EFFECTS OF VEGETATION SWITCH AND SUBSEQUENT CHANGE IN SOIL INVERTEBRATE COMPOSITION ON SOIL CARBON ACCUMULATION PATTERNS, REVEALED BY RADIOCARBON CONCENTRATIONS

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ABSTRACT. Vegetation types strongly affect soil organic carbon (SOC) accumulation in the terrestrial ecosystem through multiple factors such as litter quality and soil biodiversity. However, the roles of soil fauna in SOC accumulation remain unclear. The objectives of this study were to (1) examine how changes in litter types and soil animal communities affect SOC accumulation in continuously forested or vegetation-switched forest areas; and (2) discuss the role of soil animals in SOC accumulation in forest ecosystems. We focused on soils that have accumulated on top of a volcanic ash layer in the 268 yr since a volcanic eruption in 1739. The radiocarbon "bomb spike"' in the late 1950s and early 1960s provides a unique isotopic signature of soil carbon age. We investigated the combined effects of litter quality and soil invertebrate function on soil ^{14}C accumulation patterns. To determine the effects of vegetation types on SOC accumulation, we selected 4 types of cool temperate forests, 2 of which had undergone a vegetation switch in about 1960 (conifer to broadleaved forest, CB; broadleaved forest to conifer, BC), and 2 that had continuous forests (conifer forest, CC; broadleaved forest, BB). The Δ^{14}C values at depth intervals in CC were consistent with the expected bomb-^{14}C profile. In contrast, Δ^{14}C patterns in BB, BC, and CB differed from that of CC. Compared to CC, Δ^{14}C values of the other sites showed relatively high ^{14}C concentrations even in deeper soil layers, which suggests the bomb-induced ^{14}C has been transported to a greater depth by soil animals. Current broadleaved forests (BB and CB) had higher biomass of litter-feeding invertebrates than in current coniferous forests (CC and BC). These results suggest that carbon from leaf litter was vertically translocated to deeper soil layers by the abundant soil invertebrates in broad-leaved forests. Disagreement with the expected soil profile in BC suggests that past vegetation (broadleaved forest) has affected the present SOC accumulation pattern.

INTRODUCTION

Storage and decomposition of soil organic carbon (SOC) are significant factors in the global carbon cycle because soil is the largest carbon reservoir in terrestrial ecosystems (Körner 2000). It is still difficult to estimate the global carbon cycle accurately because of uncertainty in soil carbon dynamics (Schimel 1995). Long-term decomposition studies are essential for understanding the dynamics of soil organic matter (SOM). In the CENTURY model, Parton et al. (1987) described the effect of soil temperature and moisture on decomposition rates. Townsend et al. (1995) and Trumbore et al. (1996) examined temperature effects on the turnover time of SOM along an elevation gradient. Their findings showed that climatic factors, such as temperature, have greater effects on the decomposition process than plant litter quality on a regional scale. On a local scale, however, decomposition rates are most strongly affected by litter types (Köchy and Scott 1997). However, knowledge of long-term litter decomposition processes is limited, and few studies have assessed the changes in SOC dynamics associated with litter quality.

Decomposition rates are linked with lignin and nitrogen content of leaf litter (Melillo et al. 1982). Leaf nitrogen content is generally higher in broadleaved species than in conifers (Berg and McClaugherty 2003). Differences in litter decomposition should affect the rate of SOC accumulation. Some studies have compared decomposition rates between forested and cropped or grassland

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soils, focusing on the difference between C_4 and C_3 plants (e.g. Smith and Johnson 2004). Replacement of grasslands by woodlands has resulted in significant increases in landscape-scale ecosystem carbon stocks (Hibbard et al. 2003). In cool temperate mixed forests, the vegetation type can change from conifer to broadleaved forest (or vice versa) after disturbances such as typhoons, or as a result of artificial plantations (Hiura and Fujiwara 1999). Changes in the dominant tree species lead to changes in the quality and quantity of litter input. However, few studies have evaluated the effects of vegetation switches on SOC dynamics. The aim of this paper is to provide preliminary data on whether accumulation patterns of SOC differ after a vegetation switch, based on field investigations. The vegetation of the Tomakomai Experimental Forest (TOEF) in Hokkaido, Japan, is ideal for studying this topic, for the following reasons: (1) the period of SOC accumulation in soil is confined to the A horizon, because the A horizon has accumulated since the eruption of Mt Tarumae in 1739, and can be easily separated from the lower clastic pumice layer; and (2) recent carbon input can be identified because the tree species in each forest have been recorded at least for 60 yr, and the previous vegetation before the change was also qualitatively reported.

Several natural tracers, including carbon stable isotope ratio ($\delta^{13}C$) and ^{14}C concentrations have been used to estimate carbon dynamics. Aboveground nuclear testing in the late 1950s and early 1960s led to significantly increased levels of ^{14}C-CO_2 in the atmosphere. Thus, the "post-maximum-bomb" ^{14}C activities (1964 to the present) provide a natural isotopic signature. Because forest SOC is exclusively derived from CO_2 assimilation by plant photosynthesis, the measurement of ^{14}C concentrations in SOC can provide information about when the fraction of organic carbon was supplied to SOC. Accumulation of SOC is the result of a balance between decomposition and litter supply. Several studies have calculated turnover time of SOC fractions based on their $\Delta^{14}C$ values (e.g. Townsend et al. 1995; Trumbore 2000). Those studies were carried out assuming a steady-state decomposition process in the forest or pasture where no abrupt change in SOC accumulation, such as volcanic ash deposition, was observed.

Soil macroinvertebrates, such as earthworms and millipedes, clearly affect primary litter decomposition and SOC accumulation (e.g. Lavelle and Martin 1992; Wolters 2000; Toyota et al. 2006). Most terrestrial ecosystems contain abundant macroinvertebrates, in terms of both quantity and species diversity (Petersen and Luxton 1982). The soil invertebrate communities are associated with the accumulated plant litter (O layer) mass, which serves as food and habitat (Petersen and Luxton 1982; Takeda and Abe 2001). They directly contribute to 7–13% of carbon mineralization in European coniferous forests (Schröter et al. 2003). Soil animal activity could result in transport of carbon throughout their burrows (Don et al. 2008). Because soil animals control litter decomposition and carbon transportation, their effects on the vertical distribution of SOC may be greater than those of plant litter quality. However, little is known about the roles of soil animals in SOC accumulation, because it is difficult to determine the soil faunal effects on the fate of SOC.

