

Allocation and dynamics of C and N within plant–soil system of ash and beech

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Abstract

Forest management requires a profound understanding of how tree species affect C and N cycles in ecosystems. The large C and N stocks in forest soils complicate research on the effects of tree species on C and N pools. *In-situ* ¹³C and ¹⁵N labeling in undisturbed, natural forests enable not only tracing of C and N fluxes, but also reveal insight into the interactions at the plant–soil–atmosphere interface. *In-situ* dual ¹³C and ¹⁵N pulse labeling of 20 beeches (*Fagus sylvatica* L.) and 20 ashes (*Fraxinus excelsior* L.) allowed tracing the fate of assimilated C and N in trees and soils in an unmanaged forest system in the Hainich National Park (Germany). Leaf, stem, root, and soil samples as well as microbial biomass were analyzed to quantify the allocation of ¹³C and ¹⁵N for 60 d after labeling and along spatial gradients in the soil with increasing distance from the stem. For trees of similar heights (≈ 4 m), beech (20%) assimilated twice as much as ash (9%) of the applied ¹³CO₂, but beech and ash incorporated similar ¹⁵N amounts (45%) into leaves. The photosynthates were transported belowground through the phloem more rapidly in beech than in ash. Ash preferentially accumulated ¹⁵N and ¹³C in the roots. In contrast, beech released more of this initially assimilated ¹³C (2.0% relative ¹³C allocation) and ¹⁵N (0.1% relative ¹⁵N allocation) *via* rhizodeposition into the soil than ash (0.2% relative ¹³C, 0.04% relative ¹⁵N allocation), which was also subsequently recovered in microbial biomass. These results on C and N partitioning contribute to an improved understanding of the effects of European beech and ash on the C and N cycles in deciduous broad-leaved forest. Differences in C and N allocation patterns between ash and beech are one mechanism of niche differentiation in forests containing both species.

Key words: ¹³CO₂ labeling / Ca(¹⁵NO₃)₂ / carbon and nitrogen cycles / tree rhizodeposition / species effects / deciduous forest

Accepted January 30, 2016

1 Introduction

Soil organic C (SOC) and its turnover play a crucial role in sustainable forest management and for mitigation of greenhouse gas (CO₂) emissions. Forest C and N budgets affect tree growth, the acquisition of resources such as light, nutrients, and water, and consequently forest productivity of standing biomass and C sequestration in soil organic matter (SOM) (Litton et al., 2007). The government forestry department is conducting a broad-scale conversion of monocultures to mixed forest stands in Germany and, therefore, the impact of tree species diversity on the chemical, physical, and biological characteristics of soil is of particular interest. Where forests have been considered in previous research, the focus has been on soil biochemical properties of conifers rather than deciduous trees (Augusto et al., 2002; Berger et al., 2009a, 2009b; Mareschal et al., 2010). Mareschal et al. (2010) showed for three conifers and beech (*Fagus sylvatica* L.) that they affect various chemical properties of topsoil and have an impact on soil fertility. Schleuß et al. (2014) also

showed for the first time that mixed forests with mainly beech are superior with respect to organic C (C_{org}) stabilization in the clay and fine silt fractions of the subsoil compared with monospecific beech forests. He also pointed to a pronounced species identity and/or diversity effect on soil C storage in an old grown forest with near steady state soil conditions. However, large differences between various broad-leaved species in C and N allocations can be expected, considering their niche differentiation concerning light acquisition, nutrient uptake, interactions with mycorrhiza types, and soil morphology. The effects of these differences on C and N allocations have not been analyzed to date.

Nitrogen fluxes in the xylem of trees are regulated by three processes: remobilization from internal reserves, root uptake of N from the soil, and phloem–xylem recycling (Dambrine et al., 1995; Grassi et al., 2003). Trees are rarely C limited and tree growth is mostly limited by N availability (Millard and Grelet, 2010). Trees accumulate large amounts of C as non-structural carbohydrates and lipids (Würth et al., 2005) and



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the processes regulating the storage of an abundant resource like C are quite different from the seasonal allocation of scarce resources such as N. Remobilization of stored N in the tree biomass is essential for the growth of temperate deciduous trees, especially in spring, and the relative contributions of remobilized N and N taken up by roots to the N used for growth depends on tree age, soil fertility, and other environmental factors (Millard, 1996; Dyckmans and Flessa, 2001). Regulation of N uptake by roots could involve shoot–root cycling of N, because an inverse correlation has been found between the concentrations of amino acids and amides in phloem sap and nitrate uptake by the roots of beech (Geßler et al., 1998) and *Prunus persica* (Youssefi et al., 2000). We hypothesize that there are species-specific patterns of C and N allocation in the tree compartments due to differences in remobilization and recycling processes and, therefore, differences in the amount of rhizodeposition into the soil.

Beech and ash (*Fraxinus excelsior* L.) differ considerably in their mycorrhizal association. Beech is associated with ectomycorrhiza, while ash associates with arbuscular mycorrhiza (Meinen et al., 2009). A rapid transfer of photosynthates to ectomycorrhiza has indeed been reported (Leake et al., 2001; Esperschütz et al., 2009; Högberg et al., 2010). Callesen et al. (2013) discovered in ash and beech that the $\delta^{15}\text{N}$ pattern reflected tree species-related traits affecting the N cycling as well as site fertility and former land use, and possibly differences in N leaching. They also mentioned that the tree species $\delta^{15}\text{N}$ patterns reflected fractionation caused by uptake of N through mycorrhiza rather than nitrate leaching or other N transformation processes. We also hypothesize differences in the C and N allocation patterns between beech and ash. We also expect beech to be more rapid in its allocation of C and N through the tree compartments, belowground and more prolific in its rhizodeposition than ash. Furthermore, beech and ash differ in their root morphology. The ash root grows as a typical tap root system. Beech, in contrast, has a heart root system in which several major roots are developed, growing parallel at depth (Schütt et al., 2006). Therefore, we expect differences in the ^{13}C and ^{15}N allocation between beech and ash at different depths. We assume that beech shows depth-related rhizodeposition, while ash might exhibit a peak in C and N deposition at a certain depth.

Bauhus et al. (1998) reported that microbial biomass is on average lower in forest floor beneath conifers than beneath deciduous species and concluded that microbial variables are sensitive to tree species, stand age, and soil type. However, they did not explore to what extent the trees directly affect soil C and N turnover and microbial biomass through their rhizodeposits. Quantifying the C flux from plant shoots to roots is necessary in order to estimate the contribution of recently fixed plant photosynthates to ecosystem C cycling and microbial biomass (Ostle et al., 2000; Rangel-Castro et al., 2004). Tracing photosynthetically assimilated C after $^{13}\text{CO}_2$ labeling and observing the subsequent ^{13}C flux through rhizodeposition into soil and microbial communities is a powerful tool for the investigation of C turnover in forest soils (Potthoff et al., 2003). To date, there have been very few studies using labeling of trees under field conditions to investigate C and N turnover in soil (Högberg et al., 2008; Epron et al., 2011; Shibisto-

va et al., 2012). Nevertheless, field studies on the C flux from the tree canopy to belowground are necessary because laboratory mesocosms may not realistically reproduce the complexity and dynamics found in the field, especially in the case of forests (Högberg et al., 2008). Furthermore, long-term experiments are necessary to achieve a more complete understanding of C and N cycling in forest ecosystems.

