

NOTE

Stream food web fueled by methane-derived carbon

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ABSTRACT: Food webs driven by energy from the oxidation of methane are now recognized to be omnipresent in terrestrial and freshwater ecosystems (e.g. lakes, soils and peat bogs), as well as in deep-sea hydrothermal vents and cold seeps. However, the incorporation of methane-derived carbon into stream food webs has never been reported. Here we present the first circumstantial evidence from stable carbon and nitrogen isotope ratios that a stream food web composed of aquatic macroinvertebrates is partly sustained by methanotrophs or chemoautotrophs that gain carbon respired by methane-oxidizing bacteria. Methane-derived carbon seems to enter stream communities around anoxic habitats such as backwater pools and hyporheic zones. Because these reductive habitats exist in many streams, food webs partly sustained by methane-derived carbon are likely to be ubiquitous in lotic ecosystems.

KEY WORDS: Backwater pool · Carbon isotope ratio · Hyporheic zone · Macroinvertebrates · Methane gas · Methanotrophic bacteria · Nitrogen isotope ratio · Stream food web

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INTRODUCTION

It has long been thought that macroinvertebrate communities sustained by energy from the oxidation of methane are restricted to specific anoxic environments such as deep-sea hydrothermal vents and cold seeps (Paull et al. 1985, Childress et al. 1986, Page et al. 1990). A mytilid bivalve inhabiting deep-sea vents has been shown to obtain carbon from symbiotic methane-oxidizing bacteria (Childress et al. 1986). Some species of benthic ciliates also utilize methane-derived carbon produced in cold seep sediments (Werne et al. 2002). However, recent research increasingly recognizes the existence of methanotrophic communities in many reductive terrestrial and freshwater environments, such as lakes, soils and peat bogs (Fenchel & Finlay 1995, Brune et al. 2000). For instance, Hollander & Smith (2001) pointed out the predominance of carbon

cycling mediated by methanotrophic bacterial processes in a eutrophic lake where anoxic conditions were well developed in both water columns and sediment. In addition, some macroinvertebrates (Chironomidae spp.) collected from lake sediments were thought to be sustained by methanotrophs (Kiyashko et al. 2001, Grey 2002, Grey et al. 2004). However, the input of methane-derived carbon into stream macroinvertebrates has never been reported, even though a wide array of anoxic habitats, including hyporheic zones and backwater pools, exists in stream ecosystems (Jones & Holmes 1996, Jones & Mulholland 1998).

It is well documented that the stable carbon isotope signature ($\delta^{13}\text{C}$) of biogenic methane is highly depleted owing to the large carbon isotope fractionation associated with methanogenesis (Sugimoto & Wada 1995, Zyakun 1996). Moreover, methane-oxidizing bacteria that preferentially consume $^{12}\text{C}_4$ and

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chemoautotrophs that assimilate carbon resulting from oxidation of methane are usually characterized by further depletion in $\delta^{13}\text{C}$ values (Coleman et al. 1981, Grossman et al. 2002). As a consequence, the $\delta^{13}\text{C}$ values of the organisms that consume methanotrophs and/or chemoautotrophs are significantly depleted (usually $< -40\%$; Page et al. 1990, Conway et al. 1992), relative to organisms based on photoautotrophic sources, such as terrestrial and aquatic plants (see France 1995). The stable nitrogen isotope ratio ($\delta^{15}\text{N}$) is also informative, because methane-oxidizing bacteria often exhibit highly depleted $\delta^{15}\text{N}$ values (Kiyashko et al. 2001, Grey et al. 2004). This is probably due to the preferential assimilation of $^{14}\text{NH}_3$ by methane-oxidizing bacteria (Lee & Childress 1994). Thus, analysis of stable carbon and nitrogen isotopes is appropriate to assess the contribution of methanotrophs to food webs. Here we present the first evidence from stable carbon and nitrogen isotope ratios that some stream macroinvertebrates derive their organic tissue from carbon that was originally in methane gas.

