Plant Soil (2013) 362:67–77
DOI 10.1007/s11104-012-1245-0

REGULAR ARTICLE

# Contribution of $^{15}N$-labelled leaf litter to N turnover, nitrous oxide emissions and N sequestration in a beech forest during eleven years

**Nadine Eickenscheidt · Rainer Brumme**

Received: 24 January 2012 / Accepted: 30 March 2012 / Published online: 8 May 2012

**Abstract**

*Aims* Decomposition of leaf litterfall plays a major role for nitrogen (N) dynamics in soils. However, little is known as to which extent beech leaf litter contributes to N turnover and nitrous oxide ($N_2O$) emissions within one decade after litterfall.

*Methods* In 1997, we exchanged recently fallen leaf litter by $^{15}N$-labelled litter in a beech stand (*Fagus sylvatica*) at the Solling, Germany. Measurements were conducted 2–3 and 10–11 years after litter exchange.

*Results* Two years after litter exchange, 92 % of added $^{15}N$ was recovered in the surface 10 cm of the soil. The labelled N was primarily found in the upper part of the F layer of the moder type humus. Eleven years after litter exchange, 73 % of the added $^{15}N$ was lost and the remaining 27 % was mainly recovered in the lower part of the F layer indicating N sequestration. The remaining leaf litter N was subject to measurable N mineralisation (2–3 % of litter N) and $N_2O$ production (0.02 %). Between 0.3 % (eleventh year) and 0.6 % (second year) of total annual $N_2O$ emissions were attributed to beech leaf litter of a single year.

*Conclusions* Most of the annual $N_2O$ emissions (1.33–1.54 kg N $ha^{-1}$ $yr^{-1}$) were probably derived from older soil N pools.

**Keywords** Nitrous oxide · Beech leaf litter · $^{15}N$ isotope technique · N mineralisation · Litter N dynamics

## Introduction

Weathering of geological parent material contributes very marginally to mineral nitrogen (N) content of soils (Berg and Matzner 1997; Parton et al. 2007). Thus, decomposition of litter and mineralisation inhere particular importance for N availability in forest ecosystems (Parton et al. 2007). Decomposition has been described as a three-stage process: (i) the early stage, (ii) the late stage, and (iii) the humus-near stage, latter is characterised by a decomposition rate close to zero (Berg and McClaugherty 2003; Berg and Matzner 1997).

During the early stage, initial litter N concentrations primarily drive net N release from litter (Parton et al. 2007). A rapid mass loss of native litter N but a simultaneous net increase in N and lignin contents of the decaying litter during the early stage was generally reported (Berg and McClaugherty 2003; Laganière et al. 2010; Melillo et al. 1982; Zeller et al. 2000). Fahey

---

Responsible Editor: Per Ambus.

N. Eickenscheidt · R. Brumme
Soil Science of Tropical and Subtropical Ecosystems,
Buesgen Institute, University of Goettingen,
Buesgenweg 2,
37077 Goettingen, Germany

N. Eickenscheidt (✉)
Institute of Forest Ecology and Forest Inventory,
Johann Heinrich von Thuenen Institute,
Alfred-Moeller-Strasse 1,
16225 Eberswalde, Germany
e-mail: nadine.eickenscheidt@vti.bund.de

et al. (2011) observed a continuous decrease in native litter $^{15}$N of 20–30 % in the first year after an addition of $^{15}$N-labelled litter to a sugar maple stand. The loss, however, was balanced by a large N incorporation from external sources into the litter that resulted in an increase in N contents of 26–32 %. External N incorporation balanced N loss over a 2-years period in three European beech forest soils and net release of N was measured in the third year (Zeller et al. 2000; Zeller et al. 2001). Translocation of N from the soil to the decaying litter by fungi accounted for 25–35 % of the exogenous N, whereas atmospheric N deposition accounted for 30–50 % in a French beech forest (Zeller et al. 2000).

In the late stage of decomposition, condensation of degradation products combined with import of nutrients produces recalcitrant humic compounds, which accumulate (Berg and McClaugherty 2003). Berg and Matzner (1997) reported a rate-retarding effect of high initial litter N and soil N concentrations during the late stage of decomposition, which was ascribed to (i) the suppressing effect of N on the formation of ligninase in white-rot fungi, and (ii) condensation reactions in which lignin incorporates N, resulting in stable chemical structures. In forest ecosystems exposed to chronic high N depositions, leaching of organic N from the decaying litter but simultaneously reduced fungal N transport, due to sufficient supply of external N by depositions, may further promote N sequestration in soil organic matter (SOM; Fahey et al. 2011). In highly N enriched acid forest soils, absence of earthworms and bioturbation, which are key factors for decomposition, additionally reduce N loss (e.g. Schaefer et al. 2009). An increase in organic layer and soil N has indeed been reported from forests exposed to chronic high N and acid depositions (e.g. Meiwes et al. 2009).

Beech leaf litter is generally rich in N, water soluble compounds, tannin, and lignin (e.g. Berg and McClaugherty 2003; Lorenz et al. 2004; Melillo et al. 1982) and European beech forests have often been exposed to chronic high N and acid depositions. Thus, rapid decomposition in the early stage but slow decomposition in the late stage and temporary N sequestration may be expected (Berg and McClaugherty 2003).

Mineral N, which is released from the decaying litter, may be utilised by micro-organisms for nitrification and denitrification processes during which nitrous oxide ($N_2O$) may be produced either as a by-product or intermediate (Davidson et al. 2000). Nitrous oxide is a potent greenhouse gas and contributes to the catalytic decomposition of stratospheric ozone (Crutzen 1979; IPCC 2007). High emissions of $N_2O$ have been reported from European beech forest soils, which have been exposed to chronic high N depositions (Brumme and Beese 1992; Papen and Butterbach-Bahl 1999; Zechmeister-Boltenstern et al. 2002). However, one of our previous studies revealed that despite the high $N_2O$ emissions of 2.6 kg N ha$^{-1}$ yr$^{-1}$ from an acid beech forest soil in Germany, only 6 % of the total emissions were derived from N depositions of the recent year whereas the remaining part was derived from the soil N pool (Eickenscheidt et al. 2011). Therefore the question arises as to what extent the turnover of leaf litter N contributes to $N_2O$ emissions.

