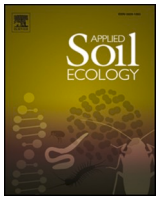
Applied Soil Ecology 195 (2024) 105234

Contents lists available at ScienceDirect

Applied Soil Ecology

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



Quantifying apparent and real priming effects based on inverse labelling

Jie Zhou [a], Yuan Wen [b], Chunyan Liu [b], Evgenia Blagodatskaya [c], Yakov Kuzyakov [d,e,h], Zhaohai Zeng [b], Davey L. Jones [f,g], Huadong Zang [b,*]

[a] College of Agriculture, Nanjing Agricultural University, Nanjing 210095, China
[b] College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, China
[c] Department of Soil Ecology, Helmholtz Centre for Environmental Research - UFZ, D-06120 Halle (Saale), Germany
[d] Agro-Technological Institute, Peoples Friendship University of Russia (RUDN University), 117198 Moscow, Russia
[e] Department of Soil Science of Temperate Ecosystems, Department of Agricultural Soil Science, Georg August University of Göttingen, D-37077 Göttingen, Germany
[f] School of Natural Sciences, Bangor University, Bangor, Gwynedd LL57 2UW, UK
[g] Centre for Sustainable Farming Systems, Food Futures Institute, 90 South St, Murdoch, WA 6150, Australia
[h] Institute of Environmental Sciences, Kazan Federal University, 420049 Kazan, Russia

ARTICLE INFO

Keywords:
Priming effect
Soil organic matter
Nitrogen fertilization
^{14}C-labeled glucose
Microbial biomass turnover

ABSTRACT

Organic inputs to soils can accelerate soil organic matter (SOM) decomposition via so-called priming effects, but at the same time microbial biomass turnover can be accelerated – that will be termed as apparent priming effect. However, only a few studies have been set up to quantify the contribution of extra CO_2 production from apparent priming. Here, we labeled the microbial biomass by ^{14}C-glucose in pre-incubation and then added labile carbon (C; ^{12}C or ^{14}C glucose) and nitrogen (N; NH_4^+) in soil and incubated over 120 days to investigate the contribution of apparent priming to total SOM priming. After 120 days of pre-incubation, 48 % of added glucose was released as CO_2, and 34 % of added glucose was recovered in microbial biomass. After glucose addition, microbial biomass and salt extractable organic C were similar between glucose and water addition. However, glucose addition increased the contribution of ^{14}C-glucose to microbial biomass by 2.5-folds and to CO_2 by 10-folds. This increased contribution of ^{14}C-glucose indicated accelerated microbial biomass turnover by labile C. Furthermore, 10 and 47 μg C g^{-1} of previously added and incorporated into microbial biomass ^{14}C and ^{12}C were replaced by new ^{14}C, which contributed to 10 % and 33 % of primed CO_2 emissions, respectively. On the contrary, N addition reduced previously added ^{14}C in both microbial biomass pool and released CO_2. This may reflect that the faster microbial turnover contributes to microbial necromass and further to stable SOM formation. Similar total apparent priming (~1.5 μg C g^{-1} in 120 days, mainly in the first 20 days) was observed after glucose and N addition, which contributed around 1–4 % of total priming. Unlike after glucose and N additions, the ^{14}C released as CO_2 (8 % of the remaining previously added ^{14}C-glucose) after water addition was mainly derived from reutilization of microbial necromass. This was supported by the absence of changes in either the ^{14}C or total C in microbial biomass. Overall, the turnover of the microbial biomass pool – the apparent priming – should not be ignored, since microbial biomass acts not only as a major determinant of SOM turnover but also as a C pool.

1. Introduction

Soil organic matter (SOM) acts as a important role in soil fertility, ecosystem function maintenance, and climate regulation (Jackson et al., 2017; Lehmann et al., 2020; Liu et al., 2023). Changes in SOM decomposition following labile substrates addition (priming effects, PEs) have been supposed to be a considerable driver of the ability of soils to function as sources or sinks of atmospheric CO_2 (Kuzyakov, 2010; Guenet et al., 2018). Priming can be separated into three parts: (i) apparent priming, which equals to changes in CO_2 originating from the turnover of microbial biomass (and preferential substrate use can also increase the amount of CO_2 released from soil without increasing rates of SOM-mineralization), (ii) i.e., real positive priming, and include (iii) real negative priming, where rates of SOM-mineralization decrease of following the addition of labile substrate, as microorganisms switch to primary feeding on labile substrate rather than SOM (Blagodatskaya and Kuzyakov, 2008; Bastida et al., 2019; Siles et al., 2022; Verbrigghe et al., 2022). Typically, SOM turnover is not determined directly but as

* Corresponding author.
E-mail address: zanghuadong@cau.edu.cn (H. Zang).

https://doi.org/10.1016/j.apsoil.2023.105234
Received 5 March 2023; Received in revised form 27 November 2023; Accepted 1 December 2023
Available online 16 December 2023

alterations in CO_2 efflux. Unfortunately, the original extra CO_2 produced (primed C) could not be easily assessed, and therefore the relative contribution of real and apparent PEs to the overall response is difficult to determine. Other processes, such as accelerated microbial turnover may also induce alterations in CO_2 efflux (Blagodatskaya and Kuzyakov, 2008; Kuzyakov et al., 2019). Hence, it remains unclear whether detected PEs are real or apparent priming, or their combination under various conditions. Understanding the mechanistic basis of priming, however, is crucial for predicting how SOM stocks will respond to future anthropogenic perturbation and the implementation of plant-soil mediated climate change mitigation options. Until now, few distinct approaches have been proposed to quantify the source of primed CO_2 originating from apparent PEs.

Although microbial biomass C has been proposed to be the significant origin of extra C mineralized in apparent PEs (Mason-Jones and Kuzyakov, 2017; Qiu et al., 2020), direct evidence is still scarce. Further, the rapid turnover of microbial biomass caused the accumulation of microbial necromass (i.e., after cell death), which is a major origin of stable SOM (Miltner et al., 2012; Buckeridge et al., 2020). Because of its heterogeneous nature, microbial necromass could serve as an available C source for living microorganisms and its reutilization could contribute to the priming of SOM decomposition (Shahbaz et al., 2017; Bore et al., 2019). Hence, the comprehensive research of microbial biomass could supply new perceptions into microbial consequences in soil C dynamics. The addition of $^{13}/^{14}$C-labeled low molecular weight substrate (i.e., glucose) or plant residues to SOM is an established method to evaluate the turnover of microbial biomass as well as its contribution to respired CO_2 (Chen et al., 2014; Shahbaz et al., 2018; Zhou et al., 2022a). However, the PEs originating from the microbial biomass was evaluated as a whole since only one C source is labeled in most previous publications (Mau et al., 2015; Qiu et al., 2020; Zhou et al., 2021). The majority of previous studies have focused on partitioning the contribution of the added C from overall CO_2 evolution (Shahbaz et al., 2018), but have failed to distinguish between CO_2 evolved from the turnover of microbial C or SOM. This has prevented the contribution of microbial biomass turnover to apparent PEs from being determined (Blagodatskaya et al., 2014; Li et al., 2018; Liang et al., 2019).

