

Functional response of the soil microbial community to biochar applications

Wenhuan Xu¹ | William B. Whitman² | Michael J. Gundale³ | Chuan-Chi Chien⁴ | Chih-Yu Chiu¹

¹Biodiversity Research Center, Academia Sinica, Taipei, Taiwan

²Department of Microbiology, University of Georgia, Athens, GA, USA

³Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, Umeå, Sweden

⁴Central Region Campus, Industrial Technology Research Institute, Nantou, Taiwan

Correspondence

Chih-Yu Chiu, Biodiversity Research Center, Academia Sinica, 128 Academia Road, Section 2, Nankang, Taipei 11529, Taiwan.

Email: bochiu@sinica.edu.tw

Funding information

Funding from Academia Sinica (AS-ITRI-109-01), Taiwan.

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,

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2020 The Authors. *GCB Bioenergy* Published by John Wiley & Sons Ltd

burying biochar can sequester atmospheric carbon dioxide (CO₂) 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/m². 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/cm³ (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 CO₂ 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:

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

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:

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

where β , π_{study} , and ε 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. β_0 was the intercept value, which reflected the overall mean lnRR at the mean *Biochar* and $\ln(\text{Period})$ (Cohen et al., 2003). β_1 and β_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, β_1 represents the response with an increase of 1% biochar, that is, the lnRR of the specific trait will increase β_1 units. Our Figure 2 results were based on β_1 and β_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 $(e^{\ln RR} - 1) \times 100\%$. 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).

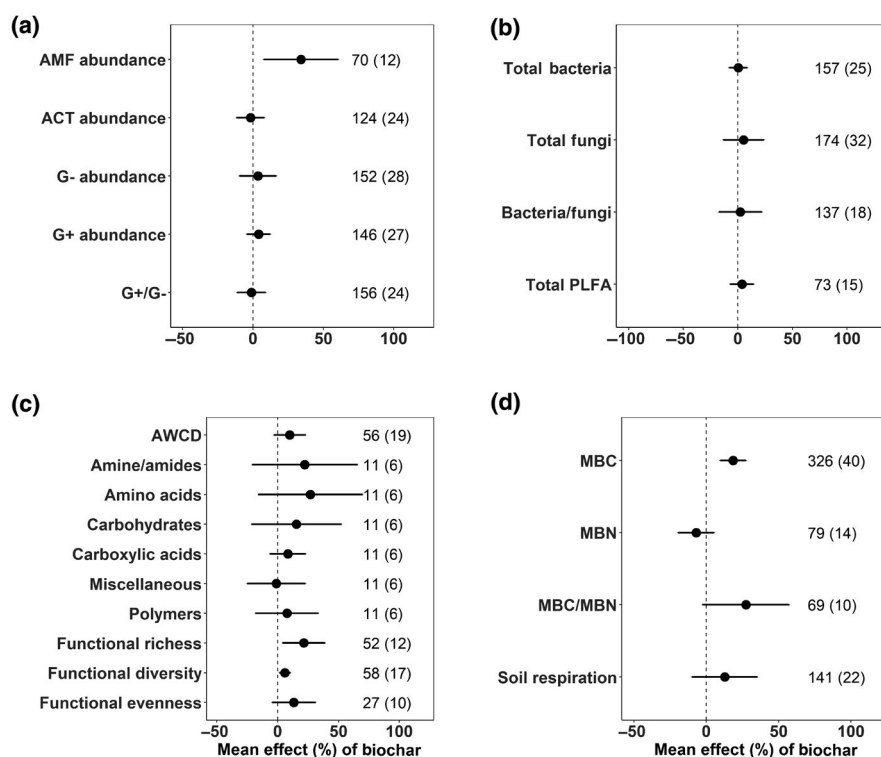


FIGURE 1 Mean effect of biochar application on soil microbial biomass and community structure and function. (a) changes in the abundance of each functional group based on their phospholipid-derived fatty acid (PLFA); (b) changes in total amount of bacteria, fungi based on the total PLFA; (c) changes 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

