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GREEN ROOF SOIL ORGANISMS: ANTHROPOGENIC ASSEMBLAGES OR NATURAL COMMUNITIES?

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ABSTRACT

Green roofs provide a range of ecosystem services, from stormwater retention to thermal insulation. They can also provide habitat for biodiversity, remediating land lost in development. However, few extensive green roofs are designed with this benefit in mind and, as such, biodiversity often does not reach its full potential. In particular, the soil ecology of green roofs is poorly understood, despite soil microorganisms having a large impact on nutrient cycling and thus plant diversity. In particular, whilst there are studies describing the soil microarthropods and microbial communities present on green roofs, little is known about how these species arrive there. This paper aims to determine how soil microarthropods and microbes colonise green roofs and which species survive post-construction, to inform green roof technosol design and to understand if remediation of impoverished green roof soils is possible. To do this, we conducted a preliminary study by analysing green roof construction materials (substrates and *Sedum* plugs) for microarthropods, bacteria and fungi before constructing a new green roof. We then monitored survival and independent colonisation over eleven months.

Whilst green roof substrates were a poor source of colonisation, *Sedum* plugs showed potential as a vehicle for colonisation by microbes and, especially, by soil microarthropods. However, the majority of the species present within *Sedum* plugs were not adapted to the harsh conditions of the green roof, resulting in high mortality. Two ubiquitist species, the Collembola species complex *Parisotoma notabilis* and a mite of the family Scutoverticidae survived in high abundance after the eleven month sample period, and the functional role of these species on a green roof should be investigated. Some species colonised independently during the study, highlighting that microarthropods and microbes in green roofs consist of a mix of anthropogenic assemblages and natural communities. Mycorrhizal fungi were extremely successful, independently colonising almost all *Sedum* plants by the end of the study. However, the absence of arbuscules suggests that this colonisation may not have a benefit to plant growth in this instance.

Demonstrating that the succession of soil organisms is influenced by the communities present in construction materials has implications for substrate design, demonstrating that soil organisms may be

inoculated onto green roofs to provide functioning technosols. In addition, the independent colonisation of mycorrhiza in this study stimulates discussion about the role of commercially applied mycorrhizal fungi in green roof construction.

KEY WORDS

Technosol; Green Roof; Microarthropod; Microbe; Mycorrhiza; Colonisation

1. INTRODUCTION

Green roofs are one of many anthropogenic habitats that could contribute to urban biodiversity by supporting local fauna and flora. Many green roofs are built following an 'extensive' design of shallow substrates planted with succulents such as *Sedum spp.* These are designed to benefit buildings from an engineering perspective by, for example, reducing stormwater runoff (VanWoert et al., 2005) or insulating buildings from seasonal temperature fluctuations (Jaffal et al., 2012) as well as improving the aesthetics of the roof. These extensive green roofs do support floral and faunal diversity, but often few features are designed specifically for this objective. As a result, biodiversity is often limited (Williams et al., 2014), overlooking the fact that many organisms directly influence, and can improve, engineering properties, providing ecosystem services (Blouin et al., 2013; de Vries et al., 2013). Below-ground biodiversity is particularly important in this regard, because soil properties and thus function are known to be altered by soil communities (Lavelle et al., 2006).

The ability of a habitat to support biodiversity relies on the colonisation ability and subsequent survival of the organisms colonising. Whilst there are now numerous studies describing aspects of green roof ecology at different stages of development (see: Williams et al., 2014), little research has been focussed on the initial stages of green roof construction or the 'virgin' green roof state, a key element of baseline data needed to understand colonisation and successional processes. It is thought that green roof substrates are virtually inert pre-construction, due to the practise of firing substrates to remove seed banks and a lack of opportunity for natural colonisation thereafter (Emilsson, 2008). But personal observations by the authors note that often substrates are then stored outside, and within *Sedum*

nurseries plants are typically grown outdoors or in non-sterile glasshouses, affording two opportunities for pre-construction colonisation of materials by microarthropods.

Many soil microarthropods are relatively immobile, particularly in terms of active transport (Wallwork, 1970), and may not be able to colonise green roofs by their own means post-build, especially considering that these roofs are not usually connected to ground level soils. Braaker et al., (2014) found that for above-ground insects, the relative inaccessibility of green roofs means that only the most mobile species are able to colonise them, suggesting that green roof communities are often driven by organism dispersal ability rather than suitability of the habitat. This could have a number of negative consequences, including green roofs acting as a sink habitat for species, either when mobility changes (e.g. when offspring are born, see: Baumann, 2006) or when environmental conditions change due to season or weather (e.g. during drought, see: Rumble and Gange, 2013), resulting in a loss of biodiversity. In addition, a collection of species colonising an environment based on their mobility, rather than on adaptations to their environment could mean that sustainable communities form slowly, or not at all, hampered by environmental conditions. Rumble and Gange (2013) investigated this within green roof substrates, finding that below-ground biodiversity was not sustainable, due to a lack of resilience in the community to drought. Studies on ground-level soils suggest that even without the challenges green roofs present to less-mobile species, microarthropod colonisation into virgin soils can take 10-20 years. This could represent more than a fifth of a green roofs overall life span (Porsche and Köhler, 2003), so mechanisms to speed this process up or biologically enhance roof technosols could be an important factor in ensuring green roofs provide maximum functionality and ecosystem service provision.

In order to produce green roofs with sustainable, diverse, soil communities it is, therefore, important to understand how species colonise green roofs and how this may be facilitated. There are two key stages in a green roof's development when soil organisms may colonise a green roof. The first is pre-build, within construction materials, which to our knowledge has not been investigated. The second is post-build via natural colonisation, for example by passive methods such as phoresy and aerial dispersal.

The latter have not been investigated for green roofs, but are well-established as dispersal methods for other habitats (Flø and Hågvar, 2013)

As extensive green roofs are designed to be low maintenance after construction, it has been suggested that the design of pre-build construction materials is key, with several papers aiming to develop green roof substrates that support sustainable plant communities from the onset (Molineux et al., 2009; Odonõ No et al., 2014). In addition, we suggest that the design of green roof components should take soil organisms into consideration, as this is potentially a key element in ensuring later sustainable development of substrates and plant communities (Wardle et al., 2004). As technosols, green roof substrates can, in theory, be designed and tailored to support desired species communities. This could benefit plant growth as well as support higher faunal trophic levels by supplying prey, improving overall green roof biodiversity. Understanding whether there is an incumbent soil community within construction materials could inform this technosol design, allowing the creation of biologically active technosols or technosols that facilitate colonisation and survival. Rumble & Gange (2013) suggest that at least some colonisation of arthropods occurs post-build, but the contribution of these species compared to those arriving in construction materials is not yet known. Understanding how soil microorganisms colonise and proliferate on green roofs could determine if healthy soil could be installed on already existing green roofs for remediation purposes, to facilitate colonisation and potentially provide refugia in times of environmental change. So little is known about how species colonise green roofs, that a preliminary study was undertaken to address these questions and to highlight areas of future research.