Microbial biomass turnover is affected by nitrogen (N) availability, since microorganisms need energy and N to balance anabolic and catabolic reactions (Sinsabaugh et al., 2016). Theoretically, N limitation caused higher C allocation to N acquisition, which subsequently reduced microbial turnover and inhibited microbial growth and PEs (Chen et al., 2014). Accordingly, as N availability increases, soil microbial turnover would be stimulated via the reduction in C:N ratio of substrates (Finn et al., 2015). Taken together, agroecosystems are experiencing increased inputs of anthropogenically derived N, which mainly originates from chemical N fertilizers and is about 10-folds >100 years ago (Canfield et al., 2010). Therefore, understanding how N fertilization impacts microbial biomass turnover and consequently PEs is becoming increasingly important within the context of the C budget in agroecosystems.

Grasslands are composed of larger soil C per unit area relative to the global average and play a major role in the global C dynamics (Riggs and Hobbie, 2016). However, the C held in these soils can also turnover rapidly and is susceptible to decrease upon climate and management changes (Crème et al., 2020; Stoner et al., 2021). Since glucose is not sorbed by soil and is very quickly incorporated almost ubiquitously across the microbial community, the use of isotopically labeled glucose represents a good approach to labeling and tracking the dynamics of the microbial biomass (Jones and Edwards, 1998; Gunina and Kuzyakov, 2015). After uptake into the microbial cell, glucose-derived C is either rapidly released as CO_2 and becomes immobilized in living biomass (short-term), or ultimately enters the necromass pool (long-term) (Glanville et al., 2016). Thus, we aimed to quantify the contribution of microbial biomass C to the PEs in a grassland soil, and further to evaluate the influence of N fertilization on apparent priming. To achieve this, we first pre-incubated soil with ^{14}C-glucose for 120 days and followed the label recovery in the microbial biomass, salt extractable organic C, and CO_2 efflux pool. After pre-incubation, labile C and N were applied to the soil and incubated for a further 120 days (inverse labeling) to assess the impact of labile C and N addition on the turnover of ^{14}C labeled microbial biomass, and consequently quantify the apparent PEs.

2. Material and methods

2.1. Site description and soil sampling

Soil samples were taken from the 0 to 10 cm (Ah horizon) of a grassland site located at the University of Hohenheim, Germany (48°43′N, 39°13′E). The soil is classified as a silty loam textured Stagnic Cambisol (WRB, 2015). The mean annual temperature and rainfall at the site are 10.4 °C and 654 mm, respectively. After collection, the soil was stored in gas-permeable polyethylene bags at 4 °C for a maximum of 4 weeks after sampling. Before use, the soil was homogenized and sieved with 2 mm, to remove fine roots and other plant residues. The soil has a pH_{H2O} of 5.1, soil organic carbon of 2.1 ± 0.2 %, total N of 0.21 ± 0.01 %, and microbial biomass of 584 mg C kg^{-1} (Zhou et al., 2022b).

2.2. Experiment design and incubation

2.2.1. ^{14}C-glucose addition (pre-incubation)

Oven-dried soil (50 g) was weighed and then put into a 100-ml glass jar (diameter 5 cm), yielding 32 jars. The soil was maintained at 50 % of the water holding capacity (WHC) with distilled water, and pre-incubated for 7 days at 20 °C. The uniformly labeled ^{14}C-glucose solution was used with a radioactivity of 32 kBq ml^{-1}. For each jar, 352 µl solution was added dropwise to the soil surface using pipette to gain a uniform distribution. The total amount of glucose-C added corresponded to 20 % of the microbial biomass (120 µg g^{-1} glucose-C). This amount of glucose is sufficient to stimulate microbial activity but insufficient to induce large amounts of microbial growth (Blagodatskaya et al., 2014; Zhou et al., 2021). After ^{14}C-glucose addition, the glass jars were sealed and incubated at 20 °C for 4 months in the dark. The amount and rate of $^{14}CO_2$ evolution from the soil was determined by placing vials containing NaOH (1.0 M, 2 ml) inside the jars. During this period the jars were maintained in an aerated condition and the soil moisture was maintained at 70 % WHC throughout. After 120 days pre-incubation, half of the jars (16 pots) were destructively harvested to measure microbial biomass C and ^{14}C activity.

2.2.2. Main incubation and CO_2 analyses (inverse labeling)

Following pre-incubation, a solution of either (i) distilled water (Control), (ii) ^{12}C-glucose (20 % of microbial biomass C, 120 µg g^{-1} glucose-C), (iii) ^{14}C-glucose (120 µg g^{-1} glucose-C), or (iv) an N solution (NH_4Cl; 125 µg N g^{-1} soil) were added dropwise to the soil surface (Fig. 1). Each treatment has four replicates, totally yielding 16 jars. The amount of N applied in the incubation was equal to 150 kg N ha^{-1}, which is the amount of mineral N fertilizer applied to local grasslands in northern Germany (Zang et al., 2016), given that the bulk density and soil depth are 1.2 g cm^{-3} and 0.1 m, respectively. Following solution addition, the glass jars were incubated in the dark at 20 °C for a further 120 days with the soil moisture maintained at 70 % WHC throughout. Subsequently, an alkali traps (2 ml, 1 M NaOH) were used to absorb the evolved CO_2 at regular intervals during the incubation. Three empty bottles (blanks) were used to eliminate the effects of CO_2 in the atmosphere during incubation. The NaOH was periodically changed after 1, 5, 9, 16, 25, 39, 55, 68, and 115 days of incubation and the solution was transferred to a conical flask with 2–3 washing of the vial with distilled water. Four glass jars for each treatment were destructively sampled at the beginning and end to measure soil microbial biomass C (MBC) concentration.

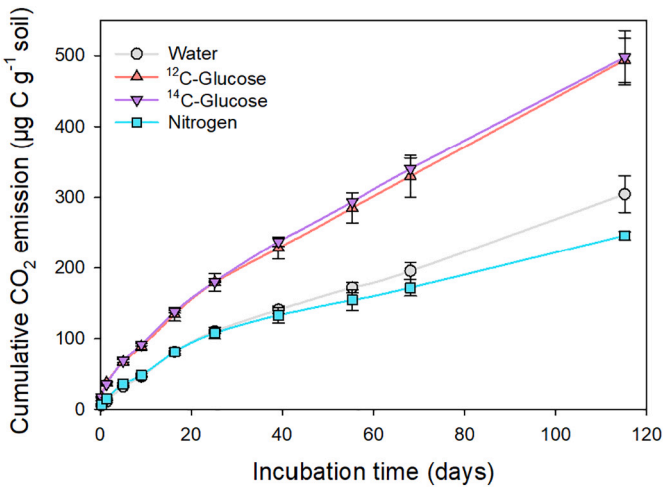


Fig. 1. Cumulative CO_2 emission after the addition of either water, glucose (^{12}C- and ^{14}C-glucose), or nitrogen to a grassland soil and incubation for 120 days of the experiment. The values represent means ± standard error ($n = 4$).

