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Global *meta*-analysis reveals positive effects of biochar on soil microbial diversity

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ABSTRACT

Biochar has gained global attention due to its potential for climate change mitigation and soil quality improvement. Yet, the consequences of biochar additions for soil microbes -the major biotic drivers of soil function- remain unknown across global environmental gradients. We aimed to explore the responses of soil bacterial communities to biochar addition, and further investigate how biochar and soil properties impact these responses. We conducted a global *meta*-analysis and found that, in general, biochar has a limited impact on the proportion of major bacterial phyla, with only *Acidobacteria* and *Gemmatimonadetes* being largely impacted: the relative abundance of *Acidobacteria* decreased by 14.6%, while that of *Gemmatimonadetes* increased by 19.8%. Also, the experimental type played a role in shaping the response of microbial community to biochar application. In addition, biochar significantly promoted the diversity of soil bacteria, i.e., genetic richness and diversity. These changes were significantly associated with biochar load, C/N ratio, pyrolysis temperature, biochar pH, as well as soil C/N ratio and pH. We further found that the impacts of biochar on functional diversity, i.e., C substrate richness consumed by soil microbes increased with the biochar load, which might relate to increased genetic richness. Our work suggests that selecting key biochar properties can improve soil quality, microbial function, and climate change mitigation while maintaining the positive impacts of biochar on soil microbial diversity. Further research is needed to link the response of soil microbial composition at the genus level to biochar addition, with microbial functions.

1. Introduction

Carbon sequestration in terrestrial ecosystems is critical as a strategy to mitigate climate change impacts worldwide (Pörtner, Roberts et al. 2022). One of the most investigated approaches to capturing carbon in terrestrial environments is the use of biochar, which has the potential to deliver emission reductions of 3.4–6.3 $PgCO_2e$ (Lehmann, Cowie et al. 2021). As a soil amendment, the application of biochar is also expected to promote soil fertility and crop production, generate renewable energy, and reduce organic agricultural waste (El-Naggar, Lee et al. 2019, Ye, Camps-Arbestain et al. 2020, Schmidt, Kammann et al. 2021, Xia, Cao et al. 2023). Much less is known, however, on how biochar additions impact soil biodiversity, especially that of microbial communities, the major biotic drivers of soil function (Chen, Jin et al. 2022, Singh, Northup et al. 2022). This knowledge is critical to support the application of biochar while maintaining sustainable ecosystems and helping to preserve soil biodiversity.

Despite their global importance, how and why biochar influences soil microorganisms is poorly understood. The current lack of knowledge exists for two main reasons. Most previous work has been done at the local scale, while much less is known about the impacts of biochar on soil microbes across global environmental gradients (Gao, DeLuca et al. 2019). Several local-scale studies have claimed that the application of biochar improves the diversity of soil bacteria (e.g., genetic variation, diversity or richness, and community composition of bacteria), while also increasing microbial biomass (Bamminger, Poll et al. 2016).

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However, neutral, and even negative influences of biochar on diversity, biomass, C metabolism, and functional diversity have also been reported (Kelly, Peltz et al. 2014, Abujabhah, Doyle et al. 2019). Second, the influence of various biochar properties and loads on bacterial diversity is still poorly understood.

The major constituents of biochar include polycondensed organic C, and mineral fraction (ashes) as biochar is produced by the thermal decomposition of organic material, such as wood or manure, under a limited supply of oxygen (O_2), and at relatively low temperatures (<700 °C) (Antal and Grønli 2003). Due to different materials and production conditions, the key properties of biochar, such as C:N ratios, often differ between studies, which can impact soil microbial communities, and therefore might shape soil microbial structure differently (Kolton, Graber et al. 2017, Li, Wang et al. 2020). However, how the wide range of biochar properties affects microbial structure and biodiversity across various soil conditions is unclear. Assessing the impacts of biochar on soil microbial diversity is critical to understanding soil health, productivity, and climate change mitigation in response to global-scale extended biochar practices.

The investigation of the genetic composition of soil microorganisms has greatly advanced recently with the development of next-generation sequencing (NGS). NGS refers to high-throughput, non-parallel DNA sequencing technologies following the Sanger DNA sequencing technique, which circumvents the requirement for the creation of time-consuming and costly amplification libraries (Egan, Schlueter et al. 2012, Yang, Xie et al. 2014). A growing body of research has investigated the effects of biochar on soil bacterial communities based on NGS, through which some studies have found biochar to increase the diversity of bacteria and modify the composition of rhizospheric microbiota (De Tender, Debode et al. 2016, Han, Xu et al. 2022). One study showed that one year after biochar application, *Gemmatimonadete* and *Acidobacteria* were found to be enriched in the biochar treatment plots (Jenkins, Viger et al. 2017). Another study found that biochar increased the abundance of *Actinobacteria, Proteobacteria,* and *Planctomycetes*; however, these increases were dependent on the type of biochar employed (Ali, Khan et al. 2019). Furthermore, several studies have suggested that the relative abundance of *Acidobacteria*, *Chloroflexi*, and *Gemmatimonatestes* decreased under biochar treatments (Xu, Tan et al. 2016, Qiu, Yinghua et al. 2020). Aside from the biochar type, the pyrolysis temperature and soil C concentration can also have variable influences. For example, *Chloroflexi* species have been shown to be more prevalent when high pyrolysis biochar is applied to low-C soil (Dai, Barberan et al. 2017).

