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Laurent Caner, Bernhard Zeller, E. Dambrine, Jean-François Ponge, M. Chauvat, et al.. Origin of the nitrogen assimilated by soil fauna living in decomposing beech litter. *Soil Biology and Biochemistry*, 2004, 36, pp.1861-1872. hal-00258686

**HAL Id: hal-00258686**

**<https://hal.science/hal-00258686>**

Submitted on 24 Feb 2008

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*Origin of the nitrogen assimilated by soil fauna living  
in decomposing beech litter*

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Accepted Version: *Soil Biology and Biochemistry*, 2004,  
**36**, 1861-1872

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## Abstract

We investigated the nitrogen source for main taxa of soil fauna in two beech forests of contrasted humus type using  $^{15}\text{N}$ -labelled beech litter and  $^{15}\text{N}$  analysis of soil fauna.  $^{15}\text{N}$ -labelled beech litter was deposited on the topsoil in December 2000 in four stands of different ages at Leinefelde (Germany) with mull humus and in one mature stand at Sorø (Denmark) with moder humus. The fate of the tracer isotope was measured in litter and soil, as well as in the soil fauna, and for each taxa, we calculated the proportion of N in the animal derived from the labelled substrate. Of the original N contained in the litter, 20% to 41% was lost after 9 months at Leinefelde, and only 10% at Sorø. This loss was counterbalanced by the incorporation of 24% to 31% external N at Leinefelde, and 31% at Sorø, partly originating from fungal colonisation of the added litter. The proportion of N assimilated from the labelled litter by the different soil animals varied in relation to their mobility and feeding preferences. Large and mobile soil animals, especially predators, derived on average less  $^{15}\text{N}$  because they were also able to feed outside the labelled litter boxes. Detritivores assimilated at most 15% of their nitrogen content at Leinefelde and 11% at Sorø from the decomposing labelled litter. The most labelled taxa at Leinefelde were small fungivorous and coprophagous species, mainly isotomid Collembola such as *Isotomiella* and *Folsomia*. At Sorø, best labelled taxa were saprophagous species such as Enchytraeidae, Glomeridae and Phthiracaroida. These low rates of  $^{15}\text{N}$  assimilation indicate that fresh litter is not directly the main N source for soil animals. The results obtained suggest that soil fauna fed preferentially upon microorganisms colonising the litter at Leinefelde (mull) and from litter itself at Sorø (moder).

**Key words:**  $^{15}\text{N}$  tracing, litter, soil fauna, detritivores, predators

## 1. Introduction

Food selection by soil fauna is generally investigated by morphological evidence of tissue degradation and gut content observations in the field and in chamber experiments (Whittaker, 1981; Behan Pelletier and Hill, 1983; Verhoef et al., 1988; Saur and Ponge, 1988; Ponge, 1991a; Klironomos et al., 1992), as well as by choice experiments (Visser and Whittaker, 1977; Shaw, 1988; Stöckli, 1990; Hendriksen, 1990). These investigations, although long and difficult, provide most reliable indications about the different ingested material. However, they do not imply that nutrients (C, N) contained in the ingested diet are effectively

assimilated by the animals. For instance, most of the N in beech litter is incorporated in polyphenol-protein compounds (Berthelin et al., 1994).

This N form is not readily available to many microorganisms and invertebrates. These difficulties might be resolved using nutrient tracers such as stable isotopes ( $^{13}\text{C}$ ,  $^{15}\text{N}$ ) which assess the long-term assimilated nutrients. Variation of nitrogen stable isotope ratios ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) in soil animals recently appeared as a new and easy tool to analyse the long-term dietary preferences and the trophic position of soil animals along food chains, because the  $\delta^{15}\text{N}$  of predators is about 3‰ higher than that of herbivore or detritivore species (Minagawa and Wada, 1984; Peterson and Fry, 1987; Schmidt et al., 1997; Neilson et al., 2000; Ponsard and Arditì, 2000; Scheu and Falca, 2000; Oelbermann and Scheu, 2002).

Because large variations in  $\delta^{15}\text{N}$  occur within each trophic level, Ponsard and Arditì (2000) and Scheu and Falca (2000) suggested that there were continuous gradients from primary to secondary decomposers, and from predators feeding predominantly on primary to predators feeding predominantly upon secondary decomposers (Scheu, 2002) rather than a theoretical trophic chain. However, results should be interpreted carefully, as  $\delta^{15}\text{N}$  may vary with age of soil fauna (Owens, 1987; Ponsard and Averbuch, 1999; Adams and Sterner, 2000; Oelbermann and Scheu, 2002) and quality of the prey (Oelbermann and Scheu, 2002) and are site-specific (Neilson et al., 2000).

Food diets and prey-predator relationships may also be studied using stable isotope labelling. Briones et al. (1999) used substrates differing in their quality and their  $\delta^{13}\text{C}$  to investigate the relative contribution of mixtures of different substrates to animal nutrition.

In a coniferous forest with moder humus, Setälä and Aarnio (2002), using soil  $^{15}\text{N}$ -labelling, showed that: 1) animals collected in surface litter layer (L layer) fed principally in this layer; 2) large and mobile fauna collected in F and H layers fed predominantly in the L layer; and 3) small sedentary taxa from F and H layers fed mostly in the layer in which they were collected. These results imply that foodwebs in moder humus were vertically stratified with little exchange between horizons, as suggested by Ponge (1999).

Based on these studies, we explored the combination of both natural isotope variation and isotopic labelling to get insight into the functional role of the soil fauna. This approach was applied to two beech forests differing in their humus form.

For this purpose,  $^{15}\text{N}$ -labelled beech litter was deposited in December 2000 at two sites. We chose a chronosequence of four beech stands with mull humus at Leinefelde (Germany) and

an old beech forest with moder humus at Sorø (Denmark). The biogeochemical cycles of carbon and nutrients at these two sites are intensively studied in the context of several European projects (FORCAST, 2000). After 9 months (September 2001), the fate of the tracer isotope was measured in litter and soil, as well as in soil animals. In parallel, natural  $^{15}\text{N}$  isotope variations in the soil fauna were measured. For each taxon, we calculated the proportion of  $^{15}\text{N}$  in the animal that was derived from the labelled substrate. Species were then ranked by the percentage of assimilated N, which was related to their mobility and their involvement in litter decomposition and N mineralisation.

## 2. Material and methods

### 2.1. Site description

The study was carried out in two beech (*Fagus sylvatica* L.) forests with contrasting humus types, the Leinefelde forest in Germany, and the Sorø forest in Denmark.

The site of Leinefelde, Thuringen (Germany), located 51°23' N; 10°19' E at an altitude of 200 m, is comprised of 4 adjacent beech stands of increasing age: 40 y (L-1), 70 y (L-2), 120 y (L-3) and 150 y (L-4). The parent material is limestone with loess deposit of varying depth. Soils vary over a short distance between loamy-clay Eutric Cambisols on limestone to loamy to clay-loamy Luvisols (FAO classification) on loess, presenting features of hydromorphy at 60–70 cm soil depth. The humus form is always a Mull. The L layer is spotted with many earthworms' casts. Soil pH in the A<sub>1</sub> horizon varies in the range 6 to 7 (Mund, personal communication), depending on the depth of the loess cover.

The site of Sorø, located 55°29' N; 11°38' E at an altitude of 20 m, is an almost pure beech stand 100 y old. Soil is a sandy-loamy Dystric Cambisol (FAO classification), with a moder humus with well-developed L, F and H layers. A more detailed description of the sites can be found in FORCAST (2000).

### 2.2. $^{15}\text{N}$ -labelled litter field experiment

$^{15}\text{N}$ -labelled senescent leaves were picked off at the end of November 1997 and 1998 from 12-year-old beech trees formerly enriched by spraying  $^{15}\text{N}$ -labelled urea on their foliage (Zeller et al., 1998). The litter collected each year was air-dried; thoroughly mixed (12 kg) and 10 samples were analysed for N content and  $\delta^{15}\text{N}$ . The nutrient content of the labelled litter was close to that of the natural litter of the sites. The litter produced in the year 1997 with a  $\delta^{15}\text{N}$  of 2580‰ was deposited at sites L-2 and L-3 and at Sorø, that collected in the

year 1998, with a  $\delta^{15}\text{N}$  of 1234‰, was deposited at sites L-1 and L-4 (Table 1). The initial N concentration in the labelled litter was 1.1% N, about 10% of the total N was water-soluble. Eighteen grams of  $^{15}\text{N}$ -labelled litter were introduced in plastic boxes (25 x 25 x 2.5 cm length x width x height) closed with a 5 mm mesh size plastic net in order to allow almost all soil invertebrates to enter and free drainage. The nets were fixed at the bottom and at the top of each box. This amount (290 g m<sup>-2</sup>) represented about 3/4 of the annual litterfall in the studied forests (FORCAST 2000). In December 2000, after main litterfall, 80 litter boxes (5 m<sup>2</sup>) were deposited at each stand in two parallel lines. Each line was 50 cm wide (two adjacent boxes) and 5 m long (20 adjacent boxes). The two lines were 1 m apart. The existing fresh L layer was carefully removed from the plot surface before deposition of the  $^{15}\text{N}$ -labelled litter. At each site, 6 litter boxes were randomly collected in June 2001 for litter decomposition studies and in September 2001 for soil fauna and litter decomposition studies. The litter layer below the boxes (Lv layer) at Leinefelde and the F and H layers at Sorø, as well as 2 soil cores (8 cm diameter) of the 0-5 cm topsoil were collected in the field. 6 litter samples and 6 soil cores were also collected outside the labelled litter area at Leinefelde to study the natural  $\delta^{15}\text{N}$  of soil fauna.

