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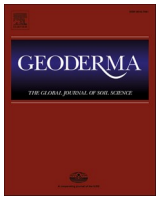

Organic carbon loading of soils determines the fate of added fresh plant-derived organic matter

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ABSTRACT

It is crucial to promote soil carbon sequestration while reducing CO_2 emissions to mitigate climate change. However, the extent of increasing actual soil carbon storage depends on the amount and composition of organic matter input, including its fate during decomposition and soil organic matter (SOM) formation via microbial transformation. With respect to the need to increase carbon sequestration in soil and sustain soil fertility, it is of great interest to better understand how soils with different organic matter content react to amendment with fresh organic matter. Here, we incubated three agricultural soils representing a gradient in C content, adding two different ^{13}C labeled plant residues varying in carbon-to-nitrogen ratio. Carbon mineralization was monitored together with the analysis of the $^{13}CO_2$ signatures. After the incubation, ^{13}C compound-specific PLFAs, microbial necromass, and enzyme activities were analyzed. This study demonstrates that the carbon return on investment, thus the amount of retained fresh carbon in relation to the amount of added organic matter, clearly depends on the amount of native soil carbon. Notably, the addition of fresh organic matter to carbon-deficient soils leads to a higher specific CO_2 release compared to soils with high carbon loading, which can be attributed to the differences in the soil microorganisms' response. The CO_2 release of the soil with the lowest C-content was 2.1 and 2.0 mg g^{-1} soil for treatment with oat and pea litter addition, respectively, whereas for the soil with the highest C-content, CO_2 release was 1.7 mg g^{-1} soil for oat treatment and 1.6 mg g^{-1} soil for pea treatment. Thus, higher SOC contents sustain a higher 'return on investment' for the fresh carbon that is amended to soils. With plant litter amendments the microbial community shifted towards a higher fungi-to-bacteria ratio (F/B). This shift in the microbial community was more pronounced (F/B ranging from 0.04 to 0.11) with the addition of oat litter (low quality) compared to pea litter (high quality). Hence, it is important to consider the fate of organic amendments with different N availability when aiming to rebuild soil carbon stocks in degraded soils. Soil management should focus on sustaining soil carbon in balance with current carbon stocks to avoid the vicious circle of soils losing carbon in conjunction with increased greenhouse gas release.

1. Introduction

Soils are vital as the largest terrestrial carbon (C) pool in global C-cycling and storage (Post et al., 1982; Scharlemann et al., 2014). Moreover, with accelerating climate change, promoting sustainable C sequestration in soils is an essential tool to store atmospheric carbon dioxide (CO_2), maintain soil multi-functionalities such as in food and fiber production or biodiversity conservation, and adapt to climate change (Kopittke et al., 2022).

Agricultural land use has drastically reduced soil C stocks over

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millennia (Sanderman et al., 2017). Thus, agricultural soils are depleted in soil organic matter (SOM), particularly in areas with a long history of intensive cultivation (Don et al., 2011; Stewart et al., 2008). Furthermore, studies have shown that a soil organic C (SOC) content of 2 % is the critical minimum threshold for soil productivity (Stockmann et al., 2015; Zvomuya et al., 2008). It is assumed that soils have a specific capacity to store a certain amount of C, which has been suggested to be defined by the amount of fine-sized soil minerals (Hassink, 1997). Based on the concept of a distinct storage capacity of soil C, soils reach C saturation with increasing amounts of stored C (Hassink, 1997; Stewart et al., 2007), with limits defined by the amount of fine-sized minerals. Thus, soils maintaining low-C saturation levels, which we also refer to as soil C loading here, exhibit a greater potential to sequester additional amounts of C. Soil management has thus to focus on either increasing soils' SOC stock, if depleted, or maintaining SOC stocks in cases of soils at high-C saturation level. As plant residues represent the main C input to soils, the key to managing soil C stocks is to know how soils with different organic matter (OM) content and thus C saturation levels react to plant litter input. And it is crucial to better understand how soils tend to convert fresh plant-derived C into more persistent SOC forms based on their C loading, and thus contribute to long-lasting soil C storage.

The amount of SOC, which is stored either as particulate OM (POM) or as mineral-associated OM (MAOM), is the result of the decomposition of plant-derived OM, mainly fresh rather easily bioavailable POM, and the parallel formation of more persistent SOM forms (Angst et al., 2023). The activity and distribution of soil microorganisms control the fate of fresh OM and, consequently, the overall soil C flow, which regulates the ratio between C mineralization (soil respiration) and persistent SOM formation. This process is achieved by building up microbial biomass, and its subsequent entombing as microbial necromass C (Liang et al., 2017; Schimel and Schaeffer, 2012). Due to its pronounced association with MAOM, necromass C is one of the main components of rather persistent SOC (Angst et al., 2021; Hall et al., 2020). Although factors such as the quantity of plant litter input and its nitrogen (N) content have been studied extensively in their effect on C mineralization (Brown et al., 2014; Dungait et al., 2012; Mendoza et al., 2022; Nguyen et al., 2016), knowledge on how the C saturation of soil affects the fate of freshly added C remains scarce.

Fungi and bacteria, the major groups of soil microbiota, are the main drivers of soil C dynamics (Six et al., 2006). Microbial habitats, which vary in C saturation level, influence the soil microorganisms' life strategies by fostering certain densities of microorganisms that a specific soil environment can support (r- vs. K-strategy) (Andrews and Harris, 1986). Thus, the life strategy of microorganisms determines C mineralization, which is reflected in soil respiration. At stoichiometric imbalanced conditions, fungi and bacteria adjust the consumption of OM sources in relation to their demand for C and nutrients to meet their needs for energy and growth. Therefore, in addition to the amount of available soil C as an energy source, the amount of N and its availability determines soil C dynamics. The carbon-to-nitrogen ratio (C/N) of plant litter input thus directly affects soil C dynamic through its impact on the microbial community (Cotrufo et al., 2013; Gentile et al., 2011; Nguyen et al., 2016). It was shown that C availability limits especially bacterial growth, whereas N limitations stronger affect fungal growth (Rousk and Baath, 2007; Wang et al., 2014). Consequently, soils comprising different C saturation levels and the addition of plant litter with varying N content lead to significant differences in the mineralization of organic matter (Parkin et al., 1997; Wang et al., 2013). However, the mechanism by which soil C saturation and the N availability of the plant litter input influence the fate of soil C is not well elucidated.

To address the mentioned research gaps, we incubated three agricultural soils with clearly differing C saturation levels together with plant residues, either with narrow or wide C to N ratio and thus a different N availability. The work is based on the following hypotheses: 1) although gains in C storage is highest in soils with low C stocks, C return on investment is higher in soils with higher C saturation level and, thus, soils with higher C loading; and 2) plant litter rich in N promotes C retention in soils. The study's findings provide potential strategies to steer the use of plant OM amendments to keep or even increase soil C storage and thus sustain soil multi-functionality, including climate change mitigation. Furthermore, it is one important key to supporting future management strategies for sustaining or increasing SOM loading, especially in view of the recovery of C-depleted soils in agroecosystems.

2. Material and methods

2.1. Soil samples

To compare soil with different OM loadings, three agricultural soils, including bare fallow, three-field crop rotation, and direct seeding, were used in this study. These three soils with a SOC gradient were collected from a long-term field experiment in Puch (southern Germany, 48°11′37.0″N, 11°12′57.4″E), which is maintained by the Bavarian State Research Center for Agriculture (LfL). All experimental sites are located at an elevation of 550 m a.s.l. with a mean annual temperature of 8.8 °C and a mean annual precipitation of 872 mm. All three soils are characterized as Luvisols derived from loess deposits (IUSS Working Group WRB, 2015) and share the same soil mineralogy due to the same parent material and the close vicinity of the plots. There were no carbonates detectable in the soils from the same experimental plots as demonstrated in a the previous study (Wu et al., 2022).

Soil material used for the incubation experiment was sampled from the upper 10 cm of the Ap horizon at random locations within each plot (five spots per plot) and mixed thoroughly to form a composite sample. As the focus was on the mechanistic understanding of the actual fate of added plant OM on soil material differing in C loading, the composite samples represent the specific management plot. The fresh soil samples were air-dried at room temperature and sieved with a 2 mm mesh size. All visible plant residues were picked out by hand. Sub-samples were taken from each soil to determine basic soil properties (Table 1). Based on the SOC contents, we referred to the soil of bare fallow as low-C soil, three-field crop rotation as medium-C soil, and soil of the direct seeding fields as high-C soil in the following study. The initial SOC level of each soil is referred to as C loading.

The total C and N contents of bulk soils pre- and post-incubation combined with ^{13}C enrichment were measured using an isotope ratio mass spectrometer (IRMS, Delta V Advantage, Thermo Fisher, Dreieich, Germany) that was coupled to an elemental analyzer (EuroEA, Eurovector, Pavia, Italy). The enrichment value is given in atom-% (at%).