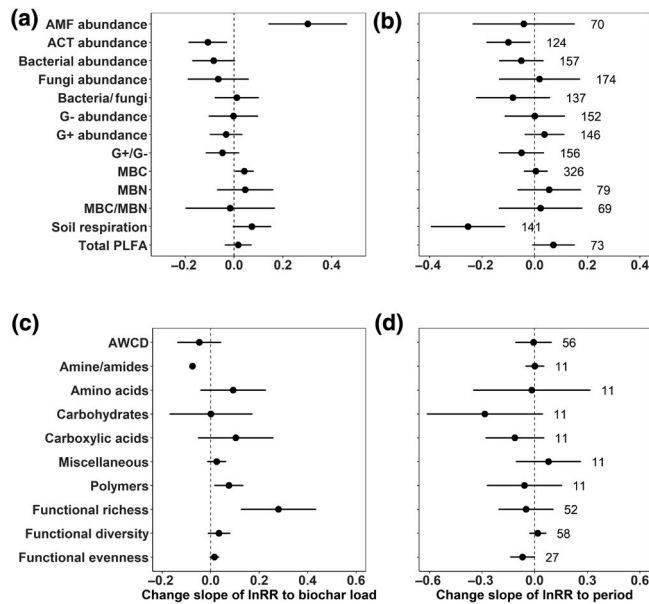


FIGURE 2 Change in the slope of lnRR in response to biochar load and time since application for soil microbial biomass and community composition (a, b) and function (c, d). The values represent changes of lnRR when increasing every 1% (w/w) of biochar and every day of application in soil microbial community 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	−0.13**	97.32	−0.16**	23.57	−0.11
Bacteria	67.59	−0.13*	115.49	−0.15**	45.12	−0.22**	20.68	−0.02
Fungi	102.64	−0.23**	128.24	−0.28**	79.57	−0.34**	34.91	0.02
Bacteria/fungi	117.2	0.14*	99.78	0.16**	104.63	0.21**	14.23	0.03
G−	72.33	−0.11	97.23	−0.19**	32.67	−0.37**	35.36	0.04
G+	64.29	−0.06	94.91	−0.09*	35.95	−0.14**	29.61	0.12
G+/G−	74.06	−0.08	117.33	−0.11**	59.61	−0.11*	22.08	0.17
MBC	135.83	−0.04	245.34	−0.04	165.63	−0.1**	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	−0.49**	2.64	0.27**	NA	NA
Polymers	4.01	−0.06	2.15	−0.41	3.36	0.15	NA	NA
Functional richness	48	0.21*	2.33	0.03	42	−0.14	47	−1.17**
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

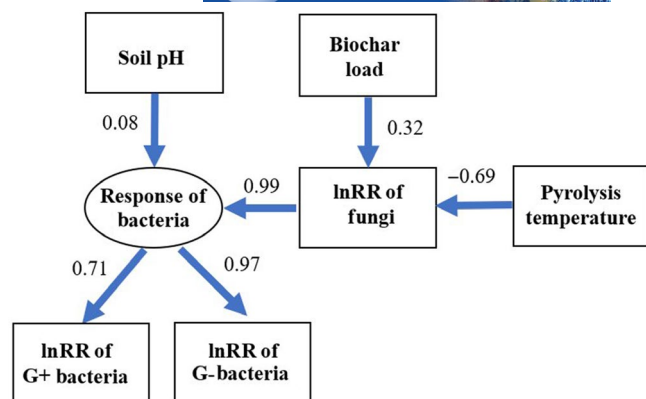


FIGURE 3 Structural equation model depicting the response of soil microbial community to biochar associated with biochar properties, application load, and soil condition. Response of bacteria is an endogenous latent variable of lnRR of gram-positive (G+) and gram-negative (G-). Arrows indicate the directionality 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,

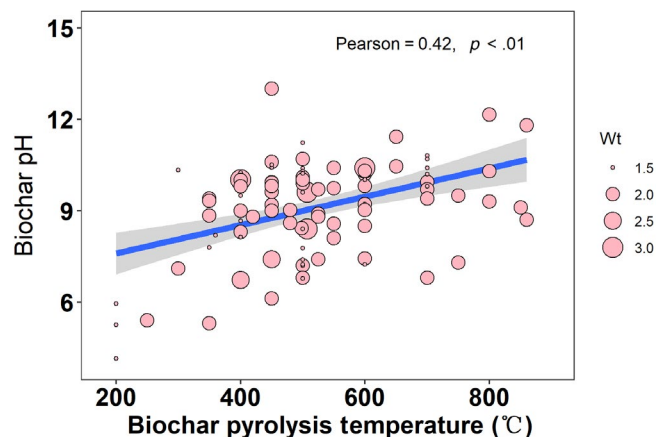


FIGURE 4 The relationship of biochar pyrolysis temperature and biochar pH with Pearson correlation coefficient and p -value. The sizes of circles represent the relative weights (Wt), which depend on the number of replicates of the treatment and control of each study

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- α -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.

ACKNOWLEDGEMENTS

We thank the authors whose works are included in the meta-analyses and editorial comments from reviewers. This study was supported by funding from Academia Sinica (AS-ITRI-109-01), Taiwan.

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.

