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3

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23   **Abstract**

24   We investigated soil microarthropod communities in 2 physically dissimilar inorganic soil materials and in a

25   mixture of these 2 materials to examine the effect of the structural complexity of a habitat on microarthropod

26   abundance and communities, teasing it out from that of nutritional factors. Mesh boxes were filled with perlite (a

27   highly porous material), similar size of granite gravels (no pores inside), or their mixture, and placed on a forest

28   floor. The boxes were collected after 8 or 20 months, and the microarthropods were extracted and identified to the

29   species level, with a focus on Collembola. We also evaluated fine-root biomass and the amount of organic matter

30   in the boxes. It was found that the mixture of perlite and granite enhanced microarthropod abundance and root

31   development. A partial redundancy analysis (pRDA) revealed that collembolan communities developed differently

32   among the substrate materials. We also found that variation in the collembolan communities was related to

33   fine-root development and the abundance of other microarthropods, implying that habitat structural complexity

34   affects collembolan communities indirectly by affecting soil food webs.

35

36   Keywords: Collembola; community; habitat complexity; soil microarthropods; soil structure.

37

Introduction

In soil, heterogeneity is the basis of biodiversity (Bardgett 2002). Communities of soil microarthropods, which are highly abundant in temperate forests (Petersen and Luxton 1982), are affected by litter types (Anderson 1975), litter decomposition stages (Hasegawa and Takeda 1995), and litter depth or strata in soil (Takeda 1978; Hågvar 1983; Ponge 2000). These factors interact to produce various biotic and abiotic environments in soil, resulting in diverse habitats for individual species.

Previous studies have highlighted the importance of habitat diversity on a small scale in soil (reviewed by Wardle 2002). Diverse combinations of litter materials provide a broad spectrum of substrates for decomposition, leading to the provision of diverse food resources for microarthropods. Similarly, in terms of physical habitat, the diversity in substrates translates to the presence of diverse habitable spaces for microarthropods. Further, because of its complexity, soil structure is thought to buffer species interactions, including competition and predation, and to contribute to the coexistence of different species (Anderson and Healey 1972; Wardle 1995). The positive correlation between the diversity of the habitat components of soil organic layers and microarthropod diversity supports these views (Anderson 1978).

In the aforementioned context, experiments have been conducted using multiple litter species as substrate materials to investigate whether habitat heterogeneity affects microarthropod communities in terms of species composition, abundance, and diversity. Studies on Collembola (Takeda 1987) and Oribatida (Hansen and Coleman 1998; Kaneko and Salamanca 1999; Hansen 2000) revealed that a mixture of litter types results in species compositions of microarthropod communities that are different from those observed when using a single litter type. Further, a mixture of litter types appears to have a positive effect on total soil microarthropod abundance (Kaneko and Salamanca 1999; Hansen 2000) and diversity (Hansen and Coleman 1998; Kaneko and Salamanca 1999; Hansen 2000). Hansen (2000) suggested that mixtures of litter types contribute to strengthening the soil physical structure, and thereby improve the properties of habitats, such as the amount and variety of habitable pore space, accumulation of fine organic particles, and development of plant fine roots and fungal hyphae, which would likely result in enhanced microarthropod communities.

In contrast, some studies on litter mixtures have failed to detect any clear effects of litter mixtures on

64   microarthropod abundance (Blair et al. 1990; Wardle et al. 2006). Wardle et al. (2006) suggested that the

65   nutritional quality of individual litter types, rather than the composition of the mixture, has a significant effect on

66   soil biota. This indicates that trophic aspects of substrate materials used when studying such mixtures contribute to

67   ambiguity in measurements of structural complexity. To elucidate the mechanisms by which the structural

68   complexity of a habitat affects the development of soil ecosystems and microarthropod communities, it is

69   necessary to exclude trophic factors of the substrate materials used in experiments analyzing such mixed

70   substrates.

71   Accordingly, we decided to use mixtures of inorganic materials instead of litter. We utilized 2 inorganic materials

72   with different physical traits: perlite, which has a porous structure, and granite, which is a non-porous material.

73   When equal volumes of the 2 materials were mixed, the physical traits of the mixture, such as pore volume,

74   surface area, and water-holding capacity, attained values that were intermediate of those of the 2 parent materials,

75   while the system became more complex and spatially heterogeneous on a small scale. We compared the

76   development of soil biota, especially collembolan communities, between mesocosms containing single materials

77   (i.e., perlite or granite) and the mixture of these materials, to examine the effect of the structural complexity of a

78   habitat.

79   We hypothesized that a mixture of inorganic materials would have positive effects on the abundance and species

80   richness of microarthropods, as well as on biotic factors such as the accumulation of organic matter and

81   elongation of plant roots in the developmental processes of ecosystems within mesocosms. An additional

82   objective was to determine how such a mixture of inorganic materials affects individual collembolan species, and

83   consequently, the collembolan community structure.

84

85   **Materials and methods**

86   The study was conducted in a temperate coniferous forest on a ridge in the Kamigamo Experimental Forest

87   (135.04°E, 35.46°N; 220 m ASL), Field Science Education and Research Center, Kyoto University, located in

88   central Honshu Island, Japan. During the study period (from 2004 to 2007), the mean temperature was 14.8°C and

89   the mean annual cumulative precipitation was 1485 mm (measured at the Kamigamo Experimental Forest office).

90 The forest canopy was dominated by Japanese cypress (*Chamaecyparis obtusa*). The understory vegetation

91 comprised *Eurya japonica*, *Evodiopanax innovans*, *Camellia japonica*, and *Cleyera japonica*. The soil humus type

92 was moder, with an organic layer approximately 4-cm thick. Fine roots of Japanese cypress formed a root mat in

93 the A_0 layer.

94 Three treatments were prepared: perlite, granite, and a 1:1 (v/v) mixture of the 2. Perlite is highly porous and

95 thus has a high water-holding capacity, unlike granite. Mean particle sizes were 4 mm and 5 mm for perlite and

96 granite, respectively. Materials were rinsed with tap water before use.

