Fate of nitrogen released from $^{15}$N-labeled litter in European beech forests

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Summary  The decomposition and fate of $^{15}$N-labeled beech litter was monitored in three European beech (Fagus sylvatica L.) forests (Aubure, France; Ebrach, Germany; and Collelongo, Italy) for 3 years. Circular plots around single beech trees were isolated from roots of neighboring trees by soil trenching, and annual litterfall was replaced by $^{15}$N-labeled litter. Nitrogen was continuously released from the decomposing litter. However, over a 2-year period, this release was balanced by the incorporation of exogenous N. Released N accumulated mainly at the soil surface and in the topsoil. Microbial biomass remained almost constant during the experiment at all sites except for considerably lower values at Ebrach. The $^{15}$N enrichment of the microbial biomass increased strongly during the first year and then remained stable. The $^{15}$N released from the decomposing litter was rapidly detected in roots and leaves of the beech trees, increasing regularly and linearly over the course of the experiment. The uptake of litter-released $^{15}$N by the trees was reduced under conditions that reduced tree growth. Under these conditions, leaves and fine roots were the dominant N sinks, and little N was allocated to other plant parts. By contrast, N uptake and N allocation from leaves to stem and bark tissues increased when tree growth was enhanced. Budgets for $^{15}$N showed that 2 to 4% of litter-released N was incorporated into the trees, about 35% remained in the litter and about 50% reached the topsoil.

Keywords: Fagus sylvatica, litter decomposition, N cycle, nitrogen, N partitioning, N uptake.

Introduction

Litter decomposition is an important nutrient recycling process that influences primary production of forest ecosystems. Plant-available nutrients are released from litter by both physical leaching and breakdown of structural organic compounds by soil biota (Scheu and Wolters 1991). An additional effect of the decomposition process is the formation of soil organic matter (SOM), which leads to the temporary sequestration of C and N in forest soils (Nadelhoffer et al. 1999a). This accumulation of C and N is generally reflected in the composition of humus, which acts as a large storage pool for both elements. Moreover, the humus type reflects soil and other site-specific parameters which determine the fate of litter N.

In an earlier study (Zeller et al. 2000), we assessed the fate of litter-released N in a slow-growing montane beech (Fagus sylvatica L.) forest on an acidic brown soil, using $^{15}$N-labeled beech litter. On this site, litter mass loss and total N variation followed the general trends observed for beech litter decomposition (Staaf 1980). Nitrogen dynamics in decomposing litter consisted of two phases that occurred simultaneously, as previously observed (Berg 1988, Blair et al. 1992, Hart et al. 1993). While litter N was released, external N from the surrounding soil was incorporated into the decomposing litter through colonization by fungi and bacteria, and from throughfall N (Downs et al. 1996, Nadelhoffer et al. 1999b). The litter N released during the early stages (i.e., the first 6 months of decomposition) was rapidly incorporated into microbial biomass and further partitioned into the beech trees. After 3 years of litter decomposition, most of the litter N accumulated in the topsoil and only a small proportion (2.5%) was detected in the trees. This suggests that, in this slow-growing forest, trees were not a major sink for litter N. However, the fate of litter N in other European beech forests with different soil types and growth conditions is unknown.

The primary purpose of this study was to compare the fate of litter N in three different European beech ecosystems with contrasting climate, soil type and tree growth (i.e., Aubure, France; Ebrach, Germany; and Collelongo, Italy). At Aubure, the fate of litter-released N was intensively studied as part of an earlier investigation (Zeller et al. 2000). In the current paper, results from Ebrach and Collelongo were compared to those from Aubure. Aims were (i) to follow N dynamics in the decomposing litter at each site, (ii) to study the progressive repartitioning of litter-released N in the solid organic-N fraction and in microbial biomass in relation to soil depth, and (iii) to determine the uptake and repartitioning of litter N by the trees. Relationships between litter N dynamics and site-specific parameters are discussed. Knowledge of the litter N dynamics in the soil–plant system will enable the development of
models to predict whether C and N are stored in the soil or transferred to the plant.

