Trade-off in key carbon-degrading enzyme activities limits long-term soil carbon sequestration with biochar addition

Running title: Biochar addition on enzyme activity and soil carbon

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Abstract Amending soil with biochar is a promising agricultural approach to abate climate change by sequestering carbon (C) into soil. Impacts of biochar addition on microbial mediated decomposition process could influence soil C sequestration, but the underlying mechanisms are uncertain. Here, we conducted a meta-analysis of 130 studies and 563 paired observations worldwide, to investigate the effects of biochar addition on key extracellular enzyme activities that constitute the rate-limiting steps of microbial decomposition. Our results showed that biochar addition significantly increased soil ligninase activity targeting complex phenolic macromolecules, but suppressed cellulase activity degrading ordered polysaccharides with simpler structure. This trade-off in C-degrading enzyme activity explained more variation in soil C content response to biochar addition than a wide range of other climatic, edaphic and microbial attributes. Specifically, soil ligninase:cellulase ratio increased with time after biochar addition, and was negatively related to changing soil C content with biochar addition. These results indicate that despite the overall promotion of soil C content by reducing cellulase activity, the greater ligninase activity and increased ligninase:cellulase ratio may contribute to the declining effects of biochar amendment on soil C sequestration over time. Our results suggest that, through shift in key extracellular enzyme activities, physiological acclimation of soil microbial metabolic activity limit the long-term consequence of biochar addition on soil C sequestration.

Keywords Biochar addition; enzyme activities; soil carbon sequestration; experimental duration; soil microorganism; meta-analysis

1. INTRODUCTION

Biochar amendments of carbon (C)-rich product from biomass pyrolysis, have increasingly been regarded as a cost-effective and environmentally friendly method of increasing soil C sequestration[1-2]. The amount of biochar amendment has substantially increased over past decades, and is currently estimated to sequester 0.3-2.0 Pg CO_2 annually[1,3-4]. Despite many studies demonstrating advantages of biochar to increasing soil C sequestration[5-7], it remains unclear if and how biochar addition affects soil C dynamics over time. Indeed, several recent studies have shown that effects of biochar addition on soil C sequestration can be positive[8-9], negative[10-11] or neutral[12-13]. Such large discrepancies illustrate a poor understanding of the underlying mechanisms. The positive effects of biochar addition on soil C sequestration can be explained by the stimulation of plant growth[2,14] with a subsequent adsorption of plant organic compounds on biochar surfaces[15]. On the other hand, biochar addition can accelerate decomposition of pre-existing soil organic C (SOC) by changing microbial community composition and activities[11,16]. However, a mechanistic understanding of the composite effects of biochar addition on SOC decomposition process is lacking, hampering the prediction of the long-term effects of biochar addition on soil C dynamics.

Although some recent studies indicated that soil C sequestration varied significantly due to variations in time after biochar addition, biochar production technologies, and site-specific conditions (e.g., climate and soil properties)[26-27], a comprehensive understanding of underlying mechanisms remain unexplored. In particular, there is no direct evidence of how biochar addition affects key enzyme activities (e.g., cellulase and ligninase) that are likely to influence long-term impacts on soil C sequestration across varying environmental conditions.

Soil extracellular enzymes catalyze the degradation of soil organic matter, deconstructing plant and microbial residues by breaking down large macromolecules into simpler molecules[17-18]. Various extracellular enzymes target different pools of SOC, including ligninase (e.g., phenol oxidase (PO) and peroxidase (PER)) targeting structurally complex polyphenolic macromolecules, and cellulase (e.g., β-1,4-Glucosidase (BG) and α-1,4-glucosidase (AG)) degrading ordered polysaccharides with simpler structure[17,19]. Biochar addition may have different impacts on ligninase and cellulase activity, partly because of variations in chemical composition of soil organic matter and also due to shifts in microbial community composition[20-22]. For instance, the condensation of cellulose and hemicellulose into humic-like macromolecules on the surface of biochar[2,23] could induce microbes to secret ligninase but suppress cellulase, as enzyme productions are commonly induced by the presence of adequate substrates[24-25]. This shift in C-degrading enzyme activity may have substantial but as yet unknown effects on soil C sequestration with biochar addition.

To address these knowledge gaps, we conducted a global meta-analysis to evaluate the responses of soil cellulase and ligninase activities to biochar addition, and how these responses may affect soil C sequestration. We compiled a database of C-degrading enzyme activities and soil C sequestration from 130 biochar addition studies and 563 paired observations worldwide (Fig. S1; Table S1). We combined the advantages of classic meta-analysis with advanced model selection analysis to quantify the relative importance of potential predictors in explaining the effects of biochar addition on enzyme activities and soil C sequestration. This approach allows us to assess the role of enzyme activities in determining changes in soil C sequestration with biochar addition across a wide range of climatic, edaphic and experimental factors. Specifically, we tested the following hypotheses: (1) Biochar addition induces shifts in C-degrading enzyme activity, enhancing ligninase activity while suppressing cellulase activity; and (2) A shift in C-degrading enzyme activities will affect soil C sequestration with biochar addition.

2. MATERIALS AND METHODS

2.1 Description of the seven kinds of enzymes included in this study

Seven kinds of extracellular enzymes involved in SOC decomposition were included in this meta-analysis (Table S2) following previous studies[17,19,28]. Specifically, cellulase included BG, AG, β-1,4-Xylosidase (BX) and β-D-Cellobiosidase (CBH). Ligninase included PO, Polyphenol oxidase (PPO) and PER. Enzyme activity assays are explained in Supplementary Note 1.

2.2 Data collection

We searched for articles on the effects of biochar addition on cellulase and ligninase activities using Web of Science (http://apps.webofknowledge.com/), Google Scholar (http://scholar.google.com/), and China National Knowledge Infrastructure (http://www.cnki.net/). The cutoff date was May 2022. The following keywords and terms were used for literature searching: (a) “biochar addition” or “biochar amendment” or “biochar application” (b) “cellulase” or “ligninase” or “glucosidase” or “xylosidase” or “cellobiosidase” or “peroxidase” or “phenol oxidase”, and (c) “soil” or “terrestrial” or “land”.

Articles included in this study had to meet the following criteria: (a) climatic, vegetation and soil attributes were similar for the control and biochar addition treatments; (b) biochar properties (biochar materials, biochar pH, biochar temperature, biochar C% and biochar N%) and application protocols (biochar application method, biochar application rate and duration) were clearly described; (c) ecosystem types were reported; and (d) standard deviation (SD) and sample size were reported or could be calculated from the data presented in the publication. Measurements with different duration of biochar addition in the same site were considered as independent observations since one of the primary purposes is to explore the duration impacts on enzyme activities and soil C dynamics. Measurements from contaminated soil were excluded to eliminate the confounding effects of pollutants on soil enzyme activities[29-30]. The PRISMA flowchart illustrating processes for the selection of the included articles is shown in Fig. S1. Based on these criteria, we obtained 563 paired observations from 130 articles worldwide (Fig. S2, Table S1 & Supporting Dataset). For results that were presented in graphs, we used Getdata Graph Digitizer (version 2.26) (http://www.getdata-graph-digitizer.com/download.php) to extract the data.

We first extracted information on cellulase and ligninase activities. If one paper reported two or more kinds of cellulase or ligninase, the sum of these enzyme activities were calculated as the overall responses of cellulase and ligninase activities, respectively[19,31]. We further collected a wide range of environmental factors, including elevation (0-1746 m), latitude (−42.95° S-55.37° N), longitude (−119.74° W-147.10° E), mean annual precipitation (MAP, 27-2500 mm), mean annual temperature (MAT, −1.0-32.3 °C), and vegetation type (cropland, grassland, forest, open area and wetland) for each site. Edaphic properties, including SOC, soil total nitrogen (N), soil C:N, soil pH and soil texture (sand, silt and clay content) were also recorded. For missing environmental and edaphic variables we searched for relevant publications by the same research group at the same study sites or contacted the corresponding authors. Alternatively, missing data of climatic (including MAT, MAP) and soil attributes (SOC, total N and soil texture) were obtained from the WorldClimate Database (http://www.worldclim.org/) and Soil grids database (https://www.isric.org/explore/soilgrids), respectively. For biochar properties and application protocols, we recorded biochar materials (i.e., wood, herb, residue, urban waste), pH, pyrolysis temperature, C and N content (%) as well as method (Field, Pot and Lab), rate (%) and duration (year) of biochar application. Additionally, we recorded SOC, total N, soil C:N, pH, microbial biomass, the abundance of fungi, bacteria, fungi:bacteria, and plant biomass for both ambient and biochar addition treatments, when these variables were reported.

2.3 Data analysis

We used meta-analysis to qualify the effect of biochar addition on soil cellulase and ligninase activities, ligninase:cellulase ratio and other edaphic and microbial variables[32-33]. Specifically, we calculated the logarithmic response ratio (LnR) of each variable using the following equation:

$$\mathrm{LnR}=\mathrm{Ln}\left(\frac{\overline{X_B}}{\overline{X_C}}\right)=\mathrm{Ln}(\overline{X_B})-\mathrm{Ln}(\overline{X_C}) \qquad (1)$$

with $\overline{X_B}$ and $\overline{X_C}$ as the arithmetic average values in the biochar addition and control treatments, respectively. The variance (V_i) of Ln-R was calculated as:

$$V_i=\frac{SD_B^2}{n_B X_B^2}+\frac{SD_C^2}{n_C X_C^2} \qquad (2)$$

with SD_B and SD_C are the standard deviations, n_B and n_C are the number of replicates, and X_B and X_C are the averaged arithmetic values of variables for biochar addition and control treatments, respectively.