The number of *meta*-analysis studies focused on biochar has been increasing recently, however, according to our literature review across 196 *meta*-analysis studies, only 24 such studies have been directly related to soil microorganisms (Table 1, Text S1, Appendix S1-2). Most of these studies have focused on microbial biomass C and N (Pokharel, Ma et al. 2021, Chagas, de Figueiredo et al. 2022), microbial activities (Chen, Jin et al. 2022, Blanchy, Bragato et al. 2023), and greenhouse gas emissions (Lyu, Zhang et al. 2022). Only a few *meta*-analyses have focused on soil microbial structure and diversity (Li, Wang et al. 2020, Xu, Whitman et al. 2021), and to date, none of these have included NGS data in the *meta*-analysis. Here, we conducted a global *meta*-analysis to synthesize scientific evidence of the responses of soil microbial communities to biochar additions and help to disentangle potential impacts of biochar on soil microbes via incorporating NGS data. We aimed to investigate how biochar loads and properties affect soil microbial structure directly and indirectly across different soil properties and experiment types. Such a *meta*-analysis might provide new information regarding the underlying mechanisms through which biochar influences soil microbes and regulates bacterial-mediated functions, which may have implications for the future direction of biochar research.

Table 1
Main attributes focused on in 24 *meta*-analysis studies related to the effects of biochar application on soil microorganism.

No.	Paper source	Attributes in the *meta*-analysis studies
1	Blanchy, Bragato et al. (2023)	soil organic matter content, soil nutrient availability, soil water-holding capacity, soil pH, microbial activity, plant growth and development, greenhouse gas emissions, carbon sequestration, and soil structure.
2	Atilano-Camino, Laborin et al. (2022)*	GHG emissions, soil metal availability, microbial activity, CO_2 emissions, N_2O emissions, soil metal concentration.
3	Chagas, de Figueiredo et al. (2022)	Total C, organic C, microbial biomass C, labile C, fulvic acid.
4	Chen, Jin et al. (2022)	Soil microbial enzyme activities for C, N and P acquisition, enzyme ratios (EC:EP, EN:EP), microbial metabolic limitation (C, N and P), and the strength of limitation caused by different types of biochar (wood and crop residue).
5	Lyu, Zhang et al. (2022)*	pH, feedstock type, pyrolysis temperature, biochar application rate, C/N ratio, microbial community, CO_2 emissions, CH4 emissions, N2O emissions, abundance of functional genes (mcrA, pmoA, amoA, nirS, nirK, nosZ)
6	Singh, Northup et al. (2022)*	Soil pH, cation exchange capacity, organic carbon, bulk density, porosity, microbial diversity, crop productivity
7	Zhang, Jing et al. (2021)	Soil total N, inorganic N, microbial biomass N, biological N-2 fixation, nitrous oxide emission, crop productivity, plant N uptake.
8	Pokharel, Ma et al. (2021)	Soil microbial biomass C and nitrogen (N), enzyme activities (urease, alkaline phosphatase, dehydrogenase, and others), soil pH, soil texture, pyrolysis temperature, and C/N ratio, crop productivity, greenhouse gas emissions
9	Schmidt, Kammann et al. (2021)	yield, root biomass, water use efficiency, microbial activity, soil organic carbon, greenhouse gas emissions.
10	Siedt, Schaffer et al. (2021)*	sorption capacity, physical and chemical soil characteristics, soil microbial communities, transformation and retention of nutrients and pesticides.
11	Xu, Whitman et al. (2021)*	arbuscular mycorrhizal fungal abundance, microbial biomass C, functional richness, soil respiration, actinomycetes (ACT) abundance, bacterial abundance, fungal abundance, C metabolic ability, soil pH, and soil microbes feeding on miscellaneous C.
12	Zhang, Jing et al. (2021)*	NH4 + and NO3– content, N mineralization, nitrification, N-2 fixation, plant N uptake, N2O emissions, N leaching, abundance of soil denitrifying/nitrifying genes (amoA, narG, nirS/nirK + S, and nosZ), proportion of N-2 fixation bacteria, and N-acetyl-glucosaminidase activity.
13	Li, Wang et al. (2020)*	Microbial biomass, diversity.
14	Pokharel, Ma et al. (2021)	Soil microbial biomass C, urease activity, alkaline phosphatase activity, dehydrogenase activity.
15	Gao, DeLuca et al. (2019)	Available phosphorus, microbial biomass phosphorus, inorganic nitrogen (NO3–-N and NH4 + -N).
16	Xiao, Rasmann et al. (2019)	MFG abundance and richness, N-cycling gene abundance (including nifH, amoA, nirK, nirS and nosZ), abundance of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB), soil pH, biochar pyrolysis temperature, fertilizer application, and cover plants.
17	Zhang, Xiang et al. (2019)	Ratios of soil fungi to bacteria (F/B), Ratios of Gram-positive bacteria to Gram-negative bacteria (G+/G-), Microbial biomass and activities, Total phospholipid fatty acids, Actinomycete activity, Soil nutrient cycling, Carbon sequestration Crop yields
18	Zhou, Zhou et al. (2017)	Soil respiration, autotrophic respiration, heterotrophic respiration, microbial biomass C, dissolved organic C, and temperature sensitivity (Q10) of respiration.