**Materials and methods**

**Site description**

The sites where the $^{15}$N-labeled litter was deposited are: Aubure, France (48°12′ N, 07°11′ E); Ebrach, Germany (49°52′ N, 10°27′ E); and Collelongo, Italy (41°52′ N, 13°38′ E). These beech forests were included in a large ecosystem study on carbon and nitrogen cycling in forests (CANIF) (Schulze 2000). Characteristics of the selected beech forests are presented in Table 1. These sites represent the range of conditions where beech forest naturally occurs: acidic sandy plains and a humid continental climate (Ebrach, northern Bavaria); acidic, coarse-textured montane soils and an oceanic climate (Aubure, eastern France); and calcareous soils and a montane Mediterranean climate (Collelongo, central Italy). The selected sites have an altitude of 400–1600 m, a mean annual temperature of 5.4–7.5 °C and precipitation of 750–1400 mm. Some soil chemical characteristics of the experimental sites are given in Table 2. At Aubure, the humus layer includes a 2–3-cm-thick L layer, a 2-cm-thick F layer and a thin, discontinuous H layer. The soil is deep, coarse-textured and acidic, with a relatively high C/N ratio in the upper soil. At Ebrach, the humus layer is composed of a 2-cm-thick L layer, a 1-cm-thick F layer and a 1-cm-thick H layer. Here, the soil is deep, sandy and acidic with low C and N contents. At Collelongo, the humus layer is composed of a 2-cm-thick L layer and a 0.5-cm-thick F layer. The soil is shallow, rocky, calcareous and rich in clay, carbon and nitrogen, with a low C/N ratio in the humus.

At all sites, the leaf litter was replaced with $^{15}$N-labeled beech litter. The $^{15}$N-labeled beech litter was obtained by foliar application of $^{15}$N-urea to 10-year-old beech trees (Zeller et al. 1998), growing on a calcisol (FAO 1988). Consequently, the N, P and Ca concentrations of the $^{15}$N-labeled litter differed from those of the original litter at each site. The $^{15}$N-labeled litter used at Aubure was produced in 1994 (0.8% N, 3.24 atom% $^{15}$N excess). There was not enough of this material for use at the other sites, where we used a $^{15}$N-labeled litter produced in 1993 with a $^{15}$N enrichment of 2.13 atom% $^{15}$N excess (0.8% N). The chemical composition of the beech litters was similar.

The Aubure litter decomposition experiment, including the sampling procedure, is described in detail by Zeller et al. (2000). Five circular plots, with an area of 4 m$^2$, were established around each of five 50-year-old beech trees. Within each plot, 200 g m$^{-2}$ of $^{15}$N-labeled litter was evenly distributed. Because trees were smaller at Collelongo and Ebrach, a circular plot of 1 m$^2$ was isolated around each of nine selected

<table>
<thead>
<tr>
<th>Location</th>
<th>Aubure (F)</th>
<th>Ebrach (G)</th>
<th>Collelongo (I)</th>
</tr>
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<tbody>
<tr>
<td>Latitude, longitude</td>
<td>48°12′ N, 07°11′ E</td>
<td>49°52′ N, 10°27′ E</td>
<td>41°52′ N, 13°38′ E</td>
</tr>
<tr>
<td>Slope (%)</td>
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<td>2</td>
<td>15</td>
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<td>Climate</td>
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<tr>
<td>Rainfall (mm)</td>
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<td>1100</td>
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<tr>
<td>Annual mean temperature (°C)</td>
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<tr>
<td>Elevation (m a.s.l.)</td>
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<td>400</td>
<td>1600</td>
</tr>
<tr>
<td>Leaf litter characteristics</td>
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</tr>
<tr>
<td>N (mg g$^{-1}$)</td>
<td>14.2</td>
<td>12.8</td>
<td>13.0</td>
</tr>
<tr>
<td>P (mg g$^{-1}$)</td>
<td>1.2</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
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<tr>
<td>Ca (mg g$^{-1}$)</td>
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<tr>
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<td>Rendzina</td>
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<td><em>F. sylvatica</em></td>
<td><em>F. sylvatica</em></td>
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<td>30</td>
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<td>Mean tree height (m)</td>
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<td>DBH (cm)</td>
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<td>4.4</td>
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<td>0.05</td>
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<td>Scattered herbs and fern</td>
<td>Scattered herbs and fern</td>
<td>Scattered herbs and fern</td>
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</tbody>
</table>
trees by root trenching and insertion of a plastic ring down to 0.3 m soil depth. In March 1996 (Ebrach) and May 1996 (Collelongo), the L layer was carefully collected at each of the nine plots, then 250 g of $^{15}$N-labeled litter was evenly distributed and a nylon net fixed above the added litter. Removal of the L layer was necessary to avoid confusion of partly decomposed litter with the added $^{15}$N-labeled litter during the experiment. The N and P concentrations in the $^{15}$N-labeled litter were lower than in the original beech litter from Ebrach and Collelongo.

