European Journal of Soil Biology 81 (2017) 100–107

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

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

Janine Sommer [a, *], Michaela A. Dippold [c], Sarah L. Zieger [b], Anika Handke [a], Stefan Scheu [b], Yakov Kuzyakov [a, c, d, e]

[a] University of Goettingen, Soil Science of Temperate Ecosystems, Büsgenweg 2, 37077 Göttingen, Germany
[b] University of Goettingen, J.F. Blumenbach Institute of Zoology and Anthropology, Berliner Str. 28, 37073 Göttingen, Germany
[c] University of Goettingen, Agricultural Soil Science, Büsgenweg 2, 37077 Göttingen, Germany
[d] Agro-Technological Institute, Peoples' Friendship University of Russia, RUDN, Miklukho-Maklaya Str., 6, Moscow, Russia
[e] Institute of Environmental Sciences, Kazan Federal University, 420049 Kazan, Russia

ARTICLE INFO

Article history:
Received 5 February 2017
Received in revised form
26 June 2017
Accepted 4 July 2017

Handling editor: Professor. Christoph Tebbe.

Keywords:
$^{13}CO_2$ labeling
Carbon cycle
Tree rhizodeposition
Species effect
Deciduous forest
PLFA

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

* Corresponding author.
E-mail address: sommer.janine@yahoo.de (J. Sommer).

http://dx.doi.org/10.1016/j.ejsobi.2017.07.001
1164-5563/

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

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],

$$[^{13}C]_{incTracer} = [C]_{component} \cdot \frac{at\%_{labelled} - at\%_{ref}}{at\%_{appliedTracer} - at\%_{ref}} \quad (1)$$

where $[C]_{component}$ is the carbon content of the component (mmol $g^{-1}_{dryComponent}$) and $[C]_{incTracer}$ is the total amount ^{13}C incorporated into the respective components, i.e. soil, leaf, stem and root, in (mmol $g^{-1}_{dryComponent}$), $at\%_{labelled}$ is the ^{13}C of the labeled sample of the seedling (leaf, stem, root, soil), $at\%_{ref}$ the ^{13}C of the unlabeled reference sample of the seedling (leaf, stem, root, soil), and at $\%_{applied\ Tracer}$ 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 ($[^{13}C]_{incTracer\text{-}PLFA}$) of the microbial community was determined according to equation (1), using the PLFA amount (μg 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 $[^{13}C]_{incTracer}$ of a certain fatty acid by the amount of C in this fatty acid (equation (2)).

$$^{13}C/^{12}C_{repl} = ([^{13}C]_{incTracer\text{-}PLFA} / \text{Total } C_{PLFA}) \text{ x } 100\% \quad (2)$$

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

Total C_{PLFA} amount of PLFA Carbon (*μmol* 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 Significance 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 μg 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. ^{13}C 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 ^{13}C into their PLFA (18:2ω6,9) (0.010–0.004% ^{13}C of applied $^{13}CO_2$) both under beech at day 5 and 20 after labeling ($p < 0.05$) and under ash (0.004–0.002% ^{13}C of applied $^{13}CO_2$) (Fig. 2). Arbuscular mycorrhizal fungi and Gram negative 2 also heavily incorporated ^{13}C under ash and incorporated more ^{13}C ($p < 0.05$) than under beech 5 and 20 days after labeling. The ^{13}C 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 ^{13}C decreased by half from day 5–20 after labeling. In contrast, in actinomycetes 1 the incorporation of ^{13}C 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 ^{13}C incorporation in ash occurred in arbuscular mycorrhizal fungi, where only one-third of the incorporation at day 5 remained at day 20.

However, the ^{13}C 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 ^{13}C in PLFAs of individual groups

Fungi in beech soil replaced more of their PLFA-C by ^{13}C than all other microbial groups (Table 1). Microbes in ash soil replaced less of their PLFA-C by ^{13}C in fungi than in beech soil. Nonetheless, more ^{13}C 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 ^{13}C 5 days after labeling. Notably, Gram positives, actinomycetes and Gram negatives replaced less ^{13}C 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 2
Mean plant biomass (±SEM) ($N = 10$), soil mass and leaf area of beech and ash.

