The tree species matters: Belowground carbon input and utilization in the myco-rhizosphere

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Abstract
Rhizodeposits act as major carbon (C) source for microbial communities and rhizosphere-driven effects on forest C cycling receive increasing attention for maintaining soil biodiversity and ecosystem functions. By in situ 13CO2 pulse labeling we investigated C input and microbial utilization of rhizodeposits by analyzing 13C incorporation into phospholipid fatty acids (PLFA) of beech- (Fagus sylvatica) and ash-associated (Fraxinus excelsior) rhizomicrobial communities. Plant compartments and soil samples were analyzed to quantify the allocation of assimilates. For 1 m high trees, ash assimilated more of the applied 13CO2 (31%) than beech (21%), and ash allocated twice as much 13C belowground until day 20. Approximately 0.01% of the applied 13C was incorporated into total PLFAs, but incorporation varied significantly between microbial groups. Saprotrophic and ectomycorrhizal fungi under beech and ash, but also arbuscular mycorrhizal fungi and Gram negative bacteria under ash, incorporated most 13C. PLFA allowed differentiation of C fluxes from tree roots into mycorrhiza: twice as much 13C was incorporated into the fungal biomarker 18:2u6,9 under beech than under ash. Within 5 days, 30% of the fungal PLFA-C was replaced by rhizodeposit-derived 13C under beech but only 10% under ash. None of the other microbial groups reached such high C replacement, suggesting direct C allocation via ectomycorrhizal symbioses dominates the C flux under beech. Based on 13CO2 labeling and 13C tracing in PLFA we conclude that ash allocated more C belowground and has faster microbial biomass turnover in the rhizosphere compared to beech.

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1. Introduction

The total forest area of the world in 2005 was estimated to be about 4 billion ha or 30% of the total land area [1]. Forests store 80% of the terrestrial aboveground biomass and thus determine the C balance of terrestrial ecosystems [2]. An estimated 73.5 t ha\(^{-1}\) of carbon (C) are stored in the soils (0–30 cm) of the world’s forests, which is more than in the living tree biomass (71.5 t ha\(^{-1}\)) [1]. The C stock in the litter horizon of European forests is estimated to be 6.1 t ha\(^{-1}\) and the C stock in mineral soil 113 t ha\(^{-1}\) [1]. Beech is the most common deciduous tree in Germany, covering an area of 1.68 million ha, which is 15.4% of the entire forest area [3]. Beech therefore is of great economic value and ecological importance in Central Europe [4]. Ash makes up ca. 10% of the forest area in Germany and is seen as a promising species for the future forestry industry [5]. These two tree species therefore are major representatives of forests in Germany and taken as model species for investigating C allocation of trees belowground [6,7]. Notably, the link between tree species identity and soil microorganisms in mixed-species forests remains little studied [8].

C allocation to roots and into the rhizosphere has received little attention in trees [9–11]. Up to 90% of the net primary production of trees enters the soil as detritus [12], where fungi and bacteria subsist on rhizodeposits and show a high metabolic versatility. The amount, composition and dynamics of rhizodeposits and their ecological functions, especially those of trees, are poorly

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investigated [13]. This calls for focusing on feedback mechanisms between rhizodeposits and the microbial community composition.

Phospholipids — biomarkers for microbial community composition — are fundamental membrane components of all living cells [2]. Certain fatty acids are marker molecules for certain microbial groups [14] and can be used to characterize microbial communities, including Gram positive and Gram negative bacteria as well as fungi. They also can be used to assess the effects of plant species diversity on soil microbial communities [2].

This study was part of a nine-year project that investigated soil organic matter (SOM) formation under broad-leaved trees dominating in Germany. SOM stocks were higher in mixed stands as compared to mono-specific stands [15,16]. SOM accumulation in the litter layer was highest under beech (0.81 kg m$^{-2}$) compared to mono-specific organic matter (SOM) formation under broad-leaved trees dominating in Germany. Saxony, Germany. The region is characterized by mild winters and differences in root morphology and mycorrhiza types [23]. Beech roots are associated with ectomycorrhizal (ECM) fungi such as Byssocoriciella atrovirens, Lactarius substilicus and Xerocomus chrysenteron, [24].

