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WHAT CAN STABLE ISOTOPES ($\delta^{15}N$ AND $\delta^{13}C$) TELL ABOUT THE FOOD WEB OF SOIL MACRO-INVERTEBRATES?

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Abstract. Animals reflect the stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) of their diet, with a slight enrichment in ^{15}N that allows the use of $\delta^{15}N$ as a trophic-level indicator. Stable isotope contents were measured for the litter, soil, and macro-invertebrates of three temperate deciduous forest sites, in spring, summer, and autumn, to study the trophic structure of this community. No distinct trophic structure could be derived from measurements of $\delta^{13}C$. In contrast, when the $\delta^{15}N$ values of all animal species were grouped together, the hypothesis of an isotopically similar diet was rejected. Therefore, the community spreads over more than one trophic level and was subdivided into detritivores and predators. The potential detritivore food sources in the forest litter and soil showed a variety of isotopic ratios. Despite this fact, the variance of the isotopic ratios of the detritivorous species was not larger than what could be expected from interspecific variability of trophic isotopic enrichment alone. This was also the case for the predators in most of the sample sets. However, in some cases this variance was significantly larger, due to a small number of species with high $\delta^{15}N$ values. The $\delta^{15}N$ values of the detritivores indicated that the mean $\delta^{15}N$ value of their food was close to that measured for the superficial litter layers. The difference in $\delta^{15}N$ between detritivores and predators was highly significant and never significantly different from the value expected for one trophic transfer (3.4‰), although often slightly higher. Most of the litter macro-invertebrate community we studied can therefore be described as belonging to two trophic levels, one feeding on the superficial litter layers (or on soil fractions that have a similar $\delta^{15}N$ value), and a second trophic level feeding on the first, with some indication of intraguild predation among the predators.

Between-site differences of up to 7‰ were found for $\delta^{15}N$ in the litter, and the $\delta^{15}N$ values of the whole animal community were shifted in accordance with the local value of the litter $\delta^{15}N$. Therefore, the trophic structure must be studied in relation to the local isotopic content of the litter. Seasonal differences in isotopic ratios of the litter or animal samples were neither large nor consistent. These findings indicate similar trophic structure of the communities at the three sites and during the three sampling periods.

Key words: $\delta^{15}N$ and $\delta^{13}C$; detrivores; food web; forest litter; intraguild predation; isotope ratios; macro-invertebrates; soil fauna; stable isotopes; trophic levels.

INTRODUCTION

Biological materials contain carbon and nitrogen with various proportions of their naturally occurring stable isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$). Animal tissues are built with atoms of the food they assimilate, and therefore grossly reflect the food's isotopic composition. This property has been used to trace carbon flows through animal communities (e.g., carbon from C_3 vs. C_4 plants (Petelle et al. 1979), and carbon of aquatic vs. terrestrial origin (Kline et al. 1993; review in Ponsard [1998]). However, it has also been shown that, for carbon (DeNiro and Epstein 1978) and above all for nitrogen (DeNiro and Epstein 1981), ''you are what you eat . . . plus a few per mil'': animal tissues tend to be slightly enriched in the heavier isotope compared with their diet. This has been confirmed in nearly all the animal species that have been thus far studied (vertebrates and invertebrates, aquatic and terrestrial). Although the underlying physiological, biochemical, and biophysical processes are not fully understood (Ponsard and Averbuch 1999), the degree of enrichment of an animal vs. its diet appears to be fairly constant among organisms for a given element.

Early attempts to use $^{13}C/^{12}C$ ratios as trophic level indicators seemed to be successful. McConnaughey and McRoy (1979) and Rau et al. (1983) examined the ^{13}C content of various organisms that had been assigned independently to a trophic level by more classical means, and found a positive correlation. However, Schwinghammer et al. (1983) reported being ''unable to detect any ^{13}C enrichment in various trophic levels,'' and, while the use of $^{13}C/^{12}C$ ratios to distinguish between C_3 and C_4 or between aquatic and terrestrial primary producers spread rapidly, very few later studies

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use them as trophic-level indicators for consumers. For this purpose, the $^{15}N/^{14}N$ ratio is widely preferred. DeNiro and Epstein (1981) and Schoeninger and DeNiro (1984) showed the $^{15}N/^{14}N$ ratio to increase much more markedly with the trophic level than the $^{13}C/^{12}C$ ratio. Later, nitrogen isotopic content was used to infer information not previously available, such as changes in the trophic position of a given species, depending on the age or the environment of the individuals. For instance, Hobson and Welch (1995) were able to detect cannibalism of the larger size classes of Arctic char on the smaller ones, Kling et al. (1992) have shown that a potentially omnivorous zooplankton species fed on different trophic levels in different lakes, and Abend and Smith (1995) have shown the same for whales in the western and eastern North Atlantic.

Real food webs typically are very complex, and it is impossible to disentangle them without some conceptual simplifications. One such approach is the notion that organisms can be grouped into trophic levels. However, the fact that some organisms feed on several levels can make it necessary to define the ''effective'' trophic level of a given species as a fractional number. One simple way to do so is to estimate it as the mean number of trophic links that relate a given species to basal species, i.e., species that have no prey (Yodzis 1989, Begon et al. 1995). Another approach that additionally takes into account the relative importance of these links is to define the trophic level as ''the mean length, weighted in proportion of energy flows, of all chains from basal species to species A'' (Yodzis 1989).

Indeed, it is desirable to give different degrees of importance to anecdotal trophic links compared to main links. Nevertheless, it is unclear from a practical point of view how the proportion of energy flow through each pathway can be assessed. Studying in detail a given species' array of prey, and their relative contributions to its diet, requires an enormous amount of work, and all this information will finally be aggregated into a single number representing the fractional trophic level of this species. The isotopic content of an organism does not tell *which* species it consumes, but directly indicates the mean number of trophic transfers that occurred between basal species and this organism, weighted in proportion to the flow of matter, i.e., the mean trophic level on which it feeds. In that, the isotopic method can be seen as a practical way of performing a weighted average of trophic links. It is relatively fast and easy, with the additional advantage of taking into account not ingested food, but food that is really assimilated. It can thus give a broad, although somewhat fuzzy, idea of some of the main features of a food web, such as the mean number of trophic transfers between the bottom and the top species, the ''stratigraphy'' of a food web (in the sense of Cohen and Luczak [1992], i.e., the proportion of species at each height above the basal species), or, if some of the basal species have isotopic signatures that are different enough, the existence of separate or confluent pathways of matter transfer.

