succession of green roofs from the point of construction. It is clear that colonisation can happen by new species very soon after construction, but in very low numbers so establishment is slow. Once again an unusual species, this time *Sminthurinus trinotatus* has been found on the roof, emphasising the unique community assemblages that could be nurtured on green roofs.

Chapter 6

Application of microbial inoculants to a mature green roof

6.1 Introduction

Evidence gathered in Chapter 3 suggests that green roofs support only a limited diversity of microarthropod life, in addition to low plant diversity and microbial mass. Extensive green roofs of this design are extremely common and, due to their affordability, one roof can provide hectares of habitat. For example, the extensive *Sedum* planted green roof installed on the Ford Motor Company factory in Michigan, USA, spans over 10 acres. However, as mentioned in Chapter 3, with a level of microarthropod diversity similar to that of a desert or glacial foreland, and far less diverse than parkland, this large roof and many others like it may not be reaching its potential in terms of diversity. Converting existing green roofs into valuable habitat is a challenge that must be further investigated if these habitats are to truly mitigate habitat loss.

One way to do this may be the application of additional nutrients, but as mentioned in Chapter 4.1, not only may this cause an increase in nutrient leaching (Berndtsson, 2010), but its necessity for reapplication could render it less sustainable than a more long-term solution. The addition of microbial inoculants could provide a better solution, especially in light of the fact that the firing process used to prepare green roof substrates could render it devoid of microbial life. In section 5.3.3.1, the substrate was subjected to PLFA analysis before being used on the green roof and it was found that it supported very little microbial life. Thus, adding microbial inoculants to an impoverished system could benefit soil communities by enhancing nutrient cycling (Molineux, 2010) and alleviating the effects of drought (Davies *et al.,* 1992), as discussed in Chapter 4.1. Ideally, these inoculants would be added during the construction of a roof as this would be the most efficient time to do so. However, with so many green roofs already installed, it is likely that the impoverished soil communities seen in Chapter 3 already exist on green roofs globally. It is therefore important to establish if microbial inoculants could be used to remediate the impoverished communities present on extensive green roofs.

In addition to improving the soil community with microbial inoculants, it was also discussed in sections 4.1 and 5.1 that the addition of microbial inoculants to green roofs could help reveal relationships between soil organisms and thus improve our knowledge of soil ecology in a green roof context.

In Chapter 5 we saw that the addition of microbial inoculants caused intense competition within the microbial community, to the detriment of the plant community. On a mature roof the resident community is likely to differ significantly in structure, as it has had more time to establish. The effect of microbial inoculants are, therefore, likely to be markedly different. This

chapter builds on the community analysis seen in Chapter 3, observing changes induced in the community by the addition of inoculants.

The hypotheses for this chapter are that the addition of microbial inoculants will alter the soil communities currently established on the green roof and enable a healthy establishment of soil flora. This will also benefit plants and microarthropods. The effects of adding microbial inoculants to a mature roof will be different to those seen in Chapter 5, when added to a new roof, due to the fact that microbial species will already be established.

6.2 Methods

6.2.1 Experimental design

Permanent plots were selected on Roof B (see section 3.2.1) in June 2011, using a random number generator. The treatments bacteria, mycorrhiza and *Trichoderma* were added to the plots in a fully factorial randomised block design, with a control to which only deionised water was added (Fig. 6.1), totalling five replicates of each treatment. Concentrations and contents of inoculants can be found in section 4.1.

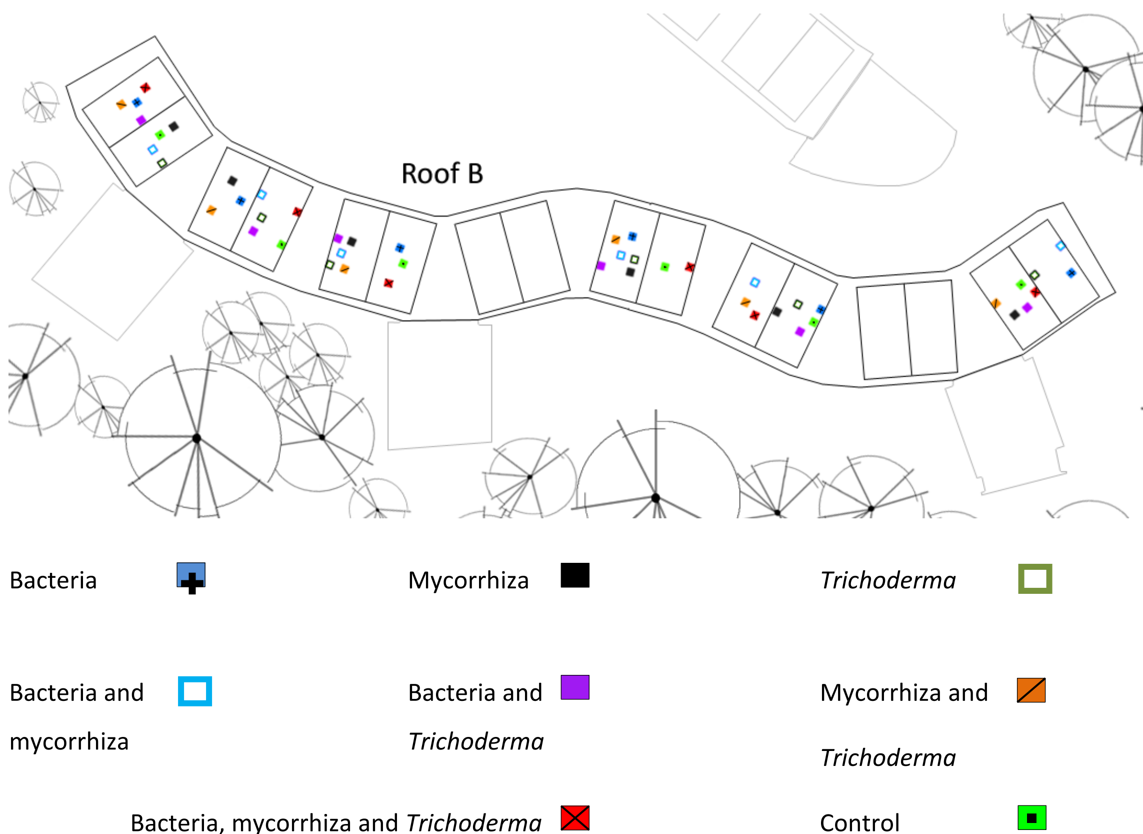

Fig. 6.1. Diagram of Roof B with permanent plots marked as coloured squares. Each colour represents a microbial treatment.

Before inoculation a portion of root was taken from one individual of *S. spurium* from each plot and this was tested for the presence of mycorrhiza as outline in section 2.3.2. Microarthropod samples were then taken every two months between September 2011 and July 2012 from each plot by inserting a 5cm diameter soil corer down to the roof lining at approximately 8cm. This was done twice in each plot, with both samples pooled to overcome problems associated with clumped microarthropod distributions (Ettema and Wardle, 2002) and resulted in a 294.5cm^3 sample from each plot. Two 3±0.05g portions of soil were removed from these samples and stored at -20˚C. These samples were then subject to PLFA analysis as described in section 2.4.2. The remainder of the soil sample was subject to microarthropod extraction as outlined in section 2.2.2. In July 2012, the same *S. spurium* individuals were once again tested for mycorrhizal analysis. In addition to this, plant surveys of each plot were carried out in January, May and July 2012 using the same method as in section 3.2.3.

6.2.2 Statistical analyses

Analysis was performed using SPSS 19.0, except PCA, which was performed using R. Shannon-Wiener indices were used to assess changes in biodiversity between September 2011 and July 2012 for all microarthropods and within microarthopod groups (collembola, mites and a group containing all other microarthropods: 'others'). Each of these groups, as well as total microarthropods, was compared using a repeated measures ANOVA with bacteria, mycorrhiza and *Trichoderma* as treatments and time as main effects. Bonferroni was used to separate differences between time points. The number of different species in each plot was also subject to the same method.

Diversity of vegetation was also measured using Shannon-Wiener and this along with cover was also tested using repeated measures ANOVA with bacteria, mycorrhiza and *Trichoderma* treatments and time as main effects.

PCA was conducted on groups of microarthropods to determine how their communities were organised, with abiotic factors added as supplementary variables to help explain the data. Significant axes were determined using parallel analysis with 1000 Monte Carlo permutations. Correlations between axes and fatty acids were obtained using the 'dimdesc' algorithm in FactoMineR (Husson *et al.*, 2013) and significant correlations are present in tables where diagrams are unclear. Month and treatment were added separately to the PCAs as qualitative supplementary variables to help explain the data, and confidence ellipses are drawn around these data at the 95% confidence level. Relationships between microarthropods, abiotic factors and vegetation cover were explored using stepwise multiple regression.

PLFA data was split into community parameters denoted by fatty acid markers for different microbe types (total microbes, bacteria, fungi, AM fungi, gram positive bacteria, gram negative bacteria and sulphate reducing bacteria), as described in section 2.4.2. Each community parameter was compared separately using repeated measures ANOVA with bacteria, mycorrhiza and *Trichoderma* treatments and time as main effects. Bonferroni was used to separate differences between time points.

PCA was conducted on the microbial community as a whole and abiotic factors were added as supplementary variables to help explain the data. Significant axes were determined using parallel analysis with 1000 Monte Carlo permutations. Correlations between axes and fatty acids were obtained using the 'dimdesc' algorithm in FactoMineR (Husson *et al.*, 2013) and significant correlations are present in tables where diagrams are unclear. Month and treatment were added separately to the PCAs as qualitative supplementary variables to help explain the data, and confidence ellipses are drawn around these data at the 95% confidence level. Relationships between microbe groups, abiotic factors and vegetation cover were explored using Stepwise Multivariate Regression.

For all data, if the assumptions of ANOVA could not be met, transformation by square root, *Ln*+1 or arcsine square root was applied as appropriate.

6.3 Results

6.3.1 Microarthropods

Class/subclass	Mean individuals (m^{-2})	Relative abundance (%)	No. sp./ morphospecies
Acarina (ad & juv)	41209.0 (±2658.6)	76.95	15[a]
Collembola (ad & juv)	10757.0 (±945.9)	20.09	4
Unidentified insect larvae	967.1 (±82.4)	1.81	12[a]
Hemiptera (ad & juv)	477.4 (±50.9)	0.89	5[a]
Thysanoptera (ad & juv)	84.3 (±18.3)	0.16	1
Aranae (ad & juv)	29.3 (±5.4)	0.05	1[a]
Chilopoda (ad & juv)	23.1 (±5.4)	0.04	1[a]
Gastropoda (ad & juv)	6.2 (±2.6)	0.01	1[a]

[a]morphospecies, as opposed to species

Table 6.1. Number of species and morphospecies of microarthropod found on the roof throughout the entire sample period. Ad = adult, juv = juvenile.

Forty species were found on the roof during the sample period. Two morphospecies of hemiptera present in the first sample period (see Chapter 3) were absent in this sample period, but a new hemiptera to the roof, of the family Tingidae, was found. One morphospecies of Thysanoptera and one species of Gastropoda (*Vallonia costata*) were also found on the roof (the latter in extremely low abundance towards the end of the sampling period). Aside from these, the key functional groups missing in the first sample period, such as Isopoda, had still not colonised (Table 6.1).

Mean microarthropods found per sample increased from the previous year. Insect larvae of Coleoptera, Diptera and Lepidoptera were still the most abundant group aside from mites and collembola. Hemiptera had increased dramatically during this sampling period, mostly due to the presence of an aphid population in summer (Table 6.1).

Total microarthropod abundance changed over time (Table 6.2, Fig. 6.1), reaching peaks in abundance in September 2011, January 2012 and March 2012 and declining throughout the summer months. January was the most variable month with regards to community structure (Fig. 6.1), whilst July and May were the least. May and November were almost identical with regards to the community present (Fig. 6.1).

Microarthropod group	Degrees of freedom	*F* value	*P* value
Total microarthropods	3.11, 124.63	48.09	**<0.001**
Mites	1.87, 74.97	28.47	**<0.001**
Collembola	3.45, 137.96	107.29	**<0.001**
Larvae	4.00, 159.97	29.49	**<0.001**
Others	2.16, 86.48	23.24	**<0.001**

Table 6.2. Results of repeated measures ANOVA with time as a main factor on microarthropods classified into different groups.

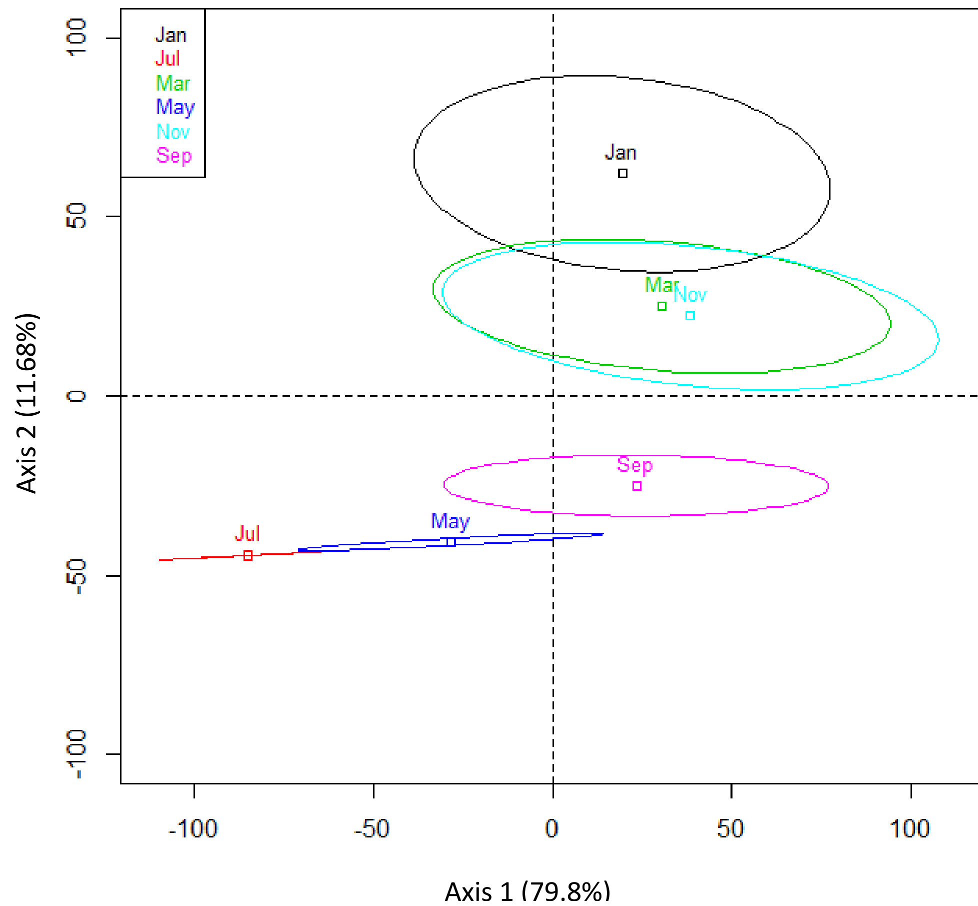

Fig. 6.2. PCA ordination plot depicting total microarthropods grouped into month. Confidence ellipses are at the 95% confidence level and individual plot values are omitted for clarity.

The total microarthropod community was higher in abundance in those plots treated with *Trichoderma* than in other treatments and the control ($F_{1,\,40} = 5.63$, $p < 0.05$) (Fig. 6.3). For total microarthropods, PCA did not depict many differences between treatments, with all treatments overlapping in community structure to some extent. However, the community present in control plots overlapped less with the other treatments, particularly in the case of the mycorrhiza treatment which shared only a small amount of its community structure with the control (Fig. 6.2). This suggests that some small differences in community structure may have resulted from the addition of inoculants.

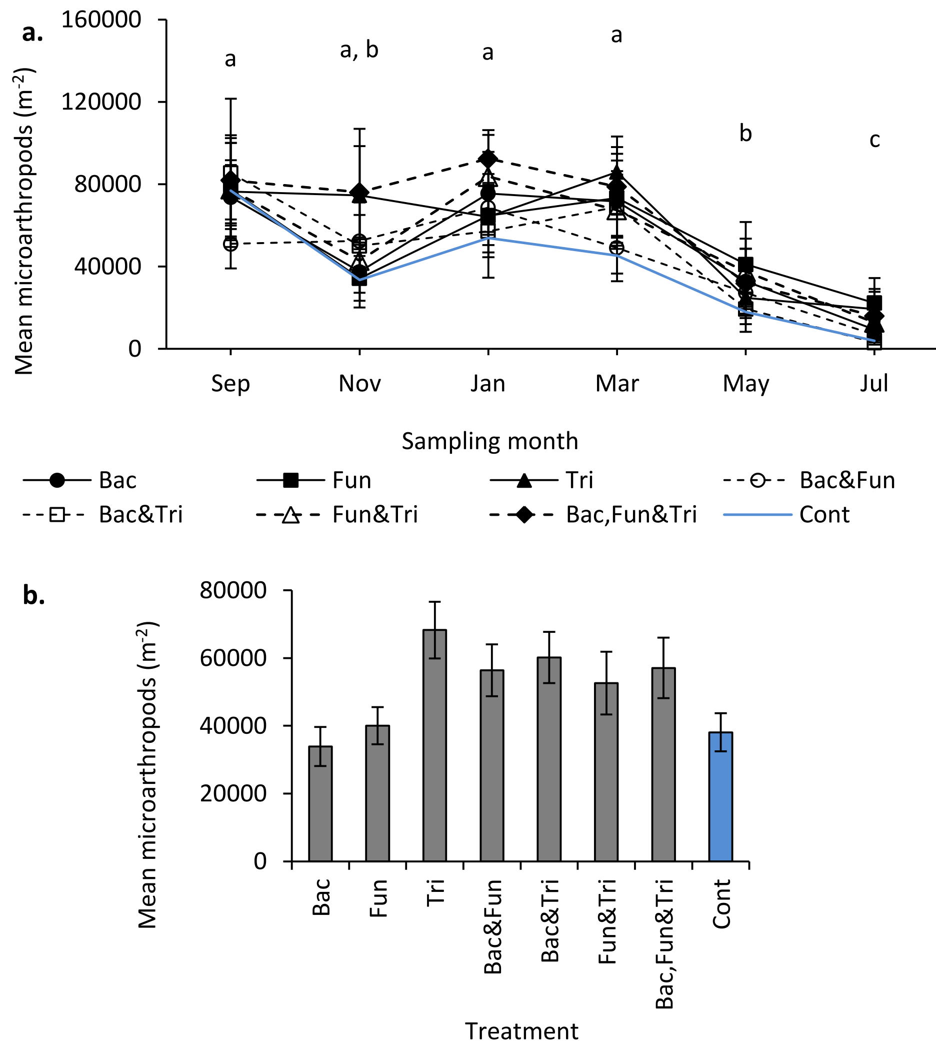

Fig. 6.3. Mean microarthropods per treatment (a) over time and (b) averaged for all time points. Letters denote statistically similar groups. Error bars represent SEM.

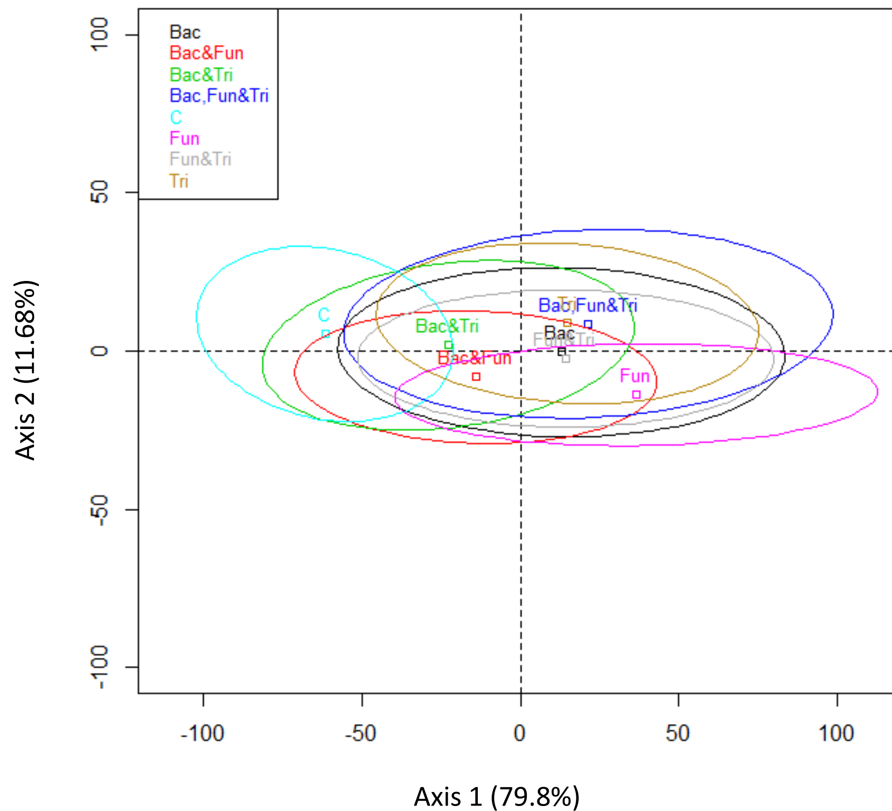

Fig. 6.4. PCA ordination plot for total microarthropods, depicting confidence intervals for each treatment based on all plots. Individual samples not shown for clarity.

Collembola were much lower in abundance than in the previous sample period (see section 3.3.3.2). 12 124 individuals were counted in total on the six sample dates and these were made up of 96.7% *S. aureus*, 2.8% *D. pallipes* and less than 1% each of *I. palustris* and *P. notabilis*. *D. bicinctus* was absent and no new species of collembola were encountered since the first sampling period. Density of collembola varied between 0 – 91 000 individuals m^{-2}, with a peak occurring in January 2012, before numbers decreased dramatically during the summer period (Fig. 6.4).

The inoculants had no main effects on collembolan abundance but those plots treated with bacteria and mycorrhiza showed a different pattern in abundance over time to other plots (Time*B*T: $F_{3.45,\ 107.29} = 2.62$, $p < 0.05$) (Fig. 6.5) with these plots peaking in January, where other plots either had less pronounced peaks, or peaked in March.

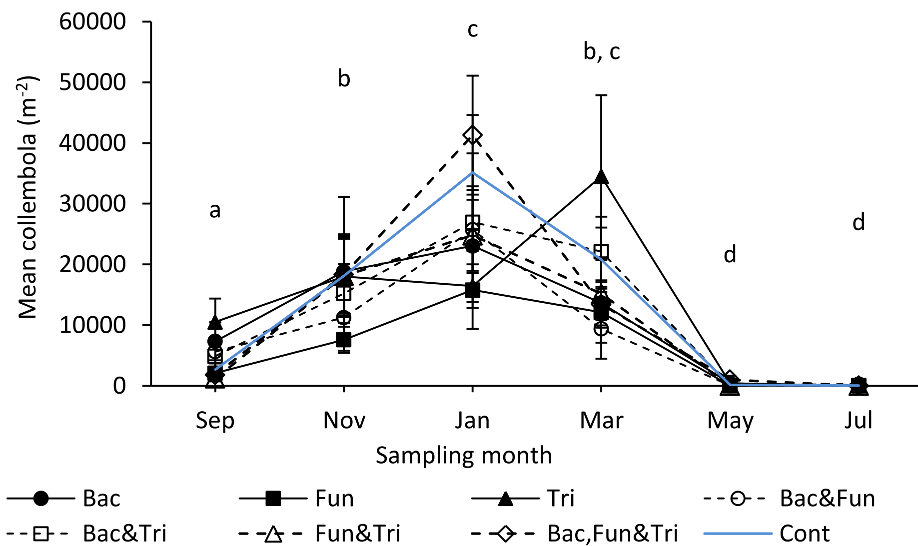

Fig. 6.5. Mean collembola in plots of each treatment. Shared letters denote statistically similar groups. Error bars represent SEM.

