

# Functional response of the soil microbial community to biochar applications

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## Abstract

Biochar has the potential to mitigate the impacts of climate change and soil degradation by simultaneously sequestering C in soil and improving soil quality. However, the mechanism of biochar's effect on soil microbial communities remains unclear. Therefore, we conducted a global meta-analysis, where we collected 2,110 paired observations from 107 published papers and used structural equation modeling (SEM) to analyze the effects of biochar on microbial community structure and function. Our result indicated that arbuscular mycorrhizal fungal abundance, microbial biomass C, and functional richness increased with biochar addition regardless of loads, time since application, and experiment types. Results from mixed linear model analysis suggested that soil respiration and actinomycetes (ACT) abundance decreased with biochar application. With the increase of soil pH, the effect of biochar on fungal abundance and C metabolic ability was lessened. Higher biochar pH associated with higher pyrolysis temperatures reduced the abundance of bacteria, fungi, ACT, and soil microbes feeding on miscellaneous C from Biolog Eco-plate experiments. SEM that examined the effect of biochar properties, load, and soil properties on microbial community indicated that fungal abundance was the dominant factor affecting the response of the bacterial abundance to biochar. The response of bacterial abundance to biochar addition was soil dependent, whereas fungi abundance was mostly related to biochar load and pyrolysis temperature. Based on soil conditions, controlling biochar load and production conditions would be a direct way to regulate the effect of biochar application on soil microbial function and increase the capacity to sequester C.

## KEYWORDS

biochar, Biolog, C utilization, functional diversity, global meta-analysis, PLFA, soil microbial community

## 1 | INTRODUCTION

Biochar is a carbon (C)-rich compound formed by the controlled pyrolysis of agricultural waste and other biomass

(Marris, 2006). It has attracted worldwide attention as a soil amendment that could sequester C and improve soil fertility (Marris, 2006; Smith, 2016; Weng et al., 2017). Because it can remain stable in soils for thousands of years,

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burying biochar can sequester atmospheric carbon dioxide (CO<sub>2</sub>) and thus mitigate climate change (Hagemann et al., 2017). In addition, biochar soil amendments improve soil quality and crop yield (Agegnehu et al., 2016, 2017). Soil degradation has also become a global issue due to a wide range of causes, such as salinization, overfertilization, contamination by industrial wastes, and alteration of precipitation patterns due to climate change. Because of its porous structure and high content of essential cations, biochar can increase soil quality and productivity by improving soil pH, cation exchange capacity, and water holding capacity (Gondek et al., 2019; Liu, Wang, et al., 2016). The costs of land degradation and global warming exceed \$231 and \$271 billion annually, respectively (Ackerman & Stanton, 2008; Nkonya et al., 2016). Thus, adoption of biochar management practices has the potential to deliver significant economic as well as ecological benefits.

Soil microbes are key drivers of soil biological and chemical processes and critical for maintaining terrestrial ecosystem stability and ecological function. These processes include C decomposition, N transformation, and nutrient uptake by plant roots (Castrillo et al., 2017; Karhu et al., 2014; Whitman et al., 1998). Based on structural and functional criteria, soil microbes are often classified into a few major functional groups. For bacteria, these groups include gram-positive (G+), gram-negative (G-), and actinomycetes (ACT), whereas major functional groups of fungi include saprotrophs, arbuscular mycorrhizal fungi (AMF), and other major mycorrhizal groups (e.g., ectomycorrhizal fungi). G+ and G- bacteria have different functions due to structural differences in their cell walls. For example, the cell walls of G+ bacteria possess teichoic acids, a family of phosphate-rich polymers that binds cations such as magnesium and sodium. Teichoic acids can also contain D-alanine ester substitutions, giving the molecule zwitterionic properties (Garimella et al., 2009). The major roles ACT play in soil micro-environment ecology include the cycling of organic matter, inhibiting the growth of seral plant pathogens in the rhizosphere, decomposing complex mixtures of polymers in the dead plant, animal, and fungal biomass, and producing many extracellular enzymes (Bhatti et al., 2017). AMF help plant roots capture nutrients, such as phosphorus, sulfur, and nitrogen (N), as well as micronutrients from the soil, while saprophytic fungi (SF) function in soil by decomposing non-living, organic matter and producing enzymes to decompose cellulose, hemicellulose, and pectin (Brundrett, 2002; Vanderwolf et al., 2013).