### 2.3. Mass loss and N dynamics

Collected litter samples were carefully cleaned from adhering soil particles and plant residues, dried at 65°C to constant mass, weighed and ground in a ball mill before chemical analyses. N content and  $^{15}\text{N}$  isotopic abundance were measured. In this experiment we did not measure the input of particles into the boxes. But no fresh litter from the stands was deposited on the labelled litter boxes during the time of the experiment (December 2000 until September 2001). In June 2002, about 200 – 500 µg white fungal hyphae were collected at the surface of decaying leaves at L-1 and L-2 sites and at Sorø.

### 2.4. Soil fauna studies

The soil mesofauna (Collembola, Oribatida, Gamasida and Enchytraeidae) and macrofauna (Diplopoda, Chilopoda, Isopoda, Araneae and Lumbricidae) were extracted from  $^{15}\text{N}$ -labelled litter boxes, underlying litter layers and the 0-5 cm topsoil as well as from unlabelled samples (litter, 0-5 cm topsoil).

Animals were extracted using a two-step procedure:

- 1) enchytraeids were first extracted by the wet funnel method (O'Connor, 1955) then preserved in 95 % ethanol;

2) remaining samples were then transferred to a funnel closed by a 1.5 mm mesh size wire net. Arthropods (including macrofauna) and Lumbricidae were extracted by heat (Macfadyen, 1962) for 10 days. Soil fauna was collected in ethylene glycol then transferred to 95% ethanol after the extraction was completed. Storage for a short period in ethylene glycol, and for longer periods in ethanol had little effect on the  $\delta^{15}\text{N}$  of soil arthropods (Fabiàn, 1998; Ponsard and Amlou, 1999).

Earthworms were also collected in the field when removing the litter boxes. Species extracted were *Lumbricus* sp. (juveniles), *Allolobophora rosea*, *Aporectodea caliginosa* at Leinefelde and *Lumbricus* sp. (juveniles), *Lumbricus castaneus*, *Lumbricus rubellus*, and *Dendrobaena octaedra* at Sorø.

Soil animals were separated under a dissecting microscope and determined to the genus or to the family level for Collembola, to the superfamily level for Oribatida and Gamasida, to the family level for Diplopoda and to the order level for Chilopoda, Isopoda and Araneae. This level of determination allowed separation of animals following a priori feeding preferences (fungivorous, coprophagous, saprophagous, herbifungivorous (pollen, micro-algae and fungal spores) within the decomposer compartment, and predators (Gunn and Cherrett, 1993; Walter and Proctor, 1999; Haq, 1981; Luxton, 1979; Behan and Hill, 1978; Poole, 1959). A list of the taxa analysed with their feeding preferences is given in the Appendix. Small microarthropods were transferred to tin capsules by pipetting them into ethanol then the alcohol was evaporated at 50°C. Large animals were dried at 50°C, ground in a ball mill then the powder was weighed in tin capsules. Capsules were stored in a desiccator until  $^{15}\text{N}$  analysis.

Fungal hyphae colonising the decomposing litter collected on June 2002 were sorted under a dissecting microscope, manually cleaned with distilled water to remove adhering litter and soil and their  $^{15}\text{N}$  content was measured.

## 2.5. $^{15}\text{N}$ analysis

$^{15}\text{N}$  contents of litter, soil and animals were measured by an elemental analyser (Carlo Erba, NA1500-NC, Milano, Italy) coupled with a gas isotope mass spectrometer (Finnigan, delta-S, Bremen, Germany) by continuous flow (EA-CF-IRMS).  $^{15}\text{N}$  abundance is expressed as  $\delta^{15}\text{N}$  units relative to atmospheric  $\text{N}_2$  as standard, according to the formula:

$$\delta^{15}\text{N} (\text{‰}) = [(R_{\text{sample}}/R_{\text{STD}}) - 1] \times 1000, \text{ where } R \text{ is the } ^{15}\text{N}/^{14}\text{N} \text{ ratio.}$$

An internal standard (labelled beech litter powder) of known isotopic composition ( $\delta^{15}\text{N} = 50\text{‰}$ ) was measured after each batch of twelve samples, and used as a working standard to

calibrate the mass spectrometer for labelled samples. Reliable measures of  $\delta^{15}\text{N}$  for soil fauna were obtained for samples containing more than 10  $\mu\text{g N}$ , thus we grouped smallest animals to obtain 10-100  $\mu\text{g N}$ . As the N content of the fauna is close to 10%, samples of 10 to 50 (depending on their size) microarthropods (Collembola, Oribatida, Gamasida and Uropodida) were needed for analyses. The animals extracted from the six replicates were bulked and three sub-samples were made when possible for isotopic analysis. For larger animals, 200-500  $\mu\text{g}$  were weighed and replicates corresponded to individuals or groups of 2-3 individuals.

Gut contents of large earthworms were removed by the filter paper method (Dalby et al., 1996) and after dissection. For small animals, as their whole body was used for isotopic analysis, the presence of non-assimilated  $^{15}\text{N}$ -labelled litter in the digestive tract may have caused a bias by artificially increasing the labelling rate of the animals.

The isotopic excess of detritivores and predators was calculated by subtracting the mean natural  $\delta^{15}\text{N}$  value of the same animals collected in non-labelled areas ( $\delta^{15}\text{N}_{\text{na}}$ ). At Leinefelde we used the natural abundance of animals from the L-2 stand. At Sorø we used the natural  $\delta^{15}\text{N}$  values of the soil fauna previously studied at the site of Fougères (France), a beech forest stand with a moder humus similar to that of Sorø.

The proportion of litter-derived  $^{15}\text{N}$  in detritivores was calculated as the ratio of the animal isotopic excess to the mean  $^{15}\text{N}$  content of the enriched litter, before deposition in the field (December 2000) and at the time of collection (September 2001) for both Leinefelde and Sorø using the following equation:

$$\text{assimilated N by detritivores} = \frac{\delta^{15}\text{N}_{\text{detritivore}} - \delta^{15}\text{N}_{\text{na detritivore}}}{\delta^{15}\text{N}_{\text{deposited litter}} - \delta^{15}\text{N}_{\text{collected litter}}} \times 100 (\%)$$

where  $\delta^{15}\text{N}_{\text{detritivore}}$  being the  $\delta^{15}\text{N}$  of the animals collected in the labelled litter boxes and  $\delta^{15}\text{N}_{\text{na detritivore}}$  being the natural  $\delta^{15}\text{N}$  of the animals collected in the non-labelled area.

For predators we calculated the proportion of litter-derived  $^{15}\text{N}$ , and the proportion of prey-derived  $^{15}\text{N}$  using mean values of micro-detritivores (Collembola, Oribatida), for micropredators (Gamasida, Uropodida and Pseudoscorpionida) and the mean value of all detritivores for macropredators (Lithobiomorpha, Geophilomorpha, Araneae) using these equations:



$$\text{assimilated N by micropredators} = \frac{\delta^{15}\text{N micropredator} - \delta^{15}\text{Nna micropredator}}{\text{mean } \delta^{15}\text{N of microdetritivores}} \times 100 \text{ (\%)}$$

$$\text{assimilated N by macropredators} = \frac{\delta^{15}\text{N macropredator} - \delta^{15}\text{Nna macropredator}}{\text{mean } \delta^{15}\text{N of detritivores}} \times 100 \text{ (\%)}$$

The proportion of litter-derived nitrogen for the different soil taxa was analysed by two-way analysis of variance using the SAS General Linear Model (SAS Institute, 1995). Contrasts were employed for each taxon to test differences according to stand age and soil depth.

### 3. Results

#### 3.1. Litter decomposition

##### 3.1.1. Leinefelde

Within nine months, beech litter had lost between 27% (L-1, L-2 and L-3) and 23% (L-4) of its original weight while the total N content slightly decreased (10%, L-1), remained almost stable (L-2 and L-3), or increased (7%, L-4) compared to the original amount (Table 2).

