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Gradual and abrupt increase in atmospheric CO2 concentrations trigger divergent responses of microbial necromass accumulation in paddy soils

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Abstract

Microbial necromass C (MNC) plays a critical role in promoting soil organic C (SOC) formation and stabilization, particularly in the context of climate change. Most investigations on the impact of elevated CO2 concentrations (eCO2) on MNC have been performed by exposing ecosystems to an abrupt increase in CO2. However, the atmospheric CO2 increase is a gradual process, and knowledge about the response of necromass to gradual increase in CO2 is lacking. We tested the hypothesis that microbial necromass would show different responses to abrupt and gradual CO2 increases. Furthermore, it is still unknown whether eCO2 will trigger similar responses between surface and subsurface soil layers. Here, we determined the MNC concentrations and their proportions in SOC in surface and subsurface soil layers via open-top chambers. CO2 treatments included ambient control, abrupt CO2 increase by 200 ppm above control, and gradual CO2 increase by 40 ppm each year until reaching 200 ppm over five years. Overall, compared with the ambient control, abrupt

eCO2 induced a significantly stronger decrease in necromass (20.3 %) when averaged across three soil layers, while gradual eCO2 led to insignificant variations in necromass (6.7 %). The necromass proportion in SOC decreased with depth under both eCO2 treatments with a significant decline occurring in abrupt eCO2 treatment. The observed greater magnitude of eCO2 effects on fungal necromass relative to bacterial necromass in subsurface layers illustrates a distinct microbial community response to climate change. Taken together, abrupt and gradual eCO2 approaches differed in their impact on MNC, and its response to eCO2 may be somewhat overestimated by abrupt eCO2 approach. Furthermore, MNC in subsurface soil witnessed a more sensitivity or vulnerability to eCO2 than that of topsoil. We suggest vigilant attention will need to be paid to the feedback of necromass C in deep soil poised by future climate change.

Keywords: Elevated atmospheric CO_2, Microbial residues, Soil organic carbon, Soil depth, Paddy soil

1. Introduction

The atmospheric CO_2 concentration has increased by >40% above its pre-industrial level and is expected to increase further even under the most ambitious scenarios of climate-change mitigation (Terrer et al., 2018; Panneerselvam et al., 2020). Soil organic carbon (SOC) is the largest terrestrial C pool and plays a vital role in mediating feedbacks in the climate-carbon cycle (Lehmann and Kleber, 2015; van Groenigen et al., 2017). Elevated atmospheric CO_2 concentrations (eCO_2) indirectly

affect soil C stocks and fluxes by increasing photosynthesis and plant growth (Kuzyakov et al., 2019; Castañeda-Gómez et al., 2020; Hu et al., 2020). No consistent pattern, however, has been found that allows us to generalize the response of SOC across different ecosystems exposed to eCO_2 (reviewed by Kuzyakov et al. (2019)). Positive, neutral, or negative effects of eCO_2 on SOC concentration have been reported (de Graaff et al., 2006; Drake et al., 2011; van Groenigen et al., 2017). The equivocal effect of eCO_2 on SOC concentration represents uncertainties about the primary mechanisms controlling the formation and persistence of SOC under eCO_2 (Bradford et al., 2016; Cotrufo and Lavallee, 2022).

The sequestration of C in soil is determined by the balance between C inputs and outputs, which is fundamentally regulated by microbial metabolic activity (Janzen, 2015; Liang et al., 2019). The mechanistic understanding of soil-C dynamics relies on a better understanding of microbially mediated C processes (Schimel and Schaeffer, 2012; Liang et al., 2017). Soil microbes degrade organic matter, leading to the loss of C from the soil, but they assimilate C for constructing biomass via anabolism and eventually contribute to SOC in the form of necromass after cell death (Miltner et al., 2012; Kallenbach et al., 2015; Liang et al., 2019). Microbial necromass plays a key role in mediating climatic feedbacks to soil-C cycles (Lehmann and Kleber, 2015; Cotrufo et al., 2019) because they are considered an important contributor to the accumulation of slow-cycling SOC by iterative cycles of microbial growth and death (Liang et al., 2017; Sokol et al., 2019), stabilized by association with the surfaces of soil minerals (Kögel-Knabner, 2012; Cotrufo et al., 2013).

These interdependent processes, conceptualized as a soil microbial C pump (Liang et al., 2017), have important implications for understanding the response of microbially driven C accumulation to global climate change, which have thus attracted considerable attention (Buckeridge et al., 2022; Cotrufo and Lavallee, 2022; Feng and Wang, 2023). Limited information, however, is available on how higher atmospheric CO_2 concentrations affect the efficiency of microbial C pumps and their contribution to SOC storage. Previous studies of the response of microbial necromass to eCO_2 have only focused on the surface soil layer, but investigation of microbial necromass C (MNC) in different soil layers is still lacking. This oversight hinders our comprehensive understanding of the microbial mediation and control over the potential for SOC sequestration and underlying mechanisms in scenarios of global eCO_2.

Rice (*Oryza sativa* L.) paddy agroecosystems, as the largest type of anthropogenic wetlands, have a high potential for sequestrating C (Liu et al., 2021a). eCO_2 represents a dominant driver of global climate change, so the response of rice-paddy soils to eCO_2 has become a major concern (Bhattacharyya et al., 2013; Hu et al., 2020). Paddy soils in China account for approximately 40% of the total potential sequestration of soil C, crucial for mitigating climate change (Pan et al., 2004; Ge et al., 2019). An increase in atmospheric CO_2 concentration may alter the belowground allocation and turnover of C by increased C inputs from plant biomass, roots, and exudates in paddy soils (Ainsworth and Long, 2005; Kuzyakov et al., 2019; Xiong et al., 2019; Hu et al., 2020). These changes may alter microbial-community structure

(Zak et al., 2000; Hu et al., 2017; Shen et al., 2023) and microbial C-use efficiency (Kallenbach et al., 2015) and consequently the production and accumulation of necromass (Liang et al., 2017). The response of microbially driven C accumulation to climate change may differ between paddy and upland soils due to the unique redox conditions of paddy soils (Xia et al., 2019). Available information on the accumulation of microbial necromass in paddy soils under eCO_2, however, is very limited (Liu et al., 2021b).

Most experimental efforts to determine the nature and magnitude of microbial responses (mainly focusing on microbial biomass and community structure) to eCO_2 have notably typically used abrupt increases (Klironomos et al., 2005; Li et al., 2022), i.e. CO_2 concentrations were directly increased by hundreds of ppm. Ecosystems, though, are experiencing a gradual increase in atmospheric CO_2 concentration. The average rate of increase in atmospheric CO_2 concentration over the last decade was 2.40 μmol mol^{-1} y^{-1} (WMO, 2021). One fundamental question that has not yet been fully addressed is whether an ecosystem exposed to a single increase in CO_2 concentration would yield similar responses to an ecosystem exposed to a gradual increase. Evidence suggests that a gradual increase and a single large increase in CO_2 concentration may have different consequences (Luo and Reynolds, 1999; Klironomos et al., 2005). These different approaches (abrupt vs gradual) of increasing atmospheric CO_2 concentrations have led to different rates of C sequestration, with higher rates for abrupt than gradual increases (Luo and Reynolds, 1999). The responses of microbial-community structure and function to eCO_2 also differed

between abrupt and gradual increases in CO_2 concentration in a model plant-soil system (Klironomos et al., 2005). The sensitivity of microbial necromass in paddy ecosystems to these different approaches, however, remains mostly unknown.

