Impact of biochar and lignite-based amendments on microbial communities and greenhouse gas emissions from agricultural soil

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Abstract
Understanding the responses of the microbial community and greenhouse gas (GHG) emissions to the incorporation of different organic amendments is essential for their proper utilization. In this study, laboratory-incubated microcosm experiments were conducted to investigate the short-term effects of pine-wood biochar and lignite-based amendment on the microbial communities and GHG emissions from agricultural soil. Soils amended at five different application rates were incubated for 19 d under the conditions of 60% water-filled pore space and 25 °C. Microbial biomass in the amended soil after incubation was measured by the solid colony counting method, and the soil microbial diversity was assayed using a Biolog EcoPlate. The biochar and lignite-based amendment had distinct effects on the soil microbial communities and GHG emissions. The microbial community growth and utilization of C sources were improved by the biochar but restrained by the lignite-based amendment in most cases. The biochar and lignite-based amendment had a minor impact on methane emissions. Carbon dioxide emissions were promoted by the biochar and inhibited by the lignite-based amendment during the short-term incubation period. Nitrous oxide emissions decreased with the application rate of biochar but increased with the rate of lignite-based amendment. The addition of biochar at a rate of 3–4% and lignite-based amendment at a rate of <1% has the potential to improve soil quality. Salt leaching is required to avoid accumulation when the biochar and lignite-based amendments are applied. The findings can provide a reference for the application of biochar and lignite-based amendment in silt loam soil.

1 INTRODUCTION

Agricultural development in arid and semiarid regions is threatened by soil salinization, fertility limitations, and water shortages (Sakadevan & Nguyen, 2010). In order to obtain high crop yields and meet the projected increase in human food demand, excessive mineral fertilizers are frequently applied in these regions. However, excessive application of...
Organic amendments are most frequently used to rebuild the soil organic matter content, provide essential nutrients (such as N, P, and K), and reestablish microbial populations....
The application of biochar to soils changes the soil physicochemical properties and stimulates the activities of soil microorganisms (Lehmann et al., 2011). Biochar amendment changes microbial habitats, directly or indirectly affects microbial metabolic activities, and modifies the soil microbial community in terms of abundance and structure (Palansooriya et al., 2019). The addition of biochar to soil often results in positive effects on the abundance of fungi (Warnock et al., 2007), but negative effects have also been observed in biochar-amended soil (Chen et al., 2016). Biochar-amended soils have also shown significant changes in the community composition and diversity of fungal, bacterial, and archaeal populations. Xu et al. (2014) found that biochar application significantly increases the diversity of soil bacteria and changes the relative abundance of some microbes related with carbon and nitrogen turnover. Q. Li et al. (2018) reported that biochar amendment increases bacterial diversity but decreases soil microbial biomass. Senbayram et al. (2019) found that biochar addition has a more distinct influence on the bacterial community composition in sandy soil than in clay soil. However, no discernible differences in the bacterial community structure or even lower diversity of archaea and fungi were found in the biochar-amended soil (Anderson et al., 2014). Therefore, the effect of biochar on microbial performance is determined by a variety of physical and chemical properties of biochar, as it may provide different habitats for microorganisms (Palansooriya et al., 2019). In contrast, research related to the influence of lignite-based amendments on microbial communities is lacking and not well understood. The incorporation of lignite-based amendments in soil may enhance microbial activity by helping to increase in soil moisture content and providing additional organic C as a substrate for microorganisms. Clouard et al. (2014) reported that the microbiological diversity in lignite-rich soil is lower than that in soil without lignite. However, Rumpel et al. (2001) reported that the presence of lignite does not have a significant impact on the microbial biomass in lignite-containing mine soils. Tran et al. (2015) found that lignite amendment has limited impacts on soil microbial communities, and lignite together with N fertilizer does not significantly stimulate microbial activities.

Agricultural soil-borne GHG emissions are primarily released through plant and microbial processes and affected by soil physical, chemical, and biological properties. Biochar and its storage in soils have been heralded as a solution to mitigate GHG emissions by sequestering C and simultaneously providing environmental and agricultural benefits (Zhang et al., 2019). Karhu et al. (2011) reported that biochar addition to agricultural soil increases CH₄ uptake. Case et al. (2015) found that biochar suppresses N₂O emissions while maintaining N availability in sandy loam soil. Xu et al. (2014) also found that biochar reduces N₂O emissions by stimulating both nitrification and denitrification. Woolf et al. (2010) estimated that biochar can sustainably offset 7–12% of anthropogenic GHG emissions on an annual basis without endangering food security or habitat or soil conservation. They clarified that the annual net emissions of CO₂, CH₄, and N₂O can be reduced by a maximum of 1.8 Pg CO₂-C equivalent (CO₂-Ce), and total net emissions can be reduced over the course of a century by 130 Pg CO₂-Ce. In contrast, Verhoeven and Six (2014) debated whether biochar mitigated field-scale N₂O emissions in a northern California vineyard. Senbayram et al. (2019) reported that the application of alkaline biochar to acidic soils can potentially increase N₂O and CO₂ emissions. There are few reports on GHG emissions from soil reclaimed by lignite-based amendments. Tran et al. (2015) found that CO₂ emissions from soils amended with lignite are inhibited in the short term.

Although a variety of studies have been conducted to investigate the effects of organic amendments on soil functions, the mechanisms of the effects on soil microbial activities and GHG emissions are still not well understood, in particular for the lignite-based amendments. The performance of amendments also varies with their feedstock, processing, and application rate, as well as with the soil type and in situ conditions. The objective of this study was to investigate the impacts of biochar and a lignite-based amendment on microbial communities and GHG emissions from agricultural soil through laboratory-incubated microcosm experiments. We hypothesize that the lignite-based amendment and biochar may have distinct effects on soil microorganisms and GHG emissions even though they have a variety of common properties. The specific purposes were (a) to investigate the effects of biochar and a lignite-based amendment on soil properties; (b) to understand the impact of the biochar and a lignite-based amendment on soil microbial communities and the diversity of microbial community functions; (c) to explore the influence of the biochar and a lignite-based amendment on GHG emissions from soil; and (d) to analyze the relationships between soil microbial activities and GHG emissions from soil with different amendments.

2 | MATERIALS AND METHODS

2.1 | Physicochemical properties of soil, biochar, and lignite-based amendment

The soil used in the incubation experiment was sampled from a maize field in the Hetao Irrigation District located in the upper reaches of the Yellow River, China. Research on the effects of irrigation and fertilization regimes on grain yield, water, N productivity, and soil-borne GHG emissions from a mulched cultivated maize field has been conducted in the sampling site (Li, Xiong, Cui et al., 2020; Li, Xiong, Huang et al., 2020). Soil was sampled from the experimental plots at
TABLE 1 The physical and chemical properties of the tested soil, biochar and lignite-based amendment

| Property                      | Soil   | Biochar | Lignite-based amendment |
|-------------------------------|--------|---------|-------------------------|
| Sand, %                       | 13.4   | –       | –                       |
| Silt, %                       | 72.6   | –       | –                       |
| Clay, %                       | 14.0   | –       | –                       |
| Texture                       | Silt loam | –     | –                       |
| Bulk density, g cm\(^{-3}\)  | 1.40   | 0.161   | –                       |
| Field capacity, cm\(^{-3}\)  | 0.32   | –       | –                       |
| pH                            | 6.17   | 7.32    | 8.48                    |
| Electric conductivity, mS cm\(^{-1}\) | 0.42 | 8.47 | 30.08 |
| Total N, %                    | 0.12   | 0.56    | 3.09                    |
| Total C, %                    | 2.06   | 43.06   | 36.63                   |
| C/N ratio                     | 16.74  | 76.46   | 11.86                   |
| \(\text{NO}_3^-\)-N, mg kg\(^{-1}\) | 22.81 | 690.4   | 2.811.15                |
| \(\text{NH}_4^+\)-N, mg kg\(^{-1}\) | 2.36 | 3.65 | 9.28 |
| Organic matter, g kg\(^{-1}\) | 22.2   | 675.7   | 976.0                   |
| Available P, mg kg\(^{-1}\)  | 14.4   | –       | –                       |
| Available K, mg kg\(^{-1}\)  | 641.66 | –       | –                       |

a depth of 0–20 cm. The undecomposed litter was removed prior to soil sampling. The sampled soils were mixed, air dried, passed through a 2-mm sieve, and stored at an ambient temperature (around 20 °C).

