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ORIGINAL PAPER

Effects of Biochar Amendment on Nitrous Oxide Emission, Bacterial and Fungal Community Composition in a Tobacco-Planting Soil

Yuan Tang[1] · Weichang Gao[2] · Yi Chen[2] · Qinghai Zhang[1] · Jianzhong Cheng[3]

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Abstract

Biochar (BC) is a promising soil amendment for mitigating nitrous oxide (N_2O) emissions. However, field experiments have reported inconsistencies in the changes in N_2O emissions, and the underlying microbial mechanisms are unclear. A tobacco (*Nicotiana tabacum* L.) field under different tobacco BC application rates (0, 1, 10, 25, and 50 t ha^{-1}) was established to investigate the changes of soil N_2O emissions and microbial community compositions. BC amendments significantly increased the cumulative N_2O emissions by 1.96–4.18 folds, mainly due to enhanced soil substrate availability under tobacco BC application. Shifts of bacterial community structure at the phylum level under BC amendment were observed, while changes in the structure of soil fungi at the genus level occurred. The abundance of denitrifying bacteria (*Bradyrhizobium* and *Pseudomonas*) and denitrifying fungi (*Trichocladium* and *Trichoderma*) was significantly increased with BC amendment, contributing to the stimulated soil N_2O emissions by affecting aerobic denitrification. The field N_2O mitigation of BC application should be reconsidered if tobacco BC is applied to upland soils.

Keywords Nitrous oxide · Bacteria · Fungi · Biochar · High-throughput sequencing

1 Introduction

Nitrous oxide (N_2O) is an important greenhouse gas that has an atmospheric lifetime of 114 years and a 298-fold greater global warming potential compared to CO_2 over 100 years (Rock et al. 2007; Thomson et al. 2012). Agricultural soil is the main source of N_2O in the atmosphere (4.2–6.0 Tg N $year^{-1}$) and is responsible for approximately 62% of the total global N_2O emissions (Thomson et al. 2012). Therefore, reducing N_2O emissions by developing effective mitigation strategies is warranted to mitigate climate change.

Biochar (BC) is a carbon-rich material produced by the pyrolysis of organic residues under a limited supply of oxygen at a relatively low temperature (< 700 °C) (Cheng et al. 2018). BC has been demonstrated to reduce N_2O emissions in a range of incubation and field-effect experiments (Yin et al. 2014; Ameloot et al. 2016) and has been considered as a tool to mitigate climate change (Mao et al. 2012). Evidence indicates that soil pH has an inverse relationship with N_2O production (Baggs et al. 2010), suggesting that the elevated soil pH after BC amendment may be the main mechanism through which BC reduces N_2O emissions (Obia et al. 2015). BC can also limit denitrification by reducing substrate availability (dissolved organic carbon (DOC) and NO_3^--N) (Yin et al. 2014; Ameloot et al. 2016). However, the results of previous studies are inconsistent. Some studies found that BC amendment had no significant influence on N_2O production (Cheng et al. 2012; Case et al. 2018), or even stimulated N_2O emission (Spokas and Reicosky 2009; Lin et al. 2017; Yoo et al. 2018). There are many factors by which BC addition might affect soil N_2O fluxes, such as BC types (the feedstock source and pyrolysis conditions), application rates, soil properties, and N fertilizer types. To date, the mechanisms

✉ Qinghai Zhang
zhqh@gmc.edu.cn

✉ Jianzhong Cheng
chengjianzhong@vip.gyig.ac.cn

[1] School of Public Health, The Key Laboratory of Environmental Pollution Monitoring and Disease Control, Ministry of Education, Guizhou Medical University, Guiyang 550025, Guizhou Province, China

[2] Guizhou Academy of Tobacco Science, Guiyang 550081, Guizhou Province, China

[3] State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550081, Guizhou Province, China

and the importance they might have in altering N_2O emissions remain controversial and are still poorly understood (Cayuela et al. 2014).

The understanding of the inconsistent effects of BC on N_2O emissions is limited by the lack of understanding of the mechanisms altering changes in community composition under BC application (Guo et al. 2020). BC amendment can enhance or reduce soil microbial biomass, depending on its type and application rate (Kolb et al. 2009; Dempster et al. 2012). However, as soil microbial biomass changes, it is unlikely that all microbial communities will undergo the same alterations (Lehmann et al. 2011). Numerous studies have demonstrated that BC amendment alters the composition of microbial communities at the phylum level. For example, the relative abundances of *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* were enhanced by BC treatments (Anderson et al. 2011; Khodadad et al. 2011; Hu et al. 2014). In contrast, other studies have shown that the relative abundances of these microbial communities decreased in BC-amended soils (Kolton et al. 2011; Hu et al. 2014; Wu et al. 2016). Similarly, BC amendment has been found to have positive or negative effects on the abundance of *Bradyrhizobiaceae*, which plays an important role in N cycling associated with the immobilization of ammonium and nitrate (Anderson et al. 2011; Cheng et al. 2019). Therefore, BC addition may alter the abundance or activity of microbial functional groups as well as the ratio of nitrifiers to denitrifiers, which regulates N_2O emission (Braker and Conrad 2011; Shi et al. 2019). However, there is still little data available on the potential links between shifts in microbial community composition and the observed soil N_2O emissions.

In this study, a field experiment was conducted using soils amended with BC to investigate changes in soil microbial response and N_2O emissions. BC was prepared using tobacco stalk, which is a typical byproduct of tobacco cultivation. Tobacco is an important economic crop in the karst regions of China, and its cultivation process mainly includes ridging, mulching, planting in holes, and transplanting. Tobacco planting techniques are different from those of other crops. Moreover, flue-cured tobacco is a crop with high nitrogen requirements. Therefore, this type of tobacco BC is usually characterized by high nitrogen content, which may be beneficial to soil fertility and carbon sequestration. We hypothesized that tobacco BC might also affect soil N_2O emissions and microbial community in farmland. Understanding the microbial mechanisms controlling N_2O emissions is critical for predicting the utility of tobacco BC as a long-term C storage medium. Therefore, five application rates of tobacco BC (0, 1, 10, 25, and 50 t ha^{-1}) were incorporated into the tobacco-planting soil to investigate soil N_2O emissions and bacterial and fungal community compositions. The objectives of this study were to explore (1) the effects of different doses of BC on N_2O emissions from a tobacco-planting soil; (2) the responses of soil bacterial and fungal communities to BC amendment; and (3) the link between N_2O emissions from BC-amended soil and the structure and function of the N-cycling microbial community.

2 Materials and Methods

2.1 Study Site

The experiment was set up on an arable field at the Pingba Tobacco Experimental Station in Guizhou province of China (26° 29′N, 106° 17 E; 1391 m a.s.l.) on yellow soil, as determined by the Chinese Genetic Soil Classification System. The site was located in a subtropical humid monsoon climate with abundant precipitation (average annual rainfall > 1000 mm). The distribution of the precipitation is very uneven, and precipitation in summer accounts for about 70% of the total annual rain. At the same time, due to the characteristics of low soil water storage capacity and strong rock leakage in karst regions, soil drought is severe and accompanied by frequent alternation of wet and dry processes. The BC used in this study was made of tobacco stalk, and had a high content of N (12.67 g kg^{-1}). The details for BC production procedures were described previously (Tang et al. 2021). The BC was ground to pass through a 1-mm mesh sieve for use in the field study. The basic properties of the soil and the BC are presented in Table 1.