The overall effects of biochar addition on different variables and 95% confidence intervals (CI) were evaluated using ‘rma.mv’ function in the ‘metafor’ package of R project[34]. We included “Publication” and “Observation” as random factors in the mixed-effect models, because some studies contributed more than one paired observation[19,35]. To facilitate the interpretation of data, the effect size was back-transformed to percentage change. The effect of biochar addition on each variable was considered significant if the 95% CI did not overlap with zero. The normality of each kind of enzyme activity was tested using the Kolmogorov-Smirnov and Shapiro-Wilk tests, except for PPO due to its small sample size.

We conducted meta-analytic models to analyze the combined effects of environmental, edaphic and experimental factors on the responses of soil cellulase activities, ligninase activities, ligninase:cellulase ratio and soil C sequestration to biochar addition. Briefly, we used a mixed-effects meta-regression model using 'glmulti' package in R[28,36-37]. The importance of different factors was evaluated using the sum of Akaike weights. The weight was considered as the overall support for each variable in all potential models. A cutoff of 0.8 was set to identify the significant predictors for each model[19,33,38]. Further, we used Spearman's rank correlation analysis to evaluate the relationships of cellulase activities, ligninase activities, and ligninase:cellulase ratio with environmental, edaphic and experimental factors. To further understand the effect of experiment duration on soil C sequestration, we conducted both linear regression and piece-wise regression models to fit the relationship between the LnR of SOC and time after biochar addition. Specifically, a piece-wise regression model was carried out using the 'segmented' R package[39]. The optimal regression model was selected by comparing regression coefficients (r) and the model was statistically significant if $P < 0.05$.

3. RESULTS

Averaged across all studies, biochar addition significantly suppressed cellulase activity by 8.3% ($P < 0.001$; Fig. 1a). Specifically, biochar addition decreased the activities of BG and BX by 7.3% and 9.3% ($P < 0.05$), respectively. In contrast, biochar addition increased ligninase activity by 7.1% on average ($P < 0.001$), with an increase of PER activity by 7.0% ($P < 0.001$). The differential responses of cellulase and ligninase activities to biochar addition led to marginally increased ratio of ligninase:cellulase activities by 10.7% ($P = 0.052$). In addition, the responses of cellulase, ligninase and ligninase:cellulase ratio were normally distributed according to both Kolmogorov-Smirnov and Shapiro-Wilk test ($P > 0.05$; Fig. 1b).

Model selection analysis showed that the effects of biochar addition on cellulase activity were best explained by biochar application rate, MAP, longitude and soil clay content (Fig. 2a). On the other hand, the responses of ligninase activity to biochar addition were mostly explained by soil N content, biochar temperature, site locations (i.e., longitude) and biochar pH. Linear regression analysis confirmed that LnR of cellulase activity was negatively correlated with biochar application rate, whereas a positive correlation was found with MAP ($P < 0.05$). Moreover, LnR of ligninase had negative relationships with soil N content and biochar pyrolysis temperature, but positive correlation to biochar pH ($P < 0.05$; Fig. 2b). Regarding the ligninase:cellulase ratio, the most important predictors were the duration of biochar addition, soil C:N, biochar C content, and biochar C:N. Specifically, the LnR-Ligninase:cellulase ratio was positively correlated with duration of biochar addition but negatively correlated with soil C:N, biochar C content and C:N ($P < 0.05$).

For studies that have reported SOC, biochar addition enhanced SOC by an average of 52.8% ($P < 0.001$) (Fig. 3a). Our piece-wise regression analysis showed that biochar-induced increases in SOC generally diminished with duration of biochar addition (Fig. 3b; $P < 0.001$). The model selection analysis showed that the response of SOC to biochar addition was best explained by LnR-Ligninase:cellulase across a wide range of variables of environmental (Longitude, MAT and MAP), edaphic (soil

N, C:N, clay and pH), experimental protocols (biochar C, biochar C:N, biochar pH, biochar temperature, biochar rate and time) and changes in microbial attributes (LnR-microbial biomass and LnR-fungi:bacteria). Specifically, changes in SOC with biochar application were negatively related to the ratio of ligninase:cellulase (Fig. 3d, $P < 0.001$).

4. DISCUSSION

Our results indicate that biochar addition significantly increased ligninase activity and decreased cellulase activity, resulting in marginally increased ligninase:cellulase ratio (Fig. 1). Changes in ligninase:cellulase explained the most variation in the response of SOC to biochar addition across a wide range of environmental, edaphic and experimental variables. Specifically, increases in ligninase:cellulase ratio were associated negatively with the responses of SOC. Moreover, the response of ligninase:cellulase ratio increased with time after biochar addition, suggesting a declining capacity for soil C sequestration with long-term addition[5,23]. Together, to the best of our knowledge, our findings provide the first evidence from soil extracellular enzymes relevant to unravelling the mechanisms controlling soil C sequestration with prolonged biochar exposure.

4.1 Differential responses of cellulase and ligninase activity with biochar addition

We propose three possible underlying mechanisms to explain different responses of cellulase and ligninase activity with biochar addition (Fig. 4). First, biochar-induced reductions in soil N availability could stimulate ligninase rather than cellulase activity.

Several lines of evidence have demonstrated reductions in soil N availability after biochar addition, perhaps driven by (1) higher plant biomass production (Fig. S3) and associated translocation of N from soil to vegetation, (2) the additional C inputs increasing bulk soil stoichiometric C:N ratios (Fig. S4a), and (3) occlusion of soil available NH_4^+ by phenolic- and lignin-like compounds through complex organo-mineral interactions on biochar surfaces (Fig. S4b). In response, soil microorganisms may increase ligninase production to stimulate the breakdown of complex phenolic- and lignin-like compounds to acquire bound N. In support of this explanation, we found that soil N content was the most important predictor (negative) of the effects of biochar addition on soil ligninase activity (Fig. 3). Indeed, this explanation supports the “microbial N mining theory”, which assumes that microorganisms invest resources to decompose complex structural macromolecules to acquire N under N limitation[40-42].

Second, biochar addition significantly altered the chemical composition of soil organic matter[11,27], which likely contributed to the trade-off responses of cellulase and ligninase. By introducing additional phenolic- and lignin-like compounds, biochar could reduce the availability of readily decomposable C compounds since they could be occluded within macromolecule assemblages through complex organo-mineral interactions on biochar surfaces[20-21]. Thus, microbial utilization of these readily decomposable C compounds could be suppressed due to limitation in substrate availability[20-21]. In contrast, the addition of lignin-like organic C would induce expression of ligninase encoding microbial genes and possibly translational upregulation of ligninase enzymes. In addition, this explanation from the perspective of chemical composition could be supported by the great importance of production temperature on predicting the responses of soil ligninase activity to biochar addition (Fig. 2a), since biochar produced at high pyrolysis temperature commonly have more C being present in aromatic compounds[43-44]. Therefore, our findings indicate that after the initial depletion and stabilization of readily decomposable C by biochar, soil microbes likely stimulate ligninase production to access more chemically recalcitrant C pools[45].

Third, shifts in microbial community composition could contribute to the opposite effects of biochar addition on cellulase and ligninase activity. Our results show positive associations between ligninase activity and microbial biomass with biochar addition, but not for cellulase (Fig. S5). These results suggest that soil microbial community composition or enzyme production efficiency may change under biochar addition. Indeed, previous studies have reported that biochar addition stimulates fungal growth such as the two most commonly occurring types of mycorrhizal (arbuscular mycorrhizal and ectomycorrhizal) fungi[46-47].Consistently, we found increased fungal abundance with biochar addition, which were positively correlated with changes in ligninase activity (Fig. S5-6). These results indicate that biochar-induced shifts toward a fungi-dominant microbial community could promote ligninase activity, possibly because fungi were more efficient in mineralizing structural complex macromolecules than bacteria and are the primary producers of phenol oxidase[49]. In addition, we observed positive relationships between

LnR-Ligninase:cellulase and LnR-fungi:bacteria (Fig. S5), further suggesting that the shifts in extracellular enzyme activities observed in this study were related to changes in microbial community composition.

4.2 Linking trade-off in soil enzyme activity to changes in SOC with biochar addition

The trade-off responses of cellulase and ligninase could exert inverse effects on soil C sequestration with biochar addition. Specifically, the suppressed cellulase activity may promote soil C sequestration with biochar by reducing the decomposition of ordered polysaccharides with simpler structure[17]. In contrast, the enhanced ligninase activity may cause increase in the decomposition of complex phenolic macromolecules, which is commonly considered as rate-limiting step of SOC decomposition[50-51]. Therefore, biochar-induced sequestration of SOC could reflect the trade-off effects of these key extracellular enzymes. In support of this, our model selection analysis results indicate that shifts in soil enzyme activity from cellulase to ligninase explained the most variations in soil C sequestration with biochar addition (Fig. 3). Our regression analysis further showed a significant negative relationship between ligninase:cellulase ratio and SOC with biochar addition (Fig. 3d), suggesting that the increased decomposition of lignin-like substrates relative to cellulose-like substrates may limit soil C sequestration with biochar addition.