While the effects of biochar on soil microbial communities have been previously examined with studies mainly focused on changes in the taxonomic groups using next-generation sequencing or phospholipid-derived fatty acid (PLFA) analyses (Nelissen et al., 2015; Steinbeiss et al., 2009; Watzinger et al., 2014), it has proven difficult to understand its effects on

function. Although the impacts are large, their nature is difficult to predict (Lehmann et al., 2011; Xu et al., 2018). For example, some studies (Luo et al., 2017; Paz-Ferreiro et al., 2015) have shown that biochar increases AMF abundance by stimulating AMF spore germination (Rillig et al., 2010), whereas other studies found that biochar decreased or had no effect on AMF abundance (Igalavithana et al., 2017; Warnock et al., 2010). The application of biochar has also been correlated with increases in the abundance of Actinobacteria and readily available C sources (Igalavithana et al., 2017). Bacterial and fungal abundances and their ratios are also responsive to biochar amendments as biochar may affect microbial C use efficiency and facilitate fungal growth (Jiang et al., 2015). Furthermore, the internal pore systems of biochar may protect fungal extraradical mycelia from grazers (Cheng et al., 2017; Keiblinger et al., 2015). Various biochar pyrolysis temperatures produce different amounts of organic residues that can differentially affect fungal relative to bacterial growth, and the biochar pyrolysis temperature has been found to be positively associated with the bacteria/fungi ratio (Zhang, Jing, et al., 2018).

The abundances of gram-positive (G+) and gram-negative (G-) bacteria and their ratios (G+/G-) are important indicators of change in the soil microbial community because they respond differently to different C sources. The effects of biochar on G+, G-, and G+/G- ratio are associated with environmental stress (Fierer et al., 2003; Zhou et al., 2017), biochar load (Ameloot et al., 2013), soil textures and nutrient conditions (Zheng et al., 2018). The high exchange capacity of some biochars promotes ion retention in some soils and is beneficial for the growth of G+ bacteria and increased G+/G- ratios (Ameloot et al., 2013). The growth of G- bacteria is also affected by biochar. G- bacteria favor soils with easily degradable organic matter and, thus, become dominant immediately after biochar application. However, with time, there is a shift toward G+ as C availability decreases (Mitchell et al., 2015).

Biochar can be a source of labile or extractable C and can also serve as a structural refugium that protects microbial growth, leading to an increase in microbial biomass C (MBC; Liu, Zhang, et al., 2016; Zhou, et al., 2017). In contrast, the effects of biochar on microbial biomass N (MBN) vary. In some experiments, the MBN declines upon biochar application as the microbial community structure responds to altered soil physicochemical properties (Albuquerque et al., 2014; Xu et al., 2018). In other experiments, the effects of biochar on MBN appear to be insignificant, and this may be related to a variety of factors, including N status of the soil or N competition by plants (Lehmann & Rondon, 2006).

The changes in the microbial community upon biochar amendments may also affect their C metabolic activity and functional diversity, which are indicators of the functional response of soil microbes. The Biolog technique is another quantitative method for determining functional diversity and C utilization

rates of microbes that have been increasingly applied to biochar research (Edenborn et al., 2017; Galazka et al., 2019; Xu et al., 2018). Recent research using the Biolog technique suggests that biochar can increase microbial metabolic activity in heavy metal contaminated soils (Hmid et al., 2015). Besides, the application of biochar has been suggested to increase utilization of miscellaneous and polymer group C substrates due to alterations of the microbial community (Tian et al., 2016). Functional richness, evenness, and diversity have also been found to increase with biochar application (Xu et al., 2018). A potential explanation for this pattern is that additions of labile C sources and microenvironment alterations caused by biochar application widen the extent of C utilization by soil microbes (Liao et al., 2016; Zhu et al., 2017).

Although the effect of biochar on soil microbes has been widely investigated, the responses of different functional groups are variable (Hardy et al., 2019; Liao et al., 2016; Luo et al., 2017), and the mechanisms by which biochar impacts soil microbial communities remain unclear (Liu, Zhang, et al., 2016; Zhang, Jing, et al., 2018). Moreover, different studies report different results for the effects of biochar on AMF, ACT, bacterial and fungal abundances, MBC, MBN, soil respiration, C metabolic ability, and functional diversity (Fernandez et al., 2014; Zhang et al., 2016; Zhu et al., 2017). For instance, some studies (Dil et al., 2014; Zhu et al., 2017) show a decrease in microbial activity, C use rate, and functional diversity after biochar application, whereas others studies have shown increases (Liao et al., 2016; Xu et al., 2015). The ambiguity and complexity of the regulating paths of biochar on soil microbes might also depend on biochar properties, production conditions (e.g., pyrolysis temperature), the properties of the soils they are added to, or the experimental design (Biederman & Harpole, 2013; Zhang, Jing, et al., 2018). The amount of biochar added, experimental duration, climate conditions, position in the soil profile, and methods of biochar application could introduce further uncertainty (Bamminger et al., 2018; Liu, Zhang, et al., 2016).