Abrupt and gradual increases in CO_2 concentration have different impacts on rice growth, biomass production, and soil physiochemical properties, and these changes can consequently affect microbially regulated processes of methanogenesis (Shen et al., 2023; Wang et al., 2021, 2023). Whether and how different scenarios of eCO_2 (gradual vs abrupt) affect the accumulation of microbial necromass and its contribution to the accumulation of SOC in paddy soil remain unknown. Does the response of microbial necromass to eCO_2 differ between surface and subsurface layers? Which component is more sensitive to eCO_2, bacterially or fungally derived C? Such information is vital for improving our understanding of the mechanisms of the microbially driven sequestration of SOC and for better predicting the dynamics of soil C in paddy ecosystems under future atmospheric conditions.

To answer these questions, we performed a field experiment using open-top chambers (OTCs) to address the effects of abrupt vs gradual eCO_2 on changes in the accumulation of MNC after four years of exposure in a subtropical paddy. To our knowledge, this study is the first to examine whether the accumulation of microbial necromass in soil would respond differently when a paddy was exposed to an abrupt or a gradual increase in CO_2 concentration.

Our first hypothesis was that the accumulation of microbial necromass and its contribution to SOC would differ significantly between gradual and abrupt eCO_2

treatments, because the different eCO_2 treatments would differ greatly in the production of plant biomass, physicochemical properties, and enzymatic activities based on a previous study at the same experimental site (Wang et al., 2021).

Our second hypothesis was that bacterial necromass would increase more than fungal necromass under eCO_2 treatments due to eCO_2-induced higher C availability in soil, because bacteria more quickly metabolize readily available resources when the availability of labile C is high (Rousk and Bååth, 2011).

Finally, studies of the effects of eCO_2 on microbial necromass have primarily focused on the topsoil (e.g. Liang et al., 2015; Liu et al., 2021b). How eCO_2 affects this source of microbial C in subsurface layers is thus mostly unknown. We compared the differences in the accumulation of microbial necromass in three soil layers (0–5, 5–10, and 10–20 cm) under abrupt and gradual eCO_2 to determine whether the response of microbial necromass to different eCO_2 treatments would differ between surface and subsurface layers. Our third hypothesis was thus that eCO_2 would promote the accumulation and contribution of microbial necromass to SOC to a larger extent in subsurface layers where labile C is relatively limited and more sensitive to fresh inputs of C (Fontaine et al., 2007; Soong et al., 2020).

2. Materials and methods

2.1. Site description

We set up open-top chambers (OTCs) at the Agricultural Meteorology and Ecology Experimental Station of Nanjing University of Information Science & Technology, Nanjing, southeastern China (32°16′N, 11°86′E). The area has a subtropical monsoon

climate with mean annual temperature and precipitation of 15.6 °C and 1110-1200 mm, respectively. The soil is classified as hydromorphic soil. The field was planted under a rice-winter wheat (*Triticum aestivum* L.) rotation, a typical cropping system in the study area.

2.2. Experimental setup and soil sampling

The CO_2-enrichment experiment was conducted during the rice growing season in 2016–2020 using OTCs (diameter of 3.75 m, height of 3 m, and bottom area of 10 m^2). Four chambers were deployed in each treatment in a completely randomized design (Fig. 1). The treatments were (i) ambient CO_2 control ('ambient CO_2'), (ii) gradually elevated CO_2 concentration ('gradual eCO_2'), and (iii) abruptly elevated CO_2 concentration ('abrupt eCO_2'). The CO_2 concentration for gradual eCO_2 was gradually increased by 40 μ mol mol^{-1} y^{-1} from 2016 to 2020, i.e. ambient CO_2 + 40, + 80, + 120, + 160, and + 200 μ mol mol^{-1}, respectively, and the CO_2 concentration for abrupt eCO_2 was increased once by 200 μ mol mol^{-1} in 2016 and maintained at the same level until 2020. Highly pure CO_2 was injected into each OTC through perforated tubes surrounding the glass wall of the OTC. The real-time concentrations of CO_2 in the OTCs were monitored using a GMM222 CO_2 sensor (Vaisala Inc., Helsinki, Finland) and maintained at the target concentrations by an automatic computerized control system. See Wang et al. (2021) for a detailed site description.

The eCO_2 experiment was performed during the growing season in 2020, and CO_2 was enriched from 20 June to 16 October. The average CO_2 concentrations over the growing season were 415 ± 18, 617 ± 24, and 613 ± 26 ppm under ambient, gradual,

and abrupt eCO_2, respectively. The rice variety was Nanjing 9108, which is a common local japonica variety. Nitrogen (N) fertilizer was supplied at a rate of 25 g N m^{-2} y^{-1} during the growing season, with 60% as a base dressing before transplanting and two 20% top dressings at the tillering stage. The paddy field was flooded midseason and then drained and reflooded. Detailed information for water and fertilization management are presented in Table S1.

Soil samples were collected in October 2020 using a ring sampler 50 mm in diameter. Five replicate cores were collected from each OTC and then divided into three layers (0–5, 5–10, and 10-20 cm). The samples from the same OTC and layer were pooled to form one composite sample. All visible roots, plant debris, and stones were carefully removed. The samples were then immediately transported on ice to the laboratory. The study analyzed a total of 27 samples (three CO_2 treatments, three soil layers, and three replicates; n = 27). All samples were divided into two parts: one part was stored at 4 °C for the analysis of chemical parameters, and the other part was air-dried and ground for the analysis of SOC, total N, and amino sugar concentrations.

2.3 Analysis of soil properties

The concentrations of SOC and total N were measured by dry combustion using a Vario EL elemental analyzer (Elementar Analysensysteme, Hanau, Germany). Dissolved organic C (DOC) was extracted from the soil with 0.5 M K_2SO_4 (1:4, w/v), and its concentration was determined using an Elementar liquid TOC II analyzer. The concentrations of ammonium (NH_4^+) and nitrate (NO_3^-) were measured using a FIAstar 5000 Automatic Flow Injection Analyzer (FOSS, Sweden) and summed as

inorganic N (IN). The DOC/IN ratio was calculated to represent the available C/N. Soil pH was assessed in a 1:2.5 soil:water suspension using a pH meter.

2.4 Amino sugar analysis and calculation of MNC concentration

The soil concentrations of amino sugars (routinely used as a biomarker of microbial necromass) were analyzed using the classical method developed by Zhang and Amelung (1996). Briefly, ~0.5 g of air-dried soil (passed through a 0.25-mm sieve) was hydrolyzed with 6 M HCl at 105 °C for 8 h. The solution was cooled to room temperature, and inositol was added as a surrogate standard. The hydrolysates were filtered, adjusted to a pH of 6.6–6.8 using 1M potassium hydroxide, and then centrifuged at 3000 rpm for 10 min. The supernatant was dried by rotary evaporation. The residues were redissolved in methanol, dried under N_2 at 45 °C, and then freeze-dried. The dried residues were derivatized using a derivatization reagent and extracted with dichloromethane. The dichloromethane phase, after the removal of excess derivatization reagent, was dried using N_2 gas. The final amino sugar derivatives were quantified on an Agilent 7890 gas chromatograph using a DB-5 column (30 m × 0.25 mm i.d., film thickness of 0.25 μm) and a flame ionization detector.