The aim of this study was to quantify C and N allocation into plant compartments of beech and ash and to investigate whether there is a species effect on the surrounding soil and microbial biomass and its C gain from the tree. Generally, two approaches for dual isotope labelling of plants are possible: (1) using natural pathways of CO_2 assimilation by photosynthesis and N uptake from soil or (2) transfer of C_{org} and N into the stem of plants by passive uptake through transpiration flow (Wichern et al., 2011). However, it is not known how the applied C and N isotopes are distributed within a deciduous tree, or whether dual isotope labeling also works for medium-sized trees under field conditions in a temperate forest ecosystem. We therefore conducted an *in-situ* pulse-labeling experiment using ^{13}C and ^{15}N to investigate the C and N allocation from the atmosphere *via* the plant compartments into soil by two broad-leaved tree species: beech and ash.

2 Material and methods

2.1 Site description

The experimental site (10°05'N, 10°30'E) was located in the SW of Weberstedt within Thuringia, Germany, in the NE part of the Hainich National Park. The Hainich, with an area of 16,000 ha, is the largest contiguous and most diverse broad-leaved forest in Germany and a part of it has been a UNESCO world natural heritage site since 2011. It has not been managed for forestry since 1990.

The mean annual temperature is 7.5°C and the mean annual precipitation is 670 mm. The mean elevation of our study site was 300 m asl. The forest site had deciduous trees of diverse ages with a long-term forest history of at least 200 y and grows on a Stagnic Luvisol (WRB, 2006) developed from loess that is underlain by Triassic limestone. Fifty trees scattered within a maximum distance of 300 m from the center of the site were chosen for their height. The light intensity was approx. the same everywhere because the beech-dominated forest had a closed leaf cover. Nevertheless, slight differences in light intensity might have been possible due to different numbers of leaf layers.

2.2 Experimental design and sampling setup

2.2.1 $^{13}\text{CO}_2$ pulse labeling

Within a regeneration area of uniform light intensity under a closed beech canopy, 50 trees (25 beeches, 25 ashes) with approx. similar height (3–4 m) were chosen scattered with a maximum distance of 300 m from the center of the site. Of these 50 trees, 20 ashes and 20 beeches were selected to perform a pulse labeling experiment, leaving 5 trees of each

species as unlabeled reference trees. The aboveground biomass is listed in Table 1. All leaves, stems, and twigs were removed, dried and weighed. Labeling was performed on four or eight trees of each species on three consecutive days (8 trees on 16.08.2011 from 11.30 to 13.30; 16 trees on 17.08.2011 from 9.30 to 12.30, and also 16 trees on 18.08.2011 from 9.30 to 11.30). The 20 trees of each species were considered as replicates, since the labeling was done on sunny days with similar light and microclimatic conditions (Table 1).

The ^{13}C was applied as $^{13}\text{CO}_2$ to the aboveground parts of the plants by simultaneously pulse labeling trees in individual chambers. The chambers were ca. 5 m high and 2.5 m in diameter consisting of transparent polyethylene film with a thickness of 80 μm , which were hung from a wooden frame and closed properly with adhesive tape to avoid gas leakage. The $^{13}\text{CO}_2$ pulse was produced by injecting 60 mL 5 M H_2SO_4 into a solution of 100 mL distilled water containing 6.85 g $\text{Na}_2^{13}\text{CO}_3$ (Cambridge Isotope Laboratories, MA, USA) enriched to 99.0 atom% ^{13}C . Polyethylene wide mouth bottles (500 mL) containing $\text{Na}_2^{13}\text{CO}_3$ were fixed to a bowl and placed inside the chamber. The chamber was then closed and H_2SO_4 acid was carefully added from the outside into the $\text{Na}_2^{13}\text{CO}_3$ solution using syringes, and the puncture holes caused by the syringes were sealed with tape. Sulfuric acid was added in fivefold excess to ensure complete evolution of $^{13}\text{CO}_2$. A fan (5–12 V) inside each chamber guaranteed a uniform distribution of $^{13}\text{CO}_2$. Samples of CO_2 inside the chambers at the beginning and end of the treatment were taken to determine the change in CO_2 concentration.

2.2.2 ^{15}N labeling

Prior to the CO_2 pulse labeling, 12 mL glass vials were used as reservoirs containing 9.7 mL of a $\text{Ca}(\text{NO}_3)_2$ solution. This ^{15}N solution was made up of 36 g $\text{Ca}(\text{NO}_3)_2$ (99.23 atom% ^{15}N , Campro Scientific GmbH, Berlin, Germany) dissolved in 1200 mL sterilized water. Three leaves of beech and three leaflets of ash with a similar area were cut 3 times on the edges to allow the uptake of the solution by the tree and then placed directly in the $\text{Ca}(\text{NO}_3)_2$ solution in the vials. Three vials were fixed on the branches at different heights in each tree. The vials were closed with Parafilm and additionally covered with a transparent bag to avoid spilling on the ground. The vials were installed on the trees 3 d before the CO_2 labeling and were removed before the CO_2 labeling with a cut behind the leaves to avoid contamination of the ground or other leaves. Sampling was done after 4, 8, 13, 23, and 63 d, respectively. Leaf application of the ^{15}N tracer was chosen because it allows the investigation of a unidirectional allocation belowground.

Table 1: Mean aboveground biomass.

Tree species	Leaf Biomass / g Mean \pm SD	Stem Biomass / g Mean \pm SD
Beech	71 \pm 5	402.8 \pm 60.7
Ash	45 \pm 3	180 \pm 10

2.3 Sampling and isotope analysis

Of the 20 labeled trees of each species, five sets of four trees along with one reference tree were selected randomly for sampling at five time intervals (1, 5, 10, 20, and 60 d after CO_2 labeling or 4, 8, 13, 23, and 63 d after ^{15}N labeling). The plastic chamber was removed after the labeling period of 2 h and samples of four beeches and four ashes and a reference to each species were taken at each of the five time intervals.

All leaves of each tree were harvested completely, mixed after drying, and subsampled. Stems were sampled 10 cm above the start of the root, in the middle of the tree and from the top part. Root samples were taken 15 cm from the main root after the tree was entirely uprooted to make sure it belongs to the labeled tree. Soil was sampled 15 cm from the tree with a split tube in three replicates. The intact core was divided into 3 depth intervals (0–10 cm; 10–20 cm; 20–30 cm) and all soil samples were sieved to 2 mm. For the analysis of leaves, stem, root and bulk soil C and N content and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, all samples were freeze-dried, ground in a ball mill (Retsch Schwingmühle MM2, Haan, Germany), and stored in a desiccator until further analysis.

Leaf and soil samples were filled into tin capsules for measurement of relative N and C isotope abundances using an elemental analyzer NA1500 (Fison-instruments, Rodano, Milano, Italy) coupled to a Delta plus isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany) through a ConFlo III interface (Thermo Electron Corporation, Bremen, Germany). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were calibrated based on co-measured certified IAEA Standards (IAEA-600, USGS26, USGS40, USGS41, IAEA-CH-6, IAEA-CH-7, NBS 18, IAEA-N-1, IAEA-N-2, and IAEA-NO-3).