ORCID

Wenhuan Xu  <https://orcid.org/0000-0002-3302-8531>

Chih-Yu Chiu  <https://orcid.org/0000-0002-6842-1253>

REFERENCES

- Ackerman, F., & Stanton, E. A. (2008). *What we'll pay if global warming continues unchecked*. Natural Resources Defense Council.
- Agegehu, G., Bass, A. M., Nelson, P. N., & Bird, M. I. (2016). Benefits of biochar, compost and biochar-compost for soil quality, maize yield and greenhouse gas emissions in a tropical agricultural soil. *Science of the Total Environment*, 543, 295–306. <https://doi.org/10.1016/j.scitotenv.2015.11.054>
- Agegehu, G., Srivastava, A., & Bird, M. I. (2017). The role of biochar and biochar-compost in improving soil quality and crop performance: A review. *Applied Soil Ecology*, 119, 156–170. <https://doi.org/10.1016/j.apsoil.2017.06.008>
- Alburquerque, J. A., Calero, J. M., Barrón, V., Torrent, J., Del Campillo, M. C., Gallardo, A., & Villar, R. (2014). Effects of biochars produced from different feedstocks on soil properties and sunflower growth. *Journal of Plant Nutrition and Soil Science*, 177, 16–25. <https://doi.org/10.1002/jpln.201200652>
- Al-Wabel, M. I., Al-Omran, A., El-Naggar, A. H., Nadeem, M., & Usman, A. R. (2013). Pyrolysis temperature induced changes in characteristics and chemical composition of biochar produced from conocarpus wastes. *Bioresource Technology*, 131, 374–379. <https://doi.org/10.1016/j.biortech.2012.12.165>
- Ambihai, S., & Gnanavelrajah, N. (2013). Improving soil productivity through charred biomass amendment to soil. *American-Eurasian Journal of Agricultural & Environmental Sciences*, 13, 1345–1350.
- Ameloot, N., Graber, E. R., Verheijen, F. G., & De Neve, S. (2013). Interactions between biochar stability and soil organisms: Review and research needs. *European Journal of Soil Science*, 64, 379–390. <https://doi.org/10.1111/ejss.12064>
- Anderson, J., & Domsch, K. (1978). A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biology and Biochemistry*, 10, 215–221. [https://doi.org/10.1016/0038-0717\(78\)90099-8](https://doi.org/10.1016/0038-0717(78)90099-8)
- Bamminger, C., Poll, C., & Marhan, S. (2018). Offsetting global warming-induced elevated greenhouse gas emissions from an arable soil by biochar application. *Global Change Biology*, 24, e318–e334. <https://doi.org/10.1111/gcb.13871>
- Bamminger, C., Zaiser, N., Zinsser, P., Lamers, M., Kammann, C., & Marhan, S. (2014). Effects of biochar, earthworms, and litter addition on soil microbial activity and abundance in a temperate agricultural soil. *Biology and Fertility of Soils*, 50, 1189–1200. <https://doi.org/10.1007/s00374-014-0968-x>
- Bargmann, I., Martens, R., Rillig, M. C., Kruse, A., & Kücke, M. (2014). Hydrochar amendment promotes microbial immobilization of mineral nitrogen. *Journal of Plant Nutrition and Soil Science*, 177, 59–67. <https://doi.org/10.1002/jpln.201300154>
- Bates, D., Maechler, M., Bolker, B., & Walker, S. (2017). Package lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-13.