(*continued on next page*)

Table 1 (*continued*)

No.	Paper source	Attributes in the *meta*-analysis studies
19	Zhou, Zhang et al. (2017)	Soil microbial biomass carbon (SMBC), soil microbial biomass nitrogen (SMBN), soil respiration quotient (RQ), microbial quotient (MQ), metabolic quotient (qCO(2)) and total soil CO2 production have been affected by biochar. The effects vary depending on the type of biochar, soil texture, soil reaction, and soil organic carbon (SOC) level.
20	Liu, Zhang et al. (2016)	Soil organic C content, soil microbial biomass C content, and soil CO2 fluxes were the attributes affected by biochar. Other factors that affected the response to biochar amendment include land-use type, soil texture and pH, biochar characteristics (application rate, pyrolysis temperature, C/N ratio, and pH), and agricultural practices (use of synthetic N fertilizer and waste compost fertilizer).
21	Novak, Ippolito et al. (2016)	pH, surface area, nutrient concentration, porosity, metal binding capacity, soil health characteristics, carbon sequestration, nutrient retention, water retention, binding of enteric microbes, enhancement of metal binding in mining-impacted soils.
22	Wang, Xiong et al. (2016)	Bioavailable portion of biochar, long-term C sequestration, labile and recalcitrant biochar C pools, priming effects on soil organic matter mineralization, feedstock, pyrolysis temperature, soil clay content, microbial activities, soil fertility.
23	Gul, Whalen et al. (2015)*	pH, cation exchange capacity, aggregation, microbial abundance, community structure of microorganisms.
24	Biederman and Harpole (2013)	Aboveground productivity, crop yield, soil microbial biomass, rhizobia nodulation, plant K tissue concentration, soil phosphorus (P), soil potassium (K), total soil nitrogen (N), total soil carbon (C), and soil pH.

Note: "*" represent the studies directly related to microbial structure and diversity.

2. Materials and methods

2.1. Literature collection

We collected literature from the Web of Science, Google Scholar, and the China National Knowledge Infrastructure (CNKI). The keywords we used to search references were [biochar application] OR [biochar addition] OR [biochar treatment] AND [soil microbes] OR [high throughput sequencing] OR [PLFA], OR [Biolog Eco plate], refers to Fig. S1 to see more details. The literature selected for this *meta*-analysis included journal papers or academic theses that described the biochar experiments with at least three replicates for each treatment. The experiment design should be in a randomized complete block design including both control and treatment groups. The control group should be in the same environmental conditions and management as the biochar treatment group but without biochar. We included field, pot, and incubation experiment types in our study. The biochar concentrations were converted to percentages of the soil weight in the top layer to simplify the presentation of results, particularly when the application rates were given as a weight per area (e.g., ton ha^{-1}, kg m^{-2}) based on a previous study (Biederman and Harpole 2013). For example, if applying 20 ton ha^{-1} to soils with a bulk density of 1.2 g cm^{-3}, and the depth is assumed 10 cm (the incorporation was provided in each study), and the weight/cm^3 of biochar can be calculated as $20/(100*10) = 0.02$ g/cm^{-3}, then the application rate can be converted to the percentage of weight: 0.02/1.2≈1.67%.

Data sources were extracted mainly from the descriptions, methods and materials, tables, figures, and appendices of research papers. We employed digitizer software -Engauge Digitizer- to extract data (https://digitizer.sourceforge.net) when they were graphically presented. In those cases where data were missing or were not presented in the articles, the data were obtained directly from the corresponding authors. The climate data of the soil sample locations used in this *meta*-analysis included the mean annual temperature (MAT, °C) and precipitation (MAP, mm yr^{-1}), which were directly derived from cited papers, or the database at https://www.worldclim.org/ using their coordinate data via R coding (Zhang, Chen et al. 2018). MAT and MAP were only applied to data from field experiments.

We collected the author's information, experiment location, and coordinates as background data. Meanwhile, we extracted the soil properties (C/N ratios, pH values), biochar application rates (% of soil weight), chemical properties (C/N ratios, pH values), and production data (material and pyrolysis temperature) as tentative explanatory variables. The response variables we included in the *meta*-analysis encompassed three components based on their determining techniques (Fig S2): 1) from the NGS data, the variables include a richness index represented by the number of operational taxonomic units (OTUs, an operational definition used to classify groups that clustered by DNA sequence similarity), two diversity indices (Chao1 and Shannon index), and the relative abundance of different bacterial phyla; 2) from the PLFA experiments, the variables were soil bacterial biomass, G + and G- bacteria, and actinomycete abundance; 3) from the Biolog Eco-plate experiments, the variables were average well color development (AWCD) as well as C metabolism diversity and richness. Among these parameters, the OTUs, Chao1, and Shannon index reflected the bacterial community richness and diversity based on specific regions of bacterial RNA or DNA sequencing data. The AWCD reflected the activity and capacity for C metabolism by bacteria, while the C utilization diversity and richness reflected the types of C sources that were well utilized.