MATERIALS AND METHODS

We measured the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of aquatic macroinvertebrates, their surrounding organic matter, and methane bubbles that were collected in a headwater reach of the Horonai Stream in the Tomakomai Experimental Forest ($42^\circ 43' \text{N}$, $141^\circ 36' \text{E}$, altitude 20 to 30 m), Hokkaido, northern Japan. This small, cold, spring-fed stream (15.4 km^2 in drainage area, 14 km in length, 2 to 5 m in width, gradient $< 1\%$) flows through a broad-leaved deciduous forest, with water temperature remaining stable throughout the year (annual range = 7 to 10°C , pH = 6.7 to 7.3). Sampling was done

in 3 backwater pools (ca. $0.5 \times 1.5 \text{ m}$ area and 10 to 15 cm depth for each pool) formed in the stream channel margins, where large amounts of coarse plant detritus such as leaves and twigs were accumulated. Aquatic macroinvertebrates and detritus, including leaf litter and fine particulate organic matter (FPOM) ($225 \mu\text{m} \leq \text{size} < 1 \text{ mm}$), were collected by hand between 5 May and 30 July 2001. In the same period, periphyton, a primary producer that constitutes the base of stream food webs, was scraped with a wire brush from 15 ceramic tiles ($20 \times 20 \text{ cm}$ area) that had been placed in the stream 3 wk before the sampling. Prior to the scraping, we gently rubbed the tile surface by hand with repeated distilled water rinses, to exclude other organic matter from the epilithic material. In addition, methane bubbles rising from backwater pools were collected according to Uzaki et al. (1991). We also collected some adult aquatic insects emerging from the stream in Malaise traps (2 m high, 1.8 m long, 1.2 m wide, 0.5 mm mesh; Townes 1972), which were placed on stream banks from May to July 2000 and in May 2001. Because the *Helodes* beetles collected in 2000 and 2001 showed quite low $\delta^{13}\text{C}$ values (see 'Results'), additional samples were taken from 29 August to 8 October 2002 to ensure a sufficient sample size.

In the laboratory, samples of macroinvertebrates collected (see Table 1) and photoautotrophic resources (leaf litter, FPOM, periphyton [concentrated on glass fiber filters]) were dried at 60°C for 48 h and ground to a fine powder. We did not remove gut contents from macroinvertebrate samples. Accordingly, the isotope ratios of macroinvertebrates were affected by those of gut contents to some extent, but the isotope ratios can still provide significant information on the original carbon source of organic tissue because the gut propor-

Table 1. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (‰) of stream macroinvertebrates used in the analyses

Taxon	n	$\delta^{13}\text{C}$ (‰)			$\delta^{15}\text{N}$ (‰)		
		Mean \pm SE	Median	Min, Max	Mean \pm SE	Median	Min, Max
Invertebrates collected from substrate surface in backwater pools							
Tipulidae spp. (larva)	6	-31.9 ± 6.3	-29.6	-44.7, -28.5	0.5 ± 3.7	1.8	-7.0, 2.4
Gammaridea spp.	4	-32.7 ± 0.8	-32.8	-33.4, -31.6	1.7 ± 1.0	1.8	0.6, 2.6
Invertebrates collected from inside detritus accumulation in backwater pools							
<i>Helodes</i> sp. (larva)	32	-55.3 ± 11.4	-57.7	-68.2, -28.2	-9.1 ± 4.9	-11.2	-13.9, 2.1
<i>Agabus</i> sp. (larva)	4	-45.6 ± 1.4	-45.9	-46.7, -43.4	-6.2 ± 1.2	-6.2	-7.6, -4.7
Chironomidae spp. (larva)	4	-36.6 ± 7.3	-37.3	-42.8, -28.8	-2.6 ± 4.4	-3.7	-6.4, 3.2
Pisidiidae sp.	2	-36.3	-36.3	-40.4, -32.2	-0.4	-0.4	-2.8, 2.0
Invertebrates collected by Malaise traps placed on stream banks							
<i>Helodes</i> sp. (adult)	4	-67.3 ± 2.4	-67.6	-69.8, -64.0	-3.7 ± 1.7	-3.5	-5.4, -2.1
Chloroperlidae spp. (adult)	18	-33.5 ± 12.8	-27.7	-61.8, -26.4	4.0 ± 2.8	5.1	-2.3, 6.2
Leuctridae spp. (adult)	10	-37.7 ± 11.0	-39.4	-51.8, -25.3	1.9 ± 1.8	1.9	-2.1, 5.1
<i>Sialis</i> sp. (adult)	3	-41.0 ± 18.8	-31.2	-62.6, -29.1	2.1 ± 2.7	1.3	-0.2, 5.1

