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Microbial inoculants as a soil remediation tool for extensive green roofs

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Abstract

Green roofs are increasingly used in the urban environment to insulate buildings, reduce stormwater runoff and remediate biodiversity lost in construction. Most common in the Northern Hemisphere are extensive green roofs, due to their low cost and low maintenance requirements. However, plant growth on these roofs is often limited and this could have implications for ecosystem service provision as well as reduce the economic feasibility of green roofs as an aesthetically successful product. In addition, the increasing popularity of green roofs as an eco-product means that a high number of these roofs, that do not reach their maximum potential in terms of plant growth, already exist, highlighting a need for a successful remediation tool post-build.

Previous studies suggest that the soil food web on green roofs, integral for nutrient cycling in soils, is also lacking and that this may be an effective aspect to target in order to improve plant establishment and success. Microbial inoculants have already been added to green roofs, but with little scientific research informing their application. In this field experiment we aimed to determine if the addition of these foundation species in green roof soil food webs, including mycorrhizas, *Trichoderma spp.* and soil bacteria, could improve the abundance and biodiversity of higher trophic species, such as microarthropods, and if this had resultant effects on plant growth on a mature green roof.

It was found that some microbial inoculants were more successful at remediating soil food webs than others, with *Trichoderma* in particular producing higher populations of some microarthropod groups. However, these changes in microarthropod community dynamics did not have a resultant positive effect on *Sedum spp.* growth. The authors hypothesise that mature extensive green roofs have an established microbial community that may limit the success of commercial inoculants. This is the first study to demonstrate multi-trophic community changes as a result of the addition of soil microbial inoculants.

Keywords

Microarthropod; Mycorrhiza; Trichoderma; Bacteria; *Bacillus;* Green Roof

1. Introduction

Green, or ‘living’, roofs (vegetated roofs) are of increasing interest to architects, city planners and civil engineers across the globe due to the multitude of benefits they can contribute to a building’s performance in areas such as energy efficiency and sustainable drainage (VanWoert et al., 2005; Jaffal et al., 2012). Extensive green roofs are common in the Northern Hemisphere, and in the UK usually comprise of a shallow substrate (no more than 10cm) consisting of crushed brick, planted with hardy plants of the genus *Sedum* (Grant, 2006). Despite their continuing prevalence, many extensive green roofs fail to establish at a satisfactory rate or, in some cases, fail to establish completely and require costly remediation (McIntyre and Snodgrass, 2010). In addition to this economic problem, poor plant establishment could also result in green roofs that are not maximised in terms of their ecosystem

services provision (Williams et al., 2014). For example, as carbon sequestration is related to plant biomass (Getter et al., 2009), the contribution to carbon savings afforded by a green roof with poor plant growth is likely to be negligible. Green roof vegetation is also expected to reduce indoor air temperatures via evapo-transpiration (Jim and Tsang, 2011) which, again, is likely to be affected by the size and health of plants on the roof. Hence the reported benefits of a green roof are inherently reliant on the success of vegetative growth.

Examining the soil biota present within the substrate of a green roof could hold the key to ensuring the success of vegetation establishment. To date, interactions between soil fauna and above-ground communities on green roofs have been largely ignored, despite above and below-ground communities at ground level having been shown to be inextricably linked (Wardle et al., 2004). Below-ground processes (or within-substrate in the case of a green roof) are key for nutrient cycling, promoting plant productivity, permitting decomposition, buffering environmental changes and improving water retention (Neher, 1999).

Much of the nutrient cycling occurring in soils relies on three things: the decomposition of plants, exudate production by living plants and inputs of inorganic nitrogen (Neher, 1999). Decomposition is facilitated by microbes, including bacteria and fungi, microarthropods, such as mites and Collembola, and macro-arthropods, such as earthworms, all of which reside in the soil (Neher, 1999). Previous research suggests that many of these key functional groups are missing or impoverished in a green roof environment (Rumble and Gange, 2013). In addition, those populations of microarthropods that are present on green roofs can experience dramatic seasonal population declines caused by drought (Rumble and Gange, 2013).

Getter and Rowe (2008) suggest that increasing the depth of green roof substrate benefits the growth of some *Sedum spp.* However, in the case of remediating green roofs that are already seen to be failing, adding substrate is far from ideal due to incurred cost, increased loading and the requirement to replant the roof. Thus, a remediation tool that is low cost and low maintenance needs to be investigated.

Green roofs are a harsh environment, typically experiencing high surface temperatures in summer and high winds throughout the year (Getter and Rowe, 2008). The microarthropod communities present reflect this, with the type of species found and their abundance similar to that of a desert, or glacial foreland (Wallwork, 1972; Kaufmann et al., 2002; Rumble and Gange, 2013) . van der Heijden et al., (2008) suggest that bacteria and fungi are responsible for the majority of decomposition taking place in soils, but this varies between habitats. In desert soils, for example, the removal of fungi from soils can cause a decrease in soil decomposition of nearly 30%, whilst the exclusion of microarthropods can reduce decomposition by over 50% (Santos and Whitford, 1981). Thus, it can be inferred, that in an impoverished green roof soil community, decomposition may be limited and therefore enhancement of the soil community could have a positive effect on plant growth.

Previous research suggests that green roof *Sedum spp.* can establish relationships with mycorrhizal fungi (Rumble and Gange, 2013), but that bacteria and free-living fungi are not present at sustainable levels in mature green roofs (Rumble, 2013). In other anthropogenic microbially-poor environments, such as amenity turf, the addition of microbial inoculants has been shown to have some beneficial effects on plant growth. For example, Butler and Hunter (2008), found that the addition of microbial inoculants to golf putting greens increased plant tolerance to stress. However, they questioned the ability of mycorrhizas to colonise roots in this environment. In general, it is recommended that testing be carried out on each new environment before industrial scale application of inoculants takes place, due to the potentially unpredictable results interacting soil microbes may proffer (Corkidi et al., 2004).

Little such testing has been carried out on green roofs, but the few studies that exist have also reported unpredictable findings. Molineux (2010) found that the addition of mycorrhizas and compost tea (liquid obtained via aerobic digestion of composts) to green roofs planted with *Plantago lanceolata* improved plant growth for the first year alone and some competitive effects between inoculants were noted. She also found that fungal and bacterial biomass on green roofs could be enhanced with the addition of microbial inoculants (Molineux et al., 2014). The need for studies such as this is pressing, as commercial inoculants, including mycorrhizas and other microbes, are already used in the green roof industry, for example on the California Academy of Sciences green roof (McIntyre and Snodgrass, 2010). This is despite the relative lack of empirical evidence to determine if they improve plant growth on green roofs, or have an effect on other green roof organisms.

