Effects of Biochar Application on Soil Nitrogen Conversion and Microbial Community Composition

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Abstract

In northeastern China, successive years of cultivation have led to a decline in soil quality, a process that is exacerbated by the over-application of chemical fertilizers to ensure staple food production. The large amount of straw produced by cultivation is difficult to effectively use in recent years. There has been an increasing amount of research on the transforming straw into biomass char, but it has often focused on the effects of biomass char addition on soil physicochemical properties, without further exploring the mechanisms of this process and its effects on soil microorganisms. Microorganisms are an important part of the soil system and the process of how biomass char addition affects microorganisms through its effect on soil physicochemical properties should not be overlooked. In this study, the effect of biochar application at different preparation temperatures (300°C, 400°C and 500°C) and addition contents (0.1% and 1%) on ammonia, nitrate and total nitrogen in soil leachates were investigated. The effect of microbial sequencing on the dynamics of carbon and nitrogen was also investigated to reveal the mechanisms contributing to the changes in nitrogen forms. The results showed that biochar had a better adsorption ability on ammonia nitrogen, and biochar promoted the conversion of ammonia nitrogen to nitrate nitrogen by nitrifying bacteria. The addition of 1% biochar (prepared at 500°C) increased nitrate-nitrogen leaching by 86.52% compared to the control treatment. The sequencing of microorganisms also revealed that biochar changed the structure and abundance of the soil microbial community, especially increasing the relative abundance of the *Helicobacter* nitrification phylum by 2.02%. These results indicate that biochar facilitated the adsorption of ammonium nitrogen and the conversion of nitrate nitrogen, and solving the problem of low nitrogen fertilizer utilization while promoting the formation of beneficial bacteria in the soil.

Introduction

The organic carbon content of the black soils of the Northeast China has been declining year on year with people's agricultural activities and the land is becoming infertile. The return of straw to the fields alleviates this situation, however, straw decay releases large amounts of CO₂, which is not conducive to carbon sequestration and emission reduction. In addition, straw may contain insect eggs, causing a massive outbreak of pests and diseases in the following year's cultivation, thus returning biochar to the fields has its own unique advantages. Biochar is usually a highly aromatic solid material produced by the pyrolytic carbonization of biomass under fully or partially anoxic conditions (Chen et al. 2021). Biochar can be classified as wood charcoal, bamboo charcoal, straw charcoal and rice husk charcoal according to its raw material source (Sahoo et al. 2021). 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 (Arabi et al. 2018; Darby et al. 2016). However, most studies tend to focus on the effect of biochar on soil physicochemical properties, and few have explored the effect of biochar on microorganisms through its effect on physicochemical properties. The effect of microorganisms on soil quality cannot be ignored if we want to improve soil quality in the long term. Therefore, this study focuses on investigating the mechanisms of biochar affecting soil microbial populations and structure by

changing soil physicochemical properties. At the same time, the properties of biochar are closely related to the feedstock and pyrolysis temperature(Cheng et al. 2012). Biomass char prepared under low temperature conditions has a higher organic matter content and higher organic nitrogen concentration (Garbuz et al. 2021; Khalid et al. 2021), while biomass char prepared under high temperature conditions contains more stable Polycyclic Aromatic Hydrocarbons structures and has better adsorption properties, therefore, it is necessary to study the effect of biomass char on the soil and its microorganisms under different preparation temperature conditions.

According to the physicochemical properties of biochar, adding biochar to soil can effectively reduce soil bulk as well as improve soil water retention and aeration(Karimi et al. 2019), thus improving soil texture(Alaoui et al. 2011; Dexter 2004). At the same time, biochar is alkaline and can effectively raise pH of soil (Tormena et al. 2017), and its surface is rich in ions, which can effectively improve the cation exchange capacity in the soil (Safadoust et al. 2014). Some studies point out that adding biochar is effective to reduce the leaching of nitrate nitrogen from soils, while others reach the exact opposite conclusion, attributing this difference only to the influence of different soil types with significant flaws and the need to consider the influence of microorganisms during leaching experiments (Lv et al. 2021). The loose and porous nature of the biochar surface can provide a good living environment for microorganisms(Han et al. 2021; Hannet et al. 2021), while its nutrient elements facilitate the growth and reproduction of microorganisms, further contributing to increasing soil nutrient retention(Wang et al. 2021), improving soil fertilizer efficiency(Wang et al. 2021). Biochar can also improve soil bacterial structure effectively (Berisso et al. 2012; de Lima et al. 2017), and contribute to the survival of beneficial bacteria, thus improving the soil in the long term(Emmet-Booth et al. 2020; İlay et al. 2020).