Several recent studies have stressed the links between energy and nutrient flows on the one hand, and the food web structure on the other, particularly in soils (e.g., Hunt et al. 1987, Moore et al. 1988, deRuiter et al. 1994). The soil food web is commonly thought to be complex, species rich, and to have a large proportion of omnivores (Bengtsson et al. 1995), but trophic links are particularly difficult to examine directly in this community, for reasons that include the small size, the cryptic life, and the great taxonomic diversity of the organisms. The feeding habits of some particular soil taxa have been studied successfully by isotopic means (Spain et al. 1990, Martin et al. 1992*a*, *b*, Schmidt et al. 1997, Spain and Le Feuvre 1997 [earthworms]; Boutton et al. 1983, Lepage et al. 1993, Tayasu et al. 1994, 1997, 1998, Tayasu 1998 [termites]), but we are aware of no study applying these techniques to the soil community as a whole.

The purpose of the present study was to apply the isotopic technique at the community level, attempting to assess the nature of trophic links and their importance in community structure. More specifically, we focused on the macro-invertebrates of temperate forest litter. It is a first approach, since a complete understanding of the soil food web would, of course, also require data about the micro- and mesofauna. Our aim was four-fold: (1) to assess whether, and under which conditions, stable isotope contents can be used to ''order'' the soil macrofauna into successive trophic levels; (2) to see how many trophic levels can be distinguished in the system under study; (3) to compare the structure of the soil community in sites of increasing richness, but that seem to be otherwise comparable; and (4) to examine possible seasonal variations in this structure. Since $^{15}N/^{14}N$ ratios seem to be more promising than $^{13}C/^{12}C$ ratios for the assessment of trophic levels, our study focused on nitrogen. However, since recent technical developments (Fry et al. 1992) made it possible to measure $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios on a single sample (i.e., for the same amount of work and money), we also examined how the data on carbon compared to those on nitrogen.

Material and Methods

Study sites

The study sites were three mixed deciduous forest sites, on the Orsay campus of University Paris-Sud (25 km south of Paris, France). The sites were located within 1 km of each other. Site 1 has a mor–moder type of humus on a neopodzolic soil, and is situated on the edge of a plateau, at an elevation of ~160 m. Sites 2 and 3 are situated on the slope leading from the plateau to the valley, at an elevation of ~100 m, with a moder type of humus on leached soils. The three sites differ further by increasing amounts of annual litter fall and

decreasing acidity. In autumn 1996, measurements of the annual litter fall were (mean ± 1 SE, $n = 8$): 250 ± 30, 271 ± 45, and 339 ± 33 g/m^2, respectively on sites 1, 2, and 3. The pH in the A_1 soil layer ($n = 10$) was 3.65 ± 0.07, 3.94 ± 0.05, and 4.03 ± 0.07. On all sites, the thickness of the A_1 layer was 10–15 cm, and that of the B layer was 50–60 cm. Anthropogenic disturbances affecting these sites include the vicinity of a few pedestrian paths, the removal of dead wood, and the diffuse urban pollution of the Paris area.

Soil samples

Five core samples (7 cm diameter × ~30 cm deep) of litter, humus, and soil layers (named, from top to bottom, L, F, H, A_1, and B, according to the nomenclature of Duchaufour [1983]) were taken in April 1997 on sites 1 and 3. The low variability observed among samples from a given layer and site lead to reduction of the number of replicates from five to three for the second and third samplings, in October 1997 and January 1998, when all three sites were sampled. The H layer was sometimes absent. In addition, we analyzed some freshly fallen dead leaves collected in October 1997, superficial mycelium scraped from the L layer, and caps of some unidentified basidiomycetes collected in October 1996.

Invertebrate samples

Invertebrates were collected during the months of July 1997, October 1997, and April 1998, either directly by hand or by pitfall traps (15 cm diameter × 20 cm deep) filled with ~2 cm of tap water to drown the animals and to prevent them from eating each other. Rotting of invertebrate tissues changes their isotopic content (Ponsard and Amlou 1999). Therefore, the pitfall traps were emptied at least every 48 h. Animals were cleaned with tap water, and frozen at −20°C awaiting identification and further processing. Samples usually consisted of 5–10 individuals of the same stage (larval/adult), species, and site, ground together. When it could not be avoided, samples of <5 individuals were also used. Gut contents were not removed. A priori, they have a lower isotopic ratio than the animals' bodies and could act as a confounding factor. On the other hand, cutting out the feeding tract would probably result in a loss of haemolymph, and starving the animals to allow gut clearance may lead to an increase of their $\delta^{15}N$ values (Hobson et al. 1993). We hypothesize that most samples are representative of the isotopic content of animals with ''average filling'' of the digestive tract. This might lead to a systematic bias if, for instance, the digestive tract represented a much larger proportion of the whole body for some taxa than for others, or if the probability that the tract was empty was very different.

Inorganic carbon, present as $CaCO_3$ in the snail shells and possibly in the exoskeleton of several invertebrates, is known to be strongly enriched in ^{13}C, and for this reason it is sometimes removed by acid washing before isotopic analysis (Gearing 1991). However, such treatment has been shown to change the nitrogen isotopic content of invertebrate tissues (shrimps, Bunn et al. 1995). Since our priority was to investigate nitrogen rather than carbon isotopic content, we avoided acid washing. Snails were much richer in ^{13}C than all the other species we analyzed, and therefore were excluded from the analyses of carbon isotope data.

Stable isotope content

The animal samples were freeze-dried and ground to a fine powder in a mortar and pestle or in a ball mill. Samples of ~1 mg were measured into 6 × 4 mm tin cups for continuous-flow isotope ratio mass spectrometry (CF–IRMS) analysis of carbon and nitrogen. Isotope analyses were carried out using a PDZ-Europa automated nitrogen carbon analyzer–new technology (ANCA–NT) 20–20 stable isotope analyzer with automated nitrogen carbon analyzer–solids/liquids (ANCA–SL) preparation module (PDZ-Europa, Crewe, UK) . As nitrogen content of animal samples is ~10%, the CF–IRMS was operated in the dual element mode, allowing carbon and nitrogen isotopic contents to be measured on the same sample.

