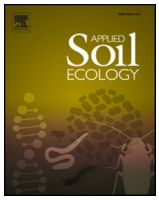
Applied Soil Ecology 191 (2023) 105052

Contents lists available at ScienceDirect

Applied Soil Ecology

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



Fate and stabilization of labile carbon in a sandy boreal forest soil – A question of nitrogen availability?

Nele Meyer [a,b,c,*,1], Outi-Maaria Sietiö [a,d,1], Sylwia Adamczyk [a,e], Per Ambus [f], Christina Biasi [g], Bruno Glaser [h], Subin Kalu [a], Angela Martin [a], Kevin Z. Mganga [a,i], Miikka Olin [j], Aino Seppänen [a], Rashmi Shrestha [k], Kristiina Karhu [a]

[a] University of Helsinki, Department of Forest Sciences, Helsinki, Finland
[b] University of Bayreuth, Department of Soil Ecology, Bayreuth, Germany
[c] Goethe University Frankfurt, Institute of Physical Geography, Frankfurt, Germany
[d] Häme University of Applied Sciences, HAMK BIO, Hämeenlinna, Finland
[e] Natural Resources Institute Finland, Helsinki, Finland
[f] University of Copenhagen, Department of Geosciences and Natural Resource Management, Copenhagen, Denmark
[g] University of Eastern Finland, Biogeochemistry Research Group, Kuopio, Finland
[h] Martin Luther University Halle-Wittenberg, Institute of Agricultural and Nutritional Sciences, Department of Soil Biogeochemistry, Halle, Germany
[i] Utrecht University, Copernicus Institute of Sustainable Development, Utrecht, Netherlands
[j] University of Helsinki, Department of Food and Nutrition, Helsinki, Finland
[k] University of Helsinki, Department of Microbiology, Helsinki, Finland

ARTICLE INFO

Keywords:
Microbial carbon pump
Microbial community
Microbial necromass
Microbial nitrogen mining
Nitrogen limitation, *Pinus sylvestris*

ABSTRACT

Labile carbon (C) fractions, such as sugars, may persist in soil due to their incorporation into microbial biomass and are ultimately stabilized as microbial necromass as part of stable soil organic matter (SOM). However, the underlying factors and mechanisms are currently highly debated. To address this knowledge gap, we conducted a 1-year greenhouse experiment including four treatments: (1) bare soil, (2) bare soil and nitrogen (N) fertilization, (3) soil planted with a tree, and (4) tree and N. The boreal forest soil was a sandy and nutrient-poor Podzol taken from 0 to 20 cm depth and trees were *Pinus sylvestris*. We hypothesized that: (1) originally labile C does not accumulate under N-deficient conditions, as microbial residues may be intensely recycled for N acquisition and (2) differences in N supply and demand change the functionality and composition of the microbial community, which will be reflected in the stabilization of microbial C. We added ^{13}C glucose to the soil and measured ^{13}C recovery to trace the fate of added C in soil, microbial biomass (MBC), dissolved organic C (DOC), phospholipid fatty acids (PLFA), and amino sugars as biomarker for microbial necromass. We also analyzed microbial community structure and enzyme activities. Around 40 % of the added C was mineralized after one day. Mineralization of the added C continued for 6 months, but stabilized thereafter. After 1 year, the treatment with both tree and N fertilization had the highest amount of added ^{13}C (34 %) remaining in soil compared to the other treatments (18 %). The recovery of ^{13}C in DOC was <1 % from the 3rd day onwards, but remained higher in MBC (2 %) and microbial necromass (1.5 %) after 1 year. N fertilization increased bacterial growth on ^{13}C-glucose and abundance of gram-positive bacteria, while trees increased the abundance of symbiotrophic fungi. The formation of more stable C in the treatment with both tree and N indicates that under those conditions, recycling of microbial necromass for N acquisition is lower and the changed microbial composition leaves behind more stable residues.

* Corresponding author at: Goethe University Frankfurt, Institute of Physical Geography, Altenhöferallee 1, 60438 Frankfurt am Main, Germany.
E-mail address: nele.meyer@em.uni-frankfurt.de (N. Meyer).
[1] Nele Meyer and Outi-Maaria Sietiö share first authorship.

https://doi.org/10.1016/j.apsoil.2023.105052
Received 1 February 2023; Received in revised form 15 May 2023; Accepted 6 July 2023
Available online 15 July 2023

1. Introduction

The fate of soil organic matter (SOM) can be manifold – part of it is degraded resulting in partly decomposed and chemically altered plant residues. Another part is assimilated by microorganisms and re-synthesized into living microbial biomass (MBC) and ultimately ends up in microbial necromass (Liang et al., 2017). It has been believed for a long time that stable soil organic carbon (SOC) mainly results from selective preservation or microbial transformation of plant residues (Martens, 2000; Tan, 2014; Zech et al., 1992). Yet, the recent microbial carbon pump theory points towards a more crucial role of microorganisms in the formation of SOC stocks (Miltner et al., 2012; Liang et al., 2019; Liang, 2020). Although MBC represents only a small portion of total SOM (0.5–4 %, Insam, 1990), their necromass may become stabilized and accumulate in soil (Glaser et al., 2004; Glaser et al., 2006; Miltner et al., 2012). In this context, even originally labile carbon (C), such as sugar-derived C, may persist in soil for a long time due to its incorporation into MBC and subsequent stabilization as necromass. Recent studies suggest that microbial residues account for 30 % - 80 % of SOC (Liang and Balser, 2011; Liang et al., 2017; Liang et al., 2019). Yet, mechanisms behind the large variability of microbial necromass accumulation are not well understood.

Since microbial necromass is assumed not to be inherently stable (Sparling et al., 1998; Buckeridge et al., 2020), its accumulation may depend on stabilization mechanisms (Liang et al., 2017). Generally, the contribution of necromass to SOC is lower in organic layers than in mineral soil (Ni et al., 2020). In this context, Li et al. (2019) and Buckeridge et al. (2020) assumed that the persistence of microbial necromass relies on adsorption to soil minerals and ultimately occlusion into microaggregates, facilitated by the “sticky” character of microbial necromass. Yet, there is not much known about the fate of labile C and microbial necromass stabilization in sandy soils with a low potential for sorption and aggregation (Six et al., 2002; Totsche et al., 2018).

Stabilization of labile C may be further affected by nutrient availability. As microbial residues are rich in nitrogen (N) (Jenkinson, 1988), they may represent an important N source at conditions where N demand is not met (Wang et al., 2020; Meier et al., 2017). Under N-deficient conditions, microbes can decompose complex and N-rich SOM, a phenomenon part of the microbial N mining theory (Craine et al., 2007; Moorhead and Sinsabaugh, 2006). This process leads to a release of N from SOM, but also accelerates C mineralization. Indeed, Cui et al. (2020) demonstrated a rapid recycling of microbial necromass at high rates of C supply, i.e. under conditions of N deficiency. In contrast, microbial necromass may preferentially accumulate in N-rich soils where the need of microorganisms to recycle microbial necromass is low (Griepentrog et al., 2014). Boreal forest soils are considered to be N-limited. In forests, microbial necromass accounts for a smaller proportion to SOC than in other land use types (i.e. 35 %, Wang et al., 2021) and microbial necromass amounts in boreal forests have been reported to be lower than in tropical forests (Chen et al., 2020). This is in line with the assumption that microbial necromass is intensely recycled in N-deficient soils.

Soil fungal and bacterial communities can participate in SOM cycling by producing various enzymes degrading SOM, and in some cases providing nutrients to each other (de Boer et al., 2005). In addition, soil bacteria participate in degradation of dead fungal mycelia (Brabcová et al., 2016; Baldrian, 2017). Interactions between soil microorganisms are important for SOM cycling, and their functionality can determine the fate of C in soils. Presence of ectomycorrhizal (ECM) fungi can slow down soil C cycling (Averill and Hawkes, 2016). Mycorrhizal fungi in general are known to harbor distinct bacterial communities, which can participate in N fixing or SOM degradation (de Boer et al., 2005; Schäfer et al., 2010; Jones, 2015; Baldrian, 2017). In addition, N limitation might restrict growth and functioning of saprotrophic fungi (Lindahl et al., 2002). Thus, estimating the magnitude of microbial necromass accumulation to SOC is challenging, since it depends on how rapidly microbial necromass is degraded by soil microorganisms, which is affected by multiple factors, such as chemical composition of the microbial necromass (Clemmensen et al., 2015; Fernandez et al., 2016; Liang et al., 2017). Especially the chemical quality of fungal necromass, such as N content, is found to determine fungal necromass decomposition rates (Fernandez and Koide, 2014; Brabcová et al., 2016; Fernandez et al., 2016; Beidler et al., 2020). Fungal necromass with high N content is observed to decay faster than necromass with lower N content (Brabcová et al., 2016; Maillard et al., 2020; Beidler et al., 2020). Also bacterial necromass decomposition rate has been found to be taxa-specific (Dong et al., 2021). Thus, microbial community structure, and factors affecting it, might be important in microbial necromass C accumulation in soil. This calls for a combined consideration of microbial communities and C stabilization mechanisms when studying the fate of labile C.

Here, we aim at investigating the fate of glucose in a sandy boreal forest soil under different levels of nutrient supply and demand. We hypothesized (1) that the fate of labile C is mainly driven by microbial N demand and supply. Specifically, we assumed that labile C is not stabilized in N-poor soils. This can be expected as newly formed microbial necromass, which likely contains large amounts of glucose-derived C, may be intensely recycled for the N acquisition. In contrast, microbial necromass becomes stabilized in soil when sufficient N supply suppresses its recycling and turnover. Further, we hypothesized (2) that differences in N supply and demand change the functionality of soil microorganisms and the microbial community composition, which will be reflected in the stabilization of microbial C in soil.

To test these hypotheses, we set up a greenhouse experiment including four treatments: (1) bare soil, (2) bare soil and nitrogen (N) fertilization, (3) soil planted with a tree, and (4) tree and N. The soil was a sandy and nutrient-poor boreal forest soil. Trees (*Pinus Sylvestris*) were supposed to induce microbial N deficiency by exuding easily degradable C and by competing with microbes for N. In order to follow the fate of labile C, we added trace amounts of ^{13}C glucose to the soil and regularly took samples throughout 1 year. Measurements of ^{13}C recovery in soil, MBC, dissolved organic carbon (DOC), amino sugars, naturally occurring charcoal particles, and PLFA were conducted. To understand the effect of microbial communities involved in labile C cycling, microbial community structure was determined with DNA-based MiSeq-sequencing and enzyme activities were measured.

2. Material and methods

2.1. Experimental setup

Mineral soil was taken with a spade from a coniferous forest Podzol in Southern Finland (60°20′58″ N, 25°00′45″ E) from 0 to 20 cm depth including the E and B horizons. The soil texture consisted of 91 % sand, 8 % silt, and 0.4 % clay (Mike Starr, unpublished data). The C/N ratio was on average 26, suspecting N limitation (Schimel and Weintraub, 2003). Due to naturally occurring forest fires, the soil contained visible charcoal pieces, which is typical for boreal forests (Ohlson et al., 2009). The soil was sieved to 6 mm, homogenized (includes mixing of E and B horizon), and filled into 32 pots (22 L, inner diameter: 35 cm, height: 20 cm), corresponding to 14.5 kg dry weight each. Pots had holes at the bottom and each pot was placed on a separate plate. The 32 pots were split into four treatments with 8 replicates each, in the following referred to as (1) Bare, (2) Bare+N, (3) Tree, (4) Tree+N.

One Scots pine tree (*Pinus sylvestris*) with a size of approximately 100 cm was planted into each pot of Tree and Tree+N treatments. The trees were acquired in autumn from a commercial nursery (Huutokoski Ltd (in 2019)). Roots were thoroughly washed with tap water before planting to remove soil originating from the plant nursery. Before starting the actual experiment, pots were kept in the greenhouse for 2.5 months to allow the trees to adjust to the new conditions.

Subsequently, Bare+N and Tree+N treatments received fertilizer in

the form of NH_4NO_3. An amount corresponding to 120 kg N ha^{-1} (3.8 g NH_4NO_3 per pot) was added to each pot with 1.5 L of Milli-Q water. To ensure a homogeneous distribution of the fertilizer, half of it was sprayed on the surface and the other half was added to the plate. The Bare and Tree treatments received Milli-Q water instead. Fertilization was repeated after 6 months.

Subsequently, trace amounts of glucose were added to the soil corresponding to 15 μg C g^{-1} soil, contributing with <0.01 % to SOC. This small amount was chosen to minimize priming effects (Karhu et al., 2016) or a shift in microbial community. Half of the 8 pots per treatment received 99 atom-% ^{13}C-labeled glucose and the other half ^{12}C glucose, the latter acting as a control for subsequent ^{13}C measurements. The glucose was dissolved in 1 L of Milli-Q water per pot. Again, half of it was sprayed to the soil surface and the other half was added to the plate and was absorbed by the soil in <1 h.