To unravel the complex effects of biochar amendments on microbial community structure, function, diversity, and C metabolic utilization, mixed linear models and structural equation modeling (SEM) were utilized to: (a) analyze the effects of biochar application on AMF, ACT, G+, G- abundance, G+/G-, C utilization based on Biolog studies; (b) quantitatively determine the change slope of the soil MBC and MBN, soil respiration, and functional diversity with different biochar loads and times since application; and (c) clarify the associations of soil properties, biochar pyrolysis temperatures, and properties with the microbial response to biochar. We hypothesized that: (a) changes in the soil microbial community after biochar addition affects microbial C utilization and metabolic activity; (b) functional attributes (C metabolic activity) of soil microbes are more sensitive to biochar load than time since application; (c) the response

of soil fungi depends on biochar properties, but the bacteria community is more depending on soil condition. Our third hypothesis stems from some observation that the mechanisms through which biochar affects bacteria and fungi are different. Specifically, bacteria have been shown to respond indirectly to biochar whereas fungi have been shown to respond directly (Castaldi et al., 2011; Chintala et al., 2014; Dai et al., 2018; Demisie et al., 2014; Jenkins et al., 2017). For example, fungal abundance is sensitive to the types of biochar used whereas bacterial abundance alteration may result from soil properties change caused by biochar addition. Better understanding of the influential factors in regulating soil microbes will allow more optimal use of biochar to improve soil quality and C sequestration.

## 2 | MATERIALS AND METHODS

### 2.1 | Literature search and data extraction

Data were collected from published papers describing the structural and functional responses of soil microbial communities to biochar application using the Web of Science, Google Scholar, and China National Knowledge Infrastructure. The studies that satisfied the following criteria were included in this meta-analysis. (a) At least three replicates in each treatment and control treatment were included. (b) The Biochar addition load was provided as a percentage in weight or in units of ton/ha or kg/m<sup>2</sup>. When the application rate was provided as mass per area, the data were converted to percentage of weight assuming a soil bulk density of 1.5 g/cm<sup>3</sup> (Biederman & Harpole, 2013). (c) Only the control and biochar application treatment data were selected if the experiment included other fertilizer additions. (d) The data of the selected variables were available or could be found or calculated from the related publications. (e) Data from studies focused on charcoal rather than biochar were not considered in this study. The data sources were extracted mainly from the text, tables, figures, and appendices of the publications. When data were presented graphically, digitizer software was used to extract effective data (<http://digitizer.sourceforge.net>; Zhang, Chen, et al., 2018). When some data were missing in the articles, they were collected from the corresponding author directly. In total, 148 studies were initially examined, but only 107 studies were included in the final meta-analysis. These papers were published from 2008 to 2019. (Appendix 1).

### 2.2 | Characteristics selection and data description

The experiment type, location, and coordinates were recorded as background data, while the biochar application loads, pH

values, pyrolysis temperature, and time since application were recorded as potentially independent variables. Soil microbial biomass (MBC, MBN) and absolute abundances of community groups (AMF, ACT, G+, and G−) were included in our analysis. Soil microbial biomass was mainly determined by the fumigation–extraction method with only a few were determined by substrate-induced respiration method (Anderson & Domsch, 1978; Lu et al., 2015; Xu et al., 2018). The abundance of different functional groups was derived from the PLFA or fatty acid methyl ester method (Moeskops et al., 2010; Schutter & Dick, 2000). Soil C metabolic activity and functional diversity including evenness and richness were measured by the Biolog technique using Eco-plate incubations. These plates test 31 kinds of C substrates (categorized into six groups: amines/amides, amino acids, carbohydrates, carboxylic acids, polymers, and miscellaneous; Table S2; Zak et al., 1994). Average well color development reflects the relative amount of C that was consumed by soil microbes and is an indicator of overall metabolic activity. Soil respiration was determined by CO<sub>2</sub> efflux measurement.

The data encompassed three experimental types: pot, incubation, and field, and the locations of the soil samples were mainly North America, Europe, Southeast Asia, and Australia (Figure S1). The final dataset included a total of seven independent variables and 23 dependent variables (Appendix 2).

### 2.3 | Data analysis and model selection

Natural log response ratio (lnRR) of 23 dependent variables was used to assess the responses of soil microbial characteristics compared between control and biochar application treatments (Hedges et al., 1999). Natural log response ratio was calculated as:

$$\lnRR = \ln\left(\frac{X_t}{X_c}\right), \quad (1)$$

where  $X_t$  and  $X_c$  are mean values of the selected microbial indicators with biochar treatment and in controls, respectively. Therefore, positive values mean that the values with biochar are larger than the control. The number of replicates was used to calculate the weighting parameter (Ma & Chen, 2016; Pittelkow et al., 2015):

$$W_t = \frac{(N_c \times N_t)}{(N_c + N_t)}, \quad (2)$$

where  $W_t$  is the weight associated with each lnRR observation,  $N_c$  and  $N_t$  are the numbers of replicates in the control and treatment, respectively.

