Hu *et al. Annals of Microbiology* (2023) 73:4
https://doi.org/10.1186/s13213-022-01708-1

Annals of Microbiology

UNIVERSITÀ DEGLI STUDI DI MILANO

ORIGINAL ARTICLE

Open Access

# The effect of biochar on nitrogen availability and bacterial community in farmland

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## Abstract

**Purpose** Nitrification and denitrification in soil are key components of the global nitrogen cycle. This study was conducted to investigate the effect of biochar application on soil nitrogen and bacterial diversity.

**Methods** Pot experiments were conducted to investigate the effects of different biochar-based rates 0% (CK), 0.5% (BC1), 1.0% (BC2), 2.0% (BC3), and 4.0% (BC4) on soil nutrient and bacterial community diversity and composition.

**Results** The results indicate that the total nitrogen (TN) and ammonium nitrogen (AN) contents in the soil increased by 4.7–32.3% and 8.3–101.5%, respectively. The microbial biomass nitrogen (MBN) content increased with increased amounts of biochar rate. The application of biochar also significantly changed the soil bacterial community composition. The copy number of 16S marker gene of related enzymes to the nitrification process in BC2 was reduced by 20.1%. However, the gene expressions of nitric oxide reductase and nitrous oxide reductase in BC3 increased by 16.4% and 16.0%, respectively, compared to those in CK. AN, nitrate nitrogen (NN), and NN/TN were the main factors affecting the structure of the soil bacterial community. In addition, the expressions of nitrite reductase, hydroxylamine, and nitric oxide reductase (cytochrome c) were also significantly correlated.

**Conclusion** Therefore, the applied biochar improved soil nitrogen availability and which ultimately resulted in an environmental risk decrease by soil nitrogen release inhibition.

**Keywords** Soil nitrogen, Microbial community composition, Bacterial diversity, Biochar, Soil bacteria

## Introduction

Since nitrogen (N) is the most limiting nutrient in the growth and development of crops, the world's consumption of nitrogen-based fertilizers is about 119.4 million tons, with an annual growth rate of 1.4%. The problem of low N use efficiency is a worldwide problem. According to statistics, the N use efficiency of China's main food crops is 27.5%, showing a gradual decline (Yang et al. 2017). Due to the high amount of N fertilization, plants grown on dry land soils, the N utilization rate of vegetable crops is only about 10% (Liu et al. 2021). Excessive N application can result in high nitrate leaching and groundwater contamination. Reducing the use of N-based fertilizers, improving the N use efficiency, and reducing N loss and its impact on the environment, with the premise of ensuring food security, are critical goals that must be addressed by China and other countries worldwide.

In recent years, the use of biochar as a soil additive to increase soil N retention and reduce nutrient leaching has increased. Research regarding the residence time of biochar in the soil and its influence on the soil N cycle

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has amplified its potential for positive regulation of soil N activities among researchers (Wang et al. 2020). As an external input material, biochar is a solid carbon-rich organic material generated by heating biomass under low oxygen or anoxic conditions. Previous studies have demonstrated that biochar addition reduces N leaching. This can be attributed to the increases in the cation and anion exchange capacities (CEC, AEC) of the soil by the biochar material (Sika and Hardie 2014). Although biochar is mostly inert, its high surface area, porous nature, and ability to adsorb soluble nutrients provide a suitable habitat for soil microorganisms and can improve the physical and chemical properties of the soil. In addition, adding biochar to the soil may change the soil microbial community composition (Xu et al. 2014). For example, biochar enhances the effectiveness of ammonia N through the adsorption of ammonium nitrogen ($NH_4^+$). Doydora et al. (2011) found that a mixed application of acidic biomass charcoal and livestock and poultry compost into the soil reduced the soil $NH_3$ loss by more than 50%. Adsorption experiments have also shown that biochar can adsorb $NH_4^+$ in the soil solution, reducing the loss of soil N and thereby reducing the risk of pollution to nearby water bodies (Chen et al. 2013).

Nitrification, the two-step conversion of ammonium ($NH_4^+$) to $NO_3^-$ via nitrite ($NO_2^-$), is generally thought to play a critical role in the N cycle. Ammonia oxidation is considered to be the rate-limiting step of nitrification and is catalyzed by ammonia monooxygenase (AMO), which is encoded by the *amoA* gene from both archaea (AOA *amoA)* and bacteria (AOB *amoA*). The short-term application of biochar has been shown to significantly alter the microbial community structure in yellow-brown soil, significantly reduce the gene expressions of ammonia synthesis-related enzymes and the abundance of ammonia-oxidizing archaea in the fluvo-aquic soil, and inhibit the ammonia oxidation of the soil (Zhang et al. 2019; Lin and Hernandez-Ramirez 2021). However, Lin et al. (2017) reported that biochar enhanced the abundance and diversity of AOB *amoA* gene copies, with biochar shifting the AOB community structure from *Nitrosospira*-dominated to *Nitrosomonas*-dominated in rice-paddy soil. Castaldi et al. (2011) did not observe any effect of biochar addition on soil microbial biomass or net nitrification activity in acidic silty-loam soils (pH = 5.4). In the process of soil denitrification, researchers have found that biochar is applied to the soil and microorganisms can inhibit the N denitrification of microorganisms by improving soil aeration; in particular, $N_2O$ release was reduced by 73% when 10 tons/ha biochar was applied (Singh et al. 2010). However, some studies have reported that the application of biochar increased the $N_2O$ emissions in the soil (Duan et al. 2018). The effect of biochar on the soil microbial community composition and soil N nutrient cycling is affected by many factors (Dangi et al. 2020). Therefore, studies are needed to evaluate the effects of different biochar types on soil microbial communities and on soil N content.

In this study, we investigated the effects of different biochar application rates on the soil microbial community diversity and structure using pot experiments. We also assessed the soil N availability properties to explore possible mechanisms that drive shifts in these bacterial communities.

