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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* $^{13}CO_2$ pulse labeling we investigated C input and microbial utilization of rhizodeposits by analyzing ^{13}C 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 $^{13}CO_2$ (31%) than beech (21%), and ash allocated twice as much ^{13}C belowground until day 20. Approximately 0.01% of the applied ^{13}C 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 ^{13}C. PLFA allowed differentiation of C fluxes from tree roots into mycorrhiza: twice as much ^{13}C was incorporated into the fungal biomarker 18:2ω6,9 under beech than under ash. Within 5 days, 30% of the fungal PLFA-C was replaced by rhizodeposit-derived ^{13}C 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 $^{13}CO_2$ labeling and ^{13}C tracing in PLFA we conclude that ash allocated more C belowground and has faster microbial biomass turnover in the rhizosphere compared to beech.

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}) and lowest in stands with highest diversity and the lowest abundance of beech (0.27 kg m^{-2}) [17]. Beech accumulates more C_{org} in the forest floor but less C_{org} in the mineral soil as compared to ash, confirming that the species-specific litter entering the soil affects major characteristics of forest soils [18]. Beech litter has high C-to-N ratio (53) and high lignin content (85 mg g^{-1} dry matter), which retards decomposition processes. Ash litter, in contrast, is characterized by high quality, low C-to-N ratio (31) and low lignin content (25.3 mg g^{-1} dry matter) [17,19,20]. Therefore, ash litter decomposes and releases nutrients faster than beech litter [21,22].

We chose to study ash and beech not only because of their different litter quality and nutrient allocation patterns, but also because of differences in root morphology and mycorrhiza types [23]. Beech roots are associated with ectomycorrhizal (ECM) fungi such as *Byssocorticium atrovirens, Lactarius subdulcis* 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 (Teroson 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 ^{12}C sodium carbonate (KMF Laborchemie Handes, Lohmar, Germany). The $^{13}CO_2$ pulse was produced by injecting 5 M lactic acid into a 0.5 M ^{13}C sodium-carbonate (Na_2CO_3) solution (Sigma-Aldrich, Traufkirchen, 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 GMM220, 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) phosphatidylcholine-dinonadecanoic acid (1 mg ml^{-1} in methanol) were added prior to extraction. Fatty acids