Moreover, ligninase:cellulase ratio increased with time after biochar addition, which could exacerbate the decline in soil C over time. Previous studies have reported that complex macromolecule C adsorbed on the surface of biochar could be used by microbial community when polysaccharides with simple ordered structure are depleted over time[22,48]. Therefore, this gradual increase in ligninase:cellulase over time may also reflect changes in the chemical composition of soil organic matter and associated shifts in microbial community composition with time after biochar addition[16,52]. These results indicate that a functional acclimation of soil microorganisms to chemical composition of organic substrate affects the response of soil C sequestration to biochar addition over time. Similar declines in soil C sequestration with the time after biochar addition have also been observed in long-term case studies, and are mainly considered the result of declining adsorption capacity of biochar over time[23,53].

However, the moderate correlations between soil C sequestration and ligninase:cellulase suggest that shifts in soil enzyme activity alone cannot fully explain the variations in soil C sequestration with biochar addition (Fig. 3d). Indeed, overall soil C sequestration is determined by interactions among at least three C pools, namely biochar, the pre-existing SOC and plant litter/root exudates[2]. Therefore, other soil processes, such as the decomposition of labile component of biochar and priming of pre-existing C in soil could also affect soil C sequestration with biochar addition[54-55]. For instance, a previous meta-analysis indicates that a small part of biochar (approximately 3%) is bioavailable with a mean residue time of 108 days[56], which may partly contribute to the initial decline in the response of soil C sequestration during the first year of biochar addition in this study. Further studies deciphering these processes (e.g., using isotopic tracers) are needed to accurately predict long-term consequences of biochar addition to soil C sequestration. In addition, the properties of original soil (e.g., SOC, pH, soil clay content) and experimental protocols (e.g., application rates) may also interactively affect soil enzyme activities and soil C sequestration with biochar addition[15,20-21]. However, such interactions are difficult to evaluate under field conditions due to high soil and experimental heterogeneities, which require further quantifications to identify maximum soil C accrual with biochar addition.

Models used to predict increases in soil C sequestration with biochar addition vary significantly, with the annual increases in SOC ranging from 0.07% to 10% per unit of biochar C addition[2,57]. Large uncertainties in estimates stem from the timescales and soil C mineralization processes simulated in different models. Existing biochar models commonly consider soil C mineralization as simple first-order reactions[57]. However, C mineralization is a complex process that combines enzyme-mediated mineralization of both fast- and slow-mineralization organic fractions[28,31]. Our results suggest that retaining inflexible microbial functional traits over the duration of biochar addition may lead to inaccurate predictions of soil C sequestration. Therefore, it is necessary to include the temporal shifts in microbial C-degrading enzyme activity to improve model predictions of soil C sequestration over time with added biochar.

Our study provides evidence for the contribution of biochar addition to enzyme-catalyzed microbial decomposition processes and soil C sequestration over wide temporal and environmental scales. Our results show that trade-off responses in cellulase and ligninase activities drive long-term impacts of biochar addition on soil C sequestration. This physiological acclimation in microbial metabolic activity has been overlooked to date, as a result of previously focusing on physical and chemical processes[6,21]. In addition, our analyses offers insights to options for regulating soil C sequestration with biochar addition across a broad range of environmental and experimental factors. Specifically, the responses of cellulase and ligninase depend on different environmental, edaphic and experimental factors (Fig. 2). Therefore, it should be possible to promote soil C accrual with biochar addition by regulating factors controlling different soil enzyme activities. For instance, biochar can be applied with N fertilizer to promote C sequestration by reducing N-mining via ligninase. Moreover, reduced response of soil ligninase activity and associated increase in soil C sequestration could also be achieved by selecting the appropriate pyrolysis temperature of biochar production. Innovative biochar management techniques to mediate different soil enzyme activities are possible, which requires collective actions of policy makers, farmers and industry at both local and regional scales.

5. CONCLUSIONS

Our synthesis identifies the trade-off responses of cellulase and ligninase to biochar addition, with important implications for soil C sequestration. We also show that biochar addition increased ligninase activity but reduced cellulase activity,with an increasing ligninase:cellulase ratio over time after biochar addition. Moreover, biochar-induced changes in ligninase:cellulase were negatively related to SOC pool size, suggesting a progressive reduction in soil C sequestration with long-term biochar addition. Different factors influenced the effects of biochar addition on cellulase and ligninase activities, which could help engineer the system to increase soil C sequestration under prolonged biochar exposure.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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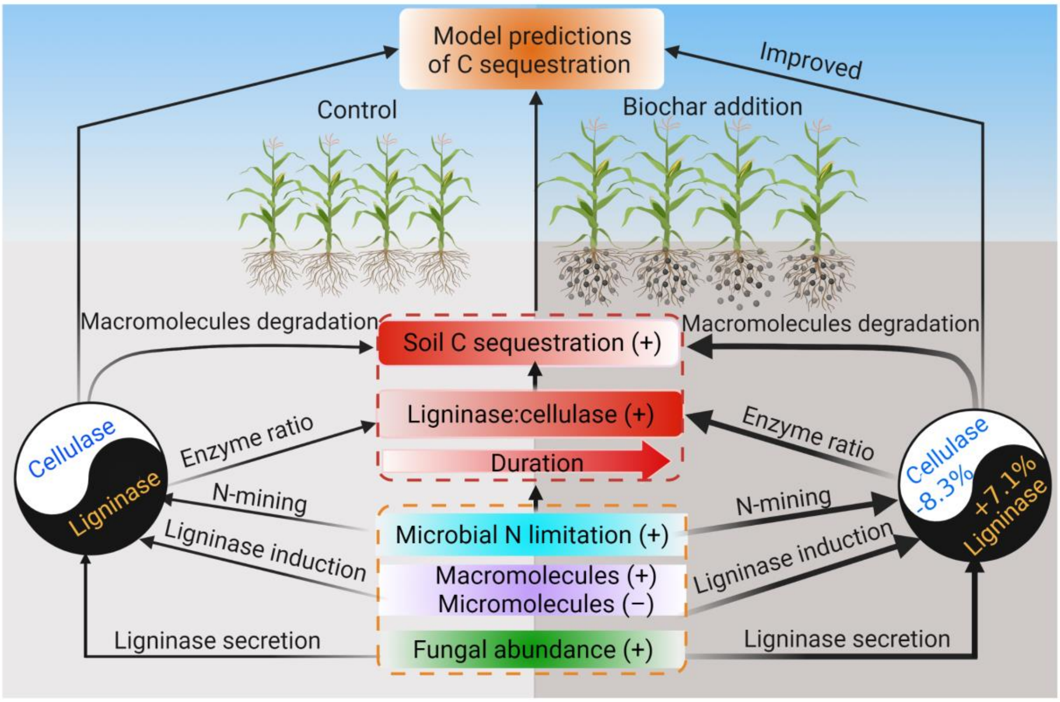

Graphical Abstract

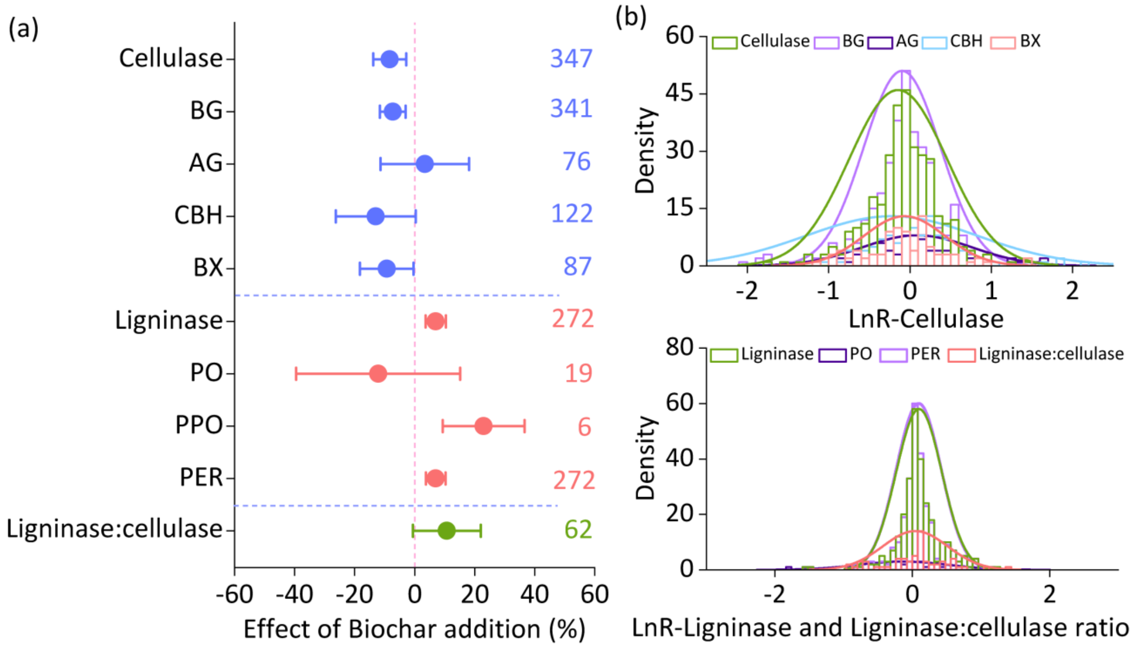

FIGURE 1 (a) Effects of biochar addition on cellulase activity, ligninase activity, and ligninase:cellulase ratio. (b) Distribution of the response ratios (log-transformed, Ln-R) of cellulase activity, ligninase activity, and ligninase:cellulase to biochar addition. The values represent the mean percentage of changes in each variable with biochar addition vs. control; error bars indicate 95% confidence intervals. The sample sizes of each variable are shown in the right column of the figure. BG, β-1,4-glucosidase, AG, α-1,4-glucosidase, CBH, β-D-cellobiohydrolase, BX, β-1,4-xylosidase, PO, phenol oxidase, PPO, Polyphenol oxidase, PER, peroxidase.