- Bhatti, A. A., Haq, S., & Bhat, R. A. (2017). Actinomycetes benefaction role in soil and plant health. *Microbial Pathogenesis*, *111*, 458–467. <https://doi.org/10.1016/j.micpath.2017.09.036>
- Biederman, L. A., & Harpole, W. S. (2013). Biochar and its effects on plant productivity and nutrient cycling: A meta-analysis. *GCB Bioenergy*, *5*, 202–214. <https://doi.org/10.1111/gcbb.12037>
- Brundrett, M. C. (2002). Coevolution of roots and mycorrhizas of land plants. *New Phytologist*, *154*, 275–304. <https://doi.org/10.1046/j.1469-8137.2002.00397.x>
- Bruun, E. W., Müller-Stöver, D., Ambus, P., & Hauggaard-Nielsen, H. (2011). Application of biochar to soil and N₂O emissions: Potential effects of blending fast-pyrolysis biochar with anaerobically digested slurry. *European Journal of Soil Science*, *62*, 581–589. <https://doi.org/10.1111/j.1365-2389.2011.01377.x>
- Cantrell, K. B., Hunt, P. G., Uchimiya, M., Novak, J. M., & Ro, K. S. (2012). Impact of pyrolysis temperature and manure source on physicochemical characteristics of biochar. *Bioresource Technology*, *107*, 419–428. <https://doi.org/10.1016/j.biortech.2011.11.084>
- Castaldi, S., Rioldino, M., Baronti, S., Esposito, F. R., Marzaioli, R., Rutigliano, F. A., Vaccari, F. P., & Miglietta, F. (2011). Impact of biochar application to a Mediterranean wheat crop on soil microbial activity and greenhouse gas fluxes. *Chemosphere*, *85*, 1464–1471. <https://doi.org/10.1016/j.chemosphere.2011.08.031>
- Castrillo, G., Teixeira, P. J., Paredes, S. H., Law, T. F., de Lorenzo, L., Feltcher, M. E., Finkel, O. M., Breakfield, N. W., Mieczkowski, P., Jones, C. D., & Paz-Ares, J. (2017). Root microbiota drive direct integration of phosphate stress and immunity. *Nature*, *543*, 513–518. <https://doi.org/10.1038/nature21417>
- Chen, L., Jiang, Y., Liang, C., Luo, Y., Xu, Q., Han, C., Zhao, Q., & Sun, B. (2019). Competitive interaction with keystone taxa induced negative priming under biochar amendments. *Microbiome*, *7*, 77. <https://doi.org/10.1186/s40168-019-0693-7>
- Chen, X. L., & Chen, H. Y. H. (2019). Plant diversity loss reduces soil respiration across terrestrial ecosystems. *Global Change Biology*, *25*, 1482–1492. <https://doi.org/10.1111/gcb.14567>
- Cheng, B.-H., Zeng, R. J., & Jiang, H. (2017). Recent developments of post-modification of biochar for electrochemical energy storage. *Bioresource Technology*, *246*, 224–233. <https://doi.org/10.1016/j.biortech.2017.07.060>
- Chintala, R., Schumacher, T. E., Kumar, S., Malo, D. D., Rice, J. A., Bleakley, B., Chilom, G., Clay, D. E., Julson, J. L., Papiernik, S. K., & Gu, Z. R. (2014). Molecular characterization of biochars and their influence on microbiological properties of soil. *Journal of Hazardous Materials*, *279*, 244–256. <https://doi.org/10.1016/j.jhazmat.2014.06.074>
- Cohen, J., Cohen, P., West, S. G., & Aiken, L. S. (2003). *Applied multiple regression/correlation analysis for the behavioral sciences* (3rd ed.). Routledge.
- Dai, Z. M., Enders, A., Rodrigues, J. L. M., Hanley, K. L., Brookes, P. C., Xu, J. M., & Lehmann, J. (2018). Soil fungal taxonomic and functional community composition as affected by biochar properties. *Soil Biology and Biochemistry*, *126*, 159–167. <https://doi.org/10.1016/j.soilbio.2018.09.001>
- Dai, Z. M., Hu, J. J., Xu, X. K., Zhang, L. J., Brookes, P. C., He, Y., & Xu, J. M. (2016). Sensitive responders among bacterial and fungal microbiome to pyrogenic organic matter (biochar) addition differed greatly between rhizosphere and bulk soils. *Scientific Reports*, *6*. <https://doi.org/10.1038/srep36101>