Stepwise multiple regression on the collembolan community determined that two factors could predict 39.1% of the variation in the community (R^2 = 0.39, $F_{2, 284}$ = 91.07, p < 0.001). Temperature had the largest effect (β = -0.92, p < 0.001) followed by substrate water content (β = -0.38, p < 0.001) where collembolan abundance decreased with increasing temperature and decreasing substrate moisture content. Fig. 6.5 (and Table 6.3) contradicts this, with increasing temperature still eliciting decreases in collembolan abundance but with increasing substrate water content increasing collembolan abundance. Though this data passed heteroscedacity tests, substrate water content and temperature were found to be highly correlated (R^2 = 0.73, p < 0.001) emphasising the importance of interpreting stepwise linear regression with care.

PCA used on species within the collembolan community organised the community along only one axis (eigenvalue: 3687.22), representing 99.33% of the variance in the community due to the dominance of *S. aureus* (Table 6.3, Fig. 6.6). However, the other two collembolan species were also positively correlated with this axis, suggesting that they are not negatively affected by the presence of *S. aureus.* Temperature, humidity and substrate water content were the abiotic factors most correlated with axis 1, suggesting they have a significant effect on *S. aureus,* with increasing humidity and water content positively correlated with *S. aureus* abundance and increasing temperature negatively correlated. Wind speed was less important but showed a positive correlation with abundance of *S. aureus*.

Species	Correlation
Axis 1	
S. aureus	1.00*
Humidity	0.47
Substrate water content	0.41
D. palippes	0.31
Wind speed	0.22
P. notabilis	0.21
Temperature	-0.60
	* 0.9999985

Table 6.3. Correlations between species and abiotic factors in PCA analysis of the collembolan community

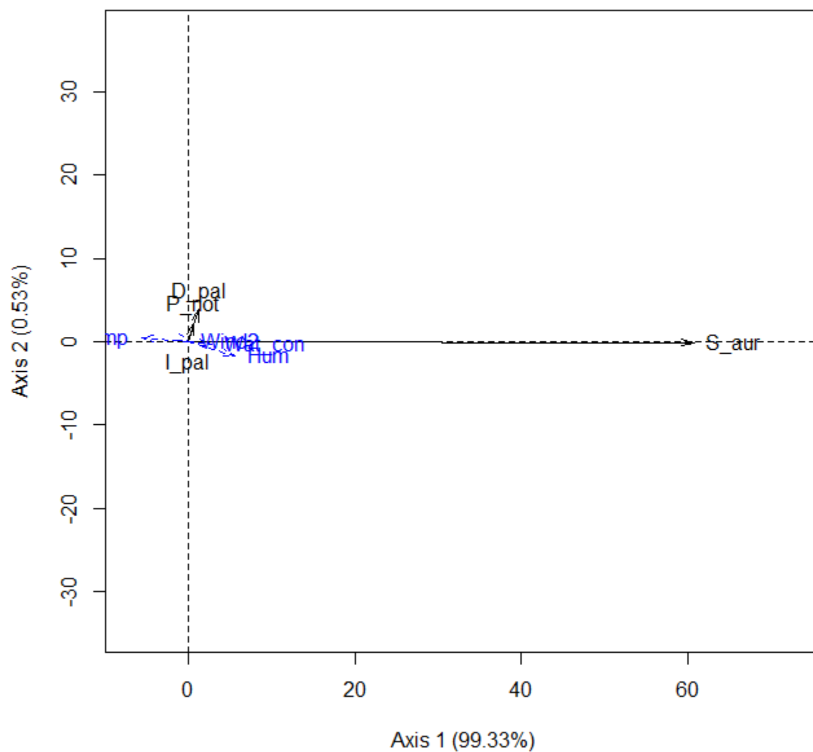

Fig. 6.6. PCA biplot depicting the collembolan community. Supplementary variables are in blue.

46 444 mite individuals were encountered on the roof, consisting of fifteen morphospecies, five of which were new from the first sampling period (five had also disappeared, see section 3.3.3.3). Scutoverticidae still dominated, making up 79.3% of the sample. *E. viridis* was no longer the second most abundant mite, making up only 1.1% of the total sample. Two unidentified mites, both of which were present in the first sampling period, were now more abundant, making up 13% and 4.7%. Mite abundance varied between 0 and 250 000 individuals m^{-2}, decreasing throughout the sample period. Mites were unaffected by any of the inoculants added, with no inoculated plots differing from the control (Fig. 6.7).

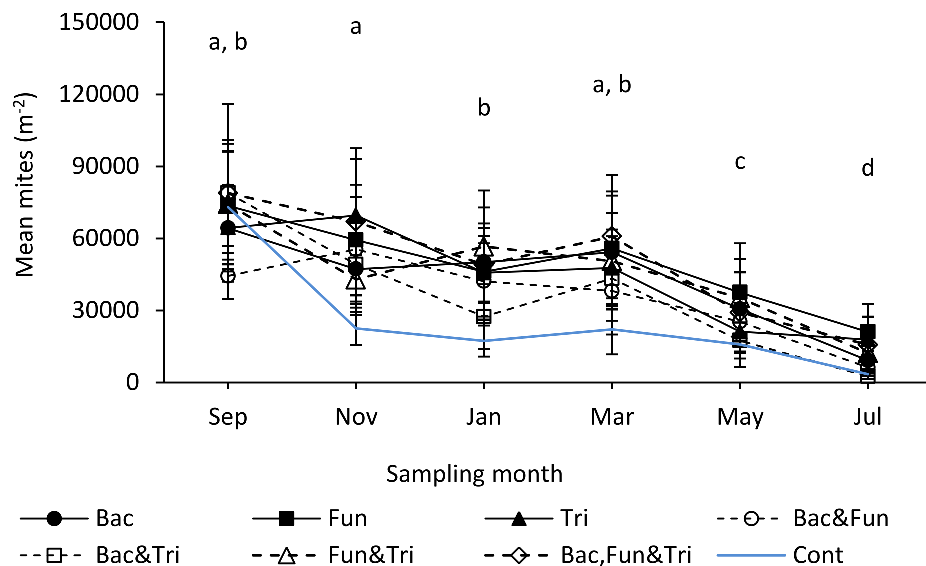

Fig. 6.7. Mean mites in plots of each treatment. Letters denote significantly similar groups. Error bars represent SEM.

Stepwise multivariate regression assigned three factors able to predict 21.1% of the variance in the mite community (R^2 = 0.21, $F_{2, 283}$ = 25.25, p < 0.001). These factors were substrate water content (β = 0.82, p < 0.001), temperature (β = 0.57, p < 0.001) and wind speed (β = 0.17, p < 0.01), where increasing water content, temperature and wind speed were correlated with increases in mite abundance.

The majority of the variance in the mite community was explained by one axis in PCA, explaining 90.53% of the variance (eigenvalue: 24 814.38). This was because one mite, (Scutoverticidae) dominated the sample (Fig. 6.8, Table 6.4). None of the mites found to be significant in the PCA were anti-correlated with this axis, suggesting that Scutoverticidae do not negatively affect any of these other morphospecies.

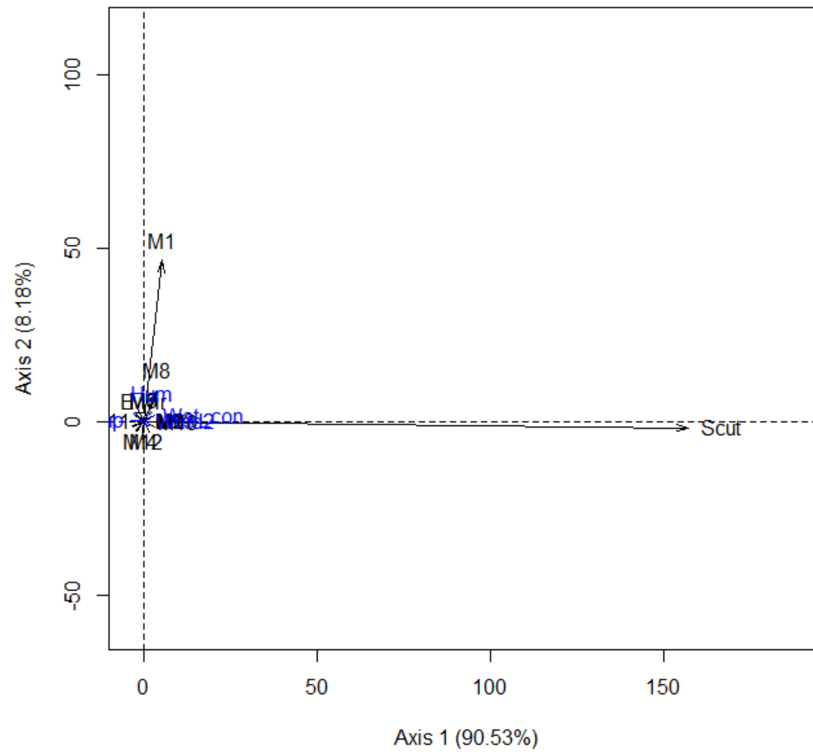

Fig. 6.8. PCA biplot depicting the mite community. Blue additions are supplementary variables.

Species/morphospecies	Correlation
Axis 1	
Scutoverticidae	1.00*
Substrate water content	0.31
Mite 8	0.20
Humidity	0.20
Mite 10	0.19
Wind speed	0.16
Mite 3	0.15
Mite 6	0.14
Temperature	-0.20

* 0.9999331

Table 6.4. Correlations between species and abiotic factors in PCA analysis of the collembolan community.

Abiotic factors were less of a driver in this PCA (with lower correlation values) suggesting that those mites significant in abundance (i.e. Scutoverticidae) were more independent of abiotic factors than the collembolan community. However, the same factors explained the majority of the variance, with humidity, water content and wind speed positively correlated with mite abundance and temperature negatively correlated.

The larval community peaked in the winter months (Fig. 6.9.) and was less dominated by one morphospecies than mites and collembola were. Flying insect larvae were lower in those plots with the bacteria treatment and the mycorrhiza treatment together ($F_{1, 40}$ = 5.92, p < 0.05) but higher in plots with the *Trichoderma* treatment ($F_{1,40}$ = 5.03, p < 0.05). Plots with all three treatments achieved abundances between these two (Fig. 6.9).

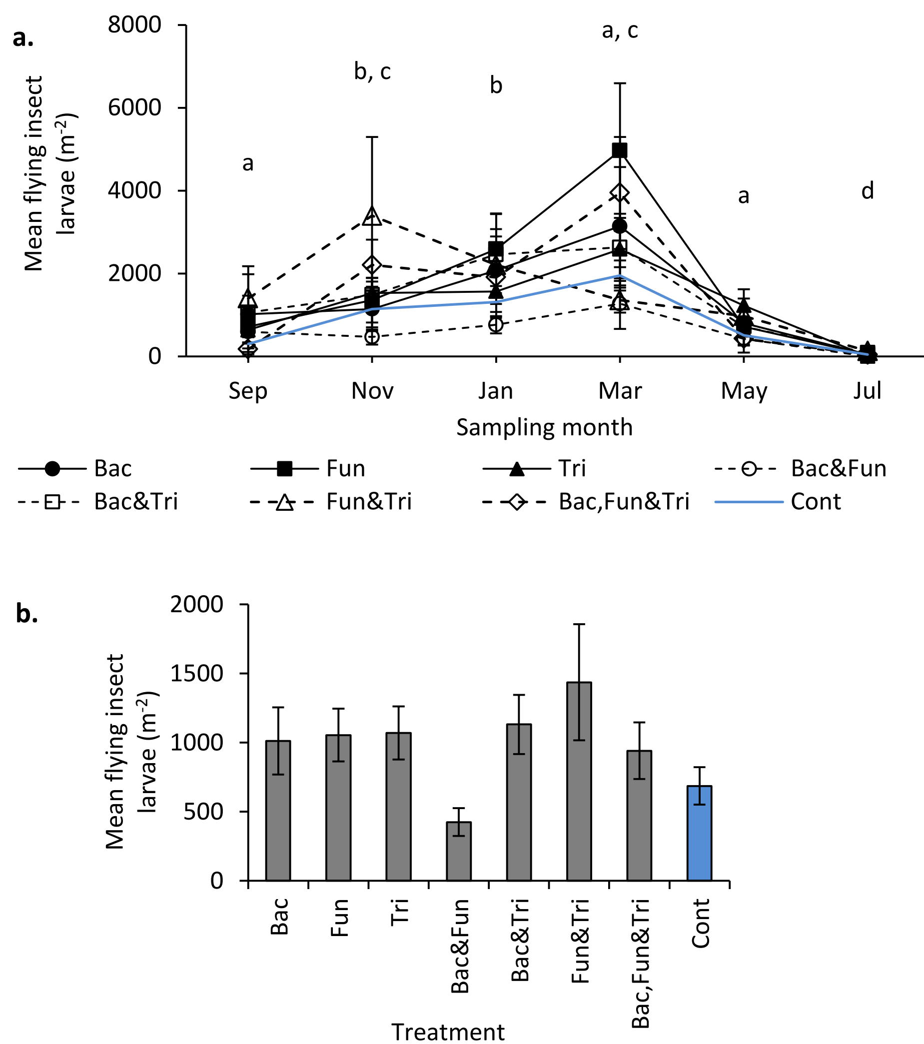

Fig. 6.9. Mean insect larvae per treatment (a) over time and (b) averaged over all treatment times. Error bars represent SEM.

Three axes were determined to be significant in describing the larval community in PCA according to parallel analysis (Table 6.5).

Axis	Eigenvalue	% variance	Cum. % variance
1	19.46	64.00	64.00
2	8.12	26.70	90.71
3	1.83	6.02	96.72

Table 6.5. Eigenvalues and their associated explained variance for axes deemed significant (by parallel analysis) to explain distributions of larval morphospecies on the green roof.

Larva species 12 had the strongest influence on axis 1 and unlike with mite and collembolan populations, there was a negative correlation between it and other larval morphospecies (Table 6.6). Larva 7 influenced the second axis, while the third axis was driven by Larva 3 (Table 6.6).

Morphospecies	Axis 1	Axis 2	Axis 3
Larvae 1	-0.01	< -0.01	0.12
Larvae 2	-0.01	< -0.01	0.03
Larvae 3	-0.01	-0.01	**0.99**
Larvae 4	< 0.01	< 0.01	< 0.01
Larvae 5	< 0.01	< -0.01	< -0.01
Larvae 6	< 0.01	< -0.01	< 0.01
Larvae 7	-0.02	**1.00***	0.01
Larvae 8	< 0.01	< -0.01	< 0.01
Larvae 9	0.01	< 0.01	0.02
Larvae 11	< -0.01	< 0.01	< -0.01
Larvae 12	**1.00****	0.02	0.01

*0.9864989781
**0.9995778725

Table 6.6. Loadings for larval morphospecies on each significant axis. Bold figures denote instances where the majority of the variance on an axis is explained by one morphospecies.

For morphospecies correlated with axis 1, humidity and wind were a driving factor, as well as temperature (Table 6.7, Fig. 6.10) but these were not strong correlations. Increasing humidity, wind speed and water content had a positive effect on the community, whilst increasing

temperature had a negative one. Larva 7, the only morphospecies significantly correlated with axis 2 was unaffected by the environmental variables measured.

Morphospecies	Correlation
Axis 1	
Larvae 12	1.00*
Humidity	0.34
Wind speed	0.28
Substrate water content	0.22
Larvae 9	0.14
Larvae 1	-0.14
Larvae 2	-0.15
Temperature	-0.32
Axis 2	
Larvae 7	1.00**

*0.9999441

**0.9996961

Table 6.7. Correlations between species and abiotic factors in PCA analysis of the larval community

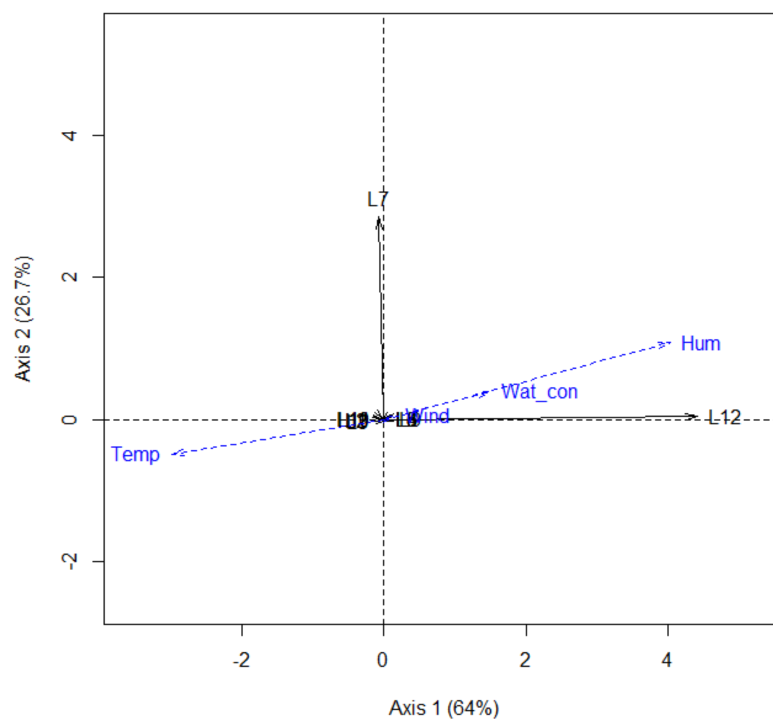

Fig. 6.10. PCA biplot depicting insect larvae. Blue values represent supplementary variables.

Other organisms present on the roof remained low throughout the sample period but reached a peak in May 2012 (Fig. 6.11). The 'all treatment' mix differed from other plots ($F_{2.16,\ 86.48}$ = 3.08, p < 0.05) in that treatments with mycorrhiza and *Trichoderma* had high mean abundances when applied singly, but these were reduced when bacteria was added to either of these inoculants. However, the 'all treatment' group did not have lower mean abundances than the mycorrhiza and *Trichoderma* mix. The 'all treatment' group also showed a difference in pattern of abundance over time, starting at higher abundances than the other mixes in September 2011 but dropping lower in July 2012 ($F_{1,40}$ = 8.3, p < 0.01) (Fig. 6.11).

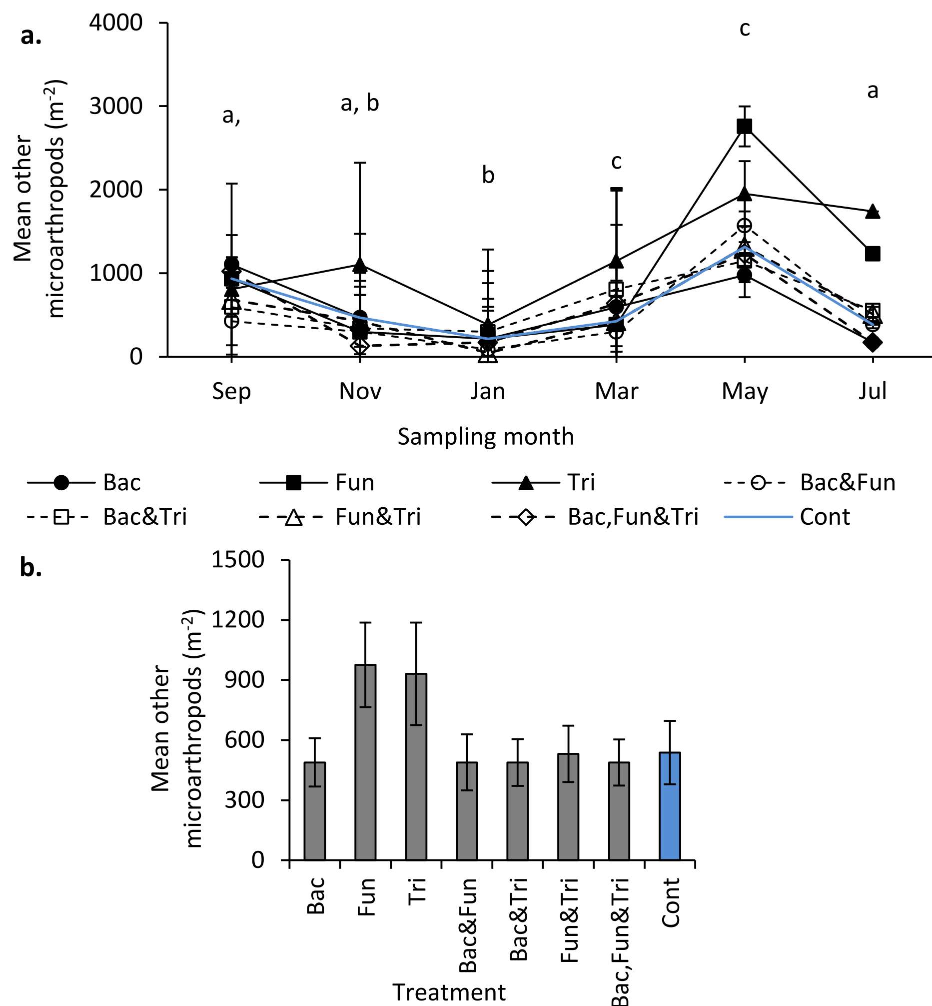

Fig. 6.11. Mean other organisms per treatment (a) over time and (b) averaged across all time points. Letters denote statistically similar groups. Error bars represent SEM.

PCA (Fig. 6.12) on the hemipteran community deemed each species to contribute to its own axis, with five significant axes produced by parallel analysis (Table 6.8). Table 6.8 however shows that beyond axis 2, very little variance is explained. This is due to the extremely low numbers of hemiptera other than the Tingida (axis 1) and Mirida (axis 2).