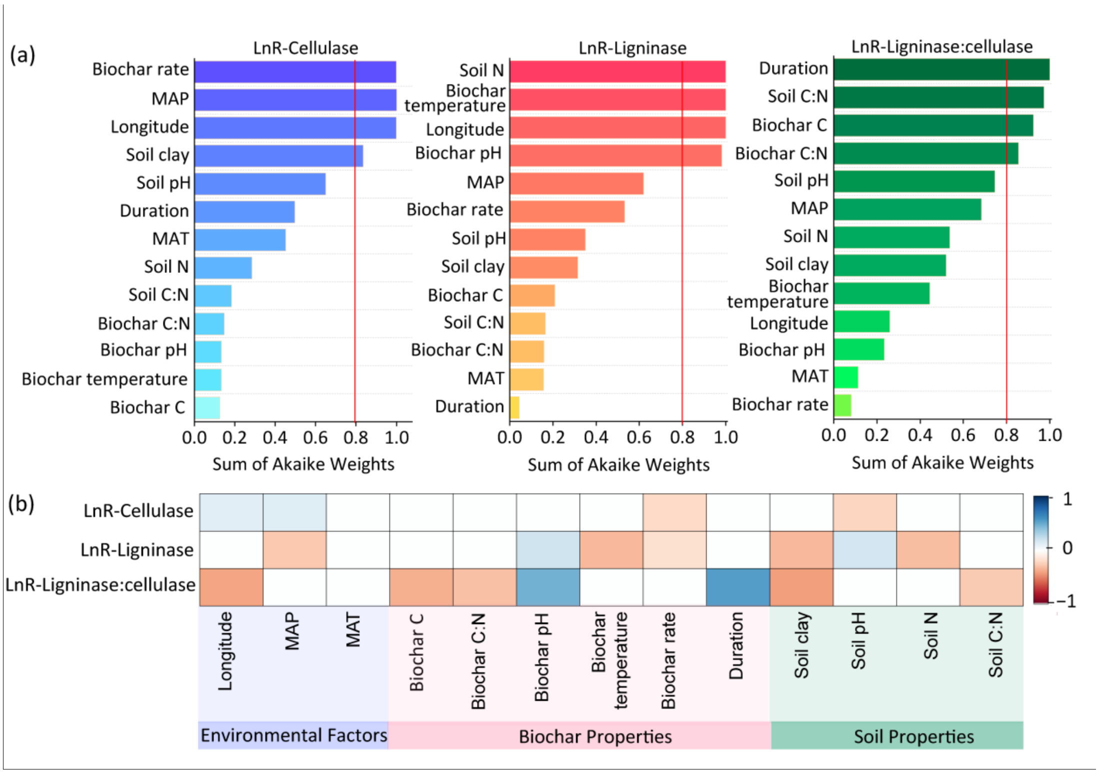

FIGURE 2 (a) Relative importance of different variables regulating the effects of biochar addition on cellulase activity, ligninase activity, and ligninase:cellulase ratio. (b) Correlations between studied variables and the responses (LnR) of cellulase activity, ligninase activity, and ligninase:cellulase to biochar addition. The relative importance is according to the sum of Akaike weights of model selection. A cutoff of 0.8 is set to differentiate the important vs. nonessential predictors. MAP, mean annual precipitation, MAT, mean annual temperature, Biochar C, the content of C (%) in biochar; Biochar C:N, the C:N of biochar, Biochar pH, the pH of biochar, Biochar temperature, the temperature of biochar production, Biochar rate, the application rate of biochar addition, Duration, the duration of biochar addition.

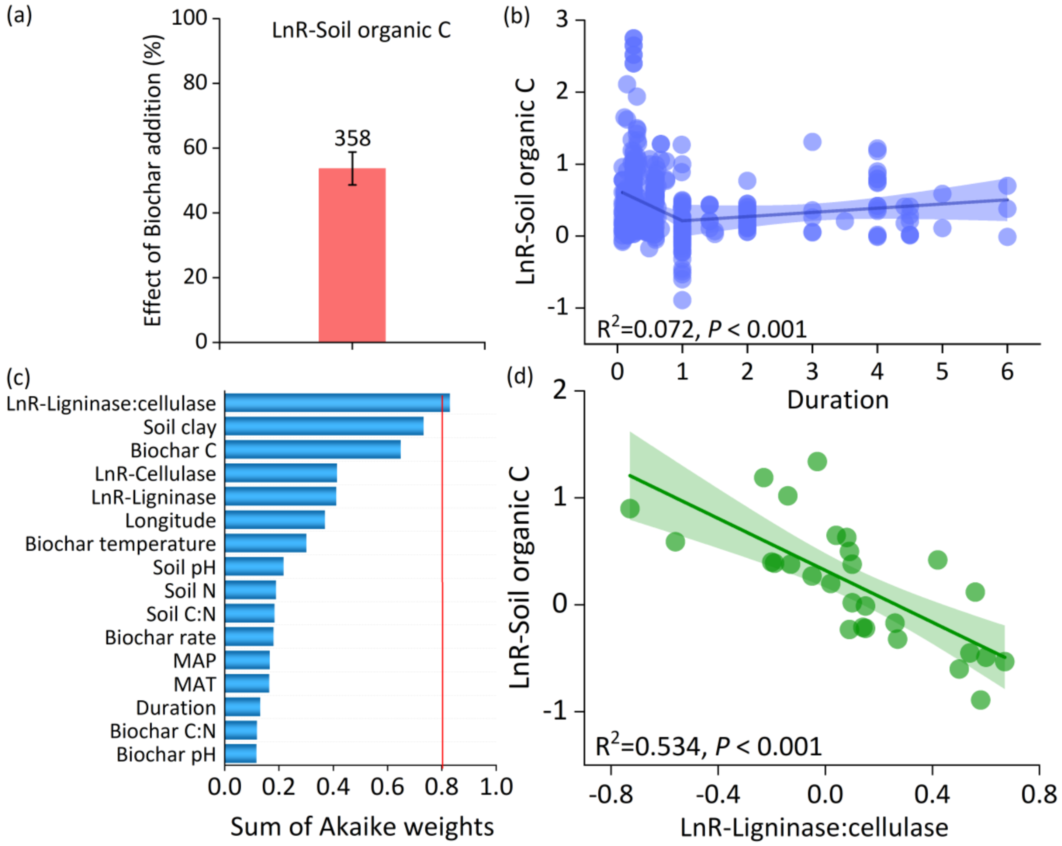

FIGURE 3 (a) Effects of biochar addition on soil organic C sequestration. (b) Relationship between duration of biochar addition and the response (LnR) of soil organic C to biochar addition. (c) Relative importance of different variables regulating the effects of biochar addition on soil organic C sequestration. (d) The relationship between the response of ligninase:cellulase and the response of soil organic C to biochar addition. The relationship between duration of biochar addition and LnR-Soil organic C was analyzed using a piece-wise regression model. Cutoff of 0.8 is set to differentiate the important vs. nonessential predictors. MAP, mean annual precipitation, MAT, mean annual temperature, Biochar C, the content of C (%) in biochar; Biochar C:N, the C:N of biochar, Biochar pH, the pH of biochar, Biochar temperature, the temperature of biochar production, Biochar rate, the application rate of biochar addition, Duration, the duration of biochar addition.