- Demisie, W., Liu, Z., & Zhang, M. (2014). Effect of biochar on carbon fractions and enzyme activity of red soil. *Catena*, *121*, 214–221. <https://doi.org/10.1016/j.catena.2014.05.020>
- Dil, M., Oelbermann, M., & Xue, W. (2014). An evaluation of biochar pre-conditioned with urea ammonium nitrate on maize (*Zea mays* L.) production and soil biochemical characteristics. *Canadian Journal of Soil Science*, *94*, 551–562. <https://doi.org/10.4141/cjss-2014-010>
- Domene, X., Hanley, K., Enders, A., & Lehmann, J. (2015). Short-term mesofauna responses to soil additions of corn stover biochar and the role of microbial biomass. *Applied Soil Ecology*, *89*, 10–17. <https://doi.org/10.1016/j.apsoil.2014.12.005>
- Edenborn, S. L., Johnson, L. M. K., Edenborn, H. M., Albarran-Jack, M. R., & Demetron, L. D. (2017). Amendment of a hardwood biochar with compost tea: Effects on plant growth, insect damage and the functional diversity of soil microbial communities. *Biological Agriculture & Horticulture*, *34*, 88–106. <https://doi.org/10.1080/01448765.2017.1388847>
- Elmer, W. H., & Pignatello, J. J. (2011). Effect of biochar amendments on mycorrhizal associations and Fusarium crown and root rot of asparagus in replant soils. *Plant Disease*, *95*, 960–966. <https://doi.org/10.1094/pdis-10-10-0741>
- Fernandez, J. M., Nieto, M. A., Lopez-De-Sa, E. G., Gasco, G., Mendez, A., & Plaza, C. (2014). Carbon dioxide emissions from semi-arid soils amended with biochar alone or combined with mineral and organic fertilizers. *Science of the Total Environment*, *482–483*, 1–7. <https://doi.org/10.1016/j.scitotenv.2014.02.103>
- Fierer, N., Schimel, J. P., & Holden, P. A. (2003). Variations in microbial community composition through two soil depth profiles. *Soil Biology and Biochemistry*, *35*, 167–176. [https://doi.org/10.1016/s0038-0717\(02\)00251-1](https://doi.org/10.1016/s0038-0717(02)00251-1)
- Galazka, A., Jonczyk, K., Gawryjolek, K., & Ciepiel, J. (2019). The impact of biochar doses on soil quality and microbial functional diversity. *BioResources*, *14*, 7852–7868.
- Garimella, R., Halye, J. L., Harrison, W., Klebba, P. E., & Rice, C. V. (2009). Conformation of the phosphate D-alanine zwitterion in bacterial teichoic acid from nuclear magnetic resonance spectroscopy. *Biochemistry*, *48*, 9242–9249. <https://doi.org/10.1021/bi900503k>
- Gondek, K., Mierzwa-Hersztek, M., Kopeć, M., Sikora, J., Głab, T., & Szczurowska, K. (2019). Influence of biochar application on reduced acidification of sandy soil, increased cation exchange capacity, and the content of available forms of K, Mg, and P. *Polish Journal of Environmental Studies*, *28*, 103–111. <https://doi.org/10.15244/pjoes/81688>
- Grace, J. B. (2006). *Structural equation modeling and natural systems*. Cambridge University Press.
- Hagemann, N., Joseph, S., Schmidt, H.-P., Kammann, C. I., Harter, J., Borch, T., Young, R. B., Varga, K., Taherymoosavi, S., Elliott, K. W., & McKenna, A. (2017). Organic coating on biochar explains its nutrient retention and stimulation of soil fertility. *Nature Communications*, *8*, 1–11. <https://doi.org/10.1038/s41467-017-01123-0>
- Hamid, A. A., Ariffin, S., & Mohamad, S. A. S. (2015). Identification and optimal growth conditions of actinomycetes isolated from mangrove environment. *The Malaysian Journal of Analytical Sciences*, *19*, 904–910.
- Hardy, B., Sleutel, S., Dufey, J. E., & Cornelis, J.-T. (2019). The long-term effect of biochar on soil microbial abundance, activity and community structure is overwritten by land management. *Frontiers in Environmental Science*, *7*, 110. <https://doi.org/10.3389/fenvs.2019.00110>

