Title:

Effect of habitat structural complexity on collembolan communities

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Abstract

We investigated soil microarthropod communities in 2 physically dissimilar inorganic soil materials and in a mixture of these 2 materials to examine the effect of the structural complexity of a habitat on microarthropod abundance and communities, teasing it out from that of nutritional factors. Mesh boxes were filled with perlite (a highly porous material), similar size of granite gravels (no pores inside), or their mixture, and placed on a forest floor. The boxes were collected after 8 or 20 months, and the microarthropods were extracted and identified to the species level, with a focus on Collembola. We also evaluated fine-root biomass and the amount of organic matter in the boxes. It was found that the mixture of perlite and granite enhanced microarthropod abundance and root development. A partial redundancy analysis (pRDA) revealed that collembolan communities developed differently among the substrate materials. We also found that variation in the collembolan communities was related to fine-root development and the abundance of other microarthropods, implying that habitat structural complexity affects collembolan communities indirectly by affecting soil food webs.

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

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

higher microarthropod taxa, accumulated organic matter, fine-root biomass, and water content. Further, a partial RDA (pRDA) was conducted, in which the effects of elapsed time and subplot were separated out. The significances of the RDA axes were then calculated using a permutation test. We excluded 7 species from this analysis because we identified fewer than 5 individuals for them or found them in only one soil sample, taking into account the recommendation made by Hasegawa (2006). Redundancy analysis and selection of the explanatory variables for the analysis were performed with the software packages “vegan” (Oksanen et al. 2010) and “packfor” (Dray 2009), respectively. The latter is based on a forward selection method described by ter Braak and Šmilauer (2002).

Results

Figure 2 displays the abundance of soil microarthropods, environmental factors, and species richness of Collembola in the experimental boxes after 8 and 20 months. The abundance of Gamasida and fine-root biomass differed among the treatments after 20 months but not after 8 months (Figs. 2c, f). Fewer Gamasida individuals appeared in perlite samples than in the other 2 samples after 20 months; no other microarthropods exhibited any differences among the treatments (Figs. 2a, b, d). Fine-root biomass was lowest in the granite samples (Fig. 2f). Water content differed among the treatments, reflecting the amount of perlite in each sample: throughout the study period, we consistently observed the highest water content in perlite and the lowest in granite (Fig. 2g). At the first sampling date, a greater amount of organic matter was detected in perlite samples than in granite samples (Fig. 2e). Collembolan species richness did not differ among treatments (Fig. 2h).

We detected a positive effect of the perlite–granite mixture on the abundance of Collembola ($P = 0.0123$) on the first sampling date (Figs. 2a, h), on the abundances of Gamasida ($P = 0.0736$) and Prostigmata ($P = 0.0352$) on the second sampling date (Figs. 2c, d), on the abundance of Oribatida on the first ($P = 0.0414$) and second ($P = 0.0772$) sampling dates (Fig. 2b), and on fine-root biomass on the first ($P = 0.0675$) and second ($P = 0.0605$) sampling dates (Fig. 2f). The effect on water content was negative on the first ($P = 0.0126$) and second ($P = 0.0023$) sampling dates (Fig. 2g).

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

individuals on at least one sampling date, are listed in Table 1. *Folsomia octoculata* was less abundant in granite than in the other 2 treatments on the first sampling date, while *Arrhopalites* sp. was the least abundant in perlite. *Onychiurus flavescens* appeared more abundant in perlite than in granite on the first sampling date, while *Entomobrya* sp. was more abundant in perlite than in granite on the second sampling date. In contrast, *Tomocerus* spp. was more abundant in granite than in perlite. With regard to the abundances of individual species, we detected a positive effect of the perlite–granite mixture on *Tomocerus* spp. (P = 0.0007), *F. octoculata* (P = 0.0046), *F. japonica* (P = 0.0552), and *Arrhopalites* sp. (P = 0.0981) on the first sampling date and *Sinella dubiosa* (P = 0.0290) and *F. japonica* (P = 0.0166) on the second sampling date.