In contrast, ash roots are associated with arbuscular mycorrhizal fungi [23] and ash presents a typical tap root system. Beech has a heart root system in which several major roots are developed, growing downward in parallel [25]. Ash exhibits fine roots of larger diameter, lower specific root area and lower specific root tip abundance than beech [23].

We hypothesized 1) the composition of microbial groups under beech to differ from that under ash, and 2) belowground C allocation and transformation to differ between microbial groups. In detail, we expected ECM to be more abundant under beech than under ash, and arbuscular mycorrhizal fungi to be more abundant under ash than under beech. We used PLFAs to investigate differences in microbial community structure under the two tree species. To analyze the incorporation of rhizodeposits into microorganisms, we pulse labeled ash and beech trees with $^{13}$CO$_2$. We quantified the allocation of rhizodeposits into various microbial groups by $^{13}$C-PLFA and inspected C incorporation into individual microbial groups, especially into mycorrhizal fungi and Gram negative bacteria.

2. Materials and methods

2.1. Site description

The experimental site — Göttinger Wald (51°35′15.39″N 9°58′57.95″E, 362 AMSL) — is located southeast of Göttingen, Lower Saxony, Germany. The region is characterized by mild winters and humid summers with an annual precipitation of 613 mm and a mean annual temperature of 8.7 °C [26]. The Göttinger Wald is a 130–145-year-old beech forest scattered with ash and maple. The soil is an Orthic Renzina with typical mull humus [27]. The pH of the topsoil varies between 4.4 and 7.0 [28,29]. Forty ash and 40 beech trees with a height of ca. 1 m (73–177 cm) were chosen in May 2012.

2.2. Experimental design and sampling

Ash and beech seedlings (approximately 1 m high) were taken from the forest with undisturbed soil, and the entire soil core was placed into 23-cm-diameter pots of a depth of 26 cm. The trees had a reestablishment time of 2 months; they were kept in a shaded area under the canopy of mature beech trees and then transferred to an outdoor greenhouse. The seedlings were irrigated regularly, and herbs were removed by cutting the shoots at the soil surface. Shortly before the start of the experiment, the pot was wrapped with plastic and closed airtight with Terostat (Teronor Terostat-VII, Henkel, Düsseldorf, Germany) to avoid $^{13}$CO$_2$ re-uptake from soil respiration [10]. An irrigation system was established consisting of PVC tubes (Deutsch & Neumann, Berlin, Germany) with an inner diameter of 6 mm and fixed with cable ties to the plastic wrapping (OBO Bettermann GmbH & Co. KG, Menden, Germany). A ventilation system was used within the plastic bags.

Seedlings were acclimatized for two days in the chamber at 400 ppm with unlabeled CO$_2$ produced by injecting 5 M lactic acid in a 0.5 M solution of $^{13}$C sodium carbonate (KMF Laborchemie Handes, Lohmar, Germany). The $^{12}$CO$_2$ pulse was produced by injecting 5 M lactic acid into a 0.5 M $^{13}$C sodium-carbonate (Na$_2$CO$_3$) solution (Sigma-Aldrich, Taufkirchen, Germany) enriched with 99 atom% $^{13}$C. The seedlings were exposed to $^{13}$CO$_2$ for three days and to $^{12}$CO$_2$ for two days for 16 h day$^{-1}$ with a maximum CO$_2$ concentration of 1800 ppm. The CO$_2$ concentration in the chamber was monitored using an infrared gas analyzer (CARBOCAP™ Serie GM220, Driesen + Kern GmbH, Bad Bramstedt, Germany). To reduce dilution of the $^{13}$CO$_2$ by plant-derived CO$_2$ at night, CO$_2$ in the chamber was absorbed by pumping the air through a 1 M NaOH solution.

The $^{13}$CO$_2$ pulse labeling was conducted on 20 ash and 20 beech tree seedlings in a chamber with a surface area of 1 × 1 m$^2$ and approximately 2 m high [30,31]. Twenty beeches and 20 ashes remained unlabeled as reference trees. Conditions in the chamber were kept at 1013 hPa, 20 °C and 70% relative humidity; light intensity was 420 μE for 16 h day$^{-1}$. The seedlings were labeled in four periods involving batches of ten seedlings each. Five beech and ash seedlings of each batch were sampled immediately after three days labeling with $^{13}$CO$_2$ and two days exposure to $^{12}$CO$_2$ and another 5 beech and ash seedlings of each batch were sampled 20 days after the start of the labeling. The reference seedlings were kept under similar conditions.