97 Pore size distributions differed between treatments with respect to fine pore spaces smaller than 2.8 mm (Fig.

98 1): perlite has a large proportion of fine pore spaces, while granite is characterized by primarily macropore spaces.

99 Their mixture exhibited intermediate characteristics. Pores larger than 44 μm are accessible for soil

100 microarthropods (Lavelle and Spain 2001). Pore size distributions were determined as follows. Material cores of

101 100 cm^3 (*n* = 3) were saturated with water, drained through a sandbed (pF = 1.8), and subsequently exposed to a

102 pressure plate (31 kPa) to determine the maximum water-holding capacity, field capacity, and lento-capillary point,

103 respectively. These correspond to pore sizes (diameters) of 2.8 mm, 44 μm, and 8.8 μm, respectively.

104 Subsequently, the materials were oven dried at 70°C. From the data, percent volumes of pores with diameters of

105 >2.8 mm, 2.8 mm–44 μm, 44–8.8 μm, and <8.8 μm were determined.

106 We arranged plastic mesh boxes (mesh size 5 mm; 12 cm [length] × 12 cm [width] × 8 cm [depth]) on the forest

107 floor after removing the organic layer from under the bottom of each box on August 31, 2005. We created 10

108 contiguous 20-m^2 subplots and arranged a set of 3 boxes (one for each treatment) in each subplot; thus, 30 boxes

109 (10 for each treatment) were established. Each box was filled with 1 L of the selected materials, and the top of

110 each box was covered with 1-mm mesh to prevent the entry of large litter.

111 We collected the boxes from half the total number of subplots after 8 months (on April 20, 2006) and the

112 remaining boxes after 20 months (on April 26, 2007). Thus, no samples were obtained from the same subplot in

113 2006 or 2007. After carefully cutting out fine roots with a knife, the boxes were placed in plastic bags and

114 transported to the laboratory for analysis. Each box was placed on a Tullgren funnel to extract soil

115 microarthropods. Extraction was performed for 7 days at a temperature of 35°C. Water content was determined by

116   comparing the weights of each box before and after extraction. Fine roots were collected using forceps, oven dried

117   at 40°C for 3 days, and weighed to 0.1 mg. Organic matter was collected with a 2-mm sieve; the carbon content

118   (mg C box^{-1}) was then measured using an NC analyzer (Sumigraph NC-900; Sumitomo Chemical Co., Osaka,

119   Japan) after milling the collected organic matter.

120   Microarthropods were identified to the species level for Collembola and to the order or suborder levels for other

121   taxa, using a microscope. Collembola were categorized into 36 taxa; *Tomocerus punctatus* and *T. varius* were

122   grouped as *Tomocerus* spp. since it was difficult to distinguish their juveniles from each other. Overall, the group

123   appeared to be dominated by *T. varius*.

124

125   **Statistical analyses**

126   We conducted a one-way ANOVA followed by a multiple comparison test with Welch's *t*-test and a Bonferroni

127   correction to analyze the differences in the mean values of the abundance of microarthropods, fine-root biomass,

128   accumulated organic matter, water content, and species richness of Collembola at each sampling date among the

129   treatments. Counts of microarthropod individuals were log-transformed before the analysis. To calculate species

130   richness, the number of species and standard errors were estimated by the first-order jackknife procedure (Smith

131   and van Belle 1984), and the estimated values were used in the ANOVA and multiple comparison tests. To

132   examine the effects of the mixture on these measured values, we tested observed and predicted values using a

133   paired *t*-test. The observed value was that of the corresponding variable in the mixture treatment, and the predicted

134   value was calculated as the mean of the 2 single-material treatments for each subplot (Blair et al. 1990; Kaneko

135   and Salamanca 1999; Wardle et al. 2006). To examine the effect of the mixture on species richness, the expected

136   number of species in the mixture treatment, based on measuring the number of observed individuals, was

137   estimated from the combined communities of the 2 single-material treatments, using a rarefaction method for each

138   subplot. Differences between the estimated values and the number of observed species in the mixture treatment

139   were then examined using a Wilcoxon signed-rank test.

140   To determine the ordination of the collembolan communities, we conducted RDA with forward selection of the

141   explanatory variables to examine the effects of environmental factors, namely, elapsed time, subplot, 3 other

142 higher microarthropod taxa, accumulated organic matter, fine-root biomass, and water content. Further, a partial

143 RDA (pRDA) was conducted, in which the effects of elapsed time and subplot were separated out. The

144 significances of the RDA axes were then calculated using a permutation test. We excluded 7 species from this

145 analysis because we identified fewer than 5 individuals for them or found them in only one soil sample, taking

146 into account the recommendation made by Hasegawa (2006). Redundancy analysis and selection of the

147 explanatory variables for the analysis were performed with the software packages "vegan" (Oksanen et al. 2010)

148 and "packfor" (Dray 2009), respectively. The latter is based on a forward selection method described by ter Braak

149 and Šmilauer (2002).

150

151 **Results**

152 Figure 2 displays the abundance of soil microarthropods, environmental factors, and species richness of

153 Collembola in the experimental boxes after 8 and 20 months. The abundance of Gamasida and fine-root biomass

154 differed among the treatments after 20 months but not after 8 months (Figs. 2c, f). Fewer Gamasida individuals

155 appeared in perlite samples than in the other 2 samples after 20 months; no other microarthropods exhibited any

156 differences among the treatments (Figs. 2a, b, d). Fine-root biomass was lowest in the granite samples (Fig. 2f).

157 Water content differed among the treatments, reflecting the amount of perlite in each sample: throughout the study

158 period, we consistently observed the highest water content in perlite and the lowest in granite (Fig. 2g). At the first

159 sampling date, a greater amount of organic matter was detected in perlite samples than in granite samples (Fig. 2e).