	Beech	Ash
Leaf biomass (g per tree) Mean ± SEM	8.7 ± 0.5	3.9 ± 0.3
Stem biomass (g per tree) Mean ± SEM	37.0 ± 1.7	21.1 ± 1.3
Root biomass (g per tree) Mean ± SEM	15.4 ± 1.0	20.3 ± 1.1
Leaf area (cm^2)	268 ± 22	566 ± 91

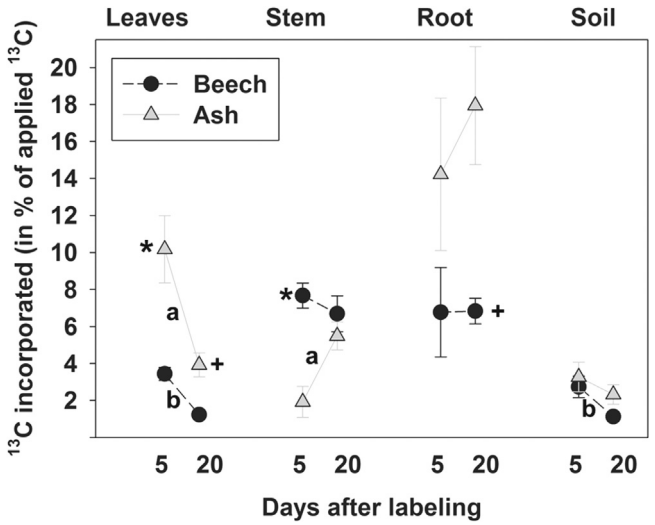

Fig. 1. Total ^{13}C incorporation (% ± SEM, $N = 10$) of the applied $^{13}CO_2$ in leaves, stem, roots and soil (0–10 cm depth) on day 5 and 20 after labeling of beech (black circles) and ash (grey triangles). * significant ($p < 0.05$) differences in ^{13}C incorporation in compartments between ash and beech on day 5,+significant differences on day 20 after labeling. Lower case letters show significant ($p < 0.05$) differences between day 5 and day 20 after labeling in beech (b) and ash (a).

Table 1
Identified microbial groups and their specific fatty acid amounts (mean ± SEM) in soil of beech and ash seedlings.

Microbial groups	Abbreviation	Fatty acids	Under beech (μg kg^{-1} dry soil)		Under ash (μg kg^{-1} dry soil)	
			day 5	day 20	day 5	day 20
Gram positive 1	G+1	i14:0 a16:0 i17:0 20:1ω9	30 ± 2	40 ± 7	32 ± 3	35 ± 2
Gram positive 2	G+2	i15:0	67 ± 1	74 ± 2	76 ± 3	74 ± 1
Gram positive 3	G+3	a15:0 a17:0	76 ± 4	69 ± 5	87 ± 7	79 ± 2
Gram positive 4	G+4	i16:0	30 ± 1	30 ± 2	28 ± 1	26 ± 1
Actinomycetes 1	Ac1	10Me16:0	22 ± 1	28 ± 3	28 ± 2	22 ± 1
Actinomycetes 2	Ac2	10Me18:0	30 ± 2	18 ± 2	28 ± 4	23 ± 1
Gram negative 1	G-1	16:1ω7 cy17:0	61 ± 1	69 ± 4	67 ± 3	40 ± 1
Gram negative 2/ Arbuscular mycorrhizal fungi	G-2	18:1ω7	114 ± 11	99 ± 10	108 ± 8	104 ± 3
Gram negative 3	G-3	cy19:0	160 ± 9	136 ± 17	137 ± 8	142 ± 5
Gram negative/ Fungi	G-/F	18:1ω9	75 ± 4	69 ± 5	58 ± 3	56 ± 2
Saprotrophic fungi/Ectomycorrhizal fungi/Animals	SF	18:2ω6,9	28 ± 1	31 ± 3	21 ± 4	24 ± 1
Arbuscular mycorrhizal fungi/Bacteria	AMF	16:1ω5	26 ± 2	28 ± 3	27 ± 2	26 ± 1
Protozoa/ Animals widespread	Pr	20:4ω6	102 ± 10	97 ± 13	128 ± 15	132 ± 4

