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Can microbial inoculants boost soil food webs and vegetation development on newly constructed extensive green roofs?

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ABSTRACT

Green roofs are a key to providing nature-based solutions in cities. However, most green roofs installed in the Northern hemisphere are shallow, stonecrop planted systems ("extensive" green roofs), which have been shown to support limited biodiversity and could be more effective at providing ecosystem services. One issue with this type of extensive green roof is that rootzones are almost sterile on construction, relying on natural colonisation to provide a soil food web. This is a slow process, meaning plant growth can also be slow. Our aim was to determine if a soil food web could be introduced when the green roof is built. We applied microbial inoculants (mycorrhizal fungi and bacteria (*Bacillus spp.*)) to a new green roof and monitored plant growth and the soil food web (bacteria, mycorrhizal fungi and microarthropods). Different inoculants altered the composition of microarthropod communities, potentially impacting later succession. In particular, bacterial inoculants increased microarthropod populations. This is one of the first studies to demonstrate that the addition of microbial inoculants impacts not only plant growth, but also faunal components of the soil food web, which could have implications for long-term resilience. Bacteria were effective at aiding mycorrhizal colonisation of plants roots, but this colonisation had no impact on the growth of our selected stonecrops, *Sedum album, Petrosedum reflexum* and *Phedimus spurius.* We suggest that if a beneficial mycorrhiza could be found to promote the growth of these specific species on green roofs, bacteria could be effective "helper" species to aid colonisation. This study enables green roof researchers and the industry to justify further exploration of the impact of microbial inoculants on green roofs.

1. Introduction

Green roofs (intentionally vegetated roofs) have the potential to deliver a number of ecosystem services in cities, such as stormwater retention and building energy reduction (Jaffal et al., 2012). These ecosystem services rely upon functions provided by either plants, soil, or both. In theory, green roofs may be constructed with deep, nutrient-rich substrates that can support complex plant life (Grant, 2006). Yet previous research suggests that some green roofs, particularly shallow, extensive green roofs (typically, roofs with a substrate of less than 10 cm), are not reaching their maximum potential in terms of plant growth or soil biodiversity (Rumble and Gange, 2013; Young et al., 2014). These extensive green roofs are, in the Northern Hemisphere, the most common type of green roof installed (Getter and Rowe, 2006; pers. obs.).

Weight is a key consideration in extensive green roof design: lightweight, free draining substrates are commonly used to reduce load on buildings (Young et al., 2014). This means they dry out quickly necessitating the planting of hardy plants such as stonecrops (typically termed as "Sedums" within the green roof industry, though taxonomically many of these species are not strictly within the genus *Sedum*), which can cope with this environment (Rumble and Gange, 2013). Extensive green roofs are also by definition shallow, ranging from 5 to 15 cm deep (GRO, 2014), again exacerbating problems with drying out. Soil moisture on green roofs can frequently be less than 1 % of substrate volume and surface temperatures of 53.5 °C have been recorded on green roofs in the UK (Rumble and Gange, 2017). As a result, in terms of abundance and diversity of microarthropods, the soil food web on extensive green roofs has been likened to a desert (Rumble and Gange, 2013) in addition to having slow community development (Rumble et al., 2018). Given that nutrient cycling is also limited on this type of extensive green roofs (Molineux et al., 2017) and that microarthropods have been shown to be responsible for almost half of the decomposition processes occurring in

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harsh environments (Santos and Whitford, 1981), there is a need to understand how to boost their populations. The green roof substrates used in these types of green roofs are also likely to be almost sterile on construction; While there are few studies investigating this, our previous research found that despite the inclusion of compost and outdoor storage of green roof substrates, microbial mass was extremely low within substrates and that they were devoid of microarthropods entirely (Rumble et al., 2018). Emilsson (2008) reports a similar issue and suggests that this is due to the practise of heat treating substrates for seed removal. Our previous research suggests that even when diverse communities are introduced to a green roof via the compost surrounding the plug plants, survival is hampered by the harsh nature of the substrate (Rumble et al., 2018). Improving conditions for the soil food web is therefore crucial and should have knock-on effects for plant growth, in terms of widening the range of plants able to be used on green roofs and improving plant health, both important aspects of green roof ecosystem service provision (Lundholm et al., 2015; Voyde et al., 2010).

The physical amelioration of green roof conditions for plants by supplying hydration gels (Young et al., 2017) or altering substrate properties and depth has been investigated (Nash, 2017; Young et al., 2017). However, the soil food web has been overlooked as a biological solution to the problem. While the efficacy of microbial inoculants is still debated, (Emmanuel and Babalola, 2020; Kaminsky et al., 2019; Santos et al., 2019) it has been shown that it is possible for commercial microbial inoculants to ameliorate growing conditions for plants, including in drought prone environments (Nadeem et al., 2014). For example, mycorrhiza can increase drought resistance in plants (Davies et al., 1992) and increased plant health can, in turn, support a healthy soil ecology by providing root exudates (Wardle et al., 2004). Bacterial inoculants could also aid in supporting and promoting a healthy soil ecology. Some species, e.g. *Bacillus spp.*, can facilitate mycorrhizal colonisation (Frey-Klett et al., 2007) and act as PGPR's (plant growth promoting rhizobacteria). PGPR's can directly aid plant growth by producing plant growth hormones such as gibberellins (Siddiqui, 2006) or indirectly by inhibiting pathogens (Persello-Cartieaux et al., 2003). Thus microbial inoculants have the potential to improve plant growth and the ecosystem services that result from this, as well as promote and support a healthy soil ecology. Impacts vary depending on the fungal and bacterial species, the species of host plant, the environmental conditions and the soil properties (Hoeksema et al., 2010; Nadeem et al., 2014) and while laboratory and greenhouse studies can provide conclusive results, field studies are far more inconsistent (Trabelsi and Mhamdi, 2013). Hence, there is a need to investigate the efficacy of microbial inoculants within the specific environment for which they are intended to be used.