**Litter sampling**

At Ebrach, all remaining $^{15}$N-labeled litter was sampled from three plots in October 1996 and October 1997. In October 1998, $^{15}$N-labeled litter was sampled from a 300-cm$^2$ area on each of three experimental plots. At Collelongo, all remaining $^{15}$N-labeled litter was collected from three plots in September 1997 and from two plots in September 1998. The collected $^{15}$N-labeled litter was dried at 65 °C, carefully cleaned of adhering soil and unlabeled organic detritus (e.g., bud scales) and weighed. Mass loss was calculated as the difference between the initial and final litter weights. At Aubure, litter mass loss in the five plots was estimated from a litterbag experiment installed nearby (Zeller et al. 2000).

**Soil sampling and analysis**

Soil $\delta^{15}$N values were measured at each site before the addition of the $^{15}$N-labeled litter. At all sites, the humus F and H layers were sampled together. At each sampling at Aubure, four soil cores per plot (0–10 cm deep) were taken from each plot, before the $^{15}$N-labeled litter had been sampled. Then two cores per plot were cut into four discs about 2.5 cm thick. From each disc, a representative soil sample was collected for analysis of solid soil $^{15}$N, then the four sub-samples were combined and sieved (4 mm) before the extraction of mineral N. Nitrate and ammonium concentrations were measured in all soil samples with a Flow Injection Analyzer (TRAACS 2000, Bran and Luebbe Inc., Buffalo Grove, IL) after extraction of 40 g soil in 200 ml of 0.05 M K$_2$SO$_4$. Microbial biomass was measured by the fumigation-extraction technique (Vance et al. 1987). Forty g of fresh soil was fumigated with alcohol-free chloroform in darkness and soluble N was extracted with 200 ml of 0.05 M K$_2$SO$_4$ (Chaussod et al. 1987). The $^{15}$N enrichment of fumigated and unfumigated extracts was measured after mineralization of 100 ml of the extract (Kjeldahl method) followed by steam distillation and evaporation of the (NH$_4$)$_2$SO$_4$ distillates adjusted to pH 4 (Bottner et al. 1998). Microbial N was calculated from the difference between fumigated and unfumigated extracts, using a correction factor $k_B = 0.68$ (Vance et al. 1987). Before total N and $^{15}$N analyses, subsamples of dry soil from each depth were pulverized.

**Tree biomass sampling**

From 1996 to 1998, 20 to 30 leaves from the upper crown of each tree were collected once in August or twice in both June and August. At the beginning of September, each tree crown was enclosed by a nylon net (mesh size 10 mm) to collect the annual litterfall. The collected leaf litter was separated from dead branches and weighed.

At Collelongo, whole trees were harvested from three selected experimental plots in 1997 and from two plots in 1998, and the stem circumference, height and annual length growth measured. Each tree was separated into the following compartments: buds, twig-bark and twig-wood (year 0), twig-bark and twig-wood (year 1), branches, stem-bark and stem-wood, mycorrhizal root tips, roots < 1 mm, roots 1–3 mm, roots > 3 mm and coarse roots. All root samples were cleaned of adhering soil particles by washing twice with distilled water. All tree biomass samples were dried and weighed before N and $^{15}$N analysis. At Ebrach, three trees were harvested in 1996 and 1997 following the same procedure as in Collelongo. In 1998 there was no whole-tree harvest, because the previous biomass determinations revealed little variation among trees. Instead, we used a nondestructive sampling procedure to estimate the biomass of all tree components. Samples of twigs,
branches, stem and root were taken and tree growth determined by measuring height growth. To determine the 15N content of each compartment, the biomass distribution of the former samplings was used.

Analysis of 15N

All samples were pulverized with a ball mill to pass a 40-mesh screen. Concentrations of N and 15N were measured with an elemental analyzer (Carlo Erba NA 1500, CE Instruments Inc., Milan, Italy) coupled to a mass spectrometer (Delta S, Finnigan Corp., San Jose, CA). Values of 15N enrichment (15N std) were calculated by subtracting 15N values from that of a control (mean of five measurements).