2.4 Calculation of ^{15}N and ^{13}C uptake

Plant uptake from sources of different isotope composition results in changes to their $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values and follows a two component mixing model according to Gearing et al. (1991), as shown in Eq. (1):

$$[C]_{incTracer} = [C]_{component} \times \frac{at\%_{labelled} - at\%_{ref}}{at\%_{applied Tracer} - at\%_{ref}}, \quad (1)$$

where $[C]_{component}$ is the C content of the component ($\text{mmol g}_{dried\ soil;leaf;stem;root}^{-1}$) and $[C]_{incTracer}$ is the total amount of ^{15}N or ^{13}C incorporated into the plant in ($\text{mmol g}_{dried\ soil;leaf;stem;root}^{-1}$), $at\%_{labelled}$ is the ^{13}C or ^{15}N values of the labeled sample of the tree (leaf, stem, root, soil), $at\%_{ref}$ the ^{13}C or ^{15}N values of the unlabeled reference sample of the tree (leaf, stem, root, soil), and $at\%_{applied Tracer}$ the ^{13}C enrichment of the added CO_2 or, respectively, ^{15}N enrichment of the added $\text{Ca}(\text{NO}_3)_2$.

2.5 Microbial biomass analysis

A portion of the 2-mm sieved soil was transferred into a plastic bag and stored at 5°C for chloroform fumigation-extraction. For the determination of microbial C and N content and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, two subsamples of 15 g were taken. One of

these were directly extracted and the other was fumigated with chloroform for 4 d in a desiccator to be able to extract C and N from the lyzed microbial cells.

Both the fumigated and non-fumigated samples were extracted with 45 ml of 0.05 M K_2SO_4 and placed on a horizontal shaker for 1.5 h. After shaking, the samples were centrifuged for 15 min at 3000 rpm, the supernatant was filtered (Rotilab® round cellulose filters, type 15A) and captured in 50 mL plastic centrifuge tubes.

Contents of C and N were measured on a TOC analyzer multi C/N® (Analytik Jena, Jena, Germany). For $\delta^{13}C$ measurements, the samples were freeze-dried and then measured on the same unit as the bulk soil samples.

Incorporation of ^{13}C and ^{15}N into fumigated and non-fumigated extracts was calculated according to the mixing model in Eq. (1). Microbial biomass C and N was calculated as the difference between fumigated and unfumigated amount, ^{13}C and ^{15}N uptake and corrected by an extraction coefficient of 0.45 for C (Wu et al., 1990) and 0.54 for N (Brookes et al., 1985).

2.6 Statistics

Field replications were corrected for outliers using the Nalimov outlier test with significance levels of 95% (when four repetitions were available). An analysis of variance (ANOVA) was calculated to quantify how single variables contributed to the observed variances of the data. Tukey HSD tests for post hoc comparison were used to compare isotope enrichment or total ^{13}C or ^{15}N uptake as dependent variables, while tree species, plant compartment and within-individual variation were used as independent variables (significance level of $p < 0.05$). The error bars show the standard error of the mean (SEM) in all graphs. Linear Regression of the relative ^{13}C and ^{15}N incorporation over time in soil (Fig. 4) and microbial biomass (Fig. 5) was fitted to the data according to a least square algorithm.

3 Results

3.1 Aboveground ^{13}C and ^{15}N dynamics

At one day after labeling, beech had taken up 20.2% and ash 9.1% of the applied $^{13}CO_2$ in all measured compartments (Fig. 1). The ^{13}C allocation has been calculated per gram dry weight; therefore, these values are comparable despite differences in tree biomass (Table 1). Beech allocated significantly more ^{13}C to its leaves, stem, and soil than ash. However, the roots did not differ in their ^{13}C allocation at day one after labeling (Fig. 1). Leaves and stem were both highly enriched in ^{13}C (Fig. 1) which reflects a rapid photosynthetic uptake in both trees, but twice as much in beech than in ash. About 80% of the ^{13}C recovered in all compartments was located in the leaves at day one and about 20% in the stem for both tree species. The ^{13}C signal was particularly pronounced for leaves and revealed a significant difference in dynamics between beech and ash. A rapid initial decrease in ^{13}C incorpo-

ration to values of about 20% of the initially incorporated $^{13}CO_2$ values was observed. However, beech showed this drop immediately and remained at the level of 20% from day 5 to day 60, whereas ash reached this 20% mark by continued decrease until 20 d after labeling. The ^{13}C signal in the stem remained constant in beech and ash from day 1 until day 60 after labeling with the exception of a significant increase of the relative ^{13}C allocation at day 60 in beech (Fig. 2A).

Whereas the highest total ^{13}C incorporation was always observed for day 1, the highest ^{15}N uptake from the applied $Ca(^{15}NO_3)_2$ into beech at 46.0% was recorded 13 d after removing the label solution. The highest ^{15}N uptake from the applied $Ca(^{15}NO_3)_2$ into ash at 45.4% was detected 8 days after the labeling (Fig. 1). This time lag in ^{15}N uptake might be due to the lack of measurement of twigs. The absolute allocation rates of the applied ^{15}N differed neither in leaves nor in the stem between the tree species. 97.5% of the assimilated ^{15}N was located in the leaves on day 13 in beech and 99.3% on day 8 in ash (Fig. 2C). For both trees, ^{15}N in the leaves de-

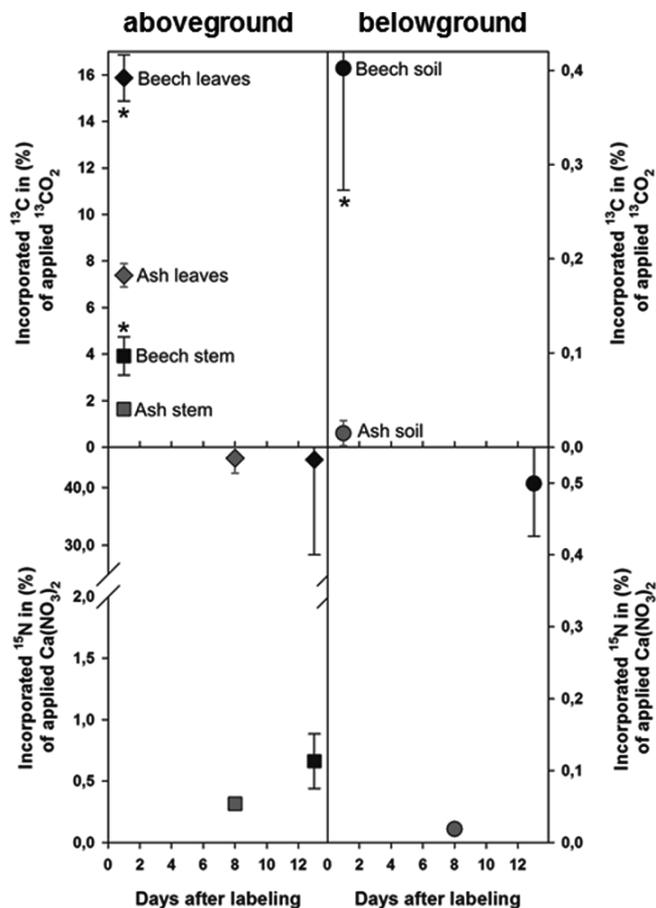


Figure 1: Upper panels: Total ^{13}C incorporation on day 1 in above- (left) and belowground (right) C pools; lower panel: Total ^{15}N allocation of the maximal incorporated $Ca(NO_3)_2$ on day 13 for beech and day 8 for ash in above- (left) and belowground (right) N pools; in 0–30 cm depth and at a distance of 15 cm radius from the tree, beech (black symbols) and ash (red symbols). Error bars show SEM. * shows significant ($p < 0.05$) differences of the different compartments between ash and beech.