The study had two primary aims. The first was to determine if current green roof building materials, i.e. *Sedum* plugs (in their residual soil) and substrate, contain soil microorganisms and microbes before a green roof is constructed. If so, these materials could act as the only source of less mobile, but functionally important, species, thereby addressing a research priority area highlighted by Braaker et al., (2014). The second aim of the study was to determine whether species within green roof building materials then go on to make up the communities found in more mature green roofs. It was hypothesized that in a green roof substrate, where there is probably little incumbent community, the foundation

community will have an important impact on the later development of the roof. In addition to these main aims, this paper builds on the work of Wanner and Dunger, (2002), developing our understanding of soil community development in virgin soils.

2. MATERIALS AND METHODS

2.1 EXPERIMENTAL SITE

In June 2011, a new green roof was constructed on a roof within the Royal Holloway grounds (London, UK; N 51.25350, W 0.33469) in the South East of England. The roof was constructed in a modular design using trays (for layout, see Supplementary Material 1). Five of these trays (replicates) were used in the current preliminary study, all other trays were part of a larger study (Rumble, 2013). Trays were of dimension 0.52m by 0.42m by 0.10m and were installed 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, Germany) to prevent leaching of particulate matter. An extensive substrate mix (Shire Green Roof Substrates, Southwater, Kent, UK), 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 et al., 2015), making comparison between studies viable. The bricks that this substrate is made from are obtained from the county of Cambridgeshire, UK,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. Mixing and packing of the substrate was supervised by the authors. Five samples of substrate each of 166cm^3 were then checked for the presence of microarthropods and a microbial community before being installed.

Trays were planted with nine *Sedum* plugs each, three of *S. album* (Linnaeus, 1753), three of *S. spurium* (Marschall von Bieberstein, 1808) and three of *S. reflexum* (Linnaeus, 1753). These had been grown in

a greenhouse by an industry supplier (*Sedum* Green Roof Ltd, Wiltshire, UK). After consultation with several green roof manufacturers (*Sedum* Green Roof Ltd, Wiltshire, UK; Shire Green Roof Substrates, Kent, UK; SkyGarden, Gloucestershire, UK) about the density at which plugs are normally planted, a distance of 0.1m between each plant was used (the quotes 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. A sample of plugs (five of each species) was checked for the presence of microarthropods and for microbial communities.

Mean, maximum and minimum temperature and average rainfall were obtained from the Met Office (public sector information licensed under the Open Government Licence v1.0).

2.2 MICROARTHROPOD SAMPLING

In order to assess microarthropod population abundance over time, every two months, from September 2011 until July 2012, two 56cm^3 substrate samples were removed from each plot. This was achieved by pushing a 3cm diameter soil corer down to the tray lining. These two samples were aggregated to overcome problems associated with clumped microarthropod distributions (Ettema and Wardle, 2002). This resulted in a 113cm^3 sample of substrate from each plot. A small portion (3g) of this substrate was removed for Phospho lipid fatty acid analysis (PLFA) analysis, to determine the composition of the microbial community. The remainder of the soil sample was weighed to obtain wet weight and then placed in Berlese Tullgren funnels at approximately 18˚C for 7 days (MacFadyen, 1953), after which the substrate was reweighed to obtain dry weight. Substrate water content at the time of sampling could then be calculated. Soil organisms were collected in 70% ethanol and stored in the dark, at room temperature, 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 restricted to the most prevalent morphospecies, identified to family level. Less common mites were assigned a

morphospecies. All Collembola and Hemiptera were identified to species level. Larvae of flying insects were identified where possible, but more commonly were assigned a morphospecies.

Collembola were identified using Hopkin (2007). Mites were identified using Strandtmann, (1971); Strandtmann and Davies, (1972); Krantz and Walter, (2009) and Walter and Proctor (2013). Hemiptera were identified using Southwood and Leston, (2005).

2.3 PHOSPHO-LIPID FATTY ACID ANALYSIS (PLFA)

3(±.05) g of soil was taken from each microarthropod substrate sample and stored at -20˚C until analysis. PLFA analysis followed a modified method of Frostegård et al., (1993). Briefly, lipid extraction was undertaken using Bligh/Dyer solvent, with phase separation performed by using chloroform as an organic solvent. Fractionation was then undertaken using normal phase silica acid columns (Cronus SPE Cartridges Si 1000mg/6ml, SMI LabHut, Gloucestershire, UK), fractioning lipid material into neutral (NLFA's), glyo- and phospholipids (PLFA's). Fatty acid methyl esters (FAMEs) were then obtained via lipid methanolysis of the PLFA fraction, using 0.2M methanolic KOH. Methyl nonadecanoate (C19:0) was added here as an internal standard. FAMEs were identified by chromatographic retention times, with bacterial PLFAs verified with a standard bacterial FAMEs mix (Sigma Aldrich, St Louis, MO, USA). Analysis was performed by a Hewlett Packard (HP) 5890 gas chromatograph equipped with a flame ionization detector and a DB-5 capillary column (30 mm x 0.25 mm i.d., film thickness 0.25 μm). 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°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. FAMEs were identified on an HP 5970 mass spectrometer.

Fatty acid nomenclature followed Frostegård et al., (1993). The abundance of individual PLFA's is expressed as equivalent responses to the internal standard, in μg g^{-1} dry weight of soil (modified from Hedrick et al., 2005). Microbial markers were used to characterize the community. The PLFAs 18:2ω6,9 (Frostegård et al., 2011) and 20:1ω9 (Sakamoto et al., 2004) were used as indicators of fungi while

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 (Zelles, 1999) were used to characterize total soil bacteria.

2.4 MYCORRHIZAL SAMPLING

Roots from the plants growing in plugs that formed the basis of the initial microarthropod baseline sample were analysed for the presence of arbuscular mycorrhizal fungi (AMF). In addition, at the end of the experiment, in July 2012, roots of plants growing in trays were also analysed for AMF.

Roots were washed with tap water and cleared in 10% potassium hydroxide (KOH) in a water bath at 80˚C for 25 minutes. Visualization of mycorrhizas in the roots was performed using a modified ink staining method of Vierheilig et al., (1998), whereby commercial ink (Quink washable blue, The Parker Pen Company, East Sussex, UK) mixed with 1% HCl and water in the ratio 0.6:15:84.4 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 the presence or absence of fungi in the form of hyphae, vesicles or arbuscules, and recorded. Approximately 100 counts were obtained from each sample.

2.5 STATISTICAL ANALYSIS

Analysis was performed using SPSS 22.0, except PCA, which was performed using R (R Core Team, 2015). Differences in total microbial mass and total microarthropod populations between initial plugs and the substrate were tested using univariate ANOVA. Univariate ANOVA was also used to test sub-groups of these variables (i.e. bacterial PLFA's, fungal PLFA's, springtails and mites) and to test mycorrhizal colonisation between different plant species.

