

1

1 *Accepted for publication on the 24th January, 2018*

2 **GREEN ROOF SOIL ORGANISMS: ANTHROPOGENIC**
3 **ASSEMBLAGES OR NATURAL COMMUNITIES?**

4 Heather Rumble^{a,b *}, Paul Finch^a and Alan C. Gange^a

5 ^a*School of Biological Sciences, Royal Holloway, University of London, Egham,*
6 *Surrey. United Kingdom. TW20 0EX.*

7 ^b*Department of Geography, University of Portsmouth, Buckingham Building, Lion*
8 *Terrace, Portsmouth. United Kingdom. PO1 3HE*

9 ^bheather.rumble@port.ac.uk, ^ba.gange@rhul.ac.uk

10

11 *Corresponding author. Tel.: +44(0)2392 846540

12 *E-mail address:* heather.rumble@port.ac.uk (H. Rumble).

13 ABSTRACT

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

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

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

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

42 KEY WORDS

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

44 1. INTRODUCTION

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

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

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

66 Many soil microarthropods are relatively immobile, particularly in terms of active transport (Wallwork,
67 1970), and may not be able to colonise green roofs by their own means post-build, especially
68 considering that these roofs are not usually connected to ground level soils. Braaker et al., (2014) found
69 that for above-ground insects, the relative inaccessibility of green roofs means that only the most mobile
70 species are able to colonise them, suggesting that green roof communities are often driven by organism
71 dispersal ability rather than suitability of the habitat. This could have a number of negative
72 consequences, including green roofs acting as a sink habitat for species, either when mobility changes
73 (e.g. when offspring are born, see: Baumann, 2006) or when environmental conditions change due to
74 season or weather (e.g. during drought, see: Rumble and Gange, 2013), resulting in a loss of
75 biodiversity. In addition, a collection of species colonising an environment based on their mobility,
76 rather than on adaptations to their environment could mean that sustainable communities form slowly,
77 or not at all, hampered by environmental conditions. Rumble and Gange (2013) investigated this within
78 green roof substrates, finding that below-ground biodiversity was not sustainable, due to a lack of
79 resilience in the community to drought. Studies on ground-level soils suggest that even without the
80 challenges green roofs present to less-mobile species, microarthropod colonisation into virgin soils can
81 take 10-20 years. This could represent more than a fifth of a green roofs overall life span (Porsche and
82 Köhler, 2003), so mechanisms to speed this process up or biologically enhance roof technosols could
83 be an important factor in ensuring green roofs provide maximum functionality and ecosystem service
84 provision.

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

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

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

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

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

120 2. MATERIALS AND METHODS

121 2.1 EXPERIMENTAL SITE

122 In June 2011, a new green roof was constructed on a roof within the Royal Holloway grounds (London,
123 UK; N 51.25350, W 0.33469) in the South East of England. The roof was constructed in a modular
124 design using trays (for layout, see Supplementary Material 1). Five of these trays (replicates) were used
125 in the current preliminary study, all other trays were part of a larger study (Rumble, 2013). Trays were
126 of dimension 0.52m by 0.42m by 0.10m and were installed at approximately 20m from ground level,
127 with 0.30m between each tray. Holes were drilled in each tray to allow water to drain freely and each
128 tray was lined with a filter sheet (ZinCo SF, ZinCo GmbH, Nürtigen, Germany) to prevent leaching of
129 particulate matter. An extensive substrate mix (Shire Green Roof Substrates, Southwater, Kent, UK),
130 consisting of crushed red brick with 10% organic matter (rough compost), was added to each tray to a
131 depth of 0.08m. This depth is within the range commonly used on extensive green roofs (FLL, 2008)
132 and has been used in previous studies (Molineux et al., 2015), making comparison between studies
133 viable. The bricks that this substrate is made from are obtained from the county of Cambridgeshire,
134 UK, where they are fired during the brickmaking process. Bricks that are not of a suitable standard are
135 crushed and stored outside in 1 tonne bags, creating the potential for seeds and microarthropods to
136 colonise prior to green roof construction. This is standard practise for green roof substrates and as this
137 experiment is designed to replicate what would happen on a real green roof, no modifications (such as
138 autoclaving) were applied to the substrate. Mixing and packing of the substrate was supervised by the
139 authors. Five samples of substrate each of 166cm³ were then checked for the presence of
140 microarthropods and a microbial community before being installed.

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

143 a greenhouse by an industry supplier (*Sedum* Green Roof Ltd, Wiltshire, UK). After consultation with
144 several green roof manufacturers (*Sedum* Green Roof Ltd, Wiltshire, UK; Shire Green Roof Substrates,
145 Kent, UK; SkyGarden, Gloucestershire, UK) about the density at which plugs are normally planted, a
146 distance of 0.1m between each plant was used (the quotes given varied between 0.1-0.2m). These plugs
147 were planted uniformly, but the order in which they were planted was random. No attempt was made
148 to remove the soil the plugs arrived in, again to replicate the normal construction of a green roof as
149 closely as possible. A sample of plugs (five of each species) was checked for the presence of
150 microarthropods and for microbial communities.

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

153 2.2 MICROARTHROPOD SAMPLING

154 In order to assess microarthropod population abundance over time, every two months, from September
155 2011 until July 2012, two 56cm³ substrate samples were removed from each plot. This was achieved
156 by pushing a 3cm diameter soil corer down to the tray lining. These two samples were aggregated to
157 overcome problems associated with clumped microarthropod distributions (Ettema and Wardle, 2002).
158 This resulted in a 113cm³ sample of substrate from each plot. A small portion (3g) of this substrate was
159 removed for Phospho lipid fatty acid analysis (PLFA) analysis, to determine the composition of the
160 microbial community. The remainder of the soil sample was weighed to obtain wet weight and then
161 placed in Berlese Tullgren funnels at approximately 18°C for 7 days (MacFadyen, 1953), after which
162 the substrate was reweighed to obtain dry weight. Substrate water content at the time of sampling could
163 then be calculated. Soil organisms were collected in 70% ethanol and stored in the dark, at room
164 temperature, until further analysis. Microarthropods were sorted to morphospecies using a dissecting
165 microscope at x100 magnification. Species identification, where possible, was then performed at higher
166 magnifications (x200-1000) using a compound microscope. In the case of mites, this was restricted to
167 the most prevalent morphospecies, identified to family level. Less common mites were assigned a

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

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

173 2.3 PHOSPHO-LIPID FATTY ACID ANALYSIS (PLFA)

174 3(\pm .05) g of soil was taken from each microarthropod substrate sample and stored at -20°C until
175 analysis. PLFA analysis followed a modified method of Frostegård et al., (1993). Briefly, lipid
176 extraction was undertaken using Bligh/Dyer solvent, with phase separation performed by using
177 chloroform as an organic solvent. Fractionation was then undertaken using normal phase silica acid
178 columns (Cronus SPE Cartridges Si 1000mg/6ml, SMI LabHut, Gloucestershire, UK), fractioning lipid
179 material into neutral (NLFA's), glyo- and phospholipids (PLFA's). Fatty acid methyl esters (FAMES)
180 were then obtained via lipid methanolysis of the PLFA fraction, using 0.2M methanolic KOH. Methyl
181 nonadecanoate (C19:0) was added here as an internal standard. FAMES were identified by
182 chromatographic retention times, with bacterial PLFAs verified with a standard bacterial FAMES mix
183 (Sigma Aldrich, St Louis, MO, USA). Analysis was performed by a Hewlett Packard (HP) 5890 gas
184 chromatograph equipped with a flame ionization detector and a DB-5 capillary column (30 mm x 0.25
185 mm i.d., film thickness 0.25 μ m). The injection temperature was 250°C and the detector temperature
186 regime started at 100°C, increasing at 20°C min⁻¹ before being held at 160°C for 5 minutes. Temperature
187 increased again at 3.5°C min⁻¹ to 280°C where it was held for 3 minutes before finally increasing at
188 20°C min⁻¹ to 320°C. Injection was splitless and helium was used as a carrier gas. FAMES were
189 identified on an HP 5970 mass spectrometer.