Pots were randomly distributed in the greenhouse and regularly watered by adding tap water to the plate, thereby avoiding leaching. Soil moisture was on average 52 ± 10 % of water holding capacity. The air temperature was 19 ± 6 °C involving a day-night cycle (20 °C daytime, 16 °C nighttime). The soil temperature was 18 ± 5 °C, measured with 2 randomly placed loggers (Tinytag Plus 2 TGP-4510, Tinytag). Pots received artificial light for 20 h per day. Plants were regularly sprayed with pyridine-based insecticide (Spruzit RTU, Neudorff) to prevent pests. During spraying, soils were covered with plastic to avoid contamination.

Soil was sampled 1 day, 3 days, 8 days, 1 month, 3 months, 6 months, 9 months, and 12 months after the glucose addition (Table S1). These sampling dates were chosen in order to capture both short-term responses at high temporal resolution but also long-term development. At each sampling date, two soil auger samples (diameter 2.5 cm, up to the bottom of the pot) were taken from opposite sides of each pot and combined to form one composite sample per pot. Sampling was always conducted 5 cm away from the edge of the pot to ensure a similar distance to the tree. Holes were subsequently filled with thoroughly washed coarse quartz sand. After sampling, soil was immediately processed for measurements of DOC, NO_3-N and NH_4-N and MBC. The remaining soil was frozen immediately (−20 °C) for DNA analysis, frozen and subsequently freeze-dried for measurements of C, δ^{13}C, PLFA and amino sugars, or dried at 105 °C to determine the water content.

2.2. Basic soil properties

Extraction for NO_3-N and NH_4-N contents was performed at all sampling dates within a few hours after soil sampling. In brief, an amount of 5 g fresh soil was extracted with 25 mL of 1 M KCl, shaken for 1 h, and filtered through a pre-rinsed paper filter (Sartorius, smooth, grade 3 hw). Measurements were conducted with an automated flow analyzer, Lachat QuikChem 8000 (Zellweger Analytics, Milwaukee, Wisconsin, United States). The pH value was measured twice, i.e., after 1 day and after 6 months in 0.01 M $CaCl_2$ with a soil:solution ratio of 1:2.5. Only two sampling dates were considered because we mainly aimed at excluding that treatment-induced changes in pH took place, which may affect microbial activity and soil C cycling. Temporal development of pH was not the scope of our study.

2.3. Recovery of ^{13}C in soil

Freeze-dried soil was milled and its ^{13}C, C, and N contents were measured for all sampling dates using an elemental analyzer (Flash 2000, Thermo Scientific, Bremen, Germany) coupled in continuous flow mode with a Thermo Delta V Advantage isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany).

The amount of glucose-derived C in soil was calculated based on a two-pool mixing model (Eq. (1)) where $C_{glucose\text{-}derived}$ is glucose-derived C (mol g^{-1} soil), at%$^{13}C_{labeled}$ is the atomic ^{13}C percentage in labeled soil, at%$^{13}C_{unlabeled}$ is the atomic ^{13}C percentage in the ^{12}C control, at %$^{13}C_{glucose}$ is the atomic ^{13}C percentage of added glucose, and $C_{labeled}$ is the C content (mol g^{-1} soil) in labeled soil.

$$C_{glucose-derived} = (at\%13C_{labeled} - at\%13C_{unlabeled}) / at\%13C_{glucose} \times C_{labeled} \quad (1)$$

Recovery of the added glucose was calculated with Eq. (2) where $C_{applied}$ is the amount of added C (mol g^{-1} soil).

$$Recovery\ (\%) = C_{Glucose-derived} / C_{applied} \times 100 \quad (2)$$

2.4. Mineralization of ^{13}C

As leaching is avoided in the experimental setup by watering the plants from below and assuming that plant C uptake from soil is negligible, we assume that glucose-derived C not recovered in the soil has been mineralized to CO_2.

2.5. Recovery of ^{13}C in MBC and DOC

Within few hours after sampling soil was processed for MBC extraction, which we assumed to represent living microbial biomass. This was carried out for the following sampling dates: 1 day, 3 days, 8 days, 1 month, 12 months. The focus was on short-term effects because microbial uptake of glucose is usually rapid (Gunina et al., 2017), i.e. no major changes were expected after 1 month. MBC was determined by the chloroform fumigation extraction method (Vance et al., 1987) as in detail described in the supplementary material. The extracts were analyzed for total organic C using a TOC-VCPH Shimadzu (Japan). For determining the ^{13}C content, the extract was subsequently freeze-dried and the residue was measured with a continuous-flow isotope ratio mass spectrometer (IRMS; Thermo Finnigan DELTA XPPlus, Bremen, Germany) interfaced with an elemental analyzer (Flash EA 1112 Series, Thermo Finnigan, Bremen, Germany) via the open split interface (Conflow III, Thermo Finnigan, Bremen, Germany). The amount of glucose-derived C in MBC (mol g^{-1} dry soil) was calculated based on Eq. (3) where *f* is fumigated, *uf* is unfumigated, *l* is labeled, and *ul* is the unlabeled control. The kEC = 0.45 is a correction factor, which was used to account for the non-extractable fraction of microbial carbon (Vance et al., 1987). The recovery was calculated by Eq. (2).

$$C_{glucose-derived}\,in\,MBC = \left(\left(at\%13C_{f,l} - at\%13C_{f,ul} \right) \times C_{f,l} - \left(at\%13C_{uf,l} - at\%13C_{uf,ul} \right) \times C_{uf,l} \right) / at\%13C_{glucose} / 0.45 \quad (3)$$

We also determined the ^{13}C recovery in unfumigated extracts with Eqs. (1) and (2), referred to as DOC.

2.6. Recovery of ^{13}C in amino sugars

Analysis of amino sugars was conducted once at the end of the experiment, i.e. after 1 year. This sampling date was chosen because we were mainly interested in stabilization of originally labile C in microbial necromass, which requires a focus on long-term treatment effects. Individual amino sugars glucosamine (GlcN), galactosamine (GalN), mannosamine (ManN), and muramic acid (MurN) were analyzed according to Zhang and Amelung (1996), as in detail described in the supplementary material. Bacterial C was calculated by multiplying MurN by a conversion factor of 45 (Appuhn and Joergensen, 2006). Fungal C was calculated with Eq. (4) in line with Appuhn and Joergensen (2006) and Faust et al. (2017), where 179.17 is the molecular weight of GlcN, 253.23 is the molecular weight of MurN, and 9 is a conversion factor from fungal GlcN to fungal necromass C.

$$\mu g\ fungal\ C\ g^{-1}\ soil = \left(\left(\left(\mu g\ GlcN\ g^{-1}\ soil / 179.17 \right) - \left(2 \times \mu g\ MurN\ g^{-1}\ soil / 253.23 \right) \right) \times 179.17 \right) \times 9 \quad (4)$$

For determination of ^{13}C in amino sugars, a separate extraction was

carried out as described in Dippold et al. (2014) and in detail described in the supplementary material. The ^{13}C in total amino sugar fraction was measured using a EURO EA Elemental Analyzer (EuroVector, Hekatech, Germany) coupled via a Conflo III Interface to an isotope ratio mass spectrometer (IRMS; Finnigen Delta V Advantage, Thermo Scientific, Bremen, Germany). Sucrose (ANU, IAEA, Vienna, Austria) and $CaCO_3$ (NBS 19, TS limestone) were used as calibration standards. Precision of ^{13}C measurements was 0.2 ‰.

The recovery of glucose-derived C in amino sugars and total fungal plus bacterial residues was calculated by Eqs. (1) and (2).

2.7. Recovery of ^{13}C in charcoal

Dried soil from the last sampling date was inspected under a microscope (up to 63× zoom) for visible charcoal pieces. Those were isolated from the soil using tweezers and their proportion was measured gravimetrically. Particles were grinded and measured with a Delta Plus isotope ratio masss spectrometer (Thermo Fisher Scientific, Bremen, Germany) coupled to a NC 2500 Elemental analyzer (CE Instruments, Milano, Italy) via a ConFlo III interface (Thermo Fisher Scientific, Bremen, Germany). The recovery of glucose-derived C in charcoal was calculated according to Eqs. (1) and (2). Recovery of charcoal was analyzed only one at the end of the experiment (i.e. after 1 year) because it was not the main scope of the study but conducted to check for other potential sorption mechanisms.

2.8. Enzyme activities

Enzyme activities were determined from frozen soil taken 3 months after glucose addition. This sampling date was chosen to reflect the longer term effect of higher N supply to microbial enzyme activities. We measured the activities of cellobiosidase (EC 3.2.1.91), β-glucosidase (EC 3.2.1.21), chitinase (EC 3.2.1.14), leucine amino-peptidase (EC 3.4.11.1), acid phosphatase (EC 3.1.3.2), β-xylosidase (EC 3.2.1.37), and *N*-acetyl-glucosaminidase (EC 3.2.1.52) using fluorometric substrates as described in Bell et al. (2013) and in detail in the supplementary material. Plates were incubated for 140 min at room temperature (20 °C) before measuring fluorescence with a plate reader (BMGLabtech, ClarioStar; excitation at 360 nm and emission at 460 nm). Quenched standard curves were built to each sample separately on 4-methylumbelliferone (MU), and 7-amino-4-methylcoumarin (AMC) for leucine amino-peptidase, by adding the same volume of soil slurry to each standard as was used in the enzyme activity assay. Enzymatic activities were expressed as nmol of MU/AMC per g^{-1} soil DW h^{-1}.

Additionally, we measured oxidative enzymes (phenol oxidase EC 1.14.18.1 and peroxidase EC 1.11.1.x) according to Marx et al. (2001). After measurement of initial absorbance (450bnm), plates were incubated in darkness for 20 h at 20 °C, and absorption was measured again. Enzymatic activities were expressed as nmol of DOPA g^{-1} soil DW h^{-1}. Plant-derived C modification related enzyme activities were calculated by summing up the activities of β-glucosidase, cellobiosidase, β-xylosidases, peroxidase, and phenoloxidase.

2.9. DNA extraction and sequencing

Total DNA was extracted from 0.5 g (fresh weight) soil taken 1 year after glucose addition. This sampling date was chosen to assess the long-term effect of higher N supply to microbial community structures. Extractions were conducted with NucleoSpin Soil Kit (Macherey-Nagel) following manufacturer's protocol with following modifications. The lysis buffer 1 was pre-warmed at 55 °C prior to extraction and 50 μL of enhancer solution was used. The samples were homogenized with shaking the tubes horizontally in vortex at maximum speed for 2.5 min. After vortexing, samples were centrifuged at 11,000 ×*g* for 2 min, and the lysate was transferred to a clean Eppendorf tube before continuing the protocol according to the manufacturer's instructions.

Concentration and purity of the extracted DNA was inspected spectrophotometrically with NanoDrop (Thermo Scientific) using wavelength of 260 nm for determining the concentration, and 260 nm/230 nm and 260 nm/280 nm ratio for determining the purity. Integrity of the extracted DNA was visually inspected with 1 % (*w/v*) agarose gel electrophoresis under UV-light with the presence of ethidium bromide. The extracted double-stranded DNA samples were stored at −20 °C before sequencing. Quantity and quality of the dsDNA was measured also at the Institute of Genomics Core Facility, University of Tartu, Estonia prior to preparation of amplicon libraries.

Sequencing of the fungal ITS2 and bacterial 16S ribosomal V3-V4 regions was performed at the Institute of Genomics Core Facility, University of Tartu, Estonia. Fungal ITS2 region was amplified from the total DNA using gITS7 and ITS4 primers (Ihrmark et al., 2012), and the bacterial 16S ribosomal V3–V4 region was amplified using 515F and 806R primers (Apprill et al., 2015; Parada et al., 2016). Amplicon libraries were generated by two-step PCR in the Institute of Genomics Core Facility, University of Tarto. The first PCR round contained 28 cycles in case of fungal primers, and 24 cycles in case of bacterial primers. The second PCR was done using Illumina Nextera XT dual index primers (Illumina Inc., San Diego, CA) to attach the indexes and sequencing adapters to the PCR products of the first PCR round. The second PCR round contained seven cycles. Sequencing of the amplicon libraries was carried out on an Illumina MiSeq System using MiSeq Reagent Kit v3 in paired end 2 × 301 bp mode.