The low temperatures and predominantly dry land in the north-east China make it suitable for applying nitrate nitrogen(Chen et al. 2014). Nitrate nitrogen is mainly found in the soil solution which is highly mobile as well as easily absorbed and used by plants(Šimanský et al. 2016). Field crops mainly use nitrate nitrogen for growth(Šimanský et al. 2017). Studies show that plants accumulate large amounts of nitrate-nitrogen during the growth period of nutrient organs(Jindo et al. 2020a). As plants continue to grow, the amount of nitrate-nitrogen in their bodies decreases(Jindo et al. 2020b). Plants take up large amounts of nutrients during the nutritional growth phase(Kamau et al. 2019), which are 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(Liu et al. 2021). 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 (Lychuk et al. 2014). 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(Moon et al. 2017), nitrate nitrogen can replace them in their osmoregulatory role, and this regulation requires less energy. Utilizing biochar can improve the efficiency of nitrate N conversion and solve a range of soil problems such as soil hardening due to excessive nitrogen fertilizer application(Zhang et al. 2019; Zhang et al. 2020), while enhancing soil quality and organic matter content(Zhang et al. 2021; Zong et al. 2021).

In this study, the effects of biochar on soil were investigated. The soil nitrogen transformation was measured by leaching experiments, the population and structure of microorganisms in the soil were also determined to reveal the intrinsic mechanism of nitrogen conversion affected by biochar application. This study would provide a theoretical basis for improving the nitrogen utilization rate of crops in northeast China, effectively using biochar and improving soil quality in the long term.

Materials And Methods

Preparation of soil and straw

Soil samples were collected in Daqing, Heilongjiang Province, China (45°46'N, 124°18'E), plant roots and visible stones in the soil samples were removed from the soil by hand.

Biochar was prepared from maize straw in the agricultural growing areas around Harbin, Heilongjiang Province. All the impurities rinsed after washing with water, into the oven drying, adding straw biomass carbon preparation this group of special equipment. The temperature of the biochar preparation equipment was set at 300°C, 400°C and 500°C respectively, and then the biochar was prepared. After removal, it is naturally air-dried and cooled for use.

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, which is mixed with 0.3 g of urea as a nitrogen source (equivalent to 240 kg of nitrogen per hectare). The soil is packed in three layers: 0-5 cm for one layer, 5-10 cm for one layer and 10-20 cm for one layer, with the biochar mixed evenly with the soil in the second layer. 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 five days and 60 mL per day for the last five days, measured in 10-day intervals and cumulatively for 60 days, with total precipitation approximately equal to the annual average precipitation in Harbin. Nine treatments was set in this study: no biochar (CK, control), 1‰ of 400°C biochar (FA), 5‰ of 400°C biochar (FB), 1% of 400°C biochar (FC), 2% of 400°C biochar (FD), 1‰ of 300°C biochar (TA), 1% of 300°C biochar (TC), 1‰ of 500°C biochar (KA), 1% of 500°C biochar (KC), and three replicates was designed for each treatment .

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).

Chemical analysis

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

Microbiological test methods

The total deoxyribonucleic acid (DNA) of the sample is extracted, primers are designed according to the conserved region, a sequencing junction is added to the end of the primer, polymerase chain reaction (PCR) amplification is performed and the product is purified, quantified and homogenized to form a sequencing library. The raw image data files obtained from high-throughput sequencing (e.g. Illumina HiSeq and other sequencing platforms) are transformed into Sequenced Reads by Base Calling analysis, and the results are stored in FASTQ (abbreviated as fq) file format, which contains the sequence information of the sequenced Reads and their The results are stored in the FASTQ file format, which contains the sequence information of the sequenced reads and their corresponding sequencing quality information.