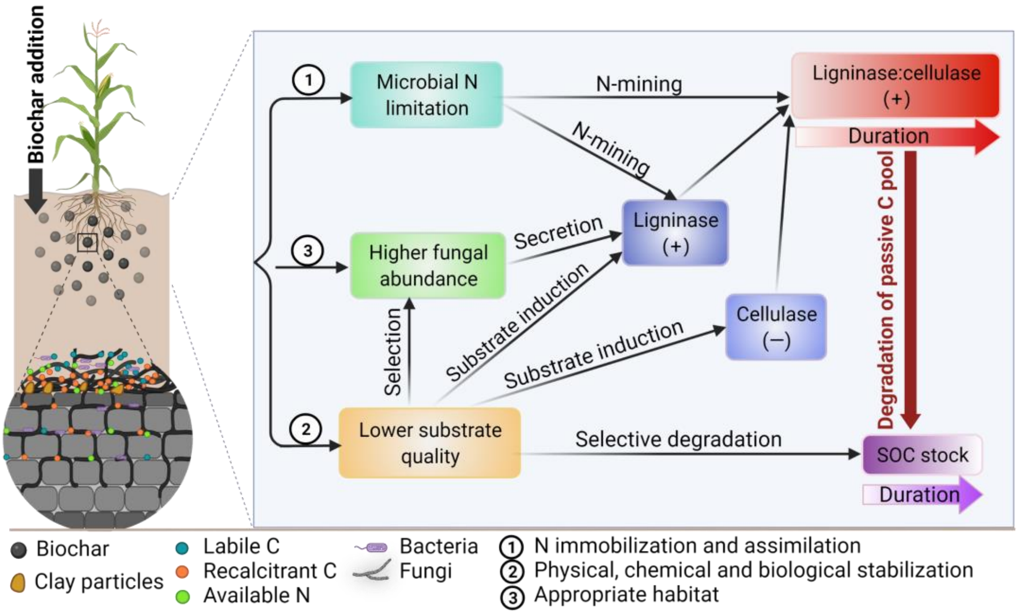

FIGURE 4 A conceptual paradigm illustrating the mechanisms of biochar addition on soil carbon-degrading enzyme activities and their impacts on soil organic carbon dynamics. Biochar addition has differential effects on soil cellulase and ligninase activities via direct and indirect effects on substrate quality, microbial community composition and soil N availability. The differential responses of cellulase and ligninase activities to biochar addition results in an increasing ligninase:cellulase ratio with duration of biochar addition, which may reduce soil organic carbon (SOC) sequestration over time.

Supporting information

Trade-off in key carbon-degrading enzyme activities limits long-term soil carbon sequestration with biochar addition

Brief description of supplementary data

TABLE S1. Overview of studies included in our meta-analysis.

TABLE S2. Overview of cellulase and ligninase included in this meta-analysis.

SUPPLEMENTARY NOTE 1 Soil carbon-degrading extracellular enzymes assays.

FIGURE S1 PRISMA flowchart illustrating the processes for the selection of articles included in this meta-analysis.

FIGURE S2 Global distribution of the biochar addition experiments selected in this meta-analysis.

FIGURE S3 (a) Effects of biochar addition on plant biomass. (b) Relationship between the response of soil organic C (SOC) and plant biomass to biochar addition.

FIGURE S4 (a) Effects of biochar addition on soil carbon:nitrogen. (b) Effects of biochar addition on soil NH_4^+. (c) Relationship between biochar-induced changes in carbon:nitrogen and the application rate of biochar addition.

FIGURE S5 Relationships between the responses of cellulase, ligninase and ligninase:cellulase with microbial attributes after biochar addition.

FIGURE S6 (a) Effects of biochar addition on fungi abundance. (b) Relationship between the response of fungi abundance and biochar carbon:nitrogen after biochar addition.

Lists of literatures included in our meta-analysis.

Data S1. Database of biochar addition studies reporting soil cellulase activity, ligninase activity and soil organic carbon sequestration that were included in our analysis.

Data S2. Database of biochar addition studies reporting individual activities of different cellulases.

Data S3. Database of biochar addition studies reporting individual activities of different ligninases.

Data S4. Database of biochar addition studies reporting soil organic carbon that were included in our analysis.

TABLE S1. Overview of studies included in our meta-analysis.

References	**Latitude (º)**	**Longitude (º)**	**Elevation (m)**	**MAP (mm)**	**MAT (ºC)**	**Land use Type**	**Experiment Type**	**Soil Depth (cm)**	**Biochar Material**
[1]Abujabhah et al. 2016	-42.95	147.10	163	744	11.5	Farmland	Field	0-15	Wood
[2]Al Marzooqo et al. 2017	24.45	54.39	2	392	28.0	Farmland	Lab Incubation	0-15	Wood
[3]Ameloot et al. 2014	53.24	-0.54	69	569	9.4	Farmland	Lab Incubation	0-10	Wood
[4]Awad et al. 2013	38.28	128.15	451	1252	7.8	Farmland	Lab Incubation	0-5	Wood
[5]Awad et al. 2018	48.71	9.19	405	744	9.2	Farmland	Lab Incubation	0-5	Wood
[6]Azlan Halmi et al. 2018	3.05	102.05	443	2500	27.0	Farmland	Field	0-20	Residue
[7]Bailey et al. 2011	46.76	-117.18	785	523	8.6	Farmland	Lab Incubation	0-5	Herb
[8]Bamminger et al. 2014	50.92	11.58	145	644	8.9	Forest	Lab Incubation	0-10	Herb
[9]Bandara et al. 2017	7.72	80.94	93	1805	27.2	Forest	Pot	0–15	Herb
[10]Benavente et al. 2018	49.32	3.48	150	651	9.9	Open area	Lab Incubation	0–15	Urban Waste
[11]Bera et al. 2016	46.26	-119.74	271	178	11.2	Farmland	Field	0-15	Wood
[12]Bera et al. 2019	29.40	-82.15	22	1299	20.7	Grassland	Lab Incubation	0-10	Wood
[13]Bhattachariya et al. 2016	29.00	79.30	244	1247	24.6	Farmland	Pot	0-15	Residue
[14]Bian et al. 2019	44.30	86.06	72	648	8.3	Farmland	Pot		Herb
[15]Chang et al. 2016	37.36	120.44	504	807	11.9	Farmland	Pot	0-20	Herb
[16]Chen et al. 2013	31.05	104.17	504	807	16.5	Farmland	Field	0-15	Herb
[17]Chen et al. 2016	31.05	104.17	38	1420	16.5	Farmland	Field	0-15	Herb
[18]Chen et al. 2017	30.25	119.72	28	1392	15.8	Farmland	Field	0-20	Wood
[19]Chen et al. 2019	28.72	112.87			17.1	Farmland	Field	0-15	Herb
[20]Chintala et al. 2014	44.21	-96.74	503	587	6.4	Farmland	Pot	0-15	Herb
[21]Cordovil et al. 2019	38.90	-9.42	106	692	15.7	Farmland	Lab Incubation	0-20	Wood
[22]Demisie et al. 2014	30.20	120.09	110	1333	16.5	Farmland	Pot	0-15	Wood

[23]Du et al. 2014	36.97	117.98	17	600	12.4	Farmland	Field	0-20	Herb
[24]Elzobair et al. 2016	42.52	-114.37	1190	251	9.4	Farmland	Field	0-30	Wood
[25]Espinosa et al. 2020	32.28	-110.94	713	305	20.7	Farmland	Field	0-5	Wood
[26]G. Gascó et al. 2016	40.12	-4.24	595	351	14.9	Farmland	Lab Incubation	0-20	Manure
[27]Gebhardt et al. 2017	31.82	-110.73	1504	559	15.6	Grassland	Pot	0-20	Wood
[28]Guo et al. 2020	30.23	119.70	49	1420	15.9	Forest	Pot	0-20	Residue
[29]Imparato et al. 2016	55.37	12.08	25	614	8.4	Farmland	Field	0-15	Herb
[30]Jegajeevagan et al. 2015	9.76	79.94	9	1221	28.1	Farmland	Lab Incubation	0-20	Residue
[31]Jenkins et al. 2017	50.98	-0.46	33	742	10.0	Farmland	Field	0-15	Herb
[32]Jin et al. 2019	28.62	116.43	26	1549	17.5	Farmland	Field	0–15	Herb
[33]Kaewpradit et al. 2019	16.21	102.82	217	1132	27.0	Farmland	Lab Incubation	0-15	Wood
[34]Karimi et al. 2020	31.31	48.66	18	209	27.3	Farmland	Lab Incubation	0-20	Residue
[35]Kumar et al. 2013	23.70	86.41	175	1171	26.0	Farmland	Pot	0-15	Herb
[36]Li et al. 2017	34.30	108.07	526	652	12.9	Farmland	Lab Incubation	0-20	Wood
[37]Li et al. 2018	30.23	119.70	150	1628	15.8	Forest	Field	0-20	Residue
[38]Li et al. 2020	30.23	119.70	150	1614	15.6	Farmland	Field	0-20	Herb
[39]Liang et al. 2014	22.68	112.90	48	1700	21.7	Farmland	Lab Incubation	0-10	Manure
[40]Liang et al. 2016	23.17	113.35	48	1700	21.7	Farmland	Lab Incubation	0-10	Residue
[41]Liang et al. 2014	40.14	116.18	50	400	11.6	Farmland	Lab Incubation	0-20	Residue
[42]Liao et al. 2016	44.30	86.03	469	175	8.3	Farmland	Lab Incubation	0-20	Herb
[43]Liu et al. 2017	23.08	114.38	105	1854	22.2	Open area	Pot	0-20	Residue
[44]Liu et al. 2016	43.45	82.90	823	194	7.5	Farmland	Pot	0-20	Wood
[45]Liu et al. 2020	30.23	119.70	49	1420	15.9	Forest	Pot	0-20	Herb
[46]Lu et al. 2020	52.52	119.69	604	1242	17.1	Farmland	Field	0-15	Herb
[47]Luo et al. 2016	22.50	114.03	0	2033	22.1	Wetland	Lab Incubation	0-2	Residue
[48]Ma et al. 2019	52.52	14.11	68	451	19.0	Farmland	Field	0-15	Wood