## Materials and methods

### Soil and biochar materials

The pot experiment uses soil collected from a typical farmland soil at a depth of 0–20 cm. Experiment Station of Danjiangkou reservoir area, Nanyang City, Henan Province, China (32°17′N, 110°53′E). The area experiences a typical northern subtropical monsoon continental climate, with an annual rainfall of 802.9 mm, and an annual temperature of 15.7 °C. The parent material for soil formation is weathered granites and gneisses. Soil samples were collected randomly from 20 tillage layers (0–20 cm) within an area of approximately 50 $m^2$. Once at the laboratory, the soil was placed in a ventilated room for one week for air-drying. Finally, the soil samples were composted, thoroughly homogenized, and sieved through a 2-mm mesh to remove small roots, plant residue, and gravel. The biochar was produced by Henan Sanli New Energy Company in China. Corn straw (*Zea mays* L.) was oven-dried (80 °C) and converted into biochar through slow pyrolysis using a furnace (Olympic 1823HE) in an $N_2$-rich environment at 400–500 °C for 4 h.

Some soil properties at the start of the experiment were as follows: organic matter, 19.9 g/kg; total N, 1.2 g/kg; alkali-hydrolyzable N, 59.2 mg/kg; available P, 6.2 mg/kg; available K, 93.3 mg/kg; and pH 7.15. Soil organic matter, alkali-hydrolyzable N, available P, and readily available K were measured using methods described by Page and Robert (1982). Biochar at the start of the experiment was as follows: C, 56.7%; H, 3.2%; O, 20.7%; N, 3.6%; ash content, 21.9%; specific surface area, 19.5 $m^2/g$; and pH 8.9. Ash content was determined by burning biochar at 750 °C for 6 h on a dry basis in an open crucible. The carbon (C), hydrogen (H), nitrogen (N), and oxygen (O) contents of biochar were measured using an elemental analyzer (vario PYRO cube, Germany). The Brunauer-Emmett-Teller specific surface area of the biochar was measured by the Micrometrics ASAP 2010 system (Micrometrics, Norcross, GA, USA) using N.

### Experimental design

In this study, different biochar application rates, including 0% (CK), 0.5% (BC1), 1.0% (BC2), 2.0% (BC3), and 4.0% (BC4), were used with the air-dried soil. In total, five treatments were made with different biochar application rates, each with four replicates. According to the analysis of the survey results of the farmers: local wheat fertilization rates were 195 kg/ha of N, 67.5 kg/ha of P and 75 kg/ha of K. Fertilizer application was mainly based on the practices of local farmers. The soil was mixed with N, P, and K fertilizers and placed in plastic pots (16 cm × 20 cm). Each pot contained 5 kg of soil. The N (urea, 0.20 g/kg soil), P (triple superphosphate, 0.15 g/kg soil), and K (potassium sulfate, 0.2 g/kg soil) fertilizers were applied in one application at planting. Sufficient water was applied to saturate the soil. The soil was allowed to dry for 3 days before sowing the wheat. The wheat (Zheng Mai 103) seeds were pre-germinated by soaking them in water before sowing. Two hills of wheat (10 seeds per hill) were planted in each pot. The stand was thinned to three plants per hill after emergence. The water content of the soil was controlled at >60% the field capacity.

### Soil samples

Soil samples were collected on the 90$^{th}$ day after wheat planting. Soil samples were collected using an auger (5 cm diameter), and approximately 10 g of soil was immediately frozen in liquid $N_2$ for DNA extraction. The rest of the composite soil sample was placed in sterilized polyethylene bags and placed on ice to be transported to the laboratory. After removing all visible roots and plant fragments, the field-moist soils were divided into two parts. One part was passed through a 2-mm sieve and stored at 4 °C. The other part was air-dried at room temperature for soil physicochemical analyses.

### Soil physicochemical properties

Total nitrogen (TN) was analyzed by the Kjeldahl method (Stanley et al. 2019). Using a flow injection automatic analyzer (Auto Analyzer 3, Germany) to determine the concentration of ammonium nitrogen (AN) and nitrate nitrogen (NN) in the soil in 1 mol/L KCl extract (1:10 w/v) (Margesin and Schinner 2005). Soil microbial biomass N (MBN) was measured using the fumigation-extraction method (Vance et al. 1987). For each column, duplicate soil samples (with a weight equivalent to a 20-g dried sample) were weighed and placed in Petri dishes. The dishes were placed in a vacuum desiccator, and a small beaker containing anhydrous ethanol chloroform was also placed in the desiccator. Then a vacuum was applied. After the chloroform was boiled for 5 min, the samples were fumigated for 24 h in the dark. Subsequently, a vacuum was applied multiple times to remove the chloroform. The samples were soaked in a 0.5-mol $L^{-1}$ $K_2SO_4$ solution for extraction, oscillated for 30 min, and then filtered. The concentrations of N in the extracts were determined by an automated total N analyzer (Multi C/N, 2100, Analytik Jena, Germany).

### Characterization of the microbial population

#### *Microbial DNA extraction and PCR amplification*

Microbial DNA from soil samples was extracted by E.Z.N.A.® soil DNA Kit (OMEGA, USA) according to the manufacturer's protocols. A soil sample (0.5 g) stored at −20°C was prepared. The final DNA concentration and purification were determined by NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis. HTS was carried out using the Illumina MiSeq PE300 platform at Majorbio Bioinformatics Technology Co., Ltd.

The V3-V4 hypervariable regions of the bacteria 16S rRNA gene were amplified with primers 338F (5′-ACT CCTACGGGAGGCAGCAG-3′) and 806R (5′- GGA CTACHVGGGTWTCTAAT-3′) by thermocycler PCR system (GeneAmp 9700, ABI, USA). The PCR reactions were conducted using the following program: 3 min of denaturation at 95 °C, 27 cycles of 30 s at 95 °C, 30s for annealing at 55 °C, and 45s for elongation at 72 °C, and a final extension at 72 °C for 10 min. PCR reactions were performed by triplicate 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase and 10 ng of template DNA. The resulted PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) according to the manufacturer's protocol (Ni et al. 2017). The PCR products were mixed at equal density ratios (Eikmeyer et al. 2013) and subjected to high-throughput sequencing.