Microarthropod data, including sub-groups tested, were square root transformed to meet the assumptions of normality. Variances were tested for heterogeneity using Levene's median test for non-

skewed data and by a non-parametric (rank) Levene's test for skewed data (Nordstokke and Zumbo, 2010). Data analysed passed the assumption of homogeneity of variances. The Shannon-Wiener Index (*H*) was calculated (using log base 10) to determine diversity of microarthropods and this was conducted for each plot.

PLFA data from the initial substrate, as well as recorded each month after planting was tested using a repeated measures ANOVA, with time as the main effect. Separate tests were performed on total PLFA's as well as on bacterial and fungal PLFA's. Greenhouse-Geisser adjustments were applied to non-spherical data and the Bonferroni post-hoc test was used to separate differences between time points.

Microarthropod data post-planting did not meet the assumptions of ANOVA. Microarthropods recorded in the initial substrate and grouped for all subsequent months were, therefore, tested using Kruskal-Wallis, as was Shannon-Wiener diversity.

PCA, using a correlation matrix, was conducted on all fatty acids in one analysis, all microarthropods in another and additionally on groups of microarthropods (Collembola, mites) to determine how their communities were organised. 95% confidence ellipses (SEM) were plotted based on data grouped into different sources/sample months. These analyses were conducted using the vegan (Oksanen et al., 2015), nFactors (Raiche and Magis, 2011) and BiodiversityR (Kindt and Coe, 2005) packages for R (R Core Team, 2015).

3. RESULTS

3.1 ABIOTIC CONDITIONS

The average temperature in the sample period was 12°C (±4), with a maximum temperature of 21°C (August 2011) and a minimum of 0°C (February 2012). Average rainfall over the sample period was 67mm (±38). Months with the highest recorded rainfall were June 2012 (134mm) and April 2012 (133mm) and the lowest recorded were in February and March 2012 (19mm and 27mm respectively) (Met Office, 2017). Substrate water content was an average of 21% (±7%) across the sample period,

with lowest values occurring in March 2012 (11%), May 2012 (17%) and November 2011 (20%). Highest values were recorded in July 2012 (30%), September 2011 (25%) and January 2012 (22%).

3.2 MICROARTHROPOD COMMUNITY

The green roof substrate was void of microarthropods on arrival. However, the plugs of each *Sedum spp*. supported microarthropod communities. Collembola were the most prevalent microarthropod ($\overline{\chi} \approx$ 11 000 (± 4000) m^{-2}), followed by mites ($\overline{\chi} \approx 2000$ (± 1000) m^{-2}). *S. album* supported a higher abundance of microarthropods than *S. reflexum* and *S. spurium* ($F_{2, 14} = 19.08$, $p < 0.001$) but was lower in species diversity ($F_{2, 14} = 8.10$, $p < 0.005$; *H*: *S. album*, 0.26; *S. reflexum*, 0.59; *S. spurium*, 0.55) as it contained high numbers of the Collembola species *Parisitoma notabilis* (Collembola: $F_{2, 14} = 22.42$, $p < 0.001$). Only three other species/morphospecies were found in the plugs, one morphospecies of the order Annelida, one aphid species, *Aphis sedi*, and one morphospecies that was identified as a larvae of a terrestrial chironomid. These morphospecies were low in abundance in all plant species.

Whilst, according to PCA (Fig 1), community composition overlapped between *Sedum spp.*, there was a suggestion that overall the communities differed. Some cosmopolitan species (e.g. *P. notabilis*), were found in all plug species, and some species were only found in plugs of one *Sedum spp.* (e.g. *S. aureus* was only present in *S. spurium* plugs). *S reflexum* also supported a higher number of species, at 12 compared to ten for *S. album* and *S. spurium.*

Microarthropod abundance in the substrate post-planting remained low compared to in the original planted plugs, reducing from an average 12980 (±4044) individuals m^{-2} down to an average of 3110 (±373) individuals m^{-2} ($\chi^2_2 = 19.07$, $P = <0.001$). Microarthropod diversity was half of that in the original plugs, reducing from an average Shannon-Wiener score of 0.47 (±0.05) down to 0.19 (±0.02) ($\chi^2_2 = 27.27$, $P = <0.001$). Colonisation rates of new species was slow. There were 15 species present in the plugs. A further two new species colonised in September 2011. No new species were sampled within the plots until May 2012 when an additional species was sampled. In July 2012, a further five new species colonised the plots, bringing the cumulative total of species sampled over the eleven months to 23.

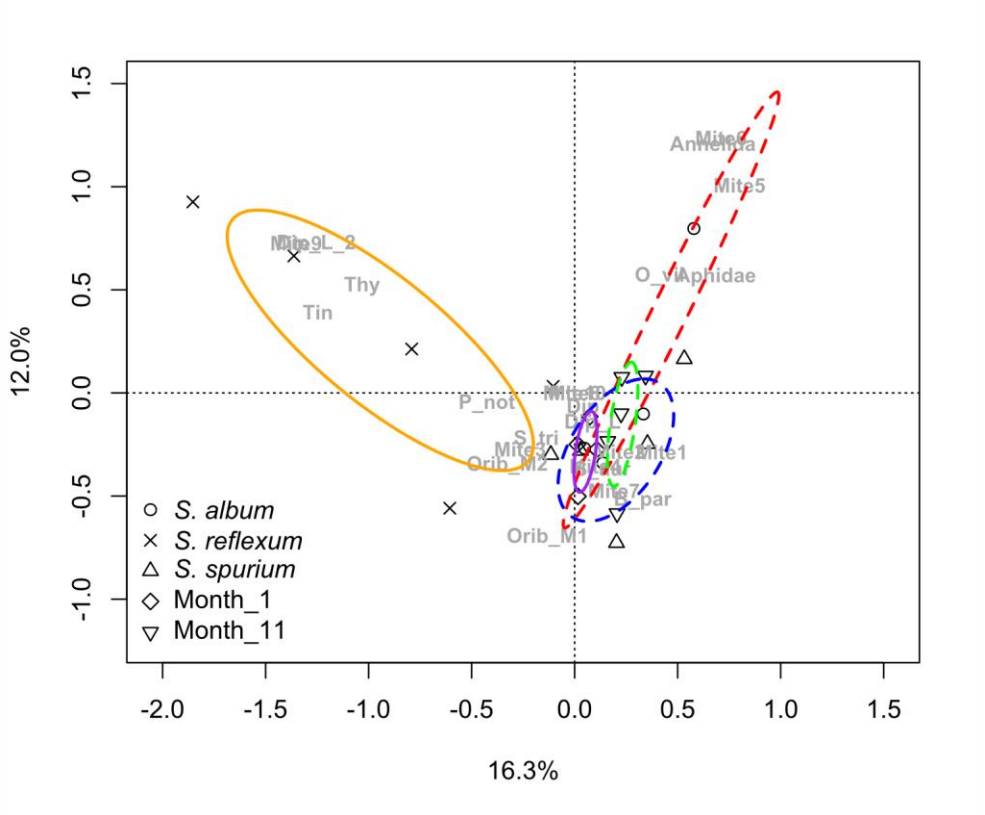

Fig. 1. PCA ordination of all microarthropods in plugs prior to planting and in the substrate post-planting in September 2011 (month one) and July 2012 (month eleven). Rings denote 95% confidence intervals; Blue dashed lines denote *S. album*, green dashed lines denote *S. reflexum*, red dashed lines denote *S. spurium*, purple solid lines denote month one and yellow solid lines denote month 11.