2.3. CO_2 emission and microbial biomass

The concentration of CO_2 trapped in the NaOH solution during pre-incubation and inverse labeling period was determined by titration. Briefly, 0.5 ml NaOH solution was titrated with 0.1 M HCl using phenolphthalein following the addition of 0.5 M $BaCl_2$ (Zang et al., 2020), and the CO_2 efflux was expressed as μg C g^{-1} soil. Microbial biomass during the pre-incubation and inverse labeling period was measured by the chloroform fumigation-extraction method. After destructive sampling, the soil from the glass jars was carefully homogenized, then 5 g soil was extracted with 20 ml of 0.05 M K_2SO_4 directly (1:5 *w/v*) (Vance et al., 1987; Wu et al., 1990). In parallel, chloroform was used to fumigate the another 5 g soil for 24 h which was then extracted in the same manner. The extracts of K_2SO_4 and non-fumigated samples were analyzed for total C content and salt extractable organic carbon (SEOC) using a 2100 TOC/TIC analyzer (Analytik Jena, Germany). MBC was calculated by the difference of K_2SO_4-extractable C between fumigated and non-fumigated soil samples using the K_{EC} factor of 0.45.

2.4. Analysis of ^{14}C activity in CO_2, and microbial biomass

^{14}C activity of NaOH traps was measured by mixing 0.5 ml of this solution with 2 ml of Rotiszint® eco plus scintillation cocktail (CarlRoth GmbH + Co. KG, Karlsruhe, Germany) and determined using a Beckman LS 6500 Liquid Scintillation Counter (Beckman Coulter Inc., USA). Before measurement, the mixture of NaOH traps and scintillation cocktail was thoroughly homogenized for 10 s using a Vortex Genie 2 (Scientific Industries Inc., USA), and kept overnight. The ^{14}C activity in fumigated and non-fumigated (^{14}C-SEOC) extracts was analyzed in 5 ml aliquots added to 15 ml of scintillation cocktail, and determined as mentioned above. The ^{14}C in the microbial biomass(^{14}C-MBC) was calculated as the difference in K_2SO_4-extractable ^{14}C between fumigated and non-fumigated soils without a correction factor (Glanville et al., 2016; Zang et al., 2020). We assumed that newly added ^{14}C incorporated into microbial biomass has the same extraction factor as the old unlabeled C in microbial biomass.

2.5. Calculations

In soils with water, ^{12}C-glucose, ^{14}C-glucose, and N addition, the CO_2 fluxes determined by titration originated from several C pools. In soil with water addition, the CO_2 fluxes originated from soil (CO_{2soil}) and previously added ^{14}C-glucose during pre-incubation ($^{14}CO_{2preincubation}$). In soil with ^{12}C-glucose addition, the CO_2 fluxes included soil-derived CO_2 (CO_{2soil}), previously added ^{14}C-glucose during pre-incubation ($^{14}CO_{2preincubation}$), and ^{12}C-glucose-derived CO_2 ($CO_{2\text{-}12C\text{-}glucose\text{-}derived}$). In soil with ^{14}C-glucose addition, the CO_2 fluxes contained soil-derived CO_2 (CO_{2soil}), previously added ^{14}C-glucose during pre-incubation ($^{14}CO_{2preincubation}$), and ^{14}C-glucose-derived CO_2 ($CO_{2\text{-}14C\text{-}glucose\text{-}derived}$). In soil with N addition, the CO_2 fluxes originated from soil (CO_{2soil}) and previously added ^{14}C-glucose during pre-incubation ($^{14}CO_{2preincubation}$).

Firstly, $^{14}CO_{2preincubation}$ (μg C g^{-1} soil) in soils added with water, ^{12}C-glucose, and N were calculated based on the radioactivity of the evolved $^{14}CO_2$ ($^{14}C_{curr}$, DPM) in the corresponding water, ^{12}C-glucose, ^{14}C-glucose, as well as N treatments, the amount of added glucose ($C_{14C\text{-}glucose}$, μg C g^{-1} soil), and the radioactivity of the applied glucose ($^{14}C_{glucose}$, DPM) in the pre-incubation:

$$^{14}CO_{2preincubation} = {}^{14}CO_{2curr} \times C_{14C-glucose} / {}^{14}C_{glucose} \quad (1)$$

Here, we assumed that the $^{14}CO_2$ was all from microbial biomass turnover. Therefore, apparent priming (μg C g^{-1} soil) – the CO_2 efflux originated from the microbial turnover – was calculated as the difference between $^{14}CO_{2preincubation}$ from ^{12}C-glucose and/or N, and water treatments.

The amount of soil-derived CO_2 in soil added with N ($(CO_{2soil})_N$, μg C g^{-1} soil) was calculated as:

$$(CO_{2soil})_N = CO_{2total} - (^{14}CO_{2preincubation})_N \quad (2)$$

Secondly, the amount of glucose-derived CO_2 in soil added with ^{14}C-glucose ($CO_{2\text{-}14C\text{-}glucose}$) was calculated based on the Eq. (1). Here should be noted that, $CO_{2\text{-}14C\text{-}glucose\text{-}derived}$ contained two parts of CO_2, one originated from the previously added ^{14}C-glucose in the pre-incubation, and the other originated from the newly added ^{14}C-glucose in the inverse labeling period. Since similar cumulative CO_2 emission was observed after both ^{12}C-glucose and ^{14}C-glucose addition in inverse labeling period (Fig. 1), we assumed the comparability of ^{14}C and ^{12}C-glucose addition and the suitability of our inverse labeling for three-source partitioning, representing (i) newly-derived microbial biomass pool from ^{12}C-glucose added in inverse labeling, (ii) old-derived microbial biomass pool from ^{14}C-glucose added in pre-incubation, and (iii) soil-derived microbial biomass pool.

Afterwards, the amount of soil-derived CO_2 in soil added with ^{14}C-glucose ($(CO_{2soil})_{14C\text{-}glucose}$) was calculated as:

$$(CO_{2soil})_{14C-glucose} = CO_{2total} - CO_{2soil-14C-glucose-derived} \quad (3)$$

where CO_{2total} is the total amount of C evolved as CO_2.

The amount of soil-derived CO_2 in soil added with water ($(CO_{2soil})_{water}$) was calculated as:

$$(CO_{2soil})_{water} = CO_{2total} - (^{14}CO_{2preincubation})_{water} \quad (4)$$

The priming effect is expressed as the difference between the soil-derived CO_2 from soil with glucose and/or N addition and CO_2 from soil with only water addition. The glucose-induced total priming effect (μg C g^{-1} soil) in unamended soils was then calculated as follows.

$$\text{Total priming effect} = (CO_{2soil})_{treatment} - (CO_{2soil})_{water} \quad (5)$$

where $CO_{2soil\text{-}treatment}$ and $CO_{2soil\text{-}water}$ indicate the cumulative CO_2 efflux in glucose ($(CO_{2soil})_{14C\text{-}glucose}$) from Eq. (3) or N-amended ($(CO_{2soil})_N$) from Eq. (2) and water-amended (control) soils, respectively.

