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RESEARCH ARTICLE

The contrasting effects of biochar and straw on N_2O emissions in the maize season in intensively farmed soil

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Abstract

This study evaluated the combined effects of biochar and straw on N_2O flux and the community compositions of nitrifiers and denitrifiers in the maize season in an intensively farmed area in northern China. The experiment consisted of four treatments: (1) CK (only chemical fertilizer application); (2) C (biochar application); (3) SR (straw application to the field); and (4) C+SR (the application of both biochar and straw). The results indicated that during the maize growing season, N_2O flux decreased by 30.3% in the C treatment and increased by 13.2% and 37.0% in the SR and C+SR treatments compared with CK, respectively. NO_3^--N, NH_4^+-N, and microbial biomass carbon (MBC) were the main soil factors affecting N_2O flux, and they were positively correlated with NO_3^--N and negatively correlated with MBC in the C treatment and positively correlated with NH_4^+-N in the SR and C+SR treatments. Both biochar addition and straw return shifted the community compositions of nitrifiers and denitrifiers. N_2O production was mainly reduced by promoting the ammonia-oxidizing bacteria (AOB) gene abundance and inhibiting the *nirK* gene abundance in the C treatment but promoted by inhibiting the AOB and *nosZ* gene abundances in the SR and C+SR treatments. *Nitrosospira* (AOB) and *Rhizobium* (*nirK*) were the main contributors among the treatments. NO_3^--N, NH_4^+-N, and MBC were the main soil factors affecting the denitrifier communities. The predominant species associated with the *nirK*, *nirS*, and *nosZ* genes were positively correlated with NO_3^--N and MBC and negatively correlated with NH_4^+-N. These results provide valuable information on the mechanism of N_2O production and reduction in biochar- and straw-amended soil under field conditions.

Keywords Biochar · Straw return · Nitrifiers · Denitrifiers

Introduction

Nitrous oxide (N_2O) is one of the most important greenhouse gases, and agricultural soil is the dominant emission source. Many studies have confirmed that more than 70% of N_2O emissions are derived from arable land where excessive nitrogen (N) fertilizer is applied (Yao et al. 2017; Tian et al. 2018; Song et al. 2020). The North China Plain is one of the main intensively farmed agricultural areas in China (Zhou et al. 2017a, b; Tan et al. 2017; Xu et al. 2019), and annual N fertilizer application can reach up to 600 kg N ha^{-1} year^{-1} in the winter wheat and summer maize rotation system in this region (Tan et al. 2017; Liu et al. 2019). This high level of N input will inevitably lead to high N_2O emissions. Earlier in situ field studies observed that the cumulative N_2O emissions in the maize season accounted for 75.2–90.0% of the annual total emissions due to the high temperature and moisture in the maize season (Liu et al. 2019; Shi et al. 2019; Xu et al. 2019). Based on this background, it is imperative to apply practical strategies to reduce N_2O emissions and better understand the mitigation mechanism in the maize season in intensively farmed agricultural areas.

Nitrification and denitrification, dominated by microorganisms, are the main pathways of N_2O emission (Hu et al. 2015). Nitrification is the oxidation of ammonium nitrogen to nitrite and then nitrate. Ammonia oxidation catalyzed by ammonia monooxygenase is the rate-limiting step of nitrification, and it

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is mainly driven by ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB). N_2O is the byproduct of incomplete NH_2OH oxidation to NO_2^- or the final product of AOB denitrification (Spokas et al. 2012; Hu et al. 2015; Caranto et al. 2016). Denitrification refers to the process by which NO_3^- or NO_2^- is gradually reduced to NO, N_2O and N_2 under the action of enzymes. The reduction of NO_2^- to NO is considered to be the rate-limiting step of denitrification, and nitrite reductase is encoded by the *nirK* and *nirS* genes, which respond differently to the soil environment (Zhang et al. 2015). The reduction step of N_2O to N_2 is the known biological sink of N_2O, and the *nosZ* gene plays an important role in coding N_2O reductase, which determines whether N_2O can be completely reduced (Yuan et al. 2019). A number of studies have shown that N_2O emissions were significantly correlated with *nirK*, *nirS*, and *nosZ* (Yao et al. 2012; Ai et al. 2013; Harter et al. 2016; Hink et al. 2018; Wang et al. 2020). But there is no established evidence of a quantitative relationship between N_2O fluxes and the denitrifiers of *nirK*, *nirS*, and *nosZ*, and which are the key drivers in the N_2O production and reduction.