creased thereafter. The first sampling at day 4 after the start of N labeling and the sampling at day 23 revealed that ash incorporated significantly more ¹⁵N into the stem (0.5% of maximal incorporated N) than beech (0.05% of maximal incorporated N). An immediate consistent increase of the relative ¹⁵N allocation into the stem in both trees over the entire experimental period could be observed. However, the increase of the relative ¹⁵N allocation from leaves to stem over time in beech was more rapid than in ash. Beech had a relative ¹⁵N allocation of 5.4% on day 63 after the labeling in comparison to ash with 3.1% (Fig. 2C).

In summary, in this experiment beech fixed twice as much ¹³C by photosynthesis as ash, but both tree species incorporated a similar ¹⁵N amount into leaves. Beech showed a more rapid transport of photosynthates through the tree and allocated twice as much of the assimilated ¹³C into the stem.

3.2 Belowground ¹³C and ¹⁵N dynamics

A slight increase of relative ¹³C incorporation observed over time in roots and soil was in accordance with the decrease in leaves and stem and reflects the belowground translocation

of assimilated ¹³C in both tree species (Fig. 2A, B). The rapid ¹³C transport in beech (Fig. 2B) showed that already at day one beech released much more ¹³C into the soil (2.0% of initially assimilated ¹³C) than ash (0.2% of initially assimilated ¹³C). This can also be observed for all soil depths in the ¹³C incorporation into microbial biomass (Fig. 3B). ¹³C allocation in the microbial biomass had already peaked one day after labeling and showed a depth-related abundance in beech (Fig. 3B). Figure 3A shows that ash displayed similar dynamics at all soil depths with a peak allocation of ¹³C into the soil at day 20, while beech had an initially higher and more constant rhizodeposition over the 60 d (Fig. 3A). On day five after labeling, ¹³C allocation to the root was higher in ash than in beech (Fig. 2B). Most microbial biomass ¹³C incorporation under ash was lower than the detection limit. Despite the fact that measurement accuracy is higher for microbial biomass than for soil (due to the high activity of microbial biomass in ¹³C incorporation and a large passive C pool in soil), there was no significant enrichment of microbial biomass under ash. The low absolute ¹³C assimilation of ash accounts for the low enrichments in microbial biomass, and there were no differences to the unlabeled reference.

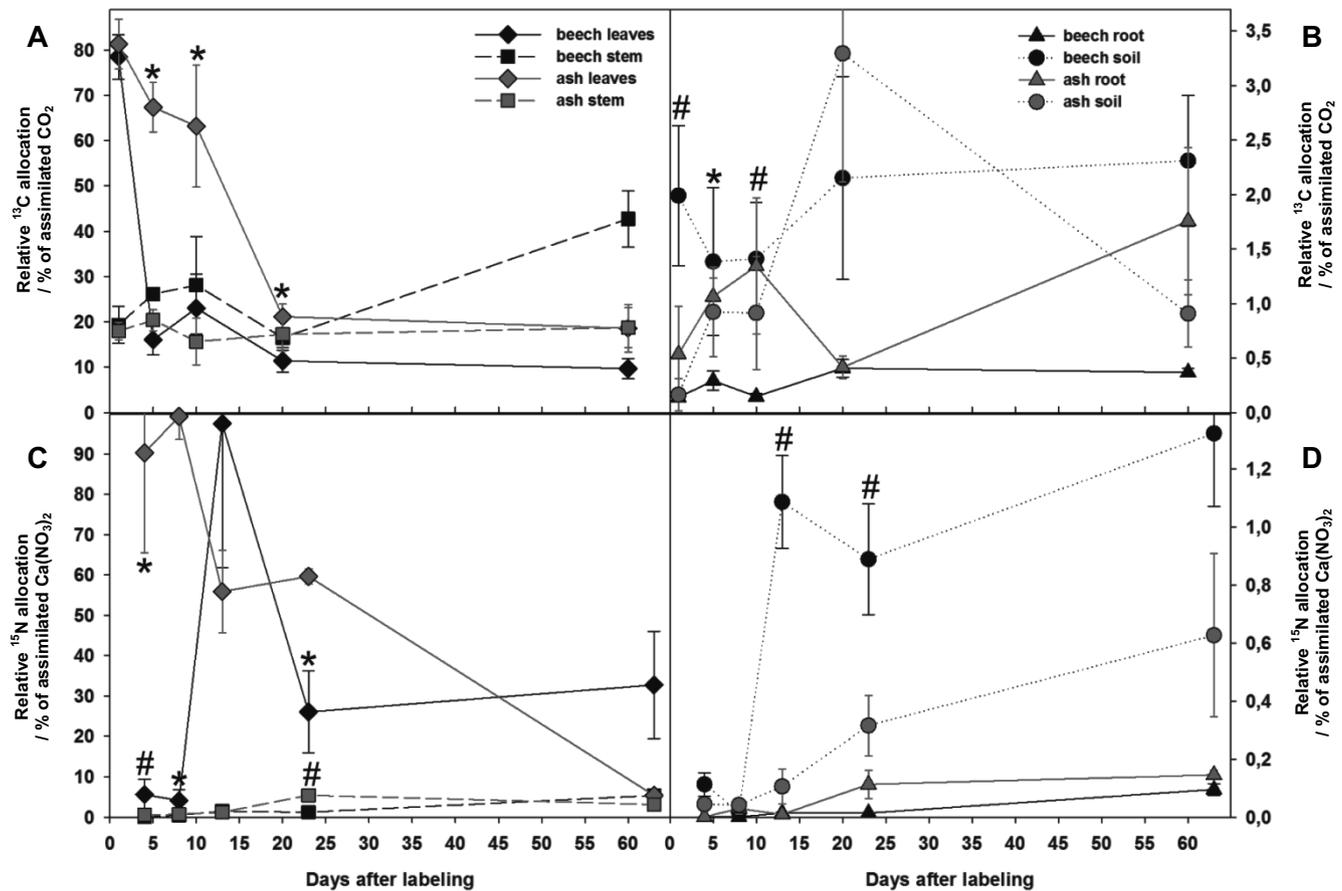


Figure 2: ¹³C allocation (in % of the initially assimilated ¹³CO₂ at day one) in (A) leaves (diamonds) and stem (squares), (B) roots (triangle) and soil (circles), 0–30 cm depth at a distance of 15 cm from the tree. Relative ¹⁵N allocation of the maximal incorporated Ca(NO₃)₂ (day 13 for beech and day 8 for ash) in (C) leaves and stem (D) roots and soil at 15 cm distance from tree at 0–30 cm depth in beech (black symbols) and ash (red symbols). Error bars show SEM. * shows significant (*p* < 0.05) differences of leaves and roots between beech and ash for individual sampling dates. # shows significant (*p* < 0.05) differences of stem and soil between beech and ash for individual sampling dates.

