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Trophic structure of macro- and meso-invertebrates in Japanese coniferous forest: Carbon and nitrogen stable isotopes analyses

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ABSTRACT

Few studies have been published on the feeding ecology of Japanese soil fauna based on stable isotope analysis. Therefore, the present work aims to use this technique for studying the trophic structure of Japanese soil fauna at two coniferous forests. Significant differences were observed between investigated sites (Arahama and Gamo) in genus richness and abundance, while for Shannon diversity indexes the difference was non-significant. The isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of the invertebrates collected at Arahama ranged from 0.3 to 6.3‰ for $\delta^{15}N$ and from −27.3 to −23.3‰ for $\delta^{13}C$. At Gamo, invertebrates $\delta^{13}C$ values ranged from −26.1 to −23.5‰ and $\delta^{15}N$ values ranged from 1.6 to 6.8‰. At both sites, invertebrates formed two distinct groups on the basis of combined C and N stable isotope ratios. The locations of these groups related to $\delta^{13}C$ values. The less enriched group ($\delta^{13}C < -25‰$) and the more enriched one ($\delta^{13}C > -25‰$). The range of $\delta^{15}N$ for the present animals exceeded two trophic levels. While, the gradual ^{15}N enrichment within the invertebrates species may indicate the dominance of omnivory in soil food webs. The differences between sites in $\delta^{15}N$ confirm the importance of studying the trophic structure of soil fauna locally.

1. Introduction

Soil-inhabiting invertebrates are essential components of the decomposer food web and nutrient cycling pathways in soil ecosystems (Edwards et al., 1970; Crossley, 1977; Seastedt, 1984). The majority of soil fauna are herbivores and detritivores. Their feeding activities play an important role in soil decomposition and nutrient cycling in terrestrial ecosystems. Soil biota consists of more species than the biota of any other environment on earth; despite their importance they are the least understood (Young and Crawford, 2004; Bardgett, 2005).

Analysis of soil invertebrate's food web trophic structure using traditional ecological methods has provided limited information. Recently, stable isotope techniques have become one of the main methodological advices in soil ecology. The isotopic signatures of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are the most commonly used ratios, and in combination can help construct the food chain of a soil system under investigation (Peterson and Fry, 1987; Rundel et al., 1989; Halaj et al., 2005). The carbon ratio can help identify the consumer's diet and ultimately trace the basal carbon source in food webs (Araujo-Lima et al., 1986; Peterson and Fry, 1987; Anderson and Polis, 1998). On the other hand, the nitrogen ratios have been used as indicators of trophic position, or the degree of intraguild predation and cannibalism among consumers (Peterson and Fry, 1987; Hobson and Welch, 1995; McNabb et al., 2001).

Stable isotope techniques have been successfully used to study trophic structure of whole below-ground animal communities (Scheu and Falca, 2000; Briones et al., 2001; Setälä and Aarnio, 2002; Schmidt et al., 2004; Dias and Hassall,

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2005; Halaj et al., 2005; Sampedro and Domínguez, 2008). Also, the feeding habits of some particular soil taxa have been studied successfully by isotopic means (Spain et al., 1990; Martin and Lavelle, 1992; Martin et al., 1992a,b; Schmidt et al., 1997; Spain and Le Feuvre, 1997; Schmidt et al., 1999; Uchida et al., 2004 [earthworms]; Scheu and Folger, 2004; Chahartaghi et al., 2005; Haubert et al., 2005; Haubert, 2006; Hishi et al., 2007; Ruess et al., 2007 [Collembola]; Boutton et al., 1983; Lepage et al., 1993; Tayasu et al., 1994, 1997, 1998; Tayasu, 1998 [termites]; Traugott et al., 2007 [Coleoptera larvae]).

