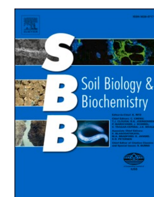
Soil Biology and Biochemistry 189 (2024) 109280

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio



Stability and carbon uptake of the soil microbial community is determined by differences between rhizosphere and bulk soil

Markus Lange [a,*,3], Mina Azizi-Rad [a,1,3], Georg Dittmann [a], Dan Frederik Lange [a,b], Alice May Orme [a,c], Simon Andreas Schroeter [a], Carsten Simon [a,d,2], Gerd Gleixner [a]

[a] Max Planck Institute for Biogeochemistry, Jena, Germany
[b] Institute for Geosciences, Friedrich Schiller University, Jena, Germany
[c] Bioorganic Analytics, Institute for Inorganic and Analytical Chemistry, Friedrich Schiller University, Jena, Germany
[d] Institute for Biogeochemistry and Pollutant Dynamics, ETH Zürich, Zürich, Switzerland

A R T I C L E I N F O

Keywords:
Microbial functioning
PLFA
NLFA
Root exudation
^{13}C
Vegetation change experiments
Soil carbon

A B S T R A C T

The interactions between plants and soil microorganisms are fundamental for ecosystem functioning. However, it remains unclear if seasonality of plant growth impacts plant-microbial interactions, such as by inducing shifts in the microbial community composition, their biomass, or changes in the microbial uptake of plant-derived carbon. Here, we investigated the stability of the microbial community and their net assimilation of plant-derived carbon over an entire growing season. Using a C3–C4 vegetation change experiment, and taking advantage of a natural ^{13}C label, we measured the plant-derived carbon in lipid biomarkers of soil microorganisms in rhizosphere and bulk soil in two soils with contrasting textures. We found that temporal stability was higher in bacterial than in fungal biomass, whereas the spatial stability of the fungal biomass was higher than that of bacterial biomass. Moreover, symbiotic AM fungi tended to be more stable in the uptake of plant-derived carbon than bacteria and saprophytic fungi. While soil texture did influence microbial community composition as expected, it had no effect on the microbial plant carbon assimilation and the differences between rhizosphere and bulk soil. In addition, the putative differences in carbon utilization between microbial groups, with the exception of AM fungi, were generally smaller than expected, reflecting opportunistic utilization of energy sources. Our results suggest that microbial uptake of plant carbon is primarily limited by plant carbon allocation rather than by environmental factors such as soil texture and seasonality. This indicates that the ongoing carbon assimilation during the growing season is supported by a functional redundancy within the microbial community, which, in turn, helps sustain ecosystem functioning.

1. Introduction

Terrestrial ecosystems greatly depend on the matter and energy supplied by plants and the respective provisioning of nutrients by microorganisms (Bardgett and van der Putten, 2014). This interaction between plants and microorganisms controls many ecosystem functions, such as the decomposition and accumulation of organic matter in the soil (Balser and Firestone, 2005; Miltner et al., 2012; Cotrufo et al., 2015; Lange et al., 2015). In the close proximity of the roots, the rhizosphere, this relationship is particularly strong, as this is the interface where plant-derived carbon such as exudates and other rhizodeposits enter the soil. The rhizosphere microbial community has direct access to plant-derived carbon as an easily available energy source, and therefore, it is the zone of the most active soil microbial community (Fierer et al., 2007; Kuzyakov and Blagodatskaya, 2015). The quantity of rhizodeposits and their accessibility for the soil microbial community (Mellado-Vázquez et al., 2016) impacts the microbial activity and thereby microbial carbon transformation and soil carbon turnover (Kuzyakov, 2002; Bird et al., 2011). However, as plants often undergo seasonal changes, such as growth after the cold season and

* Corresponding author.
E-mail address: mlange@bgc-jena.mpg.de (M. Lange).
[1] Present address: Gottfried Wilhelm Leibniz Universität Hannover, Institute of Soil Science, Germany.
[2] Present Address: Department of Analytical Chemistry, Helmholtz Centre for Environmental, Leipzig, Germany.
[3] These authors contributed equally to this work.

https://doi.org/10.1016/j.soilbio.2023.109280
Received 21 July 2022; Received in revised form 9 November 2023; Accepted 8 December 2023
Available online 12 December 2023
0038-0717/© 2023 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC license (http://creativecommons.org/licenses/by-nc/4.0/).

dieback at the end of the growing season, seasonal changes in the quantity and quality of rhizodeposition are likely. These phenological patterns of plant carbon allocation are assumed to induce a shift in the general plant-microbial interactions over the course of the growing season (Eisenhauer et al., 2018).

The proposed shift in the soil microbial community is largely attributed to phenological, i.e., temporal changes in the quantity and quality of plant carbon allocated to the soil (Habekost et al., 2008). While the first half of the growing season is presumably dominated by plant inputs of rhizodeposits, the second half is dominated by decomposition of dead plant residues, which are decomposed more slowly than rhizodeposits (Kuzyakov, 2002). However, while temporal shifts in the rhizosphere microbial community composition and their functions have gained increasing attention in recent years (e.g., Chaparro et al., 2014; Shi et al., 2015; Hannula et al., 2019; Nuccio et al., 2020), the contribution of soil- and plant-derived carbon to microbial biomass in relation to plant growth and plant dieback has rarely been considered. Therefore, our knowledge of the seasonal changes in microbial carbon sources, i.e., whether they are derived from soil or plants, is limited, hence soil carbon dynamics cannot be fully understood.

In addition to temporal shifts in the microbial community, it has also been shown that abiotic factors, such as soil texture, strongly impact the soil microbial community composition (Mellado-Vázquez et al., 2019; Johnson et al., 2003; Merckx et al., 1985). A shift in the microbial community composition could have subsequent effects on ecosystem functions, such as carbon cycling, (e.g., de Vries et al., 2006; Malik et al., 2016). The dual role of the microbial community on soil organic carbon cycling (Cotrufo et al., 2015; Lange et al., 2015; Liang et al., 2017), i.e., both the decomposition of existing soil organic matter reserves and the transfer of plant-derived carbon to a state that persists in soil is widely recognized. Shifts in the relation of the microbial community to soil organic matter cycling, namely from consumption to production of organic matter, have been reported (Lange et al., 2021). Yet, a mechanistic understanding of how the soil microbial community is shaped by phenological changes in the quantity and quality of plant-derived carbon allocated to the soil is only recently developing. For instance, Nuccio et al. (2020) reported taxonomical and functional succession of bacteria with substrate specialization depending on root growth, aging and decay. Hannula et al. (2019), nonetheless, assumed that seasonal shifts in bacterial communities do not alter overall soil functioning because the number of species that die out or migrate during the season is small. However, it is very likely that large seasonal variations in microbial biomass, microbial community composition and carbon resource availability (Zhalnina et al., 2018) will impact soil microbial processes, such as priming (Kuzyakov, 2010), re-cycling (Gleixner et al., 2002) or storage (Lange et al., 2015) of soil organic matter.

Here we take advantage of a C3–C4 vegetation change experiment to estimate the net assimilation of plant-derived carbon into different microbial groups over the course of a growing season. In addition, we examined differences between rhizosphere and bulk soils to compare the microbial community composition and their net carbon assimilation under high vs. low input of plant-derived carbon via roots and to assess the spatial stability of the entire soil community.

Microbial communities in soils and their plant-derived carbon uptake (net assimilation) can be analyzed using phospholipid fatty acids (PLFAs) and neutral lipid fatty acids (NLFAs) and their ^{13}C content (Kramer and Gleixner, 2006). The PLFA-NLFA method is of particular advantage when working at the natural isotopic abundance level, as it is highly sensitive to the shifts in the isotopic signatures of lipid biomarkers (Olsson and Johnson, 2005; Yao et al., 2015; Pett-Ridge and Firestone, 2017). The assignment of specific PLFA markers to distinguish microbial groups is comparable to the ecological concept of functional groups (Joergensen and Wichern, 2008). This is especially useful when investigating soil carbon dynamics, as the functional groups differ in their requirements for available carbon. The distinct microbial groups in soils are: Gram-positive (G+) bacteria, which are commonly considered as decomposers of soil organic matter (SOM) (Kramer and Gleixner, 2008; Bahn et al., 2013); Gram-negative (G-) bacteria, which are considered to have a high affinity for plant-derived carbon, such as root exudates and therefore preferably colonize the rhizosphere (Denef et al., 2007; Kramer and Gleixner, 2008); saprophytic fungi, which are able to decompose root exudates and plant litter as well as SOM (Treonis et al., 2004; Garcia-Pausas and Paterson, 2011; Mellado-Vázquez et al., 2019), and AM fungi, which are obligate symbionts that actively form associations with most known terrestrial plants (Smith, 2008) and actively trade carbon for nutrients with the host plant (Bonfante and Genre, 2008; Chowdhury et al., 2022).

