Biochar

ORIGINAL RESEARCH

Depth-dependent microbial necromass carbon accumulation responses to long-term biochar amendment in croplands

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Abstract

Soil microbial necromass carbon (MNC), a major contributor to stable soil organic C (SOC), is increasingly recognized as a crucial indicator of long-term C sequestration. However, the depth-dependent responses of MNC to long-term biochar amendment remain poorly understood. Here, we conducted a 12-year field experiment in two contrasting cropland soils—a C-rich Entisol and a C-poor Ultisol—to assess the effects of biochar on MNC accumulation and composition in both topsoil (0–20 cm) and subsoil (20–40 cm). In the topsoil, biochar increased MNC by 23.3% in the Entisol and 39.0% in the Ultisol, with a stronger response in fungal necromass than in bacterial necromass. Conversely, subsoil MNC decreased by 17.9–30.4% across both soils. These contrasting patterns were associated with biochar-induced changes in nutrient availability and microbial traits, including nitrogen availability, enzyme activity, metabolic quotient, and microbial biomass. To complement our findings, a meta-analysis of 85 pairs of observations from 23 peer-reviewed studies confirmed that biochar increased topsoil MNC in 83.5% of cases, with an average increase of 10.2%. The magnitude of the effect was greater in soils with low initial SOC and high sand content, and it intensified over time, peaking 10 years after application. In summary, 12-year biochar amendment enhanced MNC accumulation in the topsoil but reduced it in the subsoil, highlighting the importance of long-term and depth-specific evaluations to fully understand biochar's role in microbially mediated SOC sequestration.

Highlights

- Long-term biochar addition enhanced topsoil MNC, particularly FNC, by improving microbial biomass and nutrient availability.
- In the subsoil, biochar-induced N limitations and elevated qCO_2 constrained MNC accumulation.
- Meta-analysis revealed that biochar increased MNC accumulation, with effects shaped by initial soil properties and application duration.
- Biochar affects microbially mediated soil C dynamics, emphasizing the need to consider depth and long-term effects.

Keywords Amino sugar, Biochar amendment, Soil depth, Meta-analysis, Soil carbon sequestration

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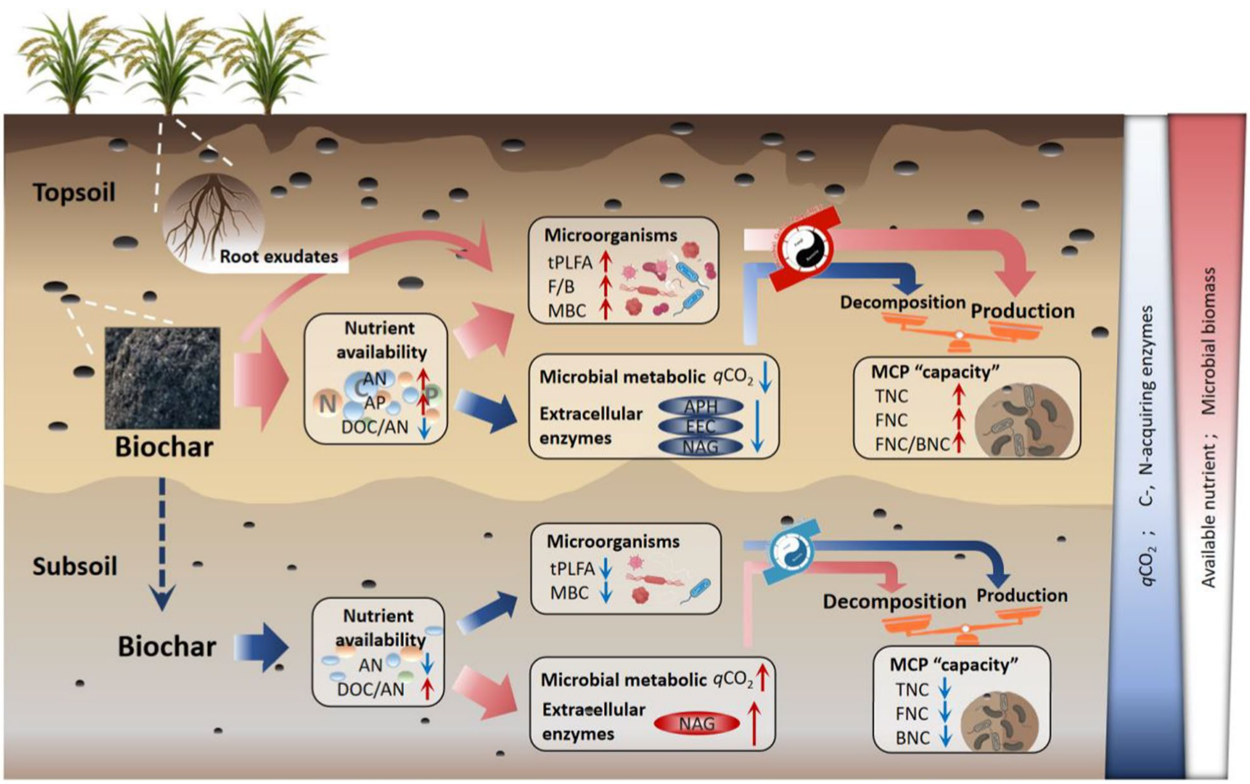
Graphical Abstract



1 Introduction

Soil organic carbon (SOC) is the largest terrestrial C reservoir, storing approximately 1550 Gt C within the top meter of soil globally (Lal 2004). It plays a critical role in regulating atmospheric CO_2 concentrations through dynamic processes of decomposition and stabilization, with soil microorganisms at the core of these mechanisms (Sokol et al. 2022). The 'microbial C pump (MCP)' framework outlines two primary microbial pathways contributing to SOC formation: ex vivo modification via extracellular enzyme activity and in vivo turnover through microbial assimilation, biosynthesis, and subsequent cell death (Liang et al. 2017). Microbial necromass C (MNC), the remnants of dead microbial cells, is increasingly recognized as a dominant and stable component of SOC, contributing 30–60% of total SOC (Liang et al. 2019; Wang et al. 2021). Due to its continuous production and slow decomposition, MNC persists in soils and plays a vital role in long-term C storage (Liang et al. 2017). Thus, promoting MNC stabilization is essential for SOC sequestration strategies aimed at mitigating climate change.

Biochar amendment is considered a particularly effective intervention for increasing SOC sequestration (Lehmann et al. 2021), with the potential to create negative feedback to mitigate climate change through multiple mechanisms: (i) direct addition of aromatic, recalcitrant C resistant to microbial decomposition (Lehmann et al. 2021), (ii) promotion of organo-mineral and organo-organic associations on biochar surfaces and within pores (Weng et al. 2022), (iii) reduction of microbial catabolism and enhancement of microbial C use efficiency (CUE) (Liu et al. 2020), and (iv) increasing belowground biomass and rhizodeposit input (Liu et al. 2021b). Recent studies emphasize that microbial necromass is a key transformation product of plant-derived C via the MCP pathway and is linked to microbial respiration (Feng and Wang 2023). Therefore, biochar may alter microbial growth and death pathways by enhancing resource availability and modifying microbial habitats (Luo et al. 2013; Chen et al. 2023a; Yang et al. 2024), thereby influencing MCP "capacity" (i.e., the absolute accumulation of MNC in the soil) and "efficacy" (i.e., the contribution of MNC to total SOC) (Liang et al. 2017; Zhu et al. 2020).

MNC accrual reflects a dynamic balance between microbial production and decomposition (Wang et al. 2022), governed by biotic and abiotic factors (Cao et al. 2023). Theoretical and empirical evidence suggests that biochar amendment can directly or indirectly induce changes in soil structure (Feng et al. 2022), nutrient (e.g., nitrogen [N] and phosphorus [P]) availability (Hossain et al. 2020), enzyme activity (Chen et al. 2019), and microbial community composition and physiological traits (Liu et al. 2020, 2023). For instance, biochar can act as a substrate and nutrient source, stimulating microbial growth (Zhang et al. 2018), and thereby promoting MNC accumulation through the entombing effect. It can also lower the microbial metabolic quotient (qCO_2) and enhance microbial CUE (Liu et al. 2019, 2020), thereby promoting the conversion of C into microbial biomass and necromass while reducing respiratory losses (Six et al. 2006). However, the low bioavailability of biochar-derived C may disrupt the soil C/N ratio. Under N-limited conditions, microbes may allocate more resources toward extracellular enzyme production to scavenge N from necromass rather than from soil organic matter (SOM), accelerating the decomposition of microbial necromass (Cui et al. 2020) and ultimately reducing the "capacity" and "efficacy" of the MCP (Zhou et al. 2023). While some studies report increased MNC following biochar addition (Zhang et al. 2022; Cheng et al. 2024), others show neutral (Kellerová et al. 2024) or even negative effects (Chen et al. 2023c). A meta-analysis of 25 pairs from 5 peer-reviewed studies showed that biochar had no significant effect on the net MNC accumulation, and even reduced the contribution of MNC to the SOC pool (Zhou et al. 2023). The different results may be due to variations in soil properties (such as initial SOC content and soil texture) and biochar characteristics (such as feedstock type and application rates). Furthermore, MNC accumulation is a slow and progressive process (Zhou et al. 2023), yet most existing studies are short-term (ranging from weeks to a few years). To better estimate the sustainability of biochar for soil C sequestration, the long-term data ($\geq$10 years) should be required (Hernandez-Soriano et al. 2016). Biochar can persist in soils for centuries to millennia (Lehmann 2007). Over time, its physical fragmentation significantly increases under field conditions, simultaneously altering the stoichiometric of soil C/N ratio (de la Rosa et al. 2018). Furthermore, our previous study found that fresh biochar addition initially promoted bacterial activity, whereas six years after biochar application the microbial community shifted to being dominated by fungi (Liu et al. 2019). Therefore, the changes in soil properties and microbial community composition may influence MNC dynamics, making the study of the long-term effects of biochar on MNC essential for more accurately assessing its C sequestration potential.