Glucosamine, galactosamine, mannosamine, and muramic acid were the detectable amino sugars. Total amino sugar concentration was calculated as the sum of the concentrations of glucosamine, muramic acid, and galactosamine but not mannosamine due to its ubiquitous origin and low concentration. Glucosamine is mainly produced by fungi, and muramic acid is exclusively produced by bacteria (Guggenberger et al., 1999; Amelung, 2001). A list of concentrations of the individual

and total amino sugars are shown in Fig. S1. Fungal and bacterial concentrations of necromass C, as indices for the capacity to sequester MNC, were calculated based on conversion factors. Specifically, the concentration of bacterial necromass C (BNC) was calculated by multiplying the concentration of muramic acid by 45, assuming that Gram-negative and Gram-positive bacteria were present at a ratio of 1:2 (Appuhn and Joergensen, 2006; Joergensen, 2018). The concentration of fungal necromass C (FNC) was calculated by subtracting the concentration of bacterially derived glucosamine from the total glucosamine concentration, assuming a muramic acid:glucosamine molar ratio of 1:2 in bacterial cells (Engelking et al., 2007; Joergensen, 2018). Total MNC concentration was estimated as the sum of the FNC and BNC concentrations. Biomarker abundances are reported as absolute concentrations (mg kg^{-1} soil) to indicate variations in soils and SOC-normalized concentrations (percentage of MNC in SOC) to indicate their relative contributions to the SOC pool.

2.5 Statistical analysis

A general linear model was fitted to test the effect of the eCO_2 treatments, soil depth, and their interaction on the soil physiochemical properties, the MNC data, and the proportion in SOC using R (version 4.0.2). The data were first checked for normality and the homogeneity of variance using the Shapiro-Wilk test and Levene's test, respectively. Tukey's honest significant difference (HSD) test was used post hoc with the "TukeyHSD" function in R. A Pearson correlation analysis was carried out to identify the relationships between the MNC data and the soil physiochemical properties. Figures were generated using SigmaPlot (version 10.0), and the correlation

heatmap was generated using R.

3. Results

3.1 Soil properties

The soil properties in the eCO_2 treatments are listed in Table 1. The eCO_2 treatments significantly interacted with depth on soil properties ($p < 0.05$). The concentrations of SOC, total N, and IN and the soil-water content (SWC) decreased significantly with depth ($p < 0.05$). The SOC and total N concentrations in the 0-5 cm surface layer did not differ significantly between the eCO_2 treatments. The concentrations of SOC and N in the 5–10 cm layer were significantly higher under gradual eCO_2 than ambient control and abrupt eCO_2 ($p < 0.05$). The SOC and total N concentrations in the 10–20 cm subsurface layer were lower under both gradual and abrupt eCO_2 than ambient control. The concentration of DOC in all three layers was 24% higher under gradual eCO_2 and 30% higher under abrupt eCO_2 than ambient control. The IN concentration decreased significantly with depth. The IN concentration in the 10–20 cm layer was significantly lower under abrupt and gradual eCO_2 than ambient control and was higher under abrupt eCO_2 than gradual eCO_2. The DOC:IN ratio was significantly higher under both eCO_2 treatments than ambient control, associated with a trend to increase with depth ($p < 0.05$). SWC was generally lower under the eCO_2 treatments than ambient control and decreased significantly with depth ($p < 0.05$). Soil pH ranged from 5.98 to 6.27 across the three layers and did not differ significantly between the eCO_2 treatments and ambient control.

3.2 Total MNC concentration

The eCO_2 treatments significantly affected the total accumulation of MNC in the paddy field (F = 20.77, p < 0.001; Table 2). The MNC concentration in the three layers averaged 6.7% lower under Gradual eCO_2 than ambient control (p > 0.05, Fig. 2a). The MNC concentration was significantly lower by 20.3% under abrupt eCO_2 than ambient control (p < 0.05). The variation of MNC concentration in the eCO_2 treatments differed significantly between the surface and subsurface layers (F = 214.98, p < 0.001), and eCO_2 strongly interacted with soil depth (F = 21.34; p < 0.001). The concentration of MNC in the 0–5 cm surface layer was higher under both eCO_2 treatments than ambient control, but not significantly (p > 0.05). By contrast, the MNC concentration in the 10–20 cm layer was significantly lower in both eCO_2 treatments (p < 0.05), particularly abrupt eCO_2 (p < 0.05). The absolute concentration of MNC in the 10–20 cm layer was 38.8 and 30.4% lower under abrupt and gradual eCO_2, respectively. The MNC concentration in the 5–10 cm layer did not tend to be lower under gradual eCO_2, unlike abrupt eCO_2, but was 23.3% higher compared with ambient control (p < 0.05).

3.3 BNC and FNC concentrations

The patterns of accumulation of BNC and FNC were significantly affected by the eCO_2 treatments, soil depth, and their interaction (Table 2). The accumulation of fungal necromass mirrored that of total MNC and was much lower in the 10–20 cm subsurface layer under the eCO_2 treatments compared with ambient control (p < 0.05, Fig. 2b). The responses of BNC concentration to eCO_2 were generally less sensitive than the responses of FNC concentration among the CO_2 treatments across the three

soil layers (Fig. 2c). The variations of the BNC and FNC concentrations in response to eCO_2 were dependent on depth. The FNC and BNC concentrations in the 5–10 cm layer were significantly higher ($p < 0.05$) under gradual eCO_2 than ambient control and abrupt eCO_2.

3.4 MNC concentration normalized to SOC concentration (MNC/SOC)

The MNC concentration normalized to the SOC concentration, i.e. the proportion of total MNC concentration in SOC (%), ranged from 43.4 to 54.8% in the three treatments (Fig. 3a). The MNC concentration normalized to the SOC concentration did not differ significantly between the eCO_2 treatments or with depth alone, but by their interaction (Table 2). The proportion of MNC in SOC was higher in the eCO_2 treatments than ambient control in the uppermost layer (0–5 cm) but lower in the two subsurface layers (Fig. 3a). The MNC/SOC ratio in the 10–20 cm layer was significantly lower under abrupt eCO_2 than ambient control ($p < 0.05$) but did not differ significantly between gradual eCO_2 and ambient control. FNC accounted for 37.5–41.5% of the total SOC pool and was approximately 5-fold higher than was BNC. The proportion of FNC in SOC averaged 8.18 and 22.3% lower across the three soil layers under gradual and abrupt eCO_2, respectively. The proportion of BNC in SOC did not differ significantly between the eCO_2 treatments and ambient control across the three layers (Fig. 3c). The ratio of fungal to bacterial necromass was significantly lower under the eCO_2 treatments than ambient control in the 10–20 cm layer but were generally similar between the 0–5 and 5–10 cm layers (Fig. 3d).

3.5. Factors influencing MNC concentration

The Pearson correlation analysis indicated that the dominant drivers of MNC concentration differed with depth (Fig. 4). The total MNC and FNC concentrations in the 0–5 cm layer were positively correlated with the DOC concentration and the DOC/IN ratio ($p < 0.05$). The total MNC, FNC, and BNC concentrations in the 5–10 cm layer were positively correlated with the SOC, total N, and IN concentrations ($p < 0.05$). The total MNC and FNC concentrations and the FNC/BNC ratio in the 10–20 cm layer were correlated positively with SWC and the SOC, total N, and IN concentrations but negatively with the DOC concentration and the DOC/IN ratio ($p < 0.05$).