2.2. Plant residues used as organic input

Since C and N are intricately linked in the fate and turnover of SOM, we used two ^{13}C-labeled plant residues differing in C/N ratio (Table 2) to study the dynamic of OM. Oat and pea plants were chosen due to their difference in C/N ratio. Plants were grown in an arable soil in the greenhouse at Rhine-Waal University and ^{13}C-leaf labeled with multiple pulses over ten days using ^{13}C-glucose solution (2 % w/v) starting four weeks after planting. This labelling approach has been shown to efficiently label plants with ^{15}N (Kanders et al., 2017) with values being in the range of other approaches albeit like all labelling approaches having some shortcomings, which have been discussed elsewhere (e.g. Hupe et al. (2016), Wichern et al. (2008), Wichern et al. (2011), and Kanders et al. (2017)). To this end, one leaf was forced into a 2 ml vial containing the sterile solution. Upon complete uptake, an additional ^{13}C-glucose solution was added to the vial. After another two weeks, the labeled leaf was removed (not further considered in this experiment), and plant stems and leaves were harvested, air-dried, and finely ground to be used in this study. We ground and homogenized the plant material prior to the experiment so that similar material was applied to all soils in each treatment. Although the plants were not homogenously labelled, it likely not strongly affected the outcome of our experiment.

Table 1
Soil characteristics of bare fallow (low-C soil), three crop rotation (medium-C soil), and direct seeding (high-C soil). The particle size distribution was determined by texture analysis, as given by Wu et al. (2022). EC stands for electrical conductivity. The SOC storage capacity for those three soil was calculated according to Feng et al. (2013) and Wiesmeier et al. (2019).

Soil	Total C content (mg g^{-1})	Total N content (mg g^{-1})	C/N	pH	EC	Clay and fine silt content (mg g^{-1})	POM (mg g^{-1})	POM C/N	SOC storage capacity (mg g^{-1})	SOC saturation level (%)
Bare fallow (low-C soil)	6.4 ± 0.1	0.8 ± 0.0	8.4 ± 0.1	6.39	54.2	368	1.8	22.4	13.5	36.8
Three crop rotation (medium-C soil)	12.5 ± 0.0	1.4 ± 0.0	9.0 ± 0.2	6.86	60.8	450	4.7	14.9	16.1	59.6
Direct seeding (high-C soil)	20.7 ± 0.0	2.1 ± 0.0	9.9 ± 0.2	7.63	67.2	501	8.9	16.4	17.7	92

2.3. Organic matter addition and incubation experiment

The amount of added plant residue was based on common management practice and thus based on the added OC to soils via the regular amounts of plant residue input (Wiesmeier et al., 2014a). The ground plant residues (oat and pea litter) were added once at the start of the incubation, and thoroughly mixed with 50 g of the respective soils. The added OM accounted for amounts of 0 mg g^{-1} (control) and 10 mg g^{-1} (equivalent to 0 and 13 Mg OC ha^{-1}) based on amounts of normally added OC via harvest residues in the area. The experiment was conducted in a 3 × 3 full factorial design with three plant residues (control, oats, and peas) and three soils (low, medium, and high-C) with five replicates, totaling 45 samples.

Each sample was incubated in polyvinyl chloride (PVC) cylinder of 50 mm in height with a 45.2 mm inner diameter (Fig. A1). Furthermore, a mono-layer mesh (100 % polyester, mesh size 55 μm) was fixed at the bottom of the cylinder with a ring (O-ring, nitrile rubber) to allow ventilation and prevent stagnating water. The cylinder was placed in a 473 ml glass jar (gas-tight, Mason Glass, KoRo) with a lid on a small metal grid to allow better gas exchange at the bottom. One tube fitting (stainless steel, Swagelok) with a septum (Standard Septa, Wagner&munz) was installed on the lid for gas sampling.

Adding water to the dry soils was considered the starting time point of the incubation. Prior to our incubation, we tested soils at water holding capacities (WHC) between 40 % and 70 % with an increment of 10 % to investigate the optimal WHC, which ensure that the soil should not be dry on the surface after 48 h of incubation. The moisture content of each sample was controlled every 2 days, and if needed, water was added to sustain constant water availability. The test yielded a moisture content of 50 % WHC as optimal water availability to sustain microbial activity in all studied soils. Thus, water was added as a fine droplet to adjust the soil moisture to 50 % of the maximal water-holding capacity. Furthermore, the incubation was carried out for 90 days in a dark thermostatic room of 22 ℃ with the jars partly opened to avoid anaerobic conditions.

After 90 days, the incubation experiment was stopped and sampled. Each soil sample was well mixed in the cylinder to ensure a homogeneous soil sample for all further analysis. An aliquot of each sample was stored at 4 ℃ for PLFA and enzyme analysis (fresh samples). Approximately 1 and 10 g of fresh soil sub-samples were obtained from each setup for PLFA and enzyme analysis, respectively. The remaining soil material of each sample was immediately frozen using liquid nitrogen to stop any biological activity and subsequently freeze-dried to yield dry soil material.

2.4. Soil respiration measurements

Table 2
^{13}C enrichment, C and N content, and the C/N ratio of oat and pea powders.

Plant powder	C content (mg g^{-1})	^{13}C atom %	N content (mg g^{-1})	C/N
Oat (*Avena sativa* L.)	440	2.18	15.9	27.8
Pea (*Pisum sativum* L.)	432	1.69	40.2	10.7

We measured soil CO_2 production and $^{13}CO_2$ signatures at seven time points during the 90 days of incubation. Gas sampling was conducted after 24 h, 72 h, 7 days, 14 days, 30 days, 60 days, and 90 days of incubation. Therefore, gas samples were taken from the headspace in each jar using a 25 ml gas-tight syringe (Model 1025 TLL, Hamilton) and injected into an evacuated vial through a septum (Exetainer 12 ml, flat bottom, Labco, UK). Prior to each gas sampling, the syringe was rinsed 3 times with argon (Ar) to remove any contamination.

At the sampling time, the jars were sealed, and 70 ml Ar was injected to avoid negative pressure in the jars due to the gas sampling. The inert gas ensured that the composition of the air in the jar remained constant. We created a higher pressure inside the vial than the air pressure by injecting a 12.5 ml gas sample into the evacuated vial to exclude the environmental gas interference in the sample.

At each gas sampling occasion, each setup's first gas sample (t_0) was taken immediately after injecting the Ar. Then, after the jars were sealed for a certain time (t_1), the second ($t_0 + t_1$), third ($t_0 + 2\ t_1$), and fourth ($t_0 + 3\ t_1$) gas samples of each jar were taken. The sealing time t_1 ranged between 40 min and 180 min, depending on the sampling day and the treatments.

The gas samples' $^{13}CO_2$ abundance and CO_2 concentration were measured using a gas chromatography (GC)-IRMS system (Delta plus, Thermo Fisher, Dreieich, Germany). The system enables injecting up to 400 μl of sample gas by trapping CO_2 with liquid nitrogen from repeated injections. The ^{13}C enrichment of the gas samples is given in atom-% ^{13}C (at%).

Using a linear regression model (Eq. (1)) of the CO_2 concentration according to the CO_2 concentration difference from the time of the four sampling points, we calculated the respired CO_2. The outliers (if any) were removed. All trend lines had $R^2 > 0.98$.

$$CO_2(ppm) = k\left(\frac{ppm}{min}\right) \times t(min) + A(ppm) \quad (1)$$

The slope $k = \frac{\Delta CO_2}{\Delta t}$, i.e., the rate with which CO_2 concentration change represents the respiration rate. The variable t is the time since the jar was sealed. A stands for the environmental CO_2 concentration calculated from the linear regression.

The respiration rate of CO_2 in the jar was calculated as follows (Eq. (2)):

$$Respiration\ CO_2\left(mg\ min^{-1}\right) = k\left(\frac{ppm}{min}\right) \times VHeadspace(mL) \times \frac{M_{CO_2}\left(\frac{mg}{mmol}\right)}{M_V\left(\frac{mL}{mmol}\right)} \times \left(\frac{10^{-6}}{ppm}\right) \quad (2)$$

VHeadspace is the available volume of the jar. M_{CO_2} is the molar weight of CO_2, and M_V is the gas molar volume at 22 ℃

The CO_2-C respiration rate was calculated using Eq. (3):

$$Respiration\ CO_2\text{-}C\left(mg\ min^{-1}\right) = Respiration\ CO_2\left(mg\ min^{-1}\right) \times \frac{M_C\left(\frac{mg}{mmol}\right)}{M_{CO_2}\left(\frac{mg}{mmol}\right)} \quad (3)$$

M_C is the molecular weight of C.

We calculated the CO_2-C respiration rate per gram of soil:

$$R_{CO_2\text{-}C}\left(mg\ min^{-1}\ g^{-1}Soil\right) = \frac{Respiration\ CO_2\text{-}C\left(mg\ min^{-1}\right)}{soil\ mass(g)} \quad (4)$$

where the soil mass is the dry weight of the soil sample per microcosm.