Previous studies have shown that the application of biochar can change soil physical and chemical properties by absorbing soil NH_4^+-N and NO_3^--N or by increasing soil carbon storage, C/N ratio, soil pH, and water holding capacity, therefore improving soil fertility (Zhang et al. 2012; Ribas et al. 2019). Based on 16S rRNA gene sequencing technology, it was found that biochar addition significantly affected the diversity of soil microorganisms involved in nitrogen conversion processes, such as nitrogen fixation, nitrification and denitrification (Chen et al. 2013; Wu et al. 2019). The activity and abundance of AOA and AOB genes were increased significantly by biochar addition (Yao et al. 2012). Lin et al. (2017) found that AOA copy numbers increased and N_2O emissions decreased after adding biochar to paddy soil. Shi et al. (2019) also found that adding biochar could significantly increase the copy numbers of AOA and AOB and that AOB was more sensitive than AOA to biochar.

Biochar significantly changes the denitrifier community composition. Previous studies have shown that biochar addition significantly changed the abundance and activity of the *nirS* and *nirK* genes, which significantly reduced N_2O emissions (Harter et al. 2014; Krause et al. 2018). Harter et al. (2016) also found that biochar improved the activity and abundance of the *nosZ* gene, thus promoting complete denitrification and reducing N_2O emissions. However, there is no clear causal relationship between soil properties and reductions in N_2O emissions in biochar-amended soils. Therefore, the effects of biochar addition on N_2O production and reduction (consumption) processes and the mechanisms are still unclear.

Straw return, as an important way of reusing agricultural waste, is strongly recommended by the Chinese government (Zhou et al. 2017a, b). Straw return is generally believed to have positive effects on the ability of soil microorganisms to hold carbon and nitrogen, increasing the total soil microbial biomass and improving the metabolic capacity and functional diversity of the soil microbial community (Peng et al. 2016; Li et al. 2018; Ma et al. 2019). However, there are different opinions on the impact of straw return on N_2O emissions. Some studies found that straw return can promote N_2O emissions (Ju et al. 2011; Cui et al. 2012; Liu et al. 2014a, b; Ma et al. 2019), while other studies confirmed that straw return can reduce N_2O emissions (Yao et al. 2017; Lin et al. 2017; Wang et al. 2020). In addition, there are few studies on the effect of straw on nitrification and denitrification microorganisms. Zhao et al. (2017) found that straw increased the copy numbers of AOA and AOB and shifted the denitrifier community composition, thus affecting N_2O generation. Wang et al. (2020) indicated that upon adding straw, the abundances of *nirK* and *nirS* decreased, and the abundance of the *nosZ* gene increased, thus reducing N_2O emissions. Therefore, it is urgent to clarify the effect of straw return on nitrification- and denitrification-mediated N_2O emissions and the associated microbial communities in agricultural soil.

In this study, through high-throughput sequencing, we investigated the N_2O emissions and the response mechanism of nitrification and denitrification in intensively farmed land following 2 years of biochar addition and straw return. The objectives of our study were (1) to compare the effects of biochar, straw, and a combination of biochar and straw on N_2O emissions; (2) to clarify the effects of biochar and straw on the community composition of nitrifiers and denitrifiers; and (3) to explore the response mechanism of N_2O emissions to biochar and straw amendment in intensively farmed land in northern China.