tion was very small. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of these prepared samples were measured using a continuous-flow isotope ratio mass spectrometer (CF/IRMS) (EA1108, Fisons; delta S, Finnigan). The $\delta^{13}\text{C}$ value of methane gas was measured with a gas-chromatograph-combustion isotope ratio mass spectrometer (GC/C/IRMS) (Finnigan MAT delta S/GC system: HP GC, GC Combustion Interface, MAT 252; Finnigan) (Sugimoto 1996). Stable isotope ratios are expressed in δ notation as the differences in parts per thousand (‰) from the standard:

$$\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$$

where X is ^{13}C or ^{15}N , R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$, and appropriate standards were PeeDee Belemnite (PDB) and atmospheric nitrogen for carbon and nitrogen, respectively. Samples were analyzed in duplicate, and analytical precision was better than $\pm 0.3\text{‰}$ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values.

RESULTS

The $\delta^{13}\text{C}$ values of leaf litter (mean \pm SE = $-28.5 \pm 0.3\text{‰}$, $n = 6$), FPOM ($-28.3 \pm 0.4\text{‰}$, $n = 18$) and periphyton ($-27.7 \pm 1.6\text{‰}$, $n = 15$) were similar to each other (Fig. 1). In contrast, the mean $\delta^{13}\text{C}$ of methane bubbles rising from the bottom of the pools ($-65.9 \pm 2.4\text{‰}$, $n = 6$) was a good deal lower than those of photoautotrophic sources (Fig. 1).

Aquatic macroinvertebrates collected in backwater pools showed large variations in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Fig. 1). Invertebrates collected from the substrate surface of backwater pools (Gammaridea spp. and Tipulid crane fly larvae) exhibited relatively high $\delta^{13}\text{C}$ values, which were close to the $\delta^{13}\text{C}$ values of photoautotrophic resources (Fig. 1, Table 1). However, most of the other invertebrates collected exhibited $\delta^{13}\text{C}$ values intermediate between photoautotrophic resources and methane gas, even though a large variation existed in their $\delta^{13}\text{C}$ values among individuals. Invertebrates collected from inside the detritus accumulation (*Helodes* sp. and *Agabus* sp.) showed unusually low $\delta^{13}\text{C}$ values (Fig. 1, Table 1). In addition, some adult Chloroperlid and Leuctrid stoneflies (collected in Malaise traps), nymphs of which were often found in hyporheic zones but not in backwater pools, also had extremely low $\delta^{13}\text{C}$ values (Fig. 1, Table 1). Some Chironomid larvae, *Sialis* sp. and Pisidiidae sp. also showed $\delta^{13}\text{C}$ values lower than those of photoautotrophic resources. It is particularly worth noting that *Helodes* beetle adults (collected in Malaise traps), larvae of which were found only in backwater pools, had the lowest $\delta^{13}\text{C}$ values (Table 1) of the organisms previously reported from terrestrial and freshwater ecosystems.