Vegetation change experiments are a common method to study the carbon flow between plants, microorganisms and SOM (e.g. Balesdent and Balabane, 1996; Gleixner, 2013). Differences in the photosynthetic pathways of C3 and C4 plants result in naturally distinct δ^{13}C values of both plant types (C3: approx. −25 ‰; C4: approx. −12 ‰; Degens, 1969; O'Leary, 1981). In such experiments, the native plant community of either C3 or C4 plants is exchanged by the respective other plant type, while the soil remains unchanged. Thus, the soil receives the isotopic imprint of the newly established plant type over time. In combination with lipid biomarkers and stable isotope ratio analysis (Evershed et al., 2006; Kindler et al., 2009; Garcia-Pausas and Paterson, 2011), these experiments are valuable to explore interactions among plants, soil and microbes (Pett-Ridge and Firestone, 2017).

In this study, we asked i) if the microbial communities of the rhizosphere and bulk soil differ in their composition and their net carbon assimilation, ii) if the microbial composition and their net carbon assimilation is affected by soil texture and iii) if phenological changes impact microbial groups differently, depending on their life strategies. We hypothesized that the rhizosphere is dominated by microbial groups with a high affinity for plant-derived carbon and that the biomass of these groups contains higher proportions of plant-derived carbon than microorganisms dominating in bulk soil. Furthermore, we hypothesized that the proportion of plant-derived carbon in rhizosphere and non-rhizosphere microorganisms is relatively stable over the growing season, but that the biomass of groups with a high affinity for plant-derived carbon would be less stable and thus reflect phenological plant carbon allocation patterns. Finally, we hypothesize that soil texture will strongly influence the microbial community composition and their net carbon assimilation.

2. Material and methods

2.1. Site description and experimental design

The C3–C4 vegetation change experiment at the Max Planck Institute for Biogeochemistry in Jena, Germany was established in 2002. Eight plots of 24 m^2 were set up directly next to each other to avoid environmental biases, such as climate. Two different types of homogenized soil were filled into two plots each to a depth of 2 m (see Mellado-Vázquez et al., 2019 for details). In short, the first one was originally derived from a forest A-horizon and had a coarse texture (50% sand, 44% silt and 6% clay; pH 6.9) and relatively higher soil organic carbon concentration. The second soil was derived from the B-horizon of a calcareous soil and had finer texture (9% sand, 75% silt and 16% clay; pH 7.8) but lower soil organic carbon concentration. Both soils originally had C3 vegetation cover. Until 2006, the entire experiment was continuously cropped with C3 vegetation (Malik et al., 2012). The selection of plant species was based on comparable biomass production and phenology (Mellado-Vázquez et al., 2019). Scorpion weed *(Phacelia tanacetifolia* BENTH.), sunflower (*Helianthus annuus* L.) and wheat (*Triticum spec.* L.) were rotationally grown on all plots as two-species communities until the end of 2005. In the spring of 2006, the vegetation change to C4 plant species started on one of the coarse soil plots, and one of the fine soil plots, while the other coarse and fine soil plots were continuously cropped with C3 plants. The rotationally grown C4 plant

species were lovegrass (*Eragrostis curvula* WOLF), maize (*Zea mays* L.), amaranth (*Amaranthus spec.* L.) and millet (*Panicum spec.* L.). To increase the replicates of the experimental plots, the vegetation change was repeated in the same way on a similar set of soils in 2013 in direct proximity to the initial experiment. This resulted in four C3 plots and four C4 plots. Although the duration of vegetation change is an important factor determining the replacement of soil organic carbon derived from the new vegetation (Balesdent and Balabane, 1996), this factor is beyond the scope of our study. Therefore, the year of the vegetation change establishment was not included in the analysis models but potential differences are discussed.

All plots were equally managed. Each autumn, the total shoot biomass was harvested and re-distributed on each plot considering the original vegetation type, i.e., C3 plots received C3 plant material und C4 plots received C4 plant material. To overcome annual differences in plant performance, each plot received equal amounts of shoot biomass. From October to April, i.e., during the non-growing season, plots were covered with a water permeable sheet that allowed plant litter decomposition but prevented weed germination. In the following spring, i.e., mid of May, the plots were re-sown with their respective vegetation type. In 2016, *Cannabis sativa* L. was grown on the C3 plot, and sunflower and Mexican aster (*Cosmos bipinnatus* CAV.) germinated from the seed bank and were not weeded, and millet and amaranth were grown on the C4 plots.

2.2. Soil sampling

Soil samples were collected during the 2016 growing season, starting in June and continuing at monthly intervals until September, resulting in four sampling dates (see Table S1 for the exact sampling dates and the corresponding development stages of the plant communities). Three soil cores (approx. 20 cm by 20 cm) were taken to 10 cm soil depth using a spade from a randomly chosen location within a plot at each sampling time. Immediately after sampling, the soil was manually separated into soil material directly attached to plant roots ("rhizosphere soil"; see Fig. S1) and soil not attached to plant roots ("bulk soil") (e.g., Gobran and Clegg, 1996; Micallef et al., 2009; Acharya et al., 2023). To do this, plants and their roots were gently shaken to remove all loosely attached soil. The attached soil was removed from the roots and collected. The non-attached soil that held bulk soil and loosely attached rhizosphere soil was also collected. The subsamples were mixed to obtain one composite sample each for the rhizosphere and bulk soil per plot. Subsequently, samples were immediately sieved (2 mm mesh) and all visible plant debris was manually removed. Soil samples were then stored at −20 °C for less than a week before lipid extraction.

2.3. Lipid analysis

Phospholipids and neutral lipids were extracted from soil according to a modified protocol of Bligh and Dyer (1959) in a Büchi SpeedExtractor (BÜCHI Labortechnik GmbH, Essen, Germany) using a mixture (2:1:0.8; V:V) of chloroform, methanol and phosphate buffer (0.05 M, pH 7.4). Afterwards, the total lipid extract was separated from water-soluble compounds using phase separation after adding chloroform. The total lipid extract was split into neutral lipids, glycolipids and phospholipids by sequential elution with chloroform, acetone and methanol from a Chromabond® silica column (Macherey Nagel GmbH & Co. KG, Düren, Germany). Phospholipids and neutral lipids were hydrolyzed and methylated using a methanolic KOH solution (0,01 g ml^{-1}) followed by phase separation after adding chloroform and drying over Na_2SO_4. The resulting water-free fatty acid methyl esters (FAMEs) were purified by eluting with a 3:1 (V:V) mixture of n-hexane and dichloromethane from a Chromabond® aminopropyl column (Macherey Nagel GmbH & Co. KG, Düren, Germany).

FAMEs were quantified using a GC-FID system (GC: HP 6890 Series, AED: G 2350 A, Agilent Technologies Inc., Santa Clara, USA) relative to the internal phospholipid and neutral lipid standard (19:0). Calculation of fatty acid mass was done according to Equation (1):

$$m_{FS} = \frac{M_{FAME}}{M_{FS}} * m_{FAME} \quad (1)$$

where m_{FA} is the mass of fatty acids, m_{FAME} the mass of fatty acid methyl esters, M_{FA} the molar mass of the fatty acids and M_{FAME} the molar mass of the fatty acid methyl esters.

Referring the fatty acid mass to the soil dry weight gives the final PLFA and NLFA concentrations for further analysis.