Nitrogen released from the litter was calculated as the difference between the initial and final amounts of 15N in the litter. Nitrogen incorporated into the litter was calculated as the difference between total N and the remaining 15N in the litter, as follows:

\[
\text{released } N = \frac{(15N_{T_i} - 15N_{T_f}) - R_{std}(N_{T_i} - N_{T_f})}{R_{litter} - R_{std}},
\]

incorporated \( N = N_{T_i} - (N_{T_f} + \text{released } N), \)

where \( R = \frac{15N(14N + 15N)}{15N} \) of the 15N-labeled litter \( (R_{litter}) \) or in the standard \( (air, R_{std}), T_0 = \text{start of the experiment and } T_f = \text{time of litter decomposition (days)}. \)

Results

Litter mass loss

Decomposition rates of the 15N-labeled beech litter deposited in the Aubure and Ebrach beech forests reached peak values during the first year and were similar at the two sites (Table 3). At Collelongo, no data were gathered in the first year. During the second year, the decomposition rate constant \( (k) \) decreased markedly at Aubure and little at Ebrach. During the third year, \( k \) decreased at Ebrach and Collelongo but increased at Aubure. Mean \( k \) values showed that litter decomposition rates were similar at Aubure and Ebrach, but lower at Collelongo.

15N-labeled litter

Increases in N content of litter were observed at all sites during the first year (Figure 1A). During the second and third years, N content decreased continuously and finally reached values lower than the initial litter N content. These temporal changes in total N content of the decomposing 15N-labeled litter occurred in two phases. First, N was immobilized as long as the total N content exceeded the initial content, and second, a net release of N occurred when the N content was lower than the initial litter N. Site-specific conditions weakly influenced the litter N dynamic, which seems to be driven by the initial litter chemistry.

The N dynamics of decomposing beech litter are summarized in Figure 1B. At all sites, litter N was continuously released from the beginning of the decomposition process. This N release was linearly related to the time of decomposition (Aubure: \( P < 0.001, r^2 = 0.90; \) Ebrach: \( P < 0.001, r^2 = 0.96; \) Collelongo: \( P < 0.001, r^2 = 0.90)\), but the slopes were slightly different for each site. Annual N release was similar at Aubure and Ebrach, but lower at Collelongo. Incorporation of external N into the decomposing beech litter occurred mainly during the first year. At Collelongo, some additional external N was incorporated during the second year. Incorporation of N in the decaying litter likely reflects rapid colonization of decomposing leaf litter by fauna, fungi and microbes.

Nutrients

At Aubure, the P content of decomposing beech litter increased sharply during the first year, then remained constant for another year before decreasing to approximately the initial content (Figure 2). Similar trends in P content occurred at Ebrach and Collelongo, but with a much lower amplitude. Because the initial P content of 15N-labeled litter was virtually the same at all sites, higher P immobilization at Aubure may have been a result of the relatively high P concentration in the topsoil. At all sites, K content in the decomposing litter decreased sharply during the first year to about 30–40% of the initial amount. During litter decomposition, Mg content decreased rapidly and continuously at Ebrach and Aubure, with a moderate decrease at Collelongo. At Collelongo, Ca accumulated in the decomposing litter, whereas at Ebrach and Aubure the Ca content decreased continuously. The different trends for Ca and Mg at Collelongo are attributed to the calcareous nature of the soil.

Soil

The pattern of 15N abundance in forest soils, before the deposition of the labeled litter at each site, was similar to that observed in previous studies (Gebauer and Schulze 1991). Forest floor \( \delta^{15}N \) values were generally negative (about –2‰), whereas mineral soil \( \delta^{15}N \) values were slightly positive (about 3‰). In the mineral soil, the \( \delta^{15}N \) values increased with soil depth; e.g., at Ebrach, mean soil \( \delta^{15}N \) values were 0.53‰ for 0–10 cm depth, 2.99‰ for 10–20 cm depth and 4.03‰ for 20–30 cm depth (Figure 3). At Aubure, the \( ^{15}N \) enrichment of the deposited litter was higher than in the litter deposited at Ebrach and Collelongo. This prevented a comparison of the time course of soil \( \delta^{15}N \) values among all sites. Nevertheless,
the repartitioning of the litter-released $^{15}$N in the soil profile remained unaffected. A similar trend was observed at all sites: litter-released N accumulated mainly at the soil surface, resulting in a progressive increase in soil $\delta^{15}$N of the topsoil. The $\delta^{15}$N values in the deeper soil at each of the three beech forests remained close to values obtained before treatment. At Aubure, $\delta^{15}$N values showed a steep gradient from the surface to a depth of 10 cm beginning in the first year after litter deposition. After 4 years, upper soil (2 cm depth) $\delta^{15}$N values increased to 60‰ (SE = 6.4). After 3 years at Ebrach and Collelongo, $\delta^{15}$N values in the upper soil (2 cm depth) increased to 33‰ (SE = 4) and 40‰ (SE = 12), respectively. At both sites, final soil $\delta^{15}$N values at each soil depth were not significantly different.