160 Collembolan species richness did not differ among treatments (Fig. 2h).

161   We detected a positive effect of the perlite–granite mixture on the abundance of Collembola ($P = 0.0123$) on the

162 first sampling date (Figs. 2a, h), on the abundances of Gamasida ($P = 0.0736$) and Prostigmata ($P = 0.0352$) on the

163 second sampling date (Figs. 2c, d), on the abundance of Oribatida on the first ($P = 0.0414$) and second ($P =

164 0.0772$) sampling dates (Fig. 2b), and on fine-root biomass on the first ($P = 0.0675$) and second ($P = 0.0605$)

165 sampling dates (Fig. 2f). The effect on water content was negative on the first ($P = 0.0126$) and second ($P =

166 0.0023$) sampling dates (Fig. 2g).

167   The dominant collembolan species, which accounted for more than 1% of the total collected collembolan

168 individuals on at least one sampling date, are listed in Table 1. *Folsomia octoculata* was less abundant in granite

169 than in the other 2 treatments on the first sampling date, while *Arrhopalites* sp. was the least abundant in perlite.

170 *Onychiurus flavescens* appeared more abundant in perlite than in granite on the first sampling date, while

171 *Entomobrya* sp. was more abundant in perlite than in granite on the second sampling date. In contrast, *Tomocerus*

172 spp. was more abundant in granite than in perlite. With regard to the abundances of individual species, we

173 detected a positive effect of the perlite–granite mixture on *Tomocerus* spp. ($P = 0.0007$), *F. octoculata* ($P =$

174 0.0046), *F. japonica* ($P = 0.0552$), and *Arrhopalites* sp. ($P = 0.0981$) on the first sampling date and *Sinella*

175 *dubiosa* ($P = 0.0290$) and *F. japonica* ($P = 0.0166$) on the second sampling date.

176 In RDA, the elapsed time, water content, abundance of oribatids, fine-root biomass, and some subplots were

177 selected as the explanatory variables using forward selection. These factors explained 65.4% of the total variation

178 among the sampled collembolan communities. The effects of elapsed time and subplot as conditional factors were

179 separated out in pRDA. The constraining factors in pRDA, namely, the abundance of oribatids, fine-root biomass

180 and water content, explained 15.3% of the variance.

181   Ordination of the collembolan communities based on RDA is shown in Figure 3a. Axes 1 and 2 explained

182 22.2% and 15.4% of the total variance, respectively. A permutation test indicated that RDA axes 1-5 were

183 significant ($P < 0.05$). The ordination revealed relatively large effects of elapsed time and subplot on the

184 collembolan communities (Fig. 3a). Arrows representing Oribatida and fine-root biomass, as well as elapsed time,

185 were oriented in the lower right quadrant of the coordinate plane. Reflecting elapsed time, the majority of the

186 communities sampled after 20 months were plotted in the lower half of the plane, whereas those sampled after 8

187 months were plotted in the upper half. Likewise, most collembolan taxa were plotted in the lower half of the plane,

188 indicating their colonization of the substrates over time. Subplot had a considerable effect on ordination,

189 indicating the effect of forest floor heterogeneity on the collembolan communities. For example, the arrows for

190 subplots d, f, and g coincided with those of the communities sampled from those subplots.

191   In pRDA (Fig. 3b), in which the effects of subplot and elapsed time were separated out, the collembolan

192 communities were plotted into 3 groups according to the treatments. Axes 1 and 2 explained 16.4% and 12.7% of

193 the residual variance, respectively. A permutation test indicated that RDA axes 1 and 2 were significant ($P < 0.05$).

194

Discussion

196   Microarthropod communities developed in our field-incubated mesocosms containing 2 inorganic materials and

197   their mixture. As hypothesized, we found a positive effect of the mixture on the abundance of microarthropods

198   (Fig. 2), similar to the results of previous studies using mixed litter (Kaneko and Salamanca 1999; Hansen 2000).

199   Enhancement was observed in the animal taxa on the first and/or second sampling dates (Figs. 2a, b, c, d). With

200   respect to Collembola, a positive effect of the mixture was detected on the abundance of some species (Table 1),

201   whereas no positive effect was detected on species richness (Fig. 2h).

202   Although we expected enhancement of the accumulation of organic matter and development of fine roots in the

203   experimental boxes, only the latter was actually observed (Figs. 2e, f). This may have contributed to the observed

204   positive effects on microarthropod abundances to some extent. These findings suggest that habitat structural

205   complexity may have a positive effect on the abundance of soil microarthropods, partly via enhancement of the

206   development of fine roots, which could increase the availability of trophic resources.

207   The responses of individual collembolan species to the composition of the substrate varied among species (Table

208   1), with linear and non-linear responses exhibited (Fig. 3).

209

Effect of habitat structural complexity on the abundance of microarthropods and abiotic/biotic factors in

the mesocosms

212   In our system, inorganic materials served primarily as the physical framework of the habitat and received organic

213   debris and fine roots from the surrounding organic layer, which likely served as energy sources in the

214   experimental system. The fact that inorganic materials neither supplied any nutrients nor decomposed in the study

215   period is important in interpreting the results of this study, compared with previous studies using mixtures of

216   organic litter species. Those studies have suggested that mixing litters with dissimilar chemical traits improves

217   food resource availability by stimulating microflora (Salamanca et al. 1998; Kaneko and Salamanca 1999), but

218   this effect was absent in the present study. It has been also suggested that the slow decomposition of mixed litter

219   makes these substrates superior at maintaining the physical structure and available pore space when compared to

220   substrates based on single litter species, leading to a relatively higher capacity to entrain small organic particles

221   (Hansen and Coleman 1998; Hansen 2000). Unlike previous studies, none of the examined material decomposed

222   in the present study. This may explain why enhancement of the accumulation of organic matter was not observed.