2.10. Bioinformatics

The raw ITS and 16S rDNA sequences were received as pre-processed from the Institute of Genomics (University of Tartu), where the adapter and barcode sequences were cut away. The raw nucleotide sequence data are available in the NCBI database under Bioproject number PRJNA841816 (SUB11971273 for fungi and SUB11971781 for bacteria). The general read quality of the obtained raw reads was checked with fastqc (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/).

The raw sequence data were filtered and clustered to operational taxonomic units (OTUs) using mothur (version 1.46.1) (Schloss et al., 2009). For filtering, denoising and clustering the bacterial sequences to OTUs, the standard operating procedure (mothur MiSeqSOP) (Kozich et al., 2013, accessed 2021-10) was followed. For filtering, denoising and clustering fungal sequences to OTUs, pipeline by Sietiö et al. (2018) was followed. For identification, bacterial sequences were aligned against mothur-formatted SILVA-database (release 132, (Quast et al., 2012; Yilmaz et al., 2014)) as described in the standard operating procedure (Kozich et al., 2013) and fungal sequences were aligned against UNITE-database (UNITE+INSD version 8.3, Abarenkov et al., 2021) in mothur with classify.seqs. With fungal dataset, all fungal sequences, which did not have Phylum-level classification, were re-aligned against NCBI database, and all non-fungal sequences or ambiguous sequences (i.e. sequences which could not be verified to be of fungal origin) were discarded from analysis (31,788 sequences, 3.8 % of total sequence count). All sequences which were suspected to belong to group of algae parasites (sequences from phyla Aphelidiomycota and Monoblepharomycota), equaling to 0.03 % of total sequence count, were removed before downstream analysis.

In both fungal and bacterial datasets, all singletons and doubletons were removed from the downstream analyses. For prediction of functionality, the obtained fungal taxa were assigned to functional guilds according to the FUNGuild database (Nguyen et al., 2016) in R (R Core Team, 2019) with metagMisc package (Mikryukov, 2017), except for yeasts and molds, which were curated manually. For acquiring a rough estimate of the distribution of bacterial sequences into Gram-positive and Gram-negative groups based on the bacterial cell wall properties, we manually assigned the different bacterial phyla as Gram-positive or Gram-negative based on literature. The assignment is shown in Table S2,

along with the relevant references.

2.11. PLFA

PLFA were extracted from soils collected 8 days after ^{13}C labeling. This sampling date was chosen to observe, which microbial groups are initial users of the added glucose in different treatments. The selected timepoint was chosen so that the microbial ^{13}C-biomass in PLFA would be the highest. PLFA extraction was conducted from 5 g freeze-dried and grinded soils according to the protocol described in Frostegård et al. (1991) with slight modifications described in Karhu et al. (2022) and in the supplementary material. Chromatographic separation and quantification of PLFA were done with gas chromatography (6850 Agilent Technologies) using Omegawax 250 Capillary GC column (30 m length, 250 μm internal diameter, 0.25 μm film thickness, Supelco Sigma-Aldrich) and helium as carrier gas (flow rate 20.0 mL min^{-1}). The GC temperature started at 150 °C with 1 min hold time, heated to 200 °C at 3 °C/min and to 250 °C at 5 °C/min (12 min hold). From the PLFA, i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7t, i17:0, a17:0, 17:0, cy17:0, 18:1ω7, and cy19:0 were used as indicators for bacterial biomass, and 18:2ω6,9 was used for fungal biomass (Frostegård et al., 1996; Klamer and Bååth, 2004). For converting bacterial and fungal PLFA into C, we used the conversion factors 12.43 μmol PLFA-C of bacterial markers per 1 mg of bacterial biomass C (Frostegård and Bååth, 1996) and 212.4 μmol PLFA-C of 18:2ω6,9 per 1 g of fungal biomass C (Klamer and Bååth, 2004), respectively.

Compound-specific stable carbon isotope ratios ($\delta^{13}C$) were analyzed on the 520 °C hydropyrolysates using a Trace GC coupled to a Delta C isotope ratio mass spectrometer via a GC combustion interface (all instruments Thermo Finnigan) by the Centre for Stable Isotope Research and Analysis, University of Göttingen, Germany. Chromatography was performed on an Agilent DB5-MS/DB1-MS column chain (30 m and 15 m length, respectively, both 250 μm internal diameter, 0.25 μm film thickness) and a split/splitless injector was used (270 °C, splitless time 0.8 min). The flow rate was 1.2 mL min^{-1}. The GC temperature started at 80 °C with 1 min hold time, heated to 171 °C at 10 °C/min, to 196 at 0.7 °C/min (4 min hold), to 210 °C at 1.5 °C/min and to 300 at 10 °C/min (10 min hold). The combustion reactor was set to 940 °C. Contribution of the ^{13}C-label to fungal and bacterial biomass C was estimated using conversion factors from Frostegård and Bååth (1996) and Klamer and Bååth (2004). Since we detected in ^{13}C-PLFA GC-C-IRMS assay only four bacterial markers with average of 16.57C atoms, we used a conversion factor on 7.82 mmol bacterial PLFA-C per gram bacterial biomass C instead of the conversion factor suggested by Frostegård et al. (1996). For converting fungal PLFA into C, we used the conversion factor 212.4 μmol PLFA-C of 18:2ω6,9 per 1 g of fungal biomass C (Klamer and Bååth, 2004). The proportion of ^{13}C used for growth was calculated individually for fungi and bacteria following the description in Karhu et al. (2022).

2.12. Statistics

Statistical analyses and figures were conducted in R (R Core Team, 2019).

For measurements that were conducted at several sampling points, we performed linear mixed effect models to test the effect of the factors tree, fertilizer, time, and their interactions. The pot ID was considered as a random effect. Data were checked for normality of residuals by visual inspection of qqplots and Shapiro-Wilk test, and for variance homogeneity with a Levene's test. If assumptions were violated, the model was repeated on log- or square-transformed data. As this did not change the overall result, and because linear mixed effect models have been shown to be robust to moderate violations of distributional assumptions (Schielzeth et al., 2020), we decided to keep the original model.

For measurements that were available for single sampling dates (e.g. enzymes, amino sugars, PLFA) we conducted ANOVA with the factors tree, fertilizer, and their interaction. Models were performed on log- or square-root-transformed data in case that normality was violated. Tukey test was used as a post-hoc test.

Significant differences in the number of observed OTUs were tested with non-parametric Kruskal-Wallis test using kruskal.test function from stats package (R Core Team, 2019). Statistical significance of the changes detected between different treatments (Bare, Bare +N, Tree, Tree+N) were tested with permutational multivariate analysis of variance (perMANOVA) with adonis function from vegan package (Oksanen et al., 2017) using 999 permutations. Prior to perMANOVA the sequence counts were normalized with the library sizes and in perMANOVA the treatments (tree or no tree, N addition or no N addition, and combined effect of tree and N addition) were used as explanatory variables.

To analyze the variance of fungal and bacterial community structures between the treatments, dissimilarity matrices were calculated from the normalized sequence counts with vegdist function from vegan (Oksanen et al., 2017). The eigenvalues for the principal coordinate analysis (PCoA), which are scaling factors that represent the proportion of variation explained by each axis, were calculated and visualized with pco and plot.pco functions from labdsv (Roberts, 2016). A number of shared and unique OTU visualizing Venn diagrams were constructed for fungi and bacteria from the presence/absence transformed data (without doubletons) with function venn from gplots (Warnes et al., 2016).

We defined significant differences as $p < 0.05$ and tendencies towards significant differences as $p < 0.1$.

3. Results

3.1. Basic soil biological and chemical properties

The SOC content decreased significantly with time (Fig. S1, from 0.98 ± 0.08 % on the first day to 0.89 ± 0.09 % after 12 months). On average, treatments with a tree had larger SOC contents (0.98 ± 0.09 %) than bare soil (0.95 ± 0.10 %; significant tree effect; Table S3). Nitrogen fertilized pots had larger SOC contents (0.98 ± 0.09) than their unfertilized counterparts (0.95 ± 0.10; significant fertilizer effect). Soil C/N values were 25.7 ± 1.5 in the Bare treatment, 21.6 ± 3.0 in the Bare+N treatment, 26.0 ± 1.9 in the Tree treatment, and 23.7 ± 1.9 in the Tree+N treatment, averaged over all sampling dates. Contents of NO_3-N and NH_4-N were significantly affected by the tree and the fertilizer and by their interaction: fertilizer addition increased the contents of mineral N and the presence of a tree induced a decrease (Table S3 and S4). NO_3-N and NH_4-N contents also decreased with time, interrupted by a second fertilizer addition after 6 months. Soil pH remained stable throughout the experiment (no significant time effect; Table S3). Trees did not change soil pH. Yet, fertilizer addition slightly and significantly increased the pH value from 4.47 to 4.57.

3.2. Fate and recoveries of added glucose

Glucose-derived C recovery in soil declined with time (significant time effect). Within one day, around 40 % of the added tracer was mineralized (Fig. 1a). A decrease in recovery mainly occurred within the first 6 months, but stabilized thereafter. The tree had a significant effect on the glucose-derived C recovery, which remained at an elevated level in comparison with the bare treatment. Nitrogen fertilizer alone had no significant effect, but there was a tendency towards a significant fertilizer x time interaction ($p < 0.1$, Table 1): within the first month of the experiment, larger amounts of glucose-derived C were lost from fertilized treatments (both Bare+N and Tree+N) than from their unfertilized counterparts. This order changed at a later stage of the experiment where more glucose-derived C remained in the fertilized pots.

In DOC, glucose-derived C was present in considerable amounts only at the first day after glucose addition. Already at the third day <1 % of the added glucose was recovered (significant time effect). Glucose-

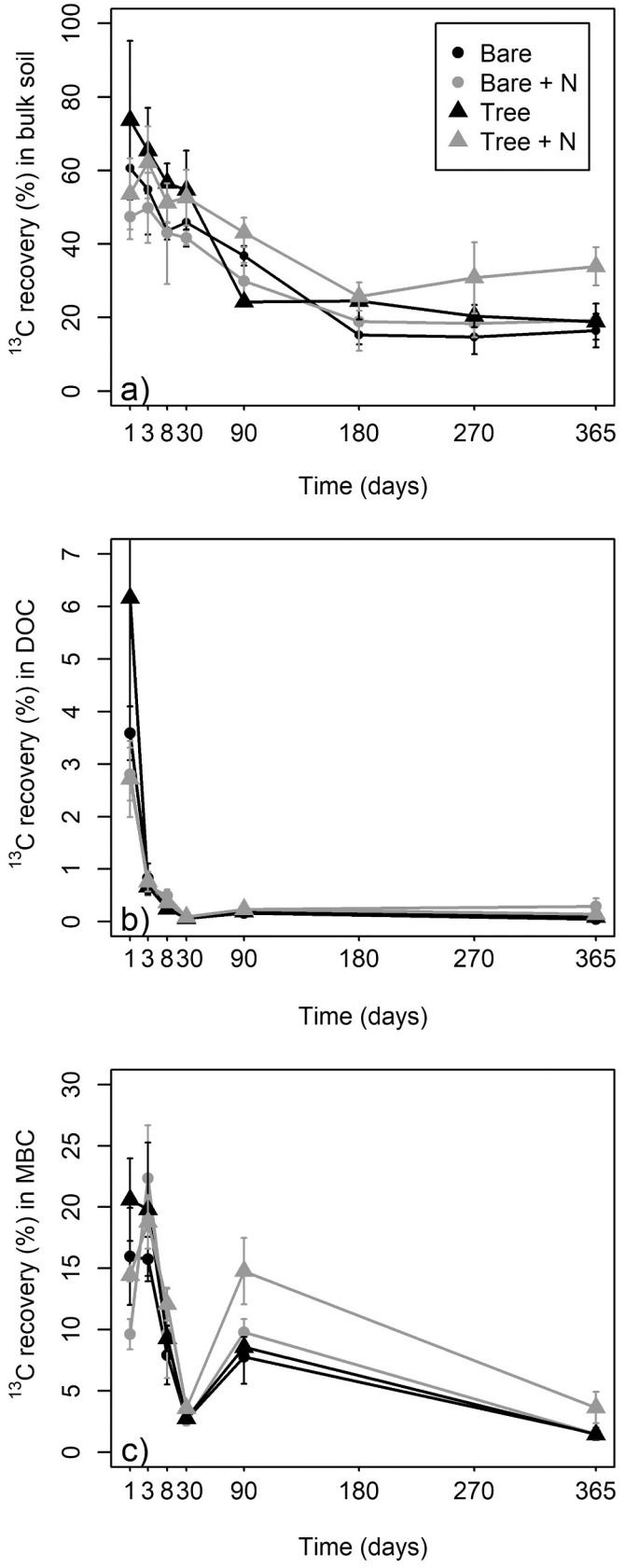


Fig. 1. Recovery of glucose-derived C in (a) bulk soil, (b) dissolved organic carbon (DOC), and (c) microbial biomass (MBC).