2.2 Experimental Design

The field experiment was conducted in a randomized complete block design, with BC application at rates of 0, 1, 10, 25, and 50 t ha^{-1} (BC_0, BC_1, BC_2, BC_3, and BC_4,

Table 1 Chemical variables of tobacco-planting soil at the depth of 0–20 cm and biochar obtained from tobacco stalk

Parameters	Soil	Biochar
pH ($_{H2O}$)	6.58	8.34
C $_{total}$ (%)	2.24	41.32
N $_{total}$ (%)	0.21	1.27
C/N mass ratio	10.68	32.63
P $_{total}$ (g kg^{-1})	0.55	4.04
K $_{total}$ (g kg^{-1})	13.00	45.95
N $_{available}$ (mg kg^{-1})	145.56	-
P $_{available}$ (mg kg^{-1})	6.27	-
K $_{available}$ (mg kg^{-1})	250.50	-

C $_{total}$, total carbon content; *N $_{total}$*, total nitrogen content; *C/N mass ratio*, carbon and nitrogen mass ratio; *P $_{total}$*, total phosphorus content; *K $_{total}$*, total potassium content; *N $_{available}$*, available nitrogen content; *P $_{available}$*, available phosphorus content; *K $_{available}$*, available potassium content

respectively) in a tobacco field. In 22 April 2018, BC was spread on the soil surface, and thoroughly plowed to a depth of 20 cm. Then, the dosage (675 kg ha^{-1}) of special base fertilizer for flue-cured tobacco (N: P_2O_5: K_2O = 10:10:25) was applied into all treatments. In 23 April 2018, ridges were set up in each plot at a height of 30 cm. Each treatment was replicated three times, and the area of each plot was 19.36 m^2. In order to prevent edge effects, a 1-m buffer zone was set between different plots. In May 2018, tobacco seedlings of *Nicotiana tabacum* L. (K326) were transplanted into the row ridges, and they matured in September 2018. The field trial lasted for 120 days, which was the growth season of flue-cured tobacco in Guizhou province.

2.3 Measurement of N_2O Flux

During the whole tobacco growing season, soil N_2O emissions were measured following the static chamber method from May 2018 to September 2018. First, chamber bases (30-cm diameter and 10-cm height) were inserted 7 cm deep into the soil for each plot. These bases remained in place throughout the entire monitoring period. For measuring soil N_2O emissions, each chamber was placed on the base and sealed by filling the base with water. The air temperature inside the chamber was recorded using a digital thermometer during sampling. An electric fan was installed to ensure the mixing of gases inside the chamber. Four gas samples were taken at 0, 8, 16, and 24 min after chamber closure. Gas samples in the pre-evacuated glass bottle were immediately transported to the laboratory, placed on a Gilson autosampler (Gilson Sample Changer 223, USA) and analyzed using a gas chromatograph (Agilent 7890A, USA) equipped with an electron capture detector to determine the N_2O concentration. Standard gases were used to calibrate the system before gas samples were analyzed. The GC was calibrated using three certified standard gases, comprising 0.28 ppm, 0.50 ppm, and 0.96 ppm N_2O (Chengdu Chenggang Messer Gas Products Co. Ltd, China). The details of the calculation of N_2O fluxes and cumulative emissions are described by Yoo et al. (2018).

2.4 Soil Collection and Analyses

Samples were taken from five different locations in each plot after removing the tobacco plants. These samples were mixed until homogeneity and sieved through 2-mm mesh to remove stones, roots, and plant residues. Sieved soil samples were divided into two parts. One subsample was immediately stored for DNA analysis. The other subsample was air-dried for the determination of soil physicochemical properties. The details of the analyses of total C (TC), total N (TN), total P (TP), total K (TK), available nitrogen (AN), available P (AP), and available K (AK) are described in the previous study (Tang et al. 2021).

2.5 Characterization of Soil Microbial Communities

DNA was extracted from soil samples (0.25 g) using the MO BIO PowerSoil DNA Isolation Kit. The quality and purity of DNA were determined using gel electrophoresis and UV spectrophotometry. The bacterial V3–V4 region of the 16S rRNA gene was amplified using 341F/806R primer pairs. The ITS1-5F region of fungi was amplified using the primers ITS5-1737F (forward primer) and ITS2-2043R (reverse primer). A total of 30 μL of polymerase chain reaction (PCR) reaction mixture comprised 15 μL of 2 × Phusion High-Fidelity PCR Master Mix, 10 μL (1 ng/μL) of template DNA, 1 μL of each primer (1 mM), and 3 μL of milli-Q water. The thermal cycling conditions included 1 min initial denaturation at 98 °C; 30 cycles of 10 s denaturation at 98 °C; 30 s annealing at 50 °C; and 30 s extension at 72 °C, followed by 5 min of final extension at 72 °C. Before Illumina sequencing (Illumina, San Diego, CA, USA), equal amounts of PCR products from different samples were mixed, purified, and quantified.

Quality filtering on the raw tags was performed to obtain high-quality clean tags using the QIIME software package. Both the barcode and the primer were filtered from the high-quality reads. Operational taxonomic units (OTUs) were clustered at a 97% similarity threshold cutoff level using the UPARSE. The representative sequence for an OTU can be obtained by determining the most frequently occurring sequence in the OTU. The taxonomies of representative OTU sequences were selected and determined using the RDP classifier and GreenGenes database from species to phylum at hierarchical levels. Then, the phylogenetic relationships and alpha and beta diversities of OTU representatives were analyzed using the MUSCLE program (Cheng et al. 2019). The raw data sequences used here have been deposited to NCBI with accession number SAMN29594028-SAMN29594042.

2.6 Statistical Analysis

Values were expressed as the mean ± standard error. Differences in N_2O fluxes among various BC treatments were assessed by performing an analysis of variance (ANOVA) with the least significant difference test using SPSS version 16.0 (SPSS Inc., Chicago, IL). Alpha and beta diversity metrics were calculated for each sample using QIIME. Alpha diversity was applied to analyze the species diversity in samples through the following indices: observed species, Chao1, abundance-based coverage estimator (ACE), Shannon, Simpson, and Good's coverage. Beta diversity based on both weighted and unweighted Unifrac distances was calculated to evaluate inter-sample species complexity. The

relationship between soil properties and the diversity and composition of soil microbes was investigated using correlation and regression analyses. Non-metric multidimensional scaling (NMDS) was used to visualize the grouping of microbial communities between different treatments. In addition, redundancy analysis (RDA) was performed to clarify the relationships between bacterial/fungal community structures and environmental factors. The level of significance for statistical testing was considered at 0.05 ($p \leq 0.05$).

3 Results

3.1 Effect of BC Amendment on Soil Moisture and N_2O Emissions

Soil moistures under different BC treatments increased with the increase of tobacco growth period (Table 2). Compared with BC_0, BC_1, BC_2, BC_3, and BC_4, treatments increased soil moisture at the root extending period (REP) by 20.56, 30.76, 32.92, and 42.29%, respectively. Moreover, there was significantly different soil moisture between BC_4 and BC_0. At the vigorous period (VP), the effect of BC amendment on soil moisture was much smaller compared to BC_0, ranging from − 9.76 to 8.08%. At the mature period (MP), the BC amendment decreased soil moisture from 1.60 to 5.57%, but the difference between treatments was not significant.

For all treatments, the soil N_2O emissions peaked at the day of tobacco transplanting, followed by a sharp decrease to low levels at day 40 (Fig. 1a). Compared to the control (BC_0), the BC_1, BC_2, BC_3, and BC_4 treatments significantly increased the peak N_2O emissions from 17.02 to 24.74, 84.57, 59.47, and 92.09 μg N m^{-2} h^{-1}, respectively, with higher decreases for the BC_3 and BC_4 treatments (4.97- and 5.41-fold) than the BC_2 treatment (1.45-fold) at day 40 after tobacco transplanting.

During the whole tobacco growing season, BC amendment significantly increased soil cumulative N_2O emissions (Fig. 1b). Compared with BC_0, the cumulative N_2O emissions significantly increased by 1.96-, 4.16-, 2.67-, and 4.18-fold in the BC_1, BC_2, BC_3, and BC_4 treatments, respectively ($p < 0.05$). For all treatments, 47.57–73.37% of the cumulative N_2O emissions originated during the first 40 days after seeding transplanting. However, there was no significant difference in cumulative N_2O emissions between the BC_2 and BC_4 treatments (Fig. 1b).

