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Stable isotope analysis indicates trophic differences among forest floor carabids in Japan

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Abstract

Differences in trophic niches among carabid beetles (Coleoptera: Carabidae) co-occurring on the forest floors of warm temperate forests in central Japan were studied using carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analyses. Different carabid species showed similar $\delta^{15}N$ values, which were higher than those of their possible invertebrate prey (herbivores and detritivores) collected from the litter layer, indicating that these species were consumers in the same trophic level. In contrast, $\delta^{13}C$ values differed among carabid species, indicating interspecific differences in prey animals. The variation in the $\delta^{13}C$ value was larger in summer than in autumn. In summer, $\delta^{13}C$ values indicated that some carabids depended highly on either grazing (low $\delta^{13}C$ values) or detrital sources (high $\delta^{13}C$ values) within the food chain [*Chlaenius posticalis* Motschulsky and *Haplochlaenius costiger* (Chaudoir), respectively], although other species with intermediate $\delta^{13}C$ values likely depended on both. The latter group of species comprised mostly two dominant genera (*Carabus* and *Synuchus*). Although congeners might have similar feeding habits, the stable isotope ratios indicated trophic niche differences between adults of different species and between adults and larvae of the same genus.

Introduction

Carabidae (Coleoptera) is a species-rich and dominant taxonomic group in invertebrate communities of temperate forest floors (Thiele, 1977; Lövei & Sunderland, 1996). Most carabid beetles are polyphagous feeders that forage on the ground surface. Because of spatial niche overlap, carabid species potentially compete for food (Niemelä, 1993). Differentiation of diet in combination with differences in seasonal activity pattern may contribute greatly to niche differentiation among co-existing carabid species in forests (Sota, 1985a,b; Loreau, 1986), although the actual importance of competition in structuring carabid assemblages has been questioned repeatedly (Niemelä, 1993; Lövei & Sunderland, 1996). However, the potential importance of trophic niche difference remains unclear in carabid assemblages, because of methodological limitations (Lövei & Sunderland, 1996). Trophic niches of carabid beetles have been traditionally evaluated by field observation or gut content analysis. However, diet composition of carabid beetles is difficult to study by field observation because they are inconspicuous and often night-active foragers (Lövei & Sunderland, 1996). Also, identification of their food items based on gut content analysis (reviewed by Thiele, 1977; Hengeveld, 1980) is not sufficient because they perform external digestion, which means that food item particles that they consume are not necessarily found in the gut. Thus, to comprehensively understand the trophic niches of the component species of a carabid assemblage, a more convenient method that is applicable to all field samples is necessary.

Stable isotope analysis is one method to determine the ratios of heavy to light isotopes in an organism's tissue and is useful in determining the trophic niche of a consumer (Bearhop et al., 2004; Layman et al., 2007). The carbon stable isotope ratio ($\delta^{13}C$) of a consumer's tissues is only slightly enriched (around 0.5‰ per trophic level) compared with that of its diet, whereas the nitrogen stable isotope ratio ($\delta^{15}N$) is more enriched (around 3‰ per trophic level; Vander Zanden & Vadeboncoeur, 2002;

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Post, 2002; McCutchan et al., 2003). Thus, the $\delta^{13}C$ of consumers reflects the basal resource of a food chain, whereas the $\delta^{15}N$ indicates the trophic level of consumers. A community-wide survey of these stable isotope ratios can reveal time-integrated resource utilizations of different consumers as was demonstrated in some insect group assemblages (e.g., termites, Tayasu et al., 1997; ants, Blüthgen et al., 2003).

In this study, we examined seasonal activity patterns of carabid beetles and their potential diets (invertebrates) inhabiting temperate broadleaf forest floors in central Japan and analyzed interspecific differences in the trophic niche among carabid species in various seasons using carbon and nitrogen stable isotope rations. Feeding habits of carabid species were estimated based on stable isotope ratios of the carabid beetles and their candidate prey animals. This study revealed different dependencies on grazing and detrital food chains among co-existing carabid species.

