APPLIED SOIL ECOLOGY 38 (2008) 91–99

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/apsoil

Stable isotope natural abundances ($\delta^{13}C$ and $\delta^{15}N$) of the earthworm *Eisenia fetida* and other soil fauna living in two different vermicomposting environments

Luis Sampedro [a,*], *Jorge Domínguez* [b]

[a] *Departamento de Ecoloxía, Centro de Investigacións Ambientais de Lourizán, Xunta de Galicia, Apartado 127, Pontevedra, Galicia E-36080, Spain*
[b] *Dpto. Ecoloxía e Bioloxía Animal, Universidade de Vigo, E-36310 Vigo, Spain*

ARTICLE INFO

Article history:
Received 10 April 2006
Accepted 12 April 2007

Keywords:
Epigeic earthworms
Soil food web
Trophic relationships
Isotopic ratios

ABSTRACT

Manure heaps and vermicomposting systems are hotspots of heterotrophic activity supporting a high-detritivore biomass where epigeic earthworms interact intensively with bacteria, fungi and other soil fauna. We carried out a prospective study of the vermicomposting food webs using the natural abundance of stable C and N isotopes in 66 samples of soil fauna and the substrates in which the animals live in two systems: (i) a high-feeding-rate vermireactor, fed with pig slurry, and (ii) a farm manure vermicomposting heap fed with cattle manure. The aims of the study were specifically (i) to test the extent to which the isotopic signals in the earthworms resemble those of the substrates in which they live, (ii) to further our knowledge of the ontogenic changes in resource utilization of the earthworm *Eisenia fetida*, and (iii) to obtain information about the relative trophic position of the soil fauna in the food web of vermicomposting systems. Tissues of earthworms were significantly ^{15}N-enriched (by 4–8‰) relative to fresh and mature manures in both vermicomposting systems. The $\delta^{13}C$ values of adult earthworms were not different from those of the fresh animal wastes in both vermicomposting systems, suggesting that adult worms preferred fresh manure than worked materials as carbon source. The little but significant enrichment in ^{15}N observed in hatchlings living in the pig slurry vermicomposting bins relative to adult tissues likely reflect different feeding strategies, not observed in the cattle manure heap. Besides, hatchlings in the cattle manure heap appeared markedly depleted in ^{13}C (by ~5‰) relative to the adult earthworms, suggesting the use of a different source of carbon in the early stage. Diptera larvae presented very low values of $\delta^{13}C$, likely suggesting a relevant role of methanotrophic bacteria in their diet. Based on the shifts in $\delta^{15}N$, a taxon in the pig slurry vermicomposting bins may be assigned at least to three relative trophic positions separated by a ^{15}N shift of 2‰, with Enchytraeida clearly in the lower position, adults and hatchlings of *E. fetida* and nematodes in an intermediate level, and Collembola at the higher position showing an enrichment of 9‰ relative to the substrate. In the cattle manure heap three trophic levels may be also identified, with larvae of Diptera and Coleoptera as the less ^{15}N-enriched level, a general detritivore group in intermediate position, and finally a predatory taxa with a +9‰ shift comprised by Staphylinidae.

---

* *Corresponding author*. Tel.: +34 986 805078; fax: +34 986 856420.
E-mail address: lsampe@uvigo.es (L. Sampedro).

0929-1393/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.apsoil.2007.10.008

1. Introduction

In nature pure epigeic earthworms, such as *Eisenia fetida*, live in fresh organic matter in litter mounds, manure heaps and herbivore dung. Such environments are hotspots of heterotrophic activity where epigeic earthworms intensively interact with microorganisms and soil fauna within the decomposer community, strongly affecting decomposition processes (e.g. Aira et al., 2006b). Vermicomposting systems also support complex biotic assemblages with a very high-detritivore biomass comprised of decomposer bacteria and fungi, protozoa, rotifera, mites, nematodes, collembolans, enchytraeids and earthworms (Monroy, 2006). The decaying organic matter in these vermicomposting systems is a spatially and temporally heterogeneous matrix of organic resources with contrasting qualities due to different degradation rates during decomposition (see Moore et al., 2004). The biotic interactions of decomposers, i.e. bacteria and fungi, and the soil fauna, such as competition, mutualism, predation, facilitation, and the rapid changes that occur in both functional diversity and in substrate quality are main properties of these systems.

Also applicable to vermicomposting systems, a continuous range from pure detritivore to pure microbivore has been proposed as feeding strategies in detritus-based food webs (Scheu, 2002), although their trophic structure and specific resource utilization are poorly known. Some important features have been achieved in the knowledge of the trophic role of endogeic and anecic earthworms (Lavelle and Spain, 2001), but the role of epigeic earthworms in organic matter matrixes remains almost unknown. It is known that pure epigeic earthworms living in manure and vermicomposting systems accelerate decomposition processes (Aira et al., 2006a, 2007a), but where they obtain their energy inputs (i.e. decaying organic matter, microorganisms, microfauna or a combination of them) remains unknown; epigeic earthworms may utilize from non-selective substrate feeding to grazers strategies, and have the ability to shift between living and non-living carbon sources (Domínguez et al., 2003a; Sampedro et al., 2006).

Stable isotope techniques have been successfully used to study belowground communities in agricultural and forest soils (Scheu and Falca, 2000; Briones et al., 2001; Setälä and Aarnio, 2002; Schmidt et al., 2004). The carbon-isotope ratio ($^{13}C/^{12}C$) of the diet into consumers' tissues was showed to present a small shift of 0.5–2‰ due to fractionation in biochemical pathways and this finding has been used in studies of animal ecology and resource exploitation (e.g. Briones et al., 1999b; Staddon, 2004). The heavy isotope of nitrogen (^{15}N) is preferentially incorporated into the tissues of the consumer from the diet, resulting in a systematic enrichment of ca. 3.4‰ in the nitrogen-isotope ratio with each trophic level (Mianagawa and Wada, 1984) which has been useful for assessing relative positions in the food chains (Robinson, 2001). Although some exceptions to the above rule have been revealed for soil food webs (e.g. Schmidt, 1999; Ruess et al., 2004), stable isotopes of nitrogen are powerful tool in soil ecology (Scheu, 2002; Schneider et al., 2004).

Vermicomposting systems and the epigeic earthworms commonly used (*Eisenia* spp.) have been employed as experimental models for studying the structure and function of detritus-based food webs and adaptive strategies in earthworms (i.e. Aira et al., 2007b, 2007c; Domínguez et al., 2003b, 2005; Monroy et al., 2005). These systems, which are easy to handle, are suitable for prospective studies and manipulative experiments addressing unresolved issues in earthworm ecology, such as resource utilization in earthworms, including possible ontogenic differences, the source of the energy that they use for reproduction, and the energetic relationships with their gut microflora.