Determining the effects of inocula addition on non-target living roof organisms, such as microarthropods, also provides clues as to how species interactions occur in green roof substrates, a factor that is completely unknown. The success of microbial inoculant addition in enhancing plant growth is reliant upon the microarthropods present, as these organisms contribute to the regulation of nutrient release from soil microbes (Bünemann et al., 2006). The relationships between and within above and below-ground organisms are difficult to determine, due to the cryptic nature of soil, so soil food web experiments have typically been conducted by adding or removing soil food web components in order to observe resultant changes in flora and fauna. For example, Chen and Wise (1999), in exploring whether soil food webs are bottom-up or top-down controlled, added nutrients to the soil in the form of mushrooms, potatoes and instant *Drosophila* medium (Formula 4-24, Carolina Biological Supply, Burlington, N. Carolina). They then studied soil arthropod communities to determine if increases in populations would result from the addition of these different nutrient sources, finding this to be true for most groups of soil fauna. Other studies testing the same nutrient addition principle have reported similar results (Kajak, 1981; Davidson and Potter, 1995). Commercial inocula could have similar effects to fertilizers, by mobilising nutrients currently unavailable to plants, enabling higher uptake (Schubert and Lubraco, 2000) and, theoretically, by providing food for higher trophic groups.

To our knowledge, analysis of higher trophic groups within the soil after the addition of microbial inocula has never been conducted to test this theory.

Commercial inocula typically consist of three major groups of soil organism: mycorrhizal fungi, bacteria (particularly *Bacillus spp.*) and *Trichoderma*, again as a mix of species within the genus (Trabelsi and Mhamdi, 2013). In addition, commercial inoculants typically contain mixes of species, in order to increase the probability that a species specific relationship can develop (Koomen et al., 1987; Gadhave et al., 2016). There is evidence to suggest that in some cases, however, an antagonistic relationship may develop between inocula species (Molineux, 2010), negating their desired effect. Here we describe a study in which three commercial inocula mixes, encompassing mycorrhizas, bacteria and *Trichoderma* were added to a mature green roof to determine if commercial inocula applied singly, or in combinations, affects the soil microarthropod community, and if this has resultant (or independent) effects on plant growth. We hypothesised that the addition of microbial inoculants to a green roof will alter the abundance and community structure of microarthropods and that this would have a resultant effect on plant growth. However, whether this effect would be positive, or negative, is not predictable based on past research.

This is not only the first study to examine such interactions on a green roof but, to the authors knowledge, is the first study to examine if the addition of soil microbes has an effect on soil microarthropod communities in a field situation. It also has direct applicability to the green roof industry, where commercial inoculants are already applied but have not been thoroughly tested.

2. Materials and methods

2.1 Study sites

Permanent plots were established in a randomised block design on a green roof situated in the grounds of Royal Holloway, University of London in July 2011. This roof was the focus of a previous study examining microarthropod communities present in 2010-11 (Roof B: Rumble and Gange, 2013). The green roof is situated on the top of a 12m high building and has an area of approximately 2240m^2 in total. It was built in 2004, so was 7-8 years old at the time of the current study. The roof substrate is comprised of approximately 75mm of a 4:1 crushed brick: to organic matter mix (respectively), planted with *Sedum album*, *S. acre*, *S. spurium*, *S. kamtschaticum* and *S. rupestre*, in proportions of approximately 3.5:3.5:1:1:1. No fertilisation, supplementary watering or removal of naturally colonising plants has ever occurred on this roof.

Each plot was 1m x 1m, with no plot closer than 1m to another in any direction. The commercial inoculants, supplied by Symbio Ltd. (Wormley, Surrey), were species mixes of bacteria (Bac), mycorrhiza (Myc) and *Trichoderma* (Tri) (Supp 1) and were applied once to the plots in July 2011 in a fully factorial randomised block design (Supp 2), resulting in a total of eight treatments including the control. They were not reapplied at a further time point. Inoculants were applied at the manufacturers

recommended concentrations. For *Trichoderma* this concentration was 2.46ml in 0.6l water m^{-2} and for bacteria it was 0.96ml in 0.6l water m^{-2}. The recommended rate for mycorrhiza was 2-3g per large plant, resulting in 6g applied to each plot (as, on average, each plot contained two large *Sedum spp.* plants). This was mixed with 0.6l of deionised water to aid equal dispersal and to ensure all plots received equal volumes of water. Deionised water alone was added to control plots. There were five replicates of each treatment. These plots were then monitored, as outlined in the following sections, for a period of twelve months, with the trial period ending in July 2012.

2.2. Abiotic factors

Mean monthly temperature and rainfall for the South-East of England was acquired from the Met Office (Met Office, Exeter, UK) and means calculated for the entire period preceding the sample date (i.e. the January value is the mean of dates taken in January pre-sample, December and November post-sample). Two dataloggers (EL-USB 2; Lascar, Salisbury, UK) were placed on the roof, one near the West end of the roof and one near the East end of the roof. These recorded surface temperatures and relative humidity every 30 minutes. The average of both dataloggers was used in the analysis.

2.3. Plant surveys

Plant surveys of each plot were carried out in January, May and July 2012. Individuals were counted and identified to species level where possible using Blamey et al., (2003). Additionally, vegetation cover was estimated by eye, with the aid of a gridded quadrat containing 100 divisions of $100cm^2$ each. Plant cover was estimated for each of these divisions and summed to obtain the total plant cover for the $1m^2$ plot.

2.4. Mycorrhizal sampling

Before inoculation, in July 2011, two subsamples of root (approximately 2g each) were taken from one individual of *S. spurium* from each plot without removing the plant, and tested for the presence of mycorrhizas. Individual *Sedum* plants on the green roof were large, but there were few individuals (approx. two per plot), so destructive sampling of the entire plant was not deemed appropriate, and no more than two subsamples could be taken. *Sedum* on the roof had extremely large root systems (approximately 3-4x larger than above-ground biomass), and the loose nature of the substrate meant that small portions of root could be removed easily. This meant that repetitive root sampling, as has been performed on trees (Moreira et al., 2006), could be implemented. Thus, in July 2012, the same *S. spurium* individuals were once again examined for mycorrhizas, by removing another portion of their root systems. Roots were washed with tap water and cleared in 10% potassium hydroxide (KOH) in a water bath at 80˚C for 25 minutes. The KOH was then disposed of and roots were thoroughly washed and dried. Visualization of mycorrhizas in the roots was performed using a modified ink staining method of Vierheilig et al., (1998), whereby commercial ink mixed with 1% HCl and water in the ratio

84.4:15:0.6 was added to the samples and heated at 80˚C in a water bath for 15 minutes. Root samples were stored in stain until ready to be analysed.

Percentage root length colonized was obtained with the cross-hair eyepiece method of McGonigle et al., (1990), whereby samples are spread evenly across a slide and observed at x200 magnification. Each root piece crossing the centre of the eyepiece, or the crosshair, is observed for 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. Microarthropod sampling

Microarthropod samples were taken from each plot, every two months between September 2011 and July 2012. A 5cm diameter soil corer was pushed down to the roof lining at approximately 7.5cm. This was repeated once in each plot and the two samples pooled to overcome problems associated with clumped microarthropod distributions (Ettema and Wardle, 2002). This resulted in a 295cm^3 sample of substrate from each plot.. 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 until further analysis. Microarthropods were sorted to morphospecies using a dissecting microscope at x100 magnification. Species identification, where possible, was then performed at higher magnifications (x200-1000) using a compound microscope. In the case of mites, this was usually restricted to the most prevalent mites, and species level identifications were rarely obtained. Less common mites were identified to the highest level possible or 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), Walter and Proctor (2001) and Krantz and Walter (2009). Hemiptera were identified using Southwood and Leston (2005).