- Hashem, A., Kumar, A., Al-Dbass, A. M., Alqarawi, A. A., Al-Arjani, A. B. F., Singh, G., Farooq, M., & Abd_Allah, E. F. (2019). Arbuscular mycorrhizal fungi and biochar improves drought tolerance in chickpea. *Saudi Journal of Biological Sciences*, 26, 614–624. <https://doi.org/10.1016/j.sjbs.2018.11.005>
- Hedges, L. V., Gurevitch, J., & Curtis, P. S. (1999). The meta-analysis of response ratios in experimental ecology. *Ecology*, 80, 1150–1156.
- Hmid, A., Al Chami, Z., Sillen, W., De Vocht, A., & Vangronsveld, J. (2015). Olive mill waste biochar: A promising soil amendment for metal immobilization in contaminated soils. *Environmental Science and Pollution Research International*, 22, 1444–1456. <https://doi.org/10.1007/s11356-014-3467-6>
- Hu, L., Cao, L., & Zhang, R. (2014). Bacterial and fungal taxon changes in soil microbial community composition induced by short-term biochar amendment in red oxidized loam soil. *World Journal of Microbiology Biotechnology*, 30, 1085–1092. <https://doi.org/10.1007/s11274-013-1528-5>
- Igalavithana, A. D., Lee, S.-E., Lee, Y. H., Tsang, D. C., Rinklebe, J., Kwon, E. E., & Ok, Y. S. (2017). Heavy metal immobilization and microbial community abundance by vegetable waste and pine cone biochar of agricultural soils. *Chemosphere*, 174, 593–603. <https://doi.org/10.1016/j.chemosphere.2017.01.148>
- Jenkins, J. R., Viger, M., Arnold, E. C., Harris, Z. M., Ventura, M., Miglietta, F., Girardin, C., Edwards, R. J., Rumpel, C., Fornasier, F., & Zavalloni, C. (2017). Biochar alters the soil microbiome and soil function: Results of next-generation amplicon sequencing across Europe. *GCB Bioenergy*, 9, 591–612. <https://doi.org/10.1111/gcbb.12371>
- Jiang, X., Deneff, K., Stewart, C. E., & Cotrufo, M. F. (2015). Controls and dynamics of biochar decomposition and soil microbial abundance, composition, and carbon use efficiency during long-term biochar-amended soil incubations. *Biology and Fertility of Soils*, 52, 1–14. <https://doi.org/10.1007/s00374-015-1047-7>
- Jucker, T., Hardwick, S. R., Both, S., Elias, D. M., Ewers, R. M., Milodowski, D. T., Swinfield, T., & Coomes, D. A. (2018). Canopy structure and topography jointly constrain the microclimate of human modified tropical landscapes. *Global Change Biology*, 24, 5243–5258. <https://doi.org/10.1111/gcb.14415>
- Karhu, K., Auffret, M. D., Dungait, J. A., Hopkins, D. W., Prosser, J. I., Singh, B. K., Subke, J. A., Wookey, P. A., Ågren, G. I., Sebastia, M. T., & Gouriveau, F. (2014). Temperature sensitivity of soil respiration rates enhanced by microbial community response. *Nature*, 513, 81–84. <https://doi.org/10.1038/nature13604>
- Keiblinger, K. M., Liu, D., Mentler, A., Zehetner, F., & Zechmeister-Boltenstern, S. (2015). Biochar application reduces protein sorption in soil. *Organic Geochemistry*, 87, 21–24. <https://doi.org/10.1016/j.orggeochem.2015.06.005>
- Lehmann, J., Rillig, M. C., Thies, J., Masiello, C. A., Hockaday, W. C., & Crowley, D. (2011). Biochar effects on soil biota – A review. *Soil Biology and Biochemistry*, 43, 1812–1836. <https://doi.org/10.1016/j.orggeochem.2015.06.005>
- Lehmann, J., & Rondon, M. (2006). Bio-char soil management on highly weathered soils in the humid tropics. *Biological Approaches to Sustainable Soil Systems*, 113, e530.
- Liao, N., Li, Q., Zhang, W., Zhou, G., Ma, L., Min, W., Ye, J., & Hou, Z. (2016). Effects of biochar on soil microbial community composition and activity in drip-irrigated desert soil. *European Journal of Soil Biology*, 72, 27–34. <https://doi.org/10.1016/j.ejsobi.2015.12.008>
- Liu, C., Wang, H., Tang, X., Guan, Z., Reid, B. J., Rajapaksha, A. U., Ok, Y. S., & Sun, H. (2016). Biochar increased water holding capacity but accelerated organic carbon leaching from a sloping farmland soil in China. *Environmental Science and Pollution Research*, 23, 995–1006. <https://doi.org/10.1007/s11356-015-4885-9>
- Liu, S., Zhang, Y., Zong, Y., Hu, Z., Wu, S., Zhou, J., Jin, Y., & Zou, J. (2016). Response of soil carbon dioxide fluxes, soil organic carbon and microbial biomass carbon to biochar amendment: A meta-analysis. *GCB Bioenergy*, 8, 392–406. <https://doi.org/10.1111/gcbb.12265>
- Lu, H., Li, Z., Fu, S., Méndez, A., Gascó, G., & Paz-Ferreiro, J. (2015). Effect of biochar in cadmium availability and soil biological activity in an anthrosol following acid rain deposition and aging. *Water, Air, & Soil Pollution*, 256. <https://doi.org/10.1007/s11270-015-2401-y>
- Luo, S., Wang, S., Tian, L., Li, S., Li, X., Shen, Y., & Tian, C. (2017). Long-term biochar application influences soil microbial community and its potential roles in semiarid farmland. *Applied Soil Ecology*, 117–118, 10–15. <https://doi.org/10.1016/j.apsoil.2017.04.024>
- Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q., & Brookes, P. C. (2011). Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. *Soil Biology and Biochemistry*, 43, 2304–2314. <https://doi.org/10.1016/j.soilbio.2011.07.020>
- Ma, Z., & Chen, H. Y. H. (2016). Effects of species diversity on fine root productivity in diverse ecosystems: A global meta-analysis. *Global Ecology and Biogeography*, 25, 1387–1396. <https://doi.org/10.1111/geb.12488>
- Marris, E. (2006). Putting the carbon back: Black is the new green. *Nature*, 442, 624–626.
- Meynet, P., Moliterni, E., Davenport, R. J., Sloan, W. T., Camacho, J. V., & Werner, D. (2014). Predicting the effects of biochar on volatile petroleum hydrocarbon biodegradation and emanation from soil: A bacterial community finger-print analysis inferred modelling approach. *Soil Biology and Biochemistry*, 68, 20–30. <https://doi.org/10.1016/j.soilbio.2013.09.015>
- Miransari, M. (2011). Interactions between arbuscular mycorrhizal fungi and soil bacteria. *Applied Microbiology and Biotechnology*, 89, 917–930. <https://doi.org/10.1007/s00253-010-3004-6>
- Mitchell, P. J., Simpson, A. J., Soong, R., & Simpson, M. J. (2015). Shifts in microbial community and water-extractable organic matter composition with biochar amendment in a temperate forest soil. *Soil Biology and Biochemistry*, 81, 244–254. <https://doi.org/10.1016/j.soilbio.2014.11.017>
- Moeskops, B., Buchan, D., Sleutel, S., Herawaty, L., Husen, E., Saraswati, R., Setyorini, D., & De Neve, S. (2010). Soil microbial communities and activities under intensive organic and conventional vegetable farming in West Java, Indonesia. *Applied Soil Ecology*, 45, 112–120. <https://doi.org/10.1016/j.apsoil.2010.03.005>
- Muhammad, N., Brookes, P. C., & Wu, J. (2016). Addition impact of biochar from different feed stocks on microbial community and available concentrations of elements in a Psammaquent and a Plinthudult. *Journal of Soil Science and Plant Nutrition*, 16, 137–153.
- Nelissen, V., Ruyschaert, G., Manka'abusi, D., D'Hose, T., De Beuf, K., Al-Barri, B., Cornelis, W., & Boeckx, P. (2015). Impact of a woody biochar on properties of a sandy loam soil and spring barley during a two-year field experiment. *European Journal of Agronomy*, 62, 65–78. <https://doi.org/10.1016/j.eja.2014.09.006>
- Nkonya, E., Mirzabaev, A., & Von Braun, J. (2016). *Economics of land degradation and improvement: A global assessment for sustainable development*. Springer International Publishing.