Of the original N contained in the litter, 41% was lost at L-1, and about 20% at L-2, L-3 and L-4. This output was counterbalanced by the incorporation of 31% (L-1), 24% (L-2, L-3) and 27% (L-4) external N, respectively (Table 2).

##### 3.1.2. Sorø

Within nine months, beech litter had lost about 28% of its original weight while its total N content increased (20%) compared to the original amount (Table 2).

Of the original N contained in the litter, 10.3% was lost. This output was counterbalanced by the incorporation of 31% of external N (Table 2).

As a consequence from this release of labelled litter N, the  $\delta^{15}\text{N}$  in the Lv and F layer switched from negative to positive values (Table 1). At Leinefelde the increase in soil  $\delta^{15}\text{N}$  varied from 3.3 ‰ at L-4 to 9.7‰ at L-3 after nine months of litter decomposition.

#### 3.2. $\delta^{15}\text{N}$ of soil fauna

Table 3 gives the natural isotopic abundance ( $\delta^{15}\text{N}$ ) of the different animal communities at the L-2 site. Values for detritivores ranged from  $-3.1\text{‰}$  (*Lumbricus* spp. juv) to  $0.00\text{‰}$  (Isopoda)

**Table 1.** N concentration, water soluble N and  $\delta^{15}\text{N}$  of the labelled litter and the organic soil layer just below the litter boxes (Lv at Leinefelde; F at Sorø).  $\delta^{15}\text{N}$  of the labelled litter and the organic soil layer (Lv; F) after nine month of litter decomposition. Standard deviation from the mean in brackets.

Site (stand age)	Labelled litter produced in	Total N (%)	Water soluble N (% of total N)	$\delta^{15}\text{N}$ (litter) (‰)	$\delta^{15}\text{N}$ (Lv; F) (‰)	$\delta^{15}\text{N}$ (litter) (‰)	$\delta^{15}\text{N}$ (Lv; F) (‰)
<b>Leinefelde-1</b> (40 y.)	1998	1.04 (0.1)	10.5	1234 (127)	-4.7 (0.05)	926 (63)	2.5 (0.9)
<b>Leinefelde-2</b> (70 y.)	1997	1.15 (0.05)	10.1	2580 (187)	-1.4 (0.19)	1983 (60)	7.6 (2.3)
<b>Leinefelde-3</b> (120 y.)	1997	1.15 (0.05)	10.1	2580 (187)	-1.6 (0.16)	1972 (84)	8.1 (4.8)
<b>Leinefelde-4</b> (150 y.)	1998	1.04 (0.1)	10.5	1234 (127)	-2.8 (0.25)	864 (47)	0.5 (0.8)
<b>Sorø</b> (100 y.)	1997	1.15 (0.05)	10.1	2580 (187)	-2.7 (0.24)	1750 (93)	10.3 (3.7)

with a mean of  $-1.9\text{‰}$  (S.E. = 0.3, n= 32) for all detritivores. Predator  $\delta^{15}\text{N}$  varied between  $1.2\text{‰}$  (Lithobiomorpha) to  $4.2\text{‰}$  (Coleoptera) with a mean of  $2.3$  (S.E.= 0.2, n= 38). (Figure 1).

In comparison to the  $\delta^{15}\text{N}$  of the litter initially deposited ( $2580\text{‰}$ ) and of the litter partly decomposed after nine months ( $1983\text{‰}$ ),  $\delta^{15}\text{N}$  values measured in the fauna extracted from labelled litter boxes at site L-2 were:

*Lepidocyrtus*: 236 to  $282\text{‰}$ , *Folsomia* 240 to  $257\text{‰}$ , *Pogonognathellus* 82 to  $229\text{‰}$ , Nothroidea 67 to  $126\text{‰}$ , Glomeridae 1 to  $287\text{‰}$ , Parasitidae 82 to  $196\text{‰}$ , Lithobiomorpha –  $1.4$  to  $13.0\text{‰}$ , Geophilomorpha –  $1$  to  $78\text{‰}$ , Araneae  $3.3$  to  $31.6\text{‰}$  (Table 3).

Measurements made on single large animals (*Pogonognathellus*, Lithobiomorpha, Geophilomorpha, and Diplopoda) showed large inter-individual variation, whereas bulk samples of small animals (*Folsomia*, *Lepidocyrtus*, Oribatid and Gamasid mites) presented smaller variation.

These results point out for almost all soil animals  $^{15}\text{N}$  contents larger than in the non-labelled area (background), implying that they ingested labelled litter or microorganisms feeding on labelled litter, and assimilated its heavy isotope nitrogen. The difference between the natural abundance and the  $^{15}\text{N}$  content in the labelled litter boxes gives an estimate of the proportion of animal N derived from litter.

**Table 2.** Variation of remaining mass and total N in the litter after nine months of decomposition, at Leinefelde (beech chronosequence, mull) and Sorø (moder). N dynamics in the <sup>15</sup>N-labelled beech litter were calculated according to changes in the <sup>15</sup>N enrichment of the litter. During the initial phase of litter decomposition the release of structural litter N is balanced by an incorporation of external N. Values in brackets correspond to the standard error (n = 6). Means followed by the same letter in the columns did not differ significantly (p < 0.01).

Site	Mass remaining	Total N (% of initial)	Released N	Incorporated N
<b>Leinefelde-1 (40 y.)</b>	71.6 b (1.7)	90.3b (1.9)	41.3a (2.7)	31.6a (1.7)
<b>Leinefelde-2 (70 y.)</b>	73.2 b (3.0)	100.8a (2.3)	22.5b (2.3)	23.4b (0.8)
<b>Leinefelde-3 (120 y.)</b>	73.8 b (2.5)	104.5a (1.3)	20.1b (1.9)	24.6b (2.1)
<b>Leinefelde-4 (150 y.)</b>	77.2 a (0.9)	107.7a (2.1)	19.3b (4.2)	27.1b (2.4)
<b>Sorø (100 y.)</b>	71.6 (1.2)	120.7 (2.8)	10.3 (2.7)	31.0 (1.7)

### 3.3. Proportion of N originating from labelled litter

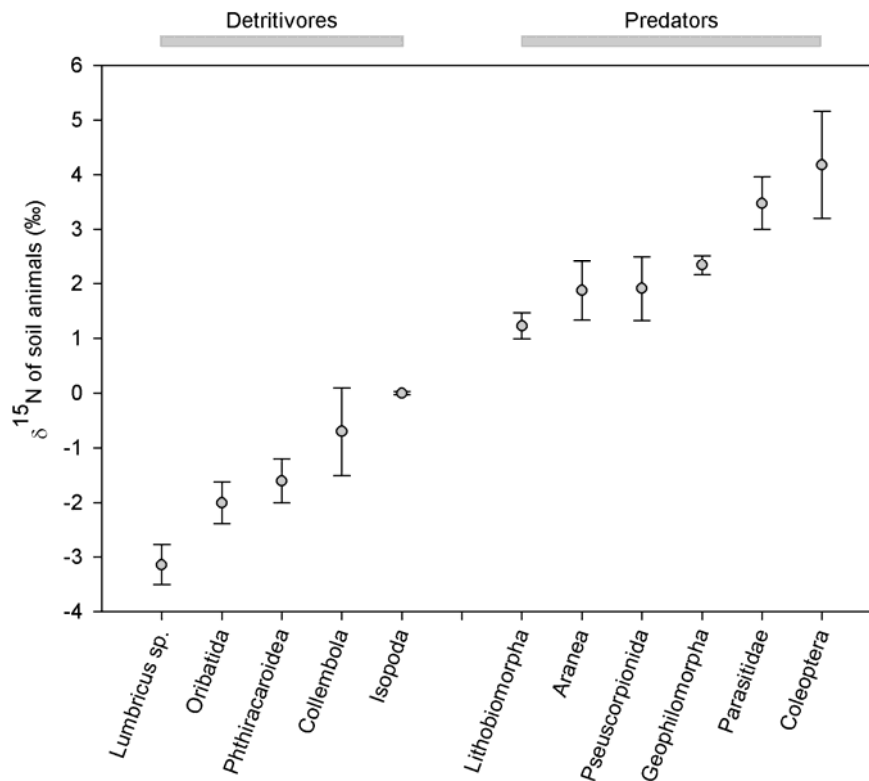
#### 3.3.1. Leinefelde

Hyphae of white-rot fungi isolated from decaying litter in June 2002 were variably enriched in <sup>15</sup>N. They had derived on average 14.0% (S.E.= 3.9, n= 4) of their nitrogen from decomposing labelled litter (Figure 2).

Faunal communities extracted from litter boxes at the four sites were largely similar, as observed outside litter boxes. However, some taxa, such as Neanuridae, *Pseudosinella*, *Pogonognathellus*, *Sminthurinus*, *Isotomiella*, *Entomobrya*, *Achipteria*, Uropodidae, Isopoda, Julidae, Geophilomorpha, were not extracted from all age classes of the chronosequence.

