Study on influence mechanism of biochar on soil nitrogen conversion
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ABSTRACT
Straw had huge annual production, burning it in the air will cause serious air pollution. Burning straw into biochar and returning biochar to the field will not only make effective use of straw resources, but also improve soil quality. Different preparation conditions and addition levels of biochar were used to investigate the ammonia nitrogen, nitrate nitrogen, and total nitrogen contents in the soil leaching solution. The results showed that biochar had a favorable adsorption effect on ammonia nitrogen, the cumulative leaching of ammonia nitrogen reduced by 80.16% in the 2% and 400°C biochar addition treatment compared to that without adding biochar. The final leaching solution electrical conductivity value of the experimental group of biochar prepared at 500°C with 1% addition was the highest at 445 μS/cm. Sequencing of microorganisms revealed that biochar altered the structure and abundance of the soil microbial community, particularly increased the relative abundance of nitrifying bacteria.

1. Introduction
Agricultural activities produced large amounts of straw, which is a serious waste of resources and a source of air pollution when directly burn. In addition, directly returning straw into the field may cause many problems, such as delay cultivation in cold regions (northeast China), release large amounts of CO₂ through decomposition, which is not conducive to carbon sequestration and emission reduction. And the possibility of insect eggs in the straw, which can lead to massive outbreaks of pests and diseases in the following year’s cultivation. Biochar is usually a highly aromatic solid material produced by the pyrolytic carbonization of biomass under fully or partially anoxic conditions [1], and it is an effective way to recycle agricultural waste [2]. Biochar has good potential to improve soil quality because of high carbon content, stability and difficulty in decomposition, loose pores, large relative area, and high cation exchange capacity [3,4]. Adding nitrogen fertilizer is indispensable in the cultivation of crops, and different preparation conditions of biochar have different effects on soil nitrogen. The properties of biochar are closely related to the feedstock and pyrolysis temperature [5]. Biochar prepared under low-temperature conditions has a higher organic matter content and higher organic nitrogen concentration [6,7], while biochar prepared under high-temperature conditions contains more stable Polycyclic Aromatic Hydrocarbons structures and has better adsorption properties. Different preparation temperatures of biochar and addition amount also have significant effects on soil nitrogen. These factors are not only limited to the adsorption of nitrogen by biochar, but also affects the microbial community of the soil, which plays a key role in soil nitrogen transformation. Therefore, this study will investigate the effects of biochar addition on soil microbial populations and structure.

As biochar is loose and porous with a large specific surface area, adding biochar into the soil can effectively reduce soil bulk, improve soil water retention and aeration [8], thus improving soil texture [9,10]. At the same time, biochar is alkaline and can effectively raise pH of soil [11], moreover, the surface of biochar is rich in ions, which can effectively improve the cation exchange capacity in the soil [12]. Some studies pointed out that adding biochar is effective to reduce the leaching of nitrate nitrogen from soils [13], while others reach the opposite conclusion [14,15]. Merely attributing this difference to the influence of different soil types had significant flaws, we should consider the influence of microorganisms during leaching experiments [16]. The large pores (more than 50 nm) on the surface of the biochar provide suitable living environment for microorganisms [17,18]; meanwhile, mesopores (2 to 50 nm) and micropores (less than 2 nm) can effectively adsorb water and nutrients [19] to improve the growth and reproduction of microorganisms environment [19]. Biochar can also effectively improve soil bacterial structure [20,21] and contribute to the survival of beneficial bacteria [22,23].
Northeast China is an important grain-producing region with low average temperatures, making it suitable for nitrate nitrogen applications [24]. Nitrate nitrogen is mainly found in the soil solution which is highly mobile as well as easily absorbed and used by plants [25]. Field crops mainly use nitrate nitrogen for growth [26]. Studies show that plants accumulate large amounts of nitrate-nitrogen during the growth period of nutrient organs [27]. As plants continue to grow, the amount of nitrate-nitrogen in their bodies decreases [28]; therefore, plants take up large amounts of nutrients during the nutritional growth phase [29], meeting the needs of current growth and for later growth. The accumulation of nitrate nitrogen in plants is a ‘reserve’ measure for plants and is required for adaptation to adversity [30]. The accumulation of nitrate nitrogen during the nutritional growth period allows plants to grow and develop well even when soil nutrients are in short supply [31]. In addition, nitrate nitrogen is an important osmoregulatory substance in the vesicles, when carbohydrate synthesis in plants and the organic matter content of the vesicles is reduced, nitrate nitrogen can replace them in their osmoregulatory role, and this regulation requires less energy [32]. Utilizing biochar can improve the efficiency of nitrate nitrogen conversion and solve a range of soil problems such as soil hardening due to excessive nitrogen fertilizer application [33,34], while enhancing soil quality and organic matter content [35,36]. Much work has been done on forest soils and agricultural black soils, but lacking research on meadow soils, which are the second-largest type of agricultural soil in northeastern China. Therefore, it is necessary to conduct research on the effect of biochar addition on meadow soils.