Table 2 Effect of biochar amendment on soil water content (%) in different tobacco growth periods

Treatments	Growth periods		
	REP (30 days)	VP (30 days)	MP (60 days)
BC_0	14.40 ± 1.80^{b}	19.68 ± 2.35^{a}	33.21 ± 1.28^{a}
BC_1	17.36 ± 1.79^{ab}	17.76 ± 2.12^{a}	31.36 ± 1.00^{a}
BC_2	18.83 ± 1.79^{ab}	20.35 ± 2.33^{a}	32.01 ± 1.01^{a}
BC_3	19.14 ± 1.80^{ab}	20.16 ± 2.78^{a}	31.73 ± 1.13^{a}
BC_4	20.49 ± 1.51^{a}	21.27 ± 2.94^{a}	32.68 ± 0.93^{a}

Values are presented as mean ± standard error ($n = 3$). Different lowercase letters within a single column indicate significant differences between treatments based on a least significant difference (LSD) test at $p \leq 0.05$. *BC_0*, biochar dose of 0 t ha^{-1}; *BC_1*, biochar dose of 1 t ha^{-1}; *BC_2*, biochar dose of 10 t ha^{-1}; *BC_3*, biochar dose of 25 t ha^{-1}; *BC_4*, biochar dose of 50 t ha^{-1}. *REP*, root extending period; *VP*, vigorous period; *MP*, mature period

3.2 Effects of BC Amendments on the Diversity of Microbes

To compare the bacterial and fungal community diversities among all the treatments, sequencing depths of 46,876 and 56,176 sequences, respectively, were randomly selected from each sample. Venn diagrams were constructed based on the shared and unique OTUs in BC-amended soils. The number of bacterial OTUs ranged from 2514 to 2709, and fungal OTUs ranged from 1595 to 2030. The common OTUs in all BC treatments for bacteria and fungi numbered 1762 and 584, respectively (Fig. 2). Good's coverage estimate for each sample exceeded 99% (Table 3), indicating that the sampling was sufficient to cover the bacterial and fungal communities. The bacterial and fungal communities of BC-amended soils had enhanced richness, with both ACE and Chao1 significantly increased in the BC-amended soils compared to the control. However, the Shannon and Simpson indices of bacteria and fungi presented considerable variation. These bacterial indices gradually decreased among all BC treatments, and an extremely low decrease trend was observed in the BC_3 treatment compared with the control. For fungi, BC increased the Shannon and Simpson indices except in the BC_4 treatment, while the maximum was observed in the BC_2 treatment.

A Pearson correlation analysis was conducted to identify the possible correlations between bacterial/fungal diversities and soil environmental factors (including soil organic matter (SOM), TC, TN, TP, TK, TS, AN, AP, AK, and soil water content (WC)) under different rates of BC application (Table 4). The indices of bacterial observed OTUs and ACE were positively associated with SOM, TC, AK, and WC ($p < 0.05$), and the PD whole tree index was positively associated with AK ($p < 0.01$). However, the diversity indices (ACE, observed OTUs, Chao1, Shannon, and Simpson) of the bacteria were not significantly affected by TN, TP, TS, AN, and AP. Like bacteria, fungal diversity was often influenced by soil parameters. For example, ACE and Simpson were positively associated with AN ($p < 0.05$). The index of Good's coverage was negatively associated with TC, TN, TP, and WC but positively associated with TS ($p < 0.05$) (Table 4).

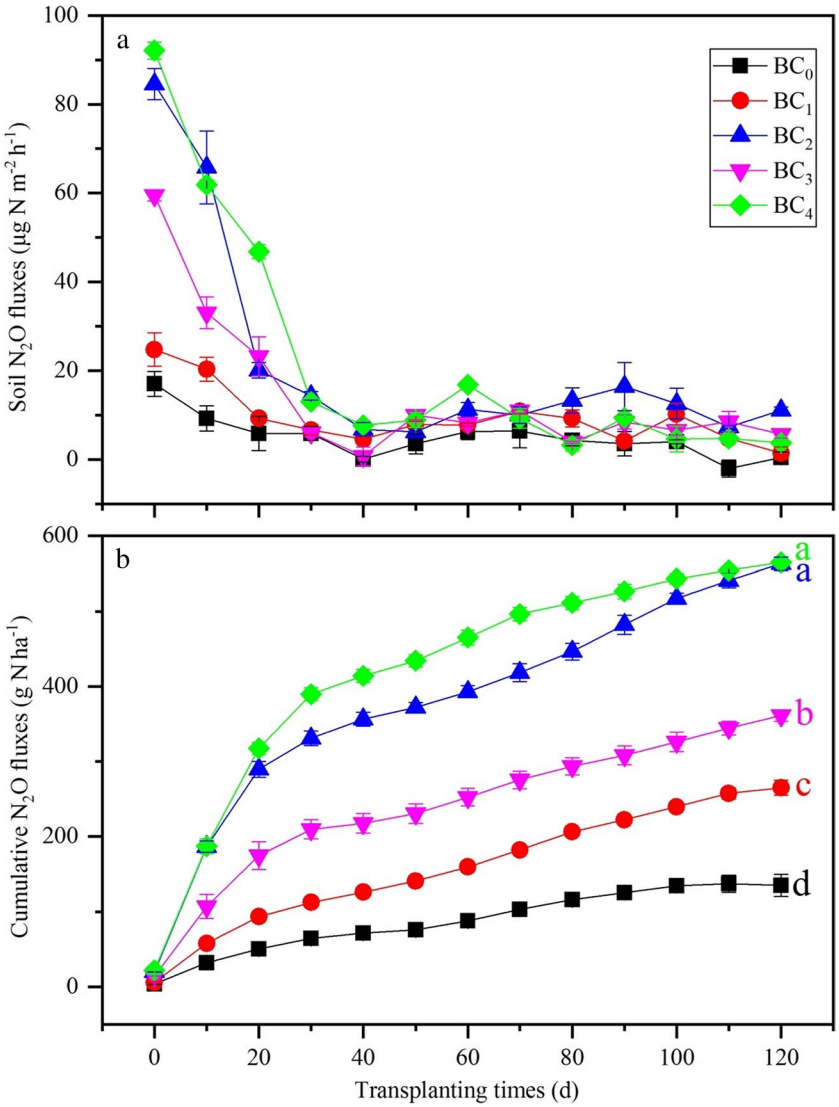

Fig. 1 N_2O fluxes (**a**) and cumulative emissions (**b**) from tobacco-planting soil amended with different doses of biochar (0, 1, 10, 25, and 50 t ha.$^{-1}$). Bars represent the mean ± standard error ($n = 3$). Different letters indicate significant differences among treatments based on a least significant difference (LSD) test at $p \leq 0.05$

3.3 Effects of BC Amendment on the Microbial Community Composition

BC addition changed the microbial community composition from the phylum to genus levels. The major bacterial phyla were *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Actinobacteria*, *Gemmatimonadetes*, *Chloroflexi*, *Verrucomicrobia*, *Thaumarchaeota*, *Nitrospirae*, and *Latescibacteria* (Fig. 3a). These dominant phyla comprised > 97% of the bacterial communities across all soil samples. As shown in Fig. 3a, *Proteobacteria* was observed to be the most sensitive phylum to BC amendment and increased from 41.7% in the BC_0 treatment to 55.2% in the BC_4 treatment. However, *Acidobacteria*, *Bacteroidetes*, and *Thaumarchaeota* were significantly decreased by BC amendment, from 17.5 to 10.0%, 10.7 to 7.8%, and 1.5 to 0.5%, respectively. There were no significant differences among BC treatments in the relative abundances of *Gemmatimonadetes* and *Chloroflexi*. For fungi, three dominant phyla of *Ascomycota*, *Mortierellomycota*, and *Basidiomycota* were identified in soil samples under BC application and accounted for more than 47% of the fungal sequences (Fig. 3b). Moreover, BC amendment increased the relative abundance of *Ascomycota* but decreased *Mortierellomycota* and *Basidiomycota*. There was a significant difference between the BC_0 and BC_4 treatments ($p < 0.05$) in the relative abundances of *Ascomycota* and *Mortierellomycota*.

