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ORIGINAL ARTICLE

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Vertical heterogeneity of a forest floor invertebrate food web as indicated by stable-isotope analysis

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Abstract Diverse populations of invertebrates constitute the food web in detritus layers of a forest floor. Heterogeneity in trophic interactions within such a species-rich community food web may affect the dynamic properties of biological communities such as stability. To examine the vertical heterogeneity in trophic interactions among invertebrates in litter and humus layers, we studied differences in species composition and variations in carbon and nitrogen stable-isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) using community-wide metrics of the forest floors of temperate broadleaf forests in Japan. The species composition differed between the two layers, and the invertebrates in the litter layer were generally larger than those in the humus layer, suggesting that these layers harbored separate food webs based on different basal resources. However, the $\delta^{13}C$ of invertebrates, an indicator of differences in the basal resources of community food webs, did not provide evidence for separate food webs between layers even though plant-derived organic matter showed differences in stable-isotope ratios according to decomposition state. The minimum $\delta^{15}N$ of invertebrates also did not differ between layers, suggesting sharing of food by detritivores from the two layers at lower trophic levels. The maximum and range of $\delta^{15}N$ were greater in the humus layer, suggesting more trophic transfers (probably involving microorganisms) than in the litter layer and providing circumstantial evidence for weak trophic interactions between layers at higher trophic levels. Thus, the invertebrate community food web was not clearly compartmentalized between the detrital layers but still showed a conspicuous spatial (vertical) heterogeneity in trophic interactions.

Keywords Compartment · Food web structure · Microhabitat

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Introduction

Understanding the structural heterogeneity within a community food web is important for predicting the dynamic properties of the community, such as a stability of species richness and composition (Paine 1980; McCann et al. 1998). May (1972, 1973) predicted that a community should be more stable when trophic interactions (consumer-resource interactions) are arranged into blocks or compartments rather than a random pattern in the food web. Subsequent studies showed that the interactions between food chains and/or the coupling of distinct energy channels by mobile consumers are important for the stability of the community (Pimm 1979; Post et al. 2000; Rooney et al. 2006). Thus, it is important to examine substructuring, such as compartmentalization, of a food web (Pimm and Lawton 1980; Raffaelli and Hall 1992). The causal factors of the compartmentalized structure of a food web may vary with the scale of the species assemblage. Within the web of a functional group, compartments can result from high-frequency interactions between particular pairs of species (Fonseca and Ganade 1996; Dicks et al. 2002; Van Veen et al. 2008). Within a whole community, the web may be compartmentalized due to spatial heterogeneity within an environment, such as the division into adjacent freshwater and terrestrial habitats (Pimm and Lawton 1980). Compartmentalization may also occur in association with differences in microhabitats such as between pelagic and benthic layers of an aquatic system (Krause et al. 2003).

The forest floor may host a food web with a compartmentalized structure. Large amounts of plant-derived organic matter accumulate, forming a stratified

structure within the forest floor with a coarse litter layer and a finer humus layer resulting from decomposition in the food web (Moore et al. 1988, Hättenschwiler et al. 2005). Such stratified microhabitats may cause compartmentalization of the forest floor food web. Setälä and Aarnio (2002) compared food webs between the litter and humus layers using ^{15}N-labeling and suggested that small-sized decomposers tend to be restricted to the humus layer while larger decomposers exploit the overlying litter layer (see also Caner et al. 2004).

Diverse invertebrate species of various sizes inhabit a forest floor and are involved in the detrital food web (Petersen and Luxton 1982). A detrital food web is often depicted as different energy channels unified by a single group of top predators in the humus layer (e.g., De Ruiter et al. 1995; Scheu 2002; Rooney et al. 2006). The ecological roles of microdecomposers (e.g., fungi and bacteria) and microarthropods (e.g., springtails and mites) in the humus layer have been studied extensively (Ingham et al. 1989; De Ruiter et al. 1998; Schröter et al. 2003). However, the roles of macroinvertebrates moving throughout the litter layer, particularly predators such as ground beetles, ants, and spiders, are not well known because they are mobile and easily omitted from sampling. Therefore, for a comprehensive understanding of forest-floor food webs, it is necessary to identify the trophic positions of these higher-order consumers more precisely.

Most studies on food webs have been based on networks of trophic interactions among species, but it is usually difficult to reconstruct a network using feeding observations or gut-content analysis for a very large number of species. Stable-isotope analyses using carbon and nitrogen stable isotopes (^{13}C and ^{15}N) provide convenient tools with which to study complex trophic interactions within a food web (Peterson and Fry 1987; Tiunov 2007). These have been used extensively to examine the food webs of forest floors (Ponsard and Arditi 2000; Scheu and Falca 2000; Scheu 2002; Halaj et al. 2005). The carbon stable-isotope ratio (δ^{13}C) is only slightly enriched in the process of consumption and assimilation, whereas the nitrogen stable-isotope ratio (δ^{15}N) of a consumer's tissues is more enriched than that of its diet (Vander Zanden and Rasmussen 2001; Post 2002). Therefore, the δ^{13}C of consumers indicates the basal resource of a food chain, while δ^{15}N indicates the trophic level of consumers, and these two stable-isotope ratios in combination can be used to simultaneously determine the trophic position of a large number of component species in a food web. Recently, community-wide metrics have been proposed to evaluate the trophic structure of a food web, which include multiple functional groups, based on the positional variation of consumers in a δ^{13}C-δ^{15}N bi-plot (Layman et al. 2007). Such metrics can be used to compare among community food webs of different systems or different sites, although empirical studies remain scarce.