Through a 60 days drenching experiment, the effect of biochar on soil nitrogen content at different preparation temperatures and different additions were investigated, the pH and EC values of the drench solution were measured, in addition, the changes in the population structure of microorganisms in the soil were determined by high-throughput sequencing. The objectives of this study were: (1) to investigate the effect of biochar addition on soil nitrogen content; (2) to investigate the effect of biochar addition on the pH and EC values of soil drench solutions; (3) to investigate the effect of biochar addition on the population structure of soil microorganisms and to relate it to changes in nitrogen content.

2. Materials and methods

2.1. Preparation of soil and straw

Soil samples were collected from Daqing, Heilongjiang Province, China (45°46’N, 124°18’E), annual average temperature 4.2°C, coldest monthly average temperature 18.5°C, plant roots and visible stones, and other non-soil matters were removed from the soil by hand. The sampling depth was 0 ~ 10 cm. The properties of the soil used are shown in the Table 1.

Biochar was prepared from maize straw in the agricultural growing areas around Harbin, Heilongjiang Province. All the impurities rinsed after washed with water and cut into small sections of less than 2 cm, then put it into our homemade machine to prepared biochar. The temperature of the biochar preparation equipment was set at 300°C, 400°C, and 500°C, respectively. Heating rate was 10°C /min, holding time was 1 h. After removal, it is naturally air-dried and cooled for use. The properties of biochar prepared at different temperatures are shown in the Table 2.

2.2. Experimental design and soil column leaching experiment

The simulations experiments were carried out using polyethylene cylindrical tubes with a bottom area of 31.17 cm² and a height of 20 cm. Three cm quartz sand (to filter the water sample) was placed on the bottom end of the tube and a nylon net was placed over the bottom opening to seal the bottom of the tube. The soil is filled with naturally air-dried soil 500 g, which is mixed with 0.3 g of urea as a nitrogen source (0.6 g N per kg of soil). The biochar was mixed evenly with the soil. When installing the soil columns, special attention was paid to compacting the soil at the edges of the column walls to ensure that no water penetrated against the walls and to minimize the edge effect. Deionized water was applied at a rate of 30 mL per day for the first 5 days and 60 mL per day for the

| Soil type     | Water content (%) | Total organic carbon (g/kg) | Weight capacity (mg/kg) | Ammonia nitrogen (mg/kg) | Nitrate nitrogen (mg/kg) | Total nitrogen (g/kg) |
|---------------|-------------------|----------------------------|-------------------------|--------------------------|--------------------------|-----------------------|
| Meadow soil   | 23%               | 23.11                      | 1.37                    | 9.43                     | 7.69                     | 2.23                  |

| Temperature (°C) | Productivity (%) | pH | EC (µS/cm) | Specific surface area (m²/g) | Content of C (%) |
|------------------|------------------|----|------------|------------------------------|-----------------|
| 300              | 50.80 ± 2.17a    | 8.70 ± 0.03a | 2432 ± 19a | 23.71 ± 2.01a | 67.30 ± 1.07a |
| 400              | 37.33 ± 1.73b    | 9.43 ± 0.08b | 2694 ± 21b | 27.69 ± 1.97b | 73.19 ± 1.55b |
| 500              | 21.33 ± 1.67c    | 10.02 ± 0.12c | 2725 ± 17c | 30.54 ± 2.12c | 79.71 ± 1.32c |