There are different relationships between soil properties and microbial communities (Fig. 4). For instance, the abundances of *Proteobacteria*, *Berkelbacteria*, *Gracilibacteria*, and *Candidatus_Kaiserbacteria* were significantly positively correlated to SOM, TN, and AK contents ($p \leq 0.05$), and extremely significantly positively correlated to TC content ($p \leq 0.01$). However, *Acidobacteria* and *Latescibacteria* showed the opposite pattern, and were significantly negatively correlated with SOM, TN, and AK contents

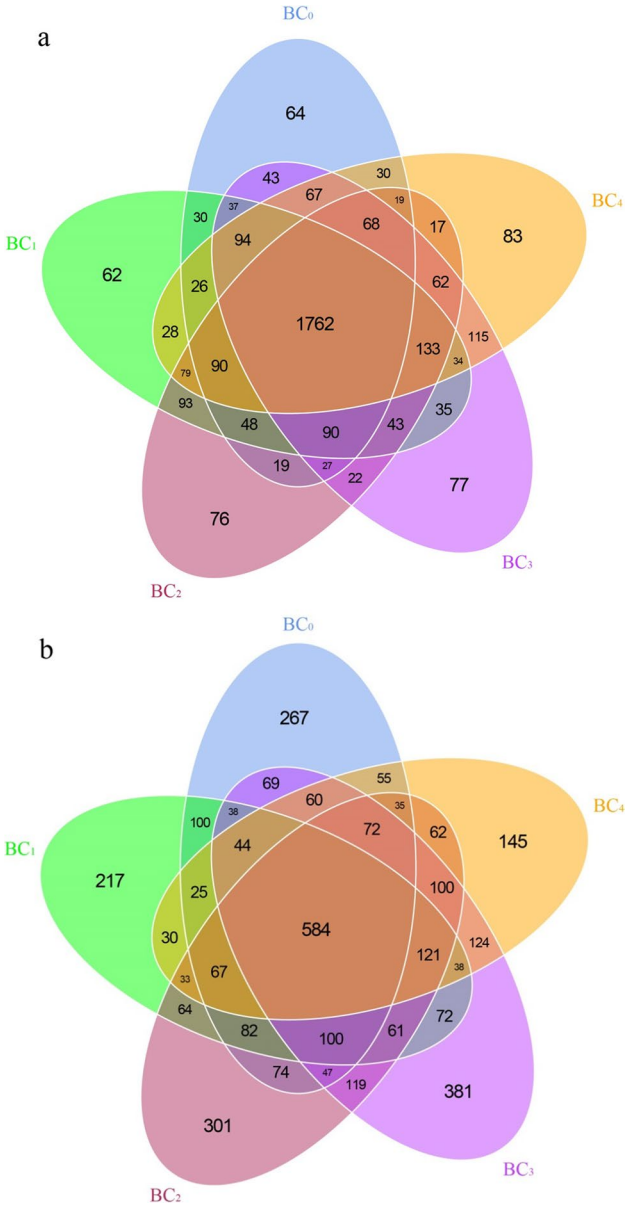

Fig. 2 Venn diagram of operational taxonomic units (OTUs): **a** bacteria, **b** fungi. BC_0, biochar dose of 0 t ha^{-1}; BC_1, biochar dose of 1 t ha^{-1}; BC_2, biochar dose of 10 t ha^{-1}; BC_3, biochar dose of 25 t ha^{-1}; BC_4, biochar dose of 50 t ha^{-1}

($p \leq 0.05$) and extremely significantly positively correlated with TS content ($p \leq 0.01$) (Fig. 4a). With regard to fungi, the abundances of *Glomeromycota* and *Monoblepharomycota* were extremely significantly negatively correlated with SOM, TC, TN, AK, and WC contents ($p \leq 0.01$) and extremely significantly positively correlated with TS content ($p \leq 0.01$). This was the opposite of the relationships between the abundances of *Aphelidiomycota*, *Zoopagomycota*, and soil properties. In addition, the abundances of *Mortierllomycota* and *Neocallimastigomycota* were also significantly negatively associated with the contents of SOM, TC, AK, and WC ($p \leq 0.05$) (Fig. 4b).

3.4 Effects of BC Amendments on the Microbial Structure

NMDS analysis showed that the bacterial communities from the BC_1 and BC_2 treatments clustered together and significantly differed from the BC_0, BC_3, and BC_4 treatments (Fig. 5a). Fungal communities from the BC_0 and BC_1 treatments clustered together and were significantly separated from the BC_2, BC_3, and BC_4 treatments (Fig. 5b). These results indicated that both bacterial and fungal community structures were clearly affected by BC amendment, whereas the low BC application rates (≤ 10 t ha^{-1}) did not exert effects that separated the bacterial community composition, and the high application rates (≥ 25 t ha^{-1}) did not separate the fungal community composition.

The RDA plots also showed that the patterns of both bacterial and fungal community compositions in different BC treatments could be explained by the rates of BC amendment (Fig. 6). Upon AMOVA, the RDA1 and RDA2 components accounted for 52.78% and 25.01% of the total bacterial variance, respectively, or 84.09% and 12.09% of the total fungal variance, respectively. BIO-ENV procedures in R were used to preselect factors with the best correlation between the microbial community variation and soil environmental factors (SOM, TC, TN, TP, TK, TS, AN, AP, AK, and WC) under different BC amendments. After filtering, AN, AP, AK, and WC were identified as variables in the RDA plot and explained 76.15% of the variation in the soil bacterial community composition. Among those factors, the AN clearly explained the largest amount of variation in the community structure (47.40%) (Fig. 6a). In addition, TP, AP, and AK could explain 65.31% of the soil fungal community composition variation, and TP explained the largest significant variation (51.35%) (Fig. 6b).

3.5 Functional Microbe Associated with N Cycling

Several functional microorganisms involved in biochemical N cycling were found in soil mixtures and their abundance was influenced by BC amendments, especially at higher dosages (Table 5). The relative abundances of *Devosia*, *Bradyrhizobiu*, *Pedomicrobium*, *Pseudomonas*, *Trichocladium*, *Trichoderma*, and *Humicola* were significantly higher after BC amendment, while significantly lower relative abundances of *Haliangium* and *Opitutus* were found in BC-amended soils. There were no significant differences in the abundances of *Mycobacterium* and *Bacillus* between different BC amendments.

Table 3 Comparison of α-diversity indices in different biochar-amended soils

	Treatments	Shannon	Simpson	Chao1	ACE	Good's coverage
Bacteria	BC_0	8.86 ± 0.04^{a}	0.991 ± 0.002^{a}	2172 ± 62^{b}	2154 ± 41^{b}	0.993 ± 0.001^{a}
	BC_1	8.71 ± 0.09^{ab}	0.984 ± 0.002^{bc}	2238 ± 17^{ab}	2246 ± 13^{ab}	0.994 ± 0.000^{a}
	BC_2	8.58 ± 0.02^{b}	0.979 ± 0.001^{c}	2247 ± 16^{ab}	2253 ± 20^{ab}	0.993 ± 0.000^{a}
	BC_3	8.86 ± 0.04^{a}	0.989 ± 0.001^{ab}	2348 ± 17^{a}	2335 ± 12^{a}	0.993 ± 0.000^{a}
	BC_4	8.57 ± 0.11^{b}	0.982 ± 0.006^{c}	2218 ± 46^{b}	2230 ± 56^{ab}	0.994 ± 0.000^{a}
Fungi	BC_0	5.31 ± 0.54^{ab}	0.920 ± 0.015^{a}	974 ± 193^{a}	994 ± 191^{a}	0.997 ± 0.001^{a}
	BC_1	5.93 ± 0.14^{a}	0.944 ± 0.012^{a}	1037 ± 36^{a}	1055 ± 43^{a}	0.997 ± 0.000^{a}
	BC_2	6.31 ± 0.08^{a}	0.958 ± 0.003^{a}	1298 ± 24^{a}	1318 ± 25^{a}	0.997 ± 0.000^{a}
	BC_3	6.01 ± 0.53^{a}	0.942 ± 0.024^{a}	1236 ± 153^{a}	1245 ± 144^{a}	0.997 ± 0.000^{a}
	BC_4	4.51 ± 0.24^{b}	0.802 ± 0.034^{b}	1070 ± 62^{a}	1101 ± 67^{a}	0.996 ± 0.001^{a}