Turnover of litter N is mostly studied through mass loss observations (e.g. Berg and McClaugherty 2003) and only few studies have used stable isotope tracers (e.g. Fahey et al. 2011; Zeller and Dambrine 2011), where the emphasis has been on the early-stage of decomposition. Long-term $^{15}$N tracer studies are required to improve our present knowledge about the processes of litter N dynamics and potential long-term N sequestration in the soil.

The objective of our study was to assess the contribution of beech leaf litter N to N mineralisation, $N_2O$ emissions, and N sequestration in the soil 2–3 years and 10–11 years after litterfall application. We conducted a $^{15}$N isotope litter exchange experiment in a beech stand exposed to chronic high N depositions at the Solling, Germany.

## Materials and methods

### Study area

The study was conducted in a 160-yr-old beech stand (*Fagus sylvatica*) at the Solling in Central Germany (51° 46′ N, 9° 34′ E, 500 ma.s.l., mean temperature: 6.97 °C, annual precipitation: 1193 mm, stand deposition: 20 kg N ha$^{-1}$ yr$^{-1}$). The soil type is a Dystric Cambisol ($pH_{H2O} \leq 3.6$; silty loam), which derived from Triassic sandstone covered by loess. The surface organic layer is a typical moder consisting of an L (litter) layer, F (fermentation) layer, and H (humus) layer with a thickness of 1–2 cm, 4–5 cm, and 2 cm, respectively. The organic layer in total stores 2790 gC m$^{-2}$ and 129 gN m$^{-2}$ (Table 1). Leaf litter production amounted to 277±25 g$m^{-2}$ $yr^{-1}$ (standard deviation sd) from 1991 to 2003 with an average N content of 1.24±0.16 % (sd), which corresponded to 3.4±0.4 gN $m^{-2}$ $yr^{-1}$ (sd; Khanna et al. 2009). The N content of live foliage was 2.46±0.11 % (sd) from 1995 to 2002 and thus, withdrawal of N of live foliage during leaf senescence amounted to 51 % (Khanna et al. 2009). A detailed site description can be found in Brumme and Khanna (2009) and in Eickenscheidt et al. (2011).

## Experimental design

In December 1997, six chamber bases (area: 0.0559 $m^2$) were installed. The bases were inserted into the organic layer. We removed the freshly fallen beech leaf litter of the recent year and replaced it by 17 g $^{15}$N-labelled beech leaf litter (0.71±0.01 %N; 3.57±0.07 atom% $^{15}$N), which corresponded to the average annual leaf litter production. The N content of the replaced litter was lower than of the nativ leaf litter, which was 1.2 %N in 1997, since the $^{15}$N-labelled litter was derived from beech trees that were grown in a greenhouse and irrigated by a $^{15}$N solution. Three of the chamber bases were only used for soil sampling in 1999 and the other three bases were used for $N_2O$ and $^{15}N$-$N_2O$ gas flux measurements in 1999 and 2008 as well as for soil sampling in 2008. Three additional chamber bases, which contained the native unlabelled litter fall (no exchange), were installed as control chambers for $^{15}N$-$N_2O$ flux measurements. Control soil samples for natural $^{15}$N abundances were taken randomly at the study site where no litter exchange was conducted.

## $N_2O$ and $^{15}N$-$N_2O$ flux measurements

We measured $N_2O$ and $^{15}N$-$N_2O$ fluxes from January 1999 to December 2000 and from May 2007 to July 2008 using the closed chamber method (chamber vol.: 5.4 L). Three $N_2O$ gas samples were collected in evacuated glass flasks (100 mL) at three regular time intervals following chamber closure (36 min at most) using a portable gas sampler equipped with a pressure sensor (Loftfield et al. 1997). Analysis was done with a gas chromatograph equipped with an EC detector (GC 14A, Shimadzu, Duisburg, Germany). Samples for $^{15}N$-$N_2O$ measurements were removed at 0.5 and 36 min (1999–2000) and at 0.5 and 20 min (2007–08) and were stored in evacuated 100 mL glass bottles. An isotope ratio mass spectrometer coupled with a preconcentration unit was used for $^{15}$N analysis (PreCon-GC-IRMS, Thermo Finnigan MAT, Bremen, Germany). Isotope abundances were expressed as absolute proportion [atom%]. We used the Keeling plot approach to calculate the $^{15}N$-$N_2O$ abundance of the $N_2O$ emitted from the soil (Tilsner et al. 2003), which was described in detail in Eickenscheidt et al. (2011).

## Soil sampling and in situ incubation

After gas flux measurements, soil sampling and an in-situ incubation of the soil was conducted within the chamber bases to determine net rates of ammonification, nitrification, and $^{15}N_{excess}$ ammonification in summer 2008. For each chamber, two soil samples of the surface 10 cm of the soil were taken with a stainless steel core (diameter: 5.5 cm). The sample included the surface organic layer and the surface mineral soil (Table 1). Soil from one core was removed and divided into 2 cm sections, of which soil extracts for mineral N ($N_{min}$=$NH_4^+$-N+$NO_3^-$-N) and $^{15}N_{min}$ analyses were prepared within few hours (t0 sample). The other soil core (t1 sample) was put into a PE bag and was returned into the hole for undisturbed in situ incubation, which lasted 16 days. During the incubation, the mean temperature in 10 cm soil depth, which was logged every 2 h, was 15.1±1.2 °C (standard deviation). After the

**Table 1** Characteristics of the surface organic layer and upper 2 cm of the $A_h$ horizon of the beech forest at Solling, Germany

| Horizon | Depth [cm] | C [%] | $N_t$ [%] | C [g $m^{-2}$] | $N_t$ [g $m^{-2}$] | C/N ratio | $^{15}N$-$N_t$ [atom%] |
|---|---|---|---|---|---|---|---|
| L | 0–2 | 46.9±0.2 | 1.67±0.04 | 379±25 | 13.9±1.0 | 27.2 | 0.3658±0.0000 |
| F | 2–6 | 45.3±0.6 | 2.01±0.04 | 848±79 | 38.4±4.2 | 22.1 | 0.3656±0.0000 |
| H | 6–8 | 25.5±4.1 | 1.24±0.19 | 1563±195 | 77.1±10.6 | 20.3 | 0.3660±0.0000 |
| $A_h$ | 8–10 | 9.1±1.7 | 0.47±0.08 | 1229±138 | 63.7±5.9 | 19.3 | 0.3669±0.0001 |