Samples of 5 beech and 5 ash seedlings and 5 reference seedlings of each species were harvested 5 and 20 days after the CO$_2$ pulse labeling. Soil was sampled next to the stem of the tree in the pot with a split tube. The intact core was sampled at depths of 0–10 cm and below 10 cm 5 and 20 days after labeling. Only the 0–10 cm depth sample was considered because the highest $^{13}$C incorporation into microbial biomass was recorded in the top 10 cm in a field experiment under beech and ash [9]. The soil was removed from the column, weighed and the water content was determined in a subsample. Each soil sample was sieved to 2 mm and stored at −20 °C until PLFA analysis.

2.3. PLFA analysis and calculation

2.3.1. Phospholipid extraction, purification, derivatization and measurement

An improved method of Frostegård et al. [32] was used to extract and purify phospholipids (for details see Ref. [33]). Six grams of soil were used for extraction and polar lipids were eluted four times with 5 ml of water-free methanol. Twenty-five milliliters of the internal standard 1 (IS 1) phosphadithylcholine-dinonadecanoic acid (1 mg ml$^{-1}$ in methanol) were added prior to extraction. Fatty acids
were saponified to free fatty acids and derivatized into fatty acid methyl esters (FAMES) [34] for measurement on a GC. Fifteen milliliters of an internal standard 2 (IS 2) tridecanoic acid methyl ester (1 mg ml\(^{-1}\) in toluene) were added before the samples were transferred to auto sampler vials. External standards consisting of the 27 fatty acids given in Supplementary Table 1 and internal standard 1 were prepared with total fatty acid contents of 1.0, 4.5, 9.0, 18.0, 24.0 and 30.0 mg, respectively, and derivatized and measured together with the samples.

### 2.3.2. Calculation of plant \(^{13}\)C uptake and \(^{13}\)C incorporation into plants and PLFA

Plant uptake from sources of different isotope composition results in changes in their isotopic signature and follows a two component mixing model [35],

\[
\frac{[^{13}\text{C}]_{\text{incTracer}}}{[^{13}\text{C}]_{\text{component}}} = \left(\frac{\%_{\text{applied Tracer}} - \%_{\text{ref}}}{\%_{\text{labelled}} - \%_{\text{ref}}}\right) \times \left(\frac{\text{C}_{\text{component}}}{\text{C}_{\text{tracer}}}\right)
\]

where \(\text{C}_{\text{component}}\) is the carbon content of the component (mmol \&kg\(^{-1}\)Component) and \(\text{C}_{\text{tracer}}\) is the total amount \(^{13}\)C incorporated into the respective components, i.e. soil, leaf, stem and root, in (mmol \&kg\(^{-1}\)Component). \%_{\text{labelled}} is the \(^{13}\)C of the labeled sample of the seedling (leaf, stem, root, soil), \%_{\text{ref}} the \(^{13}\)C of the unlabeled reference sample of the seedling (leaf, stem, root, soil), and at \%_{\text{applied Tracer}} the \(^{13}\)C enrichment of the added CO\(_2\). The incorporation is not expressed as absolute incorporation, but divided by the amount of added \(^{13}\)C to present incorporation as % of applied \(^{13}\)C.

The measurement of the FAMES, calculations and drift corrections are described in detail in Dippold and Kuzyakov [36]. Subsequently, the \(^{13}\)C incorporation into the PLFA \(\left(\frac{[^{13}\text{C}]_{\text{incTracer}}}{\text{PLFA}}\right)\) of the microbial community was determined according to equation (1), using the PLFA amount (\(\mu g\) g\(^{-1}\) dry soil) as pool size. Similar to the incorporation into tree biomass, incorporation into microbial PLFAs is presented as % of applied \(^{13}\)C.