Statistics

For data pre-processing, firstly, the Raw Reads obtained from sequencing were filtered using Trimmomatic v0.33 software; then the primer sequences were identified and removed using cutadapt 1.9.1 software to obtain high quality Reads without primer sequences; then FLASH v1.2.7 software was used to overlap each sample to obtain high quality Reads. The high quality Reads were spliced using FLASH v1.2.7 software, and the spliced sequences were Clean Reads; finally, the chimeric sequences were identified and removed using UCHIME v4.2 software to obtain the final Effective Reads.

The information is also analyzed by delineating Features (OTUs, ASVs), diversity analysis, variance analysis, correlation analysis and functional prediction analysis.

One-way analysis of variance (ANOVA) was used to test for treatment differences in ammonium nitrogen, nitrate-nitrogen and total nitrogen. Significance analysis was performed using SPSS 26.

Results And Discussion

Biochar characterization

Observed by scanning electron microscope (SEM), the surface of biochar has abundant bumps and folds accompanied by a large number of pores. The biochar prepared at a final reaction temperature of 400°C has a large pore structure and a large specific surface area, which is conducive to improving soil permeability, soil permeability and water holding capacity. It is also a suitable place for microorganisms to attach and grow in the soil.

In a comparative test, biochar from the sterilized and experimental groups were removed and microorganisms on the biochar were observed using a body microscope. As shown in the Fig 1, it is clearly observed that the biochar had a significant microbial community in the experimental treatment, but not in the sterilized and control groups, which is consistent with the experimental results.

Effects of biochar on soil nitrogen leaching

Effects of biochar on ammonium nitrogen leaching from soil

We can see from Fig. 2a 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 decreased significantly with the application of biochar at the early stage of drenching, in which the concentration of ammonium nitrogen in the soil drench solution of the group without the application of biochar was 31.47 mg/L, which was significantly higher than that of the other treatments with the application of biochar. As the leaching time increased, the ammonium nitrogen concentration in the soil solution decreased rapidly from 10 d to 30 d, and then basically reaching a stable state. On day 10, the ammonium nitrogen concentrations in treatment of 0.1% biochar prepared at 300°C, 400°C and 500°C were 5.62 mg/L, 5.44 mg/L and 6.89 mg/L, which were 82.14%, 82.71% and 78.11% lower than those in the control group respectively. In the group with addition, the ammonium nitrogen concentrations in treatment 1% biochar of 300°C, 400°C and 500°C were 5.07 mg/L, 4.44 mg/L and 5.97 mg/L, which were 83.89%, 85.89% and 81.03% lower than the control treatment respectively. The concentration of ammonium nitrogen gradually decreased with adding biochar. The concentration of ammonium nitrogen in the treatment of biochar prepared at 400°C was lower than that at 300°C and 500°C, where $FA < TA < KA$, which 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 adsorption amount and lactone group, i.e. the adsorption amount decreased with the increase of lactone group content, while the biomass char prepared at 400°C had the lowest lactone group content so the best adsorption effect was achieved at 400°C.

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

As can be seen from Fig. 2b, the cumulative ammonium nitrogen leached from the soil increased slowly with time in all treatments, with the FD treatment showing the slowest increase and the lowest final accumulation, while the control treatment showed the fastest increase and the highest final accumulation. In the control treatment, the final cumulative loss of ammonium nitrogen was 12.45 mg. In the 0.1% biochar addition treatment, the cumulative loss of ammonium nitrogen at 300°C, 400°C and 500°C was 75.90%, 77.35% and 70.44% less than the control treatment, respectively. In the 1% biochar