incubation, soil cores were subject to the same treatment as the t0 samples. Mineral N extracts were obtained after shaking 30 g fresh mineral soil with 100 mL 0.5 M $K_2SO_4$ solution or 15 g of the organic layer with 50 mL $K_2SO_4$ solution. We analysed $N_{min}$ by using continuous flow injection colorimetry (Cenco/Skalar Instruments, Breda, The Netherlands). A modified diffusion method (Corre et al. 2003; Stark and Hart 1996) was applied for the determination of $^{15}N$ in the extracted $NH_4^+$ and nitrate ($NO_3^-$). However, net $^{15}N_{excess}$ nitrification rates were not available due to failure of the isotope ratio mass spectrometer. In addition, total carbon (C) and nitrogen ($N_t$) contents (CNS Elemental Analyzer, Heraeus Elementar Vario EL, Hanau, Germany) and $^{15}N$ abundance in $N_t$ were determined in 1999 and 2008. Analyses of $^{15}N$ abundance (in $N_t$ and $NH_4^+$) were conducted with a Delta C plus IRMS (Finnigan MAT, Bremen, Germany).

### Calculation of the $^{15}N$ recovery and of the mean residence time

The recovery of the $^{15}N$ label of the exchanged leaf litter in $N_2O$, $N_t$, and net ammonification were calculated using $^{15}N_{excess}$ values. For calculations of $^{15}N_{excess}$ values, natural $^{15}N$ abundances of unlabelled samples ($N_2O$: Fig. 4; $N_t$: Table 1; $NH_4^+$: 0.3641 atom% (organic layer), 0.3666 atom% (0–5 cm mineral soil)) were subtracted from total $^{15}N$ abundances obtained from chambers with $^{15}N$-labelled leaf litter exchange. On average 69.8 mg $^{15}N_{excess}$ $m^{-2}$ were applied by the leaf litter exchange in 1997 (the natural abundance of the native leaf litter was 0.365 atom% $^{15}N$ in 1997). For recovery assessments the ratio of $^{15}N_{excess}$ masses of $N_2O$, $N_t$, and net ammonification, respectively, to masses of applied $^{15}N_{excess}$ by litter exchange were calculated.

The mean residence time (MRT) of N (and C) in the surface organic layer (0–8 cm) was assessed using the measured mass of N (C) in the surface organic layer divided by the mass of N (C) of the annual aboveground total litterfall (6.8 gN $m^{-2}$ $yr^{-1}$ and 258 gC $m^{-2}$ $yr^{-1}$; Khanna et al. 2009).

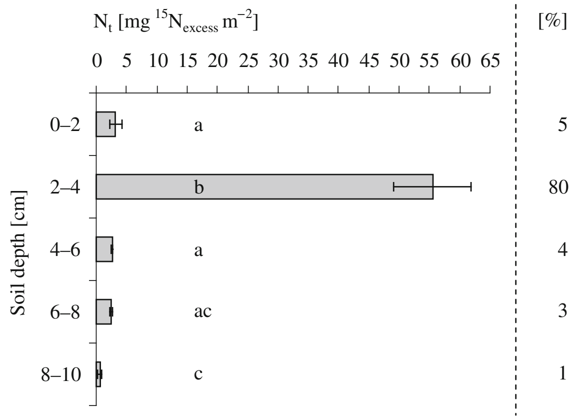
### Statistical analyses

Statistical analyses were conducted using R 2.12.0 (R Development Core Team 2010). We applied linear mixed effects models on time series data (Eickenscheidt et al. 2011). Results were given as mean±1 standard error and effects were considered significant where $P \leq 0.05$.

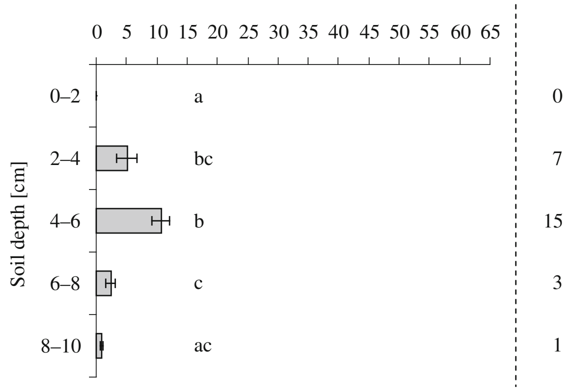
## Results

### Recovery and movement of $^{15}N$ in the soil

Two years after $^{15}N$-labelled beech litter addition, 92 % of the $^{15}N$ was recovered in the surface 10 cm of the soil, most of which (80 % of the total recovery) was found in the upper part of the F layer of the surface organic layer at 2–4 cm soil depth (Fig. 1a). Eleven years after the addition, 27 % of the $^{15}N$ label was recovered with the highest recovery (15 % of the total recovery) in 4–6 cm soil depth, the lower part of the F layer (Fig. 1b). The changes of $^{15}N$-$N_{t\text{-excess}}$ contents in the soil profile from 1999 to 2008, with a



a) 2 years after litter exchange



b) 11 years after litter exchange

**Fig. 1** Distribution of soil $^{15}N$ in the surface 10 cm of the soil 2 years (**a**) and 11 years (**b**) after $^{15}N$-labelled litter exchange in the beech stand. Among soil depths, means (± SE; $n=3$) followed by the same letter were not significantly different (ANOVA, Tukey HSD-test at $P \leq 0.05$). On the right hand side, the recovery (Rec.) of the $^{15}N$-labelled litter in soil $N_t$ is given for each layer decrease in 0–2 cm ($P$=0.038) and 2–4 cm soil depths ($P$=0.006) and an increase in 4–6 cm soil depth ($P$=0.001), were significant. The ratio of $^{15}$N-N$_{t\text{-excess}}$ to native N$_t$ ($^{14}$N+natural $^{15}$N) within the surface organic layer (0–8 cm) decreased from 0.03 % after 2 years to 0.01 % after 11 years. The mean residence time (MRT) of N derived from the annual aboveground litterfall in the surface organic layer averaged to approx. 19 years and was higher than the MRT of C (10.5 years) in the surface organic layer.