4. Discussion

4.1 Gradually and abruptly elevated CO_2 concentrations differentially altered the total accumulation of microbial necromass in the paddy soil

Four years of abruptly or gradually elevated CO_2 concentrations led to large changes in microbial necromass within the 0–20 cm soil profile compared with the ambient control. This result supported our first hypothesis that the response of microbial necromass to a gradual increase in CO_2 concentration differed from the response to an abrupt single increase in concentration. The average abundance of microbial necromass across the three layers in did not differ significantly between gradual eCO_2 and ambient control but did differ between gradual and abrupt eCO_2. This result suggested that the microbial response may not have been as strongly affected by higher atmospheric CO_2 levels as previously reported based on a single increase (Liu et al., 2021b). The necromass responded more to eCO_2 when CO_2 was

raised abruptly than when it was raised gradually, suggesting a higher sensitivity of microorganisms to a large pulse of CO_2. This finding was consistent with observations that subjecting soil organisms to a single increase in CO_2 concentration imposed a selective pressure on the microbiota, which may differ from the selective pressure exerted by an incrementally increased CO_2 concentration (Klironomos et al., 2005).

Our findings support the view that studies using a single increase in CO_2 concentration could have overestimated some responses of the microbial community (Klironomos et al., 2005; Shen et al., 2023). The complexity of soil processes hinders a straightforward interpretation of different variations of necromass accumulation under the two eCO_2 conditions, but we suggest that the gradual vs abrupt eCO_2 treatments differentially influenced microbial population growth, death, and necromass decay by directly and indirectly affecting soil properties and substrate availability. This suggestion was supported by various changes in soil properties, plant biomass, and microbes in the plots that were exposed to the abrupt vs gradual eCO_2 treatments (Wang et al., 2021; Shen et al., 2023). These factors may have conjointly influenced the microbially mediated C processes and accumulation of necromass in our study (Fig. 4).

The abundance of microbial necromass in the uppermost soil layer notably did not significantly change in response to either eCO_2 treatment, but the accumulation of necromass was highest in the 5–10 cm middle layer in the gradual treatment and was low in the abrupt treatment. This finding illustrated the different outcomes of gradual vs abrupt eCO_2 on microbial C accumulation with soil depth. We cannot clarify this

phenomenon, but it was most likely due to the differences in the spatial availability of C and N between abrupt and gradual eCO_2, indicated by the different concentrations of SOC, total N, and IN and their significant correlations with the MNC concentration. These results implied that the abrupt and gradual eCO_2 treatments probably drove distinct translocational or formational processes of microbial C at the vertical scale. These findings emphasize that caution should be taken in interpreting the magnitude of microbial responses to increases in atmospheric CO_2 levels that are much more abrupt than those that would occur in nature. Atmospheric CO_2 concentration increases gradually rather than abruptly to a high level, which should not be ignored in future studies.

Elevated CO_2 concentrations generally tended to decrease the accumulation of microbial necromass in the paddy soil compared with the ambient control, irrespective of soil layer. This result indicated that the overall capacity or efficiency of paddy soil to sustain the microbially derived, slow-cycling C fraction was hindered under eCO_2 conditions. In actuality, the dynamics of MNC in soil involves a continuous replenishing, with new formation and subsequent decomposition, and will ultimately reach a relatively steady state in soils. Our observations imply that the initial balance is perturbed by ongoing climate change and suggests an alteration of the mediation of C-cycling feedbacks by soil microbes in paddy-field ecosystems. Extraneous C entering soil is either allocated for the production of respiratory energy or for biosynthetic stabilization and growth during the microbial decomposition of organic matter (Hagerty et al., 2014).

Reductions of microbial necromass under eCO_2 is probably indicative of a decreased efficiency of use of extraneous C by microbes, despite higher inputs of plant C, i.e. a greater proportion of new C is allocated to respiration than to growth (Ziegler and Billings, 2011), consistent with the general phenomenon of accelerated SOC turnover under eCO_2 characterized by higher C inputs and higher respiration rates (van Groenigen et al., 2017; Kuzyakov et al., 2019). Glaser and Gross (2005) reported a faster turnover of microbial residues after seven years of exposure to elevated atmospheric CO_2 concentrations using a compound-specific $\delta^{13}C$ analysis of amino sugars. The elevated CO_2 concentrations in their study may have increased the rate of turnover of newly formed microbial necromass, limiting the microbial potential for sequestering soil C. Reduced microbial C-use efficiency and increased mineralization of necromass may collectively work together to reduce the efficiency of the soil microbial C pump as drivers for sequestering SOC in paddy fields under global climate change. Our results also indicate the need for more-accurate assessments of the stability of microbially derived C fractions with increased C inputs under global change to accurately project the future dynamics of C from an ecosystem perspective (Kallenbach et al., 2015).

4.2 Differential accumulation of microbial necromass with depth in response to gradually and abruptly elevated CO_2 concentrations

Investigating microbial necromass in different soil layers is important for gaining a comprehensive understanding of microbially derived C dynamics and its response to global climate change (Jia et al., 2017; He et al., 2022; Zosso et al., 2023). Whether

the effects of eCO_2 on microbial necromass will yield similar results between surface and subsurface layers is mostly unknown. Our results indicated that the direction and magnitude of the effects of eCO_2 on microbial necromass differed between the surface and subsurface layers, illustrating the heterogeneity of necromass associated with depth induced by eCO_2 exposure for four years. The effects of eCO_2 on microbial necromass were positive in the uppermost 0–5 cm layer but were not significant, consistent with a previous observation in a FACE study (Liu et al., 2021b) that elevated CO_2 concentrations stimulated the accumulation of microbial necromass in subtropical paddy soil. This phenomenon may have been due to the typical C-limitation of soil microorganisms (Schimel and Weintraub, 2003), so higher C supplies under elevated atmospheric CO_2 concentrations may have thus stimulated microbial growth and increased microbial biomass (de Graaff et al., 2006; Blagodatskaya et al., 2010; Kuzyakov et al., 2019), leading to the accumulation of necromass (Cotrufo et al., 2013; Liang et al., 2015).

By contrast, we found that the positive impacts of eCO_2 on MNC concentration decreased and became negative with depth, indicating that the responses of MNC concentration were highly dependent on depth within the 0–20 cm soil profile. Subsoil microorganisms are generally believed to be more responsive than topsoil microorganisms to the supply of labile C (Fontaine et al., 2007), so more labile C in deep layers induced by eCO_2 should have led to a higher accumulation of necromass. Our observation was contrary to this expectation. The MNC concentration in the 10–20 cm layer was significantly lower in both eCO_2 treatments compared with the

ambient control ($p < 0.05$), indicating that microbially derived C at depth was more sensitive to eCO_2 compared with C in the top layer. This difference would partially negate the effect of the higher input of plant C on the storage of C in the paddy soil.