In RDA, the elapsed time, water content, abundance of oribatids, fine-root biomass, and some subplots were selected as the explanatory variables using forward selection. These factors explained 65.4% of the total variation among the sampled collembolan communities. The effects of elapsed time and subplot as conditional factors were separated out in pRDA. The constraining factors in pRDA, namely, the abundance of oribatids, fine-root biomass and water content, explained 15.3% of the variance.

Ordination of the collembolan communities based on RDA is shown in Figure 3a. Axes 1 and 2 explained 22.2% and 15.4% of the total variance, respectively. A permutation test indicated that RDA axes 1-5 were significant ($P < 0.05$). The ordination revealed relatively large effects of elapsed time and subplot on the collembolan communities (Fig. 3a). Arrows representing Oribatida and fine-root biomass, as well as elapsed time, were oriented in the lower right quadrant of the coordinate plane. Reflecting elapsed time, the majority of the communities sampled after 20 months were plotted in the lower half of the plane, whereas those sampled after 8 months were plotted in the upper half. Likewise, most collembolan taxa were plotted in the lower half of the plane, indicating their colonization of the substrates over time. Subplot had a considerable effect on ordination, indicating the effect of forest floor heterogeneity on the collembolan communities. For example, the arrows for subplots d, f, and g coincided with those of the communities sampled from those subplots.

In pRDA (Fig. 3b), in which the effects of subplot and elapsed time were separated out, the collembolan communities were plotted into 3 groups according to the treatments. Axes 1 and 2 explained 16.4% and 12.7% of the residual variance, respectively. A permutation test indicated that RDA axes 1 and 2 were significant ($P < 0.05$).

Discussion

Microarthropod communities developed in our field-incubated mesocosms containing 2 inorganic materials and their mixture. As hypothesized, we found a positive effect of the mixture on the abundance of microarthropods (Fig. 2), similar to the results of previous studies using mixed litter (Kaneko and Salamanca 1999; Hansen 2000). Enhancement was observed in the animal taxa on the first and/or second sampling dates (Figs. 2a, b, c, d). With respect to Collembola, a positive effect of the mixture was detected on the abundance of some species (Table 1), whereas no positive effect was detected on species richness (Fig. 2h).

Although we expected enhancement of the accumulation of organic matter and development of fine roots in the experimental boxes, only the latter was actually observed (Figs. 2e, f). This may have contributed to the observed positive effects on microarthropod abundances to some extent. These findings suggest that habitat structural complexity may have a positive effect on the abundance of soil microarthropods, partly via enhancement of the development of fine roots, which could increase the availability of trophic resources.

The responses of individual collembolan species to the composition of the substrate varied among species (Table 1), with linear and non-linear responses exhibited (Fig. 3).

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

In our system, inorganic materials served primarily as the physical framework of the habitat and received organic debris and fine roots from the surrounding organic layer, which likely served as energy sources in the experimental system. The fact that inorganic materials neither supplied any nutrients nor decomposed in the study period is important in interpreting the results of this study, compared with previous studies using mixtures of organic litter species. Those studies have suggested that mixing litters with dissimilar chemical traits improves food resource availability by stimulating microflora (Salamanca et al. 1998; Kaneko and Salamanca 1999), but this effect was absent in the present study. It has been also suggested that the slow decomposition of mixed litter 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

layer (Nielsen et al. 2008). Larsen et al (2004) conducted a soil compaction experiment to reveal the importance of the amount of pores 120–300 μm in size in influencing the abundance of certain collembolan species. These studies indicated that the availability of habitable pore spaces for microarthropods could limit their abundance. In the current study, the pore size range corresponding to that in the previous studies was 44 μm–2.8 mm. The amount of this size of pore was positively correlated with the amount of perlite (i.e., perlite > mixture > granite, Fig. 1). Unlike in the previous studies, no higher animal taxon showed any correlation to these pores (Figs. 2a–d), although some individual collembolan species displayed the pattern perlite > mixture > granite in terms of abundance (Table 1). Since all the treatments had high porosities (>50%), the amount of pores was unlikely to have limited microarthropod abundances. Another possible explanation is that a large proportion of the pore spaces of the size range 44 μm–2.8 mm may have been internal and closed (or narrow-necked) in perlite particles, and therefore would have been unavailable for microarthropods.