Table 1
Statistics for recovery of glucose-derived C in soil, MBC, DOC, and amino sugars. Numbers in bold indicate effects with at least a tendency towards significance ($p < 0.1$).

Recovery		F-value	*p*-value
^{13}C recovery in soil	Intercept	563.8606	<0.0001
	Tree	6.4433	**0.0260**
	Fertilizer	0.0015	0.9693
	Time	90.4033	**<0.0001**
	Tree * Fertilizer	0.4840	0.4999
	Tree * Time	0.0053	0.9419
	Fertilizer * Time	3.8956	**0.0510**
	Tree * Fertilizer * Time	0.5881	0.4449
^{13}C recovery in MBC	Intercept	198.93683	<0.0001
	Tree	1.83673	0.2003
	Fertilizer	0.24017	0.6329
	Time	31.46684	**0.0001**
	Tree * Fertilizer	0.00231	0.9624
	Tree * Time	0.05882	0.8090
	Fertilizer * Time	0.21575	0.6437
	Tree * Fertilizer * Time	0.27834	0.5994
^{13}C recovery in DOC	Intercept	27.526063	<0.0001
	Tree	0.293839	0.5997
	Fertilizer	0.889394	0.3642
	Time	7.804835	**0.0066**
	Tree * Fertilizer	0.480000	0.5016
	Tree * Time	0.183625	0.6695
	Fertilizer * Time	0.726573	0.3967
	Tree * Fertilizer * Time	0.094335	0.7596
^{13}C recovery in amino sugars[a]	**Tree**	20.232	**0.00201**
	Fertilizer	3.801	**0.08703**
	Tree * Fertilizer	0.082	0.78207
^{13}C recovery in charcoal	Tree	1.431	0.266
	Fertilizer	2.188	0.177
	Tree * Fertilizer	1.219	0.302

[a] Log transformed data.

derived C was on average taken up faster from the DOC pool in the fertilized treatments compared with their unfertilized counterparts (Fig. 1b).

Considerable proportions of the added glucose were incorporated into MBC within one day (Fig. 1c). The recovery sharply decreased within the first month to a level of 2–4 %, which remained in the soil even after 1 year. This low level was interrupted by a second peak in MBC recovery after 3 months. There was no significant effect of tree or fertilizer addition on the recovery of glucose-derived C in MBC (Table 1). Yet, the recovery was on average larger in the unfertilized treatments in the first days while later this order reversed.

Glucose-derived C recovery in amino sugars was measured at the end of the experiment. Only small amounts of the added C (< 0.3 %) were recovered in amino sugars (Fig. 2a). Calculating total fungal and bacterial necromass C from amino sugars, the proportion increased to 0.7–3.3 %. Thus, 5–8 % of the remaining glucose-derived C that was still present in the soil after 1 year was found in microbial residues (Table S5). There was a significant effect of both tree and fertilizer: more glucose-derived C was incorporated into microbial necromass in the presence of a tree (Table 1). Fertilization significantly increased this amount in planted and bare treatments.

Visible charcoal pieces had a contribution of 0.22 % to soil mass, corresponding to 8.34 % of SOC. The recovery of glucose-derived C in charcoal was 0.46 ± 0.70 % after 1 year, accounting for <3 % of glucose-derived C still present in soil after 1 year (Table S5). There was no significant difference between treatments (Table 1).

3.3. Fungal-to-bacterial ratios of recent microbial biomass (PLFA) and long-term microbial biomass (amino sugars)

Based on PLFA measured 8 days after glucose addition, bare soils

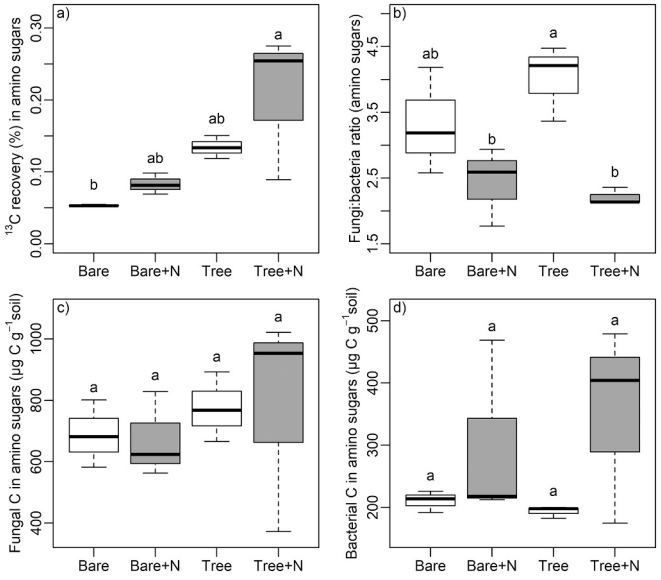


Fig. 2. Amino sugars 1 year after the ^{13}C-glucose addition, (a) recovery of glucose-derived C in amino sugars, (b) contribution of microbial necromass to SOC, (c) fungal to bacterial ratio, (d) fungal necromass, (e) bacterial necromass, and (f) quantity of microbial necromass.

without N addition had a lower fungal-to-bacteria ratio compared to treatments with tree and bare soil+N. N fertilization increased the fungal biomass-C to bacterial biomass-C ratio in bare soils ($p \leq 0.01$) but not in planted treatments (Fig. 3a). The presence of trees tended to increase the fungal biomass-C ($p \leq 0.1$; Fig. 3b), but bacterial biomass-C remained similar (Fig. 3c). Bacterial growth on ^{13}C-glucose was higher in fertilized than in non-fertilized treatments ($p \leq 0.05$, Fig. 3f). In planted treatments, fertilization had no effect on fungal growth on ^{13}C-glucose, but increased the fungal growth on ^{13}C-glucose in the non-planted treatment ($p \leq 0.05$; Fig. 3e). After 8 days from labeling, bacteria were taking up the added glucose more eagerly than fungi and utilizing it for their growth (Fig. 3f), and the bacterial growth on ^{13}C-glucose per total biomass was on average 5-times higher than the fungal growth on glucose per total biomass.

Based on amino sugar analysis conducted 12 months after glucose addition, soil fungal (Fig. 2c) and bacterial (Fig. 2d) necromass were similar between treatments. However, soil fungal-to-bacterial necromass ratio was lower in fertilized treatments ($p \leq 0.01$) when compared to non-fertilized treatments (Fig. 2b; Table S6).

3.4. Soil enzyme activities

The interaction of tree and fertilization had a significant effect on the sum of C cycling-related enzyme activities (β-glucosidase, cellobiosidase, β-xylosidase, peroxidase, and phenoloxidase). Treatments with a tree had lower plant-biomass C modification related enzyme activities than bare soil when no N was added (Fig. 4i). Fertilization resulted in a tendency ($p \leq 0.1$) towards lower plant-biomass C modification related enzyme activities in the bare soil but higher enzyme activities in planted soil (Fig. 4i). The C cycling related enzymes β-glucosidase (Fig. 4b) and cellobiosidase (Fig. 4c) were lower in treatments with a tree than in bare soil when no N was added ($p \leq 0.05$). Similarly, phosphatase activities were higher in fertilized than in non-fertilized treatments (Fig. 4d). Microbial N acquisition related chitinase and leucine aminopeptidase activities were similar in all treatments and fertilization had no significant effect on their activity.

3.5. Soil microbial community structures

We obtained all together 809,291 good quality fungal sequences without doubletons, which were divided into 1000 fungal OTUs with 97

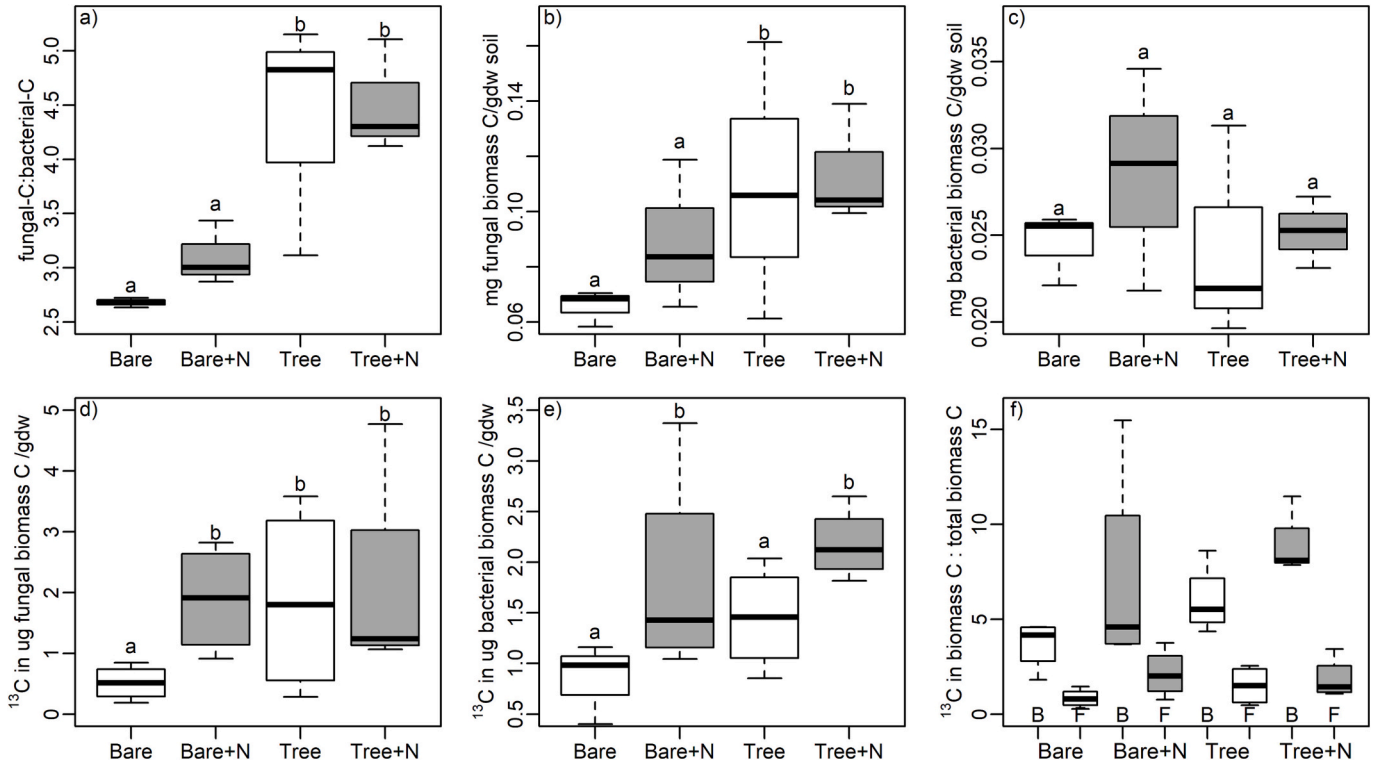


Fig. 3. Results of PLFA analysis in the different treatments after 8 days of the ^{13}C-labeling, a) Soil fungal-to-bacteria ratio, b) fungal biomass-C (mg fungal biomass C/gdw soil), c) bacterial biomass-C (mg bacterial biomass C/gdw soil), d) ^{13}C-fungal biomass (^{13}C in fungal biomass C/gdw), e) ^{13}C-bacterial biomass (^{13}C in bacterial biomass C/gdw) and f) proportion of ^{13}C of total biomass in case of bacteria (B) and fungi (F), respectively.