Subsoils (>20 cm depth), which store more than half of total SOC and play a crucial role in global C cycling (Jobbágy and Jackson 2000), are increasingly attracting attention. However, existing research on the effects of biochar on MNC has primarily focused on topsoil (0–20 cm), with little investigation into changes in MNC within the subsoil. The lower radiocarbon (^{14}C) concentrations in subsoil SOC suggest longer residence times (Rethemeyer et al. 2005; Rumpel and Kögel-Knabner 2010), indicating its potential for long-term C sequestration, especially in agricultural ecosystems (Button et al. 2022). Over time, biochar particles may fragment into micro- and nano-sized particles and migrate downward through the soil profile (Spokas et al. 2014; Obia et al. 2017), potentially influencing subsoil C dynamics and MNC accumulation. In addition, biochar can reduce nutrient leaching and limit vertical nutrient translocation (Shi et al. 2021), while simultaneously enhancing root-derived C inputs to deeper layers by improving plant growth (Liu et al. 2021b). Given that microbial activity is strongly regulated by nutrient and substrate availability (Manzoni et al. 2012), biochar-induced alterations in subsoil conditions may reshape microbial community composition and functional traits (Liu et al. 2023; Yuan et al. 2023). For instance, our recent study reported a decline in copiotrophic taxa in the subsoil after four years of biochar application (Ma et al. 2023). However, direct experimental evidence on how biochar influences MNC dynamics in the subsoil remains scarce. Addressing this knowledge gap is essential for accurately assessing the long-term C sequestration potential of biochar at the soil profile scale.

Soil C sequestration remains a critical global challenge in the context of climate change. In China, purple soils and red soils are two widely distributed cropland soil types with distinct physicochemical properties. Purple soils, derived from Jurassic and Cretaceous rocks and predominantly found in southwestern China, are classified as non-zonal soils characterized by high mineral content, coarse texture, elevated sand content, and susceptibility to erosion and SOC loss (Chen et al. 2021). In contrast, red soils are zonal soils mainly distributed south of the Yangtze River, and are highly weathered and leached, with loamy clay to clay textures, low water and nutrient retention, poor structure, and limited SOC storage capacity (Liu et al. 2018). These characteristics constrain SOC stabilization in both soil types. Biochar, with its high C and nutrient content, large specific surface area, high porosity, and low bulk density, is a promising amendment to improve soil quality and enhance crop productivity in these systems (Liu et al. 2018).

In this study, we conducted a 12-year field experiment in two contrasting agricultural soils—C-rich purple soil and C-poor red soil—to investigate the long-term effects of biochar amendment on SOC stability and MNC dynamics in both topsoil and subsoil. We hypothesize that: (i) long-term biochar application enhances MNC accumulation in the topsoil by improving nutrient availability and altering microbial community traits, whereas it may reduce MNC in the subsoil because of nutrient limitations and increased necromass decomposition; (ii) biochar promotes fungal necromass C (FNC) accumulation more strongly than bacterial necromass C (BNC), as fungi generally exhibit higher CUE than bacteria and respond more positively to biochar (Liu et al. 2020). Moreover, existing studies on the effects of biochar on MNC accumulation and its contribution to SOC are mainly concentrated on individual experimental sites, and the data available in existing meta-analysis databases remain insufficient. Consequently, a comprehensive global synthesis has yet to be conducted. To address this gap, we compiled and analyzed published studies examining biochar's impact on MNC (via amino-sugar data) in order to characterize the response of MNC to biochar application and its contribution to SOC. And we hypothesize that (iii) the response of MNC to biochar will be modulated by soil characteristics, climatic variables, and biochar properties. These findings will offer a theoretical foundation for optimizing biochar-based C management strategies in agricultural soils.

2 Materials and methods

2.1 Field manipulation experimental design

The field experiment was conducted on two types of paddy soils: a sandy loam soil from Guanghan (31°03'N, 104°10'E), Sichuan Province, located in the Chengdu Plain; and a clay soil from Yingtan (28°15′N, 116°55′E), Jiangxi Province, located in the Poyang Lake Plain. According to USDA Soil Taxonomy, these soils are classified as Entisol and Ultisol, respectively. In the Guanghan and Yingtan regions, purple soil and red soil are the typical local soil types, and the local crop rotation systems in these regions are respectively rice–wheat and rice-rice cropping. The detailed information on the initial basic soil properties of the experimental sites is presented in Table S1.

The field experiment began in May 2010. Biochar was applied at a rate of 20 t ha^{-1} to the soil surface and incorporated into the 0–20 cm by rotary tillage prior to seedling transplantation. No further biochar applications were made in subsequent years. To maintain consistency, the same tillage treatment was applied to the control plots (without biochar). Each treatment was replicated three times, with plots measuring 4 m × 5 m (20 m^2). Fertilization practices were integrated with local field management routines and adhered to the principles of controlled experiments, with consistent application rates maintained across both locations. The N fertilizer application rate was 240 kg N ha^{-1} (using urea), applied in three splits: 60% as basal, 20% at tillering, and 20% at panicle formation. P and K fertilizers were applied as basal fertilizers using single superphosphate and potassium chloride, at rates of 150 kg P_2O_5 ha^{-1} and 75 kg K_2O ha^{-1}, respectively. The water-management regime during the rice growing season followed flooding–field drying–flooding–wetting, and all other management practices conformed to local agricultural norms.

The biochar used in the experiment was provided by Sanli New Energy Company, Henan, China, and was produced by pyrolyzing wheat straw in a vertical kiln at 450 °C for 2 h under limited oxygen. The biochar was ground to pass through a 2 mm sieve and then homogenized prior to field application. The basic properties of biochar were as follows: organic C of 467 g kg^{-1}, total N (TN) of 5.9 g kg^{-1}, total P of 4.0 g kg^{-1}, pH of 10.4, and ash of 20.8%.

2.2 Soil sampling

Topsoil (0–20 cm) and subsoil (20–40 cm) samples were collected from both the control and biochar plots at each site in October 2022. Five soil cores (5 cm diameter) were randomly taken per plot using an Eijkelkamp soil sampler, pooled, and sieved through a 2-mm mesh to remove roots and debris. The composite samples were then subdivided into three portions: one portion was air-dried for testing available P (AP) and amino sugar; a second was stored at 4 °C for the determination of available N (AN), dissolved organic C (DOC), microbial biomass C (MBC), and enzyme activity; and a third portion was freeze-dried for phospholipid fatty acid (PLFA) analysis.

2.3 Soil physicochemical properties analysis

Soil bulk density was measured in situ using a 100 cm^3 cylindrical soil core. SOC was determined by the potassium dichromate external heating method, TN by the Kjeldahl method, and AP by the Olsen ($NaHCO_3$ extraction-molybdenum antimony anticolorimetric) method. AN was extracted with a 2 M KCl solution and analyzed using a continuous-flow autoanalyzer (Skalar San++, Holland). DOC was extracted with deionized water at a ratio of 1:5 (w/v), filtered through a 0.45 µm membrane filter, and measured using a TOC analyzer (multi N/C 3100; Analytik Jena AG, Germany). Soil MBC was extracted with a 0.5 M K_2SO_4 solution according to the chloroform-fumigation method (Vance et al. 1987) and measured using a TOC analyzer.

2.4 Soil C mineralization and metabolic quotient

Microbial respiration was measured as described in our previous study (Liu et al. 2019). Briefly, 60.0 g of air-dried soil was weighed into a 250 mL glass bottle and then was incubated in the dark at 60% water-holding capacity and 25 °C for 14 d. Microbial respiration rates were measured on day 1, 3, 5, 7, 10, and 14 after incubation. The CO_2 concentration was determined using a gas chromatograph (Agilent 7890A) equipped with a flame ionization detector (FID). The mineralization potential of SOC (R_{SOC}; g CO_2-C kg^{-1} SOC) was calculated by normalizing cumulative C mineralization by SOC. qCO_2 (g C kg^{-1} MBC h^{-1}) was calculated as the ratio of basal respiratory C to MBC.

2.5 Extracellular enzyme activity analysis and PLFA analysis

Four extracellular enzyme activities were measured using fluorogenically labeled substrates, including two C-acquiring enzymes (EEC; β-1,4-glucosidase [BG] and cellobiohydrolase [CBH]), one N-acquiring enzyme (β-1,4-*N*-acetylglucosaminidase [NAG]), and one P-acquiring enzyme (acid phosphatase [APH]). Enzyme activities were normalized to MBC to calculate biomass-specific enzyme activities (nmol μg^{-1} MBC h^{-1}), offering insight into the efficiency of enzyme production relative to microbial biomass (Wang et al. 2024).

Based on enzymatic stoichiometry theory, vector analysis of extracellular enzyme activities was used to assess microbial resource limitations (Moorhead et al. 2016). The vector length and vector angle were calculated as follows:

$$Vector\ length = SQRT\left(x^2 + y^2\right) \tag{1}$$

$$Vectorangle(^\circ) = DEGREES(ATAN2(y, x)) \tag{2}$$

where x=ln (BG)/ln (NAG) represents the relative activity of C-versus N-acquisition enzymes, and y=ln (BG)/ln (AP) represents the relative activity of C-versus P-acquisition enzymes. Vector length indicates microbial C limitation, with longer vectors denoting greater limitation. Vector angle reflects the balance between P-versus N-acquisition enzymes: angles < 45° indicate relative microbial N limitation, while angles > 45° indicate relative P limitation. The magnitude of N or P limitation increases as the angle deviates further below or above 45°, respectively.