Materials and methods

Study sites

The study sites were warm temperate zone, secondary forests of 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 covered predominantly with evergreen broadleaf trees, and Uryu-yama (hereafter Uryu) is situated on a mountainside and covered with various deciduous and evergreen broadleaf trees. The species composition of the carabid assemblages differed between Yoshida and Uryu. Yoshida is close to the study site of Sota (1985a), who made field observations on the activity patterns and diet of the subtribe Carabina (*Carabus* sensu lato). The life history of the subtribe Carabina was also studied at Uryu by Sota (1985b).

Seasonal occurrence of carabid beetles and prey animals

To identify the appropriate seasons for collecting carabid beetles as samples for stable isotope analysis and to list the candidate prey animals of carabid beetles, activity patterns of carabid beetles and other invertebrates were studied using pitfall traps and Tullgren apparatuses. Sampling of invertebrates was conducted every 2 weeks from May 2005 to April 2006. Pitfall traps (plastic cups of 7 cm diameter × 8 cm deep) were used to collect invertebrates inhabiting the litter layer of 200-m^2 plots (10 × 20 m) that were established at Yoshida and Uryu. At every sampling occasion, 45 pitfall traps were set at 2-m intervals in each plot, so that the rims were level with the ground surface. Each trap contained 50 ml of 20% ethanol to prevent trapped invertebrates from escaping or feeding within the traps. The pitfall sampling started at noon, and the pitfalls and trapped organisms were collected after 24 h. Tullgren apparatuses were used to collect invertebrates living in the humus layer. Within each 200-m^2 plot, 16 core samples of surface humus (25 × 25 cm, 3 cm deep) were arbitrarily taken. In the laboratory, these core samples were placed on a 3-mm-mesh sieve and heated for 48 h with incandescent light bulbs; animals that passed through the sieve fell into a solution of 70% ethanol and were immediately fixed to prevent desiccation and rotting. The collected invertebrates were sorted based on external morphology and were identified to species whenever possible (otherwise to genus or family).

Stable isotope analysis

For stable isotope analysis, carabid beetles and their candidate prey animals were collected every 2 weeks at Yoshida and Uryu in two seasons of 2006, from May to July (hereafter summer) and from October to November (hereafter autumn). For these samplings, a new quadrat of 400 m^2 was established at each site (20 × 20 m at Yoshida, 10 × 40 m at Uryu). The candidate prey animals were selected from animals abundantly collected in the survey of 2005, according to previous studies on carabid diet (Smit, 1957; Skuhravý, 1959; Habu & Sadanaga, 1961, 1965; Dawson, 1965; Penney, 1966; Hengeveld, 1980; Sota, 1985a). Prey animals included Oligochaeta (Megascolecidae and Enchytraeidae), lepidopteran larvae, dipteran larvae, Hemiptera, Orthoptera, and Collembola. Carabid beetles, Hemiptera, and Orthoptera were collected from the litter layer using pitfall traps that were used in the activity pattern investigation. An array of 25 pitfall traps was established in each sampling quadrat for 24 h, starting at noon. Each trap contained 50 ml of 20% ethanol to prevent trapped invertebrates from escaping or feeding within the traps. However, the effect of ethanol was minimized by removing the ethanol solution completely at collection. Earthworms (Megascolecidae), lepidopteran larvae, and large dipteran larvae (Bibionidae) were collected by hand from the litter layer in 16 subquadrats (50 × 50 cm) that were placed arbitrarily within a sampling quadrat. For the small dipteran larvae, Collembola, and Enchytraeidae, 16 cores of surface humus (25 × 25 cm, 3 cm deep) were sampled from each subquadrat and sorted in the laboratory using the Tullgren apparatuses that were used in the investigation of activity pattern. These collected invertebrates were kept at −30 °C for stable isotope analysis. The

procedures of stable isotope analysis are described in Okuzaki et al. (2009). Following the standard method, $\delta^{13}C$ and $\delta^{15}N$ values are expressed as the per mil (‰) deviation from international standards, calculated as:

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

where R for $\delta^{13}C$ is $^{13}C/^{12}C$ and for $\delta^{15}N$ it 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 measured for the working standard) was ± 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$.