Focusing on the patterns of species-level responses to treatments, individual collembolan species responded differently (Table 1). Four different patterns of responses to the substrate material composition gradient were observed. (1) The 2 most abundant taxa on the second sampling date (*Tomocerus* spp. and *Entomobrya* sp.) responded differently, albeit linearly, to the 2 different materials. Their abundances in the mixture treatment approximated that of the mean of the 2 single-material treatments. (2) The mixture treatment enhanced the abundances of some species: *Tomocerus* spp. on the first sampling date, *Sinella dubiosa* on the second sampling date, and *Friesea japonica* on both sampling dates. (3) Some species were abundant in one of the 2 single-material treatments and also abundant at an equivalent level in the mixture treatment: *Folsomia octoculata* was abundant in perlite and in the mixture on the first sampling date whereas *Arrhopalites* sp. was abundant in granite and in the mixture treatment on the same date. (4) Some species were not clearly affected by the treatments: all the species apart from the abovementioned ones maintained similar abundance levels among the treatments. No species responded concavely to the material composition gradient. These response patterns are very similar to those reported by Takeda (1987), whose experiments involved mixtures with different ratios of pine-needle litter (*Pinus densiflora*) and broad-leaved litter (*Alnus pendula*). That study revealed that some collembolan species responded 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

collembolan community. Water content was selected as a significant explanatory variable, likely because it was strongly correlated with the amount of perlite (Fig. 2g), representing the substrate material composition gradient among treatments to which some collembolan species linearly responded. Although pore spaces were not measured in every mesocosms and were not analyzed in the RDA, water content may have represented pore spaces because the 2 factors are closely correlated. As indicated by Larsen et al. (2004), some species may positively respond to the amount of pore spaces of a certain size, which would partially explain community differentiation.

The other factors selected by forward selection were fine-root biomass, and the abundance of oribatid mites. The pRDA axis 1 largely differentiated the communities in the treatments that included granite (i.e., G and M in Fig. 3b) from those in the perlite treatment (P), while axis 2 differentiated the communities in the treatments that included perlite (i.e., P and M) from those in the granite treatment (G). Since these 2 axes were correlated with oribatid abundance and fine-root biomass, these factors may indicate some reasons for the observed responses of the communities. Given that fine roots largely represent a labile carbon source (due to root exudates) (Bais et al. 2006) while oribatid mites can represent dead plant material and fungi (Wallwork 1983), the observed differentiation of collembolan communities may have, to some extent, reflected fast and slow cycles in soil micro-food webs (Hunt et al. 1987; Moore et al. 2003). This hypothetical interpretation is not contradictory to a recent theory that the food habits of collembolan species vary, depending on the species, from bacteria to fungi (Berg et al. 2004; Kaneda and Kaneko 2004; Chahartaghi et al. 2005), although we did not determine the amounts of bacterial or fungal biomass. Interestingly, the pRDA axis 2 was largely correlated to the natural habitat. In particular, the abundances of F layer dwelling species (*F. octoculata*, *O. yosiiana*, and *S. dubiosa*) were correlated with fine root biomass. This was consistent with Fujii (2012) who suggested the role of fine roots as a food resource, leading to resource partitioning among collembolan species.

In this study, we analyzed 3 different types of field mesocosms, 2 single inorganic material treatments, and a mixture treatment, and discovered some positive effects on the abundance of arthropods and the development of different collembolan communities, although the underlying mechanisms responsible for the differentiation 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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References

Anderson JM (1975) Succession, diversity and trophic relationships of some soil animals in decomposing leaf litter. J Anim Ecol 44: 475–495

Anderson JM (1978) Inter- and intra-habitat relationships between woodland Cryptostigmata species diversity and the diversity of soil and litter microhabitats. Oecologia 32: 341–348

Anderson JM, Healey IN (1972) Seasonal and interspecific variations in the major components of the gut contents of some woodland Collembola. J Anim Ecol 41: 359–368