To determine whether biochar application significantly increased or decreased the 23 dependent variables, mixed linear

models including random effects by studies were built, and the final model was selected based on Akaike information criterion (AIC) values. The candidate models were selected mainly based on whether predictors should be log-transformed. Compared with the models including interaction terms of biochar load and period, the models without interaction terms always had lower AIC values except for functional diversity (Table S1), which means better fitness. For consistency, the model without interaction terms was selected as follows:

$$\lnRR = \beta_0 + \beta_1 \cdot Biochar + \beta_2 \cdot \log(Period) + \pi_{study} + \varepsilon, \quad (3)$$

where  $\beta$ ,  $\pi_{study}$ , and  $\varepsilon$  are coefficients, the random effect factor of “study,” and sampling error, respectively. *Biochar* and *Period* represent the load of biochar and application period, and lnRR represents the natural log response ratio of the biochar and control groups. The random effect explicitly accounts for autocorrelation among observations within each “study.” Maximum likelihood estimation was performed using the *lme4* package (Bates et al., 2017). Except for experiment type, the other predictors were continuous predictors, that is, *Biochar*, *Period*, which were standardized or scaled to reduce statistical variance due to different units or scales.  $\beta_0$  was the intercept value, which reflected the overall mean lnRR at the mean *Biochar* and  $\ln(Period)$  (Cohen et al., 2003).  $\beta_1$  and  $\beta_2$  were the slopes of biochar load and period terms, which represent the change of lnRR when biochar and period increase by one unit. For instance,  $\beta_1$  represents the response with an increase of 1% biochar, that is, the lnRR of the specific trait will increase  $\beta_1$  units. Our Figure 2 results were based on  $\beta_1$  and  $\beta_2$ . To simplify the interpretation of the graphic result, 23 variables of lnRR and its corresponding confidence intervals were transformed back to the percentage change as  $(e^{\lnRR} - 1) \times 100\%$ . All statistical analyses were performed in R (version 3.6.3) using the code available in the Appendix.

### 2.4 | Structural equation model

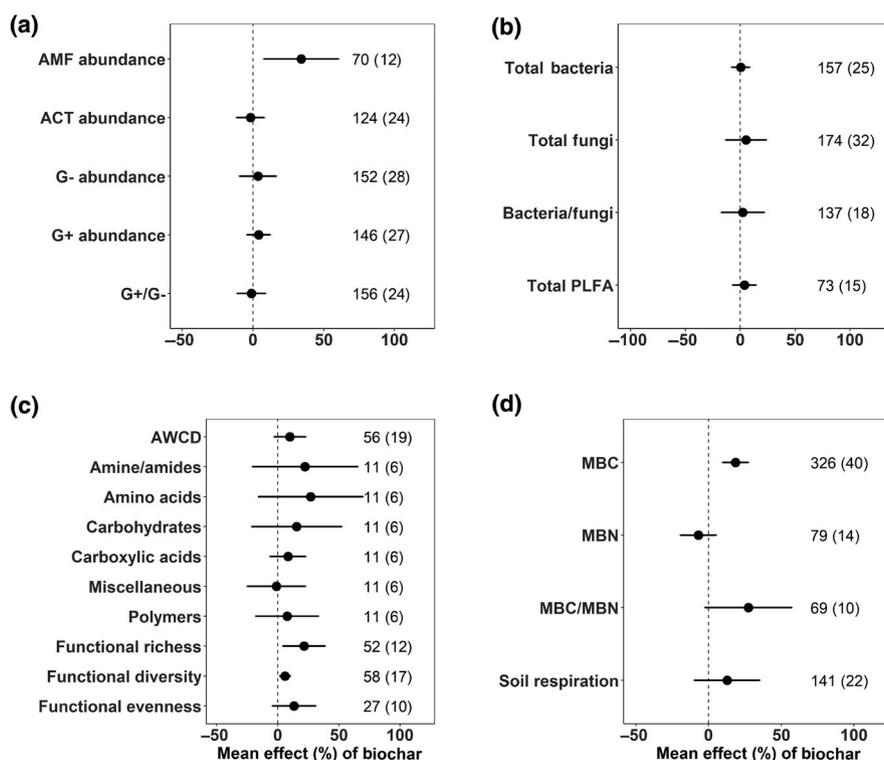
To investigate the relationship between functional response of soil microbes, soil condition, biochar properties, and application method, SEM were built based on their impact paths. The conceptual model (full model) and reduced models were performed to find a relative fit model, and the model was selected by comparing the goodness-of-fit index (GFI) and root mean square error of approximation (RMSEA; Chen et al., 2019). We chose the final model with the highest GFI and lowest RMSEA value (Chen et al., 2019; Grace, 2006). The final model comprises one endogenous latent variable and one response variable with three explanatory variables. The endogenous latent variable is the response of the bacteria, which is represented by log response ratios of G+ and G− bacteria. SEMs were implemented using the “lavvan” package (Rosseel, 2012).

### 3 | RESULTS

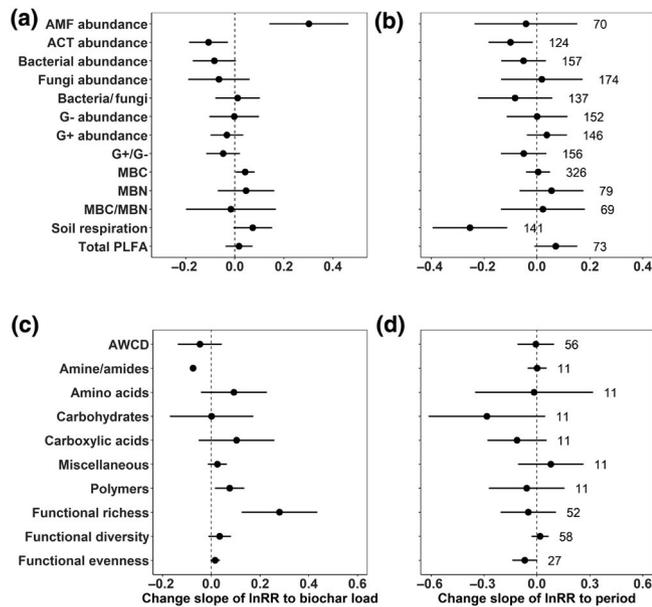
#### 3.1 | Soil microbial community and function to biochar