We aimed to determine the heterogeneity of the invertebrate community food web structure in the floors of temperate forests in Japan. Focusing on the layered structure, we discriminated between invertebrates living on and within the litter layer (ground invertebrates) and those living within the humus layer (soil invertebrates) and compared the variation of carbon and nitrogen stable-isotope ratios between ground and soil invertebrates using community-wide metrics of the stable-isotope ratios to detect heterogeneities of food-web structure between the litter and humus layers. The stable-isotope ratios of plant-derived organic matter (basal resource) increase with soil depth due to progressive decomposition (Balesdent et al. 1993; Högberg 1997; Ehleringer et al. 2000; Ponsard and Arditi 2000; Billing and Richter 2006), and these increases are reflected in the stable-isotope ratios of the detritivores (Tayasu et al. 1997; Schneider et al. 2004; Uchida et al. 2004; Chahartaghi et al. 2005; Hishi et al. 2007; Hyodo et al. 2008). Variations of isotope ratios in consumers at lower trophic levels (lower-order consumers) such as herbivores and detritivores may further be reflected in those at higher trophic levels (higher-order consumers) such as carnivores. Thus, the difference of decomposition states in basal resources of a food chain may be indicated not only by δ^{13}C values of consumers but also by their δ^{15}N values in detrital food web (Scheu 2002). We expected that if the invertebrate community food web was compartmentalized between layers, corresponding differences would appear in the stable isotope ratios, which would be summarized by community-wide metrics.

Materials and methods

Sampling

The study sites were secondary forests in the warm temperate zone with evergreen and deciduous broadleaf trees, located in the eastern part of the Kyoto Basin, central Japan: Yoshida-yama (35°01′47′′N, 135°47′14′′E; altitude 121 m) and Uryu-yama (35°02′21′′N, 135°48′09′′E; altitude 301 m). Yoshida-yama (hereafter Yoshida) is located on a small hill, and Uryu-yama (hereafter Uryu) is on a mountainside. The forest floors of both sites were covered with mull-type humic soil (Ponge 2003), consisting of litter and humus layers. The litter layer exhibited little segmentation due to rapid decomposition. Sampling quadrats of 400 m^2 (20 × 20 m and 10 × 40 m) were established at Yoshida and Uryu.

Invertebrate sampling was conducted every 2 weeks from May to July (summer) and from October to November (autumn), 2006. We defined ground invertebrates as those occurring in the litter layer and soil invertebrates as those occurring in the humic soil layer up to 3 cm depth. We used pitfall traps (7 cm diameter by 8 cm deep plastic cups) and our hands to collect ground invertebrates and Tullgren apparatuses to collect soil invertebrates. An array of 25 pitfall traps was placed so that the rims were level with the soil surface in each

quadrat at noon and were collected after 24 h. Each cup contained 50 ml of 20% ethanol to prevent trapped invertebrates from rotting or being eaten by carnivorous species until the collection. Together with the pitfall sampling, the collection by hand of ground invertebrates and the Tullgren apparatuses sampling of soil invertebrates were performed in 16 subquadrats (50 × 50 cm) placed arbitrarily within each quadrat. In each subquadrat, the ground invertebrates (Megascolecidae, lepidopteran larvae, and dipteran larvae) were collected by hand while carefully removing litter. Thereafter, to collect soil invertebrates, surface humus (25 × 25 × 3 cm deep) was brought back to the laboratory and placed in the Tullgren apparatuses. Samples were placed on a 3-mm mesh sieve and heated for 48 h by electric light bulbs; animals that fell through the sieve were immediately fixed in 70% ethanol to prevent desiccation and rotting. These collected invertebrates were identified by species as accurately as possible and kept at −30°C for stable-isotope analysis. Although ethanol preservation may alter $\delta^{13}C$ in animal tissue by removing lipid content, any such changes are small and insignificant in soil animals (Sticht et al. 2006).

Plant-derived organic matter is the basal resource of forest-floor food webs. Leaves and litter (ground basal resources) and humic soil (soil basal resource) were collected from the sampling quadrats at both Yoshida and Uryu. Five samples of each type of organic matter were prepared for stable isotope analysis per monthly sampling. Leaves were collected in May and June, and litter and humus were collected in May, June, July, and October. The leaf species sampled as foods of lepidopteran larvae were those grazed by lepidopteran larvae; individual leaves were treated as separate samples. Litter was collected from the litter layer and prepared as a bulk sample for stable isotope analysis. Humus was collected from the external soil layer in which plant tissue was completely broken down, and a cupful (9 cm in diameter, 4 cm deep) of humus was treated as a separate sample. These samples were dried at 60°C for 24 h in the laboratory immediately after collection. After desiccation, the leaf and litter samples were cut into fine pieces with scissors, and the humus was filtered through a 1-mm mesh sieve. Samples were kept at −30°C until stable-isotope analysis.