Data are means ± standard deviation, n = 3. Different letters show the significant difference according to the LSD test at 5% probability level.
last 5 days, measured in 10-day intervals and cumulatively for 60 days, with total precipitation approximately equal to the annual average precipitation in Harbin. The specific experimental design is as follows in Table 3.

Three replications were performed for each treatment. Additional sterilization experiments were carried out according to the experimental conditions of the CK, CB, CN and FC groups for comparative analysis.

2.3. Chemical analysis

Ammonia nitrogen was determined by nano reagent photometric method according to National Environmental Protection Standards of the People’s Republic of China (HJ 535–2009), nitrate nitrogen was measure by dual wavelength colorimetric method with reference to National Environmental Protection Standards of the People’s Republic of China (GB/T 346–2007) and total nitrogen was analyzed by UV spectrophotometric method with reference to National Environmental Protection Standards of the People’s Republic of China (GB11894-89). The manufacturer of the spectrophotometer (T6 New Century) is Beijing Pu-Analysis General Instrument Co.

The pH of the leachate solution was measured with a pH meter (PHS-3 G), and the electrical conductivity of the leachate solution was measured with a conductivity meter (DDS-307A).

2.4. Microbiological test methods

Soil bacterial microflora were determined by high-throughput sequencing. DNA was extracted from the samples, the primers are designed according to the conserved regions, and the sequencing junction is added at the end of the primers to perform PCR amplification, and the products are purified, quantified and homogenized to form a sequencing library, and the built library is first subjected to library quality control, and the qualified library is sequenced by Illumina Novaseq 6000. The raw image data files obtained from high-throughput sequencing (Illumina Novaseq) are transformed into Sequenced Reads by Base Calling analysis, and the results are stored in FASTQ (short for fq) file format, which contains the sequence information of Sequenced Reads and their corresponding sequencing quality information.

2.5. Statistics

Data were statistically and analytically analyzed using origin 2021 and SPSS software, analysis of variance (ANOVA) followed by the LSD test was performed with biochar addition as the main factors for the following variables: soil ammonia nitrogen, nitrate nitrogen, total nitrogen, at the significant level of $p < 0.05$.

3 Results

3.1. Biochar characterization

Observed by scanning electron microscope (SEM), the surface of biochar has abundant bumps and folds accompanied by lots of pores. The biochar prepared at a final reaction temperature of 400°C has a large pore structure and a large specific surface area.

In a comparative test, biochar from the non-sterilized and sterilized treatments were removed and observed using a microscope. As shown in Figure 1, it can be clearly observed that the biochar from the non-sterilized group had a clear microbial community, while the sterilized group did not, indicating that biochar is suitable place for microbial growth.

3.2. Effects of biochar on soil nitrogen leaching

3.2.1. Effects of biochar on ammonium nitrogen leaching from soil

It can be seen from Figure 2(a) that the concentration of ammonium nitrogen in the soil drench solution was greatly influenced by the amount of biochar applied during the experimental period, the concentration of ammonium nitrogen in the soil drench solution significantly decreased with the application of biochar at the early stage of drenching, in which the concentration of ammonium nitrogen in the soil drench solution of CN group was 31.47 mg/L, which was significantly ($p < 0.05$) higher than that of the other treatments with the application of biochar. As the leaching time increased, the ammonium nitrogen concentration in