Overall, we obtained 2588 paired observations from 136 published papers across 165 study sites worldwide with three experiment types (Appendix S1, Fig. 1). The soil C/N ratio values primarily ranged from 5 to 20, while the biochar C/N ratios ranged from 20 to 300. The soil pH ranged mostly from 4 to 8, while the biochar pH ranged from 8 to 11 (Fig. S2). The study sites were primarily located in China, Europe, North America, and Australia.

2.2. Meta-analysis

To compare the differences between the biochar treatments and control groups across different studies, a natural log response ratio (ln*RR*) (Hedges, Gurevitch et al. 1999) was employed to assess the responses of soil bacteria attributes. The metric to express effect size was $lnRR = ln(X_t/X_c)$, where X_t and X_c are the mean values of the selected bacterial attributes of the biochar applications and control groups, respectively. There are two advantages to using ln*RR* as a dependent variable to express effect size: 1) ln*RR* linearizes the metric and 2) and the distribution of ln*RR* is much more normal in small samples (Hedges, Gurevitch et al. 1999).

Similar to previous studies (Pittelkow, Liang et al. 2015, Kielak, Barreto et al. 2016), we weighted each ln*RR* observation by the replications of each study as follows: $W_t = (N_c \times N_t)/(N_c + N_t)$, where W_t is the weight associated with each ln*RR* observation, while N_c and N_t are the numbers of replications for the controls and treatments, respectively.

2.3. Statistical models and redundancy analysis

To determine the responses of bacterial communities to applied biochar loads and experiment types, we developed mixed linear models while considering the studies as random effects. Therefore, the basic model was $\ln RR = \beta_0 + \beta_1 \cdot Bio\_load + \beta_2 \cdot Etype + \pi_{study} + \varepsilon$, where ln*RR* is the response variable consisting of various bacterial attributes. "Bio_load" is the percentage of biochar applied, and "Etype" is the experiment type of each study. β_{0-2} represents the coefficient of the intercept and independent variables, while π_{study} represents the random

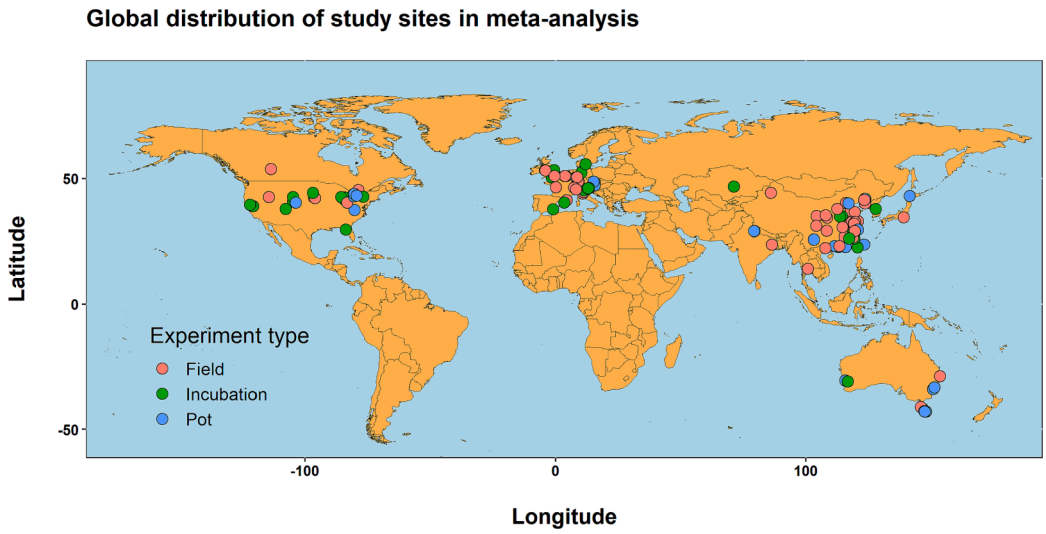

Fig. 1. Global distribution map of the 165 study sites used in the *meta*-analysis and different experiment types across all study sites.

effects of the studies, and ε represents the residue term. Standardizing the variables by scale() function was used to reduce variations and optimize the models. Thus, we developed a basic model as follows:

$$\ln RR = \beta_0 + \beta_1 \cdot scale(Bio\_load) + \beta_2 \cdot Etype + \pi_{study} + \varepsilon \quad (1)$$

Maximum likelihood estimation was performed using the *lme4* package (Bates, Mächler et al. 2014). To understand how application duration, the MAT and MAP of the soil sample location, soil C/N, pH, and biochar pH affected the responses of soil bacteria to biochar, we added each of these as dependent variables to replace the term "Etype" in equation (1). To simplify the interpretation of the graphic result, the ln*RR* values were transformed back to a percentage of the change via the equation $\left(e^{\ln RR} - 1\right) \times 100\%$.

