Shen *et al. Biochar* (2026) 8:127
https://doi.org/10.1007/s42773-026-00643-7

Biochar

ORIGINAL RESEARCH Open Access

Soil nitrogen level controls biochar's enhancement of microbial-derived carbon sequestration

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Abstract

Biochar is a carbon (C)-rich product of thermochemical conversion of organic materials that can enhance soil C sequestration. Given the tight coupling of C and nitrogen (N) cycles and the constraints of C:N stoichiometry on microbial growth, soil N level can regulate microbial growth, with implications for microbial necromass formation and soil organic carbon (SOC) accumulation. Here, we conducted a comprehensive meta-analysis of 932 paired observations globally to assess biochar effects on microbial biomass C (MBC), microbial necromass C (MNC), and SOC. Biochar increased soil C pools, with larger gains under low-N conditions (MBC + 37.4%, MNC + 14.0%, SOC + 47.9%) than under high-N conditions (MBC + 22.7%, MNC + 6.15%, SOC + 29.4%). In high-N soils, MBC accumulation is optimized under higher biochar application rates, warmer climates, and lower initial SOC/TN ratios, whereas MNC accumulation responds more strongly to longer experimental duration, higher biochar total C content, and alkaline soils. In low-N soils, MBC responds to soil properties (initial SOC and soil depth) and experimental duration, while MNC increases are mainly driven by biochar properties (biochar application rate and total C content) and experimental duration. Overall, initial soil N level dictates distinct C sequestration pathways—high-N soils favor physical protection, while low-N soils rely on microbial-mineral association. This N-dependent mechanism enables precision biochar management for climate-smart agriculture.

Highlights

- The global meta-analysis revealing that initial soil N level mediates the pathways of biochar-driven microbial C sequestration.
- Biochar increased microbial-derived C pools to a greater extent in low-N soils than in high-N soils.
- The physical protection dominates in high-N soils, while microbial-mineral association prevails in low-N soils.

Keywords Biochar, Soil N availability, Microbial biomass C, Microbial necromass C, Precision C management

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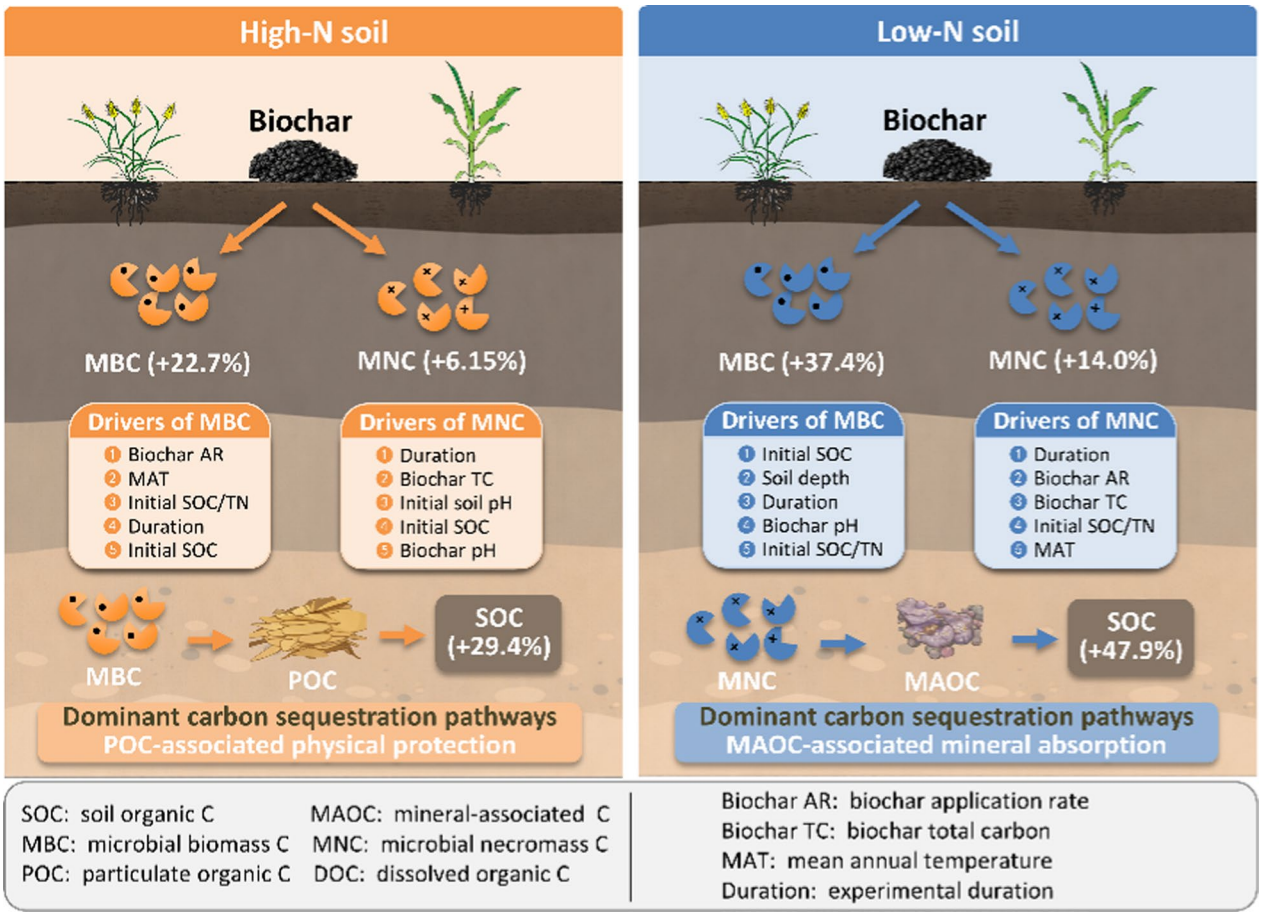
Graphical Abstract



1 Introduction

Soil organic carbon (SOC) is fundamental to climate regulation (Wang et al. 2022), nutrient cycling (Sun et al. 2025), and crop productivity (Lal 2020; Ma et al. 2023). SOC accumulation depends not only on the amount of carbon (C) entering soils, but also on how it is processed by microorganisms, which transform C inputs into distinct SOC fractions (Morrissey et al. 2023; Wu et al. 2025). Key components of the microbial-derived C pool include microbial biomass carbon (MBC), an active intermediate, and microbial necromass carbon (MNC), a more persistent metabolic product; both are critical indicators of soil health and long-term SOC sequestration (Cotrufo et al. 2015; Takele et al. 2025; Yang et al. 2025b). Dissolved organic carbon (DOC) serves as a labile substrate linking microbial activity to the accumulation of these microbial-derived C fractions (Chen et al. 2023a; Ren et al. 2024, 2025). Therefore, understanding how agricultural management practices regulate the formation and stabilization of these distinct C pools is essential for developing effective strategies to increase SOC storage in agroecosystems.

Biochar, a carbon-rich material produced by the pyrolysis of plant residues under oxygen-limited conditions (Lehmann 2007), has been widely advocated as a promising soil amendment for improving soil quality and mitigating climate change (Antonangelo et al. 2025; Luo et al. 2023). Numerous studies have reported that biochar enhances SOC sequestration, improves nutrient availability, and modifies microbial activity (Hu et al. 2025; Yuan et al. 2025). However, the effects of biochar on microbial-derived C pools remain highly variable, with findings ranging from positive to neutral or even negative outcomes. A meta-analysis reported that, compared with no biochar addition, biochar application significantly increased soil microbial biomass (Pokharel et al. 2020). However, Li et al. (2019) reported no significant effect of biochar on soil microbial biomass carbon, whereas other

studies suggested that biochar application reduced the accumulation of microbial-derived carbon (Zhou et al. 2023). Such discrepancies may stem from the heterogeneity in biochar feedstocks and production conditions, as well as their interactions with different soil properties and climatic environments.