The proportion of N derived from labelled litter ranged from 0 to 15% in the different taxa (Figure 2). The age of the beech stand had no effect on the proportion of N originating from litter (p > 0.05) for most soil fauna extracted from litter boxes, except for Glomeridae (p = 0.006), Enchytraeidae (p = 0.003), Julidae (p = 0.015) and *Eniochthonius* (p < 0.001) which derived more N from litter in the younger stand (L-1) compared to others.

In the litter under the boxes the proportion of N originating from the labelled litter in the taxa *Folsomia*, *Eniochthonius*, Onychiuridae and Uropodidae was larger in the younger stands (L-1 and / or L-2) compared to older ones, but inter-individual variation was large.



**Figure 1.** Variation in  $\delta^{15}\text{N}$  in natural abundance of soil animals at site L-2

Soil fauna of the four stands (decomposers then predators) were ranked according to the amount of  $^{15}\text{N}$  assimilated from labelled litter into litter boxes (Figure 2). Three groups of decomposers and two groups of predators were separated:

• Detritivores:

- (1) Taxa having derived more than 10% of their N from labelled litter:

The soil animals that were the most enriched in  $^{15}\text{N}$  were mainly small fungivorous (Eupodidae: 14.6%; *Pseudosinella*: 13.3%), coprophagous (*Isotomiella*: 15.1%; *Folsomia*: 11.4%) and herbifungivorous grazers (*Sminthurinus*: 13.3%).

When extracted from the litter under the boxes these animals derived less than 3% of their N from the above labelled litter.

- (2) Taxa having derived 5 to 10% of their N from labelled litter:

These taxa were mostly saprophagous of variable size (*Eniochthonius*: 8.1%, Glomeridae: 8.0%, Enchytraeidae: 7.1% and Phthiracaroida: 6.9%); fungivorous and herbifungivorous (*Lepidocyrtus*: 9.3%) and small litter browsers and suckers (Neanuridae: 9.1%)

When extracted from the litter layer under the boxes, these taxa had derived less than 3% of their N from the above labelled litter.

**Table 3:**  $^{15}\text{N}$  abundance ( $\delta^{15}\text{N}$ ) in soil fauna extracted from non labelled and labelled litter at the site L-2

Taxa	In natural litter		In the $^{15}\text{N}$ -labelled litter	
	mean $\delta^{15}\text{N}$	S.E.	mean $\delta^{15}\text{N}$	S.E.
<b>DETRITIVORES</b>				
Collembola (bulk)	-0.70	0.80 (n= 3)	268.2	14.4 (n=3)
Collembola <i>Lepidocyrtus</i>			252.3	8.7 (n=2)
Collembola <i>Folsomia</i>			146.5	44.5 (n=3)
Collembola <i>Pogonognathellus</i>				
Oribatida	-2.00	0.38 (n= 6)	155.0	22.3 (n=3)
Phthiracaroida Oribatida	-1.63	0.40 (n= 5)	100.1	(n=1)
Belboidea Oribatida			96.2	17.4 (n=3)
Nothroidea Lumbricus	-3.14	0.37 (n=11)		
Isopoda	0.00	0.03 (n= 6)		
Glomeridae			137.0	83.3 (n=3)
<b>Total detritivores</b>	<b>-1.88</b>	<b>0.28 (n= 32)</b>		
<b>PREDATORS</b>				
Parasitidae	3.48	0.48 (n= 6)	137.6	21.3 (n=5)
Araneae	1.87	0.54 (n=6)	19.6	8.4 (n=3)
Lithobiomorpha	1.23	0.24 (n= 10)	6.7	4.3 (n=3)
Geophilomorpha	2.34	0.18 (n= 10)	41.3	23.0 (n=3)
Pseudoscorpionida	1.91	0.59 (n= 3)	50.6	(n=1)
Coleoptera	4.18	0.98 (n= 3)		
<b>Total predators</b>	<b>2.26</b>	<b>0.21 (n= 38)</b>		

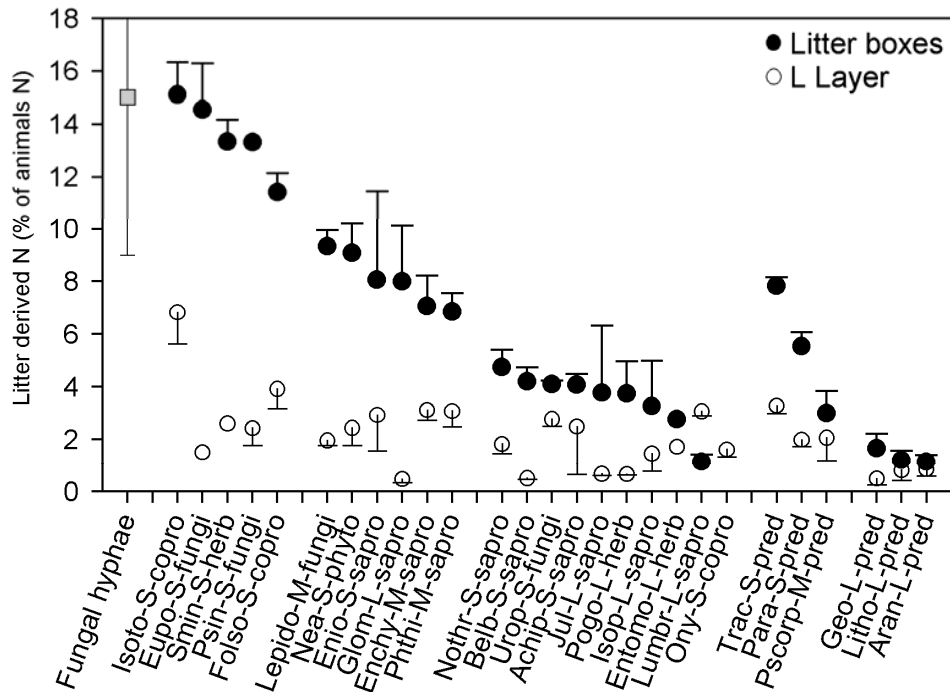
(3) Taxa having derived 1 to 5% of their N from labelled litter:

These soil animals were saprophagous Oribatida (Nothroidea: 4.7%, Belboidea; 4.2%, *Achipteria*: 4.1%), large saprophagous (Julidae: 3.8 %, Isopoda: 3.2%, and *Lumbricidae*: 1.2%), and herbifungivorous Collembola (*Entomobrya*: 2.8 %, *Pogonognathellus*: 3.8%) and Uropodidae (4.1%).

When extracted from the underlying Lv layer, these taxa had derived 0.1% (Belboidea, Julidae, *Pogonognathellus*) to 2.5% (*Achipteria*) of their N from the above labelled litter.

• Predators:

Micro-predator taxa (*Trachytes*) derived between 3% to 8% of their N from labelled litter (Figure 2). When related to the mean labelling level of micro-detritivores (their prey), micro-predators derived between 40% and 101% of their nitrogen from the prey available in the litter boxes (Table 3). Values larger than 100% are due to the fact that *Trachytes* fed on prey more labelled than average.



**Figure 2.** Litter derived N (% of total animal N) in the different soil animal taxa living in the labelled litter or in the decaying litter just below, at Leinefelde (all stands). Names, sizes and feeding preferences as in the Appendix.

Macro-predator taxa derived less than 2% of their N from labelled litter. When related to the mean label of detritivores, macro-predators derived between 15% and 22% of their nitrogen from the prey available in the litter boxes (Table 3).

Predators extracted from the decayed litter below the boxes assimilated less  $^{15}\text{N}$  but the difference was not significant ( $p > 0.05$ ).

In the 0-5 cm topsoil, detritivores and micro-predators derived a low proportion of their N from labelled litter (Collembola 1.3%, Glomeridae 1.1%, Isopoda 1.1%, Oribatida 1.0%, Enchytraeidae 0.4%, Julidae 0%, Gamasida 1.5%). For macro-predators extracted from the 0-5cm topsoil the values were close to that obtained for animals collected in the litter boxes (Lithobiomorpha 2.9%, Geophilomorpha 0.9%).

In comparison, the Lv litter layer presented  $\delta^{15}\text{N}$  values of less than 10‰ (between 0.5‰ at L-4 and 8.1‰ at L-3) and the 0-5 cm topsoil of less than of less than 5‰. Thus these horizons with  $\delta^{15}\text{N}$  lower than that of the soil fauna provided low amounts of  $^{15}\text{N}$  to the soil animals (Table 1).