We carried out a prospective study of the food webs evolving in vermicomposting systems using stable isotope natural signatures. The aims of the study were specifically (i) to test the extent to which the isotopic signal in the earthworms resemble those of the substrates in which they live, (ii) to further our knowledge of the ontogenic changes in resource utilization of the earthworm *E. fetida*, and (iii) to get insight about the relative trophic position of the soil fauna of the food webs in the vermicomposting systems.

2. Materials and methods

2.1. *Sampling, fauna extraction and sample processing*

We studied the natural abundance of stable C an N isotopes in 66 samples of soil fauna and the substrates in which the animals live in two contrasting systems: (i) high-feeding-rate vermireactors, fed with pig slurry, a non-fermenter manure, and (ii) ruminant farm manure vermicomposting heaps, fed with cattle manure with a marked natural yearly cycle. We selected (i) two 200 L experimental pig slurry-fed vermicomposting bins installed in a greenhouse, belonging to the facilities of the University of Vigo; (ii) two outdoor cattle manure heaps in a nearby cattle farm, also supporting a dense population of *E. fetida* (Monroy et al., 2006). The pig slurry used for feeding the vermireactors was the same during all the time, previously collected at a neighbouring pig farm, homogenized and preserved in 200 L containers at 2 ± 2 °C. According to the common practices in our area, the farmers used the same mixture of pasture and fodder for feeding the cows during the whole season. Three randomly distributed replicate samples (ca. 2 kg fresh weight (f.w.) each; $N = 3$) were taken from each vermicomposting bin (pH 6.8; 321 ± 50 mg C g^{-1} dry weight (d.w.); 25 ± 1 mg N g^{-1} d.w.; mean values ± S.E.M.) and from each cattle manure heaps (pH 6.9; 460 ± 63 mg C g^{-1} d.w.; 16 ± 1 mg N g^{-1} d.w.) for macro- and mesofauna extraction. Subsamples (ca. 300 g f.w.) of the whole matrix of decomposing organic material, which will be referred throughout the text "worked substrate", were immediately frozen at −30 °C for isotopic analysis. Additional samples of the fresh pig slurry used for feeding the bins (pH 7.8; 338 ± 24 mg C g^{-1} d.w.; 28 ± 4 mg N g^{-1} d.w.) and the fresh cattle manure added to the manure heap (pH 7.2; 349 ± 11 mg C g^{-1} d.w.; 20 ± 6 mg N g^{-1} d.w.) were also taken and immediately frozen at −30 °C.

The extraction of soil fauna was performed immediately and sample processing was completed within 48 h of sampling. Coleoptera (family Staphylinidade and family Geotrupidae), Diptera larvae, earthworms and cocoons (*E. fetida*) were hand sorted directly from the samples, washed in tap water, then in distilled water and gently dried on paper tissue. Coleoptera, Diptera and cocoons were frozen at −30 °C into Pyrex tubes. Earthworms, grouped into two categories according their weight (less than and greater than 100 mg f.w.), were allowed to empty their guts in sterile Petri dishes (20 h at 20 °C), and were then euthanized by brief immersion in distilled water at 60 °C and frozen at −30 °C. Hatchlings weight ranged 30–90 mg f.w., and earthworms weighing more than 100 mg (individual weight ranged 200–400 mg f.w.) were mainly mature.

Collembola and enchytraeids were obtained by accelerated wet extraction gently dispersing subsamples of manure (ca. 200 g f.w.) with 600 mL of cold distilled water for 5 min in glass beakers. Collembola (*Proisotoma minuta* (Tullberg, 1871) and *Folsomia candida* (Willem, 1902)) were separated by flotation, immediately collected from the water surface on a piece of aluminium foil, gently removed with a paintbrush, washed again with distilled water, then concentrated by decantation and frozen at −30 °C in Pyrex tubes. Enchytraeids were collected from the bottom of the beaker by brief decantation (12 h at 4 °C) and washed on a polyethylene sieve (50 μm) with cold distilled water before being frozen at −30 °C. The washing water was examined under a binocular microscope and found to contain a few broken specimens.

Nematodes were extracted from subsamples of 20 g f.w. of manure using a modified Baermann funnel method. The extracts were filtered through a polyethylene sieve (100 μm) to separate the enchytraeids, allowed to settle for 12 h at 4 °C, then rinsed on a polyethylene sieve (20 μm), and the fraction on the sieve was then carefully collected with water on glass fibre filters (Millipore GF prefilters 45 mm diameter, previously burned in a muffle furnace) and immediately frozen at −30 °C in cryotubes. All samples were checked under dissecting microscope to avoid the presence of enchytraeids or any material other than nematodes. Mites were extracted using Berlesse dry hot extraction, but they were scarce and the material obtained was not enough for isotopic analysis. The same happened for enchytraeids, nematodes and collembolans obtained from the cattle manure heaps.

In order to reduce unnecessary animal killing, subsamples for isotopic analyses were prepared randomly taken a fraction of the whole biological material extracted from each replicate sample. They consisted in 20 adult earthworms, 60 earthworm hatchlings, 30 cocoons, 6 beetles and ca. 70 mg d.w. of enchytraeid worms, Collembola and Diptera larvae. We used all the nematodes obtained after the sample extraction (ca. 20 mg d.w.). After freezing, samples were freeze-dried and the whole sample pulverised using either mortar and pestle for animal samples, or a shaking mill for manure samples.

2.2. *Isotopic analysis*

Subsamples (1–5 mg d.w.) were taken from each pulverised sample, weighted into ultra-clean tin capsules and analysed with an isotope ratio mass spectrometer Finnigan MAT Delta Plus (Finnigan Mat, Bremen, Germany) coupled to two elemental analyzers Flash EA 1112 ThermoQuest by a Finnigan MAT ConFlo II interface, in the central laboratory facilities of the University of A Coruña (http://www.sxain.udc.es/sxain/). The amount of sample was previously adjusted to ensure a C:N that allow analysis in the dual isotope mode in which C and N isotope ratios are measured simultaneously on the sample. Duplicate analyses were performed on all the samples. Calibration for ^{13}C was done against the certificated standards NBS22, sucrose and graphite (all from National Bureau of Standards, Gaithersburg, USA), and for ^{15}N against IAEA-N1, IAEA-N2 and IAEA-NO3 (International Atomic Energy Agency, Vienna, Austria). The coefficients of variation of the device in this mode for the certificated standards were less than 0.3% and 0.5% for ^{13}C and ^{15}N determinations, respectively.

Stable isotope ratios of carbon and nitrogen are expressed as the ratio of heavy-to-light carbon and nitrogen relative to V-PDB limestone ($^{13}C/^{12}C$) and atmospheric dinitrogen standards ($^{15}N/^{14}N$), using conventional delta notation ($\delta^{13}C$, $\delta^{15}N$) in parts per thousand: $\delta X‰ = (R_{sample}/R_{standard} - 1) \times 1000‰$, where $X = {}^{13}C$ or ^{15}N and $R = {}^{13}C/{}^{12}C$ or $^{15}N/^{14}N$, respectively.