addition treatment, the cumulative ammonium nitrogen leaching at 300°C, 400°C and 500°C was 78.39%, 78.96% and 76.31% lower than the control treatment, respectively. The cumulative loss of ammonium nitrogen decreased gradually with the increase of biochar application. Biomass charcoal application affected the leaching and translocation of nitrogen from the soil.(Ren et al. 2021), The soil column leaching experiment revealed that biomass charcoal application reduced the leaching of ammonium nitrogen from the soil, and for 400°C biochar addition of two percent could reduce the leaching of ammonium nitrogen by 80.08%, which was the best effect. As the amount of biomass charcoal used increased, the adsorption of ammonium nitrogen became more effective and the amount of ammonium nitrogen in the leachate solution was reduced, with the adsorption of biomass charcoal being more effective than nitrate nitrogen for high concentrations of ammonium nitrogen.

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 some of the biochar-applied groups was higher than that of the control group at the beginning of experiment (Fig. 2c), while some of the other treatments were lower than the control group, indicating that nitrification was still weak in some of the treatments at the beginning, and the nitrate-nitrogen produced was sequestered by sorption. At the highest concentration of 40 d, the nitrate-nitrogen concentrations in the treatments of biochar prepared at 300°C, 400°C and 500°C were all 1% > 0.1%, TC > TA, FC > FA, KC > KA, and overall, the nitrate-nitrogen concentrations in the soil drench solutions of the biochar application treatments were higher than those of the control treatment as time progressed, and showed an overall trend of increasing and then decreasing. 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. Urea in the soil is gradually converted to nitrate nitrogen by soil enzymes and nitrifying bacteria, with the initial nitrification rate being low and then gradually accelerating. 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 the rainfall, while in the later stages of leaching, as the nitrogen source has been consumed in large quantities, nitrification is weakened and less nitrate is converted, so the nitrate in the leachate is low.

The nitrate-nitrogen content of the soil leachate increased when adding biochar. The addition of biochar in moderate amounts can increase the nitrate-nitrogen concentration in the soil due to the increased permeability of the soil after the application of biochar and the increased rate of nitrification of the nitrogen reaction in the soil due to the fixation of ammonium nitrogen by biochar.

Effects of biochar application on the amount of accumulated nitrate-nitrogen leaching in soil leaching solution

We can see from Fig. 2d, the total amount of nitrate-nitrogen gradually increased as the leaching time increased, but the growth rate showed a tendency to increase and then decrease. The effect of applying different biomass charcoal on the cumulative nitrate-nitrogen leaching was also different, with the control treatment leaching 16.25 mg, all experimental treatments were higher than the control group. In the 400°C biochar treatment, as the amount of biochar applied increased, the cumulative loss of nitrate-nitrogen was 24.72 mg, 25.54 mg, 28.98 mg and 18.39 mg for 0.1%, 0.5%, 1% and 2% of biochar applied, respectively, with a trend of increasing and then decreasing nitrate-nitrogen accumulation. This may be due to the fact that nitrification had already reached its maximum in the FC treatment and the continuing addition of biochar could not promote nitrification, while the excessive addition of biochar played a role in adsorption. In 300°C biochar treatment, with the 0.1% and 1% of biochar application, the cumulative loss of nitrate-nitrogen was 18.25 mg and 23.30 mg, respectively, and the accumulation of nitrate-nitrogen showed an increasing trend. In the 500°C biochar treatment, with the 0.1% and 1% of biochar application, the cumulative nitrate-nitrogen loss was 26.14 mg and 30.31 mg, respectively, with an increasing trend of nitrate-nitrogen accumulation. The surface of the biochar was loose and porous (Tan and Yuan 2017), and a large amount of ammonium nitrogen was adsorbed on the surface of the biochar at the beginning of the leaching experiment (Yang et al. 2015). With the time extension, a large number of nitrifying bacteria multiplied and enriched in this part to gradually convert ammonium nitrogen into nitrate nitrogen. For 300°C and 500°C biochar, nitrate-nitrogen tended to increase as the amount of biochar applied increased, while 400°C biochar showed a trend of increasing and then decreasing nitrate-nitrogen. The addition of excess biochar significantly reduced the soil capacity and also adsorbed a significant amount of the nitrate-nitrogen produced, thus reducing the amount of nitrate-nitrogen leached. Overall, nitrate-nitrogen leaching increased and then decreased after the application of biochar, and peaked at 40 days. The slow release of nitrate-nitrogen compared to the control group also contributed to the uptake and use of nitrogen by the plants, resulting in improved nitrogen use efficiency.