Carbon and nitrogen stable isotope ratios are now widely used in ecological research. The carbon isotope ratio ($\delta^{13}C$) has been used to trace carbon sources, whereas the nitrogen isotope ratio ($\delta^{15}N$) indicates trophic level. Therefore, these ratios can be used to study the foodweb. Recent publications have described soil foodwebs using these ratios (Ponsard and Arditi 2000; Scheu and Falca 2000; Halaj et al. 2005; Tiunov 2007; Okuzaki et al. 2009). However, isotopic studies on soil foodwebs are still under development. In particular, the trophic level enrichment in $\delta^{15}N$ is still being debated (e.g. Eggers and Jones 2000).

Earthworms are one of the most prominent "ecosystem engineers" (Jones et al. 1994). Their ecological groupings have been characterized on the basis of their feeding habits as epigeic, endogeic, and anecic (Lavelle 1988). Epigeic species are litter-dwelling, they feed on fresh organic materials, and

they are important in the comminution of litter. Endogeic species live in the soil, moving horizontally through the upper mineral soil. They consume large quantities of soil and organic residues and some surface litter. Anecic species live in all strata of soil, normally in vertical burrows, and feed on and bury surface litter (Lavelle et al. 1997; Edwards 1998). Several studies have indicated that detritivores (humivores or soil-feeders), which feed on highly decomposed materials, have higher $\delta^{15}N$ values (Schmidt et al. 1997; Tayasu et al. 1997); thus, the values can be used to differentiate the feeding habits of soil fauna along the "humification gradient" (i.e. state of decay of plant material, *sensu* Tayasu et al. 1997). Tayasu et al. (2002) first demonstrated the applicability of ^{14}C in termites and used the ^{14}C-CO_2 record to estimate the "carbon retention" of the food. Hyodo et al. (2006) defined this value as the "diet age." The diet age of a consumer can be defined as the time elapsed since the carbon in its diet was fixed from atmospheric CO_2 by primary producers. Briones et al. (2005) extensively measured $\Delta^{14}C$ values of earthworms, and, after a seminal study by Scharpenseel et al. (1989), suggested that these could be applied to ecological groupings. Hyodo et al. (2008) showed that diet ages show a positive relationship with $\delta^{15}N$ values, suggesting that carbon turnover is related to decomposition processes. The diet age of soil fauna is an indicator of "active carbon" in soils. Because the carbon age, which is calculated by ^{14}C concentration in earthworms, indicates their functional position in the decomposition process, we can discuss the effects of earthworm activities on organic carbon by comparing ^{14}C concentrations between earthworms and their food resources.

We hypothesized that a change in tree species (a "vegetation switch") would affect the animal community, resulting in changes in SOC accumulation. We evaluated how a vegetation switch and the soil invertebrate composition affect SOC accumulation in the forest ecosystem. The objectives of this study were (1) to evaluate the relationship between the vertical distribution of SOC and vegetation type, (2) to investigate whether soil invertebrate composition responds to vegetation type, (3) to estimate how a vegetation switch affects the SOC accumulation, and (4) to discuss the function of invertebrates on the pattern of SOC accumulation by measuring stable isotope ratios and ^{14}C concentrations of soil invertebrates.

METHODS

Site Description

The study site was located in a cool temperate forest in the Tomakomai Experimental Forest of Hokkaido University, Japan (42°4′N, 141°4′E; 60–80 m altitude; mean annual temperature 6.0 °C; mean annual precipitation 1450 mm). The present vegetation developed after the large eruption of Mt Tarumae from 1667 to 1669 (Igarashi 1987). The organic layer (O layer) on the A layer was mainly composed of plant leaves mixed with twigs, and had an average thickness of 1.5–5 cm. The soil at the A layer, below the O layer, had an average thickness of 8 cm with a color classification of 7.5YR 2/1 (Sakuma 1987), and its soil type was a volcanic Regosol (FAO et al. 1998). The parent material of the soil is clastic pumice and sand that was deposited by eruptions of Mt Tarumae in 1667 and 1739 (Fujiwara 1987). There is a spatial decoupling between the lower clastic pumice layer and upper layers (O layer and A layer), which have accumulated since the 1739 eruption of Mt Tarumae (Sakuma 1987). These soils are well drained. Vegetation types and history have been recorded for at least 60 yr at this study site. To distinguish possible effects of a vegetation switch using the bomb-^{14}C signature, we selected 4 types of forest with different types of vegetation. In one forest area (CB), the vegetation switched from conifer (C) to broadleaved (B) forest in 1954 after a typhoon. In another forest (BC), broadleaved forest was replaced by a conifer plantation in 1963. Two control forests were CC (continuous conifer) and BB (continuous broadleaved). The BB and CB sites were within a deciduous stand dominated by Japanese oak (*Quercus crispula*) with some Japanese elm

(*Ulmus davidiana*), Japanese white bark magnolia (*Magnolia obovata*), and *Prunus ssiori.* Conifer stands were dominated by Pinaceae, *Picea jezoensis* in CC, and *Abies sachalinensis* in BC. Both conifer stands contained some Japanese oak (*Q. crispula*), Matsumurae maple (*Acer amoenum*), Japanese white bark magnolia (*M. obovata*), and Japanese lime (*Tilia japonica*). The stand basal area was estimated by measuring the diameter of trees (DBH > 5 cm) at chest height (1.3 m) in a 50×50 m plot at each site. The diameter of small trees with DBH <5 cm was not recorded.

Sample Collection and Preparation

Five litter traps (open area 0.5 m^2) were set at each site to measure litter production and ^{14}C values. The litter fallen into the traps was collected monthly from May to November 2007, oven-dried at 60 °C overnight, and weighed. Other sampling was carried out in October 2007. To estimate the mass of litter accumulation on the forest floor, 5 replicates of O layer samples (quadrate samples) were collected from each 25×25 cm quadrate. Samples were oven dried at 60 °C overnight and weighed. At the same location, soil macroinvertebrates (>2 mm) were collected by hand sorting within these O layers (25×25 cm quadrate, 5 replicates) and soil down to the deepest layer in the A horizon below these O layers. Soil fauna individuals in the Haplotaxida and Isopoda orders were identified at species level according to Easton (1981), Ishizuka (2001), and Blakemore (2003), those in the Diplopoda and Coleoptera (adults) were identified at family level, and other animals were identified at order level. The biomass of individual fauna was measured. Earthworm specimens were collected from quadrates and from surrounding areas to obtain sufficient samples for isotope analysis. For isotope analyses, soil macroinvertebrates were kept in glass containers with distilled water at 15 °C, allowing them to empty their gut contents. After 36–48 hr, they were rinsed with distilled water to remove soil and feces, and freeze-dried for 48 hr. Each specimen was ground to a fine powder using an agate mortar and pestle, and its isotope ratio was measured individually. At the BB site, casts of earthworms (Megascolecidae) were collected because there were large amounts of visible casts in the soil surface layer down to 4 cm depth.