Soil microbial community was analyzed using the PLFA method. Key biomarkers included fatty acids 16:1ω5c, 18:2ω6,9c, and 18:1ω9c for fungi, and fatty acids 15:00, 17:00, i14:0, i15:0, i16:0, i17:0, a15:0, a17:0, 16:1ω9c, 16:1ω7c, 17:1ω8c, 18:1ω7c, 18:1ω5c, cy17:0, and cy19:0 for bacteria. Under substrate scarcity, microbial stress (MS) can cause gram-negative bacteria to convert monoenoic fatty acids into cyclopropyl fatty acids (Feng and Simpson 2009). The MS was determined using the ratio: (cy17:0 + cy19:0)/ (16:1ω7c + 18:1ω7c) (Bossio and Scow 1998; Fierer et al. 2003). Detailed extraction methods for extracellular enzymes and PLFA are provided in the supplementary material 1 (Text S2, 3).

2.6 Amino sugar analysis

Amino sugars, which are biomarkers that characterize microbial necromass, were determined according to the method of Zhang and Amelung (1996), the detailed procedure of which was described in our previous study (Liu et al. 2021a). Briefly, an air-dried soil sample (containing > 0.3 mg N) was hydrolyzed with 10 mL of 6 M HCl at 105 °C for 8 h to obtain amino sugar monomers. After digestion, an internal standard (myo-inositol) was added to assess the recovery of the purification steps. Finally, amino sugars were quantified using an Agilent 7890B GC (Agilent Technologies, Santa Clara, CA, USA) equipped with an HP-5 column and a flame ionization detector.

Two types of amino sugars (glucosamine [GlcN] and muramic acid [MurA]) were determined as indicators of microbial necromass. Total necromass C (TNC) was calculated as the sum of FNC and BNC. FNC and BNC were estimated as follows (Hu et al. 2024):

$$FNC = \left(\frac{GlcN}{179.17} - 1.16 \times \frac{MurA}{251.23}\right) \times 179.17 \times 10.8 \tag{3}$$

$$BNC = MurA \times 31.3 \tag{4}$$

where 179.17 and 251.23 are the molecular weights of GlcN and MurA, respectively, and 10.8 and 31.3 are the conversion factors for fungal GlcN to FNC and bacterial MurA to BNC, respectively.

2.7 Statistical analysis

The t-test ($p < 0.05$) was used to assess differences between control and biochar treatments at each soil depth. A Mantel test, conducted using the "vegan" package in R, evaluated correlations between MNC content and environmental variables (nutrient availability, enzyme activities, and microbial properties). Random forest analysis with the "rfPermute" package identified key environmental drivers of MNC. A Spearman correlation heatmap was generated with the "corrplot" package. Variation partitioning analysis was conducted using the "vegan" package, excluding variables with variance inflation factors (VIF) > 10 to avoid multicollinearity (Fan et al. 2024). Furthermore, hierarchical partitioning analysis was used to determine the independent contributions of each predictor to MNC using the “rdacca.hp” package.

2.8 Data collection and meta-analysis

Peer-reviewed articles published before November 2025 were retrieved from Web of Science (http://apps.webofknowledge.com/) and China National Knowledge Infrastructure (https://www.cnki.net/) using the keywords: “biochar” and “amino sugars” or “microbial necromass” or “microbial residues” or “microbial-derived carbon” or “muramic acid” or “glucosamine” or “fungal residue” or “fungal necromass carbon” or “bacterial necromass carbon” or “bacterial residue” or “necromass”.

Studies were included in the meta-analysis based on the following criteria: (a) Biochar was directly applied to soil (i.e., not as part of compost), and soil samples were collected from the topsoil (depth 0–20 cm) and subsoil (depth > 20 cm). (b) Both control (free-biochar) and biochar treatments were included under the same experimental conditions (without or with other treatments, provided biochar effects could be isolated). (c) Studies reported the mean, standard deviation (SD) or standard error (SE), and sample size. Based on these criteria, 85 topsoil and 4 subsoil observation pairs were extracted from 23 peer-reviewed articles in the supplementary material 2 (Dataset Table S1). Due to the limited number of subsoil data, no further effect analysis was conducted. Data presented only in figures were digitized using GetData Graph Digitizer v2.24 (https://getdata-graph-digitizer.com/).

The effect size of biochar on each variable was assessed using the natural logarithm of the response ratio (Hedges et al. 1999):

$$\ln(\mathrm{RR}) = \ln\left(\frac{\mathrm{XB}}{\mathrm{XC}}\right) = \ln(X_B) - \ln(X_C) \quad (5)$$

where X_B and X_C represent the means of the biochar and control treatments, respectively. The weighted response ratios and 95% confidence intervals (CIs) were calculated using MetaWin 2.1 (https://metawin.software.informer.com/2.1/). The “metafor” package in R was used to implement a random effects model (REM) and perform subgroup analyses. A Q test was conducted to assess heterogeneity among subgroups, and Qm values were used to evaluate moderator effects on effect size variation. An LSD test was applied to assess within-group differences. Further methodological details are provided in the supplementary material 2.

3 Results

3.1 Effects of long-term biochar amendment on soil nutrients and C stability

Compared with the controls, long-term biochar amendment increased SOC content by 21.9% and 67.3% in the topsoil of the Entisol and Ultisol, respectively (Table 1). In the subsoil, biochar amendment increased SOC by 23.5% in the Ultisol, but had no significant effect in the Entisol. In the topsoil of both experimental plots, biochar amendment significantly altered soil nutrient status (Table 1). Specifically, biochar increased TN, AP, AN, and the C/N ratio, while decreasing the DOC/AN ratio compared to the controls. In contrast, in the subsoil, biochar reduced AN content by 14.1–21.8% and increased the DOC/AN ratio of both soils.

Although cumulative C mineralization did not differ significantly between biochar-amended and control treatments in either the topsoil or subsoil, biochar amendment reduced the R_{SOC} by 17.1% and 33.8% in the topsoil of the Entisol and Ultisol, respectively, and by 17.5% in the subsoil of the Ultisol (Fig. 1).

3.2 Changes in microbial traits under long-term biochar amendment

Regarding MBC-normalized soil enzyme activities, long-term biochar amendment reduced the activities of C-, N-, and P-acquiring enzymes by 11.3–29.8% in the topsoil of both Entisol and Ultisol (Table 1). In contrast, in the subsoil, biochar increased NAG activity by 13.0–20.7% in both soils and enhanced EEC activity by 9.5% in the Entisol. Compared to the controls, biochar increased vector length in the topsoil but decreased it in the subsoil of both soils, while vector angle was reduced only in the subsoil of the Ultisol.

Biochar increased MBC by 25.5% and 47.3% in the topsoil of Entisol and Ultisol, respectively, but decreased it by 11.5% and 16.2% in the subsoil (Table 1). In the topsoil, biochar reduced qCO_2 by 19.6% and 25.1% in the Entisol and Ultisol, respectively, whereas it increased qCO_2 by 24.5% and 21.1% in the subsoil.

Microbial community structure and PLFA profiles were also affected by biochar amendment in both Entisol and Ultisol (Table 1 and Fig. S2). In the topsoil, biochar increased total PLFA by 37.2–39.4% and elevated the fungi-to-bacteria ratio in both soils. In contrast, in the subsoil, biochar reduced total PLFA by 25.4–25.8% and had no significant effect on the fungi-to-bacteria ratio. Furthermore, MS decreased in the topsoil but increased in the subsoil of both soils following biochar treatment compared to controls.

Table 1 Effects of biochar amendment on soil biochemical properties at the end of the 12-year experiment