2.6. Statistical analysis

Analysis was performed using SPSS 22.0, except PCA, which was performed using R (R Core Team, 2015). Diversity of vegetation was measured using the Shannon-Wiener index and mycorrhizal colonisation in addition to differences in cover of *Sedum spp.* and bryophytes were tested using repeated measures ANOVA with bacteria, mycorrhiza and *Trichoderma* treatments and time as main effects.

Shannon-Wiener indices were used to assess changes in microarthropod biodiversity between September 2011 and July 2012 for all microarthropods and within microarthopod groups (Collembola, mites and larvae of flying insects). Each of these groups, as well as total microarthropod abundance,

was compared using a repeated measures ANOVA with bacteria, mycorrhiza and *Trichoderma* as treatments and time as a main effect. Greenhouse-geisser corrections were applied to non-spherical data (Greenhouse and Geisser, 1959). Bonferroni post-hoc tests were used to separate differences between time points. The number of Collembola and insect larvae present in May and July was not sufficient for inclusion into the statistical analysis.

Data were transformed using square root transformation to meet the assumptions of ANOVA, except for plant data, which met the assumptions of ANOVA untransformed. Mycorrhizal data, as count data, was ArcSine transformed. 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.

PCA was conducted on all microarthropods in one analysis and additionally on groups of microarthropods (Collembola, mites and larvae of flying insects) to determine how their communities were organised. Data were unconstrained. Additionally, each PCA was plotted twice, with 95% confidence ellipses (SEM) plotted based on microarthropods grouped into (1) different sample months and (2) different microbial treatments. This was to allow clearer visualisation of species groupings over time and within different treatments. 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).

For the months January and July, where Tingidae were present and plant surveys had been conducted, Spearman's Rank-Order Correlation was performed in SPSS. This was to determine if there was an association between bryophyte cover and the abundance of Tingidae, as some species of the family are associated with bryophyte dominated communities (Hufnagel et al., 2004), perhaps as a source of food (Gerson, 1969).

3. Results

3.1. Abiotic conditions

All mean monthly temperatures for sampled months in the current study were warmer than in the two years preceding them, with the exception of July 2012, which was approximately 2°C cooler than in 2010 and 0.3°C warmer than in 2011. Autumn and winter sample months (September, November and January) were drier than in the year preceding them, particularly in January 2012, where rainfall was approximately half that of the previous year. Summer rainfall (May and July) was considerably higher in 2012 than in both preceding years (42.5 mm and 103.6 mm respectively compared to 27 mm and 49.1 mm in 2011 and 30.2 mm and 26.1 mm in 2010).

The lowest mean sample period surface temperature was between the January and March 2012 surveys, with a mean temperature of 5.76°C (±6.15). The coldest absolute surface temperature recorded

by the dataloggers was -10.5°C, recorded in February 2012. The warmest mean sample period surface temperature was between May and July 2012 surveys, with a mean temperature of 19.96°C (±9.05). The highest surface temperature recorded on the roof was 53.5°C, recorded in May 2012.

Relative humidity ranged between 9.5% (May 2012) and 100% (frequent throughout the year), with least mean surface humidity in the May to July sample period (76.76%, ±23.18%) and highest mean surface humidity in the November to January sample period (95.50%, ±5.77%).

Substrate water content varied between 7.24% (May 2012) and 39.04% (January 2012), with total mean substrate water content recorded at 21.14% (±6.90%). The driest month sampled, according to mean substrate water content, was May 2012 (13.55%, ±3.51%) and the wettest mean substrate period was January 2012 (31.81%, ±2.33%).

3.2. Vegetation and fungi

Plant diversity was exceptionally low on the roof, with all plots dominated by *Sedum spp*. and bryophytes, with the addition of lichen, *Trifolium arvense* and few other plants. One individual of *Epilobium angustifolium* was present in March 2012. *Anthyllis vulneraria* was present sparsely throughout the year (maximum of three small individuals in any one month). Seedlings of *Acer pseudoplatanus* populated the roof in March before dying, presumably due to water stress. As such, Shannon-Wiener values for seasonal (vascular) migrants were 0 for all plots, with the exception of one plot, sampled in March, where the value was 0.3.

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

All three of the main plant species on the roof changed in abundance over time (Time vs: Sedum $F_{2, 80} = 32.70$, $p < 0.001$; Bryophytes $F_{1.46, 58.19} = 210.46$, $p < 0.001$; *T. arvense* $F_{1, 40} = 13.36$, $p < 0.01$) (Fig 1a). The plant community displayed a clear shift from winter to summer, dominated by bryophytes in January before *Sedum* became the most prevalent genus in the summer months (Fig 1a). *T. arvense* was absent in January but grew throughout the summer period. However, the decline of bryophytes in the summer was not compensated for by *T. arvense* and *Sedum,* so an overall increase in bare substrate occurred in March and July. None of the inoculants added had an effect on total plant cover, cover of *Sedum* or cover of *T. arvense* (data not shown). Lichens were too rare to analyse. The addition of *Trichoderma* to plots altered the pattern in bryophyte cover over time ($F_{1.46, 58.19} = 3.70$, $p < 0.05$). Figure 1b outlines that bryophytes performed better in plots treated with *Trichoderma* in January, but worse in the following months, though these differences are very small.

<FIGURE 1 NEAR HERE>

Colonisation of *Sedum* roots by mycorrhizal fungi at the end of the trial period in July 2012 was high, with a mean colonisation across all treatments of 75.7 (±1.6)%. The proportion of counts containing vesicles was also exceptionally high, averaging 50.2 (±2.0)% across the whole roof. 25.5(±1.3)% of counts contained hyphae only and prevalence of arbuscules was extremely low, averaging only 0.05(±0.03)% across the whole roof. All counts containing vesicles and/or arbuscules also contained hyphae.

The total percentage colonisation of roots by mycorrhizal fungi at the end of the experiment in July 2012 was unaffected by the addition of inoculants, with no significant differences between treatments and the control, and no interactions between treatments ($F_{1, 55} = 0.74$, $p > 0.05$). Vesicles and hyphae alone, when analysed separately, were not found to have been affected by any of the inoculants, nor were there any interactions between treatments. Numbers of arbuscules were too low to analyse.

3.3. Microarthropods

Forty microarthropod species were found on the roof during the sample period. Of note was a species of Hemiptera not previously recorded on this roof, in the family Tingidae, identified as *Acalypta parvula.* Another species not previously recorded on this roof was the aphid, *Aphis sedi.* One morphospecies of Thysanoptera and one species of Gastropoda (*Vallonia costata*) were also found on the roof for the first time (the latter in low abundance towards the end of the sampling period). Aside from these, key functional groups expected in ground level soils, such as Isopoda, Annelida and Formicidae, were absent.

Insect larvae of Coleoptera, Diptera and Lepidoptera (hereafter referred to as "larvae of flying insects") were the most abundant group aside from mites and Collembola. Homiptera were most abundant in summer, when an aphid population was present on the substrate surface.