Tiunov (2007), expected that the application of stable isotope method in soil ecology can lead to a breakthrough in our understanding of the soil community functioning (Scheu, 2002; Neilson et al., 2002; Moore et al., 2004). Stable isotope analysis of soil trophic webs requires a deep understanding of the composition and structure of the communities and their time-related changes and taking into account isotopic fractionation in biogeochemical processes of all levels.

While more research is needed to validate stable isotope techniques as a tool to study feeding ecology of soil animals and their trophic structures in soil food webs (Schmidt et al., 2004), few studies have been published on the feeding ecology of Japanese soil fauna based on this technique (Uchida et al., 2004; Ikeda et al., 2007; Hishi et al., 2007). Therefore, the present work aims to evaluate the patterns of $\delta^{13}C$ and $\delta^{15}N$ natural abundance in macro- and meso-invertebrates and their potential food sources at two coniferous forests, in northeastern Japan. The two forest have been selected randomly to investigate the inter and intraguild isotopic variations between two communities and determine the ability of $\delta^{13}C$ and $\delta^{15}N$ signatures to understand the trophic relations in soil food webs.

2. Materials and methods

2.1. Study sites

The present study was carried out in the northeast of Honshu Island, Japan. The sampling sites were two coniferous forests (*Pinus thunbergii*); at Arahama (38̊ 13′ N, 140̊ 59′ E) and at Gamo (38̊ 15′ N, 141̊ E) along the Sendai Bay. The sites were located within 5 km of each other and close to the shore; 12, 160 m far from the shore for Gamo and Arahama, respectively (Fig. 1). The ground vegetations of Arahama are represented by *Sedum sarmentosum*, *Carpinus laxiflora*, *Oxalis corniculata*, *Parthenocissus tricuspidata*, *Cryptotaenia Canadensis*, *Paederia scandens*, *Glechoma hederacea*, *Mazus miquelii*, *Plantago asiatica*, *Lonicera japonica*, *Chrysanthemum* sp., *Nipponanthemum nipponicum*, *Erigeron annuus*, *Dactylis glomerata*, *Gramineae* sp. and *Cephalanthera longibracteata*. While at Gamo it is represented by *Equisetum palustre*, *Duchesnea indica*, *Rosa luciae*, *Ampelopsis glandulosa*, *Oenanthe javanica*, *Galium spurium*, *Lonicera japonica*, *Taraxacum officinale* and *Festuca arundinacea*.

2.2. Sampling and sample processing

Samples were collected during the months of June and July 2007. Sampling was inside a rectangular area of 20 × 30 m within each sampling sites (Arahama and Gamo), at least 5 m away from the forest margin for collection. Invertebrates were collected either directly by hand (for stable isotope analyses) or by pitfall traps. Pitfall traps were set in the selected areas, 15 traps were used; 5 m apart from each others (three traps in five lines). Plastic container (500 ml with diameter of 7 cm and a height 15 cm) filled with 60 ml propylene glycol was used as trap. Each trap was covered by plastic sheet to protect it from litter and rain. The traps were set for 5–10 days then collected. The traps were emptied three times per site during the sampling period. The samples have been collected alternately between the two sites. The data were standardized by the equation of $N/(d^{*}\mathrm{Tr})$ where N = number of individuals in one sample in a habitat, d = number of days between two sampling and Tr = number of usable traps (some traps were destroyed). Five samples of litter, humus and soil (5 cm deep) were taken from each lines for soil factors (soil pH, water content and organic matter) and stable isotope analysis.

2.3. Measurement of ecological factors

Some ecological factors including air and soil temperatures, pH, water content (Wt.C.), organic matter (Org.M.) were recorded during sampling at studied sites. Air temperature one meter above soil surface, and soil temperature at 5 cm depth were recorded, using a normal thermometer. Soil pH was performed in a suspension of soil and distilled water with a ratio of 1:2.5 by pH meter. Water content was determined by relating the water loss 50 g soil sample to the dry weight (at 110 °C) of the sample. Muffle furnace at a temperature of 500 °C for 8 h was used to measure the organic matter content in the 5 g soil sample.