190 Fatty acid nomenclature followed Frostegård et al., (1993). The abundance of individual PLFA's is
191 expressed as equivalent responses to the internal standard, in μ g g⁻¹ dry weight of soil (modified from
192 Hedrick et al., 2005). Microbial markers were used to characterize the community. The PLFAs 18:2 ω 6,9
193 (Frostegård et al., 2011) and 20:1 ω 9 (Sakamoto et al., 2004) were used as indicators of fungi while

194 C14:0i, C15:0i, C15:0ai, C16:1i, C16:0i, C16:1 ω 7c, C16:0(10Me), C17:0i, C17:0ai, C17:0cy,
195 C17:0(10Me), C18:1 ω 9c, C18:0(10Me) and C19:0cy (Zelles, 1999) were used to characterize total soil
196 bacteria.

197 2.4 MYCORRHIZAL SAMPLING

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

201 Roots were washed with tap water and cleared in 10% potassium hydroxide (KOH) in a water bath at
202 80°C for 25 minutes. Visualization of mycorrhizas in the roots was performed using a modified ink
203 staining method of Vierheilig et al., (1998), whereby commercial ink (Quink washable blue, The Parker
204 Pen Company, East Sussex, UK) mixed with 1% HCl and water in the ratio 0.6:15:84.4 was added to
205 the samples and heated at 80°C in a water bath for 15 minutes. Root samples were stored in stain until
206 ready to be analysed.

207 Percentage root length colonized was obtained with the cross-hair eyepiece method of McGonigle et al.
208 (1990), whereby samples are spread evenly across a slide and observed at x200 magnification. Each
209 root piece crossing the centre of the eyepiece, or the crosshair, is observed for the presence or absence
210 of fungi in the form of hyphae, vesicles or arbuscules, and recorded. Approximately 100 counts were
211 obtained from each sample.

212 2.5 STATISTICAL ANALYSIS

213 Analysis was performed using SPSS 22.0, except PCA, which was performed using R (R Core Team,
214 2015). Differences in total microbial mass and total microarthropod populations between initial plugs
215 and the substrate were tested using univariate ANOVA. Univariate ANOVA was also used to test sub-
216 groups of these variables (i.e. bacterial PLFA's, fungal PLFA's, springtails and mites) and to test
217 mycorrhizal colonisation between different plant species.

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

220 skewed data and by a non-parametric (rank) Levene's test for skewed data (Nordstokke and Zumbo,
221 2010). Data analysed passed the assumption of homogeneity of variances. The Shannon-Wiener Index
222 (H) was calculated (using log base 10) to determine diversity of microarthropods and this was conducted
223 for each plot.

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

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

232 PCA, using a correlation matrix, was conducted on all fatty acids in one analysis, all microarthropods
233 in another and additionally on groups of microarthropods (Collembola, mites) to determine how their
234 communities were organised. 95% confidence ellipses (SEM) were plotted based on data grouped into
235 different sources/sample months. These analyses were conducted using the vegan (Oksanen et al.,
236 2015), nFactors (Raiche and Magis, 2011) and BiodiversityR (Kindt and Coe, 2005) packages for R (R
237 Core Team, 2015).

238 3. RESULTS

239 3.1 ABIOTIC CONDITIONS

240 The average temperature in the sample period was 12°C (± 4), with a maximum temperature of 21°C
241 (August 2011) and a minimum of 0°C (February 2012). Average rainfall over the sample period was
242 67mm (± 38). Months with the highest recorded rainfall were June 2012 (134mm) and April 2012
243 (133mm) and the lowest recorded were in February and March 2012 (19mm and 27mm respectively)
244 (Met Office, 2017). Substrate water content was an average of 21% (± 7 %) across the sample period,

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

247 3.2 MICROARTHROPOD COMMUNITY

248 The green roof substrate was void of microarthropods on arrival. However, the plugs of each *Sedum*
 249 *spp.* supported microarthropod communities. Collembola were the most prevalent microarthropod ($\bar{\chi} \approx$
 250 11 000 (± 4000) m^{-2}), followed by mites ($\bar{\chi} \approx 2000$ (± 1000) m^{-2}). *S. album* supported a higher abundance
 251 of microarthropods than *S. reflexum* and *S. spurium* ($F_{2, 14} = 19.08$, $p < 0.001$) but was lower in species
 252 diversity ($F_{2, 14} = 8.10$, $p < 0.005$; H : *S. album*, 0.26; *S. reflexum*, 0.59; *S. spurium*, 0.55) as it contained
 253 high numbers of the Collembola species *Parisitoma notabilis* (Collembola: $F_{2, 14} = 22.42$, $p < 0.001$).
 254 Only three other species/morphospecies were found in the plugs, one morphospecies of the order
 255 Annelida, one aphid species, *Aphis sedi*, and one morphospecies that was identified as a larvae of a
 256 terrestrial chironomid. These morphospecies were low in abundance in all plant species.

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

262 Microarthropod abundance in the substrate post-planting remained low compared to in the original
 263 planted plugs, reducing from an average 12980 (± 4044) individuals m^{-2} down to an average of 3110
 264 (± 373) individuals m^{-2} ($\chi^2_2 = 19.07$, $P = < 0.001$). Microarthropod diversity was half of that in the original
 265 plugs, reducing from an average Shannon-Wiener score of 0.47 (± 0.05) down to 0.19 (± 0.02) ($\chi^2_2 =$
 266 27.27, $P = < 0.001$). Colonisation rates of new species was slow. There were 15 species present in the
 267 plugs. A further two new species colonised in September 2011. No new species were sampled within
 268 the plots until May 2012 when an additional species was sampled. In July 2012, a further five new
 269 species colonised the plots, bringing the cumulative total of species sampled over the eleven months to
 270 23.