The tested soil is classified as anthropogenic-alluvial soil according to Chinese Soil Classification and Terminology (Shi et al., 2010). Texture analysis showed that the soil was silt loam according to the U.S. soil texture triangle. Two different amendments (i.e., biochar and lignite-based amendment) were separately added to the soil. The soil organic matter content was approximately 22.2 g kg\(^{-1}\) prior to the addition of amendments. The biochar was created from pine wood. The chips of pine wood were placed in ceramic crucibles, each covered with a fitting lid, and pyrolyzed in a muffle furnace under oxygen-limited conditions. The pyrolysis temperature was raised to 500 °C at a rate of 20 °C min\(^{-1}\), and the temperature was then held at 500 °C for 4 h. The lignite-based amendment was produced by the Apaxfon Biological Science and Technologies Company in China. Both the biochar and lignite-based amendment were air dried and passed through 1-mm sieves. The detailed physicochemical properties of the soil, biochar, and lignite-based amendment are presented in Table 1.

2.2 Incubation experiments

Incubation experiments were conducted to examine the responses of the microbial community and GHG emissions to the addition of the biochar and lignite-based amendment, respectively. The biochar and lignite-based amendment were separately and uniformly mixed with soil at weight proportions of 1, 2, 3, 4, and 5%, respectively. The addition quantities of these treatments were approximately equivalent to the application rates of 14, 28, 42, 56, and 70 t ha\(^{-1}\) in the field, respectively. Soil without any amendment was considered the control. Soil with different quantities of amendment was packed into a flask with a volume of 650 ml. Each flask was filled with 210 g of soil mixture, and the packing bulk density was 1.4 g cm\(^{-3}\), which was consistent with the bulk density in the field. Three replicates were conducted for each treatment. Deionized water was uniformly sprayed on the soil surface using a syringe to increase the soil water content to 40% water-filled pore space (WFPS; i.e., degree of saturation). The WFPS was calculated using the following equation:

\[
\text{WFPS} = \left( \frac{\omega \rho_b}{f \rho_w} \right) \times 100\% \quad (1)
\]

\[
f = \frac{\rho_s - \rho_b}{\rho_s} \quad (2)
\]

where \(\omega\) is the mass wetness (g\(^{-1}\)), \(\rho_b\) is the soil bulk density (g cm\(^{-3}\)), \(f\) is the soil porosity, \(\rho_s\) is the density of solids (2.65 g cm\(^{-3}\)), and \(\rho_w\) is the density of water (g cm\(^{-3}\)).

The flasks were sealed with Parafilm and pre-incubated in an incubator at 28 °C and a relative humidity of 50% for 7 d. Subsequent to the primary incubation, urea (46% N) fertilizer was dissolved in deionized water and uniformly applied to the soil surface at a rate of 0.32 g N kg\(^{-1}\). This
application was equal to the application of 250 kg ha\(^{-1}\) in the field. The final soil water content was controlled at 60% WFPS. All the flasks were incubated at 25 °C without cover except for the GHG sampling periods. The soil water content was controlled at 60% WFPS by adding deionized water through a syringe every day.

### 2.3 Greenhouse gas emissions

Gases were sampled from each flask twice with an interval of 45 min after 0.33, 0.67, 1, 2, 3, 4, 5, 7, 9, 11, 13, 15, and 19 d of incubation. Twenty milliliters gas was sampled each time. Gases were sampled using a polypropylene syringe and immediately injected into a gas container. The flask was closed during the sampling period. The concentrations of GHGs were examined within 48 h using a GC-2014 gas chromatograph (Shimadzu Scientific Instruments) equipped with an autosampler, thermal conductivity, flame ionization detector (FID), and electron capture detector (ECD). The oven temperature for gas chromatography was controlled at 50 °C. Nitrous oxide was detected with a \(^{63}\)Ni ECD at 300 °C. Methane and CO\(_2\) were detected by a FID at 200 °C.

The fluxes of CO\(_2\), N\(_2\)O, and CH\(_4\) were calculated by the slope of the linear regression between the GHG concentration and time, which is expressed as follows:

\[
F = \frac{d_c}{d_t} \cdot \frac{M}{V_0} \cdot \frac{273}{(273 + T)} \cdot V
\]  

where \(F\) is the emission flux of the gas (mg m\(^{-2}\) h\(^{-1}\)), \(d_c/d_t\) is the variation ratio of the measured gas, \(M\) is the molar mass of the gas (g mol\(^{-1}\)), \(T\) is the incubation temperature (K), \(V_0\) is the volume of measured gas under the standard condition (m\(^3\)), and \(V\) is the gas space volume of the flask (m\(^3\)).

The cumulative GHG emissions during the incubation were estimated by the following equations:

\[
G = (\bar{F} \cdot 24 \cdot d) / 100
\]

\[
\bar{F} = \sum_{i=1}^{n} \frac{F_i d_i}{d}
\]

where \(\bar{F}\) is the average emission flux of the incubation period (mg m\(^{-2}\) h\(^{-1}\)), \(d\) is the days of incubation, \(F_i\) is the measured gas emission flux for the \(i\)th sample (mg m\(^{-2}\) h\(^{-1}\)), and \(d_i\) is the number of days between the adjacent sampling events.

### 2.4 Soil biochemistry assay

The soil was collected to examine the biochemical properties after the termination of incubation. The soil electrical conductivity (EC) and pH were determined on a 1:5 saturated soil extract. The NH\(_4^+\)-N and NO\(_3^-\)-N contents of the soil were determined from 2 mol L\(^{-1}\) KCl extracts (soil to water ratio of 1:5) and measured using a continuous flow analyzer (AutoAnalyzer 3 HR, Seal Analytical). Soil organic matter was measured using the potassium dichromate volumetric-external heating method. The available K was examined using the ammonium acetate extraction-flame photometry, and the available P was measured using the sodium bicarbonate extraction-Mo-Sb colorimetry method (Pansu & Gautheyrou, 2006).

The soil microbial biomass was measured using the solid colony counting method. Ten grams of soil sample was suspended in 90 ml of sterile deionized water. Suspensions were shaken for 30 min in a vibrator and diluted with variable dilution factor according to the different types of microorganisms. Each dilution factor was determined on the basis of pre-incubation experiment. The diluted liquid was inoculated to different media by an automatic spiral plating system. The media were incubated 4–5 d in the dark at 28 °C, and then the colonies were observed. Fungi, bacteria, and actinomycetes were incubated in beef peptone agar medium, Martin’s medium, and modified Gao’s No. 1 medium, respectively. Nitrifying bacteria, NH\(_3\)-oxidizing bacteria, and denitrifying bacteria were selectively incubated in specific media for their physiological groups (Li et al., 2005).

The microbial community functional diversity of soil was assessed using a Biolog EcoPlate with 31 C sources (Garland, 1997). Soil samples were incubated for 24 h at 25 °C, and then 10 g of incubated soil was mixed with 90 ml of 0.85% (m/v) sterile NaCl solution. The mixture was shaken at 22 °C for 1 h at 200 rounds min\(^{-1}\). After 30 min of settling, 1 ml of supernatant was diluted in 9 ml of 0.85% sterile NaCl solution at 10-fold dilutions until a final 1:1000 dilution was reached (Feigl et al., 2017). Subsequently, 150 μl of suspension was added to the microplate wells and then incubated for 240 h at 25 °C in the dark. The absorbance value of 590 nm was examined every 24 h using the Biolog EcoPlate system (Biolog). All treatments had three replicates in each Biolog EcoPlate.

The microbial community functional diversity is expressed as the average well color development (AWCD). The calculation of AWCD is expressed as

\[
AWCD = \frac{\sum (C_i - R)}{n}
\]

where \(C_i\) is the optical density value at 590 nm for each well, \(R\) is the optical density value of the control group, and \(n\) is the number of C sources for the Biolog EcoPlate (31).

Substrate richness (S) was calculated by counting the total number of C substrates oxidized by individual treatments on the Biolog EcoPlate. Diversity parameters, namely the Shannon index (\(H\)), Shannon evenness index (\(E\)), Simpson index (\(D\)), and McIntosh index (\(U\)) were calculated when the value
The Shannon, Shannon evenness, Simpson, and McIntosh indices were calculated using the following equations (Staddon et al., 1997):

\[ H = - \sum p_i \ln(p_i) \]  

\[ E = \frac{H}{\ln(S)} \]  

\[ D = 1 - \sum p_i^2 \]  

\[ U = \sqrt{\sum n_i^2} \]

where \( p_i = \frac{C_i - R}{\sum(C_i - R)} \) is the ratio of the absorbance value of each well to the sum of the absorbance value of all wells, and \( n_i \) is the relative absorbance value of the \( i \)th well.

2.5 Data analysis

The daily GHG emissions, cumulative emissions, soil organic matter, soil nutrients, and diversity values were compared in different treatments by one-way ANOVA using SPSS package 20.0 (SPSS). The assumptions on normality of residuals and homogeneity of variance were checked firstly. Statistical differences among the treatments were analyzed by Duncan’s test at a significance level of \( P = .05 \). The AWCD data based on substrate utilization were analyzed using principal component analysis (PCA, Canoco 5.0).