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 (16:1 ω5) 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 ^{13}C labeling and tracing the flux of C into the soil and into microbial groups. Beech assimilated 21% and ash 30% of the applied $^{13}CO_2$ in all plant compartments 5 days after labeling. A field $^{13}CO_2$ labeling experiment with 3-4-m-tall beech trees assimilated 20% but ash trees only 9% of the applied $^{13}CO_2$ into plant compartments after a 5-h labeling period [9]. The difference in the initial $^{13}CO_2$ 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 CO_2 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 ^{13}C into ash as compared to beech. The fact that beech initially allocated twice as much of the assimilated ^{13}C 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 ^{13}C as compared to ash roots. The relative ^{13}C 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 ^{13}C 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 ^{13}C incorporation in the PLFA of actinomycetes over time might therefore also be linked to the decreased ^{13}C enrichment in fungi and might even reflect a ^{13}C 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 ^{13}C 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 ^{13}C under beech, and only a minor fraction of the root-derived ^{13}C 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 ^{13}C signature of the fungal biomarker (18:2ω6,9) might be influenced by an additional ^{13}C signal derived from linoleic acid from root hairs [44].

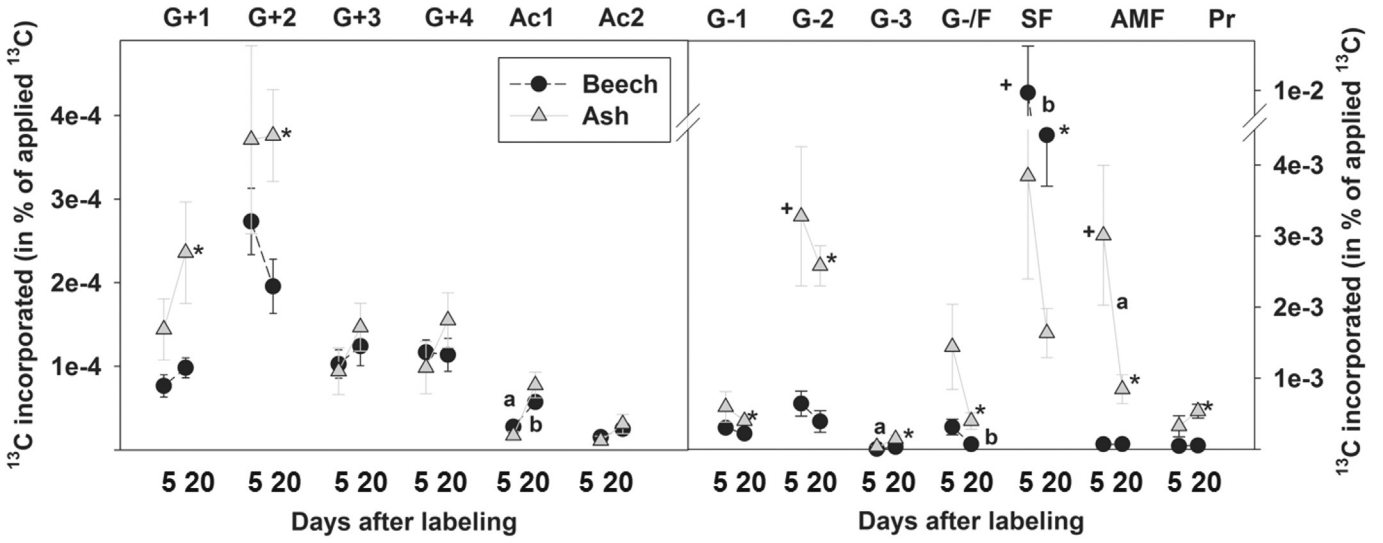

Fig. 2. ^{13}C incorporation into PLFAs (% of ^{13}C input) of applied CO_2 in microbial groups in ash (grey triangle) and beech soil (black circle). Error bars show SEM ($N = 10$);+significant ($p < 0.05$) differences between beech and ash at day 5 and * at day 20 after labeling. Lower case letters indicate significant ($p < 0.05$) differences between day 5 and day 20 after labeling in beech (b) and ash (a).