Given the central role of microbially derived C in SOC formation, these inconsistent responses point to a broader stoichiometric conundrum regarding biochar amendment. Although biochar is generally recalcitrant and has a high C:N ratio, it may increase microbial N demand, potentially limiting the conversion of available C into microbial biomass and necromass, especially in soils with low initial N levels (Kerner et al. 2023). Simple stoichiometric reasoning would therefore predict limited accumulation of microbially derived C in N-poor soils. Yet growing evidence shows that biochar can still enhance SOC accumulation under such low-N conditions (Chagas et al. 2022; Meng et al. 2023). This apparent paradox suggests that biochar-induced SOC accumulation is not governed by substrate stoichiometry alone, but also by processes that regulate microbial C retention and stabilization.

The fate of biochar-induced C likely depends on soil N status, which shapes microbial processing. C inputs to soil can be incorporated into SOC via microbial assimilation and subsequent necromass formation, or stabilized through physicochemical protection mechanisms such as mineral–organic associations and soil aggregation (Kayoumu et al. 2025; Wang et al. 2021; Zhou et al. 2025, 2026). Zhang et al. (2024a) reported that glucose-induced SOC accumulation in low-N soils was mainly associated with physicochemical protection, whereas in high-N soils it was more closely linked to microbial growth than to microbial necromass turnover. These findings suggest that soil N level can modify the relative importance of microbial growth, necromass formation, and residue stabilization during SOC accumulation. Biochar complicates this relationship because its high C:N ratio may favor microbial investment in N-acquiring enzymes over biomass production, thereby weakening the transfer of assimilated C into microbial necromass (Zhou et al. 2023). However, it remains unclear how soil N level modulates biochar-induced changes in microbial-derived C and alters the pathways through which microbial biomass and residues contribute to SOC stabilization.

Therefore, we conducted a systematic review and meta-analysis of data from peer-reviewed publications examining microbial-derived C responses to biochar additions, quantitatively comparing changes in MBC and MNC across soils spanning different initial N levels. The objectives of this study were to: (1) quantify the effects of biochar application on microbial-derived C across contrasting initial soil N levels; (2) examine how the response of microbial-derived C to biochar varies with biochar characteristics, soil properties, and experimental conditions; and (3) determine how soil N availability regulates the pathways through which microbial-derived C contributes to SOC stabilization under biochar amendment. We hypothesize that initial soil N level regulates biochar-induced SOC stabilization by shifting the dominant pathway through which microbial-derived C is stabilized. In low-N soils, biochar's high C:N ratio may shift microbial metabolism toward N acquisition, reducing growth efficiency while favoring the stabilization of the microbial residues that are produced via mineral association. In high-N soils, sufficient N is expected to favor microbial growth, leading to greater accumulation of particulate organic matter that is subsequently stabilized through physical protection mechanisms.

2 Materials and methods

2.1 Data collection

We collected data from peer-reviewed studies published prior to June 30, 2025 by searching the Web of Science (http://apps.webofknowledge.com/) and the China National Knowledge Infrastructure (CNKI) databases (http://www.cnki.net/). The keywords used for the literature search were: (Biochar OR hydrochar) AND (microbial necromass OR fungal necromass OR bacterial necromass OR microbial residue OR fungal residue OR bacterial residue OR microbial-derived carbon OR microbial biomass carbon OR microbial carbon OR microbial C). Hydrochar was included only as a search term to broaden literature retrieval. Studies using hydrochar alone were excluded during screening and were not included in the final dataset. Studies were screened according to the following inclusion criteria: (a) only studies conducted in cropland systems were included, whereas studies in grassland or forest ecosystems were excluded; (b) experiments had to include both a control (no biochar) and a biochar-amended treatment; (c) biochar had to be produced via pyrolysis, and wildfire-derived char was excluded; and (d) when the same research group reported identical results across multiple publications, the data were included only once. Based on these criteria, we compiled 932 paired observations from 173 peer-reviewed studies. Available N was reported for only a limited subset of the collected studies and initial soil total N was more frequently reported across the dataset. For studies reporting both variables, correlation analysis showed a highly significant positive relationship between initial soil total N and available N ($P < 0.001$; Fig. S6), supporting the use of total N to distinguish soils with contrasting initial soil N levels. Therefore, soils were grouped by initial total N content using 1.0 g kg^{-1} as

the threshold. This value has been used as a soil total N grouping boundary in previous agricultural meta-analyses and corresponds to the Grade III–IV boundary in the Second National Soil Survey of China (National Soil Survey Office 1998; Ren et al. 2017; Yokamo et al. 2023). Soils with initial total N < 1.0 g kg^{-1} were classified as low-N soils, whereas those with initial total N ≥ 1.0 g kg^{-1} were classified as high-N soils. Ultimately, the high-N dataset comprised 388 paired observations from 95 studies, whereas the low-N dataset comprised 544 paired observations from 78 studies. The geographic distribution of the study sites is shown in Fig. 1.

Additional information extracted from the selected studies included the geographic coordinates of the experimental sites (e.g., latitude and longitude), climatic variables (e.g., mean annual temperature (MAT) and mean annual precipitation (MAP)), initial soil properties (e.g., SOC content, pH, and the proportions of sand, silt, and clay), and biochar characteristics (e.g., pH, total C content, and pyrolysis temperature). When relevant data were presented only in graphical form, values were extracted using WebPlotDigitizer (version 4.2, USA). When studies reported standard errors (SE) rather than standard deviations (SD), SD was calculated as $SD = SE \times \sqrt{n}$. For studies that did not report MAT or MAP, the corresponding values were obtained from the WorldClim global climate database (http://www.worldclim.org/) based on the geographic coordinates of the study sites. To better explore the conditions under which microbial-derived C responds to biochar addition, experimental conditions, soil properties, and

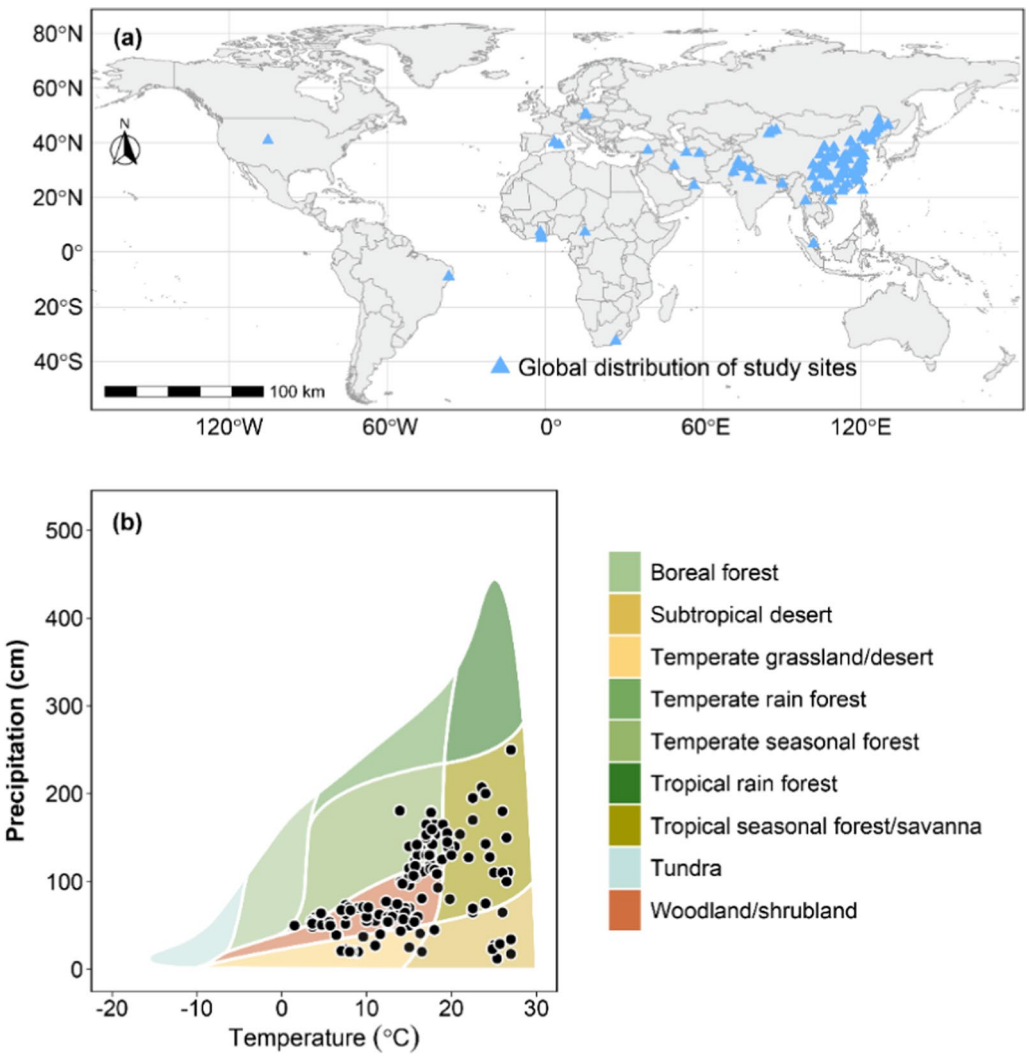


Fig. 1 The distribution of experimental sites included in the meta-analysis on microbial-derived carbon responses to biochar application (**a**), and the distribution of study sites across major biomes (**b**)

biochar characteristics were categorized. Table S1 presents the classification criteria and grouping levels for both continuous and categorical variables.