A 50-cm-long, open iron cylinder with a diameter of 110 mm (core sampler) was used to obtain a core from the surface O layer to the deepest layer of soil (A layer). We extracted 3 cylinder-shaped cores (16 or 18 cm deep) from the O layer and A layer above the volcanic ash layer in 1739 at each of the 4 sites (BC, CB, BB, and CC). The depth of these cores, i.e. the depth until the 1739 volcanic ash layer, was 16 cm in BC, CB, and BB, and 18 cm in CC. Each core was divided in 2-cm fragments along its depth for analyses, and the thickness of its O layer was measured. The samples were dried at 60 °C for 48 hr, then gravel, coarse litter, and roots were removed using a sieve (9.5-mm mesh size), and the sample was weighed. We analyzed the sample for ^{14}C content, stable isotope ratios, and organic carbon content. Because we intended to include earthworm casts (often as large as several millimeters) into examined soils for analysis of carbon and isotopes, a 9.5-mm mesh size was used for the preparation of the samples. Carbon content and stable isotope ratios were measured at each depth in each core (3 replicates). For ^{14}C analysis, however, we combined 3 samples at each depth from 3 separate cores at each site into 1 composite sample. The ^{14}C concentration in the O layer was measured in composite samples made from O layer (quadrates) samples from 5 replicates at each site, independently of the core samples. Each sample was treated with 0.5M HCl to remove carbonate, then washed with distilled water and dried.

Analyses

Organic carbon and nitrogen concentrations in the O layer, soils, and litter fall were analyzed using an NC analyzer (SUMIGRAPH NC-95A, SHIMADZU, Kyoto, Japan). Carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope ratios in soil invertebrates, earthworm casts, O layers, soils at each depth, and litter

fall were measured using the following procedure. The samples were enclosed in tin capsules and analyzed by an elemental analyzer (EA1108, Fisons, Italy) connected to an isotope ratio mass spectrometer (Delta S, Finnigan MAT™, Bremen, Germany). The precision of the on-line procedure was better than ±0.2‰ for both isotope ratios. The natural abundances of ^{13}C and ^{15}N are expressed in per mil (‰) deviation from international standards; δ^{13}C or δ^{15}N $= (R_{sample}/R_{standard} - 1) \times 1000$, where R in δ^{13}C and δ^{15}N is ^{13}C/^{12}C and ^{15}N/^{14}N, respectively. Pee Dee Belemnite and atmospheric nitrogen are the international standards for carbon and nitrogen, respectively.

For ^{14}C analysis, samples (estimated to produce about 1.5 mg of carbon) were combusted in evacuated and sealed Vycor® tubes with CuO, Cu, and Ag wire at 850 °C for 2 hr. After cooling, the Vycor tubes were cracked on a vacuum line, and the CO_2 was cryogenically purified. The purified CO_2 was graphitized under Fe catalysis at 650 °C for 6 hr (Kitagawa et al. 1993). The concentration of ^{14}C was measured by accelerator mass spectrometry (AMS) following the method of Uchida et al. (2004a) in NIES-TERRA, Japan, or by AMS in the Rafter Radiocarbon Laboratory, Institute of Geological and Nuclear Sciences, New Zealand. The ^{14}C concentrations are reported as ^{14}C values, as defined by Stuiver and Polach (1977). Positive ^{14}C values indicate the presence of bomb-induced ^{14}C, and negative values indicate the predominance of SOM with ^{14}C that has experienced significant radioactive decay (half-life 5730 yr).

Carbon Ages in Litter and Earthworms

In this paper, we define "carbon age" as the time that has elapsed since carbon (C) was fixed from atmospheric CO_2 by primary producers. The carbon age of soil animals is identical to the "diet age" defined by Hyodo et al. (2006). We expand this definition to organic matter, including litter and earthworm casts. Carbon ages were estimated by comparing the ^{14}C content of samples with records of atmospheric $^{14}CO_2$, which doubled in the early 1960s and is still decreasing after the Nuclear Test Ban Treaty. We determined the carbon ages of litter fall, O layers, and earthworms from the difference between the sample collection year (2007) and the year (t) when the ^{14}C value of the sample matched that of atmospheric CO_2. We used the data of atmospheric $^{14}CO_2$ for the Northern Hemisphere (Levin and Kromer 2004; Levin et al. 2008). The year (t) was calculated using a regression curve as follows: year (t) $= 2073.9 - 16.71 \ln (^{14}C)$ ($r^2 = 0.997$, $P < 0.0001$) (Hua and Barbetti 2004), following Hyodo et al. (2008). ^{14}C measurements of soil animals and their food resources (litter, O layer, and soil) provide important information about what fraction of organic matter the soil animals have consumed and how long the organic matter was retained in the soil. As the O layer and SOM are "unconsumed" fractions, the "consumed" fraction can be estimated only by analyzing animals that consumed (assimilated) the fraction.

Statistical Analyses

We hypothesized that vegetation types affect the abundance of soil invertebrates, resulting in changes in the soil carbon accumulation pattern. To test this hypothesis, data on litter, soils, invertebrates, and carbon ages of earthworms were tested by one-way ANOVA with vegetation types (CC, CB, BB, and BC) as the main factor. Differences among sites in O layer accumulation, soil C stock, and the biomass of soil invertebrates were assessed by one-way ANOVA, followed by Tukey-Kramer multiple comparison tests. Simple regression analysis was used to assess the effect of carbon ages on δ^{15}N values of the earthworms at each site. All analyses were performed using R environment v 2.8.0 for statistical computing (R Development Core Team 2006).

RESULTS

Litter Production and Carbon Stock at the Forest Floor

The amount of litter fall in the 4 forest types ranged from 0.17 to 0.29 dry kg/m^2 (Table 1). The total O layer carbon stock was significantly lower in current broadleaved forests (BB and CB) than in current coniferous forests (CC and BC) (Tukey-Kramer-test, $P < 0.05$). Total soil carbon did not differ significantly among the 4 sites (one-way ANOVA, $F_{3,8} = 1.84$, $P = 0.22$). The total stem basal area varied from 13.69 cm^2/m^2 (CB) to 37.59 cm^2/m^2 (BC).

Table 1 Stem basal area (BA), litter fall above ground, and total carbon in O layer and soil below ground ($n = 5$ in O layer; $n = 3$ in soil; mean ± S.D.) in each site.