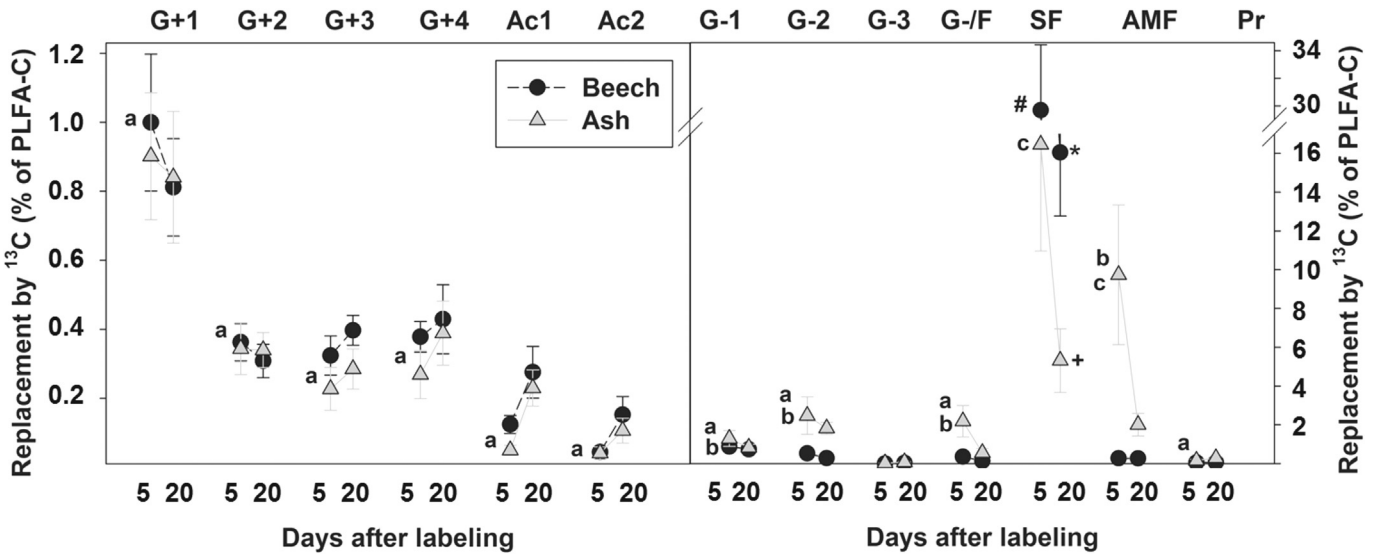

Fig. 3. ^{13}C replacement (% of PLFA-C) of microbial PLFAs in ash (grey triangle) and beech soil (black circle). Error bars show SEM ($N = 10$); symbols indicate significant differences to all other microbial groups in beech (*) and ash (+) 20 days after labeling and in beech (#) 5 days after ^{13}C labeling ($p < 0.05$). Lower case letters (a, b, c) indicate significant differences between microbial groups in ash 5 days after ^{13}C 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 ^{13}C enrichment presumably mainly reflects ^{13}C 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 ^{13}C 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 ^{13}C enriched in ash and incorporated more ^{13}C 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 ^{13}C 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 ^{13}C from their mycelium to bacterial and fungal populations in the (myco-)rhizosphere [47]. Therefore, the ^{13}C 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 ^{13}C incorporation in ash soil.

The highly significant difference in ^{13}C 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 ^{13}C by factor of 100 as compared to 18:2ω6,9 suggesting that even if eucayotic cells were coextracted their contribution to the ^{13}C enrichment was negligible. Generally, eukaryotic 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 ^{13}C 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 ^{13}C decrease is similar to the ^{13}C 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 ^{13}C 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 ^{13}C 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 ^{13}C uptake than mycorrhizal fungi (Fig. 2), but more important than most Gram positive bacteria. The ^{13}C 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 ^{13}C 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 ash also incorporated much of its assimilated ^{13}C in Gram negative 2 bacteria and partly in arbuscular 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.

Acknowledgements

We wish to thank A. Meyer, S. Enzmann and I. Ostermeyer as technical staff for their help and also R. Langel and L. Szwec for their support with the isotope analyses. The Labor für Radioisotope, especially B. Kopka, provided important technical support. This work was funded by the German Research Foundation (DFG KU-1086/13-2 and NST 186/1006-1/P).