3. Results

3.1. The effect of biochar on key bacterial community attributes

The results showed the relative abundance of *Acidobacteria* decreased by 14.6% ($p < 0.01$), on average, in response to the application of biochar, while that of *Gemmatimonadetes* increased significantly by 19.8% ($p < 0.01$)) (Fig. 2a). Other phyla were not altered to a significant degree with the application of biochar, whereas the Chao1 and richness of OTUs increased by 7.9% and 4.1% ($p < 0.05$), respectively. The G + biomass increased with biochar by10.7% ($p < 0.01$)) (Fig. 2b).

3.2. The effect of experiment type on soil microbial responses to biochar addition

We further investigated how experiment types affected the effectiveness of biochar on soil microbes (Fig. 3). The result showed ln*RR* of *Gemmatimonadetes* was greater in incubation experiment than in field and pot experiment ($p < 0.05$). For the richness of OTUs, the ln*RR* value was significantly lower in incubation than in field experiments, but there were no significant differences between field and pot experiments. The ln*RR* of *Verrucomicrobia* was the largest in the incubation experiment, while there was no difference between experiments in the field and pot.

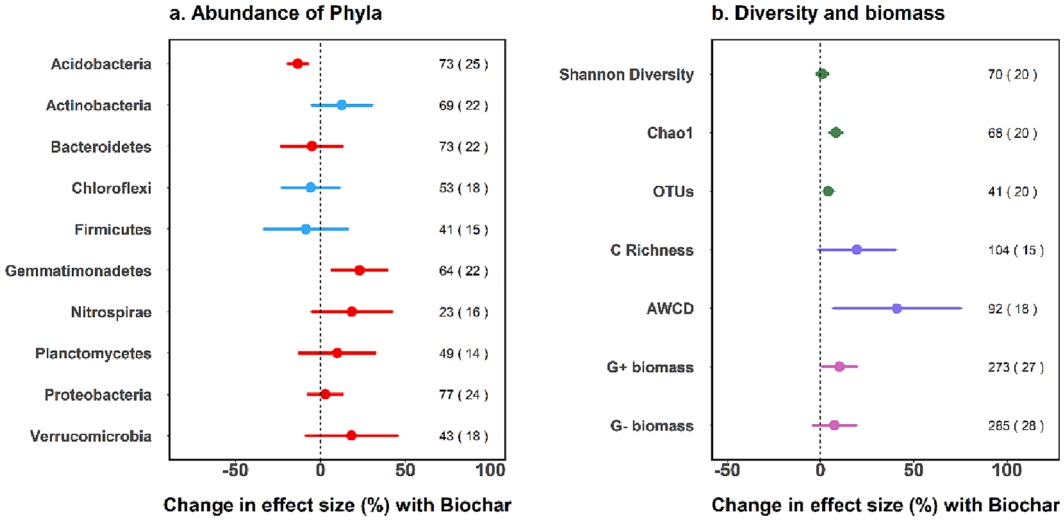

Fig. 2. Changes in effect size (%) with biochar application on (a) abundance of bacterial phyla, and (b) diversity biomass. Values are mean ± 95% confidence intervals of the percentage of effects between the biochar and control treatments. The red color in panel a represents G- phyla, the blue represents either G + bacteria phyla or mixed phyla containing both G + and G- classes, and the green represents diversity-related attributes. The dark blue represents attributes related to C metabolism, while G + and G- biomass were represented by the purple dots. The numbers of the paired comparisons were listed beside each attribute with the number of studies in the bracket. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

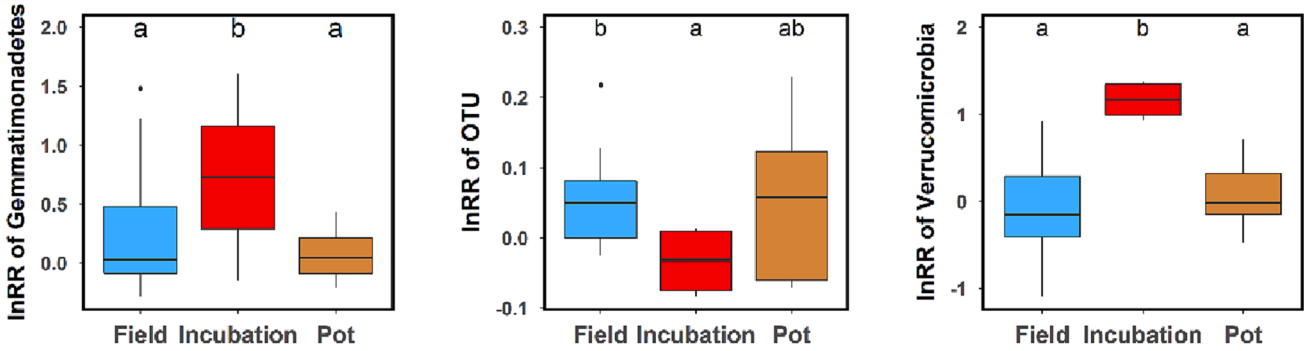

Fig. 3. Natural log response ratio (ln*RR*) of (a) *Gemmatimonadetes*, (b) OTUs and (c) *Verrucomicrobia* to different experiment types - field (blue), incubation (red), and pot (brown). Values are mean ± 95% confidence intervals of the percentages of change between the biochar and control treatments. T-tests for each paired comparison at a confidence level of p = 0.05 was conducted across the attributes, and the different letters above each bar show a significant difference between treatments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. The effect of biochar properties on soil microbial responses to biochar addition