## Mineralisation of N and $^{15}$N

Net ammonification and mineralisation, measured at about 15 °C in the field, was low in 0–2 cm soil depth (L layer), highest in 2–4 cm soil depth (upper F layer; Fig. 2a), and decreased gradually below this depth. Net NH$_4^+$ immobilisation occurred in 8–10 cm soil depth (mineral soil). Net nitrification increased with soil depth within the organic layer but differences were not statistically significant (Fig. 2a). In total, net mineralisation amounted to 58 mg N m$^{-2}$ d$^{-1}$ within the surface 10 cm of the soil, half of which was nitrified.

Net $^{15}$N$_{excess}$ ammonification in the surface 10 cm of the soil in general followed the trend of total net ammonification and in total amounted to 6±1 µg $^{15}$N m$^{-2}$ d$^{-1}$ (Fig. 2b). Half of the total $^{15}$N ammonification could be attributed to the 2–4 cm soil depth. During the incubation period, 0.0086 % of the $^{15}$N label was net ammonified in the surface 10 cm of the soil per day (Fig. 2b). Assuming a similar contribution of the $^{15}$N label to net nitrification than to net ammonification and assuming annual net N mineralisation rates between 0.7 and 1.0 gN m$^{-2}$ yr$^{-1}$ (Brumme et al. 2009), 2–3 % of the litter N was net mineralised 11 years after the litter exchange.

About 0.6 % of total net N ammonification could be ascribed to the exchanged leaf litter, assuming that the native N contained in the $^{15}$N-labelled litter participated equally in net ammonification as the $^{15}$N label. Data for net $^{15}$N$_{excess}$ nitrification were not available due to technical problems.

On average, the daily net $^{15}$N$_{excess}$ ammonification of the surface 10 cm of the soil accounted for 0.030±0.012 % of the $^{15}$N-N$_{t\text{-excess}}$. The proportion was two times higher than the proportion of net native N ammonification per day of the native N$_t$ regarding the same depth interval, which was 0.015±0.005 %. However, the differences were not statistically significant. Also, no significant differences between these proportions occurred when the different depth intervals of the soil were considered separately (Fig. 3).

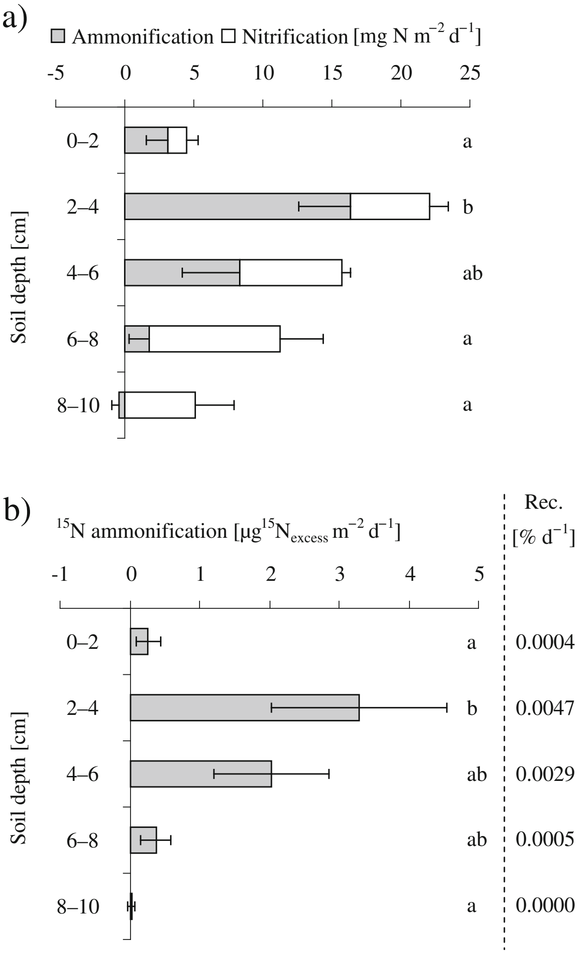


**Fig. 2** Net ammonification and nitrification rates (**a**) and net $^{15}$N ammonification rates (**b**) of the surface 10 cm of the soil in the beech stand. On the right hand side of Fig. 2b, the daily recovery (Rec.) of the $^{15}$N-labelled litter in net ammonification is given for each soil layer. The undisturbed in situ incubation lasted 16 days and was conducted within the chamber bases of the litter exchange experiment in summer 2008. Among soil depths, means (±SE; $n$=3) followed by the same letter were not significantly different (ANOVA, Tukey HSD-test at $P\leq0.05$). Letters in figure a) belong to net ammonification rates and no significant differences occurred among net nitrification rates in different soil depths. Net $^{15}$N nitrification rates were not available

## N$_2$O and $^{15}$N-N$_2$O fluxes

Fluxes of N$_2$O displayed pronounced seasonality with high emissions during the summer months and low

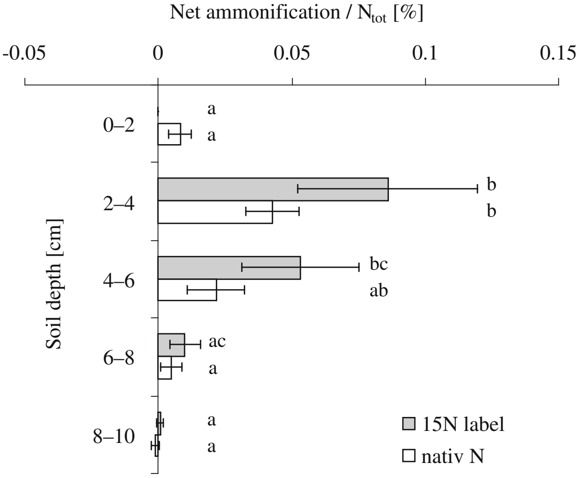


**Fig. 3** Ratio of daily net ammonification to $N_t$ given for $^{15}N_{excess}$ derived from labelled beech litter and for native N ($^{14}N$+natural $^{15}N$) for the five depth intervals. Among soil depths, means (±SE; $n=3$) followed by the same letter were not significantly different regarding $^{15}N$ or native N, respectively (ANOVA, Tukey HSD-test at $P \leq 0.05$). No significant differences occurred between $^{15}N$ and native N regarding the different soil depths (Student's *t*-test at $P \leq 0.05$)

emissions during the winter months (Fig. 4). The cumulative $N_2O$ emission amounted to 1.33±0.26 kg N ha$^{-1}$ yr$^{-1}$ in 1999, 1.54±0.88 kg N ha$^{-1}$ yr$^{-1}$ in 2000, and 1.35±0.57 kg N ha$^{-1}$ yr$^{-1}$ in 2007–08.