Soil microbial biomass

Microbial biomass N ($N_{\text{mic}}$) in the topsoil (top 10 cm) revealed large differences among sites (Figure 4). Over the entire experiment, microbial biomass N was about threefold higher at Collelongo and Aubure than at Ebrach. Annual variation in microbial biomass was generally low at all sites and N levels.

The microbial biomass $^{15}$N ($^{15}$N$_{\text{mic}}$) differed among sites. At Aubure, $^{15}$N$_{\text{mic}}$ was twice as high as at Ebrach. The difference in $^{15}$N$_{\text{mic}}$ between Ebrach and Collelongo can be attributed to
the size of the microbial biomass pool. At all sites, microbial biomass $^{15}$N increased significantly in the time span between litter deposition and the first sampling date, and then remained nearly constant for almost 3.5 years. At Aubure, microbial biomass $^{15}$N decreased sharply during the fourth year, indicating that litter-released $^{15}$N was replaced by unlabeled N.

Vegetation

Increases in $^{15}$N content were detected in beech trees on sites where $^{15}$N-labeled litter was applied. Following the first year after litter deposition, $^{15}$N$_{\text{leaf}}$ of foliage reached 2‰ at Aubure and 6‰ at Ebrach, indicating that litter-released N was rapidly taken up by the roots and transferred to leaves (Figure 5). The $^{15}$N$_{\text{leaf}}$ was calculated as:

$$^{15}\text{N}_{\text{leaf}} = \left[\frac{(R_{\text{sample}} - R_{\text{unlabeled control}})}{R_{\text{unlabeled control}}}\right] \times 1000.$$  (3)

During the following years, $^{15}$N$_{\text{leaf}}$ in the foliage increased linearly at all sites. The mean annual increase in $^{15}$N$_{\text{leaf}}$ was 5.7‰, 14.5‰ and 7‰ at Aubure, Ebrach and Collelongo, respectively. Final $^{15}$N$_{\text{leaf}}$ in the foliage was 28‰ at Aubure (57 months), 48‰ at Ebrach (41 months) and 27‰ at Collelongo (38 months). Because of the different initial $^{15}$N enrichment of the labeled litter, foliage $^{15}$N$_{\text{leaf}}$ from Aubure cannot be directly compared with the $^{15}$N$_{\text{leaf}}$ obtained at Ebrach and Collelongo. Significantly higher $^{15}$N$_{\text{leaf}}$ values ($P < 0.001$) were recorded at Ebrach than at Collelongo. At both sites, leaf biomass was similar, so that dilution of litter-derived N into the large leaf N pool cannot fully account for this difference.

Three years after deposition of the $^{15}$N-labeled litter, $^{15}$N values were higher than control tree values in all tree biomass compartments and sites, except for stem bark and wood at Collelongo (Figure 6). Large differences in $^{15}$N values among the sites were observed. At Ebrach, the $^{15}$N values in all aerial and root biomass compartments except for mycorrhizal root tips and fine roots were similar, but much higher than at Aubure and Collelongo. At Aubure and Collelongo, $^{15}$N values were highest in mycorrhizal root tips and fine roots, perhaps because both compartments were in close contact with the decomposing litter and are essential for nutrient uptake. The repartitioning of litter-released $^{15}$N in the aboveground biomass at Aubure and Collelongo followed a similar trend with the highest $^{15}$N values found in leaf litter and in the most recently formed biomass compartments (buds, twigs). Low (Aubure) or negative (Collelongo) $^{15}$N values in branches and stems suggest that these tissues are low N sinks as a result of slow wood growth at both sites. At Aubure and Collelongo, under conditions of slow growth due to montane climatic conditions (high altitude, short growth period and water stress), N demand by the trees appears to be adapted to the extreme conditions. Here, litter-released N accumulated mainly in the humus and upper soil layers, mostly in organic form.