Litter and soil samples were dried at 60°C for 48 h immediately after collection. Horizons A_1 and B were sieved through a 1 mm mesh sieve. Small branches and fruit were removed by hand in samples of horizons L and F. The samples then were ground to a fine powder in a ball mill. Due to their lower nitrogen content (<2%), litter and soil samples had to be analyzed separately for carbon and nitrogen isotopic content (single element mode). The amounts used for carbon analyses were ~1 mg for L, F, and H layers, and 10 mg for A_1 and B horizons. For nitrogen analyses, 10 mg were used for all types of soil or litter samples.

Isotopic contents were expressed in ''δ'' units as the relative difference (in parts per thousand) between the sample and conventional standards (atmospheric N_2 for nitrogen; PD-belemnite [PDB] carbonate for carbon), according to the formula $\delta^{13}C$ or $\delta^{15}N$ (‰) = $(R_{sample}/R_{standard} - 1) \times 1000$, where R is the ratio of heavy/light isotope content for the considered element.

A standard of known isotopic composition (δ_s = 1.01‰ for nitrogen and $\delta_s = -29.47$‰ for carbon), and having a C/N ratio comparable to that of animal tissues, was measured after each batch of ten samples. This standard was 1 mg leucine prepared by freeze drying 50 mL of a 20 mg/mL stock solution into tin cups, and calibrated against ''Europa flour'' (PDZ-Europa, Crewe, UK) and IAEA ammonium sulfate standards N1 and N2 (International Atomic Energy Agency, Vienna, Austria). The spectrometer was then recalibrated before the next ten measures were made. A correction for spectrometer drift was applied as follows: $\delta_i' = \delta_i + i(\delta_s^* - \delta_s)/11)$, where δ_i is the value measured for the ith sample in the batch of 10 analyses,

δ_i' is its corrected value, and δ_s^* is the value measured for the standard after the 10th sample. However, even the highest corrections applied were small: −0.18 to +0.19‰ for $\delta^{15}N$ (mean absolute value, 0.05‰), −0.15 to +0.17‰ for $\delta^{13}C$ (mean absolute value, 0.01‰).

The percent C and percent N values used for calibration were respectively 55.8% and 9.88%, based on measurements against another standard. The calibration thus yielded a C/N value of 5.65 for the standard, which is 0.51 higher than the theoretical value expected for leucine (5.14). We therefore applied a correction for spectrometer drift in the same way as for isotopic contents, and then subtracted 0.51 from the corrected value. Again, the drift was very small, since the highest corrections were −0.08% and +0.07% (mean absolute value, 0.01%).

For a random subset of the animal preparates, two samples of the same powder were analyzed separately to evaluate the homogeneity of the powders. The mean differences between replicate measures of the same sample (after correction) were $\delta^{15}N = 0.14$‰ and $\delta^{13}C = 0.24$‰ ($n = 7$). As a comparison, the standard error of the values measured for the leucine standard were 0.1‰ and 0.02‰, respectively. The samples thus were less homogenous for carbon than for nitrogen.

Data analysis

The animal species were separated a priori into two trophic categories, depending on whether they were considered as ''detritivores'' or ''predators'' in the literature (Perrier 1923, 1929, 1930, McE. Kevan 1962, Demange 1981, Petersen and Luxton 1982, Chinery 1986, DuChatenet 1990, Sueur 1995). To our knowledge, none of the predators is reported to be a specialist predator of other predators, except possibly the Trombidiidae (parasites). Some species that did not fit these categories are considered separately: Silphidae (scavengers), Elateridae and Curculionidae (root eaters), *Bombus terrestris* and *Noctua pronuta* (Nectarivores), and an undetermined heteroptere (sap sucker).

In a wide range of consumer-food pairs, the isotopic content of an animal has been found on average to be $\Delta_C = 0.4 \pm 1.4$‰ ($n = 76$) higher than that of its food for carbon (Gearing et al. 1984) and $\Delta_N = 3.4 \pm 1.1$‰ ($n = 26$) higher for nitrogen (Minagawa and Wada 1984). More recent literature reviews give similar means but no estimate of the standard deviation (Peterson and Fry 1987), or indicate only a range of values (Ehleringer et al. 1986, Gannes et al. 1997). We consider the quoted figures to be the best available estimates of the means and standard deviations of enrichment per trophic transfer in a random group of species belonging to diverse taxa. Thus, the mean number of trophic transfers that occur between the bottom of a food web and a given species was calculated as the difference of their δ values expressed in Δ units for a given element.

The isotopic homogeneity of the diet of a given group of n species was tested in the following way. If we assume that $\delta_{species} = \delta_{food} + \Delta \pm \sigma_\Delta$, where σ_Δ is the standard deviation of the values of isotopic enrichment (Δ) in various species given in Gearing et al. (1984) and in Minagawa and Wada (1984), then the null hypothesis that the interspecies variability of Δ alone can account for the observed variance of δ within the group (σ_g^2) can be tested by comparing σ_g^2 (with $n - 1$ df) to σ_Δ^2 (with 26−1 and 76−1 df, respectively for N and C) with a one-tailed F test. We tested the isotopic homogeneity of the detritivore group, the predator group, and the whole community for each site. Furthermore, we tested whether the difference between mean predator and detrivore $\delta^{15}N$ value was significantly different from Δ_N (Welch's two-tailed t' test; Zar 1999).

For all three sampling periods on a given site, and for all three sites within a given sampling period, Kendall's coefficient of concordance (W) was calculated for the ranks of the $\delta^{15}N$ and $\delta^{13}C$ values. When there were no common species, but only common genera or families (Cylindroiulidae, Tenebrionidae, Agelenidae, Lyniphidae, Trombidiidae, Lithobiidae, Myrmicinae, Carabidae, Curculionidae, and Elateridae), mean family values were also taken into account for these calculations. The significance of W was tested with the χ^2 distribution (Sokal and Rohlf 1995).

Lipids are known to have a low ^{13}C content compared to carbohydrates, and, above all, compared to proteins (DeNiro and Epstein 1976). They usually are removed from vertebrate tissues prior to carbon isotope analysis (Hobson et al. 1993, Minami et al. 1995, Smith et al. 1996). This is almost never the case for invertebrates (except Spies and DesMarais [1983]), although insect tissues, for instance, can contain as much as 25% lipids (Teeri and Schoeller 1979), and Rau et al. (1991) found a negative relationship between $\delta^{13}C$ and lipid content (estimated from C/N ratio) in marine invertebrates. However, as far as we know, the effect on $\delta^{15}N$ of chemical treatments used to remove lipids (ether, toluene, or choloroform/methanol) has never been investigated, so that ''correcting'' the $\delta^{13}C$ values by one of these methods might have biased the $\delta^{15}N$ values in an unknown way. We therefore did not remove lipids from our samples prior to isotopic analysis, but we examined a possible link between C/N ratio and carbon isotope content, separately for predators and detritivores.