Appendix A. Supplementary data

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

References

[1] FAO, FAO Forestry Paper, Global Forest Resources Assessment 2005. Progress towards Sustainable Forest Management, vol. 147, 2006, pp. 11–36.
[2] J. Esperschütz, A. Gattinger, F. Bügger, H. Lang, J.C. Munch, M. Schloter, J.B. Winkler, A continuous labelling approach to recover photosynthetically fixed carbon in plant tissue and rhizosphere organisms of young beech trees (*Fagus sylvatica L.*) using ^{13}C depleted CO_2, Plant Soil 323 (2009) 21–29.
[3] J.H.v. Thünen-Institut, Dritte Bundeswaldinventur, in. Braunschweig, 2012, pp.https://bwi.info/start.aspx.
[4] H. Rennenberg, M. Dannenmann, A. Gessler, J. Kreuzwieser, J. Simon, H. Papen, Nitrogen balance in forest soils: nutritional limitation of plants under climate change stresses, Plant Biol. 11 (2009) 4–23.
[5] M. Schön, Forstwirtschaft und Gefäßpflanzen der roten Liste: Arten - Standorte, Herbert Utz Verlag GmbH, München, 1998.
[6] T.R. Cavagnaro, L.E. Jackson, J. Six, H. Ferris, S. Goyal, D. Asami, K.M. Scow, Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production, Plant Soil 282 (2006) 209–225.
[7] P. Marschner, K. Baumann, Changes in bacterial community structure induced by mycorrhizal colonisation in split- root maize, Plant Soil 251 (2003) 279–289.
[8] M. Scherer-Lorenzen, C. Körner, E.D. Schulze, The functional significance of forest diversity: a synthesis, in: Forest Diversity and Function: Temperate and Boreal Systems, Springer, Berlin, Heidelberg, New York, 2005, pp. 377–389.
[9] J. Sommer, M.A. Dippold, H. Flessa, Y. Kuzyakov, Allocation and dynamics of C and N within plant-soil system of ash and beech, J. Plant Nutr. Soil Sci. 179 (2016) 376–387.
[10] J.-A. Subke, H.W. Vallack, T. Magnusson, S.G. Keel, D.B. Metcalfe, P. Högberg, P. Ineson, Short-term dynamics of abiotic and biotic soil $^{13}CO_2$ effluxes after in situ $^{13}CO_2$ pulse labelling of a boreal pine forest, New Phytol. 183 (2009) 349–357.
[11] Y. Kuzyakov, O. Gavrichkova, REVIEW: time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls, Glob. Change Biol. 16 (2010) 3386–3406.
[12] J. Cebrian, Patterns in the fate of production in plant communities, Am. Nat. 154 (1999) 449–468.
[13] B.W. Hütsch, J. Augustin, W. Merbach, Plant rhizodeposition-an important source for carbon turnover in soils, J. Plant Nutr. Soil Sci. 165 (2002) 397–407.
[14] L. Zelles, Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review, Biol. Fertil. Soils 29 (1999) 111–129.
[15] A. Guckland, H. Flessa, J. Prenzel, Controls of temporal and spatial variability of methane uptake in soils of a temperate deciduous forest with different abundance of European beech (*Fagus sylvatica* L.), Soil Biol. biochem. 41 (2009) 1659–1667.
[16] P.M. Schleuss, F. Heitkamp, C. Leuschner, A.C. Fender, H.F. Jungkunst, Higher subsoil carbon storage in species-rich than species-poor temperate forests, Environ. Res. Lett. 9 (2014).
[17] A. Guckland, M. Jacob, H. Flessa, F.M. Thomas, C. Leuschner, Acidity, nutrient stocks, and organic-matter content in soils of a temperate deciduous forest with different abundance of European beech (*Fagus sylvatica* L.), J. Plant Nutr. Soil Sci.-Z. Pflanzenernahr. Bodenkd. 172 (2009) 500–511.