2.4. Preparation of samples for isotope analysis

The litter samples were rinsed in distilled water and transferred into sealable plastic bags, deep-frozen (−20 °C) and stored. The animal samples were starved for 2 days to induce evacuation of their gut and then rinsed in distilled water. Later, they were freeze dried for 24 h and ground in mortar. Ten milliliters chloroform–methanol (2:1 Vol) solution was added to animal samples to remove lipids. Then they were filtered through Whatman GF/F glass-fiber filters (precombusted at 500 °C) and freeze dried again. Soil and humus samples were acidified with 1 mol/L HCl to remove carbonates before the isotope analysis. Then they were ground using a mortar.

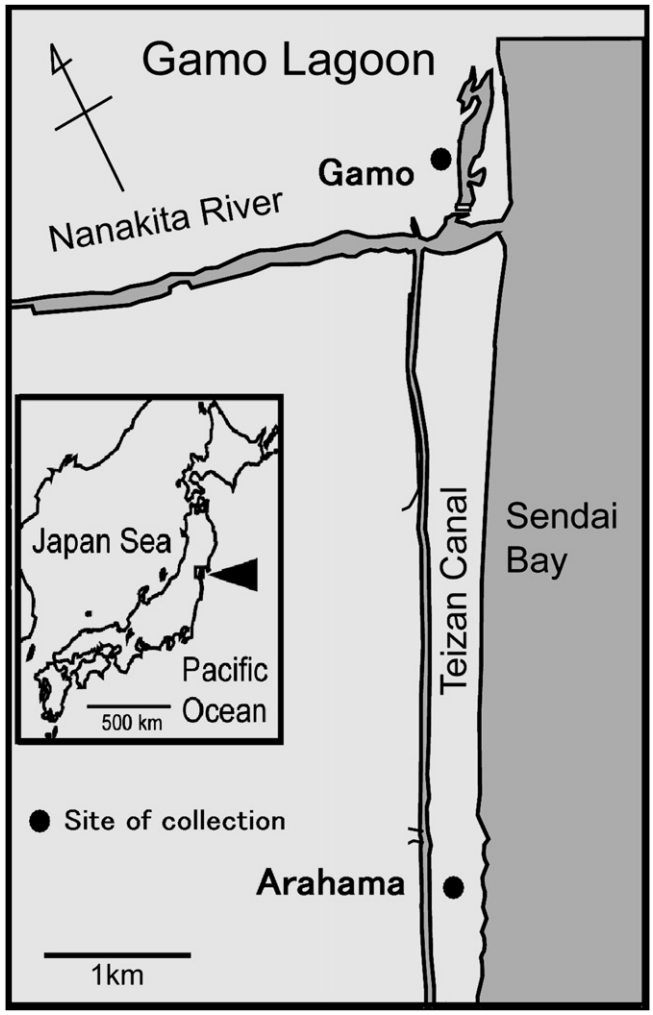

Fig. 1. Location of the sampling sites, Sendai bay in northeastern Japan.

2.5. Stable isotope ratio analysis

The carbon and nitrogen isotope ratios of the samples were measured with a mass spectrometer (DELTA plus, Finnigan Mat) directly connected to an elemental analyzer (NA-2500, CE Instruments). All the isotopic data are reported in the conventional δ notation as follows:

$\delta^{13}C \quad \delta^{15}N = (R\text{sample}/R\text{standard} - 1)1000(‰)$

where R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio for $\delta^{13}C$ and $\delta^{15}N$, respectively. Pee Dee Belemnite (PDB) was used as the $\delta^{13}C$ standard and nitrogen gas as the $\delta^{15}N$ standard. The overall analytical error was within $\pm 0.2‰$ for both $\delta^{13}C$ and $\delta^{15}N$.