The CO_2-C respiration rate was normalized to each gram of SOC per gram of soil:

$$Normalized\ R_{CO_2\text{-}C}\left(mg\ min^{-1}\ g^{-1}SOC\right) = \frac{Respiration\ CO_2\text{-}C\left(mg\ min^{-1}\ g^{-1}Soil\right)}{SOC(mg\ g^{-1}Soil)} \times \left(\frac{10^3 mg}{g}\right) \quad (5)$$

The share of plant litter-derived C (f_{plant}) in CO_2 was calculated as follows:

$$f_{plant} = \frac{^{13}C_{sample} - {}^{13}C_{soil}}{^{13}C_{plant} - {}^{13}C_{soil}} \quad (6)$$

where $^{13}C_{sample}$, $^{13}C_{soil}$, and $^{13}C_{plant}$ represent the content of ^{13}C in at% in the gas sample, soil, and plant litter, respectively.

The native SOM-derived CO_2-C is calculated using Eq. (7):

$$Native\ SOM-derived\ CO_2\text{-}C(mg\ g^{-1}soil) = \left(1 - f_{plant}\right) \times R_{CO_2-C} \quad (7)$$

The priming is calculated as follows:

$$Priming(\%) = \frac{Respiration\ CO_2\text{-}C(treatment) - Respiration\ CO_2\text{-}C(control)}{Respiration\ CO_2\text{-}C(control)} \times 100\% \quad (8)$$

As no carbonates were detected as demonstrated in a previous study using the same soils from the same research site (Wu et al., 2022), no carbonate-derived CO_2-C emission had to be taken into consideration.

2.5. Compound-specific phospholipid fatty acid analysis and enzyme assays

2.5.1. Phospholipid fatty acid analysis

Soil material from the incubated samples was analyzed for their PLFAs. PLFA extraction was adapted from Frostegård et al. (1991). According to the presence of PLFAs in different groups of microorganisms, we classified the Gram-negative (G−) bacteria (16:1ω7c, cy17:0, 18:1ω7c, cy19:0 PLFAs), Gram-positive (G+) bacteria (i15:0, a15:0, i16:0, i17:0 PLFAs), actinobacteria (Ac) (10Me16:0, 10Me17:0 and 10Me18:0 PLFAs), and fungi (18:2ω6,9 PLFAs) (Lewandowski et al., 2015; Sommer et al., 2017). The markers a14:0, 14:1w5c, 14:0, 15:0, 16:0, 17:0, 18:3w3,6,9, 18:0, and 20:0 were classified as non-specified PLFAs.

Briefly, 6 g of incubated fresh soil sample was placed in a 50 ml centrifuge tube. The internal standard 1 (25 μl, 1 μg μl^{-1}, 19:0 phospholipid) was added. A single-phase mixture of chloroform, methanol, and citric buffer (0.15 M, pH 4.0) (1:2:0.8 v/v/v) was used to extract the lipids. The solution was transferred to a silica column, to which we added chloroform, acetone, and methanol in sequence to elute the neutral-, glycol-, and phospholipids, respectively. The phospholipids were hydrolyzed by 1 M sodium hydroxide solution in methanol, followed by methylation with 0.3 M BF_3 solution in methanol. The final products were extracted in hexane and dried using an N_2 stream. This sample was re-dissolved in toluene with 15 μl of 13:0 fatty acid methyl ester (1 μg μl^{-1}). The specific compound PLFAs were measured using gas chromatography-mass spectrometry (GC–MS), equipped with 15-m HP-1 methylpolysiloxane coupled with a 30-m HP-5 (5 % phenyl)-methylpolysiloxane column. Both columns have an internal diameter of 0.25 mm and a film thickness of 0.25 μm. The He flow was 2 ml min^{-1}, and the injection volume was 1 μl. Measurement temperatures were set at 80 ℃, increased to 164 ℃ at 10 ℃ min^{-1}, followed by an increasing rate of 0.7 ℃ min^{-1} to 230 ℃ until it reached 300 ℃ at 10 ℃ min^{-1}. The quantity of PLFA was calculated based on a calibration curve of external standards, followed by correcting internal standards (Gunina et al., 2014).

The $^{13}C/^{12}C$ ratios of the PLFAs were determined using an IRMS

(Delta Plus TM, Thermo Finnigan, Germany) coupled with a GC (Trace GC 2000, Thermo Finnigan, Germany). The ^{13}C enrichment was corrected for the effect of the derivative C based on Glaser and Amelung (2002). Additionally, the share of plant-derived ^{13}C in the PLFA in analogy to gas samples was calculated using Eq. (6). The plant litter-C incorporation was calculated using Eq. (9):

$$\text{plant litter} - \text{C incorporation}(\%) = \frac{^{13}C \text{ abundance in PLFAs}}{^{13}\text{C input in the soil}} \times 100\% \quad (9)$$

A PLFA-based F/B ratio was calculated using the abundance of total fungal PLFAs and bacterial PLFAs.

2.5.2. Enzyme assays

Enzyme activities involved in C, N, and P cycles were measured based on fluorimetric microplate assays (Marx et al., 2005; Marx et al., 2001). Based on the fluorogenic substrates, including 4-methylumbelliferone (MUF) and 7-amino-4-methyl coumarin (AMC) (Sigma-Aldrich), we analyzed the activities of six enzymes: β-glucosidase (BG), β-xylosidase (BX), β-cellobiohydrolase (BC), chitinase, leucine-aminopeptidase, and acid phosphatase. The activities of C-cycling enzymes BG, BX, and BC were detected using 4-methylumbelliferone-b-D-glucoside, 4-methylumbelliferone-7-b-D-xyloside, and -methylumbelliferone-b-D-cellobioside, respectively. The N-cycle-related enzymes chitinase and leucine-aminopeptidase were determined using L-tyrosine-7-amido-4-methyl-coumarin (AMC-T) and L-leucine-7-amino-4-methyl coumarin (AMC-L). 4-Methylumbelliferyl-phosphate (MUF-P) was used to determine the P cycle's acid phosphatase activity.

Briefly, around 1 g of soil (equivalent dry weight) was dispersed in 50 ml of deionized water using low-energy ultrasonication (50 J s^{-1}, 120 s) to create a suspension. Aliquots of 50 μl suspension and 50 μl buffer (MES (pH:6.8) buffer for MUF substrate and TRIZMA (pH:7.2) buffer for AMC substrate) were assigned to 96-well microplates (black pure Grade® microplates, Brand GmbH, Wertheim, Germany). Subsequently, 100 μl of fluorogenic substrate solution was added at concentrations of 20, 40, 60, 80, 100, 200, and 400 mmol substrate g^{-1} soil. The fluorescence was measured after 0-, 60-, and 120-min assay incubation with a Victor 3 1420–050 Multi-Label Counter (Perkin Elmer, USA) at an excitation of 355 nm and an emission of 460 nm. The fluorescence was converted to the amount of MUF or AMC based on the standard solutions. The enzyme activities were reported as MUF or AMC release in nmol/h g^{-1} dry soil and calculated using the standard curve (German et al., 2011). The kinetic parameters V_{max}, maximum enzyme activity, and K_m, substrate concentration (S) at which the reaction rate equals $V_{max}/2$, were estimated using the non-linear regression model (Michaelis–Menten kinetics) (Marx et al., 2001):

$$V = (V_{max} \times S)/(K_m + S)$$

The enzyme activity ratios can be used to examine the relative allocation to energy versus nutrient acquisition (Stone et al., 2014). Total activities of C-cycling enzymes (TAC), N-cycling enzymes (TAN), and P-cycling enzymes (TAP) were used to calculate the relative C- vs. P-acquiring enzyme activities (TAC/(TAC + TAP)) and the relative C- vs. N-acquiring enzyme activities (TAC/(TAC + TAN)) (Sinsabaugh et al., 2008).

2.6. Microbial necromass

To assess the amount of microbial necromass we analyzed the content of four amino sugars, namely, glucosamine (GlcN), mannosamine, galactosamine, and muramic acid (MurA). The extraction procedure was adapted from Liang et al. (2012).

Briefly, around 400 mg of incubated freeze-dried soil sample was weighed into a hydrolysis glass (Duran, Schott) and mixed with 10 ml of 6 M hydrochloric acid (HCl). Samples were hydrolyzed at 105 ℃ for 8 h and then cooled to room temperature. Subsequently, 50 μl of 0.5 mg ml^{-1} Myo-inositol (Sigma-Aldrich) was added to each flask as an internal standard. The solution was filtered and collected in a pointed flask. A rotary evaporator (Heidolph) was used to reduce the liquid volume. The dried residue was suspended with 3 ml of deionized water.

Additionally, the pH value was adjusted to 6.6–6.8 using 1 M potassium hydroxide (KOH) solution for ion precipitation. The precipitates were separated by centrifugation at 3500 rpm for 20 min at 7 ℃. Then, the supernatant was transferred to a glass tube and dried under a nitrogen stream. Each sample received 300 μl derivation reagent containing 32 mg ml^{-1} hydroxylamine hydrochloride and 40 mg ml^{-1} 4-dimethylamino-pyridine in pyridine-methanol (4:1 v/v). The samples were vortexed thoroughly and heated at 78 ℃ for 35 min in a water bath. Overall, 1 ml acetic anhydride was given to each reaction vial after cooling to room temperature. The samples were re-vortexed thoroughly and heated at 78 ℃ in the water bath for 25 min. When the samples cooled to room temperature, 2 ml dichloromethane (DCM) was added to each sample. Overall, 1 ml of 1 M HCl was added to each sample and vortexed thoroughly to wash the samples. The samples were left to sit undisturbed to separate the two phrases. Then, the upper phase was discarded. Furthermore, the samples were washed three times with H_2O and dried under a gentle nitrogen stream. For the measurement, the residue was dissolved in 300 μl ethyl acetate/n-hexane (1:1 v/v) and transferred to GC vials with a small insert.