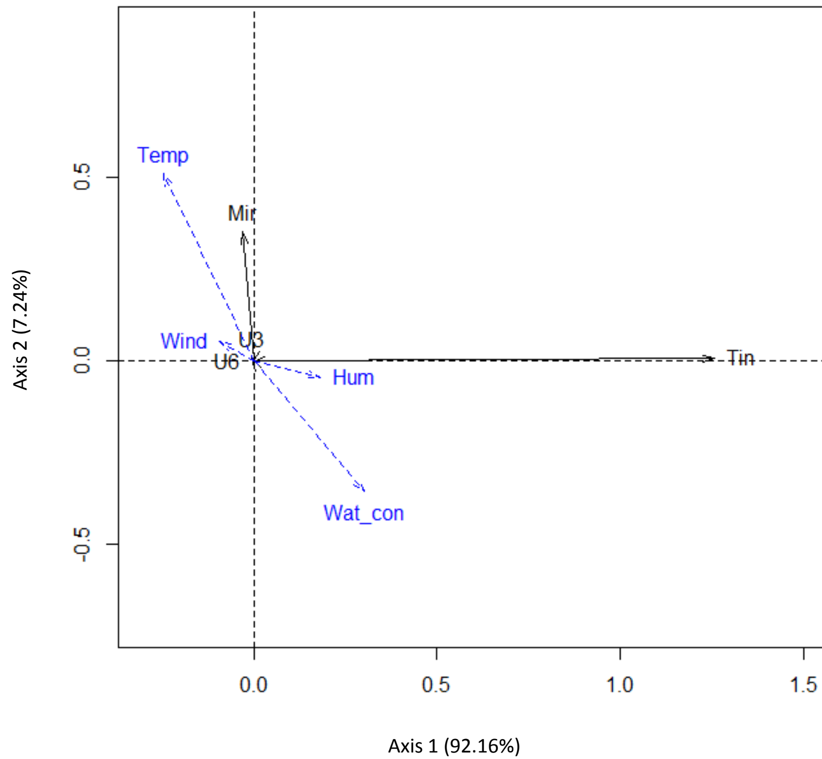

Fig. 6.12. PCA biplot depicting Hemiptera. Blue additions represent supplementary variables.

Axis	Eigenvalue	% variance	Cum. % variance
1	9.34	75.79	75.79
2	2.91	23.59	99.39
3	0.06	0.47	96.72
4	0.01	0.08	99.94
5	0.01	0.06	100.00

Table 6.8. Eigenvalues and their associated explained variance for axes deemed significant (by parallel analysis) to explain distributions of Hemiptera on the green roof.

Temperature, humidity, substrate water content and wind speed are all correlated to axis one, the former strongly. Only temperature and wind speed are related to axis two (Table 6.9), both weakly. This suggests that all four abiotic factors are more important for the Mirida populations than to the Tingida. Increases in temperature and wind speed correspond to increases in the Mirida, whereas increases in humidity and substrate water content correspond to decreases in the Mirida, contrary to most of the groups present on the roof. The

Tingida on the other hand was negatively affected by wind speed, though was still positively affected by increasing temperature.

Morphospecies	Correlation
Axis 1	
Miridae	1.00*
Temperature	0.36
Wind speed	0.29
Tingidae	-0.13
Substrate water content	-0.41
Humidity	-0.41
Axis 2	
Tingidae	0.99
Temperature	0.15
Wind speed	-0.21
	*0.9991159

Table 6.9. Correlations between species and abiotic factors in PCA analysis of the larval community.

Though both species are highly correlated with their own axis, the Tingida also showed a slight correlation with axis 1 (Table 6.9). This suggests the two species do interact and, as this is a negative correlation, that increases in the Mirida correspond with decreases in the Tingida. Data for other organisms on the roof were not high enough in abundance to perform PCA. Parallel analysis determined that no axes were significant in explaining their distribution.

6.3.2 Vegetation and fungi

All plots were dominated by *Sedum spp*. and bryophytes, with the addition of lichen, *Trifolium arvense* and few other plants (Table 6.10, Fig.6.13). Shannon-Wiener values for seasonal migrants were, in fact, 0 for all plots except one plot in March that had a value of 0.3.

On average *Sedum spp*. dominated, reaching 43.4(±1.52)% cover for the entire sample period, closely followed by bryophytes, which obtained 31.1(±2.0)% cover. *Trifolium arvense* was extremely common during the sample period, particularly in July. Over the year it obtained an average cover of 11.7(±1.3)%. On average, 15.6(±1.1)% of the plot area was bare. Lichen and seasonal migrants each accounted for less than 1% of cover (Fig. 6.13).

Plants		
Sedum		
Sedum album	*Sedum acre*	*Sedum kamtschaticum*
Sedum spurium	*Sedum rupestre*	
Seasonal colonisers		
Anthyllis vulneraria	*Cirsium arvense*	*Epilobium angustifolium*
Tree saplings		
Acer pseudoplatanus		

Table 6.10. Plant species encountered during the sample period. In addition to these were bryophytes, lichen and one species of unidentified grass.

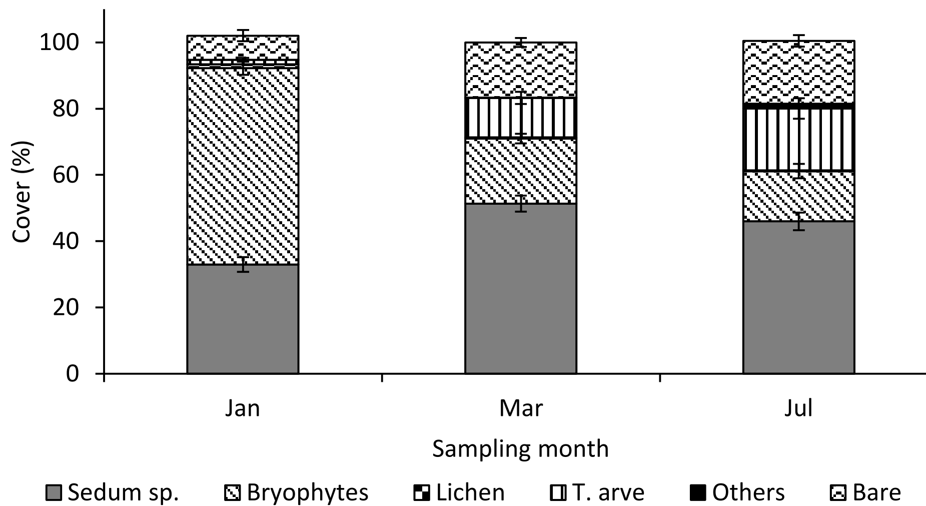

Fig. 6.13. Percentage cover of vegetation and bare substrate on the roof. *T. arve* = *T. arvense.* Error bars represent SEM.

The plant community displayed a clear shift from winter to summer, dominated by bryophytes in January before the *Sedum spp.* became the most common plants in the summer months (Fig. 6.13). *T. arvense* was absent in January but grew throughout the summer period (Fig. 6.14). However, the decline of bryophytes in the summer was not compensated for by *T. arvense* and *Sedum spp.* and so an overall increase in bare substrate occurred in March and July. None of the inoculants added had an effect on total plant cover, cover of *Sedum spp.*, cover of *T. arvense* or cover of lichen (Figs 6.14 and 6.15). However, the addition of *Trichoderma* to plots altered the pattern in bryophyte cover over time ($F_{1.46,\ 58.19}$ = 3.70, p < 0.05) (Fig. 6.15). PCA also determined there to be little difference between treatments (data not shown).

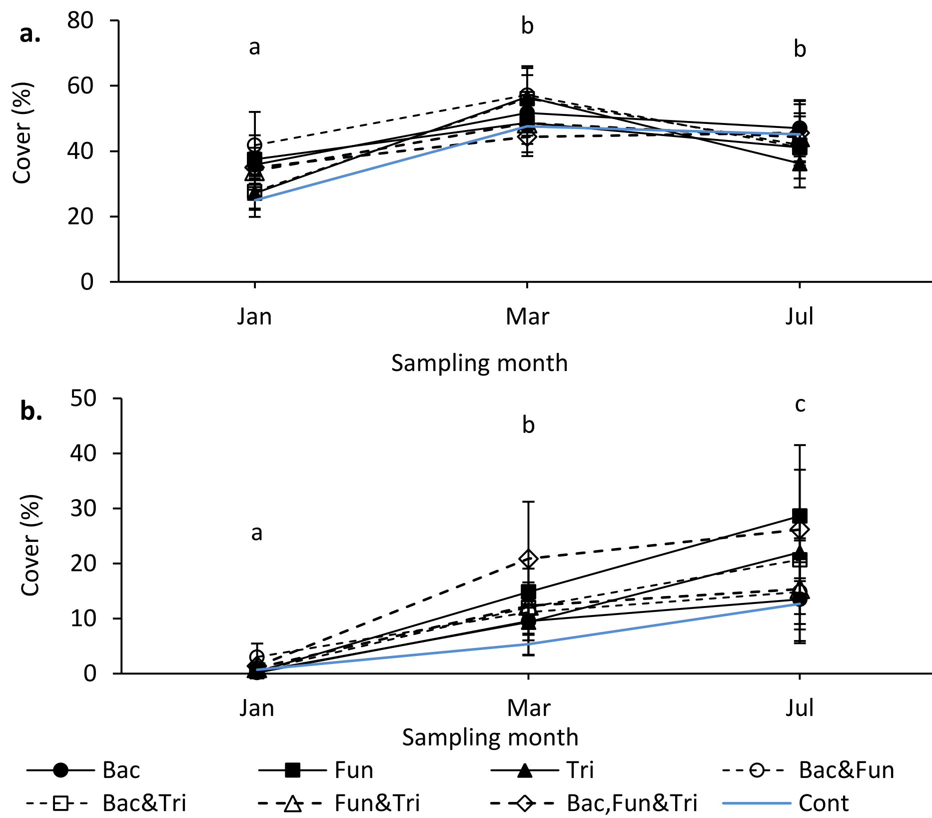

Fig. 6.14. Percentage cover per treatment of (a) *Sedum spp.* and (b) *T. arvense*. Error bars represent SEM. Letters denote statistically similar groups.

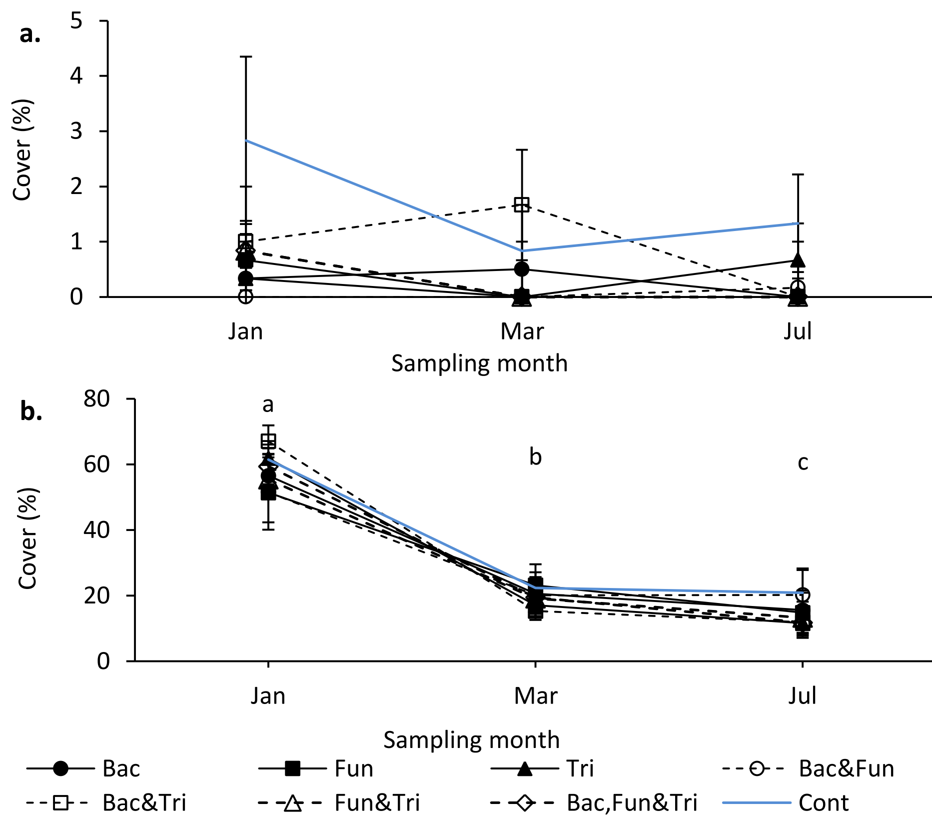

Fig. 6.15. Percentage cover per treatment of (a) lichen and (b) bryophytes. Error bars represent SEM. Letters denote statistically similar groups.

Variability was little affected by treatment for *Sedum spp.* or *T. arvense* but within the bryophyte community, variability was higher in July for the bacteria & fungi treatment (Fig. 6.16). For lichen too, the bacteria & *Trichoderma*, control and all treatment plots showed different patterns in variability than the other treatments (Fig. 6.16).

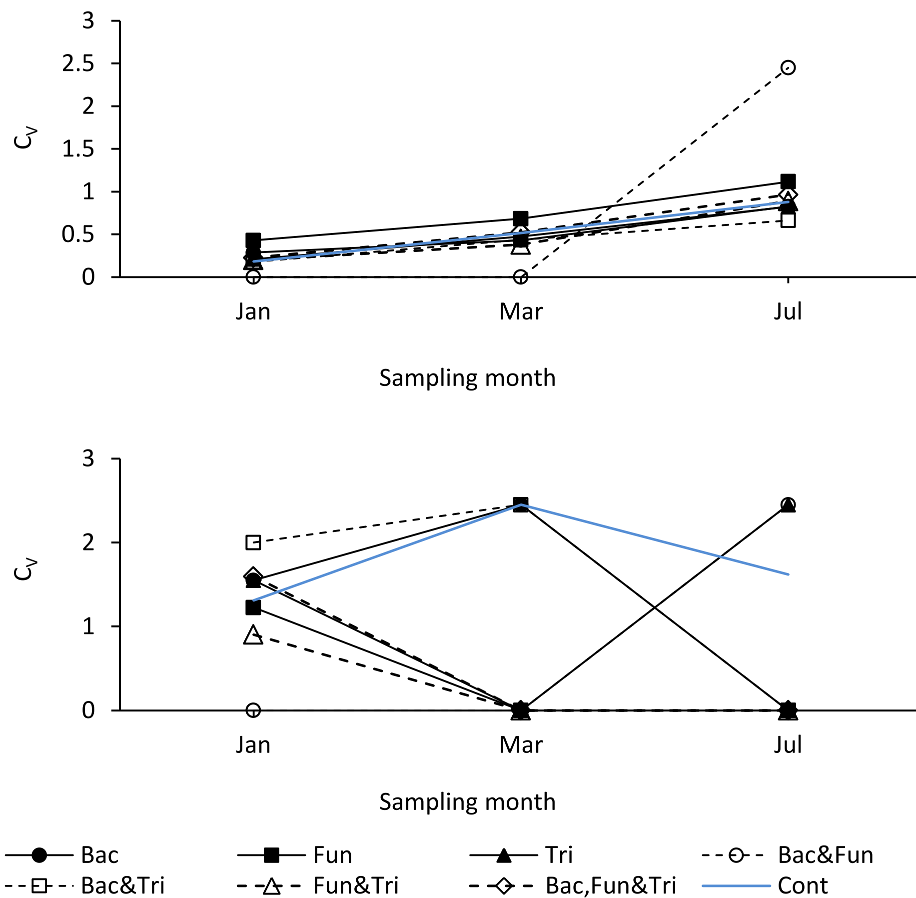

Fig 6.16. Variability (C_V) of (a) bryophytes and (b) lichens for each treatment.

Stepwise multivariate regression found variation in most plant groups to be correlated to moisture. 26.1% of the variation in the *Sedum* population could be predicted by two factors ($R^2 = 0.26$, $F_{2, 140} = 24.70$, $p < 0.001$): humidity ($\beta = -0.96$, $p < 0.001$) and substrate water content ($\beta = -0.69$, $p < 0.001$), where *Sedum spp.* cover increased when humidity and substrate water content decreased. 71% of the bryophyte variance ($R^2 = 0.71$, $F_{2, 140} = 167.00$, $p < 0.001$) was also predicted by the factors humidity ($\beta = 1.14$, $p < 0.001$) and substrate water content ($\beta = 0.34$, $p < 0.001$), though bryophytes increased with increasing humidity and increasing substrate water content. *T. arvense* variance was predicted by two factors explaining 26.8% ($R^2 = 0.27$, $F_{2, 140} = 41.91$, $p < 0.001$). These were temperature ($\beta = 0.29$, $p < 0.001$) and humidity (($\beta = 0.57$, $p < 0.001$), with increasing cover as both temperature and humidity increased. Lichen

was only very weakly affected by the variables measured, with 2.8% of its variance explained by humidity (R^2 = 0.03, $F_{1, 141}$ = 4.09, p < 0.05, β = 0.17, p < 0.05). The remainder of the plants encountered in the study period were too few to satisfactorily determine explanatory factors.

Colonisation of roots by mycorrhizal fungi was high with a mean value of infection across all treatments at 78.8(±1.7)%. The proportion of counts containing vesicles was also exceptionally high, averaging 52.4(±2.2)% across the whole roof. 26.3(±1.3)% of counts contained hyphae only and arbuscules were extremely low, averaging only 0.05(±0.03)% across the whole roof (Fig. 6.17). All counts containing vesicles and/or arbuscules also contained hyphae.

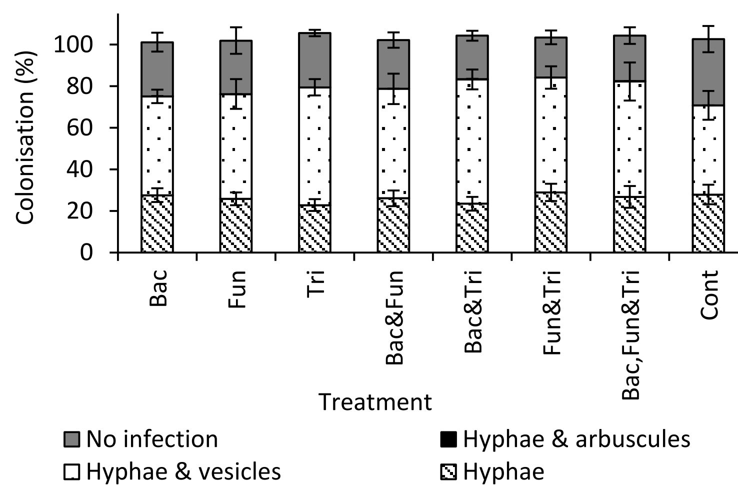

Fig. 6.17. Mean colonisation of roots of *Sedum spp.* by mycorrhizal fungi in each treatment. Hyphae & arbuscules are not visible due to extremely low prevalence. Error bars represent SEM.

The percentage of colonisation of roots by mycorrhizal fungi was higher in those treatments to which *Trichoderma* had been added ($F_{1, 48}$ = 4.34, p < 0.05) (Fig. 6.18) but treatment had no effect on the percentage colonisation by hyphae alone or on hyphae with vesicles. The plant species the root sample was taken from also had no effect on mycorrhizal colonisation. Numbers of arbuscules were too low to analyse.

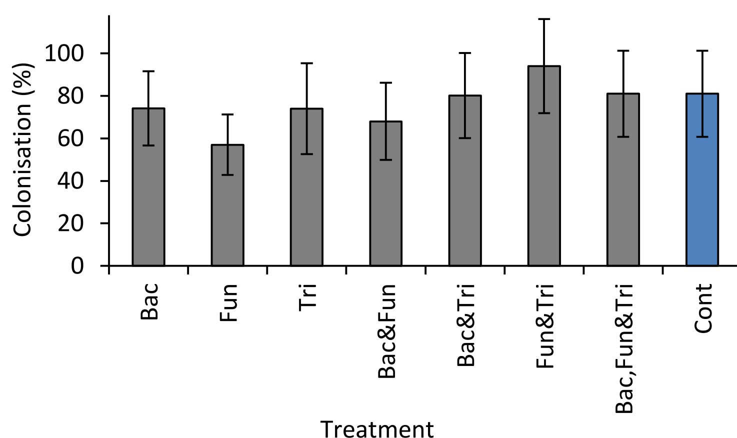

Fig. 6.18. Mean colonisation of roots of *Sedum spp.* by mycorrhizal fungi by treatment. Error bars represent SEM.

6.3.3 Interactions between microarthropods and plants

PCA conducted on the community as a whole showed it to be extremely complex, with parallel analysis on all organisms determining that four axes significantly explained their distribution. However, this still only accounted for 28.2% of the variance in the community (Table 6.11).

Axis	Eigenvalue	% variance	Cum. % variance
1	3.40	8.94	8.94
2	3.10	8.16	17.10
3	2.25	5.92	23.02
4	1.96	5.14	28.17

Table 6.11. Eigenvalues and percentage variance explained, by PCA, performed on all organisms found and plant cover for months where plant surveys were carried out.

No plants were associated along axis 1, suggesting those species well correlated with axis 1 to be independent of plant cover. Abiotic factors too have less influence on axis 1, with the exception of wind speed which was strongly positively correlated with axis 1 (Table 6.12, Fig. 6.19).

Fig. 6.19. PCA biplot for all microarthropods throughout the sample period and all plant groups throughout the sample period. Blue values are supplementary abiotic factors.

The plant groups were however, strongly correlated with axis 2 as were all the abiotic factors, suggesting plants are heavily influenced by wind speed, temperature, water content and humidity and that those organisms strongly correlated with axis 2 are also strongly influenced by these abiotic factors and/or plant cover. Of note is *S. aureus,* which shows increases with increased humidity, wind speed, water content and bryophyte cover and decreases with increasing temperature, cover of *Sedum spp.* and cover of *T. arvense* (Table 6.12, Fig. 6.19).

Species/morphospecies	Group	Correlation value
Axis 1		
Larva 3	**U. F.**	**0.79**
D. pallipes	**Collembola**	**0.71**
P. notabilis	**Collembola**	**0.70**
Larva 1	U. F.	0.60
S. aureus	Collembola	0.51
E. viridis	**Acarina**	**0.44**
Wind speed	NA	0.40
Larva 2	U. F.	0.32
Larva 10	U. F.	0.29
Mite 2	**Acarina**	**0.25**
Mirida 1	Hemiptera	0.28
Water content	NA	0.26
Mite 1	Acarina	0.25
Mite 3	**Acarina**	**0.24**
Larva 11	**U. F.**	**0.21**
Unidentified 6	**Hemiptera**	**0.20**
Arana 1	**Aranae**	**0.17**
Gastropoda 1	Gastropodae	-0.22
Temperature	NA	-0.28
Tingida 1	Hemiptera	-0.35
Axis 2		
Temperature	NA	0.69
Sedum spp	**Sedum**	**0.63**
T. arvense	**Fabales**	**0.53**
Larva 2	U. F.	0.29
Larva 10	U. F.	0.23
Larva 8	**U. F.**	**0.23**
Mite 10	**Acarina**	**0.22**
Larva 1	U. F.	0.22
Thysanoptera 1	**Apterygota**	**0.21**
Mirida 1	Hemiptera	0.21
Mite 12	**Aacarina**	**0.20**
Gastropoda 1	Gastropodae	0.19

Species/morphospecies	Group	Correlation value
Tingida 1	Hemiptera	0.17
Mite 8	**Acarina**	**0.17**
Other plants	**Assorted**	**0.16**
Mite 1	Acarina	-0.17
Mite 9	**Acarina**	**-0.19**
Mite 7	**Acarina**	**-0.24**
Lichen	**Lichen**	**-0.26**
Water content	NA	-0.52
S. aureus	Collembola	-0.57
Wind speed	NA	-0.58
Larva 12	**U. F.**	**-0.67**
Humidity	NA	-0.79
Bryophytes	**Bryophytes**	**-0.87**

Table 6.12. Correlations and *p* values for each species/morphospecies in relation to axes 1 and 2 in a PCA. Bold entries denote organisms found on only one axis. U.F = Unidentified flying insect larvae. Those grouped as NA are supplementary variables (abiotic factors).