2.6. Statistical analysis

Descriptive statistics such as mean (M) and standard deviation (SD) were calculated using SPSS and Microsoft Excel (version 2000). MANOVA was used for comparing means of studied variables between the two investigated sites. The paired t-test was used for comparing means isotopic signatures between two invertebrate groups. All statistical analyses were performed using SPSS software package (version 9).

3. Results

3.1. Abundance and diversity index

During the period of investigation, the total invertebrate samples consisted of 10,494 individuals representing 24 taxa; 6049 individuals belonging to 22 taxa were captured at Arahama site, 15 taxa and 4445 individuals at Gamo site. The traps

Table 1
Average values (±standard deviations) of the studied environmental factors in the study area.

	Arahama	Gamo
Air temperature (°C)	24.17 ± 0.42	18.95 ± 0.72
Soil temperature (°C)	21.93 ± 0.61	17.50 ± 2.01
Soil pH	6.14 ± 0.59	5.56 ± 0.38
Soil water content (%)	18.70 ± 10.56	33.32 ± 14.09
Soil organic matter (%)*	11.46 ± 0.36	10.96 ± 0.31

*Indicated significant ($p < 0.05$) differences by ANOVA.

belonging to Arahama site contained mainly Collembola, Isopoda and isopod larvae, however Isopoda, Amphipoda and ants occurred mainly in traps at the Gamo site (Table 1). Significant differences were observed between sites in invertebrates abundance ($F = 15.5$, $p = 0.004$).

3.2. Environmental factors

The mean values of the measured ecological factors; air and soil temperatures, pH, water content (Wt.C.) and organic matter (Org.M.) were demonstrated in Table 2. Statistical analysis (MANOVA) indicated a significant difference between the two sites in soil organic matter ($p < 0.05$).

3.3. Isotopic signatures of soil, humus and litter

Isotopic signature of soil, humus and litter as food sources showed a wide range of values for carbon and nitrogen at both sites, respectively (Figs. 2 and 3). Moreover, the $\delta^{15}N$ values were quite similar for the two sites, while the mean values of $\delta^{13}C$ for humus and litter at Arahama were higher than at Gamo (MANOVA: $F = 17.61$, $p = 0.006$ for humus; $F = 4.99$, $p = 0.06$ for litter). In both sites, soil exhibited higher $\delta^{15}N$ values than humus and litter (t-test, $p < 0.04$). While in the $\delta^{13}C$ values there were no significant differences among them.

3.4. Isotopic signatures of Invertebrates

The isotopic signatures of the invertebrates collected from Arahama ranged from 0.2 to 6.3‰ for $\delta^{15}N$ and from −27.3 to −23.3‰ for $\delta^{13}C$ (Fig. 2). At Gamo, invertebrates $\delta^{15}N$ values ranged from 1.6 to 6.8‰ and $\delta^{13}C$ values ranged from −26.1 to −23.5‰. The range of $\delta^{13}C$ and $\delta^{15}N$ values for invertebrates at Arahama showed relatively wider than at Gamo.

Table 2
Abundance of soil macro- and meso-invertebrates (individual/day/trap) in Arahama and Gamo sites which collected by pitfall traps (means ± standard deviations; SD).