$^{15}$N budgets (Table 4)

Recovery of litter $^{15}$N in the soil–plant compartment at each site approached 90% of applied $^{15}$N. The $^{15}$N-labeled litter contained from 32 to 41% of the applied $^{15}$N after 3 years of litter decomposition. The distribution of litter-released N in the soil profile followed similar trends at all sites. Litter-released $^{15}$N accumulated almost exclusively in the topsoil at each site. About 26–37% remained in the upper 2 cm layer, about 9–18% between 2 and 10 cm and only a few percent in...
deeper soil layers, except at Collelongo where 5% of litter-released N was transferred to depths between 10 and 30 cm. In contrast, the repartitioning of litter N in the trees occurred differently among the three sites. At Collelongo, 0.3% of litter N was incorporated into root and aboveground tree biomass, however, the same amount (0.4%) was returned to the soil through the annual litterfall. At the other two sites, incorporation of litter $^{15}$N by the trees was much greater, especially at Ebrach (3.4%). Moreover, less than 30% of the litter $^{15}$N incorporated in tree biomass returned to the soil as litterfall, indicating that litter N was used for growth.

### Discussion

Because the initial $^{15}$N enrichment of the litter at Aubure was higher than at the two other sites, $^{15}$N enrichment, expressed as $\delta^{15}$N of soil and tree biomass, cannot be compared among sites. Nevertheless, the trends in progressive $^{15}$N enrichment in soil and trees were comparable at all sites. Additionally, all $^{15}$N-labeled litter had a similar chemical composition.

Nitrogen release from decomposing $^{15}$N-labeled beech litter was strongly coupled with biological and physical breakdown of canopy litter, both factors responsible for litter mass loss. A close correlation between litter mass loss and release of litter N suggests that C and N release occurred at similar rates, first by leaching of soluble compounds and then by fragmentation by soil fauna (Cotrufo et al. 2000). While litter N was released, there was an accumulation of exogenous N in litter from throughfall, and colonization by microbes and fungi (Berg 1988, Downs et al. 1996, Zeller et al. 2000). The input of exogenous N exceeded the loss of structural litter N for about 2 years, then a net loss of N was observed (Figure 1). A similar pattern of change in beech total litter N content has been observed in earlier studies without use of isotopic tracers (Staaf 1980, Blair 1988). Although the N content of the original litter at each site was slightly higher than in the $^{15}$N-labeled litter with which it was replaced, N dynamics of the original litter may have been similar, as observed in a litter bag experiment with beech litter with an initial N concentration of 1.5% N at Aubure (Zeller et al. 2000). Litter-released $^{15}$N was rapidly taken up by the trees and transferred to the leaves during the first 6 months after litter deposition. During this period, leaf $\delta^{15}$N increased linearly at all sites, suggesting a weak but continuous flux of litter N into the trees. Furthermore, the absence of a peak in leaf $\delta^{15}$N indicated that released $^{15}$N from the different litter N fractions participated equally in tree N uptake. Incorporation by trees of readily available fertilizer $^{15}$N is high initially but low during subsequent years (Mead and Preston 1994, Preston and Mead 1994). However, the litter-released N dynamics in the soil and the fluxes between the different soil compartments remain uncertain. For example, the size of the microbial biomass varied between sites, and may have affected their impact on soil N fluxes (Figure 4).