223   We detected an enhancement of plant fine root growth. Interestingly, the amount of fine roots was equivalent

224   between perlite and mixture samples on the second sampling date. Fine roots may have responded to merely the

225   presence of fine pore spaces in perlite, which are important for root elongation (Pagliai and De Nobili 1993). The

226   significance of this response is discussed later. Fine roots may have affected microarthropods via their role in

227   supplying energy in the ecosystem (Wiggins et al. 1979, Parmelee et al. 1993). Nonetheless, variation in fine roots

228   did not fully explain the microarthropod abundances, since no higher taxon displayed similar patterns in response

229   to changes in fine root biomass (Figs. 2a–d, f). The responses of individual collembolan species to fine roots

230   appeared to be varied. This implies the presence of some other unevaluated factor(s) or mechanism(s), involved

231   with mixing perlite and granite, affecting microarthropod abundances.

232   Water content was evidently different among the treatments (Fig. 2g). Water content has been recognized to be

233   one of the most important factors regulating the abundance of soil microarthropods; this fact has been confirmed

234   in field studies (reviewed by Hopkin 1997). This is because soil fauna are generally less tolerant to desiccation

235   than those aboveground, and even live fauna move downward and thus away from the topsoil when they sense

236   desiccation (Swift et al. 1979). Nonetheless, it is the humidity in the soil pore spaces, rather than the water content,

237   that affects soil microarthropods (Vanier 1987). In spite of the variation in water content between treatments, the

238   humidity in the pore spaces of any treatment in the present study was likely at saturating levels, since we observed

239   water films even on the granite particle surfaces during sampling, notwithstanding the fact that the granite

240   treatment exhibited the lowest mean water content. Thus, we concluded that water content was unlikely to have

241   affected microarthropod abundance in terms of desiccation.

242   A correlation between the total number of microarthropods and the amount of pore spaces of a certain size has

243   been reported in the field (Vreeken-Buijs et al. 1998; Nielsen et al. 2008) and laboratory (Larsen et al. 2004). The

244   field studies reported a correlation between Collembola and the amount of pores >90 μm in size in mineral soil

245   (Vreeken-Buijs et al. 1998) and between Oribatida and the amount of pores 60–300 μm in size in the soil organic

246   layer (Nielsen et al. 2008). Larsen et al (2004) conducted a soil compaction experiment to reveal the importance

247   of the amount of pores 120–300 μm in size in influencing the abundance of certain collembolan species. These

248   studies indicated that the availability of habitable pore spaces for microarthropods could limit their abundance. In

249   the current study, the pore size range corresponding to that in the previous studies was 44 μm–2.8 mm. The

250   amount of this size of pore was positively correlated with the amount of perlite (i.e., perlite > mixture > granite,

251   Fig. 1). Unlike in the previous studies, no higher animal taxon showed any correlation to these pores (Figs. 2a–d),

252   although some individual collembolan species displayed the pattern perlite > mixture > granite in terms of

253   abundance (Table 1). Since all the treatments had high porosities (>50%), the amount of pores was unlikely to

254   have limited microarthropod abundances. Another possible explanation is that a large proportion of the pore

255   spaces of the size range 44 μm–2.8 mm may have been internal and closed (or narrow-necked) in perlite particles,

256   and therefore would have been unavailable for microarthropods.

257   Focusing on the patterns of species-level responses to treatments, individual collembolan species responded

258   differently (Table 1). Four different patterns of responses to the substrate material composition gradient were

259   observed. (1) The 2 most abundant taxa on the second sampling date (*Tomocerus* spp. and *Entomobrya* sp.)

260   responded differently, albeit linearly, to the 2 different materials. Their abundances in the mixture treatment

261   approximated that of the mean of the 2 single-material treatments. (2) The mixture treatment enhanced the

262   abundances of some species: *Tomocerus* spp. on the first sampling date, *Sinella dubiosa* on the second sampling

263   date, and *Friesea japonica* on both sampling dates. (3) Some species were abundant in one of the 2 single-material

264   treatments and also abundant at an equivalent level in the mixture treatment: *Folsomia octoculata* was abundant in

265   perlite and in the mixture on the first sampling date whereas *Arrhopalites* sp. was abundant in granite and in the

266   mixture treatment on the same date. (4) Some species were not clearly affected by the treatments: all the species

267   apart from the abovementioned ones maintained similar abundance levels among the treatments. No species

268   responded concavely to the material composition gradient. These response patterns are very similar to those

269   reported by Takeda (1987), whose experiments involved mixtures with different ratios of pine-needle litter (*Pinus*

270   *densiflora*) and broad-leaved litter (*Alnus pendula*). That study revealed that some collembolan species responded

271   linearly to the litter composition gradient, and others responded positively to the mixture treatment, while the

272   species richness values were similar among all treatments.

273   We hypothesize that the third pattern of response described above may represent a putative mechanism to explain

274   how a mixture of materials generates a positive effect on the abundance of an organism. Assuming that a material

275   is necessary for, or is preferred by, an organism but that its quantity is not limiting, such a response will be

276   non-linear to the gradient of the mixture and reveal a positive effect of the mixture. Indeed, certain biotic

277   components in the boxes were probably correlated with the presence, rather than amount, of perlite or granite. In

278   other words, it appears that the fine-root biomass on the second sampling date responded positively and equally to

279   the treatments that included perlite (perlite and mixture, Fig. 2f), while Oribatida responded positively and equally

280   to the treatments that included granite (granite and mixture, Fig. 2b). Although fine roots responded specifically to

281   the fine pore spaces contributed by perlite, as discussed earlier, it is unclear why Oribatida responded specifically

282   to the treatments that included granite. Some collembolan species also responded in this manner: *F. octoculata*

283   showed equivalent abundances between the perlite and mixture treatments but lower abundance in granite on the

284   first sampling date, while *Arrhopalites* sp. did similarly but to the treatments that included granite on the same

285   date (Table 1), although the actual factors to which they responded were not clear. More importantly, when 2 or

286   more species display this type of response, but to different materials, their total abundance can form a single peak

287   on the material composition gradient. This could be the reason for the positive effect on the total abundance of

288   Collembola observed on the first sampling date (Fig. 2a). In other words, individual species responded

289   non-linearly to different materials.