### 2.3.3. Calculation of PLFA replacement

Besides \(^{13}\)C incorporation, which yields a quantitative value for the \(^{13}\)C flux into the membranes of a specific microbial group, the \(^{13}\)C replacement was calculated. In contrast to the incorporation, the \(^{13}\)C replacement is fully independent of pool size and represents the amount of a certain fatty acid that is replaced by newly incorporated \(^{13}\)C. Although this value does not provide the quantitative relevance of C flux into this microbial group, it does contain ecological information: it shows the relevance of the rhizodeposit-derived \(^{13}\)C as a C source for the respective microbial group. Replacement of membrane lipids by \(^{13}\)C is calculated by dividing the \(^{13}\)C incorporation \(\left(\frac{[^{13}\text{C}]_{\text{incTracer}}}{\text{PLFA}}\right)\) of a certain fatty acid by the amount of C in this fatty acid (equation (2)).

\[
^{13}\text{C}_{\text{repl}} \text{[\%]} = \left(\frac{[^{13}\text{C}]_{\text{incTracer}}}{\text{PLFA}} / \text{Total C_{PLFA}}\right) \times 100\%
\]

with \(\frac{[^{13}\text{C}]_{\text{incTracer}}}{\text{PLFA}}\) amount of \(^{13}\)C incorporated into PLFA (\(\mu mol\) \(^{13}\)C per g\(^{-1}\) dry soil)

Total C_{PLFA} amount of PLFA Carbon (\(\mu mol\) C per g\(^{-1}\) dry soil).

PLFAs were classified using the amounts of the individual fatty acids for corresponding microbial groups by factor analysis with a principal component extraction method. The classified data were compared with the literature for pure culture fatty acid fingerprints to determine functional microbial groups. Incorporation of \(^{13}\)C into individual fatty acids was summed to quantify the incorporation of individual microbial groups.

### 2.4. Statistical analysis

The labeling and subsequent \(^{13}\)C analyses were done with 10 independent replicates. Field replicates were tested for normal distribution using the Kolmogorov-Smirnoff test, for homogeneous variances using Levene’s test and corrected for outliers using the Nalimov outlier test with significance levels of 95% [37]. \(^{13}\)C enrichment and PLFAs as dependent variables were investigated for significant differences between tree species using Tukey’s Honestly Significant Difference (Tukey’s HSD) post hoc test (p < 0.05) following nested one-way analysis of variance (ANOVA) using Statistica (version 7, Statsoft GmbH, Hamburg, Germany) to inspect effects of tree species on plant compartments and soil microbial groups. The error bars in graphs show standard errors of the mean (SEM) of the ten replicates. PLFAs were assigned to corresponding microbial groups by a factor analysis of C contents of the entire dataset. Fatty acids were categorized according to previous studies and combining fatty acid biomarkers and their isotopic ratios allow insight into belowground trophic interactions [14,38]. Factor analysis was performed on the normalized PLFA contents (% of total PLFA) without considering ubiquitous and plant-derived fatty acids. Based on the factor loadings microbial groups of similar statistical behavior (>0.55 or < -0.55) were determined.

### 3. Results

#### 3.1. Grouping of the individual fatty acids

Table 1 lists the grouping of the individual fatty acids based on the results of factor analysis (Suppl. Table 2), whereby each of these groups represents a microbial group with distinct ecophysiological behavior in soil. The table includes only those fatty acids that are not ubiquitous but at least partially specific for a certain microbial group.

#### 3.2. \(^{13}\)C dynamics in trees and soil

Beech had approximately twice as much leaf and stem biomass but less root biomass than ash (Table 2). Leaves, stem and roots of both species were highly enriched in \(^{13}\)C. Five days after the labeling, up to 20% and 30% of the applied \(^{13}\)CO\(_2\) were recovered in all measured compartments of beech and ash, respectively (Fig. 1). Ash assimilated 30% \(^{13}\)C in all measured compartments and recovered with 34% twice as much in leaves as beech. In the stem, beech incorporated \(8\%\) \(^{13}\)C of the applied \(^{13}\)CO\(_2\), i.e. four times more than ash. Ash significantly increased the \(^{13}\)C in the stem by three times until day 20 after labeling, while \(^{13}\)C values in beech remained constant. Furthermore, the \(^{13}\)C incorporation was particularly pronounced in ash roots: approximately 48% of the \(^{13}\)C in all compartments was recovered at day 5 in the roots (versus only 33% in beech). Approximately 10% of the \(^{13}\)C incorporated in all compartments could be recovered after 5 days in the soil under both tree species and decreased to below 5% until day 20.