Values are presented as mean ± standard error ($n = 3$). Different lowercase letters within a single column indicate significant differences between treatments based on a least significant difference (LSD) test at $p \leq 0.05$. *ACE*, abundance-based coverage estimator; BC_0, biochar dose of 0 t ha^{-1}; BC_1, biochar dose of 1 t ha^{-1}; BC_2, biochar dose of 10 t ha^{-1}; BC_3, biochar dose of 25 t ha^{-1}; BC_4, biochar dose of 50 t ha^{-1}

Table 4 Relationships between diversity indices of bacteria/fungi and soil environmental factors

	Indices	SOM	TC	TN	TP	TK	TS	AN	AP	AK	WC
Bacteria	Observed OTUs	0.59*	0.56*	0.37	0.45	0.52*	−0.33	0.48	0.36	0.66**	0.52*
	Shannon	−0.27	−0.30	−0.38	−0.21	0.24	0.47	−0.39	−0.42	−0.24	−0.16
	Simpson	−0.27	−0.28	−0.32	−0.23	0.14	0.37	−0.50	−0.49	−0.27	−0.19
	Chao1	0.45	0.41	0.29	0.41	0.36	−0.26	0.21	0.19	0.44	0.47
	ACE	0.54*	0.53*	0.37	0.46	0.42	−0.32	0.38	0.32	0.60**	0.54*
	Good's coverage	−0.14	−0.14	−0.12	−0.20	0.23	0.09	−0.06	−0.21	−0.12	−0.25
	PD whole tree	0.62*	0.58*	0.46	0.42	0.37	−0.44	0.47	0.35	0.68**	0.45
Fungi	Observed OTUs	0.27	0.26	0.30	0.36	0.11	−0.31	0.43	0.47	0.18	0.23
	Shannon	−0.14	−0.13	−0.10	0.05	0.12	0.09	0.52*	0.29	−0.20	−0.21
	Simpson	−0.15	−0.19	−0.19	−0.03	0.06	0.18	0.64*	0.33	−0.20	−0.24
	Chao1	0.33	0.31	0.36	0.42	0.18	−0.38	0.39	0.48	0.24	0.30
	ACE	0.30	0.29	0.34	0.41	0.16	−0.36	0.34	0.44	0.20	0.28
	Good's coverage	−0.46	−0.52*	−0.55*	−0.54*	−0.10	0.54*	0.03	−0.45	−0.43	−0.59*
	PD whole tree	0.22	0.25	0.26	0.29	0.10	−0.33	0.41	0.41	0.15	0.19

Significant values are shown as *$p \leq 0.05$ and ** $p \leq 0.01$. *SOM*, soil organic matter; *TC*, total carbon; *TN*, total nitrogen; *TP*, total phosphorus; *TK*, total potassium; *AN*, available nitrogen; *AP*, available phosphorus; *AK*, available potassium; *WC*, soil water content; Observed OTUs, observed operational taxonomic units; *ACE*, abundance-based coverage estimator; *PD whole tree*, phylogenetic diversity whole tree

4 Discussion

Compared with the control without BC amendment, the cumulative N_2O emissions in the BC amendment treatments showed a significant increasing trend ($p < 0.05$) over the 120-day tobacco cultivation. Similarly, some previous studies reported a significant increase of N_2O emissions after BC amendments (Chen et al. 2015; Agegnehu et al. 2016; Wei et al. 2020), while other researchers observed a decline or no significant difference in N_2O production following the application of BC prepared from different raw materials (Cayuela et al. 2014; He et al. 2017). The inconsistent effects of BC application on soil N_2O emissions largely depend on the type of BC (feedstock source and pyrolysis conditions) used and the soil physical and chemical properties (pH, water content, and C and N status) (Cayuela et al. 2014). The increasing trend of N_2O emissions observed in BC treatments in this study was attributed the higher total C and N contents under BC application. Compared with the control, the available N and total C contents of BC treatments increased by 7.40–23.06%, and 7.56–119.50%, respectively (Tang et al. 2021). The enhanced SOM induced by BC application can increase substrate availability for the growth of microorganisms, resulting in greater N_2O emissions in tobacco-planting soil. Some studies have also suggested that a positive priming

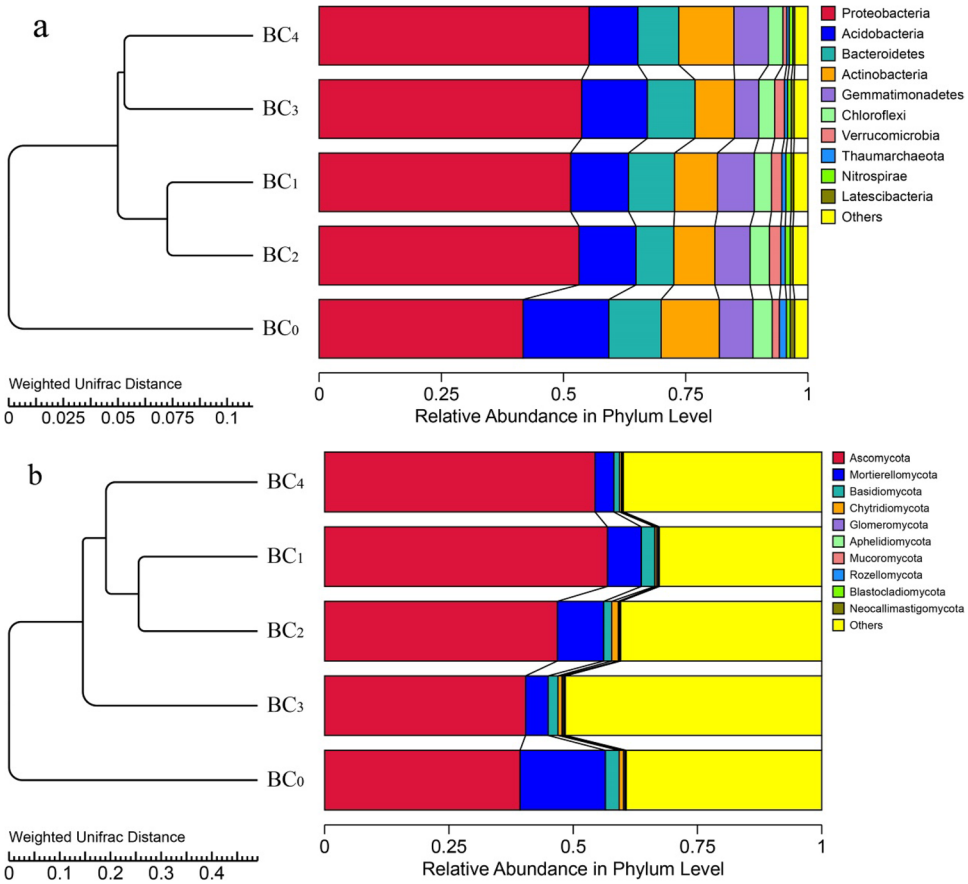

Fig. 3 Hierarchical cluster analysis of relative abundances of bacterial (**a**) and fungal (**b**) phyla under different biochar application rates. BC_0, biochar dose of 0 t ha^{-1}; BC_1, biochar dose of 1 t ha^{-1}; BC_2, biochar dose of 10 t ha^{-1}; BC_3, biochar dose of 25 t ha^{-1}; BC_4, biochar dose of 50 t ha^{-1}

effect is induced after the addition of BC with easily available substrates (labile C and available N), and the increase in microbial efficiency caused by adding substrate can induce microbial N mining through SOC mineralization (Yoo and Kang 2012; Farrell et al. 2013). Similarly, enhanced N_2O emissions were observed in the soil amended with wheat straw-derived BC with high N content (Lin et al. 2017). Therefore, the application of BC derived from different materials may produce inconsistent effects on N_2O emissions, while the application of N-rich BC to the aerobic soil of tobacco fields results in mainly positive priming effects.