290   No clear effect of the mixing of inorganic materials on the number of collembolan species was detected (Fig. 2h).

291   Studies that focused on oribatid communities (Hansen and Coleman 1998; Kaneko and Salamanca 1999; Hansen

292   2000) reported positive effects of substrate mixtures on species richness, whereas a study on collembolan

293   communities did not (Takeda 1987), suggesting that this anomaly was potentially due to the differences in life

294   histories between the 2 higher taxonomic groups. Oribatida species display distinct differences in food habits

295   among species and low fecundity (Behan-Pletier and Hill 1983; Kaneko 1988; 1989), while Collembola species

296   are omnivorous (reviewed by Hopkin 1997) and have relatively short generation times and iteroparity

297   (Takeda 1987). This characteristic of Collembola may have been responsible for the equivalent numbers of

298 species observed among the treatments, although community compositions indicated evident differences (Table

299 1).

300

301 **Effect of habitat structural complexity on collembolan communities in the experimental mesocosms**

302   Redundancy analysis revealed that the soil ecosystems and collembolan communities in the experimental boxes

303 developed progressively with time (Fig. 3a). Subplots also affected the ordination, indicating that heterogeneity in

304 the forest floor affected the collembolan communities. Because of these relatively large effects, the RDA did not

305 reveal clear treatment effects. After a pRDA was conducted to eliminate the effects of elapsed time and subplot,

306 the collembolan communities were clearly classified into 3 separate groups on the coordinate plane, based on

307 treatment (Fig. 3b). This suggested the presence of relative differences in the compositions of the collembolan

308 communities among the treatments. This also indicates that the communities that developed in the boxes with

309 mixed substrates were not merely intermediate between those in the single-material boxes, since the 3 groups

310 were graphically localized in different directions. As discussed above, this difference may have been generated by

311 the varied responses, both linear and non-linear, of the species to the material gradients. This is in agreement with

312 previous research conducted on litter. Kaneko and Salamanca (1999) and Hansen (2000) found that oribatid

313 communities developed differently in substrates based on mixed or single litters.

314   The pRDA indicated that the water content, fine-root biomass, and abundance of oribatid mites was correlated

315 with the variation among the collembolan communities. Water content was significantly different among

316 treatments (Fig. 2g). In general, it is reasonable to consider that the water content affected the composition of the

317 communities, since individual collembolan species may vary in their desiccation tolerance (Kærsgaard et al. 2004).

318 However, the fact that the 2 most abundant collembolan taxa in the present study, *Tomocerus* spp. and

319 *Entomobrya* sp., displayed contrasting responses on the second sampling date, i.e., the former was abundant in

320 granite and the latter was abundant in perlite (Table 1) could not allow such a simplistic interpretation. Because

321 both the genera can be considered to be relatively desiccation-tolerant (Takeda 1978), the variation among the

322 treatments was not simply due to desiccation. In addition, as was discussed earlier, we believe that the mesocosms

323 of all treatments were likely saturated, suggesting that water content was unlikely to have directly affected the

324   collembolan community. Water content was selected as a significant explanatory variable, likely because it was

325   strongly correlated with the amount of perlite (Fig. 2g), representing the substrate material composition gradient

326   among treatments to which some collembolan species linearly responded. Although pore spaces were not

327   measured in every mesocosms and were not analyzed in the RDA, water content may have represented pore

328   spaces because the 2 factors are closely correlated. As indicated by Larsen et al. (2004), some species may

329   positively respond to the amount of pore spaces of a certain size, which would partially explain community

330   differentiation.

331   The other factors selected by forward selection were fine-root biomass, and the abundance of oribatid mites. The

332   pRDA axis 1 largely differentiated the communities in the treatments that included granite (i.e., G and M in Fig.

333   3b) from those in the perlite treatment (P), while axis 2 differentiated the communities in the treatments that

334   included perlite (i.e., P and M) from those in the granite treatment (G). Since these 2 axes were correlated with

335   oribatid abundance and fine-root biomass, these factors may indicate some reasons for the observed responses of

336   the communities. Given that fine roots largely represent a labile carbon source (due to root exudates) (Bais et al.

337   2006) while oribatid mites can represent dead plant material and fungi (Wallwork 1983), the observed

338   differentiation of collembolan communities may have, to some extent, reflected fast and slow cycles in soil

339   micro-food webs (Hunt et al. 1987; Moore et al. 2003). This hypothetical interpretation is not contradictory to a

340   recent theory that the food habits of collembolan species vary, depending on the species, from bacteria to fungi

341   (Berg et al. 2004; Kaneda and Kaneko 2004; Chahartaghi et al. 2005), although we did not determine the amounts

342   of bacterial or fungal biomass. Interestingly, the pRDA axis 2 was largely correlated to the natural habitat. In

343   particular, the abundances of F layer dwelling species (*F. octoculata*, *O. yosiiana*, and *S. dubiosa*) were correlated

344   with fine root biomass. This was consistent with Fujii (2012) who suggested the role of fine roots as a food

345   resource, leading to resource partitioning among collembolan species.

346   In this study, we analyzed 3 different types of field mesocosms, 2 single inorganic material treatments, and a

347   mixture treatment, and discovered some positive effects on the abundance of arthropods and the development of

348   different collembolan communities, although the underlying mechanisms responsible for the differentiation

349   remain unresolved. Since potential trophic factors for soil organisms, such as the amounts of organic matter and

fine roots, differed among the treatments, the direct effect of physical habitat could not be isolated from the direct and indirect effects of trophic factors. Although some studies have emphasized the potential importance of habitat for detritivorous microarthropods as a refuge from predators (Vreeken-Buijs et al. 1998; Hansen 2000; Nielsen et al. 2008), it was not practical to examine this approach in the present mesocosms. Therefore, future studies focusing on the structural habitat for soil microarthropods should control trophic factors by, for instance, forcing initial mesocosms to contain a certain amount of organic matter and preventing fine roots from invading them.

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456

457 **Figure legends and table title:**

458 Figure 1: Pore size distribution in the experimental boxes: Filled parts of columns denote solid matter. Each

459 column represents the mean of 3 replicates. P, perlite; M, mixture; G, granite.