Site	BA (cm^2/m^2)	Litter fall (kg/m^2/yr)	O layer C[1] (kg/m^2)	Soil C (kg/m^2)
CC	35.36	0.24	1.9 ± 0.77[b]	4.3 ± 1.34[a]
BB	22.99	0.25	0.4 ± 0.15[a]	3.2 ± 0.14[a]
CB	13.69	0.17	0.3 ± 0.09[a]	3.4 ± 0.28[a]
BC	37.59	0.29	1.5 ± 0.28[b]	3.0 ± 0.29[a]

[1]Different letters following "O layer C" and "Soil C" columns indicate significant differences among sites at the 5% level (Turkey-Kramer test).

Depth Distribution of SOM and ¹⁴C

Δ^{14}C values were in the range of 5–160‰, 73–134‰, 48–118‰, and 48–112‰ for CC, BB, CB, and BC, respectively (Figure 1). Δ^{14}C values in BB, CB, and BC were relatively high at all depths. In CC, however, Δ^{14}C values of <10‰ were found in the deeper layers (12–14, 14–16 cm). These values indicate a higher amount of "pre-bomb" organic matter. The O layer (in cylinders) had an average thickness of 4.5, 1.5, 2, and 3 cm in CC, BB, CB, and BC, respectively. Carbon concentration was >30% until 4 cm depth in CC, and 2 cm depth in CB and BC, whereas it was <30% even in the top 0–2 cm depth in BB. The depths of layers in which carbon concentration was greater than 20% corresponded with the thickness of O layers (in cylinders) in CC, BB, and CB. The O layer (determined from composite quadrate samples) had Δ^{14}C values of 84‰, 81‰, 66‰, and 91‰ in CC, BB, CB, and BC, respectively.

Soil Fauna Composition

Total biomass of invertebrates differed significantly among the sites (one-way ANOVA, $F_{3,16} = 7.53$, $P = 0.002$). The total biomass was significantly higher in the current broadleaved forests (BB, 14.1 g dry/m^2 and CB, 12.8 g dry/m^2) than in the current coniferous forests (CC, 3.6 g dry/m^2 and BC, 2.1 g dry/m^2; Tukey-Kramer test, $P < 0.05$). Large Oligochaeta (earthworms) were dominant soil macroinvertebrates at all sites (Table 2). The earthworm species collected comprised Megascolecidae (*Metaphire hilgendorfi*, *Amynthas yunoshimaensis*, and *Amynthas vittatus*) and Lumbricidae (*Eisenia japonica*). Apart from in BC, Megascolecidae earthworms were dominant, especially *M. hilgendorfi*, which had the highest biomass in BB and CB. However, *M. hilgendorfi* was not found in either of the current coniferous forest sites (CC and BC). The biomass of litter-feeding soil invertebrates (Megascolecidae earthworms; *M. hilgendorfi*, *A. yunoshimaensis*, and *A. vittatus*, Isopoda; *Ligidium japonicum*, Diplopoda) was significantly higher in the current broadleaved forests (BB, 12.8 g dry /m^2; CB, 10.6 g dry/m^2) than in the current coniferous forests (CC, 2.8 g dry/m^2; BC, 2.0 g dry/m^2; Tukey-Kramer test, $P < 0.05$). In contrast, the biomass of the Lumbricidae earthworm *Eis-*
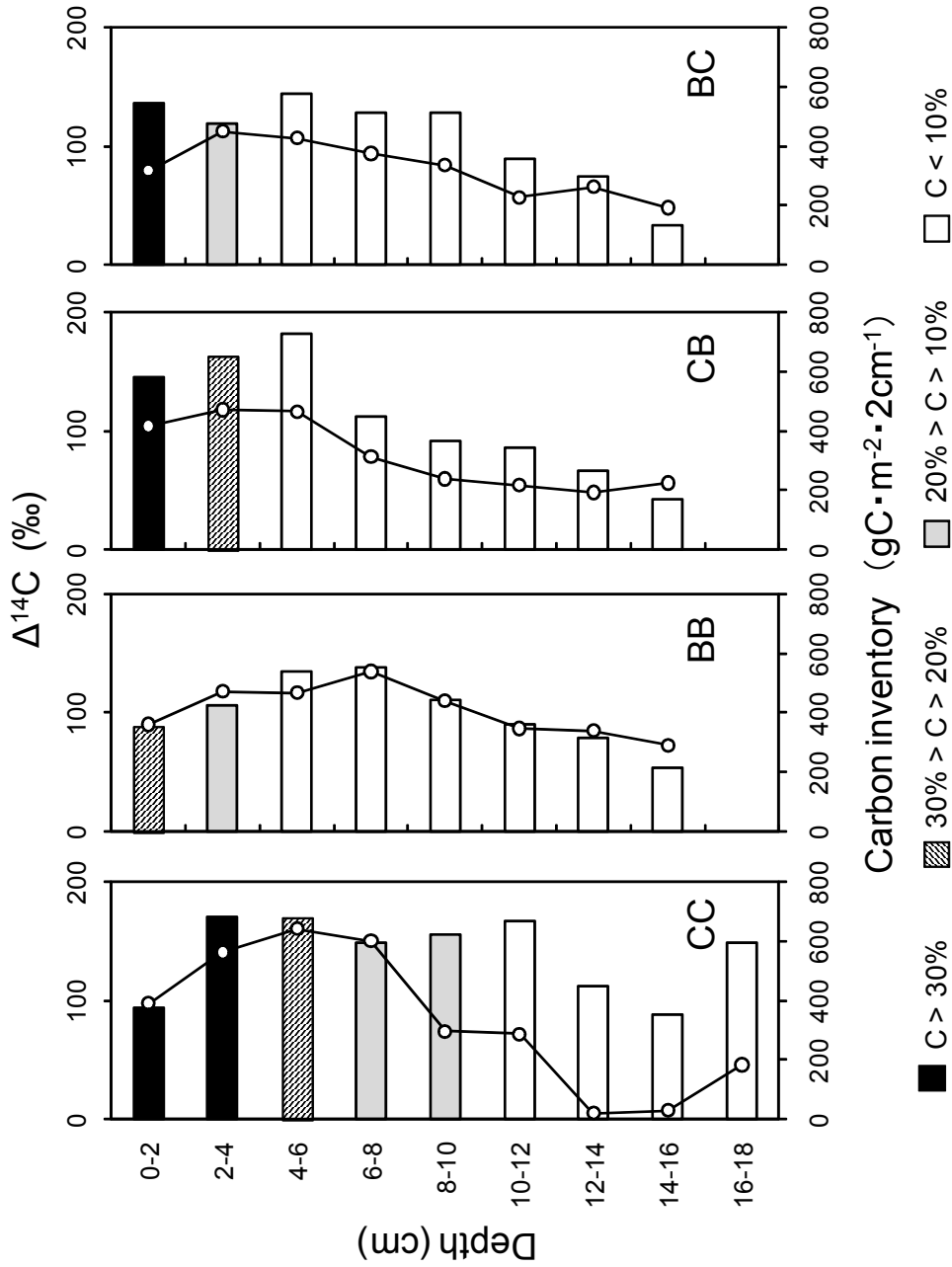
Figure 1 Carbon inventory (bars) and Δ¹⁴C values (open circles/line) with depth at CC, CB, BB, and BC sites. Zero in the depth axis indicates litter surface, and depth intervals including both O layer and mineral soil layer are measured from litter surface to volcanic ash layer. Carbon inventory at each depth interval is graded by carbon concentration on a scale of high (C >30%) to low (C <10%). The analytical uncertainty of ¹⁴C values ranged from 3.1 to 5.7‰.

enia japonica (Lumbricidae), which is categorized as a soil feeder, was not significantly different among sites (one-way ANOVA, $F_{3,16} = 0.81$, $P = 0.51$).