PLFAs and NLFAs were assigned to different microbial groups according to our in-house library (e.g., Thoms and Gleixner, 2013; Mellado-Vázquez et al., 2016; Karlowsky et al., 2018; Mellado-Vázquez et al., 2019). All monounsaturated PLFAs (16:1ω5, 16:1ω7, 17:1, 18:1ω7, 18:1ω9) were assigned to G- bacteria, while all the branched fatty acids (15:0i, 15:0a, 16:0i, 17:0i, 17:0a) were considered as G+ bacterial markers (Zelles et al., 1997; Zelles, 1999). Cyclopropyl fatty acids cy17:0 and cy19:0 represent a subgroup of G- markers. They are synthesized under environmental stress (Guckert et al., 1986; Kaur et al., 2005) and are often observed in environments richer in G+ bacteria than G- bacteria (Treonis et al., 2004; Mellado-Vázquez et al., 2016). Consequently, they were analyzed separately as "cy G- markers". In addition, 10Me17:0 and 10Me18:0 were analyzed separately as markers for G+ *Actinobacteria* (Zelles, 1999). Therefore, G+ bacteria have been analyzed and reported without the markers for *Actinobacteria*. The PLFA 18:2ω6,9 was assigned to saprophytic fungi (Zelles, 1999; Frostegård et al., 2011) and the NLFA 16:1ω5 was used as marker for AM fungi (Olsson, 1999). In addition, the PLFA marker 20:4ω6 was used as general marker for soil fauna (Ruess and Chamberlain, 2010).

Incorporation of ^{13}C from plant-derived material into the NLFA and PLFA markers for microbial biomass was determined using compound-specific ^{13}C analysis with a GC-IRMS system (HP5890 GC, Agilent Technologies, Palo Alto USA; GC combustion III and IRMS: Delta Plus XL, Finnigan MAT, Bremen, Germany), using an HP Ultra column (60 m, 0.25 mm internal diameter × 0.25 μm film thickness, Agilent Technologies, Palo Alto, USA) and helium as a carrier gas. A SATFA-mix (saturated fatty acid mix, methyl esters) was injected as an external standard before each triplicate sample measurement and fatty acid 19:0 was used for drift correction (Werner and Brand, 2001). The $\delta^{13}C$ values of SATFA were analyzed with split mode (1:10); whilst $\delta^{13}C$ values of monounsaturated and polyunsaturated fatty acids were measured with splitless mode. The initial oven temperature of 140 °C was held for 1 min, followed by an increase in temperature at a rate of 2 °C min^{-1} until reaching 260 °C. Followed by a heating rate of 30 °C min^{-1} until a final temperature of 340 °C that was held for 3 min. The software ISODAT NT 2.0 (SP 2.67, Thermo Fisher, USA) was used for data evaluation. Isotope ratios are expressed as $\delta^{13}C$ value in per mil [‰] relative to the international reference standard Vienna-PeeDee Belemnite (V-PDB) (Eq. (1)) using NBS 19 (Werner and Brand, 2001):

$$\delta^{13}C\ value[‰]_{V-PDB} = \frac{\left[\left({}^{13}C/{}^{12}C\right)_{sa} - \left({}^{13}C/{}^{12}C\right)_{std}\right]}{\left({}^{13}C/{}^{12}C\right)_{std}} \times 1000 \quad \text{(Equation 1)}$$

where $(^{13}C/^{12}C)_{sa}$ is the $^{13}C/^{12}C$ ratio of the sample and $(^{13}C/^{12}C)_{std}$ the $^{13}C/^{12}C$ ratio of the reference standard V-PDB. $\delta^{13}C$ values were also corrected for the methyl carbon added during methylation (Eq. (2); Kramer and Gleixner, 2006):

$$\delta^{13}C_{FA} = \frac{\left[(N_{FA}+1)\ \delta^{13}C_{FAME} - \delta^{13}C_{MeOH}\ \right]}{N_{FA}} \quad \text{(Equation 2)}$$

where $\delta^{13}C_{FA}$ is the isotope ratio of the lipid fatty acid (PLFAs and NLFAs), $\delta^{13}C_{FAME}$ the isotope ratio of the lipid fatty acid methyl ester, $\delta^{13}C_{MeOH}$ that of methanol used for derivatization and N_{PLFA} is the number of carbon atoms of the lipid fatty acid.

2.4. Assessing the carbon origin in PLFA

The net proportion (%) of assimilated carbon derived from plants to individual lipid fatty acids (PLFAs and NLFAs) compared to soil-derived carbon (Fp_{FA}; Eq. (3)) was calculated as adapted from Kramer and Gleixner (2006):

$$Fp_{FA} = \frac{[\delta^{13}C_{FA-C4} - \delta^{13}C_{FA-C3}]}{[\delta^{13}C_{Plant-C4} - \delta^{13}C_{Plant-C3}]} \times 100 \qquad \text{(Equation 3)}$$

where $\delta^{13}C_{FA\text{-}C4}$ and $\delta^{13}C_{FA\text{-}C3}$ represent the isotopic values of individual lipid fatty acid markers and the $\delta^{13}C_{\text{-Plant-C4}}$ and $\delta^{13}C_{\text{-Plant-C3}}$ are the isotopic values of plant material from the experiment plot with C4 vegetation change and control C3 plot, respectively.

The $\delta^{13}C$ values of root material ($\delta^{13}C_{\text{-Plant}}$) was determined on 3 mg of air-dried (40 °C) and ball-milled root material using EA-IRMS. The system was calibrated versus V-PDB using CO_2 as the reference gas (Werner and Brand, 2001). In addition, soil water content [%] was measured gravimetrically according to the method introduced by Black (1965) from 5 g of soil (wet weight) that were collected as subsamples from the soil samples taken for lipid analysis.

2.5. Statistical analysis

To analyze how the concentrations and the net assimilation of plant-derived carbon into lipid markers was impacted by roots and how both metrics would vary over the vegetation period, we employed Linear Mixed-Effects Models (LMM) using the “lmer”-function in R (R Development Core Team, 2021) library “lme4” (Bates et al., 2015). Starting from a constant null model with “plot identity” as random intercept, we extended the null model stepwise. Since more than one lipid marker is indicative for each of the bacterial subgroups, the random intercept for bacterial models was “lipid marker” fitted within “plot identity”. The fitting sequence of the fixed effects started with “rhizosphere” (rhizosphere, bulk soil) followed by “season” (time point of sampling; June, July, August, September) and “soil texture” (coarse, fine). Next, “plant type” (C3, C4) was included in the models to test the effects on lipid marker concentration. To test whether the effects of season were different in rhizosphere vs. bulk soil, the interaction term "rhizosphere × season" was included in the models additionally. The maximum likelihood method was applied and likelihood ratio tests (χ^2 ratio) were used to compare succeeding models and test for a significant model improvement by the added fixed effects (Zuur et al., 2009). To test if the composition of the lipid markers, based on their relative abundances, showed a shift over the season or was impacted by the environmental factors, a Permutational Multivariate Analysis of Variance (PERMANOVA) was performed applying the “adonis2”-function (permutations = 499) in the “vegan” package (Oksanen et al., 2020). The inverse coefficient of variation (CV^{-1} = mean/standard deviation (S.D.)) of the microbial biomass and of the microbial net assimilation of plant-derived carbon over the season was calculated to assess the temporal stability (Haddad et al., 2011; Strecker et al., 2016). Differences of the temporal stability among microbial groups and differences between rhizosphere and bulk soil were analyzed by applying LMM, similar as described above, i.e., starting from a constant null model, with lipid marker identity and with plot identity as random intercept, and stepwise extension of the null model afterwards. The fitting sequence of the fixed effects started with “microbial group” followed by “rhizosphere” and the interaction term “groups × rhizosphere”. However, in the full model, the dispersion was very high for some groups (see Fig. 3) so the interaction term "groups × rhizosphere" was not significant. Therefore, the rhizosphere effect was tested separately for all groups. These results were finally reported in Fig. 3, as they more appropriately represent the differences shown.