6.3.4 The microbial community

6.3.4.1 Seasonal patterns

PLFA community	Overall mean µg g^{-1} (±SEM)
Total	4.11 (±0.008)
Bacterial	2.18 (±0.004)
Fungal	1.93 (±0.004)
Gram positive	1.10 (±0.002)
Gram Negative	0.17 (±0.0006)
Sulphate reducing	0.31 (±0.0006)
AM fungi	0.05 (±0.0001)

Table 6.13. Overall means plus SEM for the entire sample period for each group of fatty acids.

The mass of fatty acids over the sample period was predominantly of bacterial origin, although fungal PLFAs were not much less prevalent (Table 6.13). Of the bacterial PLFAs, gram positive bacterial fatty acids were higher in abundance than sulphate reducing bacterial fatty acids and gram negative bacterial fatty acids (which were the lowest in abundance) (Table 6.13). AM fungal fatty acid mass was extremely low (Table 6.13).

Parallel analysis of the fatty acid community determined that three PCA dimensions significantly explained the majority of the variation in the community (Table 6.14). All 16 PLFAs were significantly correlated with one of the two primary axes (Table 6.14, Fig. 6.20). Unlike on

the young roof (see section 5.3.3), much of the variance in this community can be explained by the fungal fatty acids C18:1ω9 and C18:2ω6,9 (Fig 6.20) on axis 1 and the sulphate reducing bacterial PLFA C17:0(10Me) on axis 2 (Table 6.15) and the bacterial community is less well correlated with each other. This suggests that the community is more fungal dominated than bacterial dominated than the young roof in Chapter 5. C18:2ω6,9 and C17:0ai are the only PLFAs to be negatively correlated with either axis (axis 2). This slight negative correlation suggests that one or all of those PLFAs strongly correlated to axis 2 have a negative relationship with C18:2ω6,9 and C17:0ai.

Axis	Eigenvalue	Percentage variance	Cumulative percentage variance
1	0.91	72.22	72.22
2	0.21	16.70	88.92
3	0.05	4.00	92.91

Table 6.14. Eigenvalues and the percentage variance of the PLFA community explained by each axis in PCA.

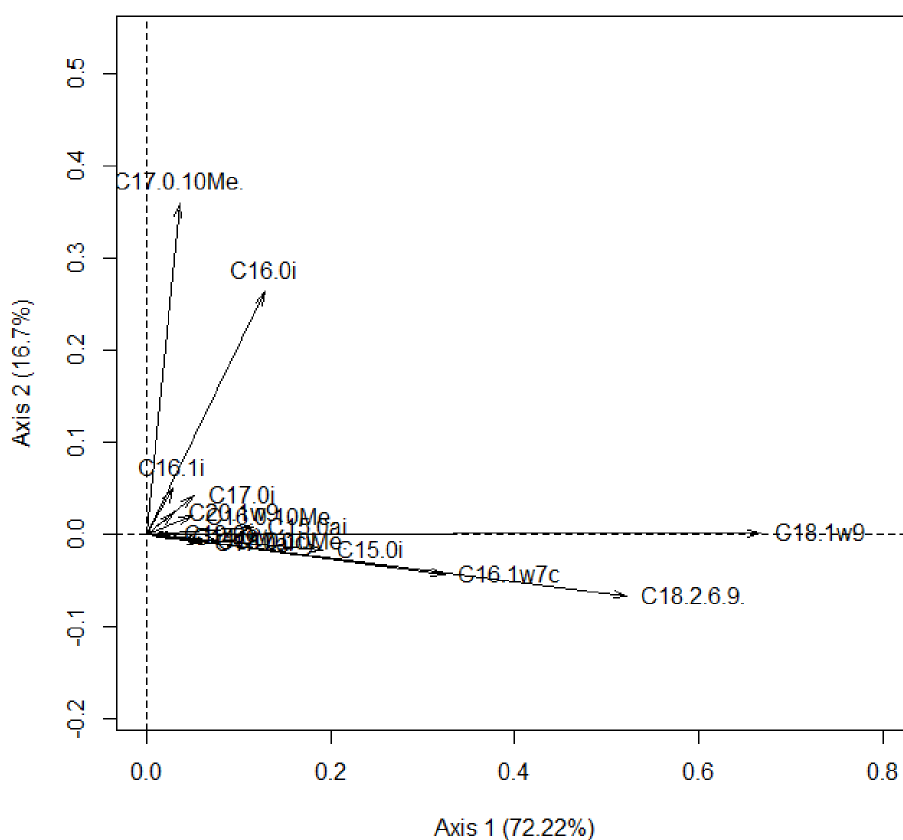

Fig 6.20. PCA biplot for all microbial fatty acids.

Fatty acid	Organism	Correlation
Axis 1		
C18:1ω9	**Fungi**	**0.98**
C18:2ω6,9	Fungi	0.95
C17:0ai	Bacteria, G+	0.86
C16:1w7c	**Bacteria**	**0.85**
C14:0i	**Bacteria, G+**	**0.82**
C15:0ai	**Bacteria, G+**	**0.79**
C16:0(10Me)	Bacteria, SR	0.76
C18:0(10Me)	**Bacteria, SR**	**0.75**
C15:0i	**Bacteria, G+**	**0.74**
C17:0cy	**Bacteria, G-**	**0.71**
C20:1ω9	Fungi, AM	0.68
C17:0i	Bacteria, G+	0.61
C16:1i	Bacteria, G+	0.48
C16:0i	Bacteria, G+	0.44
C19:0cy	**Bacteria, G-**	**0.27**
Axis 2		
C17:0(10Me)	**Bacteria, SR**	**0.99**
C16:0i	Bacteria, G+	0.89
C16:1i	Bacteria, G+	0.82
C20:1ω9	Fungi, AM	0.51
C17:0i	Bacteria, G+	0.49
C16:0(10Me)	Bacteria, SR	0.30
C18:2ω6,9	Fungi	-0.12
C17:0ai	Bacteria, G+	-0.13

Table 6.15. Correlations and their significance of PLFAs with each axis. Bold entries denote unique PLFAs for that axis. G+ denotes gram positive bacterial fatty acids, G- gram negative, SR sulphate reducing and AM arbuscular mycorrhizal fungi.

Microbial mass was highest in May 2012 for all PLFA groups (Figs 6.21 and 6.22). PLFAs were lowest in November 2011 and January 2012 for most groups (Figs 6.21 and 6.22), with gram negative bacteria disappearing altogether (Fig. 6.21). AM fungal PLFAs were lowest in March (Fig. 6.22), when all groups had the highest variability (Fig. 6.23, additional figures in Appendix IIII). PCA determined that most months were aligned along axis 1, the fungal driven axis, with the exception of May, which was equally distributed along both and highly variable (Fig. 6.24).

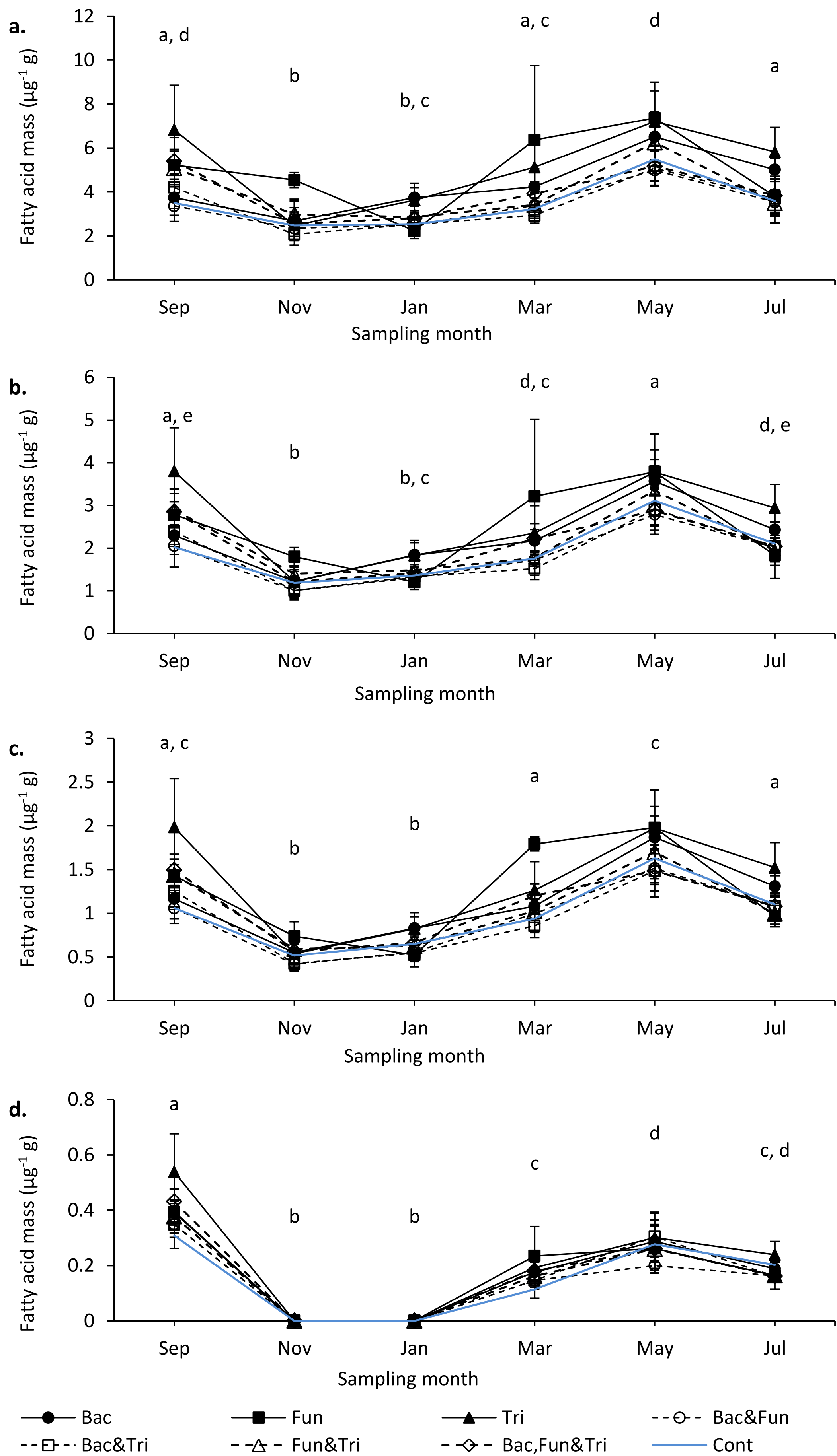

Fig 6.21. Mass of PLFAs over time for: (a) total microbes, (b) bacteria, (c), gram positive bacteria, (d) gram negative bacteria. Shared letters denote statistically similar groups. Error bars denote SEM.

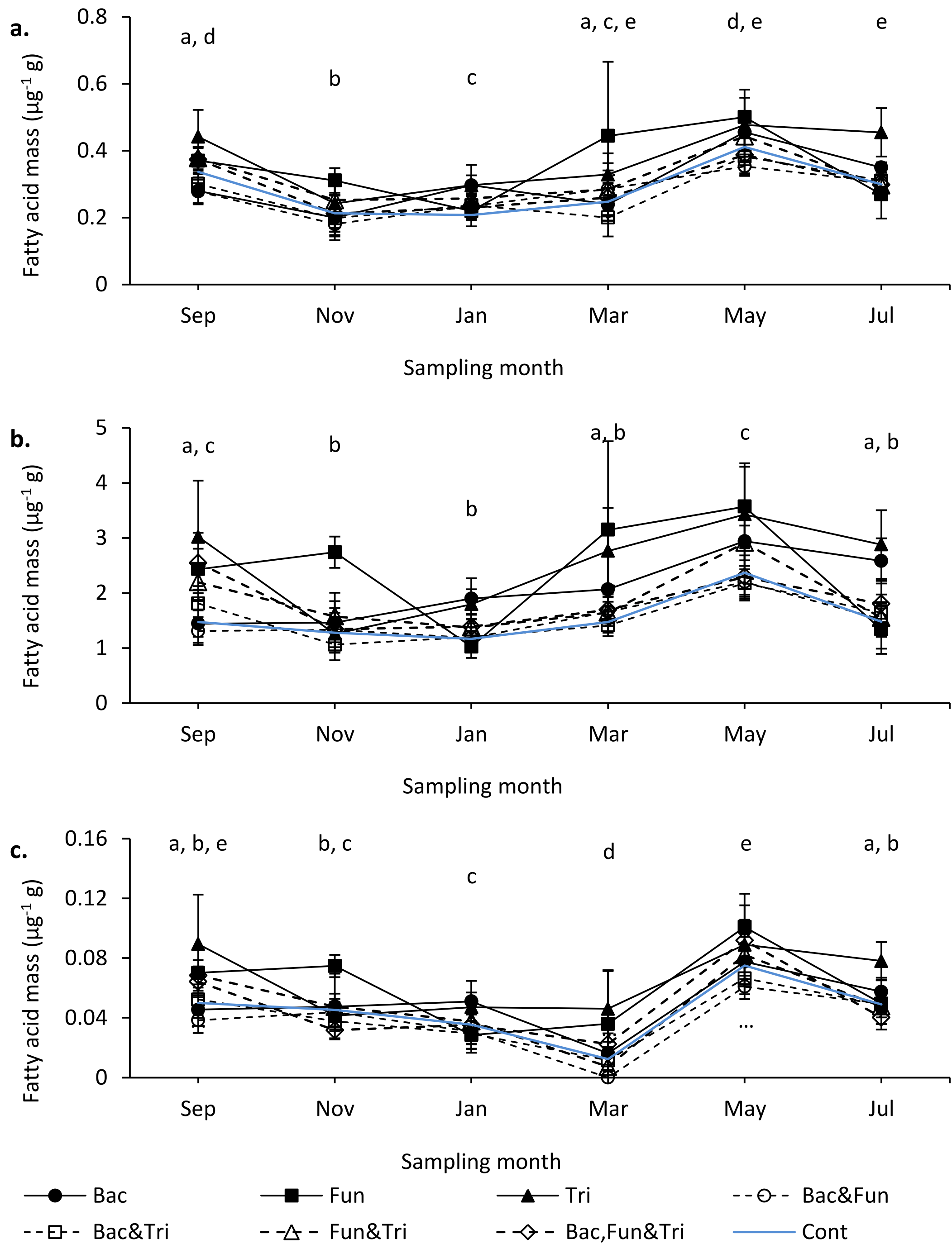

Fig 6.22. Mean abundance of PLFAs over time. (a) Sulphate reducing bacteria, (b) fungi, (c) AM fungi. Shared letters denote statistically similar groups. Error bars denote SEM.

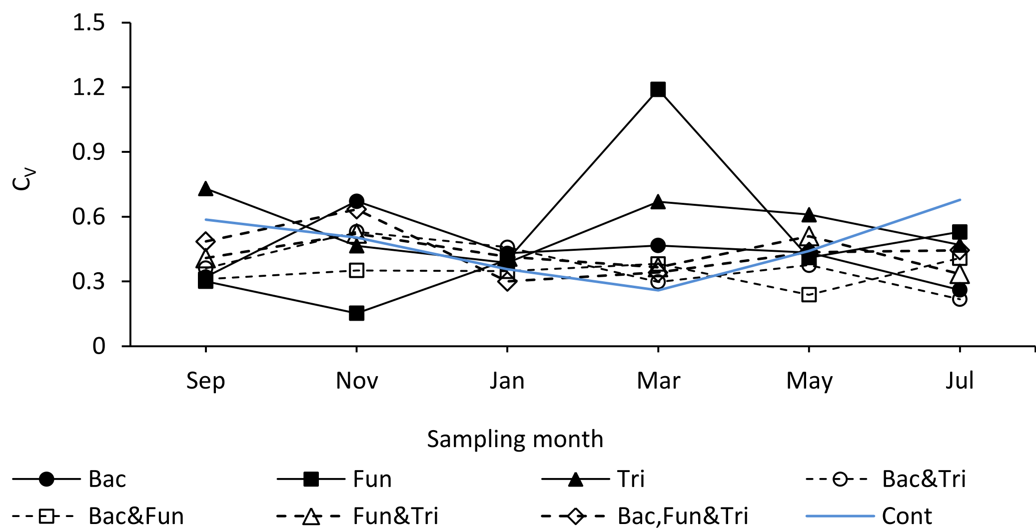

Fig 6.23. Variability (C_V) for total microbial abundance over time.

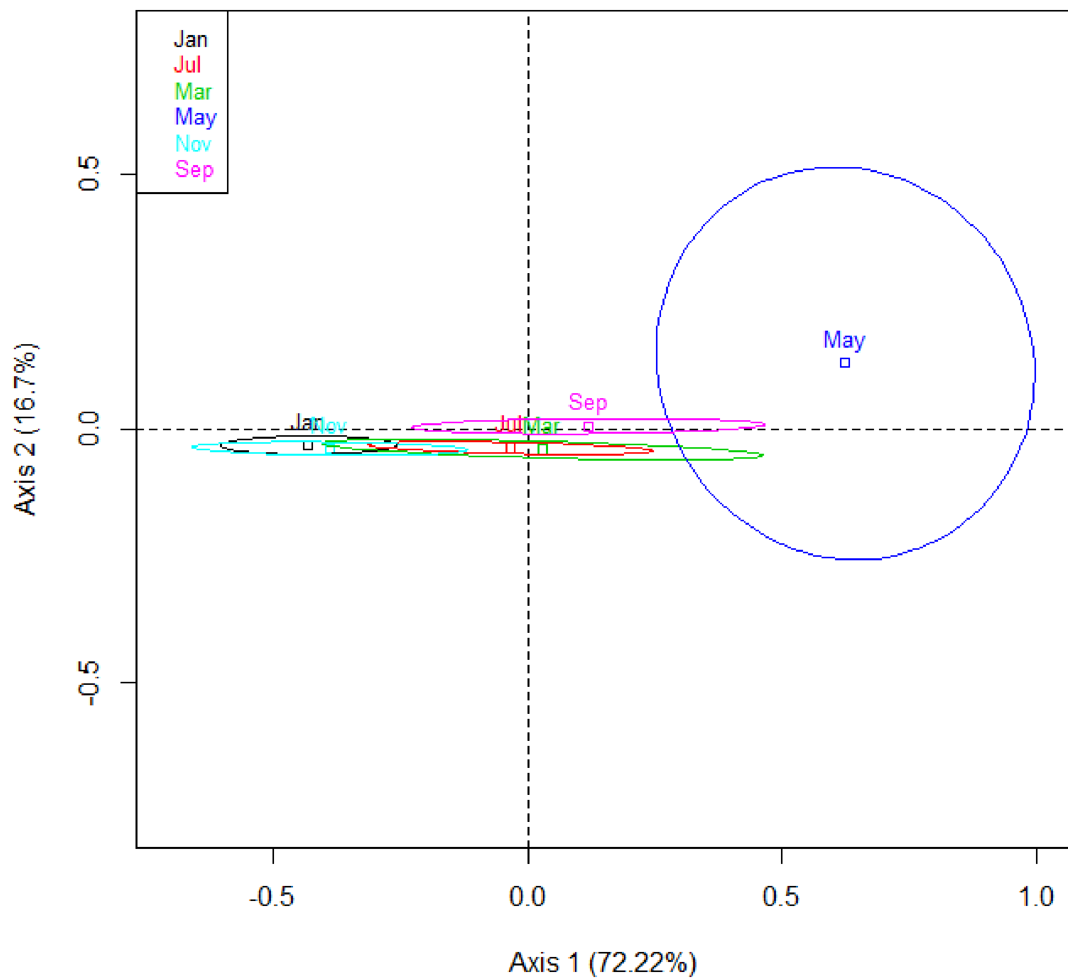

Fig 6.24. PCA ordination plot depicting organisation of total microbial PLFAs. Individual plots are omitted for clarity.

6.3.4.2 Effect of inoculants

In Figs 6.21 and 6.22 it is clear that the mycorrhizal and *Trichoderma* inoculants alone produce different patterns in microbial mass to one another and to the bacterial treatment, mixed treatments and control, which follow a similar pattern to one another. The coefficients of variation for these two treatments are also more variable than the other treatments and control (Fig. 6.23, additional figures in Appendix IIII), particularly in those communities where bacteria lowered fatty acid mass. The fungal inoculant in particular produced highly variable microbial mass, particularly in March 2012 (Fig. 6.23, additional figures in Appendix IIII). PCA also determined that the microbial community was more variable in the fungal treatment (Fig. 6.25).

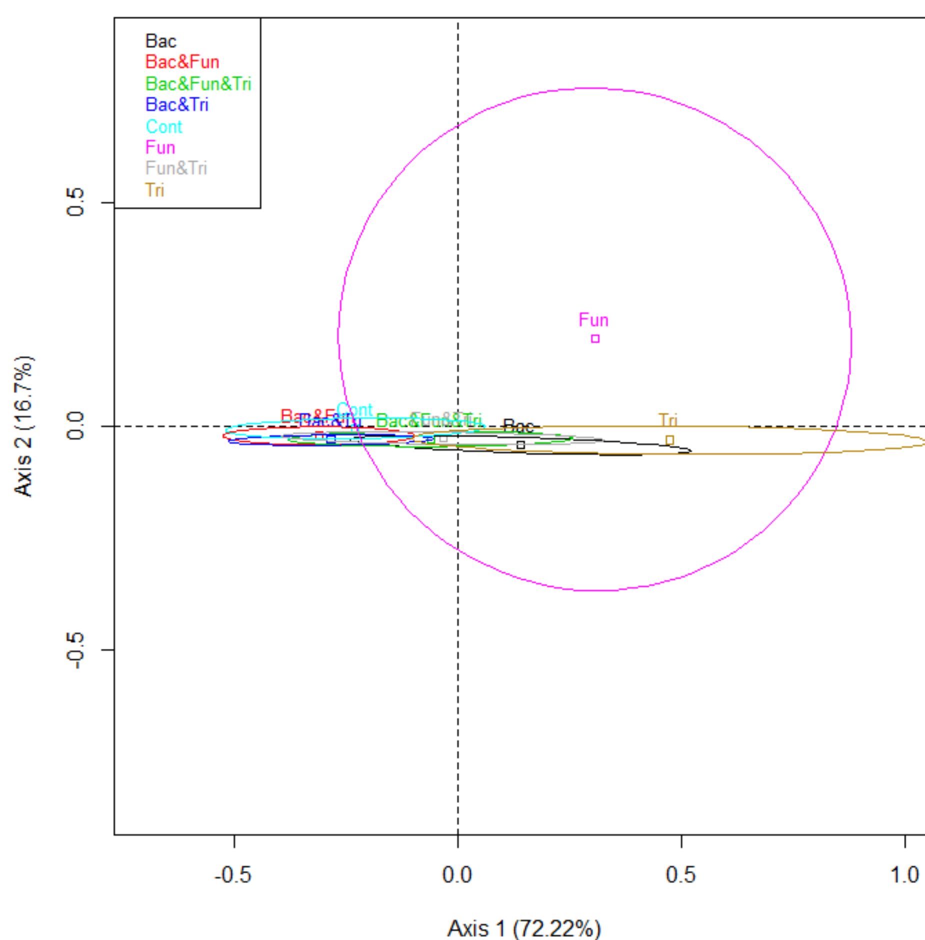

Fig 6.25. PCA ordination plot depicting plots separated by treatment. Individual plots are omitted for clarity.