- Paz-Ferreiro, J., Liang, C., Fu, S., Mendez, A., & Gasco, G. (2015). The effect of biochar and its interaction with the earthworm *Pontoscolex corethrurus* on soil microbial community structure in tropical soils. *PLoS One*, 10, e0124891. <https://doi.org/10.1371/journal.pone.0124891>
- Pittelkow, C. M., Liang, X., Linquist, B. A., Van Groenigen, K. J., Lee, J., Lundy, M. E., Van Gestel, N., Six, J., Venterea, R. T., & Van Kessel, C. (2015). Productivity limits and potentials of the principles of conservation agriculture. *Nature*, 517, 365–368. <https://doi.org/10.1038/nature13809>
- Rillig, M. C., Wagner, M., Salem, M., Antunes, P. M., George, C., Ramke, H. G., Titirici, M. M., & Antonietti, M. (2010). Material derived from hydrothermal carbonization: Effects on plant growth and arbuscular mycorrhiza. *Applied Soil Ecology*, 45, 238–242. <https://doi.org/10.1016/j.apsoil.2010.04.011>
- Rosseel, Y. (2012). Lavaan: An R package for structural equation modeling and more. Version 0.5–12 (BETA). *Journal of Statistical Software*, 48, 1–36.
- Schutter, M. E., & Dick, R. P. (2000). Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. *Soil Science Society of America Journal*, 64, 1659–1668. <https://doi.org/10.2136/sssaj2000.6451659x>
- Singh, B. P., & Cowie, A. L. (2014). Long-term influence of biochar on native organic carbon mineralisation in a low-carbon clayey soil. *Scientific Reports*, 4, 3687. <https://doi.org/10.1038/srep03687>
- Smith, P. (2016). Soil carbon sequestration and biochar as negative emission technologies. *Global Change Biology*, 22, 1315–1324. <https://doi.org/10.1111/gcb.13178>
- Steinbeiss, S., Gleixner, G., & Antonietti, M. (2009). Effect of biochar amendment on soil carbon balance and soil microbial activity. *Soil Biology and Biochemistry*, 41, 1301–1310. <https://doi.org/10.1016/j.soilbio.2009.03.016>
- Taskin, E., Brana, M. T., Altomare, C., & Loffredo, E. (2019). Biochar and hydrochar from waste biomass promote the growth and enzyme activity of soil-resident ligninolytic fungi. *Heliyon*, 5. <https://doi.org/10.1016/j.heliyon.2019.e02051>
- Tian, J., Wang, J., Dippold, M., Gao, Y., Blagodatskaya, E., & Kuzyakov, Y. (2016). Biochar affects soil organic matter cycling and microbial functions but does not alter microbial community structure in a paddy soil. *Science of the Total Environment*, 556, 89–97. <https://doi.org/10.1016/j.scitotenv.2016.03.010>
- Vanderwolf, K. J., Malloch, D., Mcalpine, D. F., & Forbes, G. J. (2013). A world review of fungi, yeasts, and slime molds in caves. *International Journal of Speleology*, 42, 77–96. <https://doi.org/10.5038/1827-806x.42.1.9>
- Vile, D., Shipley, B., & Garnier, E. (2006). A structural equation model to integrate changes in functional strategies during old-field succession. *Ecology*, 87, 504–517. <https://doi.org/10.1890/05-0822>
- Vylkova, S. (2017). Environmental pH modulation by pathogenic fungi as a strategy to conquer the host. *PLoS Pathogens*, 13. <https://doi.org/10.1371/journal.ppat.1006149>
- Wang, S., & Huang, Y. (2020). Determinants of soil organic carbon sequestration and its contribution to ecosystem carbon sinks of planted forests. *Global Change Biology*, 26, 3163–3173. <https://doi.org/10.1111/gcb.15036>
- Warnock, D. D., Lehmann, J., Kuyper, T. W., & Rillig, M. C. (2007). Mycorrhizal responses to biochar in soil—Concepts and mechanisms. *Plant and Soil*, 300, 9–20. <https://doi.org/10.1007/s11104-007-9391-5>
- Warnock, D. D., Mummey, D. L., McBride, B., Major, J., Lehmann, J., & Rillig, M. C. (2010). Influences of non-herbaceous biochar on arbuscular mycorrhizal fungal abundances in roots and soils: Results from growth-chamber and field experiments. *Applied Soil Ecology*, 46, 450–456. <https://doi.org/10.1016/j.apsoil.2010.09.002>
- Watzinger, A., Feichtmair, S., Kitzler, B., Zehetner, F., Kloss, S., Wimmer, B., Zechmeister-Boltenstern, S., & Soja, G. (2014). Soil microbial communities responded to biochar application in temperate soils and slowly metabolized ¹³C-labelled biochar as revealed by ¹³C PLFA analyses: Results from a short-term incubation and pot experiment. *European Journal of Soil Science*, 65, 40–51. <https://doi.org/10.1111/ejss.12100>