The measurement was carried out on a TRACE 1310 Gas Chromatograph (Thermo Scientific™), equipped with a fused silica column (ZB-5HT Inferno, L 60 m, ID 0.25 mm, FT 0.25 μm, Phenomenex Inc, USA). Helium was the carrier gas with a constant flow of 1 ml min^{-1}. The injection volume was 1 μl. First, the temperature was set at 120 ℃, held for 4 min, then ramped to 250 ℃ at 30 ℃ min^{-1} for 10 min, and finally to 280 ℃ at 5 ℃ min^{-1} for 10 min.

The microbial necromass C content was estimated based on the biomarker muramic acid and glucosamine, representing the maximum C content in the microbial necromass pool. The bacterial and fungal necromass C was calculated using Eq. (10) and Eq. (11), respectively, according to Appuhn and Joergensen (2006).

$$Bacterial\ necromass\ C\left(mg\ g^{-1}soil\right) = MurA(mg\ g^{-1}soil) \times 45 \quad (10)$$

$$Fungal\ necromass\ C\left(mg\ g^{-1}soil\right) = (mol\ GlcN - 2 \times mol\ MurA) \times molar\ weight\ GlcN \times 9 \quad (11)$$

The contribution of fungal or bacterial necromass C to SOC was calculated using the following:

$$Microbial\ necromass\ C\ contribution(\%) = \frac{Microbial\ necromass\ C}{Total\ SOC} \times 100\%$$

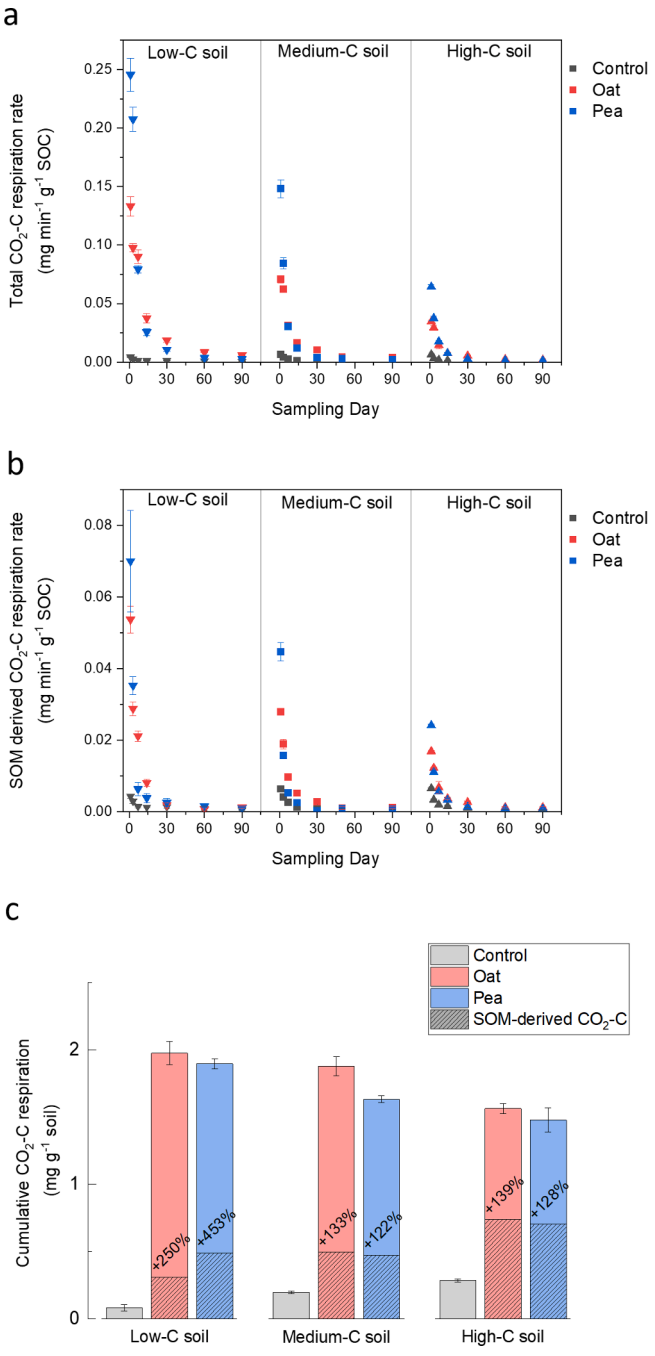

Fig. 1. a) Total CO_2-C respiration rate of low-, medium-, and high-C soil of oat and pea treatments. b) Total CO_2-C respiration rate of low-, medium-, and high-C soil of the control. c) Cumulative CO_2-C respiration of low-, medium-, and high-C soil. The numbers above the shade bars represent the priming effect. The gray, red, and blue dots and bars represent control, oat, and pea treatment, respectively. The shade bars stand for the native SOM-derived CO_2-C. Standard deviations are displayed with error bars.

2.7. Statistical analysis

Statistical analysis was carried out in Origin 2019 (OriginLab) for the datasets. First, the normality of the variance of the parameters was tested for normality using the Shapiro – Wilk test. Then, the data that passed the parametrical test were analyzed using a one-way analysis of variance (ANOVA) with Tukey's honestly significant difference test as the post hoc test. When the normality test failed, a log transformation was applied to the raw data, and the normality was tested again. Finally, for the non-normally distributed data, Kruskal – Wallis ANOVA was applied with Dunn's test as the post hoc test.

3. Results

3.1. Soil respiration

Soil CO_2-C respiration rates (Fig. 1) followed the OM loading (high-C soil > medium-C soil > low-C soil) in the control samples (without plant litter addition). However, this pattern was reversed due to the addition of plant litter. Adding pea litter led to higher soil respiration rates at the beginning of the incubation compared to adding oat litter. The total respired CO_2-C for the control samples was 0.09 ± 0.03, 0.21 ± 0.01, and 0.31 ± 0.01 mg g^{-1} for the low-, medium- and high-C soils, respectively. However, after plant litter addition, these values were 2.12 ± 0.10, 2.03 ± 0.07, and 1.69 ± 0.04 mg g^{-1} soil for oat litter addition, and 2.03 ± 0.05, 1.76 ± 0.03, and 1.60 ± 0.10 mg g^{-1} soil for soils with pea litter.

The litter addition stimulated mineralization (priming) of native SOM-derived C in all three soils, with the magnitude of soil priming varying according to the initial soil C loading. Oat litter addition in the low-C soil increased native SOM mineralization by 3.6-fold compared to control soils, whereas pea litter addition increased it by 5.7-fold. Soil priming was relatively lower in the medium-C soil, where increases were 2.3- and 2.2-fold, and in the high-C soil, with 2.4- and 2.3-fold increases, for oat and pea litter addition, respectively. The proportion of CO_2-C respiration derived from native SOM was 1 %–9 % higher in the pea litter treatment than in the oat litter treatment.

Considering respired to added plant-derived C, a higher proportion of added plant litter was mineralized in soils with lower C loading. For oat litter, 38 %, 32 %, and 20 % was respired in low-C, medium-C, and high-C soil, respectively. These numbers were 33 %, 28 %, and 19 % for pea litter. The total C recovery of added C over all samples was 101 ± 3 %.

3.2. Total organic carbon budget

Plant litter addition led to an increased SOC content after 90 days of incubation in all three soils (Fig. 2), which was relatively similar among all soils. However, compared to the present native SOC, the relative increase in SOC was negatively related. Thus, the low-, medium-, and high-C soil showed an increase in SOC content of 33 %, 15 %, and 9 % after 90 days of incubation with oat litter addition and 33 %, 14 %, and 8 % with pea litter addition, respectively.

3.3. Phospholipid fatty acids (PLFAs)

Compared to the control soils, plant litter addition resulted in a higher fungi-to-bacteria (F/B) ratio in all three soils. However, the shift of the F/B ratio (Fig. 3) was negatively related to the initial soil C loading. The change in the F/B ratio compared to the controls followed the order low-C soil > medium-C soil > high-C soil. Furthermore, oat litter addition significantly increased the F/B ratio (0.04–0.11) compared with pea litter addition, where the F/B ratio increased to 0.12 for the low-C soil, 0.11 for the medium-C soil, and 0.04 for the high-C soil.

The incorporation of oat and pea plant litter-C into different microbial groups associated with the three soils revealed different microbial consumption patterns (Fig. 4). The pea litter amendment led to higher total incorporation in microbial PLFAs than oat litter in all three soils.