[49]Manasa et al. 2018	30.76	76.77	7	604	23.5	Farmland	Pot	0-15	Herb
[50]Masto et al. 2013	23.82	86.46	353	1008	32.3	Farmland	Field	0-15	Wood
[51]Ouyang et al. 2014	23.17	112.53	193	537	21.2	Forest	Lab Incubation	0-10	Manure
[52]Pandey et al. 2016	26.89	80.98	360	1774	25.1	Farmland	Pot	0-15	Wood
[53]Paz-Ferreiro et al. 2012	40.63	-4.10	113	900	12.2	Forest	Pot	0-10	Urban Waste
[54]Paz-Ferrerio et al. 2014	22.68	112.90	986	431	21.7	Forest	Pot	0-10	Wood
[55]Pei et al. 2020	30.90	120.31	48	1700	15.9	Farmland	Lab Incubation	0-20	Residue
[56]Pokharel et al. 2018	53.42	-113.55	49	1420	2.9	Grassland	Lab Incubation	0-10	Wood
[57]Rafael et al. 2020	-25.73	32.65	353	1008	23.0	Grassland	Lab Incubation	0-20	Residue
[58]Rafael et al. 2019	-25.73	32.65	37	782	23.0	Grassland	Lab Incubation	0-20	Residue
[59]Ren et al. 2020	22.22	107.78	1200	301	22.0	Forest	Field	0-20	Herb
[60]Sanchez-Hernandez et al. 2019	39.80	-4.13	489	1610	16.6	Open area	Lab Incubation	0-15	Residue
[61]Sekaran et al. 2019	44.21	-96.74	665	27	8.0	Farmland	Field	0-7.5	Residue
[62]Sial et al. 2019a	34.33	108.40	500	580	13.0	Farmland	Lab Incubation	0-20	Residue
[63]Sial et al. 2019b	34.33	108.40	516	630	13.0	Farmland	Lab Incubation	0-20	Residue
[64]Sial. et al. 2019c	34.33	108.40	516	630	13.0	Farmland	Lab Incubation	0-20	Residue
[65]Song et al. 2016	34.78	113.66	516	630	14.4	Farmland	Pot	0-20	Herb
[66]Song et al. 2018	34.78	113.66	100	640	14.4	Farmland	Pot	0-20	Herb
[67]Song et al. 2019	34.78	113.66	100	640	14.4	Farmland	Field	0-20	Herb
[68]Song et al. 2019	30.23	119.70	100	640	16.2	Forest	Field	0-20	Residue
[69]Song et al. 2020	26.33	116.79	150	1712	18.5	Farmland	Lab Incubation	0-40	Residue
[70]Song et al. 2020	34.78	113.66	384	1464	14.4	Farmland	Field	0-20	Herb
[71]Song et al. 2020	50.63	6.99	100	640	9.1	Farmland	Lab Incubation	45-75	Herb
[72]Song et al. 2020	43.81	125.32	173	748	5.5	Farmland	Lab Incubation	0-15	Herb
[73]Teutsherova et al. 2018	40.05	-3.52	221	582	14.9	Farmland	Pot	0-10	Residue

[74]Tian et al. 2016	26.73	115.05	476	153	18.0	Farmland	Field	0-20	Wood
[75]Ventura et al. 2014	44.55	11.59	597	387	13.0	Farmland	Field	20-40	Wood
[76]Walelign et al. 2015	30.21	120.09	32	700	16.5	Farmland	Lab Incubation	0-15	Wood
[77]Wang et al. 2015	34.78	113.66	1830	1316	14.5	Farmland	Lab Incubation	0-20	Herb
[78]Wang et al. 2018	37.69	112.75	123	1333	9.5	Farmland	Lab Incubation	0-20	Herb
[79]Wu et al. 2013	52.77	-111.68	100	613	2.7	Farmland	Lab Incubation	0-6	Herb
[80]Wu et al. 2018	37.12	38.82	813	411	18.0	Farmland	Lab Incubation	0-10	Herb
[81]Wu et al. 2019	30.70	103.85	670	393	16.3	Farmland	Pot	0-15	Herb
[82]Wu et al. 2020	30.77	111.33	467	439	16.5	Farmland	Lab Incubation	0-15	Herb
[83]Yang et al. 2018	33.35	120.16	536	964	14.6	Open area	Pot	0-20	Residue
[84]Yadav et al. 2019	26.80	80.90	97	1100	24.0	Farmland	Field	0-10	Herb
[85]Yang et al. 2020a	34.26	121.09	2	950	15.5	Farmland	Field	0-10	Herb
[86]Yang et al. 2020b	34.26	121.09	129	53	15.5	Farmland	Field	0-20	Herb
[87]Yi et al. 2019	22.26	112.83	23	1097	22.6	Farmland	Pot	0-15	Residue
[88]Yoo et al. 2012	36.82	127.11	7	2066	11.5	Farmland	Lab Incubation	0-10	Herb
[89]Yoo et al. 2015	37.20	126.83	51	961	11.6	Farmland	Lab Incubation	0-10	Herb
[90]Zhai et al. 2015	40.22	116.26	54	1283	11.2	Farmland	Lab Incubation	0-20	Herb
[91]Zhang et al. 2014	36.97	111.55	5	1265	9.8	Farmland	Field	0-20	Herb
[92]Zheng et al. 2016	31.05	104.17	44	511	16.5	Farmland	Field	0-15	Herb
[93]Zheng et al. 2019	40.80	123.55	146	483	7.5	Farmland	Field	0-20	Herb
[94]Zhou et al. 2020	36.07	112.10	695	36	11.0	Forest	Field	0-20	Residue
[95]Chang et al. 2016	34.27	112.70	598	1722	14.2	Farmland	Field	0-20	Wood
[96]Chen et al. 2015	34.26	108.07	1254	600	12.9	Farmland	Field	0-20	Wood
[97]Chen 2019	38.02	106.60	313	641	8.6	Grassland	Pot	0-10	Herb
[98]Duan 2020	24.75	109.85	1357	231	19.0	Forest	Field	20-30	Wood
[99]Gao et al. 2020	34.07	117.55	234	1875	14.8	Farmland	Field	0-15	Herb

[100]Guo 2018	46.83	127.04	36	830	3.0	Farmland	Field	0-20	Herb
[101]Han 2017	36.21	116.69	695	736	13.1	Forest	Field	0-20	Herb
[102]Hu et al. 2019	26.47	118.78	156	531	19.4	Forest	Lab Incubation	0-20	Wood
[103]Jia et al. 2016	36.45	113.25	295	670	9.5	Open area	Pot	0-20	Herb
[104]Lei 2016	27.05	118.15	486	1501	18.0	Forest	Pot	0-15	Wood
[105]Li 2016	33.25	112.90	970	533	15.0	Farmland	Field	0-30	Residue
[106]Li et al. 2016	33.27	113.03	457	1747	15.0	Farmland	Field	0-30	Herb
[107]Meng 2016	28.06	110.03	157	848	17.2	Farmland	Pot	5-20	Herb
[108]Meng et al. 2018	44.43	121.83	157	732	18.5	Farmland	Field	10-20	Herb
[109]Song 2014	34.60	112.42	471	619	13.6	Farmland	Pot	0-20	Residue
[110]Sun 2015	39.00	115.00	459	619	12.0	Farmland	Lab Incubation	0-20	Herb
[111]Wang et al. 2017	40.87	117.76	150	630	8.8	Farmland	Field	0-20	Herb
[112]Wang 2018	36.04	103.74	18	550	8.7	Farmland	Lab Incubation	0-20	Herb
[113]Wei 2019	30.13	116.63	1620	345	17.0	Farmland	Lab Incubation	0-20	Herb
[114]Wei & Hong 2019	36.47	113.02	1750	470	10.2	Farmland	Field	0-20	Herb
[115]Wu et al. 2018	34.14	113.81	889	599	703.5	Farmland	Pot	0-30	Residue
[116]Xu et al. 2019	30.25	120.30	17	1050	15.9	Farmland	Field	0-20	Herb
[117]Zhang 2013	45.70	126.64	82	15	4.7	Farmland	Pot	0-20	Herb
[118]Zhang et al. 2017	37.69	112.64	65	418	10.0	Farmland	Pot	0-20	Herb
[119]Zhao et al. 2015	24.31	103.40	65	759	21.2	Farmland	Field	30-40	Herb
[120]Zhao et al. 2016	34.26	108.07	65	418	13.0	Farmland	Field	0-20	Wood
[121]Zhao 2016	34.29	108.07	1432	1446	13.0	Farmland	Field	0-20	Wood
[122]Zhao 2018	40.94	122.06	452	621	10.5	Farmland	Pot	0-20	Herb
[123]Zheng et al. 2018	20.05	110.20	452	621	24.3	Open area	Lab Incubation	10-100	Residue
[124]Azeem et al. 2020	33.98	-117.33	523	264.00	17.60	Grassland	Field	0-15	Wood
[125]Chen et al. 2021	30.39	119.87	73	1553.00	17.00	Farmland	Field	0-20	Residue

[126]Ghosh et al. 2021	23.86	86.34	270	1215.00	25.40	Open area	Lab Incubation	0-15	Herb
[127]Halmi et al. 2021	3.05	102.05	349	1219.00	29.40	Open area	Lab Incubation	0-20	Residue
[128]Hou et al. 2021	43.51	124.81	187	550.00	4.50	Farmland	Field	0-20	Herb
[129]Sial et al. 2022	34.33	108.40	420	627.00	13.10	Farmland	Lab Incubation	0-20	Residue
[130]Zhang et al. 2021	29.93	118.85	150	15.90	1450.00	Forest	Lab Incubation	0-20	Residue

TABLE S2. Overview of cellulases and ligninases included in this meta-analysis.