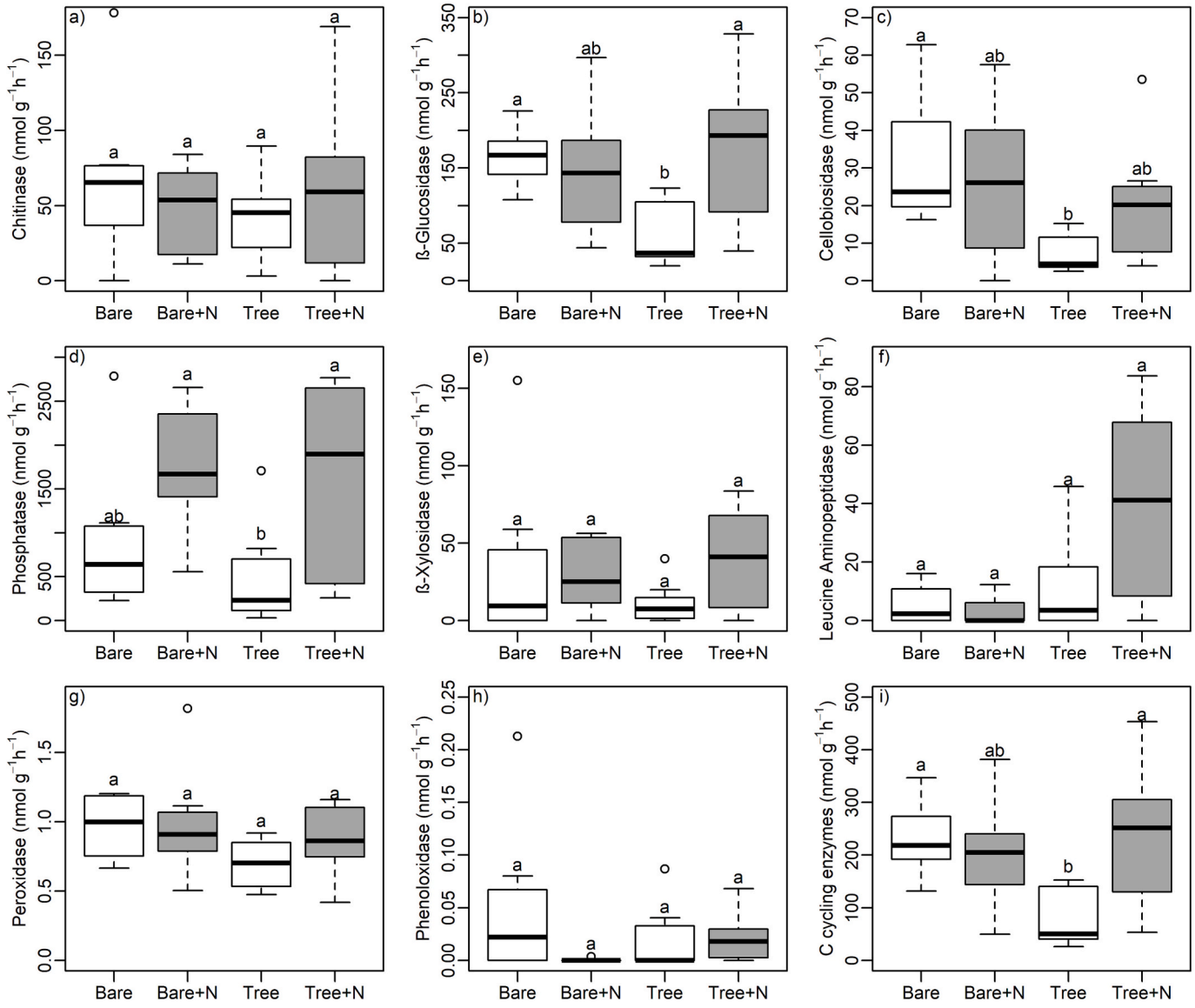


Fig. 4. Enzyme activities measured 3 months after the ^{13}C-labeling. a) chitinase, b) β-glucosidase, c) cellobiosidase, d) phosphatase, e) β-xylosidase, f) leucine-aminopeptidase, g) peroxidase, h) phenoloxidase, and i) sum of C cycling related enzymes. Different letters indicate significant differences between treatments ($p < 0.05$).

% similarity. The fungal sequences could be divided into nine fungal phyla, from which the most dominant were Ascomycota (52 % of all sequences), Basidiomycota (29 % of all sequences) and Mucoromycta (14 % of all sequences) (Table S7). Other phyla, Rozellomycota, Mortiellomycota, Chytridiomycota, Glomeromycota, Kickxellomycota, and Olpidiomycota, were in minority containing all together 5 % of all sequences. Fertilization tended to increase the richness estimates in both bare soil and tree treatments ($p \leq 0.1$) (Fig. S2).

The tree and fertilization had both significant effects on soil fungal community structures ($p \leq 0.001$, F-model = 8.32 for interaction of tree and fertilization with perMANOVA) (Fig. 5a). The communities in tree treatments had higher proportion of potentially symbiotrophic fungi, such as member of Tylosporaceae family, *Hyaloscypha finlandica* (0.01 % in Bare soil treatments and 0.1 % in Tree treatments), *Suillus bovinus* (<0.001 % in Bare soil treatments and 0.05 % in Tree treatments), *Wilcoxina mikolae* (<0.001 % in Bare soil treatments and 0.06 % in Tree treatments), and Serendipita (<0.001 % in Bare soil treatments and 0.04 % in Tree treatments) (Table S7). The Tree+N treatment had higher proportion of *Wilcoxina mikolae* (0.06 % in Tree and 0.11 % in Tree+N treatments), *Telephora terrestris* (<0.001 % in Tree and 0.08 in Tree+N treatments), Laccaria (<0.001 % in Tree and 0.06 % in Tree+N treatments) than the non-fertilized Tree treatment. The presence of trees increased the proportion of symbiotrophic fungi ($p \leq 0.001$ with Kruskal Wallis) but decreased the proportion of saprotrophic fungi, soil molds and yeast ($p \leq 0.01$ for saprotrophs, $p \leq 0.001$ for molds and $p \leq 0.1$ for yeasts with ANOVA) (Fig. 6). Fertilization increased the proportion of soil molds and decreased the proportion of saprotrophic fungi of the total community in both Bare and Tree treatments ($p \leq 0.001$ for molds and $p \leq 0.01$ for saprotrophs with ANOVA).

We obtained all together 728,187 good quality bacterial sequences without singletons, which were divided into 4867 bacterial OTUs with 97 % similarity. Bacterial sequences could be divided into 30 phyla, form which the most dominant ones were: Proteobacteria (27 % of all sequences), Acidobacteria (25 % of all sequences), Chloroflexi (15 % of all sequences), and Actinobacteria (8 % of all sequences) (Table S2). Fertilization decreased the richness estimates in bare soils ($p \leq 0.01$), but had no effect on the richness estimates in the presence of a tree (Fig. S2). Tree and fertilization had both significant effects on soil bacterial community structures ($p \leq 0.001$, F-model = 4.95 for interaction of tree and fertilization with perMANOVA) (Fig. 5b). Fertilization

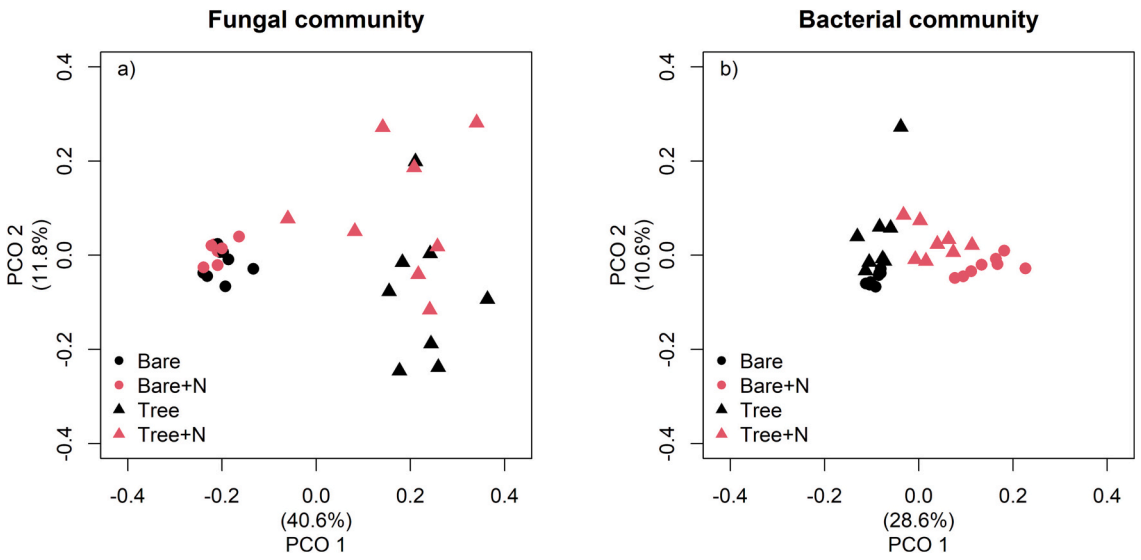


Fig. 5. a) PCoA of the fungal OTUs, and b) PCoA of the bacterial OTUs in soil taken 1 year after glucose addition.

increased the proportion of class Actinobacteria in the total bacterial community ($p \leq 0.001$ with Kruskal Wallis), and also the presence of tree caused a slight increase to the proportion of Actinobacteria ($p \leq 0.05$ with Kruskal Wallis). Overall, fertilization tended to increase the proportion of Gram-positive bacteria ($p \leq 0.001$ with Kruskal Wallis, Fig. 7b), and decreased the proportion of Gram-negative bacteria ($p \leq 0.01$ with Kruskal Wallis, Fig. 7a), when the relative abundances of mainly Gram-positive phyla were summed up.

4. Discussion

We studied the fate and stabilization of originally labile C in soil. Our study demonstrated that considerable amounts of labile C can become stabilized in the long-term, even in such sandy soils as in our study. We found considerably larger proportion of the added ^{13}C remaining in the combined treatment with tree and N fertilization in comparison with bare soil or unfertilized soil. Larger stabilization of originally labile C was likely caused by low recycling rates of microbial biomass and necromass in the absence of N limitation, and by changes in the microbial community structure, possibly towards a community with more recalcitrant residues.

4.1. General observations on fate and stabilization of labile C

The microbial uptake of the added glucose was rapid and 10 %–20 % of it was recovered in the pool of MBC after 24 h. Likewise, it almost disappeared from the pool of DOC. Other mechanisms than microbial utilization that may remove glucose from the soil solution, such as sorption on clay minerals, can rather be excluded in this sandy soil. Even for more clayey soils, microbial uptake of glucose has been reported to outcompete sorption on minerals (Fischer et al., 2010). Further, as carbon use efficiencies have been reported to range between 0.3 and 0.5 (Spohn et al., 2016), total uptake by microorganisms may be 3 times higher than measured in MBC. This is also confirmed by the finding that 35–50 % of the added C was lost from the soil after one day, i.e. it had been mineralized. Moreover, Geyer et al. (2020) observed recovered glucose-derived C in microbial residues already 6 h after amendment, indicating that parts of the primary consumers already passed the stage of living MBC. In summary, despite the comparatively small recovery of glucose-derived C in MBC, we conclude that almost all of the added glucose cycled through the microorganisms within 1 day.

Despite the fast uptake of glucose by microorganisms, considerable proportions (16–34 %) of glucose-derived C persisted in soil after 1 year. Specifically, the recovery hardly decreased anymore from month six on until the end of the experiment, as similarly observed by Basler et al. (2015). We suggest that incorporation into MBC converted originally labile C into less labile compounds.

Since all the added ^{13}C-glucose cycled through the microbial biomass, we assume that the ^{13}C remaining in soil after one year is present either in MBC, amino sugars or microbial metabolites. However, it is still debated whether microbial residues are stable per se or whether their stabilization requires physical or biochemical protection mechanisms. Recent studies have reported critical involvement of minerals in necromass stabilization (Buckeridge et al., 2020; Li et al., 2019; Wang et al., 2020). Despite the low potential for stabilization mechanisms in our soil, we found surprisingly similar recoveries of glucose-derived C like those reported in studies on less sandy soil (Kallenbach et al., 2015; Basler et al., 2015). In this context, Buckeridge et al. (2020) showed that necromass can also become stabilized by adhering to other necromass or SOM. Also our results suggest that microbial necromass accumulation does not entirely rely on aggregation or associations with clay and silt minerals.

Charcoal is common in boreal forests due to natural fires (Ohlson et al., 2009). Charcoal has a large sorption capacity and may protect C from decomposition (Glaser et al., 2002; Pietikäinen et al., 2000). Yet, <1 % of glucose-derived C was recovered in charcoal. The actual relevance of charcoal is underestimated in our study as we only focused on larger charcoal pieces. Still, the negligible recovery suggests that labile C can be stabilized in sandy soils also through other mechanisms than stabilization with mineral or biochar surfaces.