Mean microarthropod abundance for all treatments changed over the sample period (Time: $F_{3.12, 124.67} = 48.09$, $p > 0.001$) (Fig 2a), peaking in September 2011, steadily declining until March 2012 and steeply declining in the summer sample months (Fig 2a). The total number of microarthropods sampled was 60,357 (±35). Parallel analysis determined that the first six PCA axes explained the majority of the variance within the microarthropod community. These six axes accounted for 30.78% of the variance (axis 1 = 7.87%, axis 2 = 5.87%). Confidence ellipses suggested that microarthropod communities were different each month. The most notable seasonal patterns highlighted by PCA are the groupings of Collembola and the mite *Eupodes viridis* associated with the March confidence ellipse and axis 1 and the groupings of mites, spiders and centipedes associated with the September ellipse ("M#", "Chi", "Ara") and axis 2 (Fig. 2b). Collembola, particularly *Sminthurinus aureus*, were abundant in January and March before declining in summer months. Most mites were abundant in September and November, before populations declined rapidly. This is with the exception of Scutoverticidae, which declined to a

lesser extent in the summer months. Tingidae were prevalent throughout the year, with the exception of March and May 2012, whilst Aphididae were only present in large numbers in May 2012.

<FIGURE 2 NEAR HERE>

The mean microarthropod community was higher in abundance in those plots treated with *Trichoderma* than in other treatments and the control ($F_{1, 40} = 5.6$, $p < 0.05$) (Fig. 3a). No interactions between microarthropod abundance over time and treatment could be detected. For mean microarthropods, PCA confidence intervals did not depict clear separations between treatments, with all treatments overlapping in community structure to some extent. However, the community present in plots treated with *Trichoderma* showed a more variable microarthropod community (Fig. 3b), with the *Trichoderma* confidence interval aligning more with axis 1 than other treatments. This axis was influenced by Collembola (*S. aureus* in particular), the mite *E. viridis* and a number of larvae of flying insects (Fig. 3b).

<FIGURE 3 NEAR HERE>

As a group, Collembola were extremely low in abundance, with only 12,124 (±35) individuals encountered in total on the six sample dates, making up approximately 20% of the microarthropod population. The roof was dominated by one species, *S. aureus*, which made up 96.7% of the collembolan population. Other species were present in low abundance, including *Deuterosmithurus pallipes* (2.8%) and less than 1% each of *Isotomurus palustris* and *Parisotoma notabilis*. The density of Collembola varied between 0 – 91 000 individuals m^{-2} throughout the sample period (Time: $F_{3, 120} = 34.60$, $p > 0.001$). Peak abundance was in January 2012, before numbers decreased dramatically during the summer period. The inoculants had no effect on Collembola as a group. The mycorrhizal treatment affected the pattern of collembolan abundance over time (Time*Mycorrhiza: $F_{3.0, 120.0} = 2.90$, $p < 0.05$). Figure 4a suggests that collembolan abundance was significantly lower in mycorrhiza treated plots than in other treatment plots and the control in September and March, but not in other months. The plots with bacteria and *Trichoderma* added together also had a combined effect with time (Time*Bacteria**Trichoderma*: $F_{3.0, 120.0} = 2.90$, $p < 0.05$). Figure 4b suggests that the abundance of , Collembola in January within these treatment plots was higher than in other treatments, but this was not the case in other months (Fig 4b). The lack of diversity of Collembola meant that PCA added little value to data analysis (data not shown).

<FIGURE 4 NEAR HERE>

46,444 (±53) mite individuals were encountered on the roof, consisting of fifteen morphospecies, five of which had not been found on the roof previously. Mites were the most common group on the roof, representing 77% of the total microarthropod abundance. A mite of the family Scutoverticidae dominated, making up 79.3% of the mite population. Mite abundance varied between 0 and 250,000 individual's m^{-2}, decreasing throughout the sample period (Time: $F_{1.87, 74.97} = 28.47$, $p > 0.001$). Mites

were unaffected by any of the inoculants added, with no inoculated plots differing from the control (data not shown). PCA also suggested that community structure did not vary between treatments (data not shown).

The community of larvae of flying insects peaked in the winter months (Time: $F_{3, 120} = 12.78$, $p > 0.001$; data not shown) and was less dominated by one morphospecies than mites and Collembola were. In total 1,092 (±2) larvae were encountered, 2% of the total microarthropod population. Larvae were lower in abundance in those plots where the bacterial treatment and the mycorrhizal treatment had been added together ($F_{1, 40} = 5.20$, $p < 0.05$) but higher in plots with the *Trichoderma* treatment ($F_{1,40} = 4.84$, $p < 0.05$) (Fig 5a). Parallel analysis determined that the first four PCA axes explained the majority of the variance within the larvae of flying insect community. These four axes accounted for 48.49% of the variance (axis 1 = 14.97%, axis 2 = 13.31%). PCA suggested that the community present in plots treated with *Trichoderma* was more variable than in other treatments and the control plots (Fig 5b) and that this community may be aligned with axis 2. Axis 2 was dominated by two larval species, a Chironomid midge and a species belonging to the superfamily Mycetophiloidea ("L5", "L6").

<FIGURE 5 NEAR HERE>

Other organisms present on the roof (Hemiptera and Gastropoda) remained low throughout the sample period but reached a peak in May 2012 (data not shown). However, the Tingid, *Acalypta parvula*, was negatively correlated with bryophyte cover during the sample period ($r_s = -0.28$, $p < 0.01$).

4. Discussion

4.1. G*reen Roof Development*

After eight years of development, the green roof had switched from a bryophyte dominated community structure (see: Rumble and Gange, 2013) to a *Sedum spp.* dominated community, achieving just over 40% total cover of the *Sedum* genus. It is unclear, with limited long term studies in similar climates, whether this is representative of other green roofs, but studies in northern Europe by Emilsson (2008) report similar slow rates of development. If complete vascular plant cover is a design aim for a green roofs, accelerating this process may be a research priority. Other colonising vascular plants had reduced dramatically in both number and cover since the 2010-11 sample period (see Rumble and Gange, 2013) despite more favourable weather conditions prevailing. It is not known whether this was due to conditions at the time of germination or due to competitive exclusion by *Sedum.* The presence of the legume *T. arvense* as one of the few colonising vascular plants, along with the high level of mycorrhizal colonisation in *Sedum spp.* could be indicative of a nutrient limited environment, where specialists could dominate.

Succession in the microarthropod community, although slow, had progressed in terms of abundance, though not diversity, increasing from the previous sample period (see: Rumble and Gange,

2013). Whilst the abundance of microarthropods as a whole increased in response to inoculants, this population growth was not equal across all faunal groups. In 2011, the population of Collembola had decreased dramatically due to two drought events (see: Rumble and Gange, 2013). Over a year later, despite higher average rainfall, the absence of extreme drought and generally more stable temperatures, the abundance of Collembola was still very low in this study. This demonstrates the long term detrimental effect of drought on some green roof microarthropods, even if the weather is more favourable in subsequent years, and reveals how fragile these communities are. In this instance, the addition of inoculants did not help this faunal group recover from the previous year's unfavourable conditions, highlighting that as a remediation tool, the success of inoculants is dependent on the starting population.