In the present study, the relative abundances of *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Thaumarchaeota* decreased under BC treatment, while *Proteobacteria* increased. *Proteobacteria* was the most abundant phylum in all BC treatments, which suggested that BC retained nutrients and improved soil biological properties, thereby enhancing the growth of *Proteobacteria* (Liu et al. 2019). *Acidobacteria* usually live in an acidic environment and play an important role in the biogeochemical cycling of carbon (Jiang et al. 2017). However, tobacco BC is alkaline and can considerably modify the microbial living environment by correcting soil acidity, which is not favorable for *Acidobacteria*. Therefore, BC amendment decreased the abundance of *Acidobacteria*. Similar results were reported by other studies (Xu et al. 2016; Ali et al. 2022). Therefore, BC addition to soil can not only change the characteristics of the habitat in which bacteria colonize but also affect soil bacterial activity and nutrient cycling.

Consistent with previous studies (Chen et al. 2013; Yao et al. 2017b), the present study found that BC application had an influence on the relative abundances of the fungal community at the phylum level. For example, the abundances of *Ascomycota*, *Mortierellomycota*, and *Basidiomycota* significantly changed with BC application. Within the *Ascomycota* phylum, the relative abundances of *Humicola* and *Microthecium* increased significantly, while *Fusarium* and *Alternaria* decreased significantly under BC addition. *Alternaria* and *Fusarium* are fungal genera ubiquitous in the environment, and many species are known as plant pathogens, causing root rot (Elmer and Pignatello 2011; Arfi et al. 2012). In the present study, the decreased *Alternaria* and *Fusarium* abundances in BC-amended soils may be beneficial for protecting plants against pathogens and disease. The decline patterns were consistent with those reported by Lehmann et al. (2011), who indicated that BC addition suppressed some crop diseases. In addition, the relative abundance of the phylum *Zygomycota* increased with the increase of the BC application rate, and similar results were also reported in other studies (Yao et al. 2017b; Zhang et al. 2018). *Zygomycota* can not only degrade different organic pesticides but also promote the

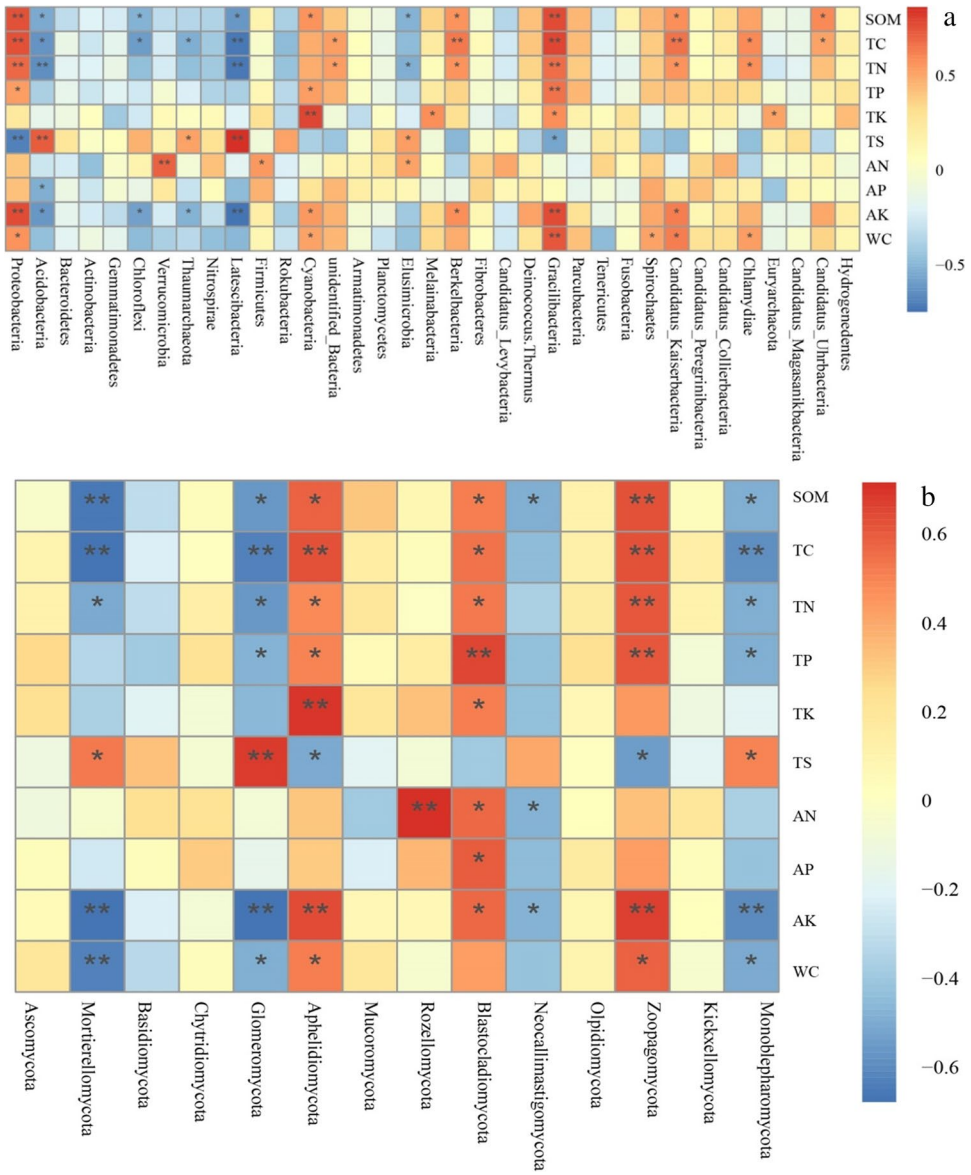

Fig. 4 Correlation heatmap of soil properties with the bacteria (**a**) and fungi (**b**) at the phylum level. Different colors at the extreme right indicate the different *r* values. Significant results are shown as $*p \leq 0.05$ and $**p \leq 0.01$. SOM, soil organic matter; TC, total carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AN, available nitrogen; AP, available phosphorus; AK, available potassium; WC, soil water content

recycling of soil nutrients and crop growth (Neumann et al. 2014). Therefore, BC application will stimulate special functional fungi, thereby promoting nutrient cycling and disease resistance (Lehmann et al. 2011).

The effects of BC amendment on changes in soil microbial community structure remain controversial (Castaldi et al. 2011; Hu et al. 2014; Zhang et al. 2018). Castaldi et al. (2011) reported that BC amendment had no or little effect on soil microbial community structures. However, some studies demonstrated that both bacterial and fungal community structures were markedly altered due to BC application, with both bacteria and fungi mainly altered at the phylum or genus level (Hu et al. 2014; Zhang et al. 2018). In the present study, changes in community structure for bacteria could be observed at the phylum level under BC amendment, while the shifts in fungi could occur at the genus level. These inconsistent results might have been caused by several factors, such as the diverse parent microbial communities, soil conditions, and BC types and application rates (Lehmann 2007), which collectively influenced the microbial dynamics and distribution (Tsiamis et al. 2012). Taking these studies together, it can be concluded that BC applications can affect soil microbes through both indirect and abiotic functions. The changes in soil properties, microbial community structure, and function caused by BC amendment may affect the biogeochemical cycling of soil nutrient elements (Zhang et al. 2018).