In 1999–2000 $^{15}N$-$N_2O_{emitted}$ abundances were significantly higher in the litter exchange chambers than in the control chambers (Table 2, Fig. 4; $P=0.036$). Cumulative $^{15}N$-$N_2O_{excess}$ emissions were 0.29 g $^{15}N$ ha$^{-1}$ yr$^{-1}$ in 2000 which was 0.04 % of the added $^{15}N$ in leaf litter. Eleven years after the litter application, the recovery of $^{15}N$ in emitted $N_2O$ decreased to 0.13 g $^{15}N$ ha$^{-1}$ yr$^{-1}$ in 2007–08, which was 0.02 % of the added $^{15}N$ in leaf litter. However, the abundances of $^{15}N$ in $N_2O$ emitted from the soil in 2007–08 did not significantly differ between the litter exchange chambers and the control chambers (Table 2, Fig. 4).

Assuming that native N contained in the exchanged leaf litter participated equally in $N_2O$ emissions as the $^{15}N$ label, then 0.3 % (2007–08) and 0.6 % (1999–00) of total annual $N_2O$ emissions were derived from total N of a single litterfall year.

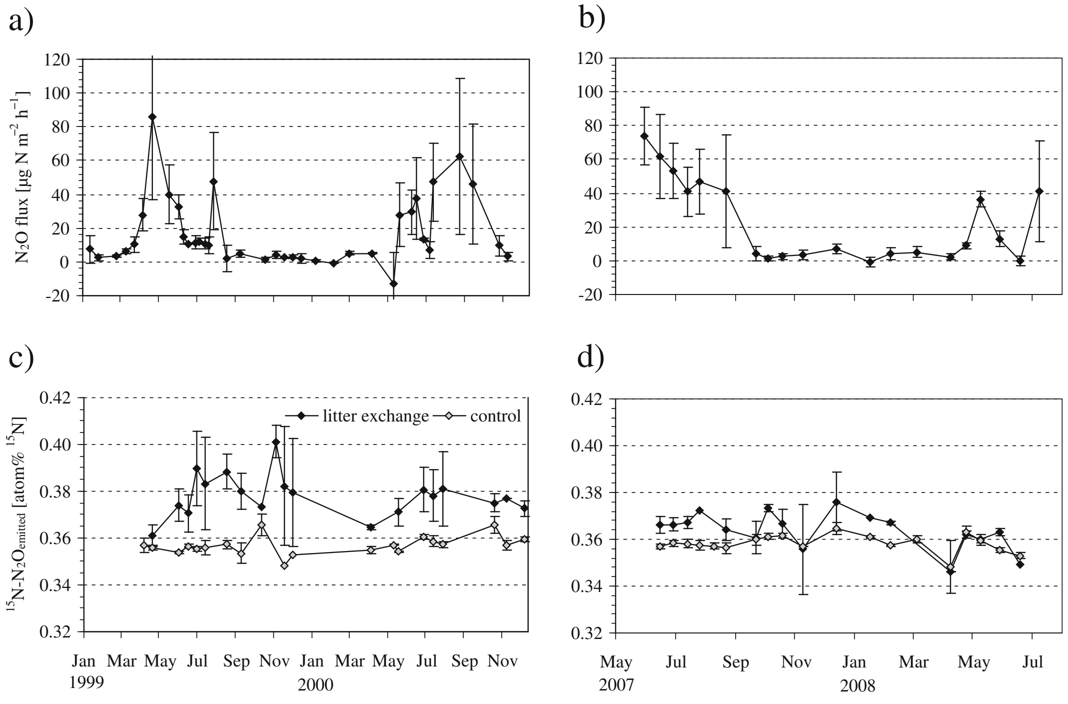


**Fig. 4** Mean (± SE; $n=3$) $N_2O$ fluxes in the litter exchange experiment of the Solling beech stand from January 1999 to December 2000 (**a**) and from May 2007 to July 2008 (**b**) and mean (±SE; $n=3$) $^{15}N$ abundances of $N_2O$ emitted from the soil from the chambers where litter was exchanged and from the control chambers for the measurements in 1999–2000 (**c**) and in 2007–08 (**d**)

**Table 2** Abundances of $^{15}N$ in $N_2O$ emitted from the soil of the beech stand during the measurements in 1999–2000 and 2007–08

| | $^{15}N\text{-}N_2O_{emitted}$ [atom%] | | | |
|---|---|---|---|---|
| | Control | | Litter exchange | |
| 1999–2000 | 0.3574 | (0.0006)$^{a}$ | 0.3782 | (0.0090)$^{b}$ |
| 2007–2008 | 0.3582 | (0.0012)$^{ab}$ | 0.3636 | (0.0024)$^{ab}$ |

Among treatments, means (± SE; $n=3$) followed by the same letter were not significantly different (ANOVA, Tukey HSD-test at $P \leq 0.05$)

In 2007–08, the daily $^{15}N\text{-}N_2O_{excess}$ emissions amounted to 0.0002 % of $^{15}N\text{-}N_{t\text{-}excess}$ of the surface 10 cm of the soil, which was the same as the ratio of native $N_2O$ emissions to native $N_t$ of the surface 10 cm of the soil assuming $N_2O$ only derived from the top 10 cm of the soil.