**Table 3:**  $^{15}\text{N}$  abundance ( $\delta^{15}\text{N}$ ) in soil fauna extracted from non labelled and labelled litter at the site L-2

Taxa	In natural litter		In the $^{15}\text{N}$ -labelled litter	
	mean $\delta^{15}\text{N}$	S.E.	mean $\delta^{15}\text{N}$	S.E.
<b>DETRITIVORES</b>				
Collembola (bulk)	-0.70	0.80 (n= 3)		
Collembola <i>Lepidocyrtus</i>			268.2	14.4 (n=3)
Collembola <i>Folsomia</i>			252.3	8.7 (n=2)
Collembola <i>Pogonognathellus</i>			146.5	44.5 (n=3)
Oribatida	-2.00	0.38 (n= 6)	155.0	22.3 (n=3)
Phthiracaroida				
Oribatida Belboidea	-1.63	0.40 (n= 5)	100.1	(n=1)
Oribatida Nothroidea			96.2	17.4 (n=3)
Lumbricus	-3.14	0.37 (n=11)		
Isopoda	0.00	0.03 (n= 6)		
Glomeridae			137.0	83.3 (n=3)
<b>Total detritivores</b>	<b>-1.88</b>	<b>0.28 (n= 32)</b>		
<b>PREDATORS</b>				
Parasitidae	3.48	0.48 (n= 6)	137.6	21.3 (n=5)
Araneae	1.87	0.54 (n=6)	19.6	8.4 (n=3)
Lithobiomorpha	1.23	0.24 (n= 10)	6.7	4.3 (n=3)
Geophilomorpha	2.34	0.18 (n= 10)	41.3	23.0 (n=3)
Pseudoscorpionida	1.91	0.59 (n= 3)	50.6	(n=1)
Coleoptera	4.18	0.98 (n= 3)		
<b>Total predators</b>	<b>2.26</b>	<b>0.21 (n= 38)</b>		

### 3.3.2. Sorø

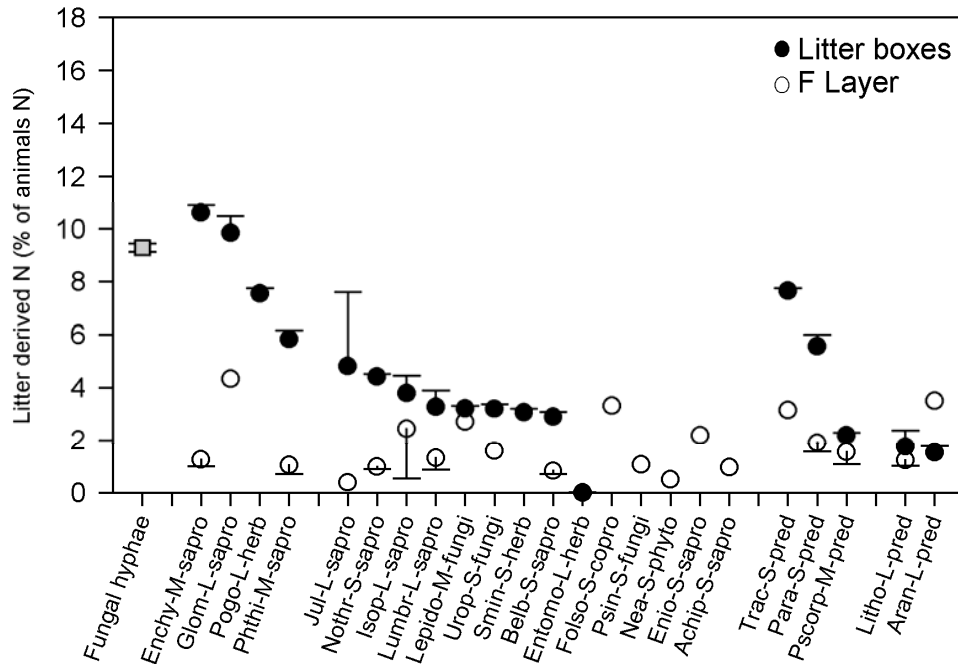
Hyphae of white-rot fungi isolated from decaying litter in the boxes collected in June 2002 had derived on average 9.3% (S.E. = 0.16, n=3) of their nitrogen from decomposing litter (Figure 3).

Soil fauna were ranked according to the amount of  $^{15}\text{N}$  assimilated from the labelled litter, separately for decomposers and predators (Figure 3). Taxa derived on average less nitrogen from labelled litter than at Leinefelde. Two groups of decomposers and two groups of predators were separated:

- Detritivores:

- (1) Taxa having derived 5 to 11% of their N from labelled litter:

These soil animals were mostly saprophagous of variable size (*Enchytraeidae*: 10.8% *Glomeridae*: 10.1%, and *Phthiracaroida*: 6.1%), herbivorous *Collembola* (*Pogonognathellus*: 7.8 %).



**Figure 3.** Litter derived N (% of total animal N) in the different soil animal taxa living in the labelled litter or in the decaying litter just below, at Sorø. Names, sizes and feeding preferences as in the Appendix.

When extracted from the F layer these taxa derived 1 to 4.5% of their N from the above placed labelled litter.

(2) Taxa having derived 0.1 to 5% of their N from labelled litter:

These soil animals were mostly herbivorous and fungivorous Oribatida (Nothroidea: 4.7%; Belboidea: 3.2%), fungivorous, and herbifungivorous Collembola (*Lepidocyrtus*: 3.5%, *Sminthurinus*: 3.3%; *Entomobrya*: 1.4%) and Uropodidae (3.2%).

When extracted from the F layer these soil animals derived between 1% and 3% of their N from the above placed labelled litter.

- Predators:

Micro-predator taxa derived between 2% to 7.6% of their N from labelled litter (Figure 3). When related to the mean labelling level of micro-detritivores, micro-predators derived between 50% and 177% of their nitrogen from prey available in the litter boxes (Table 4).

Values larger than 100% are due to the fact that Parasitidae and *Trachytes* fed on prey more labelled than the average. Macro-predator taxa derived less than 2% of their N from labelled litter (Figure 3). When related to the mean labelling level of detritivores, macro-predators derived between 21% and 27% of their nitrogen from prey available in the litter boxes (Table 4).



**Table 4:** Percentage of nitrogen derived from prey in predators collected in the litter boxes. Mean and standard error in brackets

	Parasitidae*	<i>Trachytes</i> *	Pseudo-scorpionida*	Lithobio-morpha <sup>#</sup>	Geophilo-morpha <sup>#</sup>	Araneae <sup>#</sup>
Leinefelde	68.1 (5.6) n = 13	101.3 (6.5) n = 4	37.8 (9.4) n = 9	15.1 (4.1) n = 12	22.0 (8.6) n = 5	15.7 (3.6) n = 10
Sorø	130.0 (16.1) n = 4	177.2 (3.7) n = 2	59.7 (4.1) n = 3	21.5 (14.1) n = 3	- -	27.9 (7.8) n = 3

\*: calculation made using the mean labelling of microdetritivores (Collembola, Oribatida), see equations in the text. #: calculation made using the mean labelling of all detritivores

Predators extracted from the F layer assimilated less  $^{15}\text{N}$  but the difference was not significant ( $p > 0.05$ ).

In the H layer detritivores derived 0.8 to 1.6%, and predators 0.3% to 3.0% of their N from labelled litter. In the 0-5 cm soil layer, Enchytraeidae and Oribatida derived respectively 0.8 and 0.7%, and predators derived 0.6 to 1.2% of their N from labelled litter. For micropredators the values are not significantly different from those of the F layer.

F and H layers showed  $\delta^{15}\text{N}$  values of 10.4‰ and 4.1‰, respectively which are lower to the animals ones; thus they provided a low amount of  $^{15}\text{N}$  to the soil fauna extracted in these layers, as in the Leinefelde site.

#### 4. Discussion

During the course of litter decomposition, N release is balanced by the accumulation of external N through several processes such as immigration of fauna and fungi and throughfall deposition (Berg 1988; Zeller et al., 2000). The role of white-rot fungi in the input of external N was clearly shown by Zeller et al. 2000 using combined  $^{15}\text{N}$  and ergosterol analysis. It is confirmed in this experiment by the relatively low label of the white fungi compared to that of the decaying labelled litter. It is likely that a large part of the fungal biomass present in the boxes derived its nitrogen from external sources, especially basidiomycetes which may exploit a large volume of litter and soil (Brownlee et al., 1983; Thompson, 1984).

Results in natural  $^{15}\text{N}$  abundance showed that earthworms are the most  $^{15}\text{N}$  depleted soil animals, which is in agreement with their classification as primary decomposers. Collembola and Oribatida showed a large range of values in relation with their feeding behaviour; they feed on substrates with different  $^{15}\text{N}$  content such as leaves, faecal pellets, decomposer fungi

hyphae, mycorrhiza fungal hyphae, and bacteria. The predators, as expected, are more enriched in  $^{15}\text{N}$ . Gamasida and Coleoptera are the most enriched soil animals from this site.