2.2 Meta-analysis

Microbial necromass carbon was defined as the sum of bacterial necromass carbon and fungal necromass carbon. The quantification of bacterial and fungal necromass carbon was based on the concentrations of muramic acid (MurA) and glucosamine (GluN), respectively (Zhu et al. 2020).

$$\text{Microbial necromass C} = \text{MurA} \times 45 + \left(\frac{\text{GluN}}{179.2} - 2 \times \frac{\text{MurA}}{251.2}\right) \times 179.2 \times 9 \quad (1)$$

where 45 and 9 are the conversion factors used to estimate bacterial- and fungal- necromass carbon from muramic acid (MurA) and glucosamine (GluN), respectively; 251.2 and 179.2 represent the molecular weights of MurA and GluN (Zhu et al. 2020). A random-effects model was employed in the meta-analysis to investigate the effect of biochar on microbial-derived carbon (Hedges et al. 1999). The effect size was calculated as the natural logarithm of the response ratio (ln*R*), as shown in Eq. (2):

$$\ln R = \ln(X_t/X_c) \quad (2)$$

where X_t and X_c represent the mean values of the treatment group (biochar application) and the control group, respectively. The variance (V) for individual observations was calculated using Eq. (3):

$$V = \frac{S_t^2}{n_t X_t^2} + \frac{S_c^2}{n_c X_c^2} \quad (3)$$

where S_t and S_c represent the standard deviations of the biochar treatment group and the control group, respectively, and n_t and n_c represent the number of replicates in the biochar treatment and control groups, respectively. The overall weighted response ratio ($\ln R_{++}$) was calculated using Eq. (4):

$$\ln R_{++} = \frac{\sum(W_i \times \ln R_i)}{\sum W_i} \quad (4)$$

where W_i indicates the weighting factor and $\ln R_i$ refers to the response ratio for the *i*th observation; the weight (W_i) of each observation was calculated as the inverse of its total variance (Eq. (5)).

$$W_i = \frac{1}{V_i} \quad (5)$$

The standard error of $\ln R_{++}$ was calculated using Eq. (6), and the 95% confidence interval (CI) was calculated using Eq. (7):

$$s(RR_{++}) = \sqrt{\frac{1}{\sum W_i}} \quad (6)$$

$$95\%\,CI = RR_{++} \pm 1.96 \times s(RR_{++}) \quad (7)$$

If the 95% confidence interval does not overlap with zero, the effects of biochar on the variable were considered significant (Hedges et al. 1999). To facilitate interpretation, the effect size ($\ln R_{++}$) was converted to the relative percentage change using Eq. (8):

$$\text{Effect size}\,(\%) = (e^{\ln R_{++}} - 1) \times 100\% \quad (8)$$

2.3 Statistical analysis

All statistical analyses were performed using the 'metafor' package in R 4.3.3. Publication bias was evaluated using Egger's regression test and Rosenberg's fail-safe number method (Hedges et al. 1999; Rosenberg 2005). If the *p*-value from Egger's test exceeded 0.05 or the fail-safe number was greater than 5N +10 (where N represents the sample size), the results were considered robust and not affected by publication bias. In this study, the observed results met these criteria, indicating no evidence of publication bias (Table S2). Linear regression analyses were conducted to assess the relationships among DOC, MBC, MNC, SOC, mineral-associated organic carbon (MAOC), and particulate organic carbon (POC) in high-N soils and low-N soils. Random forest models were used to evaluate the responses of MBC and MNC to multiple factors (e.g., mean annual temperature, mean annual precipitation, soil organic carbon content, experimental duration, and biochar total carbon content) in high-N soils and low-N soils. The analysis was conducted using the "randomForest" package, and model evaluation was performed with the "A3" package in R version 4.3.3.

3 Results

3.1 Responses of soil MBC, DOC, and MNC to biochar in high-N and low-N soils

Overall, biochar addition increased the concentrations of SOC, MBC, DOC, and MNC in both high-N and low-N soils (Fig. 2a, c). In low-N soils, SOC, MBC, DOC, and MNC increased by 47.9%, 37.4%, 29.7%, and 14.0%,