Results

Litter and soil

Fig. 1 shows the $\delta^{15}N$ and $\delta^{13}C$ values of the different litter and soil layers in January, April, and October, and some additional data for fungi. The profiles were similar among sites and among sampling periods, showing an enrichment with depth in the heavier isotope for both elements, although much stronger for nitrogen than for carbon. The nitrogen profile seemed

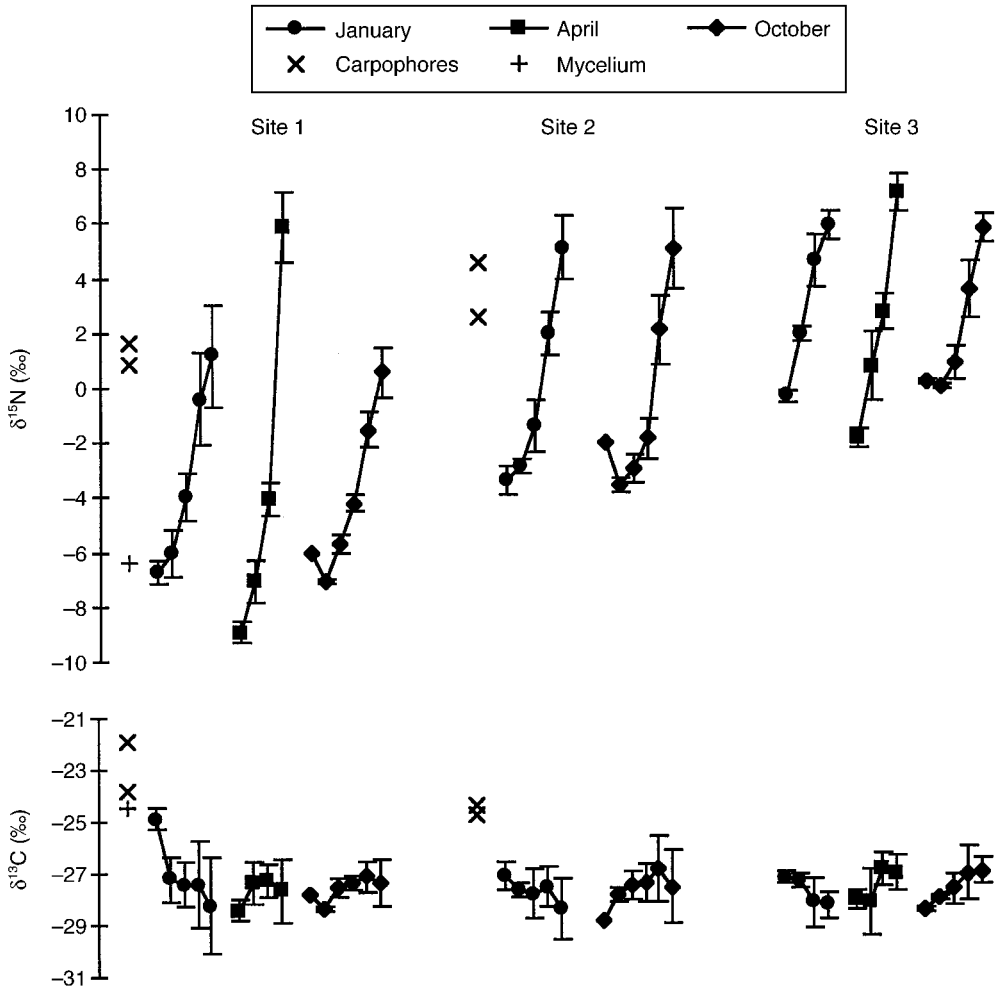

FIG. 1. The $\delta^{15}N$ and $\delta^{13}C$ values (± 1 SD) of different litter and soil layers in January 1998 (sites 1, 2 and 3; $n = 3$), April 1997 (sites 1 and 3; $n = 5$), and October 1997 (sites 1, 2 and 3; $n = 3$; except for annual litter fall, $n = 1$ on sites 1 and 2, $n = 2$ on site 3). Additional data on carpophores and mycelium collected in October 1996 are shown. For a given month and site, the layers are from the left to the right: freshly fallen leaves (in October only), L, F, H (practically absent in the samples of site 1 in April, and in all samples of site 3), A_1, and B.

to be steeper in April: the upper layers seemed to be less enriched, and the B layer seemed to be more enriched in ^{15}N in April than in January and October. No such seasonal trend could be detected for carbon. Moreover, the $\delta^{13}C$ values were quite similar for all three sites, while the $\delta^{15}N$ baseline was very different between them.

The mushroom caps were strongly enriched in ^{15}N compared to litter. The mycelium had a $\delta^{15}N$ value that was close to that of the L layer of the litter in October. Both caps and mycelium had $\delta^{13}C$ values 1.5–5‰ higher than the highest value observed for any litter or soil layer.

Invertebrates

General features of the trophic community.—Fig. 2 shows the $\delta^{15}N$ and $\delta^{13}C$ values measured for all the animal species caught on each site and during each season, together with the values of the L litter found for sampling periods some weeks or months before the animal sampling (January litter values and April animal values; April litter and July animals; October litter and October animals). Each point represents one species. The number of species per site for a given sampling period ranges 26–43. The list of species and their trophic category are given in the Appendix. The main detritivore taxa were woodlice, millipedes, insects, and gastropods. The main predator taxa were spiders, coleoptera, centipedes, and ants.

A general among-site difference in the $\delta^{15}N$ of the animals could be observed that paralleled the shift of the $\delta^{15}N$ values of the litter (Fig. 2). Among-site differences were very small for $\delta^{13}C$ and did not seem related to the (also very small) differences in litter $\delta^{13}C$.