Stable-isotope analysis

We determined the carbon and nitrogen stable-isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) for all invertebrate species for which we had collected approximately 1 mg dry weight (the measurable amount) at the two sites. The sample size for stable isotope measurements per species was 1–15 (see Electronic supplementary material). All invertebrate and organic matter samples were dried at 60°C for 48 h prior to stable-isotope analysis. Individual large invertebrates (> 3 mm body length; body length was defined as maximum distance between the distal ends of a body) and a number of small invertebrates (< 3 mm body length) were ground into a fine powder using an agate mortar and pestle. Small invertebrate taxa, of which we collected only a few individuals, were used directly for analysis. When both larvae and adults were sampled for a species, only adults were analyzed for hemimetabolous insects, whereas both larvae and adult holometabolous insects were analyzed separately (e.g., Coleoptera, Lepidoptera, and Diptera). For social insects in the groups Formicidae and Isoptera, only workers were analyzed. For Oligochaeta (Enchytraeidae and Megascolecidae) and lepidopteran larvae (Geometridae and Noctuidae), the gut contents were surgically removed before desiccation. Samples of approximately 1 and 4 mg of invertebrate and organic matter, respectively, were placed in a tin cup for combustion.

The $\delta^{13}C$ and $\delta^{15}N$ values were measured using a mass spectrometer (Finnigan MAT Delta S, Bremen, Germany) coupled with an elemental analyzer (Fisons EA1108, Milan, Italy) at the Center for Ecological Research, Kyoto University. Their values are expressed as the per mil (‰) deviation from international standards, calculated as follows:

$$\delta^{13}C \text{ or } \delta^{15}N = \left(R_{\text{sample}}/R_{\text{standard}} - 1\right) \times 1,000,$$

where R for $\delta^{13}C$ is $^{13}C/^{12}C$ and for $\delta^{15}N$ is $^{15}N/^{14}N$. The international standards were Pee Dee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. DL-Alanine ($\delta^{13}C$: −23.47‰, $\delta^{15}N$: −1.66‰) was also analyzed as a working standard. The analytical precision (standard error of the values measurements for the working standard) was ±0.2‰ for $\delta^{13}C$ and ±0.2‰ for $\delta^{15}N$.

Comparison of community and trophic structures

To compare the species compositions between sites, seasons, and microhabitats, non-metric multidimensional scaling (NMDS; Clarke 1993) was performed for the presence/absence data of trophic species using MASS packages (sammon) of R version 2.5.1. Adults and larvae of the same species were treated as separate trophic species if appropriate (Briand and Cohen 1984).

Differences in invertebrate communities and the trophic structures between sites (Yoshida and Uryu), seasons (summer and autumn), and microhabitat (litter and humus layer) were examined in terms of ten metrics with species richness and stable-isotope ratios. Invertebrate species richness was evaluated as the number of trophic species per microhabitat. Layman et al. (2007) proposed several metrics of trophic diversity applicable to bi-plot data with mean $\delta^{13}C$ and $\delta^{15}N$ values of each trophic species in a community: $\delta^{15}N$ range (NR), $\delta^{13}C$ range (CR) and total area (TA). NR and CR are the distances between the two species with the most enriched and most depleted $\delta^{15}N$ and $\delta^{13}C$ values, respectively; TA is the convex hull area encompassed by all species in the $\delta^{13}C$-$\delta^{15}N$ bi-plot space (see Layman et al. 2007

for details). We also compared the mean, maximum, and minimum values of $\delta^{13}C$ and $\delta^{15}N$ (C_{mean}, C_{max}, C_{min}, N_{mean}, N_{max}, and N_{min}) of the component communities to evaluate their relative positions on the bi-plot. To examine the effects of microhabitat, site, and season on the ten community-wide metrics, a three-way analysis of variance (ANOVA) was performed using JMP version 5 (SAS Institute Inc., Cary, NC, USA). In addition, the overlap of trophic niche of invertebrate community between microhabitats was assessed by the percentage of overlapping area in the total area of convex hulls of the two microhabitats, i.e., 100 × [overlapping area of convex hulls]/([TA of litter layer] + [TA of humus layer] − [overlapping area of convex hulls]). If the food web was compartmentalized between litter and humus layers, the values of community-wide metrics would differ between two layers and the overlap of trophic niche between two layers would be small.