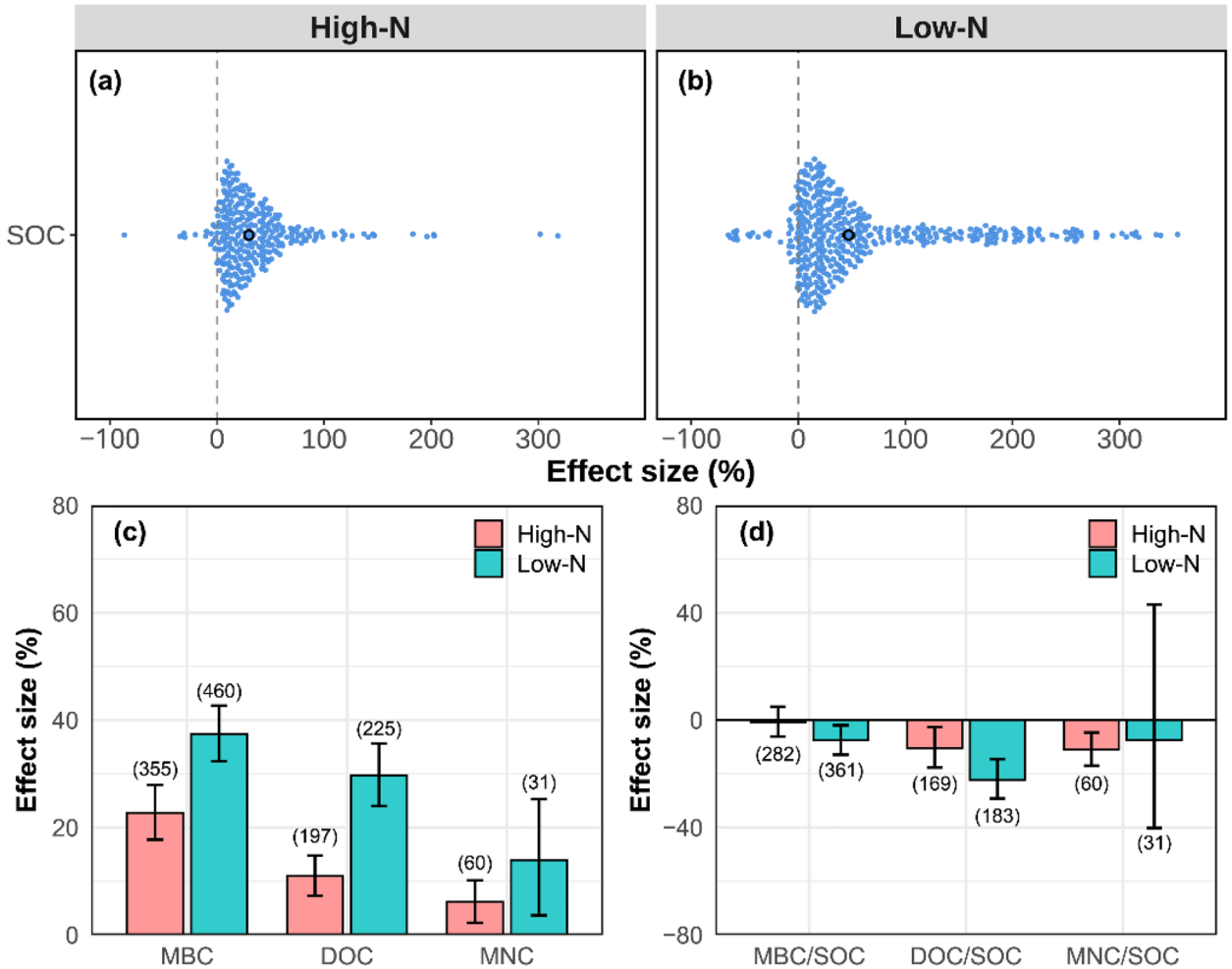


Fig. 2 Overall effects of biochar on SOC, MBC, DOC, and MNC in high-N and low-N soils (**a**–**c**), as well as on the ratios of MBC/SOC, DOC/SOC, and MNC/SOC (**d**). Error bars indicate 95% confidence intervals (CIs), and an effect is deemed significant when the CI does not include zero. Sample sizes are shown above or below the CIs. SOC: soil organic carbon; MBC: microbial biomass carbon; DOC: dissolved organic carbon; MNC: microbial necromass carbon

respectively. In contrast, the corresponding increases in high-N soils were smaller, with SOC, MBC, DOC, and MNC rising by 29.4%, 22.7%, 11.0%, and 6.15%, respectively. Regarding the contribution of C fractions to SOC, biochar decreased MBC/SOC, MNC/SOC, and DOC/SOC in low-N soils by 7.52%, 7.50%, and 22.2%, respectively (Fig. 2d). Similarly, in high-N soils, biochar decreased MBC/SOC, MNC/SOC, and DOC/SOC by 0.757%, 11.0%, and 10.4%, respectively (Fig. 2d).

3.2 Responses of soil MBC and MNC to biochar depending on climatic and experimental conditions

In high-N soils, MBC accumulation was optimized under warmer and low-precipitation conditions (MAT ≥20 °C and MAP ≤400 mm, Fig. 3a). Moreover, biochar significantly enhanced MNC accumulation under intermediate climatic conditions (10 °C < MAT < 20 °C and 400 mm < MAP < 800 mm, Fig. 3b). Notably, field experiments showed significant increases in both MBC (Fig. 3a) and MNC (Fig. 3b) following biochar addition, whereas laboratory experiments exhibited a significant response only for MBC (Fig. 3a). Additionally, biochar produced the greatest increase in MBC when experiment duration was 2–5 years, whereas experiment duration ≥5 years showed a significant increase in MNC accumulation.

In low-N soils, biochar increased MBC irrespective of variations in climatic factors and experimental conditions (Fig. 3c). Moreover, biochar addition increased MNC by 71.9% at MAT ≤ 10 °C, by 20.0% at MAP of 400–800 mm, by 15.7% in field experiments, and by 87.6% and 39.3% when experiment duration was 2–5 years and ≥5 years, respectively (Fig. 3d).

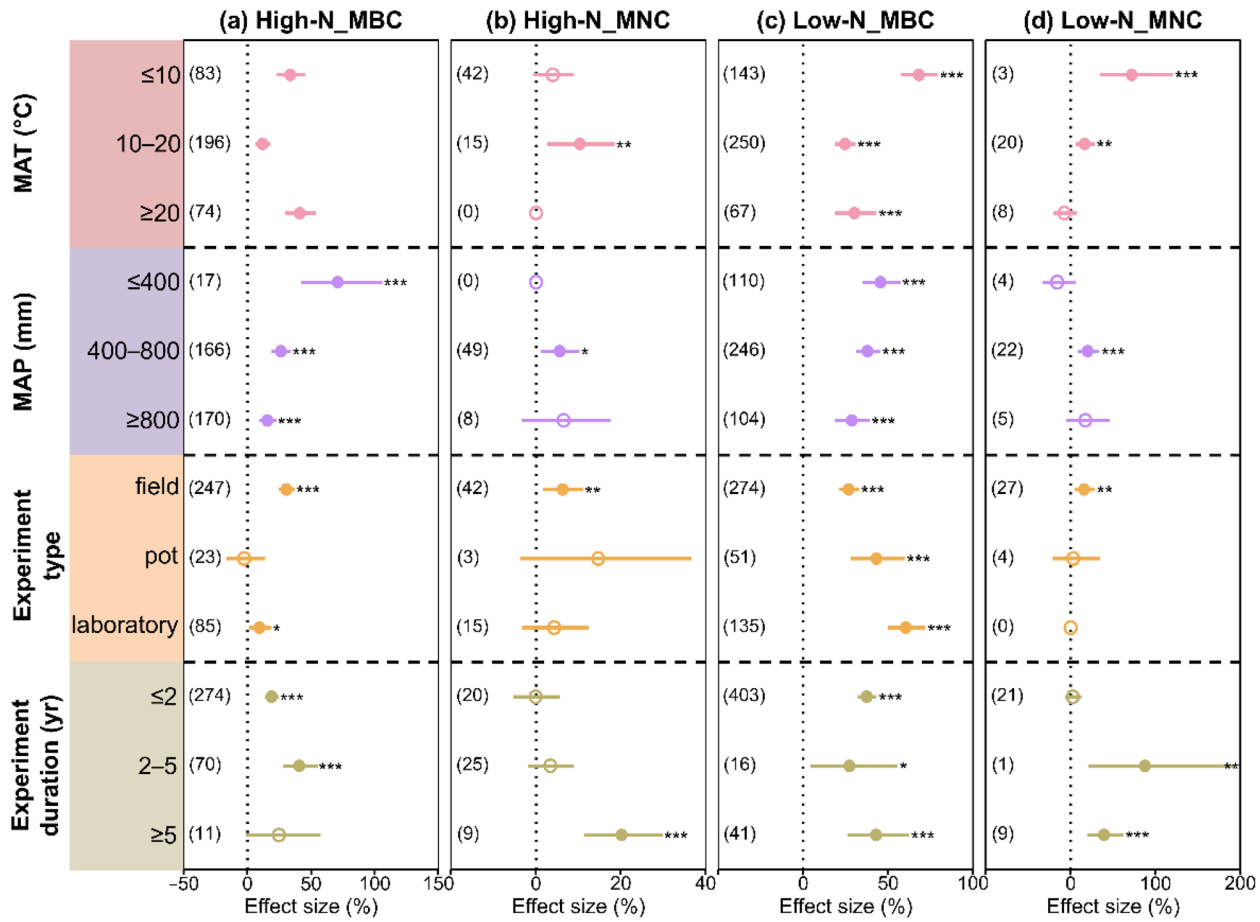


Fig. 3 Effects of experimental conditions on the responses of MBC and MNC to biochar under high-N and low-N soils. MBC: microbial biomass carbon; MNC: microbial necromass carbon; MAT: mean annual temperature; MAP: mean annual precipitation. Numbers in parentheses indicate the number of observations. Error bars represent 95% confidence intervals (CIs). The closed and open symbols indicate significant and nonsignificant effects, respectively. Asterisks indicate significant differences (*, ** and *** denote $P < 0.05$, 0.01 and 0.001, respectively)

3.3 Responses of soil MBC and MNC to biochar depending on soil properties

In high-N soils, biochar-induced changes in microbial-derived C varied with soil properties. When SOC was ≤10 g kg^{-1}, biochar increased MBC and MNC by 43.5% and 23.1%, respectively (Fig. 4a, b); similarly, positive responses of both MBC and MNC to biochar were observed when SOC/TN was <8 (Fig. 4a, b). Acidic soils exhibited the strongest positive response of MBC to biochar addition, whereas MNC accumulation was significantly enhanced in alkaline soils (Fig. 4a, b). Biochar increased MBC accumulation regardless of soil texture (Fig. 4a), whereas MNC was enhanced in loam and sandy soils (Fig. 4b). The positive effect of biochar on MBC was more pronounced with increasing soil depth (Fig. 4a); meanwhile, the limited observations from topsoil (≤20 cm) indicated a significant increase in soil MNC following biochar addition (Fig. 4b).