3 RESULTS

3.1 Soil physical and chemical properties

The soil pH was 6.17 prior to incubation and reached 6.36 after incubation for the control. The soil pH nonlinearly increased with the addition of biochar and the lignite amendment (Figure 1). The soil pH of the lignite-amended soil was slightly higher (average of 0.17 units) than that of the corresponding biochar-amended soil.

The soil EC linearly increased with the application rate of biochar and the lignite-based amendment, but the magnitude (or slope) was evidently different. The increase in the EC of the soil amended with the lignite-based amendment was clearly larger than that of the biochar-amended soil. The soil EC reached 1,020.0 \( \mu \text{S cm}^{-1} \) and 2,396.7 \( \mu \text{S cm}^{-1} \) for the treatments with 5% biochar and lignite-based amendment, respectively. In contrast, the soil EC was 765.7 \( \mu \text{S cm}^{-1} \) for the control.

The soil organic matter also significantly \( (P < .05) \) increased with the addition of different amendments (Figure 2). The content of organic matter in the soil with lignite-based amendment was clearly higher than that with biochar after the incubation as organic matter content in the original lignite-based amendment (976.0 g kg\(^{-1}\)) was much higher than that in the biochar (675.7 g kg\(^{-1}\)). The soil organic matter contents reached 53.48 and 95.86 g kg\(^{-1}\) for the 5% biochar
FIGURE 2 Soil organic matter varied with the amount of amendments (a) biochar and (b) lignite-based amendment

and lignite-amended soils, respectively. In contrast, the soil organic matter content was 22.2 g kg\(^{-1}\) in the nontreated soil.

The total C, NO\(_3^–\)–N, NH\(_4^+\)–N, and available P and K in the soil after incubation with different treatments are presented in Table 2. The soil total C significantly (\(P < .05\)) increased with the addition of both the biochar and lignite-based amendment. The highest total C contents were 4.86 and 4.41% for the 5% biochar and lignite-based amendment treatments, respectively. The total N in the incubated soil was not significantly (\(P > .05\)) different for the different biochar treatments. The total N in the soils amended with different amounts of biochar varied from 0.18 to 0.20%, which was similar to that in the control (0.19%). In contrast, the soil total N significantly (\(P < .05\)) increased from 0.22 to 0.31% with the addition of the lignite-based amendment.

The soil NO\(_3^–\)–N content significantly (\(P < .05\)) increased as the application rate of biochar and lignite-based amendment increased. The increase in the soil NO\(_3^–\)–N content of the lignite-based amendment treatments was higher than that of the biochar treated one. Although the soil NH\(_4^+\)–N content in the biochar-treated soils was less than that in the control, the difference was not significant (\(P > .05\)). The addition of the lignite-based amendment had no significant effect (\(P > .05\)) on soil NH\(_4^+\)–N when the application rate was lower than 3%. However, the soil NH\(_4^+\)–N content significantly increased when the application rate was higher than 4%.

Soil available P generally increased with the application of biochar. However, the difference was not significant (\(P > .05\)) among different biochar treatments when the application rate was lower than 4%. Soil available P significantly (\(P < .05\)) increased with the addition of the lignite-based amendments. The available P in the soil amended with 5% lignite-based amendment was approximately fivefold higher than that in the control.

The addition of both biochar and lignite-based amendments increased the soil available K. However, statistical analysis indicated that the increase in soil available K in different biochar treatments was not significantly (\(P > .05\)) different from that in the control. A significant increase in soil available K was detected with the addition of lignite-based amendments. Soil available K increased from 1,005.43 mg kg\(^{-1}\) with the 1% lignite-based amendment to 2,058.42 mg kg\(^{-1}\) (5% lignite-based amendment).

3.2 Microbial communities in soils amended with the biochar and lignite-based amendment

Table 3 presents the categories and number of microorganisms in the soil after incubation with different additions of biochar and lignite-based amendment. The number of fungi in the biochar-amended soils generally increased with the increase in the biochar application rate, but decreased when the rate was beyond 4%. The largest fungi population (2.43 \(\times\) 10\(^4\) colony-forming units [CFU] g\(^{-1}\)) was obtained in the 3% biochar treatment. In contrast, the number of fungi cultures decreased with the addition of lignite-based amendment (except for in the 1% treatment), but the difference was not significant (\(P > .05\)). The number of fungi in 1% lignite-based amendment increased by 40.6%, whereas the number of fungi in the treatments with more than 1% lignite-based amendment was reduced on average by 54.1% compared with that of the control.
The number of actinomycetes nonmonotonically varied with the application rate of biochar. The addition of biochar with the ratio of 3 and 4% promoted the growth of actinomycetes. The number of actinomycetes in the other biochar treatments was less than that in the control. The maximum number of actinomycetes (9.09 × 10^5 CFU g^-1) was obtained in the 3% biochar treatment. The addition of the lignite-based amendment generally reduced the number of actinomycetes in the soil. The greater the addition of lignite-based amendments, the less the actinomycetes cultures grew. The number of actinomycetes cultures in the 5% lignite-based amendment treatment was reduced by 36% compared with that of the control.

The population of bacterial cultures also nonmonotonically increased with the increase in the biochar application rate. The bacteria populations in the 1 and 2% biochar treatments were lower than those in the control (1.34 × 10^6 CFU g^-1), and those in the 3–5% biochar treatments were significantly (P < .05) higher than that in the control. The largest bacteria population (1.97 × 10^6 CFU g^-1) was obtained in the 3% biochar treatment. The addition of the lignite-based amendment decreased the bacteria population for most treatments (except for the 2% treatment). The greater the addition of lignite-based amendment (>2%), the lower the bacteria population obtained in the soils. Soil amended with the 5% lignite-based amendment had only 37% of the population of bacteria compared with that in the control.

The number of nitrifying bacteria increased nonmonotonically with the application rate of biochar. The number of nitrifying bacteria increased by 15.1–82.2% in different biochar treatments compared with that in the control. The largest number of nitrifying bacteria was obtained in the 5% biochar treatment with 1.95 × 10^7 CFU g^-1. In contrast, the addition of the lignite-based amendment inhibited the growth of nitrifying bacteria. The number of nitrifying bacteria in all the lignite-based amendment treatments was less than that in the control. The lowest number of nitrifying bacteria was obtained in the treatment with 3% lignite-based amendment, and the highest number of nitrifying bacteria was obtained in the 1% lignite-based amendment.

The number of denitrifying bacteria was three orders of magnitude less than the number of nitrifying bacteria in different treatments. Denitrifying bacteria increased with the addition of biochar. The largest number of denitrifying bacteria was obtained in the 2% biochar treatment. The addition of lignite-based amendment had no significant (P > .05) effects on the denitrifying bacteria. The mean number of denitrifying bacteria was 5.98 × 10^4 CFU g^-1 in the soils amended with the lignite-based amendment.

Biochar addition significantly (P < .05) increased the number of soil NH₃-oxidizing bacteria. Soil treated with 4% biochar had the most NH₃-oxidizing bacteria. In contrast, the presence of the lignite-based amendment had limited impact.

| TABLE 2 | Soil total C, diverse forms of N, and available P and K after incubation for different amendment treatments (mean ± standard deviation) |
|---------|-------------------------------------------------------------------------------------------------|
| Treatments | Total C | NH₄⁺-N | NO₃⁻-N | Available P | Available K |
| Control | 15.75 ± 0.66f | 15.19 ± 0.01ef | 4.19 ± 0.01ef | 0.19 ± 0.01ef | 0.66f |
| Biochar (1%) | 16.96 ± 0.11e | 16.96 ± 0.01ef | 5.36 ± 0.04ed | 0.19 ± 0.01ef | 0.19 ± 0.01ef |
| Biochar (2%) | 18.00 ± 0.78ad | 21.33 ± 0.04b | 4.20 ± 0.01ef | 0.19 ± 0.01ef | 0.19 ± 0.01ef |
| Biochar (3%) | 17.43 ± 0.87d | 17.43 ± 0.01ef | 4.12 ± 0.01eb | 0.19 ± 0.01ef | 0.19 ± 0.01ef |
| Biochar (4%) | 15.54 ± 1.06d | 15.54 ± 0.02ed | 4.12 ± 0.01eb | 0.19 ± 0.01ef | 0.19 ± 0.01ef |