Results

In total, 212 trophic species (187 species, including 124 and 147 species from Yoshida and Uryu, respectively) were collected from both sites and analyzed for stable-isotope analysis (see ESM for detail). The species composition differed greatly between the litter and humus layers. NMDS ordination showed that species compositions of invertebrate communities were clearly distinguished between litter and humus layers by axis 1 (Fig. 1). Only 17 trophic species (8.0%) were collected from both layers. Of these, we estimated three ant species

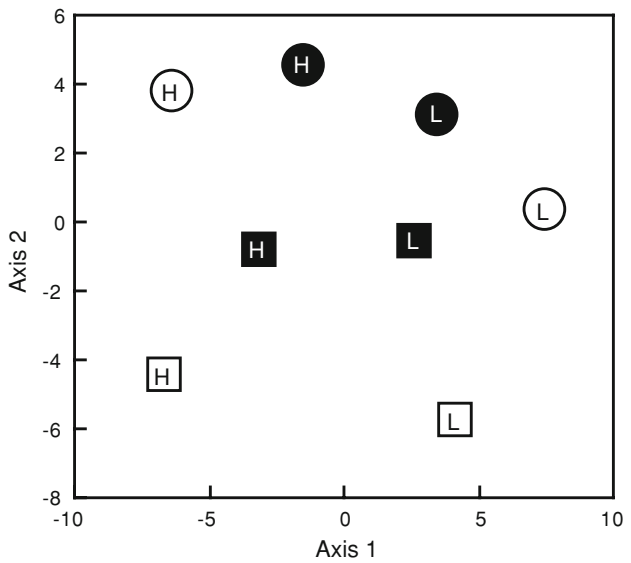

Fig. 1 Non-metric multidimensional scaling ordination for species compositions of eight invertebrate communities in forest floor. Final stress of this ordination was 0.043. Invertebrate communities are distinguished by site (*circle* Yoshida, *square* Uryu), season (*open* summer, *closed* autumn) and microhabitat (*L* litter layer, *H* humus layer)

to be foragers in both layers, and the other species were classified as either ground or soil invertebrates according to the layer in which their respective adults were collected, because adults were used in the stable-isotope analysis. For all trophic species, body length ($\log_{10}$-transformed) was significantly greater for ground invertebrates than soil invertebrates, although body length did not differ between seasons or between sites (three-way ANOVA: microhabitat, $F_{1,399} = 274.0$, $p < 0.0001$; season, $F_{1,399} = 0.94$, $p = 0.33$; site, $F_{1,399} = 1.0$, $p = 0.32$; interactions among three factors were excluded because they were not significant; necrophagous and coprophagous consumers were excluded as is mentioned below; ESM).

The $\delta^{13}C$ and $\delta^{15}N$ values of plant-derived organic matter differed significantly among three types of organic matter (leaves, litter, and humus) and did not change by seasons or site (three-way ANOVA for $\delta^{13}C$: type, $F_{2,94} = 96.2$, $p < 0.0001$; season, $F_{1,94} = 0.005$, $p = 0.95$; site, $F_{1,94} = 1.7$, $p = 0.20$: three-way ANOVA for $\delta^{15}N$: type, $F_{2,94} = 43.5$, $p < 0.0001$; season, $F_{1,94} = 2.3$, $p = 0.13$; site, $F_{1,94} = 1.4$, $p = 0.24$; interactions among three factors were excluded because they were not significant: Fig. 2 and 3). The stable-isotope ratios increased progressively according to the decomposition process (i.e., in the order of leaves, litter, and humus). The least square means (±SE) of leaves, litter, and humus were −31.0 ± 0.2, −29.6 ± 0.1, and −28.2 ± 0.1‰, respectively, for $\delta^{13}C$, and −4.4 ± 0.2, −4.0 ± 0.1, and −2.5 ± 0.1‰, respectively, for $\delta^{15}N$.

Figures 2 and 3 show the bi-plots of $\delta^{13}C$ and $\delta^{15}N$ for trophic species of ground and soil invertebrate communities in summer and autumn at Yoshida and Uryu. Not shown are species of Nicrophorinae, Scarabaeidae (except Melolonthinae), Histeridae, and Staphylinidae (except soil species) collected in summer, most of which had much higher $\delta^{15}N$ values than carnivores (4.1–13.5‰ at Yoshida; 2.6–11.0‰ at Uryu; see ESM for details). These were considered necrophagous and coprophagous consumers that scavenge vertebrate carcasses or excrement (Ikeda et al. 2007) and were excluded from the statistical analyses.

Of the ten community-wide metrics of food-web structure by site, season, and microhabitat, only the number of trophic species was significantly different between sites (Table 1), and the isotopic profile of invertebrate communities did not differ between sites. The number of trophic species, NR, and TA decreased significantly from summer to autumn, indicating a seasonal decrease in trophic diversity (Table 1). The number of trophic species in summer was approximately twice that in autumn at both sites.