4.2. Fate and stabilization of labile C as affected by N demand and supply

The study relies on the assumption that differences in N demand and supply exist between treatments and that the soil was primarily N limited. We detected that fertilization increased C cycling related enzyme activities in treatments with a tree, but lowered enzymatic activities in bare soils. The lower C cycling related enzyme activity in planted treatments might indicate that microbes utilize the C from rhizodeposition rather than SOM. This is further supported by the higher abundance of symbiotrophs and overall microbial biomass in treatments with a tree. The addition of N relieved the N-limitation of microbes, and the relative C-limitation of microbes increased, which probably made them to allocate some of the excess N for C cycle related enzyme production and growth. Similarly, fertilization caused an increase in phosphatase activities. This suggests that N was the most limiting

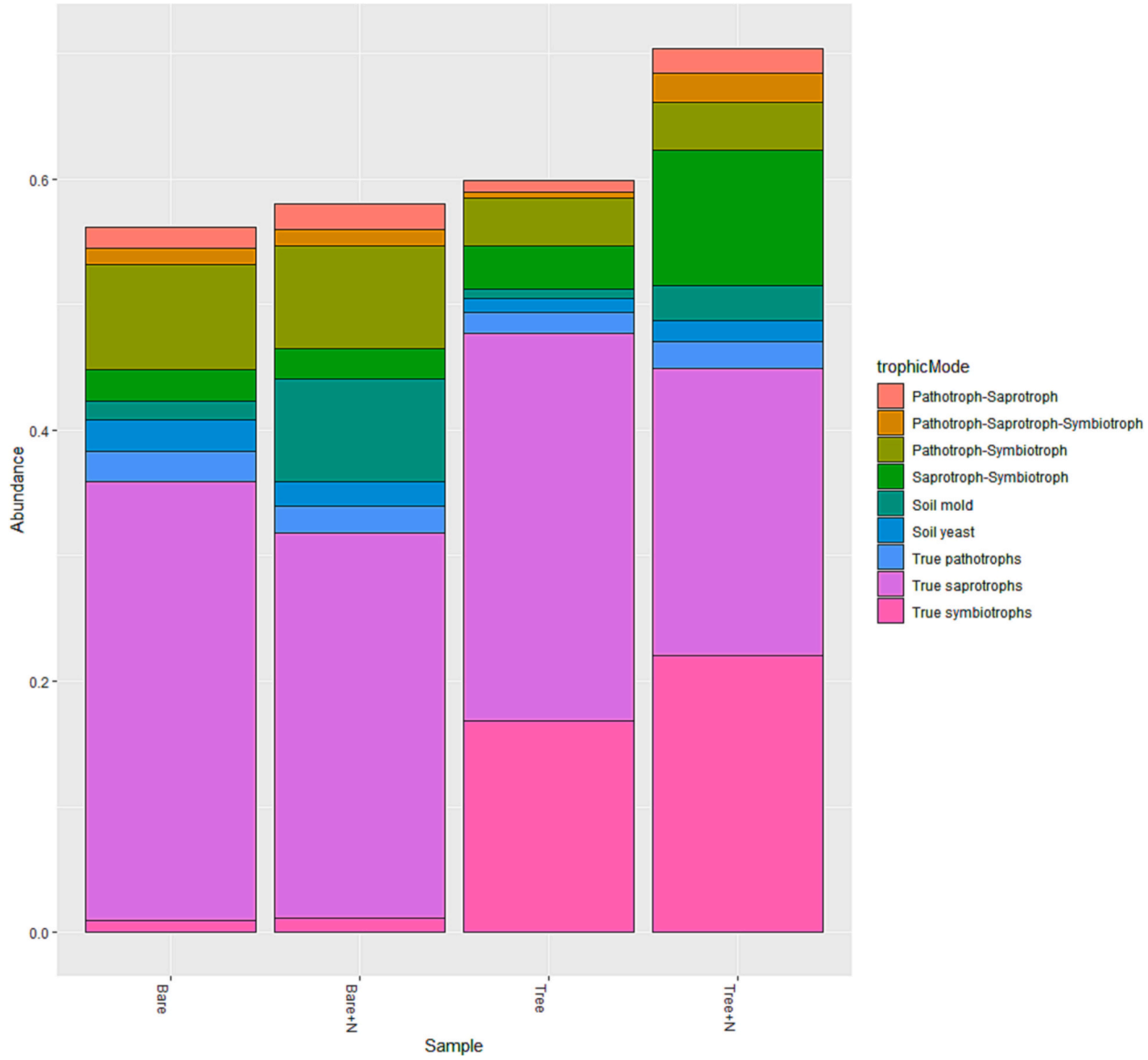


Fig. 6. Division of detected fungal species to different functional guilds according to the prediction made with FUNGuild database. Soil was taken 1 year after glucose addition. 'True' symbiotroph, 'True' saprotroph and 'True pathotroph' refer to fungi with only one known ecological function. Classification of fungi to dual or trial role reflects the ability of those fungi to have different lifestyle depending on environmental conditions.

nutrient in planted treatments, and that phosphorus was the most limiting nutrient when N limitation was alleviated (Soong et al., 2020). In bare soil, in contrast, the weak response of enzyme activities to N addition suggests that C may be the main limiting element for microbial growth. Hence, our treatments created differences in microbial N demand and supply, and also C supply from tree to soil microbes.

Based on the assumption that microbial necromass is rich in N (Jenkinson, 1988), we hypothesized that glucose-derived C is less intensely recycled when N demand is met. Surprisingly, N addition stimulated the turnover of glucose-derived C in the first month, i.e. it induced a rapid loss of the tracer in comparison with the corresponding non-fertilized treatment. This short-term effect of N has also been demonstrated in incubation experiments with durations of few days, in which N addition usually stimulated glucose mineralization (Meyer et al., 2018; Nordgren, 1992). A possible explanation is that immediate microbial growth on glucose is preferentially performed by fast growing *r*-strategists. This was also supported by PLFA analyses conducted 8 days after glucose addition (for details see Section 4.3). Here, bacterial growth on ^{13}C-glucose per total biomass was higher than the fungal growth, which indicates that as fast growing *r*-strategists, bacteria were initial uptakers of the added glucose. Their activity critically depends on N availability (Fontaine et al., 2003; Lindahl et al., 2002).

Yet, we found that N addition had a time-dependent effect on the turnover of glucose-derived C. While fertilization induced a rapid loss of glucose-derived C in the short-term, a larger percentage remained in fertilized treatments in the long-term. In long-term, part of the glucose-derived C can become stabilized, for example in necromass. As necromass and other microbial metabolites, such as proteins, represent an important N source, slow growing *K*-strategists may decompose it to acquire N (Cui et al., 2020; Meier et al., 2017; Fontaine et al., 2003). Indeed, unfertilized soils had lower amino sugar concentrations than fertilized soils and also a lower recovery of glucose-derived C therein. This difference was especially prominent in the presence of a tree, where N limitation in the unfertilized treatment was largest. Other studies have also demonstrated that fertilization increases necromass accumulation (Fan et al., 2020; Hu et al., 2022). Overall, our findings support the hypothesis that originally labile C does not stabilize in soil and microbial necromass when microbial N demand is not met.

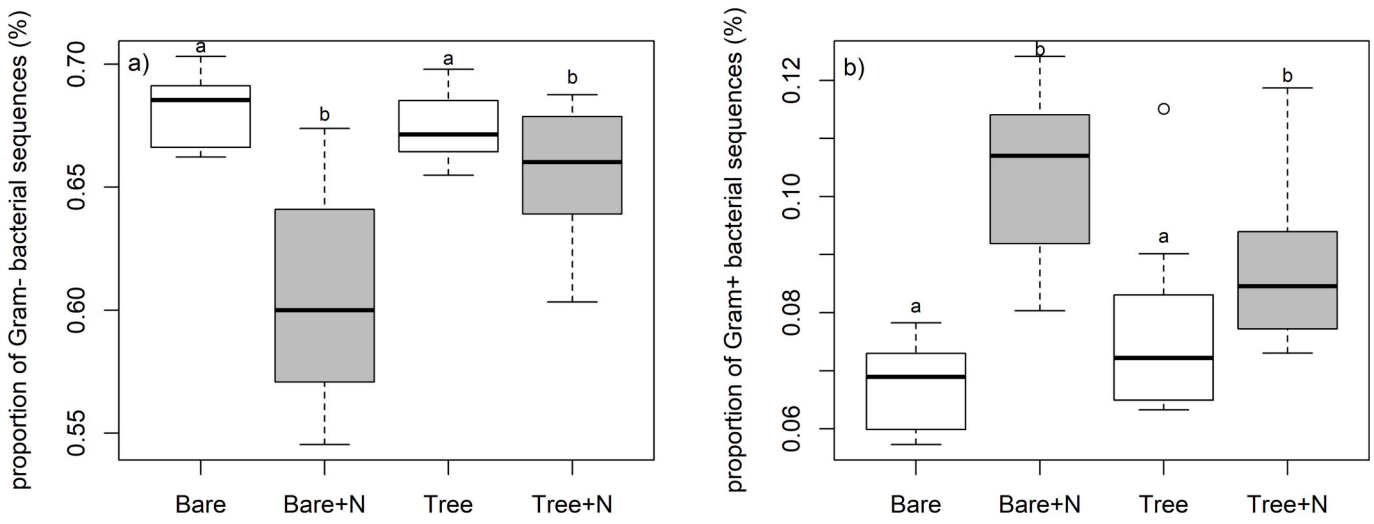


Fig. 7. Relative abundance of a) Gram-negative bacteria, and b) Gram-positive bacteria in soil 1 year after glucose-addition.

4.3. Microbial functionality and community response to differences in N demand and supply

We hypothesized that N supply affects the soil microbial community. Indeed, fertilization increased the live fungal biomass as estimated with PLFA and the soil fungal-to-bacterial ratio in bare soil. This suggests that N addition was favoring growth of those fungi, which were able to utilize the added N to their growth more efficiently than for instance bacteria, which might have been also limited by the C availability. However, the effect of fertilization on the fungal and bacterial biomass was not as clear in the treatments with a tree. This was most likely due to the higher N demand of the tree, and symbiotic microbes might have transported the excess N for the tree in exchange for photosynthetic C (Stuart and Plett, 2020). Interestingly, bacterial short-term growth on ^{13}C-glucose (8 days after its addition) per total biomass was higher than the fungal growth on glucose in all the four treatments. This indicates that as fast growing *r*-strategists, bacteria were initial uptakers of the added glucose in short-term (8 days after its addition) whereas fungi were likely secondary utilizers of the glucose. Hence, N addition might have in short term increase the bacterial metabolic activity, which was reflected as rapid growth on added ^{13}C-glucose.

Also in necromass, the fungal-to-bacterial C ratio was lower in fertilized than in non-fertilized treatments. Since the C in fungal necromass was rather similar in all treatments, the observed decline in fungal-to-bacteria ratio due to fertilization was caused by higher bacterial necromass-C. In forests, N addition has been demonstrated to increase bacterial residues in soils but not fungal residues (Hu et al., 2022). This is possibly due to higher N demand of bacteria than fungi (Strickland and Rousk, 2010). Together with the higher ^{13}C-glucose utilization of bacteria in our fertilized treatments, this suggests that N availability was limiting the bacterial growth in our soils.

We assumed that fertilization affects microbial community structures and that this effect is clearer in the presence of a tree. Following our assumption, we discovered that fertilization and presence of a tree changed both fungal and bacterial community structures compared to the non-fertilized control. Presence of a tree, favored symbiotrophic fungi over soil molds and yeasts. However, fertilization favored saprotrophic fungi and soil molds in both bare soils and tree treatments. Ectomycorrhizal fungi might compete with saprotrophic microorganisms for nutrients, especially for organic N (Gadgil and Gadgil, 1971). Although this competition is considered to occur mainly in organic horizons, the addition of N to soils with a tree might have to some extent relieved the saprotrophic microbes from the competition. Ectomycorrhizal fungi typically receive sufficient supply of C from their host plant (Smith and Read, 2008) and may degrade SOM only for acquiring N and other nutrients (Read and Perez-Moreno, 2003; Talbot and Treseder, 2010). Thus, the presence of ectomycorrhizal fungi most likely contributed to the higher accumulation of microbial remains in tree treatments and the fertilization might have additionally lowered the need of scavenging N from soils.

Bacterial community structure is found to change in time and response to the belowground C allocation from the plant roots (Baldrian, 2017b). This was observed also in our study where the soil bacterial community structure changed in response to the presence of tree. In the bacterial community, bacteria from class Actinobacteria responded to fertilization treatment positively. Some species of Actinobacteria are known to be capable of degrading plant-derived organic matter and utilizing it as sole source of energy in soils (Lewin et al., 2016). It might be that in our study this class was more dominant in N fertilized soils because N fertilization relieved it from N-deficiency, and it was able to grow over other species which were more reliable on plant exudates. Since also the proportion of saprotrophic fungi increased in N fertilized treatments, it might be that the bacteria from class Actinobacteria have also benefitted from the metabolites the saprotrophs have exuded during the organic matter decomposition process.