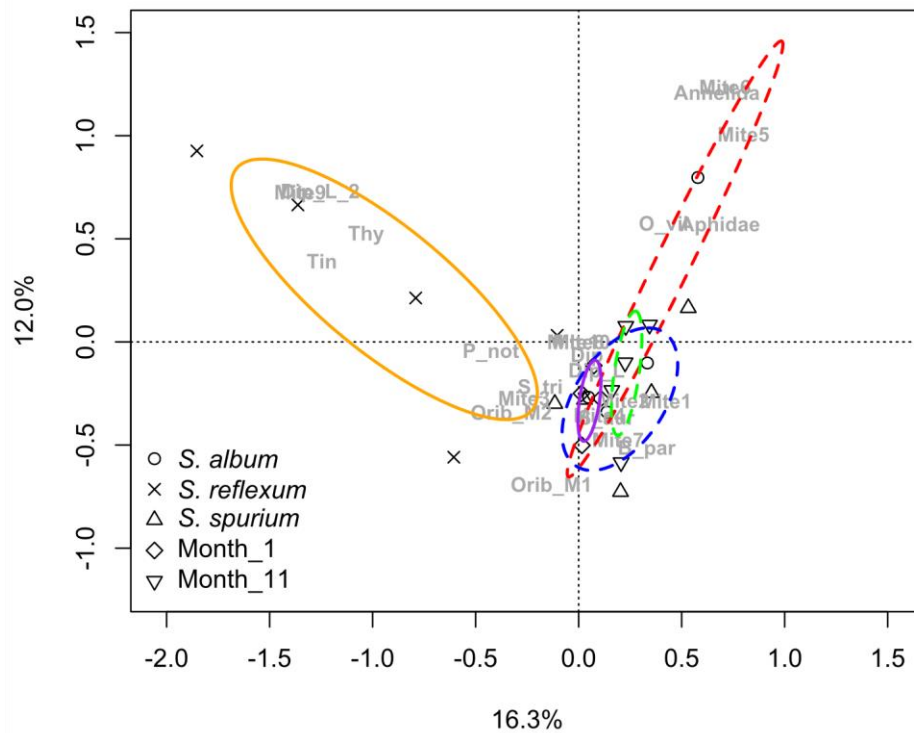
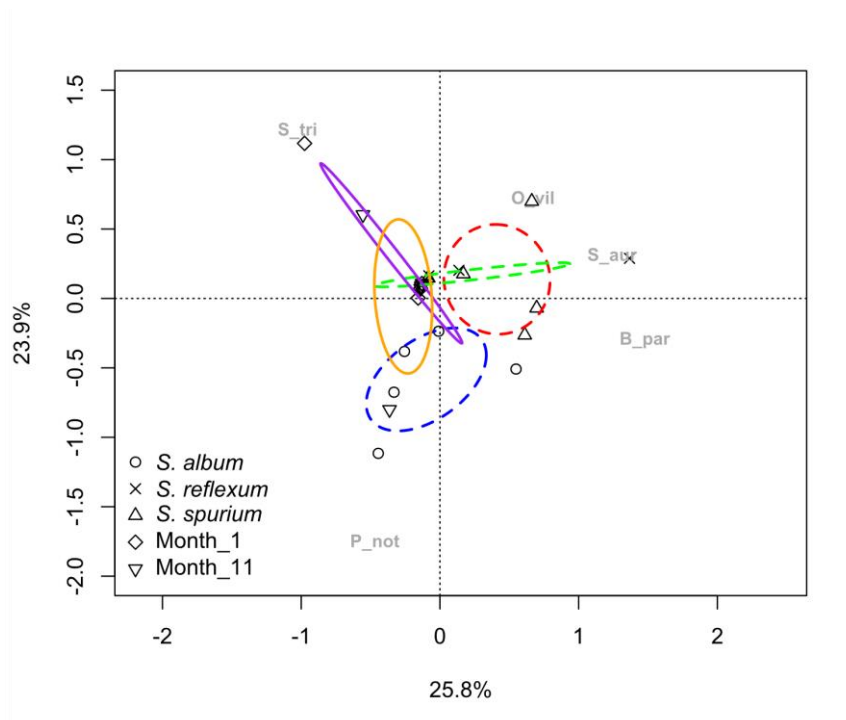


Fig. 1. PCA ordination of all microarthropods in plugs prior to planting and in the substrate post-planting in September 2011 (month one) and July 2012 (month eleven). Rings denote 95% confidence intervals; Blue dashed lines denote *S. album*, green dashed lines denote *S. reflexum*, red dashed lines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

271

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

a.)



b.)

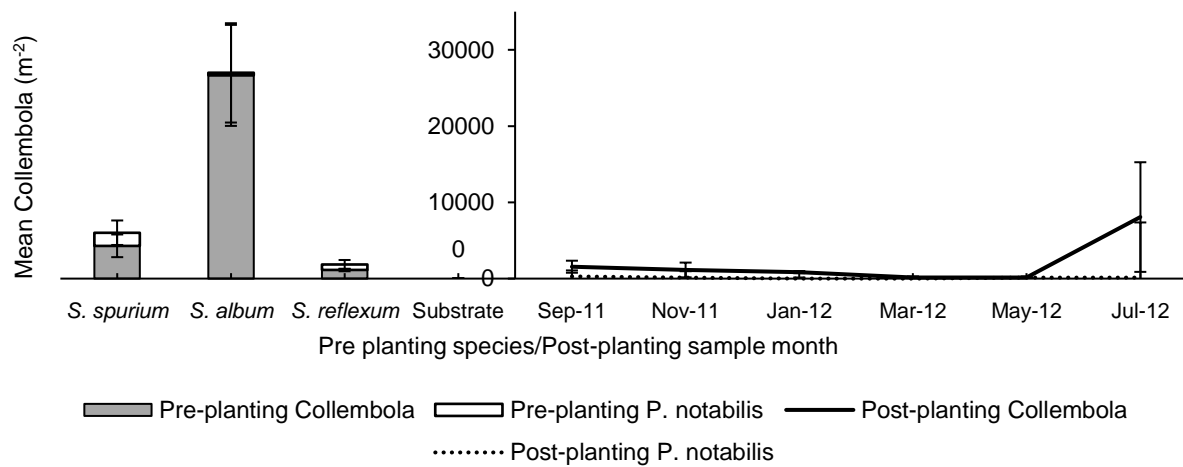
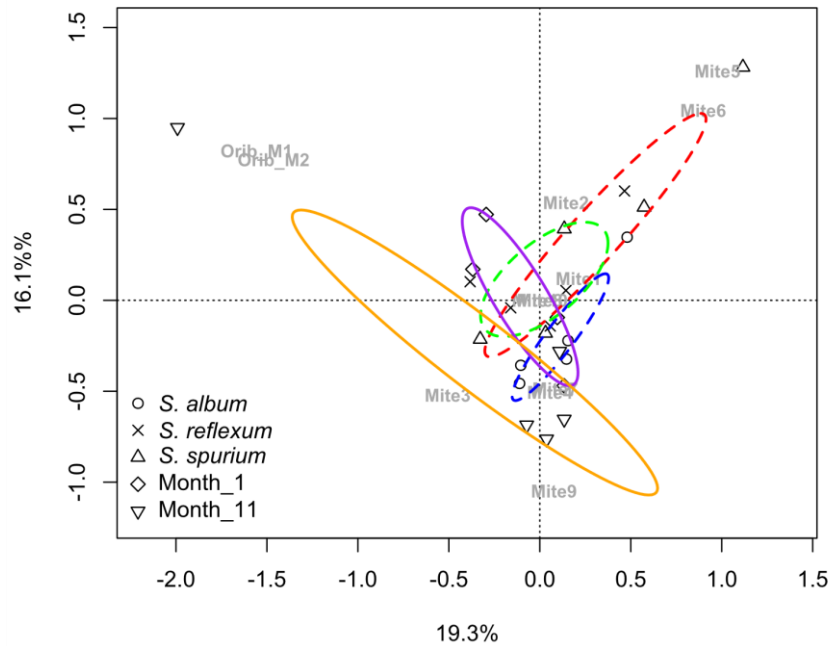


Fig 2. (a) PCA ordination of Collembola in plugs prior to planting and in the substrate post-planting in September 2011 (month one) and July 2012 (month eleven). Rings denote 95% confidence intervals; Blue dashed lines denote *S. album*, green dashed lines denote *S. reflexum*, red dashed lines denote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

a.)



b.)