The addition of the bacterial inoculant significantly lowered the total mass of fatty acids, mass of bacterial and fungal fatty acids and mass of sulphate reducing bacterial fatty acids (Table 6.16, Fig. 6.26).

PLFA community	*F* value	Degrees of freedom	*p* value
Total	5.91	5, 200	**<0.05**
Bacterial	4.63	3.94, 157.79	**<0.05**
Fungal	4.93	5, 200	**<0.05**
Gram positive	3.68	3.92, 156.73	>0.05
Gram negative	0.91	5, 200	>0.05
Sulphate reducing	8.15	3.54, 141.56	**<0.01**
AM fungi	3.84	3.71, 148.22	>0.05

Table 6.16. *F* and *p* values describing the effect of the bacterial inoculant on the mass of PLFAs (μg^{-1}) attributed to different microbial communities. Bold entries denote significant values.

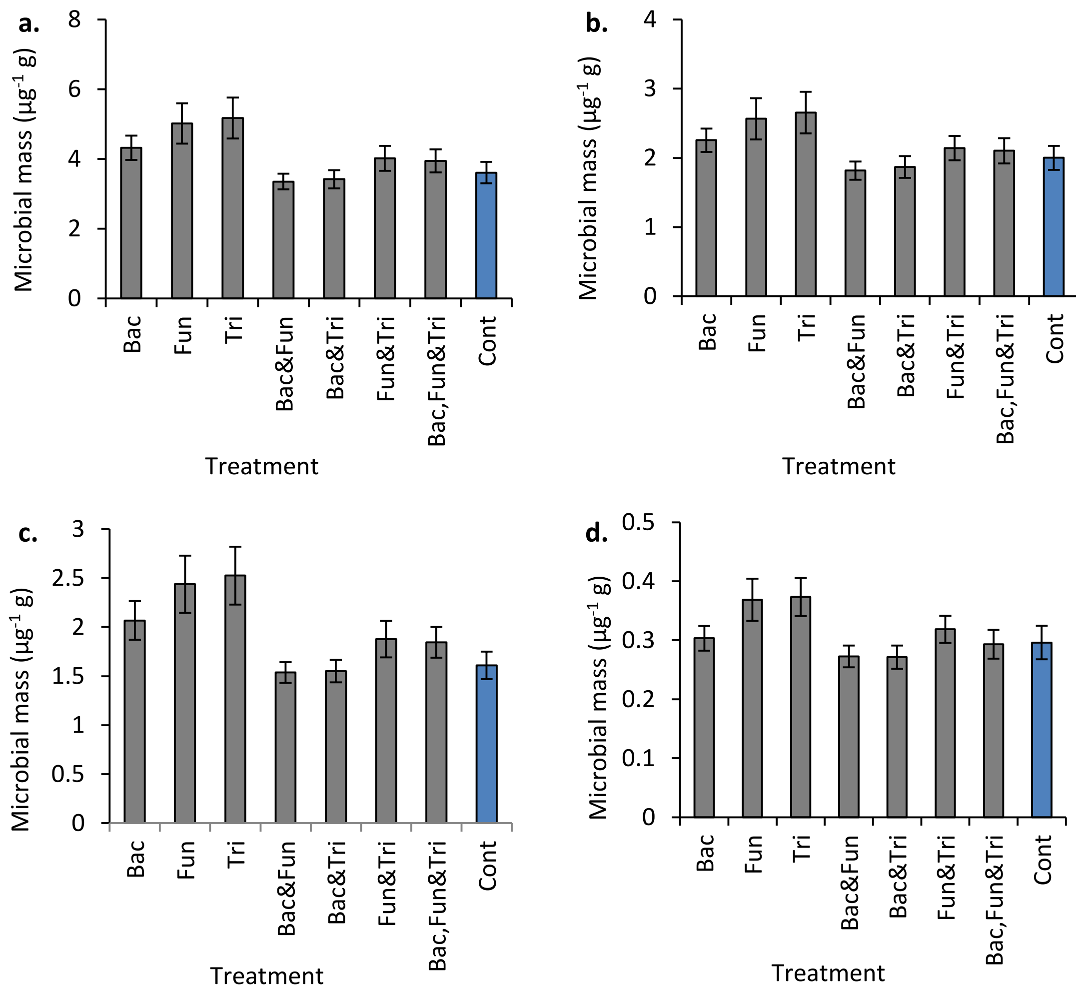

Fig. 6.26. Microbial mass averaged over all time points. (a) Total microbial mass, (b) bacteria, (c) fungi, (d) sulphate reducing bacteria. Error bars represent SEM.

The fungal and *Trichoderma* inoculants had no effect on any of the microbial community parameters. However, when all three treatments were applied together, an interaction occurred in all of the microbial community parameters (Table 6.17). The microbial community

was higher in the 'all treatment mix' and dual mycorrhiza and *Trichoderma* mix than in the mycorrhiza and *Trichoderma* treatments, when applied either singly or together. These treatments were also higher than when bacteria was added to mycorrhiza and *Trichoderma* when together or alone (Figs 6.26 and 6.27).

PLFA community	*F* value	Degrees of freedom	*p* value
Total	14.60	5, 200	**<0.001**
Bacterial	9.23	3.94, 157.79	**<0.01**
Fungal	16.79	5, 200	**<0.001**
Gram positive	8.73	3.92, 156.73	**<0.01**
Gram negative	5.60	5, 200	**<0.05**
Sulphate reducing	5.78	3.54, 141.56	**<0.05**
AM fungi	7.43	3.71, 148.22	**<0.01**

Table 6.17. *F* and *p* values describing the interaction between the bacterial, fungal and *Trichoderma* inoculants on the mass of PLFAs (μg^{-1}) attributed to different microbial communities. Bold entries denote significant values.

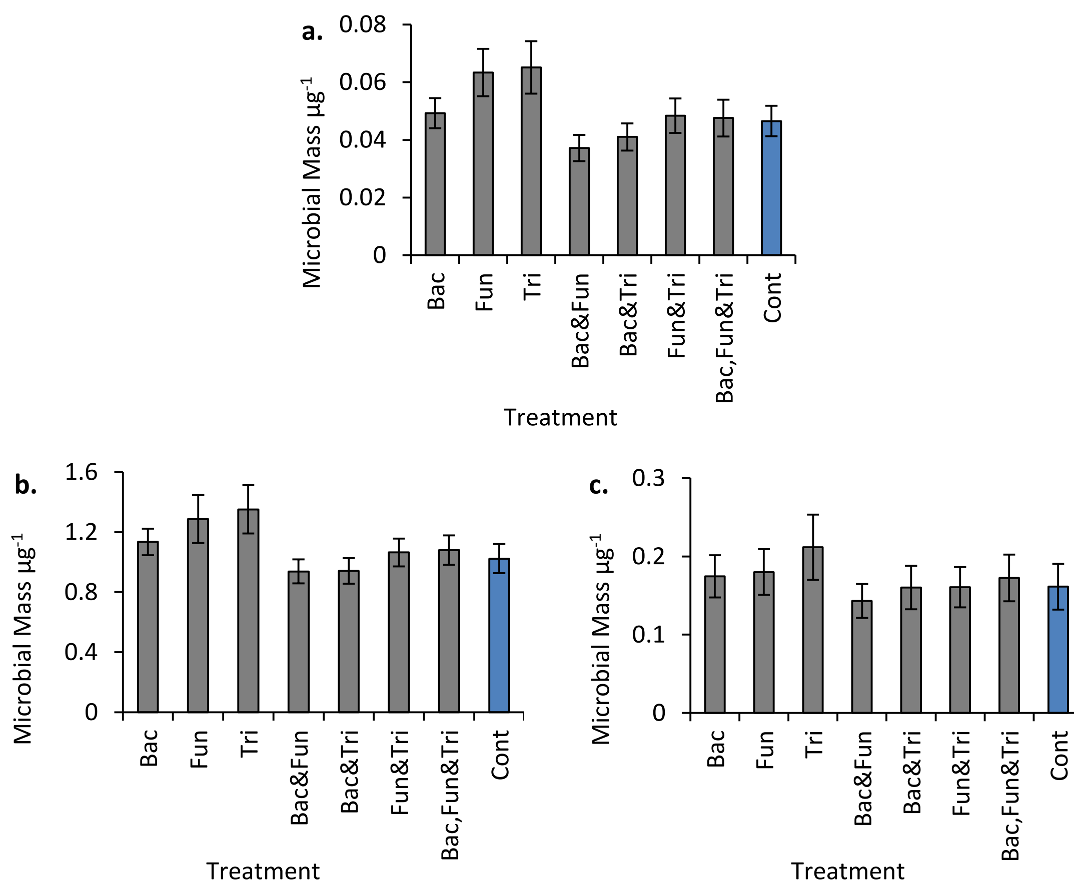

Fig. 6.27. Microbial mass averaged over all time points. (a) AM Fungi, (b) Gram positive bacteria, (c) Gram negative bacteria. Error bars represent SEM.

6.3.4.3 Interactions with abiotic factors

Stepwise multiple linear regression determined that mean daily temperature alone explained a small proportion of the variance in some of the PLFA groups. Overall, mean daily temperature accounted for 14.9% of variance in the total microbial population, 28.3% in the bacterial population and 13.3% in the population of sulphate reducing bacteria (Table 6.18, Fig. 4.28).

For the fungal community, only 10.7% of the variance could be explained by abiotic factors, the majority of which was explained by mean daily humidity but some too by average wind speed. Slightly more could be explained for AM fungi alone, with three factors accounting for 18.9% of the variation within the community. These factors were temperature, wind speed and humidity (Table 6.18).

The gram positive bacterial PLFAs were explained by humidity, temperature and substrate water content, which together accounted for 29.5% of the variation in the community. Gram negative bacterial PLFAs could be explained by two variables accounting for 46.0% of variation within the communtiy. These variables were temperature and substrate water content (Table 6.18).

Community parameter	R^2	*F* value	*p* value	Factor	*β*	*p* value
Total microbes	0.15	49.87	<0.001	Temperature	0.39	<0.001
Bacteria	0.28	71.94	<0.001	Temperature	0.45	<0.001
Fungi	0.11	17.08	<0.001	1. Humidity	0.34	<0.001
				2. Wind speed	0.12	<0.05
Gram positive bacteria	0.30	39.68	<0.001	1. Humidity	-0.43	<0.001
				2. Temperature	0.43	<0.001
				3. Water content	0.34	<0.01
Gram negative bacteria	0.46	121.43	<0.001	1. Temperature	1.20	<0.001
				2. Water content	0.71	<0.001
Sulphate reducing bacteria	0.13	43.99	<0.001	Temperature	0.37	<0.001
AM fungi	0.19	22.06	<0.001	1. Temperature	0.71	<0.001
				2. Wind speed	0.20	<0.01
				3. Humidity	0.34	<0.01

Table 6.18. R^2, *F* and *p* values for stepwise multiple linear regressions perfomed on different PLFA communtiy parameters (left) and *β* weights with their respective *p* values for explanatory factors.

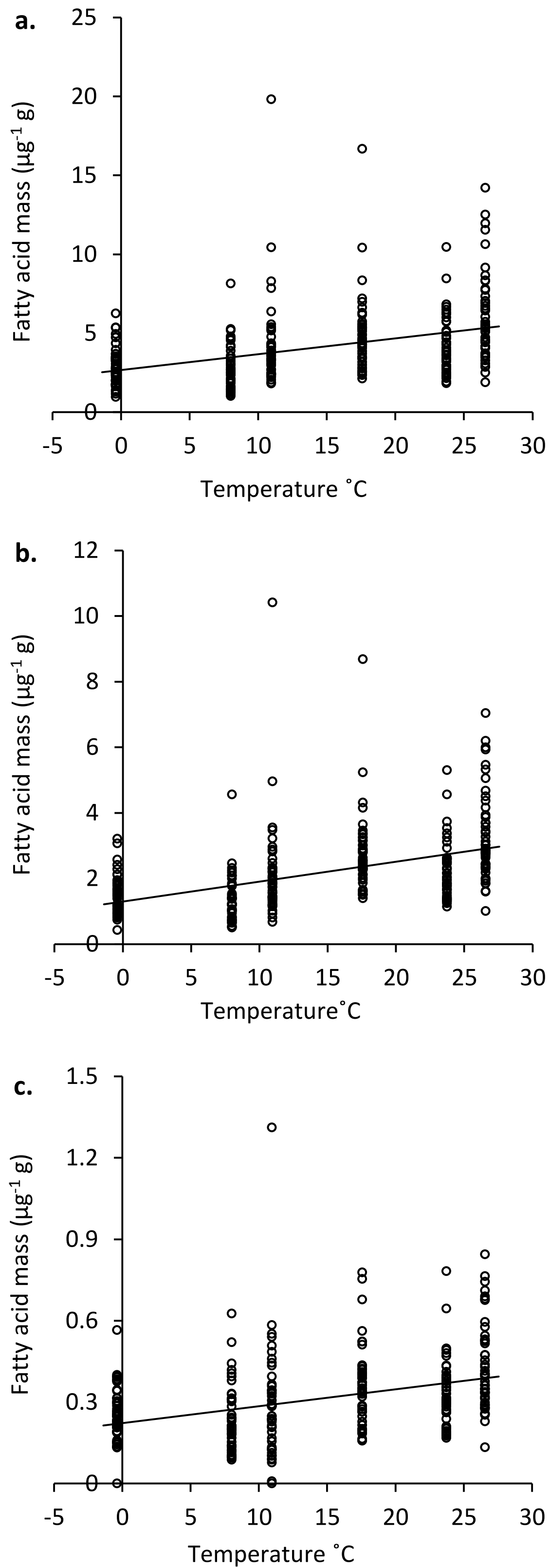

Fig 6.28. PLFAs against mean daily temperature for (a) Total microbial PLFAs, (b) bacterial PLFAs and (c) sulphate reducing bacterial PLFAs.

PCA found mean daily temperature, substrate water content and mean daily humidity to be correlated with axis 1 (the latter negatively) and wind speed to be correlated with neither axis (Table 6.19).

Abiotic factor	Correlation	*p* value
Axis 1		
Temperature	0.31	<0.001
Substrate water content	-0.26	<0.001
Humidity	-0.32	<0.001

Table 6.19. Correlations and *p* values of abiotic factors added as supplementary variables in the PLFA community PCA

6.4 Discussion

6.4.1 Microbial community changes

Overall the microbial community had changed substantially since the 2010/2011 sample period (see section 3.3.4). Saprotrophic fungi was now more common than gram negative bacteria; Sulphate reducing bacteria also explained a large proportion of the variance. This shift from a bacterially dominated to a fungal dominated microbial community is expected over a successional gradient (Ohtonen *et al.,* 1999). Sulphate reducing bacteria have also been found to increase in mass with vegetation cover (Kaštovská *et al.,* 2007; Zhang *et al.,* 2006) and mycorrhizal colonisation (Amora-Lazcano and Azcón, 1996), agreeing with the observations in the current study. Some bacterial parameters, such as C16:0i, were much more dominant than they had been previously. These in particular drove variability in May when fungal microbes were also at their most abundant. This suggests that although fungi had a greater influence on the microbial community than in the previous year, the successional process towards a fungal dominated community was still underway and bacteria still had a large influence on the soil community.

Mass of all microbial parameters had increased, apart from bacterial mass which did not reach the levels it had the previous year. However, the pattern of abundance over time broadly matched that of the previous year. However, as the maxima of bacterial mass occurred in summer of the previous year and it was this period that had fewer samples taken in this year, it is likely that in year two maximum values were missed. Patterns in abundance of fungal, including AM fungal, parameters were also the same in both years with maxima occurring in spring.

Changes in the community composition suggest that over a relatively short time scale, dominant microbial groups can change. The roof in this sampling year was bacteria dominated, but fungi were only just lower in abundance. All groups showed consistent seasonal patterns with the previous year, suggesting that abiotic factors continue to be a major driving force in microbial community structure.

6.4.2 The microbial community and inoculants

The bacterial treatment significantly lowered microbial mass of most of the community parameters measured (total mass, bacterial mass, fungal mass and sulphate reducing bacterial mass), and those that were not significant showed the same pattern, suggesting that a higher sample size may have produced the same result for all parameters. The interaction between all treatments suggests this suppressant effect of bacteria was lessened in the presence of both mycorrhiza and *Trichoderma*.

Although *Bacillus spp.* have been shown to stimulate mycorrhizal activity in some cases (Medina *et al.,* 2003) and to be negatively affected by fungi in other cases (Wu *et al.,* 2005), they have also been shown to decrease the survival rate of fungal mycelia (*B. pumilis* and *B. licheniformis*, as inferred from ergosterol and chitin levels in the soil) (Probanza *et al.,* 2001; Domenech *et al.,* 2004). Of these two species, *B. licheniformis* is most likely to have caused this decrease in fungal mycelia as it is patented as an anti-fungal agent (Neyra and Sadasivan, 1996), without any mention of non-target effects on non-pathogenic fungi. Xavier and Germida (2003) found that *B. licheniformis* had an inhibitory effect on spore germination of *G. clarum.* On the other hand, *B. pumilis* has been found to have stimulatory effects on the mycorrhiza *G. deserticola* (Medina *et al.,* 2003). Differences in this effect could be dependent on concentrations of the two, as has been seen between other bacteria species and ectomycorrhizal fungus (Frey-Klett *et al.,* 1999). Studies with *G. intraradices* and bacterial inocula obtained using soil filtering also found that soil bacteria inhibited fungal growth but that hyphal exudates benefitted bacterial growth. This suggests that bacteria in our inocula could take advantage of hyphal exudates to further increase their competitive ability against the fungi.

Although of the many species present in our inocula some may have had a beneficial effect, the evidence suggests that overall they did not and that a restriction in the resident fungal population occurred, probably due to the proliferation of *B. licheniformis*. As fungi were significant drivers in this soil to begin with, it can be surmised that many of the resident bacterial species present were also reliant on a fungal dominated community assemblage. Perturbation of the fungal community by *Bacillus spp.* could, therefore, extend towards the

associated bacterial community as well, producing effects matching those seen here (Andrade *et al.,* 1997). It can be deduced from this that bacterial mixes added to an already complex soil food web can have unpredictable and perhaps negative consequences, making this 'off the shelf' bacterial inoculant unsuitable for use on mature green roofs. Analysis of the species present and tailoring inoculation to these species would be necessary to produce effective bacterial inoculants. As previously mentioned, combinations of some species such as *G. deserticola* and *B. pumilis,* can indeed stimulate mycorrhizal colonisation (Medina *et al.,* 2003).

Another important difference to be noted when comparing microbial mass in *Trichoderma* and mycorrhiza treatments is one of persistence. Although the effect of bacterial inoculants is thought to be long lived due to the impacts they have on successional development (Probanza *et al.,* 2002), the increased mass of bacteria is short lived, with studies reporting a drop in bacterial mass 60 days after inoculation (Domenech *et al.,* 2004). The opposite is true of fungal inoculants, with mass increasing as establishment over time increases. Thus, over the period of this experiment, fungal inoculants would have increased microbial mass due to proliferation of the additives themselves, whereas bacterial inoculants would not. This emphasises the importance of looking at PLFA community structure, as well as abundance, to determine inoculant effects as well as simple microbial mass.

Although not statistically significant, it is worth noting that there was a small rise in microbial mass under the *Trichoderma* and mycorrhiza treatments. Analysis of the variability (C_V) for each treatment on each microbial community parameter also determined that the mycorrhiza and *Trichoderma* treatments had high variability over the sample period compared to bacteria treated plots. This suggests that for some of the microbial community parameters, these treatments may have had an effect, though a variable one.

The mycorrhizal treatment in particular produced variable microbial mass for all parameters, especially in March. The variability of mycorrhizal fungi between seasons is well documented, with higher colonisation rates often occurring in spring (Allen, 1983; Lopez-Sanchez and Honrubia, 1992; Mohammad *et al.,* 1998) although this varies depending on climate, plant type and growth year of the plant (Gay *et al.,* 1982; Merryweather and Fitter, 2008). The abiotic factors measured did not explain fungal abundance and so it could be that competition between resident and added fungi caused the variability in fungal PLFAs, as already seen in section 5.4.3. Fungal mass peaked in May, after high variability in March, so perhaps competition increased as the active period of the fungi began, but a competitor had dominated and stabilised fungal mass by May. PCA also showed that the community was more influenced by the gram positive bacterial fatty acid, C16:0i, in mycorrhiza treated plots in May,

perhaps suggesting that a symbiotic bacteria was able to proliferate once fungal mass had stabilised. Marschner and Baumann (2003) found that mycorrhizal colonisation of roots changed the community structure of bacteria in non-rhizosphere soil due to a reduction in plant root exudates and changes in soil pH. This phenomenon varied in its effect depending on species, with *G. mosseae* having a greater effect than *G. intraradices*. Andrade et al (1997) suggest that the nature of the exudates produced by AM fungi can influence bacterial community structure and that the AM fungal species is more important in influencing the community than the amount of mycorrhizal development. Thus, an increase in AM fungi produces corresponding differences in the microbial community, explaining the findings in the current study.

The *Trichoderma* treatment was less variable than the mycorrhiza treatment, but more variable than all the other treatments. However, the pattern of variability in *Trichoderma* was different to that in mycorrhiza, staying consistently variable rather than peaking in March. Again, this is likely due to competition with resident fungi (Benhamou and Chet, 1997), as well as competition between the inoculants (Widden and Hsu, 1987), with microbial mass benefitting in some plots but not in others. This competitive ability of *Trichoderma*, in some cases supressing pathogenic soil fungi, is the very reason for applying it (Papavizas, 1985). However, these results suggest that in established field soils, the success of this disease dampening may vary. Additionally, *Trichoderma* species are known to have a heterogeneous distribution when nutrients are also spatially heterogeneous (Regalado *et al.*, 1996). It is recommended that future studies in this field include nutrient analysis to elucidate more about the spatial patterns of soil microbes, as this too could be a source of variability in microbial mass induced by the addition of *Trichoderma*.