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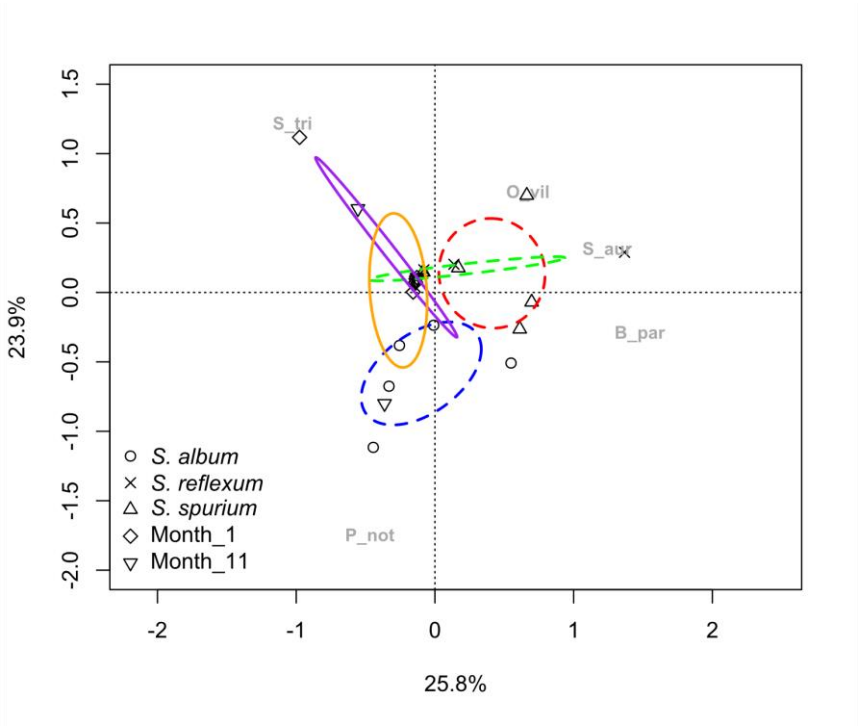
272 The Collembola population in the plugs consisted of the species complex *Parisotoma notabilis* (Porco
273 et al., 2012) and three further species: *Orchesella villosa*, *Brachystomella parvula* and *Sminthurinus*
274 *aureus*. *S. album*, with a high dominance of *P. notabilis*, supported a different community to the other
275 two plug species, which were similar to one another according to PCA (Fig 2a). Post-planting the
276 collembolan community shifted dramatically in terms of species composition. *B. parvula* died out post-
277 planting and *S. aureus* was not recorded after September 2011. *O. villosa* abundance greatly reduced
278 post-planting, below recordable levels until May 2012. *P. notabilis* remained the most common
279 Collembola throughout the study period. One collembolan, *S. trinotatus* colonised post-planting ($\overline{\chi} \approx$

a.)



b.)

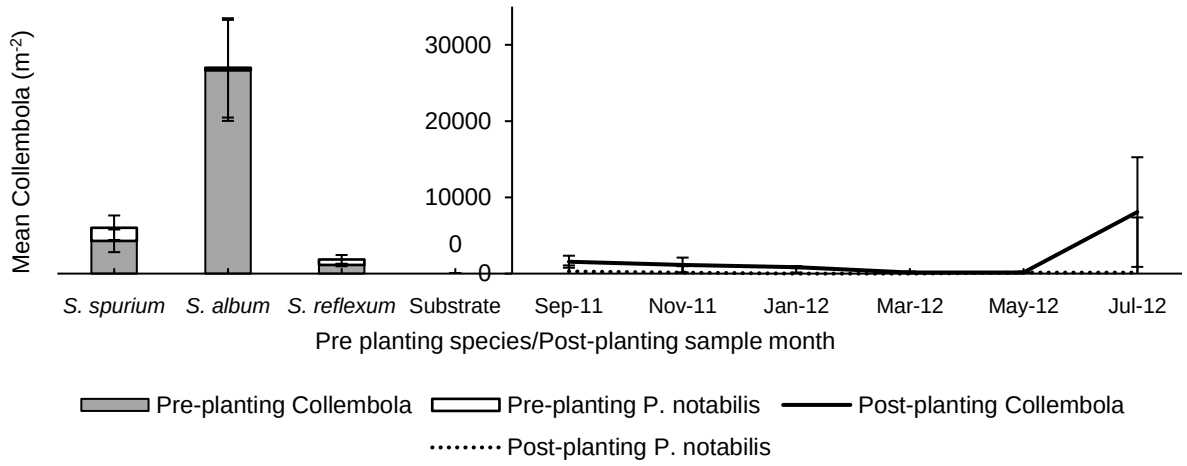


Fig 2. (a) PCA ordination of Collembola in plugs prior to planting and in the substrate post-planting in September 2011 (month one) and July 2012 (month eleven). Rings denote 95% confidence intervals; Blue dashed lines denote *S. album*, green dashed lines denote *S. reflexum*, red dashed lines denote *S. spurium*, purple solid lines denote month one and yellow solid lines denote month 11. (b) Mean Collembola m^{-2} pre and post-planting. Grey bars and dashed lines represent the contribution made by *P. notabilis*. Error bars represent SEM.

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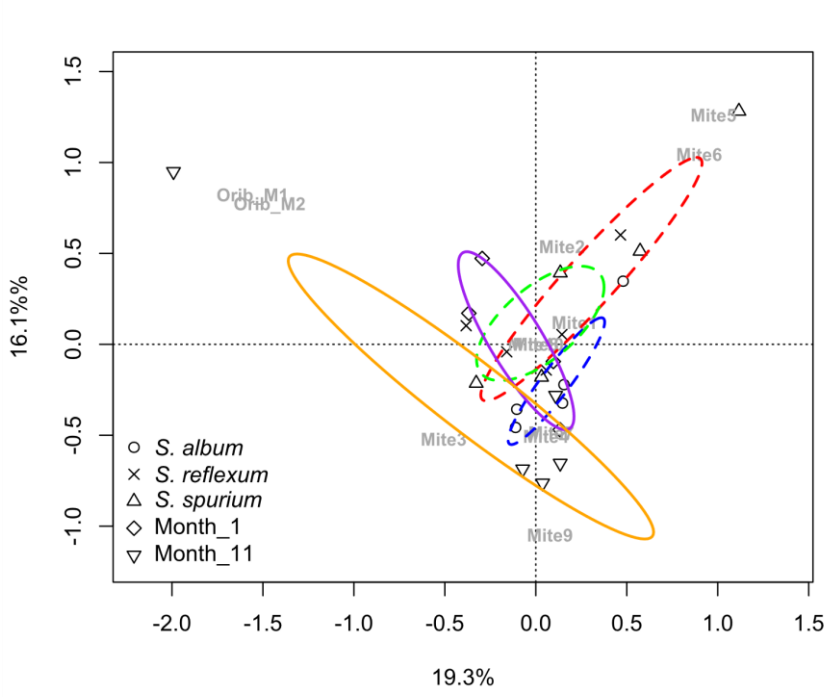
a.)