Luo and Reynolds (1999) reported that N nutrient demand would increase to balance the additional C sink associated with the lower N availability under eCO_2. Decreased N availability may inhibit the rate of microbial growth (Blagodatskaya et al., 2010) and thus the production of microbial biomass (Butterly et al., 2016), so less necromass would accumulate in N-limited ecosystems (Jia et al., 2017; Ding et al., 2019; He et al., 2022). The microorganisms in our study may also have been N-limited when the amounts of labile C were higher in the deeper subsurface layer, so the lower accumulation of necromass would likely represent inhibited microbial anabolism. This interpretation was supported by the lower availability of N relative to labile C (i.e. IN/DOC) in the deep subsurface layer, indicated by the positive correlation between the IN and MNC concentrations (Fig. 4, $p < 0.05$). By comparison, the IN and MNC concentrations were not significantly correlated in the surface layer.

The competition between plants and microbes for nutrients stimulated by eCO_2 likely promoted the mineralization or reuse of microbial necromass due to the low C:N:P ratios (Kaiser et al., 2014). The supply of more labile C may thus have promoted microbial growth and activity in the deep soil layers, thus intensifying the requirement for N (Blagodatskaya et al., 2010) and leading to the remineralization of MNC to alleviate such stoichiometric constraints (He et al., 2011; Khan et al., 2016;

Jia et al., 2017). Microbial necromass in our study was much less abundant under abrupt eCO_2, where the DOC/IN ratio was significantly higher at depth relative to gradual eCO_2. The lower accumulation of microbial necromass and the lower IN concentration at our eCO_2 site agreed well with the higher N demand due to the abrupt increase in CO_2 concentration (Luo and Reynolds, 1999). Our results also provide supportive evidence that the efficiency of accumulating soil microbial necromass relies mostly on the tradeoff between C sequestration and nutrient supply (Feng and Wang, 2023). Similarly, the divergent accumulation of microbial necromass in the surface and subsurface layers in response to climate change have been reported (Jia et al., 2017; Ding et al., 2019; Wen et al., 2023). These findings emphasize vigilant attention will need to be paid to the feedback of, or threat, to the fraction of stable C in deep soil posed by global climate change (Chen et al., 2023).

The role and capacity of microbially mediated C sequestration may be potentially altered, and the changing magnitude may vary with soil depth under future CO_2 scenarios. The sequestration of C in soils with rising CO_2 concentrations is notably limited by progressively lower N availability in natural ecosystems (Luo et al., 2004; Terrer et al., 2018). For example, a meta-analysis by Cotrufo et al. (2019) found that C sequestration via microbial anabolism in grassland soil was highly persistent but required a lot of N. We found that eCO_2 could potentially trigger N limitation and weaken the accumulation of microbial C in subsoil layers even in agricultural systems receiving extraneous inputs of N fertilizers. Managing the accumulation of C in paddy soils should therefore target harmonizing the nutrient supply and the potential

sequestration of C driven by the microbial C pump under scenarios of increasing CO_2 concentrations.

4.3 Differential responses of fungal and bacterial necromasses to gradually and abruptly elevated CO_2 concentrations

Elevated atmospheric CO_2 concentrations have been reported to induce changes in microbial communities responsible for the turnover of organic matter in soil (reviewed in (Pritchard, 2011; Butterly et al., 2016; Sun et al., 2021). Different microbial-community responses to climate change could lead to corresponding accumulations of necromass (Liang et al., 2015). Our results demonstrated differing effects of eCO_2 on the accumulation of fungal and bacterial necromasses, partially in agreement with our second hypothesis. Fungal necromass, however, was more sensitive than bacterial necromass to eCO_2, especially in the abrupt eCO_2 treatment, partially not supporting our hypothesis. Abruptly increasing the CO_2 concentration decreased fungal necromass by 42.4% relative to the ambient control, which was significantly higher than gradual eCO_2 with a decrease of 32.6% in the 10–20 cm deep layer, suggesting a higher sensitivity or vulnerability of fungally derived C at depth to eCO_2. Our results were consistent with the finding by van Groenigen et al. (2007) that eCO_2 decreased fungally derived residues in a grassland system with high rates of N fertilization, indicating that eCO_2 did not favor a fungal pathway of decomposition. Similarly, Klironomos et al. (2005) reported that some fungi were sensitive to a pulse of C in an abrupt eCO_2 treatment, which reduced the richness of fungal species. The large decrease in the abundance of fungal necromass represented

an overall dwindling contribution of fungal necromass to the subsoil microbial C pool induced by an increase in CO_2 concentration. This may have been primarily responsible for the significant decrease in the total accumulation of microbial C in the deeper layers in our study, which accounted for approximately 80% of the total MNC pool.

Most bacteria are *r*-strategists that quickly metabolize available substrates (Blagodatskaya and Kuzyakov, 2008) and are expected to multiply and outcompete fungi with increased concentrations of available C under elevated CO_2 conditions. We found, however, that bacterial necromass was less affected than fungal necromass by eCO_2 across the three layers, contrary to our hypothesis. Several lines of evidence have suggested that bacterial residues are quickly turned over under N-limited conditions (Kindler et al., 2006; He et al., 2011; Zheng et al., 2021). Smaller variations of bacterial necromass may thus be partially due to the faster turnover under eCO_2 conditions that counteract bacterial C accumulation.

Increased C availability induced by elevated CO_2 concentrations is also generally associated with a higher abundance of Gram-negative than Gram-positive bacteria (Janus et al., 2005; Sun et al., 2021). The increase in abundance of Gram-negative bacteria may not strongly affect bacterial necromass due to their thinner peptidoglycan layer in the periplasm compared to Gram-positive bacteria (Kögel-Knabner, 2002), so the effects of eCO_2 would probably take much longer to manifest. The divergent accumulation of fungal and bacterial necromasses under eCO_2 would also lead to a large change in the ratio of fungal to bacterial necromass in subsoil

layers, indicating an alteration of fungally and bacterially mediated inputs of C to the soil. These changes may affect the long-term sequestration of C in paddy ecosystems, because fungal necromass is more recalcitrant than bacterial necromass in soil (Schweigert et al., 2015; Khan et al., 2016; Zheng et al., 2023). We again highlight the importance of separately considering individual soil layers for accurately predicting microbially mediated C dynamics under eCO_2 because the sensitivity of microbial C varies with soil depth.

4.4 Contribution of microbial necromass to the SOC pool under different eCO_2 conditions

Microbial necromass contributed significantly to SOC (~50%) at our site, indicating that the accumulation of microbial necromass is an important process contributing to the accumulation of SOC in agroecosystems (Miltner et al., 2012; Kallenbach et al., 2015; Liang et al., 2019). Our findings suggested that elevated atmospheric CO_2 concentrations may lead to larger changes in the contribution of microbial necromass to SOC in subsoil compared to topsoil. The proportion of microbial C in the SOC pool increased in the topsoil layer, indicating a more intensive processing of microbial C in the uppermost layer that received the most input of plant litter. More importantly, we found that eCO_2 greatly decreased the proportion of necromass in SOC under both the gradual and abrupt eCO_2 treatments in the 10–20 cm subsurface layer, indicating the preferential loss of this microbially derived fraction of C in the sequestration of subsoil organic C in scenarios of higher CO_2 concentrations. The abrupt eCO_2 treatment decreased the MNC concentration normalized to the SOC concentration in

the deep layers more than did the gradual eCO_2 treatment, but the difference was not significant.