3. Results

3.1. Microbial biomass and community composition over the growing season

The concentrations of lipid biomarkers differed strongly among microbial groups. Across all samples, the average concentrations of the AM fungi biomarker (NLFA 16:1ω5) was highest with 3.7 μgC g^{-1} (±3.5 S.D.) followed by the G- bacteria (2.5 ± 1.7 μgC g^{-1}) and saprophytic fungi (1.5 ± 1.0 μgC g^{-1}). Markers of G+ bacteria had an average concentration of 1.3 ± 0.7 μgC g^{-1}, for *Actinobacteria* it was 1.4 ± 0.6 μgC g^{-1} and for cy G- markers it was 1.5 ± 0.8 μgC g^{-1}. Besides the average concentration of the lipid markers, their temporal patterns differed as well. While the concentration for AM fungi was lowest in July and increased sharply towards the end of the growing season, the marker concentrations of saprophytic fungi and most bacterial markers were highest in early summer (July) and decreased towards the end of the growing season. This pattern was particularly pronounced in the rhizosphere (Fig. 1). These seasonal effects were not clearly related to soil moisture, which slightly increased from June (12% soil water content) to July (15%) and decreased again towards August (9%) and September (11%). Including soil moisture into the statistical models did not, or at most slightly reduced, the explained variation by season (Table S2), most likely because the concentration of individual lipid markers was inconsistently correlated to changes in soil moisture (Table S3).

The average concentration of all markers was higher in rhizosphere soil than in bulk soil (Table 1, S2). However, this difference was only significant for the markers assigned to saprophytic fungi (concentration increased by 48% from bulk to rhizosphere soil), G- bacteria (27% higher concentration in rhizosphere soil than in bulk soil), and G+ bacteria (16% higher, Table 1). Although overall significantly different, the marker concentrations of G- bacteria and G+ bacteria did not differ in August (Fig. 1) as indicated by the significant interaction term “rhizosphere × season” (Table 1). Soil texture had an impact on most of the microbial groups except cy G- markers. In contrast, plant type (photosynthetic pathways C3 vs. C4) had no impact on most of the microbial groups, except on cy G- markers. The lipid marker assigned to soil fauna was strongly affected by the season: It constantly decreased from spring towards the end of the growing season; and its concentration was higher in rhizosphere than in bulk soil. Furthermore, in groups to which multiple lipid markers were assigned, the largest effect was the factor “marker” itself, showing that the concentrations of the individual markers greatly differed from one another, even if they were assigned to the same group (Table S3). The composition of the lipid markers was mostly explained by differences in soil, followed by season and the distance to the roots (Table 1). This indicates that effects of soil texture, season and distance to the roots differently impacted the individual organism groups.

3.2. Microbial carbon assimilation over the growing season

Similar to the concentrations of the lipid biomarkers, the proportion of plant-derived carbon of the lipid biomarkers varied among microbial groups. It was on average highest in the NLFA marker assigned to AM fungi (80.5 ± 9.3 %), followed by saprophytic fungi (59.4 ± 25.6 %). The proportion of plant-derived carbon in markers of bacterial groups was lower but in a similar range to that of saprophytic fungi, being highest in G- bacteria (46.6 ± 20.6 %) and lowest in *Actinobacteria* (37.2 ± 20.4 %). The plant-derived carbon in the marker for soil fauna was on average 43.1 ± 34.1 %. The net assimilation of plant-derived carbon depended strongly on whether it was measured in rhizosphere or bulk soil and was expectedly greater in the rhizosphere (Fig. 2). Except for the AM fungi marker, which did not differ between rhizosphere and bulk soil (Table 2). The seasonal patterns among all groups were slightly different, however, they all had the greatest contents of plant-derived

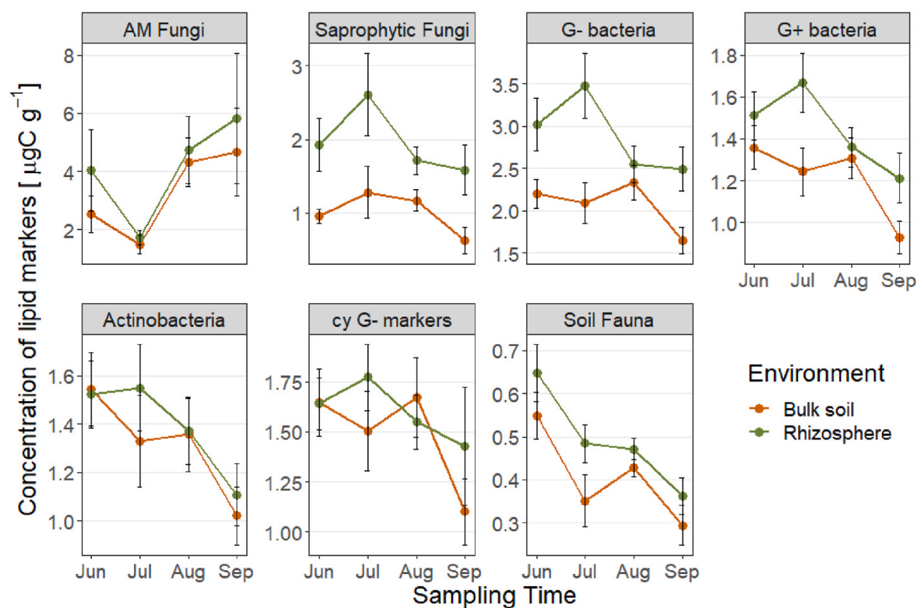


Fig. 1. Average concentrations of lipid markers (μg carbon per g soil) assigned to AM fungi and saprophytic fungi, bacteria (G- bacteria, G+ bacteria, *Actinobacteria*, cyclopropyl markers of G- bacteria) and soil fauna over the growing season in rhizosphere and bulk soil. Error bars represent the standard error (S.E.). Note the different scaling among sub-plots.

Table 1
Results of linear effects models testing differences between rhizosphere and bulk soil ("Rhizosphere") the growing season ("Season"), differences in C3 and C4 vegetation ("Plant type"), soil texture ("Soil texture") and the interaction between rhizosphere and the growing season ("Rhizo × Season") on the concentrations of lipid markers assigned to different microbial groups. Asterisks indicate the level of significance ("***" < 0.001, "**" < 0.01, "*" < 0.05, "." < 0.1) of Chi-squared tests.

	Rhizosphere		Season		Plant type		Soil texture		Rhizo × Season	
	Chi^2	R^2	Chi^2	R^2	Chi^2	R^2	Chi^2	R^2	Chi^2	R^2
Actinobacteria	1.5	0.00	**39.8 *****	0.08	0.3	0.01	1.6	0.07	3.3	0.01
G+ bacteria	**33.3 *****	0.03	**72.0 *****	0.05	1.0	0.02	0.8	0.01	**17.8 *****	0.01
G- bacteria	**51.2 *****	0.06	**24.8 *****	0.03	0.6	0.01	**8.1 ****	0.12	**16.0 ****	0.02
cy G- markers	1.6	0.01	**12.9 ****	0.04	1.0	0.04	0.3	0.01	4.9	0.02
AM Fungi	0.9	0.01	**11.5 ****	0.16	0.4	0.01	**5.2 ***	0.07	0.5	0.01
Saproph. Fungi	**18.2 *****	0.22	**9.3 ***	0.09	0.8	0.02	**7.0 ****	0.11	2.2	0.02
Soil Fauna	**4.5 ***	0.07	**30.1 *****	0.35	4.6 *	0.04	2.1	0.02	1.3	0.01
Community Composition	**8.7 *****	**0.09**	**4.1 ****	**0.13**	*2.4.*	*0.02*	**14.6 *****	0.15	0.7	0.02

carbon in early summer (July; Fig. 2).

The net assimilation based on the soil faunal marker showed a similar pattern to the microbial markers described above. However, this marker showed the greatest variation in net assimilation of plant-derived carbon during the season (e.g., in the rhizosphere 90% in July and 30% in September). Furthermore, and contrary to the concentrations of the lipid markers, soil texture had no effect on the assimilation of plant-derived carbon by any of the microbial groups (Table 2). Moreover, the differences in the plant carbon assimilation between rhizosphere and bulk soil were not affected by soil texture (Fig. S3).

3.3. Stability of microbial biomass and assimilation of plant-derived carbon over the growing season

The temporal stability of the biomass, calculated as the inverse coefficient of variation (see Methods), differed among microbial groups. It was significantly lower in AM fungi and saprophytic fungi than in all bacterial groups and the soil fauna (Fig. 3a). Furthermore, the concentration of markers of G- bacteria, G+ bacteria and *Actinobacteria* showed a higher temporal stability in the rhizosphere than in bulk soil. This pattern was similar in saprophytic fungi by trend ($p < 0.01$), while the temporal stability of the AM fungi marker, the cy G- markers and the markers assigned to soil fauna was statistically not different between rhizosphere and bulk soil (Fig. 3a).