2.6. Statistical analysis

The values shown in the figures are given as means ± standard error (mean ± SE). Before the analysis of variance (ANOVA), the data was checked for normality and homogeneity of variance with Shapiro-Wilk

($p > 0.05$) and Levene-test ($p > 0.05$), respectively. One-way ANOVA was carried out to evaluate the effect of glucose and N addition for all parameters at the end of inverse labeling. Repeated-measures ANOVA was used to assess the cumulative priming effect during incubation in soil with glucose and N addition. All statistical analyses were conducted using SPSS 22.0 for Windows (SPSS Inc., Chicago), and all the figures were made using Sigmaplot (12.5; Systat Software, Inc., San Jose, Ca, USA) unless otherwise specified.

3. Results

3.1. Cumulative CO_2 efflux and priming after glucose and N addition

The cumulative CO_2 effluxes from soil was similar (~500 μg C g^{-1}) when measured following the addition of ^{12}C- or ^{14}C-glucose in inverse labeling stage (Fig. 1). Compared with glucose addition, the cumulative CO_2 loss was 40 % and 54 % lower in soil amended with water (control) and inorganic N, respectively ($p < 0.05$, Fig. 1). In the inverse labeling stage, the cumulative CO_2 emission from previously added ^{14}C-glucose was 7.1 μg C g^{-1} after water addition (Fig. 2A). The cumulative $^{14}CO_2$ emission was similar between soil added with ^{12}C-glucose and those receiving N addition over the 120 days incubation in inverse labeling stage ($p < 0.05$, Fig. 2A), whilst it was larger than after water addition.

In the inverse labeling stage, the apparent priming after ^{12}C-glucose addition was ca.1.8 μg C g^{-1} in the first 20 days, which was larger than that after N addition. In the later 100 days, the apparent priming was similar between ^{12}C-glucose and N addition treatments, which was around 1.5 μg C g^{-1} ($p > 0.05$, Fig. 2B). Overall, the priming of SOM in response to glucose addition was positive and produced up to 150 μg C g^{-1} after 120 days incubation (Fig. 2C). In contrast, N addition induced a reduction (negative priming) in SOM mineralization over the same period (−68 μg C g^{-1}). Furthermore, the contribution of apparent to total priming was larger after N as compared to glucose addition (Fig. 4D).

After 120 days pre-incubation, the ^{12}C and ^{14}C incorporated into microbial biomass were 551 and 41 μg C g^{-1} (Fig. 3B). Although the size of the microbial biomass did not change markedly between the water and glucose addition ($p > 0.05$, Fig. 3B), the proportion of previously added ^{14}C to MBC decreased by 3.4 %. And 38 μg C g^{-1} of new ^{14}C was incorporated into the microbial biomass under glucose addition in the inverse labeling stage (Fig. 3B). Similarly, N addition also did not change the size of the microbial biomass ($p > 0.05$). However, N decreased the amount of previously added ^{14}C in MBC pool by 22.6 % as compared to water addition in the inverse labeling stage ($p < 0.05$, Fig. 3B). Furthermore, the ^{12}C incorporated into microbial biomass under glucose addition was 6.2 % and 9.6 % lower as compared with water and N additions, respectively.

3.2. Fate of previously added ^{14}C before and after incubation

After the initial 120 days incubation phase in pre-incubation, 48 % of the added glucose-C was released as CO_2, 34 % was incorporated into MBC, 2 % entered the SEOC pool, and the remaining 16 % was assumed to be immobilized in the soil or microbial necromass (Fig. 4). After adding water in inverse labeling stage, 4.2 % of the previously added ^{14}C was respired as CO_2, and the ^{14}C in the soil pool decreased by 0.9 % compared with pre-incubation. After glucose addition, the previously added ^{14}C immobilized in the MBC pool decreased by 2.3 % compared to water addition, whilst the soil pool increased by 4.1 %. Similarly, N fertilization decreased the previously added ^{14}C in the MBC pool but increased in the SOM pool relative to water addition ($p < 0.05$, Fig. 4).

4. Discussion

After the input of easily metabolizable C, primed CO_2 can originate from SOM mineralization and/or from the stimulated turnover of microbial biomass, i.e., real and apparent priming effects (Kuzyakov, 2010). In our case, the apparent priming (1.8 μg C g^{-1}) was found within 20 days after glucose addition (Fig. 2B), which contributed to around 4.0 % of total priming (45 μg C g^{-1}). This was consistent with

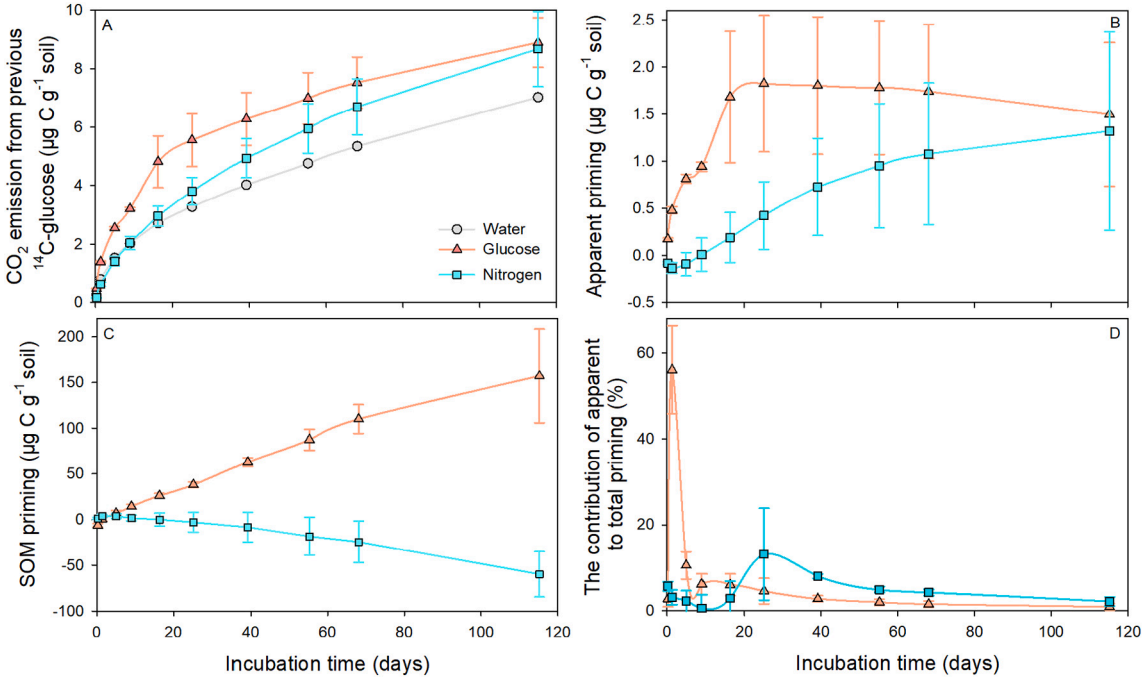


Fig. 2. Cumulative CO_2 emission from previous ^{14}C-glucose (A), apparent priming (B), and soil organic matter (SOM) priming (C), as well as the contribution of apparent to total priming (D) after glucose and nitrogen addition in grassland over 120 days incubation. The values represent means ± standard error (n = 4). Since similar cumulative CO_2 emission was observed after both ^{12}C-glucose and ^{14}C-glucose addition in the inverse labeling period (Fig. 1), we therefore confirmed the comparability of ^{14}C and ^{12}C-glucose addition and the suitability of our inverse labeling. Here should be noted that the glucose addition in the figure indicated the ^{14}C-glucose treatment.