Taxon	Arahama		Gamo	
	Total Indv.	Mean ± SD	Total Indv.	Mean ± SD
Lasius japonicus (Ant)	186	1.32 ± 0.57	313	2.77 ± 3.31
Stenamma sp. (Ant)	57	0.41 ± 0.19	0	–
Porcellio scaber (Isopoda)	1033	6.59 ± 4.16	2685	9.89 ± 2.02
Isopod larvae	1764	11.89 ± 5.48	0	–
Armadillidium vulgare (Isopoda)	18	0.13 ± 0.10	2	0.02 ± 0.04
Platorchestia japonica (Amphipoda)	5	0.03 ± 0.07	1041	7.21 ± 3.14
Dryophthorus japonicus (Beetle)	–	–	6	0.012 ± 0.01
Pterostichus microcephalus (Beetle)	8	0.06 ± 0.04	43	0.83 ± 0.90
Eusilpha japonica (Beetle)	44	0.36 ± 0.44	0	–
Carabus insulicola kita (Beetle)	10	0.08 ± 0.08	0	–
Ocypus lewisius (Beetle)	3	0.02 ± 0.02	0	–
Gonocephalum japanum (Beetle)	12	0.10 ± 0.12	0	–
Ryuthela sp. (Spider)	164	1.09 ± 0.54	271	1.307 ± 0.94
Xysticus saganus (Spider)	117	0.89 ± 1.01	0	–
Lycosa sp. (Spider)	67	0.53 ± 0.55	50	0.525 ± 0.49
Allolobophora sp. (Earthworm)	3	0.03 ± 0.04	9	0.079 ± 0.10
Cryptops japonicus (Centiped)	4	0.03 ± 0.03	2	0.035 ± 0.07
Bothropolys rugosus (Centiped)	1	0.01 ± 0.02	1	0.017 ± 0.04
Lithobius pachypedius (Centiped)	1	0.01 ± 0.02	3	0.006 ± 0.01
Tomocerus sp. (Collembola)	2540	17.78 ± 3.12	11	0.022 ± 0.01
Neotrichophorus junicor (Insect larvae)	9	0.07 ± 0.07	0	–
Gonolabis marginalis (ear wing)	2	0.01 ± 0.02	0	–
Chiromantes haematocheir (crab)	1	0.01 ± 0.02	6	0.041 ± 0.05
Chiromantes dehaani (crab)	0	–	2	0.004 ± 0.01
Total	6049	1.883 ± 1.490	4445	1.518 ± 1.152

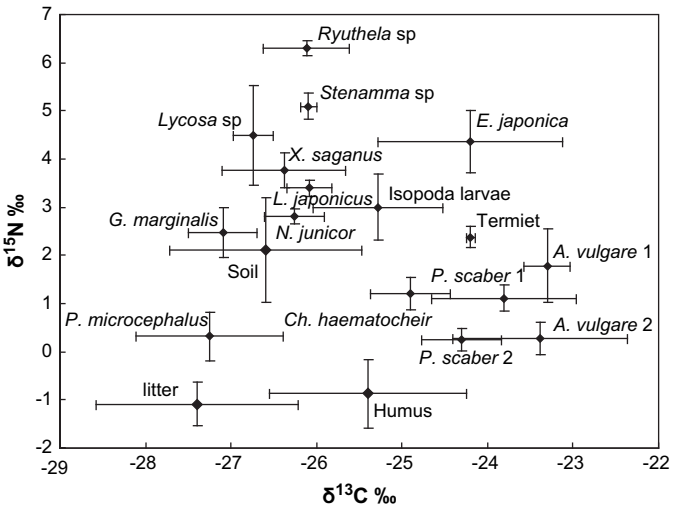

Fig. 2. Mean values of carbon and nitrogen stable isotopes ratios for soil macro- and meso-invertebrates, soil, humus and litter at Arahama. Note *P. scaber* and *A. vulgare* (Isopoda) have two forms; 1: light and 2: dark forms.

Invertebrates in both investigated sites formed two distinct trophic groups on the basis of combined C and N isotope ratios ($F = 123.98$, $p = 0.001$ for Arahma; $F = 71.69$, $p < 0.0001$ for Gamo). The locations of these groups related to $\delta^{13}C$ values. The less enriched group ($\delta^{13}C < -25‰$) and the more enriched one ($\delta^{13}C > -25‰$) (Figs. 2 and 3). The two groups separated by about 0.8‰ for $\delta^{13}C$. At Arahama the less enriched group included beetles (*Pterostichus microcephalus*), insect larvae, earwigs, ants, spiders and the second group consists of isopods, beetles (*Eusilpha japonica*) and crabs. While at Gamo the first group included earthworms, amphipods, centipedes (*Cryptops japonicus*), beetles and ants. The second group comprised of isopods, centipedes (*Bothropolys rugosus*) and crabs.