Statistical analysis of trophic niche differences

To examine intra- and interspecific variation in trophic niches among carabid species, ANOVA was performed on the $\delta^{13}C$ or $\delta^{15}N$ values at each site (Yoshida and Uryu) in each season (summer and autumn). Species whose sample size was only one were excluded from the analysis. After ANOVA, Tukey's HSD test for post-hoc comparisons of all carabid species pairs was performed. In addition, the effects of season and species on the $\delta^{13}C$ and $\delta^{15}N$ values of carabid species were examined by two-way ANOVA using species with more than three samples in both seasons at each site. JMP version 5 (SAS Institute, Cary, NC, USA) was used for all analyses.

Results

A total of 269 and 333 invertebrate species were collected from the forest floors at Yoshida and Uryu, respectively (Table 1; see Tables S1–S4 for monthly abundance of each taxon). The number of collected species was larger in summer than in autumn, especially in the litter layer where it was one-half as rich in autumn compared with summer. Seven and 16 carabid species were recorded at Yoshida and Uryu, respectively (Table 1; Figure 1). The number of carabid species collected in the litter layer was almost unchanged from summer (six) to autumn (five) at Yoshida, whereas it decreased from 13 to 7 at Uryu.

Adults of the genus *Carabus* (subgenus *Ohomopterus*) were active from April to October, and larvae were collected from the litter layer in June through August (Figure 1). By contrast, adults of *Leptocarabus* and *Synuchus* were collected in two separate seasons, from May to July and from September to January (Figure 1). *Synuchus* larvae were collected from the humus in November to March. For *Carabus* and *Synuchus*, three or more species co-occurred, and the seasonal activity was synchronized among the congeners. Of the other six carabid species collected from the litter layer, *Chlaenius posticalis* Motschulsky, *Haplochlaenius costiger* (Chaudoir), *Pterostichus microcephalus* (Motschulsky), and *Anisodactylus tricuspidatus* Morawitz were active in summer. The humus-dwelling adults of *Perigona nigriceps* Dejean appeared throughout the year, but were most abundant in autumn.

Among the candidate prey animals collected from the litter layer, earthworms (Megascolecidae) occurred from June to November, and were most abundant in June and July (Figure 1). Lepidopteran larvae (Geometridae and Noctuidae) occurred throughout the year, except during February, and were most abundant from May to July. At Yoshida, dipteran larvae (Bibionidae) and Orthoptera were abundant from October to December and from July to November, respectively. The small dipteran larvae, Collembola, and Enchytraeidae occurred throughout the year and were abundant from May to August.

Stable isotope analyses were performed for seven carabid species from Yoshida and 12 species from Uryu, as well as for the candidate prey animals (Figure 2). The sample size was 1–25 per taxon (for details, see Table S5). The $\delta^{13}C$ and $\delta^{15}N$ values of the prey animals showed large variations across taxa, but most of the litter-dwelling prey had lower $\delta^{15}N$ values than the carabids. Primary consumers involved in the grazing chain (i.e., lepidopteran larvae,

Table 1 Numbers of invertebrate species collected at Yoshida and Uryu from the litter layer in pitfall traps and from the humus layer using Tullgren apparatuses from May 2005 to April 2006

		Summer (May–July)			Autumn (October–November)			Year-round		
Site	Layer	All	Carabid	Prey	All	Carabid	Prey	All	Carabid	Prey
Yoshida	Litter	87	6	8	42	5	5	125	6	15
	Humus	132	0	42	116	1	37	193	1	50
	Total	198	6	49	142	6	41	269	7	60
Uryu	Litter	104	13	10	55	7	8	152	15	19
	Humus	163	1	46	141	1	44	228	1	60
	Total	243	14	55	181	8	51	333	16	75