Extracellular enzyme	EC[1]	Class	Type	Target
β-1,4-Glucosidase (BG)	3.2.1.21	Cellulase	C-targeting hydrolysis	Cellulose
α-1,4-glucosidase (AG)	3.2.1.20	Cellulase	C-targeting hydrolysis	Cellulose
β-1,4-Xylosidase (BX)	3.2.1.37	Cellulase	C-targeting hydrolysis	Hemicellulose
β-D-Cellobiosidase (CBH)	3.2.1.91	Cellulase	C-targeting hydrolysis	Cellulose
Phenol oxidase (PO)	1.10.3.2	Ligninase	Recalcitrant C oxidation	Lignin and other complex compounds
Polyphenol oxidase (PPO)	1.14.18.1	Ligninase	Recalcitrant C oxidation	Lignin and other complex compounds
Peroxidase (PER)	1.11.1.7	Ligninase	Recalcitrant C oxidation	Lignin and other complex compounds

[1] EC: Enzyme Commission Numbers; abbrevations used in manuscript for each enzyme are shown in parentheses.

SUPPLEMENTARY NOTE 1 Soil carbon-degrading extracellular enzymes assays.

Multiple methods have been used to measure enzyme activities based on assessments of substrate concentrations or products over time at certain temperatures[1-3]. Most studies measured soil cellulase activity using fluorimetric methods with fluorescent 4-methylumbelliferone substrates[1,4], and assessed soil ligninase activity by colorimetric methods using L-3,4-dihydroxy-phenylalanine as substrate[2]. We acknowledge that methods and incubation conditions often varied among studies. However, we only consider the logarithmic response ratio of enzymes in each individual study, in which experiment condition such as the type and concentration of substrates, buffer pH, incubation temperature and time etc. were the same for each paired observations. Therefore, differences in measurement methods should have minimum influence on the biochar effect on enzyme activities in this meta-analysis[5-6].

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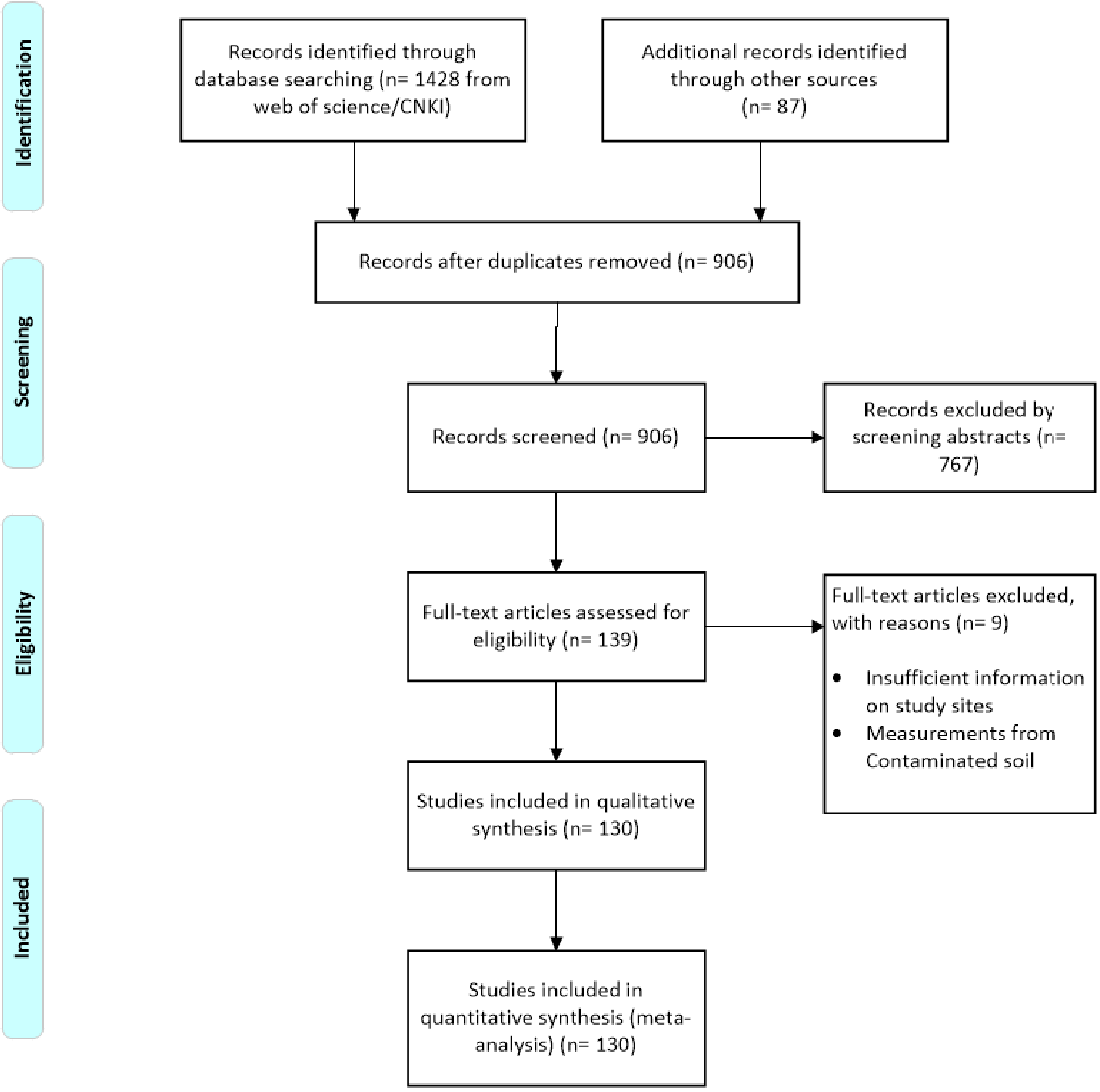

FIGURE S1 PRISMA flowchart illustrating the processes for the selection of articles included for the meta-analysis.

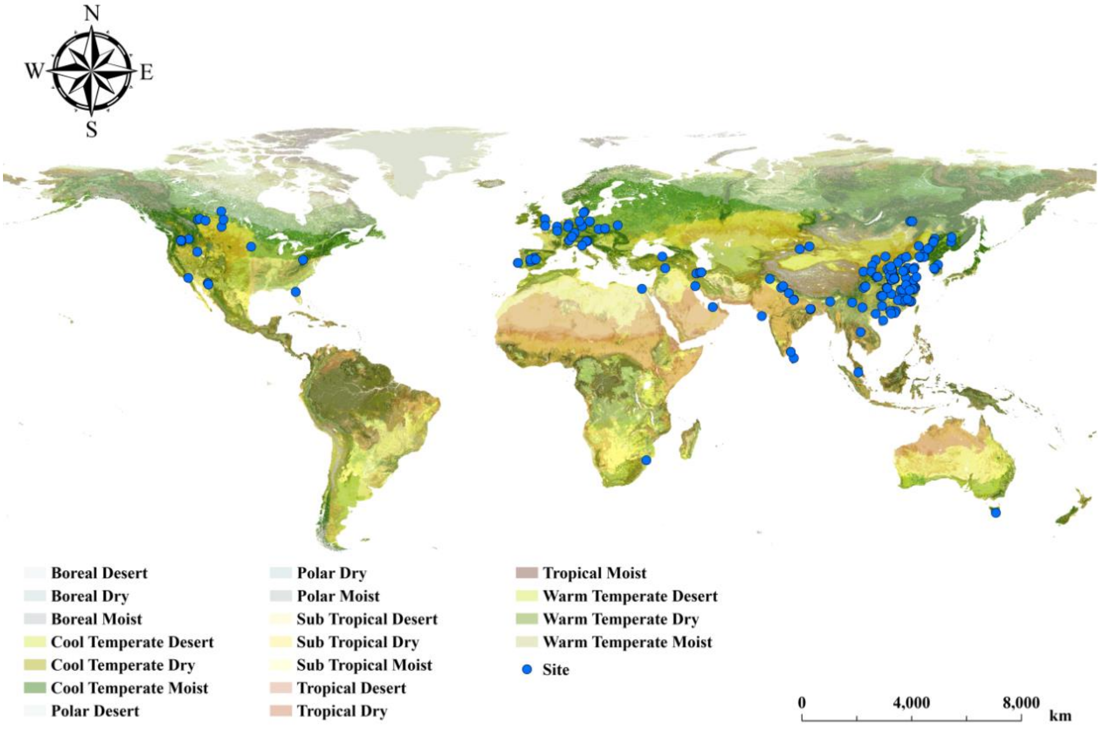

FIGURE S2 Global distribution of the biochar addition experiments selected for this meta-analysis.