4.2. Inoculant Addition

There was no evidence that application of any of the commercial inoculants to a mature green roof had any effect on vascular plants. However, bryophytes, whilst unaffected by the addition of bacteria or mycorrhiza, were affected by the addition of *Trichoderma* in different ways at different times of the year. Cover in January 2012 was higher in plots treated with *Trichoderma* than in other plots, but subsequently, in March and July, the rate of bryophyte cover was less in *Trichoderma* plots than in others.

In vascular plants, *Trichoderma* may increase plant tolerance to disease (Papavizas, 1985; Mousseaux et al., 1998; Cuevas, 2011) and abiotic stress (Mastouri et al., 2010), enabling enhanced growth. However, this has not been studied extensively in bryophyte species. In terms of the reduction in bryophyte growth in *Trichoderma* treated plots in spring and summer, we do not suggest that there is a direct effect of the *Trichoderma*. Whilst *Trichoderma* are commonly found within decaying or senescent bryophyte tissues in natural environments (Osono et al., 2012; Scheirer and Dolan, 1983) it has rarely been reported that *Trichoderma* cause specific harm to bryophyte species. As saprotrophic fungi, they are thought to decompose bryophyte tissues at later decomposition stages than other fungi (Thormann et al., 2003; Akita et al., 2011). Akita et al. (2011), suggested, after extensive laboratory testing, that it is likely that *Trichoderma* only damage bryophyte tissues once some form of decomposition has already occurred. Thus, in the current study, it is likely that another factor, such as infection by a more virulent fungal pathogen, grazing from herbivores or abiotic stress, caused initial senescence in bryophyte tissues. Thus, the application of *Trichoderma* to green roofs, where bryophyte communities are already stressed, may exacerbate these effects. In terms of nutrient cycling, *Trichoderma* are clearly performing as successful decomposers in this environment when added as an inoculant, potentially increasing nutrient availability for other species.

The addition of *Trichoderma* also caused an increase in the total abundance of microarthropods compared to other treatments and the control. Community analysis highlighted differences in

community structure in plots treated with *Trichoderma*, with a cluster of larval species, Collembola (*S. aureus, D. pallipes* and *P. notabilis*) and the mite *E. viridis* driving this pattern. Larvae of flying insects were also found to be present in higher abundances in plots treated with *Trichoderma* and the larval community structure also differed in *Trichoderma* treated plots compared to other treatments, driven by "L5" (of the Mycetophiloidea) and "L6" (a Chironomid larvae). The likeliest explanation for this is an addition of food source for fungal feeding species on addition of *Trichoderma.* Many soil microarthropods and, in particular, those separated by the current PCA, are known to be fungal feeders. Observations of fungal feeding are recorded for both *S. aureus* and *P. notabilis* (Walter, 1987; Gillet and Ponge, 2005). The diet of *E. viridis* specifically is not well known but Walter, (1987) inferred from laboratory feeding experiments that mites of the *Eupodes* genus do feed on fungi, including *Trichoderma*. The separation of *E. viridis* from other mites in the study, suggests that it may have a unique ecology amongst green roof mite species, more akin to Collembola. Larval species of the superfamily Mycetophiloidea are also known to feed primarily on fungi (Krivosheina and Zaitzev, 2008), so may directly benefit from *Trichoderma* addition. Chironomid larvae, which grouped with fungal feeders in the PCA, do not feed directly on fungi but on faecal matter (Ponge, 1991), and may have indirectly benefitted from the increase in abundance of other microarthropods as a result of *Trichoderma* addition. To the author's knowledge, this is the first demonstration that changes in microarthropod abundance can occur as a result of the addition of free-living saprophytic fungi to soils, demonstrating a multi-level food web impacted by the addition of commercial inoculants. Sibi and Anandaraj (2008) found that, when adding a range *T. harzianum* amplifiers (e.g. manure), Sorghum residues in particular enhanced *T. harzianum* in the rhizosphere of black pepper, *Piper nigrum*, which, as a result, increased populations of mycophagous mites and their associated predators. Thus, with a longer development time or repeated applications, higher order trophic responses to the addition of these microbial inoculants may also be seen, improving microarthropod diversity on green roofs by encouraging the colonisation of predatory arthropods.

The impacts of the addition of *Trichoderma* on the microarthropod community suggest that species population numbers are not only limited by water, as highlighted by Rumble and Gange (2013), but also, in more favourable weather spells, by nutrient availability. This has implications for the long-term sustainability of green roofs; the addition of water or water reservoirs, as suggested as a solution for impoverished microarthropod communities by Rumble and Gange (2013), may not boost populations as much as is possible in this environment. As highlighted in Rumble and Gange (2013), populations of Collembola are limited below a critical threshold of substrate water content (approximately 10%), after which there are other factors controlling population growth. Beyond this threshold, the addition of nutrients, whether it be in the form of inoculants such as *Trichoderma* or in terms of slow release nutrient supplies, could promote a more sustainable soil community. Along with the prevalence of detrital and fungal feeding species on the roof and the lack of predators encountered, this supports the

463 hypothesis that population dynamics on these roofs are primarily controlled by resources from the
464 bottom-up, rather than top-down by predators (Chen and Wise, 1999). Thus, if the resource base on
465 green roofs could be sufficiently improved, increased abundances of not only soil dwelling
466 microarthropods would be seen, but also their above ground predators, such as spiders and wasps (Chen
467 and Wise, 1999) contributing to a more diverse ecosystem overall.

468 Not all species or microarthropod families responded to inoculants in the same way. As a group
469 studied independently, mites were unaffected by any of the inoculants, either in terms of abundance or
470 community structure. As the mite community was dominated by the hardy, xerophillic mite order,
471 Scutoverticidae, this is perhaps unsurprising. Scutoverticidae are thought to be generalist feeders, so
472 could be expected to shift diet depending on food availability (Smrž, 2006). In addition, whilst this
473 order is thought to be associated with moss (Schäffer et al., 2010), the effect of *Trichoderma* on the
474 green roof bryophyte community did not translate to the mite community, suggesting that these
475 organisms are robust to changes in their environment.

476 Some inoculants had more complex, or even negative effects on microarthropod groups. For
477 example, Collembola in bacteria treated plots were higher in abundance than in other plots in the month
478 of January, but in subsequent months were less abundant. Whilst bacteria are a dietary component for
479 some Collembola species (Gillet and Ponge, 2005) the increased mass of bacteria after inoculation is
480 short lived, with studies reporting a drop in bacterial mass 60 days after inoculation (Domenech et al.,
481 2004). Instead bacterial inoculants are thought to have long lived impacts on successional development
482 (Probanza et al., 2002). *Bacillus spp.,* for example, have been shown to decrease the survival rate of
483 some fungal mycelia (Probanza et al., 2001; Domenech et al., 2004). Thus, the particular seasonal
484 patterns in bacterial effects on Collembola populations could be due to a long term lowering of
485 collembolan food sources.

486 Further microbial-microarthropod interactions were observed in this study that are difficult to
487 explain without further research. For example, when bacteria and mycorrhiza were added together, the
488 group of insect larvae decreased in abundance, though no community changes or enhancement of
489 mycorrhizal colonisation was demonstrated. Mycorrhizal colonization can reduce the growth of
490 rhizophagous insect larvae (Johnson and Rasmann, 2015), but as no difference in mycorrhizal
491 prevalence was noted, perhaps this is dependent on mycorrhizal species rather than abundance. Without
492 higher resolution data for both fungal and insect groups, it is difficult to speculate further on the
493 mechanism involved.