To date, there are few studies that have investigated the efficacy of microbial inoculants on green roofs. Rumble and Gange (2017) tested their use on a mature green roof to determine if they could remediate poorly established green roofs. A fungal inoculant, *Trichoderma,* had a positive impact on the soil food web but little impact on plant growth. Bacterial and mycorrhizal inoculants had little effect on either the soil community or on plant growth and the authors suggested that this likely was due to the fact that the mature roof already had a resilient microbial population, which was difficult to perturb. Molineux et al. (2017) found that the application of compost tea could enhance fungal biomass within green roof substrates, but that this could have an antagonistic effect on AM fungi. Hoch et al. (2019) also found that soil fungal communities could be altered with the addition of inoculants; In their case the inoculants were obtained from existing green roofs and applied within a glasshouse setting. The current study aims to investigate whether microbial inoculants, specifically PGPR bacteria and mycorrhizae, are successful at promoting plant and soil food web development when applied to a new green roof. The first year of succession is critical in determining subsequent plant establishment on green roofs (Kinlock et al., 2016) and so it is important to understand if the successional process can be influenced by considering the ecological design more carefully at green roof construction. Practically, extensive green roofs are also often designed to be low maintenance once installed, with potential access difficulties after construction, so application of soil inoculants at the construction phase could be the most practical time to do so.

There are two avenues for microbes to be introduced to a novel green roof substrate: Within the substrate itself or in the soil (plugs) surrounding plants on construction. The substrates are impoverished on construction, containing trace bacterial populations and little else (John et al., 2014; Rumble et al., 2018), perhaps due to being fired before storage. Rumble et al. (2018) found that stonecrop plugs do harbour microbes and microarthropods but that most of this component of the soil food web dies out very quickly in the initial months after installation, so in addition to creating a new soil food web using microbial inoculants, these inoculants could support the survival of incoming communities.

We hypothesised that, due to the almost sterile nature of green roof substrates, inoculants will be able to establish more effectively on a newly built green roof than a mature roof, because there is no incumbent microbial community present. We aimed to determine if the addition of inoculants on a newly constructed green roof has an impact on the diversity and abundance of the soil food web and whether plant growth is altered by these inoculants in the important first year of development, to determine if microbial inoculants can speed up green roof development, which is important for the delivery of ecosystem services. In addition, we aimed to determine if microbial inoculants in the green roof substrate can facilitate the survival of microarthropods and microbes that are installed on green roofs from stonecrop plugs, as outlined by Rumble et al. (2018). The paper challenges the current practise of installing a sterile habitat and waiting for it to be colonised naturally, instead investigating ways to install soil ecology with substrates, with the aim of speeding up succession on green roofs where this is desired.

2. Methodology

2.1. Experimental design

A modular green roof, using 30 separate trays as plots, was constructed in June 2011 on an unplanted roof at Royal Holloway, University of London, UK. This site is in Southern England, experiencing a mean annual temperature of 11.5 °C and mean annual rainfall of less than 650 mm, with four distinct seasons ("UK regional climates - Met Office," n.d.). The roof was approximately 20 m from ground-level, with no overhanging vegetation or shade.

The trays were 0.52 m by 0.42 m by 0.10 m with 0.30 m between each tray. All 30 trays were distributed randomly in a 6 × 5 grid across the roof (Supplementary material: Fig.S1). Dataloggers (EL-USB-2, Lascar Electronics Ltd., UK) were placed in trays 9, 15 and 26 to record temperature and humidity at the soil level every 30 min.

Holes were drilled in each tray to allow water to drain, and each tray was lined with a filter sheet (ZinCo SF, ZinCo GmbH, Germany) to prevent particular matter leaching. An extensive substrate (Shire Green Roof Substrates, UK) of crushed red brick with 10 % (by volume) organic matter (rough compost), was added to each tray to a depth of 80 mm. This is within the range commonly used on extensive green roofs (FLL, 2008) and has been used in previous studies (Molineux et al., 2009).

Twenty of the trays were planted with nine stonecrop plugs each, three *Sedum album,* three *Phedimus spurius* and three *Petrosedum reflexum* (except tray 22, which had four *P. spurius* and two *S. album* due to a supply error) distributed randomly, with 0.1 m between each plant. These plants were chosen because they are commonly planted on green roofs. *Plants* had been grown in a greenhouse (Sedum Green Roof Ltd, UK). The soil the plugs arrived in was not removed and no weeding occurred during the study. Ten trays were left unplanted and were not analysed as part of the current study.

2.2. Inoculants

Inoculation took place one week after construction. Inoculants were provided by Symbio Ltd (UK). The bacterial inoculant consisted of PGPR bacteria: *Bacillus laterosporus, B. licheniformis, B. megaterium, B. pumilis* and *B. subtilis*. The mycorrhizal inoculant consisted of *Gigaspora margarita, Glomus aggregatum, Gl. brasilianum, Gl. clarum, Gl. deserticola, Gl. etunicatum, Gl. intraradices, Gl. monosporus* and *Gl. mosseae*. Concentrations were as per the manufacturers recommended concentrations; 1.5 g/m^2 (0.3 g per small plant) for mycorrhiza and 0.96 ml of bacterial solution, mixed with 0.6 l of deionised water. Five of the trays were inoculated with the bacterial inoculant, five with the mycorrhizal fungi inoculant, five with both treatments and five with only deionised water (control). Inoculants were not reapplied at a further time point.

2.3. Temporal surveys

Two soil samples from each plot were taken in alternate months between September 2011 and July 2012, inclusive, using a 1.5 cm diameter core borer driven down to the plot lining (8 cm). Samples from each plot were summed together to overcome problems of clumped microarthropod distributions (Ettema and Wardle, 2002), equating to a total sample area of 3.5 cm^2 and volume of 28 cm^3. 3(± 0.05)g of this soil was removed for PLFA analysis (Section 2.5). The remainder was used for microarthropod extraction by Berlese-Tullgren funnel (Section 2.4).