	Entisol				Ultisol			
	Topsoil		Subsoil		Topsoil		Subsoil	
	Control	Biochar	Control	Biochar	Control	Biochar	Control	Biochar
SOC (g kg^{-1})	19.6 ± 0.9	23.9 ± 1.0 **	6.3 ± 0.4	6.5 ± 0.3	7.6 ± 0.6	12.7 ± 0.7 ***	4.7 ± 0.3	5.8 ± 0.5 *
TN (g kg^{-1})	1.79 ± 0.05	1.92 ± 0.03 *	0.46 ± 0.02	0.45 ± 0.02	0.64 ± 0.06	0.86 ± 0.03 **	0.34 ± 0.03	0.36 ± 0.05
BD (g cm^3)	1.13 ± 0.04	1.00 ± 0.06 *	1.18 ± 0.03	1.16 ± 0.04	1.18 ± 0.03	1.09 ± 0.04 *	1.24 ± 0.04	1.21 ± 0.02
pH	5.95 ± 0.12	6.29 ± 0.06 *	6.35 ± 0.22	6.58 ± 0.10	5.41 ± 0.13	5.72 ± 0.05 *	5.96 ± 0.12	6.17 ± 0.09
C/N	11.0 ± 0.4	12.5 ± 0.5 *	13.9 ± 0.6	14.4 ± 0.7	11.9 ± 0.3	14.8 ± 0.9 **	14.0 ± 0.6	16.3 ± 1.0 *
DOC (mg kg^{-1})	124.1 ± 8.5	140.0 ± 9.3	47.2 ± 2.6	47.0 ± 3.8	93.5 ± 5.1	115.8 ± 10.6 *	57.4 ± 6.2	53.4 ± 3.1
AP (mg kg^{-1})	14.2 ± 0.6	18.0 ± 1.5 *	3.8 ± 0.2	3.7 ± 0.1	6.9 ± 0.8	10.4 ± 0.9 **	4.0 ± 0.5	5.8 ± 0.9 *
AN (mg kg^{-1})	44.8 ± 4.3	57.3 ± 3.4 *	7.5 ± 0.3	6.4 ± 0.5 *	25.8 ± 2.1	36.0 ± 2.1 **	12.0 ± 1.0	9.4 ± 0.5 *
DOC/AN	2.8 ± 0.1	2.4 ± 0.1 *	6.3 ± 0.6	7.3 ± 0.1 *	3.6 ± 0.1	3.2 ± 0.1 *	4.8 ± 0.1	5.7 ± 0.4 *
MBC (mg kg^{-1})	305.8 ± 28.5	383.9 ± 20.7 *	79.0 ± 3.8	69.9 ± 3.9 *	151.4 ± 18.9	222.9 ± 17.2 **	92.0 ± 6.0	77.1 ± 5.0 *
qCO_2 (g C kg^{-1} MBC h^{-1})	3.29 ± 0.11	2.65 ± 0.08 **	7.0 ± 0.38	8.72 ± 0.51 **	4.73 ± 0.25	3.54 ± 0.25 **	5.12 ± 0.18	6.20 ± 0.25 **
EEC (nmol μg^{-1} MBC h^{-1})	0.73 ± 0.03	0.65 ± 0.03 *	1.26 ± 0.05	1.38 ± 0.05 *	0.89 ± 0.06	0.75 ± 0.02 *	1.02 ± 0.04	1.10 ± 0.06
NAG (nmol μg^{-1} MBC h^{-1})	0.16 ± 0.01	0.12 ± 0.01 *	0.32 ± 0.01	0.36 ± 0.02 *	0.25 ± 0.02	0.19 ± 0.01 *	0.26 ± 0.01	0.31 ± 0.02 **
APH (nmol μg^{-1} MBC h^{-1})	1.34 ± 0.19	1.00 ± 0.07 *	2.03 ± 0.18	2.41 ± 0.25	1.90 ± 0.17	1.33 ± 0.11 **	1.88 ± 0.13	1.61 ± 0.06 *
Vector length	1.59 ± 0.02	1.63 ± 0.01 *	1.61 ± 0.01	1.58 ± 0.01 *	1.53 ± 0.02	1.58 ± 0.01 *	1.61 ± 0.01	1.59 ± 0.01 *
Vector angle (∘)	56.98 ± 0.46	56.85 ± 0.12	57.53 ± 0.54	57.79 ± 1.01	57.35 ± 0.12	56.79 ± 0.68	58.39 ± 0.45	56.59 ± 0.38 **
tPLFA (nmol g^{-1})	68.0 ± 9.3	94.9 ± 5.3 *	25.1 ± 2.9	18.7 ± 2.2 *	42.7 ± 4.1	58.5 ± 7.2 *	28.6 ± 2.2	21.3 ± 3.4 *
Fungi/Bacteria	0.21 ± 0.01	0.24 ± 0.02 *	0.16 ± 0.01	0.16 ± 0.01	0.29 ± 0.02	0.34 ± 0.02 *	0.22 ± 0.01	0.22 ± 0.01
MS	1.04 ± 0.07	0.84 ± 0.03 *	1.61 ± 0.03	1.71 ± 0.04 *	1.36 ± 0.02	1.18 ± 0.06 **	1.42 ± 0.05	1.56 ± 0.05 *

Data are presented as means ± standard deviation (n = 3). The asterisks indicate the significance level: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. *SOC* soil organic carbon, *TN* total nitrogen; *BD* bulk density, *C/N* the ratio of SOC to TN, *DOC* dissolved organic carbon, *AP* available phosphorus, *AN* available nitrogen, *MBC* microbial biomass carbon, qCO_2 metabolic quotient, *EEC* extracellular carbon-acquiring enzyme, *NAG* β-1,4-*N*-acetylglucosaminidase, *APH* acid phosphatase, *tPLFA* total phospholipid fatty acid, and *MS* microbial stress

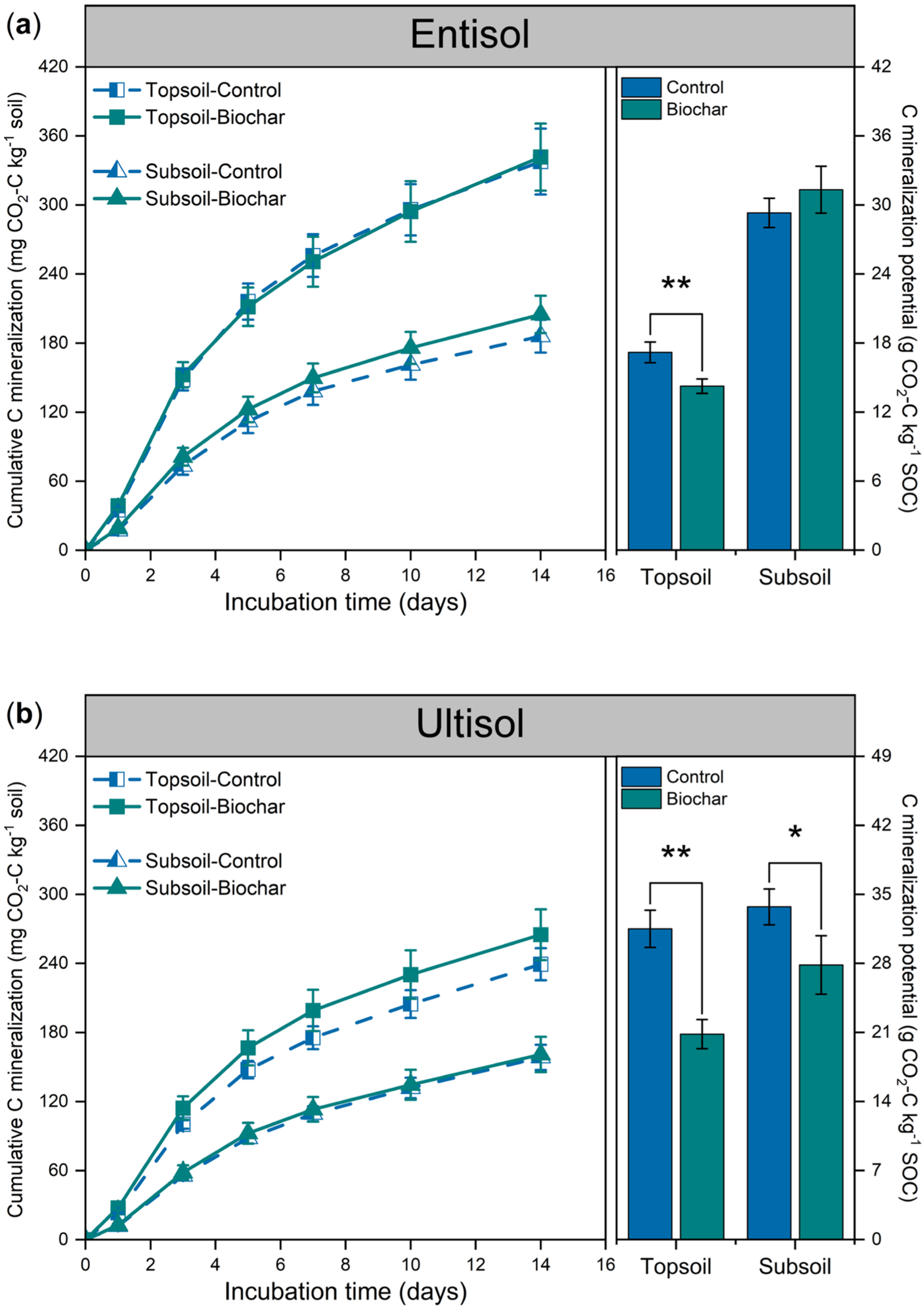


Fig. 1 Effects of biochar on C mineralization and its mineralization potential in Entisol (**a**) and Ultisol (**b**). Data are presented as means ± standard deviation (n = 3). The asterisks indicate the significance level: *$p < 0.05$; **$p < 0.01$

3.3 Effects of biochar amendment on MNC accrual and composition

In the topsoil, long-term biochar amendment significantly increased TNC and FNC content by 23.3% and 26.6% in the Entisol, and by 39.0% and 42.3% in the Ultisol, respectively, compared to the controls (Fig. 2).