In contrast to the stability of biomass over the season, the proportion of plant-derived carbon was most stable in AM fungi markers and least stable in cy G- markers (Fig. 3b). The stability of assimilation of plant-derived carbon in other groups was lower but not significantly different from AM fungi markers. The temporal stability of plant-derived carbon assimilation did not differ between rhizosphere and bulk soil for the faunal and all bacterial markers. In contrast, while the stability plant-derived carbon in the saprophytic fungi marker was on a very low level, the stability in the bulk soil was even lower (Fig. 3b). In the AM fungi, there was a trend ($p < 0.1$) that the temporal stability of the plant-derived carbon was higher in the bulk than in the rhizosphere soil.

4. Discussion

In this study, we used a C3–C4 vegetation change experiment and induced $\delta^{13}C$ changes to investigate how the biomass of different microbial groups and their net assimilation of plant-derived carbon change over the season, as affected by rhizosphere and bulk soil, as well as by the soil texture.

4.1. Rhizosphere effects

In line with our first hypothesis, we found that functional microbial groups with a high affinity for plant-derived carbon, i.e., G- bacteria and

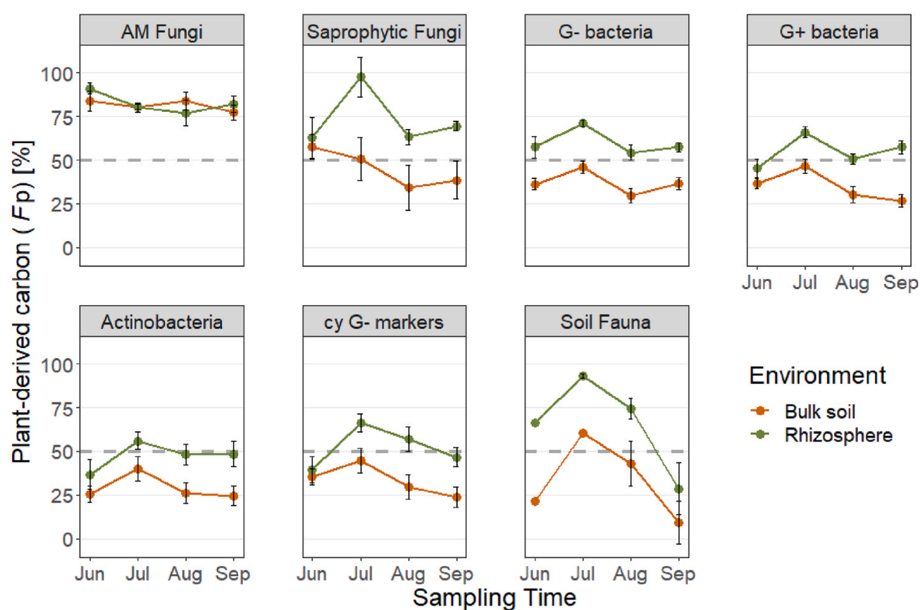


Fig. 2. Weighted average of *F*p value (plant-derived carbon weighted on concentration of the lipid marker) of AM fungi and saprophytic fungi, bacteria (G- bacteria, G+ bacteria, *Actinobacteria*, cyclopropyl markers of G- bacteria) and soil fauna over the growing season in rhizosphere and bulk soil. Error bars represent the S.E. The gray dashed line provides a guide at 50%. See Supplementary Fig. S2 for the net assimilation of plant-derived carbon in the microbial biomass in µgC g^{-1}.

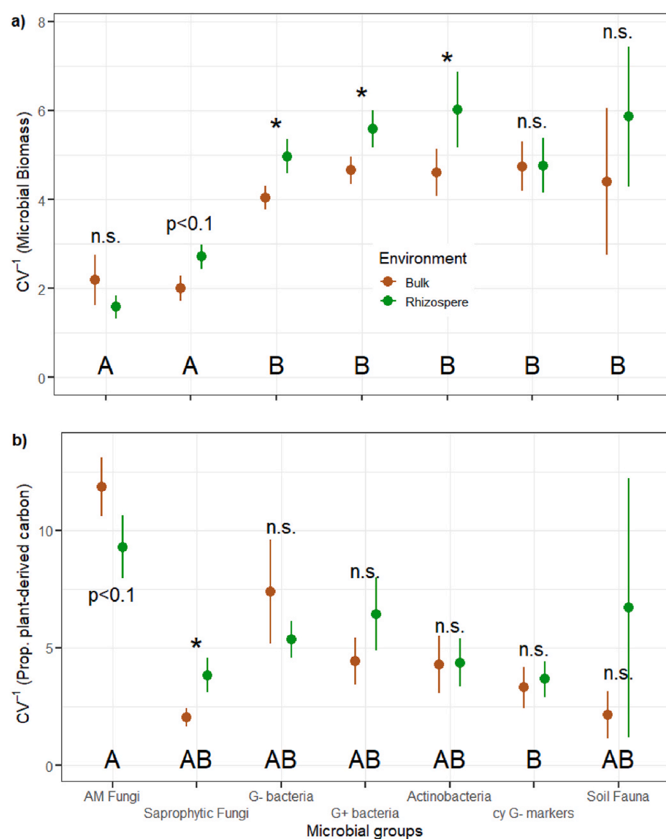


Fig. 3. Temporal stability (inverse coefficient of variation, CV^{-1}) of a) the microbial biomass and b) the proportion of plant-derived carbon in lipid markers over the growing season in rhizosphere and bulk soil. Given are the average values of stability and the error bars represent S.E. See Supplementary Table S4 for the exact values on which this graph is based.

saprophytic fungi, were most abundant in the rhizosphere. The largest biomass difference between rhizosphere and bulk soil was observed in the fungal marker, while the differences between G- and G+ bacteria were not as strong as might have been expected based on their supposed preference for plant or soil-derived carbon (Denef et al., 2007; Kramer and Gleixner, 2008; Bahn et al., 2013). In line with our results, saprophytic activity of G- bacteria was reported in recent years (Bai et al., 2016; Mellado-Vázquez et al., 2019), while the G+ bacteria assimilate large portions of new photosynthates, if available (Mellado-Vázquez et al., 2016; Karlowsky et al., 2018; Mellado-Vázquez et al., 2019; Mielke et al., 2022).

The higher uptake of plant-derived carbon in the rhizosphere by most of the microbial groups was expected because of the higher availability of plant-derived carbon (Bais et al., 2006; Philippot et al., 2013; Pausch and Kuzyakov, 2018). Greater quantities of easily decomposable energy sources (rhizodeposits) lead to higher microbial activity and growth in the rhizosphere compared to bulk soil (Kuzyakov and Blagodatskaya, 2015). This was clearly confirmed by the general differences in microbial biomass. However, some groups showed no clear response in biomass between rhizosphere and bulk soil. For example, although very different in their life strategies, the biomass of AM fungi and *Actinobacteria* were not different between rhizosphere and bulk soil. AM fungi demonstrated their obligatory dependence on plant-derived carbon (Bago et al., 2000; Chowdhury et al., 2022) since the proportion of plant-derived carbon in the specific lipid marker was not different between rhizosphere and bulk soil. In contrast, non-impacted biomass of *Actinobacteria* likely indicates a slow growth, which is independent of the availability of easily decomposable plant-derived carbon sources and confirms a general, oligotrophic life-style of these organisms (Liu et al., 2018).

Our data show that most microbial groups utilize both plant- as well as soil-derived carbon, and are flexible in choosing these sources although they have different preferences for carbon sources. All groups considered here exhibited an opportunistic carbon use as shown by the significantly higher plant-derived carbon proportion in the rhizosphere, except for AM fungi which also incorporated high quantities of plant-derived carbon in bulk soil. In our study, this opportunistic carbon use is possible because no substrate limitation appears to occur in either rhizosphere or bulk soil as indicated by equally low concentrations of

Table 2
Results of linear effects models testing differences between rhizosphere and bulk soil ("Rhizosphere"), the growing season ("Season"), soil texture ("Soil texture") and the interaction between rhizosphere and the growing season ("Rhizo × Season") on the proportion of plant-derived carbon (*F*p) in lipid markers assigned to different microbial groups. Asterisks indicate the level of significance ("***" < 0.001, "**" < 0.01, "*" < 0.05, "." < 0.1) of Chi-squared tests.