In low-N soils, the largest increase in MBC occurred in soils with SOC ≥20 g kg^{-1}, SOC/TN ≥8, and a clay texture (Fig. 4c), which contrasted with the pattern observed in high-N soils. Furthermore, biochar increased MNC more at SOC 10–20 g kg^{-1} by 16.2%, SOC/TN ≥8 by 11.8%, neutral soils by 23.1%, loam soils by 71.9%, sandy soils by 14.9%, and soil depth ≤20 cm by 14.0%, compared to no biochar addition (Fig. 4d).

3.4 Responses of soil MBC and MNC to biochar depending on biochar characteristics

In high-N soils, MBC and MNC exhibited the highest increase and reached a significant level when biochar pH was ≥9. Biochar addition increased MBC irrespective of changes in its total carbon content (Fig. 5a), and a significant increase in MNC was observed only when the total carbon content of biochar was 45–65% (Fig. 5b). The effect of biochar was only significant

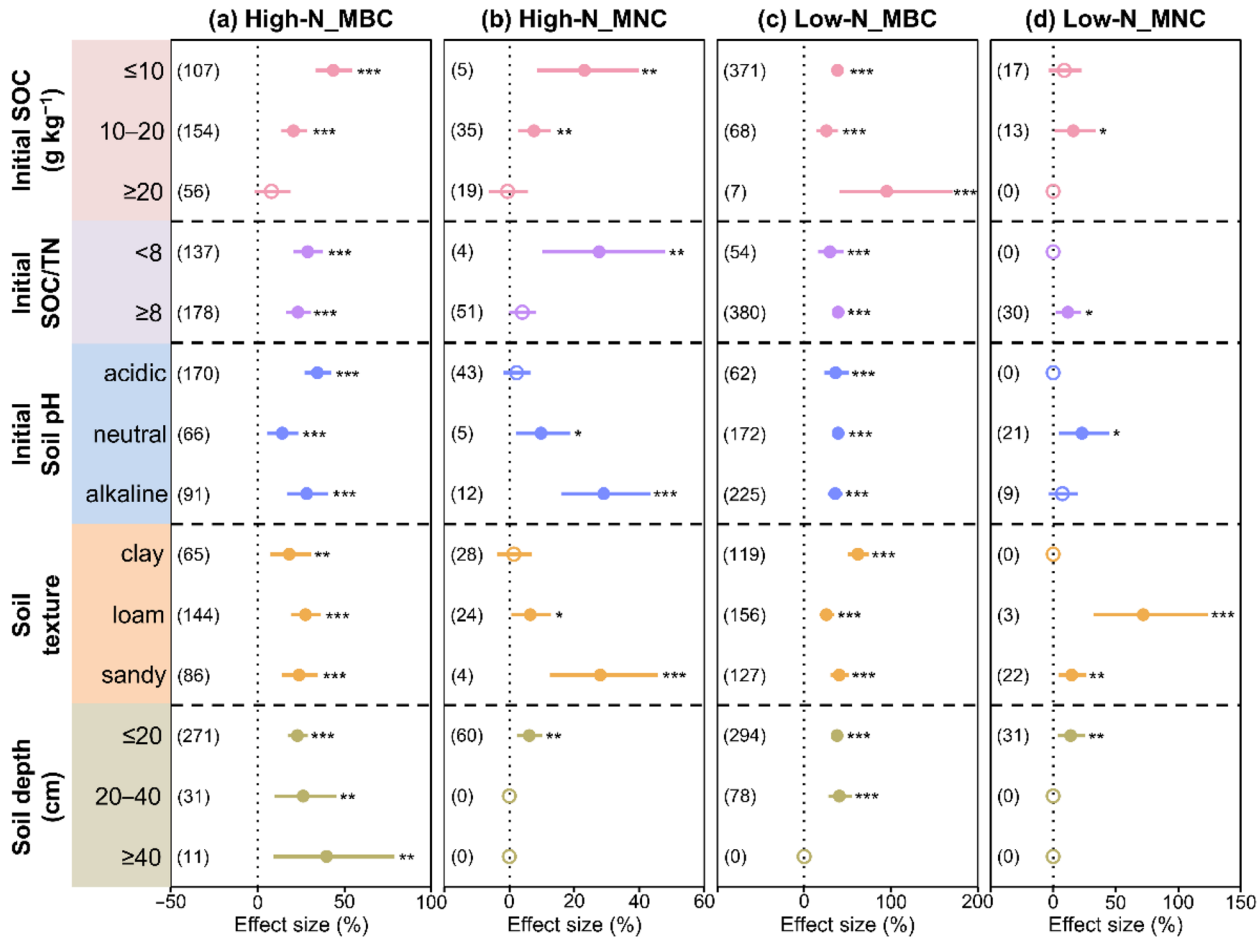


Fig. 4 Effects of soil properties on the responses of MBC and MNC to biochar under high-N and low-N soils. MBC: microbial biomass carbon; MNC: microbial necromass carbon; Initial SOC: Initial soil organic carbon; Initial SOC/TN: Initial soil organic carbon to total nitrogen ratio. Numbers in parentheses indicate the number of observations. Error bars represent 95% confidence intervals (CIs). The closed and open symbols indicate significant and nonsignificant effects, respectively. Asterisks indicate significant differences (*, ** and *** denote $P < 0.05$, 0.01 and 0.001, respectively)

when the biochar feedstock was crop residue, but not in other feedstocks (Fig. 5a, b). In addition, significant accumulation of MBC and MNC was observed when the pyrolysis temperature of biochar was ≥500 °C (Fig. 5a, b). MBC increased with the amount of biochar applied, and significant accumulation of MNC occurred when the biochar application rate was < 10 t ha^{-1} and 20–40 t ha^{-1} (Fig. 5a, b).

In low-N soils, regardless of changes in biochar pH, total carbon content, or pyrolysis temperature, MBC was significantly accumulated (Fig. 5c). In contrast to the results in high-N soils, the highest accumulation of MBC in low-N soils was observed when biochar pH ≤ 7, and total carbon content was <45% (Fig. 5c). Moreover, MBC accumulation showed a decreasing trend with increasing biochar application rate (Fig. 5c). In the results, MNC accumulated significantly when biochar pH > 7, total carbon content ≥ 65%, feedstock was crop residue, and the application rate was <10 t ha^{-1} (Fig. 5d).

3.5 Interrelationship between microbial-derived carbon and soil organic carbon fractions

Linear regressions showed that the associations between microbial-derived C and SOC fractions differed between high-N and low-N soils (Fig. 6 a–h). In high-N soils, MBC showed a significant positive relationship with POC (Fig. 6c, $P < 0.05$), and POC was strongly correlated with SOC (Fig. 6h, $P < 0.001$). This relationship indicates that microbial biomass was closely associated with particulate C accumulation under high-N conditions.

In low-N soils, MNC showed a significant positive relationship with MAOC (Fig. 6f, $P < 0.05$), while MAOC was positively correlated with SOC (Fig. 6g, $P < 0.01$). These relationships indicate a close linkage between microbial