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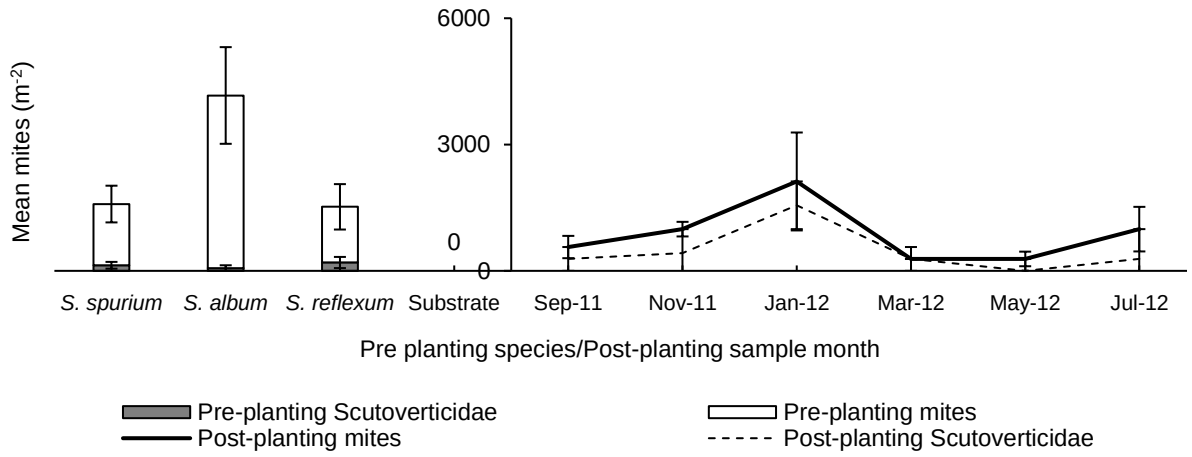

Fig 3. (a) PCA ordination of mites in plugs prior to planting and in the substrate post-planting (Post) in September 2011 (month one) and July 2012 (month eleven). Rings denote 95% confidence intervals; Blue dashed lines denote *S. album*, green dashed lines denote *S. reflexum*, red dashed lines denote *S. spurium*, purple solid lines denote month one and yellow solid lines denote month

11. (b) Mean mites m^{-2} pre and post-planting. Grey bars and dashed lines represent the contribution made by Scutoverticidae. Error bars represent SEM.

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71 (± 52) m^{-2}). In general, Collembola remained low in abundance until July 2012, when *P. notabilis* and *S. trinotatus* vastly increased in number (Fig 2b).

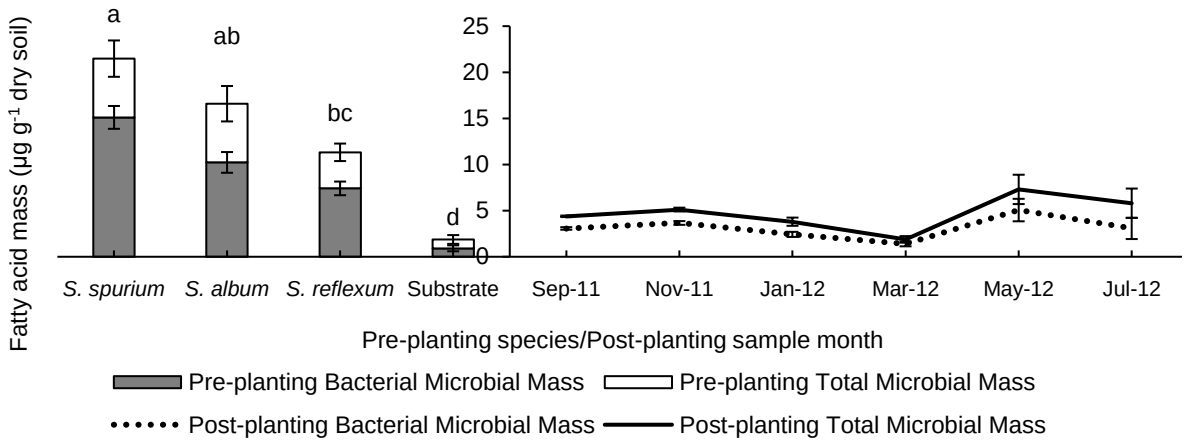
No mites were present in the bare substrate and only *S. album* plugs supported significantly higher numbers of mites than the substrate ($F_{3, 16} = 6.64$, $p < 0.001$). Eight morphospecies of mite including one oribatid of the family Scutoverticidae and one species in the Bdellidae family were present within plugs. The mite community changed in species composition post-planting, with two morphospecies disappearing but three morphospecies of mite colonising (Fig 3a). The Scutoverticid, present both pre and post planting, was extremely successful post-planting, peaking in January 2012 to levels comparable to those in the original plugs (Fig 3b).

Aphis sedi and a terrestrial chironomid larva were present both pre and post planting. The Annelida morphospecies was not found post-planting. Diptera, their larvae and Thysanoptera colonised post-planting, all in low abundance until July 2012 when they reached a peak. In terms of community structure there was little difference between plugs and sample dates post-planting, except in July 2012 (data not shown).

3.3 MICROBIAL COMMUNITY

Bare green roof substrate before construction was almost inert, with very little microbial mass detected by PLFA (0.3 to 2.2μg g^{-1} dry soil). Plant plugs contained significantly more microbial mass than the substrate ($F_{3, 18} = 33.16$, $p < 0.001$). In addition, microbial mass was higher in *S. spurium* plugs than in *S. reflexum* plugs ($p < 0.05$) and there was a suggestion that *S. album* also supported more microbial mass than *S. reflexum* ($p < 0.076$) (Fig. 4a). *S. spurium* supported the most microbial abundance in terms of bacterial markers (*S. spurium;* vs. *S. album*: $p < 0.05$; vs *S. reflexum:* $p < 0.001$) and all plugs supported significantly more bacterial mass than the substrate (Substrate; vs. *S. spurium:* $p < 0.001$; *S. album:* $p < 0.001$; *S. reflexum:* $p < 0.01$). Mass of bacterial PLFA's was higher than mass of fungal PLFA's in all plugs and fungal mass did not vary between plug species ($p > 0.05$ for all plug species). All plugs supported more mass of fungal PLFA's than the substrate (Substrate; vs. *S. spurium:* $p < 0.001$; *S. album:* $p < 0.001$; *S. reflexum:* $p < 0.05$). Post-planting, the surrounding substrate remained

a.)