#### *Bioinformatic analysis of sequencing data*

The histogram of species composition in the article was based on the data table, and the R language tool was used to plot the difference in species composition between treatments. Alpha diversity index (Shannon, Chao, Ace, Simpson and Coverage) was analyzed by the mothur index, and the difference test method between index groups is used using Student's *T* test. Beta diversity uses R language Principal Component Analysis (PCA) statistical analysis and mapping.

Rarefaction curves were plotted by randomly selecting operational taxonomic units (OTUs) under a similarity

level of 97%. The Mothur software (version 7.0) was employed to calculate Community richness and Community diversity indices (Guan et al. 2018). Based on the clustering of OTU analysis results, Alpha diversity (Shannon, Chao, Ace, Simpson and Coverage) and species community results at different classification levels were analyzed to determine the bacterial community. OTUs of bacteria were classified using the SILVA (Release128) database, and they were denominated at the domain, phylum, class, order, family, and genus levels (Yang et al. 2019). The 16S function prediction uses PICRUSt (a bioinformatics software package designed to predict metagenome functional content from marker gene (e.g., 16S rRNA) surveys and full genomes) to eliminate the influence of the copy number of 16S marker genes in the species genome and compares with KEGG to obtain metabolic information at each level of the metabolic pathway and the number copies of related enzymes (Langille et al. 2013). The Illumina MiSeq sequencing data were deposited in the Sequence Read Archive of the National Center for Biotechnology Information database (accession number: PRJNA752436).

### Statistical analyses

Statistical analyses were performed using Statistical Product and Service Solutions 22.0 (SPSS Inc., Chicago, IL, USA). Significant differences were obtained by a one-way analysis of variance (ANOVA), with means compared using Duncan's multiple range test ($p$<0.05). Principal Component Analysis (PCA) was used to compare the soil bacterial community composition between the different treatments. Redundancy analysis (RDA) and Monte Carlo permutation tests were conducted using Canoco 5.0.

## Results

### Soil N availability and microbial biomass

Different biochar application rates significantly affected soil N availability (Table 1). Compared with CK, biochar application increased the soil TN content by 4.7–32.3%. Soil TN in BC3 and BC4 increased significantly ($p < 0.05$) and were significantly ($p < 0.05$) higher than in BC1 and BC2. NN contents in BC1, BC2, BC3, and BC4 were 6.4%, 9.5%, 11.6%, and 12.5% lower, respectively, than in CK. AN contents in BC2, BC3, and BC4 were 57.4%, 58.9%, and 101.5% higher, respectively, than in CK. MBN and AN trends were consistent for all treatments.

### Effects of biochar on soil microbial diversity and community structure

#### *Microbial richness and diversity indices*

We observed 74,1965 quality sequences, with an average of 22,752 sequences per sample. The average base length was 416 bp for the bacterial 16S rRNA. The coverage index of soil amended with biochar was 97%, indicating that the dataset included all sequences between V3 and V4 regions and that the sequence data volumes were reasonable (Fig. 1). The number of public OTUs processed by each treatment was 2039, 70.0%, 66.6%, 65.2%, 67.2%, and 66.0% of the total OTUs from CK, BC1, BC2, BC3, and BC4, respectively.

The alpha diversity of bacteria communities was positively affected by the application of biochar rates (Table 2), and biochar treatments significantly increased the Ace, Chao, and Shannon indices. Compared with CK, the Ace and Chao indices increased in BC1 were 11.1% and 11.5%, respectively. With increased biochar application, the Shannon index of the soil bacteria increased. In contrast, the biochar treatments significantly decreased the Simpson index related to CK. The Simpson indices in treatments BC3 and BC4 were significantly lower by 72% and 60%, respectively, than in CK.

#### *Effects of biochar on soil bacterial community composition*

Analyses based on the 16S rRNA data indicate that the main bacterial phyla in the soil samples were *Proteobacteria*, *Actinobacteria*, *Chloroflexi*, *Acidobacteria*, and *Bacteroidetes*. Their total relative abundance was 81.60–84.93%. The relative abundances of *Proteobacteria*, *Actinobacteria*, *Chloroflexi*, *Acidobacteria*, and *Bacteroidetes* were 28.78–32.26%, 24.92–32.67%, 5.96–10.84%, 4.98–8.97%, and 5.53–7.14%, respectively (Fig. 2). The relative abundance of *Proteobacteria* in BC3 was 4.4% higher than in CK.

**Table 1** Effects of different biochar application rates on soil nitrogen availability

| Treatment | TN(g kg$^{-1}$) | NN(mg kg$^{-1}$) | AN(mg kg$^{-1}$) | MBN(mg kg$^{-1}$) | NN/TN | AN/TN | MBN/TN |
|---|---|---|---|---|---|---|---|
| CK | 2.32±0.03 b | 132.38±2.45 a | 8.64±0.52 c | 51.35±1.02 c | 57.64±0.01 a | 3.73±0.85 b | 22.15±0.26 ab |
| BC1 | 2.43±0.20 b | 128.91±4.23 b | 9.63±0.76 c | 53.20±1.16 c | 52.03±0.01 b | 3.84±1.68 ab | 23.90±1.25 a |
| BC2 | 2.50±0.09 b | 119.80±4.87 bc | 13.60±0.90 b | 61.24±3.27 b | 48.85±0.03 b | 4.44±2.61 ab | 23.97±0.82 a |
| BC3 | 3.07±0.08 a | 116.95±4.79 c | 13.73±1.43 b | 62.80±1.00 ab | 38.64±0.01 c | 4.35±1.80 ab | 20.44±0.79 b |
| BC4 | 3.04±0.01 a | 115.81±2.56 c | 17.41±1.05 a | 64.84±1.30 a | 38.16±0.01 c | 4.75±1.04 a | 21.79±0.45 b |