BC addition can change soil microbial communities by regulating soil properties (Sheng and Zhu 2018). In the present study, RDA showed that AN, AP, and AK were significantly related to changes in bacterial community structures. Previous studies indicated that BC addition resulted

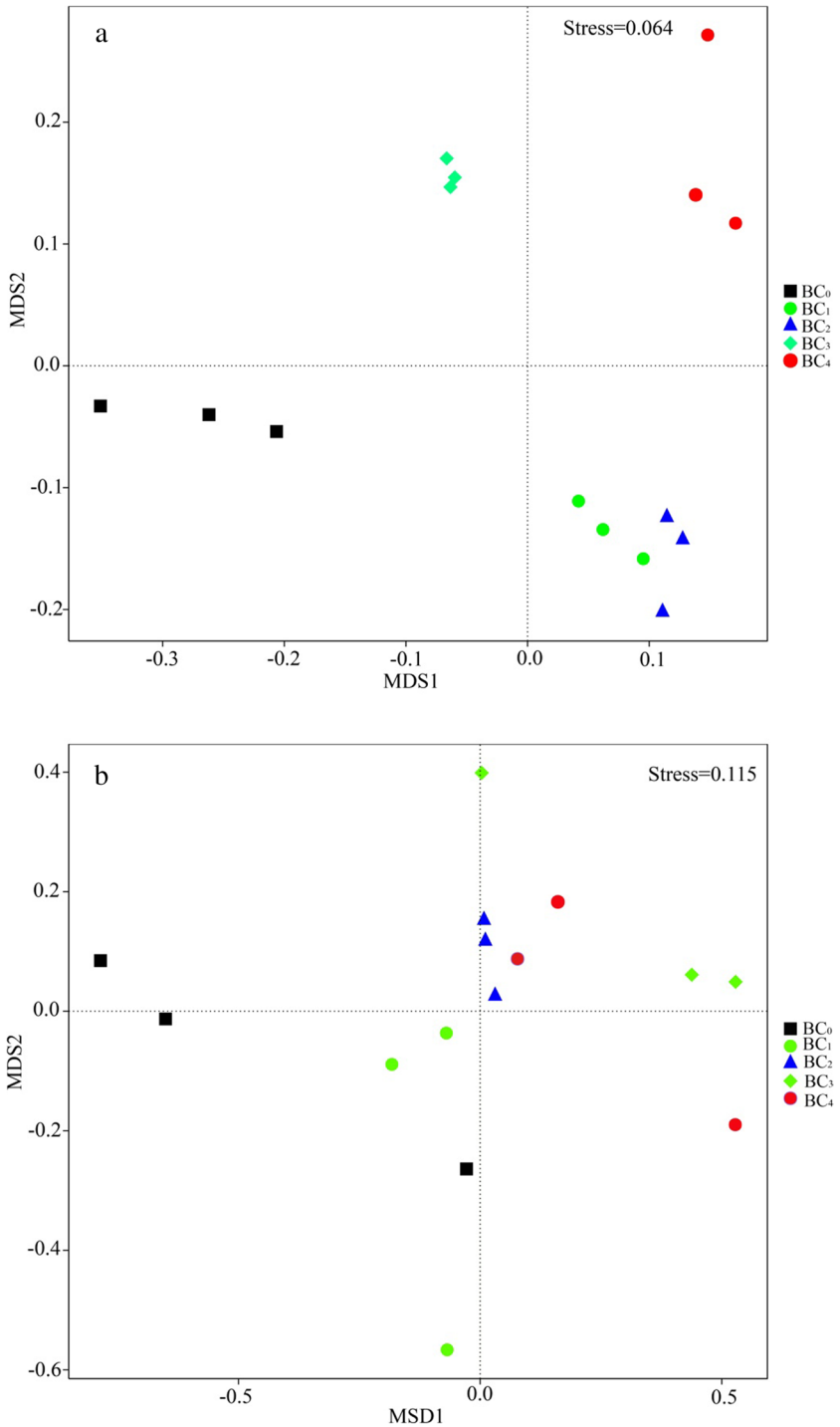

Fig. 5 Nonmetric multidimensional scaling of the communities of bacteria (**a**) and fungi (**b**) in soils amended with different biochar application rates. BC_0, biochar dose of 0 t ha^{-1}; BC_1, biochar dose of 1 t ha^{-1}; BC_2, biochar dose of 10 t ha^{-1}; BC_3, biochar dose of 25 t ha^{-1}; BC_4, biochar dose of 50 t ha^{-1}

in significant changes in bacterial community composition, which was mainly affected by pH, TC, and TN contents (Yao et al. 2017a; Sheng and Zhu 2018). Therefore, changes in microbial community composition after BC addition in some cases were attributed to BC-induced changes in pH. However, the tobacco-planting soil from the karst area is close to neutral (pH = 6.58), and adding BC to neutral soils had little effect (0.22–0.89 pH units). Therefore, changes in community composition could not confidently be attributed to pH effects caused by BC amendment in the present study. The contents of AN, AP, and AK, rather than TN, TP, and TK, were significantly related to the structure of the bacterial community, indicating that the bacteria in tobacco-planting soils were more likely to use bioavailable N, P, and K. Similarly, RDA showed that the composition of the soil fungal community changed with the TP, AP, and