δ^{15}N and δ^{13}C of Organic Matter and Soil Animals

Overall, the data set showed that the δ^{15}N and δ^{13}C values ranged from –4.7 to +5.4‰ and –30.8 to –22.3‰, respectively (Figure 2). On the basis of combined δ^{15}N and δ^{13}C values, 3 ecological categories of animals were established: litter-feeding invertebrates (Megascolecidae and Isopoda, *L. japonicum*), soil-feeding earthworms (Lumbricidae, *E. japonica*), and predators (Araneae, Carabidae, and Chilopoda). Litter-feeding invertebrates had lower δ^{15}N values than soil-feeding earthworms and predators (Figure 2). The soil-feeding earthworm *E. japonica* had δ^{15}N values similar to those of the invertebrates that were categorized as predators in CC and BB, respectively. In contrast to CC and BB, *E. japonica* in BC had slightly higher δ^{13}C and δ^{15}N values than the litter-feeding earthworms. *M. hilgendorfi* in CB tended to have higher δ^{13}C and δ^{15}N values than the other litter-feeders. Stable isotope ratios of *E. japonica* in CB could not be measured because of their low biomass (Table 2).

Carbon Age of Litter and Earthworms

The differences between the carbon age of litter fall and that of the O layer (quadrate samples) were consistent among the sites (Figure 3). δ^{15}N values of earthworms significantly increased with carbon age in CC ($P < 0.01$) and BC ($P < 0.05$), where *E. japonica* (Lumbricidae) assimilated older carbon (CC, 10–15 yr old, BC, 5–15 yr old) than the litter-feeding (Megascolecidae) earthworms did (Figure 3). In BB, the carbon age of litter-feeding earthworms (Megascolecidae) was similar to that of a soil-feeding *E. japonica* earthworm (around 10 yr), however their δ^{15}N values varied widely. In CB, carbon ages of the earthworm *M. hilgendorfi* varied widely (>15 yr at the maximum), while its δ^{15}N values were very similar.

DISCUSSION

Effects of Litter Types and Animal Communities on SOC Accumulation in Continuous Forests

Previous studies have reported that biological mixing by soil macroinvertebrates, known as "bioturbation" (Scheu 1987), homogenizes the ^{14}C concentration in soils by comparing the ^{14}C data of soils in the presence or absence of earthworms (Stout and Goh 1980). In the case of the TOEF depth intervals, if there were no bioturbation, the vertical profile of Δ^{14}C values should have been higher in surface layers, maximizing in the layer of 1960s, and decreasing to almost zero at the bottom because the SOC had accumulated for 268 yr after the eruption of Mt. Tarumae in 1739. The Δ^{14}C values at depth intervals in CC showed good agreement with the expected profile, and the profile was consistent with those reported previously for forest soils (Trumbore 2000). Low Δ^{14}C values (<10‰) in the deeper 2 layers (12–14 and 14–16 cm) in CC indicate that the post-bomb signal (at least higher than 50‰) has contributed little to these layers because the oldest carbon in the soil had Δ^{14}C values around zero (theoretically in 1739: before the Suess effect). However, the deepest soil layer analyzed (16–18 cm) in CC had a higher Δ^{14}C value (~50‰) and carbon concentration (5.4%) than the upper 2 layers (12–14 and 14–16 cm). This indicates that the post-bomb signal has contributed little to the 2 deeper layers (12–14 and 14–16 cm) and the carbon input at the deepest layer (16–18 cm) cannot be explained without considering the contribution of root carbon. Although plant roots are thought to increase Δ^{14}C values in soils, few studies have demonstrated their effects. In this study, we detected the effects of plant roots on the Δ^{14}C pattern because the accumulated carbon was less than 268 yr old and older carbon does not exist. However, at the other sites (BC, CB, and BB), carbon input to SOC by root production cannot be distinguished from carbon input by litter fall.

Table 2 Biomass and density of soil macroinvertebrates (>2 mm) at the 4 study sites. Values represent the mean value of 5 replicates. Feeding habits of earthworms and other organisms are also listed.

Taxa			Feeding habit[1]	Biomass (mg dry wt/m²)				Density (ind./m²)			
Class	Order	Species or family		CC	BB	CB	BC	CC	BB	CB	BC
Arachnida	Acari			0.1				6.4			
Arachnida	Araneae		P	60.6	164.0	38.5	13.6	108.8	76.8	16.0	9.6
Arachnida	Opiliones			0.7				3.2			
Arachnida	Pseudoscorpionida								3.2		
Chilopoda	Geophilomorpha		P	30.0	34.3	0.2	5.9	54.4	32.0		9.6
Chilopoda	Lithobiomorpha		P	53.7	26.6	13.7	49.9	80.0	19.2	6.4	22.4
Diplopoda	Merocheta	Paradoxosomatidae	LF	41.2	25.2			9.6	9.6		
Entognatha	Collembola			10.1	2.5		0.9	57.6	12.8	3.2	3.2
Entognatha	Diplura			0.9				19.2			
Entognatha	Protura			0.4				6.4			
Gastropoda				127.8	397.7			6.4	19.2		
Insecta	Coleoptera		P	508.9	116.3	733.3	39.2	60.8	28.8	48.0	6.4
Insecta	Diptera			3.6		9.4	2.8	28.8	3.2	6.4	3.2
Insecta	Hymenoptera			11.1	28.1	7.3		19.2	3.2	3.2	
Insecta	Lepidoptera				456.7	1426.6			6.4	6.4	
Malacostraca	Isopoda	*Ligidium japonicum*	LF	80.0	137.7	124.8	44.5	38.4	73.6	35.2	25.6
Oligochaeta	Annelida	Enchytraeidae		1.9	11.0			19.2	19.2		
Oligochaeta	Haplotaxida	*Amynthas vittatus*	LF		1682.9				3.2		
Oligochaeta	Haplotaxida	*Amynthas yunoshimaensis*	LF	1460.5	3486.6	3665.3		6.4	16.0	9.6	
Oligochaeta	Haplotaxida	*Metaphire hilgendorfi*	LF?		5721.4	6588.6			9.6	12.8	
Oligochaeta	Haplotaxida	*Eisenia japonica*	SF	1236.8	1818.3	214.5	1916.1	9.6	19.2	3.2	25.6
Total				3628.2	14,109.3	12,822.7	2072.9	534.4	352.0	150.4	105.6