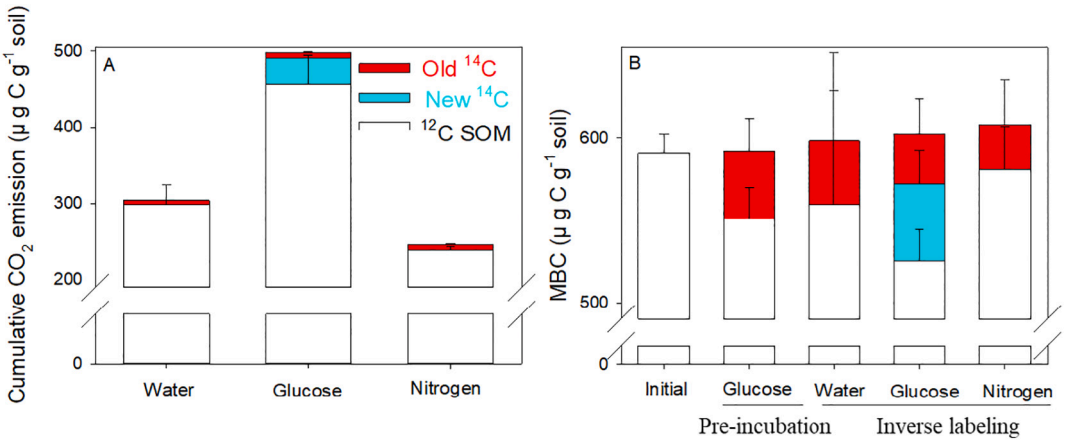


Fig. 3. Cumulative CO_2 emission (A) and soil microbial biomass C (MBC, B) derived from three sources (i.e., soil, previously added glucose, and newly added glucose) after the addition of either water, glucose, or nitrogen addition in a grassland soil after 120 days incubation (pre-incubation), and after 120 days incubation with water, glucose, or nitrogen addition in grassland in inverse labeling stage. The values represent means ± standard error (n = 4). Since similar cumulative CO_2 emission was observed after both ^{12}C-glucose and ^{14}C-glucose addition in inverse labeling period (Fig. 1), we therefore confirmed the comparability of ^{14}C and ^{12}C-glucose addition and the suitability of our inverse labeling. Here should be noted that the glucose addition during pre-incubation indicated the ^{12}C-glucose treatment, whilst the glucose addition during inverse labeling stage indicated ^{14}C-glucose treatment.

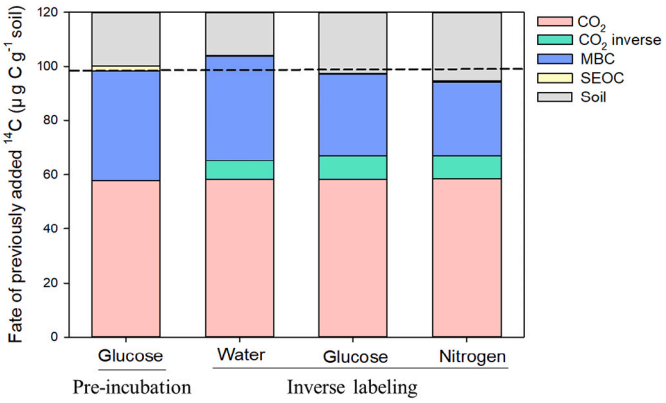


Fig. 4. Fate of previously added ^{14}C-glucose either lost as CO_2, or incorporated into the microbial biomass, salt extractable organic C pool, soil, and CO_2 inverse pool after 120 days incubation (pre-incubation), and after 120 days incubation with water, glucose, or nitrogen addition in grassland (inverse labeling stage). Since similar cumulative CO_2 emission was observed after both ^{12}C-glucose and ^{14}C-glucose addition in inverse labeling period (Fig. 1), we therefore confirmed the comparability of ^{14}C and ^{12}C-glucose addition and the suitability of our inverse labeling. Here should be noted that the glucose addition during pre-incubation indicated the ^{12}C-glucose treatment, whilst the glucose addition during inverse labeling stage indicated ^{14}C-glucose treatment.

Blagodatsky et al. (2010) who suggested that apparent priming increased much faster - within days to few weeks. This was because microorganisms accelerated their biomass turnover when a large amount of labile substrates passed through their biomass in a short time (Liang et al., 2015; Sun et al., 2022). The greater mineralization of previously added ^{14}C from soil microbial biomass or necromass, as well as increased incorporation of new ^{14}C into microbial biomass while microbial biomass C did not change between ^{14}C-glucose and water added soils in inverse labeling stage (Fig. 2B) confirmed the fast turnover of microbial biomass (Liang et al., 2019; Zhou et al., 2021). The input of labile C favored the maintenance of the activated microorganisms and consequently resulted in the prolonged turnover of microbial biomass (Wang et al., 2021). Remarkably, the larger amount of newly ^{14}C incorporated into microbial biomass after glucose addition relative to water, which could also be due to the increased dominance of fast-growing microorganisms benefiting from the addition of labile C (Fontaine et al., 2003; Zhou et al., 2022b). Microorganisms increased the mineralization of recalcitrant C since their N requirements were not met. Therefore, *exo*-enzymes were stimulated to mine N from SOM (Chen et al., 2014; Zhou et al., 2022c), thus causing depolymerization of SOM and the subsequent increase of decomposition to CO_2 (Sawada et al., 2021), i.e., a positive PEs (Figs. 2C, 5). Here, 10 and 47 μg C g^{-1} of previously added glucose in pre-incubation and ^{12}C-SOM in CO_2 efflux were replaced by new ^{14}C incorporation after ^{14}C-glucose addition (Fig. 3B), which contributed to 10 % and 33 % of primed CO_2 emission, respectively (Fig. 2B). Therefore, the stronger positive PEs was the result of the SOM decomposition and the faster turnover of the microbial biomass (Chen et al., 2014). However, the magnitude of apparent PEs was lower (Fig. 2B) than that from previous studies (220 % of CO_2 efflux in the control), while real PE reached 50 % (Blagodatskaya and Kuzyakov, 2008; Blagodatsky et al., 2010). This may be explained by the applied amount of labile C and the initial soil C levels. Therefore, more studies are required to evaluate the contribution of apparent to total PEs depending on incubation conditions and basic properties of soils.