| Table 3. Test condition setting. |
|----------------------------------|
| Simple | Addition of Nitrogen (g) | Temperature of biochar (°C) | Addition of biochar (g) |
| CK     | -                      | -                           | -                   |
| CB     | -                      | 400                         | 5                   |
| CN     | 0.3                    | -                           | -                   |
| TA     | 0.3                    | 300                         | 0.5                 |
| TC     | 0.3                    | 300                         | 5                   |
| FA     | 0.3                    | 400                         | 0.5                 |
| FB     | 0.3                    | 400                         | 1                   |
| FC     | 0.3                    | 400                         | 5                   |
| FD     | 0.3                    | 400                         | 10                  |
| KA     | 0.3                    | 500                         | 0.5                 |
| KC     | 0.3                    | 500                         | 5                   |
the soil solution continued to decrease, and eventually reached a steady state. On day 10, the ammonium nitrogen concentrations in treatment of TA, FA and KA were 5.62 mg/L, 5.44 mg/L and 6.89 mg/L, respectively. The ammonium nitrogen concentrations in treatment of TC, FC, and KC were 5.07 mg/L, 4.44 mg/L and 5.97 mg/L, respectively.

3.2.2. Effects of biochar application on the amount of accumulated ammonium nitrogen leaching in soil leaching solution

As can be seen from Figure 2(b), the cumulative ammonium nitrogen leached from the soil increased slowly with time in all treatments, FD treatment showed the slowest increase and the lowest final accumulation, while CN treatment showed the fastest increase and the highest final accumulation. In CN treatment, the final cumulative loss of ammonium nitrogen was 12.45 mg. In treatment of TA, FA and KA, the cumulative loss of ammonium nitrogen was 3.00 mg/L, 2.82 mg/L and 3.68 mg/L, respectively. In treatment of TC, FC and KC, the cumulative loss of ammonium nitrogen was 2.69 mg/L, 2.62 mg/L and 2.96 mg/L, respectively.

3.2.3. Effects of biochar on nitrate nitrogen concentration in soil leaching solution

Unlike the situation of ammonium nitrogen leaching, the nitrate-nitrogen concentration in the soil leaching solution of the biochar-applied treatments was higher than that of CN treatment at the beginning of experiment (Figure 2(c)), while other treatments were lower than CN treatment, indicating that nitrification was still weak in
3.2.4. Effects of biochar application on the amount of accumulated nitrate nitrogen leaching in soil leaching solution

As can be seen from Figure 2(d), the total amount of nitrate nitrogen gradually increased as the leaching time, but the growth rate showed a tendency to increase and then decrease. The effect of applying biochar on the cumulative nitrate nitrogen leaching was also different, all treatments were higher than group CN (16.25 mg). In FA, FB, FC and FD treatment, as the amount of biochar applied increased, the cumulative loss of nitrate nitrogen were 24.72 mg, 25.54 mg, 28.98 mg and 18.39 mg, respectively, with a trend of increased and then decreased nitrate nitrogen accumulation. This may be the fact that nitrification had already reached maximum in FC treatment and the continuing addition of biochar could not promote nitrification due to the ammonia content, while the excessive addition of biochar played a role in adsorption. In treatment of TA and TC, the cumulative loss of nitrate nitrogen were 18.25 mg and 23.30 mg, respectively, and the accumulation of nitrate nitrogen showed an increasing trend. In treatment of KA and KC, the cumulative nitrate nitrogen loss were 26.14 mg and 30.31 mg, respectively, and the accumulation of nitrate nitrogen showed an increasing trend.

For treatment of CK and CB without nitrogen addition, the cumulative nitrate nitrogen loss was 3.80 mg and 5.53 mg, respectively, which was significantly lower than that of the other nitrogen addition treatments. This indicates that although the addition of biochar improved soil permeability and enhanced the activity of some aerobic microorganisms, the increase in the cumulative nitrate nitrogen leaching was mainly due to the enhancement of nitrification by biochar addition rather than organic nitrogen mineralization.