Overall, the ln*RR* of *Acidobacteria* decreased with biochar load ($p < 0.05$), while C richness consumed by microbes increased considerably with biochar loads (both $p < 0.01$) (Fig. 4b 4). From the result of the spearman correlation heatmap, besides *Gemmatimonadetes,* soil C/N was also correlated with *Proteobacteria* and *Acidobacteria*. Biochar C/N was related to the ln*RR* of *Bacteroidetes*, and *Planctomycetes*. Biochar load was related to half of the dominant bacterial phyla (Fig. 4c). The ln*RR* of *Gemmatimonadetes* increased with soil C/N ($p < 0.01$), while the ln*RR* of the Chao1 index increased with soil pH (Fig. S3). The effect of biochar application duration on microbial attributes was also tested and only *Actinobacteria* was significantly affected (Table S1). For field experiment, there was no significant effect of MAT and MAP (Table S1).

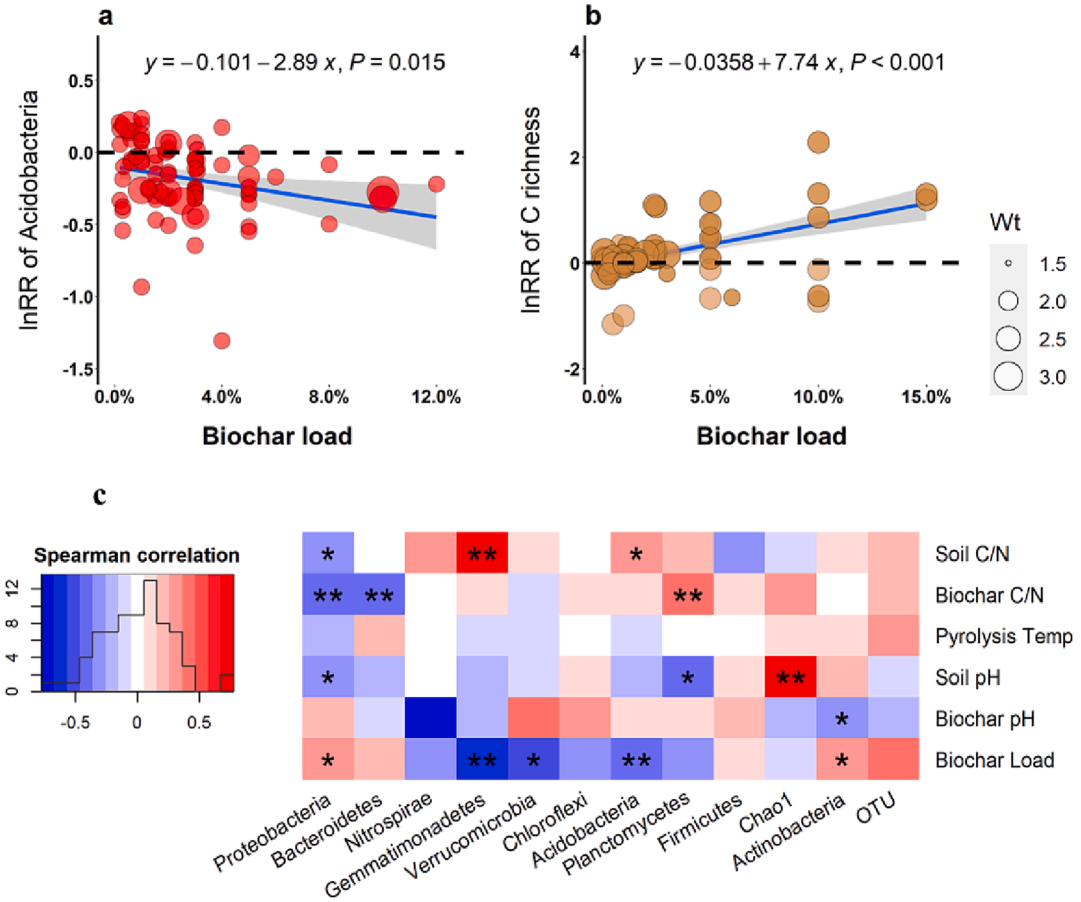

Fig. 4. Natural log response ratio (ln*RR*) of microbial attributes (in red) as a function of biochar load (a), and ln*RR* of diversity attributes (in brown) as a function of biocharload (b). Fitted regression lines and their 95% confidence intervals (shaded) are presented. The size of the dots represents the relative weights by the number of replications of corresponding observations. The Spearman correlation heatmap shows the relationship between microbial attributes and biochar, and soil properties with significant levels (c). Colors from dark blue to red represent negative to positive correlations, respectively. The darker colors represent stronger correlations between the two variables. The boxes with "*" stand for $p < 0.05$, while that with "**" stands for $p < 0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

4.1. The effect of biochar on key bacterial community attributes

The application of biochar is expected to help support long-term carbon sequestration, however, its impact on soil microbial communities is still unclear, especially across global environmental conditions and in response to contrasting biochar properties (Li, Wang et al. 2020, Pokharel, Ma et al. 2020, Zhang, Jing et al. 2021). We show that biochar has a limited impact on most investigated microbial phyla, with only *Acidobacteria* and *Gemmatimonadete* being largely impacted. Such changes were associated with biochar properties and their impacts on soil conditions suggesting that the impact of biochar on soil microbial diversity can be managed by its properties.