Means followed by a common letter are not significantly different at the .05 level by Duncan’s test.
The category and number of microorganisms in soil after incubation for different biochar and lignite-based amendment treatments (mean ± standard deviation)

| Treatment       | Fungi  | Actinomycetes | Total   | Nitrifying bacteria | Denitrifying bacteria | Ammonia-oxidizing bacteria |
|-----------------|--------|---------------|---------|---------------------|-----------------------|----------------------------|
|                 | × 10⁶ CFU g⁻¹ | × 10⁶ CFU g⁻¹ | × 10⁷ CFU g⁻¹ | × 10⁷ CFU g⁻¹ | × 10⁹ CFU g⁻¹ | × 10⁶ CFU g⁻¹ |
| Control         | 1.33 ± 0.25c  | 7.71 ± 0.92bc | 1.34 ± 0.04bcd | 1.07 ± 0.31bc | 5.83 ± 0.84d | 1.25 ± 0.01de |
| Biochar (1%)    | 1.53 ± 0.12bc | 6.80 ± 0.24cd | 0.81 ± 0.03def | 1.22 ± 0.20b  | 6.47 ± 0.12cd | 1.61 ± 0.10c  |
| Biochar (2%)    | 1.77 ± 0.40bc | 6.35 ± 0.28de | 1.23 ± 0.04de  | 1.79 ± 0.41a  | 8.13 ± 0.12a  | 2.24 ± 0.01b  |
| Biochar (3%)    | 2.43 ± 0.65a  | 9.09 ± 3.16a  | 1.97 ± 0.03a   | 1.26 ± 0.19b  | 7.87 ± 0.05ab | 2.06 ± 0.05b  |
| Biochar (4%)    | 2.10 ± 0.26ab | 8.47 ± 1.07ab | 1.84 ± 0.04ab  | 1.79 ± 0.33a  | 7.40 ± 0.14abc| 2.69 ± 0.08a  |
| Biochar (5%)    | 1.40 ± 0.30c  | 5.86 ± 1.46def| 1.57 ± 0.04abc | 1.95 ± 0.34a  | 6.67 ± 0.06bcd| 2.27 ± 0.02b  |
| Lignite-based (1%) | 1.87 ± 0.45abc | 6.63 ± 0.27cd | 1.27 ± 0.02cde | 0.87 ± 0.10cd | 6.50 ± 0.08cd | 1.65 ± 0.19c  |
| Lignite-based (2%) | 0.57 ± 0.23d  | 6.84 ± 0.32cd | 1.53 ± 0.02abc | 0.73 ± 0.04d  | 5.77 ± 0.08d  | 1.23 ± 0.13de |
| Lignite-based (3%) | 0.70 ± 0.17d  | 5.30 ± 0.58ef | 1.06 ± 0.03cde | 0.28 ± 0.03e  | 6.13 ± 0.03cd | 1.15 ± 0.24e  |
| Lignite-based (4%) | 0.60 ± 0.10d  | 5.70 ± 0.08def| 0.76 ± 0.01ef  | 0.34 ± 0.07e  | 5.53 ± 0.04d  | 1.26 ± 0.07de |
| Lignite-based (5%) | 0.57 ± 0.12d  | 4.97 ± 0.04f  | 0.49 ± 0.01f   | 0.47 ± 0.11e  | 6.00 ± 0.08d  | 1.39 ± 0.07d  |

*CFU, colony-forming units.

*Means followed by a common letter are not significantly different at the .05 level by Duncan’s test.

on the soil NH₃-oxidizing bacteria (except for the 1% lignite-based amendments treatment). The number of NH₃-oxidizing bacteria was 1.65 × 10⁶ CFU g⁻¹ in the 1% lignite-based treatment, and the mean number of NH₃-oxidizing bacteria was 1.26 × 10⁶ CFU g⁻¹ in the other lignite-based treatments.

The community-level physiological profile was characterized by the AWCD and principal components (PCs). Figure 3 illustrates the AWCD in the biochar and lignite-based amendment treatments during the incubation period. The AWCD increased as the incubation period increased for the biochar treatments. The 3 and 4% biochar treatments consistently exhibited higher AWCD than the control. The 4% biochar treatment had the highest AWCD at all sampling periods. The other biochar treatments had a higher AWCD than the control after 168 h of incubation, but a lower AWCD than the control after 192 h of incubation. In contrast, the AWCD increased with the incubation period for the 1 and 2% lignite-based treatments but did not substantially change for the 3–5% treatments. After 120 h of incubation, all the lignite-based amendment treatments had a lower AWCD than the biochar treatments after 240 h of incubation at a temperature of 25 °C. This indicated that the addition of lignite-based amendment inhibited the activity of soil microbial communities.

Principal component analysis of 31 C sources revealed the different patterns of potential C utilization and different microbial communities. Five PCs with eigenvalues >1 were identified. The percentages of PC1, PC2, PC3, PC4, and PC5 were 48.04%, 22.61%, 12.33%, 7.26%, and 5.31%, respectively. The cumulative contribution of the first and second PCs was 70.65% (Table 4), so the first two PCs were used to represent the microbial community functional diversity in different treatments. The PCA showed distinct differences among the biochar and lignite-based treatment (Figure 4). Biochar addition increased the utilization of amides, amino acids, carbohydrates, and carboxylic acids (Figures 4a and 4b). In general, treatments with the same amendment clustered together except for 2% biochar treatment (Figure 4a). Biochar and lignite-based amendment treatments were widely separated from each other on the PC1 axis. Most of the biochar treatments and the control were positively correlated with all the loading variables on PC1, whereas all the lignite-based
amendment treatments were negatively correlated with all the loading variables on PC1. Both PC1 and PC2 separated the treatments during the incubation period, but the degree of separation was different. Distribution along PC2 did not reveal distinct patterns during the incubation period. This indicated that the differences in the metabolism of C sources among biochar and lignite-based amendment treatments were mainly reflected in PC1.

The addition of biochar significantly increased the richness when the addition proportion of biochar was 4%, whereas there was no significant effect on the richness for the other biochar treatments (Table 5). The richness decreased with the increase in lignite-based amendment. The richness in the 5% lignite-based amendment decreased by 9.67 compared with that in the control.

The Shannon index quadratically ($R^2 = .94$) increased with the application rate of biochar. The Shannon index in the 3 and 4% biochar treatments was higher than that in the control. The highest Shannon index was obtained in the 4% biochar treatment (Table 5). The Shannon index decreased linearly ($R^2 = .97$) with the increase in lignite-based amendment. The Shannon index in the 5% lignite-based amendment treatment decreased by 32.3% compared with that in the control. There was no significant difference ($P > .05$) in the Shannon evenness among the biochar treatments. The Shannon evenness increased quadratically ($R^2 = .78$) with lignite-based amendment addition. The largest value of Shannon evenness was obtained in the 3% lignite-based amendment treatment. No significant difference in the Simpson index was found in both the biochar and lignite-based amendment treatments. Only the addition of 3 and 4% biochar significantly increased the McIntosh index, and the other biochar treatments significantly decreased the value of McIntosh index. A higher McIntosh index was obtained with the lower application rate of the lignite-based amendment. Overall, biochar addition at rates of 3 and 4% enhanced the diversity and evenness indexes of the microbial communities.

### 3.3 Greenhouse gas emissions from amended soils

The temporal dynamic variation in GHG fluxes during the incubation period is presented in Figure 5. The CH$_4$ fluxes were generally very small for all treatments. A peak in the CH$_4$ flux occurred after 2 d of incubation for all treatments. The peak values were positive for the control and most biochar treatments. The largest peak (0.37 mg m$^{-2}$ h$^{-1}$) was obtained in the 4% biochar treatment. In contrast, the peak values of CH$_4$ flux were negative and of much smaller magnitude for most of the lignite-based amendment treatments. After 3 d of incubation, the CH$_4$ fluxes approached 0 for all treatments.

Biochar addition clearly enhanced the soil CO$_2$ emissions. The CO$_2$ fluxes increased with the incubation time, approached the peak value after 5 d of incubation, and then decreased gradually to steady levels after 15 d of incubation for all the biochar treatments. The peak values of CO$_2$ fluxes varied from 253 to 316 mg m$^{-2}$ h$^{-1}$ for different biochar treatments. In contrast, the addition of the lignite-based amendment inhibited the soil CO$_2$ emissions compared with the control. The CO$_2$ fluxes decreased with the increase in the lignite-based amendment. The peaks of CO$_2$ fluxes occurred after 4–5 d of incubation for all lignite-based amendment treatments. The peaks of CO$_2$ fluxes varied from 170 to 217 mg m$^{-2}$ h$^{-1}$ for different lignite-based amendment treatments.
TABLE 4  Carbon substrates loaded on the first five principle components (PCA) in analysis of Biolog-EcoPlate data