The microhabitat was the main factor responsible for heterogeneities of food web structure. The number of trophic species, NR, N_{mean}, N_{max}, C_{mean} and TA differed significantly between litter and humus layers, although the overlap of trophic niche as indicated by the proportion of overlap between convex hulls was large (Table 1). The difference in N_{mean} and C_{mean} indicated

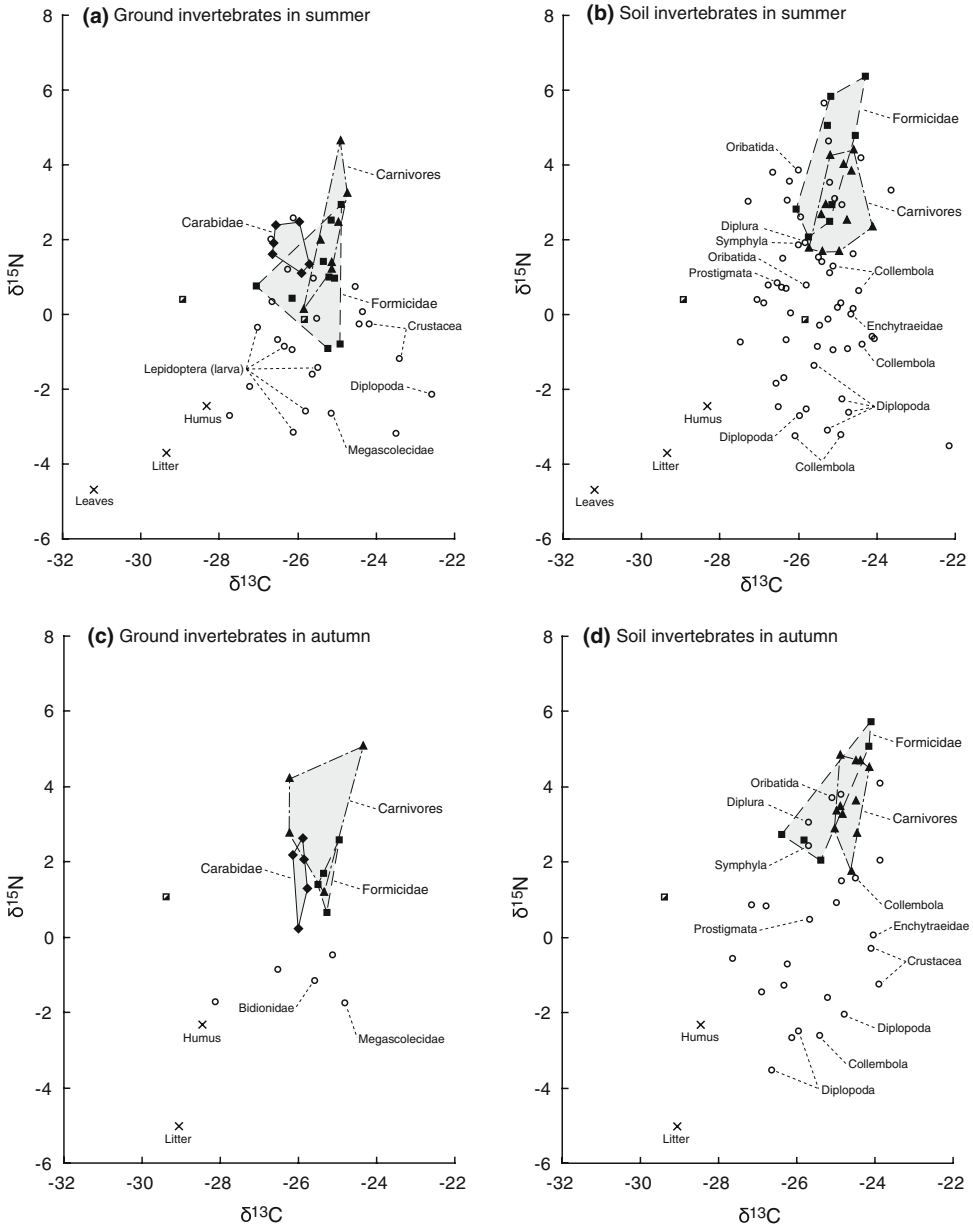

Fig. 2 $\delta^{13}C$ and $\delta^{15}N$ values of invertebrate communities at Yoshida in summer (**a** ground invertebrates collected from the litter layer; **b** soil invertebrates collected from the humus layer) and in autumn (**c** ground invertebrates; **d** soil invertebrates). Each point represents mean $\delta^{13}C$ and mean $\delta^{15}N$ of each trophic species. *Filled diamonds*, *squares*, and *triangles* represent Carabidae, Formicidae, and carnivores (Chilopoda, Araneae, Opiliones, Pseudoscorpiones, and Gamasida), respectively. *Open circles* represent other species. Species of Formicidae collected from both layers are represented by *half-filled squares*

that soil invertebrates had higher overall $\delta^{13}C$ and $\delta^{15}N$ values than ground invertebrates. Trophic diversity was higher in soil invertebrates than in ground invertebrates as was indicated by the larger number of trophic species and the higher NR and TA values in soil invertebrates than in ground invertebrates (Table 1).