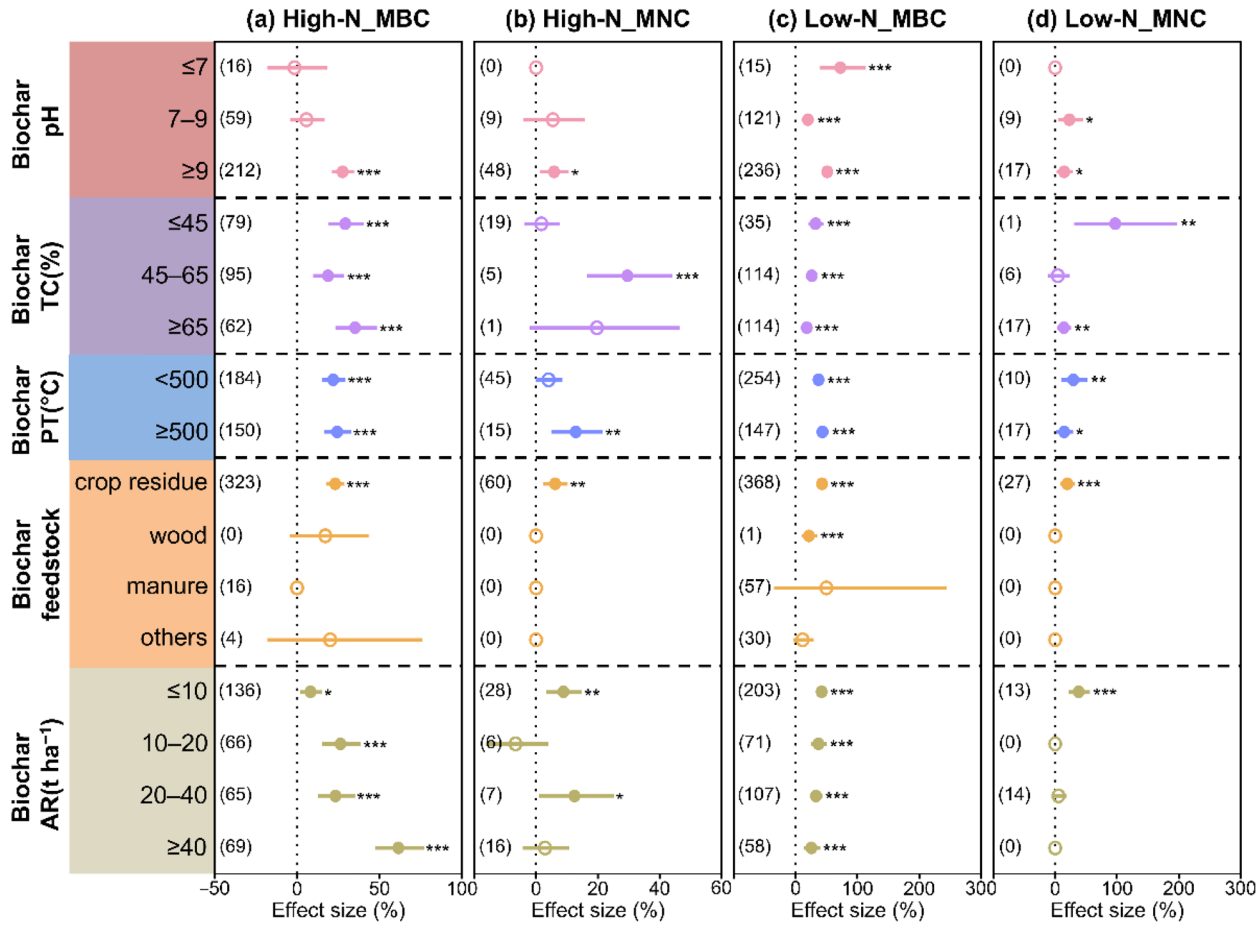


Fig. 5 Effects of biochar characteristics on the responses of MBC and MNC to biochar under high-N and low-N soils. MBC: microbial biomass carbon; MNC: microbial necromass carbon; Biochar TC: biochar total carbon; Biochar PT: biochar pyrolysis temperature. Numbers in parentheses indicate the number of observations. Error bars represent 95% confidence intervals (CIs). The closed and open symbols indicate significant and nonsignificant effects, respectively. Asterisks indicate significant differences (*, ** and *** denote $P < 0.05$, 0.01 and 0.001, respectively)

necromass and the mineral-associated C pool under low-N conditions.

3.6 Relative importance of explanatory factors

Random forest analysis showed that in high-N soils, biochar application rate, mean annual temperature (MAT), and initial SOC/TN were the top three factors influencing the biochar effects on MBC (Fig. 7a); whereas experimental duration, biochar total carbon content, and initial soil pH were the top three factors affecting MNC (Fic. 7c). In low-N soils, biochar-driven increases in MBC were mainly explained by initial SOC, soil depth, and experimental duration (Fig. 7b), whereas increases in MNC were primarily associated with experiment duration, biochar application rate, and biochar total carbon (Fig. 7d). Notably, in high-N soils, biochar characteristics were the most important contributors to the increase in MBC (34.7%; Fig. 7e), whereas experimental conditions contributed most to the increase in MNC (36.4%; Fig. 7f). In low-N soils, soil properties were the dominant drivers of MBC accumulation (50.5%; Fig. 7g), while biochar characteristics explained the largest share of variation in MNC increase (38.9%; Fig. 7h).

4 Discussion

4.1 Initial soil N level regulates biochar-driven accumulation of microbial-derived C

It is widely reported that biochar amendment can increase SOC (Ding et al. 2023; Gao et al. 2019; Yu et al. 2025). Consistent with this, our results showed that SOC increased by 29.4% in high-N soils (Fig. 2a) and by 47.9% in low-N soils (Fig. 2b), indicating a markedly stronger C sequestration response under N-poor conditions. Soil C sequestration and turnover are largely mediated by microbial growth and activity (Feng et al. 2024; He et al. 2025), and microbial responses to C inputs are strongly conditioned by initial N availability (Zhang et al. 2024a). Therefore, the larger SOC gain in low-N soils may reflect

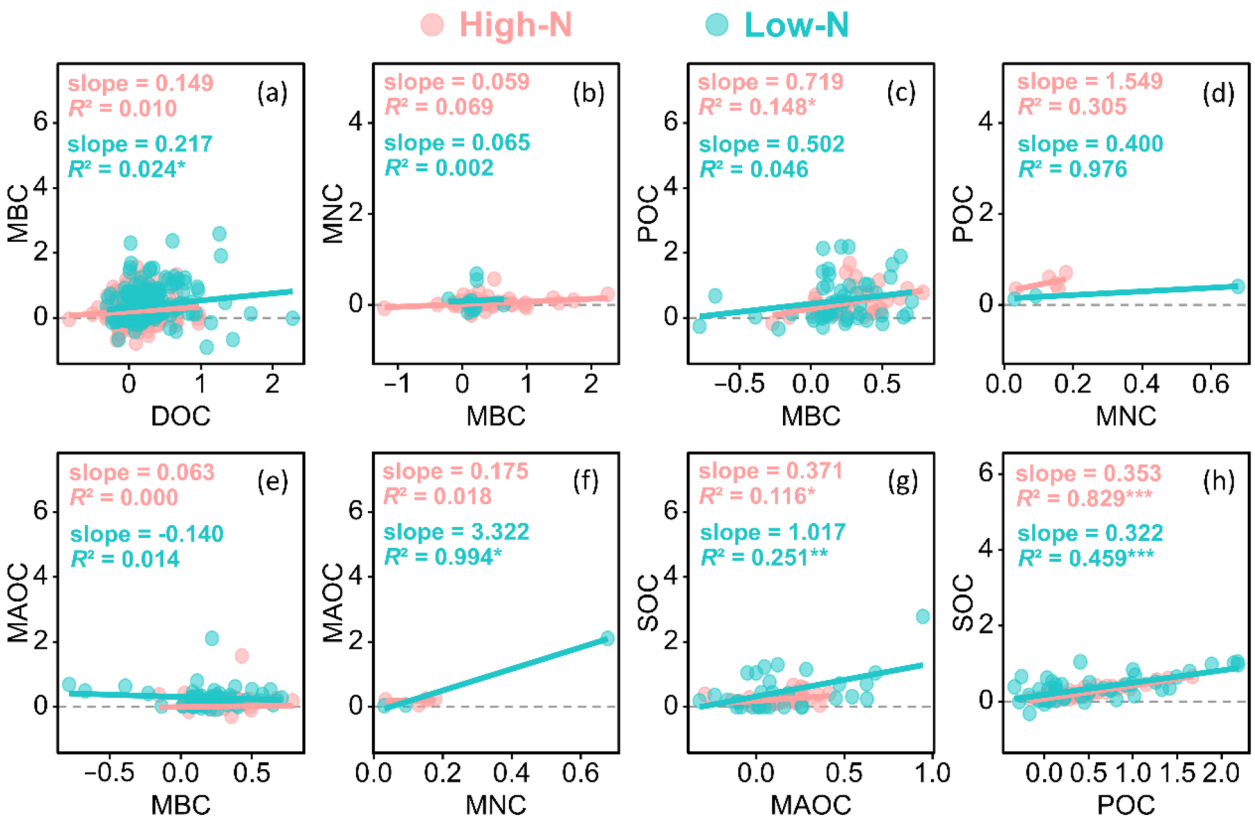


Fig. 6 Correlations of the response ratios of DOC with MBC (**a**), MBC with MNC (**b**), MBC with POC (**c**), MNC with POC (**d**), MBC with MAOC (**e**), MNC with MAOC (**f**), MAOC with SOC (**g**), POC with SOC (**h**). Linear regressions are shown as straight lines. Asterisks indicate significant differences (*, ** and *** denote $P < 0.05$, 0.01 and 0.001, respectively). DOC: dissolved organic carbon; MBC: microbial biomass carbon; MNC: microbial necromass carbon; POC: particulate organic carbon; MAOC: mineral-associated organic carbon; SOC: soil organic carbon