These results indicate that the qualitative assessment of the contribution of microbial C to the sequestration of SOC at different depths may be more important than focusing only on changes in the topsoil layer. Together with the absolute concentration of microbial necromass per unit of soil, our observations again highlight that the nature and magnitude of microbial responses to eCO_2 are probably overestimated to some extent when using abrupt increases.

FNC made consistently larger contributions to SOC across the three layers, and its concentration was strongly affected by the eCO_2 treatments. The proportion of bacterial necromass in SOC, however, was marginally affected by eCO_2, indicating an asynchronous contribution of microbial C from different microbial groups to the accumulation of paddy SOC under eCO_2. These results also infer that the composition or source (fungi vs bacteria; microbe vs plant) of SOC is undergoing an alteration driven by elevated CO_2 concentrations, so the direction and magnitude of rice-paddy feedbacks to enhanced atmospheric CO_2 concentrations may be potentially altered. Microbial necromass is believed to be an important component of the stable pool of soil C, playing an important role in regulating ecosystem C stocks under global change (Liang et al., 2017; Sokol et al., 2019), so even a small change in this pool has large consequences for altering atmospheric composition (Liang et al., 2015). The sensitivity of the MNC concentration to elevated CO_2 levels in our study may indicate the possible vulnerability of, or changes in, the long-term stability of the C pool of

rice-paddy ecosystems and may prove important in the prediction of the impacts of global change on the storage of soil C.

We are aware that we only investigated soil depth down to 20 cm, so deeper layers (>20 cm) should be considered in future studies due to the large amount of C they store and to their higher sensitivity to global climate change (Zosso et al., 2023). Any change in climate or agricultural practice that increases the supply of fresh C along the soil profile could stimulate large losses of ancient C buried in deep soil (Lal, 2004). Our collective findings nonetheless reinforce the importance of investigating microbially mediated C processes in subsoil layers in response to environmental changes, which may be more susceptible than surface layers to the changes (Chen et al., 2023; Zosso et al., 2023). Care must also be exercised when interpreting the changing magnitude of microbial responses to increases in atmospheric CO_2 concentrations that are much more abrupt than those that would occur in nature. Experimental efforts to better simulate real-world environmental scenarios are thus necessary to address critical issues associated with gradual changes in natural ecosystems (Luo and Reynolds, 1999; Wang et al., 2023) Future studies should consider that the atmospheric CO_2 concentration is gradually, rather than abruptly, increasing to a high constant, which will affect the sequestration of C mediated by microorganisms.

5. Conclusions

Our study found that microbially mediated C processes in response to a large, abrupt increase in CO_2 concentration elicited changes that differed from those in response to

a gradual increase in CO_2 concentration. The abrupt eCO_2 treatment induced a larger variation in the accumulation of microbial necromass compared with the gradual eCO_2 treatment, indicating that the role or capacity of the microbial mediation of C sequestration via anabolism was differentially altered in the paddy soil under different eCO_2 scenarios. The impacts of eCO_2 on the accumulation of microbial necromass was highly contingent on soil depth, with marginal accumulation in the surface layer but substantial depletion in the subsurface layers. Our findings indicated that an elevated atmospheric CO_2 concentration may lead to larger changes in the contribution of microbial necromass to the subsoil SOC pool than the topsoil SOC pool. Our study demonstrated that the abrupt and gradual eCO_2 increases differed in their impact on the accumulation of microbial necromass, which may further differentially alter the microbial contribution to the accumulation of SOC in paddy fields. We cannot be certain that the differential responses of microbial necromass to abrupt vs gradual increases in CO_2 concentrations we detected will not occur in other ecosystems. We therefore suggest that CO_2 concentration be gradually rather than abruptly increased to a high constant in further studies, because gradual increases will most likely occur in most ecosystems in the real world. Equal attention will need to be paid to identifying the response of microbial C in deeper soil layers if we are to accurately project the future dynamics of soil C in specific ecosystems under scenarios of a changing climate.

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References

Ainsworth, E.A., Long, S.P., 2005. What have we learned from 15 years of free-air CO_2 enrichment (FACE)?: A meta-analytic review of the responses of photosynthesis, canopy. New Phytologist 165, 351-371.

Amelung, W., 2001. Methods using amino sugars as markers for microbial residues in soil, In: Lal, R., Kimble, J.M., Follett, R.F., Stewart B.A., eds. Assessment Methods for Soil Carbon. CRC/Lewis Publishers, Boca Raton, FL.

Appuhn, A., Joergensen, R.G., 2006. Microbial colonisation of roots as a function of plant species. Soil Biology & Biochemistry 38, 1040-1051.

Bhattacharyya, P., Roy, K.S., Neogi, S., Dash, P.K., Nayak, A.K., Mohanty, S., Baig, M.J., Sarkar, R.K., Rao, K.S., 2013. Impact of elevated CO_2 and temperature on soil C and N dynamics in relation to CH_4 and N_2O emissions from tropical flooded rice (*Oryza sativa* L.). Science of the Total Environment 461, 601-611.

Blagodatskaya, E., Blagodatsky, S., Dorodnikov, M., Kuzyakov, Y., 2010. Elevated atmospheric CO_2 increases microbial growth rates in soil: results of three CO_2 enrichment experiments. Global Change Biology 16, 836-848.

Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biology and Fertility of Soils 45, 115-131.

Bradford, M.A., Wieder, W.R., Bonan, G.B., Fierer, N., Raymond, P.A., Crowther, T.W., 2016. Managing uncertainty in soil carbon feedbacks to climate change. Nature Climate Change 6, 751-758.

Buckeridge, K.M., Creamer, C., Whitaker, J., 2022. Deconstructing the microbial

necromass continuum to inform soil carbon sequestration. Functional Ecology 36, 1396-1410.

Butterly, C.R., Phillips, L.A., Wiltshire, J.L., Franks, A.E., Armstrong, R.D., Chen, D.L., Mele, P.M., Tang, C.X., 2016. Long-term effects of elevated CO_2 on carbon and nitrogen functional capacity of microbial communities in three contrasting soils. Soil Biology & Biochemistry 97, 157-167.

Castañeda-Gómez, L., Walker, J.K.M., Powell, J.R., Ellsworth, D.S., Pendall, E., Carrillo, Y., 2020. Impacts of elevated carbon dioxide on carbon gains and losses from soil and associated microbes in a *Eucalyptus* woodland. Soil Biology & Biochemistry 143, 107734.

Chen, J., Luo, Y.Q., Sinsabaugh, R.L., 2023. Subsoil carbon loss. Nature Geoscience 16, 284-285.

Cotrufo, M.F., Lavallee, J.M., 2022. Soil organic matter formation, persistence, and functioning: A synthesis of current understanding to inform its conservation and regeneration, In: Sparks, D.L. (Ed.), Advances in Agronomy. Elsevier Academic Press Inc, San Diego, pp. 1-66.

Cotrufo, M.F., Ranalli, M.G., Haddix, M.L., Six, J., Lugato, E., 2019. Soil carbon storage informed by particulate and mineral-associated organic matter. Nature Geoscience 12, 989-994.

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Global Change Biology 19, 988-995.

de Graaff, M.A., van Groenigen, K.J., Six, J., Hungate, B., van Kessel, C., 2006. Interactions between plant growth and soil nutrient cycling under elevated CO_2: a meta-analysis. Global Change Biology 12, 2077-2091.