In both sites, the invertebrate communities show a wide range of ^{15}N enrichments. There is a significant difference in $\delta^{15}N$ signatures between the collected invertebrates at each site. They are mainly subdivided into decomposers, which feed on organic materials on the whole humification process from dead plant tissues to soil organic matter and predators. Decomposers are generally composed of primary (lower trophic level) and secondary (higher trohpic level) decomposers. At Arahama, *Porcellio scaber* and *Armadillidium vulgare* (isopods), *Pterostichus microcephalus* (beetle), and *Chiromantes haematocheir* (Juvenile crab) showed the lowest $\delta^{15}N$ values which can be considered as primary decomposers in this food chain. While insect and isopod larvae, *Gonolabis marginalis* (ear wing), termites and *Lasius japonicus* (ant) had overlapping values of $\delta^{15}N$ and grouped at an intermediate trophic position between the primary decomposers and predators. Three spider species and *Eusilpha japniceonica* (beetle) represented the predatory group in this trophic chain (Fig. 2). In the other side, at Gamo the primary decomposers were represented by earthworm, *Porcellio scaber* (isopods) and *Platorchestia japonica* (amphipods). *Cryptops japonicus* and *Bothropolys rugosus* (centipedes), *Pterostichus microcephalus* (beetles) and *Lasius japonicus* (ants) had an intermediate position between primary consumer and predators and could be classified as secondary decomposers. While

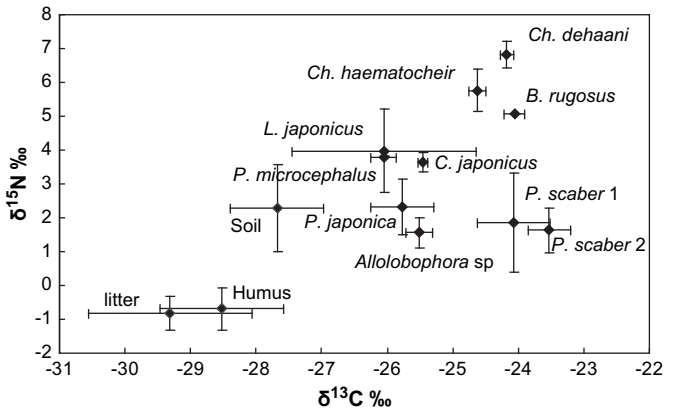

Fig. 3. Mean values of carbon and nitrogen stable isotopes ratios for soil macro- and meso-invertebrates, soil, humus and litter at Gamo. Note *P. scaber* (Isopoda) have two forms; 1: light and 2: dark forms.

Chiromantes haematocheir and *Chiromantes dehaani* (crabs) showed the highest $\delta^{15}N$ values as the predators of this trophic chain (Fig. 3).

4. Discussion

In food web analysis, carbon and nitrogen isotope ratios have commonly been used to estimate the food sources and the relative trophic levels of animals, respectively. However, the studies on soil macroinvertebrates have shown that increased degree of decomposition of food sources also results in the $\delta^{15}N$ of the soil animals (Tayasu et al., 1997; Ponsard and Arditi, 2000; Scheu and Falca, 2000; Schmidt et al., 2004; Uchida et al., 2004; Hishi et al., 2007). As a result, the wide range of ^{15}N enrichment in soil organisms was commonly found along the humification gradient of diets (Hyodo et al., 2008). In the present results, the $\delta^{15}N$ values of the two invertebrate communities spanned a gradient of 5.2 and 6.1‰. A similar range of values had been recorded for soil invertebrates communities (Ponsard and Arditi, 2000; Scheu and Falca, 2000; McNabb et al., 2001; Chahartaghi et al., 2005). Assuming that a mean value of $\delta^{15}N$ increase for one trophic transfer was approximate 2.3–3.4‰ (Minagawa and Wada, 1984; McCutchan et al., 2003; Vanderklift and Ponsard, 2003), the range of nitrogen ratios of the present animals exceeded two trophic levels. Halaj et al. (2005) reported that the macroarthropod community at temperate forest in southern Oregon, USA consists of at least two to three trophic levels with a considerable amount of variation in the ^{15}N enrichment among detritivores and predators.