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions

376 with plants and other organisms. Ann Rev Plant Biol 57: 233–266
377 Bardgett RD (2002) Causes and consequences of biological diversity in soil. Zoology 105: 367–374
378 Behan–Pelletier VM, Hill SB (1983) Feeding habits of sixteen species of Oribatei (Acari) from an acid peat bog,
379 Glenamoy, Ireland. Rev Ecol Biol Sol 20: 221–267
380 Berg MP, Stoffer M, van den Heuvel HH (2004) Feeding guilds in Collembola based on digestive enzymes.
381 Pedobiologia 48: 589–601
382 Blair JM, Parmelee RW, Beare MH (1990) Decay rates, nitrogen fluxes, and decomposer communities of single-
383 and mixed-species foliar litter. Ecology 71: 1976–1985
384 Chahartaghi M, Langel R, Scheu S, Ruess L (2005) Feeding guilds in Collembola based on nitrogen stable isotope
385 ratios. Soil Biol Biochem 37: 1718–1725
386 Dray S, Legendre P, Blanchet G (2009) packfor: Forward Selection with permutation (Canoco p.46). R package
387 version 0.0-7/r58. http://R-Forge.R-project.org/projects/sedar/
388 Fujii S (2012) Effect of fine roots on the organization of collembolan community in a forest ecosystem. Doctoral
389 thesis. Kyoto University, Kyoto. 92 pp
390 Hågvar S (1983) Collembola in Norwegian coniferous forest soils II: Vertical distribution. Pedobiologia 25: 383–
391 401
392 Hansen RA (2000) Effects of habitat complexity and composition on a diverse litter microarthropod assemblage.
393 Ecology 81: 1120–1132
394 Hansen RA, Coleman DC (1998) Litter complexity and composition are determinants of the diversity and species
395 composition of oribatid mites (Acari: Oribatida) in litterbags. Appl Soil Ecol 9: 17–23
396 Hasegawa M (2006) Application of ordination in community ecology of soil animals. Edaphologia 80: 35–64 (in
397 Japanese with English summary)
398 Hasegawa M, Takeda H (1995) Changes in feeding attributes of four collembolan populations during the
399 decomposition process of pine needles. Pedobiologia 39: 155–169
400 Hopkin SP (1997) Biology of the Springtails (Insecta: Collembola). Oxford University Press, Oxford. 330 pp
401 Hunt HW, Coleman DC, Ingham ER, Ingham RE, Elliott ET, Moore JC, Rose SL, Reid CPP, Morley CR (1987)

402 The detrital food web in a shortgrass prairie. Biol Fertil Soils 3: 57–68
403 Kaneda S, Kaneko N (2004) The feeding preference of a collembolan (*Folsomia candida* Willem) on
404 ectomycorrhiza (*Pisolithus tinctorius* (Pers.)) varies with mycelial growth condition and vitality. Appl Soil Ecol
405 27: 1–5
406 Kaneko N (1988) Feeding habits and cheliceral size of oribatid mites in cool temperate forest soils in Japan. Rev
407 Ecol Biol Sol 25: 353–363
408 Kaneko N (1989) Life histories of four oribatid mite species in a mull type soil in a cool temperate forest in Japan.
409 Pedobiologia 33: 117–126
410 Kaneko N, Salamanca N (1999) Mixed leaf litter effects on decomposition rates and soil arthropod communities
411 in an oak-pine forest stand in Japan. Ecol Res 14: 131–138
412 Kærsgaard CW, Holmstrup M, Malte H, Bayley M (2004) The importance of cuticular permeability, osmolyte
413 production and body size for the desiccation resistance of nine species of Collembola. J Insect Physiol 50: 5–15
414 Larsen T, Schjonning P, Axelsen J (2004) The impact of soil compaction on euedaphic Collembola. Appl Soil Ecol
415 26: 273–281
416 Lavelle P, Spain AV (2001) Soil Ecology. Kluwer Academic Publishers, Dordrecht. 654 pp
417 Moore JC, McCann K, Setälä H, De Ruiter PC (2003) Top-down is bottom-up: Does predation in the rhizosphere
418 regulate aboveground dynamics? Ecology 84: 846–857
419 Nielsen UN, Osler GHR, van der Wal R, Campbell CD, Burslem DFRP (2008) Soil pore volume and the
420 abundance of soil mites in two contrasting habitats. Soil Biol Biochem 40: 1538–1541
421 Oksanen J, Blanchet FG, Kindt R, Legendre P, O'Hara RB, Simpson GL, Solymos P, Henry M, Stevens H,
422 Wagner H (2010) vegan: Community Ecology Package. R package version 1.17–2. URL http://CRAN.R–
423 project.org/package=vegan
424 Pagliai M, De Nobili M (1993) Relationships between soil porosity, root development and soil enzyme activity in
425 cultivated soils. Geoderma 56: 243–256
426 Parmelee RW, Ehrenfeld JG, Tate RL (1993) Effects of pine roots on microorganisms, fauna, and nitrogen
427 availability in two soil horizons of a coniferous forest spodosol. Biol Fertil Soils 15: 113–119