We calculated 95% CI and  $p$ -value of each attribute and defined statistical significance as  $p < .05$ . The lnRR of AMF abundance increased on average by 0.29 units (which was 34.1% after conversion,  $p = .02$ ) with biochar application, while ACT, G+, G- abundance, total PLFA did not significantly change with biochar (Figure 1a,b). Biochar addition increased the lnRR of MBC by 0.17 units, or 18.5% ( $p < .01$ ), with a marginal increase of the lnRR of MBC/MBN ratio by 0.24 units, or 27% ( $p = .1$ ; Figure 1d). Also, the lnRR of functional richness and diversity increased significantly on average by 0.20 and 0.058, or 21.6% and 6.0% ( $p < .01$ ), respectively (Figure 1c). Furthermore, in the analysis of the magnitude of change of microbes to biochar addition load and period, we calculated the slope of biochar

and period, and calculated the 95% CI with considering the “study” as a random effect (Equation 3). The lnRR of AMF abundance increased by 0.37 units ( $p < .01$ ) per percentage addition of biochar, while ACT abundance decreased by 0.1 units ( $p = .01$ ; Figure 2a). Except for MBC increasing by 0.04 ( $p = .03$ ), the rest of the microbial attributes did not change significantly with biochar load (Figure 2a). Among the functional responses, the lnRR of amine consumption by microbes decreased by 0.07 ( $p < .01$ ) with % biochar addition, while polymer utilization increased by 0.08 ( $p = .04$ ; Figure 2c). Functional richness was another indicator that significantly increased upon % biochar addition, that is, 0.33 ( $p < .01$ ) per percent addition. With biochar application time, lnRR of ACT abundance and soil respiration decreased by 0.1 ( $p = .02$ ) and 0.2 ( $p < .01$ ) per day, respectively (Figure 2b). None of the functional attributes showed a significant trend with the application period except for the lnRR of functional evenness, which marginally decreased by 0.02 ( $p = .06$ ) per day (Figure 2d).



**FIGURE 1** Mean effect of biochar application on soil microbial biomass and community structure and function. (a) changes in the abundance of each functional group based on their phospholipid-derived fatty acid (PLFA); (b) changes in total amount of bacteria, fungi based on the total PLFA; (c) changes in C metabolic attributes based on Biolog Eco-Plate studies and (d) based on microbial biomass and soil respiration measurements. Solid circles and bars are the means  $\pm$  95% CIs, respectively, of the percentage effects between the biochar treatment and control. The numbers shown without parentheses are the total observation numbers, while the numbers in parentheses are the numbers of the publications surveyed. ACT, AMF, G+, G-, MBC, and MBN represent actinomycetes, arbuscular mycorrhizal fungi, gram-positive bacteria, gram-negative bacteria, microbial biomass C, and microbial biomass N. Amine/amides, amino acids, carbohydrates, carboxylic acids, miscellaneous, and polymers are six categories of C sources in Biolog Eco-plates based on their optical density (OD) values. Functional richness, diversity, and evenness represent C utilization status of soil microbes. ACT, actinomycetes; AMF, arbuscular mycorrhizal fungi; AWCD, average well color development representing C metabolic ability of microbes in Biolog incubations; G+, gram-positive; G-, gram-negative; MBC, microbial biomass C; MBN, microbial biomass N



**FIGURE 2** Change in the slope of lnRR in response to biochar load and time since application for soil microbial biomass and community composition (a, b) and function (c, d). The values represent changes of lnRR when increasing every 1% (w/w) of biochar and every day of application in soil microbial community and function. See Figure 1 for explanations of the notation. The numbers on the (b) and (d) plots are the number of replicates for individual variables. The number of replicates for plots (a) and (c) is the same as (b) and (d), respectively

### 3.2 | The effect of soil and biochar properties and experiment types

The effects of soil pH, biochar pH, and pyrolysis temperature and experimental types on microbial attributes were tested. The lnRR of bacterial and fungi abundance decreased with soil pH, while the bacteria/fungi ratio and functional richness showed an upward trend (Table 1). With the increase of biochar pH, lnRR of ACT, bacteria, and fungi decreased by 0.13 (13.8%,  $p < .01$ ), 0.15 (16.2%,  $p < .01$ ), and 0.28 (32.3%,  $p < .01$ ) units, respectively, while the bacteria/fungi ratio increased by 0.16 units (17.3%,  $p < .01$ ). Also, G-, G+, G+/G- ratio, miscellaneous C utilization showed negative correlations with biochar pH. A similar pattern was also found in biochar pyrolysis temperature. The majority of microbial attributes were not affected by experimental design, while only the lnRR of functional richness was lower in incubation than field experiments (Figure S2).