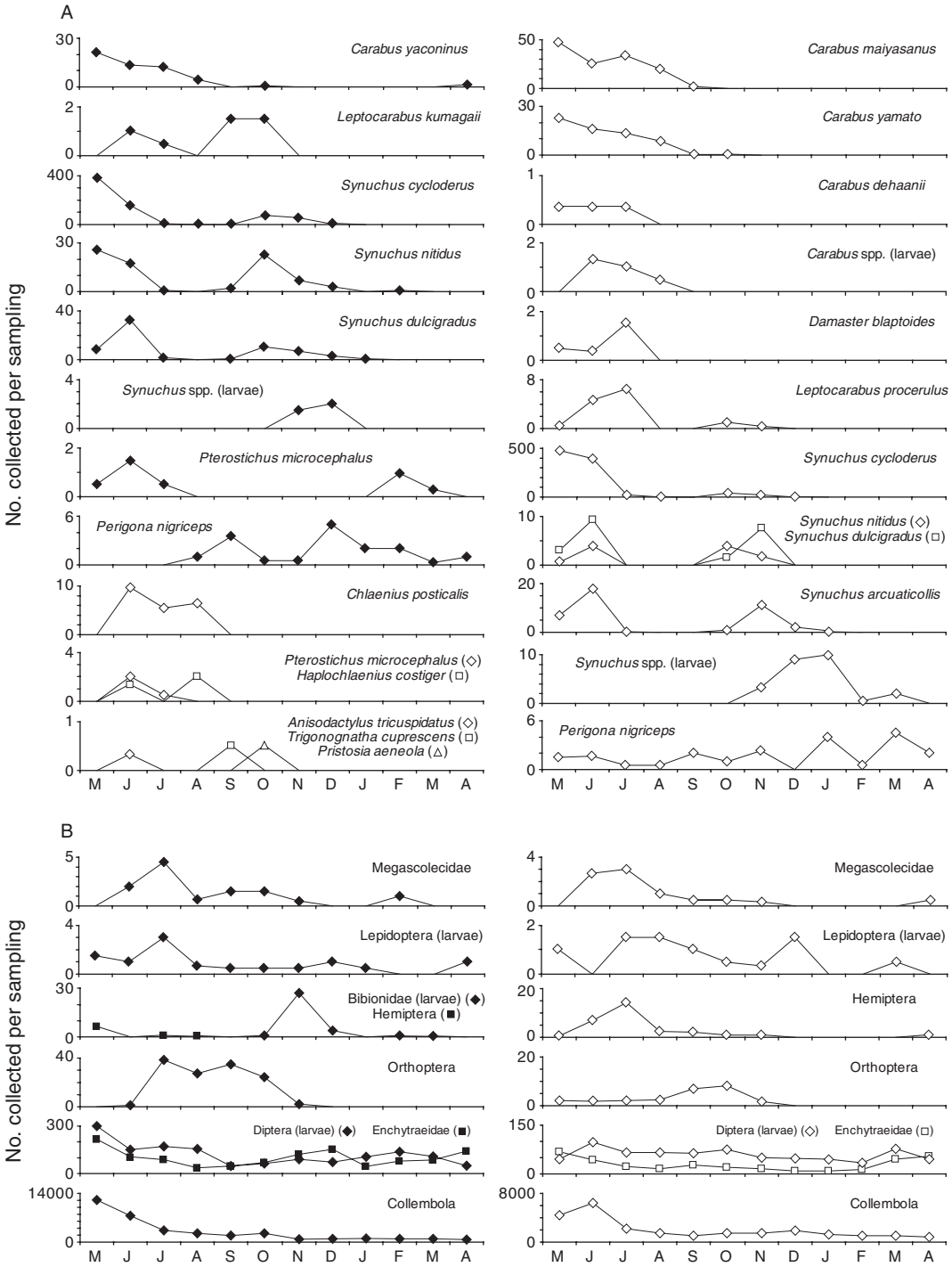

Figure 1 Mean number of individuals collected per sampling time of (A) carabid beetles and (B) their candidate prey animals at Yoshida (solid symbols) and Uryu (open symbols) in each month, from May 2005 to April 2006. Carabids (except larvae of *Synuchus* spp. and *Perigona nigriceps*), Megascolecidae, larvae of Lepidoptera, Bibionidae, Hemiptera, and Orthoptera were collected from a 200-m^2 plot in the litter layer by 45 pitfall traps. Larvae of *Synuchus* spp. and *P. nigriceps*, small dipteran larvae, Enchytraeidae, and Collembola were collected from a 0.03-m^3 core of the humus layer by Tullgren apparatuses.