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

As shown in Fig. 2c, the total nitrogen concentration in the drench solution showed general patterns of increasing, then decreasing. The lowest total nitrogen concentration in the control treatment was 5.32 mg/L on 60 d. The rest of the experimental treatments were significantly higher than the control treatment, indicating that the application of biochar to alkaline, nitrogen-supplying soils significantly increased the total nitrogen concentration in their drench solutions.

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 Fig. 2f, that as the dripping time increased, the amount of total nitrogen leached

from the experimental groups gradually increased. For the control group the total nitrogen loss was 127.94 mg, for the 300°C and 400°C biochar treatments the total nitrogen accumulation increased as the amount of biochar applied increased, while for the 500°C biochar treatment the total nitrogen accumulation decreased as the amount of biochar applied increased. This is 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. At higher carbonization temperatures (500°C-700°C), the biochar contains more stable PAH structures and therefore has better sorption properties, which may lead to higher total N leaching from the low temperature biochar soil. Whereas the opposite was true for the predominance of sorption at higher temperatures (Sun et al. 2021). Biochar (500°C) was effective in reducing total nitrogen leaching and was the most effective, whereas for low temperature biochar at 300°C and 400°C, total nitrogen leaching was less than the control treatment for small additions and gradually increased with the addition of more biochar.

Analysis of sterilization experiment

Control treatment was carried out on disinfected and virgin soils under the condition of added nitrogen sources. The results showed that in the first two cycles of the trial, the concentrations of ammonium nitrogen leachate in the sterilized soil were 98.89 mg/L and 21.86 mg/L, respectively, while the concentrations of ammonium nitrogen leachate in the original soil were 21.34 mg/L and 1.14 mg/L, respectively. In the first two cycles of the trial, the concentrations of nitrate leachate in the sterilized soil were 16.17 mg/L and 2.42 mg/L, respectively, while the concentrations of nitrate leachate in the original soil were 23.06 mg/L and 221.45 mg/L respectively. In the control treatment without the addition of carbon and nitrogen sources, the concentrations of ammonium nitrogen drench solution were 0.31 mg/L and 0.76 mg/L and nitrate nitrogen drench solution concentrations were 5.28 mg/L and 11.47 mg/L, respectively. In the first two cycles, the concentrations of ammonium nitrogen in the soil drench were 3.63 and 18.18 times higher than those in the original soil, while the concentrations of nitrate nitrogen were 1.42 and 90.51 times higher than those in the original soil, respectively.

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

As shown in Fig. 3a, the conductivity of the drench solution tended to decrease as the drenching time increases. The slope of the conductivity curve of the drench solution decreased the most from the beginning of the drenching to the day 30, after which the slope of the decrease gradually decreases, which was reflected in the rapid decrease of the conductivity of the drench solution at the beginning, while the rate of decrease gradually slows down afterwards. The raw material used for this experiment was maize straw, which is rich in nutrients and minerals, and after charring the ions of minerals such as K, Ca and Na were retained in the biochar. On the 10th day, the soil leachate added with 2% biochar at 400°C has the highest electrical conductivity of 3244 $\mu\text{S}/\text{cm}$, and the electrical conductivity of control treatment was 2160 $\mu\text{S}/\text{cm}$. The surface rich in ions and the application of a certain amount of biochar

to the soil can increase the soluble state salinity. 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 251µs/cm of the control treatment.