460

461 Figure 2: Abundance of soil microarthropods (a-d), environmental factors (e-g), and species richness of

462 Collembola (h) in the experimental soil boxes after 8 and 20 months: White, gray, and black columns indicate

463 the values for perlite, mixture, and granite treatments, respectively. Bars indicate 1 standard error ($n = 5$). The

464 number of species and standard errors were estimated by the first-order jackknife procedure. Subscripts indicate

465 significant differences in the mean values on the same sampling date ($P < 0.05$). Note that the multiple

466 comparison test was conducted when the effect of treatment was revealed to be significant in a one-way analysis

467 of variance (ANOVA). Asterisks indicate statistically significant differences in the observed and predicted

468 values for the mixture treatment: $+P < 0.10$; $*P < 0.05$; $**P < 0.01$. Arrows indicate a negative effect.

469

470 Figure 3: Ordination of collembolan communities that developed in perlite, granite, and their mixture: Ordination

471 was based on (a) redundancy analysis (RDA) and (b) partial RDA (pRDA) in which the effects of elapsed time

472 and subplot were separated. Codes for the communities (black) are as follows: the first letter (P, G, or M)

473 indicates the treatment (perlite, granite, or mixture, respectively); the number in the middle (1 or 2) indicates the

474 sampling date (after 8 or 20 months, respectively); and the third letter (a-j) indicates the subplot. For example,

475 M2d indicates the collembolan community for treatment M in subplot d, sampled 20 months after the

476 experimental soil boxes were established. Arrows represent the factors (abundance of microarthropods, fine-root

477 biomass, accumulated organic matter, water content, subplot, and elapsed time). The 15 dominant collembolan

478 taxa (each accounting for >1% of the total number of individuals on at least one sampling date) are shown in red.

479 Abbreviations used for the collembolan taxa are as follows: Arpl, *Arrhopalites* sp.; Dsri, *Desoria* sp.3; Etmb,

480 *Entomobrya* sp.; Flsm, *Folsomia octoculata*; Frsj, *Friesea japonica*; Istm, *Isotoma carpenteri*; Lpdc,

481 *Lepidocyrtus* sp.; Lpgt, *Lophognathella choreutes*; Mglt, *Megalothorax minimus*; Ocpd, *Oncopodura yosiiana*;

482 Onyf, *Onychiurus flavescens*; Smth, *Sminthurinus* sp.; Snld, *Sinella dubiosa*; Tmcr, *Tomocerus* spp.; Ttrc,

483   *Tetracanthella sylvatica.*

484

485   Table 1: Abundances of dominant collembolan species in the experimental boxes after 8 and 20 months.

486   A total of 15 taxa (each accounting for >1% of the total number of collembolan individuals on at least one

487   sampling date) are shown. Values indicate the number of individuals per box. Parenthetical values indicate 1

488   standard error ($n = 5$). Superscripts indicate significant differences among the mean values of the treatments (P

489   < 0.05). Note that the multiple comparison test was conducted when the effect of treatment was revealed to be

490   significant in a one-way analysis of variance (ANOVA). Data on the habitats of individual species in the soil

491   organic layer are from Takeda (1978): L, F, and H represent litter layer, fermented layer, and humus layer,

492   respectively. Hyphenated letters L-F and F-H indicate that the species inhabit(s) both layers. Asterisks indicate

493   statistically significant differences between the observed and predicted values: $+P < 0.10$; $*P < 0.05$; $**P <$

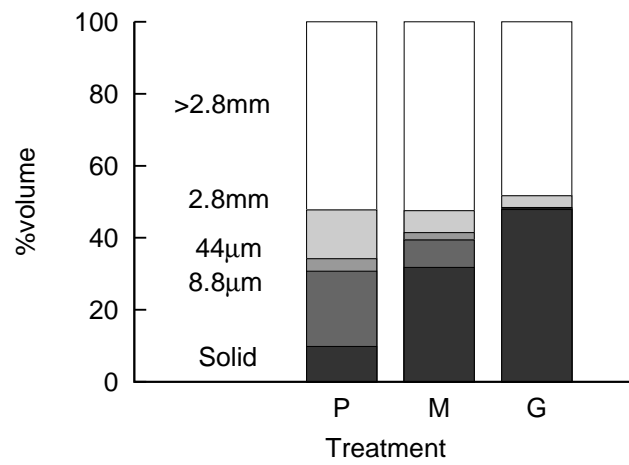494 0.01; $***P < 0.001$; ns, not significant.