Schmidt et al. (2004) mentioned that dual $\delta^{13}C$, $\delta^{15}N$ analysis can have the power to separate soil fauna to groups. While, Ponsard and Arditi (2000) suggested that no distinct trophic structure could be derived from measurements of $\delta^{13}C$. The present data shows that the $\delta^{13}C$ values can separate invertebrate community into two distinct groups at both investigated sites. This means these groups belong to two different food chains. Denies (1980) showed that the $\delta^{13}C$ values depend on the C fixation pathway (i.e. −20 to −35‰ for C3-plants and −9 to −14‰ for C4-plants). Since the present $\delta^{13}C$ values within the range of −20 to −35‰, therefore the C sources of these two food chains depend on the C3-plants. In addition, Wedin et al. (1995) found an increase in $\delta^{13}C$ values of C3-plant material during decomposition. Therefore, the early-decomposed C3-plant (litter) is the basic food source for one food chain while the late-decomposed C3-plant (humus) is the basic food source for the other chain. This led us to conclude that the $\delta^{13}C$ analysis can have the power to separate the food web to different food chains. More research is required to confirm this conclusion.

From the trophic structure of the soil invertebrate of the two studies communities, it can conclude that the soil decomposers do not form distinct trophic levels. They appear to be gradient from primary to secondary decomposers. The same conclusion was obtained by Scheu and Falca (2000) for community of invertebrates in two temperate beech forests. Hyodo et al. (2008) showed that the gradual enrichment of ^{15}N with the humification of diets brought the increase in the $\delta^{15}N$ values of soil organisms with diet age in the below-ground food web. This probably explains the variation in $\delta^{15}N$ values of decomposers, which was previously thought to show a continuum from primary to secondary decomposers (Scheu and Falca, 2000; Schmidt et al., 2004). Overall, The gradual ^{15}N enrichment within the present invertebrates species may indicate the dominance of omnivory in soil food webs. Hishi et al. (2007) mentioned that a gradual $\delta^{15}N$ enrichment has been observed not only in collembola, but also in the broader soil organism community (Scheu and Falca, 2000), and may indicate the dominance of omnivory in soil food webs (Eggers and Jones, 2000).

In the present investigated sites, primary decomposers seemed to have $\delta^{13}C$ values as well as $\delta^{15}N$ values closer to litter than secondary decomposers. However, primary decomposers except for *Pterostichus microcephlus* in our study sites have $\delta^{13}C$ values more separate from the litter and showed a pronounced enrichment shift (up to 6‰) from the litter. Similar trophic shift in $\delta^{13}C$ and $\delta^{15}N$ between consumers and their diets had been reported in terrestrial ecosystems (Ponsard and Arditi, 2000; McCutchan et al., 2003; Schmidt et al., 2004; Uchida et al., 2004; Halaj et al., 2005; Traugott et al., 2007). These results suggested that the primary decomposers selectively assimilated ^{13}C-enriched organic carbon pools from the humus, since the humus would contain ^{13}C-enriched components more than the litter. Some fungus hyphae have higher $\delta^{13}C$ values than vascular plants (Ponsard and Arditi, 2000). Tayasu et al. (1997) showed that the fungus-associated termite Acanthotermes acanthothorax was higher in $\delta^{13}C$, but values of $\delta^{15}N$ was comparable with those in wood-feeding termites. In addition, Schmidt et al. (2004) reported a enchytraid species, *Enchytraeus buchholzi*, was enriched in ^{13}C ($\delta^{13}C$ −24.4‰) than other soil invertebrates, and suggested the contribution of soil algae to its diet. Since our study sites located near seashore, strong winds often splashed the sea water about, and the micro algae in the splash could grow in the wet condition of soil. In the present investigated sites fungus hyphae and soil algae in the bulk of humus may contribute to the diet of primary consumers as the ^{13}C-enriched component. Plant generally contains a higher proportion of complex carbohydrates with lower $\delta^{13}C$, such as cellulose and lignin than microorganisms such as algae and fungi (Benner et al., 1987; Wedin et al., 1995). Primary decomposers are prevented from assimilating the more ^{13}C-poor organic components of the litter and soil, because they lack the enzymes necessary to digest them (Lavelle et al., 1995), and therefore preferentially assimilate ^{13}C-rich organic carbon pools (Ponsard and Arditi, 2000). There is a need for more research on the ^{13}C-rich diets of the primary consumers. At Arahama, one primary decomposer species, *Pterostichus microcephlus*, had similar $\delta^{13}C$ values to the litter, suggesting it mainly assimilated ^{13}C-poor litter-derived organic matter.