The relative abundance of *Acidobacteria* was found to decrease with the addition of biochar. Many species of *Acidobacteria* are acidophilic (Eichorst, Kuske et al. 2011), which means that they thrive under highly acidic conditions; however, biochar is typically alkaline, due to base cations (e.g. Ca and Mg) that accumulate on its surface during pyrolysis (Mitchell, Simpson et al. 2015). Thus, our analysis suggested biochar was detrimental to the growth of many *Acidobacteria*. Since *Acidobacteria* actively participate in key carbon, nitrogen, and sulfur biogeochemical cycling through decomposition of biopolymers, exopolysaccharide secretion, etc., (Kalam, Basu et al. 2020), the application of biochar could affect soil functioning due to microbial composition change.

We found a general increase in *Gemmatimonadetes* in response to biochar, which is consistent with previous local-scale studies (Xu, Wang et al. 2014, Whitman, Pepe-Ranney et al. 2016). This increase may be related to the higher abundance of the two classes within the *Gemmatimonadetes* phylum - *Gemm-1* and *Gmm-3* classes (t) - under the application of biochar (Whitman, Pepe-Ranney et al. 2016). Moreover, *Gemmatimonadetes* generally are found to be more abundant in dryer soils (Fawaz 2013), and biochar appeared to enhance this phylum in clay soils, potentially due to the positive effect of biochar on macroporosity and water permeability. The positive effect of biochar on *Gemmatimonadetes* abundance may positively enhance rhizosphere functioning, as *Gemmatimonadetes* are associated with plants and the rhizosphere (Mujakić, Piwosz et al. 2022).

We further show that biochar increased the diversity of bacterial communities (OTUs richness and Chao1), which might be explained by the C sources and ash inputs from the biochar, as novel C sources might provide substrates for different bacterial phyla or species (Luo, Durenkamp et al. 2013, Mitchell, Simpson et al. 2015, Wang, Fonte et al. 2017, Xu, Wang et al. 2018). In addition, although biochar constitutes a small portion of ash, it contains mineral elements including Ca, Mg, K, Na, Si, P, S, Fe, Al, and trace elements, etc., contingent on the raw materials, which might provide energy sources (e.g., new redox couples) and nutrition for a greater variety of bacteria (Mitchell, Simpson et al. 2016, Cole, Zandvakili et al. 2019). While Chao1 and OTU richness increased in response to biochar, the Shannon diversity did not change. This discrepancy might be explained by the evenness of different bacterial taxonomic groups. The Shannon diversity reflects both taxonomic richness and evenness, while Chao1 and OTU index is more influenced by richness. Thus, biochar may increase bacterial populations, while the relative dominant phyla or classes of bacteria might remain unchanged.

In addition, the G + bacterial biomass increased under biochar treatments, which was consistent with other studies showing that the increased bacterial biomass might be accounted for by the introduction of labile or extractable C via biochar (Luo, Durenkamp et al. 2013, Xu, Wang et al. 2018). Furthermore, biochar can provide microbes with suitable habitats for growth and protection; thus, promoting their biomass (Zhang, Dijkstra et al. 2014).

4.2. The effect of experiment type on soil microbial responses to biochar addition

The observed differences in the relative abundance of *Gemmatimonadetes*, *Verrucomicrobia,* and OTUs across different experiment types suggested that the experimental conditions may play a role in shaping the microbial community response to biochar application (Zhang, Liu et al. 2016). Our finding that biochar increased the relative abundance of *Gemmatimonadetes* and *Verrucomicrobia* mainly in incubation experiments might be because incubation experiments provide a more controlled environment with less variation in soil physicochemical properties, which may favor the growth of some dominant phyla, such as *Gemmatimonadetes* and *Verrucomicrobia* (Rousk, Brookes et al. 2009, Zhang, Liu et al. 2016). Also, laboratory studies generally use higher dosages of biochar than field or pot studies, which may favor some specific phyla (Trivedi, Delgado-Baquerizo et al. 2016, Xu, Whitman et al. 2021).

Moreover, the lower richness of OTUs observed in incubation experiments compared to field experiments may be due to the more controlled conditions such as temperature, moisture, and nutrient availability in laboratory conditions (Harter, Weigold et al. 2016). This indicates that the homogeneity of soil and environment conditions in incubation experiments may limit the diversity of soil microbes, resulting in lower microbial richness (Lehmann and Joseph 2015). But for field experiments, they are subject to more natural variation in soil properties, which may lead to inclusion of a greater range of microbial niches (Guo, Chen et al. 2018). Increased soil heterogeneity may limit the dominance of one or two specific phyla, which could result in greater increases in the overall richness of bacterial taxa after biochar application.