#### 3.3. Abundance of microbial groups in ash and beech soil

Many fatty acids within the Gram positives displayed a deviating pattern, resulting in a subdivision into four groups. Similarly, four groups of Gram negatives and two groups of actinomycetes were separated by PCA (Supplementary Table 1). The lowest amounts of microbially derived fatty acids under both tree species were detected for actinomycetes, arbuscular mycorrhizal fungi and fungi. In general, the PLFA amount in both tree species differed by only approximately 0.01 \(\mu g\) g\(^{-1}\) dry soil between species and between sampling dates. The fatty acids
derived from the Gram negative/fungi group were significantly higher under beech 5 days after labeling. In contrast, putative protozoa-derived fatty acids were higher under beech and displayed the highest amount of fatty acids (0.16 μg g⁻¹ dry soil). Similarly, the sum of fatty acids under beech and ash did not differ significantly, reflecting steady state of the microbial biomass.

3.4. ¹³C incorporation into PLFAs of individual groups

In general, the incorporation into Gram negatives, fungi and protozoa exceeded the incorporation into Gram positives and actinomycetes by a factor of 10. Fungi heavily incorporated ¹³C into their PLFA (18:2ω6,9) (0.010–0.004% ¹³C of applied ¹³CO₂) both under beech at day 5 and 20 after labeling (p < 0.05) and under ash (0.004–0.002% ¹³C of applied ¹³CO₂) (Fig. 2). Arbuscular mycorrhizal fungi and Gram negative 2 also heavily incorporated ¹³C under ash and incorporated more ¹³C (p < 0.05) than under beech 5 and 20 days after labeling. The ¹³C incorporation into the microbial groups under ash exceeded that into the respective groups under beech by factors of 2–12.

In the fungi and Gram negative/fungi group the incorporation of ¹³C decreased by half from day 5–20 after labeling. In contrast, in actinomycetes 1 the incorporation of ¹³C incorporation increased by a factor of two from day 5–20 after labeling in beech soil (in ash by a factor of four). The strongest drop in the ¹³C incorporation in ash occurred in arbuscular mycorrhizal fungi, where only one-third of the incorporation at day 5 remained at day 20.

However, the ¹³C labeling picked up in the arbuscular mycorrhizal fungi/Gram negative 2 group indicates the presence of arbuscular mycorrhizal fungi rather than confirming mycorrhization.

3.5. Replacement of ¹³C in PLFAs of individual groups

Fungi in beech soil replaced more of their PLFA-C by ¹³C than all other microbial groups (Table 1). Microbes in ash soil replaced less of their PLFA-C by ¹³C in fungi than in beech soil. Nonetheless, more ¹³C in PLFAs were replaced in fungi than in all other microbial groups in ash soil 20 days after labeling (Fig. 3). Three microbial groups in ash soil also revealed major differences in the replacement of their PLFA-C by ¹³C 5 days after labeling. Notably, Gram positives, actinomycetes and Gram negatives replaced less ¹³C in their PLFAs than fungi and arbuscular mycorrhizal fungi.

The 30% carbon replacement of the fungal PLFA 18:2ω6,9 within 5 days indicates fast PLFA turnover and suggests that tree-derived C

### Table 1

<table>
<thead>
<tr>
<th>Microbial groups</th>
<th>Abbreviation</th>
<th>Fatty acids</th>
<th>Under beech (μg kg⁻¹ dry soil)</th>
<th>Under ash (μg kg⁻¹ dry soil)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>day 5</td>
<td>day 20</td>
</tr>
<tr>
<td>Gram positive 1</td>
<td>G+1</td>
<td>i14:0, a16:0, i17:0</td>
<td>30 ± 2</td>
<td>40 ± 7</td>
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<tr>
<td></td>
<td></td>
<td>20:1ω9</td>
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<td></td>
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<tr>
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<td>G+2</td>
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<tr>
<td></td>
<td></td>
<td>20:1ω9</td>
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<tr>
<td>Gram positive 3</td>
<td>G+3</td>
<td>i16:0, a16:0, a17:0</td>
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<td></td>
<td></td>
<td>20:1ω9</td>
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<tr>
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<td>10Me16:0</td>
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<td></td>
<td></td>
<td>22 ± 1</td>
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<tr>
<td>Gram negative/Fungi</td>
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<td>18:1ω9</td>
<td>75 ± 4</td>
<td>69 ± 5</td>
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<td>Saprotrophic fungi</td>
<td>SF</td>
<td>18:2ω6,9</td>
<td>28 ± 1</td>
<td>31 ± 3</td>
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<td>Arbuscular mycorrhizal fungi/Bacteria</td>
<td>AMF</td>
<td>16:1ω5</td>
<td>26 ± 2</td>
<td>28 ± 3</td>
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<td>Protozoa</td>
<td>Pr</td>
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<td>102 ± 10</td>
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<td>Animals widespread</td>
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![Table 2](image)