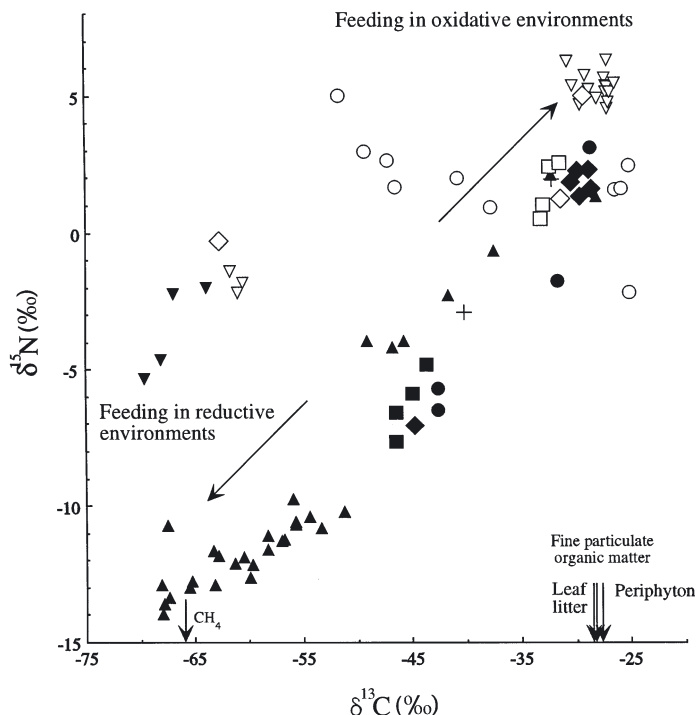


Fig. 1. Carbon and nitrogen isotope signatures of aquatic macroinvertebrates collected in a northern Japanese headwater stream. Each symbol corresponds to 1 individual, except Pisidiidae, where the mean δ -value of 10 individuals is represented. Arrows show the mean $\delta^{13}\text{C}$ values of bubble methane and photoautotrophic resources. ■: *Agabus* sp. (larva); ●: Chironomidae spp. (larva); ▽: Chloroperlidae spp. (adult); □: Gammaridea spp.; ▼: *Helodes* sp. (adult); ▲: *Helodes* sp. (larva); ○: Leuctridae spp. (adult); +: Pisidiidae sp. (Bivalvia); ◇: *Sialis* sp. (adult); ◆: Tipulidae spp. (larva)

The positive linear function fitted as a significant model of the relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of macroinvertebrates across species ($\delta^{15}\text{N} = 0.428\delta^{13}\text{C} + 14.2$, $r = 0.97$, $p < 0.0001$, $n = 52$). In this analysis, adult aquatic insects were excluded because the metamorphosis of insects from larvae/nymphs into adults is known to greatly increase the $\delta^{15}\text{N}$ value (Scrimgeour et al. 1995). The result indicated that stream macroinvertebrates with lower $\delta^{13}\text{C}$ values tended to show lower $\delta^{15}\text{N}$ values (Fig. 1).

DISCUSSION

The present study showed that many aquatic macroinvertebrates collected in the headwater stream had highly depleted $\delta^{13}\text{C}$ (Fig. 1). In particular, invertebrates found inside detritus accumulation in backwater pools (Table 1) and those that spend part of their life cycle in hyporheic zones (Chloroperlid and Leuctrid stoneflies) tended to show more depleted $\delta^{13}\text{C}$ values.

Because the $\delta^{13}\text{C}$ values of photoautotrophic resources were ca. -28% , and there is little shift in $\delta^{13}\text{C}$ associated with trophic transfer of organic carbon (usually less than $\pm 1.0\%$, DeNiro & Epstein 1978), the existence of macroinvertebrate $\delta^{13}\text{C}$ values $\ll -30\%$ can be attributed to the incorporation of non-photoautotrophic carbon sources into these organisms.

Researchers have empirically deduced that the high depletion of $\delta^{13}\text{C}$ ($< -40\%$) in macroinvertebrates found at or near oxic/anoxic interfaces can be attributed to the incorporation of ^{13}C -depleted methane-derived C via methane-oxidizing bacteria (Paull et al. 1985, Childress et al. 1986, Page et al. 1990, Vacelet et al. 1996, Colaco et al. 2002). Although we did not measure the $\delta^{13}\text{C}$ values of methane-oxidizing bacteria, they are expected to be extremely low ($\leq -66\%$) because they may preferentially use $^{12}\text{CH}_4$ and thus may have isotopically lighter carbon than the residual methane gas we measured (Coleman et al. 1981, Grossman et al. 2002). Thus, macroinvertebrate $\delta^{13}\text{C}$ values $\ll -30\%$ seem to be due at least in part to the carbon input through the consumption of methane-oxidizing bacteria (Fig. 2).