Fp value	Rhizosphere		Season		Soil texture		Rhizo × Season	
	Chi^2	R^2	Chi^2	R^2	Chi^2	R^2	Chi^2	R^2
Actinobacteria	**24.4** ***	0.20	**21.2** ***	0.11	0.0	0.00	5.7	0.02
G+ bacteria	**62.5** ***	0.22	**57.2** ***	0.13	0.0	0.00	**18.7** ***	0.04
G- bacteria	**53.9** ***	0.25	**57.2** ***	0.13	0.0	0.00	**18.7** ***	0.04
cy G- markers	**19.9** ***	0.21	**21.0** ***	0.15	0.0	0.00	**10.7** *	0.06
AM Fungi	0.1	0.00	**9.0** *	0.19	0.1	0.00	2.7	0.05
Saproph. Fungi	**9.7** **	0.27	**11.0** *	0.22	0.0	0.00	4.5	0.07
Soil Fauna	**4.1** *	0.19	**18.6** ***	0.49	1.0	0.02	2.7	0.03

the cy G- markers, indicative of stress conditions such as substrate limitation (Guckert et al., 1986; Kaur et al., 2005).

Moreover, the relatively large proportion of microbially assimilated plant carbon in the bulk soil is remarkable. On the one hand, this practical and commonly used method of separating the rhizosphere soil from the bulk soil (e.g., Gobran and Clegg, 1996; Micallef et al., 2009; Acharya et al., 2023) cannot fully exclude that the bulk soil is completely free of rhizosphere soil. However, the mixing of rhizosphere soil into the bulk soil can be assumed as negligible, given the huge amount of bulk soil collected. Instead, the relatively large proportion of plant-derived carbon in lipid markers in the bulk soil indicates microbial utilization of plant litter and rhizosphere soil of the previous year, which was homogeneously distributed and tilled at the end of the growing season (see Methods). In addition, at the beginning of the growing season, the proportion of plant carbon was similar between the rhizosphere and the topsoil in most microbial groups. This indicates that, at this timepoint, rhizodeposition was of minor importance, which is likely related to the early development of the plants (Table S1). However, as the plant-derived carbon in the bulk soil is not constant over the growing season, it is also likely that there is a certain transfer of rhizodeposits from the rhizosphere to the bulk soil by abiotic processes, such as water movement, or through biological processes such as transportation through fungal hyphal networks (e.g., Butler et al., 2003; Parihar et al., 2020; Chowdhury et al., 2022).

4.2. Changes over the growing season

While differences between rhizosphere and bulk soil explained the largest differences in net assimilation of plant-derived carbon, differences in microbial biomass were largely explained by the timepoint of sampling. These temporal effects on the microbial biomass, however, were not or only weakly related to changes in soil moisture (Suppl. Table S1). Instead, it is likely that the temporal patterns are directly related to root exudation, which in turn might reflect plant requirements driven by environmental conditions such as water or nutrient availability (Maurer et al., 2021). For example, the sharp decrease in AM fungi biomass in July, when the biomass of most other free-living microorganisms peaked, suggests altered root exudation patterns. Most notably, saprophytic fungal biomass and the proportion of plant-derived carbon in that group increased in the rhizosphere in July. Similar to our study, an opposite pattern in the net assimilation of plant-derived carbon between AM fungi and saprophytic fungi has been reported (Chowdhury et al., 2022). This strongly indicates a directed root exudation, which might be related to environmental conditions such as plant water availability (Gargallo-Garriga et al., 2018). The effect of soil water content on microbial biomass might therefore also be indirectly mediated through plants via root exudation.

The net assimilation of plant-derived carbon of the soil fauna was very variable throughout the growing season. This could indicate either 1) that with time, different animals of different feeding guilds are reflected by the C20:4ω6 marker or 2) that the soil fauna changed their feeding habit during the season. As this marker is unspecific for soil fauna (Ruess and Chamberlain, 2010) and showed relatively large concentration shifts during the vegetation period, it is more likely that variation in plant-derived carbon in the soil fauna marker reflects a community shift of the soil fauna from fungivore or herbivores in early summer to bacterivores and humus-feeding fauna later.

Contrary to our second hypothesis, there was no clear pattern that the microbial biomass of groups with a high affinity for plant-derived carbon was less stable over the growing season compared to groups with a preference for soil carbon. In contrast, the stability of the biomass of both AM fungi and saprophytic fungi was significantly lower than the biomass stability of all bacterial groups. This is primarily due to the sharp increase and decrease, respectively, in both groups during the July sampling (as discussed above) and likely reflects differences in the life strategies and growth forms between fungi and bacteria (Ho et al., 2017). However, the higher temporal stability of bacterial biomass in the rhizosphere (all groups except cy G- markers) compared to the bulk soil was unexpected, as we considered an entire growing season from the early development of the plants to their senescence. This indicates a more constant carbon supply for bacteria close to the roots during the growing season than in bulk soil, including those groups that presumably prefer to utilize soil carbon.

In line with our second hypothesis, the stability of the net assimilation of plant-derived carbon in bacteria was not different between rhizosphere and bulk soil. In contrast, saprophytic fungi showed a significantly lower stability of their plant-derived carbon assimilation in bulk soil than close to the roots. Nuccio et al. (2020) reported a strong difference in bacterial communities between rhizosphere and bulk soil, while the fungal community differed more strongly between treatments with root carbon amendments. The relatively high temporal stability of plant-derived carbon in bacterial groups, being not different between rhizosphere and bulk soil, might thus be explained by different bacterial communities that are adapted to the different availability of plant-derived carbon over the season in both zones. In contrast to bacteria, the hyphal growth of saprophytic fungi would allow a similar portion of plant-derived carbon in rhizosphere and bulk soil, comparable to AM fungi. Therefore, the strong differences in the net assimilation of plant carbon by fungi between rhizosphere and bulk soils are likely attributable to different fungal communities, with the rhizosphere community taking advantage of constant carbon supply from roots, resulting in more stable net assimilation of plant carbon. Nuccio et al. (2020), further suggested that a functional succession of the rhizosphere community takes place, depending on the type and age of plant material provided. With regards to the bacterial community two aspects of our study support this hypothesis: 1) The relatively high temporal stability of bacterial groups compared to saprophytic fungi (Figs. 3), and 2) temporal stability being not different between rhizosphere and bulk soil. A compositional community succession that enables the decomposition of different compound types of plant-derived carbon over the growing season would lead to a relatively constant net assimilation of plant-derived carbon, as observed in our study. Finally, this also supports the suggestion by Hannula et al. (2019) that seasonal shifts in bacterial communities are unlikely to change the overall functioning of

soil.

4.3. Impact of soil texture and duration of the experiment

It is well known that soil texture strongly influences microbial composition (e.g., Johnson et al., 2003; Merckx et al., 1985). This is in line with earlier findings of this vegetation change experiment (Mellado-Vázquez et al., 2019), and our results are consistent with these. However, contrary to our hypothesis, the proportion of plant-derived carbon assimilated by soil microorganisms was not affected by soil texture. In addition, the stability of both the microbial biomass, and the proportion of plant-derived carbon assimilated by the microbial groups, was not affected or not consistently affected by soil texture (Fig. S3). This indicates that the allocation of plant-derived carbon to the microbial community is most strongly influenced by plants and less so by environmental factors such as soil texture.

The different years of establishing the vegetation change (2006 and 2013) did not impact the rhizosphere effect on microbial biomass and plant-derived carbon or their stability over time (Fig. S4). However, the duration of the vegetation change influenced the isotopic signature of soil organic carbon (Balesdent and Balabane, 1996). Specifically, in 2016, the year of the study, the $\delta^{13}C$ values of the soil organic carbon in the top 5 cm on the C4 plots established in 2006 was on average −24.0‰ (±2.2) and −25.4‰ (±2.0) on the C4 plots established in 2013. The $\delta^{13}C$ values on the C3 plots were for both years of establishment −27.6‰ (±0.9). Because the temporal shift in $\delta^{13}C$ values in the soil of the C4 plots impacts the $\delta^{13}C$ values of the lipid markers, it also influences the calculation of the *F*p values. In our study, the plant-derived carbon was higher in the plots established in 2006 than in 2013 for most of the considered lipid biomarkers (Fig. S4). In addition, we see an increased stability for both the microbial biomass and the plant-derived carbon for most groups considered, in particular in all biomarkers assigned to bacteria in the older plots than in the more recently established plots (Fig. S4). The increased stability with time for microbial biomass and microbial processes has already been reported earlier (Strecker et al., 2016) and might be attributed to a tightening of the plant-microbial interactions over time (e.g., Agrawal, 2001; Lange et al., 2019).