a
ab
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25
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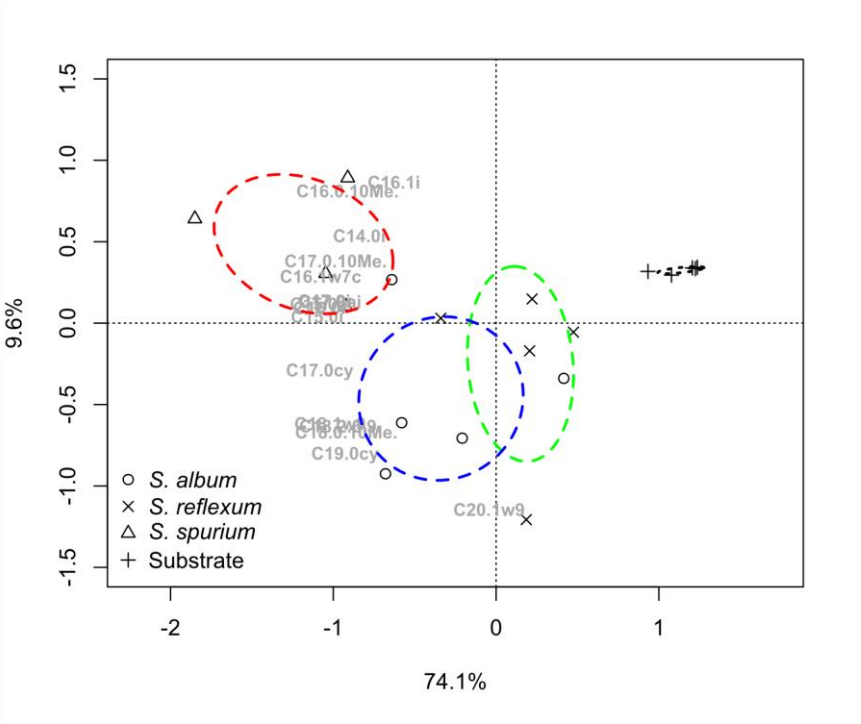
Fatty acid mass (µg g-1 dry soil)
S. spurium
S. album
S. reflexum
Substrate
Sep-11
Nov-11
Jan-12
Mar-12
May-12
Jul-12
Pre-planting species/Post-planting sample month
Pre-planting Bacterial Microbial Mass
Pre-planting Total Microbial Mass
Post-planting Bacterial Microbial Mass
Post-planting Total Microbial Mass

b.)


1.5
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-0.5
-1.0
-1.5
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-2
-1
0
1
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C16.1i
C14.0i
C17.0cy
C19.0cy
C20.1w9
S. album
S. reflexum
S. spurium
Substrate

c.)

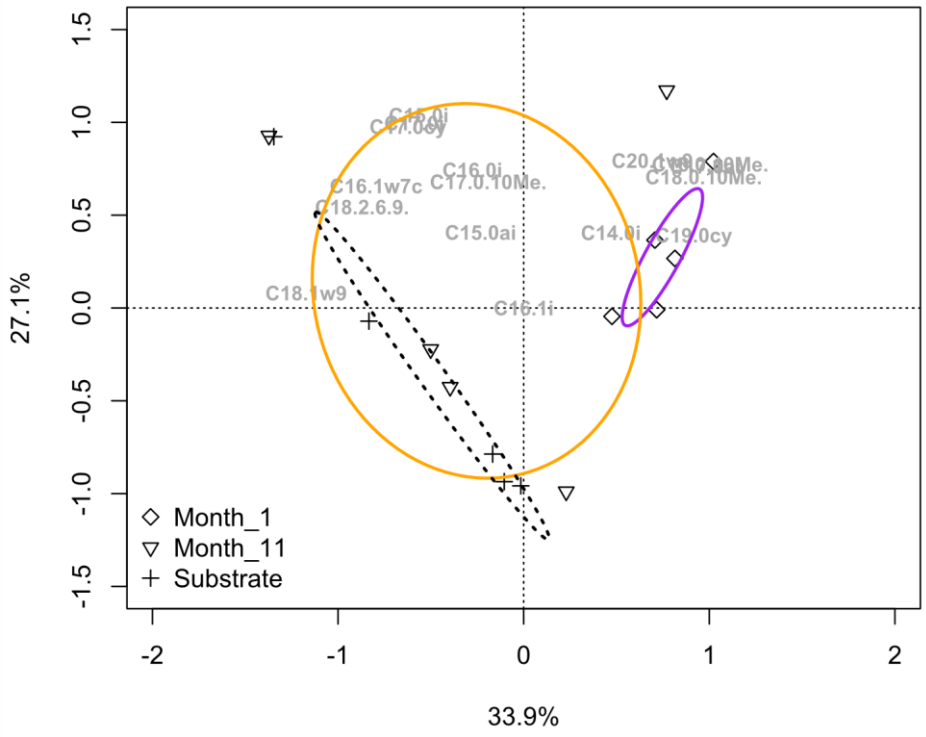

Fig 4. (a) Mean abundance of PLFA's (μg g^{-1} dry soil) in plugs and substrate prior to planting and in the substrate post-planting over time. Grey shaded bar portions and dashed lines represent bacterial biomass, whilst the unshaded bar portion represents fungal biomass. Error bars represent SEM. Letters represent statistical differences between pre-planting source materials (plugs and substrate) and post-planting monthly samples for total microbial mass; PCA ordination of PLFA's in substrate prior to planting, with (b) plugs and (c) post-planting in September 2011 (month one) and July 2012 (month eleven). Rings denote 95% confidence intervals; Blue dashed lines denote *S. album*, green dashed lines denote *S. reflexum*, red dashed lines denote *S. spurium*, black dotted lines denote the substrate, purple solid lines denote month one and yellow solid lines denote month 11.

low in microbial mass, with no month statistically different to the initial substrate. In May 2012, eleven months after planting, a slight but very modest increase in fatty acid mass was seen, but this was not statistically significant (Fig. 4a).

312 The microbial community structure also differed between plug species, particularly in the case of *S.*
313 *spurium* and the bare substrate, which were clearly separated by PCA (Fig. 4b). Post-planting, the
314 community resembled that of the bare substrate (Fig. 4c).

315 No mycorrhizal structures were observed in the roots of any of the plugs pre-planting. However,
316 mycorrhizal colonisation took place during the eleven months of the experiment. By month eleven,
317 hyphal counts varied between 0-45% root length colonised (RLC) in the total sample area and more
318 than 92% of plants had been colonised by mycorrhiza. Mean RLC was 21% (±2%). 72% of plants
319 contained vesicles. Mean % of counts with vesicles was 4% (±0.7%). Arbuscules were extremely rare,
320 ranging between 0 and 2%, with 95% of plants containing no arbuscules in the roots. No arbuscules or
321 vesicles were present in the absence of hyphae. Mean RLC for *S. album* was 21% (±5%), *S. spurium*
322 16% (±4%) and *S. refluxum* 16% (±3%). RLC did not differ between plant species ($F_{2, 15} = 0.54$, $p =$
323 0.60), and neither did vesicle number ($F_{2, 15} = 0.12$, $p = 0.988$).