Due to the small corer size, a pilot study was undertaken comparing three samples taken with a 1.5 cm and three taken with an 8 cm corer. No significant differences were found in terms of total microarthropods extracted p/g between the corer sizes ($F_{1,3} = 3.93$, $p > 0.05$), nor for any sub-group of microarthropod except oribatid mites ($F_{1,3} = 7.99$, $p = 0.048$), which were present in higher abundances in samples taken with the small corer. It was therefore deemed appropriate to use the small corer, ensuring longevity of the plots and a high temporal resolution of samples.

Plant cover is a key metric informally used by the green roof industry as a proxy of installation success (pers. observation). This was analysed every other month; photographs were taken and analysed using Adobe Photoshop (version 9, Adobe Systems Inc., USA). Uncached histograms counted the pixels in each plant and the total pixels in each tray. Using the known area of the tray in m^2, the area per pixel was calculated. These data were used to calculate plant cover and relative ratios of each plant to total cover and to initial plant cover. This was performed for each individual plant that had been planted at construction, as well as for colonising plants contributing to more than 1% of plot cover. Naturally colonising species were identified to species level using Blamey et al. (2003) and consisted of only new individuals of stonecrop (which had spread via fragments of planted species), *Stellaria media* and *Festuca rubra.*

2.4. Microarthropod analysis

Each sample was weighed and subject to extraction by Berlese-Tullgren funnel for five days at approximately 18°C (MacFadyen, 1953). Soil was then reweighed to determine water lost. Microarthropods were stored in 70% ethanol and identified, to species level, or sorted into morphospecies where this was not possible, using dissecting and compound microscopes. Collembola were identified using Hopkin (2007).

2.5. Phospho-lipid fatty acid analysis (PLFA)

Three (± 0.05) 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). Lipid extraction was via Bligh/Dyer solvent and phase separation used chloroform. Fractionation was undertaken using normal phase silica acid columns (Cronus SPE Cartridges Si 1000 mg/6 ml, SMI LabHut, UK), fractioning lipid material into neutral, glyco- and phospholipids (PLFA's). Fatty acid methyl esters (FAMEs) were then obtained via lipid methanolysis of the PLFA fraction, using 0.2 M methanolic KOH. Methyl nonadecanoate (C19:0) was added at this point as an internal standard. FAMEs were identified by chromatographic retention times, with bacterial PLFAs verified with a bacterial FAMEs mix (Sigma Aldrich, St Louis, USA). Analysis was performed by a Hewlett Packard (HP) 5890 gas chromatograph equipped with a flame ionization detector and DB-5 capillary column (30 mm × 0.25 mm i.d., film thickness 0.25 μm). The injection temperature was 250°C. The detector regime was: Start 100 °C> increase at 20 °C min^{-1} > hold 160 °C for 5 min> increase at 3.5 °C min^{-1} > hold 280 °C for 3 min> increase at 20 °C min^{-1} > Finish at 320 °C. Injection was splitless using helium as a carrier gas. FAMEs were identified on an HP 5970 mass spectrometer.

Fatty acid nomenclature followed Frostegård et al. (1993). The abundance of 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 soil bacteria.

2.6. Destructive survey

One year after inoculation, the original stonecrops were removed from all plots. Roots and shoots were separated, and fresh weight of shoots recorded. Roots were washed to remove soil and excess water was squeezed out. The plugs had not dispersed into the substrate 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. After weighing for fresh root weight, a small portion of the new growth roots was removed, weighed and placed in 70 % ethanol for mycorrhizal analysis (Section 2.7). Roots and shoots were then 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.

2.7. Mycorrhizal analysis

Roots were cleared in 10% KOH in a water bath at 80°C for 25 min. Visualization of mycorrhizas was performed using a modified method of Vierheilig and Wyss (1998), whereby commercial ink (Quink washable blue, The Parker Pen Company, UK) mixed with 1 % HCl and water in the ratio 0.6:15:84.4 was added to samples and heated for 15 min in an 80°C water bath. Percentage root length colonized (RLC) was obtained with the cross-hair eyepiece method of McGonigle et al. (1990), whereby samples are spread evenly across a slide and observed at ×200 magnification. Each root piece crossing the crosshair is observed for the presence or absence of fungi in the form of hyphae, vesicles or arbuscules. One-hundred counts were obtained from each sample.

2.8. Statistical analysis

All analysis was performed using SPSS 25.0, except PCA and parallel analysis, which were performed with R (R Core Team, 2015) using vegan (Oksanen et al., 2015) and hornpa (Huang, 2015) respectively. Coefficient of variance (Cv) was calculated for all variables.

Relative plant cover was calculated as the cover in July 2012 compared to their cover at construction. Due to the size of the plant 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.

Repeated measures ANOVA and Bonferroni post-hoc tests were used to analyse microbial mass (total PLFA's), bacterial PLFA's, fungal PLFA's and total microarthropods. None of these data were spherical, so Greenhouse-Geisser adjustments were applied. Fungal PLFA's were Ln+1 transformed and total microarthropod data was square root transformed to meet the assumption of normality. For microarthropods, May did not approximate a normal distribution and included very low species counts (only one observation) so could not be included in the statistical analysis. July did approximate a normal distribution ($D = 20, p = 0.046$) so was included in the analysis, as ANOVA is robust to violations of the assumption of normality (Schmider et al., 2010) and these data did satisfy homogeneity of variances.

Kruskall-Wallis ANOVA was used to analyse all stonecrop plant parameters (relative cover, growth rate, fresh and dry shoot and root weight) mycorrhizal PLFA's, collembola and mites (each month was compared) in addition to mycorrhizal RLC (each plant species and mycorrhizal structure was compared).

PCA's were performed on PLFA and microarthropod data. In all PCA's, month and treatment were added as supplementary qualitative variables and confidence ellipses were drawn at the 95% confidence level.

3. Results

3.1. Microbial community

Mean microbial mass changed over time ($F_{2.7,43.2} = 8.41, p < 0.001$), with lowest mean mass occurring in January 2012 and mean mass increasing thereafter towards May 2012 (Fig. 1a.). Bacterial PLFA's changed in microbial mass each month, with no clear overall pattern. Fungal PLFA's increased over time, particularly in March 2012 to July 2012 (Fig. 1a.).