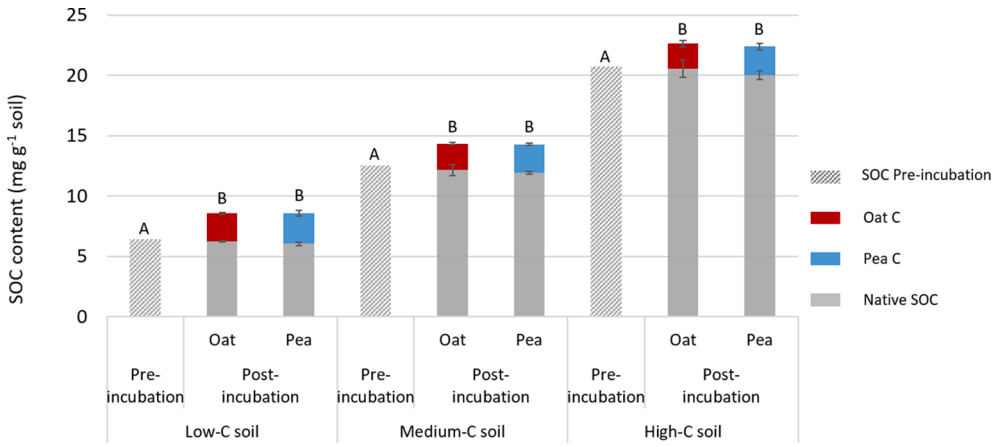

Fig. 2. Total carbon content in low-, medium-, and high-C soils pre- and post-incubation. Standard deviations are displayed with error bars. For a given soil, the capital letters represent significant differences between treatments.

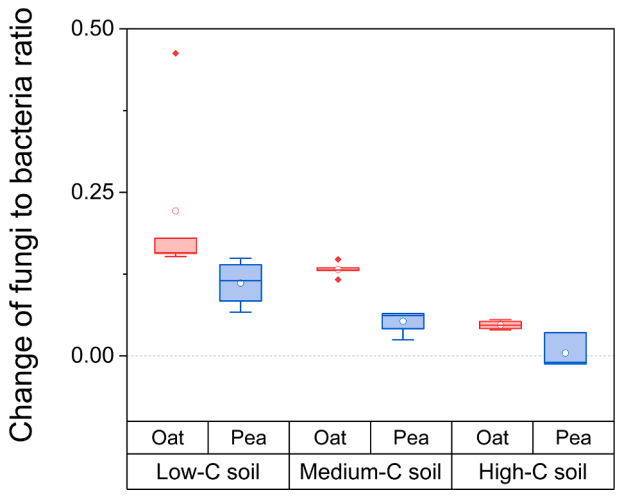

Fig. 3. Change in fungi-to-bacteria ratio (F/B) of oat treatment and pea treatment of low-, medium-, and high-C soil, calculated based on PLFA biomarker contents. The box plot represents the first quartile, the median, the mean, the outliers, and the third quartile range of the data. The change in the fungi-to-bacteria ratio was calculated by subtracting the F/B of the control from the F/B of the treatment.

This trend was more pronounced in the low- (1.2 %) and medium-C soils (1.4 %) than in the high-C soil (0.8 %). Overall, total oat-derived C incorporation in PLFAs was significantly higher ($p < 0.05$) in the high- and medium-C soils than in the low-C soil, with the high-C soil further higher than the medium-C soil. For incorporation of pea-derived C into microbial biomass, no significant differences were detected between the three soils; however, slightly lower incorporation of pea-derived C into PLFAs was shown in low-C soils. Most plant litter-C was incorporated into bacteria (actinobacteria, and G+ and G− biomarkers), varying from 51 % to 66 % of incorporated litter-C, which demonstrated that bacteria are the dominant microbial group incorporating fresh plant-derived C. This incorporation of plant C into bacterial PLFA was more pronounced with pea addition compared to oat addition.

In contrast, oat litter addition resulted in higher plant litter-C incorporation in saprotrophic fungi than in soils with pea amendment. Saprotrophic fungi contributed less than 22 % of the plant-C incorporation in PLFAs. The plant litter-C incorporated in the other PLFAs was between 18 % and 29 %.

3.4. Microbial necromass

Fig. 5a presents the microbial necromass calculated based on the amino sugar content. The microbial residue C in control soils significantly differed among the low-, medium-, and high-C soils, with 5.33 ± 2.47, 8.98 ± 2.57, and 10.54 ± 3.61 mg g^{-1} soil, respectively, thus following the soil C loading. The addition of oat litter increased the microbial residue C content by 2.77 ± 0.32, 1.43 ± 2.64, and 4.95 ± 3.94 mg g^{-1} for the low-, medium-, and high-C soils, respectively. Pea amendment slightly increased microbial residue C content in the low- (1.87 ± 0.19 mg g^{-1} soil) and medium-C soils (+0.77 ± 0.17 mg g^{-1} soil) but reduced microbial residue C content in the high-C soil (1.02 ± 1.31 mg g^{-1} soil). The content of bacterial necromass C showed significant differences between oat and pea treatments for all three soils. However, the fungal necromass C content significantly differed between oat and pea treatments in the low- and high-C soils but not in the medium-C soil. Microbial necromass C contributed more than 80 % of the SOC in the low-C soil, a lower value of about 70 % in the medium-C soil, and approximately 50 % in the high-C soil (Fig. 5b). Fungal C residues were more dominant than bacterial ones. Therefore, fungal residues contributed substantially more to the overall SOC pool. The fungal C residues also contributed more than 60 % to SOC in all soils, particularly in soils with oat litter addition, whereas bacterial residues contributed less than 8 %. Conversely, pea addition increased the contribution of bacterial residue C to SOC compared with the unamended control soils, which ranged from 10 % to 15 %; however, the contribution was still on a lower level than that of fungal residue C.

3.5. Enzyme vectors

Fig. 6 shows the enzyme activities translated into vectors for soils under either C or nutrient limitations (here N and P). The distance between the data points and the coordinate origin shows the strength of the relative C limitation. Interestingly the high-C soil presented a higher relative C limitation than the medium-C and low-C soils. The data points in the left upper area of the diagonal line show the P > N limitation, whereas those in the right lower part are under the N > P limitation. The control samples demonstrated that the low-C soil is more N and P-limited than the other two soils. Additionally, plant litter addition shifted the enzyme vectors toward the diagonal line and/or the

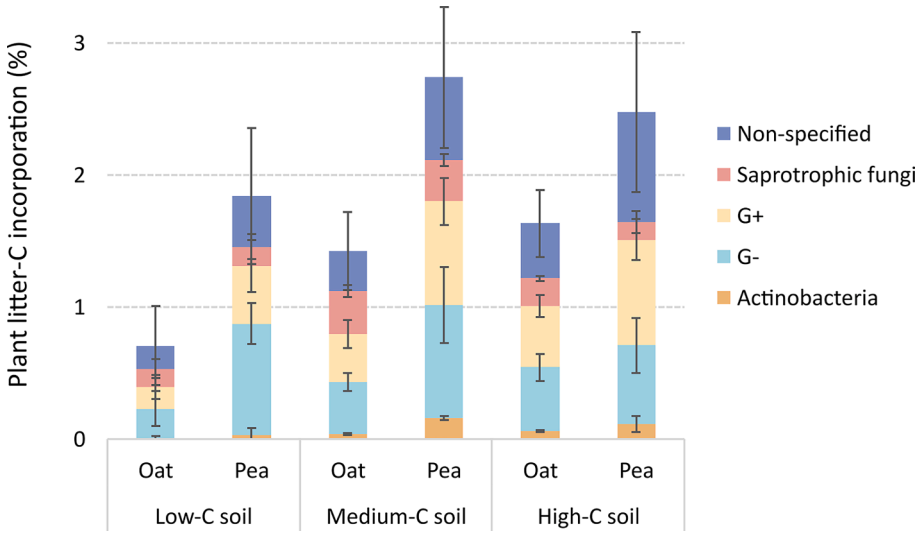

Fig. 4. Plant litter-C incorporated in the microbial group actinobacteria, Gram-negative bacteria (G−), Gram-positive bacteria (G+), saprotrophic fungi, and non-specified PLFAs. Standard deviations are displayed with error bars.

coordinate origin, indicating the alleviation of the C and/or N and P limitation in all soils. The pea treatment alleviated relative limitations more than the oat treatment.

4. Discussion

4.1. C saturation level determines OC dynamic

Besides soil properties, including mineralogy and texture, soil management, and thus organic matter input, are the main determinants of the total amount of OC sequestered in agricultural soils. For soils with comparable texture, the soil C loading, which results from the soil "management legacy", is shown to be the primary regulator of the sequestration of plant litter. In the present study, the overall C storage capacity of the three soils is comparable (Table 1), and thus, management with different OC inputs determines soil C storage and retention. At the average C saturation level of around 50 % in arable soils of South East Germany (Wiesmeier et al., 2014b), the three soils studied here nicely represent the low-, medium-, and high-C saturation level. In the absence of inputs of fresh plant-derived OM (the control soils), the magnitude of soil respiration directly mirrors the soil C content (as shown in Fig. 1b); the higher the SOC content, the higher the released CO_2 from heterotrophic respiration. Generally, increasing fresh OM input results in a corresponding increase in CO_2 release (Don et al., 2013; Mendoza et al., 2022; Rui et al., 2016; Wang et al., 2013). In this 90 days incubation study, we are able to demonstrate that soil C loading, which reflects the management legacy, directly affects this relationship (Fig. 1). Soils with very low-C loading show a higher CO_2 release after adding plant litter than well-managed OC-rich soils.