Petersen H, Luxton M (1982) A comparative analysis of soil fauna populations and their role in decomposition processes. Oikos 39: 287–388

Ponge JF (2000) Vertical distribution of Collembola (Hexapoda) and their food resources in organic horizons of beech forests. Biol Fertil Soils 32: 508–522

Salamanca EF, Kaneko N, Katagiri S (1998) Effects of leaf litter mixtures on the decomposition of *Quercus serrata* and *Pinus densiflora* using field and laboratory microcosm methods. Ecol Eng 10: 53–73

Smith EP, van Belle G (1984) Nonparametric estimation of species richness. Biometrics 40: 119–129

Swift MJ, Heal OW, Anderson JM (1979) Decomposition in terrestrial ecosystems. Blackwell Scientific Publications, London. 384 pp

Takeda H (1978) Ecological studies of collembolan populations in a pine forest. II. Vertical distribution of Collembola. Pedobiologia 18: 22–30

Takeda H (1987) Dynamics and maintenance of collembolan community structure in a forest soil system. Res Popul Ecol 29: 291–346

ter Braak CJF, Šmilauer P (2002) CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (version 4.5). Microcomputer Power, New York. 500 pp.

Vannier G (1987) The porosphere as an ecological medium emphasized in Professor Ghilarov's work on soil animal adaptations. Biol Fertil Soils 3: 39–44

Vreeken–Buijs MJ, Hassink J, Brussaard L (1998) Relationships of soil microarthropod biomass with organic matter and pore size distribution in soils under different land use. Soil Biol Biochem 30: 97–106

Wallwork JA (1983) Oribatids in forest ecosystems. Ann Rev Entomol 28: 109–130

Wardle DA (1995) Impacts of disturbance on detritus food webs in agro–ecosystems of contrasting tillage and weed management strategies. Adv Ecol Res 26: 105–185

Wardle DA (2002) Communities and ecosystems: linking the aboveground and belowground components. Princeton University Press, Princeton. 392 pp

Wardle DA, Yeates GW, Barker GM, Bonner KI (2006) The influence of plant litter diversity on decomposer abundance and diversity. Soil Biol Biochem 38: 1052–1062

454 Wiggins EA, Curl EA, Harper JD (1979) Effects of soil fertility and cotton rhizosphere on populations of
455 Collembola. Pedobiologia 19: 75–82
456

Figure legends and table title:

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.