Mean microbial mass was higher in plots treated with mycorrhiza, either singly or in a mix with the bacterial inoculant ($F_{1,16} = 4.61$, $p < 0.05$; Fig. 1b.). Analysing constituent PLFA groupings, higher mean masses of both bacterial ($F_{1,16} = 4.49$, $p = 0.05$; Supplementary material: Fig. S2a) and fungal ($F_{1,16} = 4.99$, $p < 0.05$; Supplementary material: Fig. S1b). PLFA's were present in plots treated with the mycorrhizal treatment, both singly and in combination with the bacterial inoculant, over the control plots. There was no impact of any of the inoculants on PLFA's relating to arbuscular mycorrhizal fungi, but these values were extremely low during the sample period (data not shown).

PCA determined that three axes explained most of the variance in the PLFA community, explaining 34 %, 20 % and 11 %. PCA showed very little difference between treatments overall (Supplementary material: Fig. S3a). The slight differences between treatments grouped the plots treated with bacteria and control plots together, whilst there was a second overlapping group that related to plots treated with mycorrhiza and plots with the mixed treatment (Supplementary material: Fig. S3b).

3.2. Arbuscular mycorrhizal fungi

Hyphal counts varied between 0 % and 63 % RLC 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. Plants that contained vesicles ranged from 0 % to 24 %, with most (98 %) plants having counts of fewer than 50% vesicles. Arbuscules were extremely rare, ranging between 0 % and 2 %, with 95 % of plants containing no arbuscular counts. There were no plant species-specific differences in mycorrhizal colonisation (data not shown).

There were no significant effects of the addition inoculants on mean RLC overall, nor on hyphae alone (Supplementary material: Fig. S4). There was a positive additive effect of adding mycorrhiza and bacteria together on the mean number of vesicles counted ($F_{3,165} = 4.24$, $p < 0.05$) compared to single inoculants (though not to the control). Single inoculants produced no differences in mean vesicle number compared to the control (Fig. 2).

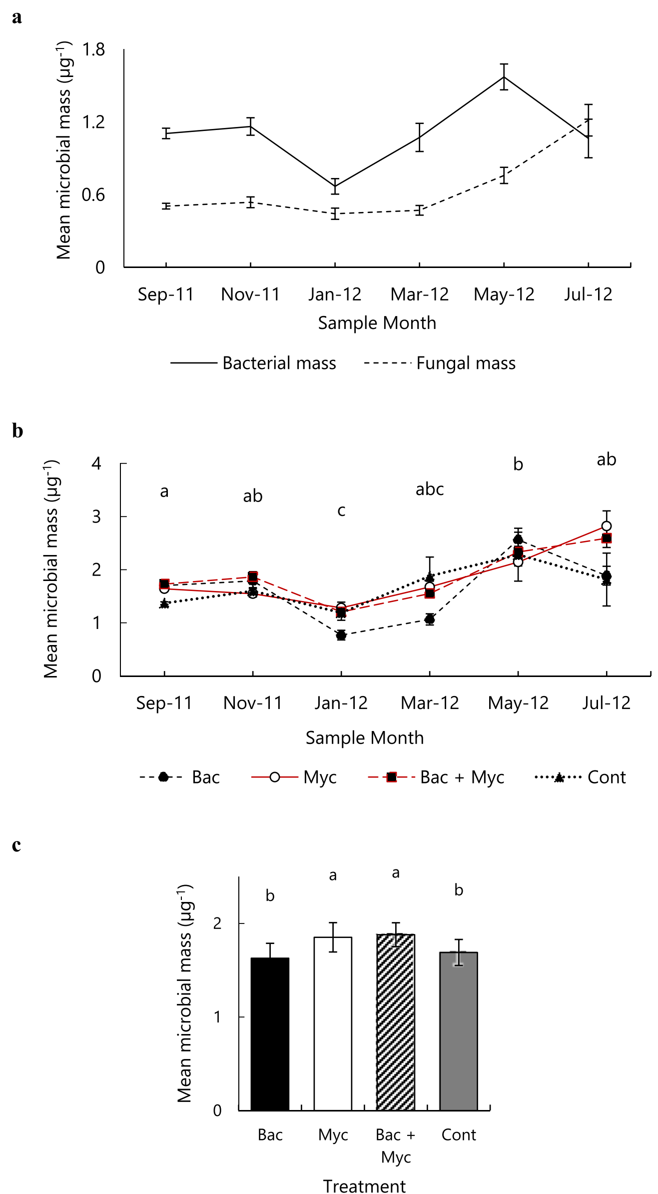

Fig. 1. Mean microbial mass of (a) bacterial and fungal PLFA's in all treatments over time; (b) all PLFA's split out by treatment over time; (c) all PLFA's split out by treatment across all time points. Error bars represent standard error of the mean. Letters denote statistically similar (a & b) months or (c) treatments. Red dashed lines denote statistically significant treatments.

Presence of hyphae also differed between species ($\chi^2(2) = 7.57$, $p < 0.05$), with higher colonisation in *S. album* than in *P. spurius*. Vesicle presence did not differ between stonecrop species (data not shown).

3.3. Plant Performance

Mean relative cover of *S. album* and *P. reflexum* was not affected by inoculants, though there was a suggestion that bacteria may have had a positive impact on *S. album* growth ($\chi^2(3) = 6.58$, $p > 0.05$; Fig. 3a). There was a suggestion that mean relative cover of *P. spurius* demonstrated an additive negative effect of adding both inoculants together ($\chi^2(3) = 7.68$, $p = 0.053$; Fig. 3b).