The stability of bacterial necromass-C in soils has been found in some cases to be taxa-specific (Buckeridge et al., 2020; Dong et al., 2021). However, the taxonomic information is not the only factor determining the recalcitrance of necromass. For example, remains of Gram-positive bacteria are discovered to be rather slowly decomposable (Buckeridge et al., 2020; Dong et al., 2021) while Gram-negative bacteria are found to be decomposed relatively fast in soils (Buckeridge et al., 2020; Dong et al., 2021). In our study, the fertilization caused an increase to proportion phyla Actinobacteria and Firmicutes, which contain mainly Gram-positive bacteria, and decrease in Gram-negative bacteria harbouring phyla. Also, the bacterial necromass-C increased in fertilized pots. Gram-negative bacteria harbor less murein compared to Gram-positive bacteria (Glaser et al., 2004). Thus, the increase of Gram-positive and decrease of Gram-negative bacteria due to fertilization has contributed to the observed bacterial necromass accumulation in fertilized treatments. The higher proportion of Gram-positive bacterial DNA might also reflect that these bacterial groups have been decomposed more slowly in our study, since part of the observed bacterial sequences might be from deceased cells (Carini et al., 2016).

5. Conclusions

Our study demonstrated that considerable amounts of labile C become stabilized even in such sandy soils as in our study. The proportion of stabilized C was regulated by N demand and supply. The underlying mechanisms behind lower labile C stabilization under N deficient conditions may be the need of microbes to decompose and recycle microbial necromass for the N acquisition. Yet, different conditions of N demand and supply and the presence of trees also greatly change the microbial community with different taxa having different recalcitrance of their necromass. Especially the presence of trees in combination with N fertilizer seem to favor a microbial community with more recalcitrant residues. Thus, stabilization of glucose-derived C in fertilized and planted soil was induced by a combination of less need for N mining and more recalcitrant microbial residues. Our study also highlights distinct differences between short- and long-term effects of fertilization on C mineralization and stabilization. We conclude that N demand and supply regulate the fate of labile C in soil and that N availability is critically important for its long-term stabilization especially under N deficient conditions, like those occurring close to trees in boreal forest soils. This is relevant in predicting SOC changes in fertilized forest soils.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2023.105052.

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 are available in the NCBI database under Bioproject number PRJNA841816.

Acknowledgements

This research was supported by funds from the Academy of Finland (grant numbers 319952, 316401, 348824), Helsinki Institute of Life Science (HiLIFE) (a HiLIFE Fellow Grant to K.K. covering e.g. the salary of N.M.) and Maj and Tor Nessling Foundation (personal grant to O.-M. S.). We thank Marjut Wallner and Yi Xu for their assistance with laboratory analysis, Daniel Richterich for his support in the greenhouse, Marianne Benesch for conducting $\delta^{13}C$ measurements of amino sugars, Gerhard Gebauer (BayCEER - Laboratory of Isotope Biogeochemistry, Bayreuth) for conducting $\delta^{13}C$ measurements of charcoal, and Jussi Heinonsalo for providing the *P. sylvestris* seedlings. The CSC IT Center for Science, Finland is acknowledged for providing computational resources for processing the sequencing data.

References

Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R.H., Kõljalg, U., 2021. UNITE Mothur Release for Fungi 2. Version 10.05.2021. Unite Community. https://doi.org/10.15156/BIO/1265829.

Apprill, A., McNally, S., Parsons, R., Weber, L., 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat. Microb. Ecol. 75, 129–137. https://doi.org/10.3354/ame01753.

Appuhn, A., Joergensen, R.G., 2006. Microbial colonisation of roots as a function of plant species. Soil Biol. Biochem. 38, 40–51. https://doi.org/10.1016/j.soilbio.2005.09.002.

Averill, C., Hawkes, C.V., 2016. Ectomycorrhizal fungi slow soil carbon cycling. Ecol. Lett. 19, 937–947. https://doi.org/10.1111/ele.12631.

Baldrian, P., 2017. Microbial activity and the dynamics of ecosystem processes in forest soils. Curr. Opin. Microbiol. 37, 128–134. https://doi.org/10.1016/j.mib.2017.06.008.

Baldrian, P., 2017b. Forest microbiome: diversity, complexity and dynamics. FEMS Microbiol. Rev. 41, 109–130. https://doi.org/10.1093/femsre/fuw040.

Basler, A., Dippold, M., Helfrich, M., Dyckmans, J., 2015. Microbial carbon recycling – an underestimated process controlling soil carbon dynamics – part 1: a long-term laboratory incubation experiment. Biogeosciences 12, 5929–5940. https://doi.org/10.5194/bg-12-5929-2015.

Beidler, K.V., Phillips, R.P., Andrews, E., Maillard, F., Mushinski, R.M., Kennedy, P.G., 2020. Substrate quality drives fungal necromass decay and decomposer community structure under contrasting vegetation types. J. Ecol. 108, 1845–1859. https://doi.org/10.1111/1365-2745.13385.

Bell, C.W., Fricks, B.E., Rocca, J.D., Steinweg, J.M., McMahon, S.K., Wallenstein, M.D., 2013. High-throughput fluorometric measurement of potential soil extracellular enzyme activities. J. Vis. Exp. 81, e50961 https://doi.org/10.3791/50961.

de Boer, W., Folman, L.B., Summerbell, R.S., Boddy, L., 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol. Rev. 29, 795–811. https://doi.org/10.1016/j.femsre.2004.11.005.

Brabcová, V., Nováková, M., Davidová, A., Baldrian, P., 2016. Dead fungal mycelium in forest soil represents a decomposition hotspot and a habitat for a specific microbial community. New Phytol. 210, 1369–1381. https://doi.org/10.1111/nph.13849.

Buckeridge, K.M., La Rosa, A.F., Mason, K.E., Whitaker, J., McNamara, N.P., Grant, H.K., Ostle, N.J., 2020. Sticky dead microbes: rapid abiotic retention of microbial necromass in soil. Soil Biol. Biochem. 149, 107929 https://doi.org/10.1016/j.soilbio.2020.107929.

Carini, P., Marsden, P.J., Leff, J.W., Morgan, E.E., Strickland, M.S., Fierer, N., 2016. Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. Nat. Microbiol. 2, 1–6. https://doi.org/10.1038/nmicrobiol.2016.242.

Chen, G., Ma, S., Tian, D., Xiao, W., Jiang, L., Xing, A., Zou, A., Zhou, L., Shen, H., Zheng, C., Ji, C., He, H., Zhu, B., Liu, L., Fang, J., 2020. Patterns and determinants of soil microbial residues from tropical to boreal forests. Soil Biol. Biochem. 151, 108059 https://doi.org/10.1016/j.soilbio.2020.108059.

Clemmensen, K.E., Finlay, R.D., Dahlberg, A., Stenlid, J., Wardle, D.A., Lindahl, B.D., 2015. Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. New Phytol. 205, 1525–1536. https://doi.org/10.1111/nph.13208.

Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. Ecology 88, 2105–2113. https://doi.org/10.1890/06-1847.1.

Cui, J., Zhu, Z., Xu, X., Liu, S., Jones, D.L., Kuzyakov, Y., Shibistova, O., Wu, J., Ge, T., 2020. Carbon and nitrogen recycling from microbial necromass to cope with C:N stoichiometric imbalance by priming. Soil Biol. Biochem. 142, 107720 https://doi.org/10.1016/j.soilbio.2020.107720.

Dippold, M.A., Boesel, S., Gunina, A., Kuzyakov, Y., Glaser, B., 2014. Improved $\delta^{13}C$ analysis of amino sugars in soil by ion chromatography-oxidation-isotope ratio mass spectrometry. Rapid Commun. Mass Spectrom. 28, 569–576. https://doi.org/10.1002/rcm.6814.

Dong, W., Song, A., Yin, H., Liu, X., Li, J., Fan, F., 2021. Decomposition of microbial necromass is divergent at the individual taxonomic level in soil. Front. Microbiol. 12, 1674. https://doi.org/10.3389/fmicb.2021.679793.

Fan, Y., Yang, L., Zhong, X., Yang, Z., Lin, Y., Guo, J., Chen, G., Yang, Y., 2020. N addition increased microbial residual carbon by altering soil P availability and microbial composition in a subtropical Castanopsis forest. Geoderma 375, 114470. https://doi.org/10.1016/j.geoderma.2020.114470.

Faust, S., Heinze, S., Ngosong, C., Sradnick, A., Oltmanns, M., Raupp, J., Geisseler, D., Joergensen, R.G., 2017. Effect of biodynamic soil amendments on microbial communities in comparison with inorganic fertilization. Appl. Soil Ecol. 114, 82–89. https://doi.org/10.1016/j.apsoil.2017.03.006.

Fernandez, C.W., Koide, R.T., 2014. Initial melanin and nitrogen concentrations control the decomposition of ectomycorrhizal fungal litter. Soil Biol. Biochem. 77, 150–157. https://doi.org/10.1016/j.soilbio.2014.06.026.

Fernandez, C.W., Langley, J.A., Chapman, S., McCormack, M.L., Koide, R.T., 2016. The decomposition of ectomycorrhizal fungal necromass. Soil Biol. Biochem. 93, 38–49. https://doi.org/10.1016/j.soilbio.2015.10.017.

Fischer, H., Ingwersen, J., Kuzyakov, Y., 2010. Microbial uptake of low-molecular-weight organic substances out-competes sorption in soil. Eur. J. Soil Sci. 61, 504–513. https://doi.org/10.1111/j.1365-2389.2010.01244.x.

Fontaine, S., Mariotti, A., Abbadie, L., 2003. The priming effect of organic matter: a question of microbial competition? Soil Biol. Biochem. 35, 837–843. https://doi.org/10.1016/S0038-0717(03)00123-8.

Frostegård, A., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol. Fertil. Soils 22, 59–65. https://doi.org/10.1007/BF00384433.

Frostegård, Å., Tunlid, A., Bååth, E., 1991. Microbial biomass measured as total lipid phosphate in soils of different organic content. J. Microbiol. Methods 14, 151–163. https://doi.org/10.1016/0167-7012(91)90018-L.

Frostegård, Å., Tunlid, A., Bååth, E., 1996. Changes in microbial community structure during long-term incubation in two soils experimentally contaminated with metals. Soil Biol. Biochem. 28, 55–63. https://doi.org/10.1016/0038-0717(95)00100-X.

Gadgil, R.L., Gadgil, P.D., 1971. Mycorrhiza and litter decomposition. Nature 233, 133. https://doi.org/10.1038/233133a0.

Geyer, K., Schnecker, J., Grandy, A.S., Richter, A., Frey, S., 2020. Assessing microbial residues in soil as a potential carbon sink and moderator of carbon use efficiency. Biogeochemistry 151, 237–249. https://doi.org/10.1007/s10533-020-00720-4.

Glaser, B., Lehmann, J., Zech, W., 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal - a review. Biol. Fertil. Soils 35, 219–230. https://doi.org/10.1007/s00374-002-0466-4.

Glaser, B., Turrión, M.B., Alef, K., 2004. Amino sugars and muramic acid - biomarkers for soil microbial community structure analysis. Soil Biol. Biochem. 36, 399–407. https://doi.org/10.1016/j.soilbio.2003.10.013.

Glaser, B., Millar, N., Blum, H., 2006. Sequestration and turnover of bacterial- and fungal-derived carbon in a temperate grassland soil under long-term elevated atmospheric pCO_2. Glob. Chang. Biol. 12, 1521–1531. https://doi.org/10.1111/j.1365-2486.2006.01186.x.

Griepentrog, M., Bodé, S., Boeckx, P., Hagedorn, F., Heim, A., Schmidt, M.W.I., 2014. Nitrogen deposition promotes the production of new fungal residues but retards the decomposition of old residues in forest soil fractions. Glob. Chang. Biol. 20, 327–340. https://doi.org/10.1111/gcb.12374.

Gunina, A., Dippold, M., Glaser, B., Kuzyakov, Y., 2017. Turnover of microbial groups and cell components in soil: ^{13}C analysis of cellular biomarkers. Biogeosciences 14, 271–283. https://doi.org/10.5194/bg-14-271-2017.

Hu, J., Huang, C., Zhou, S., Liu, X., Dijkstra, F.A., 2022. Nitrogen addition increases microbial necromass in croplands and bacterial necromass in forests: a global meta-analysis. Soil Biol. Biochem. 165, 108500 https://doi.org/10.1016/j.soilbio.2021.108500.

Ihrmark, K., Bödeker, I.T.M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K.E., Lindahl, B.D., 2012. New primers to amplify the fungal ITS2 region–evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiol. Ecol. 82, 666–677. https://doi.org/10.1111/j.1574-6941.2012.01437.x.

Insam, H., 1990. Are the soil microbial biomass and basal respiration governed by the climatic regime? Soil Biol. Biochem. 22, 525–532. https://doi.org/10.1016/0038-0717(90)90189-7.