N fertilization decreased the cumulative CO_2 emissions by 19 % and induced a pronounced suppression in SOM turnover (negative PEs; Fig. 2C). This finding is in agreement with previous short-term studies that found inorganic N fertilization reduced CO_2 by 8–42 % relative to soil not receiving N (Zang et al., 2016). Although previous research suggested that mineral N additions can reduce microbial biomass by 15–20 % (Li et al., 2018; Chen et al., 2022), we did not observe any changes in this study ($p > 0.05$; Fig. 3B). This might reflect sufficient labile C added in pre-incubation that was readily available for microorganisms (Kuzyakov, 2010). However, the previously added ^{14}C incorporation into microbial biomass was decreased by 33 % after N addition (Fig. 3B), which can be explained by accelerated microbial turnover of the available C when N is not restricted (preferential substrate use) (Zhou et al., 2022b). This has been proposed as the explanation for the reduced SOM decomposition under N fertilization (Zhou et al., 2020). As microbial biomass pool did not alter after N addition (Fig. 3B), the lower catabolic CO_2 release in the N fertilized soils led to lower qCO_2 (the ratio of CO_2 and MBC), which indicated a higher microbial C use efficiency (Spohn et al., 2016). In other words, less C was used to invest in enzyme production for SOM mineralization (Malik et al., 2020), and consequently induced lower priming under N fertilization. Taken together, only 10 % of ^{14}C-MBC under N fertilization was released as primed CO_2 (Fig. 4), which reflected the faster microbial turnover may contribute to microbial necromass and furthermore stable

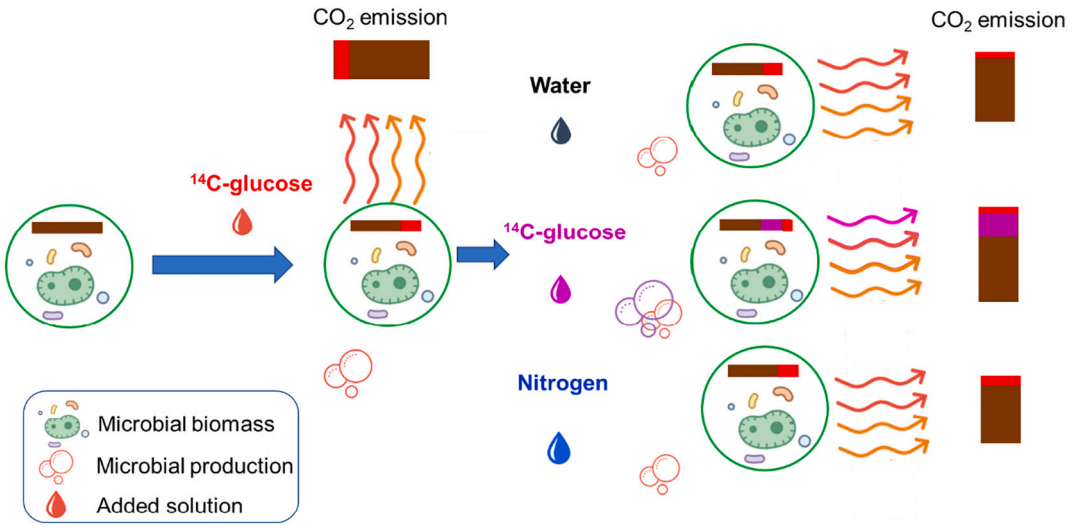


Fig. 5. Schematic representation of the whole experiment and carbon (C) sources partition in microbial biomass and CO_2 emission. The green circles represent the microbial cells and the rectangles represent the contribution of glucose and soil organic matter (SOM) derived C. The bending arrows represent CO_2 losses and the rectangle nearby represent the contribution of glucose and soil organic matter (SOM) derived C. The brawn, red, and pink color represents the contribution of SOM, first added glucose, and second added glucose. The combination of several circles represents the microbial production from first and second added glucose. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

SOM formation (Liang et al., 2019).

Unlike glucose and N treatments, water addition in inverse labeling stage only caused around 5 μg C g^{-1} $^{14}CO_2$ release (Fig. 1), which accounted for 8 % of the remaining previously added ^{14}C-glucose (Fig. 3A). Since there were no changes in either the ^{14}C part or total C in microbial biomass ($p > 0.05$; Fig. 3B), the ^{14}C released as CO_2 after water addition was mainly derived from reutilization of microbial necromass rather than from microbial biomass turnover (Chen et al., 2019; Cui et al., 2020).

<10 % of total ^{14}C-glucose added during pre-incubation was released as CO_2 after water, glucose, or N addition in inverse labeling stage (Fig. 4). This reflected the formation of relatively stable C pools from glucose after long time incubation, which may store in microbial biomass or SOM, and not intensively involved in the C turnover (Moreno-Cornejo et al., 2015). Under such circumstances, microorganisms relying on available C would stimulate their growth rate and shorten their life span (Reischke et al., 2014; Cui et al., 2020), consequently boosting necromass production. However, labile C induced stronger apparent priming relative to labile N addition, especially in the first 20 days (Fig. 2B). This may be explained by that labile glucose requires low activation energy as compared with microbial residues for decomposition. On the other hand, glucose can also be easily undertaken by microorganisms without much relying on enzymatic activities to decompose them (Spohn et al., 2016). Furthermore, it is likely that microorganisms sustained their metabolic ability by feeding on dead microbial biomass (Kindler et al., 2006; Chen et al., 2018), as a consequence causing stronger apparent PEs after glucose addition. This was also supported by Gunina et al. (2014) who indicated that glucose entering glycolysis is preferentially incorporated into microbial biomass, i.e., recycled.

We acknowledge that 16 % of added glucose-C in the pre-incubation remained may incorporated into soil or microbial necromass, which was not tested in the current study. Further researches needs to consider C incorporation in microbial necromass and soil for quantifying the apparent priming effect. We assumed that the $^{14}CO_2$ was mainly from microbial biomass turnover rather than soil organic matter or microbial residue mineralization after 120-day incubations. However, microbial necromass would gradually accumulate in soils over time, and ^{14}C would be redistributed among living microbial groups (Cui et al., 2020). Due to the lack of isotopic data on the added glucose-derived C into soil and microbial necromass, we could not get the knowledge on the contribution of microbial biomass turnover on soil C sequestration and subsequent recycled by microorganisms, and further would overestimate the apparent priming effect induced by labile C and N. Despite these limitations, our study constitutes the biggest effort to understand the contribution of apparent to total priming under labile C and N input based on the case study.

5. Conclusions

We used an inverse labeling approach to quantify the relative contribution of real and apparent priming in grassland soil. We labeled the microbial biomass using ^{14}C-glucose in pre-incubation and then added ^{12}C or ^{14}C (glucose) and N and incubated the soil for 120 days. The retention of microbial biomass C in soil and its loss via mineralization were influenced by both labile C and N addition. A positive priming effect was observed after glucose addition, which was caused by the faster turnover of microbial biomass, leading to accelerated mineralization of microbial necromass and SOM. This in turn resulted in overall less glucose-derived C being recovered in soil C pools compared with the soil with water addition. N fertilization did not affect the size of the microbial biomass but decreased CO_2 emissions and caused negative priming effect. We therefore conclude that N fertilization resulted in a larger retention of added ^{14}C from glucose in soil than in the soil without N. We attributed the larger ^{14}C retention to the intensive microbial reutilization and thus microbial necromass formation, as a consequence facilitating soil C sequestration. Apparent priming happened within 20 days after glucose (1.8 μg C g^{-1}) and N (0.55 μg C g^{-1}) addition, which contributes to around 4 % and 1 % of total priming over 120 days, respectively. Although >95 % of the observed priming effect after both C and N addition originated from SOM mineralization (real priming), the microbial biomass turnover (apparent priming) should not be ignored (especially over a short period as a few days to weeks), since microorganisms act not only as a major determinant of SOM turnover but also as a microbial C pool.

CRediT authorship contribution statement

Jie Zhou: Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing – original draft, Writing – review & editing.