2.3. *Statistical analysis*

To study the modifications of the isotopic signature of *E. fetida* through its life cycle (namely adults, hatchlings and cocoons) we conducted GLM analysis on data from the two pig slurry vermicomposting bins and the two cattle manure heaps separately for each manure type. As the data matrix was unbalanced due to the absence of cocoons in some sites, we used a Main effects ANOVA in the GLM procedure (Hill and Lewicki, 2006). The study of the stable isotope signatures in the soil fauna of the food webs was also performed separately for each manure type using the complete dataset from only one pig slurry vermicomposting bin and from one cattle manure heap. To better identify the relative trophic positions in the food web as revealed by the expected increase in ^{15}N up through the food chain, we assumed that the basic energy source for the whole food web in the vermicomposting system was the decaying organic matter, i.e. the substrate of the bins and heaps, which at the same time is the physical matrix supporting the decomposer community. In accordance with this assumption, we calculated the $\Delta^{15}N$ values relative to this baseline in both animal wastes. Values are shown as mean ± S.E.M. Tukey HSD test for separation of means was used at $\alpha = 0.01$ in all cases. All the analyses were conducted using Statistica 6.0 (StatSoft, Tulsa, OK).

3. Results

3.1. *Isotopic signals in decomposing manures*

The nature of the manure applied to each system strongly influenced the shift in the C stable isotopic composition between the fresh manure and the vermicomposts that the fauna live in (Fig. 1). The worked substrate in the pig slurry bins appeared depleted in the heavy C isotope, by ~3‰, relative to the fresh pig slurry, indicating fractionation during the decomposition process, whereas in the cattle manure heaps no significant changes in $\delta^{13}C$ were observed. Both manures showed significant enrichment in ^{15}N during decomposition following the expected trend, and this process was more evident in pig slurry ($\Delta^{15}N = +3‰$) than in cattle manure ($\Delta^{15}N = +1.8‰$). This shift in $\delta^{15}N$ appeared to be proportional to the N concentration in the worked substrates ($R^2 = 0.45$; $P < 0.001$; $N = 15$).

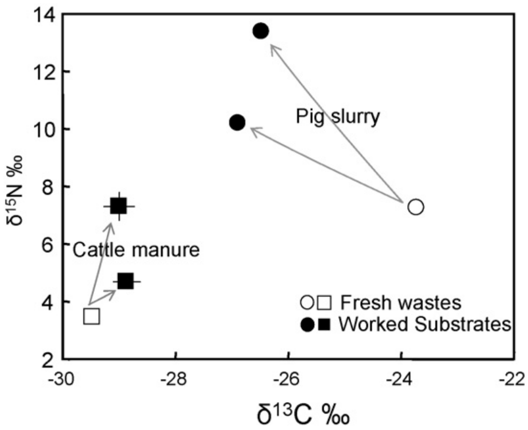

Fig. 1 – Dual plot of δ^{13}C and δ^{15}N patterns in the worked substrates (dark symbols) of vermicomposting bins feed with pig slurry (two sites) and cattle manure heaps (two sites), and in the corresponding fresh organic wastes (open symbols). Mean ± S.E.M.; $N = 3$. Analyses were performed separately for each kind of animal waste, with two sites each. Within each organic waste, all changes significantly differ at $P < 0.01$, unless changes in δ^{13}C for cattle manure ($P > 0.05$).

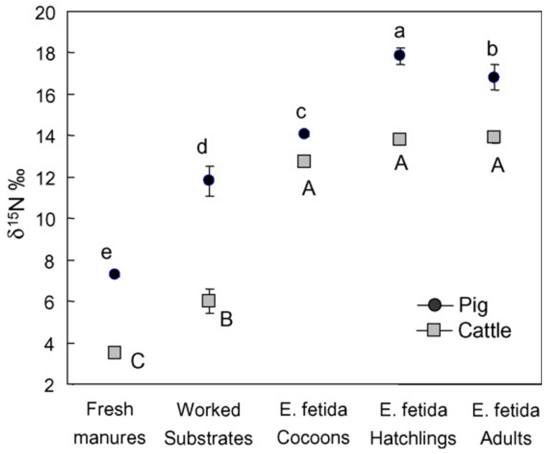

Fig. 2 – δ^{15}N signatures at three stages of the life cycle of the epigeic earthworm *Eisenia fetida*, the worked substrates in which they live, and the fresh manure applied to feed the vermicomposting bins and the cattle manure heaps. Analyses were performed separately for each kind of animal waste, with two sites each, and main effects were $F_{(4,18)} = 344$; $P < 0.001$ for pig slurry and $F_{(4,15)} = 231$; $P < 0.001$ for cattle manure. Different letters indicate significant differences within the same waste (capital and lower case letters), Tukey HSD, $\alpha = 0.01$. Error bars indicate the S.E.M.; sample size differs between categories, being 3 or 6.

3.2. Isotopic ratios through the life cycle of the earthworm E. fetida

The N isotopic signatures in the life stages of *E. fetida* revealed different trends in the non-fermenter pig slurry vermicomposting bins and the ruminant manure heaps (Fig. 2). Earthworm tissues were significantly enriched in ^{15}N (by 4–8‰) relative to fresh manure and worked substrate in both pig and cattle systems (Fig. 2). In the two pig slurry vermicomposting bins the hatchlings of *E. fetida* showed a δ^{15}N significantly greater than the adult earthworms ($\Delta = +1$‰; $P < 0.01$) whereas in the cattle manure heaps the difference was not significant ($P > 0.05$).

The ANOVA performed on the δ^{13}C in the two pig slurry vermicomposting bins showed that hatchlings and adult earthworms were enriched in ^{13}C relative to the signature of the worked substrate by at least 2‰, but did not differ significantly from the fresh pig slurry. However, hatchlings in the cattle manure heaps were markedly depleted in ^{13}C (by ~5‰) relative to the adult earthworms, which did not differ from the available food sources (Fig. 3).

The δ^{13}C for cocoons in the pig slurry vermicomposting bins was the same as for hatchlings and adult earthworms, but the cocoons in the cattle manure heaps were enriched by 2‰. Cocoons were significantly depleted in ^{15}N (by 2.7‰) relative to the adult earthworms in the pig slurry vermicomposting bins but not in the cattle manure heaps (Fig. 3).

3.3. Vermicomposting food web

Soil fauna differed notably in the pig slurry vermicomposting bins and the cattle manure heaps. The pig vermicomposting bins, placed in a greenhouse, lacked coleopteran other than small Staphylinidae beetles (ca. 500 mg d.w. individual weight), and displayed high densities of enchytraeids, nematodes and large collembolans; the cattle manure heap presented an active beetle population, a high density of Diptera larvae, no enchytraeids, and low densities of nematodes and collembolans.