495

Figure 1: Pore size distribution in the experimental boxes: Filled parts of columns denote solid matter. Each column represents the mean of 3 replicates. P, perlite; M, mixture; G, granite.
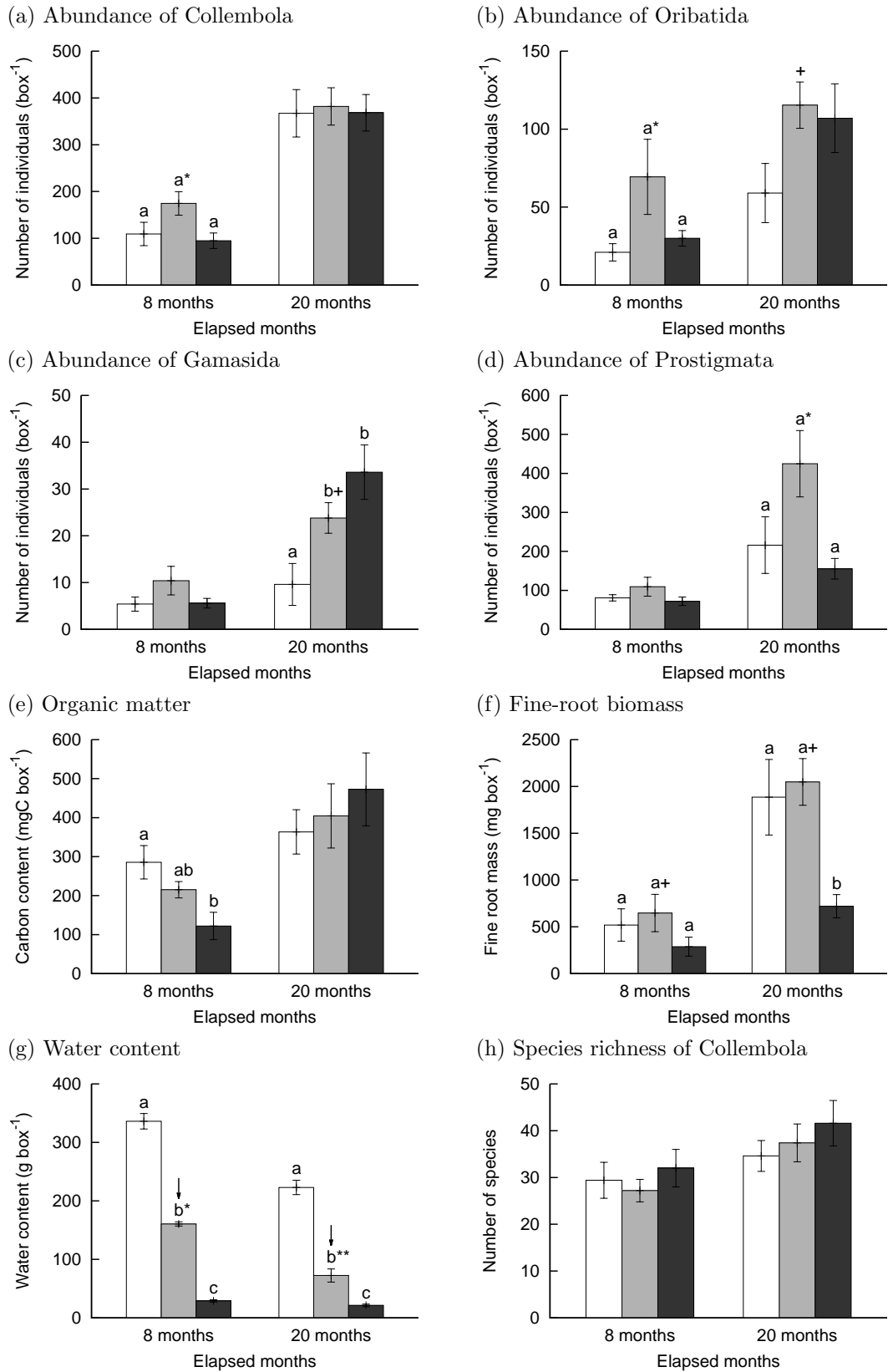
Figure 2: Abundance of soil microarthropods (a-d), environmental factors (e-g), and species richness of Collembola (h) in the experimental soil boxes after 8 and 20 months: White, gray, and black columns indicate the values for perlite, mixture, and granite treatments, respectively. Bars indicate 1 standard error ($n = 5$). The number of species and standard errors were estimated by the first-order jackknife procedure. Subscripts indicate significant differences in the mean values on the same sampling date ($P < 0.05$). Note that the multiple comparison test was conducted when the effect of treatment was revealed to be significant in a one-way analysis of variance (ANOVA). Asterisks indicate statistically significant differences in the observed and predicted values for the mixture treatment: $+P < 0.10$; $*P < 0.05$; $**P < 0.01$. Arrows indicate a negative effect
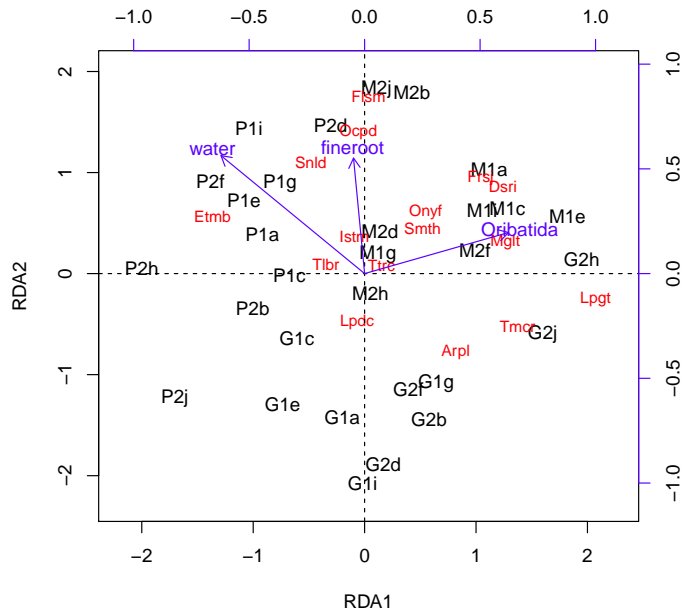
(a) RDA
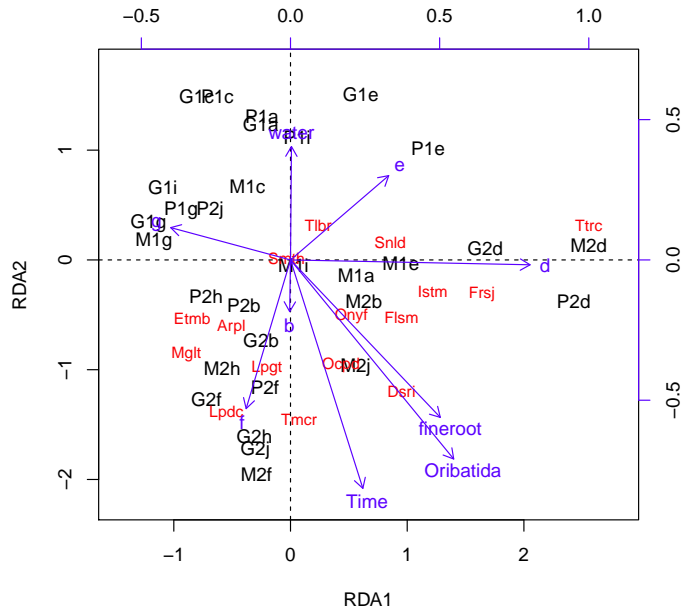
(b) pRDA