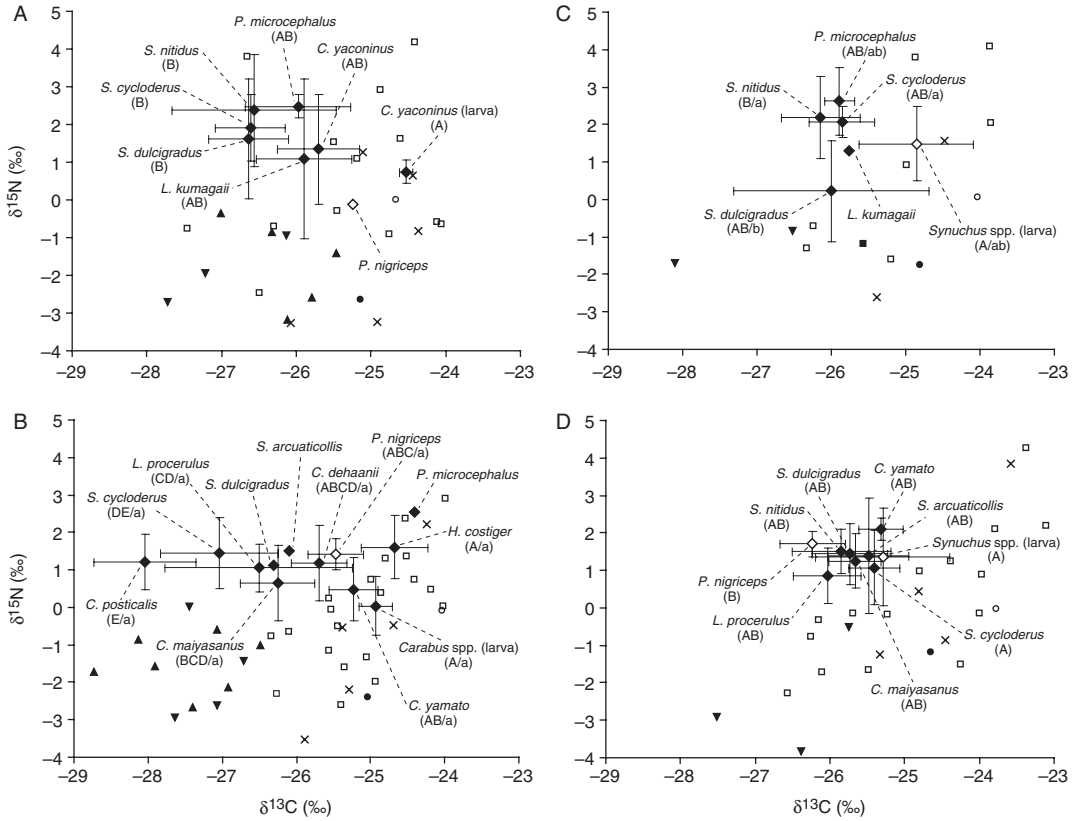

Figure 2 Stable isotope ratios of carabid beetles and their candidate prey animals in the summer at (A) Yoshida and (B) Uryu, and in the autumn at (C) Yoshida and (D) Uryu. Mean $\delta^{13}C$ and $\delta^{15}N$ values from different taxa are represented by different symbols: diamonds, Carabidae; circles, Oligochaeta; squares, dipteran larvae; triangles, lepidopteran larvae; inverted triangles, Hemiptera and Orthoptera; and crosses, Collembola. Filled and open symbols distinguish between animals collected from the litter and humus layers, respectively. For carabid species, horizontal and vertical bars represent SD values for $\delta^{13}C$ and $\delta^{15}N$ values, respectively. Significant differences in the isotope ratios between carabid species are indicated by different letters (Tukey's HSD: $P<0.05$); capital and lower case letters represent differences in $\delta^{13}C$ and $\delta^{15}N$ values, respectively.

Table 2 Range of $\delta^{13}C$ and $\delta^{15}N$ values in carabids and candidate prey animals, and the results of an ANOVA of $\delta^{13}C$ and $\delta^{15}N$ values of carabid species

		Carabid		Prey			$\delta^{13}C$ of carabid		$\delta^{15}N$ of carabid	
Season	Site	CR	NR	CR	NR	d.f.	F-value	P-value	F-value	P-value
Summer	Yoshida	2.1	3.2	3.7	7.4	6,43	4.52	0.001	0.95	0.471
	Uryu	3.6	2.5	4.7	6.4	8,51	16.31	<0.0001	2.27	0.037
Autumn	Yoshida	1.3	2.4	4.3	6.7	4,24	2.69	0.056	3.93	0.014
	Uryu	0.9	1.3	4.4	8.1	8,61	2.77	0.011	0.78	0.618

CR and NR represent the ranges of species mean values (‰) for $\delta^{13}C$ and $\delta^{15}N$, respectively (including adults and larvae of the same carabid species).