The trophic shifts in δ^{15}N observed in each group of soil fauna, measured as Δ^{15}N with regard to the corresponding fresh vermicompost are summarized in Fig. 4. In the pig slurry vermicomposting bins, the GLM analysis revealed four separate groups ($P < 0.01$) distanced by about 2‰ Δ^{15}N shift (Fig. 4(a)). The group more enriched with ^{15}N was the grazer collembolans, followed by nematodes and earthworm hatchlings forming a separate group. Adult earthworms and enchytraeids showed more depleted δ^{15}N values. The less ^{15}N-enriched isotopic signature corresponded to Staphylinidae. In the cattle manure heap, three possible trophic levels were identified. The lower level was occupied by Diptera larvae and Coleoptera, enriched in ^{15}N by 3‰ relative to the worked substrate in which they live. The next level showed a Δ^{15}N ca. 7‰ and included dung beetles (family Geotrupidae) and both young and adult earthworms (Fig. 4(b)). The higher trophic level revealed by ^{15}N analysis in the cattle manure heap corresponded to the beetles belonging to the family Staphylinidae. The Δ^{15}N values of these beetles in the cattle manure were similar to those of the collembolans living in the pig slurry vermicomposting bins (Fig. 4(b)).

The study of the δ^{13}C signatures in the soil fauna communities of pig slurry and cattle manure systems showed contrasting trends (Fig. 5). In the pig slurry food web (Fig. 5(a)), the δ^{13}C values of all the groups appeared within the range of

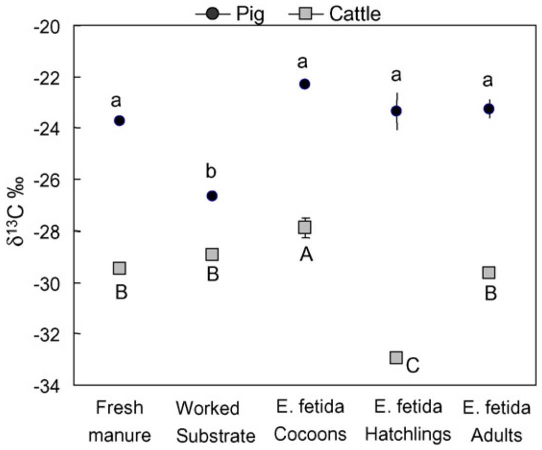

Fig. 3 – δ^{13}C signatures at three stages of the life cycle of the epigeic earthworm *E. fetida*, the worked substrates in which they live, and the fresh manure applied to feed the vermicomposting bins and the cattle manure heaps. Analyses were performed separately for each kind of animal waste, with two sites each, and main effects were $F_{(4,18)} = 23$; $P < 0.001$ for pig slurry and $F_{(4,15)} = 68$; $P < 0.001$ for cattle manure. Different letters indicate significant differences within the same waste (capital and lower case letters), Tukey HSD, $\alpha = 0.01$. Error bars indicate the S.E.M.; sample size differs between categories, being 3 or 6.

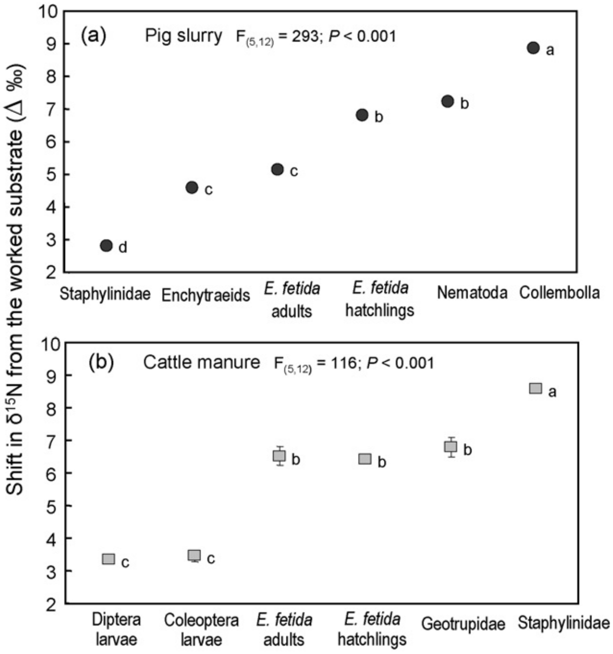

Fig. 4 – Shift in the δ^{15}N (expressed as Δ‰) of some components of the micro- and mesofauna of two vermicomposting systems with regard to the worked substrates in which they live. (a) A pig slurry vermicomposting bin and (b) a cattle manure heap. Different letters denote significant differences at $\alpha = 0.01$, Tukey HSD test. Error bars indicate the S.E.M.; $N = 3$.

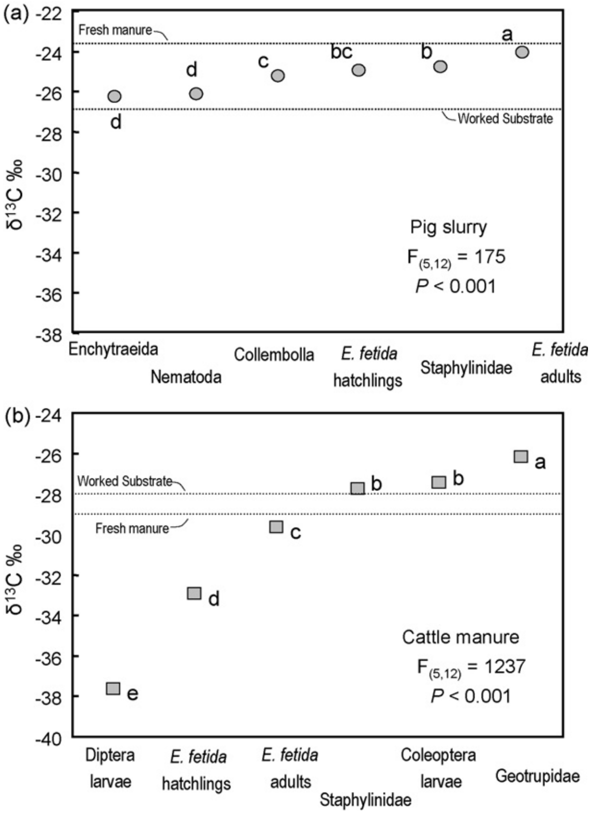

Fig. 5 – δ^{13}C signatures of some components of the micro- and mesofauna in (a) a pig slurry vermicomposting bin and (b) a cattle manure heap. Different letters denote significant differences at $\alpha = 0.01$, Tukey HSD test. Error bars indicate the S.E.M.; $N = 3$. The doted lines show the δ^{13}C signatures of each worked substrate and fresh manure.

isotopic signatures in the fresh vermicompost and in the fresh pig slurry, a narrow range of 2‰ δ units. The δ^{13}C for the adult earthworms was the less depleted and the closest to that in fresh pig slurry, whereas nematodes and enchytraeids showed the more depleted carbon composition, quite close to the isotopic signature of the worked substrate.