5. Conclusions

Except AM fungi, being obligate symbionts with plants, all soil microbial groups were opportunistic in the energy sources (plant- and/or soil-derived) they utilized over the course of the growing season. Namely, the proportion of plant-derived carbon they assimilated varies during the growing season. However, among different functional soil microbial groups, the ones which were more closely associated with the rhizosphere (in particular saprophytic fungi) showed higher variations in the net assimilation of plant-derived carbon, highlighting the importance of plant inputs for soil functioning. Yet, irrespective of the variation in net assimilation of plant-derived carbon, the constant assimilation during the growing season suggests that succession in the microbial community might sustain soil biogeochemical cycling despite varying plant inputs, and we propose functional redundancy provided by a succession of the microbial community as a mechanistic explanation.

Authors' contributions

ML and GG conceived this study; MAR conducted the sampling and did the laboratory work. ML analyzed the data and wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment

We sincerely thank Agnes Fastnacht, Stefanie Hempel and Uta Gerighausen for maintaining and weeding the experimental site, Maria Wittwer and Steffen Rühlow for assistance with the GC-FID and GC-IRMS systems. We would also like to thank all former members of the Molecular Biogeochemistry research group who helped with the annual harvest of the experimental site. This study was financially supported by the Max Planck Institute for Biogeochemistry, Jena. Markus Lange gratefully acknowledges funding by the Zwillenberg-Tietz Foundation. Georg Dittmann, Dan Frederik Lange, Alice May Orme, Simon Andreas Schroeter and Carsten Simon would like to thank the International Max Planck Research School for Biogeochemical Cycles (IMPRS-gBGC) for funding. Simon Andreas Schroeter gratefully acknowledges the CRC 1076 AquaDiva, funded by the Deutsche Forschungsgemeinschaft (DFG).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2023.109280.

References

Acharya, S.M., Yee, M.O., Diamond, S., et al., 2023. Fine scale sampling reveals early differentiation of rhizosphere microbiome from bulk soil in young Brachypodium plant roots. ISME Communications 3, 54.
Agrawal, A.A., 2001. Ecology - phenotypic plasticity in the interactions and evolution of species. Science 294, 321–326.
Bago, B., Pfeffer, P.E., Shachar-Hill, Y., 2000. Carbon metabolism and transport in arbuscular mycorrhizas. Plant Physiology 124, 949–958.
Bahn, M., Lattanzi, F.A., Hasibeder, R., et al., 2013. Responses of belowground carbon allocation dynamics to extended shading in mountain grassland. New Phytologist 198, 116–126.
Bai, Z., Liang, C., Bodé, S., et al., 2016. Phospholipid 13C stable isotopic probing during decomposition of wheat residues. Applied Soil Ecology 98, 65–74.
Bais, H.P., Weir, T.L., Perry, L.G., et al., 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology 57, 233–266.
Balesdent, J., Balabane, M., 1996. Major contribution of roots to soil carbon storage inferred from maize cultivated soils. Soil Biology and Biochemistry 28, 1261–1263.
Balser, T.C., Firestone, M.K., 2005. Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. Biogeochemistry 73, 395–415.
Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. Nature 515, 505–511.
Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67, 1–48.
Bird, J.A., Herman, D.J., Firestone, M.K., 2011. Rhizosphere priming of soil organic matter by bacterial groups in a grassland soil. Soil Biology and Biochemistry 43, 718–725.
Black, C.A., 1965. Methods of Soil Analysis: Part I Physical and Mineralogical Properties. American Society of Agronomy, Madison, Wisconsin, USA.
Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911–917.
Bonfante, P., Genre, A., 2008. Plants and arbuscular mycorrhizal fungi: an evolutionary-developmental perspective. Trends in Plant Science 13, 492–498.
Butler, J.L., Williams, M.A., Bottomley, P.J., Myrold, D.D., 2003. Microbial community dynamics associated with rhizosphere carbon flow. Applied and Environmental Microbiology 69, 6793–6800.
Chaparro, J.M., Badri, D.V., Vivanco, J.M., 2014. Rhizosphere microbiome assemblage is affected by plant development. The ISME Journal 8, 790–803.
Chowdhury, S., Lange, M., Malik, A.A., et al., 2022. Plants with arbuscular mycorrhizal fungi efficiently acquire Nitrogen from substrate additions by shaping the decomposer community composition and their net plant carbon demand. Plant and Soil 475, 473–490.
Cotrufo, M.F., Soong, J.L., Horton, A.J., et al., 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nature Geoscience 8, 776–779.