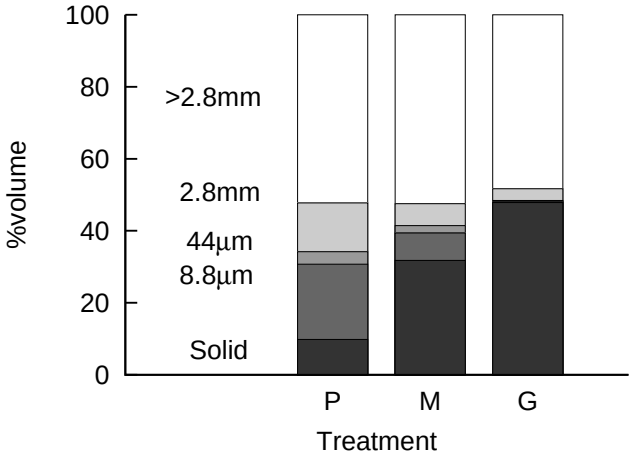
Figure 2: Abundance of soil microarthropods (a-d), environmental factors (e-g), and species richness of Collembola (h) in the experimental soil boxes after 8 and 20 months: White, gray, and black columns indicate the values for perlite, mixture, and granite treatments, respectively. Bars indicate 1 standard 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.

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.

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.



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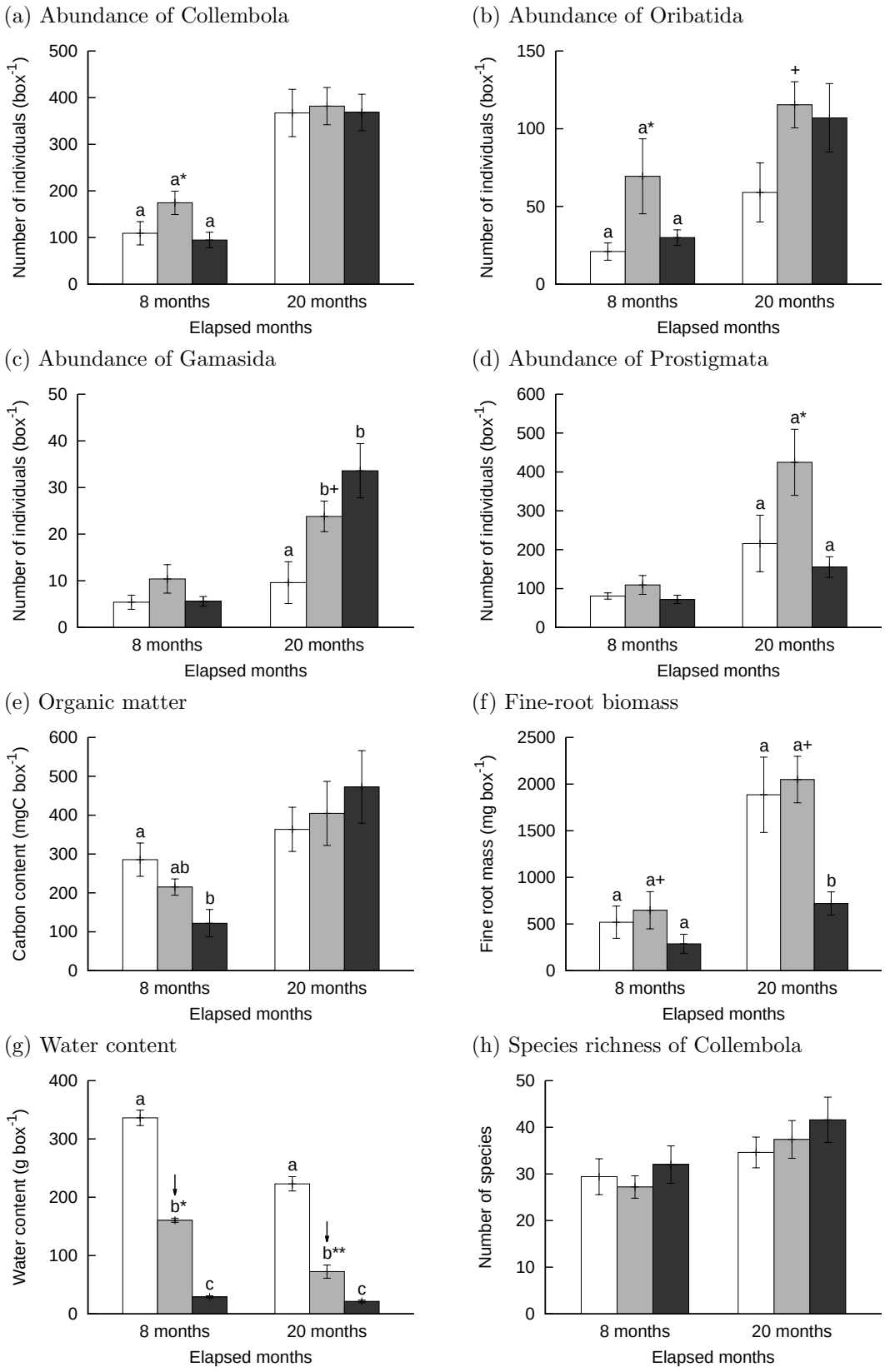
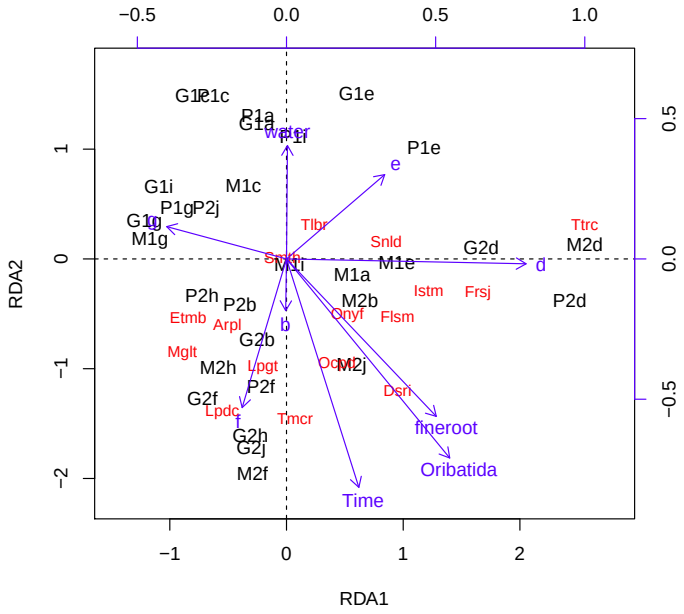