- Weng, Z. H., Van Zwieten, L., Singh, B. P., Tavakkoli, E., Joseph, S., Macdonald, L. M., Rose, T. J., Rose, M. T., Kimber, S. W., Morris, S., & Cozzolino, D. (2017). Biochar built soil carbon over a decade by stabilizing rhizodeposits. *Nature Climate Change*, 7, 371–376. <https://doi.org/10.1038/nclimate3276>
- Whitman, W. B., Coleman, D. C., & Wiebe, W. J. (1998). Prokaryotes: The unseen majority. *Proceedings of the National Academy of Sciences of the United States of America*, 95, 6578–6583. <https://doi.org/10.1073/pnas.95.12.6578>
- Xu, W., Deng, F., Shuiyuan, F., Wang, G., Ruan, H., & Guohua, C. (2016). Effect of biochar on soil microbial biomass and the diversity of carbon source metabolism in poplar plantation. *Journal of Nanjing Forestry University (Natural Sciences Edition)*, 40, 14–20.
- Xu, W., Wang, G., Deng, F., Zou, X., Ruan, H., & Chen, H. (2018). Responses of soil microbial biomass, diversity and metabolic activity to biochar applications in managed poplar plantations on reclaimed coastal saline soil. *Soil Use and Management*, 34, 597–605. <https://doi.org/10.1111/sum.12460>
- Xu, W., Zhang, Y., Wang, G., & Ruan, H. (2015). Response of carbon metabolism by soil microbes to different fertilization regimes in a poplar plantation in coastal area of northern Jiangsu, China. *Chinese Journal of Ecology*, 34, 1791–1797.
- Zak, J. C., Willig, M. R., Moorhead, D. L., & Wildman, H. G. (1994). Functional diversity of microbial communities: A quantitative approach. *Soil Biology and Biochemistry*, 26, 1101–1108. [https://doi.org/10.1016/0038-0717\(94\)90131-7](https://doi.org/10.1016/0038-0717(94)90131-7)
- Zhang, L., Jing, Y., Xiang, Y., Zhang, R., & Lu, H. (2018). Responses of soil microbial community structure changes and activities to biochar addition: A meta-analysis. *Science of the Total Environment*, 643, 926–935. <https://doi.org/10.1016/j.scitotenv.2018.06.231>
- Zhang, T. A., Chen, H. Y., & Ruan, H. (2018). Global negative effects of nitrogen deposition on soil microbes. *The ISME Journal*, 12, 1817–1825. <https://doi.org/10.1038/s41396-018-0096-y>
- Zhang, Y., Peng, S., Song, Q., Wenwen, Z., Jiaojiao, G., Wang, G., & Ruan, H. (2016). Effects of different fertilizers regimes on the functional diversity of soil microbes under poplar plantation. *Journal of Nanjing Forestry University (Natural Sciences Edition)*, 40, 1–8.
- Zheng, H., Wang, X., Luo, X., Wang, Z., & Xing, B. (2018). Biochar-induced negative carbon mineralization priming effects in a coastal wetland soil: Roles of soil aggregation and microbial modulation. *Science of the Total Environment*, 610, 951–960. <https://doi.org/10.1016/j.scitotenv.2017.08.166>
- Zhou, H., Zhang, D., Wang, P., Liu, X., Cheng, K., Li, L., Zheng, J., Zhang, X., Zheng, J., Crowley, D., & van Zwieten, L. (2017). Changes in microbial biomass and the metabolic quotient with biochar addition to agricultural soils: A Meta-analysis. *Agriculture, Ecosystems Environment*, 239, 80–89. <https://doi.org/10.1016/j.agee.2017.01.006>

- Zhou, Z., Wang, C., Zheng, M., Jiang, L., & Luo, Y. (2017). Patterns and mechanisms of responses by soil microbial communities to nitrogen addition. *Soil Biology and Biochemistry*, 115, 433–441. <https://doi.org/10.1016/j.soilbio.2017.09.015>
- Zhu, L. X., Xiao, Q., Shen, Y. F., & Li, S. Q. (2017). Microbial functional diversity responses to 2 years since biochar application in silt-loam soils on the Loess Plateau. *Ecotoxicology and Environmental Safety*, 144, 578–584. <https://doi.org/10.1016/j.ecoenv.2017.06.075>
- Zimmerman, A. R., Gao, B., & Ahn, M.-Y. (2011). Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. *Soil Biology Biochemistry*, 43, 1169–1179. <https://doi.org/10.1016/j.soilbio.2011.02.005>

SUPPORTING INFORMATION

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

How to cite this article: Xu W, Whitman WB, Gundale MJ, Chien C-C, Chiu C-Y. Functional response of the soil microbial community to biochar applications. *GCB Bioenergy*. 2020;00:1–13. <https://doi.org/10.1111/gcbb.12773>