Yuan Wen: Writing – review & editing. **Chunyan Liu:** Formal analysis, Visualization, Writing – review & editing. **Evgenia Blagodatskaya:** Formal analysis, Methodology, Writing – review & editing. **Yakov Kuzyakov:** Methodology, Writing – review & editing. **Zhaohai Zeng:** Writing – review & editing. **Davey L. Jones:** Formal analysis, Methodology, Writing – review & editing. **Huadong Zang:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Supervision, 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.

Acknowledgments

This study was financially supported by the National Natural Science Foundation of China (42207388, 32101850), the Young Elite Scientists Sponsorship Program by CAST (2020QNRC001), and the RUDN University Strategic Academic Leadership Program. The contribution of Yakov Kuzyakov was supported by the Strategic Academic Leadership Program "Priority 2030" of the Kazan Federal University. The contribution of Davey Jones was supported by the UK Global Challenges Research Fund (GCRF) project awarded to Bangor University by the UK Natural Environmental Research Council (NE/V005871/1). The authors would like to thank Karin Schmidt for her laboratory assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2023.105234.

References

Bastida, F., García, C., Fierer, N., Eldridge, D.J., Bowker, M.A., Abades, S., Delgado-Baquerizo, M., 2019. Global ecological predictors of the soil priming effect. Nat. Commun. 10, 3481.
Blagodatskaya, E., Blagodatsky, S., Anderson, T.H., Kuzyakov, Y., 2014. Microbial growth and carbon use efficiency in the rhizosphere and root-free soil. PLoS One 9, e93282.
Blagodatskaya, E.V., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biol. Fertil. Soils 45, 115–131.
Blagodatsky, S., Blagodatskaya, E., Yuyukina, T., Kuzyakov, Y., 2010. Model of apparent and real priming effects: linking microbial activity with soil organic matter decomposition. Soil Biol. Biochem. 42, 1275–1283.
Bore, E.K., Kuzyakov, Y., Dippold, M.A., 2019. Glucose and ribose stabilization in soil: convergence and divergence of carbon pathways assessed by position-specific labeling. Soil Biol. Biochem. 131, 54–61.
Buckeridge, K.M., Mason, K.E., McNamara, N.P., Ostle, N., Puissant, J., Goodall, T., Griffiths, R.I., Stott, A.W., Whitaker, J., 2020. Environmental and microbial controls on microbial necromass recycling, an important precursor for soil carbon stabilization. Commun. Earth Environ. 1, 1–9.
Chen, J., Seven, J., Zilla, T., Dippold, M.A., Blagodatskaya, E., Kuzyakov, Y., 2019. Microbial C: N: P stoichiometry and turnover depend on nutrients availability in soil: a ^{14}C, ^{15}N and ^{33}P triple labelling study. Soil Biol. Biochem. 131, 206–216.
Chen, L.Y., Liu, L., Qin, S.Q., Yang, G.B., Fang, K., Zhu, B., Kuzyakov, Y., Chen, P.D., Xu, Y.P., Yang, Y.H., 2018. Regulation of priming effect by soil organic matter stability over a broad geographic scale. Nat. Commun. 10, 5112.
Chen, R., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X., Blagodatskaya, E., Kuzyakov, Y., 2014. Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. Glob. Chang. Biol. 20, 2356–2367.
Canfield, D.E., Glazer, A.N., Falkowski, P.G., 2010. The evolution and future of Earth's nitrogen cycle. Science 330 (6001), 192–196.
Chen, X., Lin, J., Wang, P., Zhang, S., Liu, D., Zhu, B., 2022. Resistant soil carbon is more vulnerable to priming effect than active soil carbon. Soil Biol. Biochem. 168, 108619.
Crème, A., Rumpel, C., Malone, S.L., Saby, N.P.A., Vaudour, E., Decau, M.L., Chabbi, A., 2020. Monitoring grassland management effects on soil organic carbon-a matter of scale. Agronomy 10, 2016.
Cui, J., Zhu, Z., Xu, X., Liu, S., Jones, D.L., Kuzyakov, Y., Shibistova, O., Wu, J.S., Ge, T., 2020. Carbon and nitrogen recycling from microbial necromass to cope with C:N stoichiometric imbalance by priming. Soil Biol. Biochem. 142, 107720.
Finn, D., Page, K., Catton, K., Strounina, E., Kienzle, M., Robertson, F., Dalal, R., 2015. Effect of added nitrogen on plant litter decomposition depends on initial soil carbon and nitrogen stoichiometry. Soil Biol. Biochem. 91, 160–168.
Fontaine, S., Mariotti, A., Abbadie, L., 2003. The priming effect of organic matter: a question of microbial competition? Soil Biol. Biochem. 35, 837–843.
Glanville, H.C., Hill, P.W., Schnepf, A., Oburger, E., Jones, D.L., 2016. Combined use of empirical data and mathematical modelling to better estimate the microbial turnover of isotopically labelled carbon substrates in soil. Soil Biol. Biochem. 94, 154–168.
Guenet, B., Camino-Serrano, M., Ciais, P., Tifafi, M., Maignan, F., Soong, J.L., Janssens, I. A., 2018. Impact of priming on global soil carbon stocks. Glob. Chang. Biol. 24, 1873–1883.
Gunina, A., Kuzyakov, Y., 2015. Sugars in soil and sweets for microorganisms: review of origin, content, composition and fate. Soil Biol. Biochem. 90, 87–100.
Gunina, A., Dippold, M.A., Glaser, B., Kuzyakov, Y., 2014. Fate of low molecular weight organic substances in an arable soil: from microbial uptake to utilisation and stabilisation. Soil Biol. Biochem. 77, 304–313.
Jackson, R.B., Lajtha, K., Crow, S.E., Hugelius, G., Kramer, M.G., Piñeiro, G., 2017. The ecology of soil carbon: pools, vulnerabilities, and biotic and abiotic controls. Annu. Rev. Ecol. Evol. Syst. 48, 419–445.
Jones, D.L., Edwards, A.C., 1998. Influence of sorption on the biological utilization of two simple carbon substrates. Soil Biol. Biochem. 30, 1895–1902.
Kindler, R., Miltner, A., Richnow, H.H., Kästner, M., 2006. Fate of gram-negative bacterial biomass in soil-mineralization and contribution to SOM. Soil Biol. Biochem. 38, 2860–2870.
Kuzyakov, Y., 2010. Priming effects: interactions between living and dead organic matter. Soil Biol. Biochem. 42, 1363–1371.
Kuzyakov, Y., Horwath, W.R., Dorodnikov, M., Blagodatskaya, E., 2019. Review and synthesis of the effects of elevated atmospheric CO_2 on soil processes: no changes in pools, but increased fluxes and accelerated cycles. Soil Biol. Biochem. 128, 66–78.
Lehmann, J., Hansel, C.M., Kaiser, C., Kleber, M., Maher, K., Manzoni, S., et al., 2020. Persistence of soil organic carbon caused by functional complexity. Nat. Geosci. 13, 529–534.
Li, L.J., Zhu-Barker, X., Ye, R., Doane, T.A., Horwath, W.R., 2018. Soil microbial biomass size and soil carbon influence the priming effect from carbon inputs depending on nitrogen availability. Soil Biol. Biochem. 119, 41–49.
Liang, C., Gutknecht, J.L., Balser, T.C., 2015. Microbial lipid and amino sugar responses to long-term simulated global environmental changes in a California annual grassland. Front. Microbiol. 6, 385.
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.
Liu, E., Zhou, J., Yang, X., et al., 2023. Long-term organic fertilizer-induced carbonate neoformation increases carbon sequestration in soil. Environ. Chem. Lett. 21, 663–671.
Malik, A.A., Martiny, J.B., Brodie, E.L., Martiny, A.C., Treseder, K.K., Allison, S.D., 2020. Defining trait-based microbial strategies with consequences for soil carbon cycling under climate change. ISME J. 14, 1–9.
Mason-Jones, K., Kuzyakov, Y., 2017. "Non-metabolizable" glucose analogue shines new light on priming mechanisms: triggering of microbial metabolism. Soil Biol. Biochem. 107, 68–76.
Mau, R.L., Liu, C.M., Aziz, M., Schwartz, E., Dijkstra, P., Marks, J.C., Price, L.B., Keim, P., Hungate, B.A., 2015. Linking soil bacterial biodiversity and soil carbon stability. ISME J. 9, 1477–1480.
Miltner, A., Bombach, P., Schmidt-Brücken, B., Kästner, M., 2012. SOM genesis: microbial biomass as a significant source. Biogeochemistry 111, 41–55.
Moreno-Cornejo, J., Zornoza, R., Doane, T.A., Faz, Á., Horwath, W.R., 2015. Influence of cropping system management and crop residue addition on soil carbon turnover through the microbial biomass. Biol. Fertil. Soils 51, 839–845.
Qiu, G., Zhu, M., Xu, J., Brookes, P.C., 2020. Evaluating C sources and microbial biomass dynamics involved in the triggering response with soil depth. Soil Biol. Biochem. 149, 107958.
Reischke, S., Rousk, J., Bååth, E., 2014. The effects of glucose loading rates on bacterial and fungal growth in soil. Soil Biol. Biochem. 70, 88–95.
Riggs, C.E., Hobbie, S.E., 2016. Mechanisms driving the soil organic matter decomposition response to nitrogen enrichment in grassland soils. Soil Biol. Biochem. 99, 54–65.
Sawada, K., Inagaki, Y., Toyota, K., 2021. Priming effects induced by C and N additions in relation to microbial biomass turnover in Japanese forest soils. Appl. Soil Ecol. 162, 103884.
Shahbaz, M., Kuzyakov, Y., Sanaullah, M., Heitkamp, F., Zelenev, V., Kumar, A., Blagodatskaya, E., 2017. Microbial decomposition of soil organic matter is mediated by quality and quantity of crop residues: mechanisms and thresholds. Biol. Fertil. Soils 53, 287–301.
Shahbaz, M., Kumar, A., Kuzyakov, Y., Borjesson, G., Blagodatskaya, E., 2018. Priming effects induced by glucose and decaying plant residues on SOM decomposition: a three-source $^{13}C/^{14}C$ partitioning study. Soil Biol. Biochem. 121, 138–146.
Siles, J.A., Díaz-López, M., Vera, A., Eisenhauer, N., Guerra, C.A., Smith, L.C., Bastida, F., 2022. Priming effects in soils across Europe. Glob. Chang. Biol. 28, 2146–2157.