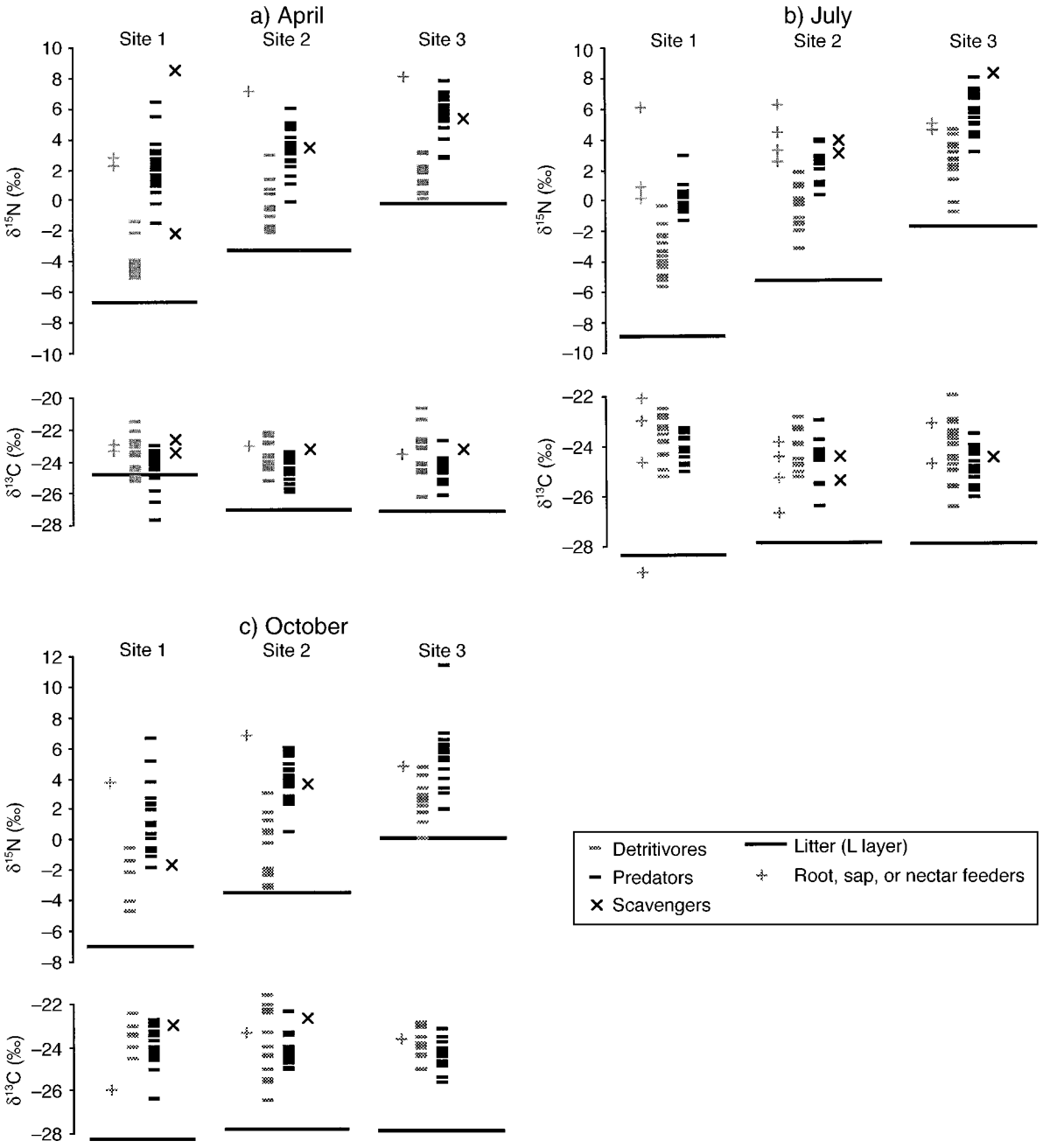

FIG. 2. The $\delta^{15}N$ and $\delta^{13}C$ values of the L litter horizon and of detritivorous, predatory, and other invertebrate soil species collected in (a) April, (b) July, and (c) October at three deciduous forest sites. Each point represents one species. The April value for the L layer on site 2 (direct measurement not available) has been estimated by subtracting 1.9‰ from $\delta^{15}N$ and adding 0.08‰ to the $\delta^{13}C$ values measured on this site in October. This calculation was based on the shifts of the isotopic content of the L layer between April and October observed on sites 1 and 3, which showed remarkably close values (+1.85‰ and +1.90‰, respectively, for $\delta^{15}N$; and −0.09 and −0.08‰ for $\delta^{13}C$).

Seasonal differences were neither large nor consistent among sites. The coefficient of concordance between $\delta^{15}N$ values of sets of identical taxa from different sites in the same sampling period, or from the same site over different sampling periods, was usually highly significant (Table 1). The coefficient of concordance between $\delta^{13}C$ values was always lower than that between the $\delta^{15}N$ values, and it was nonsignificant twice (Table 1). There were 8–23 common species for each comparison, representing ~50% of the total number of species caught on a given site at a given time. Globally, the macrofauna communities were thus comparable on the

TABLE 1. Kendall's coefficient of concordance (W) between the isotopic ranks of identical taxa trapped at a given time on different sites or on a given site at different sampling periods.

Date or site	df	W ($\delta^{15}N$)	W ($\delta^{13}C$)
April	22	0.88**	0.70**
July	17	0.79**	0.65*
October	10	0.68*	0.57 NS
Site 1	7	0.96**	0.76*
Site 2	10	0.91**	0.73*
Site 3	10	0.86**	0.59 NS

* $P < 0.05$; ** $P < 0.01$; NS, nonsignificant.

three sites with respect to species composition, isotopic ranking, and absolute $\delta^{13}C$ values, but not with respect to absolute $\delta^{15}N$ values.

On all three sites, $\delta^{15}N$ values of the predators were higher than those of the detritivores. Five values were excluded from subsequent calculations: one earwig sample (*Forficula auricularia*) from site 1 in April, one spider captured on site 2 in July (*Harpactea* sp.), two slug species (Limacidae) captured on site 3 in July, and one isopod sample (*Porcellio laevis*) captured on site 2 in October. *Harpactea* showed $\delta^{15}N$ and $\delta^{13}C$ values much lower than those of all other predators, and even lower than those of all detritivores at this site. If this is not an artifact, it means that this species is specialized on a particular set of prey, with a very different isotopic content from that of the typical detritivores. The earwig, slug, and woodlice samples had $\delta^{15}N$ values that were below even the lowest litter value measured on their respective sites. These low values were likely to have been artifacts, since samples of the same or similar species on other sites or sampling periods showed $\delta^{15}N$ values that were within the range found for other detritivores. A contamination of the samples by gut contents is possible, but cannot account for values lower than even the lowest value of the potential food sources considered here. If they are not artifacts, these low values indicate that these species either use food sources other than those measured here, or that they feed selectively on some depleted components of the litter. These species also had $\delta^{15}N$ values that were ~6‰ below that of the ''lowest'' predator, which would indicate that either they are not an important food source for any predator, or that we did not catch the predator(s) that feed on them. Scavenger species had $\delta^{15}N$ values that were usually within the range of the values found for predators. The species feeding on living plant parts (roots, sap, or nectar) had $\delta^{15}N$ values that were usually higher than those of the detritivores.