[18] C. Langenbruch, M. Helfrich, H. Flessa, Effects of beech (*Fagus sylvatica*), ash (*Fraxinus excelsior*) and lime (*Tilia* spec.) on soil chemical properties in a mixed deciduous forest, Plant Soil 352 (2012) 389–403.
[19] M. Jacob, K. Viedenz, A. Polle, F.M. Thomas, Leaf litter decomposition in temperate deciduous forest stands with a decreasing fraction of beech (*Fagus sylvatica*), Oecologia 164 (2010) 1083–1094.
[20] A.M. Kooijman, E. Cammeraat, Biological control of beech and hornbeam affects species richness via changes in the organic layer, pH and soil moisture characteristics, Funct. Ecol. 24 (2010) 469–477.
[21] L. Vesterdal, I.K. Schmidt, I. Callesen, L.O. Nilsson, P. Gundersen, Carbon and nitrogen in forest floor and mineral soil under six common European tree species, For. Ecol. Manag. 255 (2008) 35–48.
[22] M. Jacob, N. Weland, C. Platner, M. Schaefer, C. Leuschner, F.M. Thomas, Nutrient release from decomposing leaf litter of temperate deciduous forest trees along a gradient of increasing tree species diversity, Soil Biol. biochem. 41 (2009) 2122–2130.
[23] C. Meinen, D. Hertel, C. Leuschner, Biomass and morphology of fine roots in temperate broad-leaved forests differing in tree species diversity: is there evidence of below-ground overyielding? Oecologia 161 (2009) 99–111.
[24] L.B. Shi, M. Guttenberger, I. Kottke, R. Hampp, The effect of drought on mycorrhizas of beech (*Fagus sylvatica* L.): changes in community structure, and the content of carbohydrates and nitrogen storage bodies of the fungi, Mycorrhiza 12 (2002) 303–311.
[25] P. Schütt, H. Weisberger, H.J. Schuck, U.M. Lang, B. Stimm, A. Roloff, Enzyklopädie der Laubbäume, Nikol Verlagsgesellschaft mbH & Co KG, Hamburg, 2006.
[26] S. Scheu, G. Poser, The soil macrofauna (Diplopoda, Isopoda, Lumbricidae and Chilopoda) near tree trunks in a beechwood on limestone: indications for stemflow induced changes in community structure, Appl. Soil Ecol. (1996) 115–125.
[27] M. Maraun, J. Alphei, P. Beste, M. Bonkowski, R. Buryn, S. Migge, M. Peter, M. Schaefer, S. Scheu, Indirect effects of carbon and nutrient amendments on the soil meso- and microfauna of a beechwood, Biol. Fertil. Soils 34 (2001) 222–229.
[28] S. Scheu, M. Falca, The soil food web of two beech forests (*Fagus sylvatica*) of contrasting humus type: stable isotope analysis of a macro- and a mesofauna-dominated community, Oecologia 123 (2000) 285–296.
[29] L. Ebrecht, Vegetation, Standortverhältnisse und Ausbreitungsbiologie von Pflanzen auf Rückegassen und Waldwegen im Göttinger Wald und im Solling, in: Fakultät für Forstwissenschaften u, Waldökologie, Cuvillier Verlag Göttingen, 2005.
[30] J. Pausch, J. Tian, M. Riederer, Y. Kuzyakov, Estimation of rhizodeposition at field scale: upscaling of a ^{14}C labeling study, Plant Soil 364 (2013) 273–285.
[31] M. Riederer, J. Pausch, Y. Kuzyakov, T. Foken, Partitioning NEE for absolute C input into various ecosystem pools by combining results from eddy-covariance, atmospheric flux partitioning and $^{13}CO_2$ pulse labeling, Plant Soil 390 (2015) 61–76.
[32] Å. Frostegård, A. Tunlid, E. Bååth, Microbial biomass measured as total lipid phosphate in soils of different organic content, J. Microbiol. Methods 14