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, although the exact effect varied. In this experiment, the application of biochar also facilitated nitrification as the nitrogen source was provided and nitrification was active in the soil, and at the same time the nitrification reaction reduced the pH of the soil. The pH of the soil leachate gradually stabilized as the leaching time increased (Fig. 3b), on day 60, the pH of all treatments was higher than that of the control treatment (8.48), which was caused by the higher pH of the biochar than the soil. The surface of the biochar riching in nutrients and its short-term application to the soil can significantly increase the salinity of the soil, which helps plant growth and development(Song et al. 2019). The significant change of pH at the beginning of the application of biochar on the basis of the nitrogen source was due to the alkaline nature of the biochar itself, but the nitrification that occurred during the experiment reduced the soil pH As the duration of the leaching experiment increased, the pH levels levelled off and eventually all treatments had a higher pH than the control treatment due to the alkaline nature of the biochar, which in the long run will increase the pH of the soil when applied to the soil.

The abundance and diversity of bacteria and fungi in soil

Table 1 Analysis of bacterial and fungal abundance and diversity

Sample ID	OTUs	ACE index	Chao1 index	Shannon index	Coverage index
B1	1237	1367.18	1388.23	7.90	0.9965
B2	1360	1394.05	1399.64	9.04	0.9988
F1	476	1069.53	656.17	6.55	0.9994
F2	399	1832.13	931.0	7.65	0.9993

It can be seen from the Table 1 that the Coverage index in the samples all reached above 0.980. The OTUs in the 1% biochar treatment were higher than those in the control treatment, with an increase of 9.94%. The Shannon index of the experimental treatment was 1.14 units higher than that of the control treatment. Meanwhile, the Chao index of the experimental treatment was 11.41 units higher than that of the control treatment, 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 the Table1, the Coverage Index in the samples all reached above 0.999. The OTUs in the F2 applied 1% biochar treatment were lower than those in the control treatment, with a decrease of 16.18. The Shannon index of the experimental group was 1.10 units higher than that of the control group.

Meanwhile, the Chao index of the experimental group was 274.83 units higher than that of the control treatment, and the ACE index and Chao index were in agreement with each other.

Community composition of bacteria and fungi in soil

Table 2 Bacterial and fungal community composition.

Sample	Kindom	Phylum	Class	Order	Family	Genus
B1	1	24	70	167	295	502
B2	1	30	83	185	312	506
F1	1	9	24	59	100	163
F2	1	7	17	46	95	140

According to the results of the OUT division and taxonomic status identification, the specific composition of microorganisms in soil at each taxonomic level is as follows (Table2). The experimental group had the highest level of microbial communities at all taxonomic levels, with the phylum, order, family and genus levels containing more microorganisms than the control group.

According to the OUT division and taxonomic status identification results, the specific composition of microorganisms in the soil at each taxonomic level is as follows (Table2), the experimental treatment is at the lowest level of microbial communities at each taxonomic level, where the microorganisms contained at the phylum, order, family and genus levels are lower than those of the control group.

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

As can be seen from Fig. 4a, there are 11 groups in the horizontal bacterial community of phylum, and the relative abundance of bacteria in the control group and the experimental group are as follows: Proteobacteria (40.56%, 31.65%), Actinobacteria (22.43%, 8.95%), Acidobacteria (5.91%, 19.52%), Gemmatimonadetes (5.78%, 10.88%) and Bacteroidetes (5.78%, 10.88%) Idetes (6.86%, 7.85%), Chloroflexi (3.64%, 8.72%), Firmicutes (8.21%, 1.16%), Patescibacteria (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, Blastomonas, 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. The increase in the abundance of Helicobacter nitrophilus phylum indicated that the application of biochar helped the reproduction of nitrifying bacteria and promoted the occurrence of nitrification reactions. It was also shown that the entry

of biochar into the soil under the provision of a nitrogen source affected the horizontal community structure of the bacterial phylum, but there were differences in the effects produced on various bacteria, indicating that different bacteria differed in their adaptation to the soil after the application of biochar.

It can be seen from Fig. 4b that there are 10 groups of fungal community at the level of phylum, and the relative abundance of bacteria in the control treatment and the experimental treatment 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%, 0.66%), Mucoromycota (0.24%, 0.50%), Olpidiomyota (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, Olpidiomyota and Blastocladiomycota was lower than that of the control treatment, with decreases of 12.46%, 0.18% and 0.06%. The results indicated that biochar entering 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 to the soil after biochar application.