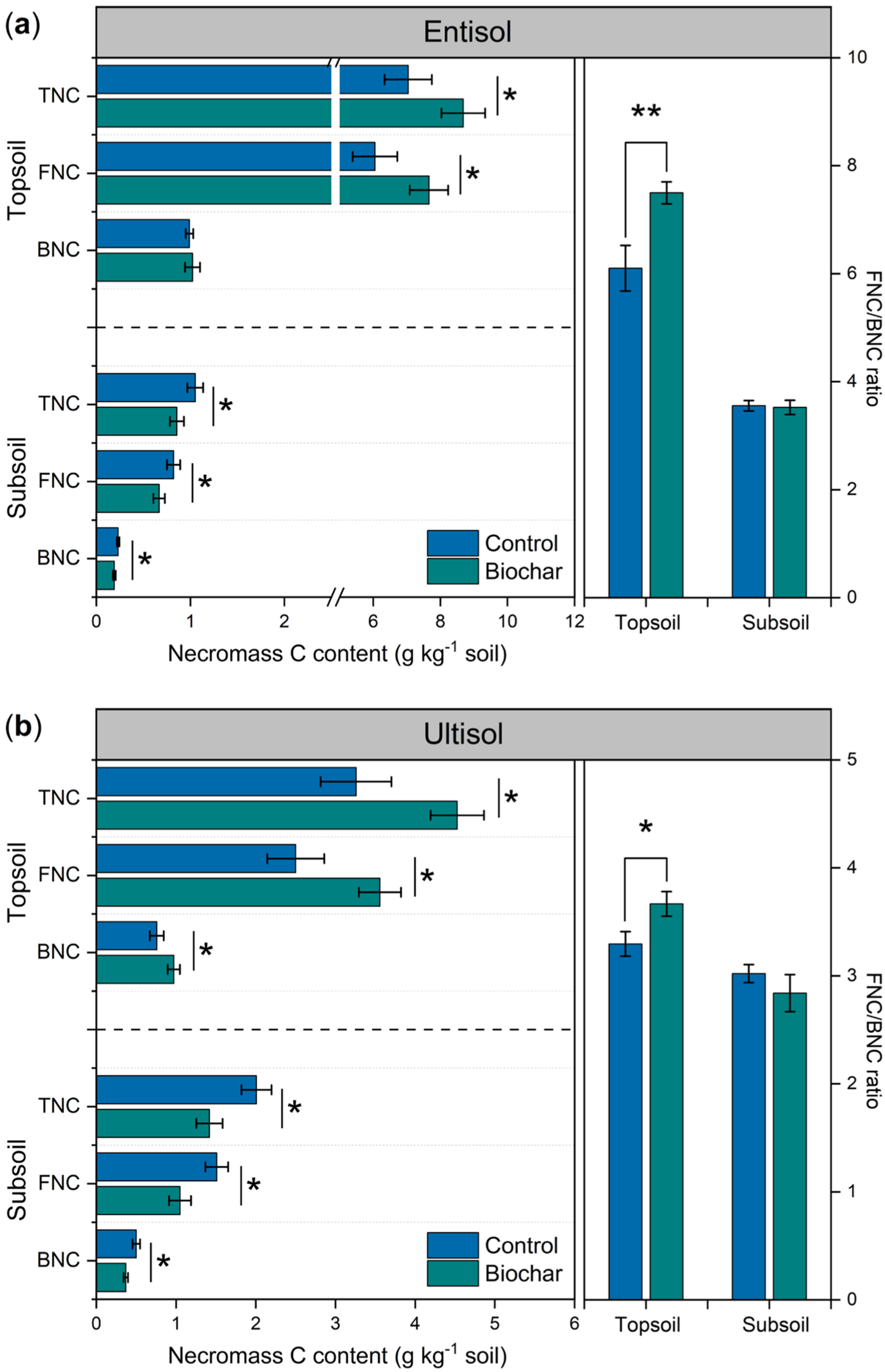


Fig. 2 Effects of biochar on microbial necromass carbon (MNC) content and the fungal necromass carbon (FNC)/ bacterial necromass carbon (BNC) ratio in Entisol (**a**) and Ultisol (**b**). *TNC* total necromass carbon. Data are presented as means ± standard deviation (n = 3). The asterisks indicate the significance level: *$p < 0.05$; **$p < 0.01$

However, biochar had no effect on the contributions of TNC or FNC to SOC in the Entisol, and even reduced their contributions by 14.7–16.7% in the Ultisol (Fig. S1). In contrast, in the subsoil, biochar decreased MNC contents by 17.9–18.7% in the Entisol and 26.2–30.4% in the Ultisol, resulting in reductions in the contribution of MNC to SOC by 20.6–21.4% and 40.3–43.8%, respectively. Furthermore, biochar significantly increased the FNC/BNC ratio in the topsoil but had no significant effect in the subsoil compared to the controls.

Frequency analysis showed that biochar application induced positive responses in TNC, FNC, BNC, and the FNC/BNC ratio in 83.5%, 82.4%, 61.2%, and 61.2% of the total 85 observations, respectively (Fig. 3a). Meta-analysis further confirmed significant increases in TNC (10.2%), FNC (12.1%), and BNC (4.5%) following biochar application, along with a significant increase in the FNC/BNC ratio (Fig. 3b).

3.4 Correlations between MNC accumulation and soil abiotic and biotic factors

Random forest analysis and Mantel test elucidated the relationships between MNC accumulation and environmental variables (Figs. 4 and S3). The correlations between TNC, FNC, and BNC with various environmental factors were broadly consistent across both soil layers. Specifically, MNC accumulation was significantly positively correlated with AN and MBC, and negatively correlated with DOC/AN, EEC, NAG, qCO_2, and MS in both soil layers (all $p < 0.05$). Notably, the relationships differed between soil layers: in the topsoil, MNC accumulation was also significantly positively correlated with DOC, AP, vector length, and total PLFA, whereas in the subsoil, it was positively correlated only with the fungal/bacterial PLFA ratio. Variation partitioning analysis showed that the combined effects of nutrient availability, enzyme activities, and microbial properties explained 75.0% and 49.0% of the variation in MNC in the topsoil and subsoil, respectively (Fig. 5a). The individual effect of nutrient availability was higher in the subsoil (28.0%) than in the topsoil (0.5%). Hierarchical partitioning analysis further indicated that the factors of total PLFA, DOC/AN, DOC, MS, and vector length were the primary drivers of MNC in the topsoil, whereas in the subsoil, DOC/AN, EEC, qCO_2, total PLFA, MS, and DOC played dominant roles (Fig. 5b).

The responses of MNC to biochar amendment depended on climatic conditions (Fig. 6). Biochar increased TNC more effectively in climates with mean annual temperature (MAT) ≥ 10 ℃ (12.5%) than in those with MAT < 10 ℃ (8.0%), and in regions with mean annual precipitation (MAP) ≥ 550 mm (13.7%) than in those with MAP < 550 mm (7.1%). Furthermore, the effects of biochar amendment on MNC varied with initial soil properties (Fig. 6a–d). In soils with low initial SOC (< 12 g kg^{-1}), biochar increased TNC and FNC by 13.7% and 16.7%, respectively, compared with increases of 6.4% and 8.3% in soils with high SOC (≥ 20 g kg^{-1}). MNC,

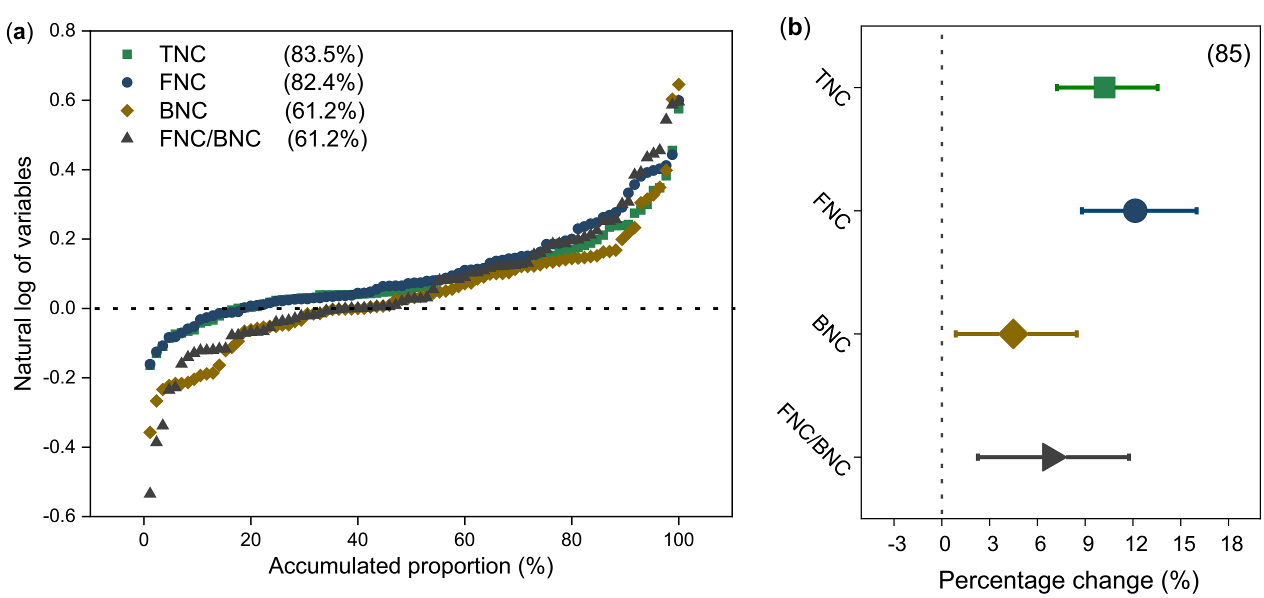


Fig. 3 Results of frequency-analysis (**a**) and overall effect sizes (**b**) of biochar on microbial necromass carbon (MNC). Positive and negative values indicate promotive and inhibitory effects, respectively. Percentages in (**a**) indicate the proportion of promotive effects. Numbers in parentheses in (**b**) indicate the sample size