(1991) 151–163.
[33] A. Gunina, M.A. Dippold, B. Glaser, Y. Kuzyakov, Fate of low molecular weight organic substances in an arable soil: from microbial uptake to utilisation and stabilisation, Soil Biol. biochem. 77 (2014) 304–313.
[34] D.R. Knapp, Handbook of Analytical Derivatization Reagents, wiley, 1979.
[35] P.J. Gearing, J.N. Gearing, J.T. Maughan, C.A. Oviatt, Isotopic distribution of carbon from sewage sludge and eutrophication in the sediments and food web of Estuarine ecosystems, Environ. Sci. Technol. 25 (1991) 295–301.
[36] M.A. Dippold, Y. Kuzyakov, Direct incorporation of fatty acids into microbial phospholipids in soils: position-specific labeling tells the story, Geochimica Cosmochimica Acta 174 (2016) 211–221.
[37] W. Gottwald, Statistik für Anwender, WILEY-VCH Verlag GmbH, 2000.
[38] L. Zelles, Q.Y. Bai, R. Rackwitz, D. Chadwick, F. Beese, Determination of phospholipid-derived and lipopolysaccharide-derived fatty acids as an estimate of microbial biomass and community structures in soils, Biol. Fertil. Soils 19 (1995) 115–123.
[39] S. Cesarz, A.C. Fender, F. Beyer, K. Valtanen, B. Pfeiffer, D. Gansert, D. Hertel, A. Polle, R. Daniel, C. Leuschner, S. Scheu, Roots from beech (*Fagus sylvatica* L.) and ash (*Fraxinus excelsior* L.) differentially affect soil microorganisms and carbon dynamics, Soil Biol. Biochem. 61 (2013) 23–32.
[40] I. Callesen, I. Stupak, P. Georgiadis, V.K. Johannsen, H.S. Østergaard, L. Vesterdal, Soil carbon stock change in the forests of Denmark between 1990 and 2008, Geoderma Reg. 5 (2015) 169–180.
[41] S. Oostra, H. Majdi, M. Olsson, Impact of tree species on soil carbon stocks and soil acidity in southern Sweden, Scand. J. For. Res. 21 (2006) 364–371.
[42] J. Lacey, Actinomycetes in Soils, Compost and Fodders, Academic Press, London, 1973.
[43] L. Thirup, K. Johnsen, V. Torsvik, N.H. Spliid, C.S. Jacobsen, Effects of fenpropimorph on bacteria and fungi during decomposition of barley roots, Soil Biol. Biochem. 33 (2001) 1517–1524.
[44] J. Pausch, S. Kramer, A. Scharroba, N. Scheunemann, O. Butenschoen, E. Kandeler, S. Marhan, M. Riederer, S. Scheu, Y. Kuzyakov, L. Ruess, Small but active – pool size does not matter for carbon incorporation in below-ground food webs, Funct. Ecol. (2016) 479–489.
[45] L. Ruess, P.M. Chamberlain, The fat that matters: soil food web analysis using fatty acids and their carbon stable isotope signature, Soil Biol. biochem. 42 (2010) 1898–1910.
[46] P.A. Olsson, E. Baath, I. Jakobsen, B. Soderstrom, The use of phospholipid and neutral lipid fatty-acids to estimate biomass of arbuscular mycorrhizal fungi in soil, Mycol. Res. 99 (1995) 623–629.
[47] B. Drigo, A.S. Pijl, H. Duyts, A. Kielak, H.A. Gamper, M.J. Houtekamer, H.T.S. Boschker, P.L.E. Bodelier, A.S. Whiteley, J.A. van Veen, G.A. Kowalchuk, Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO_2, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 10938–10942.
[48] A. Frostegard, E. Baath, The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil, Biol. Fertil. Soils 22 (1996) 59–65.
[49] C. Apostel, M. Dippold, B. Glaser, Y. Kuzyakov, Biochemical pathways of amino acids in soil: assessment by position-specific labeling and ^{13}C-PLFA analysis, Soil Biol. Biochem. 67 (2013) 31–40.
[50] E. Baath, Growth rates of bacterial communities in soils at varying pH: a comparison of the thymidine and leucine incorporation techniques, Microb. Ecol. 36 (1998) 316–327.
[51] J. Rousk, E. Bååth, Fungal biomass production and turnover in soil estimated using the acetate-in-ergosterol technique, Soil Biol. Biochem. 39 (2007) 2173–2177.
[52] P.L. Staddon, C.B. Ramsey, N. Ostle, P. Ineson, A.H. Fitter, Rapid turnover of hyphae of mycorrhizal fungi determined by AMS microanalysis of C-14, Science 300 (2003) 1138–1140.
[53] C. Apostel, M. Dippold, Y. Kuzyakov, Biochemistry of hexose and pentose transformations in soil analyzed by position-specific labeling and ^{13}C-PLFA, Soil Biol. Biochem. 80 (2015) 199–208.