The mean rate at which *S. album* achieved cover was not impacted by

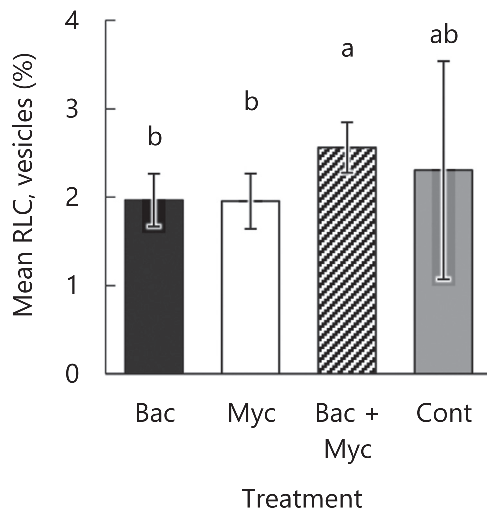

Fig. 2. Mean percentage RLC of vesicles in all plant species in plots treated with bacteria, mycorrhiza, bacteria + mycorrhiza and untreated (controls). Error bars represent standard error of the mean. Letters denote statistically similar results.

inoculant addition. For *P. spurius* there was an additive negative effect of adding mycorrhiza and bacteria ($\chi^2(3) = 9.04$, $p < 0.05$; Fig. 3c). There was also an additive negative effect of adding both inoculants on *P. reflexum* ($\chi^2(3) = 8.10$, $p < 0.05$; Fig. 3d), though unlike in P. *spurius* this did not reduce the rate of growth below that of the control. Cv was higher for all plants treated with mycorrhiza alone than for the control or other treatments, with several high growth rate outliers present in *P. reflexum* and *P. spurius* plants treated with mycorrhiza (Supplementary material: Fig. S5).

The addition of bacterial and fungal inoculants had no effect on mean fresh or dry shoot weight for any of the plant species (Supplementary material: Fig. S6a & 6b).

3.4. Microarthropods

Twenty-two species of microarthropod were extracted during the sample period, all at low abundances. Parallel analysis determined that the community was extremely complex. The first two axes were deemed to be retained, but this only explained 23.2 % of the variance, with a further 17 axes identified. The most prevalent group of microarthropods were collembola, totalling 235,669 individuals/m^2 over the sample period.

These were made up of five species: *Brachystomella parvula, Orchesella villosa, Parisotoma notabilis, Sminthurinus aureus* and *Sminthurinus trinotatus*. Mites were the second most abundant group, totalling 141,543 individuals/m^2 over the sample period. Present were 12

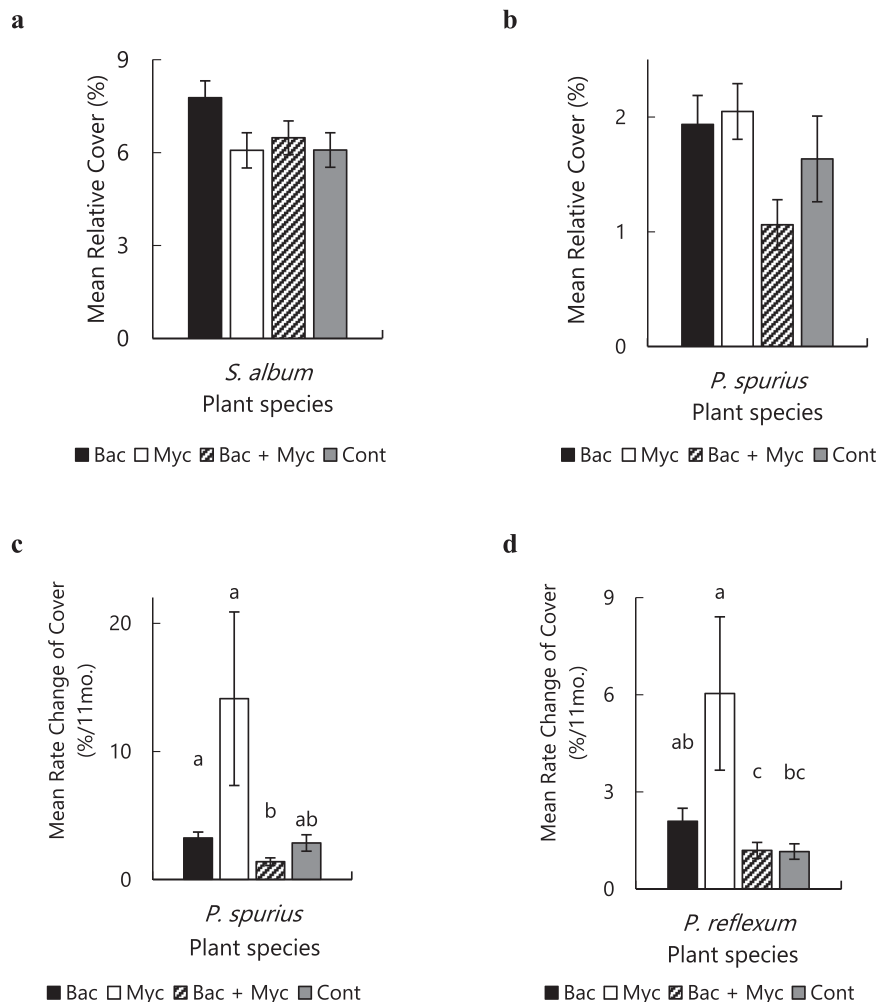

Fig. 3. Mean relative cover of (a) *S. album* and (b) *P. spurius* under different inoculant treatments and mean rate of change in cover of *P. Spurius* and *P. reflexum*. Error bars represent standard error of the mean. Letters denote statistically similar results.

morphospecies of mite including two oribatid mites, two Tydeids and one species of the family Bdellidae. Organisms not belonging to collembola or mite families remained in low abundance throughout most of the sample period ($\overline{X}$ =700 ± 10), only reaching more than 1000 individuals/m^{-2} in June 2012 ($\overline{X}$=2784 ± 633). These were made up of four families: Aphidae, Diptera (chironomids and other fly larvae), and Thysanoptera.