| Chemical guild | Substrates | Chemical formula | PCA1, 48.04% | PCA2, 22.61% | PCA3, 12.33% | PCA4, 7.26% | PCA5, 5.31% |
|----------------|------------|------------------|--------------|--------------|--------------|--------------|--------------|
| Miscellaneous  | Pyruvic acid methyl ester | C_4H_6O_3       | 0.641        | —            | 0.412        | 0.299        | 0.304        |
|                | Glucose-1-phosphate | C_6H_12O_6P     | 0.885        | 0.225        | —            | —            | —            |
|                | D,L-α-Glycerol phosphate | C_3H_2O_5       | 0.372        | 0.901        | —            | —            | —            |
| Polymers       | Tween 40    | —                | 0.644        | —            | —            | —            | 0.425        |
|                | Tween 80    | —                | 0.842        | —            | —            | —            | —            |
|                | α-Cyclodextrin | C_{60}H_{60}O_{30} | 0.808      | 0.418        | 0.003        | —            | —            |
|                | Glycogen | (C_{6}H_{10}O_{5})_n | 0.791      | 0.487        | 0.264        | —            | —            |
| Carbohydrates  | D-Cellobiose | C_{12}H_{12}O_{11} | 0.825        | —            | —            | —            | 0.084        |
|                | α-D-Lactose | C_{12}H_{12}O_{12} | 0.345        | 0.377        | 0.263        | 0.236        | 0.775        |
|                | β-Methyl-D-glucoside | C_{6}H_{12}O_{6} | 0.783        | —            | 0.122        | 0.517        | —            |
|                | D-Xylose | C_{6}H_{10}O_{5} | 0.289        | —            | —            | 0.074        | —            |
|                | L-Erythritol | C_{6}H_{12}O_{4} | 0.725        | —            | —            | 0.247        | 0.365        |
|                | D-Mannitol | C_{6}H_{12}O_{6} | 0.880        | —            | 0.105        | —            | —            |
|                | N-Acetyl-D-glucosamine | C_{12}H_{18}NO_{6} | 0.855      | —            | —            | 0.077        | 0.024        |
| Carboxylic acids | D-Glucosaminic acid | C_{6}H_{12}NO_{6} | 0.850        | 0.185        | —            | —            | —            |
|                | D-Galactonic acid latone | C_{6}H_{10}O_{6} | 0.421        | —            | 0.821        | 0.036        | —            |
|                | D-Galacturonic acid | C_{6}H_{9}O_{5} | 0.404        | —            | 0.373        | —            | —            |
|                | 2-Hydroxy benzoic acid | C_{6}H_{12}O_{3} | 0.280        | 0.851        | —            | 0.328        | —            |
|                | 4-Hydroxy benzoic acid | C_{6}H_{12}O_{3} | 0.924        | 0.179        | —            | —            | —            |
|                | γ-Hydroxy butyric acid | C_{3}H_{6}O_{3} | 0.958        | —            | —            | —            | 0.100        |
|                | Itaconic acid | C_{5}H_{8}O_{3} | 0.687        | 0.500        | 0.490        | —            | —            |
|                | α-Keto butyric acid | C_{4}H_{8}O_{3} | 0.208        | 0.807        | —            | 0.501        | —            |
|                | D-Malic acid | C_{4}H_{8}O_{3} | 0.626        | 0.040        | —            | 0.341        | —            |
| Amino acids    | L-Arginine | C_{6}H_{13}N_{2}O_{2} | 0.712        | —            | —            | —            | 0.321        |
|                | L-Asparagine | C_{6}H_{11}N_{2}O_{3} | 0.844        | —            | 0.166        | 0.249        | —            |
|                | L-Phenylalanine | C_{6}H_{11}N_{2}O_{2} | 0.708        | 0.517        | 0.405        | —            | 0.029        |
|                | L-Serine | C_{6}H_{11}NO_{3} | 0.864        | 0.312        | 0.196        | —            | —            |
|                | L-Threonine | C_{6}H_{11}NO_{3} | 0.593        | —            | —            | 0.529        | —            |
|                | Glycyl-L-glutamic acid | C_{12}H_{22}N_{2}O_{5} | 0.478      | —            | 0.716        | 0.388        | —            |
| Amides         | Phenylethylamine | C_{6}H_{12}N | 0.733        | 0.490        | 0.038        | —            | 0.157        |
|                | Putrescine | C_{6}H_{12}N_{2} | 0.477        | 0.506        | 0.474        | 0.229        | 0.175        |

FIGURE 4  Principal component analysis (PCA) of soil microbial community function diversity. LB, lignite-based amendment; BC, biochar
Biochar hindered the soil N$_2$O emissions compared with the control. A peak in N$_2$O fluxes occurred after 16 h of incubation in all biochar treatments, and then decreased gradually to the steady levels (4 d after incubation). The peak values of N$_2$O fluxes varied in the range of 3.9 to 19.6 mg m$^{-2}$ h$^{-1}$ in different biochar treatments. The N$_2$O fluxes in the treatments of lignite-based amendment decreased steadily over time and stabilized after 4 d of incubation. The main emission periods of N$_2$O occurred in the first 4 d of incubation for different treatments with biochar and lignite-based amendment adding. However, the N$_2$O fluxes in the biochar treatments were much smaller than those in the lignite-based amendment treatments in the initial incubation stage (2 d after incubation).

The cumulative GHG emissions during the incubation period are presented in Figure 6. The cumulative CH$_4$ emissions were approximately 0.01 kg ha$^{-1}$ in biochar treatments and ~0.002 kg ha$^{-1}$ in lignite-based amendment treatments. The cumulative CO$_2$ emissions nonlinearly increased with the rate of biochar addition. The maximum cumulative CO$_2$ emissions were obtained in the 4% biochar treatment, which increased by 60.6% compared with the control. In contrast with biochar treatment, the cumulative CO$_2$ emissions generally decreased with the addition of lignite-based amendment. The cumulative CO$_2$ emissions from soil amended with 5% lignite-based amendment decreased by 19.6% compared with the control. In addition, the cumulative CO$_2$ emissions in biochar treatments were larger than those in the lignite-based amendment treatments. The addition of biochar generally decreased the cumulative N$_2$O emissions, but the cumulative N$_2$O emissions increased with the application rate of the lignite-based amendment. The maximum cumulative N$_2$O emissions reached 1.94 kg ha$^{-1}$ (5% lignite-based amendments) after 19 d of incubation.

### DISCUSSION

This study investigated the effects of biochar and lignite-based amendment addition on soil physicochemical properties, microbial communities, and GHG emissions through laboratory-incubated microcosm experiments. Soil physicochemical properties varied with the addition of biochar and lignite-based amendment. The soil microbial abundance and utilization ability of C sources were improved by the biochar in general but restrained by the lignite-based amendment in most cases. The GHG emission features were distinct between the biochar and lignite-based amendment treatments due to the different influences on soil microbial communities.

#### 4.1 Impact of organic amendments on soil properties

A large amount of research shows that soil physicochemical properties are improved with the biochar incorporation (He et al., 2020; Lehmann et al., 2011; McHenry, 2011). Most of these findings have been obtained from pot and field experiments, which are influenced by a variety of factors such as climate, soil, crop, and field management (Gul et al., 2015; Laird et al., 2010; Yao et al., 2017). In the present study, we investigated soil physicochemical properties response to the biochar and lignite-based amendment through laboratory-incubated microcosm experiments. The addition of the biochar and lignite-based amendment resulted in the increase in soil pH, EC, organic matter, and available nutrients. However, the increase magnitude in lignite-based amended soils was clearly larger than that in the biochar-amended ones due to its original high background.
Soil C/N ratio increased with biochar addition but decreased with the application of lignite-based amendment. The addition of biochar with a high C/N ratio resulted in a high C/N ratio in the biochar-amended soil, which is consistent with the findings of Case et al. (2012) and Hu et al. (2014). The decreased C/N ratio in soil with lignite-based amendment addition is different from the findings of Zhong et al. (2010), who found that N-modified lignite with a C/N ratio of 76.2 significantly increased the soil microbial biomass C/N ratio (the original C/N is 8.5).
FIGURE 6  Cumulative greenhouse gas emissions during the incubation period in different biochar and lignite-based amendment treatments. Bars with a common letter are not significantly different at the .05 level by Duncan’s test.

ratio in the present study is attributed to the original low C/N ratio that is lower than the tested soil. The difference of biochar and lignite-based amendment in soil C/N ratio is attributed to the original C/N ratio (Table 1). As shown in Gelsomino et al. (2006), the C/N ratio in the amended soil is highly dependent on the original C/N ratio of the amendments.