Values are presented as mean ± SD ($n = 4$), and data with different lowercase letters are significantly different at $p < 0.05$ according to Duncan's multiple range test

*Abbreviations*: *TN* total inorganic N, *NN* nitrate nitrogen, *AN* ammonium nitrogen, *MBN* microbial biomass of nitrogen

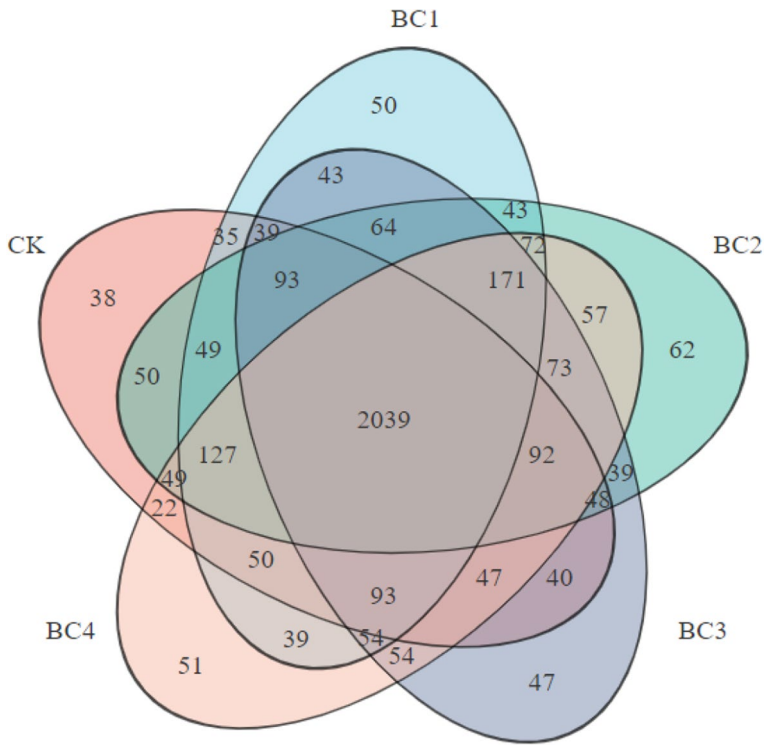


**Fig. 1** Venn diagram of the OTUs of soils bacterial communities from each treatment: CK, BC1, BC2, BC3, and BC4, in which the biochar dosages were 0%, 0.5%, 1%, 2%, and 4%

**Table 2** Effects of biochar application rates on the alpha diversity of the bacterial community

| Treatment | Ace | Chao | Shannon | Simpson | Coverage |
|---|---|---|---|---|---|
| CK | 2932±116 b | 2911±105 b | 5.93±0.34 b | 0.025±0.016 a | 0.977±0.002 a |
| BC1 | 3257±127 a | 3245±117 a | 6.31±0.06 a | 0.012±0.002 ab | 0.975±0.002 a |
| BC2 | 3157±182 ab | 3117±193 ab | 6.32±0.12 a | 0.012±0.004 ab | 0.974±0.004 a |
| BC3 | 3083±209ab | 3106±173 ab | 6.39±0.11 a | 0.007±0.001 b | 0.974±0.007 a |
| BC4 | 3050±78 ab | 3052±96 ab | 6.39±0.12 a | 0.010±0.003 b | 0.972±0.004 a |

Values are mean plus standard deviation ($n = 3$), and data with different lowercase letters are significantly different at $p < 0.05$ according to Duncan's multiple range test. Treatments CK, BC1, BC2, BC3, and BC4 had biochar dosages of 0%, 0.5%, 1%, 2%, and 4%

Compared to CK, the relative abundance of *Proteobacteria* was significantly reduced in BC4. The relative abundances of *Chloroflexi* and *Acidobacteria* increased significantly with increased biochar application. Compared to CK, BC4 increased the relative abundances of these two phyla by 79.0% and 61.9%, respectively.

### *Effect of biochar on the principal components of soil bacterial communities*

A PCA was performed on the soil bacterial communities with regard to the different biochar application rates, from which two principal factors were extracted (Fig. 3). The total interpreted amount was 80.47%, of which PC1 and PC2 comprised 51.23% and 29.24%, respectively. The soil samples from CK were distributed in the negative areas of PC2. BC1, BC2, BC3, and BC4 gradually changed from the negative area to the positive area of PC2 and were mainly distributed in the positive area of PC2.

### Effect of biochar on predictive functional profiling of bacterial communities related to soil nitrification and denitrification using 16S rRNA marker gene sequences

N cycling processes in the soil need to be coordinated by various enzymes in each branch (data URL: https://www.genome.jp/). According to the N metabolism pathway