de Vries, F.T., Hoffland, E., van Eekeren, N., et al., 2006. Fungal/bacterial ratios in grasslands with contrasting nitrogen management. Soil Biology and Biochemistry 38, 2092–2103.
Degens, E.T., 1969. Biogeochemistry of stable carbon isotopes. In: Eglington, G.M.M.T.J. (Ed.), Organic Geochemistry. Elsevier, New York, pp. 304–329.
Denef, K., Bubenheim, H., Lenhart, K., et al., 2007. Community shifts and carbon translocation within metabolically-active rhizosphere microorganisms in grasslands under elevated CO_2. Biogeosciences 4, 769–779.
Eisenhauer, N., Herrmann, S., Hines, J., et al., 2018. The dark side of animal phenology. Trends in Ecology & Evolution 33, 898–901.
Evershed, R.P., Crossman, Z.M., Bull, I.D., et al., 2006. 13C-Labelling of lipids to investigate microbial communities in the environment. Current Opinion in Biotechnology 17, 72–82.
Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354–1364.
Frostegård, Å., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in soils. Soil Biology and Biochemistry 43, 1621–1625.
Garcia-Pausas, J., Paterson, E., 2011. Microbial community abundance and structure are determinants of soil organic matter mineralisation in the presence of labile carbon. Soil Biology and Biochemistry 43, 1705–1713.
Gargallo-Garriga, A., Preece, C., Sardans, J., et al., 2018. Root exudate metabolomes change under drought and show limited capacity for recovery. Scientific Reports 8, 12696.
Gleixner, G., 2013. Soil organic matter dynamics: a biological perspective derived from the use of compound-specific isotopes studies. Ecological Research 28, 683–695.
Gleixner, G., Poirier, N., Bol, R., Balesdent, J., 2002. Molecular dynamics of organic matter in a cultivated soil. Organic Geochemistry 33, 357–366.
Gobran, G.R., Clegg, S., 1996. A conceptual model for nutrient availability in the mineral soil-root system. Canadian Journal of Soil Science 76, 125–131.
Guckert, J.B., Hood, M.A., White, D.C., 1986. Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of Vibrio cholerae: increases in the trans/cis ratio and proportions of cyclopropyl fatty acids. Applied and Environmental Microbiology 52, 794–801.
Habekost, M., Eisenhauer, N., Scheu, S., et al., 2008. Seasonal changes in the soil microbial community in a grassland plant diversity gradient four years after establishment. Soil Biology and Biochemistry 40, 2588–2595.
Haddad, N.M., Crutsinger, G.M., Gross, K., et al., 2011. Plant diversity and the stability of foodwebs. Ecology Letters 14, 42–46.
Hannula, S.E., Kielak, A.M., Steinauer, K., et al., 2019. Time after time: temporal variation in the effects of grass and forb species on soil bacterial and fungal communities. mBio 10, e02635, 02619.
Ho, A., Di Lonardo, D.P., Bodelier, P.L.E., 2017. Revisiting life strategy concepts in environmental microbial ecology. FEMS Microbiology Ecology 93.
Joergensen, R.G., Wichern, F., 2008. Quantitative assessment of the fungal contribution to microbial tissue in soil. Soil Biology and Biochemistry 40, 2977–2991.
Johnson, M.J., Lee, K.Y., Scow, K.M., 2003. DNA fingerprinting reveals links among agricultural crops, soil properties, and the composition of soil microbial communities. Geoderma 114, 279–303.
Karlowsky, S., Augusti, A., Ingrisch, J., et al., 2018. Land use in mountain grasslands alters drought response and recovery of carbon allocation and plant-microbial interactions. Journal of Ecology 106, 1230–1243.
Kaur, A., Chaudhary, A., Kaur, A., et al., 2005. Phospholipid fatty acid - a bioindicator of environment monitoring and assessment in soil ecosystem. Current Science 89.
Kindler, R., Miltner, A., Thullner, M., et al., 2009. Fate of bacterial biomass derived fatty acids in soil and their contribution to soil organic matter. Organic Geochemistry 40, 29–37.
Kramer, C., Gleixner, G., 2006. Variable use of plant- and soil-derived carbon by microorganisms in agricultural soils. Soil Biology and Biochemistry 38, 3267–3278.
Kramer, C., Gleixner, G., 2008. Soil organic matter in soil depth profiles: distinct carbon preferences of microbial groups during carbon transformation. Soil Biology and Biochemistry 40, 425–433.
Kuzyakov, Y., 2002. Review: factors affecting rhizosphere priming effects. Journal of Plant Nutrition and Soil Science 165, 382–396.
Kuzyakov, Y., 2010. Priming effects: interactions between living and dead organic matter. Soil Biology and Biochemistry 42, 1363–1371.
Kuzyakov, Y., Blagodatskaya, E., 2015. Microbial hotspots and hot moments in soil: concept & review. Soil Biology and Biochemistry 83, 184–199.
Lange, M., Eisenhauer, N., Sierra, C.A., et al., 2015. Plant diversity increases soil microbial activity and soil carbon storage. Nature Communications 6, 6707.
Lange, M., Koller-France, E., Hildebrandt, A., et al., 2019. How Plant Diversity Impacts the Coupled Water, Nutrient and Carbon Cycles, Mechanisms Underlying the Relationship between Biodiversity and Ecosystem Function. Academic Press, pp. 185–219.
Lange, M., Roth, V.-N., Eisenhauer, N., et al., 2021. Plant diversity enhances production and downward transport of biodegradable dissolved organic matter. Journal of Ecology 109, 1284–1297.
Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology 2, 17105.
Liu, Y.-R., Delgado-Baquerizo, M., Wang, J.-T., et al., 2018. New insights into the role of microbial community composition in driving soil respiration rates. Soil Biology and Biochemistry 118, 35–41.
Malik, A., Scheibe, A., Lokabharathi, P.A., Gleixner, G., 2012. Online stable isotope analysis of dissolved organic carbon size classes using size exclusion chromatography coupled to an isotope ratio mass spectrometer. Environmental Science and Technology 46, 10123–10129.
Malik, A.A., Chowdhury, S., Schlager, V., et al., 2016. Soil fungal:bacterial ratios are linked to altered carbon cycling. Frontiers in Microbiology 7.
Maurer, D., Malique, F., Alfarraj, S., et al., 2021. Interactive regulation of root exudation and rhizosphere denitrification by plant metabolite content and soil properties. Plant and Soil 467, 107–127.
Mellado-Vázquez, P.G., Lange, M., Bachmann, D., et al., 2016. Plant diversity generates enhanced soil microbial access to recently photosynthesized carbon in the rhizosphere. Soil Biology and Biochemistry 94, 122–132.
Mellado-Vázquez, P.G., Lange, M., Gleixner, G., 2019. Soil microbial communities and their carbon assimilation are affected by soil properties and season but not by plants differing in their photosynthetic pathways (C3 vs. C4). Biogeochemistry 142, 175–187.
Merckx, R., den Hartog, A., van Veen, J.A., 1985. Turnover of root-derived material and related microbial biomass formation in soils of different texture. Soil Biology and Biochemistry 17, 565–569.
Micallef, S.A., Shiaris, M.P., Colón-Carmona, A., 2009. Influence of Arabidopsis thaliana accessions on rhizobacterial communities and natural variation in root exudates. Journal of Experimental Botany 60, 1729–1742.
Mielke, L., Taubert, M., Cesarz, S., et al., 2022. Nematode grazing increases the allocation of plant-derived carbon to soil bacteria and saprophytic fungi, and activates bacterial species of the rhizosphere. Pedobiologia 90, 150787.
Miltner, A., Bombach, P., Schmidt-Bruecken, B., Kaestner, M., 2012. SOM genesis: microbial biomass as a significant source. Biogeochemistry 111, 41–55.
Nuccio, E.E., Starr, E., Karaoz, U., et al., 2020. Niche differentiation is spatially and temporally regulated in the rhizosphere. The ISME Journal 14, 999–1014.
O'Leary, M.H., 1981. Carbon isotope fractionation in plants. Phytochemistry 20, 553–567.
Oksanen, J., Blanchet, F.G., Friendly, M., et al., 2020. Vegan: Community Ecology Package, vol. 2, pp. 5–7.
Olsson, P.A., 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. FEMS Microbiology Ecology 29, 303–310.
Olsson, P.A., Johnson, N.C., 2005. Tracking carbon from the atmosphere to the rhizosphere. Ecology Letters 8, 1264–1270.
Parihar, M., Rakshit, A., Meena, V.S., et al., 2020. The potential of arbuscular mycorrhizal fungi in C cycling: a review. Archives of Microbiology 202, 1581–1596.
Pausch, J., Kuzyakov, Y., 2018. Carbon input by roots into the soil: quantification of rhizodeposition from root to ecosystem scale. Global Change Biology 24, 1–12.
Pett-Ridge, J., Firestone, M.K., 2017. Using stable isotopes to explore root-microbe-mineral interactions in soil. Rhizosphere 3, 244–253.
Philippot, L., Raaijmakers, J.M., Lemanceau, P., van der Putten, W.H., 2013. Going back to the roots: the microbial ecology of the rhizosphere. Nature Reviews Microbiology 11, 789–799.
R Development Core Team, 2021. R: A Language and Environment for Statistical Computing, 4.1.1 ed. The R Foundation for Statistical Computing, Vienna, Austria.
Ruess, L., Chamberlain, P.M., 2010. The fat that matters: soil food web analysis using fatty acids and their carbon stable isotope signature. Soil Biology and Biochemistry 42, 1898–1910.
Shi, S., Nuccio, E., Herman, D.J., et al., 2015. Successional trajectories of rhizosphere bacterial communities over consecutive seasons. mBio 6, e00746, 00715.
Smith, S.E., 2008. Mycorrhizal Symbiosis, third ed. Academic Press, Amsterdam.
Strecker, T., Mace, O.G., Scheu, S., Eisenhauer, N., 2016. Functional composition of plant communities determines the spatial and temporal stability of soil microbial properties in a long-term plant diversity experiment. Oikos 125, 1743–1754.
Thoms, C., Gleixner, G., 2013. Seasonal differences in tree species' influence on soil microbial communities. Soil Biology and Biochemistry 66, 239–248.
Treonis, A.M., Ostle, N.J., Stott, A.W., et al., 2004. Identification of groups of metabolically-active rhizosphere microorganisms by stable isotope probing of PLFAs. Soil Biology and Biochemistry 36, 533–537.
Werner, R.A., Brand, W.A., 2001. Referencing strategies and techniques in stable isotope ratio analysis. Rapid Communications in Mass Spectrometry 15, 501–519.
Yao, H., Chapman, S.J., Thornton, B., Paterson, E., 2015. 13C PLFAs: a key to open the soil microbial black box? Plant and Soil 392, 3–15.
Zelles, L., 1999. Identification of single cultured micro-organisms based on their whole-community fatty acid profiles, using an extended extraction procedure. Chemosphere 39, 665–682.
Zelles, L., Palojärvi, A., Kandeler, E., et al., 1997. Changes in soil microbial properties and phospholipid fatty acid fractions after chloroform fumigation. Soil Biology and Biochemistry 29, 1325–1336.
Zhalnina, K., Louie, K.B., Hao, Z., et al., 2018. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. Nature Microbiology 3, 470–480.
Zuur, A., Ieno, E.N., Walker, N., et al., 2009. Mixed Effects Models and Extensions in Ecology with R. Springer, New York, NY.