The picture of the soil fauna living in the cattle manure heap changed markedly (Fig. 5(b)). The three Coleoptera groups were lightly ^{13}C-enriched (up to +3‰) relative to the cattle manure. The lower δ^{13}C values for the isotopic label of adult earthworms were similar to the values of the worked substrate in which they live and feed. However, the Diptera larvae were strongly depleted (by 9‰) relative to the food that they supposedly ingest, whereas the δ^{13}C values corresponding to earthworm hatchlings were not as low but also much more depleted than that of the fresh manure.

4. Discussion

4.1. *Isotopic signals in decomposing manures*

The progressive depletion of ^{13}C observed in the pig slurry vermicomposting bins corresponded to the expected pattern of a progressive increase in the concentration of ^{13}C-depleted lignin-rich materials. However the absence of changes in the cattle manure heap was not consistent with this trend, suggesting the existence of positive fractioning effects that counterbalance the generally observed depletion during decomposition (Schweizer et al., 1999). Both enrichment and depletion in ^{13}C have also been observed during the decomposition of litter from different plant species in arid ecosystems (Connin et al., 2001; Crow et al., 2006).

One important factor affecting δ^{13}C in soil organic matter (SOM) is that isotopic fractionation during respiratory activity (mainly of microbial origin) favours the mineralization of light carbon, leading to increasingly negative δ^{13}C values (^{13}C enrichment) in the remaining decomposing organic matter over time (Schweizer et al., 1999; Neilson et al., 2002). Moreover, easily available compounds are usually ^{13}C-enriched, whereas the more recalcitrant remaining material such as lignin is usually ^{13}C-depleted by 2–6‰ compared to the bulk plant material and by 4–7‰ relative to cellulose (Benner et al., 1987).

Furthermore, selective resource utilization by soil heterotrophs, such as fungi and bacteria using recalcitrant and more labile C, respectively, may largely affect the evolution of δ^{13}C in decomposing OM. Thus, the effect of selective resource utilization may even counterbalance the effects of the metabolic fractionation (Santruckova et al., 2000). We could speculate that the decomposition of ruminant manure rich in recalcitrant material would be dominated by fungi, whereas decomposition of the more labile non-fermenter pig slurry would be dominated by bacteria. This would favour selective resource utilization of ^{13}C-depleted materials in the former and a metabolic fractionation in the latter, leading to enrichment and depletion in the remaining OM, respectively (Santruckova et al., 2000).

As regards the N isotope ratio, ^{15}N enrichment of SOM with depth and substrate age is commonly observed in forest, grassland and agricultural ecosystems (e.g. Ponsard and Arditi, 2000, and references therein). Both pig slurry and cattle manure showed a consistent trend of progressive enrichment, which is in agreement with the pattern observed in the ageing SOM, probably due to microbial discrimination (Steele et al., 1981; Nadelhoffer and Fry, 1988). Nevertheless, as in the case of ^{13}C, there may be several processes involved in the changes and we cannot generalize the observed trends due to the lack of isotopic studies in decomposing organic wastes.

4.2. *Isotopic ratios through E. fetida life cycle*

4.2.1. *Adult earthworms*

Our results show a consistent enrichment of ^{15}N in the earthworm tissues with δ^{15}N values of ~+8‰ in earthworms in both vermicomposting systems; the δ^{13}C values corresponded to enrichment of ca. 5‰ in earthworms fed on pig slurry, and depletion of 1–5‰ in earthworm living in cattle manure. Uchida et al. (2004) reported enrichment of ca. 5‰ for ^{13}C and 3–10‰ for ^{15}N in *Eisenia japonica* living in three different types of litter types in the only other relevant study concerning this genus. Other authors studying soil feeding and litter-feeding earthworms also reported earthworm tissues being enriched in ^{15}N by 4–11‰ (e.g. Schmidt et al., 1997; Hendrix et al., 1999; Briones et al., 1999a), and a consistent δ^{13}C enrichment of ~+3‰ in earthworm species relative to the dominant vegetation and dietary sources (Spain et al., 1990; Hendrix et al., 1999; Neilson et al., 2000). Fractionation towards lighter C isotopes is known to occur during OM respiration, leading to progressive enrichment by the heavy isotope in soil fauna tissues (Staddon, 2004), although the observed shift in ^{13}C may also be explained by preferential assimilation of certain ^{13}C-enriched labile-fractions by the earthworms (Martin et al., 1992) or selective consumption of microbial biomass, which is ^{13}C-enriched relative to bulk soil (Gregorich et al., 2000). The origin of the unexpected depletion in ^{13}C in earthworm tissues observed in *E. fetida* living in cattle manure should be studied in future experiments.

The finding that the δ^{13}C values in adult earthworms in both systems did not differ from those in fresh manures, indicating the origin of their C source, is in agreement with the common observation that adult earthworms move through the matrix of decomposing manure feeding preferentially on the fresh manure deposited on the surface of the vermicomposting beds.

4.2.2. *Hatchlings*

The slight but significant enrichment in ^{15}N observed in hatchlings living in the pig slurry vermicomposting bins (Fig. 3) relative to adult tissues, reflecting the possibility of a higher trophic position of the younger individuals, and the depletion in ^{13}C observed in the cattle manure heaps (Fig. 4), evidencing the use of a different source of carbon, likely reflect different feeding strategies. A possible explanation for the carbon fractionation in earthworm hatchlings may occur through selective feeding on live or dead organic matter derived from ^{13}C-depleted fractions. Other authors reported that juvenile soil feeding earthworms were enriched in ^{13}C relative to adults (Lachnicht et al., 2002). However, N isotopic ratios appeared to be greater in adult *A. longa* and *L. terrestris* than in juvenile individuals (Schmidt, 1999; Scheu and Falca, 2000). According to the model by Ponsard and Averbuch (1999), "there should be no difference in δ^{15}N values between a growing individual and an adult feeding on the same diet". Thus, assuming similar resource allocation and assimilation rates in both hatchlings and adult earthworms, the difference in N isotopic content may be explained by the use of different N sources or by feeding on a nitrogen-poor diet (Ponsard and Averbuch, 1999).

4.2.3. *Cocoons*

We observed opposing trends of isotopic values in *E. fetida* cocoons depending on the isotope and depending on the manure considered. The results for δ^{13}C suggest that earthworms may adopt different reproductive strategies depending on the availability of resources. The divergent strategies (depleted/enriched) observed in the different life-cycle stages of *E. fetida* that depend on the nature of the organic residue highlight the plasticity in the feeding and reproductive behaviour in relation to the habitat and the food source. Little is known on isotopic values for earthworm cocoons, especially for epigeic earthworms. The only available study considered soil feeding earthworm species and it related enrichment in both ^{13}C and ^{15}N in cocoons relative to the adult worm tissues (Lachnicht et al., 2002). Nevertheless opposed shifts in the isotopic signatures between the two vermicomposting environments may also reflect contrasting habitat heterogeneity (Ponsard and Arditi, 2000; Neilson et al., 2000), and/or a broad range of isotopic signatures among the diverse chemical compounds within each organic waste (Ågren et al., 1996).