Figure 2: Abundance of soil microarthropods (a-d), environmental factors (e-g), and species richness of Collembola (h) in the experimental soil boxes after 8 and 20 months: White, gray, and black columns indicate the values for perlite, mixture, and granite treatments, respectively. Bars indicate 1 standard 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

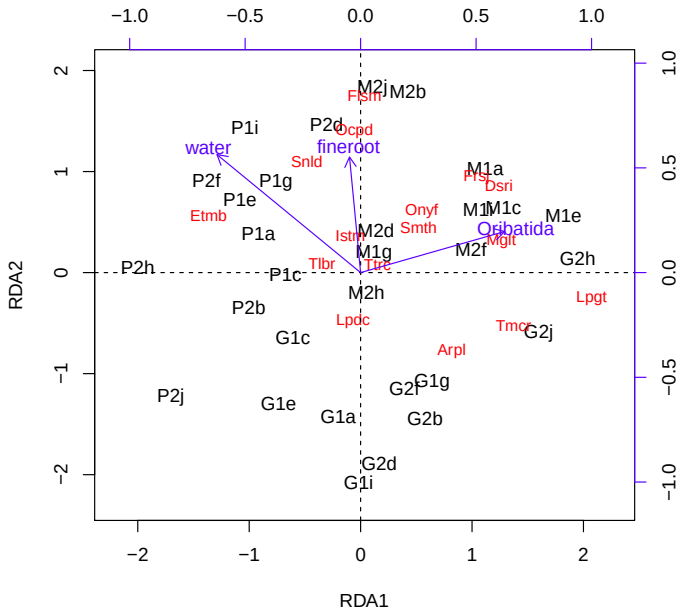


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Table 1: Abundances of dominant collembolan species in the experimental boxes after 8 and 20 months.

			8 months				20 months			
			Treatment			Effect of	Treatment			Effect of
Family	Species or species group	Habitat	Perlite	Mixture	Granite	mixture	Perlite	Mixture	Granite	mixture
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
Pseudoachorutidae	*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.