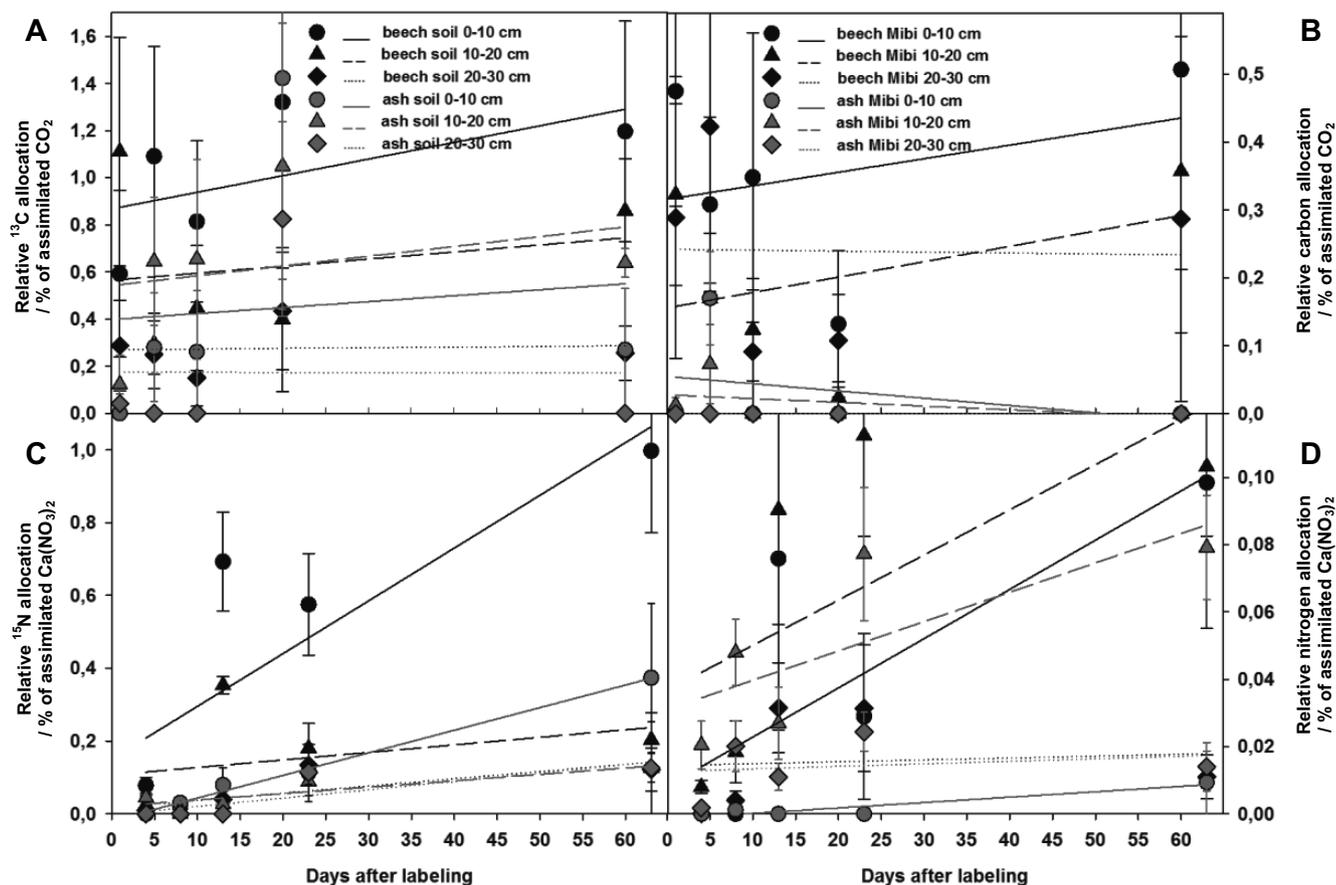


Figure 3: Relative ¹³C allocation (% of the initially assimilated ¹³CO₂ at day one) in (A) soil at 0–10 cm depth (circle), 10–20 cm depth (triangles), 20–30 cm depth (diamonds), and (B) microbial biomass depending on depth and time at 15 cm distance from the tree and in beech (black symbols) and ash (grey symbols). Relative ¹⁵N allocation of the maximal incorporated Ca(NO₃)₂ (day 13 for beech and day 8 for ash) in (C) soil and (D) microbial biomass depending on depth and time. Error bars show SEM. * shows significant (*p* < 0.05) differences between beech and ash at one depth of 0–10 cm, # at a depth of 10–20 cm; small letters show differences between depths in one tree species: (a) shows significant (*p* < 0.05) differences between beech soil at a depth of 0–10 cm to both other depths for individual sampling dates (b) shows differences between ash soil at a depth of 10–20 cm to both other depths for individual sampling dates.

Thirteen days after the N labeling, a significant difference in the ¹⁵N signal was measured in the soil sampled at 15 cm distance from beech (0.1% of the maximal incorporated ¹⁵N) and ash (0.04% of the maximal incorporated ¹⁵N) (Fig. 2D). Beech allocated more of the incorporated ¹⁵N to soil but less ¹⁵N to its roots—a behavior which is similar to the ¹³C partitioning between soil and roots (Fig. 2B). Beech also displayed a significantly depth-related rhizodeposition with a maximum in the 0–10 cm soil segment starting at day 13 after labeling (Fig. 3C). The rapid transport of assimilates in beech is also noticeable in the N allocation. While the ¹⁵N signal is evident in the soil of beech at day 13, it takes until day 65 to show a pronounced signal in ash soil. This peak on day 13 for beech is also reflected in the N allocation to the microbial biomass. Beech allocated only 10% of its incorporated ¹⁵N to the microbial biomass, whereas for ash almost all was taken up into microbial biomass. ¹⁵N incorporation into microbial biomass was significantly higher in soil of 10–20 cm soil depth under ash than at other depths for ash and the same depth for beech (Fig. 3D).

In summary, beech has an initially higher and more constant rhizodeposition than ash and beech also displays a higher ¹³C uptake by microbial biomass than ash. However, ash provides almost all of its exudated ¹⁵N for uptake into microbial biomass in comparison to beech. Only 10% of the exudated ¹⁵N was taken up by microorganisms under beech.