4.3. *Vermicomposting food web*

On the basis of shifts in δ^{15}N, the taxa in the pig slurry vermicomposting bins may be assigned to at least three relative trophic positions, with general detritivores (Enchytraeida and adult earthworms) in the lower position; hatchlings of *E. fetida* and nematodes, which could be identified as a microbivore group at an intermediate level; the grazer Colembolla clearly in the highest position, showing an enrichment of 9‰. In the cattle manure heap, three trophic levels were identified, with Diptera and Coleoptera larvae the less ^{15}N-enriched level, a general detritivore group showing a 6–7‰ shift, and finally a predatory level with a +9‰ shift corresponding to Staphylinidae.

The low position of enchytraeids is not consistent with the results of the exhaustive study carried out by Scheu and Falca (2000), who reported δ^{15}N values for enchytraeids similar to those for endogeic earthworm species, at a higher trophic level (by about +3‰) than epigeic and anecic litter-feeding earthworms. Collembola are assumed to ingest a broad range of materials such as fungi, algae, litter material and even other soil fauna (Scheu and Falca, 2000; Ruess et al., 2005a,b). In agreement with these findings, a higher trophic position was assigned to Collembola than to *Lumbricus* spp. ($\Delta\delta^{15}$N = +2‰) in a beech forest food web (Caner et al., 2004). According to Chahartaghi et al. (2005), the large collembolans found in the pig slurry vermicomposting bins could be assigned to the category of secondary decomposers, feeding predominantly on fungi, which is consistent with previous observations for large collembolans that usually live in vermicomposting beds (Monroy, 2006). However, predatory behaviour may also be consistent with the observed δ^{15}N values, because Collembola feeding on nematodes showed a enrichment from nematode tissues of Δ^{15}N = +0 to 6‰ when reared in several diets at the laboratory (Ruess et al., 2004). Although nematodes in vermicomposting beds are known to be mainly fungivorous and bacterivorous (Domínguez et al., 2003a; Monroy, 2006), the isotopic signatures observed in the whole nematode group were affected by the trophic diversity of the nematode community. Our results show that nematodes could be close to earthworm hatchlings both in carbon resource use and in trophic position.

Beetles belonging to Staphilinidae are recognized as facultative predators and they have been observed to occur at a high-trophic level among the soil macrofauna (Scheu and Falca, 2000). But there are also some non-predatory groups, such as saprovorous and even fungal feeders growing elsewhere (see in instance Ashe, 1984). A likely explanation to the different δ^{15}N signatures of Staphilinidae in pig and cattle manure is that we are considering two different trophic groups of these coleopterans, with major predatory habits in the field-conditions cattle manure heap, while fungal feeders dominating in the warm, humid and plenty of resources pig slurry vermireactors. The lack of more detailed taxonomic information is limiting the interpretation of the trend in this case.

The shift in δ^{15}N detected in earthworm hatchlings living in the pig slurry vermicomposting bins was not sufficient to assign a higher level to hatchlings than to adult earthworms. Assignation to different trophic levels was established among earthworm species with δ^{15}N signatures separated significantly by 1.5–4% (Schmidt et al., 1997, 2004; Scheu and Falca, 2000). On the other hand, earthworms may change their feeding behaviour, and the isotopic signatures would then reflect feeding guilds rather than trophic groups, as proposed by Scheu (2002). Minor changes, such as higher rates of assimilation of the earthworm gut microflora could lead to higher δ^{15}N and functional appearance at higher trophic levels (Sponheimer et al., 2003).

The observed shifts in ^{15}N were sufficient to discriminate clearly between groups, but were generally lower than the expected 3.4‰, in contrast to the findings of Ponsard and Arditi (2000), who reported equal or higher δ^{15}N shift (than the expected 3.4‰) between detritivores and predators belonging to forest soil macrofauna. Our results may indicate the existence of complex feeding strategies, probably in a continuum from detritus feeder to pure microbivory, in accordance with the observations that omnivory is common in detritus-based food webs (Ponsard and Arditi, 2000, 2001; Eggers and Jones, 2000). Most of the macro- and mesofauna living in manure heaps at least partially ingest the surrounding worked substrate, irrespective of their position in the detritivore–microbivore range of trophic level, and they possibly obtain some energy from the decaying organic matter. On the other hand, the progressive changes in δ^{15}N values observed do not provide evidence of intralevel predation.

Most of the analyzed fauna in the pig slurry vermicomposting bin showed δ^{13}C values within those of fresh waste and worked substrate, indicating that all the groups were likely sharing, at least in part, the same original carbon resources, in spite of their position in the detritivore–predator range of feeding strategies. However, in the heterogeneous cattle manure heap the δ^{13}C values of food web evidenced a strong alimentary fractionation, likely indicating selective feeding on depleted C sources, although particular features related to the digestive systems of these species and the use of an exogenous or non-sampled carbon source cannot be discounted.

One striking feature observed was that the lowest trophic position in the cattle manure heap, as indicated by δ^{15}N values, was occupied by Diptera and Coleoptera larvae, but δ^{13}C signatures clearly suggested that they used different carbon resources. Although Coleoptera larvae appeared to assimilate the whole worked substrate, Diptera larvae showed a strong fractionation, indicating (i) more ^{13}C-depleted substrates, which usually correspond to the more recalcitrant C fractions, as potential C sources, or (ii) the presence of other biogenic fractionation not previously accounted for. In this sense, recent studies have reported much depleted δ^{13}C values in detritivorous Diptera larvae of lake systems (namely chironomids) as low as 64‰ (Bunn and Boon, 1993; Kiyashko et al., 2001; Kelly et al., 2004). Bunn and Boon (1993) and Kelly et al. (2004) postulated that the low δ^{13}C signatures result from the ingestion of methanotrophic bacteria and subsequent incorporation of the biogenic ^{13}C-depleted methane-derived C released from anoxic sediments. The re-introduction of some of this extremely light C into metazoan food webs via the consumption of methanotrophic bacteria could likely be a reason for the low $\delta^{13}C$ values observed in Diptera larvae, implying a very interesting specific digestive function involving methanogenic fermentation that should be further studied.

A main shortcoming of the present study is the grouping of the fauna by major taxonomic groups, assuming that close taxonomic position imply similar resource utilization, a simplification that is widely used to reduce the experimental effort to acceptable levels. Although the diversity within the selected taxa in composting heaps is really low, with one or two major species accounting for most of the biomass within each group (Monroy, 2006), such a simplification is probably highly unrealistic for soil food webs (Scheu and Falca, 2000; Scheu, 2002).

In summary, the taxonomic units we analysed were grouped by their ^{15}N signatures into discrete assemblages, providing evidences that consistent trophic structures exists, but that the trophic role can change depending on life-cycle stage and probably on resource quality. Nevertheless, the observed patterns in isotopic composition should be validated experimentally for application to trophic studies. Furthermore, our results revealed interesting features regarding the feeding habits of some groups such as Diptera larvae and earthworm hatchlings. This initial approach provides relevant information for designing future manipulative experiments exploring the trophic guilds in highly dynamic heterotrophic systems, and also for studies investigating the strategies of resource allocation in earthworms.