As well as competition within fungal types, competition between mycorrhiza and *Trichoderma* is also well documented. *T. harzianum* and *G. intraradices* are known to compete, with the former inhibiting root colonisation by the latter whilst the latter inhibits growth of the former (Green *et al.,* 1999) and *T. harzianum* has been observed perforating and damaging extraradical hyphae of AM fungi (Rousseau *et al.,* 1996). AM fungi also reduce metabolic activity in the soil, which is detrimental to saprotroph growth (McAllister *at al*., 1994a). McAllister *et al*. (1994b) found that *G. mosseae* reduced the success of saprotrophic fungi by influencing plant growth but that *T. koningii* inhibited *G. mosseae* in its extrametrical hyphae, particularly inhibiting spore germination, and that this effect was due to exudates produced by the saprotroph. Not all interactions between mycorrhiza and saprotrophic fungi are negative though, with Calvet *et al*. (1993) finding stimulation of growth in *G. mosseae* by the addition of *T. viride*. The addition of both *Trichoderma* and mycorrhiza in our study did not produce a

significant decrease in soil microbes, and, in fact, the *Trichoderma* treatment increased colonisation levels of mycorrhizas. Competition and mutualisms varying between resident fungal types may explain the variability in microbial mass produced by these inoculants. Increased growth of fungal hyphae may also cause corresponding escalations in senescence (Anaya, 2013), a major source of soil organic carbon in mycorrhizal soils (Bending *et al.,* 2006). Again, this stimulation of mycorrhizal growth is likely to have profound impacts on the soil community, but is also likely to be spatially heterogeneous (Bending *et al.,* 2006).

The hypothesis that resident microbes on a mature green roof would influence a different pattern of results on inoculation of a mature green roof was correct. The variability of mycorrhiza and *Trichoderma*, caused by competition between some species but mutualisms between others emphasises the importance of researching specific species interactions and tailoring inocula to be harmonious with resident populations. As seen with abundance, the variability of *Trichoderma* and mycorrhiza also seemed to be suppressed by the addition of the bacterial inoculant. As mentioned previously, this is likely due to the bacterial inoculants anti-fungal properties, reducing competition between fungi by suppressing the fungal community as a whole.

6.4.3 Vegetation and inoculants

Contrary to the hypothesis that microbial inoculants would improve plant growth, there was no evidence that application of these inoculants to a mature green roof had any effect on the cover potential of *Sedum spp.,* or on migrant species. It seems, therefore, that there is little value in the use of these inoculants for improved *Sedum* growth, as in a mature roof we see no effect and on a new roof, we see a negative effect (see sections 5.3.2.3 and 5.3.2.4). However, the addition of *Trichoderma* to plots did change the pattern of bryophyte abundance over time, increasing cover in January but decreasing cover more than other treatments in March and July. The reasons for this are unclear, but the importance of bryophytes for some of the green roof species, such as Scutoverticidae and *A. parvula*, mean that this property should be investigated further.

Trichoderma polysporum has been found to colonise dead epidermal cells of mosses (Osono *et al.,* 2011). Additionally, *Trichoderma spp.* have been found in both living and senescent tissues of mosses (Scheirer and Dolan, 1983). However, there is no evidence that *Trichoderma* either harms or benefits healthy moss growth. Akita *et al.,* (2011) hypothesise that fungi that cause disease in vascular plants are likely to have the same effect on bryophytes. As *Trichoderma* is known as a disease suppressant in vascular plants (Harman *et al.,* 2004), perhaps the same

could be assumed for mosses, i.e. the addition of *Trichoderma* supressed fungal pathogens harmful to bryophytes.

6.4.4 Microarthropod community changes

Microarthropod abundance, but not diversity, had increased compared to the previous year, suggesting some limited development of the community. Shure and Ragsdale (1977) found that abundance of microarthropods increased over the successional development of granite outcrops. They also found that diversity increased over time but then slowed as succession reached the most complex habitat development, a herb-shrub vegetation system. This mirrors the conclusions from section 3.5, that the succession of organisms is either halted or extremely slow in the green roof environment. New species do colonise the roof slowly and the saturation point of similar habitats, such as glacial forelands, has been found at 40 years (Kaufmann *et al.,* 2002). In addition, even extremely harsh environments such as deserts can be invaded by new species exploiting new niches (Lobo, 1996). This suggests that a slow succession on green roofs is more likely than a halted one.

Peak collembola numbers had slightly decreased from the previous year and one of the two common species (*D. pallipes*) was much less common. Substrate water content and temperature were still found to be important driving factors in collembolan abundance, so one hypothesis for this is that numbers did not recover from the drought in April of the previous year, where collembola numbers were seen to decrease dramatically. Temperature and substrate water content remained much more stable than in the previous year, demonstrating the long term effect of drought on the populations even if the weather is milder in subsequent years. As in the previous year, collembola peaked in the winter months, most probably due to an increase in soil moisture at this time.

Most of the increase in microarthropod abundance could be attributed to a rise in mite numbers, and these were now the most dominant organism. At their peak they numbered over twice that of the previous year. Once again this community was dominated by the xerophillic oribatid of the family Scutoverticidae. Mite numbers decreased over the sample period and the characteristic population explosions of the first year were absent. However, as with the bacterial community in section 6.4.1, it could be that sampling every two months was not at a resolution high enough to capture these peaks in population, as they were often only present for a month at a time. The mite community was once again relatively independent of abiotic factors due to the prevalence of the hardy Scutoverticid. However, even without the abiotic pressures exerted by temperature and drought, the mite population declined over time, returning to levels seen in 2009/2010 (section 3.3.3.3). PCA found Scutoverticidae to be

independent not only of the abiotic factors measured, but also to the plant species present, suggesting another limiting factor, such as food availability must be regulating oribatid mite populations. There was a suggestion that microbial inoculants supported a slightly higher abundance of these mites, perhaps alleviating some of the nutrient stress occurring over time, but it was not enough to overcome the general decline in numbers.

The larval community peaked in winter, as they had the previous year. This is common in soil dwelling Diptera larvae (of which most of the larvae belonged to) as emergence takes place in spring and summer (Frouz, 1999). PCA indicated that chironomid midge larva had a negative relationship with other insect larvae. Frouz (1999) suggests that soil dwelling fly larvae are limited by two factors, drought and food availability. Therefore this interaction could be a result of competition or could be an artefact of differing food preferences which vary in their abundances throughout the year.

Few new species were found in this study period compared to the previous year (see section 3.3.3), demonstrating the slow development of microarthropod communities on this green roof. However, one such species was a member of the family Tingidae, *Acalypta parvula*. Tingidae have been reported on green roofs before (McCaffrey, 2011), but *Acalypta* is a genus that is mainly associated with mossy habitats (Rédei *et al.,* 2004). The presence of this species as yet another moss specialist on the green roofs emphasises the importance of moss communities as a habitat on green roofs.

A species belonging to the order Thysanoptera was also a new addition to the roof. The order has a wide range of dietary preferences and so without identification it is difficult to ascertain how this species may be influenced by factors on the roof. Another new addition to the roof was a species of Gastropoda, *Vallonia costata,* found only in the summer months on the roof. This species is a common UK species of dry, open habitats including scree and stone walls (Kerney *et al.,* 1979), again demonstrating the harsh conditions on the roof. How these snails dispersed to the roof is puzzling as generally land snails have low powers of dispersal (Douris *et al.,* 1998) and in previous green roof studies it was assumed that these arrived in the plugs for this reason (Kadas, 2006). However, they have been known to be spread by humans (Cowie, 1998) and many land snails are also dispersed by birds (Myšák & Horsák, 2011), both possible sources. The presence of snails in this study and high abundances reported by Kadas (2006) on green roofs suggests that green roofs may provide a valuable urban habitat for these creatures.

Sampling every two months, rather than the monthly samples taken in the first year, may mean that short-term population changes have been missed from this analysis. The addition of

inoculants seems to have produced a more variable community and so this would have been useful to elucidate how these treatments have affected the soil communities present. It is recommended that more regular sampling is conducted in future experiments if possible.

6.4.5. Microarthropods and inoculants

The addition of *Trichoderma* caused a subsequent increase in the total number of microarthropods, supporting in part the hypothesis that a change in microbial community would influence microarthropod communities. No effect was seen of *Trichoderma* on plant growth, but the addition of *Trichoderma* did increase mycorrhizal colonisation of plant roots. It can be assumed that changes in the microarthropod community were a result of direct feeding on the saprotrophic or mycorrhizal fungi. The most abundant collembola present, *S. aureus* is known to mainly feed on fungi (Gillett and Ponge, 2005), although preferentially avoids mycorrhizal fungi if other sources are available (Gange, 2000). The dominant mite, a member of the Scutoverticidae, is thought to be a generalist feeder so may also feed on fungi (Smrž, 2006). The latter is also associated with moss (Schäffer *et al*., 2010b) and so may have been affected by the seasonal changes seen in the moss community on addition of *Trichoderma*, although it did not peak in January when we saw the *Trichoderma* have the most effect on moss. Sibi *et al*., (2008) found that increases of *T. harzianum* in the rhizobia of Sorghum not only increased populations of mycophagous mites but also their associated predators, emphasising the potential of *Trichoderma* as a food source for soil food webs. Neither the collembola or mite group increased as a result of treatment when analysed separately, suggesting that the effect was small but consistent for both mites and collembola. Interestingly, patterns in mite abundance suggest that all inoculants produced abundance higher than the control and that the latter returned abundance to levels similar to that of the previous year. Perhaps the addition of inoculants enables the peaks we saw in mite abundance in the previous year to remain higher for longer than non-inoculated plots, possibly due to an increase in food availability.

The only individual group to show a significant change in abundance on addition of the inoculants was the ‘other’ group, which was increased by the addition of *Trichoderma*. This group consisted largely of diptera larvae, most notably chironomid midge larvae and a larva belonging to the superfamily mycetophiloidea. The latter is known to feed primarily on fungi (Krivosheina and Zaitzev, 2008), so may directly benefit from *Trichoderma* addition. Chironomids are thought to feed on faecal matter (Ponge, 1991) and so may be indirectly benefitting from the increase in abundance of other microarthropods as a result of *Trichoderma* addition.

6.5 Conclusions

The microbial community had changed since the previous year, with bacteria still dominating but the fungal community playing a much more significant role. The bacterial inoculant negatively affected soil microbes, suggesting that this may not be a suitable remediant for mature green roofs. Though the addition of mycorrhiza and *Trichoderma* lessened this effect, they did not affect the overall abundance of microbes. They did, however, produce extremely variable microbial abundances and the mycorrhizal treatment significantly altered the microbial community structure. This suggests that these inoculants have the potential to increase microbial mass in green roof substrates but that their success is extremely heterogeneous. Further studies into dose amounts could determine if these inoculants have the potential to remediate impoverished microbial communities.

The plant community was little affected by inoculants, although there was an interaction between *Trichoderma* and moss. Investigating the relationship between these two factors is important as many of the green roof species, such as *Acalypta parvula,* are moss specialists. Therefore any future application of *Trichoderma* to green roofs should ensure that monitoring of the bryophyte community takes place in tandem.

Overall the microarthropod community had changed little between 2010 and 2012, emphasising the slow successional process. However, the addition of the saprophytic *Trichoderma* increased microarthropod abundance overall, suggesting its potential as a green roof supplement. This is encouraging as the addition of this inoculant may be able to be applied to existing green roofs that have an impoverished soil community. Experiments applying different concentrations of *Trichoderma* should now be conducted to determine if this is a dose specific response.

As seen in Chapter 5, one application of inoculants was enough to see resulting changes in the subterranean community. It seems that in a mature green roof the potential for use of fungal inoculants in particular may be greater than on a new green roof. Larger effects may be seen with stronger doses or repeat applications, but overall mycorrhiza and *Trichoderma* seem to be promising microbial inoculants for the remediation of impoverished mature green roof soil communities.

Chapter 7

General Discussion

7.1 Biodiversity on green roofs

One of the aims of these studies was to determine the value of green roofs as a habitat for subterranean arthropods. The kinds of species found varied from cosmopolitan ground level species, such as the collembolans *P. notabilis* and *S. aureus* (Hopkin, 2006), to specialist moss inhabiting species such as *Acalypta sp.*(Gerson, 1969). Those organisms that were identified are summarised in Table 7.1 and demonstrate that the microarthropod community on green roofs is extremely unusual and, though it is missing some of the organisms we would expect at ground level (Santorufo *et al.*, 2012), it also harbours some unusual species, which are worth conserving. This mirrors findings by Kadas (2006) who also found rare and unusual above-ground insects on green roofs. We found few predators but *P. notabilis* and chironomid larvae are both coprophagous (Ponge, 1991), alluding to the fact that green roofs are capable of supporting secondary consumers.

During the initial sampling on the mature roof, constructed five years previously, the number of microarthropods present was low. Additionally, new species were encountered infrequently. Only 42 different species were found in the entire sample period, and only one or two new species were encountered each month. The species assemblage present and abundances of microarthropods were similar to that of a desert (Wallwork, 1972) or a glacial foreland (Kaufmann *et al.*, 2002), with some species crashing in abundance during the year. This produced the hypothesis that the roof was in a state of interrupted, slow succession due to the extremely harsh conditions. In the second year of sampling the rate at which new species were found slowed further. Only three new species were found throughout the sampling period and, once again, population crashes occurred in the summer months. Caution must be prescribed in comparing different years here, as the sampling technique was changed and only one roof, rather than two was sampled. However, it can generally be stated with confidence that few new species colonised these green roofs over the two year sampling period, so the impoverished state described in Chapter 3 persisted into the 8th year of the roofs existence (Chapter 6). The next question, however, was whether the rate of colonisation of green roofs is always this slow, or whether on the mature roof the community has simply reached carrying capacity. The latter hypothesis was unlikely, due to the incredibly low abundances compared to ground level soils (Santorufo *et al.*, 2012; Hartley *et al.*, 2008) and because the abundances of the species that were present increased in year two, with mites four times more prevalent in the second year of sampling, insect larvae ten times more prevalent, hemiptera twenty times more prevalent and Aranae and Chilopoda two and three times more prevalent respectively (collembola, however, had halved in abundance and this will

be discussed later in the chapter). The new roof built in Chapter 5 answered some of these questions, as well as determining the source of the microarthropods present.

Species/morphospecies	Plugs	New Roof	Mature Roof	Life history	Status
Sminthurinus aureus	Moderate	Rare	Common	Cosmopolitan[1]	Very common[1]
Deuterosminthurinus pallipes	None	None	Common	Cosmopolitan[1]	Common[1]
Parisotoma notabilis	Common	Common	Rare	Cosmopolitan[1]	Extremely common[1]
Bourletiella hortensis	None	None	Rare	Cosmopolitan[1]	Common[1]
Deuterosminthurinus bicinctus	None	None	Rare	Cosmopolitan[1]	Moderately common[1]
Isotomurus palustris	None	None	Rare	Cosmopolitan[1]	Very common[1]
Orchesella villosa	Common	Rare	None	Cosmopolitan[1]	Common[1]
Brachystomella parvula	Common	None	None	Cosmopolitan[1]	Common[1]
Sminthurinus trinotatus	None	Moderate	None	Unknown[*1]	Rarely recorded[1]
Eupodes viridis	None	None	Common	Cosmopolitan[†]	Unknown[†]
Scutoverticidae species	Common	Common	Common	Xerophile[‡2]	Common[‡2]
Acalypta sp.	None	None	Seasonally common	Moss specialist[3]	Unknown, common in Europe[4]
Vallonia costata	None	None	Rare	Xerophile[5]	Common[6]
Aphis sedi	Common	Common	None	Sedum specialist[7]	Common[7]

[*]Records from flower pots, litter on roofs and greenhouses (Hopkin, 2007; Shaw, 2013)

[†]Records range from agricultural land (Purvis and Curry, 1980) to sub-Antarctic islands (Strandtmann and Davies, 1972) but an overall picture is unclear. In these environments this species is common.

[‡]Some species

Authorities:

[1]Hopkin, 2007; [2]Schäffer *et al.*, 2010; [3]Gerson, 1969; [4] Bosmans, 1980; [5]Kerney *et al.*, 1979; [6]Wilkinson, 2011; [7]Alford, 2012

Table 7.1. Species sampled in this study that were identified, and their UK status.

Though a source population was present in the plugs, not all these organisms survived post-planting. Most of the species recorded on the new roof were present within the first two months. Thereafter colonisation rates were similar to those on the mature roof.

Throughout the 12 month sample period on the new roof, 23 species were encountered, half the number encountered throughout the whole two years on the mature roof. With the exception of the flying insects, the majority of microarthropods that later went on to be dominant on the young roof were present in the initial plugs, most notably *P. notabilis* and the Scutoverticid mite. The latter was also abundant on the mature green roof. Perhaps, therefore, the ubiquitous nature of this species means that it would have colonised anyway, especially in light of the fact that it is a common species of bare roofs (Schäffer *et al.*, 2010). *P. notabilis*, however, though found on the mature roof, was in extremely low abundance and *S. aureus* was dominant. Does this mean a shift of dominant species occurred during the history of the roof? Or, as there is no way of knowing the species assemblage of the mature roof plugs, does the community present in the plugs determine the later succession of the collembolan community? Further long term studies with plugs of different origin are the only way to determine this. In either case, it is likely that the conditions under which *Sedum* was grown, and thus the community present in the plugs, has a profound effect on the development of the roof. It is conceivable, therefore, that improvements to the microarthropod community could be made at the farm level, before planting has even begun.

However, it is important to note that though some of the species in the plugs survived and later became dominant, many species did not survive at all. Presumably this was due to the very different habitat presented in plugs grown in a greenhouse to an exposed and dry green roof. In Chapter 3 we saw that conditions on the green roof were unfavourable at certain times of the year, even to those organisms that had managed to colonise successfully, such as *S. aureus*. Thus, a further addition to the conclusion of Chapter 3, that ameliorating the conditions on the roof would increase the persistence of these organisms, would be to also ensure that those organisms initially present in the plugs are adapted to the green roof conditions. After all, *Sedum* is chosen for planting on green roofs because it is drought adapted (Grant, 2006). We should, therefore, apply the same reasoning to other organisms we want to encourage onto green roofs. This could potentially be achieved by growing *Sedum* in a similar environment to the roof, i.e. in the green roof substrate. It has been hypothesised that ground level soil has high levels of functional redundancy (Setälä *et al.*, 2005), enabling rare species to become more abundant in times of environmental change (Liiri *et al.*, 2002). It is therefore conceivable that drought adapted species are present in UK soils in low abundances, and that if *Sedum* was grown in the substrate later to be used, but exposed to a diverse source population, these species may be able to colonise the plugs more easily at the ground level. This could be achieved for both *Sedum* mats and plugs, with the former grown with a layer of substrate (placed on top of a ground-level field soil) that should also be installed on the roof,

and the latter grown in green roof substrate (again placed on ground-level soils). The movement of Scutoverticidae from non-sterile to sterile pots in section 4.3 suggests that this migration of specialist species is possible. These changes in *Sedum* farming practise would be a minor adjustment to current standard practise (personal observation) but could potentially vastly improve the source microarthropod population.

S. album supported the highest abundance of microarthropods before planting, although the lowest diversity. Moreover, *P. notabilis* was extremely abundant in these plugs and became one of the most successful species on the roof later. This is important to take into consideration when determining what plants support the best community for later survival, where diversity may not be the best indicator of a successful source population. *P. notabilis* is clearly very successful in this environment, where many of the microarthropods present in plugs did not survive post planting. As a coprophage and detritivore (Ponge, 1991) it could also be a key nutrient cycler on the roof. Thus, perhaps *S. album* supporting this species could be an argument for designing plug patterns for maximum survival of those microarthropods that can withstand the roof conditions.

Conditions post-planting are important not only for those organisms arriving with the plugs but also for those microarthropods that colonised later. The species of insect larvae found, particularly the chironomids, were common to both the young and mature roof suggesting that this habitat is consistently chosen by the adults of these species, that they are present from early on in the roofs life and that they remain in the long term. This is the only case in this study, therefore, where we can confidently conclude that this habitat is chosen for colonisation by these organisms, rather than a chance colonisation, from the construction of a green roof. There was also a collembolan species present on the new roof early on that had self-colonised, *S. trinotatus*, which has also been found in the litter of bare roofs (Shaw, 2013). There are very few records of this species in the UK and little is known about its life history, but it may be the case that this species is the equivalent of the Scutoverticid mite and may be reasonably common in unusual, and perhaps primarily urban, areas. Previous records note it in flower pots and greenhouses (Hopkin, 2007). This species could be a ubiquitous roof dweller, and an example of the kind of specialist species we may see as green roof organisms in the future, with little modification to the current design.

Some species that had colonised the mature roof clearly did not form sustainable populations. In the first year of sampling, drought caused collembola populations to crash and they were slow to recover. In year two, *D. pallipes* had decreased significantly, and overall collembola numbers had halved, despite the fact that the summer had been less harsh. This highlights the

impact of the staccato succession discussed in Chapter 3. Even a year after the population crashed, with favourable conditions, the collembolan community had not recovered from the severe drought the year before. Srivastava (2002) notes that in unsaturated environments, where there are many available niches, lost species are difficult to replace and ecosystem function may suffer as a result. This appears to be the case with collembola on the green roof and emphasises the need to improve diversity. However, with most species from plugs dying out post-planting and few species colonising later on, many improvements to the green roof construction process would be necessary to achieve this.

To finalise this section, I conclude that the species composition present in the initial plugs planted into green roofs could influence the community much later on. However, colonisation post planting is also important, particularly in the first few months of a roofs life. Beyond this, colonisation is slow and a lack of species persistence means that the community structure varies over time, though the total number of species present on green roofs varies little. The abundance of most groups increases over time, but collembola, a key driver of soil nutrient cycles, are particularly badly affected by abiotic conditions. Overall, the community is comprised of a mixture of survivors from the source population, specialist colonisers capable of withstanding the conditions and generalist colonisers incapable of withstanding the conditions. For these latter species, green roofs act as a sink habitat, incapable of supporting sustainable populations.

7.2 Vegetation and mycorrhiza

Little development of the mature roof vegetation occurred over the two years, with a mix of moss and *Sedum spp.* dominating throughout. No perennials persisted and those annuals that did well in spring and autumn mainly consisted of leguminous plants. A prevalence of legumes could suggest the roof is nitrogen (N) limited (Ritchie and Tilman, 1995); further supporting studies on green roofs have shown crushed roof tile substrates to be low in N (Emilsson *et al.*, 2007; Emilsson, 2008). The lack of persistence of self-colonising species is no doubt a result of drought in the summer and cold in the winter. However despite this lack of persistence, I hypothesise that the ruderal plants benefit the *Sedum* by supplying N, otherwise lacking, to the soil. Legumes have been shown to increase N pools in soils, although this is site and species specific and is also affected by soil P (Spehn *et al.*, 2002). Anecdotally, when *M. officinalis* died in the summer on the green roofs studied, those *Sedum* plants surrounding it seemed to grow larger and have a deeper colour than those not in proximity of *M. officinalis*. Aside from this, any ruderal species dying off in summer and winter, not just legumes, provide an input of C to

the soil that can also be utilised by *Sedum*. Incorporating nutrient analyses into future studies could help draw a clearer picture of the vegetative dynamics of green roofs.

Sedum has often been criticised in the green roof industry for producing 'monocultures'[1] on green roofs (Bousselot *et al.*, 2011) but qualitative evidence from our green roofs suggest that in such a harsh environment, these plants can shelter seedlings and fungi. Mushrooms of *Melanoleuca polioleuca* and *Omphalina pyxidata* were often seen growing from the centre of *Sedum* individuals, but not in bare areas, and *T. arvense* also appeared denser around the *Sedum spp.* in its initial stages of growth. Therefore, unless the conditions on a roof are improved for plant life, *Sedum* may be necessary for the establishment of other plants and fungi. However, in Chapter 5 it was also determined that *Sedum* has the potential to competitively exclude ruderal species for space. Experimenting with the spacing of plugs could help determine the right balance in this scenario to have maximum effect on plant growth, determining how to best shelter ruderal species whilst providing them with the space to develop. *Sedum* mats, which provide no space to colonise at all could potentially prevent ruderal species from germinating altogether as found by Emilsson (2008).