The application of biochar to the soil significantly increases the diversity of bacterial microorganisms and enhances the abundance of bacterial microbial communities (Hua et al. 2021), this effect is not significant for fungi. 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 (Jiang et al. 2015). The relative abundance of the Bacillus phylum and the Green Curvilinear phylum was also slightly elevated, with Bacillus having functions such as strong moisturising properties, good decomposition of organic matter, production of abundant metabolites, bacterial inhibition, pest control and deodorisation. Green bacterium can fix CO_2 , oxidise CO, oxidise CH_4 and degrade cellulose, etc. It also participates in the second part of nitrification, i.e. the oxidation of NO_2^- , and it also has some oxidation ability for S element. The adsorption of ammonia nitrogen by the biomass charcoal led to changes in the structure of the surrounding bacterial flora, and a higher abundance of bacteria from the Helicobacter nitrification family was detected than in the control group, with the nitrifying bacteria gradually oxidising ammonia nitrogen to nitrate nitrogen (Su et al. 2019). Meanwhile, the application of biochar reduced the soil bulk density and improved the soil water retention rate, which was conducive to the absorption and utilization of nitrate nitrogen by plants (Maienza et al. 2017). In conclusion, the application of biomass charcoal to the soil can improve the physical and chemical properties of the soil and improve the quality of the soil, affect the transformation of soil nitrogen and help plants absorb and utilize it, while increasing the abundance of microbial communities in the long term and promoting the long-term improvement of the soil by microorganisms.

Applied biochar soil bacterial biological function prediction

Gene function prediction based on KEGG and COG revealed that biochar-applied soil microorganisms accounted for the highest percentage of metabolic functions in the first pathway 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.

Conclusions

In this study, the effects of different preparation conditions (300°C, 400° C and 500°C) and contents (0.1% and 1%) of biochar on the morphological changes of nitrogen and microbial community composition in the soil were investigated, based on the provision of nitrogen source. It was found that the addition of biochar significantly increased the electrical conductivity and pH of the soil leach solution and reduced the amount of ammonium nitrogen leached out, while promoting the conversion of ammonium nitrogen to nitrate nitrogen, with the best effect of biochar prepared at 500° C with 1% addition increasing 86.52% compared to the control treatment. This confirmed the great potential of biochar in improving nitrogen utilization. At the same time, the abundance and diversity of microbial communities, especially bacteria, increased significantly, indicating that the addition of biochar improved the mycological environment of the soil. One of the main reasons why the soils in northeast China have become increasingly poor in recent years is the low nitrogen utilization rate and the heavy application of chemical fertilizers. The addition of biochar can solve this problem by promoting the conversion of ammonium nitrogen to nitrate nitrogen, which helps crops to absorb and utilize it without rapidly decomposing and releasing large amounts of carbon dioxide. Biochar also has broad application prospects for improving the soil microbial environment and improving soil quality in the long term.

Declarations

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Author contribution

Pingnan Zhao: Conceptualization, Methodology, Data curation, Writing - original draft, Visualization, Investigation. Shen Wang: Methodology, Data curation. Dong Liu: Visualization, Resources. Hongxu Li: Conceptualization–Data curation. Song Han: Supervision, Writing - review & editing, Funding acquisition. Ming Li: Writing - review & editing.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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Figures

Figure 1

Biochar characterization of (a) SEM, (b) contrast, (c) sterilization, (d) experiment.

Figure 2

Effects of biochar on nitrogen leaching of (a) Concentration of ammonium nitrogen in leachate, (b) Cumulative leaching loss of ammonium nitrogen, (c) Nitrate nitrogen concentration in leachate, (d) Cumulative leaching loss of nitrate nitrogen, (e) Total nitrogen concentration of leachate, (f) Cumulative leaching loss of total nitrogen.

Figure 3

Effects of biochar on EC value and pH value of (a) EC value, (b) pH value.

Figure 4

Effects of microorganisms on soil community structure of (a) bacteria, (b) fungus.

Figure 5

Predictive diagram of bacterial biological function.

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