### 3.3 | SEM depicts the microbial response to multiple variables

Based on previous studies, the variables affecting the microbial response to biochar were mainly related to biochar

properties, biochar application load, and soil properties. After a comparison of few models, the final model indicated that the response of bacteria to biochar application was mainly related to soil pH and lnRR of fungi with standardized coefficients of  $r = .08$  and  $.99$ , respectively (Figure 3). Biochar pyrolysis temperature had direct negative effects ( $r = -.69$ ) on the lnRR of fungi, while biochar load had relatively weak positive effects ( $r = .32$ ). The standardized coefficients of G+ and G- bacteria abundance to the latent variable of response of bacteria were 0.71 and 0.97, respectively.

## 4 | DISCUSSION

### 4.1 | Response of soil microbial community structure and function to biochar

These analyses tested three hypotheses regarding the role of biochar in soils. The first hypothesis proposed that changes in microbial abundance and community structure upon biochar addition affected C utilization by microbes. This hypothesis was not strongly supported. Despite increases in AMF abundance and MBC upon biochar addition, the utilization of the six categories of C sources was unaffected by biochar treatment. Presumably, the changes in the type of C utilization did not cluster in one of the six categories but were spread out and scattered among those six categories (Liao et al., 2016; Xu et al., 2016; Zhang et al., 2016). Besides, since there were only a limited number of studies providing the C utilization data using Biolog technique (11 pairs of data from six articles), which are very small replication numbers compared to other categories, these results were possibly biased by a lack of replication and analysis power of the meta-analysis.

However, biochar additions were significantly correlated with increases in functional richness, which suggested that more types of C sources were consumed by soil microbes after biochar application. This result would be expected because the ash and labile C introduced by biochar would facilitate the growth of microbes that feed on more types of C sources and would result in a more diversified C utilization pattern (Singh & Cowie, 2014). This inference is supported by the increase of MBC, indicating that biochar facilitated microbial growth (Ambihai et al., 2013; Bargmann et al., 2014; Bruun et al., 2011; Domene et al., 2015) and increased the possibility that microbes feed on different types of C substrates. In addition, this also depends on the time since application, as the majority of biochar C is recalcitrant and not directly used by the microbial community (Jenkins et al., 2017; Zhu et al., 2017). In fact, our data showed that the increased functional richness mainly occurred at the early stage of application (within 132 days). Functional richness was lower in incubation studies than field and pot experiments (Figure S2). Thus, the exogenous C brought by biochar

**TABLE 1** Significant factors accounting for the variance of microbial attributes

Attribute	Soil pH		Biochar pH		Biochar PyT		Etype	
	df	Coef	df	Coef	df	Coef	df	Coef
AMF	27.44	-0.02	58	-0.02	57.49	-0.05	13.27	-0.04
ACT	38.31	-0.05	88.59	<b>-0.13**</b>	97.32	<b>-0.16**</b>	23.57	-0.11
Bacteria	67.59	<b>-0.13*</b>	115.49	<b>-0.15**</b>	45.12	<b>-0.22**</b>	20.68	-0.02
Fungi	102.64	<b>-0.23**</b>	128.24	<b>-0.28**</b>	79.57	<b>-0.34**</b>	34.91	0.02
Bacteria/fungi	117.2	<b>0.14*</b>	99.78	<b>0.16**</b>	104.63	<b>0.21**</b>	14.23	0.03
G-	72.33	-0.11	97.23	<b>-0.19**</b>	32.67	<b>-0.37**</b>	35.36	0.04
G+	64.29	-0.06	94.91	<b>-0.09*</b>	35.95	<b>-0.14**</b>	29.61	0.12
G+/G-	74.06	-0.08	117.33	<b>-0.11**</b>	59.61	<b>-0.11*</b>	22.08	0.17
MBC	135.83	-0.04	245.34	-0.04	165.63	<b>-0.1**</b>	42.5	0
MBN	6.54	-0.08	25.02	0.11	13.33	0.04	7.25	-0.19
MBC/MBN	6.29	0.18	43.74	-0.1	19.55	-0.17	5.97	0.39
Soil respiration	82.07	-0.14	107.72	-0.02	123.46	-0.07	23.99	-0.47
Total PLFA	61.13	-0.06	65.89	0	23.4	0	12.1	0.08
AWCD	17.71	-0.01	10.26	0.24	11.37	-0.09	14.09	-0.53
Amine/amides	4.05	-0.19	3.03	-0.04	4	0.11	NA	NA
Amino acids	3.54	-0.28	1.63	-0.35	3.26	-0.01	NA	NA
Carbohydrates	3.46	-0.08	2.41	-0.52	3.11	0.2	NA	NA
Carboxylic acid	4.61	-0.03	6	-0.25	7	0.12	NA	NA
Miscellaneous	3.94	0.07	2.18	<b>-0.49**</b>	2.64	<b>0.27**</b>	NA	NA
Polymers	4.01	-0.06	2.15	-0.41	3.36	0.15	NA	NA
Functional richness	48	<b>0.21*</b>	2.33	0.03	42	-0.14	47	<b>-1.17**</b>
Functional diversity	8.16	-0.03	7.08	0.02	7.96	0.01	19.91	-0.14
Functional evenness	6.57	-0.13	6.59	0.05	7.78	0.14	9.42	-0.16

Note: Amine/amides, amino acids, carbohydrates, carboxylic acids, miscellaneous, and polymers are the six categories of C sources in Biolog Eco-plate.