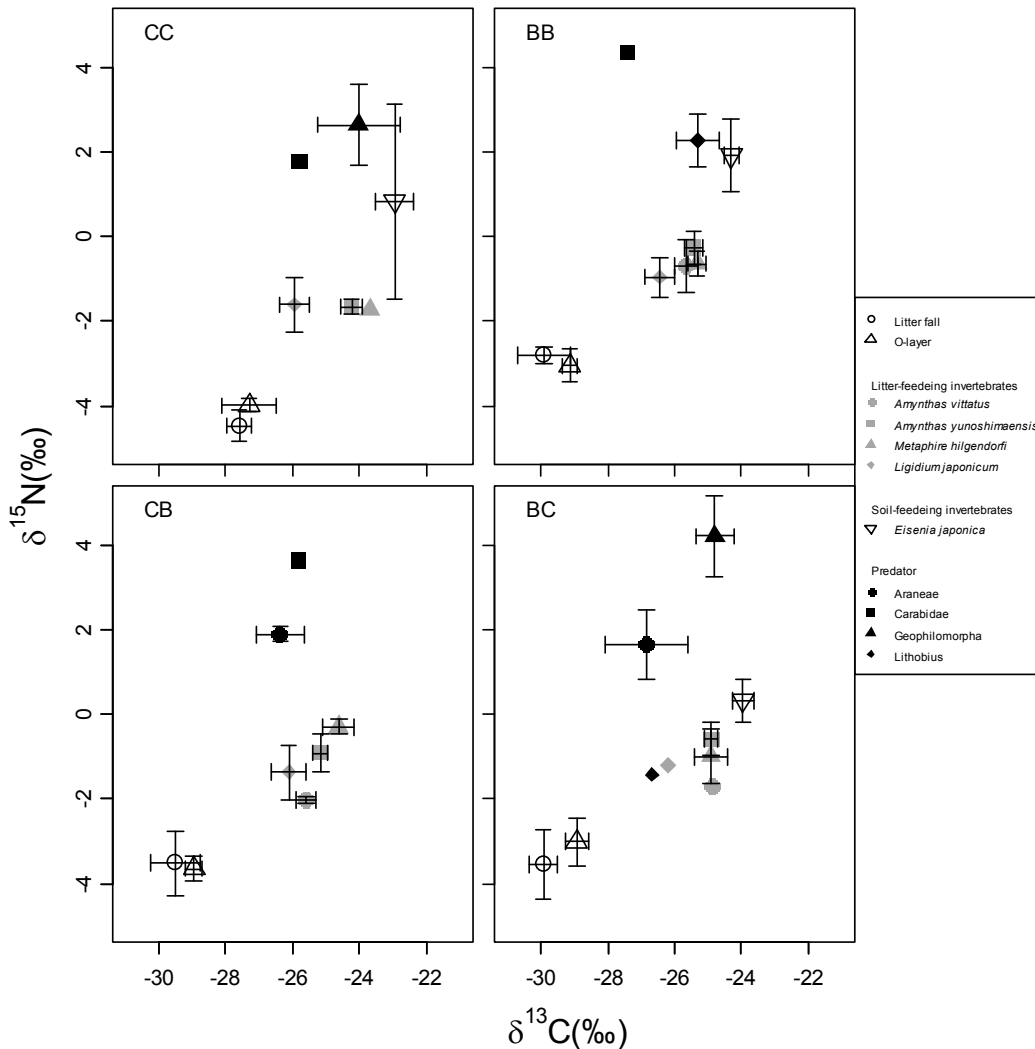[1] Abbreviations: P = predator; LF = litter feeder; SF = soil feeder.

Figure 2 Carbon and nitrogen stable isotope ratios of soil invertebrates and their food sources (litter fall and O layer; quadrate samples). Symbols indicate average value of replicates (*n* = 2 to 12) of each individual species or taxon. Error bars represent standard deviation (error bars not shown when *n* = 2).