By studying soils varying in their C loading due to specific long-term soil management practices, we also demonstrated that the intricate connection of the fate of added fresh plant litter and native OM is closely related to the specific soil C loading. It was expected that the soil with the lowest C loading and therefore, the assumed greatest C retention potential (Stewart et al., 2007) would accommodate the highest C retention from plant litter. However, although low-C soils can gain considerable C, we demonstrated that a higher proportion of the plant litter was mineralized in the soil with the lowest initial C loading after 90 days of incubation. The highest total CO_2 release (Fig. 1) pointed to a rather vulnerable native SOM in the C-depleted soil, with a 3.6- and 5.7-fold increase in CO_2 release from native SOC. This increased release of native OC was due to enhanced microbial priming from the freshly added oat and pea litter. This points to the fact that besides the amount of freshly added C, the actual soil C storage and its legacy determine the C sequestration potential of a soil. Increasing the C input to soils with low-C loading may initially lead to less efficient C retention. However, increasing C storage over time by continuous C addition increases the efficiency of the retention of added C (Fig. 2). In general a positive feedback of C retention was demonstrated as a result of litter OM addition on the overall C storage.

Irrespective of the initial C loading and C mineralization, all studied soils retained C in response to the addition of easily bioavailable plant-derived OM (Fig. 2). Due to the lower natural C content the overall gain in added C in soils with lower C loading was high. Nonetheless, the mineralization rates of freshly added C in soils with low C stocks was also high (Fig. 1). The relative C sequestration potential, which implies the gain of added C to native C, remains greater in soils with lower C saturation levels (Fig. 2) (Mendoza et al., 2022; Stewart et al., 2008; Wu et al., 2022).

4.2. Microbial activities determine the OC dynamic

Soil C loading can influence the microbial growth strategy with microorganisms of either fast or slow growth rate (Chen et al., 2014; Fontaine et al., 2004). We assume that it is more likely that microorganisms with a fast growth rate (r-strategists) are fostered in soils with low-C availability. However, in soils with higher C saturation levels, microorganisms with slow growth rates (k-strategists) may thrive since higher amounts of SOC foster stronger niche separation (Fig. 4). The assumed link of higher C mineralization rates to higher growth rates is also supported by previous studies (Buckeridge et al., 2020b; Zheng et al., 2019). Moreover, the different nutrient availability of oat vs. pea litter regulated the microbial activity and thus, the overall consumption of added plant litter. The relative C limitation of the microbial activity was more marked in soils with higher C loading, whereas the relative nutrient limitation was more obvious in soils depleted in C (Fig. 6). Low-C loading drives microorganisms to mine higher amounts of plant litter for nutrients (Fontaine et al., 2004) to meet their stoichiometric

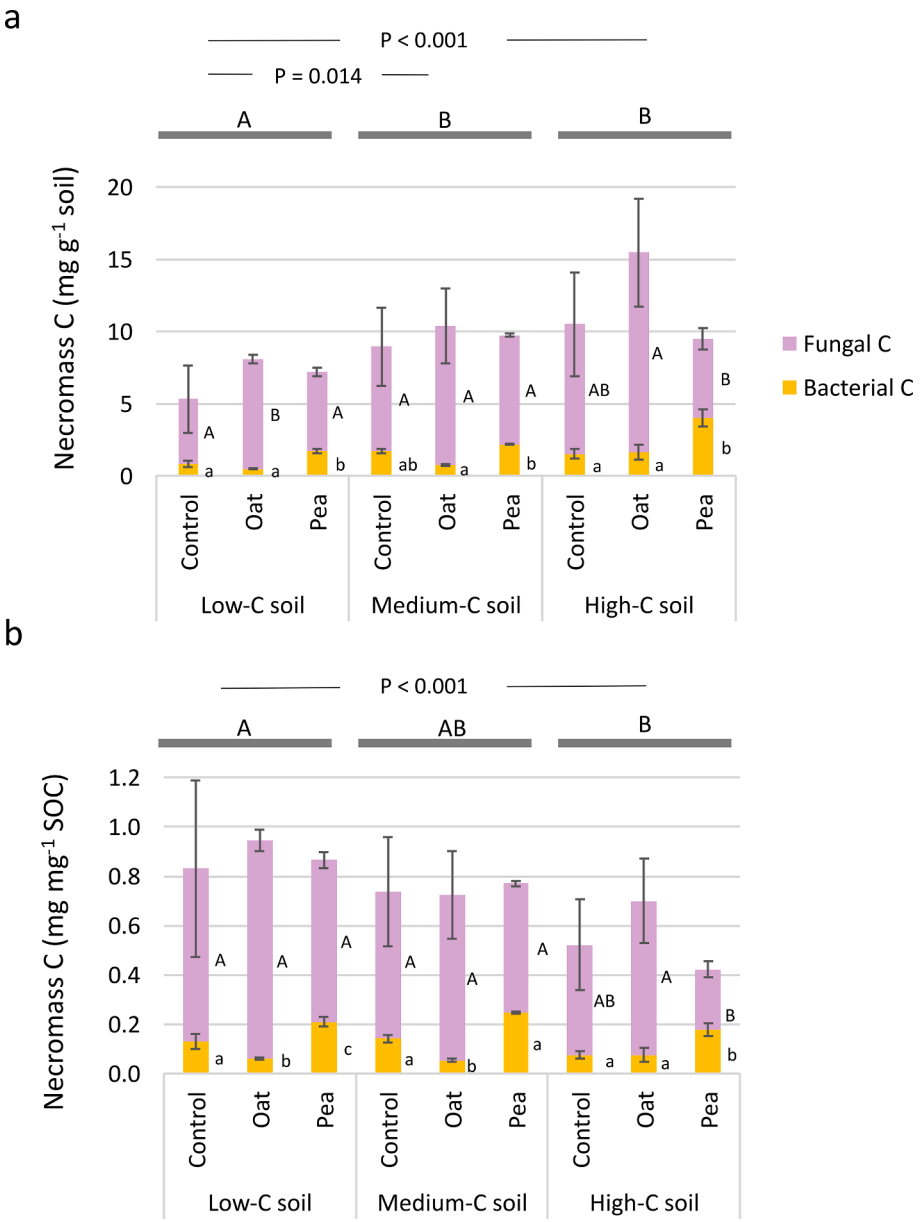

Fig. 5. a) Microbial residue c content in low-, medium-, and high-c soils of control, oat, and pea treatments as calculated from the amino sugar content (eq. (10) and Eq. (11)). b) Contribution of fungal C and bacterial C to SOC in control, oat, and pea treatments of low-, medium-, and high-C soils. The capital letters above the bars indicate significant differences in total necromass C of each soil. Standard deviations are displayed with error bars. For a given soil, the capital and lowercase letters represent significant differences between fungal and bacterial Cs of the treatments in each soil, respectively.

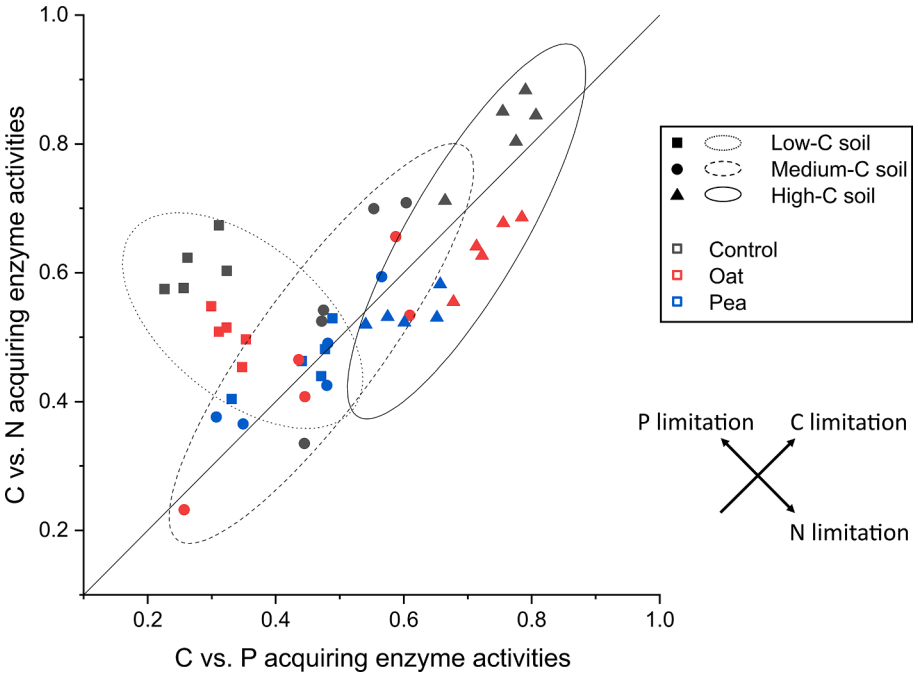

Fig. 6. C- vs. N- and C- vs. P-acquiring enzyme activities in low-, medium-, and high-C soils for control, oat, and pea treatments. The ellipses are confidence ellipses of low-, medium-, and high-C soils. The diagonal line demonstrated the C, N and P balanced condition.