Jenkinson, D.S., 1988. The determination of microbial biomass carbon and nitrogen in soil. In: Wilson, J.R. (Ed.), Advances in Nitrogen Cycling in Agricultural Ecosystems. CAB International, Wallingford, UK, pp. 368–386.

Jones, R.T., 2015. A comprehensive survey of soil rhizobiales diversity using high-throughput DNA sequencing. In: de Bruijn, F.J. (Ed.), Biological Nitrogen Fixation. Wiley Online Books, pp. 769–775. https://doi.org/10.1002/9781119053095.ch76.

Kallenbach, C.M., Grandy, A.S., Frey, S.D., Diefendorf, A.F., 2015. Microbial physiology and necromass regulate agricultural soil carbon accumulation. Soil Biol. Biochem. 91, 279–290. https://doi.org/10.1016/j.soilbio.2015.09.005.

Karhu, K., Hilasvuori, E., Fritze, H., Biasi, C., Nykänen, H., Liski, J., Vanhala, P., Heinonsalo, J., Pumpanen, J., 2016. Priming effect increases with depth in a boreal forest soil. Soil Biol. Biochem. 99, 104–107. https://doi.org/10.1016/j.soilbio.2016.05.001.

Karhu, K., Alaei, S., Li, J., Merilä, P., Ostonen, I., Bengtson, P., 2022. Microbial carbon use efficiency and priming of soil organic matter mineralization by glucose additions in boreal forest soils with different C:N ratios. Soil Biol. Biochem. 167, 108615 https://doi.org/10.1016/J.SOILBIO.2022.108615.

Klamer, M., Bååth, E., 2004. Estimation of conversion factors for fungal biomass determination in compost using ergosterol and PLFA 18:2ω6,9. Soil Biol. Biochem. 36, 57–65. https://doi.org/10.1016/j.soilbio.2003.08.019.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl. Environ. Microbiol. 79, 5112–5120. https://doi.org/10.1128/AEM.01043-13.

Lewin, G.R., Carlos, C., Chevrette, M.G., Horn, H.A., McDonald, B.R., Stankey, R.J., Fox, B.G., Currie, C.R., 2016. Evolution and ecology of actinobacteria and their bioenergy applications. Annu. Rev. Microbiol. 8, 235–254. https://doi.org/10.1146/annurev-micro-102215-095748.

Li, L., Wilson, C.B., He, H., Zhang, X., Zhou, F., Schaeffer, S.M., 2019. Physical, biochemical, and microbial controls on amino sugar accumulation in soils under long-term cover cropping and no-tillage farming. Soil Biol. Biochem. 135, 369–378. https://doi.org/10.1016/j.soilbio.2019.05.017.

Liang, C., 2020. Soil microbial carbon pump: mechanism and appraisal. Soil Ecol. Lett. 2, 241–254. https://doi.org/10.1007/s42832-020-0052-4.

Liang, C., Balser, T., 2011. Microbial production of recalcitrant organic matter in global soils: implications for productivity and climate policy. Nat. Rev. Microbiol. 9, 75. https://doi.org/10.1038/nrmicro2386-c1.

Liang, C., Schimel, J., Jastrow, J., 2017. The importance of anabolism in microbial control over soil carbon storage. Nat. Microbiol. 2, 17105. https://doi.org/10.1038/nmicrobiol.2017.105.

Liang, C., Amelung, W., Lehmann, J., Kästner, M., 2019. Quantitative assessment of microbial necromass contribution to soil organic matter. Glob. Chang. Biol. 25, 3578–3590. https://doi.org/10.1111/gcb.14781.

Lindahl, B.O., Taylor, A.F.S., Finlay, R.D., 2002. Defining nutritional constraints on carbon cycling in boreal forests – towards a less "phytocentric" perspective. Plant Soil 242, 123–135. http://www.jstor.org/stable/24120910.

Maillard, F., Schilling, J., Andrews, E., Schreiner, K.M., Kennedy, P., 2020. Functional convergence in the decomposition of fungal necromass in soil and wood. FEMS Microbiol. Ecol. 96, fiz209. https://doi.org/10.1093/femsec/fiz209.

Martens, D.A., 2000. Plant residue biochemistry regulates soil carbon cycling and carbon sequestration. Soil Biol. Biochem. 32, 361–369. https://doi.org/10.1016/S0038-0717(99)00162-5.

Marx, M.C., Wood, M., Jarvis, S.C., 2001. A microplate fluorimetric assay for the study of enzyme diversity in soils. Soil Biol. Biochem. 33, 1633–1640. https://doi.org/10.1016/S0038-0717(01)00079-7.

Meier, I.C., Finzi, A.C., Phillips, R.P., 2017. Root exudates increase N availability by stimulating microbial turnover of fast-cycling N pools. Soil Biol. Biochem. 106, 119–128. https://doi.org/10.1016/j.soilbio.2016.12.004.

Meyer, N., Welp, G., Rodionov, A., Borchard, N., Martius, C., Amelung, W., 2018. Nitrogen and phosphorus supply controls soil organic carbon mineralization in tropical topsoil and subsoil. Soil Biol. Biochem. 119, 152–161. https://doi.org/10.1016/j.soilbio.2018.01.024.

Mikryukov, V., 2017. metagMisc: Miscellaneous Functions for Metagenomic Analysis.

Miltner, A., Bombach, P., Schmidt-Brücken, B., Kästner, M., 2012. SOM genesis: microbial biomass as a significant source. Biogeochemistry 111, 41–55. https://doi.org/10.1007/s10533-011-9658-z.

Moorhead, D.L., Sinsabaugh, R.L., 2006. A theoretical model of litter decay and microbial interaction. Ecol. Monogr. 76, 151–174. https://doi.org/10.1890/0012-9615(2006)076[0151:ATMOLD]2.0.CO;2.

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 20, 241–248. https://doi.org/10.1016/j.funeco.2015.06.006.

Ni, X., Liao, S., Tan, S., Wang, D., Peng, Y., Yue, K., Wu, F., Yang, Y., 2020. A quantitative assessment of amino sugars in soil profiles. Soil Biol. Biochem. 143, 107762 https://doi.org/10.1016/j.soilbio.2020.107762.

Nordgren, A., 1992. A method for determining microbially available N and P in an organic soil. Biol. Fertil. Soils 13, 195–199. https://doi.org/10.1007/BF00340575.

Ohlson, M., Dahlberg, B., Økland, T., Brown, K.J., Halvorsen, R., 2009. The charcoal carbon pool in boreal forest soils. Nat. Geosci. 2, 692–695. https://doi.org/10.1038/ngeo617.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2017. vegan: Community Ecology Package.

Parada, A.E., Needham, D.M., Fuhrman, J.A., 2016. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ. Microbiol. 18, 1403–1414. https://doi.org/10.1111/1462-2920.13023.

Pietikäinen, J., Kiikkilä, O., Fritze, H., 2000. Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. Oikos 89, 231–242. https://doi.org/10.1034/j.1600-0706.2000.890203.x.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596. https://doi.org/10.1093/nar/gks1219.

R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Read, D.J., Perez-Moreno, J., 2003. Mycorrhizas and nutrient cycling in ecosystems - a journey towards relevance? New Phytol. 157, 475–492. https://doi.org/10.1046/j.1469-8137.2003.00704.x.

Roberts, D.W., 2016. labdsv: Ordination and Multivariate Analysis for Ecology.

Schäfer, J., Jäckel, U., Kämpfer, P., 2010. Development of a new PCR primer system for selective amplification of Actinobacteria. FEMS Microbiol. Lett. 311, 103–112. https://doi.org/10.1111/j.1574-6968.2010.02069.x.

Schielzeth, H., Dingemanse, N.J., Nakagawa, S., Westneat, D.F., Allegue, H., Teplitsky, C., Réale, D., Dochtermann, N.A., Garamszegi, L.Z., Araya-Ajoy, Y.G., 2020. Robustness of linear mixed-effects models to violations of distributional assumptions. Methods Ecol. Evol. 11, 1141–1152. https://doi.org/10.1111/2041-210X.13434.

Schimel, J.P., Weintraub, M.N., 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biol. Biochem. 35, 549–563. https://doi.org/10.1016/S0038-0717(03)00015-4.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09.

Sietiö, O.-M., Tuomivirta, T., Santalahti, M., Kiheri, H., Timonen, S., Sun, H., Fritze, H., Heinonsalo, J., 2018. Ericoid plant species and *Pinus sylvestris* shape fungal communities in their roots and surrounding soil. New Phytol. 218, 738–751. https://doi.org/10.1111/nph.15040.

Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant Soil 241, 155–176. https://doi.org/10.1023/A:1016125726789.

Smith, S.E., Read, D., 2008. Mycorrhizal Symbiosis. Academic Press, London.

Soong, J.L., Fuchslueger, L., Marañon-Jimenez, S., Torn, M.S., Janssens, I.A., Penuelas, J., Richter, A., 2020. Microbial carbon limitation: the need for integrating microorganisms into our understanding of ecosystem carbon cycling. Glob. Chang. Biol. 26, 1953–1961. https://doi.org/10.1111/gcb.14962.

Sparling, G., Vojvodić-Vuković, M., Schipper, L.A., 1998. Hot-water-soluble C as a simple measure of labile soil organic matter: the relationship with microbial biomass C. Soil Biol. Biochem. 30, 1469–1472. https://doi.org/10.1016/S0038-0717(98)00040-6.

Spohn, M., Pötsch, E.M., Eichorst, S.A., Woebken, D., Wanek, W., Richter, A., 2016. Soil microbial carbon use efficiency and biomass turnover in a long-term fertilization experiment in a temperate grassland. Soil Biol. Biochem. 97, 168–175. https://doi.org/10.1016/j.soilbio.2016.03.008.

Strickland, M.S., Rousk, J., 2010. Considering fungal: bacterial dominance in soils—methods, controls, and ecosystem implications. Soil Biol. Biochem. 42, 1385–1395. https://doi.org/10.1016/j.soilbio.2010.05.007.

Stuart, E.K., Plett, K.L., 2020. Digging deeper: in search of the mechanisms of carbon and nitrogen exchange in ectomycorrhizal symbioses. Front. Plant Sci. 10, 1658. https://doi.org/10.3389/fpls.2019.01658.

Talbot, J.M., Treseder, K.K., 2010. Controls over mycorrhizal uptake of organic nitrogen. Pedobiologia 53, 169–179. https://doi.org/10.1016/j.pedobi.2009.12.001.

Tan, K.H., 2014. Humic Matter in Soil and the Environment: Principles and Controversies, 2nd edition. CRC Press, New York.

Totsche, K.U., Amelung, W., Gerzabek, M.H., Guggenberger, G., Klumpp, E., Knief, C., Lehndorff, E., Mikutta, R., Peth, S., Prechtel, A., Ray, N., Kögel-Knabner, I., 2018.

Microaggregates in soils. J. Plant Nutr. Soil Sci. 181, 104–136. https://doi.org/10.1002/jpln.201600451.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707. https://doi.org/10.1016/0038-0717(87)90052-6.

Wang, B., An, S., Liang, C., Liu, Y., Kuzyakov, Y., 2021. Microbial necromass as the source of soil organic carbon in global ecosystems. Soil Biol. Biochem. 162, 108422 https://doi.org/10.1016/j.soilbio.2021.108422.

Wang, C., Wang, X., Pei, G., Xia, Z., Peng, B., Sun, L., Wang, J., Gao, D., Chen, S., Liu, D., Dai, W., Jiang, P., Fang, Y., Liang, C., Wu, N., Bai, E., 2020. Stabilization of microbial residues in soil organic matter after two years of decomposition. Soil Biol. Biochem. 141, 107687 https://doi.org/10.1016/j.soilbio.2019.107687.

Warnes, G.R., Bolker, B., Bonebakker, L., Gentleman, R., Huber, W., Liaw, A., Lumley, T., Maechler, M., Magnusson, A., Moeller, S., Schwartz, M., Venables, B., 2016. gplots: Various R Programming Tools for Plotting Data.

Yilmaz, P., Parfrey, L.W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J., Ludwig, W., Glöckner, F.O., 2014. The SILVA and "all-species living tree project (LTP)" taxonomic frameworks. Nucleic Acids Res. 42, D643–D648. https://doi.org/10.1093/nar/gkt1209.

Zech, W., Ziegler, F., Kögel-Knabner, I., Haumaier, L., 1992. Humic substances distribution and transformation in forest soils. Sci. Total Environ. 117–118, 155–174. https://doi.org/10.1016/0048-9697(92)90084-6.

Zhang, X., Amelung, W., 1996. Gas chromatographic determination of muramic acid, glucosamine, mannosamine, and galactosamine in soils. Soil Biol. Biochem. 28, 1201–1206. https://doi.org/10.1016/0038-0717(96)00117-4.