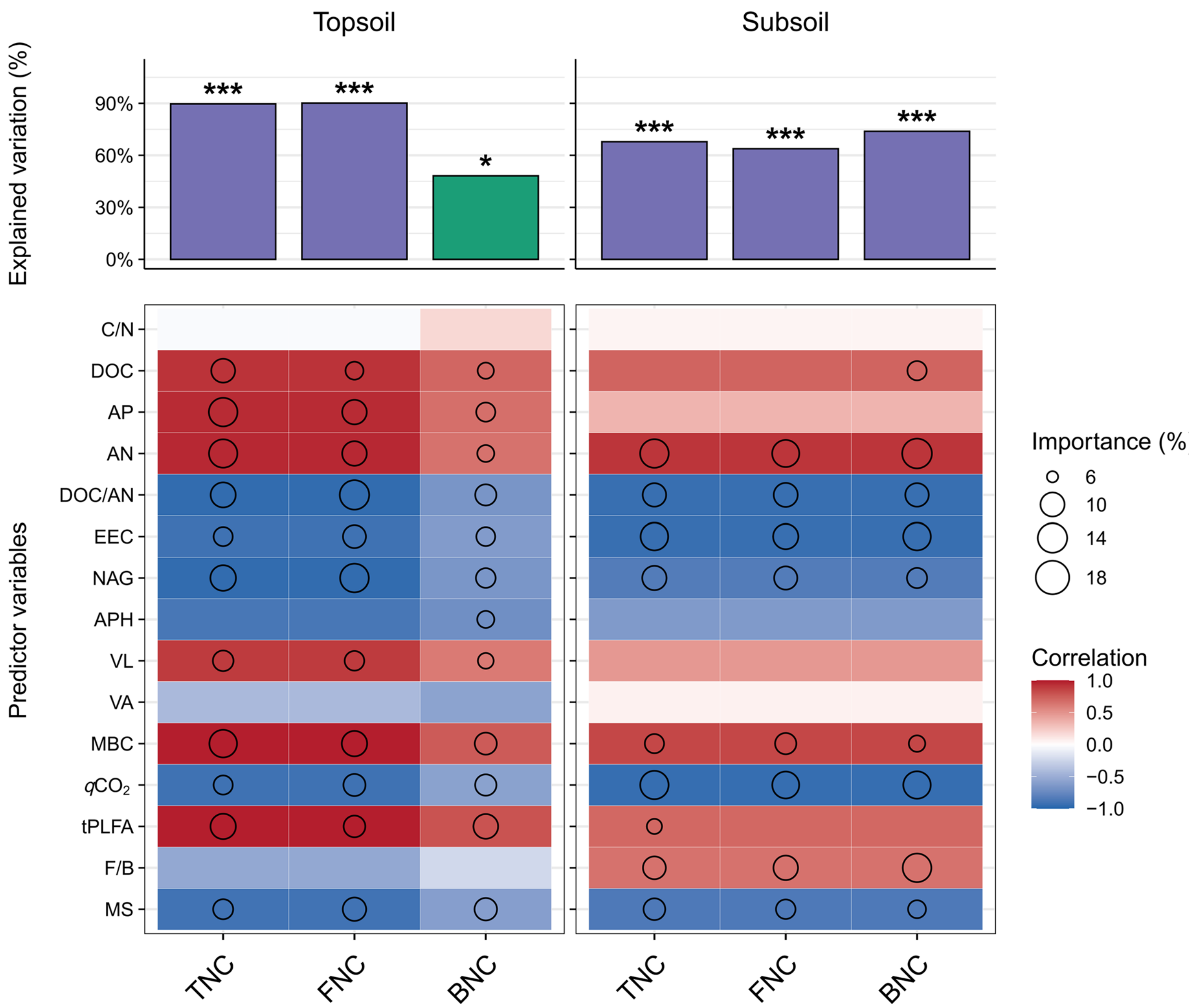


Fig. 4 Random forest and correlation analysis identify abiotic and biotic drivers of microbial necromass carbon (MNC) in topsoil and subsoil. Circle sizes reflect the relative importance of significant variables ($p < 0.05$; mean square error [MSE] increase percentage) in the random forest model, while colors indicate Spearman's correlation. Asterisks denote significance: *$p < 0.05$; ***$p < 0.001$. *TNC* total necromass carbon, *FNC* fungal necromass carbon, *BNC* bacterial necromass carbon, *C/N* the ratio of soil organic carbon to total nitrogen, *DOC* dissolved organic carbon, *AP* available phosphorus, *AN* available nitrogen, *EEC* extracellular carbon-acquiring enzyme, *NAG* β-1,4-*N*-acetylglucosaminidase, *APH* acid phosphatase, *VL* vector length, *VA* vector angle, *MBC* microbial biomass carbon, *qCO_2* metabolic quotient, *tPLFA* total phospholipid fatty acid, *F/B* fungal to bacterial PLFA ratio, and *MS* microbial stress

particularly FNC, responded more positively to biochar amendment in alkaline soils than in acid soils. TNC accumulation was most pronounced in sandy loam soils (18.6%), accompanied by increases in FNC (23.3%) and BNC (20.0%). In addition, the FNC/BNC ratio exhibited a positive response to biochar application in silty loam and clay loam soils, whereas no significant effect was observed in sandy loam soils.

The response of MNC accumulation was influenced by biochar properties (Fig. 6e–g). Biochar derived from crop residues significantly enhanced MNC, with stronger effects primarily attributable to increases in FNC. When the biochar application rate was < 20 t ha^{-1} and 20–40 t ha^{-1}, MNC increased by 11.4% and 11.6%, respectively; in contrast, when the application rate exceeded 40 t ha^{-1}, MNC increased by only 5.9%. In addition, long-term biochar application (≥ 10 years) resulted in significantly greater MNC accumulation than mid- and short-term applications (< 10 years), with similar increases observed in both FNC and BNC.

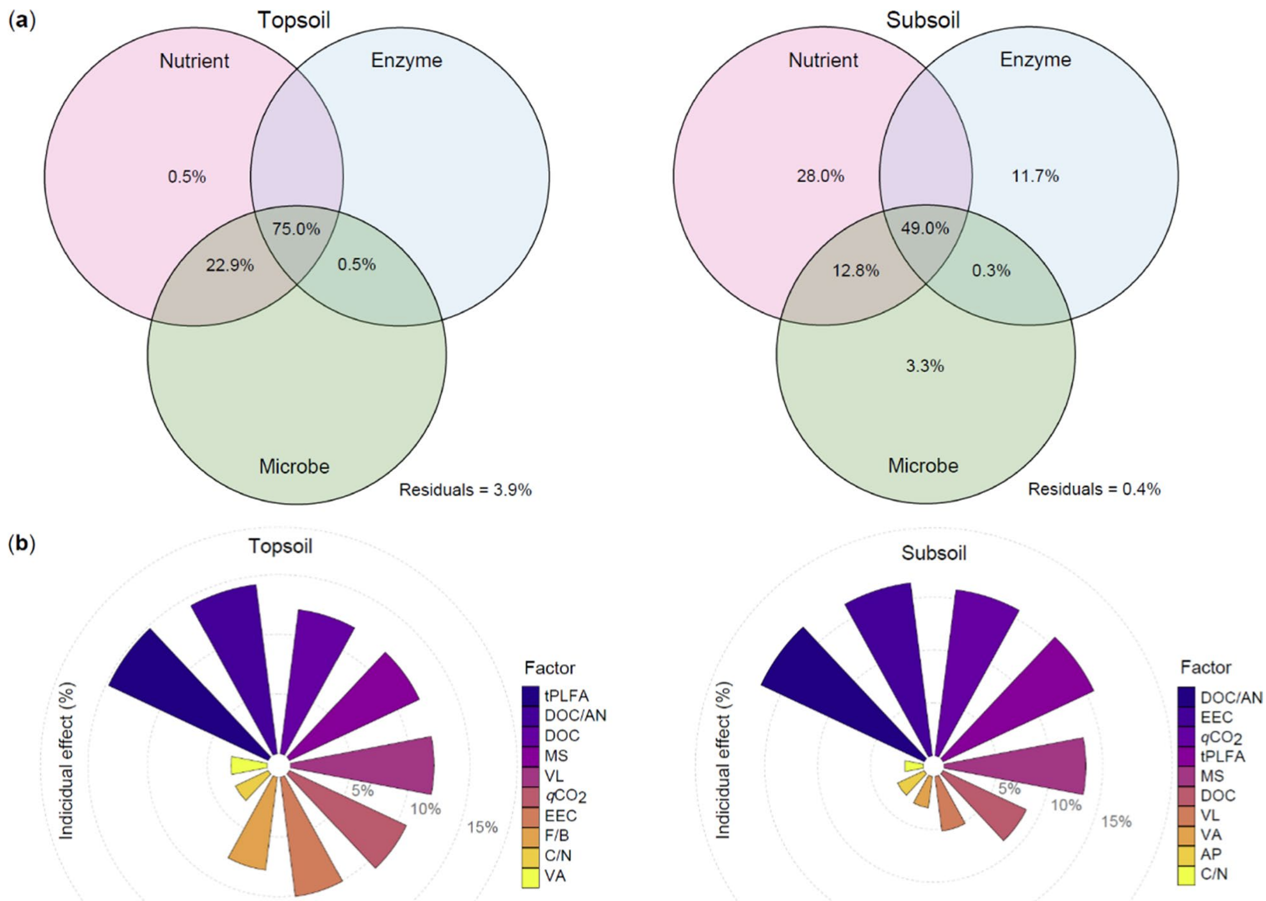


Fig. 5 Relative importance of soil abiotic and biotic factors for microbial necromass carbon (MNC) content based on variation partitioning analysis (**a**) and hierarchical partitioning analysis (**b**). Numbers in the figures indicate the proportion of variance explained. Soil variables include abiotic factors (C/N, DOC, AP, and DOC/AN) and biotic factors (EEC, NAG, qCO_2, tPLFA, F/B, and MS). *C/N* the ratio of soil organic carbon to total nitrogen, *DOC* dissolved organic carbon, *AP* available phosphorus, *AN* available nitrogen, *EEC* extracellular carbon-acquiring enzyme, *NAG* β-1,4-*N*-acetylgluco saminidase, qCO_2 metabolic quotient, *tPLFA* total phospholipid fatty acid, *F/B* the ratio of fungal to bacterial PLFA, *VL* vector length, *VA* vector angle, and *MS* microbial stress

4 Discussion

4.1 Long-term biochar amendment enhances SOC sequestration

The SOC stock is governed by the balance between exogenous C input, such as organic amendments, plant residues, and rhizodeposits, and C loss through the decomposition of these inputs and native SOC (Chen et al. 2023a). In this study, we found that long-term biochar amendment significantly increased SOC content in the topsoil at both sites (Table 1), consistent with previous findings (Liu et al. 2020; Lu et al. 2020). These results support the view that biochar application is an effective management strategy to enhance SOC sequestration. In addition to the direct C input from biochar, several mechanisms likely contributed to the observed SOC increase in biochar-amended soils, such as the suppression of R_{SOC} (Fig. 1), enhanced plant root biomass (Fig. S4), greater rhizodeposit input (Liu et al. 2023), and increased MNC accumulation (Fig. 2).