3.2.5. Effects of biochar application on total nitrogen concentration in soil leaching solution

As shown in Figure 2(e), the total nitrogen concentration in the leaching solution showed two
general patterns of increased, then decreased and finally levelled off. The lowest total nitrogen concentration in group CN was 5.32 mg/L on 60 d, indicating that the application of biochar into alkaline and nitrogen-supplying soil significantly increased the total nitrogen concentration in the drench solution.

3.2.6. Effects of biochar on cumulative total nitrogen leaching in soil leaching solution

The total nitrogen in the leaching solution was the sum of organic and inorganic nitrogen in the aqueous solution. As shown in Figure 2(f), as the dripping time increased, the amount of total nitrogen leached from the experimental groups gradually increased. The total nitrogen loss
in CN was 127.94 mg, while the total nitrogen accumulated in treatment of TC, FC and KC were 156.52 mg, 145.54 mg, and 103.10 mg, respectively.

3.3. Analysis of sterilization experiment

In this study, treatment of CN and FC were selected to supplementing the sterilization test for comparative analysis of the microbial effects on soil nitrogen. The results showed that the concentrations of ammonium nitrogen leachate in sterilized soil in treatment of CN and FC were 98.89 mg/L and 21.86 mg/L on 20 d, respectively, while the concentrations of ammonium nitrogen leachate in original soil in treatment of CN and FC were 21.34 mg/L and 1.14 mg/L. On 20 d, the concentrations of nitrate nitrogen leachate in sterilized soil in treatment of CN and FC were 6.17 mg/L and 2.42 mg/L, respectively, while the concentrations of nitrate nitrogen leachate in original soil in treatment of CN and FC were 13.06 mg/L and 22.15 mg/L, respectively. It could be seen that the concentration of ammonium nitrogen in the leaching solution of the non-sterilized soil without the addition of biochar is 4.63 times higher than that in the sterilized soil. The concentration of ammonium nitrogen in the leaching solution of non-sterilized soil was 19.18 times higher than that of sterilized soil when adding biochar. The nitrate nitrogen concentration in the leaching solution of sterilized soil without adding biochar is 2.12 times higher than that of non-sterilized soil. The nitrate nitrogen concentration in the leaching solution of the sterilized soil was 9.15 times higher than that of the non-sterilized soil when adding biochar.

3.4. Effects of biochar application on EC value and pH of soil leaching solution

As shown in Figure 3(a), the conductivity of the leaching solution tended to decreased as the leaching time. The slope of the conductivity curve of the drench solution was the largest from the beginning of the drenching to the day 30, after which the slope gradually less. The raw material used in this experiment was maize straw, which is rich in nutrients and minerals, the ions of minerals such as K, Ca and Na were retained in the biochar [37]. On day 10, group FD has the highest electrical conductivity of 3244 μs/cm, and the electrical conductivity of group CN was 2160 μs/cm. This trend became less pronounced as the application of biochar increased the void fraction of the soil and reduced the soil bulk density, but eventually the conductivity of the soil drench was higher than the 251 μs/cm of group CN.

The application of biochar not only significantly increased the pH of the soil leachate, but also had a significant effect on the pH of the soil. In this experiment, the application of biochar also facilitated nitrification as the nitrogen source was provided and nitrification was active in the soil, meanwhile, the nitrification reaction reduced the pH of the soil. The pH of the soil leachate gradually stabilized as the leaching time increased (Figure 3(b)), on day 60, the pH of nitrogen addition treatments was higher than group CN (8.48).