324 DISCUSSION

325 4.1 SOURCE POPULATIONS IN BUILDING MATERIALS

326 In the current study the substrate was completely sterile in terms of soil microarthropods. Emilsson
327 (2008) suggests that this is due to the practise of heat treating for seed removal, butour substrate was
328 stored outside after heat treatment and contained rough compost. This indicates that even when
329 opportunities for colonisation are available, recycled aggregates as substrates may be too inhospitable
330 to support life when unaccompanied by plants. This presents a wasted opportunity; by volume, as
331 substrate far outweighs any soil introduced with plants. In terms of soil microbes, the substrate was not
332 sterile, but microbial mass was extremely low, with less that 2.2µg g^{-1} total fatty acid mass (less than
333 one sixtieth of that reported in forest soils (Ascher et al., 2012)).
334 Residual soil around plug plants, as we hypothesised, did contain microbes and microarthropods,
335 allowing us to test the hypothesis that soil can be 'inoculated' into a green roof habitat. Analysis of the
336 *Sedum spp.* plugs highlighted that different plant species supported distinct microarthropod

communities, as well as distinct microbial communities. This implies that the choice of plant assemblage on a green roof may also affect the development of soil communities.

There were very few specialists of the harsh conditions found on a green roof (Rumble and Gange, 2013) in the *Sedum* plugs, perhaps unsurprising given the good quality soil and conditions in which plugs grow. Personal observations of green roof nurseries suggest that the main source of microarthropods to *Sedum* plugs are from the surrounding habitat, often a nursery of other plants, field or garden. Many microarthropods in these ground-level soils have mechanisms to withstand short periods of drought, such as synchronised emergence in collembola (Alvarez et al., 1999). Thus if *Sedum* was grown in the substrate later to be used, but exposed to this diverse source population more deliberately, these species may be able to colonise substrate, as well as plugs, at the ground level, removing the barrier of an inaccessible rooftop. Whilst Rumble and Gange (2013) suggested that ameliorating conditions on green roofs may benefit microarthropods, the current study also suggests that ensuring organisms transplanted onto a green roof are adapted to its harsh conditions already, as we practise with plant species, could be another successful strategy in building resilient soil communities on green roofs. Potentially, this could be achieved with only a minor change to current *Sedum* farming practise.

4.2 SURVIVAL OF SOURCE POPULATIONS POST-PLANTING

Within one month of the construction of the green roof, the substrate, which had not supported any microarthropods prior to planting, supported some of the species present in plugs, demonstrating that microarthropods are able to move from plugs into the surrounding substrate. At this stage the substrate also supported a slightly higher microbial mass than it had done before planting, suggesting that microbes were also able to colonise the substrate quickly from the source plugs. Whilst this would suggest that it could be possible to implant soil communities into green roof substrates, in this particular instance the impact of these new colonising communities into the substrate was short lived. Community analysis of the microbial population suggested that, over time, whilst microbial mass increased, the mix of fatty acids present became less diverse over time. By month eleven the microbial community had

become more similar to that observed in the initial substrate than to the plugs. In addition, with the exception of a few species, microarthropod abundance also declined after month one. Thus in terms of microarthropods and microbes, we suggest that few organisms present in plugs were able to survive long-term in the substrate. This is despite the characteristic droughts observed in Rumble & Gange (2013) being absent within this study period. In terms of microbes, abundance at month eleven was similar to that reported in comparable young green roofs by Molineux et al., (2014), suggesting that this is a recurring phenomenon and our findings can be generalised to other extensive green roofs of this design.

Whilst microarthropod abundance and diversity declined over time, some of the plug species were able to survive and contribute significantly to the population at month eleven, including the Scutoverticid mite and collembolan *P. notabilis*. This suggests that the species present in initial plugs affect later successional development of green roof soil communities and demonstrate that *Sedum* plugs can be an effective vehicle for introducing soil microarthropods to green roofs and that attention should be paid to the species present.

Both of the two successful microarthropods observed in this study, the cosmopolitan springtail *P. notabilis* and the mite family Scutoverticidae have been observed on green roofs before (Schrader and Böning, 2006; Rumble and Gange, 2013). They are both ubiquitists, tolerant of a wide range of conditions (Porco et al., 2012; Schäffer et al., 2010) and both are successful colonisers of primary successional habitats (Hågvar, 2010; Lehmitz et al., 2011). Their adaptations to a variety of conditions no doubt explains their prevalence on this, and other, green roofs. This sparks a wider question within green roof ecology and urban technosol development in general: Are these generalist species capable of functioning as the primary nutrient cyclers in this habitat, in the absence of a more biodiverse community? As a coprophage and detritivore (Ponge, 1991) *P. notabilis* could be a key nutrient cycler in this environment, but future research into the precise function of this species in terms of nutrient cycling would be useful. In terms of developing a functioning soil, it is possible that a few key organisms are as beneficial as a diverse community, with some authors suggesting high levels of functional redundancy in ground level soils (Setälä et al., 2005). While such species-poor communities

may not be the most desirable (Williams et al., 2014), it may be that this is all that is possible in some green roof technosols. In addition, Wardle et al., (2004) suggest that other soil organisms, such as protozoa and nematodes can also have significant impacts on soil nutrient cycling processes, especially in the absence of earthworms (as is the case on this green roof) and so these organisms also merit future research on green roofs.

4.3 INDEPENDENT COLONISATION POST-PLANTING

Some species of microarthropod colonised post-planting. However, this was a rare occurrence and at the end of this experiment these species were in extremely low abundance, highlighting the need to provide a large, robust population at construction or to make conditions more favourable for later colonisers. Of note was that the majority of new colonisers to the roof were already present within the first two months, suggesting that colonisation by microarthropod species is rapid, even if survival rates are low.

The larvae of terrestrial chironomid midges persisted in fairly high abundances on this young green roof, having colonised post-planting. These larvae were also found in high abundances in the substrates of the mature roofs studied by Rumble and Gange (2013). Their presence here early on in a green roofs development suggests that this organism is well suited to this habitat and consistently chooses to use it, perhaps due to the abundance of open patches for oviposition (Frouz, 1999). Along with the Scutoverticid mite, this species is perhaps an example of a specialist 'roof dweller'. Another of these may be the springtail *Sminthurinus trinotatus*, found in low abundances but consistently on the green roof and also recorded on bare roofs by (Shaw, n.d.). Hopkin (2007) suggests that this is a rare species in the UK, but it may be that this is an overlooked species of infrequently studied habitats.

4.4 MYCORRHIZAL FUNGI POST-PLANTING

One group of species that colonised extremely rapidly and successfully post-planting, having been absent in plug plants, was arbuscular mycorrhizal fungi (AMF). Rumble and Gange (2013) found that *Sedum* on mature green roofs was highly mycorrhizal, but whether this mycorrhiza had been transplanted to the roof in plug plants or had colonised later was not known. McGuire et al., (2013) also

416 confirm that AMF are present within mature green roof plants. In the current study, as in John et al.,
417 (2014), plugs were not mycorrhizal before planting. However, within eleven months 90% of plant roots
418 tested contained some mycorrhizal colonisation. These results suggest that mycorrhizas are capable of
419 colonising the three *Sedum* species tested here rapidly and without inoculation. This is an important
420 finding for two reasons.