Notably, both R_{SOC} and qCO_2 were higher in the topsoil of the Ultisol, which had low initial SOC, compared to the Entisol with higher initial SOC content (Fig. 1; Table 1). When SOC content falls below 1%, C sequestration becomes inefficient, as soil microorganisms exhibit high respiratory losses to acquire limiting nutrients (Clayton et al. 2021), thereby reducing their growth efficiency and resource-use capacity (Malik et al. 2019). Interestingly, the increase in topsoil SOC content due to biochar addition was greater in the Ultisol (5.12 g C kg^{-1}; a 67.3% increase) than in the Entisol (4.31 g C kg^{-1}; a 21.9% increase). Moreover, biochar also increased subsoil SOC in the Ultisol (1.10 g C kg^{-1}; a 23.5% increase). These results highlight the importance of initial SOC content in shaping the soil's response to biochar addition. The Ultisol, with its low initial SOC and high clay

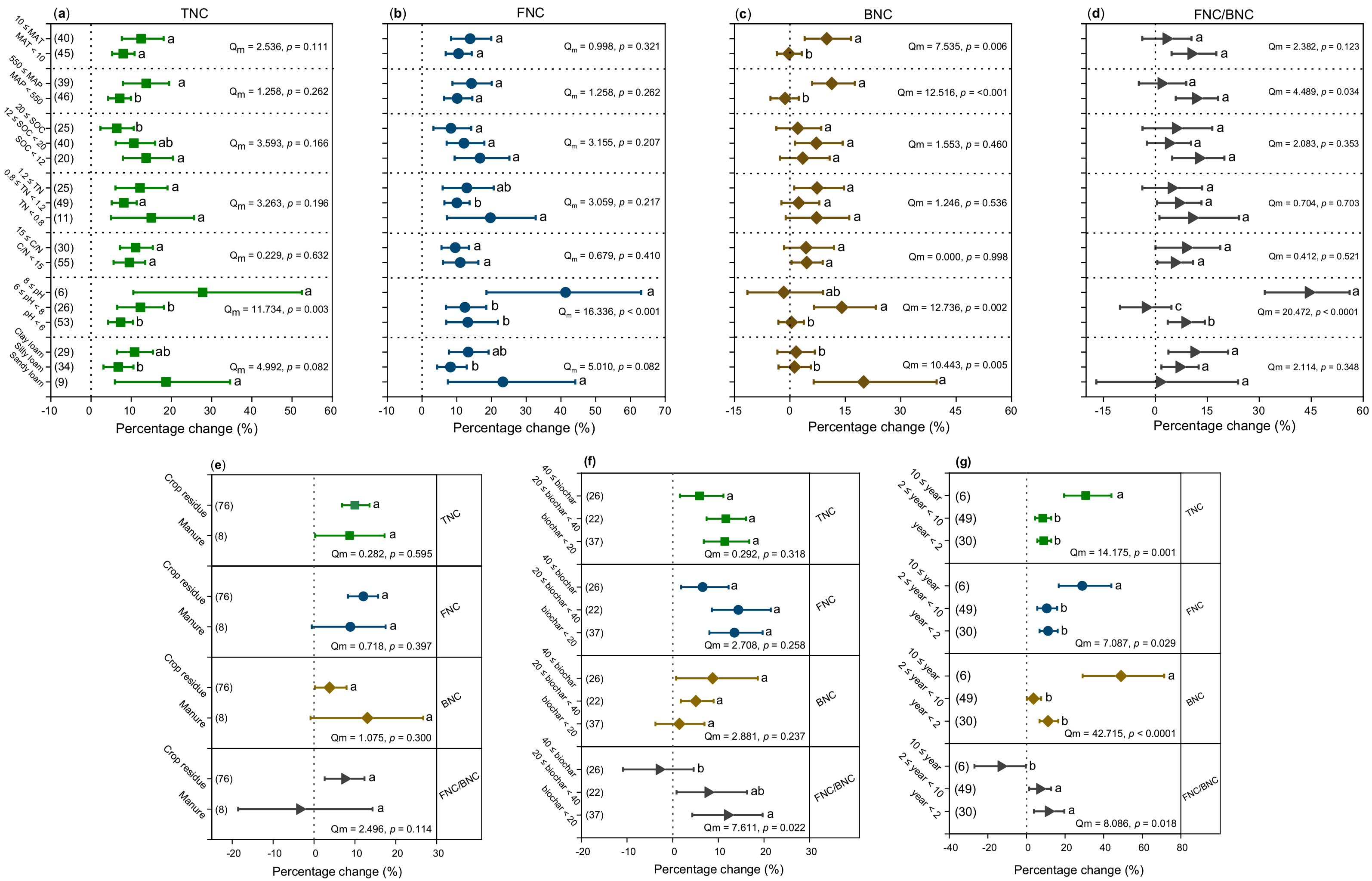


Fig. 6 Overall effect sizes of biochar on total necromass carbon (TNC; **a**), fungal necromass carbon (FNC; **b**), bacterial necromass carbon (BNC; **c**), and the FNC/BNC ratio (**d**) depending on climate factors and different soil categories explanatory variables. Effect sizes of biochar characters, including feedstock type (**e**), application rate (**f**), and amendment duration (**g**), on microbial necromass carbon. Graphs display mean values with 95% confidence intervals (CIs), numbers in parentheses indicate sample size, Qm is the heterogeneity of the weighted effect size associated with moderators, and $p < 0.05$ indicates significant differences among different groups under same moderators. Lowercase letters indicate pairwise comparisons of significant differences between groups under same moderators ($p < 0.05$). *SOC* soil organic carbon, *TN* total nitrogen, and *C/N* the ratio of SOC to TN, *MAT* mean annual temperature, *MAP* mean annual precipitation

content, likely contains unoccupied mineral surfaces primed for stabilizing exogenous C, suggesting that these soils have greater potential and efficiency for C sequestration through biochar addition. This finding supports the C saturation deficit hypothesis, which proposes that soils with low SOC content and degraded conditions may have a higher potential to store added C because they are further from their saturation threshold (Stewart et al. 2008).

4.2 Effects of long-term biochar amendment on microbial necromass accumulation in soil profiles

4.2.1 In the topsoil

In this study, long-term biochar amendment significantly increased MNC accumulation in the topsoil at both sites, primarily driven by fungal-dominated transformation of relatively stable C compounds (Fig. 2). Microbial activity and metabolic pathways are key determinants of MNC formation and persistence (Zhang et al. 2024). Consistent with this, biochar increased microbial biomass in the topsoil (Table 1), and MNC showed a strong positive correlation with microbial biomass as indicated by PLFA analysis (Fig. S6b). Hierarchical partitioning analysis further indicated that changes in topsoil microbial biomass were the primary factor controlling MNC accumulation (Fig. 5b).

Biochar promoted microbial growth through multiple pathways. On the one hand, biochar enhanced nutrient (N, P) availability in the topsoil and increased root biomass inputs (Table 1; Fig. S4), thereby enhancing substrate supply for microbial proliferation (Liu et al. 2023). On the other hand, the high C/N ratio and the abundance of recalcitrant C compounds in biochar preferentially favored fungal growth (Lyu et al. 2025). Enhanced fungal dominance promoted soil aggregate formation and stabilization (Fig. S5) through extensive mycelial networks that physically bind soil particles and modify aggregate surface adsorption properties (Angulo et al. 2024). Improved aggregate stability not only enhances nutrient retention but also provides physical protection for SOM, thereby slowing microbial necromass decomposition (Han et al. 2025). Collectively, these processes contributed to the increased MNC accumulation in the topsoil, with a particularly strong enhancement of FNC.

High microbial metabolic efficiency (i.e., lower qCO_2) and reduced enzyme investment generally indicate greater CUE, whereby a larger substrate proportion of C is assimilated into microbial biomass rather than respired as CO_2 (Deng et al. 2024). In this study, biochar significantly reduced the activities of C-, N-, and P-acquiring enzymes and lowered qCO_2 in the topsoil of both soils. This likely decreased SOM decomposition potential, as reflected by lower R_{SOC}, thereby promoting MNC accumulation. Notably, NAG, a key enzyme involved in the degradation of microbial necromass components such as fungal-derived chitin and bacterial-derived peptidoglycan (Sinsabaugh et al. 2008), was negatively correlated with MNC (Table 1 and Fig. S6a). Reduced NAG activity following biochar likely suppressed microbial necromass decomposition in the topsoil, facilitating its accumulation. This mechanism is consistent with previous findings showing that decreased NAG activity contributed to MNC accumulation in biochar-amended soils (Chen et al. 2023a).

4.2.2 In the subsoil

In contrast to the topsoil, biochar amendment resulted in a significant reduction in MNC accumulation in the subsoil of both soils. This divergent response highlights the strong depth-dependency of necromass C dynamics under biochar amendment, likely driven by biochar-induced alterations in substrate quality and nutrient distribution that reshape microbial community structure and metabolic activity, thereby regulating soil C cycling (Han et al. 2024; Zhang et al. 2024).

In the subsoil, reduced nutrient availability emerged as the dominant factor constraining MNC accumulation following biochar application (Fig. 5b). The highly porous structure and large specific surface area of biochar confer strong nutrient adsorption capacity, which enhances nutrient retention in the topsoil while limiting nutrient translocation to deeper soil layers through surface adsorption and organo-mineral interactions within biochar-mineral-fertilizer aggregates (Shi et al. 2020; Liu et al. 2023). Consequently, diminished nutrient availability in the subsoil suppressed microbial growth, thereby limiting MNC accumulation.

According to ecological stoichiometry theory, imbalances between microbial nutrient demands and environmental nutrient supply constrain microbial metabolism (Cui et al. 2022; Han et al. 2024). Under nutrient-limited conditions, microbes typically increase extracellular enzyme production to acquire limiting nutrients from existing organic matter, thereby maintaining internal C:N:P homeostasis (Cui et al. 2022). In the subsoil, microbes experienced pronounced stoichiometric imbalance following biochar amendment and consequently shifted their metabolic strategies toward intensified organic matter "mining". This shift was evidenced by increased NAG activity and elevated qCO_2 in the subsoil, indicating enhanced microbial turnover and reutilization of existing necromass to meet nutrient demands. Such intensified necromass decomposition ultimately led to reduced MNC accumulation in the subsoil.