Hemiptera, and Orthoptera) had lower $\delta^{13}C$ values than those organisms involved in the detrital chain (i.e., earthworms and large dipteran larvae). The humus-dwelling prey (small dipteran larvae, Collembola, and Enchytraeidae) tended to have higher $\delta^{13}C$ and $\delta^{15}N$ values than the litter-dwelling prey, and about half of them showed more enriched isotope ratios than the carabids.

In summer, $\delta^{13}C$ values for the carabid beetles differed significantly among species at both Yoshida and Uryu (Table 2; Figure 2A,B). At Uryu, the carabid assemblage

with 11 species showed a larger variation for $\delta^{13}C$ values than that at Yoshida with six species, as did the range of $\delta^{13}C$ values in prey animals between two sites (Table 2). *Chlaenius posticalis* Motschulsky had the lowest $\delta^{13}C$ values, whereas *H. costiger*, *Carabus yamato* (Nakane), and *Carabus* larvae had comparatively high $\delta^{13}C$ values. Compared with these extremes, species of *Synuchus*, *Carabus*, and *Leptocarabus* showed intermediate $\delta^{13}C$ values. The smaller carabid assemblage with seven species at Yoshida had less variation in the $\delta^{13}C$ values than Uryu (Table 2; Figure 2A). However, as at Uryu, the isotope ratios of *Synuchus*, *Carabus*, and *Leptocarabus* species were similar to each other. *Carabus* larvae had higher $\delta^{13}C$ values than adults at both sites. The $\delta^{15}N$ values among the carabid species were significantly different at Uryu (ANOVA; Table 2), although there was no species difference detected by post-hoc comparison, probably because of insufficient sample sizes.