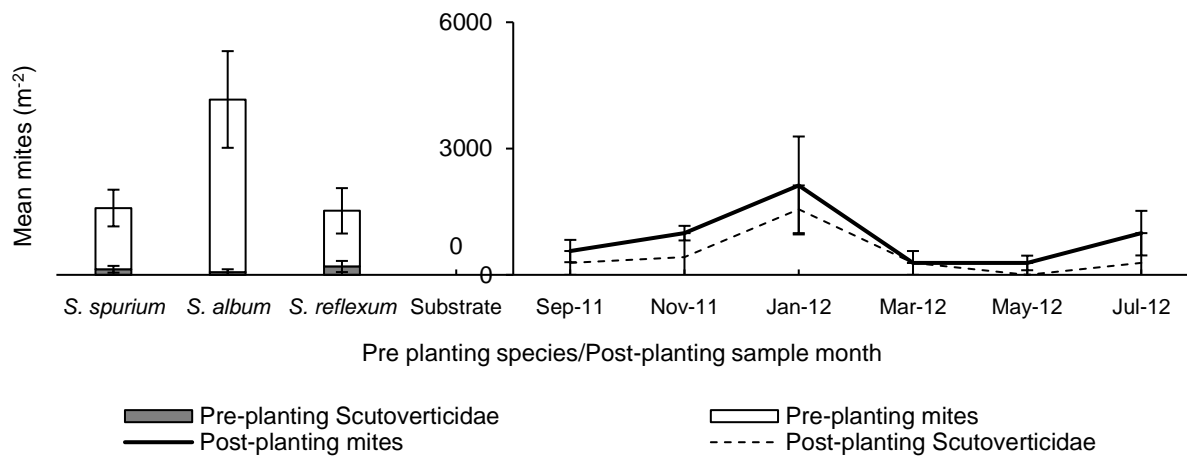


Fig 3. (a) PCA ordination of mites in plugs prior to planting and in the substrate post-planting (Post) in September 2011 (month one) and July 2012 (month eleven). Rings denote 95% confidence intervals; Blue dashed lines denote *S. album*, green dashed lines denote *S. reflexum*, red dashed lines denote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

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

282 71 (\pm 52) m⁻²). In general, Collembola remained low in abundance until July 2012, when *P. notabilis*
283 and *S. trinotatus* vastly increased in number (Fig 2b).

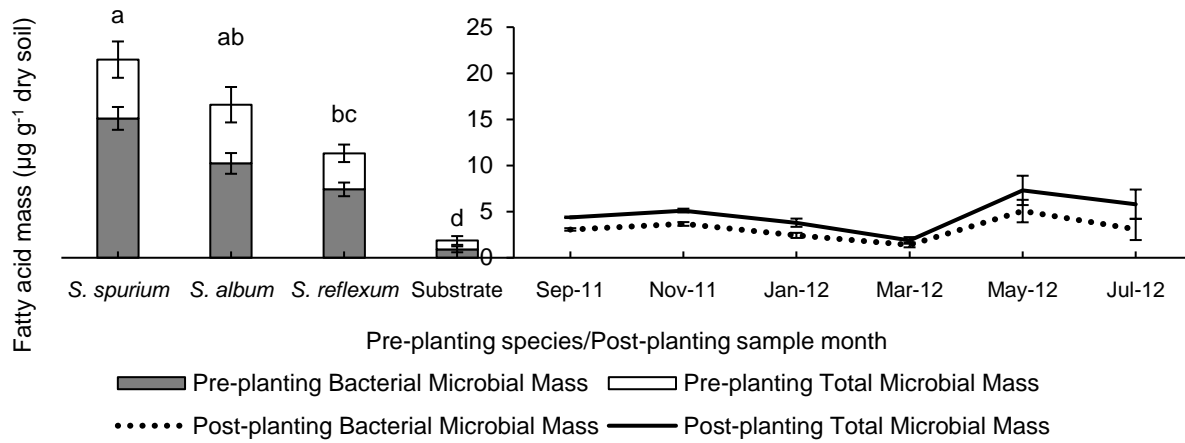
284 No mites were present in the bare substrate and only *S. album* plugs supported significantly higher
285 numbers of mites than the substrate ($F_{3,16} = 6.64$, $p < 0.001$). Eight morphospecies of mite including
286 one oribatid of the family Scutoverticidae and one species in the Bdellidae family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

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

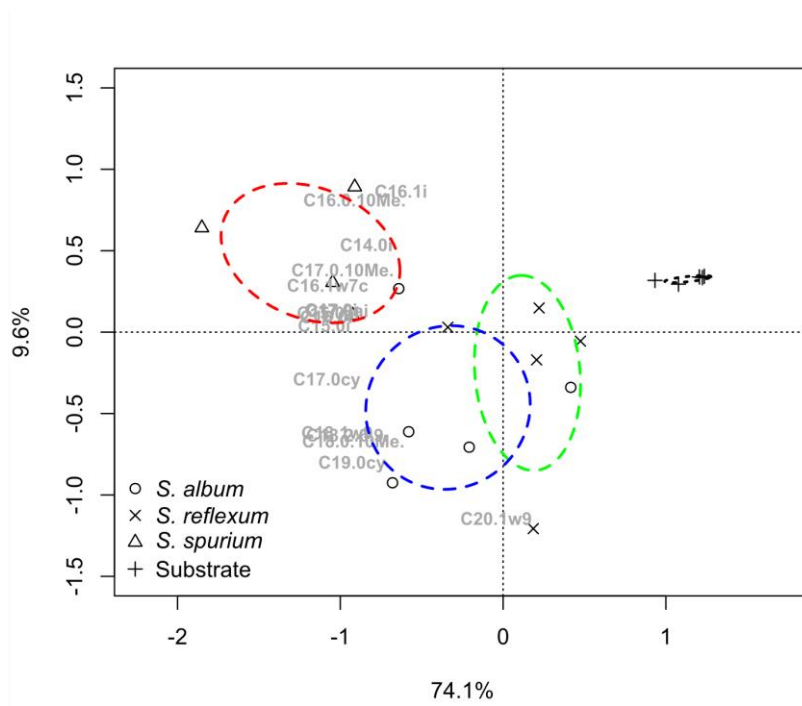
296 3.3 MICROBIAL COMMUNITY

297 Bare green roof substrate before construction was almost inert, with very little microbial mass detected
298 by PLFA (0.3 to 2.2 $\mu\text{g g}^{-1}$ dry soil). Plant plugs contained significantly more microbial mass than the
299 substrate ($F_{3,18} = 33.16$, $p < 0.001$). In addition, microbial mass was higher in *S. spurium* plugs than in
300 *S. reflexum* plugs ($p < 0.05$) and there was a suggestion that *S. album* also supported more microbial
301 mass than *S. reflexum* ($p < 0.076$) (Fig. 4a). *S. spurium* supported the most microbial abundance in
302 terms of bacterial markers (*S. spurium*; vs. *S. album*: $p < 0.05$; vs *S. reflexum*: $p < 0.001$) and all plugs
303 supported significantly more bacterial mass than the substrate (Substrate; vs. *S. spurium*: $p < 0.001$; *S.*
304 *album*: $p < 0.001$; *S. reflexum*: $p < 0.01$). Mass of bacterial PLFA's was higher than mass of fungal
305 PLFA's in all plugs and fungal mass did not vary between plug species ($p > 0.05$ for all plug species).
306 All plugs supported more mass of fungal PLFA's than the substrate (Substrate; vs. *S. spurium*: $p <$
307 0.001 ; *S. album*: $p < 0.001$; *S. reflexum*: $p < 0.05$). Post-planting, the surrounding substrate remained

a.)



b.)



c.)