There was a high proportion of moss on the mature roof, but none on the new roof, suggesting that this is a later development of a green roof. The moss layer supported more specialist species (*Acalypta sp.,* Scutoverticidae) than the *Sedum,* being a more common host species in the UK, so is a valuable contributor to supporting green roof biodiversity. It was also found that even those species that do not interact with the moss layer directly, could benefit from secondary properties of the moss layer, such as the water retention qualities associated with the crust (Chamizo *et al.*, 2012). This applied to *S. aureus,* so without the moss layer it is conceivable that our two most abundant organisms (*S. aureus* and Scutoverticidae) would not be supported. Aside from direct benefits to microarthropods provided by the moss, magpies (*Pica pica*) and crows (*Corvus corone corone*) were observed turning moss over and feeding on the associated microarthropods. This attractant property of the moss for bird species may have a profound impact on the rest of the plant community as it is likely that these birds are a source of the legumes prevalent on the roof. Some legume seeds, such as *M. officinalis*, are particularly attractive to birds and small mammals (Ogle *et al.*, 2008) and are also large, making wind dispersal to such heights unlikely (Turkington *et al.*, 1978).

The value of biodiverse roofs left to self-seed can also be assessed with our findings. On the new roof, forbs and grasses achieved higher levels of cover in unplanted plots, but plant

[1] As most green roofs are multi-species assemblages of *Sedum,* this term is inaccurate (Snodgrass and McIntyre, 2010)

diversity overall was no higher in these plots. There was also no evidence of an association between plant diversity and microarthropod diversity. These species also died off during the summer and winter, which though no doubt provided nutrients to the soil, also left these plots exposed in the harshest months. Therefore, although biodiverse roofs may be good for ruderal plants, species relying on these that need all year cover will likely suffer. Collembola in particular were found in lower abundances in unplanted plots, and as these are a key organism in nutrient cycling (Bardgett and Chan, 1999), this could have a detrimental effect on the future development of the roof. However, some organisms, such as the chironomid larva, benefitted from the unplanted plots. Therefore, incorporating both self-seeding biodiverse strategies and *Sedum* plug planted strategies into the design of one roof could provide bare areas for ruderal species and specialists of open environments, whilst maintaining all year *Sedum* cover for those species more vulnerable to harsh abiotic conditions.

This is the first study to the author's knowledge to state such high levels of mycorrhizal colonisation in *Sedum* and the first to note that *S. spurium, S. reflexum* and *S. album* form mycorrhizal associations at all. Levels were extremely high in not only the mature roof, but also the new roof, suggesting that *Sedum* grown for the industry must have a source of inocula. Finding out what these species are would be valuable as it is unknown if this species is common or rare, generalist or specialist. Initial tests (unpublished data) suggest that the mycorrhiza present in the *Sedum* improves the growth of ruderal species colonising the roof. This emphasises that *Sedum* species are a valuable addition to the green roof planting regime. As discussed later in this chapter, identifying these mycorrhizal species could allow tailored green roof inoculants to be manufactured, to the benefit of both *Sedum* and self-colonising ruderal species.

7.3 Soil Microbes

The microbial community in all three roofs was dominated by bacteria, but on the mature roof during the second year of sampling, mass of fungi was comparable to mass of bacteria, which had changed little over the sample period. This suggests a shift towards a fungal dominated community and confirms that green roofs up to this point in their development follow normal microbial succession (i.e. moving from a bacteria dominated to a fungal dominated community (Bardgett *et al.*, 2002; Ohtonen *et al.,*1999)). Masses of microbial PLFAs on the new roof were surprisingly high compared to the mature roof, with total microbial mass ranging from 1-6μg^{-1} in the first year of sampling the mature roof, whilst the new roof ranged between 1-3μg^{-1}. As with the microarthropods, this suggests that, at least as far as abundance is concerned, a vast majority of the soil microbial mass is obtained in the first year of construction. This indicates

that if amendments are to be applied to green roofs, the first year of construction is a key time to do this. This could be particularly important for fungi as the maximum abundance obtained in the first year of sampling on the mature roof was 2μg^{-1}, only 0.5μg^{-1} higher than that obtained on the new roof.

All microbial communities on the roof showed two clear separations in community structure, with bacterial fatty acids grouped separately to saprophytic fungi. The latter explained a large amount of the variance within the community. This emphasises the importance of fungi in this community, even at the early stages of the development of a green roof. However, AM fungi, denoted by the presence of the fatty acid 20:1ω9, was always the lowest parameter in abundance. This PLFA is very specific, having been shown *in vitro* to correspond with the presence of the species *Gigaspora rosea* only (Sakamoto *et al.*, 2004), and may signal low abundances of this particular fungi. In addition, roots were removed from the soil samples taken, meaning the presence of *G. rosea* would be via extraradical hyphae only. This would also reduce the chances of it being present in samples. Other ground level soils have also been reported as having low amounts of the signature fatty acid 20:1ω9 (Fitzsimons and Miller, 2010; Schnecker *et al.*, 2012). However, there were some changes in abundance of the marker 20:1ω9, despite it generally being present in low abundance. There was an increase in 20:1ω9 between the first and second year of sampling on the mature roof and its presence on the new roof was lower than at any time on the mature roof. The former suggests an increase of extraradical hyphae occurs over time. Generally, the practicality of this PLFA as a marker for AM fungi is questionable due to its specificity to detect the species *Gigaspora rosea*.

The two fungal parameters measured did not differ in their community composition throughout the sample period, with AM fungi always rare and saprophytic markers always common and the two correlated with one another. However, the bacterial community showed distinct shifts. On the new roof, gram positive, gram negative and sulphate-reducing bacteria all showed different seasonal patterns. The latter two parameters were at the same abundance as one another (gram positive bacteria were highest in abundance on all roofs). On the mature roof, bacteria showed broadly the same seasonal patterns but the community shifted; gram negative bacteria declined over time, whilst saprotrophic fungi and sulphate-reducing bacteria increased. This suggests that the community assemblage of bacteria are all similarly adapted to abiotic conditions, but that their abundance may be affected by another factor, such as organic carbon as discussed in section 6.4.1.

Fungi were largely independent of abiotic factors, but bacteria were not. Although those factors deemed significant by regression analysis varied, broadly bacteria were always

negatively affected by increased moisture factors, such as humidity and substrate water content, but positively affected by either rising temperature or rising wind speed. This suggests that the bacterial community on green roofs is adapted to the harsh conditions present right from the construction of the roof. These correlations were stronger in the mature roof community than in the new roof community, suggesting that this process develops over time. This may also explain shifts in seasonal patterns on the young roof compared to the old roof, as the community has not yet reached a sustainable climax level of adapted species. It seems overall that the microbial community assembled on the roof is far more robust and species may be better adapted for green roof life than the microarthropod species present.

7.4 The effects of microbial inoculants

In vitro the addition of microbial inoculants had no effect on plant growth, though the addition of mycorrhizas did translate into increased soil mycorrhiza. This may have been due to *Sedum's* slow growing nature, with growth differences not translating into plant mass at this early stage. However, there were differences in plant growth between sterile and non-sterile soils, suggesting that the soil community does affect *Sedum* growth to some extent.

This leads onto the hypothesis that microbial inoculants would behave differently if added at different stages of the roofs development, as the soil community changes. This hypothesis was correct, though results were mixed regarding the suitability of these particular inoculants on either a new or mature roof. With regards to the microbial community, the addition of mycorrhizal fungi had the biggest effect on the young roof, whilst bacteria affected the mature roof most. Exploring the details of these interactions clearly shows that this is driven by the difference in dominance of either bacteria or fungi on each roof.

On the young roof, we see that the addition of mycorrhizal inoculants had a positive effect on fungi in the soil, whereas no effect was seen on the mature roof. Much of the evidence in Chapter 5, on the new roof, points to intense competition occurring between resident and added fungal species, suggesting that the mature roof had time to develop a more resilient resident fungal community to invading fungi. Vierheilig (2004) found that at a critical level of root colonisation, roots with resident fungi were less likely to be colonised by a new species. This is supported by the fact that *Sedum* on the mature roof displayed a higher level of root length colonised than those roots in the new roof.

Helper bacteria (of mycorrhizas) were also more effective in the young roof. This highlights the fact that timing is important for the addition of inoculants to be successful. The level of root

colonisation in the mature roof may be sufficient to prevent colonisation by added fungi, even when helper bacteria are added. Additionally, competitive interactions may also occur between resident bacteria and added bacteria, again reducing the likelihood of successful inoculation. To date the topic of whether competitive interactions between helper bacteria exist has been largely neglected due to the difficulties of monitoring bacterial species assemblages (Young and Crawford, 2004).

Though it is clear that there is an optimum condition of a soil community in which to add inoculants, in Chapter 5 there was a decrease in *Sedum* cover with the addition of both mycorrhiza and bacteria, suggesting that the more successful establishment of microbial inoculants on new roofs may not be beneficial overall. Additionally, in initial pot experiments it was found that even though the mycorrhizal inoculant increased mycorrhizal colonisation, there was no effect on plant growth. This suggests that even in cases where the added mycorrhiza can establish in *Sedum,* it is of no benefit to the plant. As discussed in Chapter 5, this is likely due to a need for more species specific assemblages in this environment.

In the mature roof there were no negative effects on plant growth, but the bacterial inoculant negatively affected the resident fungal population. Perhaps, with larger applications, the same negative effect on plant growth on the mature roof as was observed on the young roof would be seen, but at current doses the established community was harder to perturb than on the new roof. Both fungal inoculants seemed to have a slight positive effect on the microbial community, suggesting that these inoculants may have greater potential on established green roofs than bacterial inoculants though, again, more research into doses would be needed to determine if this would be significantly beneficial. In the case of all the microbial inoculants tested, it would seem that in their current form they are not suitable for application to new green roofs to enhance plant growth, but with further research may benefit established roofs. Mycorrhizal inoculants on new roofs in particular could even limit plant cover. Although mixes of species in commercial inoculants are designed so that at least one species combination has positive effects (Koomen, 1987), it is reasonable to assume that at least one combination may produce a negative effect. It seems that this is the most likely explanation for the microbial interactions observed on the new roof. Future studies aimed at inoculating new roofs should focus on amending the soil with single species inoculants to determine which ones have positive and negative effects. Generalist species are unlikely to produce positive effects due to the harsh nature of the roof, so amplifying what is already present in the green roof soil, or producing inoculants suited to the same sort of conditions may be a more successful strategy. On mature green roofs it seems that bacterial inoculants are detrimental to subterranean communities but that fungal based inoculants have the potential to boost microbial

populations. The observed effects at their current application rate, however, are too small to determine if this would have knock-on effects for other organisms.

Though these inoculants may be harmful to the plant and microbial community on green roofs, the case for microarthropods seems quite different. This is the first study to the authors' knowledge where primary consumers i.e. bacteria and fungi, have been added to a soil to determine their effects on the entire microarthropod community, although other papers have experimented with adding additional nutrients (Chen and Wise, 1999; Maraun *et al.*, 2001; Scheu and Schaeffer, 1998) and looked at certain organisms within a community (Muturi *et al.*, 2011). Sibi *et al.*., (2008) indirectly increased *T. harzianum* populations in soil by introducing additional organic material to soil and saw corresponding increases in mites and nematodes. On the new roof, collembola benefitted significantly from the addition of bacteria, whilst the addition of both mycorrhiza and bacteria negatively affected the mite population. On the mature roof, the addition of *Trichoderma* increased total microarthropod abundance, particularly the group that comprised mainly of insect larvae. *Trichoderma* has been shown to increase collembola abundance in Kenyan farms (Muturi *et al.*, 2011) and this was determined to be due to an increase in available food. Chen and Wise (1997) found that enhancing the detritus in litter layers also saw subsequent increases in collembola, fungus gnats and oribatid mites, all species that feed on detritus and/or fungi, supporting the theory that the addition of microbes to the green roof substrate subsequently enhanced the detritus available to microarthropods. Along with the prevalence of detrital feeding species on the roof and the lack of predators, this suggests that population dynamics on these roofs are primarily controlled by resources from the bottom-up, rather than top-down by predators (Chen and Wise, 1999).

The reason for the negative effect of some combinations of inoculants on mite populations on the new roof is unclear. Oribatid mites have been shown to decrease when nutrient amendments were added to forest soils, and this has been attributed to higher earthworm activity under these treatments (Maraun *et al.*, 2001; Salamon *et al.*, 2006). Earthworms are absent from the current system, but the mechanisms by which Maraun *et al.,*(2001) suppose they disturb mites could conceivably also apply to collembola, namely that available space and food is reduced by the other species. This could be a direct effect of the presence of *P. notabilis* specifically as this would explain why this negative effect was not seen on the mature green roof. This is an avenue that needs to be further explored. Oribatid mites feeding on fungi are also known to have close mutualisms with extra-intestinal bacteria, assisting in chitin breakdown (Smrž and Čatská, 2010), and these assemblages may change with differing habitats (Wolf and Rockett, 1984). Though the diet of the oribatid mites in this study is

unknown, it could be that manipulation of the microbial community impacted this mutualistic relationship to the detriment of the mite population. Oribatid mites are also a diverse group where diet specialisms are prevalent (Ponge, 1991) and are, therefore, likely to be perturbed by changes to the diet available to them. Collembola are much more capable of diet shifting (*S. aureus*, for example) (Ponge, 1991) and so can take advantage of abundant food sources, including those supplied by inoculants. Yang (2006) hypothesises that in ecosystems where pulses of nutrients are input into the soil generalists are likely to be successful due to their opportunistic nature. Nutrient pulses have been manufactured in the current study by adding inoculants, in addition to the likely pulses of nutrient inputs created by the biannual dying off of ruderal plants. This could explain the success of collembola in this scenario and their subsequent negative effect on the more specialist oribatids.

Inoculants that stimulate proliferation of microbial mass are likely to benefit generalist organisms, without the need for the specific species interactions we see with microbial communities and plants. On the bacterially dominated new roof, these positive effects were seen with the addition of bacteria, whilst on the mature roof, which was more fungal, the addition of fungi produced this positive effect. Amplifying the abundant food sources on the roof, therefore, seems to have a beneficial effect on the resident microarthropods. The hypothesis that microarthropods were likely limited by food source as well as water is therefore true for the majority of species, and the use of inoculants to improve the microarthropod community is conceivable. However, the negative effect seen on more specialist organisms on the new roof, such as the mites, highlights that care must be taken and that more specific inoculants, as mentioned in the preceding discussion, would likely be a better option as they would benefit both generalist and specialist species. If the resource base on green roofs could be sufficiently improved, increased abundances of not only soil dwelling microarthropods would be seen, but also their above ground predators, such as spiders and wasps (Chen and Wise, 1999), contributing to a more diverse ecosystem overall.

7.5 Wider implications

Chapter 3 concluded that the similarity between the two roofs studied suggest that it is possible that urban monocultures of soil communities could be created if roofs of the same construction type are built within short distances of one another, within a similar climate. Chapter 5 supports this hypothesis, with the new roof sharing many common organisms with the mature roofs in Chapter 3, as well as a similarly impoverished soil community. Although it is not known that the roofs in the current study are directly translatable to other crushed brick roofs, it can be inferred by other evidence: The plant community present, for example, was

extremely similar to that cited in the literature in Sweden (Emilsson *et al.,* 2007; Emilsson, 2008). These too seem to be drought driven, with a high proportion of drought tolerant moss and few other wild colonisers. In addition, species that were encountered as a "by catch" in the current study have also been found in studies using pitfall traps and, again, many xerophilic species were found (Kadas, 2006; Jones, 2002). Qualitatively, the dry nature of the roofs in the current study have also been observed by the author on roofs built globally, such as those in Chicago, throughout London and in Denmark (Plate 7.1).

Plate 7.1 A green roof in Copenhagen, Denmark (Rumble, 2012). *Sedum spp.* in the foreground are coloured red, indicative of drought stress (Teeri *et al.,* 1986). The difference in plant morphology seen in the mid-ground corresponds with a change in substrate depth, with the section furthest away shallower in depth.

In the wider ecological landscape, it has been observed that the green roof community is not dissimilar to that of a glacial foreland (Kaufmann *et al.,* 2002) or Antarctic soil (Convey and Smith, 1997; Caruso and Bargagli, 2007). These communities have well documented successional patterns that may differ in species composition, but provided the abiotic conditions are the same tend to be predictable in their community structure. Therefore, if green roofs are built to the same formula worldwide, it is plausible that community development will be similar between roofs (in the same climatic zones) and thus will share the impoverished communities seen in the current study. This emphasises the importance of continual experimentation with substrates and the structure of green roofs. Schrader and

Böning (2006) for example studied roofs with a different substrate to crushed brick and found a far less impoverished community than the current study.

Species composition, however, is likely to differ between locations. Differences in species composition between the new roof and the mature roof were present in the current study; therefore it is conceivable that although community development is similar between roofs, species composition is determined by plant source as well as local sources of mobile populations. This is an important factor to consider as if, for example, source populations local to green roofs have a higher proportion of drought tolerant, mobile species, the community composition present may be considerably improved. Though it is clearly important to research the soil communities on green roofs across the globe based on this research, it may be prudent to focus on arid regions foremost, to determine if this hypothesis is true.

In ground-level habitats, broadly speaking, heterogeneity of habitat concurrently produces heterogeneous wildlife communities due to the larger numbers of niches to fill (until a habitat is saturated, Kadmon and Allouche (2007)). Thus, increasing heterogeneity at a local and landscape level is extremely valuable for urban wildlife. In the case of the current study, the extensive roofs built are not achieving their full potential with regards to providing habitat. Although ruderal communities are valuable as a habitat in themselves, harbouring rare species, the large area proposed to be taken up by extensive green roofs to remediate habitat loss far outweigh the natural levels of these kind of habitats, particularly as these roofs tend to be built to the same specification all over the UK. Taking into account the fact that the community presented in the current study is comparable to a desert environment, using green roofs as a habitat remediation tool for habitat lost in construction is unconvincing. Thus, it is important for researchers and industry to work together to improve the delivery of living roofs. Urban habitats are often, by nature, drier and hotter (Santamouris and Georgakis, 2003) and so special consideration needs to be given to ameliorating these effects. This needs to be done at a local level to provide refugia for less mobile species on each roof. The economics of implementing these changes needs to be considered, as currently price is a common barrier to producing more effective green roofs (WGRC 2012 discussion panel, pers. comm.). The current study suggests that microhabitats, such as logs, stones and deeper areas of substrate, could be a starting point, in addition to ensuring drought resistant species are present in *Sedum* plugs.

Green roof heterogeneity also needs to be thought of at a landscape level. Colonisation rates to green roofs are extremely slow, suggesting that higher connectivity with ground level habitats or intensive green roofs would improve soil communities by providing source populations. In addition, for more mobile species such as bees and bats, the surrounding

habitat seems to be an important factor in deciding whether green roofs are an acceptable habitat or not. High quality habitat surrounding green roofs may make them more acceptable as a habitat for mobile species, as converse to popular belief green roofs are less like oases and more like the surrounding desert.

Intensive green roofs have been built to mimic ground-level habitats such as calcareous grasslands and American prairies (section 1.1). The same level of imagination could be applied to extensive green roofs, viewing the economic limitations as a challenge rather than a barrier. The industry needs to be more adventurous to allow for greater experimentation in green roof design, which coupled with careful monitoring to determine the effect of these differences, will make green roofs a true habitat remediation tool, rather than the greenwash they are in danger of becoming.

7.6 Conclusions

- At the microbial level, extensive green roofs support a community adapted to the harsh abiotic conditions, though in low abundance. At the microarthropod level, the community is impoverished, dominated by xerophiles and unsustainable collembola populations. This may effect above-ground organisms by limiting nutrient cycling.
- Extensive green roofs support some unusual and less common microarthropods, such as the collembola *S. trinotatus*.
- Green roofs are likely to be dominated by bottom-up processes, as demonstrated by a lack of predators.
- Microbial inoculants have differing effects depending on the life stage of the green roof they are added to. The effects of these on new roofs are negative for microbes and specialist organisms such as mites, but can benefit generalist feeders such as collembola. On mature roofs, fungal additives may benefit both microbial and microarthropod communities. Plants were seen to be negatively affected or not affected at all by microbial inoculants.
- Competitive interactions between microbes on addition of some commercial inoculants at the early stages of a roofs life can prevent them from having the intended positive effect. At later stages the resident community is harder to perturb. Amplifying the specialist community already present in the green roof soil may be the most successful inoculation strategy.
- In order for green roofs to become effective habitat remediation tools, refugia for soil organisms in times of drought need to be provided as do mechanisms for drought amelioration. This should be applied at both local and landscape level.