The values obtained at the Leinefelde site showed a gradient from primary decomposers and secondary decomposers to primary and secondary predators (Figure 1), similar to the gradients observed in two previous studies on natural  $^{15}\text{N}$  abundance of soil animals (Scheu and Falca, 2000; Ponsard and Arditti, 2000).

The 4.2‰ difference obtained between the mean of the detritivores (-1.9‰) and the mean of predators (2.3‰) is larger than 3.4‰ which is defined as the theoretical increase between two successive trophic levels (Minagawa and Wada, 1984) suggesting that there is more than one trophic level between detritivores and predators or that animals had various ages. This feature is also in good agreement with findings by Ponsard and Arditti (2000) and Scheu and Falca (2000) who attributed it either to intra-guild predation or to the fact that predators predominantly feed on secondary decomposers.

The range of  $^{15}\text{N}$  content of soil animals collected in the labelled litter suggest that they assimilated nitrogen from labelled litter to different extent in relation to their mobility and diet. The suggestion that the proportion of assimilated nitrogen can be used as an indicator of the involvement of soil animals in the decomposition of litter is based on the following assumptions:

- the life-span of most soil animals is shorter than 9 months (Van der Drift, 1951), although this may not be true for earthworms and oribatids. Hence, N assimilated from labelled litter should not be diluted in a pool of unlabelled N assimilated before deposition of labelled litter.
- the mobility of soil animals of the same size and feeding behaviour is comparable. In fact, the labelling of animals with different mobilities cannot be easily compared because more mobile forms may have fed partly outside the labelled litter boxes.
- animals that ingest and digest more N originating from  $^{15}\text{N}$ -labelled litter will present larger enrichment in  $^{15}\text{N}$ . Ingestion without digestion should not increase the animal label.

At the Leinefelde site, during the time of the experiment, detritivores derived up to 15.1 % (for the Collembolan *Isotomiella*) of their nitrogen from decomposing litter and on average 9.4 % (S.E.=0.6, n=44) for Collembola, 7.1 % (S.E.=1.2, n=10) for Enchytraeidae, 6.8 % (S.E.=1.7, n=14) for Diplopoda, 5.5 % (S.E.=0.5, n=39) for oribatid mites and 3.3 % (S.E.=1.7, n=5) for Isopoda.

At the site of Sorø, during the time of the experiment, soil fauna derived a lower proportion of their nitrogen from decomposing beech litter, most labelled taxa (Enchytraeidae) deriving at most 11.7% of their N from labelled litter. The average proportion of litter derived nitrogen was 10.8% (S.E.= 2.9, n=3) for Enchytraeidae, 8.0% (S.E.= 1.9, n= 5) for Diplopoda, 4.6% (S.E.= 0.4, n= 11) for Oribatida, 4.1% (S.E.= 0.6, n=3) for Isopoda and 4.0% (S.E.=0.8, n=11) only for Collembola.

Earthworms derived a low amount of their nitrogen from labelled litter in both sites. This low  $^{15}\text{N}$  content might be related to their weight, which implies the ingestion of a large amount of labelled litter before their  $\delta^{15}\text{N}$  increases above background level, but also to their wandering movements which make them able to ingest food taken in different horizons (Bernier, 1998). Hence, fresh litter does not appear to be directly the main N source for the soil animals collected in the boxes as they derived less than 15 % of their nitrogen from the labelled fresh litter present in the boxes during the nine months of the experiment.

Firstly, this surprisingly low rate of assimilation of N might be related to the mobility of the fauna. Labelled litter covered about 50% of the square meter area surrounding each litter box. The effect of mobility is very clear in the predator group at both Leinefelde and Sorø. However, this holds only for large detritivores and macro-predators, as small animals collected under the boxes were distinctly less labelled, pointing to a low level of vertical displacement. The  $\delta^{15}\text{N}$  of small predators was high and on average close to that of their prey, which indicated that the immigration rate of small predators was low. Contrastingly, the  $\delta^{15}\text{N}$  of large predators was very low and extremely variable from one to another individual, probably because some of these animals fed outside the litter boxes. For the same reason, the  $\delta^{15}\text{N}$  of large detritivores was lower than that of small detritivores with similar feeding preferences.

The main reason for the low rate of  $^{15}\text{N}$  labelling of soil fauna could be found in the selection of food resources within the boxes:

- At the Leinefelde site on mull humus the soil animals which derived the largest proportion of their N from labelled litter were small fungivorous and saprophagous species. The  $^{15}\text{N}$  content of these animals was close to that of fungal hyphae collected in June 2002.
- In contrast, at the site of Sorø, on moder humus, the taxa which derived a larger proportion of their nitrogen from decomposing litter were saprophagous species such as

Enchytraeidae, Diplopoda, Phthiracaroida and other Oribatida which eat fresh and decomposed litter (Hayes, 1965; David, 1987; Ponge, 1999).

These results suggest that the detritivore community used different sources and to a different extent in these two contrasted humus types:

- At the mull site (Leinefelde) it is likely that the whole community of detritivores assimilated nitrogen preferentially from microorganisms colonising fresh litter or from faecal pellets of non-litter origin, rather than from litter itself.
- At the moder site (Sorø), litter-feeding taxa derived the larger proportion of nitrogen directly from the decomposing litter or from faecal pellets of litter origin.

These differences can be explained by the relative contribution of mesofauna, compared to macrofauna, in the disintegration of litter in both humus types (Bocock, 1964; Herlitzius, 1987; Staaf, 1987; Ponge et al., 1997).

In mull humus, most litter is processed by large animals such as earthworms, slugs, millipedes and woodlice, which are actively moving throughout litter and soil and thus diluted the heavy isotope in our experiment as mentioned by Binet and Trehen (1992), and by saprophagous fungi (Toutain, 1987). In this humus type, small animals mainly browse on microbial colonies and tunnel faeces of macroinvertebrates (Zachariae, 1965; Toutain et al., 1982; Didden, 1990).

In moder humus, most litter is processed by small animals, which may pass through several animal guts before being incorporated into stable humus, forming the H layer (Ponge, 1991b).

The feeding preference of detritivores is still a matter of debate, as contradictory observations have been made. It has been often shown that saprophagous species feed principally on microorganisms colonising the litter (Luxton, 1966; Dash and Cragg, 1972; Mitchell and Parkinson, 1976; Visser and Whittaker, 1977; Parkinson et al., 1979; Whittaker, 1981; Verhoef et al., 1988; Ponge, 1991a; Kaneko et al., 1995; Maraun et al., 1998; Scheu and Schaeffer, 1998). On the other hand, Ponsard and Arditì (2000) and Scheu and Falca (2000), using natural isotope ratios, showed that the natural  $\delta^{15}\text{N}$  of detritivores was close to that of beech litter of the L layer, and suggested that structural components of the litter (L, F) layers rather than hydrosolubles were likely the main food source for soil fauna. Ponge (1991a, 1999), using the direct observation of gut contents of animals collected in different litter horizons, considered enchytraeids as primary consumers of needle and leaf litter in moder humus, contrary to the opinion expressed by Zachariae (1965) who considered them as secondary decomposers. Briones and Ineson (2002) using  $^{14}\text{C}$  dating of enchytraeids to

investigate their feeding behaviour in mor humus showed that they assimilated carbon from organic matter 5-10 years old.

Our experiment showed that soil fauna assimilated low N from the decaying litter and rather suggest that N was assimilated from fungi colonising the litter or faecal pellets as their  $\delta^{15}\text{N}$  was close to that of the soil fauna. In comparison, soil predators appeared closely linked for their N supply to detritivores onto which they prey. The above mentioned contradiction can be resolved by considering that the litter found in the L layer is not only made of raw plant material, since a large part has been already transformed by the internal microflora of decomposing beech leaves (Reisinger et al., 1978). In particular, foliar nitrogen, which is immobilised by N-demanding microorganisms, becomes rapidly incorporated into the microbial biomass (Berg and Söderström, 1979). Thus the identity between the natural  $\delta^{15}\text{N}$  of beech leaves of the L layer and that of litter-feeding animals cannot give evidence that they have consumed beech litter.

Soil fauna from the mull sites assimilated a larger proportion of nitrogen from the decomposing litter than fauna from the moder site whereas mass losses were in the same range. We may thus suggest that mull humus release more efficiently N than moder humus during decomposition and that the passage of nitrogen from plant biomass to animal biomass is faster in mull than in moder. This is in accordance with ideas developed by Staaf (1987), Schaefer and Schauer mann (1990) and Ponge (2003) on the faster circulation of nutrients in mull humus compared to moder humus.

Finally, the soil foodwebs of the studied forests appeared to be vertically stratified, at least for microdetritivores and for micropredators, as animals found beneath the experimental boxes derived less nitrogen from labelled litter than animals found in the boxes. This does not mean that no exchange took place between fresh litter and underlying horizons, since all animals collected under the boxes exhibited a  $^{15}\text{N}$  enrichment compared to the horizon into which they were collected. However, we cannot know whether this enrichment was due to movements of animals or to translocation of  $^{15}\text{N}$  by fungal mycelia.