## Discussion

### Relocation and sequestration of litter N in the soil

Recovery of 92 % of the $^{15}N$ label 2 years after the $^{15}N$-labelled beech litter application was in line with other studies and pointed to low N losses during the first years of decomposition. Zeller et al. (2000) found between 80 % and 85 % of $^{15}N$-labelled beech leaf litter 3 years after the litter application in the surface 30 cm of an acid soil that had a poorly developed moder type humus. Fahey et al. (2011) reported a recovery of over 90 % for $^{15}N$ derived from sugar maple leaf litter application in the surface organic layer and surface 10 cm of the soil 1 year after litter application. However 11 years after litter application, 73 % of the initially added $^{15}N$ had been mineralised, taken up by trees, leached, or emitted as gaseous N compounds in our study. Uptake of litter N by trees probably represented the main sink for litter N, since total annual N uptake by trees amount to approximately 11.0 gN $m^{-2}$ compared to N leaching of 0.17 g and gaseous N emissions of 0.19 g $N_2O$-N (Brumme and Khanna 2009) and of <0.05 g $NO_x$-N (unpublished data) at the Solling beech stand. The low turnover rate of the N pool of the surface organic layer expressed by a high MRT of 19 years, in contrast to a MRT of 10.5 years for C, indicated high recovery of 27 % of the $^{15}N$ of exchanged litter 11 years after litter addition. The annual amount of litter-N sequestration thus equals to about 0.9 gN $m^{-2}$ $yr^{-1}$ and significantly contributes to the mean total N sequestration of 2.1 g N $m^{-2}$ $yr^{-1}$ in the surface organic layer observed over a period of 35 years at the studied site (Meiwes et al. 2009). The site is characterized by high load of acid and nitrogen deposition (Meesenburg et al. 2009), which has led to an N-enriched but very acid soil with a low decomposition of the litter material and the absence of deep borrowing earthworms (Meiwes et al. 2009; Schaefer et al. 2009). Although the C/N ratio of the surface organic layer was constant over the last 35 years (Meiwes et al. 2009) the soil still is accumulating N as indicated by the low leaching losses of 0.17 gN $m^{-2}$ $yr^{-1}$ and input–output balances of 2.1 gN $m^{-2}$ $yr^{-1}$ (Brumme and Khanna 2009). The most reasonable explanation is the stabilisation process which was induced by N depositions either directly by microbial N immobilisation or indirectly by plant uptake and the production of N-enriched litter including a big increase in fructification products (Khanna et al. 2009; Brumme and Khanna 2009).

Only limited amount of the applied $^{15}N$ had been relocated to the lower part of the F layer (Fig. 1), despite the huge amount of 2.8 kg C $m^{-2}$ and 0.07 kg N $m^{-2}$ which had been deposited through total aboveground litterfall since the beginning of the labelling experiment (Khanna et al. 2009). The surprisingly constant accumulation of $^{15}N$ in the H layer over the 11 years of observation revealed the lack of bioturbation, though leaching of soluble organic and inorganic compounds between the F and H layer would have occurred. Labelled $^{15}N$ accumulated in the H layer was probably derived from initially high leaching of soluble organic N during the early-stage decomposition (first 2 years) as was also observed by Zeller et al. (2000). However, the downward movement of $^{15}N$ into the lower F layer might primarily be caused by litter deposition and decomposition. Limited relocation of litter $^{15}N$ from the organic horizons to the mineral soil within 4–5 years after litter application was also reported from three European beech forest soils which accumulated 0.7–1.3 kg C on the mineral soil (Zeller and Dambrine 2011; Zeller et al. 2001). The slow incorporation rate of litter N into the mineral-bound soil fraction was attributed to the absence of earthworms. Bird and Torn (2006) recovered very little $^{13}C$ and $^{15}N$ from pine needle litter below a

8 cm thick O-horizon in which the litter was placed 2 years after the beginning of the experiment. In contrast, Fahey et al. (2011) measured a comparable fast relocation of litter $^{15}$N in the soil of a sugar maple forest with low earthworm activities. In their study, already 1 year after litter application the greatest proportion of recovered $^{15}$N was found in 0–5 cm mineral soil depth. The mineral soil was covered by only 0.36 kg C indicating a rapid incorporation of litter into the mineral soil.

Beside downward movement there is evidence that fungal hyphae may be responsible for an upward movement of N and C (Zeller et al. 2000). We observed that a significant proportion of applied $^{15}$N was still present in the L layer 2 years after litter exchange (Fig. 1a). This may be due to fungal hyphae transporting N from decaying $^{15}$N-labelled litter to fresh litter. Fungal transport of N amounted to 25–35 % of exogenous N that was incorporated into decaying litter in an acid beech forest soil with low throughfall N depositions of 6–8 kg ha$^{-1}$ yr$^{-1}$ whereas 30–50 % of the incorporated N might have come from deposited N (Zeller et al. 2000). In the sugar maple stand investigated by Fahey et al. (2011), 18 % of external incorporated N in fresh litter was derived from the litter of the previous year. Bird and Torn (2006) suggested that as a result of fungal N translocation, organic horizons may provide a sink for leachable litter N. However, 11 years after litter exchange this process was of less importance in the present study as was indicated by a non-significant $^{15}$N enrichment in the L layer (Fig. 1b).

## Mineralisation of leaf litter N

The summed net N mineralisation of the surface 10 cm of the soil amounted to 58 mg N m$^{-2}$ d$^{-1}$ (Fig. 2a), similar as was reported for this site in previous studies (Brumme et al. 2009). The surface organic layer of the investigated stand was the primary horizon for N mineralisation and contributed to 77 % to total net N mineralisation of the surface 20 cm of the soil (Brumme et al. 2009). The present study showed an increased in the net ammonification and mineralisation rates within the soil profile from the L layer (0–2 cm) to the upper F layer (2–4 cm; Fig. 2), which was the most important layer for N turnover. The change in the N turnover could be attributed to the decrease in the C/N ratio from 28 to 22 (Table 1). Litter-bag studies with $^{15}$N labelled beech litter by Zeller et al. (2000) showed that the shift from prevailing immobilisation to net mineralisation of N occurred after the first 2 years of decomposition when the C/N ratio had lowered to 25. In the present study ammonification and mineralisation continuously decreased with increasing soil depth below 2–4 cm depth (Fig. 2). Due to small changes in the C/N ratio other mechanisms might be responsible for this decrease. Respiration measurements conducted with surface organic matter of an adjacent beech stand showed a strong decrease in the release of $CO_2$ from 59 μg C ha$^{-1}$ g$^{-1}$ in the L layer to 12 and 3.9 μg C ha$^{-1}$ g$^{-1}$ in the F and H layer, respectively (Chodak et al. 2003). This clearly indicated a considerably decreased of mineralisation in the lower part of the surface organic layer probably caused by chemical stabilisation processes since physical stabilisation processes should be of minor importance in organic horizons. At the same beech stand, $^{15}$N pool dilution studies conducted by Corre et al. (2003) revealed a gross mineralisation rate that was approx. four times higher than our net mineralisation rate indicating a high microbial recycling of N. Similar results were reported from other forest soils but their relevance for the N sequestration is still not clear (Venterea et al. 2004).