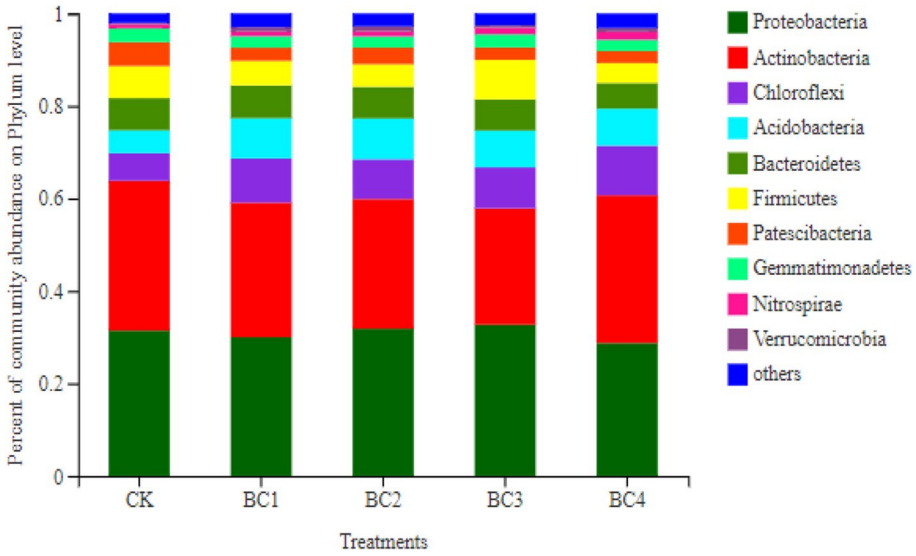


**Fig. 2** Relative abundances and community compositions of the dominant bacterial phyla in soils from each biochar treatment (phylum level). Treatments CK, BC1, BC2, BC3, and BC4 had biochar dosages of 0%, 0.5%, 1%, 2%, and 4%

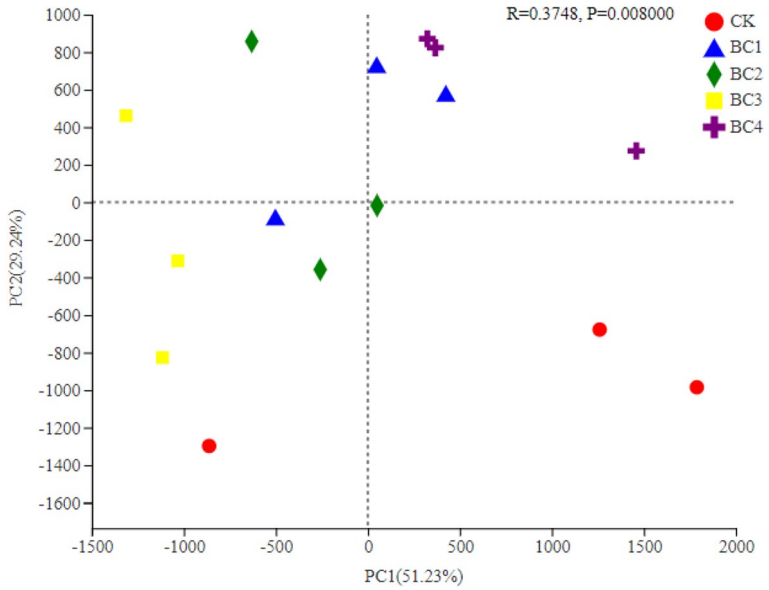


**Fig. 3** Principal component analysis of the soil bacterial community structure. Axis 1 (51.23%) and axis 2 (29.24%) explained the variations based on the Bray-Curtis dissimilarities. Treatments CK, BC1, BC2, BC3, and BC4 had biochar dosages of 0%, 0.5%, 1%, 2%, and 4%

diagram, the corresponding relationship between the enzymes and genes related to N metabolism can be obtained. In addition, the enzymes involved in the soil nitrification and denitrification can be obtained by comparing sequence data to enzyme nomenclature (Table 3). We found that ammonia monooxygenase (1.14.99.39) and hydroxylamine dehydrogenase (1.7.2.6), which are involved in the ammonia oxidation process, were significantly different. Compared to CK, the ammonia oxygenase (1.14.99.39) in BC2 decreased by 20.1%, while BC3 and BC4 had increased gene expressions of 19.3% and 22.9%, respectively. In the N denitrification process, the copy number of 16S marker gene of related nitrite reductase, nitric oxide reductase nitrite reductase (1.7.2.1), nitric oxide reductase (1.7.2.5), and nitrous oxide reductase (1.7.2.4) in BC3

**Table 3** Number of copies of 16S marker gene-related enzymatic functions to nitrification and denitrification processes in the soil treatments (gene copies/g soil)

| Enzyme name | Enzyme commission (EC) number | CK | BC1 | BC2 | BC3 | BC4 |
|---|---|---|---|---|---|---|
| Ammonia monooxygenase | 1.14.99.39 | 274±17 b | 243±28 b | 219±10 c | 327±23 a | 337±38 a |
| Hydroxylamine dehydrogenase | 1.7.2.6 | 258±26 ab | 229±17 b | 223±30 b | 311±39 a | 322±54 a |
| Nitrite reductase (NO-forming) | 1.7.2.1 | 3546±131 b | 3242±134 b | 3409±244 b | 4031±166 a | 3318±128 b |
| Nitrate reductase | 1.7.99.4 | 24968±480 a | 25178±505 a | 25469±915 a | 25044±405 a | 26160±886 a |
| Nitric-oxide reductase (cytochrome c) | 1.7.2.5 | 2095±88 c | 2121±98 bc | 2322±77 a | 2439±108 a | 2282±74 ab |
| Nitrous-oxide reductase | 1.7.2.4 | 1803±85 bc | 1739±24 c | 1897±82 b | 2092±99 a | 1899±72 b |

Values are mean plus standard deviation ($n = 3$), and data with different lowercase letters are significantly different at $p < 0.05$ according to Duncan's multiple range test. Treatments CK, BC1, BC2, BC3, and BC4 had biochar dosages of 0%, 0.5%, 1%, 2%, and 4%

were 13.7%, 16.4%, and 16.0% greater, respectively, than in CK.

### Correlations between soil bacterial community composition, soil N availability, and related enzyme gene expression

A redundant analysis (RDA) was used to analyze the correlations between soil N availability, the related enzyme functions, and the bacterial community composition. The first and second ordination axes explained 38.6% and 19.9% of the total variability, respectively (Fig. 4a). Regarding soil N availability, the main factors influencing the first ordination axis were NN (-0.5821), AN (0.5327), and NN/TN (-0.5312). Regarding enzymatic functions, the first and second ordination axes explained 26.5% and 24.9% of the total variability, respectively (Fig. 4b). The main factors influencing the first ordination axis were nitric oxide reductase (cytochrome c) (−0.5245), nitrous-oxide reductase (−0.2721), and ammonia monooxygenase (0.1272). The main factors influencing the second ordination axis were nitrite reductase (0.7263) and hydroxylamine (0.6929).