For the experimental periods (1994–1998), our results indicate that trees are not major sinks for litter-released N at the two high elevation sites. Three years after the $^{15}$N-labeled litter deposition, between 0.7 and 3.4% of litter N was recovered in the trees, mainly in leaves, twigs, bark and fine roots (i.e., growing tissues). In a similar experiment with $^{15}$N-labeled alder leaves, Swanston and Myrold (1997) detected 2.7% of the litter-released $^{15}$N in the vegetation. These results suggest that early release of N from decomposing litter plays a minor role in the N nutrition of forest trees. Higher recovery of deposited or fertilized $^{15}$N in forest trees has been observed (Mead and Preston 1994, Chang et al. 1996, Nadelhoffer et al. 1999a); however, partitioning of the incorporated N among the tree compartments was similar to the results from the present study (Figure 6). The sink strength of growing tissues for N and other nutrients is the same for soil-borne and deposited nitrogen; however, it seems that deposited N is more readily available for uptake by trees. Litter-released N accumulated mainly as organic N in the topsoil and was consequently diluted into the large soil organic N pool (Figure 3). Here, litter N was initially mineralized and then taken up by the trees. Therefore, higher recovery of $^{15}$N from deposited or fertilizer N in trees can be attributed to the different sizes of soil organic and mineral N pools. However, it was not possible to determine the form (NO$_3^-$, NH$_4^+$, organic N) in which litter-released N was taken up. High $\delta^{15}$N values in mycorrhizal root tips found in a parallel study (Zeller et al. 2000) suggest a direct uptake of organic N compounds. This hypothesis is certainly favored by the growth of extramatrical ectomycorrhizal hyphae in the decaying leaves. In contrast to non-mycorrhizal roots, ectomycorrhizal roots are able to use organic N sources (Nåsholm et al. 1998). Nevertheless, mineral N uptake is dominant in these temperate beech forests (George and Stober 2000).
Reduced tree growth, as observed at Collelongo (Table 1), showed that about 50% of incorporated N returned to the soil by way of the annual litterfall. At Aubure and Ebrach, where tree growth was higher, about 30 and 50% of incorporated N, respectively, was allocated to branches, stemwood and bark. Our data suggest that utilization of litter N by beech trees depends on tree growth, which is related to site-specific conditions. Annual increment in tree height and diameter is very low at the Collelongo site, so that the sink function of growing tissues is quite small. Water stress, particularly during the summer months, seems the most probable reason for reduced growth and it may also have influenced N mineralization and incorporation of litter N into tree biomass.

After 3 years of litter decomposition, almost all litter-released $^{15}$N accumulated in the soil. At all sites, the greatest quantity of litter N was recovered in the topsoil (0–2 cm) and much less in deeper soil horizons (2–30 cm). Higher $^{15}$N accumulation in deeper horizons at Ebrach, and especially at Collelongo, can be attributed to the activity of earthworms. Both the activity and abundance of earthworms are generally higher in beech forest soils with high Ca concentration and pH (Ponge et al. 1997). An alternative, but less likely, possibility is that increased leaching of highly enriched mineral N may increase the $\delta^{15}$N values at the 15- and 25-cm soil depths. This hypothesis can be ruled out for the Aubure beech forest, because lysimeter samplings showed almost no nitrate leaching (Jussy et al. 1999). Dissolved organic N was not directly measured at the study sites. However, for each extraction of microbial biomass N, organic N was measured in the unfumigated control and ranged from 10 to 20% of total extractable N. Finally, decomposition of fine roots in deeper soil will add some $^{15}$N to the soil organic matter, but the contribution of this source to the $^{15}$N enrichment in deeper layers is low compared with the highly enriched litter.

Various single pulse or continuous tracer application studies in forests have shown that soils (including forest floors) are the dominant sinks for deposited or fertilizer N additions (Preston et al. 1990, Preston and Mead 1994, Preston and Mead 1995, Buchmann et al. 1996, Gundersen 1998). It seems likely that the litter layer acted as a buffer at low N deposition rates (Downs et al. 1996), whereas, with increasing deposition, N was lost from N-saturated forests mainly by leaching (Durka et al. 1995). At Aubure, about 30% of the ex-
ternal N incorporated in decaying litter can be attributed to fungal biomass, whereas throughfall N seems the most probable secondary source for external N incorporation (Zeller et al. 2000). Additionally, low losses of nitrate have been reported for this forest site over a 10-year period (Dambrine et al. 2000). Decomposition of straw has demonstrated that almost all residue N has been recovered in different particle size fractions of organic matter (Stemmer et al. 1999).

In conclusion, N release from decomposing beech litter is closely connected with litter mass loss, as a result of physically and biologically mediated breakdown of the litter. At all sites, this 1:1 relation between mass loss and N release suggests a direction for further studies on N dynamics in decomposing litter. Litter-released N was rapidly incorporated in the microbial biomass, taken up by the roots by way of mycorrhizae and further transferred to N sinks in the trees. However, uptake of litter N by trees was small and almost all litter N became part of the soil N pool. Little is known about the further fate of N in mineralization or humification in beech forests.

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