The difference in trophic diversity (NR and TA) between litter and humus layers was attributed to the difference in the higher trophic level, because N_{max} was significantly larger in soil than ground invertebrates, whereas N_{min} did not differ between microhabitats (Table 1, Figs. 2 and 3). Based on $\delta^{15}N$ values, Carabidae, Formicidae, and carnivores (mainly Araneae) were higher-order consumers in the litter layer, although some ant species (Formicidae: *Camponotus*) exhibited low $\delta^{15}N$ values (approximately $-1‰$). In the humus layer, the representative higher-order consumers were Formicidae and carnivores (mainly Chilopoda and Gamasida). For these consumers at higher trophic levels, soil invertebrates tended to have higher $\delta^{15}N$ values than ground species (Figs. 2 and 3). The most enriched $\delta^{15}N$ values were approximately $3‰$ for most of ground higher-order consumers (mainly Carabidae and Formicidae), although some ground carnivores (Araneae) showed higher $\delta^{15}N$ values similar to soil higher-order consumers. In contrast, the most enriched $\delta^{15}N$ values were approximately $6‰$ for soil higher-order consumers. On the other hand, lower-order consumers consisted of lepidopteran larvae, Magascolecidae, Diplopoda, and Crustacea in the litter layer, and Diplopoda, Crustacea, and a portion of Collembola in the humus layer. No clear difference in $\delta^{15}N$ between litter and humus layers

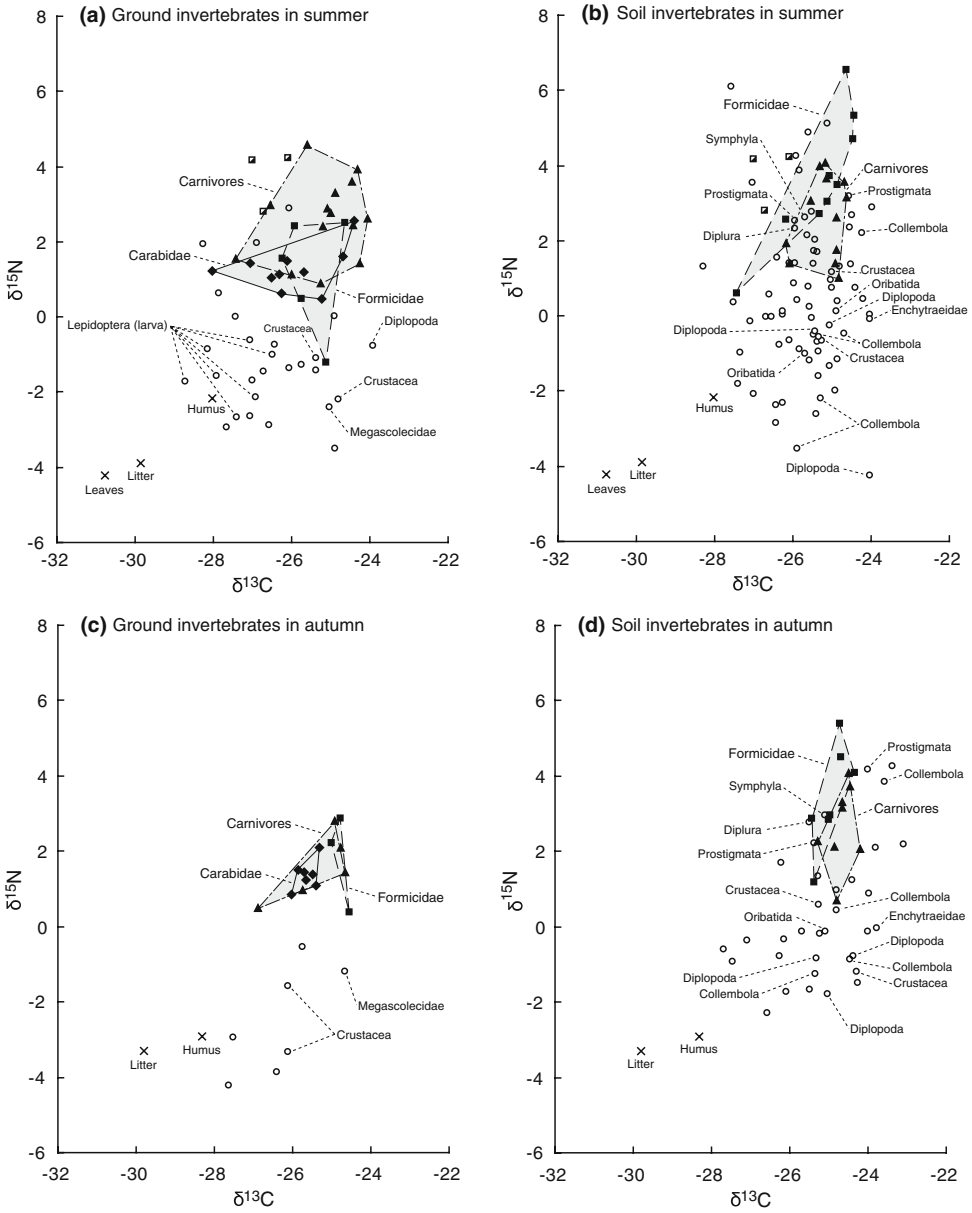

Fig. 3 δ^{13}C and δ^{15}N values of invertebrate communities at Uryu in summer (**a** ground invertebrates, **b** soil invertebrates) and in autumn (**c** ground invertebrates, **d** soil invertebrates). See legend of Fig. 1 for explanation

was apparent for these lower-order consumers at lower trophic levels (Figs. 2 and 3).