Ding, X.L., Chen, S.Y., Zhang, B., Liang, C., He, H.B., Horwath, W.R., 2019. Warming increases microbial residue contribution to soil organic carbon in an alpine meadow. Soil Biology & Biochemistry 135, 13-19.

Drake, J.E., Gallet-Budynek, A., Hofmockel, K.S., Bernhardt, E.S., Billings, S.A.,

Jackson, R.B., Johnsen, K.S., Lichter, J., McCarthy, H.R., McCormack, M.L., Moore, D.J.P., Oren, R., Palmroth, S., Phillips, R.P., Pippen, J.S., Pritchard, S.G., Treseder, K.K., Schlesinger, W.H., DeLucia, E.H., Finzi, A.C., 2011. Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO_2. Ecology Letters 14, 349-357.

Engelking, B., Flessa, H., Joergensen, R.G., 2007. Shifts in amino sugar and ergosterol contents after addition of sucrose and cellulose to soil. Soil Biology & Biochemistry 39, 2111-2118.

Feng, X.J., Wang, S.M., 2023. Plant influences on soil microbial carbon pump efficiency. Global Change Biology 29, 3854-3856.

Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450, 277-U210.

Ge, T.D., Luo, Y., He, X.H., 2019. Quantitative and mechanistic insights into the key process in the rhizodeposited carbon stabilization, transformation and utilization of carbon, nitrogen and phosphorus in paddy soil. Plant and Soil 445, 1-5.

Glaser, B., Gross, S., 2005. Compound-specific $\delta^{13}C$ analysis of individual amino sugars - a tool to quantify timing and amount of soil microbial residue stabilization. Rapid Communications in Mass Spectrometry 19, 1409-1416.

Guggenberger, G., Frey, S.D., Six, J., Paustian, K., Elliott, E.T., 1999. Bacterial and fungal cell-wall residues in conventional and no-tillage agroecosystems. Soil Science Society of America Journal 63, 1188-1198.

Hagerty, S.B., van Groenigen, K.J., Allison, S.D., Hungate, B.A., Schwartz, E., Koch, G.W., Kolka, R.K., Dijkstra, P., 2014. Accelerated microbial turnover but constant growth efficiency with warming in soil. Nature Climate Change 4, 903-906.

He, H.B., Zhang, W., Zhang, X.D., Xie, H.T., Zhuang, J., 2011. Temporal responses of soil microorganisms to substrate addition as indicated by amino sugar differentiation. Soil Biology & Biochemistry 43, 1155-1161.

He, M., Fang, K., Chen, L.Y., Feng, X.H., Qin, S.Q., Kou, D., He, H.B., Liang, C., Yang, Y.H., 2022. Depth-dependent drivers of soil microbial necromass carbon across Tibetan alpine grasslands. Global Change Biology 28, 936-949.

Hu, Z.K., Chen, X.Y., Yao, J.N., Zhu, C.W., Zhu, J.G., Liu, M.Q., 2020. Plant-mediated effects of elevated CO_2 and rice cultivars on soil carbon dynamics in a paddy soil. New Phytologist 225, 2368-2379.

Hu, Z.K., Zhu, C.W., Chen, X.Y., Bonkowski, M., Griffiths, B., Chen, F.J., Zhu, J.G., Hu, S.J., Hu, F., Liu, M.Q., 2017. Responses of rice paddy micro-food webs to elevated CO_2 are modulated by nitrogen fertilization and crop cultivars. Soil Biology & Biochemistry 114, 104-113.

Janus, L.R., Angeloni, N.L., McCormack, J., Rier, S.T., Tuchman, N.C., Kelly, J.J., 2005. Elevated atmospheric CO_2 alters soil microbial communities associated with trembling aspen (*Populus tremuloides*) roots. Microbial Ecology 50, 102-109.

Janzen, H.H., 2015. Beyond carbon sequestration: soil as conduit of solar energy. European Journal of Soil Science 66, 19-32.

Jia, J., Feng, X.J., He, J.S., He, H.B., Lin, L., Liu, Z.G., 2017. Comparing microbial carbon sequestration and priming in the subsoil versus topsoil of a Qinghai-Tibetan alpine grassland. Soil Biology & Biochemistry 104, 141-151.

Joergensen, R.G., 2018. Amino sugars as specific indices for fungal and bacterial residues in soil. Biology and Fertility of Soils 54, 559-568.

Kaiser, C., Franklin, O., Dieckmann, U., Richter, A., 2014. Microbial community dynamics alleviate stoichiometric constraints during litter decay. Ecology Letters 17, 680-690.

Kallenbach, C.M., Grandy, A.S., Frey, S.D., Diefendorf, A.F., 2015. Microbial physiology and necromass regulate agricultural soil carbon accumulation. Soil Biology & Biochemistry 91, 279-290.

Khan, K.S., Mack, R., Castillo, X., Kaiser, M., Joergensen, R.G., 2016. Microbial biomass, fungal and bacterial residues, and their relationships to the soil organic matter C/N/P/S ratios. Geoderma 271, 115-123.

Kindler, R., Miltner, A., Richnow, H.H., Kästner, M., 2006. Fate of gram-negative bacterial biomass in soil-mineralization and contribution to SOM. Soil Biology & Biochemistry 38, 2860-2870.

Klironomos, J.N., Allen, M.F., Rillig, M.C., Piotrowski, J., Makvandi-Nejad, S., Wolfe, B.E., Powell, J.R., 2005. Abrupt rise in atmospheric CO_2 overestimates community response in a model plant-soil system. Nature 433, 621-624.

Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biology & Biochemistry 34, 139-162.

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 Biology & Biochemistry 128, 66-78.

Lal, R., 2004. Soil carbon sequestration impacts on global climate change and food security. Science 304, 1623-1627.

Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. Nature 528, 60-68.

Li, S.C., Xie, S., Zhang, S.J., Miao, S.L., Tang, S.M., Chen, H.Y., Zhan, Q.W., 2022. Global patterns and controls of the soil microbial biomass response to elevated CO_2. Geoderma 428, 116153.

Liang, C., Amelung, W., Lehmann, J., Kästner, M., 2019. Quantitative assessment of microbial necromass contribution to soil organic matter. Global Change Biology 25, 3578-3590.

Liang, C., Gutknecht, J.L.M., Balser, T.C., 2015. Microbial lipid and amino sugar responses to long-term simulated global environmental changes in a California annual grassland. Frontiers in Microbiology 6, 335.

Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology 2, 17105.

Liu, Y.L., Ge, T.D., van Groenigen, K.J., Yang, Y.H., Wang, P., Cheng, K., Zhu, Z.K., Wang, J.K., Li, Y., Guggenberger, G., Sardans, J., Penuelas, J., Wu, J.S.,

Kuzyakov, Y., 2021a. Rice paddy soils are a quantitatively important carbon store according to a global synthesis. Communications Earth & Environment 2, 154.

Liu, Z.W., Liu, X.X., Wu, X.L., Bian, R.J., Liu, X.Y., Zheng, J.F., Zhang, X.H., Cheng, K., Li, L.Q., Pan, G.X., 2021b. Long-term elevated CO_2 and warming enhance microbial necromass carbon accumulation in a paddy soil. Biology and Fertility of Soils 57, 673-684.