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<tr>
<th>Microbial groups</th>
<th>Abbreviation</th>
<th>Fatty acids</th>
<th>Under beech (μg kg⁻¹ dry soil)</th>
<th>Under ash (μg kg⁻¹ dry soil)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>day 5</td>
<td>day 20</td>
</tr>
<tr>
<td>Beech</td>
<td></td>
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</tr>
<tr>
<td>Leaf biomass</td>
<td>8.7 ± 0.5</td>
<td>3.9 ± 0.3</td>
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<tr>
<td>Stem biomass</td>
<td>37.0 ± 1.7</td>
<td>21.1 ± 1.3</td>
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</tr>
<tr>
<td>Root biomass</td>
<td>15.4 ± 1.0</td>
<td>20.3 ± 1.1</td>
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<td></td>
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<tr>
<td>Leaf area</td>
<td>268 ± 22</td>
<td>568 ± 91</td>
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![Fig. 1](image)
is the major C source for ECM fungi in beech soil. Ash mycorrhizal fungi (or bacteria producing the same PLFA) replaced only 10% of their fatty acid (16:1ω5) C by tree-derived C during the same period. None of the other microbial groups reached a similarly high C replacement in such a short time as these fungal groups. This underlines that the direct C allocation via mycorrhizal symbioses dominates the nutrition of these fungal groups.

4. Discussion

4.1. C allocation within the plant-soil system as affected by tree species

Much attention has been given in the last decade to belowground C and the related soil processes under temperate tree species [9,21,39,40]. Here, we quantified the link between canopy C assimilation and belowground processes by 13C labeling and tracing of the assimilated 13C into the soil and into microbial groups. Beech assimilated 21% and ash 30% of the applied 13CO2 in all plant compartments 5 days after labeling. A field 13CO2 labeling experiment with 3–4-m-tall beech trees assimilated 20% but ash trees only 9% of the applied 13CO2 into plant compartments after a 5-h labeling period [9]. The difference in the initial 13CO2 uptake between those experiments could be the labeling duration, reflecting the more rapid photosynthate transport in beech as compared to ash.

Tree size and age may also affect CO2 uptake and distribution [9]. The 1-m-high ash trees have only half of the leaf biomass of beech but twice the leaf area. This might explain the 30% higher incorporation of the applied 13C into ash as compared to beech. The fact that beech initially allocated twice as much of the assimilated 13C into the stem than ash supports the results of Sommer et al. [9]. Beech has 25% less root biomass (<5 mm) in 0–20 cm depth than ash [23,41]. Ash also has more fine roots and a more vigorous root growth [23,39]. All these differences in root morphology and biomass help explain why beech roots incorporated only one fourth of the assimilated 13C as compared to ash roots. The relative 13C allocation into roots was significantly higher in ash than in beech 5 days after the labeling in a field experiment [9]. Evidently, ash has a higher root biomass than beech and preferentially invests the assimilated 13C into roots, at least partially explaining its higher rhizodeposition (Fig. 1).

4.2. Microbial community structure

The microbial fatty acids (Table 2) were generally similar in soil under beech and ash, as well as between 5 and 20 days after labeling. Beech, however, was associated with less Gram negative bacteria and fungi. Consequently, the ratio of fungal-to-bacterial biomass increased in the soil under beech [39] and our study confirms this: the fungal-to-bacterial ratio was higher under beech than under ash (Table 1). Table 1 illustrates that similar and rather low amounts of actinomycete fatty acids (Ac1, Ac2) were present in soil of both trees. This indicates that actinomycetes depend less on rhizodeposits than other microorganisms. Actinomycetes can degrade complex organic polymers and are positioned late in the microbial reaction chain [42]. Accordingly, they might not compete well for the large amount of easily degradable SOM initially released from decaying roots [43]. The significant increase in the 13C incorporation in the PLFA of actinomycetes over time might therefore also be linked to the decreased 13C enrichment in fungi and might even reflect a 13C flow from fungi to actinomycetes from day 5–20.