## Discussion

### Effect of biochar application rate on soil N availability

The soil N transformation process is an important part of the N cycle. Ammonium and nitrate N is a major factor determining plant growth and microorganisms (Sun et al. 2019). After biochar is applied to a field, it affects the transformation, migration, and distribution of soil N through its physical and chemical properties or by interacting with the soil (Li et al. 2020). The amount of biochar applied and the soil type are important factors that affect the migration, distribution, and leaching of soil N (Kumuduni et al. 2019). In this study, the application of biochar increased soil TN and AN contents by 4.7–32.3% and by 11.5–58.9%, respectively, indicating that the application of biochar can significantly increase the soil N content, mainly because the biochar has a rich pore structure and a large specific surface area, which can adsorb and hold soil N, reduce soil N leaching loss, and increase the soil N nutrient content (Abujabhah et al. 2018). Soil MBN is the most active component of soil organic N and plays an important role in regulating soil organic–inorganic N conversion and N cycling. Wardle et al. (2008) studied forest soils in northern Sweden and found that the addition of biochar promoted the growth of microorganisms, but Durenkamp et al. (2010) showed that the addition of biochar reduced the soil MBN content. The reason for this phenomenon is closely related to the test soil texture, original microbial biomass and nutrients, and the type of biochar. In this study, we found that the increase of biochar application rate increased MBN which can improve the availability of C in soil, thereby promoting the growth of microorganisms in the soil. In addition, although the application of biochar increased the soil TN and AN contents, it decreased the NN content by 2.6–12.5%. This is inconsistent with the results of Wang et al. (2012), in which the pot experiments showed that soil NN and AN contents increased significantly with an increased biochar application rate. The main reason for this is that the application of biochar loosens and ventilates the soil and transmits light, which is conducive to the growth of crop roots (Liu et al. 2021). We also found that the application of biochar increased the N absorption of the wheat roots by 15.3–65.2%.

### Effect of biochar application rate on soil bacterial diversity and community composition

The changes of soil microbial community structure were affected by soil type, biochar type and biochar application amount (Dai et al. 2021). In this study, we found that with an increase in the amount of biochar applied, the diversity of the soil bacterial community initially increased, then decreased (Table 4). This is because increasing the amount of biochar will promote or inhibit the growth

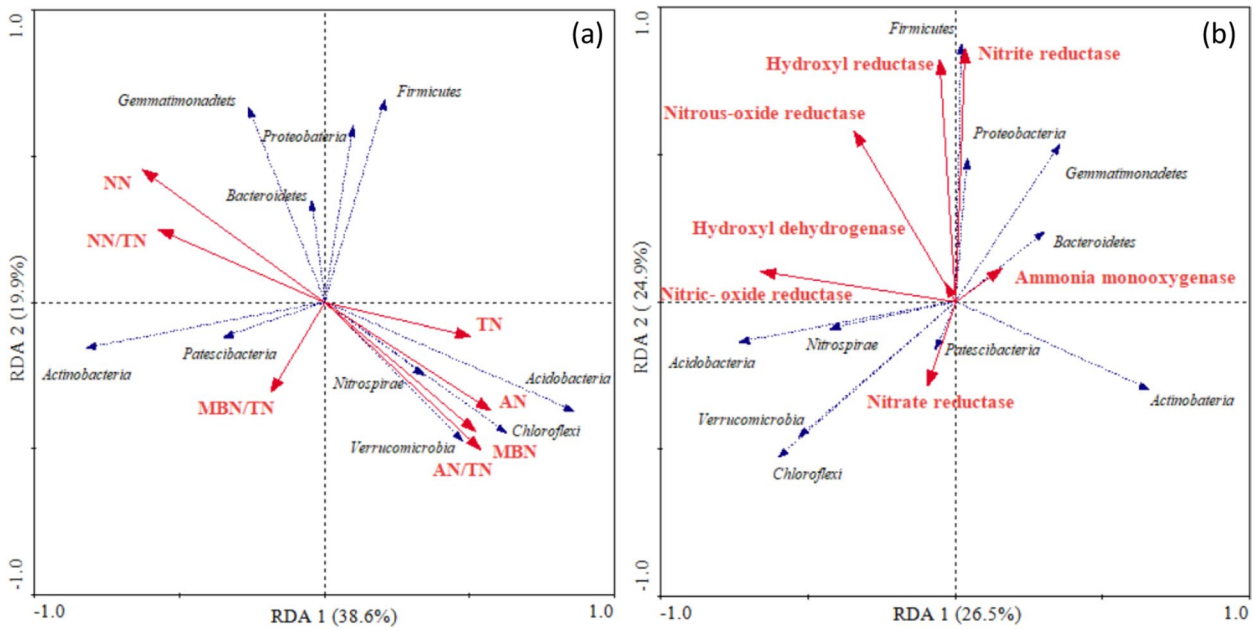


**Fig. 4** Redundancy analysis (RDA) of the composition of the soil bacterial community and **a** soil nitrogen availability and **b** the related enzyme gene expression (phylum level)

of certain types of bacteria, resulting in changes in the structure of the soil bacterial community (Zhang et al. 2017). In addition, the residence time of biochar in the soil will also affect the variations in the microbial community structure. In this study, with increased biochar application, the relative abundances of *Chloroflexi* and *Actinobacteria* increased, while the relative abundance of *Acidobacteria* decreased. In addition, addition of biochar increased the relative abundance of *Nitrospirace*, but decreased the relative abundances of *Gemmatimonadetes* and *Patescibacteria*. *Acidobacteria* mostly belong to the oligotrophic group, and the eutrophication state of the soil is not suitable for the growth of this group (Castro et al. 2013). The addition of biochar improves the nutrient status of the soil and increases the effective N content, thereby inhibiting the growth of *Acidobacteria*.