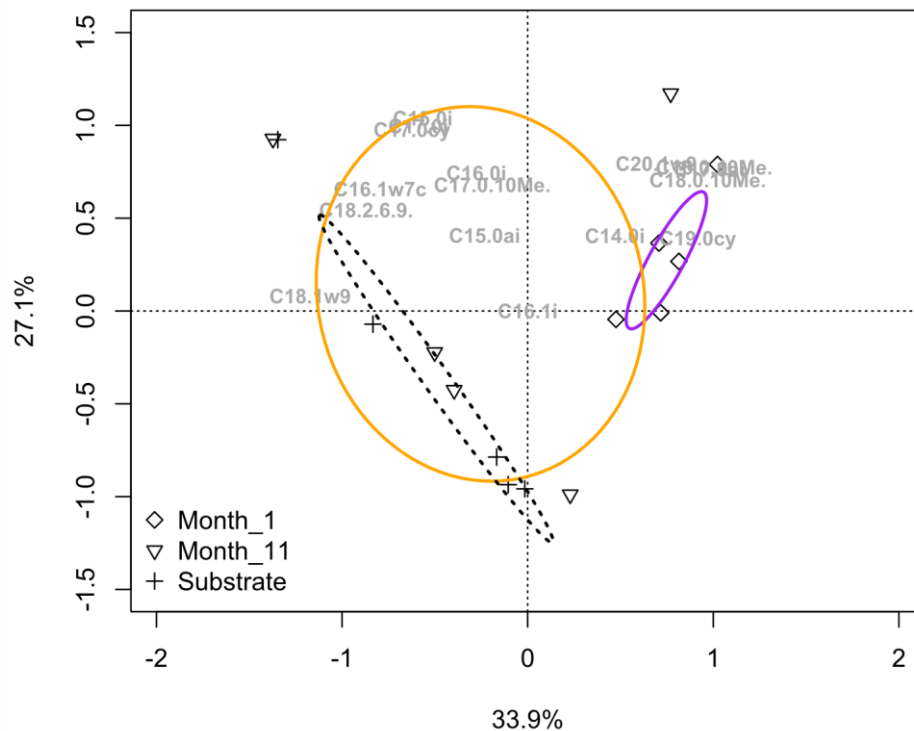


Fig 4. (a) Mean abundance of PLFA's ($\mu\text{g g}^{-1}$ dry soil) in plugs and substrate prior to planting and in the substrate post-planting over time. Grey shaded bar portions and dashed lines represent bacterial biomass, whilst the unshaded bar portion represents fungal biomass. Error bars represent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

308

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

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

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

324 DISCUSSION

325 4.1 SOURCE POPULATIONS IN BUILDING MATERIALS

326 In the current study the substrate was completely sterile in terms of soil microarthropods. Emilsson
327 (2008) suggests that this is due to the practise of heat treating for seed removal, but our substrate was
328 stored outside after heat treatment and contained rough compost. This indicates that even when
329 opportunities for colonisation are available, recycled aggregates as substrates may be too inhospitable
330 to support life when unaccompanied by plants. This presents a wasted opportunity; by volume, as
331 substrate far outweighs any soil introduced with plants. In terms of soil microbes, the substrate was not
332 sterile, but microbial mass was extremely low, with less than $2.2\mu\text{g g}^{-1}$ total fatty acid mass (less than
333 one sixtieth of that reported in forest soils (Ascher et al., 2012)).

334 Residual soil around plug plants, as we hypothesised, did contain microbes and microarthropods,
335 allowing us to test the hypothesis that soil can be 'inoculated' into a green roof habitat. Analysis of the
336 *Sedum spp.* plugs highlighted that different plant species supported distinct microarthropod

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

339 There were very few specialists of the harsh conditions found on a green roof (Rumble and Gange,
340 2013) in the *Sedum* plugs, perhaps unsurprising given the good quality soil and conditions in which
341 plugs grow. Personal observations of green roof nurseries suggest that the main source of
342 microarthropods to *Sedum* plugs are from the surrounding habitat, often a nursery of other plants, field
343 or garden. Many microarthropods in these ground-level soils have mechanisms to withstand short
344 periods of drought, such as synchronised emergence in collembola (Alvarez et al., 1999). Thus if *Sedum*
345 was grown in the substrate later to be used, but exposed to this diverse source population more
346 deliberately, these species may be able to colonise substrate, as well as plugs, at the ground level,
347 removing the barrier of an inaccessible rooftop. Whilst Rumble and Gange (2013) suggested that
348 ameliorating conditions on green roofs may benefit microarthropods, the current study also suggests
349 that ensuring organisms transplanted onto a green roof are adapted to its harsh conditions already, as
350 we practise with plant species, could be another successful strategy in building resilient soil
351 communities on green roofs. Potentially, this could be achieved with only a minor change to current
352 *Sedum* farming practise.

353 4.2 SURVIVAL OF SOURCE POPULATIONS POST-PLANTING

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

363 become more similar to that observed in the initial substrate than to the plugs. In addition, with the
364 exception of a few species, microarthropod abundance also declined after month one. Thus in terms of
365 microarthropods and microbes, we suggest that few organisms present in plugs were able to survive
366 long-term in the substrate. This is despite the characteristic droughts observed in Rumble & Gange
367 (2013) being absent within this study period. In terms of microbes, abundance at month eleven was
368 similar to that reported in comparable young green roofs by Molineux et al., (2014), suggesting that this
369 is a recurring phenomenon and our findings can be generalised to other extensive green roofs of this
370 design.