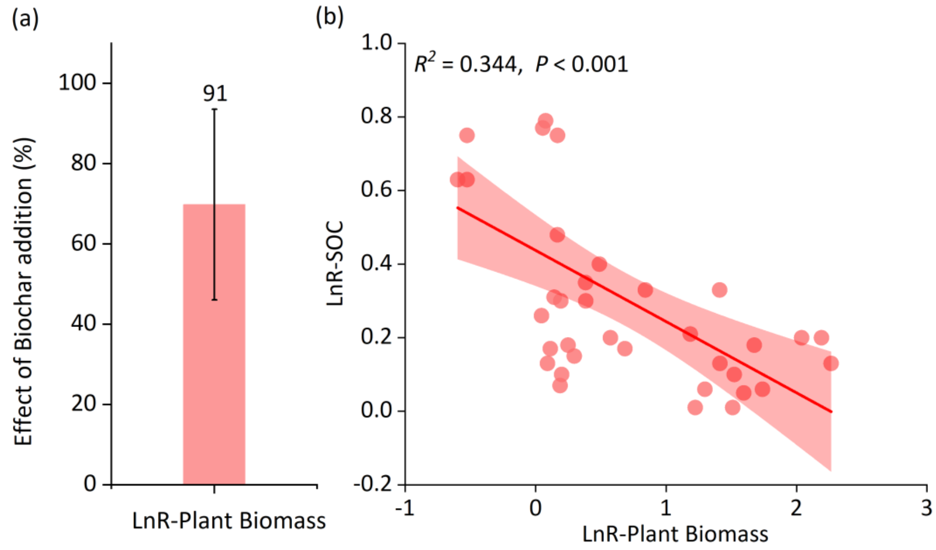

FIGURE S3 (a) Effects of biochar addition on plant biomass. (b) Relationship between the response of soil organic C (SOC) and plant biomass to biochar addition.

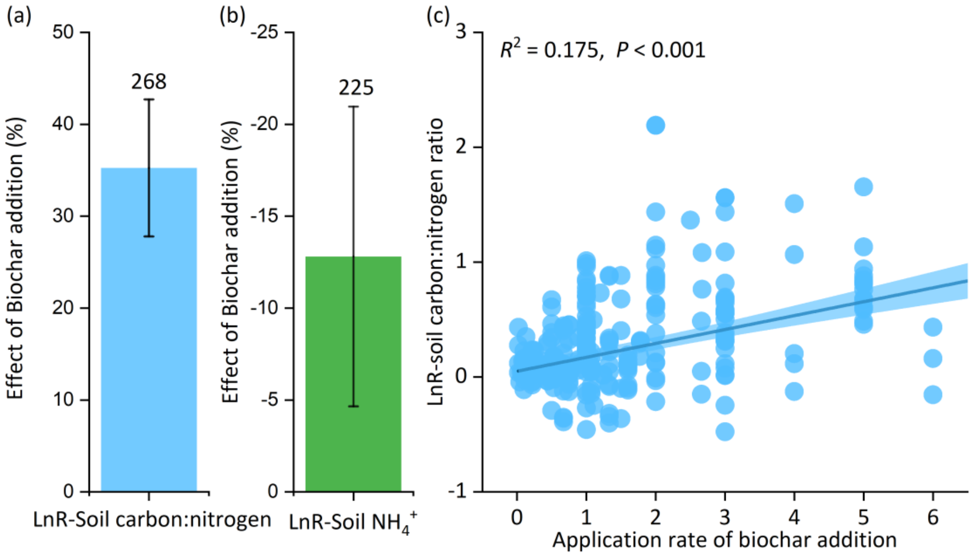

FIGURE S4 (a) Effects of biochar addition on soil carbon:nitrogen. (b) Effects of biochar addition on soil NH_4^+. (c) Relationship between biochar-induced changes in carbon:nitrogen and the application rate of biochar addition.

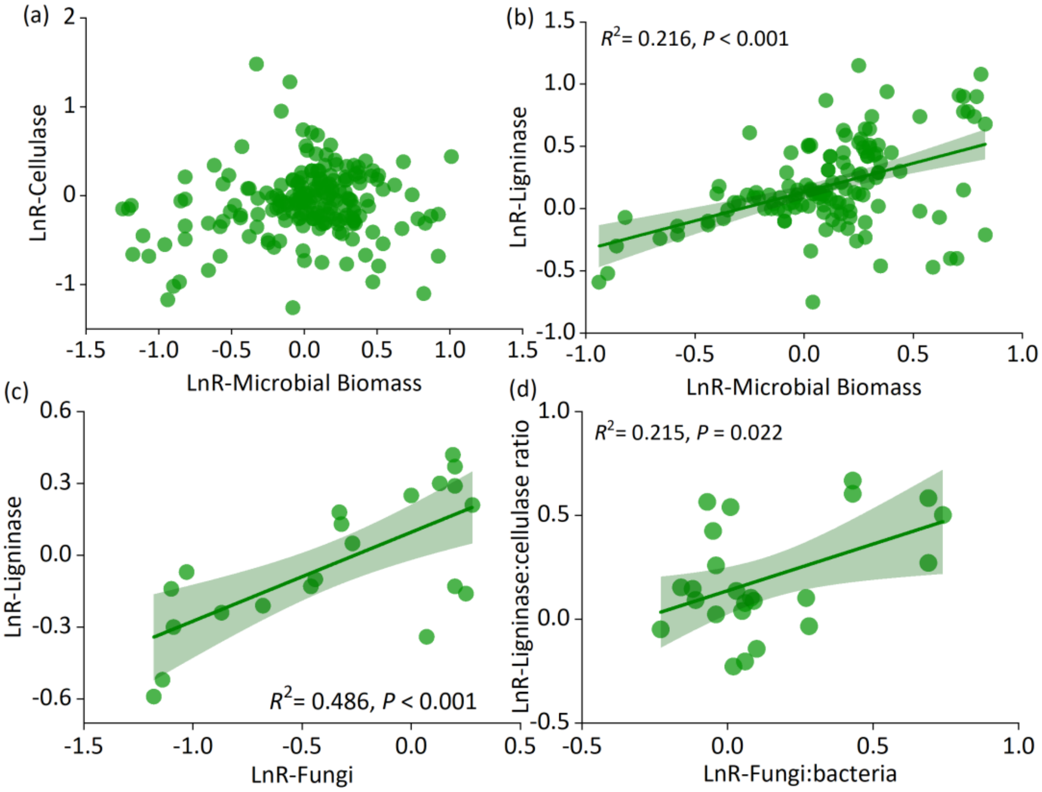

FIGURE S5 Relationships between the responses of cellulase, ligninase and ligninase:cellulase with microbial attributes after biochar addition. (a) Soil microbial biomass vs. soil cellulase. (b) Soil microbial biomass vs. soil ligninase. (c) Soil fungi abundance vs. ligninase. (d) Soil fungi:bacteria vs. ligninase:cellulase.

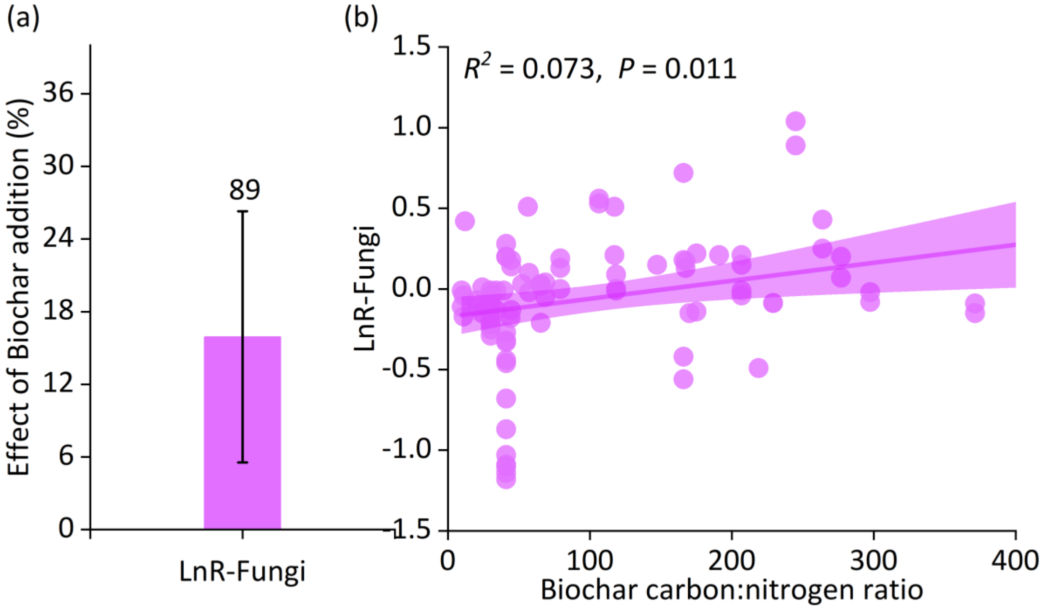

FIGURE S**6** (a) Effects of biochar addition on fungi abundance. (b) Relationship between the response of fungi abundance and biochar carbon:nitrogen after biochar addition.

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