The $\delta^{15}N$ values of the different species usually were distributed as a continuum rather than grouped into clear-cut trophic levels. There was overlap between detritivore and predator $\delta^{15}N$ values in most cases. The hypothesis that a given group of species was not isotopically more heterogeneous than expected from the variability of Δ alone was submitted to one-tailed F tests (Table 2). For $\delta^{15}N$, this hypothesis was rejected once for the detritivores, and in three of nine cases for the predators. It was always rejected when both categories were lumped together. The differences between

TABLE 2. Test of the isotopic homogeneity of the detritivores, the predators, and of the set of both detritivorous and predatory species in each site.

		April		July		October	
Group	Site	F	No. species	F	No. species	F	No. species
$\delta^{15}N$							
Detritivores	1	<1	14	1.6 NS	17	2.3 NS	6
	2	1.8 NS	13	1.6 NS	14	3.2**	13
	3	<1	11	1.8 NS	17	1.5 NS	11
Predators	1	2.8**	23	<1	14	5.0**	18
	2	1.7 NS	21	<1	11	1.8 NS	22
	3	1.1 NS	24	1.2 NS	21	3.7**	19
Detritivores + predators	1	9.8**	37	4.0**	31	6.7**	24
	2	4.6**	34	2.8**	25	6.5**	35
	3	3.9**	35	3.8**	38	4.2**	30
$\delta^{13}C$							
Detritivores	1	<1	13	<1	15	<1	6
	2	<1	12	1.5 NS	12	1.1 NS	13
	3	<1	10	<1	16	<1	11
Predators	1	<1	23	<1	14	<1	18
	2	<1	21	<1	11	<1	22
	3	<1	24	<1	20	<1	19
Detritivores + predators	1	<1	36	<1	29	<1	24
	2	<1	33	<1	23	<1	35
	3	<1	34	<1	36	<1	30

Notes: The within-group variance of isotopic content (σ^2_g) is compared to a literature estimate of interspecies variance of trophic enrichment ($\sigma^2_{\Delta N} = 1.21$, $n = 26$; $\sigma^2_{\Delta C} = 1.96$, $n = 76$) by one-tailed F tests ($F = \sigma^2_g/\sigma^2_\Delta$). When F is significantly greater than unity, the hypothesis that all species of the group feed on the same diet is rejected.

** $P < 0.01$; NS: nonsignificant.

TABLE 3. Difference between isotopic content ($\delta^{15}N$ and $\delta^{13}C$) of consecutive trophic levels (Δ_{D-L}, mean detritivores [D] minus litter layer [L]; Δ_{P-D}, mean predators [P] minus mean detritivores).

Site	April		July		October	
	Δ_{D-L}	Δ_{P-D}	Δ_{D-L}	Δ_{P-D}	Δ_{D-L}	Δ_{P-D}
$\delta^{15}N$ (‰)						
Site 1	2.6	6.2 (5.8)‡ [$P = 0.09$]	5.3	3.6 [$P = 0.85$]	4.7	3.9 (3.1)† [$P = 0.83$]
Site 2	2.9	3.8 [$P = 0.80$]	4.9	2.6 [$P = 0.55$]	3.1	4.1 [$P = 0.69$]
Site 3	1.9	4.0 [$P = 0.69$]	3.4	3.3 [$P = 0.96$]	2.3	2.7 (2.4)† [$P = 0.70$]
$\delta^{13}C$ (‰)						
Site 1	1.3	−0.7	5.3	−1.1	4.8	−0.4
Site 2	3.4	−0.7	4.1	−0.8	3.9	−0.2
Site 3	3.8	−1.1	4.0	−0.7	4.0	−0.5

Note: Level of significance, *P*, is the result of Welch's two-tailed *t*′ test for differences between Δ_{P-D} and Δ_N. The isotopic content of the L layer in April was measured for sites 1 and 3 and estimated for site 2 (see legend of Fig. 2).

† In the cases when some species with exceptionally high $\delta^{15}N$ values were found, values are given with (and without) including them in the predator mean.

the mean detritivore and the mean predator $\delta^{15}N$ values were highly significant in all sites and for all sampling periods (*t* test, $P \leq 0.001$). Their values (Table 3) were higher than the mean value expected for one trophic transfer (3.4‰) in six cases, and lower in only three cases, but they were never significantly different from the mean value expected.

Contrary to values for $\delta^{15}N$, the $\delta^{13}C$ values were not higher for predators than for detritivores (Fig. 2). Their range (excluding the $\delta^{13}C$ values obtained for snails) was smaller than that of the $\delta^{15}N$ values. The hypothesis of a homogenous diet could not be rejected for either detritivores or predators, nor could it be rejected for the total set of species (Table 2). The mean value of predator $\delta^{13}C$ was usually *lower* than the mean detritivores value (Table 3), contrary to what would be expected under the hypothesis of a mean +0.4‰ enrichment in ^{13}C per trophic transfer. Surprisingly, the $\delta^{13}C$ values of all animal species were several per mil higher than the values found for the litter (Table 3).

Biochemical confounding effect.—There was no significant correlation between $\delta^{13}C$ and $\delta^{15}N$ values of the species in any of the sites, during any of the trapping sessions. This is due, at least in part, to the fact that predators have higher mean $\delta^{15}N$ values, but *lower* $\delta^{13}C$, than do the detritivores. There was a significant negative correlation between $\delta^{13}C$ and C/N within the predator group ($r = -0.40$, $P < 0.01$), which indicates that lipid content probably lowers the $\delta^{13}C$ of these species. On the contrary, there was no correlation between C/N and $\delta^{13}C$ among the detritivores ($r = -0.11$, NS).