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

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

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

395 4.3 INDEPENDENT COLONISATION POST-PLANTING

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

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

411 4.4 MYCORRHIZAL FUNGI POST-PLANTING

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

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

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

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

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

442 plant growth (Sutton, 2008; Young et al., 2015), but, aside from studies on *S. alfredii* (Hu et al., 2013a,
443 2013b) (not a common green roof species), there are no empirical studies to our knowledge testing
444 whether mycorrhiza benefits *Sedum* species growth. As the vast majority of green roofs are planted with
445 at least some *Sedum* spp. this aspect of green roof ecology should not be overlooked. The commercial
446 inoculants used are often species mixes and it is not known which of these are able to establish within
447 *Sedum* roots, nor whether they enhance plant growth as a result. Olsson and Tyler (2004) suggest that,
448 as species of harsh, rocky environments, *Sedum* spp. may not be able to afford to donate photosynthates
449 to associated AMF and that AMF may well be more important in mediating competition between plants
450 than enhancing tolerance to abiotic stresses. Relationships between mycorrhizas and plants, as well as
451 between mycorrhizal species can produce extremely varied outcomes in terms of plant growth (Jin et
452 al., 2017). Outcomes on plant growth are also reported as being highly variable depending on fungal
453 community composition, both in the limited field trials that have been conducted (van der Heijden et
454 al., 2015) and in single and multi-species inoculations, with multiple species often failing to produce
455 any measureable effects on plants (Owen et al., 2015). Molineux et al., (2014) also highlight that
456 substrate properties themselves can determine how these communities develop and interact. Thus, if an
457 incumbent mycorrhizal community exists via this natural colonisation, the efficacy of mycorrhizal
458 additions may be more or less successful than if applied to a virgin substrate. In addition, applying any
459 mycorrhiza at all to green roofs where *Sedum* is planted could reduce plant growth by producing a
460 parasitic mycorrhiza/plant relationship. There is much research to be done in this area in terms of
461 determining which of these species is most beneficial for *Sedum* growth, as has been studied in
462 agricultural applications (Fester and Sawers, 2011), and whether the timing of the application of
463 inoculants alters success. In addition, AMF display species-specific intolerances to abiotic conditions,
464 such as drought (Klironomos et al., 2001), another aspect that is important for persistence on green
465 roofs and needs further research. The current study suggests that *Sedum* is very easily colonised by one
466 or more mycorrhiza species, very soon after planting.

467 4. CONCLUSIONS

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

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

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

491

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

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

501 ACKNOWLEDGEMENTS

502 This research was funded by The Natural Environment Research Council, within grant number
503 NE/G012482/1 and by CASE partners Symbio Ltd and Laverstoke Park. Thanks to Sedum Green Roof
504 Ltd for donating our plants and to Shire Green Roof Substrates Ltd for donating our green roof substrate.
505 Thanks to Neil Morley for assistance in the construction of the green roof and to Stephan Tietz for
506 proof-reading the manuscript.

507 **Conflict of Interest Statement**

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

512

513 References

- 514 Alvarez, T., Frampton, G.K., Goulson, D., 1999. The effects of drought upon epigeal Collembola
515 from arable soils. *Agric. For. Entomol.* 1, 243–248. doi:10.1046/j.1461-9563.1999.00032.x
- 516 Ascher, J., Sartori, G., Graefe, U., Thornton, B., Ceccherini, M.T., Pietramellara, G., Egli, M., 2012.
517 Are humus forms, mesofauna and microflora in subalpine forest soils sensitive to thermal
518 conditions? *Biol. Fertil. Soils* 48, 709–725. doi:10.1007/s00374-012-0670-9
- 519 Baumann, N., 2006. Ground-Nesting Birds on Green Roofs in Switzerland: Preliminary Observations.
520 *Urban Habitats* 4, 1–14.
- 521 Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Brussaard, L., Butt, K.R., Dai, J., Dendooven,
522 L., Peres, G., Tondoh, J.E., Cluzeau, D., Brun, J.-J., 2013. A review of earthworm impact on soil
523 function and ecosystem services. *Eur. J. Soil Sci.* 64, 161–182. doi:10.1111/ejss.12025
- 524 Braaker, S., Ghazoul, J., Obrist, M.K., Moretti, M., 2014. Habitat connectivity shapes urban arthropod
525 communities: the key role of green roofs. *Ecology* 95, 1010–1021. doi:10.1890/13-0705.1
- 526 Collins Johnson, N., Rowland, D., Corkidi, L., Egerton Warburton, L.M., Allen, E.B., 2003. Nitrogen
527 enrichment alters mycorrhizal allocation at five mesic semiarid grasslands. *Ecology* 84, 1895–
528 1908. doi:10.1890/0012-9658(2003)084[1895:NEAMAA]2.0.CO;2
- 529 Collins Johnson, N., T Wilson, G.W., Bowker, M.A., Wilson, J.A., Michael Miller, R., David Tilman,
530 by G., 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *Proc.*
531 *Natl. Acad. Sci. U. S. A.* 107, 2093–2098. doi:10.1073/pnas.0906710107
- 532 de Vries, F.T., Thébault, E., Liiri, M., Birkhofer, K., Tsiafouli, M.A., Bjørnlund, L., Bracht Jørgensen,
533 H., Brady, M.V., Christensen, S., de Ruiter, P.C., d’Hertefeldt, T., Frouz, J., Hedlund, K.,
534 Hemerik, L., Hol, W.H.G., Hotes, S., Mortimer, S.R., Setälä, H., Sgardelis, S.P., Uteseny, K.,
535 van der Putten, W.H., Wolters, V., Bardgett, R.D., 2013. Soil food web properties explain
536 ecosystem services across European land use systems. *Proc. Natl. Acad. Sci. U. S. A.* 110,

- 537 14296–301. doi:10.1073/pnas.1305198110
- 538 Egan, C., Li, D.-W., Klironomos, J., 2014. Detection of arbuscular mycorrhizal fungal spores in the
539 air across different biomes and ecoregions. *Fungal Ecol.* 12, 26–31.
540 doi:10.1016/J.FUNECO.2014.06.004
- 541 Emilsson, T., 2008. Vegetation development on extensive vegetated green roofs: Influence of
542 substrate composition, establishment method and species mix. *Ecol. Eng.* 33, 265–277.
543 doi:10.1016/j.ecoleng.2008.05.005
- 544 Ettema, C.H., Wardle, D.A., 2002. Spatial soil ecology. *Trends Ecol. Evol.* 17, 177–183.
545 doi:doi:10.1016/S0169-5347(02)02496-5
- 546 Fester, T., Sawers, R., 2011. Progress and challenges in agricultural applications of arbuscular
547 mycorrhizal fungi. *CRC. Crit. Rev. Plant Sci.* 30, 459–470. doi:10.1080/07352689.2011.605741
- 548 FLL, 2008. Richtlinie für die Planung, Ausführung und Pflege von Dachbegrünungen. Bonn.
- 549 Flø, D., Hågvar, S., 2013. Aerial dispersal of invertebrates and mosses close to a receding Alpine
550 glacier in Southern Norway. *Arctic, Antarct. Alp. Res.* 45, 481–490. doi:10.1657/1938-4246-
551 45.4.481
- 552 Frostegård, A., Tunlid, A., Bååth, E., 1993. Phospholipid Fatty Acid composition, biomass, and
553 activity of microbial communities from two soil types experimentally exposed to different heavy
554 metals. *Appl. Environ. Microbiol.* 59, 3605–17.
- 555 Frostegård, Å., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in soils. *Soil*
556 *Biol. Biochem.* 43, 1621–1625. doi:10.1016/j.soilbio.2010.11.021
- 557 Frouz, J., 1999. Use of soil dwelling Diptera (Insecta, Diptera) as bioindicators: a review of ecological
558 requirements and response to disturbance. *Agric. Ecosyst. Environ.* 74, 167–186.
559 doi:10.1016/S0167-8809(99)00036-5
- 560 Hågvar, S., 2010. Primary Succession of Springtails (Collembola) in a Norwegian Glacier Foreland.