demand. As shown by the enzyme activity (Fig. 6), N and P are major constraints to microorganisms in soils with lower C loading, and the availability of C majorly constrains microorganisms in soils with high C loading. Plant litter addition accelerates fungal growth in C-deficient soils, whereas bacterial growth is fostered in soils with sufficiently high C loading. This result agrees with previous findings, which show that nutrient availability limits fungal and C limits bacterial growth (Rousk and Baath, 2007; Wang et al., 2014). Plant litter amendment with higher N content (here, pea litter) created more balanced conditions with weaker stoichiometry constraints, thus reducing the need for microorganisms to mine for nutrients. This trend resulted in lower consumption of plant litter-derived OM, which contributes more to C retention after 90 days. However, considering the C availability from both native and freshly added OM, the bioavailability of OM for microorganisms using either one of the two pools also depends strongly on the spatial OM distribution (Kravchenko et al., 2019; Lehmann et al., 2020; Steffens et al., 2017). In soils with low C loading, more microorganisms and available native soil C might be spatially disconnected; however, proximity to available native soil C may foster priming in the high-C soil (Fig. 1). This result shows that a prerequisite of priming might be the spatial accessibility of the native OM, which is higher in the higher C-content soils.

4.3. Necromass in OC dynamic

In this study, the contribution of microbial necromass C to SOC decreased with the increasing C saturation level of the studied soils (Fig. 5). This indicated a higher retention of unprocessed freshly added plant litter, probably as POM, in soils with higher C loading. It was previously demonstrated that higher soil C saturation levels go along with increasing amounts of POM in the soil (Cotrufo et al., 2019), and presumably lower contribution of microbial necromass and MAOM.

Microbial consumption of plant-derived OM and the buildup of microbial biomass, and ultimately microbial necromass, lead to distinct ratios between microbial residues and plant-derived OM between the soils. Thus, differences in the amount of OM input and microbial decomposition are marked in the ratio between POM and microbial necromass. In this study, organic matter-depleted soils (low-C soil) with longer management of very low to neglectable OM inputs showed a very high contribution of microbial necromass to the overall soil C storage (Fig. 5b) (Liang et al., 2019; Wang et al., 2021). In contrast, soils with a continuously high plant OM input demonstrate a lower contribution of microbial necromass to the overall soil C storage, especially due to higher loads of mainly plant-derived POM. This lower contribution of microbial residues to overall C storage is also demonstrated by the increasing amount of the C/N from low-C soils to higher C loading soils, which is rich in less degraded plant-derived POM (Khan et al., 2016) (Table 1).

It can be assumed that microbial necromass and especially fungal residues, if associated with mineral surfaces as MAOM, is comparably more persistent than rather labile plant-derived OM (Cotrufo et al., 2019; Hannula and Morriën, 2022; Wang et al., 2021). The lowest specific respiration rates of respired CO_2-C per unit SOC in the control soils demonstrated this persistence (Fig. 1), with a dominant contribution of microbial necromass to the overall soil C (Fig. 5). The addition of plant litter particularly facilitated an increase in fungal biomass (Fig. 3) after 90 days, with more distinct increases in the F/B ratio in the soils with lower C loading. This effect is especially pronounced with the addition of plant residues low in N, which in this study was the oat material with rather wide C/N ratios. We demonstrated that fungal necromass particularly increases owing to plant litter addition. Therefore, this might lead to the dominance of fungal OM, which is stored as more persistent OM due to the chemical composition of the fungal necromass itself (Buckeridge et al., 2022; Sylvia et al., 2005) and has lower bioavailability due to organo-mineral associations (Hannula and Morriën, 2022). Overall, fungi made better use of low N litter (oat),

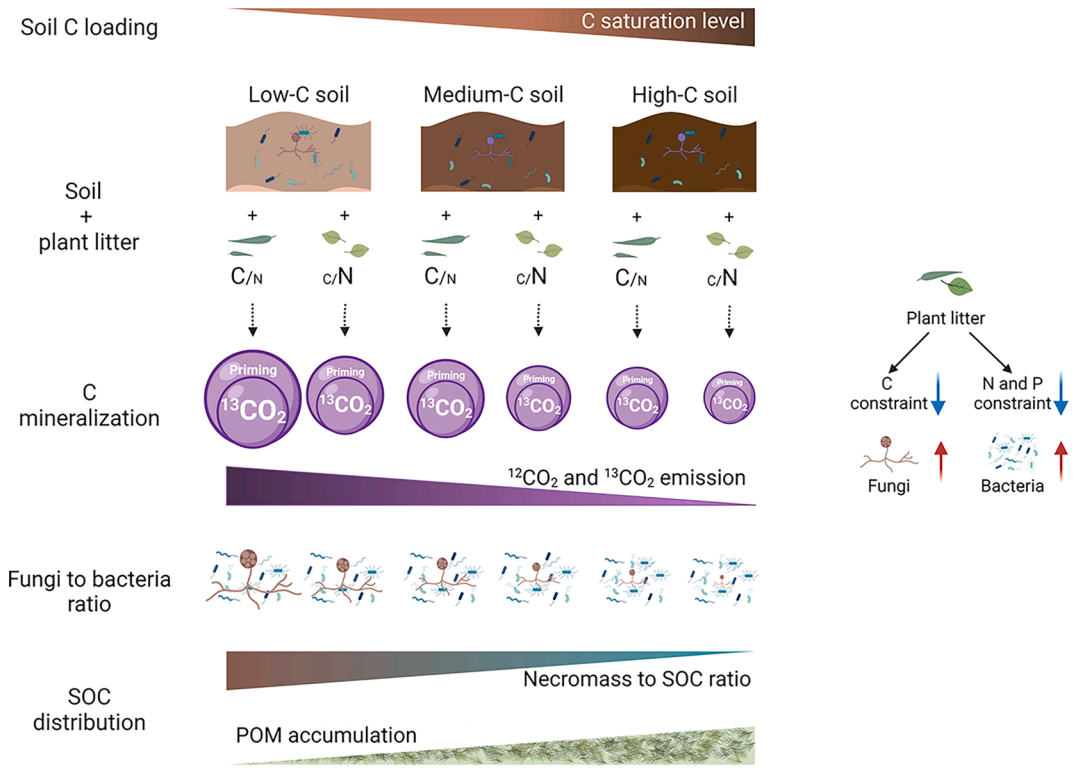

Fig. 7. C dynamics of soils with different C saturation levels.

whereas bacteria increased with high N litter addition (pea), as demonstrated by Rousk and Baath (2007) in a previous study. The different respiration rates may have indicated the differences in C use efficiency between fungi and bacteria, which can be assumed to be due to the lower efficiency of fungi in using C compared to bacteria (Anderson and Domsch, 1975; Rousk and Frey, 2015; Soares and Rousk, 2019).

However, microbial residues themselves are likely not a C sink that is persistent on a rather long timescale since both fungi and bacteria can and do consume microbial necromass to gain energy (Beidler et al., 2020; Buckeridge et al., 2020a). Consequently, microbial necromass could be considered as a rather active SOM pool used as a C and nutrient source by soil microorganisms. We demonstrate that adding plant-derived OM low in N leads to marked consumption of bacterial-derived necromass by fungi (Fig. 5). Therefore, the quantity of microbial necromass is determined by the N content of the active SOM pool, including microbial residues and plant litter input that regulates microbial activity through C and nutrient availability in the soil (Fig. 5).

4.4. Implications for soil organic carbon storage

It is essential to foster soil management strategies that ensure high soil POM contents fed by sustained plant OM input as a precursor for forming more persistent OM forms, i.e., MAOM (van Wesemael et al., 2019). This process will help avoid the vicious circle of soil losing C concomitantly with increased greenhouse gas release.

In soils with high soil C saturation levels, freshly added OM is retained in an efficient loop of microbial activity that uses both fresh and native OM while reducing overall CO_2 emissions. In soils with deficient C loading, microorganisms use the added fresh OM more inefficiently, which increases the use of native C due to priming for nutrients acquisition by the microorganisms. To enhance the "return on investment" for C addition to deficient soils, the C/N ratio of the added OM plays a major role. By adding OM with a lower N availability to C-depleted soils, the early-stage SOC retention is stimulated by an increased fungal activity, which reveals a lower C use efficiency. The C storage legacy of soil directly regulates the functioning and composition of the microbial communities that regulate the fate of freshly added OM and thus, the overall trajectory of soil C retention.