In contrast, Δ^{14}C values of BB showed relatively high ^{14}C concentrations even in the deepest soil layer, which strongly suggests that the bomb-induced ^{14}C has been mixed by soil invertebrates. The biomass of earthworms (litter- and soil-feeding forms) and litter-feeding invertebrates was higher in BB than in CC (Table 2). Bioturbation has been reported for endogeic earthworms in general (e.g. Scheu 1987), because litter-feeding earthworms mainly inhabit the litter and surface soil layers (Edwards 1998). However, the biomass and density of the endogeic earthworm *E. japonica* were similar between CC and BB (Table 2). The δ^{13}C and δ^{15}N values of litter-feeding earthworms were markedly lower than those of soil feeders. This result is consistent with many previous reports (e.g. Schmidt et al. 2004). In this study, *M. hilgendorfi*, *A. yunoshimaensis*, and *A. vittatus* were categorized as litter-feeders (epigeic species), whereas *E. japonica* was categorized as a soil-feeder (endogeic species) based on their δ^{13}C and δ^{15}N values (Figure 2). These results are consistent with
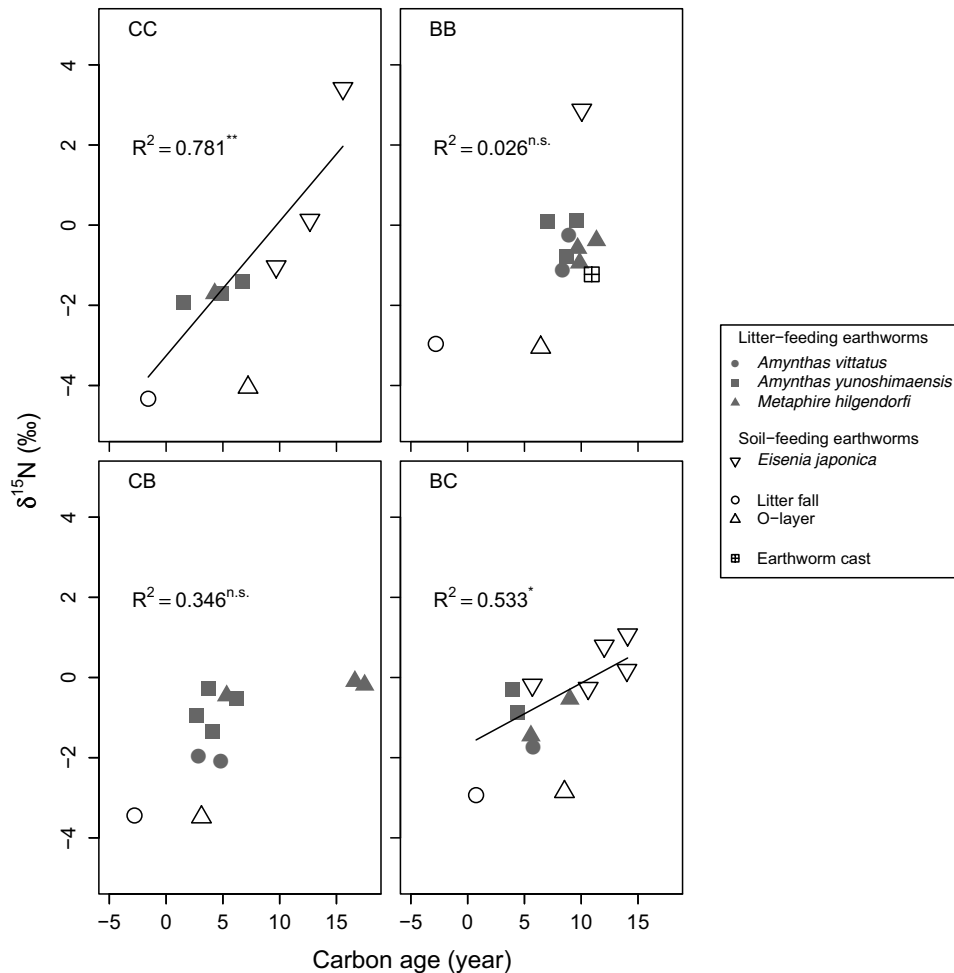
Figure 3 Relationship between carbon ages calculated from Δ^{14}C values (see text) and nitrogen stable isotope ratios of earthworms, cast, litter fall, and O layer (quadrate samples). Solid lines represent significant relationships in earthworms. Coefficients of determination and significance levels (*$P < 0.05$, **$P < 0.01$, ns = not significant) are shown. Analytical uncertainty of carbon ages ranged from 0.4 to 1.5 yr (equivalent to 2.8 to 6.0‰ in Δ^{14}C).

those reported previously for *M. hilgendorfi*, *A. vittatus*, and *E. japonica* at this study site (Uchida et al. 2004b). On the other hand, Uchida et al. (2004b) reported that in broadleaved forests in the TOEF, the gut contents of the litter-feeding earthworm *M. hilgendorfi* contained >30% inorganic materials. Therefore, these earthworms are not restricted to the O layer but burrow into soil layers. In this way, they may transfer carbon from litter to soil by digesting leaf litter and producing casts in broadleaved forests. Thus, the reason why Δ^{14}C profiles were not mixed in CC may have been because of a lack of the litter-feeding earthworm *M. hilgendorfi* (Table 2). In addition, Bonkowski et al. (1998) reported that under microcosm experimental conditions, the growth of soil-feeding earthworms increased when litter-feeding millipedes were also present. They suggested that litter consumption by litter-feeding invertebrates, such as earthworms and millipedes, provides food resources for soil-feeding earthworms. In our results, the carbon age of fresh casts of litter-feeding earthworms in BB was similar to that of the soil-feeding earthworm *E. japonica*, and the δ^{15}N value of *E. japonica* was ~4‰ higher than that of the casts (Figure 3). These results suggest that the soil-

feeding earthworm *E. japonica* assimilated the casts of litter-feeding earthworms in broadleaved forest. The coexistence of litter-feeding invertebrates and soil-feeding earthworms may accelerate vertical mixing of recent SOC into deeper soil layers.

Although the amount of litter fall was similar in BB and CC, less carbon accumulated in the O layer of BB than in the O layer of CC (Table 1). These results supported the hypothesis that more carbon is supplied from the O layer to soil in BB than in CC. This is consistent with the general pattern that the carbon stock in O layers of coniferous forests is greater than that in O layers of broadleaved forests in cool temperate regions (Cole and Rapp 1981). Using a litter-bag technique in which earthworms were excluded, Miyamoto and Hiura (2008) demonstrated that oak leaf litter was lost significantly faster than the fir needle litter in the O layer in this region. Their results also indicated that litter types were responsible for O layer accumulation patterns. On the other hand, litter loss in the O layer depends not only on litter types, but also on soil invertebrate activities in the temperate zone (Wall et al. 2008). In the TOEF broadleaved forest, experimental removal of litter-feeding earthworms decreased the disappearance of the O layer. Furthermore, soil carbon accumulation in the treatment without earthworms was lower than that in the treatment with earthworms, because a large proportion of litter consumed by litter-feeding earthworms is not assimilated (Kawaguchi et al., unpublished results). The global distribution pattern of soil animals shows that the abundance of soil animals in the temperate deciduous forests is higher than that in the temperate coniferous forests (Petersen and Luxton 1982; Fierer et al. 2009). In this study, the biomass of litter-feeding invertebrates in BB was also higher than that in CC. In addition, the biomass of earthworms was large in broadleaved forests (BB), in fact, twice as high as the average biomass in temperate deciduous forests (Petersen and Luxton 1982). The high biomass of earthworms would have a considerable effect on the reduction of O layer mass and the supply of carbon from litter to soil in broadleaved forests.

Effects of Changes in Litter Types and Animal Communities on SOC Accumulation in Vegetation-Switched Forests

In the vegetation-switched sites (CB and BC), the bomb-^{14}C peak was less prominent than that in CC (Figure 1). The differences in soil fauna (Table 2), especially the significant difference of litter-feeding invertebrates between current coniferous (CC and BC) and broadleaved forests (BB and CB), suggest that the vegetation switch resulted in changes in the soil animal community. As population dynamics of litter-feeding invertebrates are sensitive to litter quality (Loranger-Merciris et al. 2008), the absence of litter-feeding earthworms in BC would have been caused by the vegetation switch from broadleaved forest to coniferous forest. The amount of carbon accumulation in O layers was also explained by current vegetation type even in vegetation-switched sites (Table 1). The mass of the O layer was greater in BC than in CB; this would have resulted from the low decomposition rate of recent needle litter and the low biomass of litter-feeding invertebrates in BC. In contrast, the Δ^{14}C values were high across all depth intervals (minimum Δ^{14}C is 48‰) in BC, indicating the occurrence of bioturbation. This appears to contradict the lower biomass of litter-feeding invertebrates in BC than that in the current broadleaved forest (CB and BB). However, it is probable that the biomass of litter-feeding invertebrates in BC may have been higher in 1963 when the vegetation was broadleaved species, before conversion to coniferous forest. The litter-feeding invertebrates such as the earthworm *M. hilgendorfi* may have mixed the bomb ^{14}C in soil under the broadleaved forest in the early 1960s when bomb ^{14}C was being produced.