The addition of biochar and lignite-based amendment enhanced soil EC. The increase in EC in lignite-based amended soil was greater than that in biochar amended ones as the higher initial background (Table 1). In addition, the lignite-based amendment had a much higher organic matter content (976.0 g kg⁻¹) than the biochar (675.7 g kg⁻¹), so the decomposition of organic matter may release ions and increase the soil EC (Abdelhafez et al., 2014). The increase in soil EC with the addition of biochar and lignite-based amendment was also detected in previous field and incubation experimental studies (C. Li et al., 2018; Masto et al., 2013; Zolfi-Bavariani et al., 2016). A high salt content may be harmful to crops and soil microorganisms. In the short-term incubation
Effects of the biochar and lignite-based amendments on soil microbial communities

The species abundance and structure of the soil microbial community varied with the application of different organic amendments at different application rates (Tables 3 and 4). The application of biochar at a rate of 3–4% obtained the largest abundance of soil microbial biomass. The addition of biochar promoting the development of microbiomes may occur through providing favorable soil water, nutrients, and aeration environment and increasing organic C and the C/N ratio. Biochar contains abundant macro- and micropores; thus, the addition of biochar increases the total surface area and pore size distribution (Jindo et al., 2014). The improvement of soil surface area and pore structure provide microhabitats for microorganisms (Ameloot, Graber, et al., 2013; Kravchenko et al., 2019), as many soil microorganisms are specialists living in microhabitats that provide resources for their specific metabolic needs (Lehmann et al., 2011). Biochar also contains a small amount of labile organic C and micro- and macronutrients, which are likely to increase microbial abundance (Zavalloni et al., 2011). In addition, biochar addition significantly increased the soil C/N ratio, which could stimulate microorganism growth (Liu et al., 2009). Liang et al. (2017) has reported that the addition of substrate with high C/N ratio can result in a greater priming effect. These direct and indirect factors enhance the microbial abundance after biochar application. On the other aspect, salt and other toxic compounds (such volatile organic compounds, polycyclic aromatic hydrocarbons, and dioxins, and heavy metals, etc.) were added into the soil in companion with biochar application (Zheng et al., 2019), which might have inhibited microbial community growth (Rath et al., 2016). Excessive addition of biochar may inhibit the microbial growth due to the toxicity effect of salt and heavy metals (Shi et al., 2020). In the present study, the numbers of total bacteria decreased when the biochar addition rate was <3%. Ameloot, Graber, et al. (2013) found that a high rate of biochar application (90 t ha−1) reduces the number of soil microorganisms.

In contrast, the addition of lignite-based amendment significantly decreased the abundance of the soil microbial community in most cases. Although the soil nutrients increased with the increase in lignite-based amendment addition, soil EC also increased significantly. As many soil microorganisms are negatively affected by salinity owing to water availability restriction as a result of low osmotic potentials in soils and through ion toxicity (Rath & Rousk, 2015). Moreover, a large number of compounds such as phenols, phthalates, polycyclic aromatic hydrocarbons, benzenes, and aliphatic may be applied to the soil (Tran et al., 2015). Some of these components are known to have toxic effects on cellular metabolism (Maharaj et al., 2014). In addition, lignite-based amendment addition generally decreased the soil C/N ratio, which might have constrained the growth of microorganisms. The limited impacts of lignite-based amendments on the microbial abundance are consistent with the findings of Tran et al. (2015).

The abundance of denitrification bacteria was much lower than that of nitrifying and NH3-oxidizing bacteria (Table 3). Nitrification is the main N turnover process when the soil WFPS is in the range of 35–60%, whereas denitrification is the main N turnover process when the soil WFPS is >70% (Yoo et al., 2018). The WFPS was controlled at 60% during the experimental period; thus, the abundance of bacteria in the nitrification process was higher than that in the denitrification process.

The Biolog EcoPlate data clearly showed that there was a significant successional shift in the microbial community structure owing to the addition of biochar and lignite-based amendment (Figure 4a). The addition of the biochar and lignite-based amendment may change the substrate use patterns of microorganisms and influence the structure of the soil microbial community (Pietikäinen et al., 2000). In the present study, biochar addition enhanced the ability of soil microorganisms to utilize C sources, whereas the addition of lignite-based amendment inhibited the microbial utilization of soil C sources (Figure 4b). Microbial groups are generally considered to rapidly adapt to changes in soil environmental conditions (Spyrou et al., 2009). However, some research reported that the addition of stable C sources alters the structure of the soil microbial community rather than the total biomass of microbial communities, and a new equilibration may be achieved again after long-term incubation (Anders et al., 2013; Tran et al., 2015). Therefore, the long-term effects of biochar and lignite-based amendment addition on the microbial community structure are desirable.

Influence of organic amendments on greenhouse gas emissions

The addition of amendment altered the soil physico-chemical properties and microbial communities, thereby
influencing soil-borne GHG emissions. The CH$_4$ emissions were very small during the incubation period, and soil even acts as a CH$_4$ sink for some treatments (Figures 5 and 6). Strictly anaerobic condition is required for methanogenesis through different pathways. As soil moisture was maintained at 60% WFPS for all treatments during incubation, the soils were not strictly anaerobic; thus, the methanogenesis was hindered. In addition, the optimum methanogenesis temperature is between 30 and 35 °C (James et al., 1996). After incubation at 25 °C, the activities of methanogens decreased and CH$_4$ production was reduced.

The addition of biochar significantly increased the cumulative CO$_2$ emissions at different application rates, whereas lignite-based amendment addition generally decreased the cumulative CO$_2$ emissions (Figure 6). The increase in soil respiration is usually attributed to the increased concentration of labile organic C with the addition of amendment and the enhanced soil microbial activities (Chen et al., 2018). In contrast, the decrease in soil respiration is often explained by the toxic effect of organic amendments (Zimmerman et al., 2011) and reduction in substrate availability, microbial abundance, and enzymatic activity (Lehmann et al., 2011). Biochar addition increased the microbial abundance (Table 3), altered the microbial community structure, and enhanced the soil C source utilization ability of soil microorganisms (Figure 4). Therefore, biochar addition improved the soil respiration and increased the soil CO$_2$ emissions. This result is consistent with the findings of Zhou et al. (2017) and Czekala et al. (2016). In contrast with biochar, although organic matter and available nutrients were added to the soil with lignite-based amendment, a large number of salts are also applied to the soil simultaneously (Figure 1). Both bacterial growth and decomposition are directly inhibited by high salt concentrations, so that soil respiration is constrained (Rath et al., 2019). The addition of lignite-based amendment significantly reduced the microbial abundance in most cases and inhibited the ability of microorganisms to utilize C sources, thereby reducing the soil CO$_2$ efflux. This is consistent with the findings of Tran et al. (2015), who found that the application of Victorian lignite reduces CO$_2$ emissions. Schefe et al. (2008) also found that the application of lignite can suppress soil respiration in the short term.

The addition of biochar generally decreased the N$_2$O emissions, whereas lignite-based amendment addition increased the N$_2$O emissions for most cases (Figure 6). Nitrous oxide emissions decreased in biochar-amended soils and increased in lignite-based amendment soil are consistent with the findings of Bruun et al. (2011) and Sun et al. (2016). Nitrous oxide is a product of microbial metabolism during the process of nitrification and denitrification. The conditions necessary for N$_2$O emission in soils include (a) a supply of decomposable organic C, (b) a supply of mineral N, (c) low pH, and (d) the presence of denitrifying organisms (Ameloot, De Neve, et al., 2013). Biochar addition decreased the N$_2$O emissions as a result of several possible mechanisms. First, although biochar addition increased the number of nitrification bacteria (Table 3), the NH$_4^+$–N content decreased with biochar application (Table 2). The low NH$_4^+$–N content could not provide sufficient substrate for the nitrification process. Second, denitrification rates are usually assumed to decrease with pH increasing (Simek & Cooper, 2002). The increase in soil pH with the addition of biochar decreased the N$_2$O emissions (Figures 1 and 6). Third, the porous structure of biochar may increase soil aeration and suppress denitrification (Case et al., 2012). A fourth mechanism might be the absorption of NO$_3^–$-N on the biochar surface leading to the decrease in the N$_2$O emissions (Karhu et al., 2011). Although lignite-based amendment addition generally decreased the number of nitrification bacteria (Table 3). The soil NH$_4^+$–N content significantly increased, especially in the higher lignite-based amendment treatments which promoted N$_2$O emissions (Figure 6). The increase in N$_2$O emissions with lignite-based amendment addition might be caused by the decoupling of either nitrification or denitrification processes because of the significant increase in soil EC (Low et al., 1997). A high salt concentration in soil may inhibit the activity of N$_2$O reductase, which results in N$_2$O accumulation from denitrification under saline conditions (Menyailo et al., 1997). In addition, biochar addition significantly increased the soil C/N ratio, whereas the addition of lignite-based amendment generally decreased the soil C/N ratio (Table 2). Nitrous oxide emissions are influenced by the soil C/N ratio, since soil N compounds are primary terminal electron acceptors and C acts as an electron donor in the denitrification process (Thangarajan et al., 2013). In general, N$_2$O emissions are negatively correlated with the C/N ratio of soil (Huang et al., 2004; Thangarajan et al., 2013). The addition of biochar increased the soil C/N ratio and the incorporation of lignite-based amendment decreased the soil C/N (Table 2). Therefore, N$_2$O emission was promoted in lignite-based amendment treated soil but decreased in biochar-amended soil.