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Appendix I – List of plant species found to self-colonise on green roofs

Species	Authority	Country	Species	Authority	Country
Acer campestre	K; E	G; S	*Malva sylvestris*	D	E
Acer negundo	K	G	*Medicago lupulina*	K; D	G; E
Acer pseudoplatanus	D	E	*Medicago sativa*	K	G
Acerlat	K	G	*Melilotus alba*	K	G
Agropyron repens	K	G	*Melilotus officinalis*	K	G
Agrostis stolonifera	D	E	*Myositis arvensis*	K; D	G; E
Agrostis vinealis	E	S	*Oenothera biennis*	K	G
Amaranthus retrofle us	K	G	*Onobrychis montana*	K	G
Ambrosia artemisifolia	K	G	*Papavar rhoeas*	D	E
Antennaria dioica	E	S	*Papaver agremone*	K	G
Anthemis tinctoria	K	G	*Plantago media*	D	E
Anthyllis vulneraria	K	G	*Poa alpine*	E	S
Apera spica-venti	K	G	*Poa annua*	K; E	G; S
Arabidopsis thaliana	E	S	*Poa compressa*	K	G
Arenaria serpyllifolia	E	S	*Poa pratensis*	E	S
Arenaria serpyllifolia	K; E	G; S	*Poa sp.*	E	S
Artemisia vulgaris	K	G	*Poa trivialis*	K; D	G; E
Berteroa incana	K	G	*Poa palustris*	K	G
Betula sp.	E	S	*Polygonum aviculare*	K	G
Bromus hordeaceus	K	G	*Prunus padus*	K	G
Bromus sterilis	K	G	*Quercus robur*	K	G
Bromus tectorum	K	G	*Ranunculus repens*	D	E
Bromus thominii	K	G	*Robinia pseudacacia*	K	G
Buddleia davidii	D	E	*Rumex crispus*	D	E
Capsella bursa-pastoris	K	G	*Rumex obtusifolius*	D	E
Cerastium fontanum	E	S	*Sagina procumbens*	D	E
Cerastium glomeratum	E	S	*Salix caprea*	D	E
Cerastium pumilum	E	S	*Sambucus nigra*	D	E
Cerastium semidecandrum	K; E	G; S	*Saxifraga granulate*	E	S
Chenopodium album	K	G	*Saxifraga tridactylites*	E	S
Chrysanthemum segetum	K	G	*Scabiosa atropururea*	K	G
Cirsium arvense	D	E	*Senecio jacobea*	D	E
Conyza canadensis	K	G	*Senecio vernalis*	E	S
Coronilla varia	K	G	*Senecio viscosus*	K	G
Cotoneaster spp	D	E	*Senecio vulgaris*	K; D; E	G; E; S
Crataegus monogyna	K; D	G; E	*Setaria viridis*	K	G
Crepis tectorum	K; E	G; S	*Silene alba*	K	G
Deschampsia cespitosa	D	E	*Sisymbrium loeselii*	K	G
Diplota is tenuifolia	K	G	*Solanum nigrum*	K	G
Echinochola crus-galli	K	G	*Sonchus oleraceus*	D	E
Epilibium montanum	D	E	*Sorbus x intermedia*	D	E
Epilobium sp.	E	S	*Stellaria media*	E	S
Erigeron annuus	K	G	*Taraxacum*	E	S

Species	Authority	Country	Species	Authority	Country
Erodium cicutarium	K	G	*Taraxacum officinale*	K; D	G; E
Erophila verna	E	S	*Tricitum aestivale*	K	G
Erysimum cheiranthoides	K	G	*Trifolium arvense*	K	G
Euonymus europaeus	K	G	*Trifolium aureum*	K	G
Euphorbiaeplus	K	G	*Trifolium repens*	K; D	G; E
Festuca glauca	K	G	*Trifolium pratense*	K	G
Festuca ovina	K	G	*Tripleurospermum inodorum*	K	G
Festuca rubra	K	G	*Urtica dioica*	D	E
Frageria x ananassa	D	E	*Veronica arvensis*	E	S
Fraxinus excelsior	D	E	*Veronica chamaedrys*	D	E
Galinsoga ciliata	K	G	*Veronica persica*	D	E
Galinsoga arviflora	K	G	*Vicia angustifolia*	K	G
Galium aparine	D	E	*Vicia hirsuta*	D	E
Geranium molle	K	G	*Vicia sepium*	K	G
Hedera helix	D	E	*Viola arvensis*	K	G
Helianthus annuus	K	G	*Viola tricolor arvensis*	K	G
Hieracium pilosella	K; E	G; S	*Zea mays*	K	G
Holucs latanus	D	E			
Hypericum perforatum	K	G	Bryophytes		
Lactuca serriola	K	G	*Ceratodon purpureus*	K; E	G; S
Lamium ample icaule	K	G	*Mnium stellare*	K	G
Lamium pupureum	K	G	*Scleropodiumurum*	K	G
Lapsana communis	K	G	*Amblystegium juratzkanum*	K	G
Leucanthemum vulgare	K	G	*Polytrichum formosum*	K	G
Linaria vulgaris	K	G	*Cladonia coniocrea*	K	G
Lolium perenne	K	G	*Marchantia spec*	K	G

Table A1. Species list of plants and bryophytes found self-colonising on extensive green roofs in Europe. Species found by Köhler (2006) and Emilsson (2008) were found on roofs planted with *Sedum* only, species found by Dunnett *et al.,* (2008) were found on roofs where *S. acre* was a component with other drought tolerant plants. Bryophytes were not recorded by Dunnett *et al.,* (2008).
K= Köhler (2006), D = Dunnett *et al.,* (2008), E = Emilsson (2008)
G = Germany, E = England, S = Sweden

Appendix II – Review of inoculant efficacy presented in the literature

MYCORRHIZAE Authority	Type of application	Species added	Manufacturers guide/amount applied	Propagules p/gram	Applied to	Positive effects	Negative effects
Ganry *et al.,* (1982)	Root fragments	*G. mosseae*	12 mg root, spore, hyphae per bead	?	15 beads p/plant	Increased N fixation	Did not increase infectivity
Corkidi *et al.,* (2004)	Granular	AM fungi	1 tsp	?	656ml pot	Quickly colonised (4 weeks), 20-50% col. In smaller pots (160ml)	
	Granular	*G. intraradices*	10g	?		Quickly colonised (4 weeks), 20-50% col. In smaller pots (160ml)	
	Granular	*G. intraradices*	1 tsp	?		Colonised (six weeks), 20-50% col. In smaller pots (160ml)	
	Granular	*G. intraradices*	1 g	?		Colonised (six weeks), 20-50% col. In smaller pots (160ml)	
	Granular	*G. intraradices*	2 tbsp	?		Colonised (six weeks)	Less than 5% col. In smaller pots (160ml)
	Liquid	*G. intraradices*	30.5ml	?			No colonisation in large pot. Less than 5% col. In smaller pots (160ml)

MYCORRHIZAE Authority	Type of application	Species added	Manufacturers guide/amount applied	Propagules p/gram	Applied to	Positive effects	Negative effects
Corkidi *et al.,* (2004)	Granular	*Glomus* and *Gigaspora* s	¼ tsp	?		Colonised (six weeks)	Less than 1% col. In smaller pots (160ml)
	Granular	One or more AM fungal sp.	2.9g	?			No colonisation
	Granular	Endo/ecto myccorrhiza	1 tsp	?			No colonisation
	Granular	*G. intraradices*	1 tsp	?			No colonisation
Wiseman *et al.*, (2009)	Granular	Organic potting mix with 6 x *Glomus s* , 1 x *Gigaspora sp.*	3g/L	131	164ml	Shoot dry weight increased in maize at eight weeks. Increased dose dependently.	5.5% colonisation in maize after four weeks. Increased conc. had no effect. Improved soil fertility. Maple smaller than controls (already colonised). No effect on magnolia.
	Granular	3 x *Glomus s*	3g/L	152		Shoot dry weight increased with higher applications. Improved soil fertility.	3% colonisation in maize after two weeks. Increased concentration had no effect. Maple smaller than controls (already colonised). No effect on magnolia

MYCORRHIZAE Authority	Type of application	Species added	Manufacturers guide/amount applied	Propagules p/gram	Applied to	Positive effects	Negative effects
Wiseman *et al.*, (2009)	Granular	6 x *Glomus s* , 1 x *Gigaspora sp.*, 1 x *Paraglomus sp.*	1.8g/L	76 spores/g		Shoot dry weight increased in maize at two weeks. Increase dose dependent. Improved soil fertility.	Increased concentration had no effect. Maple smaller than controls (already AMF colonised). No effect on magnolia
	Granular	Compost, 3 x *Glomus s* , *Trichoderma s*	3g/L	44		Shoot dry weight increased in maize at four weeks. Improved soil fertility.	Maple smaller than controls (already colonised). No effect on magnolia. Increased concentration was toxic.
	Granular	6 x *Glomus s* , 1 x *Gigaspora sp.*, 9 x Ectomycorrhizas, *Trichoderma s ,* biostimulants, plant growth enhancers, bacteria, micronutrients, hydrogel	1.2g/L	?		Shoot dry weight increased in maize at eight weeks. Dose dependent. Improved soil fertility.	Increased concentration had no effect. Maple smaller than controls (already colonised). No effect on magnolia

MYCORRHIZAE Authority	Type of application	Species added	Manufacturers guide/amount applied	Propagules p/gram	Applied to	Positive effects	Negative effects
Wiseman *et al.*, (2009)	Granular	2 x *Glomus s* , 5 x ectomycorrhizas	1.2g/L	22 endo, 52 ecto		Shoot dry weight increased in maize at two weeks. Increased dose dependently. Improved soil fertility.	Increased concentration had no effect. Maple smaller than controls (already colonised). No effect on magnolia
	Granular	Biostimulants, soil amendments, bacteria, AM fungal spores	3g/L	88 spores/g		Improved soil fertility.	Increased concentration had no effect. Maple smaller than controls (already AMF colonised). No effect on magnolia
	Liquid	5 x ecto/endo mycorrhizas, biocatalysts, hydrogel	Mix with water	?		Improved soil fertility.	Increased concentration had no effect. Maple smaller than controls (already AMF colonised). No effect on magnolia
	Liquid	AM fungal spores with humic acid, biostimulants, bacteria, kelp, yucca and hydrogel	Mix with water	?		Improved soil fertility.	Increased concentration had no effect. Maple smaller than controls (already colonised). No effect on magnolia

BACTERIA Authority	Type of application	Species added	Manufacturers guide/amount applied	CFU p/ml	Applied to	Positive effects	Negative effects
Freitas *et al.,* (1996)	Seed application	*Bacillus brevis, B. megaterium, B. polymyxa, B. sphaericus, B. thuringiensis* and *Xanthomonas maltophilia*	N/A	10^5-10^6 p/seed	Canola seeds	*B. sphaericus, B. brevis* and *X. maltophilia* increased plant height. *B. megaterium, B sphaericus, B. polymyxa, B. brevis* and *B. thuringiensis* increased pod number, *B. thuringiensis* increased pod weight. *B. polymyxa* and *B. thuringiensis* increased seed production	P not solubilized by these bacteria in soil despite doing so *in vitro*. Suggests they produce metabolites using P, which help plant.
Kloepper *et al.,* (2004)	Media applied	*B. subtiliis* and *B. amyloliquefaciens*		Trial one: $4X10^{10}$ per/litre Trial two: 10^9p/litre at 1:40 bac:media	Vegetables – Tomato, bell pepper and cucumber & tobacco	Increased disease resistance in tomatoes. PGPR + chitosan increased growth parameters. May reduce transplant shock?	Didn't test PGPR alone in relation to growth
Adesemoye, *et al.,* (2009)	Seed application	*B. amyloliquefaciens* + *B. pumilis* and *Glomus intraradices*		10^5	Tomatoes	Plants with less fertiliser, PGPR's and AM fungi grew as well as in higher concentrations of fertiliser. Best result was PGPR + AM fungi +70% fertiliser	

BACTERIA Authority	Type of application	Species added	Manufacturers guide/amount applied	CFU p/ml	Applied to	Positive effects	Negative effects
Probanza *et al., (*2001)	Soil app, with AM fungi	*B. licheniformus + B. Pumilis* and *Pisolithus tinctorus*		10^2 p/gram	*Pinus pinea*	Mixture of AM fungi and bacteria increased plant growth.	

TRICHODERMA Authority	Type of application	Species added	Manufacturers guide/amount applied	Conc.	Applied to	Positive effects	Negative effects
Naseby *et al.*, (2000)		*Trichoderma harzanium*: TH1, T4, T12, N47 *T. pseudokoningii*: To10		10^6 spores ml^{-1}, then in 0.75 guar gum solution	Pea	All except To10 significantly suppressed *Pythium* damage (esp. T4 & N47). TH1, T4, N47 and T12 increased fresh shoot weights in both presence and absence of *Pythium*. TH1, T4, T12 increased dry weight. All but To10 increased root length with *Pythium*	
Harman *et al.*, (2004)	Dust	*T. harzianum* T22				Larger roots and shoots	
Meyer *et al.,* (2001)		*T. virens* G1-3		10^4 CFU	Bell peppers		Not a lot of differences

TRICHODERMA Authority	Type of application	Species added	Manufacturers guide/amount applied	Conc.	Applied to	Positive effects	Negative effects
Sivan *et al.,* (1984)		*T. harzanium, T. hamatum*		$5x10^{9}$ conidia p/ ml	Peas, cucumbers, tomatoes, peppers, gypsophila	Reduced *Pythium* success in cucumbers, peas and tomatoes. Broadcast worked better than seed coating.	
Yedidia *et al.,* (2001)		*T.harzianum* T203		10^{8} cfu g^{-1}		Emerged earlier. Increased shoot length, dry weight and leaf area. Mineral content higher.	
Ousley *et al.,*(1993)		*T.harzianum, T. viride*				Increased shoot weights and flower bud number	
Rabeendra *et al.*, (2006)		*T.longipile, T.tomentosum*		10^{7} conidia/ml	Cabbage, lettuce	Leaf area and shoot dry weight increased. No effect on shoot:root ratio in cabbage. In lettuce, harvestable heads were larger.	Inconsistent effects

Table A2. Table summarising previous findings relating to the efficacy of commercial inoculants.

Appendix III – Pilot: Microarthropod abundance in green roof materials and *Sedum* farms (Chapter 5)

AIII.I Introduction

Microarthropods are vital to soil functioning. Microarthropods are present within green roof soils, yet it is unknown how they arrive there. This pilot aims to determine if there is a source population of microarthropods present in *Sedum* plugs and mats before they are planted, substrate before a roof is constructed and in soils near *Sedum* growing areas.

AIII.II Method

Two *Sedum* farms were visited in 2010. The first, a farm growing *Sedum* for a large green roof manufacturer (SkyGarden, Cheltenham, Gloucestershire) was situated near Gloucester and stored *Sedum* mats and plugs outside, on top of agricultural soil. Four samples were taken from this site, two portions of *Sedum acre* mat, one plug of *S. acre* and one plug of *S. kamtschaticum.* In addition a small portion of agricultural soil beneath the *Sedum* mats was taken and a small portion of green roof substrate.

The second field site (Carp Farm) was a small *Sedum* farm in Wiltshire (Sedum Green Roof Ltd, East Knoyle, Wiltshire), the same farm that would later supply the plugs used in Chapter 5. Here *Sedum* mats were grown outside, on agricultural soil and *Sedum* plugs in an open ended greenhouse. Two *S. acre* plugs were taken from this site.

Plugs and soil samples were processed an analysed as described in Section 2.1.2.

AIII.III Results

A range of mites, collembola and other microarthropods were extracted from all samples, with the exception of the green roof substrate and the agricultural soil from SkyGarden (Table A3). These were not standardised and samples were of different sizes.

Species	Sample site	Sample type	No. individuals recorded
Mites			
Oribatid mite 1	SkyGarden	*S. acre* plug, *S. acre* mats, CF *S. acre* plugs	6, 34, 1
Oribatid mite 2	SkyGarden	*S. acre* plug, *S. acre* mats	9, 1
Mesostigmatid mite	SkyGarden, Carp Farm	*S. acre* plug, *S. kamtschaticum* plug *S. acre* mats, CF *S. acre* plugs	6, 4, 40, 3
Bdellidae sp.	SkyGarden	*S. acre* mats	3
Unknown mite 1	Carp Farm	CF *S. acre* plugs	3
Collembola			
Entomobrya multifasciata	SkyGarden	*S. acre* plug	45
Deuterosminthurus bicinctus	SkyGarden	*S. acre* plug	1
Parisotoma notabilis	SkyGarden	*S. acre* mats	8
Xenylla boerneri	Carp Farm	CF *S. acre* plugs	28
Folsomia candida	Carp Farm	CF *S. acre* plugs	29
Other species			
Chlamydatus evanescens	SkyGarden	*S. acre* plug, *S. kamtschaticum* plug, *S. acre* mats	46, 33, 44
Thysanoptera	SkyGarden	*S. acre* plug, *S. kamtschaticum* plug, *S. acre* mats	26, 97, 51
Diptera larvae	SkyGarden	*S. kamtschaticum* plug	1
Centipede	SkyGarden	*S. kamtschaticum* plug	2
Unknown species 1	SkyGarden	*S. acre* plug, *S. kamtschaticum* plug	11, 47
Unknown species 2	SkyGarden	*S. acre* plug, *S. acre* mats	1, 48
Unknown species 3	SkyGarden	*S. kamtschaticum* plug	3

Table A3. Species recorded from samples of *Sedum spp.* obtained from *Sedum* farms

Appendix IIII – Analysis of covariance (C_V) of microbial parameters over time when inoculants were added to a mature green roof (Chapter 6)

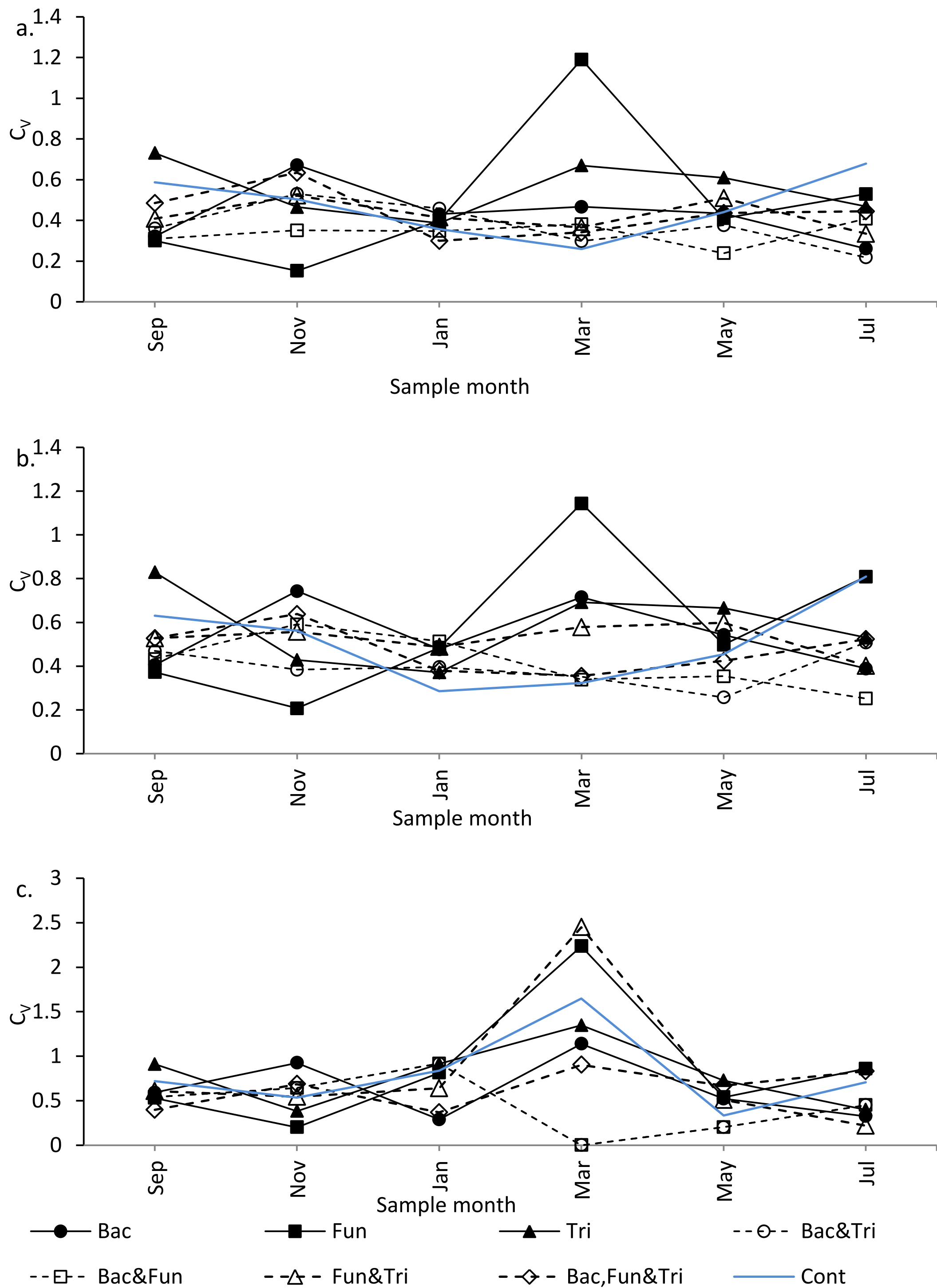

Fig. A1. C_V for a.) total microbial mass, b.) fungal mass and c.) AM fungal mass

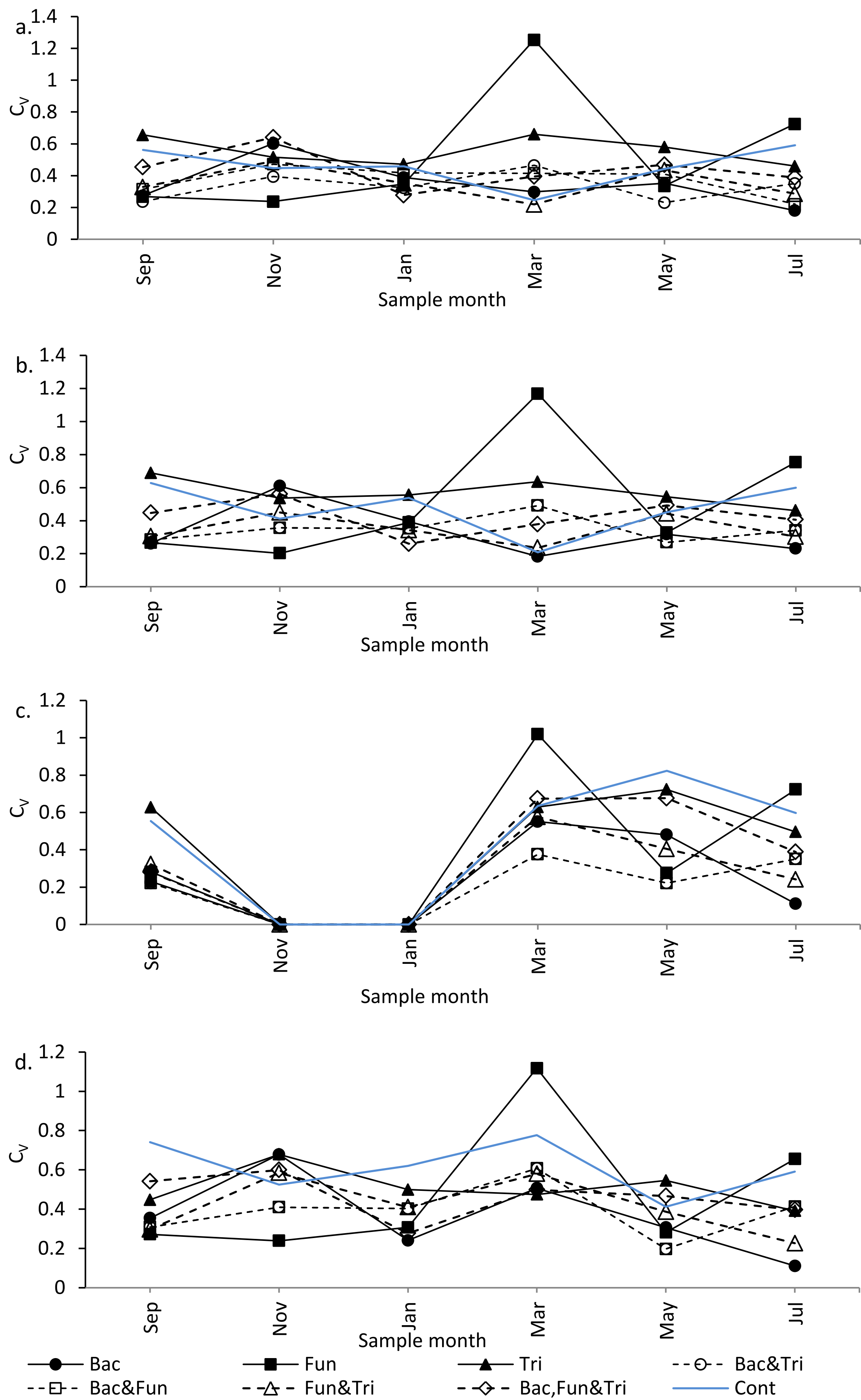

Fig A2. C_V for a.) bacterial mass, b.) Gram positive bacterial mass, c.) Gram negative bacterial mass and d.) Sulphate-reducing bacterial mass