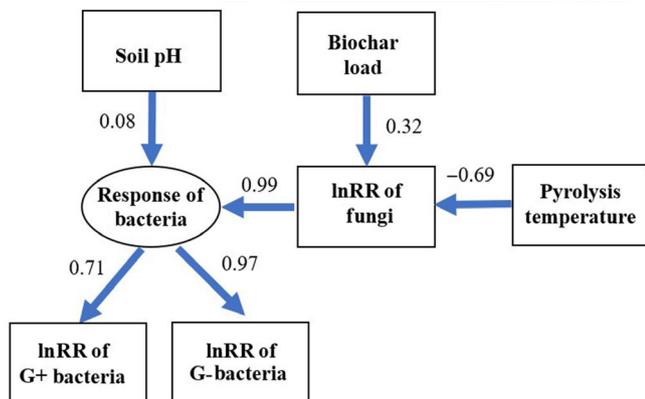
Bold values indicate  $p < .05$ , the values with "\*" means  $p < .05$ , while with "\*\*" means  $p < .01$ .

Abbreviations: ACT, actinomycetes; AMF, arbuscular mycorrhizal fungi; AWCD, average well color development; Biochar PyT, biochar pyrolysis temperature; Coef, coefficient of factors in mixed linear models, which means the extent of change of lnRR per unit of the factors' increase; df, degree of freedom in each linear mixed effects models; Etype, experimental type or design; G-, gram-negative; G+, gram-positive; MBC, microbial biomass C; MBN, microbial biomass N; NA, no appropriate value; PLFA, phospholipid-derived fatty acid.

resulted in microbial growth and a diversified C utilization pattern in the early stage.

The second hypothesis proposed that the functional responses of soil microbes were more sensitive to biochar load than time since application, which was supported by our results. There were six attributes (AMF, ACT, MBC, amines/amides, polymers, and functional richness) significantly related to biochar load, while only two attributes (ACT and soil respiration) were related to time since application (Figure 2). The observation that only two microbial attributes significantly changed with time since application does not mean that other attributes did not change. Their changes might have occurred in the early stage of biochar application but did not continue with time. In fact, some studies found that in

the early stage of biochar application, the microbial community structure and function changed rapidly but then became stable after a short time (Hu et al., 2014; Xu et al., 2016). This phenomenon might be due to a priming effect where the introduction of exogenous organic matter from biochar led to a sudden change of nutrient supply, the rapid growth of microbes, and increases in soil respiration immediately following amendment (Luo et al., 2011; Zimmerman et al., 2011). This effect might explain the observed decrease in soil respiration and ACT abundance with the application period. Also, there was a significant increase in AMF abundance with biochar load, possibly related to the sorption of signaling compounds, detoxification of allelochemicals, and indirect effects of other soil microbial populations caused



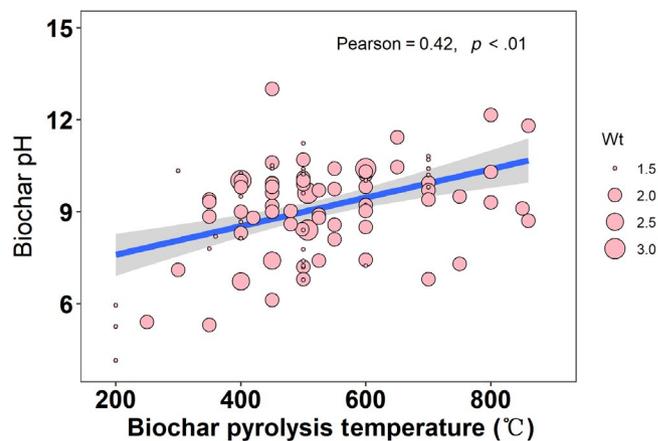
**FIGURE 3** Structural equation model depicting the response of soil microbial community to biochar associated with biochar properties, application load, and soil condition. Response of bacteria is an endogenous latent variable of lnRR of gram-positive (G+) and gram-negative (G-). Arrows indicate the directionality of the relationship, among which single-headed arrows represent directional influences of one variable upon another. The numbers between arrows are standardized coefficient ( $r$ ). All fitted coefficients are significant at  $p < .05$

by biochar application (Elmer & Pignatello, 2011; Warnock et al., 2007).