Bacteria benefit more from adding plant residues with higher N availability, which results in higher C use efficiency and reduced CO_2 emissions. Although the fresh OM input is less efficiently retained in soils lower in C (C-depleted soils), there is an increasing potential (self-enhancing positive feedback) of soils to retain SOC derived from added fresh litter over time with increasing C loading. This trend indicates that management has to focus on sustaining soil C levels to avoid rebuilding soil C stocks while suppressing further CO_2 emissions in the future. This knowledge offers some potential for ensuring the multi-functionality of soils especially facing drastic climate change and the need for mitigation. Thus, while currently well-managed medium- to high-C soils may act as C sinks, applying plant residues with higher N content to low-C soils may assist in converting those soils into valuable future C sinks more efficiently. The locally often limited amounts of available plant residues to be used as soil amendments can thus be used more efficiently for C retention. This approach might help ensure that correctly managed agricultural soils contribute to long-term terrestrial C storage.

5. Conclusion

In the present study we focused on the fate of freshly added plant

derived OC as regulated by different microbial consumption in soils with different soil carbon loading. Plant litter amendment to the soils changed the microbial community composition with a shift towards higher F/B ratios. This shift was more pronounced when adding lower-quality plant litter and in soils with low OM loading, indicating that fungi are especially limited by C availability in soils with low C loading. The addition of high-quality plant litter reduced C and nutrient limitations and thus allowed bacteria to thrive, which was more pronounced in high soils with high C loading. Further, plant litter amendments accelerated the C mineralization and fostered positive priming of inherited SOC in all three soils. However, the higher-quality plant litter amendment demonstrated less C mineralization than the lower-quality plant litter, with more material retained in the soils. Soils with higher OM-loading, regardless of the already high SOC saturation level, retained in total more freshly added OM even though priming of SOC was higher. Therefore, to obtain a better 'return on investment' of stored SOC in relation to added fresh C from OM amendments, soil management should consider sustaining soil carbon storage via balancing the input of fresh C with the current soil carbon loading.

CRediT authorship contribution statement

Tianyi Wu: Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Visualization, Writing – original draft. **Florian Wichern:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing. **Martin Wiesmeier:** Methodology, Resources, Validation, Writing – review & editing. **Franz Buegger:** Data curation, Formal analysis, Methodology, Writing – review & editing. **Lingling Shi:** Methodology, Software, Writing – review & editing. **Michaela A. Dippold:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Carmen Höschen:** Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. **Carsten W. Mueller:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing – review & editing.

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.

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Appendix

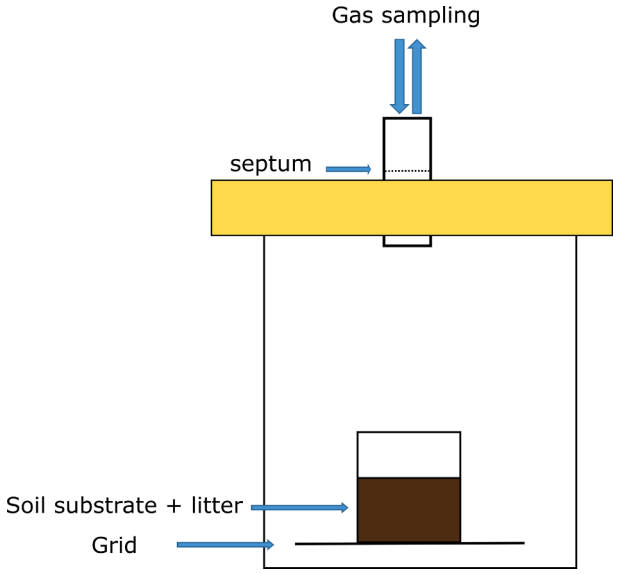

Fig. A1. Experimental setup.

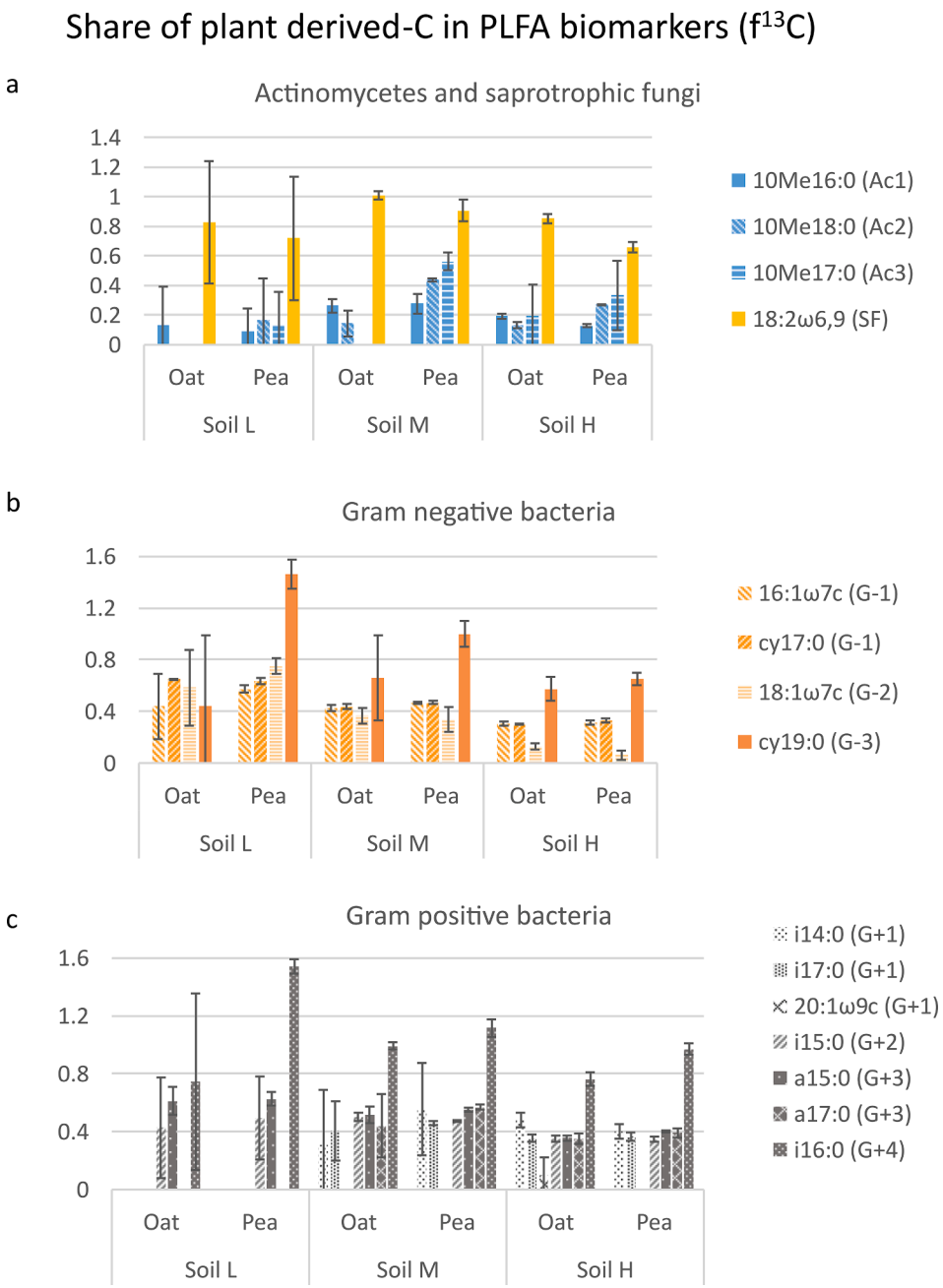

Fig. A2. Share of plant-derived C ($f^{13}C$) in actinobacteria, saprotrophic fungi, Gram-negative bacteria (G–), and Gram-positive bacteria (G+) of oat and pea treatments of low-, medium-, and high-C soils.

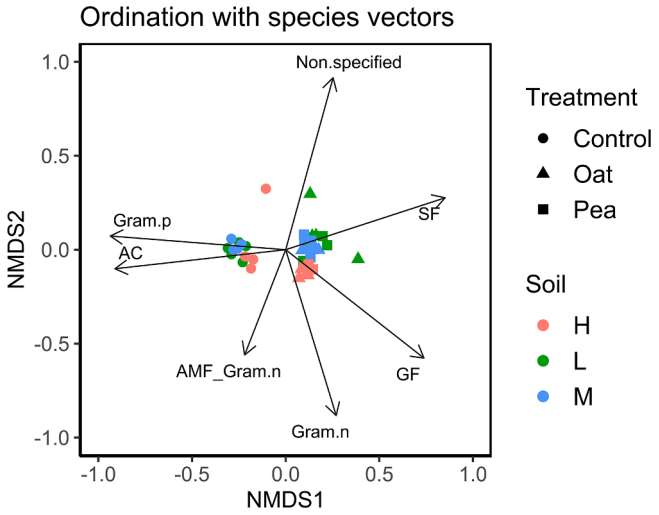

Fig. A3. Non-metric multidimensional scaling (NMDS) of the low- (L), medium- (M), and high-C (H) soils under control, oat, and pea treatments, ordinated with species vectors of actinobacteria (AC), marker 16:1ω5c (AMF), saprotrophic fungi (SF), Gram-negative or fungi (GF, marker 18:1ω9c), Gram-negative bacteria (Gram. n), Gram-positive bacteria (Gram.p), and non-specified (Non.specfied) markers is shown for all treatments.

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