Sinsabaugh, R.L., Turner, B.L., Talbot, J.M., Waring, B.G., Powers, J.S., Kuske, C.R., Follstad Shah, J.J., 2016. Stoichiometry of microbial carbon use efficiency in soils. Ecol. Monogr. 86 (2), 172–189.
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.
Stoner, S.W., Hoyt, A.M., Trumbore, S., Sierra, C.A., Schrumpf, M., Doetterl, S., Baisden, W.T., Schipper, L.A., 2021. Soil organic matter turnover rates increase to match increased inputs in grazed grasslands. Biogeochemistry 156, 145–160.
Sun, T., Zhou, J., Shi, L., Feng, W., Dippold, M.A., Zang, H., Kurganova, I., de Gerenyu, V. L., Kalinlina, O., Giani, L., Kuzyakov, Y., 2022. Microbial growth rates, carbon use efficiency and enzyme activities during post-agricultural soil restoration. Catena 214, 106226.
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.
Verbrigghe, N., Meeran, K., Bahn, M., Fuchslueger, L., Janssens, I.A., Richter, A., Vicca, S., 2022. Negative priming of soil organic matter following long-term in situ warming of sub-arctic soils. Geoderma 410, 115652.
Wang, B., An, S.S., Chao, L., Liu, Y., Kuzyakov, Y., 2021. Microbial necromass as a source of organic carbon in global ecosystems. Soil Biol. Biochem. 162, 108422.
WRB, 2015. World reference base for soil resources 2014, update 2015. International soil classification system for naming soils and creating legends for soil maps. In: World Soil Resources Reports No, 106. FAO, Rome.
Wu, J., Joergensen, R.G., Pommerening, B., 1990. Measurement of soil microbial biomass by fumigation-extraction-an automated procedure. Soil Biol. Biochem. 20, 1167–1169.
Zang, H., Wang, J., Kuzyakov, Y., 2016. N fertilization decreases soil organic matter decomposition in the rhizosphere. Appl. Soil Ecol. 108, 47–53.
Zang, H., Zhou, J., Marshall, M., Chadwick, D., Wen, Y., Jones, D., 2020. Microplastics in the agroecosystem: are they an emerging threat to the plant-soil system? Soil Biol. Biochem. 148, 107926.
Zhou, J., Zang, H., Loeppmann, S., Gube, M., Kuzyakov, Y., Pausch, J., 2020. Arbuscular mycorrhiza enhances rhizodeposition and reduces the rhizosphere priming effect on the decomposition of soil organic matter. Soil Biol. Biochem. 140, 107641.
Zhou, J., Wen, Y., Shi, L., Marshall, M.R., Kuzyakov, Y., Blagodatskaya, E., Zang, H., 2021. Strong priming of soil organic matter induced by frequent input of labile carbon. Soil Biol. Biochem. 152, 108069.
Zhou, J., Shao, G.D., Kuma, A., Shi, L.L., Kuzyakov, Y., Pausch, J., 2022a. Carbon fluxes within tree-crop-grass agroforestry system: ^{13}C field labeling and tracing. Biol. Fertil. Soils 58, 733–743.
Zhou, J., Guillaume, T., Wen, Y., Blagodatskaya, E., Shahbaz, M., Zeng, Z., Peixoto, L., Zang, H.D., Kuzyakov, Y., 2022b. Frequent carbon input primes decomposition of decadal soil organic matter. Soil Biol. Biochem. 175, 108850.
Zhou, J., Gube, M., Holz, M., Song, B., Shan, I., Shi, L., Kuzyakov, Y., Dippold, M.A., Pausch, J., 2022c. Ectomycorrhizal and non-mycorrhizal rhizosphere fungi increase root-derived C input to soil and modify enzyme activities: a ^{14}C pulse labelling of Picea abies seedlings. Plant Cell Environ. 45, 3122–3133.