About 0.6 % of total net N ammonification could be ascribed to the exchanged leaf litter and approximately 2–3 % of the litter N was net mineralised 11 years after litterfall, indicating that the (labelled) leaf litter N still participated in measureable amounts in N turnover. Compared to the native N twice as much labelled N was mineralised from the labelled N pool in the lower F layer and the H layer (Fig. 3). This demonstrated that the N from the comparably new $^{15}$N-labelled beech litter was not preferentially stabilised compared to “older” native N, which was decomposed more slowly. Results from three European beech forests, where mineralisation of litter $^{15}$N was 2–4-fold higher than mineralisation of native soil N 4–5 years after litter application, were in line with our findings (Zeller and Dambrine 2011). The $^{15}$N released from the decaying litter was mainly recovered in the coarse particulate organic matter fraction (>200 μm; Zeller and Dambrine 2011). Bird et al. (2008) also reported that 58 % of the $^{15}$N of needle litter was recovered in the light fraction, which represents the SOM fraction with the highest turnover, in a

Scots pine forest during the initial 1.5 years of their study.

### $N_2O$ emissions and contribution of leaf litter N

Emissions measured during our experiment were similar to the mean value of a 10-year measuring period at the Solling beech stand, which amounted to 1.9 kg N $ha^{-1}$ $yr^{-1}$ (Brumme and Borken 2009). The beech stand belonged to the seasonal emission type where due to low oxygen availability in the soil, caused by high microbial and root respiration and low gas diffusivity in the surface organic layer, denitrification was promoted during the summer months (Brumme et al. 1999; Wolf and Brumme 2002). Low gas diffusivity was the result of laminar undecomposed beech leaves in the L layer of the surface organic layer combined with high precipitation rate (Ball et al. 1997; Brumme and Borken 2009). Leaching of $NO_3^-$ to the transitional zone between the H layer and the surface mineral soil presumably provided the substrate for denitrification. Brumme (1995) reported that high $N_2O$ emissions occurred at temperatures of >10 °C and water tensions <200 hPa. Hence, years with high summer precipitation, as was the case for 2007 but not for 2008, exhibit high $N_2O$ emissions (compare annual fluxes of Fig. 4).

The $^{15}N$ experiment indicated that the $^{15}N$-labelled beech litter N measurably contributed to total $N_2O$ fluxes. The contribution decreased from the 2–3 years measurement to the 10–11 years measurement after litter exchange due to the decrease in $^{15}N$-labelled beech litter mass. Eleven years after the litter exchange, about 1 % of the net mineralised litter N was emitted as $N_2O$. Probably less than 1 % of the litter N was lost as $N_2O$ during 2 and 11 years after litter exchange.

The proportions of $N_2O$-N of total N were the same for labelled $^{15}N$ and native N in the surface 10 cm of the soil, despite the two times higher relative ammonification of labelled $^{15}N$ than that of native N 11 years after the exchange. This result may be due to a significant contribution of native $N_2O$ produced below 10 cm depth. However, we have no information about the proportion of $^{15}N_{excess}$ nitrification of $^{15}N\text{-}N_{t\text{-}excess}$ but which probably could further clarify the observation.

Beech leaf litter N of a single year contributed between 0.3 % (eleventh year) and 0.6 % (second year) of the total annual $N_2O$ emissions after litterfall. Thus, accumulated leaf litter N of the second to eleventh litterfall year only contributed approx. 5 % to total annual $N_2O$ emissions using an average emission of 0.46 % (harmonic mean) for a single litterfall year.

The observation that neither leaf litter N during the 2–11 years period nor inorganic N depositions of the recent year (contribution of 6 % to total $N_2O$ emissions; Eickenscheidt et al. 2011) were primarily responsible for high $N_2O$ emissions measured at the Solling beech stand, still raised the question of the origin of these emissions. The other sources of $N_2O$ emissions may include the first year litterfall N with possibly a rapid initial release of $^{15}N$ as also was reported by Zeller et al. (2000). However, we suppose that the older N pool of accumulated older litter material and accumulated deposited N may represents the major source for $N_2O$ emissions from this soil.

## Conclusions

Our study demonstrated that one quarter of the $^{15}N$ label of initially added beech leaf litter was still present in the surface 10 cm of the soil 11 years after litter addition. The high recovery of added $^{15}N$ indicated a tight microbial N cycle. Movement of beech leaf litter N through the surface organic layer was very slow. Loss of litter N from the chamber bases was mainly attributed to tree uptake whereas losses by gaseous $N_2O$ and $NO_x$ emissions and by N leaching probably accounted for less than 1 % of the litter N during the 10 years of observation. The $^{15}N$-labelled beech leaf litter still participated measurably in N turnover and $N_2O$ production and obviously was not completely stabilised 11 years after litter exchange. The contribution of beech leaf litter of a single year to total annual $N_2O$ emissions ranged between 0.3 % (eleventh year) and 0.6 % (second year). We suggest that the high $N_2O$ emissions observed in the investigated beech stand resulted from accumulated N in older humus or soil N pools, since neither leaf litterfall of the last 2 to 11 years nor N depositions of the recent year represented a significant source for $N_2O$.

**Acknowledgment** We thank the German Research Foundation for financial support (BR 1524/6).

## References

Ball BC, Smith KA, Klemedtsson L, Brumme R, Sitaula BK, Hansen S, Prieme A, MacDonald J, Horgan GW (1997) The influence of soil gas transport properties on methane oxidation in a selection of northern European soils. J Geophys Res 102(D19):23309–23317

Berg B, Matzner E (1997) Effect of N deposition on decomposition of plant litter and soil organic matter in forest systems. Environ Rev 5:1–27

Berg B, McClaugherty C (2003) Plant litter: decomposition, humus formation, carbon sequestration. Springer, Berlin, 286 pp

Bird JA, Torn MS (2006) Fine roots vs. needles: a comparison of $^{13}$C and $^{15}$N dynamics in a ponderosa pine forest soil. Biogeochemistry 79:361–382

Bird JA, Kleber M, Torn MS (2008) $^{13}$C and $^{15}$N stabilization dynamics in soil organic matter fractions during needle and fine root decomposition. Org Geochem 39:465–477

Brumme R (1995) Mechanisms of carbon and nutrient release and retention in beech forest gaps. Plant Soil 168–169 (1):593–600