Another possible carbon pathway to macroinvertebrates with highly depleted $\delta^{13}\text{C}$ values is via chemoautotrophs that assimilate respired carbon. Chemoautotrophic bacteria that use ^{13}C -depleted CO_2 resulting from oxidation of biogenic methane are characterized by significant depletion in $\delta^{13}\text{C}$ values (Hollander & Smith 2001). Because our sampling was done in backwater pools where partially anoxic conditions may occur and the production of CO_2 respired from methanotrophs is expected to be high (Sweets et al. 1991, Hollander & Smith 2001), $\delta^{13}\text{C}$ depletion of chemoautotrophic bacteria and its consumers (e.g. macroinvertebrates) can also be expected. In addition, respiratory CO_2 from aerobic decomposition of photoautotrophic organic carbon can contribute to the $\delta^{13}\text{C}$ depletion of macroinvertebrates via chemoautotrophic bacteria (France et al. 1997, France & Schlaepfer 2000). In systems with strong input from respiration (e.g. humic lakes and wetlands), $\delta^{13}\text{C}$ values for dissolved inorganic carbon may approach -20% (Peterson & Fry 1987), and chemoautotrophic bacteria that further fractionate during carbon uptake (e.g. ca. 25% of sulfur-oxidizing bacteria, Ruby et al. 1987) can, in practice, measure ca. -50 to -40% . However, this carbon pathway cannot fully explain the unusually low $\delta^{13}\text{C}$ values ($\ll -50\%$) in macroinvertebrates. Therefore, although we could not assess the relative importance of these microbially mediated carbon pathways in macroinvertebrate carbon production, it can be considered that a stream food web composed of aquatic macroinvertebrates is partly sustained by carbon that was originally in the methane gas (Fig. 2). The large individual varia-

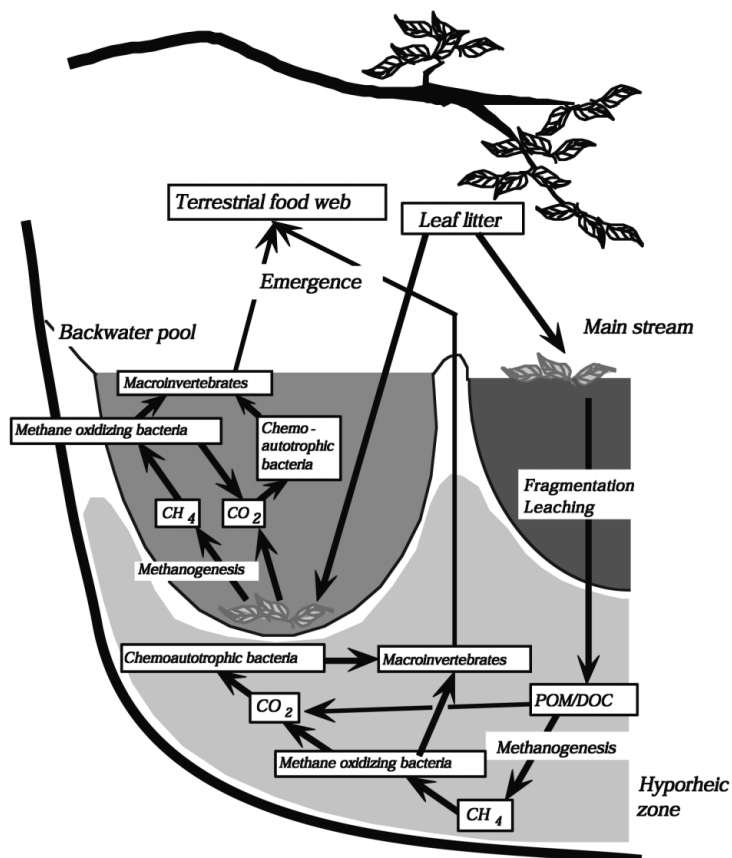


Fig. 2. Input of methane-derived energy into macroinvertebrate communities in stream and terrestrial ecosystems. Organic and inorganic carbon in rectangular boxes and arrows indicate the carbon pools and directions of carbon flow, respectively

tion in $\delta^{13}\text{C}$, found even within the same taxon (Fig. 1, Table 1), might have resulted from the difference in the dependence of macroinvertebrates on bacterial communities fueled by methane-derived carbon.