494 In general, bacterial and mycorrhizal inoculants were relatively unsuccessful, with no enhancement
495 to plant growth when added singly, or in conjunction with one another. In addition, root colonization
496 by mycorrhizal fungi was not higher in plants treated with these two inoculants. Whilst little is known
497 about the microbial community in green roofs, this particular roof was already mycorrhizal at the

beginning of the study, suggesting an incumbent microbial community. Thus, it is possible that the generalist species added to this habitat either could not establish, due to competition with native soil microbes, perhaps exacerbated by addition in a volume too low to contribute to this community, or were not specific enough to successfully establish with the plants present. Whilst there are still significant difficulties in monitoring bacterial species assemblages in complex ecosystems, there is evidence to suggest that soil bacterial biodiversity may prevent new species entering an ecosystem by utilising more available resources (van Elsas et al., 2012). There is also evidence to suggest that resident mycorrhizal populations greatly influence bacterial communities (Nuccio et al., 2013) and that incumbent mycorrhizal communities may prevent new mycorrhizal species from establishing in a new habitat (Vierheilig et al., 2000; Vierheilig, 2004). Thus, inoculant addition when a roof is constructed, when there is no prior microbial community present, may have very different results to those resulting from application to a mature green roof. In addition, amplifying the microbial community already present may be a more successful approach.

Whilst the microarthropod community was altered by the addition of *Trichoderma*, these population increases may have been too modest to affect plant growth. Microarthropods play an important role in nutrient regulation in soils (Wardle et al., 2004), but no resultant effect of their increase in abundance was seen in plant cover in this study. This suggests that the increases in abundance were not sufficient (at these concentrations of inocula, or within the time scale studied) to translate into increased plant cover. In addition, colonisation of plant species to the roof and therefore diversity, was not facilitated by an enhanced soil food web. Whilst we have demonstrated that green roof faunal biodiversity can be altered via the addition of inoculants, more research is needed to determine if the same can be achieved to facilitate plant growth on green roofs.

4.3. Applicability

Trichoderma has been shown in this study to be a promising inoculant to enhance microarthropod abundance on mature extensive green roofs, whilst bacterial and mycorrhizal inoculants have been shown to have little effect. In particular, *Trichoderma* could be useful for the extensive green roofs in temperate climates, of which many are bryophyte and *Sedum* dominated habitats (Schrader and Böning, 2006; Emilsson et al., 2007; Emilsson, 2008).

More research, however, is still needed. Whilst the abundance of some microarthropods was enhanced by the addition of *Trichoderma*, overall abundance was still very low, considerably lower than that expected in other urban soils (Hartley et al., 2008; Santorufo et al., 2012). Diversity was also unchanged compared to the previous sample period. Thus, whilst this inoculant has shown promise, further measures, such as providing refugia for soil microarthropods, as well as experimenting with concentrations of inocula, must be explored to remediate impoverished green roofs to a satisfactory level and to determine if resultant improvements of the plant community can occur. Sequential additions

of microbial inoculants at different times in the year may also prove to be more successful than single inoculation in affecting microbial populations (Molineux et al., 2014).

In addition, species composition of microarthropods is likely to differ locally, and this may be a factor that alters the success of microbial inoculants. In the current study, inoculants enhanced a certain trophic group (mycophages) but this extended only to organisms that were already present on the roof, it did not facilitate colonisation of new species of microarthropods. Presumably this was due to a lack of an appropriate nearby source population or due to a barrier to colonisation ability. Green roofs have been shown be less favourable for less mobile faunal species within urban habitat corridors (Braaker et al., 2014), so improving habitat connectivity, allowing local sources of less mobile species access to green roofs, may enhance the benefits afforded by the addition of inoculants further. In terms of testing microbial inoculants as a remediation tool, artificially removing barriers to dispersal for plants by planting wildflower mixes to begin with, could further establish if microbial inoculants can have resulting impacts on plant diversity.

5. Conclusions

In conclusion, microbial inoculants applied in this study have not been shown to enhance plant diversity or cover on green roofs, but *Trichoderma* could be a promising microbial inoculant for the remediation of impoverished mature green roof soil faunal communities, particularly in terms of mycophagous species. In the long term, whether this benefits plant growth or not, animal species occupying higher trophic levels may be better able to survive on green roofs as a result and thus improve overall faunal biodiversity. However, the effects of the addition of soil inoculants vary between soil groups and some inoculants may produce negative, or deleterious, effects, emphasising that thorough testing needs to occur before application. At higher doses or in conjunction with other green roof remediation techniques, *Trichoderma* could contribute to enhancing the biodiversity of this often overlooked group of organisms, increasing the value of green roofs to the urban landscape.

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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 and commercial products to test. They had no influence on the study; its design, delivery or interpretation.

Glossary

Arbuscular mycorrhizal fungi: Distinct group of species of mycorrhizal fungi that penetrate the roots of their host plants

Arbuscule: Organ responsible for nutrient transfer in arbuscular mycorrhizal fungi

Extensive (green roof): Green roof with often shallow substrate and low organic matter. Usually planted with hardy succulents.

Inoculant: The introduction of a microorganism or substance into a new habitat and/or organism

Microarthropod: Small invertebrates in the phylum Arthropoda

Morphospecies: Groups of organisms that differ in appearance, but may not be genetically distinct species

Mycorrhiza: Fungal group that associates with the roots of plants

Trichoderma: Genus of free-living soil fungi

Vesicle: Fungal storage organ, storing, for example, lipids

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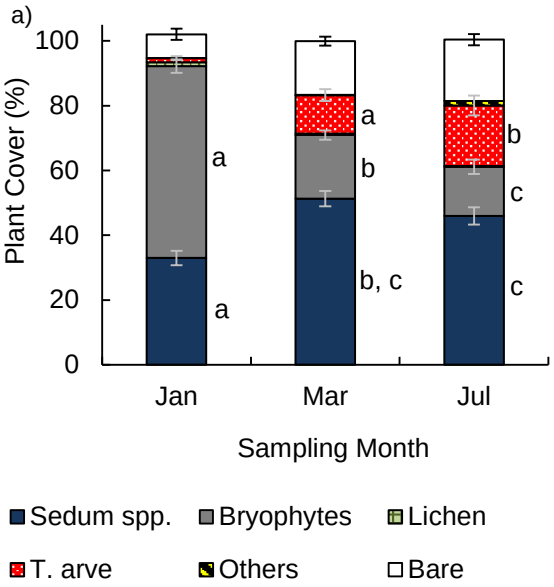
774 FIGURES AND CAPTIONS