5 CONCLUSIONS

The effects of biochar and lignite-based amendment on soil physicochemical properties, microbial communities, and GHG emissions from agricultural soil were investigated through laboratory-incubated microcosm experiments. The soil pH and EC clearly increased with the addition of biochar and lignite-based amendment. The values of pH and EC in the soil amended with the lignite-based amendment were higher than those in the soil with the same rate of biochar. The incorporation of biochar and lignite-based amendment can improve soil fertility by enhancing the soil organic matter and available nutrients. The C/N ratio in the amended soil
is highly dependent on the original C/N ratio of the amendments. The biochar and lignite-based amendment had distinct effects on the soil microbial communities and GHG emissions. The soil microbial abundance and utilization ability of C sources were improved by the biochar but restrained by the lignite-based amendment in most cases. The CH$_4$ emissions were very small during the incubation period, and soil even acts as a CH$_4$ sink for some treatments. Carbon dioxide emissions were promoted by biochar and inhibited by the lignite-based amendment during the short-term incubation period. Nitrous oxide emissions decreased with the addition of biochar but increased with the application of lignite-based amendment. As the soil EC significantly increased with the addition of amendments, particularly for the lignite-based amendment. Thus, salt leaching is required to avoid salt accumulation when the biochar and lignite-based amendment are applied in the field.

The findings of this research can provide a reference for the application of biochar and lignite-based amendment in silt loam soil. However, this research focused on the individual effects of biochar and lignite-based amendment, the synergistic effects of two organic amendments are still not known. In addition, the long-term effects of the amendments on soil properties, biological processes, and GHG emissions still need to be determined.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

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REFERENCES
Abdelhafez, A. A., Li, J., & Abbas, M. H. (2014). Feasibility of biochar manufactured from organic wastes on the stabilization of heavy metals in a metal smelter contaminated soil. Chemosphere, 117, 66–71. https://doi.org/10.1016/j.chemosphere.2014.05.086
Ameloot, N., De Neve, S., Jegjeevagan, K., Yildiz, G., Buchan, D., Funkuin, Y. N., … Sleutel, S. (2013). Short-term CO$_2$ and N$_2$O emissions and microbial properties of biochar amended sandy loam soils. Soil Biology and Biochemistry, 57, 401–410. https://doi.org/10.1016/j.soilbio.2012.10.025
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
Amini, S., Ghadiri, H., Chen, C., & Marschner, P. (2015). Salt-affected soils, reclamation, carbon dynamics, and biochar: A review. Journal of Soils and Sediments, 16, 939–953. https://doi.org/10.1007/s11368-015-1293-1
Anders, E., Watzinger, A., Rempt, F., Kitzler, B., Wimmer, B., Zehetner, F., … Soja, G. (2013). Biochar affects the structure rather than the total biomass of microbial communities in temperate soils. Agricultural and Food Science, 22, 404–423. https://doi.org/10.23986/afsci.8905
Anderson, C. R., Hamonts, K., Clough, T. J., & Condron, L. M. (2014). Biochar does not affect soil N-transformations or microbial community structure under ruminant urine patches but does alter relative proportions of nitrogen cycling bacteria. Agriculture, Ecosystems & Environment, 191, 63–72. https://doi.org/10.1016/j.agee.2014.02.021
Baiamonte, G., Crescimanno, G., Parrino, F., & De Pasquale, C. (2019). Effect of biochar on the physical and structural properties of a sandy soil. Catena, 175, 294–303. https://doi.org/10.1016/j.catena.2018.12.019
Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J. L., Harris, E., Robinson, B., & Sizmur, T. (2011). A review of biochars’ potential role in the remediation, revegetation and restoration of contaminated soils. Environmental Pollution, 159, 3269–3282. https://doi.org/10.1016/j.envpol.2011.07.023
Blanco-Canqui, H. (2017). Biochar and soil physical properties. Soil Science Society of America Journal, 81, 687–711. https://doi.org/10.2136/ssaj2017.01.0017
Brassard, P., Godbout, S., & Raghavan, V. (2016). Soil biochar amendment as a climate change mitigation tool: Key parameters and mechanisms involved. Journal of Environmental Management, 181, 484–497. https://doi.org/10.1016/j.jenvman.2016.06.063
Bruun, E., Müller-Stöver, D., Ambus, P., & Hauggaard-Nielsen, H. (2011). Application of biochar to soil and N$_2$O 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
Burrell, L. D., Zehetner, F., Rampazzo, N., Wimmer, B., & Soja, G. (2016). Long-term effects of biochar on soil physical properties. Geoderma, 282, 96–102. https://doi.org/10.1016/j.geoderma.2016.07.019
Case, S. D., McNamara, N. P., Reay, D. S., Stott, A. W., Grant, H. K., & Whitaker, J. (2015). Biochar suppresses N$_2$O emissions while maintaining N availability in a sandy loam soil. Soil Biology and Biochemistry, 81, 178–185. https://doi.org/10.1016/j.soilbio.2014.11.012
Case, S. D., McNamara, N. P., Reay, D. S., & Whitaker, J. (2012). The effect of biochar addition on N$_2$O and CO$_2$ emissions from a sandy loam soil: The role of soil aeration. Soil Biology and Biochemistry, 51, 125–134. https://doi.org/10.1016/j.soilbio.2012.03.017
Chen, J., Sun, X., Li, L., Liu, X., Zhang, B., Zheng, J., & Pan, G. (2016). Change in active microbial community structure, abundance and
carbon cycling in an acid rice paddy soil with the addition of biochar. *European Journal of Soil Science*, 67, 857–867. https://doi.org/10.1111/jeSS.12388

Chen, J., Sun, X., Zheng, J., Zhang, X., Liu, X., Bian, R., Li, L., Cheng, K., Zheng, J., & Pan, G. (2018). Biochar amendment changes temperature sensitivity of soil respiration and composition of microbial communities 3 years after incorporation in an organic carbon-poor dry cropland soil. *Biology and Fertility of Soils*, 54, 175–188. https://doi.org/10.1007/s00374-017-1253-6

Clouard, M., Criquei, S., Borschneck, D., Ziarelli, F., Marzaaoli, F., Balesdent, J., & Keller, C. (2014). Impact of lignite on pedogenetic processes and microbial functions in Mediterranean soils. *Geoderma*, 232–234, 257–269. https://doi.org/10.1016/j.geoderma.2014.05.009

Czekala, W., Malinška, K., Cáceres, R., Janczak, D., Dach, J., & Lewicki, A. (2016). Co-composting of poultry manure mixtures amended with biochar: The effect of biochar on temperature and C-CO₂ emission. *Bioresource Technology*, 200, 921–927. https://doi.org/10.1016/j.biortech.2015.11.019

Detman, A., Bucha, M., Simineit, B. R. T., Mielecki, D., Piwowarczyk, C., Chojnacka, A.,… Sikora, A. (2018). Lignite biodegradation under conditions of acidic molasses fermentation. *International Journal of Coal Geology*, 196, 274–287. https://doi.org/10.1016/j.coal.2018.07.015

Ezrin, M. H., Amin, M. S. M., Anuar, A. R., & Aimrun, W. (2010). Relationship between rice yield and apparent electrical conductivity of paddy soils. *American Journal of Applied Sciences*, 7, 63–70. https://doi.org/10.3844/ajassp.2010.63.70

Feigl, V., Ujaczki, É., Vaszita, E., & Molnár, M. (2017). Influence of red mud on soil microbial communities: Application and comprehensive evaluation of the Biolog EcoPlate approach as a tool in soil microbiological studies. *Science of the Total Environment*, 595, 903–911. https://doi.org/10.1016/j.scitotenv.2017.03.266

Garland, J. L. (1997). Analysis and interpretation of community-level physiological profiles in microbial ecology. *FEMS Microbiology Ecology*, 24, 289–300. https://doi.org/10.1111/j.1574-6941.1997.tb00446.x

Gelsomino, A., Badalucco, L., Landi, L., & Cacco, G. (2006). Soil carbon, nitrogen and phosphorus dynamics as affected by solarization alone or combined with organic amendment. *Plant and Soil*, 279, 307–325. https://doi.org/10.1007/s11104-005-2155-1

Głąb, T., Palomowska, J., Zaleski, T., & Gondek, K. (2016). Effect of biochar application on soil hydrological properties and physical quality of sandy soil. *Geoderma*, 281, 11–20. https://doi.org/10.1016/j.geoderma.2016.06.028

Gul, S., Whalen, J. K., Thomas, B. W., Sachdeva, V., & Deng, H. (2015). Physico-chemical properties and microbial responses in biochar-amended soils: Mechanisms and future directions. *Agriculture, Ecosystems & Environment*, 206, 46–59. https://doi.org/10.1016/j.agee.2015.03.015

He, K., He, G., Wang, C., Zhang, H., Xu, Y., Wang, S., … Hu, R. (2020). Biochar amendment ameliorates soil properties and promotes Miscanthus growth in a coastal saline-alkali soil. *Applied Soil Ecology*, 155, 1–10. https://doi.org/10.1016/j.apsoil.2020.103674

Hu, Y. L., Wu, F.-P., Zeng, D.-H., & Chang, S. X. (2014). Wheat straw and its biochar had contrasting effects on soil C and N cycling two growing seasons after addition to a Black Chernozemic soil planted to barley. *Biology and Fertility of Soils*, 50, 1291–1299. https://doi.org/10.1007/s00374-014-0943-6