Discussion

We found that species composition differed with only a slight overlap between the ground and soil invertebrate communities inhabiting litter and humus layers (Fig. 1). The humus layer harbored more species than the litter layer (Table 1), but we need a caution about the effect of different sampling methods between the layers. The body sizes of ground invertebrates were generally larger than those of soil invertebrates. Body size can restrict movement between two layers with differing detrital particle sizes (Setälä and Aarnio 2002). Thus, the faunal and body size difference implied that the invertebrate food web is compartmentalized between litter and humus layers. However, the interpretation of the differences found in stable-isotopic values between layers is not straightforward (Table 1).

We examined the effects of site, season, and microhabitat (detrital layer) for nine metrics with stable-isotopic values (Table 1). Site exhibited no effect, whereas season had a significant effect only on NR and TA, probably due to the seasonal change in the number of trophic species. Microhabitat exhibited significant effects on five metrics: NR, N_{mean}, N_{max}, C_{mean} and TA. In general, the δ^{13}C metrics are indicators of the differences in the basal resources of a community food web. However, the difference in mean δ^{13}C (C_{mean}) was only 0.1–0.7‰ between microhabitats, and there was no

Table 1 Community-wide metrics for species richness and trophic structure with analyses of variances for the effects of site (Yoshida and Uryu), season (summer and autumn), and microhabitats (litter layer and humus layer)

Site	Yoshida				Uryu				Three-way ANOVA		
Season	Summer		Autumn		Summer		Autumn		*F*-value (df = 1, 4 for each effect)		
Microhabitat	Ground	Soil	Ground	Soil	Ground	Soil	Ground	Soil	Site	Season	Microhabitat
Number of trophic species	46	75	18	41	56	95	22	50	13.6*	132.4***	66.1**
δ^{15}N											
Range (NR)	7.8	9.9	6.9	9.2	8.1	10.8	7.1	7.7	0.01	7.9*	14.2*
Mean (N_{mean})	0.3	1.2	1.2	1.5	0.5	1.1	0.2	1.2	2.9	1.8	11.5*
Max (N_{max})	4.7	6.3	5.1	5.7	4.6	6.5	2.9	5.4	1.6	2.5	12.2*
Min (N_{min})	−3.2	−3.5	−1.8	−3.5	−3.5	−4.3	−4.2	−2.3	0.7	1.0	0.1
δ^{13}C											
Range (CR)	5.2	5.3	3.8	3.8	4.8	4.3	3.1	4.6	0.6	6.9	0.5
Mean (C_{mean})	−25.5	−25.4	−25.7	−25.2	−26.0	−25.5	−25.7	−25.0	0.4	2.5	10.3*
Max (C_{max})	−22.6	−22.1	−24.4	−23.9	−23.9	−24.0	−24.5	−23.1	1.5	2.2	1.1
Min (C_{min})	−27.7	−27.5	−28.1	−27.6	−28.7	−28.3	−27.6	−27.7	1.3	0.9	0.8
TA	24.3	34.1	16.1	21.1	28.7	35.8	12.2	20.2	0.03	69.0**	21.6**
Overlap in TA (%)	57.9		57.9		62.4		37.1				

In three-way ANOVA, interactions among three factors were excluded because they were not significant. Percentage overlaps between convex hulls (TAs) of the two microhabitats are also shown. Necrophagous and coprophagous consumers and ant species collected from both litter and humus layers are not included in the statistics

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

difference in the range of δ^{13}C (CR, C_{min}, and C_{max}). Therefore, contrary to our expectation, no differences in basal resources that were used by consumers could be detected between litter and humus layers based on δ^{13}C value. This lack of clear difference could in part be due to small differences in the δ^{13}C values among types of plant organic matter. In addition, detritivores of the two layers could utilize the same detrital resource, as suggested by the observation that detritivores (Magascolecidae, Diplopoda, and Crustacea) in the litter layer exhibited similar δ^{13}C values to those of small lower-order consumers (Diplopoda, Crustacea, and a portion of Collembola) in the humus layer. Conversely, given that herbivores (mainly lepidopteran larvae) with lower δ^{13}C values than detritivores occurred in the litter layer, the small difference in C_{mean} between the layers may be a result of the presence of herbivores and their predators in the litter layer.