3.3 Spatial gradients of ¹⁵N and ¹³C allocation around the trees

The allocation of ¹³C at increasing distances from the tree was investigated at day 5 after ¹³C labeling (day 8 after the start of ¹⁵N labeling). At any given soil depth there was no change in ¹⁵N or ¹³C exudation along the investigated 60 cm distance. However, there were differences between the soil depths, with ash allocating significantly more ¹³C to the 10–20 cm depth than to any other depth throughout the 60 cm distance from the tree (Fig. 4). For beech, ¹³C and ¹⁵N enrichment was always highest in the top 10 cm compared to the other soil depths. However, due to the large C pools in these forest soils, the ¹³C enrichment was too low to draw

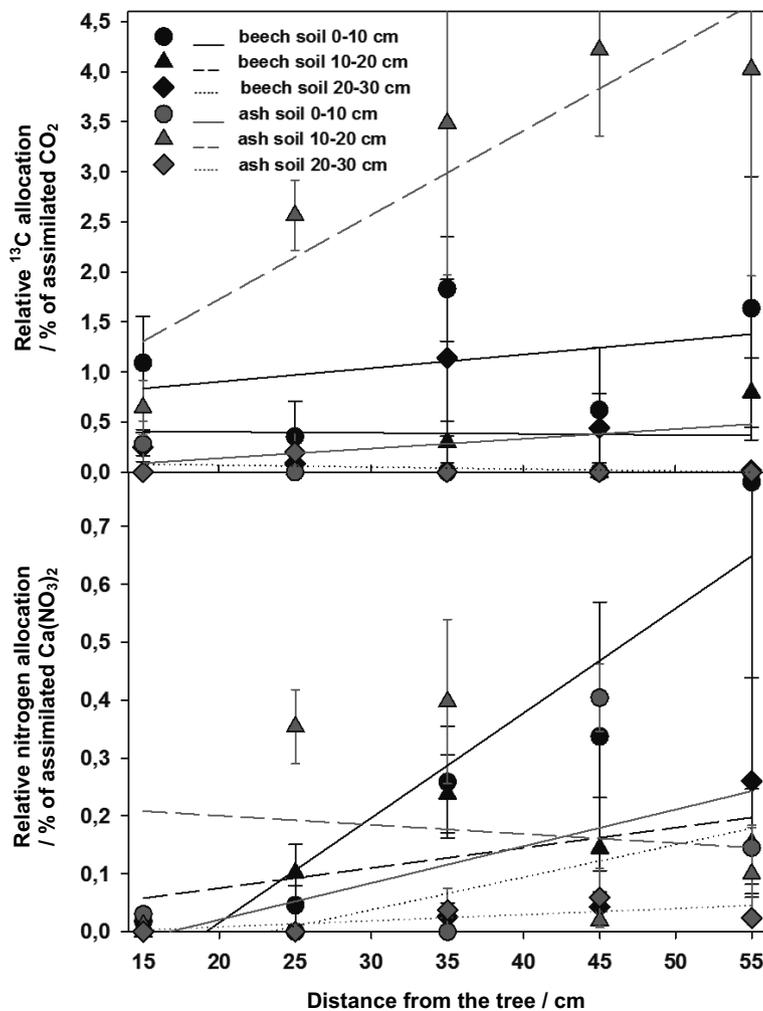


Figure 4: Relative ^{13}C allocation (in % of the initially assimilated $^{13}\text{CO}_2$) with linear fits in soil at 0–10 cm depth (circle/solid line), 10–20 cm depth (triangles/long dash line), 20–30 cm depth (diamonds/dotted lines) and distance from the tree at day 5 after labeling and in beech (black symbols/lines) and ash (grey symbols/lines). Relative N allocation of the maximal incorporated ^{15}N (day 13 for beech and day 8 for ash) with linear fits in soil depending on depth and distance from the tree. Error bars show SEM.

conclusions on the 20–30 cm depth for either tree species or to see any clear effects of increasing distance from the trees.

For both tree species, the ^{13}C allocation to soil displayed a positive trend with increasing distance from the tree, related to the increase of the rooted soil volume with distance. For both tree species, the highest ^{13}C and ^{15}N incorporation into the microbial biomass was found in the top 10 cm (Fig. 5). Thus, spatial allocation to microbial biomass does not reflect the distribution of allocation to soil, which might be connected with root distribution or might indicate that the amount of C input is not controlling microbial ^{13}C incorporation. The distance effect is less pronounced in the ^{15}N allocation in both tree species (Figs. 4 and 5).

In summary, a depth-related rhizodeposition ^{13}C and ^{15}N was evident under beech. Ash allocated more ^{13}C to the 10–20 cm soil depth. And at any given soil depth there was

no change in ^{15}N or ^{13}C exudation along the investigated 60 cm distance.

4 Discussion

4.1 Carbon allocation within plant-soil system depending on tree species

Given the major importance of belowground C and N allocation for soil processes, we used a quantitative method for investigating the coupling of canopy C assimilation with belowground processes. Therefore, we were able to trace directly the C fluxes into the soil by two tree species. This study revealed that beech assimilated twice as much of the applied $^{13}\text{CO}_2$ as ash, but beech and ash incorporated similar ^{15}N amounts into leaves. The photosynthates were transported belowground through phloem more rapidly in beech than in ash and ash preferentially accumulated ^{15}N and ^{13}C in the roots. However, beech released more ^{13}C and ^{15}N via rhizodeposition into the soil than ash, which was also subsequently recovered in microbial biomass. This approach enables tracing of above- and belowground C and N allocation and consequently can reveal the controlling influence of individual tree species on broad leaf forest C and N cycles. Pulse-labelling of trees with $^{13}\text{CO}_2$ and ^{15}N allows quantification of at least three important aspects of whole-plant C and N metabolism: (1) the portion of assimilated C and incorporated N, (2) the rate of transfer of C and N between compartments, and (3) the residence time of C and N in these compartments. Only a few *in-situ* labelling experiments on tall trees have been reported (Andersen et al., 2010; Carbone et al., 2007; Högberg et al., 2008; Keel et al., 2012; Shibistova et al., 2012) and even fewer experiments have provided quantitative insights into C residence times in short-lived storage pools and of transfer rates among plant compartments and between plants, soil, and the atmosphere (Plain et al., 2009; Epron et al., 2011; Warren et al., 2012). The many differences between individual tree species cause tremendous challenges in tree labeling studies—especially if focused on time-series allocation patterns. The necessity to harvest the entire tree to receive a full isotopic budget means that over time series not only other time points but also other tree individuals have to be compared. This introduces high variability to the data as can be seen in Figs. 2 and 3. Nevertheless, such tree labeling studies in the field offer unique opportunities to trace C and N allocation patterns under natural conditions.

The transfer time of photosynthates to ecosystem respiration has been indirectly estimated by tracing natural ^{13}C fluctuations related to climate-induced variations in ^{13}C discrimination during photosynthesis. Time lags were observed for tall Douglas fir trees (Bowling et al., 2002; McDowell et al., 2004), mixed hardwood forest (Mortazavi et al., 2005) and