In contrast to BC, earthworm biomass in CB was 4 times higher than in CC. The result suggested that the soil layers of CB could have been mixed by earthworms after the vegetation switch, because *M. hilgendorfi*, which is a potential soil mixer, dominated in CB. The difference in Δ^{14}C distribution in depth between CB and CC indicated that the vegetation change from coniferous forest to broad-

leaved forest had greatly modified the SOC accumulation patterns after the vegetation switch. In contrast, the similar patterns of Δ^{14}C values between BB and BC suggested that the vegetation change from broadleaved forest to coniferous forest in BC had little influence on their Δ^{14}C patterns. These results suggest that the presence of broadleaved forest, whether past or present, is an important factor in the distribution of Δ^{14}C across soil depth. It is still unclear whether or not changes in litter type after a vegetation switch lead to changes in SOC accumulation rate. Our results indicate that the abundant soil invertebrates disturbed the SOC accumulation pattern in broadleaved forests. Therefore, future studies should consider the roles of soil fauna in SOC dynamics, and should analyze the effects of soil invertebrates and litter type separately.

Roles of Invertebrates in SOC Accumulation Patterns

All of the examined earthworms showed carbon ages of <20 yr (Figure 3). Because the vegetation switches occurred in 1954 and 1963 at the CB and BC sites, respectively, this result implies that earthworms assimilated the dietary matter derived from the current vegetation litter. Therefore, the earthworms are closely related to the current vegetation type. There was a significant correlation between the carbon ages and δ^{15}N values in earthworm bodies in the current coniferous forests (CC and BC) (Figure 3). In particular, *E. japonica* showed higher values of carbon ages and δ^{15}N compared with those of other earthworms. The isotopic ratio of the body reflects characteristics of the dietary materials assimilated by the animals. Because δ^{15}N values increase with humification of organic matter in soils (Tayasu et al. 1997), higher values of δ^{15}N of *E. japonica* indicate that they utilized and assimilated highly decayed organic materials. On the other hand, the low values of diet ages and δ^{15}N in the other earthworms with litter-feeding habits suggest that they assimilated relatively "fresh" organic matter in CC and BC. Therefore, we concluded that the positive correlation between carbon ages and δ^{15}N in the current coniferous forests probably resulted from humification process of dietary materials, i.e. the decomposition of SOM over time.

A similar trend was reported by Hyodo et al. (2008), who reported on the relationship among termites and earthworms in the tropics (Ivory Coast). This is the first report of a positive relationship between carbon age and δ^{15}N values of soil animals in a cool temperate forest. In contrast to CC and BC, there was no significant correlation between carbon age and δ^{15}N in the current broadleaved forests (BB and CB). The reason is unclear, however; the relationships were dependent on current vegetation types. The carbon ages of *M. hilgendorfi* in CB varied widely, ranging from 5 to 17 yr, although the δ^{15}N values were almost constant (Figure 3). Carbon ages of the other litter-feeding earthworms were similar to those of the O layers (Figure 3). Neilson et al. (2000) showed that stable isotope ratios of epigeic (litter-feeding) earthworms were different from endogeic (soil-feeding) earthworms, although anecic (deep-burrowing) earthworms were rather site-dependent. Thus, it is probable that *M. hilgendorfi* in CB is an intermediate group between epigeic and anecic forms. Further studies are required to determine the generality of the relationships between the carbon ages and δ^{15}N values of earthworms, and the carbon utilization of earthworms.

Total soil carbon did not differ significantly among sites (Table 1). However, the biomass of litter-feeding invertebrates was higher in the current broadleaved forest than in the coniferous forest (Table 2), suggesting that current broadleaved forest had more rapid litter input from the O layer to the soil layer. In a 1-yr incubation experiment, Martin (1991) found that aging casts of the endogeic earthworm *Millsonia anomala* (Megascolecidae, Oligochaeta) retained recalcitrant carbon compounds, and the mineralization rate of carbon in earthworm casts was much lower (3% yr^{-1}) than that in the control soil without earthworms (11% yr^{-1}). Wolters (2000) also pointed out that soil invertebrates promote stabilization of SOM. In our results, we did not detect positive effects of earthworms on the rate of SOC accumulation (Table 1), although we did observe a considerable reduction of O

layer accumulation. In addition, ^{14}C data across depth intervals suggest that carbon was translocated from the O layer to the soil layer under broadleaved vegetation by soil invertebrates.

Earthworm activities can have the opposite effects on soil, i.e. they can stabilize SOM or enhance decomposition. For example, in a 127-day microcosm experiment, Wolters and Schaefer (1993) found that an endogeic earthworm, *A. caliginosa*, enhanced carbon mineralization in beech forest soils. Furthermore, the addition of cellulose regulated the stability of organic carbon in the deep soil layer by stimulating microbial respiration and growth (Fontaine et al. 2007). In this study, in broadleaved forests, litter-feeding earthworms may supply fresh litter continuously to deeper soil layers, resulting in enhancement of microbial decomposition activity. Previous studies based on laboratory experiments showed that earthworms contribute to SOC dynamics (e.g. Martin 1991; Wolters and Schaefer 1993; Wolters 2000). Changes in SOC dynamics are difficult to detect in field conditions even when there is a high biomass of earthworms. This is probably because both positive and negative effects on the turnover of SOC occur simultaneously. Further studies are required to elucidate the long-term effects of soil animals on SOC accumulation under field conditions. These studies would improve our understanding of SOC dynamics.

CONCLUSIONS

We report, for the first time, both the profiles of SOC and the carbon ages of soil animals based on ^{14}C data. Our results showed that soil ^{14}C profiles differ according to vegetation type and the composition of soil invertebrates. A coniferous forest that has not undergone a vegetation switch showed an isotopic signal close to the atmospheric ^{14}CO$_2$ inventory. In contrast, the Δ^{14}C values in BC, CB, and BB forests were relatively high at all soil depths. These results suggest that the existence of broadleaved forest may be an important factor in SOC mixing because the high biomass of litter-feeding invertebrates in broadleaved forests would provide litter carbon from the O layer to the soil layer over time.

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