3.5. The abundance and diversity of bacteria and fungi in soil

B1 is the result of bacterial testing of soil samples from group CN; B2 is the result of bacterial testing of soil samples from group FC; F1 is the result of fungal testing of soil samples from group CN; B2 is the result of fungal testing of soil samples from group FC. It can be seen from Table 4 that the Coverage index in the samples all reached above 0.980. The OTUs in B2 were higher than B1, with an increase of 9.94%. The Shannon index in B2 was 1.14 units higher than B1. Meanwhile, the Chao index in B2 was 11.41 units higher than B1, and the ACE index and Chao index were consistent in their patterns. The results indicate that applying biochar to the soil would significantly increase the diversity of bacterial microorganisms, as well as increase the abundance of bacterial microbial communities. As can be seen from Table 4, the Coverage Index in the samples all reached above 0.999. The OTUs in F2 were lower than F1, with a decrease of 16.18. The Shannon index in F2 was 1.10 units higher than F1. Meanwhile, the Chao index in F2 was 274.83 units higher than F1, and the ACE index and Chao index were consistent in their patterns.

3.6. Effects of biochar application on bacterial and fungal community structure in soil

As can be seen from Figure 4(a), there are 11 groups in the horizontal bacterial community of phylum, and the relative abundance of bacteria in group CN and group FC are as follows: proteobacteria (40.56%, 31.65%), actinobacteria (22.43%, 8.95%), acidobacteria (5.91%, 19.52%), gemmatimonadetes (5.78%, 10.88%) and bacteriodes (5.78%, 10.88%) idetes (6.86%, 7.85%), chloroflexi (3.64%, 8.72%), firmicutes (8.21%, 1.16%), patesicibacteria (1.98%, 3.13%), planctomycetes (1.49%, 1.32%), nitrospirae (0.30%, 2.32%) and Others (2.85%, 4.52%). The relative abundance of acidobacteria, bacteroidetes, Chlorobacteria, patella, and Nitrohelicobacteria in the experimental treatment was higher than that in the control treatment, increasing by 13.61%, 5.10%, 0.99%, 5.08%, 1.15%, 2.02%, respectively. However, the relative abundance of Proteobacteria, Actinobacteria, Firmicutes, and Lycophyta in the experimental treatment was lower than that in the control treatment, and the reduction rates were 8.91%, 13.48%, 7.05%, and 0.17%, respectively.
It can be seen from Figure 4(b) that there are 10 groups of fungal community at the level of phylum, and the relative abundance of bacteria in group CN and group FC are as follows: ascomycota (80.34%, 67.88%), basidiomycota (10.46%, 19.63%), mortierellomycota (1.58%, 3.07%), chytridiomycota (0.79%, 1.31%), rozellomycota (0.42%, 0.75%), globulomycota (0.31%,

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0.66%), mucoromycota (0.24%, 0.50%), olpidiomycota (0.18%, 0%), blastocladiomycota (0.06%, 0%), unclassified (0.56%, 0.62%). The relative abundance of Basidiomycota, Mortierellomycota, Chytridiomycota, Rozellomycota, Globulomycota, and Mucoromycota was higher than that of the control treatment, with increases of 9.17%, 1.49%, 0.52%, 0.33%, 0.35%, and 0.26%, while the relative abundance of Ascomycota, Olpidiomycota, and Blastocladiomycota was lower than that of the control treatment, with decreases of 12.46%, 0.18%, and 0.06%.

### 3.7. Applied biochar soil bacterial biological function prediction

As can be seen from Figure 5, KEGG-based gene function prediction revealed that biochar-applied soil microorganisms had the highest percentage of metabolic functions in the first pass at 41.53%. Among the metabolic functions of the second pathway, amino acid transport and metabolism were the strongest, followed by energy conversion and production, carbohydrate transport and metabolism, coenzyme transport and metabolism, lipid transport and metabolism, and nucleotide transport and metabolism, respectively. The abundance on the functions of energy conversion and production, transport, and metabolism of amino acids, transport, and metabolism of nucleotides, and transport and metabolism of coenzymes was significantly ($p < 0.01$) higher than that of the control treatment, while the abundance on the functions of transport and metabolism of carbohydrates, transport, and metabolism of lipids, transport, and metabolism of inorganic ions, and metabolism of secondary metabolites was significantly ($p < 0.01$) lower than that of the control treatment.