Mean microarthropod abundance on the roof changed over time ($F_{1.71,27.30}$ = 8.72, $p < 0.01$), with mean abundance peaking in July 2012 and to a lesser extent January 2012 (Fig. 4a). All groups drastically declined in number in the March sample, with almost no microarthropods sampled in May. PCA determined that the July sample consisted of a different community than previous months, aligning most with Axis 1, rather than with Axis 2 as September to March do. Thysanoptera, *S. trinotatus,* Tingidae and two species of mite align most with Axis 1 (Fig. 4b).

The bacterial treatment supported significantly more microarthropods than other plots when applied singly and in combination with mycorrhiza ($F_{1,\ 16}$ = 4.64, $p < 0.05$; Fig. 5a). Seasonal changes in mean abundance were not affected by the addition of inoculants (Fig. 5b).

The variability of microarthropods compared to the mean (C_V) was less over time in all inoculated plots than in control plots

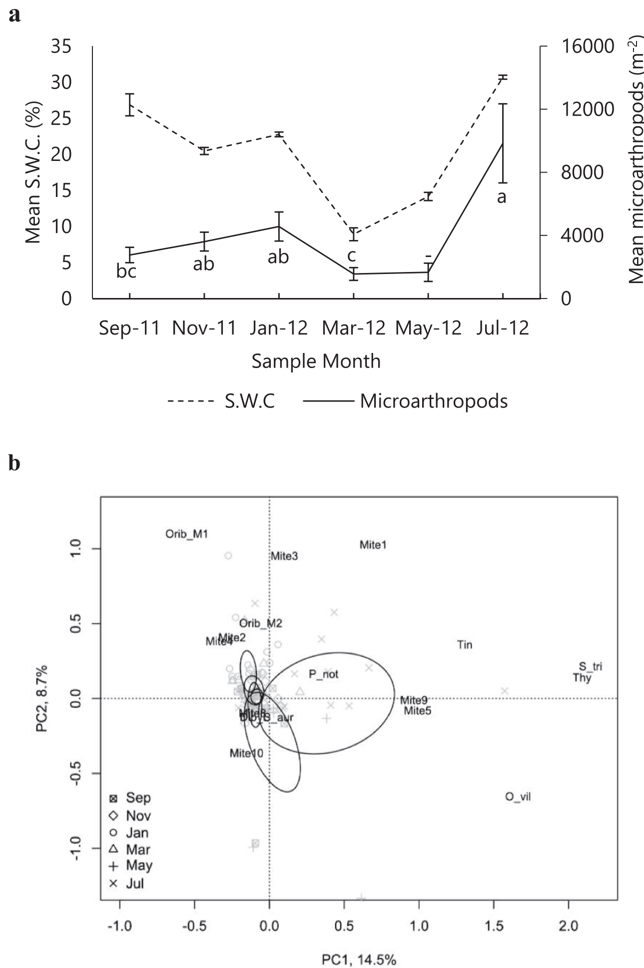

Fig. 4. (a) Mean microarthropod abundance over time, with percentage substrate water content (S.W.C) plotted on a secondary axis. Error bars represent standard error of the mean. Letters denote statistically similar groups. (b) zoomed PCA ordination plot (see Supplementary material: Fig. S6 for unzoomed plot) of the total microarthropod community for all time points and treatments. Confidence ellipses separate time points.

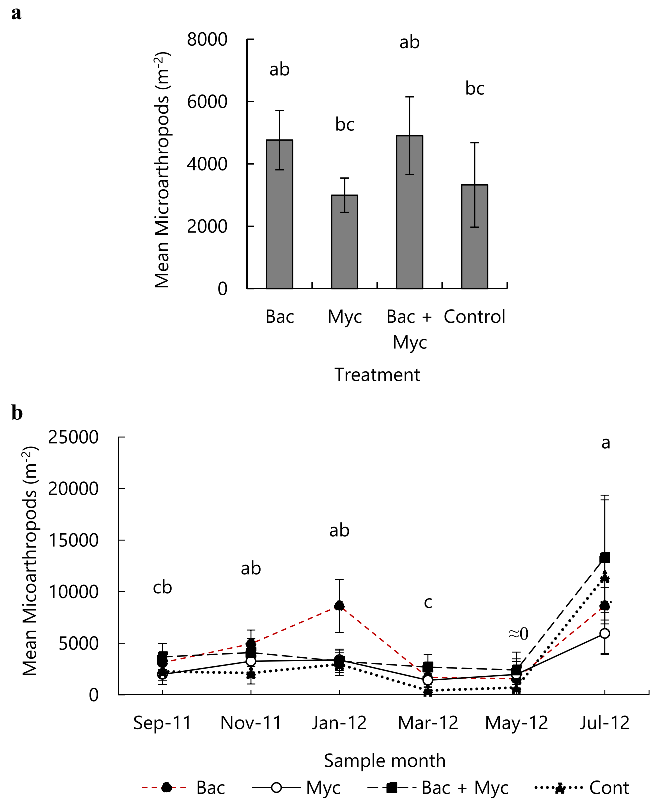

Fig. 5. Mean microarthropod abundance (m^{-2}) (a) across all time points per treatment and (b) per treatment each month. Letters denote statistically similar groups. Error bars represent standard error of the mean. Red dashed lines represent statistically significant treatments.

(Supplementary material: Fig. S7). Overall, the community varied less in plots inoculated with only fungi than in control plots and plots inoculated with bacteria (Supplementary material: Fig. S8).

PCA determined that there were few differences in microarthropod community composition between treatments and the control (Supplementary material: Fig. S9a & b). The small differences that were present within the PCA grouped the single inoculants together along PC1, along with Thysanoptera, *S. trinotatus,* Tingidae and some species of mite. The mixed treatment and the control correlated with PC2 with fly larvae and some mites, including Scutoverticidae (Supplementary material: Fig. S9a & b).

Numbers of individual microarthopod groups were very low and therefore challenging to produce robust statistics for. Kruskal-Wallis tests across all time points suggested that none of the inoculants had any statistically significant impact on either mites or collembola (Supplementary material: Fig. S10a & b).