In conclusion, the use of  $^{15}\text{N}$  enriched litter and  $^{15}\text{N}$  analysis of soil fauna appears as an interesting tool to trace the fate of nitrogen within the soil food web. The data so far obtained enabled us to discuss the “functional role” of soil microbes and fauna in the transformation of litter nitrogen. However, conclusions of this experiment were not as strong as we expected because the limited area covered by the labelled litter allowed mobile soil animals (predators) to feed outside the labelled area. Improvements could be obtained by experimenting in controlled conditions. Because extraction and identification of soil fauna needs a lot of time,

we did it only once and studied the whole range of soil animals. Another suggestion from this experiment would be to select a few important taxa and follow the progressive incorporation, assimilation and transformation of litter N with time. The combination of this method with population studies on soil fauna should help us to get further insight into the transfer of nutrients and energy processing in the soil foodweb during litter decomposition.

### **Acknowledgements**

This study received financial support from the EC through the project FORCAST (EVK2-CT1999-00035). We thank two anonymous reviewers for their constructive comments on the manuscript.

### **References**

- Adams, T.S., Sterner, R.W., 2000. The effect of dietary nitrogen content on trophic level  $^{15}\text{N}$  enrichment. *Limnology and Oceanography* 45, 601-607.
- Behan, V.M., Hill, S. B., 1978. Feeding habits and spore dispersal of oribatid mites in the North American Arctic. *Revue d'Ecologie et de Biologie du Sol* 15, 497-516.
- Behan Pelletier, V.M., Hill, S. B. 1983. Feeding habits of sixteen species of Oribatei (Acari) from an acid peat bog, Glenamoy, Ireland. *Revue d'Écologie et de Biologie du Sol* 20, 221-267.
- Berg, B. 1988. Dynamics of nitrogen ( $^{15}\text{N}$ ) in decomposing Scots pine needle litter. Long term decomposition in a Scots pine forest. VI. *Canadian Journal of Botany* 66, 1539-1546.
- Berg, B., Söderström, B. 1979. Fungal biomass and nitrogen in decomposing Scots pine needle litter. *Soil Biology and Biochemistry* 11, 339-341.
- Bernier, N., 1998. Earthworm feeding activity and development of the humus profile. *Biology and Fertility of Soils* 26, 215-223.
- Berthelin, J., Leyval, C., Toutain, F. (1994) *Biologie des sols. Rôle des organismes dans l'altération et l'humification*. In: Bonneau, M. et Souchier, B. (eds) *Pédologie 2. Constituants et propriétés du sol*. Masson, Paris, pp 143-237.
- Binet, F. Trehen, 1992. Experimental microcosm study of the role of *Lumbricus terrestris* (Oligochaeta: Lumbricidae) on nitrogen dynamics in cultivated soils. *Soil Biology and Biochemistry* 24, 1501-1506.

- Bocock, K.L., 1964. Changes in the amounts of dry matter, nitrogen, carbon and energy in decomposing woodland leaf litter in relation to the activities of the soil fauna. *Journal of Ecology* 52, 273-284.
- Briones, M. J. I., Ineson, P. 2002. Use of  $^{14}\text{C}$  carbon dating to determine feeding behaviour of enchytraeids. *Soil Biology and Biochemistry* 34, 881-884.
- Briones, M.J.I., Ineson, P., Sleep, D. 1999. Use of  $\delta^{13}\text{C}$  to determine food selection in collembolan species. *Soil Biology and Biochemistry* 31, 937-940.
- Brownlee, C., Duddridge, J.A., Malibari, A., Read, D.J., 1983. The structure and function of mycelial systems of ectomycorrhizal roots with special reference to their role in forming inter-plant connections and providing pathways for assimilate and water transport. *Plant and Soil* 71, 433-443.
- Dalby, P.R., Baker, G.H., Smith, S.E., 1996. "Filter paper method" to remove soil from earthworm intestine and to standardise the water content of earthworm tissue. *Soil Biology and Biochemistry* 28, 685-687.
- Dash, M.C., Cragg, J.B., 1972. Selection of microfungi by Enchytraeidae (Oligochaeta) and other members of the soil fauna. *Pedobiologia* 12, 282-286.
- David, J.F., 1987. Consommation annuelle d'une litière de chêne par une population adulte du diplopode *Cylindroiulus nitidus*. *Pedobiologia* 30, 299-310.
- Didden, W.A.M., 1990. Involvement of Enchytraeidae (Oligochaeta) in soil structure evolution in agricultural fields. *Biology and Fertility of Soils* 9, 152-158.
- Fabián, M., 1998. The effects of different methods of preservation on the  $^{15}\text{N}$  concentration in *Folsomia candida* (Collembola). *Applied Soil Ecology* 9, 101-104.
- FORCAST, 2000. Forest Carbon - Nitrogen Trajectories.  
[http://www.bgc.mpg.de/public/carboeur/Forcast/index\\_for.html](http://www.bgc.mpg.de/public/carboeur/Forcast/index_for.html).
- Gunn, A., Cherrett, J.M., 1993. The exploitation of food resources by soil meso- and macro invertebrates. *Pedobiologia* 37, 303-320.
- Haq, M.A., 1981. Feeding habits of ten species of oribatid mites (Acari: Oribatei) from Malabar, South India. *Indian Journal of Acarology* 6, 39-50.
- Hayes, A.J., 1965. Studies on the distribution of some phthiracarid mites (Acari, Oribatidae) in a coniferous forest soil. *Pedobiologia* 5, 252-261.
- Hendriksen, N.B., 1990. Leaf litter selection by detritivore and geophagous earthworms. *Biology and fertility of Soil* 10, 17-21.

- Herlitzius, H., 1987. Decomposition in five woodland soil, relationships with some invertebrate populations and with weather. *Biology and Fertility of Soils* 3, 85-89.
- Kaneko, N., McLean, M.A., Parkinson, D., 1995. Grazing preference of *Onychiurus subtenuis* (Collembola) and *Oppiella nova* (Oribatei) for fungal species inoculated on pine needles. *Pedobiologia* 39, 538-546.
- Klironomos, J.N., Widden, P., Deslandes, L., 1992. Feeding preferences of the collembolan *Folsomia candida* in relation to microfungal succession on decaying litter. *Soil Biology and Biochemistry* 24, 685-692.
- Luxton, M., 1966. Laboratory studies on the feeding habits of saltmarsh Acarina, with notes on their behaviour. *Acarologia* 8, 163-175.
- Luxton, M., 1979. Food and energy processing by oribatid mites. *Revue d'Ecologie et de Biologie du Sol* 16, 103-111.
- Macfadyen, A., 1962. Soil arthropod sampling. *Advances in Ecological Research* 1, 1-34.
- Maraun, M., Migge, S., Schaeffer, M., Scheu, S., 1998. Selection of microfungal food by six oribatid mite species (Oribatida, Acari) from two different beech forests. *Pedobiologia* 42, 232-240.
- Minagawa, M., Wada, E., 1984. Stepwise enrichment of  $^{15}\text{N}$  along food chains, further evidence and the relation between  $\delta^{15}\text{N}$  and animal age. *Geochimica et Cosmochimica Acta* 50, 2143-2146.
- Mitchell, M., Parkinson, D., 1976. Fungal feeding of oribatid mites (Acari: Cryptostigmata) in an aspen woodland. *Ecology* 57, 302-312.
- Neilson, R., Boag, B., Smith, M., 2000. Earthworm  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analyses suggest that putative functional classifications of earthworms are site-specific and may also indicate habitat diversity. *Soil Biology and Biochemistry* 32, 1053-1061
- O'Connor, F.B., 1955. Extraction of enchytraeid worms from a coniferous forest soil. *Nature* 175, 815-816.
- Oelbermann, K., Scheu, S., 2002. Stable isotope enrichment ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) in a generalist predator (*Pardosa lugubris*, Araneae: Lycosidae): effects of prey quality. *Oecologia* 130, 337-344.
- Owens, N.J.P., 1987. Natural variations in  $^{15}\text{N}$  in the natural environment. *Advances in Marine Biology* 24, 389-451.
- Parkinson, D., Visser, S., Whittaker, J.B., 1979. Effects of Collembola grazing on fungal colonization of leaf litter. *Soil Biology and Biochemistry* 11, 529-535.



- Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18, 293-320.
- Ponge, J.F., 1991a. Food resources and diets of soil animals in a small area of Scots pine litter. *Geoderma* 49, 33-62.
- Ponge, J.F., 1991b. Succession of fungi and fauna during decomposition of needles in a small area of Scots pine litter. *Plant and Soil* 138, 99-113.
- Ponge, J.F., 1999. Horizons and humus forms in beech forests of the Belgian Ardennes. *Soil Science Society of America Journal* 63, 1888-1901.
- Ponge, J.F., 2003. Humus forms in terrestrial ecosystems: a framework to biodiversity. *Soil Biology and Biochemistry* 35, 935-945.
- Ponge, J.F., Arpin, P., Sondag, F., Delecour, F., 1997. Soil fauna and site assessment in beech stands of the Belgian Ardennes. *Canadian Journal of Forest Research* 27, 2053-2064.
- Ponsard, S., Amlou, M., 1999. Effects of several preservation methods on the isotopic content of *Drosophila* samples. *Comptes Rendus de l'Académie des Sciences, Série III*, 322, 35-41.
- Ponsard, S., Arditi, R., 2000. What can stable isotopes ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) tell about the food web of soil macro-invertebrates? *Ecology* 81, 852-864.
- Ponsard, S., Averbuch, P., 1999. Should growing and adult animals fed on the same diet show different  $\delta^{15}\text{N}$  values? *Rapid Communications in Mass Spectrometry* 13, 1305-1310.
- Poole, T.B., 1959. Studies on the food of Collembola in a Douglas fir plantation. *Proceedings of the Zoological Society of London* 132, 71-82.
- Reisinger, O., Toutain, F., Mangenot, F., Arnould, M.F., 1978. Étude ultrastructurale des processus de biodégradation. I. Pourriture blanche des feuilles de hêtre (*Fagus sylvatica* L.). *Canadian Journal of Microbiology* 24, 725-733.
- SAS Institute, 1995. *Statistical Analysis System User's Guide, Version 6.10*. SAS Institute Inc., Cary.
- Saur, E., Ponge, J.F., 1988. Alimentary studies on the Collembolan *Paratullbergia callipygos* using transmission electron microscopy. *Pedobiologia* 31, 355-379.
- Schaefer, M., Schauer mann, J., 1990. The soil fauna of beech forests: comparison between a mull and a moder. *Pedobiologia* 34, 299-314.
- Scheu, S., 2002. The soil food web: structure and perspectives. *European Journal of Soil Biology* 38, 11-20.

- 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 a mesofauna-dominated community. *Oecologia* 123, 285-296.
- Scheu, S., Schaeffer, M., 1998. Bottom-up control of the soil macrofauna community in a beech wood on limestone: manipulation of food resources. *Ecology* 79, 1573–1585.
- Schmidt, O., Scrimgeour, C.M., Handley, L.L., 1997. Natural abundance of  $^{15}\text{N}$  and  $^{13}\text{C}$  in earthworms from a wheat and a wheat-clover field. *Soil Biology and Biochemistry* 29, 1301-1308.
- Setälä, H., Aarnio, T., 2002. Vertical stratification and trophic interactions among organisms of a soil decomposer food web: a field experiment using  $^{15}\text{N}$  as a tool. *European Journal of Soil Biology* 38, 29-34.
- Shaw, P.J.A., 1988. A consistent hierarchy in the fungal feeding preferences of the Collembola *Onychiurus armatus*. *Pedobiologia* 31, 179-187.
- StAAF, H., 1987. Foliage litter turnover and earthworm populations in three beech forests of contrasting soil and vegetation types. *Oecologia* 72, 58-64.
- Stöckli, H., 1990. Das Unterscheidungsvermögen von *Porcellio scaber* (Crustacea, Isopoda) zwischen Blättern einer Baumart, unter Berücksichtigung der makroskopisch sichtbaren Verpilzung. *Pedobiologia* 34, 191-205.
- Thompson, W., 1984. Distribution, development and functioning of mycelial cord systems of decomposer basidiomycetes of the deciduous woodland floor. In: Jennings, D.H., Rayner, A.D.M. (Eds.), *The ecology and physiology of the fungal mycelium*. Cambridge University Press, Cambridge, UK, pp. 185-214.
- Toutain, F. 1987. Activité biologique des sols, modalités et lithodépendance. *Biology and Fertility of Soils* 3, 31-38.
- Toutain, F., Villemin, G., Albrecht, A., Reisinger, O., 1982. Étude ultrastructurale des processus de biodégradation. II. Modèle enchytraeides-litière de feuillus. *Pedobiologia* 23, 145-156.
- Van der Drift, J., 1951. Analysis of the animal community in a beech forest floor. *Tijdschrift voor Entomologie* 49, 1-168.
- Verhoef, H.A., Prast, J.E., Verweij, R.A., 1988. Relative importance of fungi and algae in the diet and nitrogen nutrition of *Orchesella cincta* (L.) and *Tomocerus minor* (Lubbock) (Collembola). *Functional Ecology* 2, 195-201.
- Visser, S., Whittaker, J.B., 1977. Feeding preference for certain fungi by *Onychiurus subtenuis* (Collembola). *Oikos* 29, 320-325.

- Walter, D.E., Proctor, H.C., 1999. Mites: Ecology, Evolution and Behaviour, University of NSW Press/CABI, Sydney/Wallingford, 322 pp.
- Whittaker, J.B., 1981. Feeding of *Onychiurus subtenuis* (Collembola) at snow melt in aspen litter in the Canadian Rocky Mountains. *Oikos* 36, 203-206.
- Zachariae, G., 1965. Spuren tierischer Tätigkeit im Boden des Buchenwaldes. *Forstwissenschaftliche Forschungen* 20, 1-68.
- Zeller, B., Colin-Belgrand, M., Dambrine, E., Martin, F., 1998.  $^{15}\text{N}$  partitioning and production of  $^{15}\text{N}$ -labelled litter in beech trees following [ $^{15}\text{N}$ ]urea spray. *Annales des Sciences Forestières* 55, 375-383.
- Zeller, B., Colin-Belgrand, M., Dambrine, E., Martin, F., Bottner, P., 2000. Decomposition of  $^{15}\text{N}$ -labelled beech litter and fate of nitrogen derived from litter in a beech forest. *Oecologia* 123, 550-559.
- Zeller, B., Colin-Belgrand, M., Dambrine, E., Martin, F. 2001. Fate of nitrogen released from  $^{15}\text{N}$ -labelled litter in European beech forests. *Tree Physiology* 21, 153-162.

## Appendix

List of the taxa identified at Leinefelde and Sorø with indication  
of their size range and their feeding preferences

Taxa	Size	Feeding preferences	Abbreviation	Identification	
				Leinefelde	Sorø
<b>Enchytraeidae</b>	Medium	Saprophagous	Enchy sapro	+	+
<b>Lumbricidae</b>	Large	Saprophagous	Lumbr sapro	+	+
<b>Chilopoda</b>					
Geophilomorpha	Large	Predators	Geophil pred	+	+
Lithobiomorpha	Large	Predators	Litho pred	+	-
<b>Diplopoda</b>					
Glomeridae	Large	Saprophagous	Glom sapro	+	+
Julidae	Large	Saprophagous	Jul sapro	+	+
<b>Oribatida</b>					
Phthiracaroida	Medium	Saprophagous	Phthi sapro	+	+
Nothroidea	Small	Saprophagous	Nothr sapro	+	+
Belboidea	Small	Saprophagous	Belb sapro	+	+
<i>Eniochthonius</i>	Small	Saprophagous	Enio sapro	+	+
<i>Achipteria</i>	Small	Saprophagous	Achip sapro	+	+
<b>Gamasida</b>					
<i>Trachytes</i>	Small	Predators	Trac pred	+	+
Parasitidae	Small	Predators	Para pred	+	+
Uropodidae	Small	Fungivorous	Urop fungi	+	+
<b>Eupopodidae</b>	Small	Fungivorous	Eupo fungi	+	+
<b>Araneae</b>	Large	Predators	Aran pred	+	+
<b>Pseudoscorpionida</b>	Medium	Predators	Pscorp pred	+	+
<b>Isopoda</b>	Large	Saprophagous	Isop sapro	+	+
<b>Collembola</b>					
<i>Pogonognathellus</i>	Large	pollen, algae, fungi spora herbifungivorous	Pogo herb	+	+
<i>Entomobrya</i>	Large	pollen, algae, fungi spora herbifungivorous	Entomo herb	+	+
<i>Sminthurinus</i>	Small	pollen, algae, fungi spora herbifungivorous	Smin S herb	+	+
<i>Lepidocyrtus</i>	Medium	fungi hyphae, algae herbifungivorous	Lepido M herb	+	+
<i>Folsomia</i>	Small	Coprophagous	Folso S copro	+	+
<i>Isotomiella</i>	Small	Coprophagous	Isoto S copro	+	-
<i>Pseudosinella</i>	Small	Fungivorous	Psin S fungi	+	+
Neanuridae	Small	Phytophagous, litter browser and sucker	Nea S phyto	+	+
Onychiuridae	Small	Coprophagous	Ony S copro	+	-