Acknowledgements

We thank to Fernando Monroy for his guidance with the fauna extraction and to one anonymous referee for his critical review of the manuscript. Pascal Querner has determined collembola to species level. We thank to Castor Guisande for the use of his freeze-drier. This research was financially supported by grants from the CICYT (AGL2003-01570) and Xunta de Galicia (PGIDIT03PXIB30102PR).

REFERENCES

Ågren, G.I., Bosatta, E., Balesdent, J., 1996. Isotope discrimination during decomposition of soil organic matter: a theoretical analysis. Soil Sci. Soc. Am. J. 60, 1121–1126.

Aira, M., Monroy, F., Domínguez, J., 2006a. Changes in microbial biomass and microbial activity of pig slurry after the transit through the gut of the earthworm *Eudrilus eugeniae* (Kinberg, 1867). Biol. Fertil. Soils 42, 371–376.

Aira, M., Monroy, F., Domínguez, J., 2006b. *Eisenia fetida* (Oligochaeta, Lumbricidae) activates fungal growth, triggering cellulose decomposition during vermicomposting. Microb. Ecol. 52, 738–746.

Aira, M., Monroy, F., Domínguez, J., 2007a. *Eisenia fetida* (Oligochaeta: Lumbricidae) modifies the structure and physiological capabilities of microbial communities improving carbon mineralization during vermicomposting of pig manure. Microb. Ecol. 54, 662–671.

Aira, M., Domínguez, J., Monroy, F., Velando, A., 2007b. Stress promotes changes in resource allocation to growth and reproduction in a simultaneous hermaphrodite with indeterminate growth. Biol. J. Linean Soc. 91, 593–600.

Aira, M., Monroy, F., Domínguez, J., 2007c. Microbial biomass governs enzyme activity decay during aging of worm-worked substrates through vermicomposting. J. Environ. Qual. 36, 448–452.

Ashe, J.S., 1984. Major features of the evolution of relationships between gyrophaenine staphylinid beetles (Coleoptera: Staphylinidae: Aleocharinae) and fresh mushrooms. In: Wheeler, Q., Blackwell, M. (Eds.), Fungus–Insect Relationships; Perspectives in Ecology and Evolution. Columbia University Press, New York, pp. 227–255.

Benner, R., Fogel, M.L., Sprague, E.K., Hodson, R.E., 1987. Depletion of ^{13}C in lignin and its implications for stable isotope studies. Nature 329, 708–710.

Briones, M.J.I., Bol, R., Sleep, D., Sampedro, L., Allen, D.A., 1999a. A dynamic study of earthworm feeding ecology using stable isotopes. Rapid Commun. Mass Spectrom. 13, 1300–1304.

Briones, M.J.I., Ineson, P., Sleep, D., 1999b. Use of $\delta^{13}C$ to determine food selection in collembolan species. Soil Biol. Biochem. 31, 937–940.

Briones, M.J.I., Bol, R., Sleep, D., Allen, D., Sampedro, L., 2001. Spatio-temporal variation of stable isotope ratios in earthworms under grassland and maize cropping systems. Soil Biol. Biochem. 33, 1673–1682.

Bunn, S.E., Boon, P.I., 1993. What sources of organic carbon drive food webs in billabongs? A study based on stable isotope analysis. Oecologia 96, 85–94.

Caner, L., Zeller, B., Dambrine, E., Ponge, J.F., Chauvat, M., LLanque, C., 2004. Origin of the nitrogen assimilated by soil fauna living in decomposing beech litter. Soil Biol. Biochem. 36, 1861–1872.

Chahartaghi, M., Langel, R., Scheu, S., Ruess, L., 2005. Feeding guilds in Collembola based on nitrogen stable isotope ratios. Soil Biol. Biochem. 37, 1718–1725.

Connin, S.L., Feng, X., Virginia, R.A., 2001. Isotopic discrimination during long-term decomposition in an arid land ecosystem. Soil Biol. Biochem. 33, 41–51.

Crow, S., Sulzman, E., Rugh, E.W., Bowden, W.D., Lajtha, R.D.K, 2006. Isotopic analysis of respired CO_2 during decomposition of separated soil organic matter pools. Soil Biol. Biochem. 38, 3279–3291.

Domínguez, J., Parmelee, R.W., Edwards, C.A., 2003a. Interactions between *Eisenia andrei* (Oligochaeta) and nematode populations during vermicomposting. Pedobiologia 47, 53–60.

Domínguez, J., Velando, A., Aira, M., Monroy, F., 2003b. Uniparental reproduction of *Eisenia fetida* and *E. andrei* (Oligochaeta: Lumbricidae): evidence of self-insemination. Pedobiologia 47, 530–534.

Domínguez, J., Ferreiro, A., Velando, A., 2005. Are *Eisenia fetida* (Savigny, 1826) and *Eisenia andrei* Bouché, 1972 (Oligochaeta, Lumbricidae) different biological species? Pedobiologia 49, 81–87.

Eggers, T., Jones, H.T., 2000. You are what you eat... or are you? Trends Ecol. Evol. 15, 265–266.

Gregorich, E.G., Liang, B.C., Drury, C.F., Mackenzie, A.F., McGill, W.B., 2000. Elucidation of the source and turnover of water soluble and microbial biomass carbon in agricultural soils. Soil Biol. Biochem. 581–587.

Hendrix, P.F., Lachnicht, S.L., Callahan Jr., M.A., Zou, X., 1999. Stable isotopic studies of earthworm feeding ecology in tropical ecosystems of Puerto Rico. Rapid Commun. Mass Spectrom. 13, 1295–1299.

Hill, T., Lewicki, P., 2006. STATISTICS Methods and Applications. StatSoft, Tulsa, OK.

Kelly, A., Jones, R.I., Grey, J., 2004. Stable isotope analysis provides fresh insights into dietary separation between

Chironomus anthracinus and C. plumosus. J. N. Am. Benthol. Soc. 23, 287–296.

Kiyashko, S.I., Narita, T., Wada, E., 2001. Contribution of methanotrophs to freshwater macroinvertebrates: evidence from stable isotope ratios. Aquat. Microb. Ecol. 24, 203–207.

Lachnicht, S.L., Hendrix, P.F., Zou, X., 2002. Interactive effects of native and exotic earthworms on resource use and nutrient mineralization in a tropical wet forest soil of Puerto Rico. Biol. Fertil. Soils 36, 43–52.

Lavelle, P., Spain, A.V., 2001. Soil Ecology. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Martin, A., Balesdent, J., Mariotti, A., 1992. Earthworm diet related to soil organic matter dynamics through ^{13}C measurements. Oecologia 91, 23–29.

Mianagawa, M., Wada, E., 1984. Stepwise enrichment of 15-N along food chains: further evidence and the relation between ^{15}N and animal age. Geochim. Cosmochim. Acta 48, 1135–1140.