- 561 Arctic, Antarct. Alp. Res. 42, 422–429. doi:<https://doi.org/10.1657/1938-4246-42.4.422>
- 562 Harley, J.L., Harley, E.L., 1987. A check-list of mycorrhiza in the British flora. *New Phytol.* 105, 1–
563 102. doi:[10.1111/j.1469-8137.1987.tb00674.x](https://doi.org/10.1111/j.1469-8137.1987.tb00674.x)
- 564 Hedrick, D.B., Peacock, A., White, D.C., 2005. Interpretation of fatty acid profiles of soil
565 microorganisms, in: *Monitoring and Assessing Soil Bioremediation*. Springer-Verlag,
566 Berlin/Heidelberg, pp. 251–259. doi:[10.1007/3-540-28904-6_12](https://doi.org/10.1007/3-540-28904-6_12)
- 567 Hopkin, S.P., 2007. *A key to the Collembola (Springtails) of Britain and Ireland*. FSC Publications,
568 Shrewsbury.
- 569 Hu, J., Chan, P.T., Wu, F., Wu, S., Zhang, J., Lin, X., Wong, M.H., 2013a. Arbuscular mycorrhizal
570 fungi induce differential Cd and P acquisition by Alfred stonecrop (*Sedum alfredii* Hance) and
571 upland kangkong (*Ipomoea aquatica* Forsk.) in an intercropping system. *Appl. Soil Ecol.* 63, 29–
572 35. doi:[10.1016/j.apsoil.2012.09.002](https://doi.org/10.1016/j.apsoil.2012.09.002)
- 573 Hu, J., Wu, S., Wu, F., Leung, H.M., Lin, X., Wong, M.H., 2013b. Arbuscular mycorrhizal fungi
574 enhance both absorption and stabilization of Cd by Alfred stonecrop (*Sedum alfredii* Hance) and
575 perennial ryegrass (*Lolium perenne* L.) in a Cd-contaminated acidic soil. *Chemosphere* 93,
576 1359–1365. doi:[10.1016/j.chemosphere.2013.07.089](https://doi.org/10.1016/j.chemosphere.2013.07.089)
- 577 Jaffal, I., Ouldboukhitine, S.-E., Belarbi, R., 2012. A comprehensive study of the impact of green
578 roofs on building energy performance. *Renew. Energy* 43, 157–164.
579 doi:[10.1016/j.renene.2011.12.004](https://doi.org/10.1016/j.renene.2011.12.004)
- 580 Jin, L., Wang, Q., Wang, Q., Wang, X., Gange, A.C., 2017. Mycorrhizal-induced growth depression
581 in plants. *Symbiosis* 72, 81–88. doi:[10.1007/s13199-016-0444-5](https://doi.org/10.1007/s13199-016-0444-5)
- 582 John, J., Lundholm, J., Kernaghan, G., 2014. Colonization of green roof plants by mycorrhizal and
583 root endophytic fungi. *Ecol. Eng.* 71, 651–659. doi:[10.1016/j.ecoleng.2014.08.012](https://doi.org/10.1016/j.ecoleng.2014.08.012)
- 584 Kindt, R., Coe, R., 2005. *BiodiversityR*.

- 585 Klironomos, J.N., Hart, M.M., Gurney, J.E., Moutoglou, P., 2001. Interspecific differences in the
586 tolerance of arbuscular mycorrhizal fungi to freezing and drying. *Can. J. Bot.* 79, 1161–1166.
587 doi:10.1139/b01-099
- 588 Krantz, G.W., Walter, D.E., 2009. *A manual of acarology*, 3rd ed. Texas Tech University Press,
589 Lubbock.
- 590 Lavelle, P., Decaëns, T., Aubert, M., Barot, S., Blouin, M., Bureau, F., Margerie, P., Mora, P., Rossi,
591 J.-P., 2006. Soil invertebrates and ecosystem services. *Eur. J. Soil Biol.* 42, S3–S15.
592 doi:10.1016/j.ejsobi.2006.10.002
- 593 Lehmitz, R., Russell, D., Hohberg, K., Christian, A., Xylander, W.E.R., 2011. Wind dispersal of
594 oribatid mites as a mode of migration. *Pedobiologia (Jena)*. 54, 201–207.
595 doi:10.1016/J.PEDOBI.2011.01.002
- 596 MacFadyen, A., 1953. Notes on methods for the extraction of small soil arthropods. *J. Anim. Ecol.* 22,
597 65–77. doi:10.2307/1691
- 598 McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, J.A., 1990. A new method which
599 gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi.
600 *New Phytol.* 115, 495–501. doi:10.1111/j.1469-8137.1990.tb00476.x
- 601 McGuire, K.L., Payne, S.G., Palmer, M.I., Gillikin, C.M., Keefe, D., Kim, S.J., Gedalovich, S.M.,
602 Discenza, J., Rangamannar, R., Koshner, J.A., Massmann, A.L., Orazi, G., Essene, A., Leff,
603 J.W., Fierer, N., 2013. Digging the New York City Skyline: Soil Fungal Communities in Green
604 Roofs and City Parks. *PLoS One* 8, e58020. doi:10.1371/journal.pone.0058020
- 605 Molineux, C.J., Connop, S.P., Gange, A.C., 2014. Manipulating soil microbial communities in
606 extensive green roof substrates. *Sci. Total Environ.* 493, 632–8.
607 doi:10.1016/j.scitotenv.2014.06.045
- 608 Molineux, C.J., Fentiman, C.H., Gange, A.C., 2009. Characterising alternative recycled waste

- 609 materials for use as green roof growing media in the U.K. *Ecol. Eng.* 35, 1507–1513.
610 doi:10.1016/j.ecoleng.2009.06.010
- 611 Molineux, C.J., Gange, A.C., Connop, S.P., Newport, D.J., 2015. Using recycled aggregates in green
612 roof substrates for plant diversity. *Ecol. Eng.* 82, 596–604. doi:10.1016/j.ecoleng.2015.05.036
- 613 Nordstokke, D.W., Zumbo, B.D., 2010. A new nonparametric Levene test for equal variances.
614 *Psicologica* 31, 401–430.
- 615 Odonõ No, S., Bastida, F., Moreno, J.L., 2014. Microbiological and biochemical properties of
616 artificial substrates: A preliminary study of its application as Technosols or as a basis in Green
617 Roof Systems. *Ecol. Eng.* 70, 189–199. doi:10.1016/j.ecoleng.2014.05.003
- 618 Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O’Hara, R.B., Simpson, G.L.,
619 Solymos, P., Stevens, M.H.H., Wagner, H., 2015. *vegan: Community Ecology Package*.
- 620 Olsson, P.A., Tyler, G., 2004. Occurrence of non-mycorrhizal plant species in south Swedish rocky
621 habitats is related to exchangeable soil phosphate. *J. Ecol.* 92, 808–815. doi:10.1111/j.0022-
622 0477.2004.00912.x
- 623 Owen, D., Williams, A.P., Griffith, G.W., Withers, P.J.A., 2015. Use of commercial bio-inoculants to
624 increase agricultural production through improved phosphorus acquisition. *Appl. Soil Ecol.* 86,
625 41–54. doi:10.1016/j.apsoil.2014.09.012
- 626 Peter Shaw, n.d. (397SNtri) *Sminthurinus trinotatus* (Axelson, 1905) [WWW Document]. URL
627 [http://urweb.roehampton.ac.uk/collembola/taxonomy/%28397SNtri%29 Sminthurinus trinotatus](http://urweb.roehampton.ac.uk/collembola/taxonomy/%28397SNtri%29+Sminthurinus+trinotatus)
628 .html (accessed 2.13.17).
- 629 Ponge, J., 1991. Food resources and diets of soil animals in a small area of Scots pine litter. *Geoderma*
630 49, 33–62. doi:10.1016/0016-7061(91)90090-G
- 631 Porco, D., Potapov, M., Bedos, A., Busmachiu, G., Weiner, W.M., Hamra-Kroua, S., Deharveng, L.,
632 2012. Cryptic diversity in the ubiquitous species *Parisotoma notabilis* (Collembola, Isotomidae): A