4.3. The effect of biochar properties on soil microbial responses to biochar addition

Generally, the abundance of *Acidobacteria* decreased with biochar load, which may be due to the soil pH change with biochar application (Kleinsteuber, Muller et al. 2008). We further divided the properties of biochar and soil into a few groups to see how soil microbial composition was affected by biochar additions (Fig. S4). We found that *Acidobacteria* decreased significantly in response to biochar when the biochar load was larger than 2%, while the difference was not significant with biochar loads $< 2\%$ (Fig. S4). This may be because soils are well buffered against small amounts of inputs of biochar, which leads to less change in *Acidobacteria* abundance when the biochar load is low (Jones, Robeson et al. 2009, Kielak, Barreto et al. 2016).

The C richness reflects the diversity of C sources that can be consumed by soil microbes, and we found ln*RR* of C richness increased with biochar loads. This indicated that, quantitatively, additional soil C sources were utilized by bacteria after the application of biochar. This suggests biochar application not only affected the structure of bacterial communities, but also had influences C utilization patterns by bacteria (Bamminger, Poll et al. 2016, Xu, Wang et al. 2018).

Many studies have found that the soil C/N ratio has a strong influence on soil bacterial communities, which indicates the change of soil C/N in response to biochar application may be one of the main mechanisms of how biochar regulates bacterial communities (Bai, Ye et al. 2021, Milkereit, Geisseler et al. 2021, Rodríguez-Berbel, Soria et al. 2021). Our result showed that *Proteobacteria*, *Gemmatimondetes* and *Acidobacteria* responded to an interaction between biochar and soil C/N, which suggests the effectiveness of biochar application varies in different contexts. Since biochar C/N is normally higher than soil C/N, we expected the impact of biochar on soil C/N, and thus bacterial communities, to be greatest when soil C/N values were lower. However, we found the increase of *Gemmatimonadetes* in response to biochar was stronger as soil C/N increased. One possible explanation is that *Gemmatimonadetes* may have specific adaptations to soils with higher C/N ratios and may be

better able to utilize the carbon and nitrogen resources provided by biochar in these soils (Fawaz 2013, Whitman, Pepe-Ranney et al. 2016). Alternatively, the increase in *Gemmatimonadetes* may reflect changes in other environmental factors, such as pH or moisture content, that are correlated with the soil C/N ratio. As *Gemmatimonadetes* were positively related to vegetation restoration, plant richness, and soil nutrients (such as C, N, and P in soil) (Mujakić, Piwosz et al. 2022), the application of biochar in high soil C/N may maximize its benefit to plant growth.

Our finding that the ln*RR* of the Chao1 index increased with soil pH, and that most of the values were positive, suggested that microbial diversity overall increased with biochar addition, especially for soil with intermediate to high pH. The increased microbial genetic diversity might enhance soil functioning, since different taxa may exhibit complementarity in their C metabolisms. For example, *Acidobacteria* has been associated with the decomposition of biopolymers, and exopolysaccharide secretion while *Gemmatimonadetes* are known to specialize in rhizosphere carbon cycling with plants (Kalam, Basu et al. 2020, Xu, Whitman et al. 2021). The increased Chao1 index under higher biochar loads corresponded to our finding that richness of C substrate utilization increased in response to biochar, suggesting that an increase in genetic diversity may be a key mechanism by which the bacterial community responds to a greater diversity of carbon substrate metabolism.

5. Conclusion

We conducted a global *meta*-analysis and found that *Acidobacteria* and *Gemmatimonadetes* responded to the applied biochar in different ways, with *Acidobacteria* generally responding negatively and *Gemmatimonadetes* responding positively. Both the properties of biochar and soil conditions shaped the changes in *Acidobacteria* and *Gemmatimonadetes* under the application of biochar. Specifically, *Acidobacteria* decreased with higher biochar loads, while *Gemmatimonadetes* increased with soil C/N. The bacterial richness and C richness gradually increased with soil pH and biochar load. Given that different phyla might exert different ecological functions, further research into the mechanisms of biochar that influence soil properties and bacterial communities may be beneficial for optimizing its application. This improved understanding may maximize the use of biochar for improving soil quality and enhancing soil microbial diversity in both genetic and functional aspects for mitigating climate change. However, due to data limitations in the original studies (e.g., some soil and biochar properties were not presented, and focused microbial attributes were different across studies), it was not possible to construct an effective structure equation model (SEM) to disentangle between the latent variables and observed variables. Meanwhile, it remains unclear how the response of soil microbial community structure (including fungi composition) at the genus level to biochar addition regulates microbial functions, and our *meta*-analysis highlights that further research describing these linkages is needed.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix

Appendix S1. Literature information of 196 biochar *meta*-analysis studies.

Appendix S2. Literature information of 57 biochar *meta*-analysis studies related to soil microbes.

Appendix S3. References used in the *meta*-analysis.

Appendix S4. Source of data from references.

Appendix S5. R code for plot and statistic analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2023.116528.

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