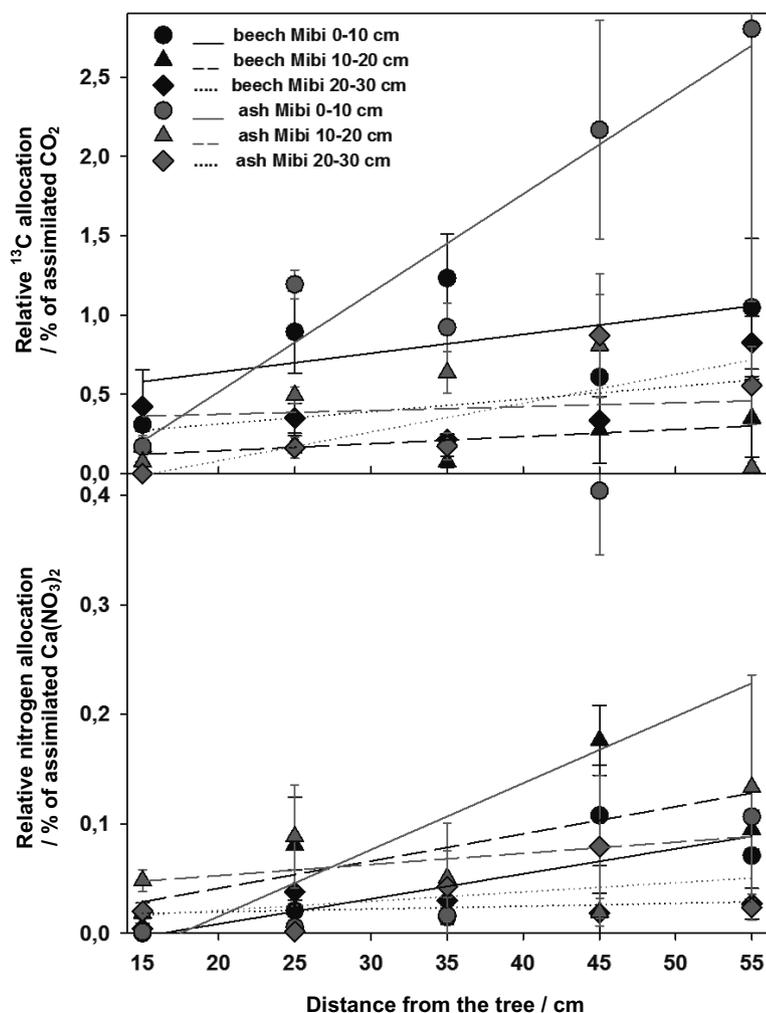


Figure 5: Relative ¹³C allocation (in % of the initially assimilated ¹³CO₂) with linear fits in microbial biomass at 0–10 cm depth (circle/solid line), 10–20 cm depth (triangles/long dash line), 20–30 cm depth (diamonds/dotted lines) depending on depth and distance from the tree at day 5 after labeling and in beech (black symbols/lines) and ash (red symbols/lines). Relative N allocation of the maximal incorporated ¹⁵N (day 13 for beech and day 8 for ash) with linear fits in microbial biomass depending on depth and distance from the tree. Error bars show SEM.

beech and oak (Epron et al., 2012). Epron et al. (2012) came to the conclusion that the relationship between the rate of C transfer and the time lag of peak ¹³CO₂ efflux from the soil differs between broad-leaved species (oak and beech) and pine. Dannoura et al. (2011) and Wingate et al. (2010) stated that the distinctly different transfer time of ¹³C belowground between two broad-leaved species and pine is caused by differences in the velocity of photosynthate transport via the phloem sap. In our study, we just compared two broad-leaved species and a rapid photosynthetic uptake in beech was detected, but also a species-specific time lag between beech and ash in the ¹³C allocation belowground. The higher photosynthetic ¹³CO₂ fixation can be partially attributed to the higher leaf biomass ($p < 0.01$) and, thus, photosynthetically active tissue in beech than in ash. However, the leaf area, not measured in this study, may be an even more accurate physiological tree parameter to characterize photosynthetic capability. Nevertheless, it can be stated that the CO₂ fixation capacity

of beech is significantly higher than of ash trees of similar tree height. Tree size and age may also affect CO₂ uptake and distribution. However, in this study we could unfortunately not repeat the measurements on the trees of different sizes, although that would be an interesting approach for further studies.

Already at day 1 after labeling, the relative ¹³C allocation of the assimilated CO₂ was about 80% in the leaves and about 20% in the stem in both tree species. Beech and ash transported about 75% of the initially incorporated C away from the leaves which resulted in the clear peak of ¹³C allocation in the stem of beech. Ash showed a slower decrease of C in the leaves and just a slight increase in the stem, which might be explained by leaf respiration of most of the assimilated C. Thus, there was no time lag difference detectable in the uptake of the ¹³C between the two tree species, but there was already a difference in time lag noticeable in the allocation into the stem. However, there might also be a difference in the velocity of photosynthate transport via the phloem sap, which causes the time lag of the C transport observed for ash and beech.

Ryan et al. (1996) showed that the dark respiration from foliage and fine roots at night was linearly related to biomass and N content, but N was a better predictor of CO₂ efflux than plant biomass. They concluded that the higher the N content the greater the CO₂ efflux produced. Langenbruch et al. (2012) showed in the same forest that ash litter has a higher N content than beech, which would support our findings for dark respiration with regard to the results of Ryan et al. (1996). Therefore, our study leads to the conclusion that ash respire most of the assimilated C directly in the leaves, presumably at night, whereas beech allocates more of the assimilated C belowground.

Trees like beech accumulate more C_{org} in the forest floor and less C_{org} in the mineral soil compared to ash (Langenbruch et al., 2012). This property of beech was mainly attributed to the high C:N and lignin:N ratio in litter, which slows decomposition (Guckland et al., 2009; Kooijman and Cammeraat, 2010). However, Vesterdal et al. (2008) showed that the forest floor and litter fall C:N ratios were not related, whereas the C:N ratio of mineral soil (0–30 cm) better indicated N status of the tree under beech and ash on rich soil. They already suggested that deciduous tree species in Europe differ in C and N sequestration rates within forest floor and mineral soil. However, Callesen et al. (2015) recently stated that the average forest SOC stock remains unchanged over decades for soils < 4.1% C in the top mineral soil, whereas the sink/source status of very C-rich and organic soils remains uncertain. Thus, besides the slow litter decomposition under beech, rhizodeposition could be another reason explaining the higher C accumulation. Nevertheless, Meinen et al. (2009) and Cesarz et al. (2013) showed that ash has more fine roots and a more vigorous root growth than beech, which

should also lead to more rhizodeposition. Ash also preferentially invested the assimilated ^{13}C into the root biomass in our study. Already 5 d after the labeling, the relative ^{13}C allocation into the root of ash was significantly higher than in beech. However, our experiment also revealed rapid ^{13}C transport through the beech tree and also significantly higher ^{13}C allocation *via* the roots into the soil in beech. At day 1 after labeling, beech had already allocated 2% of the assimilated $^{13}\text{CO}_2$ into the soil. This is 10 times more C than observed for ash. Beech also showed an initially higher and more constant rhizodeposition over the 60 d period than ash. These findings demonstrate that the $^{13}\text{CO}_2$ labeling technique applied in our experiment is suitable to investigate C rhizodeposition into soil under tall trees in deciduous forests *in situ*.

Comparison of various ecosystems revealed that the relative belowground translocation of assimilated C for trees is smaller than, for example, for grasses (Kuzyakov and Domanski, 2000). Our results demonstrate clearly that there is a species effect in the rhizodeposition even if two deciduous tree species are compared. This finding has to be considered in further labeling experiments. Only a small proportion of the rhizodeposits remains in the soil because most of the C rhizodeposits are decomposed to CO_2 by microorganisms (Kuzyakov and Larionova, 2006; Werth et al., 2006; Jones et al., 2009). Microbial biomass is composed of a large number of various microorganisms and includes the extraradical mycelium of mycorrhizal fungi. A rapid transfer of photosynthate to ectomycorrhiza has indeed been reported (Leake et al., 2001; Esperschütz et al., 2009; Högberg et al., 2010). The results in our experiment corroborate those findings. Beech is associated with ectomycorrhiza and ash roots with arbuscular mycorrhiza (Meinen et al., 2009). For beech, ^{13}C allocation into the microbial biomass peaked already at day 1 after the labeling and showed a depth-related abundance. This can be explained by the fast transfer of C from roots to mycorrhizal fungi in beech.