Notably, the levels of the putative arbuscular mycorrhizal fungal PLFA 16:1ω5 in beech and ash treatments were similar. This suggests that the putative arbuscular mycorrhizal fungal PLFA at least in part is derived from other microorganisms than arbuscular mycorrhizal fungi, presumably Gram negative bacteria [33,46] and thus 13C enrichment in arbuscular mycorrhizal fungi in fact is much lower.

4.3. Incorporation and replacement of root C into the microbial community

Fungi incorporated most 13C under beech, and only a minor fraction of the root-derived 13C was transferred into bacteria. Arbuscular mycorrhizal fungi had no enrichment under beech because its roots are associated with ECM, whereas ash roots are associated with arbuscular mycorrhiza [23]. The 13C signature of the fungal biomarker (18:2ω6,9) might be influenced by an additional 13C signal derived from linoleic acid from root hairs [44].

Fig. 2. 13C incorporation into PLFAs (% of 13C input) of applied CO2 in microbial groups in ash (grey triangle) and beech soil (black circle). Error bars show SEM (N = 10); * significant (p < 0.05) differences between beech and ash at day 5 and + at day 20 after labeling. Lower case letters indicate significant (p < 0.05) differences between day 5 and day 20 after labeling in beech (b) and ash (a).
Fig. 3. 13C replacement (% of PLFA-C) of microbial PLFAs in ash (grey triangle) and beech soil (black circle). Error bars show SEM (N = 10); symbols indicate significant differences to all other microbial groups in beech (+) and ash (×) 20 days after labeling and in beech (#) 5 days after 13C labeling (p < 0.05). Lower case letters (a, b, c) indicate significant differences between microbial groups in ash 5 days after 13C labeling (p < 0.05).

However, the pool size of fungal-derived linoleic acid is sufficiently high to use it as biomarker even if plant roots are much larger (but have a lower surface area-to-volume ratio) than fungal hyphae [45]. Therefore, the contribution of root tissue to the soil PLFA pool is relatively small and the 18:2ω6,9 13C enrichment presumably mainly reflects 13C incorporation into ectomycorrhizal fungi. This may explain why fungi, which include saprotrophic and ECM fungi in beech soil, had a much higher replacement of their PLFA-C by 13C than all other microbial groups.

The PLFAs of arbuscular mycorrhizal fungi (16:1ω5), Gram negative 2 (18:1ω7) and fungi (18:2ω6,9) were highly 13C enriched in ash and incorporated more 13C than in beech. However, as stated above, the PLFA 16:1ω5 is not specific for arbuscular mycorrhizal fungi but may also be derived from Gram negative bacteria [33,46]. However, the replacement of 13C differed between the specific Gram negative PLFA markers and 16:1ω5, which had very high enrichment 5 days after labeling and decreased significantly at day 20. This suggests that 16:1ω5 was derived from another microbial group than Gram negative bacteria presumably arbuscular mycorrhizal fungi. Arbuscular mycorrhiza fungi release plant-fixed 13C from their mycelium to bacterial and fungal populations in the (myco-)rhizosphere [47]. Therefore, the 13C enrichment in the Gram negative 2 and fungi under ash might be due to the uptake of C released from the roots by saprotrophic fungi and bacteria.

These results support the finding of Frostegard and Baath [48] and Zelles [14] that the 18:2ω6 9 biomarker is not specific for ectomycorrhiza and also includes saprotrophic fungi as indicated by 13C incorporation in ash soil.

The highly significant difference in 13C incorporation in beech and ash soil consequently is not dominated by plant origin but more likely is due to an ecophysiological difference between the tree species, such as mycorrhization. Furthermore, the 20:0 fatty acid, an unspecific eucaryotic fatty acid, not only has low amounts suggesting low contamination of soil samples by root cells, but also was less enriched in 13C by factor of 100 as compared to 18:2ω6,9 suggesting that even if eucaryotic cells were coextracted their contribution to the 13C enrichment was negligible. Generally, eucaryotic groups such as fungi cannot take up as much low molecular weight C from the soil solution as do prokaryotes because the turnover of the larger, more complex biomass of eukaryotes is slower than that of prokaryotes [49–51]. Under both tree species, the 13C incorporation into mycorrhizal fungal PLFA decreased from day 5 to day 20. This indicates that root-derived C is rapidly incorporated into mycorrhizal hyphae and that the incorporated C is turned over fast, at least in their membranes. Fungi and the Gram negative/fungi group decreased by more than half from day 5–20 after labeling. As this 13C decrease is similar to the 13C decrease in arbuscular mycorrhizal fungi, the 18:1ω9 fatty acid is probably at least partially also indicative for mycorrhizal fungi. Overall, the results suggest that PLFA analysis is a valuable tool to differentiate C fluxes from trees to different mycorrhiza types.