Nitrification is generally performed by ammonia-oxidizing bacteria and nitrite-oxidizing bacteria. Ammonia oxidation is the first and rate-limiting step of the nitrification process, which is mainly promoted by

**Table 4** Relative abundances and community compositions of the dominant bacterial phyla in soils from each biochar treatment (phylum level) (%)

| Dominant bacterial phyla | CK | BC1 | BC2 | BC3 | BC4 |
|---|---|---|---|---|---|
| Proteobacteria | 31.38±1.52 ab | 30.08±0.62 bc | 31.74±0.84 a | 32.75±0.16 a | 28.74±0.54 c |
| Actinobacteria | 32.36±1.50 a | 29.30±0.64 b | 28.08±0.25 b | 25.18±1.41 c | 31.88±1.25 a |
| Chloroflexi | 6.00±0.78 c | 9.55±0.87 ab | 8.65±0.43 b | 8.89±1.32 ab | 10.74±1.54 a |
| Acidobacteria | 4.96±0.52 b | 8.60±0.82 a | 8.81±1.94 a | 7.97±1.94 a | 8.03±0.21 a |
| Bateroidetes | 6.89±0.80 a | 7.09±0.61 a | 6.82±0.28 a | 6.68±0.43 a | 5.53±0.06 b |
| Firmicutes | 7.00±0.59 b | 5.27±0.44 c | 4.91±0.38 c | 8.53±0.64 a | 4.37±0.37 c |
| Patescibacteria | 5.21±0.53 a | 2.83±0.18 b | 3.59±0.98 b | 2.66±0.61 b | 2.56±0.81 b |
| Gemmatimonadetes | 3.01±0.62 a | 2.77±0.44 a | 2.45±0.23 a | 2.86±0.36 a | 2.45±0.18 a |
| Nitrospirae | 0.86±0.22 b | 0.99±0.03 b | 1.07±0.09 b | 1.44±0.37 a | 1.68±0.10 a |
| Verrucomicrobia | 0.26±0.17 | 0.89±0.22 a | 1.06±0.55 a | 0.51±0.38 ab | 0.79±0.10 ab |
| others | 1.50±0.35 b | 2.19±0.24 a | 2.11±0.29 a | 2.14±0.39 a | 2.38±0.12 a |

Values are mean plus standard deviation ($n = 3$), and data with different lowercase letters are significantly different at $p < 0.05$ according to Duncan's multiple range test. Treatments CK, BC1, BC2, BC3, and BC4 had biochar dosages of 0%, 0.5%, 1%, 2%, and 4%

ammonia-oxidizing microorganisms (Yao et al. 2017). In this study, predictive functional profiling of bacterial communities related to soil nitrification and denitrification using 16S rRNA marker gene sequences. We found that, compared to CK, the number of copies of ammonia monooxygenase and hydroxylamine dehydrogenase in BC2 were significantly reduced, indicating that small amounts of biochar application had an impact on the growth and reproduction of AOA and AOB. In the nitrification process, the study of the 16S rRNA AOB gene sequence shows that AOB is mainly divided into the Proteus β subgroup and γ subgroup *Nitrosospira* (Shen et al. 2008). Studies have also shown that a "complete nitrifying bacteria" of the genus *Nitrospirillum* can directly oxidize $NH_3$ to $NO_3^-$. This strain has functional genes encoding the ammonia oxidation and nitrite oxidation processes. However, the relative abundance of *Nitrosospira* in BC2 had little effect, indicating that it can inhibit the nitrite oxidation process, reduce the nitrification potential, and reduce nitrate leaching loss.

Denitrification is an important link that affects the global N cycle. According to the results, the number of copies of nitrification-related enzymes in BC3 and BC4 increased by 19.3% and 22.9%, respectively. It shows that high biochar application rate stimulates nitrification. This is consistent with the findings of Ball et al. (2010). Due to the porous characteristics of biochar, it provides a good environment for microorganisms, protects beneficial soil microorganisms, and accelerates the soil nitrification process. However, biochar also adsorbs phenolic compounds in the soil and inhibits the growth of nitrifying bacteria, thereby indirectly promoting soil nitrification. Studies have shown that adding biochar to farmland soil can improve soil aeration, inhibit the denitrification of anaerobic denitrifying microorganisms, and reduce nitrous oxide emissions. Harter et al. (2016) found that in slightly alkaline sandy soils, although biochar addition stimulated denitrification gene expression and increased denitrification, this is because biochar adsorbs nitrous oxide in the soil pores under water saturation. Cayuela et al. (2013) found that biochar generally reduces the proportion of nitrous oxide emissions in 15 different agricultural soils, indicating that biochar stimulates the final step of denitrification to reduce nitrous oxide to N. The results of this study, nitric oxide reductase (cytochrome c) and nitrous oxide reduction during the denitrification process increased in BC3 and BC4 by 16.4% and 16.0%, respectively ($p<0.05$), compared to CK. This result is consistent with previous results, indicating that the addition of biochar could promote the expression of denitrification genes and increases denitrification. This may be because the addition of biochar stimulates the bacterial nitrous oxide reductase activity encoded by nosZ and other reducing agents to reduce nitrous oxide (Harter et al. 2016).