The mean C/N value of the detritivores, calculated over all sites and trapping seasons, was 5.6, compared to 4.7 for the predators. Therefore, the fact that detritivores had higher $\delta^{13}C$ values than predators cannot be accounted for by lipid content, when C/N values are interpreted as lipid content indices, since the $\delta^{13}C$ should be lower for species with a higher lipid content. A more detailed analysis of the detritivore data shows that two groups could be distinguished, one consisting of woodlice, millipedes, and slugs, with a mean $\delta^{13}C$ value of −23.6‰ and a mean C/N of 5.8; and the other consisting of insects, with a mean $\delta^{13}C$ value of −24.9‰ and a mean C/N of 5.0. Differences in both $\delta^{13}C$ and C/N of the two groups were highly significant (one-tailed Student *t* test, $P << 0.0001$). The higher C/N of species in the first group was probably due to the fact that they have a higher inorganic carbon proportion in their exoskeleton than the insects. Inorganic carbon has higher $\delta^{13}C$ values than organic carbon, which can explain the difference in $\delta^{13}C$ between the groups. The $\delta^{13}C$ values of the detritivores of the first group are thus probably biased by the inorganic carbon they contained. The mean $\delta^{13}C$ value of the predators was −23.4‰. When only insect detritivores are considered, the difference between mean $\delta^{13}C$ values of detritivores and predators was highly significant ($P < 0.001$, one-tailed student *t* test), and its value (+0.5‰) was very close to the mean of +0.4‰ expected for one trophic transfer. Both inorganic carbon and lipids may have acted as confounding factors in the present study.

DISCUSSION

Isotopic signals in the soil and litter

Intrasite differences.—The soil is not isotopically homogeneous. The increase with depth we found for $\delta^{15}N$ and, to a lesser extent, for $\delta^{13}C$ is a pattern commonly observed in forest soils ($\delta^{13}C$, Balesdent et al. 1993; $\delta^{15}N$, Högberg 1997, and references therein). Whereas at least one global model has been proposed to account for the $\delta^{13}C$ values in different soil compartments (Ågren et al. 1996), $\delta^{15}N$ values are less well understood. They are the net result of several factors of various signs and amplitudes discussed in Nadel-

hoffer and Fry (1994) and Högberg (1997). In short, the $\delta^{15}N$ gradient observed in soils seems to be due to discrimination against heavy isotopes during mineralization processes in deeper soils, leaving the latter enriched, while plants would take up relatively ''light'' nitrogen pools. Although plant uptake by itself does not seem to involve any isotopic discrimination, this makes plants relatively depleted in ^{15}N compared to the organic matter remaining in the soil. The gradient would be maintained by the fact that the litter falling onto the surface reflects the relatively light nitrogen taken up by the plants.

Food items other than leaves are available for detritivores in the litter. Most of them probably have higher $\delta^{15}N$ values than the litter, be it feces (Steele and Daniel 1978, Checkley and Entzeroth 1985, Checkley and Miller 1986), dead/dying animals, fungi (Taylor et al. 1997), or plant parts other than leaves (Leavitt and Long 1982). Detritivores could also preferentially assimilate microorganisms that grow on the litter, rather than the leaves themselves. The litter and soil samples analyzed in the present study included both original plant material and microorganisms that have grown on it. One can only hypothesize about the possible isotopic differences between both fractions, since it seems practically impossible to isolate them for separate isotopic analyses.

The isotopic content of microorganisms depends on that of their substrate: on assimilatory fractionation, but also on the availability of the substrate (Mariotti 1982). They are probably enriched in ^{13}C relative to litter (Blair et al. 1985, Gleixner et al. 1993, Ågren et al. 1996; but see also Creach [1995]), but it is difficult to generalize about nitrogen enrichment, since contradictory results have been found (Macko and Estep 1984, Ziemans et al. 1984, Macko et al. 1987, Melillo et al. 1989, Handley and Scrimgeour 1997). For now, we can hypothesize that microorganisms are a potential food source for detritivores that is enriched in ^{13}C, compared to original leaf tissues in the litter, but it is not possible to say whether they are enriched or depleted in ^{15}N. Nadelhoffer and Fry (1988) showed that different biochemical components of the litter had very similar $\delta^{15}N$ values. On the contrary, various biochemical components of the litter (lignin, Benner et al. 1987) are strongly depleted in ^{13}C, compared to whole-leaf. Food items with various isotopic values are thus available to detritivores in the soil, and could possibly lead to differences in isotopic content between detritivores in the case of specialized species.

Inter-site differences.—The $\delta^{13}C$ values for a given horizon in different sites usually differed by <1 SD. On the contrary, there were substantial between-site differences in absolute $\delta^{15}N$ values of a given layer, although the relative differences between equivalent layers at a given site were similar. The lowest $\delta^{15}N$ values were found on site 1 and the highest on site 3. Similarly, Garten (1993) observed a gradient of $\delta^{15}N$ values in soils, soil solution and plants along a slope, with high $\delta^{15}N$ values near valley bottoms, and low values on ridges and slopes. His hypothesis to explain this gradient is that plants relied mainly on relatively ^{15}N-rich inorganic soil nitrogen near valley bottoms, while on ridges and slopes they used a higher proportion of nitrogen from ^{15}N-poor atmospheric deposition. There might be a similar trend at our study sites, since site 1 is situated at the edge of a plateau, and could therefore be more affected by nitrogen deposition than both other sites, situated at 50–60 m lower elevation.

Whatever the cause of the intersite differences in litter and soil $\delta^{15}N$, they clearly show that, even on a small spatial scale (<1 km), it is necessary to estimate the $\delta^{15}N$ value of litter at the bottom of the food web, and it is only *relative* to this baseline (not from their absolute values) that animal $\delta^{15}N$ values can be fruitfully interpreted. Some early studies (Schoeninger and DeNiro 1984) had shown that $\delta^{15}N$ values of marine species from various geographic origins corresponded well with their known trophic levels. While this might be done for very broad comparisons, our results suggest that, in the soil, only site-specific data are meaningful.

General structure of the trophic community

$\delta^{13}C$ values.—In all study sites, the detritivores and the predators had homogeneous $\delta^{13}C$ values, compared to the variance expected from interspecific differences of Δ_C. However, the entire set of species of a given site also constituted a homogeneous group. The variance of $\delta^{13}C$ of the group was often even smaller than $\sigma^2_{\Delta C}$. This could mean that $\sigma^2_{\Delta C}$ is overestimated in the literature. When only the detritivores with low inorganic carbon content were taken into account, and without correction for lipid content, the difference between mean detritivore and mean predator $\delta^{13}C$ values ($+0.5‰$) was close to $\Delta_C = +0.4‰$. However, the facts that $\sigma^2_{\Delta C}$ was large compared to Δ_C, that several biochemical confounding effects must be taken into account, and that $\delta^{13}C$ values were not correlated with $\delta^{15}N$, which is widely recognized as a better trophic-level index, suggest that $\delta^{13}C$ values cannot be interpreted in terms of trophic levels here (and maybe generally).