- 633 long-used chimeric species? PLoS One 7, e46056. doi:10.1371/journal.pone.0046056
- 634 Porsche, U., Köhler, M., 2003. Life cycle costs of green roofs, in: RIO 3 - World Climate & Energy
635 Event. Rio de Janeiro, pp. 461–467.
- 636 R Core Team, 2015. R: A language and environment for statistical computing.
- 637 Raiche, G., Magis, D., 2011. nFactors.
- 638 Rinaudo, V., Bàrberi, P., Giovannetti, M., van der Heijden, M.G.A., 2010. Mycorrhizal fungi suppress
639 aggressive agricultural weeds. Plant Soil 333, 7–20. doi:10.1007/s11104-009-0202-z
- 640 Rumble, H., 2013. Quantifying the soil community on green roofs. Royal Holloway, University of
641 London.
- 642 Rumble, H., Gange, A.C., 2017. Microbial inoculants as a soil remediation tool for extensive green
643 roofs. Ecol. Eng. 102, 188–198. doi:10.1016/j.ecoleng.2017.01.025
- 644 Rumble, H., Gange, A.C., 2013. Soil microarthropod community dynamics in extensive green roofs.
645 Ecol. Eng. 57, 197–204. doi:10.1016/j.ecoleng.2013.04.012
- 646 Sakamoto, K., Iijima, T., Higuchi, R., 2004. Use of specific phospholipid fatty acids for identifying
647 and quantifying the external hyphae of the arbuscular mycorrhizal fungus *Gigaspora rosea*. Soil
648 Biol. Biochem. 36, 1827–1834. doi:10.1016/j.soilbio.2004.04.037
- 649 Schäffer, S., Pfungstl, T., Koblmüller, S., Winkler, K.A., Sturmbauer, C., Krisper, G., 2010.
650 Phylogenetic analysis of European Scutovertex mites (Acari, Oribatida, Scutoverticidae) reveals
651 paraphyly and cryptic diversity: A molecular genetic and morphological approach. Mol.
652 Phylogenet. Evol. 55, 677–88. doi:10.1016/j.ympev.2009.11.025
- 653 Schrader, S., Böning, M., 2006. Soil formation on green roofs and its contribution to urban
654 biodiversity with emphasis on Collembolans. Pedobiologia (Jena). 50, 347–356.
655 doi:10.1016/j.pedobi.2006.06.003

- 656 Setälä, H., Berg, M.P., Hefin Jones, T., 2005. Trophic structure and functional redundancy in soil
657 communities, in: Bardgett, R.D., Hopkins, D.W., Usher, M.B. (Eds.), *Biological Diversity and*
658 *Function in Soils*. Cambridge University Press, Cambridge, p. 411.
- 659 Southwood, T.R.E., Leston, D., 2005. *Land and water bugs of the British Isles*. Pisces Conservation,
660 New Milton.
- 661 Strandtmann, R.W., 1971. The Eupodid mites of Alaska (Acarina: Prostigmata). *Pacific Insects* 13,
662 75–118.
- 663 Strandtmann, R.W., Davies, L., 1972. Eupodiform mites from Possession with a key to the species of
664 *Eupodes* (Acarina: Prostigmata). *Pacific Insects* 14, 39–56.
- 665 Sutton, R., 2008. Media modifications for native plant assemblages on extensive green roofs, in: *The*
666 *Sixth Annual International Rooftops for Sustainable Communities Conference*.
- 667 van der Heijden, M.G.A., Martin, F.M., Selosse, M.-A., Sanders, I.R., 2015. Mycorrhizal ecology and
668 evolution: the past, the present, and the future. *New Phytol.* 205, 1406–1423.
669 doi:10.1111/nph.13288
- 670 VanWoert, N.D., Rowe, D.B., Andresen, J.A., Rugh, C.L., Fernandez, R.T., Xiao, L., 2005. Green
671 roof stormwater retention: effects of roof surface, slope, and media depth. *J. Environ. Qual.* 34,
672 1036–1044. doi:10.2134/jeq2004.0364
- 673 Verbruggen, E., El Mouden, C., Jansa, J., Akkermans, G., Bücking, H., West, S.A., Kiers, E.T., 2012.
674 Spatial structure and interspecific cooperation: theory and an empirical test using the
675 mycorrhizal mutualism. *Am. Nat.* 179, 133–146. doi:10.1086/665032
- 676 Vierheilig, Coughlan, Wyss, Piche, 1998. Ink and vinegar, a simple staining technique for arbuscular-
677 mycorrhizal fungi. *Appl. Environ. Microbiol.* 64, 5004–5007.
- 678 Wallwork, J.A., 1970. *Ecology of Soil Animals*, Ecology of soil animals. McGraw-Hill, London.
- 679 Walter, P., Proctor, H., 2013. *Mites in Soil-Orders, Suborders, Cohorts*. [WWW Document]. URL

- 680 <http://keys.lucidcentral.org/keys/cpitt/public/mites/Soil Mites/Index.htm> (accessed 11.21.17).
- 681 Wang, B., Qiu, Y.-L., 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants.
- 682 *Mycorrhiza* 16, 299–363. doi:10.1007/s00572-005-0033-6
- 683 Wanner, M., Dunger, W., 2002. Primary immigration and succession of soil organisms on reclaimed
- 684 opencast coal mining areas in eastern Germany. *Eur. J. Soil Biol.* 38 SRC-, 137–143.
- 685 doi:[https://doi.org/10.1016/S1164-5563\(02\)01135-4](https://doi.org/10.1016/S1164-5563(02)01135-4)
- 686 Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H., Wall, D.H., 2004.
- 687 Ecological linkages between aboveground and belowground biota. *Science* 304, 1629–33.
- 688 doi:10.1126/science.1094875
- 689 Williams, N.S.G., Lundholm, J., Scott MacIvor, J., 2014. Do green roofs help urban biodiversity
- 690 conservation? *J. Appl. Ecol.* 51, n/a-n/a. doi:10.1111/1365-2664.12333
- 691 Young, T., Cameron, D.D., Phoenix, G.K., 2015. Using AMF inoculum to improve the nutritional
- 692 status of *Prunella vulgaris* plants in green roof substrate during establishment. *Urban For. Urban*
- 693 *Green.* 14, 959–967. doi:10.1016/j.ufug.2015.08.012
- 694 Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation
- 695 of microbial communities in soil: a review. *Biol. Fertil. Soils* 29, 111–129.
- 696 doi:10.1007/s003740050533
- 697

698 SUPPLEMENTARY MATERIAL 1: LAYOUT OF GREEN ROOF EXPERIMENTAL TRAYS

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