Luo, Y., Su, B., Currie, W.S., Dukes, J.S., Finzi, A.C., Hartwig, U., Hungate, B., McMurtrie, R.E., Oren, R., Parton, W.J., Pataki, D.E., Shaw, M.R., Zak, D.R., Field, C.B., 2004. Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. Bioscience 54, 731-739.

Luo, Y.Q., Reynolds, J.F., 1999. Validity of extrapolating field CO_2 experiments to predict carbon sequestration in natural ecosystems. Ecology 80, 1568-1583.

Miltner, A., Bombach, P., Schmidt-Brücken, B., Kästner, M., 2012. SOM genesis: microbial biomass as a significant source. Biogeochemistry 111, 41-55.

Pan, G.X., Li, L.Q., Wu, L.S., Zhang, X.H., 2004. Storage and sequestration potential of topsoil organic carbon in China's paddy soils. Global Change Biology 10, 79-92.

Panneerselvam, P., Kumar, U., Senapati, A., Parameswaran, C., Anandan, A., Kumar, A., Jahan, A., Padhy, S.R., Nayak, A.K., 2020. Influence of elevated CO_2 on arbuscular mycorrhizal fungal community elucidated using Illumina MiSeq platform in sub-humid tropical paddy soil. Applied Soil Ecology 145, 103344.

Pritchard, S.G., 2011. Soil organisms and global climate change. Plant Pathology 60, 82-99.

Rousk, J., Bååth, E., 2011. Growth of saprotrophic fungi and bacteria in soil. Fems Microbiology Ecology 78, 17-30.

Schimel, J.P., Schaeffer, S.M., 2012. Microbial control over carbon cycling in soil. Frontiers in Microbiology 3, 348.

Schimel, J.P., Weintraub, M.N., 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil

Biology & Biochemistry 35, 549-563.

Schweigert, M., Herrmann, S., Miltner, A., Fester, T., Kästner, M., 2015. Fate of ectomycorrhizal fungal biomass in a soil bioreactor system and its contribution to soil organic matter formation. Soil Biology & Biochemistry 88, 120-127.

Shen, L.D., Ren, B.J., Jin, Y.H., Liu, X., Jin, J.H., Huang, H.C., Tian, M.H., Yang, W.T., Yang, Y.L., Liu, J.Q., Geng, C.Y., Bai, Y.A., Hu, Z.H., 2023. Effects of abrupt and gradual increase of atmospheric CO_2 concentration on methanotrophs in paddy fields. Environmental Research 223, 115474.

Sokol, N.W., Sanderman, J., Bradford, M.A., 2019. Pathways of mineral-associated soil organic matter formation: Integrating the role of plant carbon source, chemistry, and point of entry. Global Change Biology 25, 12-24.

Soong, J.L., Phillips, C.L., Ledna, C., Koven, C.D., Torn, M.S., 2020. CMIP5 models predict rapid and deep soil warming over the 21st Century. Journal of Geophysical Research-Biogeosciences 125, e2019JG005266.

Sun, Y., Wang, C.T., Yang, J.Y., Liao, J.H., Chen, H.Y.H., Ruan, H.H., 2021. Elevated CO_2 shifts soil microbial communities from *K*- to *r*-strategists. Global Ecology and Biogeography 30, 961-972.

Terrer, C., Vicca, S., Stocker, B.D., Hungate, B.A., Phillips, R.P., Reich, P.B., Finzi, A.C., Prentice, I.C., 2018. Ecosystem responses to elevated CO_2 governed by plant-soil interactions and the cost of nitrogen acquisition. New Phytologist 217, 507-522.

van Groenigen, K.-J., Six, J., Harris, D., Van Kessel, C., 2007. Elevated CO_2 does not favor a fungal decomposition pathway. Soil Biology & Biochemistry 39, 2168-2172.

van Groenigen, K.J., Osenberg, C.W., Terrer, C., Carrillo, Y., Dijkstra, F.A., Heath, J., Nie, M., Pendall, E., Phillips, R.P., Hungate, B.A., 2017. Faster turnover of new soil carbon inputs under increased atmospheric CO_2. Global Change Biology 23, 4420-4429.

Wang, Y.Y., Hu, Z.H., Shen, L.D., Liu, C., Islam, A., Wu, Z.R., Dang, H.H., Chen, S.T., 2021. The process of methanogenesis in paddy field sunder different

elevated CO_2 concentrations. Science of the Total Environment 773, 145629.

Wang, Y.Y., Hu, Z.H., Shen, L.D., Lu, G.H., Cao, R., Zheng, K.Z., Liu, C., Wu, Z.R., He, S.Q., 2023. Different characteristics of soil CH_4 emissions and methanogenic communities in paddy fields under gradually and abruptly elevated CO_2 concentrations. Soil Biology & Biochemistry 180, 108993.

Wen, S.H., Chen, J.Y., Yang, Z.M., Deng, L., Feng, J., Zhang, W., Zeng, X.M., Huang, Q.Y., Delgado-Baquerizo, M., Liu, Y.R., 2023. Climatic seasonality challenges the stability of microbial-driven deep soil carbon accumulation across China. Global Change Biology 29, 4430-4439.

WMO, 2021. The State of Greenhouse Gases in the Atmosphere Based on Global Observations through 2020. WMO Greenhouse Gas Bulletin, Switzerland.

Xia, Y.H., Chen, X.B., Hu, Y.J., Zheng, S.M., Ning, Z., Guggenberger, G., He, H.B., Wu, J.S., Su, Y.R., 2019. Contrasting contribution of fungal and bacterial residues to organic carbon accumulation in paddy soils across eastern China. Biology and Fertility of Soils 55, 767-776.

Xiong, L., Liu, X.Y., Vinci, G., Spaccini, R., Drososa, M., Li, L.Q., Piccolo, A., Pan, G.X., 2019. Molecular changes of soil organic matter induced by root exudates in a rice paddy under CO_2 enrichment and warming of canopy air. Soil Biology & Biochemistry 137, 107544.

Zak, D.R., Pregitzer, K.S., King, J.S., Holmes, W.E., 2000. Elevated atmospheric CO_2, fine roots and the response of soil microorganisms:: a review and hypothesis. New Phytologist 147, 201-222.

Zhang, X.D., Amelung, W., 1996. Gas chromatographic determination of muramic acid, glucosamine, mannosamine, and galactosamine in soils. Soil Biology & Biochemistry 28, 1201-1206.

Zheng, T.T., Miltner, A., Liang, C., Nowak, K.M., Kästner, M., 2021. Turnover of gram-negative bacterial biomass-derived carbon through the microbial food web of an agricultural soil. Soil Biology & Biochemistry 152, 108070.

Zheng, T.T., Miltner, A., Liang, C., Nowak, K.M., Kästner, M., 2023. Turnover of bacterial biomass to soil organic matter via fungal biomass and its metabolic

implications. Soil Biology & Biochemistry 180, 108995.

Ziegler, S.E., Billings, S.A., 2011. Soil nitrogen status as a regulator of carbon substrate flows through microbial communities with elevated CO_2. Journal of Geophysical Research-Biogeosciences 116, G01011.

Zosso, C.U., Ofiti, N.O.E., Torn, M.S., Wiesenberg, G.L.B., Schmidt, M.W.I., 2023. Rapid loss of complex polymers and pyrogenic carbon in subsoils under whole-soil warming. Nature Geoscience 16, 344-348.