## 4.2 | The effect of soil and biochar properties

The third hypothesis proposed that properties of biochar are more influential than biochar application load or period in regulating the response of the soil microbial community structure and function. For instance, important biochar properties might include biochar pH, biochar pyrolysis temperature, and experimental design (Zhang, Jing, et al., 2018; Zhou, et al., 2017). ACT, bacteria, fungi, G+, G- abundance, and miscellaneous C utilization were simultaneously affected by biochar pH and pyrolysis temperature (Table 1), which suggests that biochar pH was positively correlated to its pyrolysis temperature. In fact, the Pearson coefficient between the two was very significant, and every 100°C increase in the pyrolysis temperature increased the biochar pH by 0.46 (Figure 4). Similar results have been reported by others (Al-Wabel et al., 2013; Cantrell et al., 2012).

The lnRR of bacteria and fungi decreased with increases in soil pH, indicating that high soil pH suppressed the differences of bacterial and fungal abundance between the control and biochar treatments. Because biochar usually has a higher pH than soil, its effect would be accentuated in more acidic soil where the change in pH would be more dramatic after biochar application (Paz-Ferreiro et al., 2015; Xu et al., 2018). However, the increase in the bacteria/fungi ratio with soil pH might due to the fact that fungi, in general,



**FIGURE 4** The relationship of biochar pyrolysis temperature and biochar pH with Pearson correlation coefficient and  $p$ -value. The sizes of circles represent the relative weights (Wt), which depend on the number of replicates of the treatment and control of each study

inhabit mildly acidic environments (Vylkova, 2017); and an increase of soil pH after biochar application might lead to a decline in the total amount of fungi. In fact, high biochar pH was correlated with a decreased fungal abundance.

High biochar pH and pyrolysis temperature decreased ACT abundance. This is not surprising as ACT favor acidic and neutral pH environments (Hamid et al., 2015; Vylkova, 2017). In addition, fungal abundance decreased faster than that of bacteria with biochar pH and pyrolysis temperature. Since pyrolysis temperature would also affect the porous structure of biochar (Al-Wabel et al., 2013), the faster decrease in fungi might be related to changes in the porous structure of biochar. High pyrolysis temperatures enhance the micro surface area and pore volume, while the growth of fungal hypha benefits from small micro surface area and pore volume (Muhammad et al., 2016; Rillig et al., 2010), so the increased size of the pores after high pyrolysis temperature might be less hospitable to fungi. Also, biochar pH affects G- and G+ and G+/G- ratios indicating that biochar pH changes the structure of soil bacterial community. Lastly, miscellaneous C utilization declined with increased biochar pH indicating that the three types of C belonging to this category: d, l- $\alpha$ -glycerol phosphate, glucose-1-phosphate, and pyruvic acid methylester were less utilized.

## 4.3 | SEM depicts the microbial response to multiple variables

Structure equation modeling has been widely used to depict the multivariate relationship between multiple dependent and independent variables in ecology and environment research (Jucker et al., 2018; Vile et al., 2006; Wang & Huang, 2020). In addition, meta-analysis combined with SEM analysis can

help disentangle multivariate variables affecting soil microbial attributes on a global scale (Chen & Chen, 2019; Zhang, Chen, et al., 2018). While our results showed that the response of bacteria to biochar was influenced by soil pH and fungal abundance, the influence by fungi was much stronger than that of soil pH. This indicates that biochar affects the bacterial community via regulating fungi abundance. The mechanisms and possible soil ecological processes might be that biochar affects mycorrhizal fungi, which affect root exudates and then affect the bacterial structure in the rhizosphere. Specifically, the processes are related to the interaction of mycorrhiza fungi and plant growth promoting rhizobacteria, mycorrhizal helper bacteria, nitrogen fixing bacteria, or deleterious bacteria under biochar (Hashem et al., 2019; Miransari, 2011). In addition, biochar load and pyrolysis temperature had direct effects on fungal rather than bacterial abundance, which indicates fungi were more sensitive to biochar per se than bacteria. This might suggest that biochar application overall had direct effects on fungal community (Dai et al., 2018), but indirect effects on bacterial community structure (Dai et al., 2016; Meynet et al., 2014).

Biochar load had positive effects on the lnRR of fungi, which was consistent with other studies (Bamminger et al., 2014; Taskin et al., 2019). This suggests that when biochar concentration increases, the growth of fungi increases. This is not surprising as fungal hypha benefits from porous structures generated by biochar, and an increasing biochar load would then lead to an increased soil porosity. However, higher pyrolysis temperature was detrimental for fungal growth, which is consistent with our second result. Since the response of fungi abundance to biochar properties results in altered responses of the bacterial community, controlling biochar material and production conditions is one essential method to regulate soil microbial communities and their functioning.

Overall, soil microbial community structure and function are sensitive to interferences that change soil properties and introduce C by adding biochar (Bamminger et al., 2018; Hu et al., 2014). In addition, the response of microbial community to biochar application is dependent on the soil conditions and affected by biochar properties. Better recognition of how biochar affects the microbial community structure and function will assist in the efficient use of biochar technology to mitigate soil degradation and climate change.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material of this article.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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