4.3 Biochar's effects on microbial necromass depending on initial soil properties and biochar characteristics

To assess the generality of our findings, we conducted a meta-analysis of 85 pairs of observations from 23 peer-reviewed studies. It is important to note that due to the scarcity of available data on subsoil MNC responses to biochar in the existing literature, our meta-analysis focused primarily on topsoil observations. This limitation highlights a critical knowledge gap regarding deep soil C dynamics and underscores the novelty of our field experiment in revealing the divergent responses between topsoil and subsoil.

In this study, the meta-analysis revealed that biochar had a significant positive effect on MNC accumulation (overall mean = 10.2%, 95% CI: 3.0–3.3%; Fig. 3b) but decreased the contribution of MNC to the total SOC pool (Fig. S7). These findings are not entirely consistent with those reported by Zhou et al. (2023), who concluded that biochar had no significant impact on MNC accumulation while it reduced the MNC/SOC ratio. Indeed, the increment in SOC induced by biochar application exceeds that of MNC, thereby diminishing the relative contribution of MNC to the SOC pool (Chen et al. 2024). Biochar, being C-rich and recalcitrant to degradation, increases the direct contribution to the SOC pool as a C source (Lehmann et al. 2021). Additionally, biochar can enhance the stabilization and accrual of SOC by promoting soil aggregate formation (Liu et al. 2023). Therefore, the dilution effect of MNC in the SOC pool was primarily attributed to the nonlinear accumulation of MNC upon biochar application (Chen et al. 2024). However, this discrepancy may primarily stem from two key differences: (i) the duration of biochar application; and (ii) the number of studies included in the database. Specifically, all peer-reviewed articles in Zhou's database lasted less than two years, whereas our study found that the effects of biochar strengthened with longer application durations (Fig. 6g). Our database covers a broader range of study durations, comprising six long-term studies (≥ 10 years), 49 medium- to long-term studies (2–10 years), and 30 short-term studies (< 2 years). These results also indicate that MNC accumulation is a slow and gradual process (Zhou et al. 2023), and short-term experiments may underestimate the positive effects of biochar on MNC accumulation. In addition, our analysis is based on 85 independent observations from 23 studies, whereas Zhou's dataset included only five studies, suggesting that the smaller sample size in Zhou's analysis may also contribute to the observed differences.

4.3.1 Climate conditions

Climate conditions (MAT and MAP) are the dominant environmental factors regulating MNC dynamics, as they not only control microbial growth and proliferation but also influence the turnover and reutilization of microbial necromass, thereby affecting MNC accumulation (Zhou et al. 2023). Under biochar amendment, MNC accumulation was more pronounced in warm and humid environments than in cold or water-limited regions (Fig. 6). Generally, higher MAT and MAP enhance substrate accessibility, thereby stimulating microbial growth and biomass production (Lyu et al. 2025). However, increase in MAT and MAP can also intensify microbial metabolism and enzyme activity (Li et al. 2024a), accelerating organic matter decomposition and aggravating N limitation (Zhou et al. 2023), which may otherwise constrain MNC accumulation. Biochar, characterized by high porosity and strong water-holding capacity, can mitigate these adverse effects by physically protecting SOC from microbial decomposition. Moreover, under humid climatic conditions, biochar more effectively provides soluble nutrients, thereby alleviating nutrient constraints and promoting MNC accumulation (Lyu et al. 2025; Fei et al. 2026).

In addition, the positive effect of biochar on BNC was more pronounced under warm and humid conditions than under cold or water-limited environments. Compared to fungi, bacteria are more likely to gain a competitive advantage at higher temperature and moisture conditions due to their faster growth rates and greater efficiency in exploiting readily available resources (Hu et al. 2023), which likely explains the pronounced increase in BNC following biochar amendment.

4.3.2 Initial soil properties (SOC and texture)

Our data indicated that the response of net MNC accumulation to biochar application was higher in soils with low initial SOC compared to soils with higher initial SOC (Fig. 6a). When the initial SOC content is low, microorganisms experience greater respiratory losses, accelerating nutrient consumption, which results in a more pronounced response of plants and microorganisms to biochar application (Clayton et al. 2021). The application of biochar provides additional nutrients for microorganisms, stimulating their growth and turnover, thereby increasing the accumulation of MNC. In contrast, in soils with higher SOC, the existing organic matter provides a rich nutrient pool, reducing the relative impact of external nutrients (Lyu et al. 2025). Li et al. (2024b) reported that conservation tillage did not improve MNC accumulation in soils with SOC < 1%, likely due to altered competition between bacteria and fungi that limited fungal necromass formation (Murugan et al. 2014). Biochar application significantly increases SOC content, and more favors fungal biomass because fungi preferentially utilize recalcitrant C substrates over bacteria (Brant et al.

2006); thus greater response in MNC accumulation, particularly FNC, was observed in low-SOC soils (Fig. 6b). This finding highlights the potential of biochar amendment to enhance C storage in low-fertility croplands.

Soil texture plays a critical role in determining MNC accumulation following cropland management (Li et al. 2024b). The meta-analysis showed that the increase of MNC induced by biochar was greater in sandy soils (Fig. 6) and diminished with increasing clay content (Fig. S8). Sandy soils generally have poor water and nutrient retention, and lack clay minerals that protect organic C, limiting microbial growth and necromass production. Biochar amendment can improve these soils by enhancing aggregation, water retention, and nutrient availability. For instance, biochar has a more significant effect on increasing plant-available water in sandy soils compared to clay soils (Zhang et al. 2021), further supporting microbial biomass and MNC accumulation (Chen et al. 2023b). Moreover, in clay- and silt-rich soils, limited soil aeration restricts MNC accumulation, particularly FNC, as fungi are more aerobic than many bacteria (Moritz et al. 2009). Biochar's porous structure and low density can reduce bulk density, increase porosity, and improve aeration (Chen et al. 2023b), thereby enhancing fungal activity and increasing the FNC/BNC ratio (Fig. 6d).

4.3.3 Biochar characteristics

The accrual and composition of MNC were also influenced by biochar characteristics, including feedstock type, application rate, and amendment duration (Fig. 6e–g). Compared with manure-derived biochar, crop residue biochar not only contains comparable nutrient levels but also has a higher lignin content and C/N ratio, which may influence microbial community composition and favor fungi that specialize in decomposing recalcitrant C sources (Zhang et al. 2018), leading to more efficient accumulation of FNC. Biochar addition rates below 40 t ha^{-1} had a more pronounced positive effect on MNC than higher rates. Higher biochar application rates (e.g., 40 t ha^{-1}) may disrupt colloid kinetics through chemical perturbation, such as the release of monovalent cations, a significant increase in pH, or negative clay particle surface charges, thereby weakening soil aggregate stability (Pituello et al. 2018). Excessive application can introduce large amounts of recalcitrant, non-bioavailable C, resulting in C/N imbalances that restrict microbial growth and necromass production. Furthermore, under N-limited conditions, microbes may preferentially decompose N-rich microbial necromass to access nutrients (Cui et al. 2020), thereby offsetting the benefits of biochar amendment. In addition, the slow decomposition rate of biochar constrains its microbial utilization (Zhou et al. 2023). The positive effect of biochar on MNC accumulation increased over time, reaching a peak after 10 years (Figs. 6g and S8b), indicating that MNC accrual is a slow and progressive process. This finding underscores the necessity of long-term experiments for accurately assessing the effects of biochar on microbial necromass accumulation, as short-term studies may underestimate its gradual yet substantial contribution to soil C sequestration.

4.3.4 Limitations and prospects

Through long-term biochar application experiments at two sites, combined with meta-analysis, we have explored the effects of biochar on MNC and its underlying mechanisms. However, there are still some limitations in this study. For example, there is a lack of more extensive, multi-timepoint sample collection. Future research should place greater emphasis on long-term continuous monitoring, increasing the temporal and spatial replication of experimental samples. Additionally, it should better integrate high-resolution molecular technologies (such as metagenomics, transcriptomics, and metabolomics) to further elucidate the regulatory mechanisms of biochar on microbial community structure and function. Moreover, the in situ monitoring methods (e.g., in situ biochar burial and retrieval) along with isotope tracer techniques to trace the direct and indirect effects of biochar on MNC in the vertical soil profile should also be better understood, thereby providing a more comprehensive understanding of the soil C sequestration mechanisms of biochar.

5 Conclusion

Our findings indicate that long-term biochar amendment substantially influences MNC accumulation, with distinct depth-dependent responses. In the topsoil, biochar enhanced nutrient availability, suppressed NAG activity and qCO_2, and stimulated microbial growth, collectively promoting MNC accumulation, particularly fungal necromass. In contrast, in the subsoil, biochar reduced nutrient availability, thereby decreasing MNC by elevating NAG activity and qCO_2 while suppressing microbial growth. The response of MNC to biochar was strongly influenced by initial SOC content, soil texture, climate, and biochar properties (e.g., feedstock type, application rate, and amendment duration). These results underscore the importance of considering both depth-specific and site-specific soil characteristics when predicting SOC dynamics in response to biochar, and highlight the need to account for long-term effects when implementing biochar-based strategies to enhance C sustainability in agricultural systems. Overall, our findings advance the understanding of biochar-driven MNC accumulation and SOC dynamics, and can help optimize biochar management for improving soil C accumulation and function.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s42773-026-00577-0.

Additional file1 (DOCX 1172 kb)

Additional file2 (XLSX 39 kb)

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Author contributions
All authors contributed to the study conception and design. Material preparation, experiments and date analysis were performed by Kaiyue Song. The first draft of the manuscript was written by Kaiyue Song and Zhiwei Liu, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability
The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interests
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.

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