A striking feature of the data was that all the animal species examined (even those with $\delta^{13}C$ values unlikely to be biased by inorganic carbon) showed $\delta^{13}C$ values several per mil above the values found for the soil and litter, whereas animal tissues are expected to be enriched by only 0.4‰, compared to their diet. A similarly high difference between animal tissues and soil or litter $\delta^{13}C$ has been reported repeatedly for earthworms (Spain et al. 1990, Martin et al. 1992*a*, *b*, Spain and Le Feuvre 1997). Our results indicate that this feature is not limited to earthworms, but is common to soil detritivores in general. As suggested by Martin et al. (1992*a*), a possible explanation for these high $\delta^{13}C$ values is that detritivores are prevented from assimi-

lating the more ^{13}C-poor organic components of the litter and soil (e.g., lignin; Benner et al. 1987), because they lack the enzymes necessary to digest them (Lavelle et al. 1995), and therefore preferentially assimilate ^{13}C-rich organic carbon pools.

δ^{15}N values.—There were no recognizable groups of species that shared similar isotopic values and that were clearly separated from others with different values (Fig. 2), as would be expected in the case of a food chain with distinct trophic levels. On the contrary, there seemed to be a continuum of species exhibiting a gradual change of $\delta^{15}N$ values. This cannot be due to random variability of the $\delta^{15}N$ of a given species, since the $\delta^{15}N$ values of species trapped on different sites or at different periods consistently ranked approximately in the same order (Table 1).

The hypothesis that all sampled species form a homogeneous trophic group feeding, ''on average,'' on the same food sources also was rejected (Table 2). Therefore, the species we sampled spread over more than one trophic level.

Despite the presence of food sources with different $\delta^{15}N$ values in the litter and soil, the variance of the $\delta^{15}N$ values among detritivorous species was small enough for them to be considered as a single trophic group from the isotopic point of view, given the estimates we have of $\sigma_{\Delta N}$, except once (October, in site 2). If we subtract 3.4‰ from the mean detritivore $\delta^{15}N$ value, we find values between those found for the L and F layers. The detritivores thus seem either to feed on a superficial and rather young litter, or to feed selectively on some particular biochemical components that have a $\delta^{15}N$ value close to that of the superficial litter layers. It is not possible to determine whether the variability among species is due to interspecific differences of Δ, or whether it reflects true differences in diet, but the detritivores are homogeneous enough to be considered as a single trophic level.

The set of predatory species also had homogeneous $\delta^{15}N$ values, except in site 1 in April, and sites 1 and 3 in October. In all three cases, the rejection of the hypothesis of homogeneity was due to one or two species that showed a strong positive departure from the general mean. These species were spiders (two samples of juvenile *Araneus* sp., one of adult *Lepthyphantes minutus*, and one of adult *Atypus affinis*), as well as one Staphilinid beetle (*Philonthus cognatus*). However, during other sampling periods, the $\delta^{15}N$ values of *Lepthyphantes minutus* and *Atypus affinis* were similar to those of the other predators. Therefore, it is possible that these few exceptionally high values are artifacts, or that the sampled individuals were in a life stage in which cannibalism is high (this is especially likely for the juvenile spiders). However, it is also possible that these species truly occupy a higher position than the second trophic level. Even when the hypothesis of homogeneity was not rejected for the predators as a group, some species nevertheless showed relatively strong departures from the mean $\delta^{15}N$ values of the others. For instance, in July, one spider on site 1 (*Haplodrasus sylvestris*) had a $\delta^{15}N$ value $\sim$3‰ higher than the median value of all other predatory species on this site. Large spiders and several other taxa occupied comparably high positions on sites 2 and 3. The frequency of such species in our collection, and their departures from mean predator values, were not always large enough to reject the hypothesis that the latter group was isotopically homogeneous. However, it is difficult to decide whether some particular species with the highest $\delta^{15}N$ values should be considered as the upper end of the second trophic level, or as typical representatives of a third level. Either there are two distinct predator trophic levels (the second being poorly represented in our samples), or there is a continuum that ranges from predators feeding mainly on detritivores to predators that include a significant proportion of other predators in their diet.

Cabana and Rasmussen (1994) have stated that increased omnivory (in the sense of feeding on more than one level *below* ones' own) should decrease the value of $\delta^{15}N$ enrichment between consecutive ''trophic levels'' compared to a linear trophic chain. On the contrary, intraguild predation should *increase* it, since it means an increase of the number of trophic transfers (i.e., ''detours'' rather than ''short cuts'') in the pathways leading matter from the bottom to the top of the food web. In the present study, the difference between mean detritivore and mean predator $\delta^{15}N$ values was often $>$3.4‰. This still holds when the exceptionally high predator values are not taken into account. Although the difference between the mean detrivore and predator $\delta^{15}N$ values was significantly different from 3.4‰ only once, this may suggest that, rather than two distinct trophic levels of predators, there could be some degree of intraguild level predation among the predators. A further study specifically focused on predators, attempting to sample these species more exhaustively than we did, and maybe considering different life stages separately, would help clarifying this question. Note, however, that this pattern is consistent with the finding of Gunn and Cherrett (1993), who estimated by direct observation that there are 2.2 trophic levels in the soil macrofauna.

In sum, the $\delta^{15}N$ values of the litter macro-invertebrate species studied here suggest that they can be described as belonging to two trophic levels, one feeding on the superficial litter layers (or on soil fractions that have a similar $\delta^{15}N$ value), and the second feeding on the first, with some indication of intratrophic-level predation among the predators. This pattern was observed consistently in all three sites and all three seasons we studied. Cohen (1994) mentions isotopic studies as one of the ''new technologies'' that could be used to ''refine and extend the description and analysis of food webs'' in the future. Actually, they can probably bring much more ''extension'' than ''refinement'' to our knowl-

edge. They are, indeed, unable to provide a detailed picture of the trophic links between individual species in a food web. Nevertheless, they give a useful general description of the trophic structure of a community. One of the main limiting factors in the progress of food web studies has always been the tremendous amount of work needed to gather the necessary data. Since isotopic techniques are much faster and easier to use than classical ones, their extensive application to a variety of ecosystems could lead to a considerable increase in the number of documented food webs.

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APPENDIX

A list of species studied on each site, along with a priori diet, is available in ESA's Electronic Data Archive: *Ecological Archives* E081-009.