The values of $\delta^{15}N$ for the predators in both investigated sites were more than 4‰, but they have different $\delta^{13}C$ values, which means that they eat different prey. Some investigations suggested that the spread of $\delta^{15}N$ values of predators likely reflects the diversity of potential prey species among detritivores and a varying degree of intraguild predation among different species (Ponsard and Arditi, 2000; Scheu and Falca, 2000; Halaj et al., 2005). In Arahama, predatory spiders and an

ant species seemed to be the top of litter based food chain and *Eusipha japonica* (beetle) seemed to be the top of food chain based on the ^{13}C-rich organic materials in humus. Isopoda larvae as a secondary decomposer had intermediate δ^{13}C values between these two food chains and would assimilate both microbes decomposing litter and ^{13}C-rich organic materials. In Gamo, secondary decomposers (*Pterostichus microcephalus*, *Lasius japonicus* and *Cryptops japonicus*) had also intermediate δ^{13}C values between these two food chains and the top predators (*Chiromantes dehaani*, *Chiromantes haematocheir* and *Botoropolys rugosus*) mainly belong to a food chain based on the ^{13}C-rich organic materials. In Gamo, the ^{13}C-rich organic materials would be main basal food for the epizoic soil community, though secondary consumers tended to assimilate both microbes decomposing litter and ^{13}C-rich organic materials.

Eusilpha japonica (beetle) appeared to be predator or carnivores at Arahama food chain. Ikeda et al. (2007) showed that this species has flight muscles, and they suggested that such a kind of beetle species were mainly feeding on soil invertebrates, because most flight species showed higher nitrogen isotopic ratios than the flightless species. On the other hand, the juvenile *Chiromantes haematocheir* (crab) was decomposer at Arahama while the adult was predator at Gamo food chain. Tiunov (2007) mentioned that the trophic enrichment could depend on the animal age and life cycle stage (Oelberbermann and Scheu, 2002; Scheu and Folger, 2004; Haubert et al., 2005; Ponsard and Averbuch, 1999).

Hishi et al. (2007) mentioned that enriched stable carbon isotope ratios δ^{13}C and δ^{15}N of soil animals in detrital food webs increase along decomposition gradients of their diet (Tayasu et al., 1997; Schmidt et al., 2004), probably reflecting the fact that soil organic matter subjected to an increased degree of microbial processes show higher isotopic values (Billings and Richter, 2006). While the quality and quantity of soil organic matter changes according to the above-ground vegetations and animals. Therefore, the higher range of δ^{15}N values for invertebrates at Arahama than Gamo may be due to the differences between sites in ground vegetations and invertebrates communities and soil organic matter content. Such differences between sites in δ^{15}N according to the community structure confirm the importance of studying the trophic structure of soil fauna locally.

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