4. Discussion

The results suggest that on newly constructed green roofs, bacteria can be used as effective "helper" species to facilitate mycorrhizal colonisation, but that this does not translate to enhanced plant growth. When applied singly, bacterial inoculants also increased the abundance of microarthropods on our new green roof, suggesting that inoculation at green roof construction can alter the later community dynamics within the soil of a green roof. These results were similar to those we found on a mature green roof, reported in Rumble and Gange (2017), where *Trichoderma spp.* altered microarthropod communities, but without resultant impacts for plant growth. In the current study, we wanted to understand if a new green roof, with no incumbent soil food web, would respond more favourably to microbial inoculants. While we

have demonstrated again that microbial inoculants can alter other trophic levels in the soil food web, we have also again noted that this has had limited impacts on plant growth. Thus we suggest that there are other factors that limit the success of bacterial and mycorrhizal inoculants on green roofs, regardless of the timing of inoculation.

4.1. Soil microbial community

Total microbial mass over the study period was within the range reported on other young green roofs by Molineux (2010), characterised by a bacterially dominated microbial community, with all PLFAs low in abundance at the start of the experiment. Fungal PLFAs increased over time. Recent research suggests that plants can play a surprisingly minor role as drivers of microbial succession in new soils (Brown and Jumpponen, 2014) and that bacteria and fungi may have different and separate successional trajectories depending on a number of factors, including propagule availability (Schmidt et al., 2014) and parent material (Alfaro et al., 2017). As green roof substrates have a unique parent material and are exposed to a unique urban propagule mix, it is unclear what microbial succession looks like in this environment, and this merits further research to determine the most effective time to apply inoculants.

4.2. Mycorrhizal colonisation, inoculation and plant growth

All species of plant were mycorrhizal in this study, including those in the control plots where no mycorrhiza was added. Wild *P. reflexum* and *P. spurius* are not reported as being mycorrhizal (Harley and Harley, 1987), yet have been found to be consistently mycorrhizal in our previous studies (Rumble et al., 2018; Rumble and Gange, 2017). A sub-sample of plugs analysed on delivery (Rumble et al., 2018) suggested that these were mycorrhizal before being planted on the roof although no inoculant was added at the plant nursery (Sedum Green Roof, pers. comm.). This together with the presence of mycorrhizas in the control plants suggests that a wider range of stonecrops than previously thought may form spontaneous mycorrhizal associations. Studies on mycorrhizal associations with stonecrops are rare, so these species have probably been overlooked.

Despite the fact that stonecrop. plugs were mycorrhizal even where inoculants were not added, we do think that our inoculants also colonised our plants because we observed morphological differences in mycorrhizae post-inoculation: Vesicle presence was higher in all three plant species in plots where the mixed treatment had been applied. There are two possible reasons we hypothesise for these morphological changes. Either the dominant VAM species changed, or a stress response, expressed in an increased number of vesicles, was elicited.

In the case of a species change, a change in mycorrhizal species in mixed plots but not in plots where mycorrhiza alone was added suggests that inoculated mycorrhiza, aided by bacteria, colonised the roots. Bacterial inoculants have been shown to be effective helper species for mycorrhizal colonisation, with the addition of both together shown to increase plant yield (Medina et al., 2003; Vivas et al., 2003b,a). This effect is likely to be species specific, both in terms of the bacterial and fungal species involved as well as the host plant species (Jäderlund et al., 2008; Xie et al., 2018); While we detected a reduction in rate of plant cover for *P. spurius* and *P. reflexum*, no changes in the biomass of these plants was observed; As they are not creeping species, like *S. album,* the cover of these species is likely to be less important as an indicator of plant growth, our conclusion is that in this instance, no significant impacts on plant growth were seen. Stonecrops are habitat specialists of rocky environments: Habitat specialists are often classified as non-mycorrhizal, with infection by mycorrhizae assumed to be opportunistic and commensal (Brundrett and Tedersoo, 2018). It has been hypothesised that these types of plant species may have less need of fungal associations due to the low competition environments in which they live (Olsson and Tyler, 2004) and may therefore be more conservative in terms of which fungal species they associate with. Moreover, we did not analyse the soil chemistry of our green roofs, which means we do not know if phosphorous (P) was limited or not. Mycorrhiza have been shown to be more beneficial to plant development if P is limited (Treseder and Allen, 2002) and several authors suggest that green roof substrates may be high in P, ascertained from the high volumes of P found in green roof leachates (Aitkenhead-Peterson et al., 2011; Harper et al., 2015).

It is clear that for our selected stonecrop species, species-specific relationships between mycorrhiza and the host plant need to be investigated, rather than using commercial species mixes. Hoch et al. (2019) have successfully demonstrated soil microbial species composition changes when inoculants created from green roof parent soils are applied under glasshouse conditions; this suggests that more appropriate inoculants for green roof plant assemblages could be designed and applied, potentially creating more resilient green roofs. Our results suggest that if beneficial mycorrhizas can be found for specific stonecrop species., helper bacteria may be an effective aide to their colonisation.

While we did not find any impact on plant growth of adding bacterial PGPR's without mycorrhizal fungi, (Xie et al., 2020) found that some bacterial PGPR's, particularly *R. irregularis* and *B. amyloliquefaciens*, did have positive impacts on plant growth, focussing on ruderal species (e.g. *Trifolium repens*) on green roofs. This adds further support for the idea that commercial inoculants may be successful on green roofs, but need to be tested in a wide variety of substrates with a wide variety of plant/inoculant pairings.

4.3. Microarthropod community dynamics

The density of all species was low throughout the sample period. While these densities were similar to other studies on green roof microarthropods (Rumble and Gange, 2013) and to other ruderal habitats (Wanner and Dunger, 2002) and, as expected considering the young age of the roof, lower than some other studies of mature green roofs (e.g., Jacobs et al., 2022). The species found were also similar to other studies, particularly in the case of the common, cosmopolitan collembolans *S. aureus* and *P. notabilis*, which have been found in several previous studies of green roof substrates (Jacobs et al., 2022; Joimel et al., 2022; Rumble and Gange, 2013; Schrader and Böning, 2006).