Brumme R, Beese F (1992) Effects of liming and nitrogen fertilization on emissions of $CO_2$ and $N_2O$ from a temperate forest. J Geophys Res 97(D12):12851–12858

Brumme R, Borken W (2009) Chapter 18: $N_2O$ emission from temperate beech forest soils. In: Brumme R, Khanna PK (eds) Functioning and management of European beech ecosystems. Springer, Berlin, pp 353–367

Brumme R, Khanna PK (2009) Functioning and management of European beech ecosystems. Springer, Berlin Heidelberg, 490 pp

Brumme R, Borken W, Finke S (1999) Hierarchical control on nitrous oxide emission in forest ecosystems. Global Biogeochem Cycles 13(4):1, 137–131,148

Brumme R, Priess J, Wang CP, Raubuch M, Steinmetz G, Meyer H (2009) Chapter 13: Nitrogen and carbon transformations. In: Brumme R, Khanna PK (eds) Functioning and management of European beech ecosystems. Springer, Berlin, pp 231–251

Chodak M, Khanna PK, Beese F (2003) Hot water extractable C and N in relation to microbiological properties of soils under beech forest. Biol Fertil Soils 39:123–130

Corre MD, Beese F, Brumme R (2003) Soil nitrogen cycle in high nitrogen deposition forest: changes under nitrogen saturation and liming. Ecol Appl 13(2):287–298

Crutzen PJ (1979) The role of NO and $NO_2$ in the chemistry of the troposphere and stratosphere. Annu Rev Earth Planet Sci 7(1):443–472

Davidson EA, Keller M, Erickson HE, Verchot LV, Veldkamp E (2000) Testing a conceptual model of soil emissions of nitrous and nitric oxides. BioScience 50(8):667–680

R Development Core Team (2010) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, available at: http://www.R-project.org

Eickenscheidt N, Brumme R, Veldkamp E (2011) Direct contribution of nitrogen deposition to nitrous oxide emissions in a temperate beech and spruce forest - a $^{15}$N tracer study. Biogeosciences 8:621–635

Fahey TJ, Yavitt JB, Sherman RE, Groffman PA, Fisk MC, Maerz JC (2011) Transport of carbon and nitrogen between litter and soil organic matter in a Northern Hardwood Forest. Ecosystems 14:326–340

IPCC (2007) Changes in atmospheric constituents and in radiative forcing. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) Climate change 2007: the physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

Khanna PK, Fortmann H, Meesenburg H, Eichhorn J, Meiwes KJ (2009) Chapter 11: biomass and element content of foliage and aboveground litterfall on the three long-term experimental beech sites: dynamics and significance. In: Brumme R, Khanna PK (eds) Functioning and management of European beech ecosystems. Springer, Berlin, pp 183–205

Laganière J, Paré D, Bradley RL (2010) How does a tree species influence litter decomposition? Separating the relative contribution of litter quality, litter mixing, and forest floor conditions. Can J For Res 40:465–475

Loftfield N, Flessa H, Augustin J, Beese F (1997) Automated gas chromatographic system for rapid analysis of atmospheric trace gases methane, carbon dioxide, and nitrous oxide. J Environ Qual 26:560–564

Lorenz K, Preston CM, Krumrei S, Feger K-H (2004) Decomposition of needle/leaf litter from Scots pine, black cherry, common oak and European beech at a conurbation forest site. Eur J Forest Res 123:177–188

Meesenburg H, Eichhorn J, Meiwes KJ (2009) Chapter 15: Atmospheric deposition and canopy interactions. In: Brumme R, Khanna PK (eds) Functioning and management of European beech ecosystems. Springer, Berlin, pp 265–302

Meiwes KJ, Meesenburg H, Eichhorn J, Jacobsen C, Khanna PK (2009) Chapter 4: Changes in C and N contents of soils under beech forests over a period of 35 years. In: Brumme R, Khanna PK (eds) Functioning and management of European beech ecosystems. Springer, Berlin, pp 49–63

Melillo JM, Aber JD, Muratore JF (1982) Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63(3):621–626

Papen H, Butterbach-Bahl K (1999) A 3-year continuous record of nitrogen trace gas fluxes from untreated and limed soil of a N-saturated spruce and beech forest ecosystem in Germany - 1. $N_2O$ emissions. J Geophys Res 104 (D15):18487–18503

Parton W, Silver WL, Burke IC, Grassens L, Harmon ME, Currie WS, King JY, Adair EC, Brandt LA, Hart SC, Fasth B (2007) Global-scale similarities in nitrogen release patterns during long-term decomposition. Science 315:361–364

Schaefer M, Migge-Kleian S, Scheu S (2009) Chapter 12: The role of soil fauna for decomposition of plant residues. In: Brumme R, Khanna PK (eds) Functioning and management of European beech ecosystems. Springer, Berlin, pp 207–230

Stark JM, Hart SC (1996) Diffusion techniquefor preparing salt solutions, Kjeldahl Digests, and persulfate digests for nitrogen-15 analysis. Soil Sci Soc Am J 60(6):1846–1855

Tilsner J, Wrage N, Lauf J, Gebauer G (2003) Emission of gaseous nitrogen oxides from an extensively managed grassland in NE Bavaria, Germany II. Stable isotope natural abundance of $N_2O$. Biogeochemistry 63(3):249–267

Venterea RT, Groffman PA, Castro MS, Verchot LV, Fernandez IJ, Adams MB (2004) Soil emissions of nitric oxide in two forest watersheds subjected to elevated N inputs. For Ecol Manag 196(1):335–349

Wolf I, Brumme R (2002) Contribution of nitrification and denitrification sources for seasonal $N_2O$ emissions in acid German forest soil. Soil Biol Biochem 34:741–744

Zechmeister-Boltenstern S, Hahn M, Meger S, Jandl R (2002) Nitrous oxide emissions and nitrate leaching in relation to microbial biomass dynamics in a beech forest soil. Soil Biol Biochem 34(6):823–832

Zeller B, Dambrine E (2011) Coarse particulate organic matter is the primary source of mineral N in the topsoil of three beech forests. Soil Biol Biochem 43:542–550

Zeller B, Colin-Belgrand M, Dambrine E, Martin F, Bottner P (2000) Decomposition of $^{15}N$-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 $^{15}N$-labeled litter in European beech forests. Tree Physiol 21:153–162