421 Firstly, the incidence of mycorrhiza within *Sedum* spp. in general is not well recorded, with several
422 sources suggesting some *Sedum* species to be non-mycorrhizal or rarely mycorrhizal (Harley and
423 Harley, 1987; Wang and Qiu, 2006). John et al., (2014) found that *S. spurium* can be highly mycorrhizal,
424 whilst *S. acre* (not studied here, but common on green roofs) is consistently reported as being non-
425 mycorrhizal (Olsson and Tyler, 2004; John et al., 2014). The current study suggests that there are AMF
426 species that will readily colonise some *Sedum spp.* post-planting on a green roof, which could have been
427 present in the substrate as spores or may have colonised aerially (Egan et al., 2014), though this was
428 not investigated.

429 John et al., (2014) suggest that the presence of AMF on green roofs could aid the establishment, survival
430 and ecosystem services provision of other, non-*Sedum*, green roof plants and we suggest that future
431 areas of research should include exploring the relationship between *Sedum*, their AMF and other
432 colonising/desired plants. In this study very few arbuscules were noted within the *Sedum* roots, whilst
433 vesicles were common. Several authors suggest that the relationship between AMF and plant can be
434 inferred by the ratio of arbuscules to other mycorrhizal structures (Collins Johnson et al., 2003), and
435 that a lack of arbuscules suggests a lack of nutrient exchange between plant and AMF, particularly in
436 terms of available P (Collins Johnson et al., 2010; Rinaudo et al., 2010; Verbruggen et al., 2012). Thus,
437 it could be possible that whilst AMF colonise *Sedum* very successfully, perhaps due to an absence of
438 other plants on a green roof, there is little in the way of nutrient exchange occurring and perhaps little
439 benefit to the plant.

440 This leads to the second important conclusion drawn from the high levels of AMF occurrence in these
441 *Sedum* spp. Mycorrhizal fungi have been applied to green roofs, with the aim of improving (non-*Sedum*)

plant growth (Sutton, 2008; Young et al., 2015), but, aside from studies on *S. alfredii* (Hu et al., 2013a, 2013b) (not a common green roof species), there are no empirical studies to our knowledge testing whether mycorrhiza benefits *Sedum* species growth. As the vast majority of green roofs are planted with at least some *Sedum* spp. this aspect of green roof ecology should not be overlooked. The commercial inoculants used are often species mixes and it is not known which of these are able to establish within *Sedum* roots, nor whether they enhance plant growth as a result. Olsson and Tyler (2004) suggest that, as species of harsh, rocky environments, *Sedum* spp. may not be able to afford to donate photosynthates to associated AMF and that AMF may well be more important in mediating competition between plants than enhancing tolerance to abiotic stresses. Relationships between mycorrhizas and plants, as well as between mycorrhizal species can produce extremely varied outcomes in terms of plant growth (Jin et al., 2017). Outcomes on plant growth are also reported as being highly variable depending on fungal community composition, both in the limited field trials that have been conducted (van der Heijden et al., 2015) and in single and multi-species inoculations, with multiple species often failing to produce any measureable effects on plants (Owen et al., 2015). Molineux et al., (2014) also highlight that substrate properties themselves can determine how these communities develop and interact. Thus, if an incumbent mycorrhizal community exists via this natural colonisation, the efficacy of mycorrhizal additions may be more or less successful than if applied to a virgin substrate. In addition, applying any mycorrhiza at all to green roofs where *Sedum* is planted could reduce plant growth by producing a parasitic mycorrhiza/plant relationship. There is much research to be done in this area in terms of determining which of these species is most beneficial for *Sedum* growth, as has been studied in agricultural applications (Fester and Sawers, 2011), and whether the timing of the application of inoculants alters success. In addition, AMF display species-specific intolerances to abiotic conditions, such as drought (Klironomos et al., 2001), another aspect that is important for persistence on green roofs and needs further research. The current study suggests that *Sedum* is very easily colonised by one or more mycorrhiza species, very soon after planting.

4. CONCLUSIONS

This preliminary study aimed to give some insight into the colonisation of green roofs by soil organisms to establish where future research in this area is needed. Our primary aim was to determine if green roof building materials are a vehicle for soil organisms finding that plugs of *Sedum* do support microarthropod and microbial communities, but substrate only supports the latter. Mycorrhizal fungi was absent from plugs.

Our second aim was to determine if these species could survive post-planting and make up a significant element of the later community. Our results suggest that microarthropod communities surviving over the course of the study consisted of a mix of species from construction materials colonising via human-mediated means (plug plants) and species that had colonised independently, post-construction. The microbial community also seemed to change post-construction. Thus soil microarthropods and possibly microbes in green roof substrates post-planting are made up of a combination of anthropogenic assemblages and natural communities. Mycorrhizas, however, colonised independently, rapidly and in high abundance.

These results suggest that species composition in the source materials of green roofs (in this instance plugs of *Sedum*) affected the subsequent community composition within the soil and that future research into how these plugs could be more effectively used as vehicles for soil organisms is needed. Moreover, we observed that independent colonisation by new species, whilst slow, was important due to the high levels of mortality experienced by transplanted microarthropods and, we suggest, for microbes as well. The high mortality of transplanted species suggests that green roofs could be acting as a sink community for some species, incapable of supporting them in the long-term, highlighting a second important area of research: ameliorating conditions for those organisms colonising independently. Mycorrhizal fungi seemed to be extremely successful at colonisation post-planting, and understanding the function of these species on green roofs should be a priority area of research.

The ability of microorganisms to colonise the green roof substrate from the plugs was encouraging for the development of technosols. However, species inoculated into green roofs in this manner need to be

adapted to these conditions from the onset to ensure their survival and maximise their impact. If this could be achieved, green roof soil communities could not only be improved on new installations, but groups of specialist soil organisms could be inoculated onto mature green roofs that are already impoverished, expanding the reach of green roof soil remediation to the many roofs that have already been built. Whilst this is already being tested for more traditional biological inoculants, such as AMF and soil bacteria (Rumble and Gange, 2017), we propose that more research is needed to understand if inoculation could be broadened to include other beneficial soil organisms, such as microarthropods.

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Conflict of Interest Statement

The authors are unaware of any conflicts of interest that may have impacted the delivery or content of this manuscript. Project partners (Symbio Ltd and Laverstoke Park) provided financial support; *Sedum* Green Roof Ltd and Shire Green Roof Substrates Ltd donated construction materials. They had no influence on the study; its design, delivery or interpretation.

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SUPPLEMENTARY MATERIAL 1: LAYOUT OF GREEN ROOF EXPERIMENTAL TRAYS

Experimental design for the new roof experiment (not to scale). Trays were 0.52x0.42x0.10m and placed 0.30m apart. Green, bold outlined plots denote plots planted with *Sedum spp.* and used in the current study. Plots not outlined in bold were not used in the current study.