Populations crashed in March and May 2012, when substrate water content was at its lowest, supporting our previous findings that substrate water content is a limiting factor for microarthropods on green roofs, particularly collembola (Rumble and Gange, 2013), though we must acknowledge that, unlike Rumble and Gange (2013), which took place on a large green roof, our small trays likely dried out quicker than a real green roof would.

Microarthropod populations recovered from drought quickly, by the July sample. However, PCA determined that the community had changed, consisting of more plant eating microarthropods, such as Tingidae and Thysanoptera, likely due to increasing vegetative matter and fewer decomposers, such as collembola and mites, likely due to drought, than in previous months. This suggests that community composition can functionally change not only as plant succession occurs but also because of drought. Given the increasing likelihood of drought in urban areas due to climate change (Revi et al., 2014), there is a need to understand this process better, to identify how best to mediate this environmental challenge. Soil microarthropods are key to providing resilience in soil communities, regulating soil decomposition (Cragg and Bardgett, 2001) and C:N ratios (Yang et al., 2012). Based on the current study and that of Rumble and Gange (2013), the succession of these species seems to be continually halted and altered by drought events (albeit more often in the current study due to the small tray size), potentially reducing microarthropod populations and limiting their important functions in this environment.

4.4. Impact of inoculants on microarthropod populations

Whereas microbial mass was highest in plots treated with mycorrhizal inoculant, the abundance of microarthropods was higher in those plots treated with the bacterial inoculant. The most common species on the green roof, the collembola *P. notabilis,* preferentially feeds on amorphous organic material (Gillet and Ponge, 2003). It is plausible that the bacteria added in the current study 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, another common source of food for *P. notabilis* (Gillet and Ponge, 2003). Though most other collembola are predominantly fungal feeders, there is evidence to suggest that their diet varies depending on the availability of food. *O. villosa,* and *S. aureus* are both documented diet-shifters (Haubert et al., 2009) and *P. notabilis* is a generalist species found in a wide variety of environments (Porco et al., 2012). In the case of a direct relationship between the bacterial inoculant and collembola, the absence of an increase in bacterial PLFA's in the bacteria treated plots could be due to grazing by these collembola (Chamberlain et al., 2006). This hypothesis would also suggest that the fungal community is limited in its ability to support collembolan communities on green roofs 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.

Molineux et al. (2017) stated that green roofs are an N limited environment, limiting plant growth. This emphasises the importance of collembola on green roofs, as they are a key regulator of C/N ratios (Verhoef and Brussaard, 1990). Moreover, this is especially true in dry environments, such as a green roof, because unlike many other decomposers (e.g. nematodes, bacteria) collembola are active in a range of environmental conditions (Filser, 2002), making them a decomposer species that is suited to hostile conditions and resilient to environmental variability; Filser (2002) hypothesises that this is due to the ability of collembola to shift diet under drought conditions, from microfauna (e. g., bacteria) to fungi, which are also more drought tolerant. Thus, increasing their populations could enable greater availability of soil nutrients for uptake by plants. It should be noted though that repeated drought events, as we observed in Rumble and Gange (2013) can erode springtail communities; Peguero et al. (2019) suggest that climate change induced droughts will be a key driver of this in the future, limiting nutrient cycling as a result.

For all groups of microarthropods, we saw only very small differences in species composition between treatments. Most of the species we recorded on the green roof were cosmopolitan, generalist species with a high dispersal ability, therefore we must assume that, to some extent, species were able to move between our sample plots, which may have homogenised the plots over time. However, there were differences in community composition that aligned with treatment type with control and mixed plots grouped differently to single inoculant plots, suggesting that this potential homogenisation was not great enough to outweigh the impacts of the inoculations. Diptera and some mites, including Scutoverticidae, seemed to be driving the control/mixed plot axis, while collembola and other groups such as Thysanoptera and *Tingidae spp.* drove the single inoculant axis. This result suggests, as with the microbial community, that species composition can be altered by inoculation and that although differences were small at this stage, the trajectory of succession could be altered by the addition of inoculants. Srivastava (2002) notes that in unsaturated, ruderal environments such as this, functional loss can be extremely difficult to mitigate, emphasising the importance of each specific species present in this environment. While microarthropod populations were boosted by the bacterial inoculant, the lack of an interaction over time and the overall population crashes in all treatments in March and May suggests this was not sufficient to overcome the impacts of drought, which can also be an issue on full size green roofs (Rumble and Gange, 2013).

While we specifically studied the impact of microbial inoculants, these results also suggest that other inputs of microbes, such as different types of compost, may impact the later development of a green roof if applied at construction. Joimel et al. (2018) suggest that this is a key source population of collembola on green roofs. While our specific starting substrates, including the compost element, supported little biodiversity and we saw poor survivorship of species coming from compost around the plug plants (Rumble et at., 2018), different types of compost could act in a similar way to our microbial inoculants; We recommend that this should be investigated as another potential ecological design element on green roofs.

5. Conclusion

The paper adds to our sparse knowledge of plant-soil interactions on green roofs. It demonstrates that a soil food web can be installed during a green roof's construction, rather than waiting for natural colonisation, though further work is needed to design the optimum components of this soil food web: Bacterial inoculants benefitted microarthropod populations, particularly collembola, but this did not translate into better plant growth. Mycorrhizas can be effective at colonising green roof plants, especially in the presence of helper bacteria, but the mix of species used in the current study had no impact on plant growth for our selected stonecrop species. Future research should investigate inoculants that are more specific to the planting regime and should also investigate whether other methods, such as applying microbial-rich compost at construction could facilitate soil community development. Our results were similar to those observed on a mature green roof, suggesting that the timing of inoculation may not be an important factor in enhancing plant growth.

CRediT authorship contribution statement

Heather Rumble: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Project administration, Writing – original draft, Writing – review & editing. **Paul Finch:** Investigation. **Alan C. Gange:** Funding acquisition, Methodology, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ufug.2022.127684.

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