Assuming this differentiation of mycorrhizal partners is at least partially possible; the two-point measurement of 13C dilution following the pulse labeling allows estimation of hyphal turnover based on a linear relationship. Such calculations suggest that 46% of ectomycorrhizal membrane lipids (18:2ω6,9) were replaced within the 14 days between the two time points under beech and that 80% of the arbuscular mycorrhizal fungi membrane lipid 16:1ω5 were replaced within the 14 days by unlabeled C. We are aware that these values are speculative and the assumption of a linear kinetic as well as the unknown contribution of other organisms membranes to the fatty acid signature both might contribute to an overestimation of these values. However, such fast decrease in 13C enrichment confirms previous data on fast turnover of mycorrhizal hyphae stating approximately 6 days [52] for arbuscular mycorrhizal fungi and suggest that EMF might have a slightly slower turnover of their hyphal biomass than arbuscular mycorrhizal fungi.

Although Gram negative bacteria take up root exudates fast and show a rapid turnover [49], they do not exchange their biomass (and thus their PLFA-C) as rapidly as mycorrhiza. None of the other microbial groups reached a similarly high and fast C replacement as the fungal groups. Therefore, the direct C flux into mycorrhiza is highly efficient and dominates the C nutrition of these fungal groups. Gram negative bacteria are less important in 13C uptake than mycorrhizal fungi (Fig. 2), but more important than most Gram positive bacteria. The 13C incorporation into Gram negatives, fungi (18:2ω6,9) and protozoa was higher by as much as a factor of 10 than into Gram positive bacteria and actinomycetes. The 13C incorporation and enrichment did not differ for most Gram positive
bacteria (including actinomycetes) between ash and beech soil. Actinomycetes are a subgroup of Gram positive bacteria and some studies suggest that they incorporate less C from dissolved C sources than free-living prokaryotic Gram positive bacteria [53]. Our results support these findings and suggest that most of the C taken up by prokaryotes is provided as soluble root exudates by the trees. Gram positive bacteria have been suggested to mainly rely on old soil organic matter and complex compounds [8]. Accordingly, uptake of C from complex rhizodeposits may explain the significant increase from day 5–20 after labeling of the $^{13}$C incorporation into the PLFA of the actinomycetes 1 group in beech and ash soil.

Amino acid labeling in soil revealed Gram negative bacteria with the highest $^{13}$C incorporation indicating that Gram negative bacteria react fast to low molecular weight organic substances [49]. In beech soil Gram negative bacteria incorporated almost no $^{13}$C, but in ash soil incorporation of $^{13}$C into Gram negative 2 was the highest of all microbial groups 20 days after labeling. This suggests that ash released higher amounts of low molecular weight organic substances into the rhizosphere than beech.

5. Conclusions

Our results highlight the importance of microbial communities and especially mycorrhizal communities for belowground C fluxes in forest soil. The microbial community structure was similar but the C utilization differed in many respects between microbial groups in beech and ash soil. The higher belowground C allocation by ash affects the $^{13}$C incorporation and faster C replacement in various microbial groups. Fungi (18:2ω6.9) under beech incorporated the most $^{13}$C with the incorporation decreasing from day 5–20. This decrease in $^{13}$C incorporation also occurred in ash, but also ash incorporated much of its assimilated $^{13}$C in Gram negative 2 bacteria and partly in arbucular mycorrhizal fungi. The results suggest that combining tree species with different root systems, rhizodeposition and mycorrhiza types will increase ecological functions and improve the resilience of forest ecosystems which is of increasing importance in face of global change.

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Appendix A. Supplementary data

Supplementary data related to this chapter can be found at http://dx.doi.org/10.1016/j.ejsoi.2017.07.001.

References


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References