775 Fig 1. Size: 1 column if stacked, 1.5-2 column if horizontal



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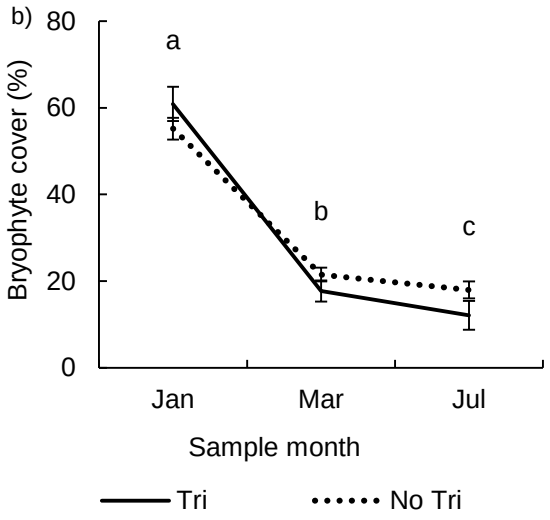
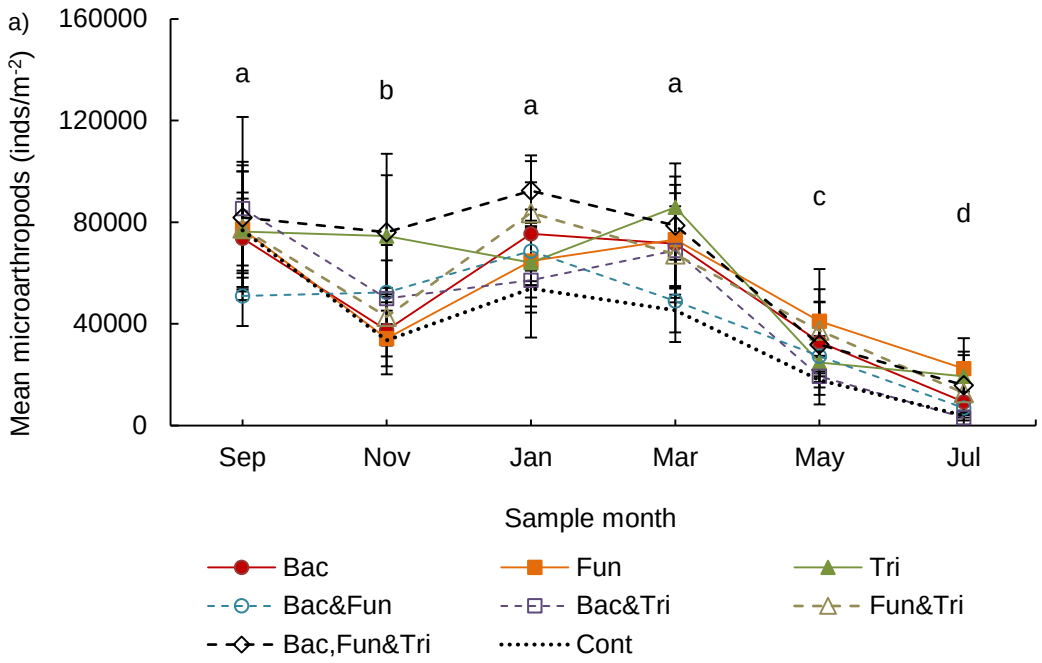


777

778 Fig 1. (a) Percentage cover of vegetation and bare substrate on the roof. *T. arve* = *T. arvense.* (b)
779 Bryophyte cover over the three sample periods in plots treated with *Trichoderma* (singly or as a
780 combination) and in all plots that did not contain the *Trichoderma* inoculant. Error bars represent
781 SEM. Tri = *Trichoderma* treatment.

782 Fig 2. Size: 1.5 column stacked

783 Fig 2a relates to interactive plot data: RumbleGange_2016_Remediation_2ndSub_IntFig2a



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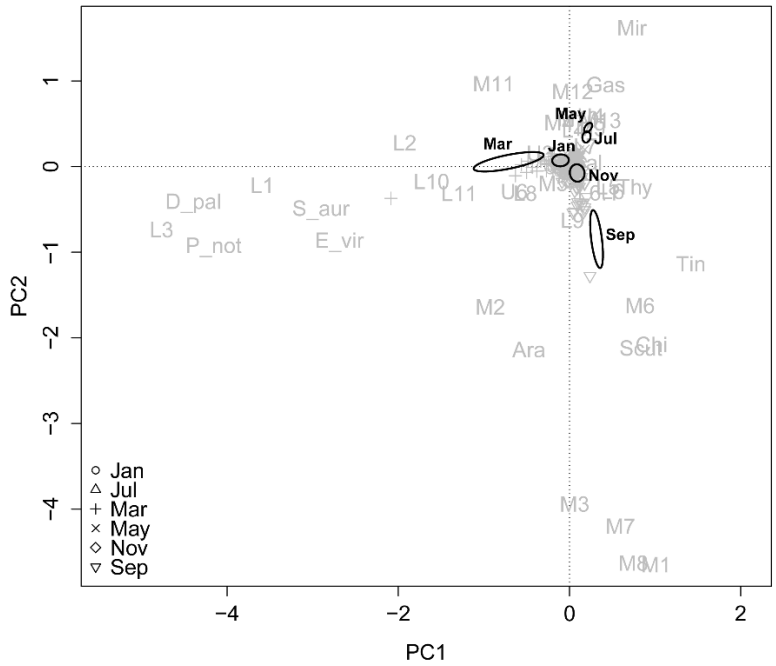
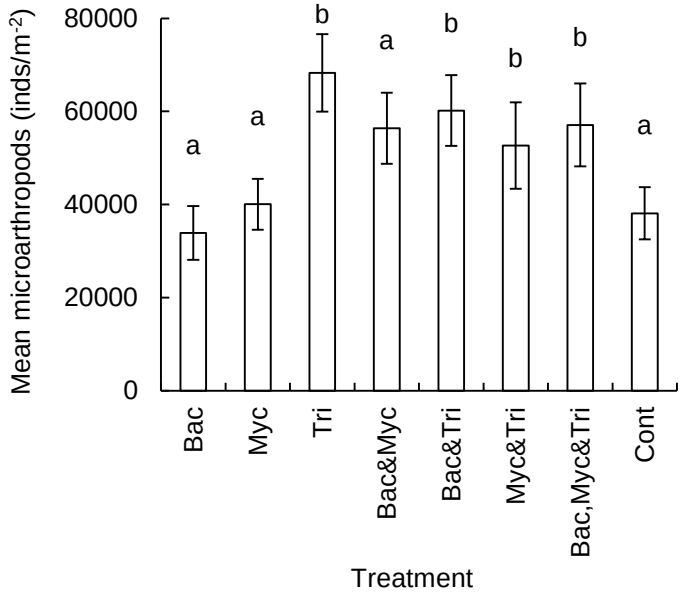


785

786 Fig 2 (a) Mean microarthropods m^{-2} for all sample points. Error bars denote SEM, (b) PCA ordination
787 plot depicting microarthropod communities throughout the sample period. Confidence ellipses are
788 drawn at the 95% confidence level (SEM), using month as a factor. Bac = bacterial treatment; Myc =
789 mycorrhizal treatment; Tri = *Trichoderma* treatment; Cont = control.