a more pronounced biochar-induced, microbially driven carbon accumulation pathway. This inference is supported by our observation that biochar increased microbial-derived C (MBC and MNC) in both high-N and low-N soils, with consistently stronger increases under low-N conditions (Fig. 2c). Together, these results highlight initial soil N level as a key moderator that should be explicitly considered when evaluating biochar-driven, microbially mediated C sequestration.

To further interpret this N-dependent response, previous studies have shown that microbial responses to biochar tend to be stronger in nutrient-depleted soils (Kerner et al. 2023; Kumar et al. 2025; Pokharel et al. 2020). This is consistent with our observation that biochar induced smaller increases in MBC in high-N soils than in low-N soils (Fig. 2c). As a C-rich amendment, biochar can increase the availability of labile C while providing porous habitats and sorptive surfaces that offer microbial refugia, potentially stimulating microbial growth and turnover under N-limited conditions (Chen et al. 2023b; Tang et al. 2025). Such microbial stimulation may be accompanied by a shift in resource allocation toward extracellular enzyme production and nutrient acquisition, potentially easing microbial nutrient constraints in low-N soils (Hansen et al. 2026; Iturbe-Espinoza et al. 2025). Consequently, a greater share of labile C may be incorporated into microbial biomass. Through subsequent microbial turnover, this microbial biomass can further contribute to microbial residue formation and accumulation (Kalu et al. 2024; Mason-Jones et al. 2023).

Biochar-induced increases in MNC may arise from both enhanced microbial assimilation and improved physical protection of microbial residues. On the one hand, biochar can promote the conversion of labile substrates into microbial products by increasing substrate availability and microbial carbon use efficiency (Kalu et al. 2024; Shi et al. 2025; Zhang et al. 2025b). On the other hand, biochar's porous structure can reduce residue accessibility via sorption, pore entrapment, and aggregate occlusion, thereby slowing further decomposition (Schlüter et al. 2022; Weng et al. 2022). This dual mechanism is supported by isotope-tracing evidence: a ^{13}C-glucose labeling study showed that

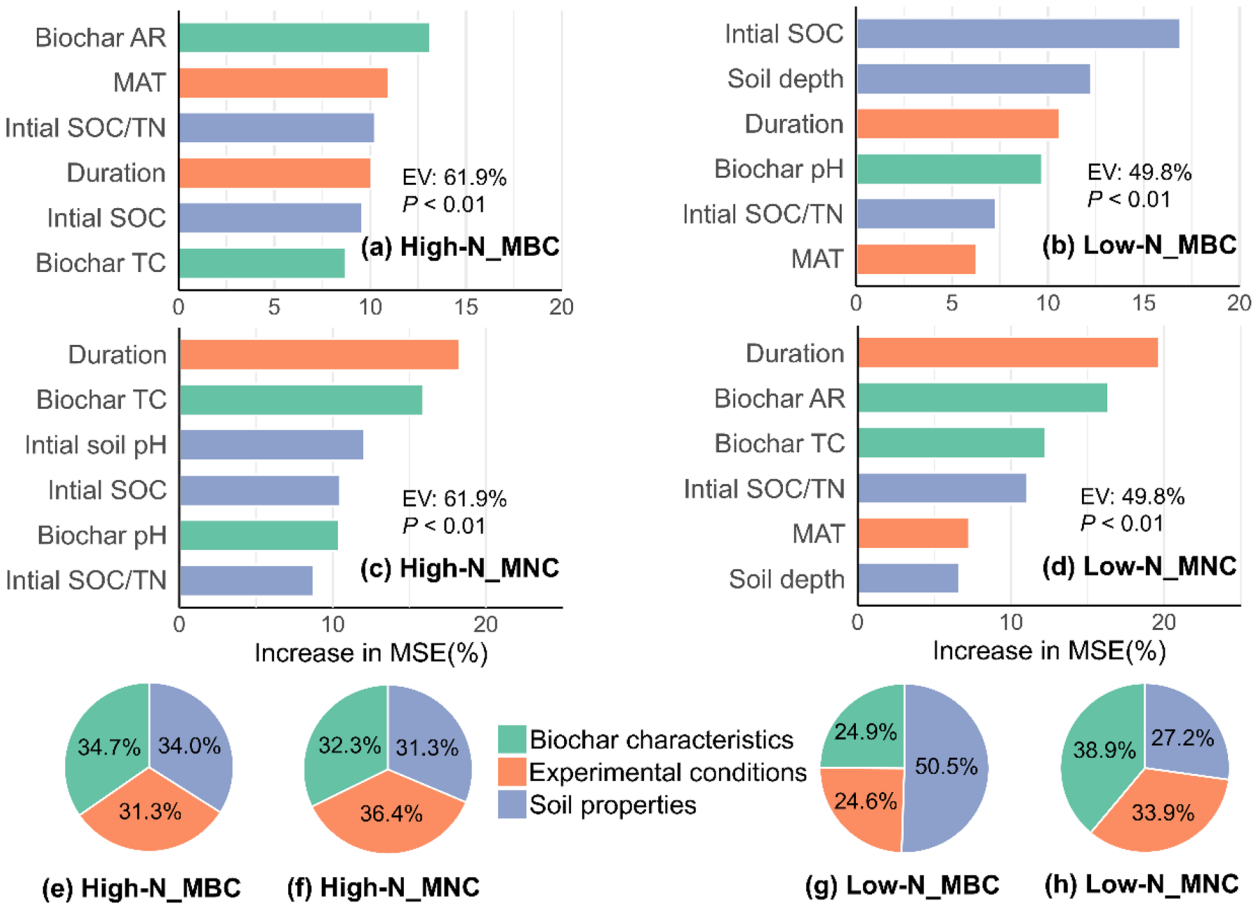


Fig. 7 Relative importance of explanatory variables for biochar-induced changes in MBC and MNC under high-N and low-N soils. Fig. 7**a-d** show the relative importance of explanatory factors for MBC in high-N soils (**a**), MBC in low-N soils (**b**), MNC in high-N soils (**c**), and MNC in low-N soils (**d**). Fig. 7**e-h** show the aggregated relative contributions of biochar characteristics, experimental conditions and soil properties to change in MBC in high-N soils (**e**), MNC in high-N soils (**f**), MBC in low-N soils (**g**), and MNC in low-N soils (**h**). MBC: microbial biomass carbon; MNC: microbial necromass carbon; Biochar AR: biochar application rate; Biochar TC: biochar total carbon; Initial SOC: initial soil organic carbon; MAT: mean annual temperature; Duration: experimental duration. MSE: mean square error; EV: explained variance

biochar increased microbial carbon use efficiency, and that MNC accumulation was associated with entrapment and physical blockage within biochar pores (Kalu et al. 2024). The stronger MNC response we observed in low-N soils than in high-N soils is also consistent with previous evidence, given that enhanced microbial biomass and enzyme activity can increase the production and turnover of microbial residues that contribute to MNC formation. In soils with lower total N, biochar more strongly enhances microbial biomass and related enzyme activities (Pokharel et al. 2020). In contrast, field evidence indicates that biochar-induced increases in microbial biomass progressively weaken as initial soil total N increases (Kumar et al. 2025). Together, these patterns suggest that when microbial N limitation is largely alleviated in high-N soils, the marginal benefits of biochar are correspondingly reduced.