The ranges of $\delta^{13}C$ and $\delta^{15}N$ values in the carabid assemblages in autumn were smaller than those in summer at both sites (Table 2). The decrease of range of $\delta^{13}C$ values corresponded to seasonal changes in species composition and $\delta^{13}C$ values in the carabid assemblages. In autumn, species with extreme $\delta^{13}C$ values (i.e., *C. postocalis* and *H. costiger*) did not appear. Moreover, species of *Synuchus*, *Carabus*, and *Leptocarabus* showed significantly higher $\delta^{13}C$ values in autumn than in summer (Table 3), with large increases from summer to autumn in species of *Synuchus* and *Leptocarabus* (Figure 2). Thus, $\delta^{13}C$ values of the carabid species converged to a smaller range in autumn. However, significant differences in isotope ratios among carabid species were detected for $\delta^{13}C$ values at Uryu and $\delta^{15}N$ values at Yoshida in autumn (Table 2; Figure 2C,D). Exceptionally, *Synuchus dulcigradus* (Bates) showed a significantly lower $\delta^{15}N$ value than other carabid species at Yoshida (Figure 2C). Larvae of *Synuchus* showed higher $\delta^{13}C$ values than their adults at both sites.

Discussion

The species richness of both carabid beetles and other invertebrates was higher at Uryu than at Yoshida (Table 1). The invertebrate community at Uryu may allow more carabid species to co-exist than that at Yoshida by providing more diverse food or trophic niches. The difference in species diversity between the sites may be attributed to differences in habitat and vegetation. This is probable because Yoshida is made up of a small hill that is mostly covered with evergreen broadleaf trees, whereas Uryu is located on a mountainside with more diverse vegetation than Yoshida.

The larvae of the *Carabus* subgenus *Ohomopterus* are reported to only feed on earthworms (Sota, 1985a,b; note that this specialization is found only in *Ohomopterus* and a few other related subgenera; see Sota & Ishikawa, 2004). In our study, the larvae of *C. yaconinus* showed 0.6‰ and 3.4‰ higher values for $\delta^{13}C$ and $\delta^{15}N$, respectively, than earthworms collected from the litter layer at Yoshida. At Uryu, *Ohomopterus* larvae showed 0.1‰ and 2.4‰ higher values for $\delta^{13}C$ and $\delta^{15}N$, respectively, than the earthworms. These fractionation values are comparable with the previously reported ones; on average, $\delta^{13}C$ and $\delta^{15}N$ values of consumers are 0.5‰ and 3.0‰ higher, respectively, than the values of their food (Vander Zanden & Rasmussen, 2001; Post, 2002; but see also McCutchan et al., 2003 for variation in the fractionation values). Therefore, the stable isotope analysis appears to confirm that the larvae of *Ohomopterus* species fed on earthworms exclusively in the field.

The $\delta^{15}N$ values did not differ much among carabid species. In addition, no species showed a $\delta^{15}N$ value as low as the candidate prey animals collected from the litter layer (Figure 2). Therefore, the carabid beetles studied were all considered consumers within the same trophic level. Food composition of those carabids wandering around the litter layer probably largely consisted of prey animals inhabiting

Table 3 ANOVA for the effect of season and species on $\delta^{13}C$ and $\delta^{15}N$ values of carabid species collected in both summer and autumn (*Synuchus nitidus*, *S. cycloderus*, and *S. dulcigradus* at Yoshida and *S. cycloderus*, *Carabus maiyasanus*, *C. yamato*, and *Leptocarabus procerulus* at Uryu)

			$\delta^{13}C$		$\delta^{15}N$	
Site	Effect	d.f.	F-value	P-value	F-value	P-value
Yoshida	Season	1,43	7.3	0.01	1.84	0.18
	Species	2,43	0.13	0.88	4.67	0.01
	Season*species	2,43	0.26	0.77	1.53	0.23
Uryu	Season	1,50	11.05	0.002	2.92	0.09
	Species	3,50	4.9	0.005	0.59	0.62
	Season*species	3,50	3.34	0.03	3.14	0.03

the litter layer. This is because about half of the candidate prey animals collected from the humus layer had higher $\delta^{13}C$ and $\delta^{15}N$ values than the carabids, and were thus unlikely to be their prey.