Huang, Y., Zou, J., Zheng, X., Wang, Y., & Xu, X. (2004). Nitrous oxide emissions as influenced by amendment of plant residues with different C:N ratios. *Soil Biology and Biochemistry*, 36, 973–981. https://doi.org/10.1016/j.soilbio.2004.02.009

James, A. G., Watson-Craik, I. A., & Senior, E. (1996). Use of a three-stage laboratory model to determine the effects of temperature on individual trophic groups of methanogenic associations isolated from anoxic landfill. *Journal of Chemical Technology and Biotechnology*, 67, 333–338. https://doi.org/10.1002/(SICI)1097-4666(199612)67:4<333::AID-JCTB584>3.0.CO;2-A

Jeffery, S., Meinders, M. B. J., Stooi, C. R., Bezemder, T. M., van de Voorde, T. F. J., Mommen, L., & van Groenigen, J. W. (2015). Biochar application does not improve the soil hydrological function of a sandy soil. *Geoderma*, 251–252, 47–54. https://doi.org/10.1016/j.geoderma.2015.03.022

Jeffery, S., Verheijen, F. G., van der Velde, M., & Bastos, A. C. (2011). A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. *Agriculture, Ecosystems & Environment*, 144, 175–187. https://doi.org/10.1016/j.agee.2011.08.015

Jindo, K., Mizumoto, H., Sawada, Y., Sanchez-Monedero, M. A., & Sonoki, T. (2014). Physical and chemical characterization of biochars derived from different agricultural residues. *Biogeoosciences*, 11, 6613–6621. https://doi.org/10.5194/bg-11-6613-2014

Karhu, K., Mattila, T., Bergström, I., & Regina, K. (2011). Biochar addition to agricultural soil increased CH₄ uptake and water holding capacity: Results from a short-term pilot field study. *Agriculture, Ecosystems & Environment*, 140, 309–313. https://doi.org/10.1016/j.agee.2010.12.005

Kraychenko, A. N., Guber, A. K., Razavi, B. S., Koestel, J., Quigley, M. Y., Robertson, G. P., & Kuzyakov, Y. (2019). Microbial spatial foot-print as a driver of soil carbon stabilization. *Nature Communications*, 10. https://doi.org/10.1038/s41467-019-11057-4

Kwiatkowska, J., Provenzano, M., & Senesi, N. (2008). Long term effects of a brown coal-based amendment on the properties of soil humic acids. *Geoderma*, 148, 200–205. https://doi.org/10.1016/j.geoderma.2008.10.001

Laird, D. A., Fleming, P., Davis, D. D., Horton, R., Wang, B., & Karlen, D. L. (2010). Impact of biochar amendments on the quality of a typical midwestern agricultural soil. *Geoderma*, 158, 443–449. https://doi.org/10.1016/j.geoderma.2010.05.013

Larney, F. J., & Angers, D. A. (2012). The role of organic amendments in soil reclamation: A review. *Canadian Journal of Soil Science*, 92, 19–38. https://doi.org/10.4141/cjss2010-064

Lee, Y., Park, J., Ryu, C., Gang, K. S., Yang, W., Park, Y.-K., … Hyun, S. (2013). Comparison of biochar properties from biomass residues produced by slow pyrolysis at 500°C. *Bioresource Technology*, 148, 196–201. https://doi.org/10.1016/j.biortech.2013.08.135

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.soilbi.2011.04.022

Li, C., Xiong, Y., Cui, Z., Huang, Q., Xu, X., Han, W., & Huang, G. (2020). Effect of irrigation and fertilization regimes on grain yield, water and nitrogen productivity of mulching cultivated maize (Zea mays L.) in the Hetao Irrigation District of China. *Agricultural Water Management*, 232, 106065. https://doi.org/10.1016/j.agwat.2020.106065
Sproul, I. M., Karpouzas, D. G., & Menkissoglu-Spiroudi, U. (2009). Do botanical pesticides alter the structure of the soil microbial community? Microbial Ecology, 58, 715–727. https://doi.org/10.1007/s00248-009-9522-z

Staddon, W., Duchesne, L., & Trevors, J. (1997). Microbial diversity and community structure of postdisturbance forest soils as determined by sole-carbon-source utilization patterns. Microbial Ecology, 34, 125–130. https://doi.org/10.1023/A:1005050624787

Sun, J., Bai, M., Shen, J., Griffith, D. W., Denmead, O. T., Hill, J., … Chen, D. (2016). Effects of lignite application on ammonia and nitrous oxide emissions from cattle pens. Science of the Total Environment, 565, 148–154. https://doi.org/10.1016/j.scitotenv.2016.04.156

Thangarajan, R., Bolan, N. S., Tian, G., Naidu, R., & Kunhikrishnan, A. (2013). Role of organic amendment application on greenhouse gas emission from soil. Science of the Total Environment, 465, 72–96. https://doi.org/10.1016/j.scitotenv.2013.01.031

Tran, C. K. T., Rose, M. T., Cavagnaro, T. R., & Patti, A. F. (2015). Lignite amendment has limited impacts on soil microbial communities and mineral nitrogen availability. Applied Soil Ecology, 95, 140–150. https://doi.org/10.1016/j.apsoil.2015.06.020

Verhoeven, E., & Six, J. (2014). Biochar does not mitigate field-scale N₂O emissions in a Northern California vineyard: An assessment across two years. Agriculture, Ecosystems & Environment, 191, 27–38. https://doi.org/10.1016/j.agee.2014.03.008

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

Woolf, D., Amonette, J. E., Street-Perrott, F. A., Lehmann, J., & Joseph, S. (2010). Sustainable biochar to mitigate global climate change. Nature Communications, 1. https://doi.org/10.1038/ncomms1053

Xu, H. J., Wang, X. H., Li, H., Yao, H. Y., Su, J. Q., & Zhu, Y. G. (2014). Biochar impacts soil microbial community composition and nitrogen cycling in an acidic soil planted with rape. Environmental Science & Technology, 48, 9391–9399. https://doi.org/10.1021/es5021058

Yao, Q., Liu, J., Yu, Z., Li, Y., Jin, J., Liu, X., & Wang, G. (2017). Three years of biochar amendment alters soil physiochemical properties and fungal community composition in a black soil of northeast China. Soil Biology and Biochemistry, 110, 56–67. https://doi.org/10.1016/j.soilbio.2017.03.005

Yoo, G., Lee, Y. O., Won, T. J., Hyun, J. G., & Ding, W. (2018). Variable effects of biochar application to soils on nitrification-mediated N₂O emissions. Science of the Total Environment, 626, 603–611. https://doi.org/10.1016/j.scitotenv.2018.01.098

Zavalloni, C., Alberti, G., Biasiol, S., Delle Vedove, G., Fornasier, F., Liu, J., & Peressotti, A. (2011). Microbial mineralization of biochar and wheat straw mixture in soil: A short-term study. Applied Soil Ecology, 50, 45–51. https://doi.org/10.1016/j.apsoil.2011.07.012

Zhang, C., Zeng, G., Huang, D., Lai, C., Chen, M., Cheng, M., … Wang, R. (2019). Biochar for environmental management: Mitigating greenhouse gas emissions, contaminant treatment, and potential negative impacts. Chemical Engineering Journal, 373, 902–922. https://doi.org/10.1016/j.cej.2019.05.139

Zheng, H., Liu, B., Liu, G., Cai, Z., & Zhang, C. (2019). Potential toxic compounds in biochar: Knowledge gaps between biochar research and safety. In Y. S. Ok, D. C. W. Tsang, N. Bolan, & J. M. Novak (Eds.), Biochar from biomass and waste (pp. 349–384). Amsterdam, the Netherlands: Elsevier. https://doi.org/10.1016/B978-0-12-811729-3.00019-4

Zhong, Z., Yang, H., Bai, R., & Maria, W. (2010). Studies on properties of N-modified brown coal and its application in afforestation in semiarid degraded soil. Journal of Soil and Water Conservation, 24, 213–216. https://doi.org/10.13870/j.cnki.sjtscc.2010.04.050

Zhou, G., Zhou, X., Zhang, T., Du, Z., He, Y., Wang, X., … Xu, C. (2017). Biochar increased soil respiration in temperate forests but had no effects in subtropical forests. Forest Ecology and Management, 405, 339–349. https://doi.org/10.1016/j.foreco.2017.09.038

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 and Biochemistry, 43, 1169–1179. https://doi.org/10.1016/j.soilbio.2011.02.005

Zolfi-Bavariani, M., Ronaghi, A., Ghasemi-Fasaei, R., & Yasrebi, J. (2016). Influence of poultry manure-derived biochars on nutrients bioavailability and chemical properties of a calcareous soil. Archives of Agronomy and Soil Science, 62, 1578–1591. https://doi.org/10.1080/03650340.2016.1151976

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