We conclude that ^{13}C labelling of trees offers the unique opportunity to trace the fate of labelled CO_2 into the tree and its release to the soil and the atmosphere in forests *in situ*. Thus, pulse labelling enables the quantification of C partitioning in forests and the assessment of the role of C and N partitioning for growth of individual tree compartments, resource acquisition and C sequestration in soils dependent on season and tree growth stage (Epron et al., 2012).

4.2 Nitrogen allocation within plant-soil system depending on tree species

To investigate the partitioning of nitrogen among soil, litter, below- and aboveground biomass, ^{15}N -nitrate ($^{15}\text{NO}_3^-$) as well as ^{15}N -ammonium ($^{15}\text{NH}_4^+$) has been added to the soil in predominantly coniferous forest ecosystems (Preston and Mead, 1994; Buchmann et al., 1995; Tietema et al., 1998; Perakis and Hedin, 2001; Compton and Boone, 2002). The results from those studies showed that inorganic N allows a rapid and localized investigation of the N partitioning between plants and microorganisms from soil (Kuzyakov and Xu, 2013). In this experiment, N tracer was added to leaves, in contrast to the other studies.

The highest ^{15}N uptake from the applied $\text{Ca}(^{15}\text{NO}_3)_2$ was observed 13 d after the start of the N labeling from beech trees, at 46.0% (Fig. 1). For ash the relative incorporation was similar, but this maximum occurred earlier, *i.e.*, 8 d after the labeling. Thus, almost half of the applied ^{15}N could be recovered in the trees. However, the delay in the ^{15}N incorporation maximum was unexpected as the highest total ^{13}C recovery was observed for day 1 after labelling in both tree species. This delay in the maximum of N recovery might be due to the labeling of a limited number of leaves, leading to N allocation from the labelled leaves through twigs to other leaves. However, our results revealed that the N redistribution in the beech crown proceeds faster than in ash.

Fine roots of ash are almost absent in some parts of the soil profile but are clustered in other parts, forming 'hot spots' within the profiles (Pausch and Kuzyakov, 2011; Schütt et al., 2006). Beech in contrast has a heart root system in which several major roots are developed, growing in parallel into the depth (Schütt et al., 2006). These differences in root distribution within the pedon can account for the depth-related ^{15}N rhizodeposition of beech with a maximum in the topsoil, whereas ash, with lower absolute ^{15}N allocation into soil, showed a more homogenous ^{15}N distribution in our study. The fact that roots in different soil depths show altered physiological activities might be the reason for ash displaying significantly higher ^{15}N incorporation in the microbial biomass in 10–20 cm in comparison to the topsoil and to beech at the same depth. ^{15}N of the ash allocated to the soil was almost completely incorporated into the microbial biomass. In contrast, for beech only 10% of its N was allocated to microbial biomass. This indicates that ash N exudates are more microbially available, presumably in the form of N-rich, low-molecular weight root exudates like amino acids. In contrast, such monomeric substances may only make up a small proportion of the N released by beech: beech rhizodeposition may be dominated not by root exudates but by more stable structural compounds like proteins.

Another explanation for the contrasting ^{15}N distribution between ash and beech in soil and microbial biomass could be the types of mycorrhiza the tree species associate with. Beech is associated with ectomycorrhiza and ash roots with arbuscular mycorrhiza (Meinen et al., 2009). The type of mycorrhizal association may also affect root activity. Meinen et al. (2009) showed by microscopic inspection of beech and ash that the mycorrhizal colonization rate was significantly higher in beech than in ash roots (Cesarz et al., 2013). This may account for the higher ^{15}N allocation into soil and microbial biomass in beech in our experiment.

Ash has more fine roots with a more vigorous root growth than beech and in general fine roots tend to contain more N (Meinen et al., 2009; Cesarz et al., 2013). Our study showed that ash incorporated more ^{15}N into roots and had less rhizodeposition into the soil, which can be explained by the morphology of the ash root system. The results suggest that beech and ash differentially impact soil processes: ash preferentially invests allocated C and N in root biomass formation whereas beech affects the belowground system *via* root exudates and associated changes in rhizosphere microorgan-

isms and C dynamics. Therefore, the individual strategies of C and N allocation of beech and ash are representative examples of niche strategies of two broad-leaved trees in deciduous forests. Although we cannot conclude which distinct advantages beech and ash achieve from their individual niche strategies, our results suggest that differences in C and N allocation patterns between ash and beech provide a higher diversity of soil functions. Especially in species-poor systems like broad-leaved forests in Central Europe, tree diversity will increase functional diversity in soils and thus improve ecosystem stability compared to forests with pure stands of a single tree species.

5 Conclusions

Tree species effects on C and N allocation and dynamics above- and belowground were investigated for beech and ash. Beech assimilates more CO₂ than ash trees of similar size, presumably due to a higher amount of photosynthetically active leaf biomass. Ash stores more C and N in the plant biomass, at least in August. Beech had a faster belowground transfer of photosynthate including a faster release of C in root exudates to microorganisms feeding on rhizodeposition. A lower and slower C transfer belowground by ash compared to beech was discovered and all C released by ash into soils was recovered in microbial biomass.

The labeling with Ca(¹⁵NO₃)₂ clearly revealed different N allocation patterns for beech and ash: whereas ash allocated 0.2% of the maximal incorporated ¹⁵N belowground mainly for root growth after 63 d, beech distributed only 0.1% of the maximal incorporated ¹⁵N into the root. Nevertheless, of the ¹⁵N allocated belowground, beech allocated the greater proportion to the soil (1.3% of the total ¹⁵N recovery), while only 0.6% were released by ash after 63 d. However, no methods currently exist to differentiate between direct translocation from root to mycorrhizal hypha and exudation and microbial uptake from soil solution.

Furthermore, for other tree species dominant in various forest ecosystems, the composition of root exudates and the fate of C in the soil microbial community need to be investigated to be able to understand C and N cycles. This knowledge is essential to (1) assess the ability of forest ecosystems to sequester C above- and belowground and (2) to improve conservation projects in deciduous forest systems and estimate their influence on the global C cycle.

Acknowledgements

We wish to thank Sarah Zieger, Ronny Thoms, and Michaela Rath (University of Göttingen) for their contribution to the field experiment as well as Kay Schünke and Steffen Wolf for helping in the field and also the KOSI for their continuous support with the isotope analyses and the Hainich National Park for the work permission. I am also very grateful to my student assistants Anika Handke and Anna Meyer and the technical staff of the Soil Science of Temperate Ecosystems, especially Ingrid Ostermeyer and Susann Enzmann, for their help in laboratory as well as to Maire Holz, who conducted a Bachelor

thesis in this project. Financial support for this work was provided by the German Research Foundation (DFG). This investigation contributes to the project B5 of the DFG Graduiertenkolleg 1086.

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