The $\delta^{13}C$ values could have large variations among carabid species and could also be an effective indicator of differences in trophic niche, because they tend to be lower in prey animals from the grazing chain and higher in those from the detrital chain. Variation in the $\delta^{13}C$ values was large at Uryu in the summer. *Chlaenius postocalis* showed the lowest $\delta^{13}C$ values, suggesting that this species largely depended on the grazing food chain. On the other hand, *H. costiger* showed the highest $\delta^{13}C$ values and was thought to largely depend on the detrital food chain. On the other hand, *Carabus*, *Leptocarabus*, and *Synuchus* adults had intermediate $\delta^{13}C$ values. The diet of *Carabus* and *Leptocarabus*, which belong to the subtribe Carabina, has been well studied (Sota, 1985a,b), and adults of these genera are polyphagous predators that mostly consume earthworms and herbivorous and detritivorous insect larvae (Sota, 1985a). *Synuchus* adults were also considered to be polyphagous predators. Thus, their intermediate $\delta^{13}C$ values probably reflected their dependence on both the grazing and detrital chains. For adults of *Carabus*, the dependency on the grazing and detrital chains might differ among species of *Carabus* adults at Uryu, because $\delta^{13}C$ values differed between *Carabus maiyasanus* Bates and *C. yamato*.

Variations in the $\delta^{13}C$ and $\delta^{15}N$ values for the carabid assemblages decreased from summer to autumn. These seasonal changes in carabid assemblages may be a result of the decrease in species richness of prey animals (Table 1). In particular, both the species number and abundance of candidate prey animals involved in the grazing chain (e.g., lepidopteran larvae) decreased in autumn. Lepidopteran larvae are main food items for *Carabus* and *Leptocarabus* in summer (Sota, 1985a). The increase of the $\delta^{13}C$ value in *Carabus*, *Leptocarabus*, and *Synuchus* from summer to autumn might be attributed to the decrease of lepidopteran larvae in their diet (Table 3). In autumn, *S. dulcigradus* had a lower $\delta^{15}N$ value than other *Synuchus* species, and therefore might frequently feed on plant materials. *Synuchus* larvae may depend mainly on prey from the humus layer because these larvae inhabited the humus layer and showed relatively high $\delta^{13}C$ values.

In summary, this study revealed that there are some trophic niche differences between carabid species occurring on the temperate forest floor. This was more evident in the assemblage with more species in summer than in autumn, and in pairs of different genera than in pairs of congeners. Moreover, trophic niches of carabid species could be evaluated by the relative dependence on grazing and detrital chains based on $\delta^{13}C$ values. However, the stable isotopic profiles cannot discriminate between different food resources with similar stable isotope ratios. After all, carabid species with similar isotopic profiles may not necessarily use the same food resources. Further evidence is necessary to conclude that a species with similar intermediate isotope ratios used the same food resources. Recently, DNA bar-coding analysis of gut contents has become available for food identification in carabid beetles (e.g., Zhang et al., 2007; Berg et al., 2008). Although it requires prior information on the DNA sequences of potential food items and can only detect food items that were recently consumed, DNA bar-coding analysis may provide useful information when combined with stable isotope analysis. Thus, despite some limitations in resolving food composition, stable isotope analysis is a suitable method for determining the approximate trophic niche of consumers, which has been difficult to directly examine in the field, and can be a useful method for evaluating the contribution of trophic niche differences to the co-existence of species.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Activity patterns of invertebrates in litter layer at Yoshida.

Table S2 Activity patterns of invertebrates in litter layer at Uryu.

Table S3 Activity patterns of invertebrates in humus layer at Yoshida.

Table S4 Activity patterns of invertebrates in humus layer at Uryu.

Table S5 Number of individuals used for stable isotope analysis of carabid beetles and their candidate prey animals at Yoshida and Uryu.

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