We found that most *Helodes* beetle larvae and adults were extremely depleted in ^{13}C . The beetle larvae are thought to graze on rotting leaves and decaying vegetation around the redox boundary (Doyen & Ulrich 1996). However, their $\delta^{13}\text{C}$ values were significantly lower than the leaf litter $\delta^{13}\text{C}$ ($p < 0.0001$ by t -test), suggesting that ^{13}C -depleted microbial aggregates (methanotrophs and/or chemoautotrophs) associated with terrestrial detritus contributed a substantial fraction of the assimilated carbon of *Helodes* beetles. Previous studies revealed that stream invertebrate detritivores often prefer detrital resources that have been conditioned by microbial colonization in comparison to uncolonized detritus (see review by Allan 1995). This is because many detritivorous macroinvertebrates show negligible enzymatic activity toward cellulose and other plant structural polysaccharides, and thus their carbon needs are often met by microbial production, as

well as vascular plant detritus itself (Allan 1995). Considering that microbial carbon accounts for <2% of the detrital standing stock of carbon in headwater streams (Findlay et al. 2002), carbon pathways mediated by methanotrophic or chemoautotrophic bacterial processes may play a disproportionately large role in sustaining many *Helodes* beetles in backwater pools.

We found that macroinvertebrates with lower $\delta^{13}\text{C}$ values tended to show lower $\delta^{15}\text{N}$ values. In particular, *Helodes* beetles (both larvae and adults) and *Agabus* sp. showed highly depleted $\delta^{15}\text{N}$ values (Table 1), which were lower than those of the surrounding organic matters (leaf litter, mean \pm SE = $-3.3 \pm 0.4\%$, $n = 6$; FPOM, $1.0 \pm 0.8\%$, $n = 18$; periphyton, $2.2 \pm 1.3\%$, $n = 15$). Considering that consumers are generally enriched in ^{15}N by 1.0 to 5.0‰ relative to their food resource (Minagawa & Wada 1984, Peterson & Fry 1987), macroinvertebrates with $\delta^{15}\text{N}$ values $\leq -4.0\%$ should have derived their nitrogen from non-photoautotrophic resources. The depletion in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ has also been detected in methanotrophic communities in hydrothermal vents (Conway & Capuzzo 1989, Conway et al. 1992, Lee & Childress 1994, Colaco et al. 2002) and in lake chironomid larvae (Grey et al. 2004). This is due to the large nitrogen isotope fractionation during the assimilation of DIN (ammonium and nitrate) by methanotrophic and chemoautotrophic bacteria (Hoch et al. 1992, Lee & Childress 1994). Therefore, stream macroinvertebrates with highly depleted $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are likely to have also depended upon methanotrophic and/or chemoautotrophic bacterial productions as their nitrogen sources.

The aerobic consumption of photoautotrophic resources (e.g. periphyton and terrestrial plant litter) has long been considered the major carbon pathway that supports headwater stream food webs (Vannote et al. 1980, Finlay 2001). However, our study suggests that biogenic methane produced by the anaerobic decomposition of photoautotrophic organic carbon fuels some stream macroinvertebrate production through the methanotrophic and/or chemoautotrophic bacterial pathways (Fig. 2). This carbon input into macroinvertebrate communities occurs in backwater pools and hyporheic zones (Fig. 2) because these habitats mineralize a large amount of accumulated organic matter with limited oxygen availability, producing a large amount of methane gas (Findlay 1995, Jones & Holmes 1996, Schindler & Krabbenhoft 1998). In addition, because most aquatic insects metamorphose into adults that emerge from freshwater to terrestrial environments for reproduction and are often consumed by terrestrial predators (Jackson & Fisher 1986), methane-derived carbon may also enter terrestrial food webs (Fig. 2). Thus, to more properly understand the com-

munity dynamics in headwater ecosystems including main channels, backwater pools, hyporheic zones and riparian landscapes, we need further studies to quantify the relative contribution of methane-derived carbon to stream and terrestrial food webs.

Acknowledgements. We are indebted to J. Urabe, T. Narita and T. Miyajima for their critical comments on the early manuscripts of this study. We also thank the students at the Tomakomai Research Station, Hokkaido University, for their logistical support. This work was supported by grants-in-aid from the Research for the Future Program (JSPS-TFTF 97100602) funded by Japan Society for the Promotion of Science, from MEXT Creative Basic Research (09NP1501), and from CREST, Japan Science and Technology Agency.

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Editorial responsibility: John Dolan,
Villefranche-sur-Mer, France

Submitted: January 20, 2004; Accepted: May 3, 2004
Proofs received from author(s): June 21, 2004