790 Fig 3. Size: 1.5 column if stacked, 2 column if horizontal



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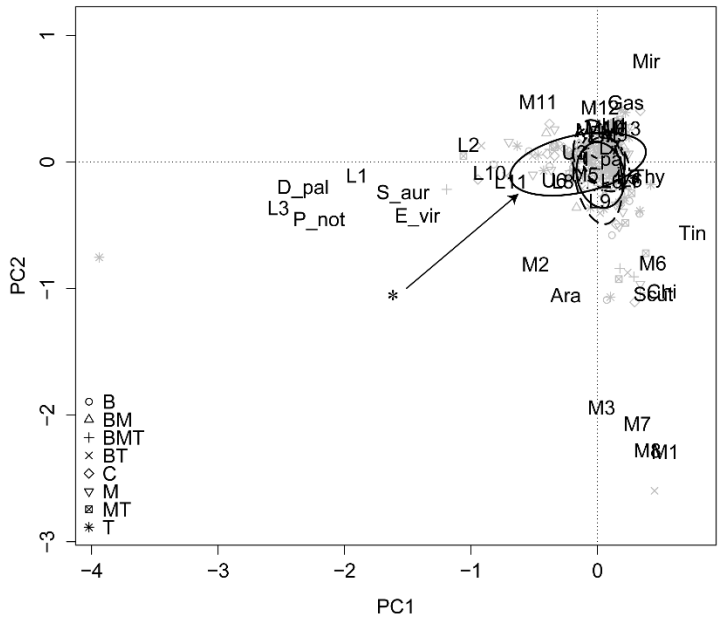
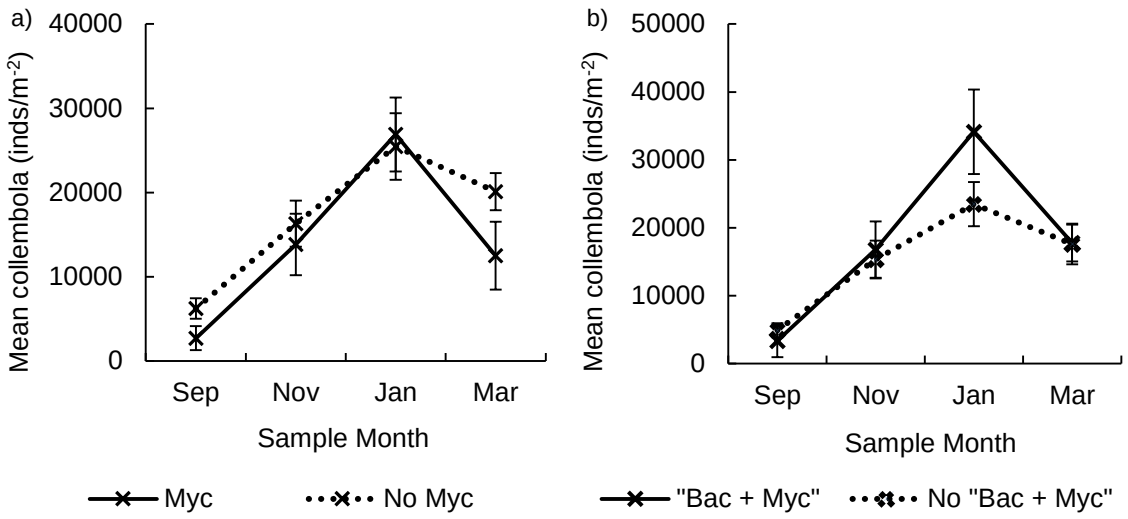


792

793 Fig 3 (a) Mean microarthropods per treatment averaged for all time points. Letters denote statistically
794 distinct groups. Error bars represent SEM. (b) PCA ordination plot for all microarthropods, depicting
795 95% confidence intervals (SEM) for each treatment based on all plots. Starred confidence interval
796 denotes communities in *Trichoderma* inoculated plots. Bac = bacterial treatment; Myc = mycorrhizal
797 treatment; Tri = *Trichoderma* treatment; Cont = control.

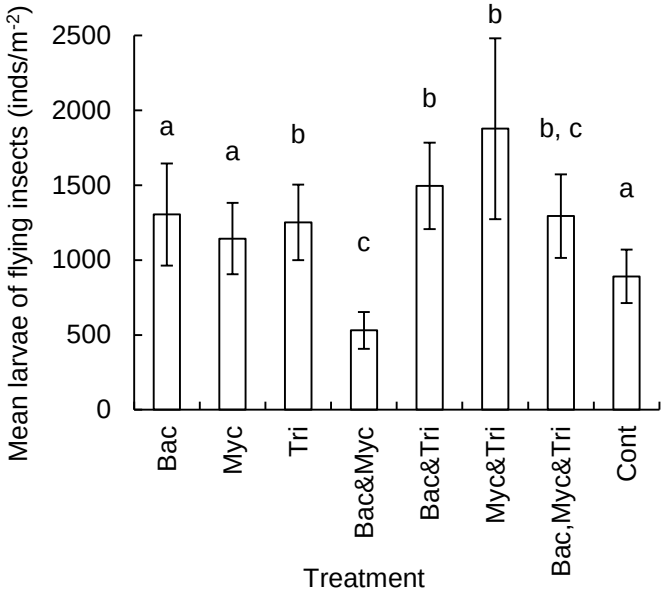
798 Fig 4. Size: 1 column if stacked, 1.5-2 column if horizontal



799

800 Fig 4. Mean collembola over time, in (a) all plots where the mycorrhizal inoculant was added
801 (including as part of a mix) and in all plots where the mycorrhizal inoculant was not added (including
802 mixes and the control) and in (b) all plots where the mycorrhizal inoculant was added in addition to
803 bacteria (including as part of a larger mix) and in all plots where these two inoculants were not added
804 together (including mixes and the control). Error bars denote SEM. Bac = bacterial treatment; Myc =
805 mycorrhizal treatment.

806 Fig 5. Size: 1.5 column if stacked, 2 column if horizontal



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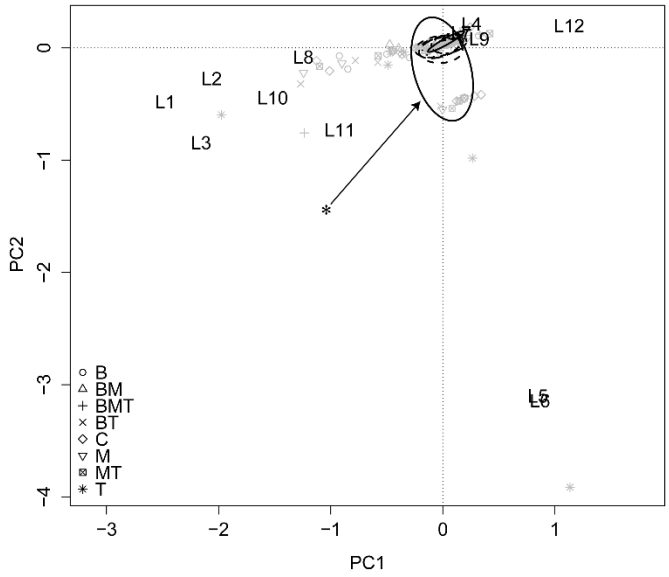

808

809 Fig 5 (a) Mean larvae of flying insects per treatment, averaged over all treatment times (excluding
810 May and July samples). Letters denote statistically distinct groups. Error bars represent SEM. (b)
811 PCA ordination plot for larvae of flying insects alone, depicting 95% confidence intervals for each
812 treatment based on all plots. Starred confidence interval denotes the community in plots inoculated
813 with *Trichoderma.*