![Figure 4. Effects of microorganisms on soil community structure of (a) bacteria, (b) fungus.](image-url)

![Figure 5. Predictive diagram of bacterial biological function.](image-url)
4. Discussions

We can see that the concentration of ammonium nitrogen gradually decreased with adding biochar, which was also consistent with the other findings [38–40]. The cumulative loss of ammonium nitrogen gradually decreased with biochar application [41]. The soil column leaching experiment revealed that biochar application reduced the leaching of ammonium nitrogen from the soil, this is also consistent with other findings [42–44], and FD treatment reduced the leaching of ammonium nitrogen 80.08%, which was the best effect. The order of cumulative leaching of ammonium nitrogen was the 400°C, 300°C, and 500°C biochar treatment (FA < TA < KA). This may be due to the significant correlation between the adsorption amount of corn straw biochar and the surface functional group carboxyl group and lactone group. There was a very strong negative correlation between the ammonium nitrogen adsorption amount and lactone group, the ammonia nitrogen adsorption amount decreased with lactone group content, while the biochar prepared at 400°C had the lowest lactone group content, so the best ammonia nitrogen adsorption effect was that biochar prepared at 400°C [45].

Urea in the soil is gradually converted to nitrate nitrogen by soil enzymes and nitrogenizing bacteria [46], with the initial nitrification rate being low and then gradually accelerating [47]. Throughout the leaching process, the concentration of nitrate-nitrogen increased and then decreased. As the leaching volume increased, the nitrate in the soil was gradually lost with leaching, while in the later stages of leaching, as the ammonium nitrogen has been consumed in large quantities, nitrification is weakened and less nitrate is converted, so the nitrate in the leachate is low. The surface of the biochar was loose and porous [48], and a large amount of ammonium nitrogen was adsorbed on the surface of the biochar at the beginning of the leaching experiment [49]. With the time extension, lots of nitrogenizing bacteria multiplied and enriched in this part to gradually convert ammonium nitrogen into nitrate nitrogen [50]. This may be related to the migration characteristics of nitrate ions in the soil, as nitrate is negatively charged and the soil colloidal particles are also negatively charged, so nitrate ions are not easily adsorbed by the soil colloids and will run off with the leachate [51].

The effect of preparing biochar at different temperatures on the cumulative nitrate nitrogen leaching was also different [52], with TA treatment < FA treatment < KA treatment and TC treatment < FC treatment < KC treatment, probably because the higher the temperature the more micropores and mesopores the biochar had better adsorb water and nutrients [53], providing a suitable living environment for nitrifying bacteria to convert to more nitrate nitrogen. The addition of biochar effectively increased the nitrate nitrogen content of the cumulative leachate, which plants used to grow, and the addition of biochar promotes the uptake of nitrate nitrogen by plants [54]. Due to the low charring temperature and high organic matter content of the biochar, and the high concentration of organic nitrogen in the biochar resulting in a high total nitrogen content in the leachate [55,56]. At higher carbonization temperatures (500°C–700°C), the biochar contains more stable PAHS structures and therefore has better sorption properties [57,58], which may lead to higher total N leaching from the low-temperature biochar soil [59]. The results of the sterilization experiments confirm that biochar has an adsorption effect on ammonia nitrogen, and that microorganisms especially nitrifying bacteria can effectively convert ammonia nitrogen to nitrate nitrogen. And biochar addition can effectively facilitate this process, which further confirms the previous conclusions [60,61]. Liu [62] synthesized that biochar addition to paddy soils improved N use efficiency, and the results of Zheng [63] showed that biochar addition to acidic soils promoted soil nitrification to improve N use efficiency, which is the same as our conclusion. Related studies have shown that MgCl2-modified biochar contributes to the slow release of ammonia and nitrate nitrogen and improves nitrogen utilization [64], while the higher the temperature of biochar preparation, the higher the pH and EC values [65], which is the same as the conclusion of our study. The addition of biochar to soil can effectively improve pH and EC values, and the higher the temperature of preparing biochar the more obvious the enhancement effect is, which is due to the higher the temperature of preparing biochar, the higher the pH and EC values of biochar [66], which is the same as the conclusion of our study.