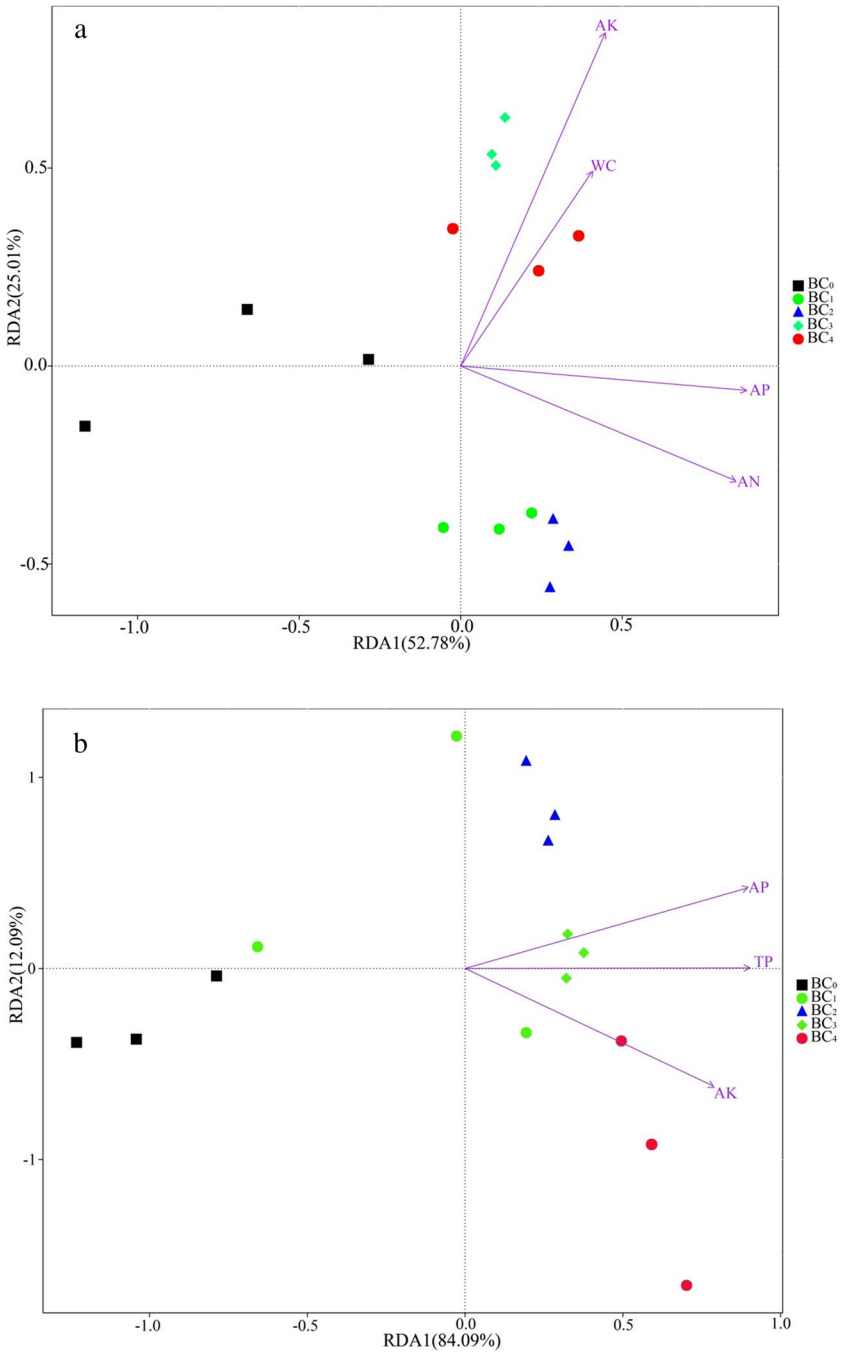

Fig. 6 Redundancy analysis (RDA) of bacterial (**a**) and fungal (**b**) community changes with soil physicochemical characteristics under different BC application rates. TP, total phosphorus; AN, available nitrogen; AP, available phosphorus; AK, available potassium; WC, soil water content. BC_0, biochar dose of 0 t ha^{-1}; BC_1, biochar dose of 1 t ha^{-1}; BC_2, biochar dose of 10 t ha^{-1}; BC_3, biochar dose of 25 t ha^{-1}; BC_4, biochar dose of 50 t ha^{-1}

AK contents. In summary, changes in fungal community composition induced by BC amendment may also regulate nutrient cycling and plant growth. The above results provide valuable insights into the response of soil properties to microbial community composition. However, it is unclear whether the profound BC-induced changes in the microbial community composition impact its activity and function.

The availability of soil N (NH_4^+ or NO_3^-) is an important factor that should be considered to control N_2O fluxes, because the N availability is an effective substrate for microbial growth (Ju and Zhang 2017). Moreover, most denitrifying bacteria are heterotrophic and therefore require an organic carbon source to maintain cellular activity, which is also an important factor regulating soil N_2O emissions (Wang et al. 2018). In the present study, soils treated with BC had higher available substrates (such as TC, TN, and AN) for denitrifying microbes, which indicated that these substrates were the main factors affecting of N_2O emission. In addition, several microbes involved in biochemical N cycling were found in soil mixtures, and their abundance was influenced by BC amendments, such as species of the genera *Devosia*, *Bradyrhizobiu*, and *Trichocladium*. More specifically, BC application

Table 5 Relative abundance (%) of functional microbial genera related to biochemical nitrogen cycling

Genera	BC_0	BC_1	BC_2	BC_3	BC_4
Devosia	0.18 ± 0.09^{c}	0.20 ± 0.04^{bc}	0.22 ± 0.01^{bc}	0.34 ± 0.03^{b}	0.57 ± 0.05^{a}
Bradyrhizobium	0.91 ± 0.17^{c}	1.58 ± 0.07^{a}	1.63 ± 0.04^{a}	1.02 ± 0.05^{bc}	1.37 ± 0.17^{ab}
Haliangium	1.25 ± 0.07^{a}	1.21 ± 0.05^{a}	0.98 ± 0.03^{b}	0.87 ± 0.07^{b}	0.53 ± 0.03^{c}
Rhodoplanes	1.02 ± 0.04^{b}	0.83 ± 0.05^{c}	0.88 ± 0.04^{bc}	1.22 ± 0.02^{a}	1.00 ± 0.08^{b}
Bacillus	0.17 ± 0.07^{a}	0.25 ± 0.04^{a}	0.29 ± 0.05^{a}	0.30 ± 0.06^{a}	0.21 ± 0.11^{a}
Mycobacterium	0.13 ± 0.05^{a}	0.09 ± 0.01^{a}	0.09 ± 0.01^{a}	0.05 ± 0.01^{a}	0.09 ± 0.02^{a}
Opitutus	0.07 ± 0.01^{a}	0.05 ± 0.01^{ab}	0.06 ± 0.00^{a}	0.05 ± 0.01^{ab}	0.03 ± 0.00^{b}
Pedomicrobium	0.31 ± 0.04^{b}	0.30 ± 0.00^{b}	0.26 ± 0.02^{b}	0.48 ± 0.02^{a}	0.45 ± 0.06^{a}
Pseudomonas	0.03 ± 0.01^{c}	0.14 ± 0.03^{bc}	0.08 ± 0.01^{bc}	0.55 ± 0.13^{a}	0.27 ± 0.07^{b}
Trichocladium	2.12 ± 1.20^{b}	12.83 ± 6.10^{b}	13.05 ± 1.01^{b}	12.88 ± 3.38^{b}	33.92 ± 10.80^{a}
Trichoderma	0.08 ± 0.07^{b}	0.11 ± 0.01^{b}	0.15 ± 0.01^{ab}	0.10 ± 0.02^{b}	0.28 ± 0.08^{a}
Humicola	0.05 ± 0.03^{b}	0.60 ± 0.50^{b}	2.18 ± 0.73^{a}	0.23 ± 0.02^{b}	0.35 ± 0.14^{b}

Values are presented as mean ± standard error ($n = 3$). Different letters indicate significant differences among treatments based on a least significant difference (LSD) test at $p \leq 0.05$. BC_0, biochar dose of 0 t ha^{-1}; BC_1, biochar dose of 1 t ha^{-1}; BC_2, biochar dose of 10 t ha^{-1}; BC_3, biochar dose of 25 t ha^{-1}; BC_4, biochar dose of 50 t ha^{-1}

significantly increased the abundances of *Bradyrhizobium* and *Pseudomonas*, several of which are known as *nirK*- and *nirS*-containing denitrifying bacteria that perform denitrification in aerobic or low-oxygen conditions (Ji et al. 2014; Sanchez and Minamisawa 2018). The cultivation of tobacco as implemented requires farming activities such as ridging. This allows the environment in the tobacco-planting soil to become almost aerobic (Pisa et al. 2022), which may explain why BC amendment increased soil N_2O emissions by affecting the aerobic denitrification of those bacteria. Moreover, *Trichocladium* and *Trichoderma* possess the *nirK* functional genes, and those genera were previously described as major sources of fungal species capable of fungal denitrification and the distinct ability to produce N_2O (Shoun et al. 2012; Xu et al. 2019). Compared to the control, the relative abundance of fungal *nirK*-containing denitrifiers (*Trichocladium* and *Trichoderma*) increased in the tobacco-planting soil under BC application. This could be another mechanism to explain the promotion in soil N_2O emissions observed under BC amendment. Therefore, BC could act as a soil conditioner, playing an important role in supporting the proliferation and interactions between these microbes, possibly because it supplies nutrient elements that these microbes can easily utilize, thereby moderating N-cycling dynamics and N_2O fluxes (Anderson et al. 2011).

5 Conclusions

Biochar significantly increased soil N_2O emissions by enhancing soil available nutrients and the relative abundance of denitrifying bacteria (*Bradyrhizobium* and *Pseudomonas*) and denitrifying fungi (*Trichocladium* and *Trichoderma*), and these effects depended on the biochar types and soil conditions. It must be taken into account that the findings presented here are based on an aerobic and fertilized field amended with tobacco biochar. Further research including long-term field trials on different cultivated soils is crucial to broaden the understanding of the impact of different biochar on soil N-cycling functional genes (fungal *nirK* and bacterial *nirK*, *nirS*, and *nosZ*) and pathways of N_2O production.

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Data Availability The raw data sequences used in this study are freely accessible via NCBI (https://www.ncbi.nlm.nih.gov/) with accession number SAMN29594028-SAMN29594042. This repository provides open access and long-term digital preservation of all data.

Declarations

Competing Interests The authors declare no competing interests.

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