Figure 3: Ordination of collembolan communities that developed in perlite, granite, and their mixture: Ordination was based on (a) redundancy analysis (RDA) and (b) partial RDA (pRDA) in which the effects of elapsed time and subplot were separated. Codes for the communities (black) are as follows: the first letter (P, G, or M) indicates the treatment (perlite, granite, or mixture, respectively); the number in the middle (1 or 2) indicates the sampling date (after 8 or 20 months, respectively); and the third letter (a-j) indicates the subplot. For example, M2d indicates the collembolan community for treatment M in subplot d, sampled 20 months after the experimental soil boxes were established. Arrows represent the factors (abundance of microarthropods, fine-root biomass, accumulated organic matter, water content, subplot, and elapsed time). The 15 dominant collembolan taxa (each accounting for > 1% of the total number of individuals on at least one sampling date) are shown in red. Abbreviations used for the collembolan taxa are as follows: Arpl, *Arrhopalites* sp.; Dsri, *Desoria* sp.3; Etmb, *Entomobrya* sp.; Flsm, *Folsomia octoculata*; Frsj, *Friesea japonica*; Istm, *Isotoma carpenteri*; Lpdc, *Lepidocyrtus* sp.; Lpgt, *Lophognathella choreutes*; Mglt, *Megalothorax minimus*; Ocpd, *Oncopodura yosiiana*; Onyf, *Onychiurus flavescens*; Smth, *Sminthurinus* sp.; Snld, *Sinella dubiosa*; Tmcr, *Tomocerus* spp.; Ttrc, *Tetracanthella sylvatica*.

Table 1: Abundances of dominant collembolan species in the experimental boxes after 8 and 20 months.

Family	Species or species group	Habitat	8 months				20 months			
			Treatment			Effect of mixture	Treatment			Effect of mixture
			Perlite	Mixture	Granite		Perlite	Mixture	Granite	
Entomobryidae	*Entomobrya* sp.	L layer	17.4 (11.5)	20.8 (12.8)	19.0 (17.6)	ns	146.2 (42.6)^a	49.0 (33.8)^{ab}	23.8 (15.4)^b	ns
	Lepidocyrtus sp.	L layer	6.6 (3.2)	5.2 (3.7)	9.4 (7.4)	ns	33.2 (11.9)	50.6 (28.3)	39.6 (13.7)	ns
	Sinella dubiosa	F layer	3.6 (2.3)	0.8 (1.3)	0.2 (0.4)	ns	1.2 (1.0)	7.6 (3.7)	0.2 (0.2)	*
Isotomidae	*Desoria* sp.3	L-F layer	0.2 (0.4)	4.0 (4.1)	2.8 (5.7)	ns	24.2 (15.6)	30.8 (20.4)	7.6 (1.1)	ns
	Folsomia octoculata	F layer	15.8 (8.2)^a	20.8 (17.3)^a	2.0 (2.0)^b	**	19.8 (6.8)	23.4 (5.7)	10.2 (2.2)	ns
	Isotoma carpenteri	L-F layer	1.4 (1.7)	0.8 (1.8)	0.6 (0.9)	ns	4.4 (2.0)	3.0 (1.8)	3.8 (2.4)	ns
	Tetracanthella sylvatica	L-F layer	11.6 (25.4)	9.8 (18.7)	12.8 (25.4)	ns	23.2 (21.0)	21.8 (21.1)	11.0 (10.3)	ns
Neelidae	*Megalothorax minimus*	F-H layer	5.4 (9.3)	7.6 (8.5)	3.0 (4.1)	ns	2.8 (1.9)	3.0 (1.8)	10.4 (4.9)	ns
Oncopoduridae	*Oncopodura yosiiana*	F layer	17.4 (12.6)	18.2 (13.8)	6.2 (5.5)	ns	37.8 (6.2)	32.8 (11.7)	27.2 (8.6)	ns
Onychiuridae	*Lophognathella choreutes*	NA	0.4 (0.9)	12.2 (18.5)	7.6 (9.8)	ns	2.2 (1.5)	20.6 (14.8)	9.6 (4.1)	ns
	Onychiurus flavescens	F-H layer	2.8 (1.8)^a	3.8 (3.8)^{ab}	0.4 (0.9)^b	ns	3.0 (1.3)	3.6 (1.0)	6.4 (2.5)	ns
Pseudachorutidae	*Friesea japonica*	NA	2.0 (2.7)	10.4 (16.1)	0.2 (0.4)	+	9.4 (8.9)	23.4 (15.2)	6.4 (2.8)	*
Sminthuridae	*Arrhopalites* sp.	L-F layer	1.0 (0.7)^a	4.8 (1.8)^b	4.2 (3.4)^b	+	3.8 (1.0)	4.0 (3.3)	13.2 (4.8)	ns
	Sminthurinus sp.	L-F layer	2.0 (2.8)	2.8 (3.6)	2.4 (2.8)	ns	0.8 (0.4)	4.0 (3.0)	1.2 (0.5)	ns
Tomoceridae	*Tomocerus* spp.	L layer	17.0 (14.0)	48.2 (14.6)	20.0 (12.9)	***	46.4 (18.9)^a	85.8 (14.8)^{ab}	183.2 (32.4)^b	ns

A total of 15 taxa (each accounting for >1% of the total number of collembolan individuals on at least one sampling date) are shown. Values indicate the number of individuals per box. Parenthetical values indicate 1 standard error ($n = 5$). Superscripts indicate significant differences among the mean values of the treatments ($P < 0.05$). Note that the multiple comparison test was conducted when the effect of treatment was revealed to be significant in a one-way analysis of variance (ANOVA). Data on the habitats of individual species in the soil organic layer are from Takeda (1978): L, F, and H represent litter layer, fermented layer, and humus layer, respectively. Hyphenated letters L-F and F-H indicate that the species inhabit(s) both layers. Asterisks indicate statistically significant differences between the observed and predicted values: $+P < 0.10$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; ns, not significant.