4.2 Experimental conditions, biochar, and edaphic controls on microbial-derived carbon depending on initial soil nitrogen level

The random forest analysis showed that differences in initial soil N status altered the relative importance of the factors driving changes in MBC (Fig. 7). In low-N soils, microbial growth is likely constrained by N limitation and may also be influenced by substrate availability. Consequently, initial SOC, as the primary source of carbon and energy (Cao et al. 2025), emerges as the most critical factor driving changes in MBC (Fig. 7b). By contrast, in high-N soils, biochar application rate was the most important variable influencing MBC (Fig. 7a). This may be because high-N soils are often associated with acidification and nutrient imbalance, whereas the biochar application rate governs the extent of ion retention and pH buffering, thereby improving the physicochemical conditions for microbial activity (Zhang et al.

2025a). Additionally, mean annual temperature (MAT) also shaped the response pattern of MBC (Fig. 7a). Specifically, MBC responses were greatest at MAT ≥20 °C in high-N soils, whereas the strongest responses occurred at MAT ≤10 °C in low-N soils (Fig. 3a, c). This pattern suggests that biochar may partially alleviate temperature-related constraints on microbial activity, including reduced activity under cold conditions and stress associated with high temperatures (Chi et al. 2024; Fang et al. 2014; Weng et al. 2022). Moreover, across both high-N and low-N soils, experimental duration exerted a pronounced influence on MBC responses (Fig. 7a, b). Over longer time scales, sustained biochar inputs can modify microbial activity by improving nutrient availability and enhancing stress tolerance across both acidic and alkaline soils, thereby promoting increases in MBC (Yang et al. 2025a).

The relative importance of the factors associated with MNC accumulation also differed with initial soil N status. In high-N soils, microbial growth is typically less limited by N availability, potentially increasing its sensitivity to carbon supply (Chen et al. 2018). Accordingly, applying biochar with a high total C content implied a larger carbon input, accelerating microbial growth and turnover, and thereby increasing MNC accumulation (Chagas et al. 2022; Prommer et al. 2019). Meanwhile, high-N soils were frequently associated with acidification and ionic imbalance (Qiao et al. 2018). In such contexts, high-pH biochar alleviated acid stress, improved nutrient availability, and enhanced the soil microenvironment, thereby promoting microbial assimilation and biomass turnover (Ali et al. 2025; Bolan et al. 2023). It is worth noting that, regardless of high-N or low-N soils, experimental duration was a key factor influencing biochar-induced increases in MNC (Fig. 7c, d). This is likely because biochar can remain stable in soils over the long term, improving soil structure and providing sustained physical protection to microbial residues (Ernest et al. 2024). Biochar enhances microbial interactions and accelerates microbial turnover, thereby promoting the accumulation and stabilization of microbial residues over time (Zhang et al. 2024c). Consequently, longer experimental durations allow greater microbial turnover and more time for residue stabilization, resulting in higher overall MNC accumulation (Chen et al. 2024; Tian et al. 2024).

4.3 Initial soil N level regulates microbial-derived carbon pathways underpinning SOC stabilization

In low-N soils, MNC was positively related to MAOC (Fig. 6f, $P < 0.05$), and MAOC was further related to SOC (Fig. 6g, $P < 0.01$), suggesting that microbial necromass contributed to SOC mainly through mineral-associated stabilization. This interpretation is consistent with the microbial carbon pump and MEMS frameworks, which emphasize that microbial residues can form persistent SOC when protected by mineral surfaces and organo-mineral associations (Cotrufo et al. 2013; Kallenbach et al. 2016; Liang et al. 2017; Zhu et al. 2020). Mineral surfaces can retain microbial-derived compounds through sorption, ligand exchange, cation bridging, and co-precipitation processes, thereby increasing their persistence in soil (Sokol et al. 2022; Zhou et al. 2023). Although microbial community structure was not directly assessed, this pathway may also reflect a greater role of resource-efficient K-strategists under low-N conditions, whose conservative metabolism and thicker cell walls may favor the production of persistent necromass with stronger affinity for mineral protection (Malik et al. 2020; Yang et al. 2024). Biochar may further support this pathway by providing sorption sites and promoting interactions among organic compounds, minerals, and microbial residues (Lehmann et al. 2021; Weng et al. 2022; Yang et al. 2022).

In high-N soils, MBC was positively related to POC (Fig. 6c, $P < 0.05$), and POC was strongly related to SOC (Fig. 6h, $P < 0.001$), suggesting that microbial biomass was more closely connected with particulate C accumulation under high-N conditions. Higher initial N levels may alleviate microbial nutrient constraints and support faster microbial growth, potentially favoring Y-strategists with rapid turnover and greater production of extracellular polymeric substances (Malik et al. 2020; Yang et al. 2024). These microbial products, together with fungal hyphae, can act as binding agents that promote soil aggregation and the physical protection of particulate organic matter (Costa et al. 2018; Zhang et al. 2024b). Biochar may further enhance aggregation through its porous architecture and surface functional groups, which provide microbial habitats and reactive sites for organo-mineral interactions (Blanco-Canqui 2017; Deshoux et al. 2023; Lehmann et al. 2011). Aggregate occlusion can limit decomposer and extracellular enzyme access to particulate organic matter, thereby slowing POC turnover and enhancing SOC persistence (Ding et al. 2025; Rocci et al. 2024).

4.4 Limitations, uncertainties, and perspectives

This study synthesized 932 paired observations to examine how biochar regulates microbial-derived C and SOC stabilization across contrasting initial soil N levels. The results indicate that initial soil N level shapes distinct SOC stabilization pathways, with MNC more closely linked to MAOC in low-N soils and MBC more closely linked to POC in high-N soils. However, uncertainties remain because key mechanistic variables, including microbial community composition,

enzyme activity, mineral composition, biochar aging, and microbial residue chemistry were not consistently reported across the compiled studies. Long-term field experiments combining isotope tracing, microbial profiling, mineralogical analysis, and SOC fractionation are needed to test the proposed mechanisms. Additionally, standardized reporting of soil properties, biochar characteristics, microbial indicators, and SOC fractions is also needed to improve cross-study synthesis and refine site-specific biochar management strategies.

5 Conclusions

This global meta-analysis indicates that the initial soil N status is a pivotal factor regulating the effects of biochar on microbial-derived C pools. Overall, both MBC and MNC respond more strongly to biochar in low-N soils than in high-N soils. The influence of biochar is jointly shaped by experimental conditions, soil properties, and biochar characteristics, with distinct regulatory mechanisms emerging between different N contexts. Specifically, in high-N soils, MBC increases are greatest under higher biochar application rates, warmer climates, and lower initial SOC/TN ratios. In contrast, MNC accumulation is stronger with longer experimental duration, higher biochar total C content, and alkaline soil conditions. In low-N soils, MBC accumulation is further promoted by higher initial soil organic carbon content, deeper soil layers, and longer experimental duration. Meanwhile, MNC accumulation depends more strongly on longer experimental duration, lower biochar application rates, and higher biochar total C content. Notably, our findings suggest divergent SOC sequestration pathways between soils with different initial N levels. In high-N soils, SOC accumulation appears to rely more on aggregate-mediated physical protection of POC, whereas in low-N soils it appears to depend more on a microbially mediated mineral-association pathway that enhances MAOC formation. In summary, these insights highlight the need to tailor biochar design and application strategies to initial soil N status to maximize SOC sequestration and associated soil health benefits.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s42773-026-00643-7.

Supplementary material 1.

Supplementary material 2.

Acknowledgements
We are grateful to every reviewer for reviewing this manuscript.

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Funding
This work was supported by the National Key R&D Program of China (2023YFD1900600), the Science and Technology Major Project of Ordos, China (ZD20232320), and the Hebei Province Full-time Introduction of National High-Level Innovative Talents Program (2024HBQZYCXY037).

Data availability
The data used in this study are available in the online supplementary material.

Declarations

Competing interests
The authors declare that they have no known competing financial interests or personal relationships that could appear to influence the work reported in this paper.

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Received: 13 March 2026 Revised: 20 May 2026 Accepted: 15 June 2026
Published online: 07 July 2026

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