Interestingly, the δ^{13}C fractionation from basal resources to lower-order consumers on the forest floor, approximately 2–4‰, was larger than the empirical value of δ^{13}C fractionation, approximately 0–1‰ (Vander Zanden and Rasmussen 2001; Post 2002). The large fractionation in δ^{13}C was reported in detrital food webs (Tayasu et al. 1997; Uchida et al. 2004; Hishi et al. 2007). In terrestrial ecosystems, the δ^{13}C fractionation from plant-derived organic matter to herbivores and detritivores might be larger than that from herbivores and detritivores to carnivores due to differences in chemical composition of food and/or assimilation rate.

With respect to δ^{15}N, there was a clear difference in N_{max} with associated differences in NR and TA (trophic diversity indices) between the layers. N_{max} and N_{mean} were larger in the humus layer than in the litter layer, and this was due to a difference in food resources of higher-order consumers (Figs. 2 and 3). In contrast, N_{min} did not differ between microhabitats, providing further evidence for the sharing of basal resource by ground and soil lower-order consumers. Therefore, the wide range of δ^{15}N in the humus layer suggested that there were a larger number of trophic transfers (trophic levels) than in the litter layer. It is possible that the involvement of microorganisms in the detrital chain contributed to an increase in the δ^{15}N values in the humus layer.

Some non-predatory species in the humus layer showed very high δ^{15}N values like the higher-order consumers (Figs. 2 and 3). For example, the highest δ^{15}N value in Collembola was 3.7–6.7‰ higher than that of humus. Similarly, Symphyla exhibited 4.3–5.9‰ higher δ^{15}N values than humus. These enrichment factor values are much higher than the ordinary isotopic enrichment factor for δ^{15}N, which is approximately 3‰ per trophic level (Minagawa and Wada 1984; Vander Zanden and Rasmussen 2001; Post 2002; McCutchan et al. 2003; Vanderklift and Ponsard 2003). Therefore, the above consumers might be secondary decomposers (see Scheu and Falca 2000) that feed selectively on microdecomposers such as fungi, which would have higher δ^{15}N values than substrate feeders (Kohzu et al. 1999; Dijkstra et al. 2006; Hart et al. 2006). By consuming these secondary decomposers, the δ^{15}N values of soil higher-order consumers (i.e., Formicidae and Gamasida) would become higher than those of ground higher-order consumers such as Carabidae and Formicidae, which would not consume secondary decomposers derived from the humus layer. A part of ground carnivores of Araneae showed very high δ^{15}N values and might partly consume secondary

decomposers inhabiting the humus layer. Although microdecomposers also inhabit the litter layer (Scheu and Schaefer 1998), and hence secondary decomposers as well, our results with the δ^{15}N profile in the ground invertebrates suggest that the trophic transfer through secondary decomposers in the litter layer was less important than that in the humus layer. However, further study is needed to examine the importance of secondary decomposers in the litter layer, because we did not use Tullgren apparatuses for the ground invertebrates and our sampling might not be sufficient.

The difference in δ^{15}N of higher-order consumers between the litter and humus layers also suggests that the ground and soil invertebrate food web was segregated at higher trophic levels, because if higher-order consumers (e.g., predators) could consume prey in opposite layers, isotopic differences would disappear. Given that ground higher-order consumers are generally large, they may not be able to capture small prey in the humus layer. Thus, one factor contributing to vertical heterogeneity of the forest floor food web might be the restriction of vertical movements by large body sizes (Setälä and Aarnio 2002). In addition, small soil higher-order consumers likely cannot capture large prey animals in the litter layer. If this segregation is true, the detrital food-web structure of invertebrate community is more complex than previously appreciated, since it is commonly assumed that top predators couple different energy channels starting from detritus (De Ruiter et al. 1995; Scheu 2002; Rooney et al. 2006). In the layered habitat, each layer was thought to have a distinct top predator group, and the coupling of energy channels may be effective within layers but not between layers. For example, different channels are coupled by carabid beetles feeding on invertebrates from both grazing and detrital chains in the litter layer (Sota 1985) and by soil higher-order consumers feeding on organisms of various functional groups in the humus layer (De Ruiter et al. 1998). However, our study did not involve large animals (e.g., mammals and birds), which feed on the ground at the study sites. These animals might feed relatively large soil invertebrates (e.g., insect larvae and Chilopoda) and couple distinct energy channels derived from the litter and humus layers.

In conclusion, our study using stable-isotope analysis did not clearly show that the two layers in the temperate forest floors harbor distinct invertebrate community food webs based on the organic matter of different decomposition states. Nevertheless, we did find heterogeneity in trophic structure associated with differences in species composition and δ^{15}N values of invertebrates between the layers. This finding may be useful for future analyses of the dynamic properties of invertebrate communities in detrital food webs. In detrital food webs, trophic positions of organisms and their functions in decomposition process may be evaluated by δ^{15}N more properly than by δ^{13}C. The use of community-wide metrics with stable isotope data in comparing food web structure is still in its infancy, and further accumulation of data will facilitate more substantial analyses of community food web variations.

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