Monroy, F., Aira, M., Velando, A., Domínguez, J., 2005. Size-assortative mating in the earthworm *Eisenia fetida* (Oligochaeta, Lumbricidae). J. Ethol. 23, 69–70.

Monroy, F., 2006. Efecto das miñocas (clase Oligochaeta) sobre a comunidade descompoñedora durante o proceso de vermicompostaxe. PhD Thesis. Universidade de Vigo, Spain.

Monroy, F., Aira, M., Domínguez, J., Velando, A., 2006. Seasonal population dynamics of *Eisenia fetida* (Savigny, 1826) (OligochaetaLumbricidae) in the field. Comptes Rendus Biologies 329, 912–915.

Moore, J.C., Berlow, E.L., Coleman, D.C., de Ruiter, P.C., Dong, Q., Johnson, N.C., McCann, K.S., Melville, K., Morin, P.J., Nadelhoffer, K., Rosemond, A.D., Post, D.M., Sabo, J.L., Scow, K.M., Vanni, M.J., Wall, D.H., 2004. Detritus, trophic dynamics and biodiversity. Ecol. Lett. 7, 584–600.

Nadelhoffer, K.F., Fry, B., 1988. Controls on natural ^{13}N and ^{12}C abundances in forest soil organic-matter. Soil Sci. Soc. Am. J. 52, 1633–1640.

Neilson, R., Boag, B., Smith, M., 2000. Earthworm δ^{13}C and δ^{15}N analyses suggest that putative functional classifications of earthworms are site-specific and may also indicate habitat diversity. Soil Biol. Biochem. 32, 1053–1061.

Neilson, R., Hamilton, D., Wishart, J., Marriott, C.A., Boag, B., Handley, L.L., Scrimgeour, C.M., McNicol, J.W., Robinson, D., 2002. Stable isotope natural abundances of soil, plants and soil invertebrates in an upland pasture. Soil Biol. Biochem. 30, 1773–1782.

Ponsard, S., Arditi, R., 2000. What can stable isotopes (δ^{15}N and δ^{13}C) tell about the food web of soil macro-invertebrates? Ecology 81, 852–864.

Ponsard, S., Arditi, R., 2001. Detecting omnivory with δ^{15}N: comment from Ponsard & Arditi. Trends Ecol. Evol. 16, 20–21.

Ponsard, S., Averbuch, P., 1999. Should growing and adult animals fed on the same diet show different δ^{15}N values? Rapid Commun. Mass Spectrom. 13, 1305–1310.

Robinson, D., 2001. δ^{15}N as an integrator of the nitrogen cycle. Trends Ecol. Evol. 16, 153–162.

Ruess, L., Häggblom, M.M., Langel, R., Scheu, S., 2004. Nitrogen isotope ratios and fatty acid composition as indicators of animal diets in belowground systems. Oecologia 139, 336–346.

Ruess, L., Tiunov, A., Haubert, D., Richnow, H.H., Häggblom, M.M., Scheu, S., 2005a. Carbon stable isotope fractionation and trophic transfer of fatty acids in fungal based soil food chains. Soil Biol. Biochem. 37, 945–953.

Ruess, L., Schütz, K., Haubert, D., Häggblom, M.M., Kandeller, E., Scheu, S., 2005b. Application of lipid analysis to understand trophic interactions in soil. Ecology 86, 2075–2082.

Sampedro, L., Jeannotte, R., Whalen, J.K., 2006. Trophic transfer of fatty acids from gut microbiota to the earthworm *Lumbricus terrestris*. Soil Biol. Biochem. 28, 3188–3198.

Santruckova, H., Bird, M.I., Frouz, J., Sustr, V., Tajovsky, K., 2000. Natural abundance of ^{13}C in leaf litter as related to feeding activity of soil invertebrates and microbial mineralisation. Soil Biol. Biochem. 32, 1793–1797.

Scheu, S., Falca, M., 2000. The soil food web of two beech forests (*Fagus sylvatica*) of contrasting humus type: stable isotope analysis of a macro- and mesofauna-dominated community. Oecologia 123, 285–296.

Scheu, S., 2002. The soil food web: structure and perspectives. Eur. J. Soil Biol. 38, 11–20.

Schmidt, O., 1999. Intrapopulation variation in carbon and nitrogen stable isotope ratios in the earthworm *Aporrectodea longa*. Ecol. Res. 14, 317–328.

Schmidt, O., Curry, J.P., Dyckmans, J., Rota, E., Scrimgeour, C.M., 2004. Dual stable isotope analysis (δ^{13}C and δ^{15}N) of soil invertebrates and their food sources. Pedobiologia 48, 171–180.

Schmidt, O., Scrimgeour, C.M., Handley, L.L., 1997. Natural abundance of ^{15}N and ^{13}C in earthworms from a wheat and a wheat-clover field. Soil Biol. Biochem. 29, 1301–1308.

Schneider, K., Migge, S., Norton, R.A., Scheu, S., Langel, R., Reineking, A., Maraun, M., 2004. Trophic niche differentiation in soil microarthropods (Oribatida, Acari): evidence from stable isotope ratios (^{15}N/^{14}N). Soil Biol. Biochem. 36, 1769–1774.

Schweizer, M., Fear, J., Cadisch, G., 1999. Isotopic (^{13}C) fractionation during plant residue decomposition and its implications for soil organic matter studies. Rapid Commun. Mass Spectrom. 13, 1284–1290.

Setälä, H., Aarnio, T., 2002. Vertical stratification and trophic interactions among organisms of a soil decomposer food web—a field experiment using ^{15}N as a tool. Eur. J. Soil Biol. 38, 29–34.

Spain, A.V., Saffigna, P.G., Wood, A.W., 1990. Tissue carbon sources for *Pontoscolex corethrurus* (Oligochaeta: Glossoscolecidae) in a sugarcane ecosystem. Soil Biol. Biochem. 22, 703–706.

Sponheimer, M., Robinson, T., Ayliffe, L., Roeder, B., Hammer, J., Passey, B., West, A., Cerling, T., Dearing, D., Ehleringer, J., 2003. Nitrogen isotopes in mammalian herbivores: hair δ^{15}N values from a controled feeding study. Int. J. Osteoarchaeol. 13, 80–87.

Staddon, P.L., 2004. Carbon isotopes in functional soil ecology. Trends Ecol. Evol. 19, 148–154.

Steele, K.W., Saunders, W.M.H., Wilson, A.T., 1981. Nitrogen isotope ratios in surface and subsurface horizons of New-Zealand improved grassland soils. N. Z. J. Agric. Res. 24, 167–170.

Uchida, T., Kaneko, N., Ito, M.T., Futagami, K., Sasaki, T., Sugimoto, A., 2004. Analysis of the feeding ecology of earthworms (Megascolecidae) in Japanese forests using gut content fractionation and δ^{15}N and δ^{13}C stable isotope natural abundances. For. Ecol. Manage. 27, 153–163.