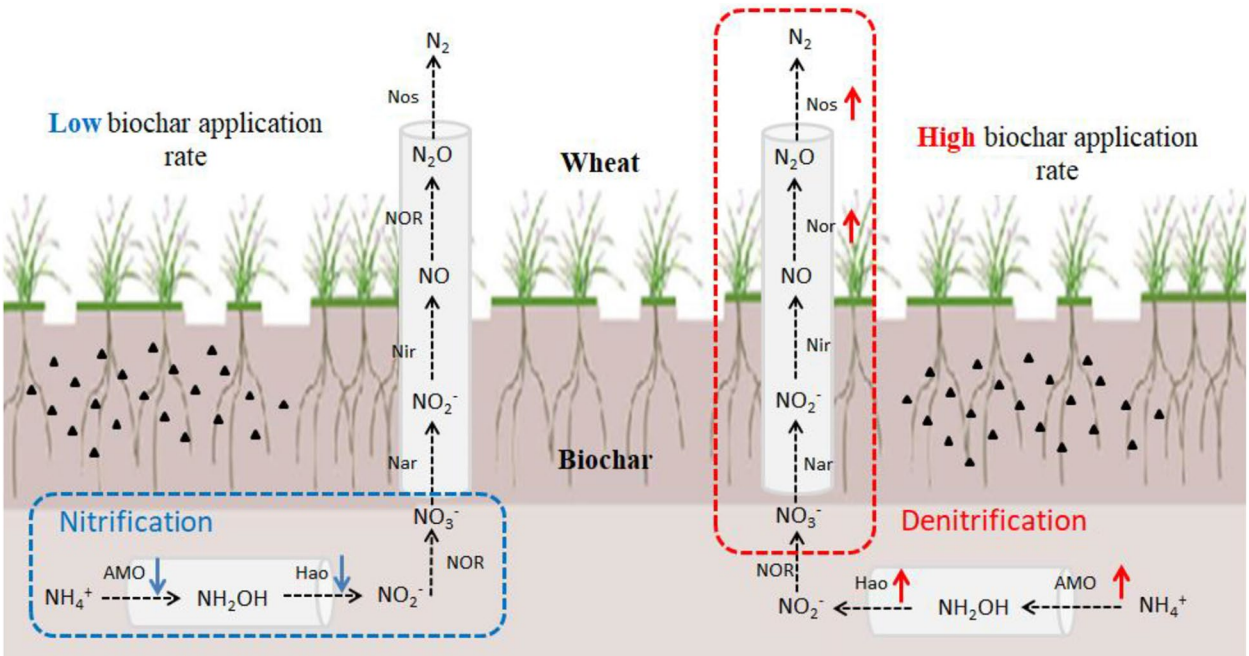


**Fig. 5** Biochar regulation mechanism on soil nitrogen availability. AMO: Ammonia monooxygenase; Hao: Hydroxylamine oxidoreductase; NOR: Nitrite oxidoreductase; Nar: Nitrate reductase; Nir: Nitrite reductase; Nor: Nitric oxide reductase; Nos: Nitrous oxide reductase

### Mechanism analysis of the effectiveness of biochar in regulating soil N

The soil microenvironment is closely related to the growth of soil microorganisms. Changes in soil nutrients, moisture, aeration, and other properties can cause changes in the composition and structure of soil bacterial communities (Zhou et al. 2019). The RDA conducted in this study found that AN, NN, and NN/TN in the soil were the main factors affecting of the soil bacterial community. In addition, the number of copies of the nitrite reductase, hydroxylamine, and nitric oxide reductase (cytochrome c) genes was also correlated with the abundance of some bacteria involved in the N cycle. Therefore, this study explored and inferred the biochar regulation mechanism on soil N content (Fig. 5): a low biochar application rate can improve the availability of N, which is mainly through reducing the expressions of ammonia monooxygenases and hydroxylamine dehydrogenase genes involved in the ammonia oxidation process, and affects the growth and reproduction rate of AOB in the soil, thereby inhibiting ammonia oxidation and a high biochar application rate can also improve the N utilization efficiency, mainly by increasing the expressions of nitric oxide reductase and nitrous oxide reductase in the denitrification process, stimulating the process of reducing $N_2O$ and NO to $N_2$ during the last two steps of the denitrification process (Cayuela et al. 2013). The 16S function prediction level did not fully represent the activity of the soil microorganisms.

Therefore, biochar improved the availability of soil N and promoted the absorption of N by crops, mainly increasing the AN and NN contents. A low application rate biochar mainly inhibits the ammonia oxidation process, and a high application rate may improve soil N utilization efficiency by promoting nitrification and denitrification processes and which ultimately resulted in environmental risk decrease by soil nitrogen release inhibition.

#### Abbreviations

| | |
|---|---|
| AOA | Ammonia-oxidizing archaea |
| AOB | Ammonia-oxidizing bacteria |
| TN | Total inorganic N |
| NN | Nitrate nitrogen |
| AN | Ammonium nitrogen |
| MBN | Microbial biomass |
| OUT | Operational taxonomic units |
| AMO | Ammonia monooxygenase |
| Hao | Hydroxylamine oxidoreductase |
| NOR | Nitrite oxidoreductase |
| Nar | Nitrate reductase |
| Nir | Nitrite reductase |
| Nor | Nitric oxide reductase |
| Nos | Nitrous oxide reductase |

#### Acknowledgements

We would like to thank Editage (www.editage.cn) for English language editing.

#### Authors' contributions

All the authors collaborated for the completion of this work. Tian Hu designed and accomplished the first draft. Jun Zhang provided valuable insights and suggestions for this article. Jiating Wei, Li Du, and Jibao Chen were involved in the initial writing and editing of the manuscript. The authors have all read and approved the final manuscript for publication.

#### Funding

This work was supported by the Henan Provincial Science and Technology Department Project (212102310076), Key research and development projects of Henan Province (221111520600) and Postgraduate Education Reform and Quality Improvement Project of Henan Province (YJS2021JD17).

#### Availability of data and materials

The Illumina MiSeq sequencing data were deposited in the Sequence Read Archive of the National Center for Biotechnology Information database (accession number: PRJNA752436)

## Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

All listed authors consented to the submission of this manuscript for publication.

#### Competing interests

The authors declare that they have no competing interests.

Received: 13 October 2022 Accepted: 12 December 2022
Published online: 11 January 2023

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## Publisher's Note