Our study has also been demonstrated that biochar surfaces are rich in ions and applied a certain amount of biochar to the soil can increase the salinity of the soluble state [67,68]. Some studies indicated that the higher the preparation temperature of the biochar the higher value of EC, but the results of this experiment did not lead to this conclusion [69], which may be due to the uptake and use of this soluble salt ion by microorganisms in the soil. Due to the alkaline nature of biochar, which finally will raise the pH of the soil when applied to the soil [70]. The surface of biochar is rich in nutrients, adding to the soil can significantly increase the concentration of soluble salts in the soil in the short term [71], with the appropriate increase in salinity helping plant growth and development [72].

Biochar addition effectively increased the abundance and diversity of soil bacterial communities. Our results also indicated that adding biochar into the soil under the provision of nitrogen source would affect the horizontal community structure of the fungal phylum, but there were differences in the effects produced by various fungi, indicating that
different fungi differed in their adaptation in the soil after biochar application [73]. Our study showed the relative abundance of Acidobacteria, which degrade plant residues, participate in iron cycling, have photosynthetic capacity and are involved in the metabolism of nitrogen and carbon compounds, was elevated [74]. The relative abundance of the Bacillus and the Chloroflexi was also slightly elevated, with Bacillus having functions such as strong moisturizing properties [75], good decomposition of organic matter, production of abundant metabolites, bacterial inhibition, pest control and deodorization. Chloroflexi can fix CO₂, oxidize CO and CH₄, and degrade cellulose [76], and so on. It also participates in the second part of nitrification (the oxidation of NO₂⁻), and it also has some oxidation ability for S element [77]. The adsorption of ammonia nitrogen by biochar-induced changes in the structure of the surrounding flora, and the relative abundance of nitrite bacteria (Nitrosomonadaceae, Nitrosomonas, Nitrosospira) was detected to increase from 0.3% to 2.5%, and the relative abundance of nitrifying bacteria (Nitrospirae, Nitroloancea) increased from 0.3% to 2.3%. Nitrification consisted of the oxidation of ammonia nitrogen to nitroso-nitrogen by nitrite bacteria and the oxidation of nitroso-nitrogen to nitrate by nitrifying bacteria [78,79]. It is possible that the addition of biochar increased the relative abundance of nitrification-related functional flora [80], which was possibly because the addition of biochar adsorbed ammonium nitrogen and water [81], while lowering the soil bulk and improving soil permeability, providing a suitable living environment for microorganisms and helped the related flora to reproduce and grow [82]. In conclusion, the application of biochar to the soil can improve the physical and chemical properties of the soil [78,83], enhance the quality of the soil, adsorb ammonium nitrogen, increase the relative abundance of nitrite bacteria, nitrifying bacteria and promote the conversion of ammonium nitrogen to nitrate nitrogen.

5. Conclusions
In this study, we investigated the effects of different preparation conditions (300°C, 400°C and 500°C) and additions (0.1%, 0.5%, 1% and 2%) of biochar on ammonia and nitrate nitrogen and microbial community composition in the soil, based on the provision of nitrogen source. It was found that the addition of biochar significantly increased the conductivity and pH of the soil leaching solution and reduced the leaching of ammonium nitrogen and increased the leaching of nitrate nitrogen. The addition of biochar increased the abundance and diversity of soil bacteria, with a significant increase in the relative abundance of nitrifying bacteria, but no significant effect on the abundance of fungi. Our study analyses the effect of biochar addition on soil nitrogen transformation, particularly from a microbial perspective, which provides a theoretical basis and data support for the practical returning biochar into the field.

Disclosure statement
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