Material and methods

Location description

The field experiment was located at the ecology and sustainability research station (36° 58′ N, 117° 59′ E, 17 m a.s.l) in Huantai County, Shandong Province, China. This site has a warm, temperate, continental monsoon climate with a mean annual temperature of 11.8–12.9 °C. The mean annual precipitation is approximately 550 mm, with the majority (70%) falling from June through September (the summer maize growing season). Winter wheat/summer maize rotation is the most important cropping system in this region. Winter wheat is usually sown in early October and harvested in early June of the next year, and maize is sown in early June and matures in late September. The fluvo-aquic soil is a sandy loam with a bulk density of 1.50 g cm^{-3}. The soil pH was 8.1, the soil organic carbon (SOC) content was 10.8 g kg^{-1}, the total N

content was 0.7 g kg^{-1}, the available N content was 48.0 mg kg^{-1}, the available P content was 11.5 mg kg^{-1}, and the available K content was 210.0 mg kg^{-1}.

Biochar characterization

The biochar was purchased from Shandong Mingchen Sanitation Equipment Co., Ltd. It was made from cotton straw by slow pyrolysis at 800 °C under an oxygen-free atmosphere. The density of the biochar was 0.30 g cm^{-3}, the pH value was 8.6, the C content was 68.7%, the N content was 0.33%, the available P was 0.12%, the available K was 1.60%, the ash content was 25.4%, and the specific surface area and pore volume of it were 12.5 m^2 g^{-1} and 1.9 mL g^{-1}.

Field experiment

The field experiment started in the winter wheat season (October 2017). Four treatments with three replications were established, and each plot (6 m × 6 m) was randomly distributed. The four treatments were as follows: (1) CK (only chemical fertilizer); (2) C (biochar, 9.0 t ha^{-1} $year^{-1}$); (3) SR (all straw returned to the field); and (4) C+SR (biochar plus straw). All treatments received the same amounts of N (200 kg ha^{-1} $year^{-1}$), P_2O_5 (55.0 kg ha^{-1} $year^{-1}$), and K_2O (40.0 kg ha^{-1} $year^{-1}$), for which the N fertilizer was urea, the P fertilizer was superphosphate, and the K fertilizer was potassium sulfate. The biochar and fertilizer were distributed equally between the wheat and maize. Half of the urea and all of the P and K fertilizers were uniformly applied as the base fertilizer, and the other half of the urea was used as topdressing in the wheat and maize seasons. Biochar and the base fertilizers were broadcasted onto the soil surface by hand and then immediately incorporated into the soil by rotary tillage. In the SR treatment, all the wheat straw or maize straw produced in the plot was mechanically chopped into 5–10 cm pieces and incorporated into the soil by rotary tillage in the following growth season. The supplemental urea fertilizer was applied as topdressing and washed into the soil with flood irrigation to prevent NH_3 volatilization.

N_2O flux measurements

Field samples were collected from June 24 to September 26, 2019, throughout the maize season due to the high N_2O emissions in this period. N_2O flux was measured by the static chamber gas chromatograph (GC) method once a week, as well as once a day for approximately one week after fertilization, irrigation or rainfall. A total of 17 samples were collected during the maize growing season. The sampling chamber consisted of a top chamber (0.4 m width × 0.4 m length × 0.5 m height) and a stainless steel square base. An additional chamber (0.5 or 1.0 m height) was added to the top if the height of the crops exceeded 50 cm. Before sampling, a 15 cm deep base was placed on the soil surface and kept for sampling in each plot. The upper edge of the base had a groove (5 cm deep) for water filling to seal the edge of the chamber with the horizontal surface. Distilled water was injected into the groove to seal the entire system during sampling. The top chamber was equipped with a circulating fan to ensure gas uniformity and was wrapped with a layer of sponge and aluminum foil to reduce the influence of solar radiation on the inner air temperature.

Gas sampling was carried out from 9:00 to 11:00 am; four gas samples were extracted from the top of the chamber at 0, 8, 16, and 24 min with a three-way stopcock using a 60 mL airtight syringe after enclosure, and 60 mL gas was pumped into pre-evacuated gas bags for analysis. During gas sampling, the atmospheric temperature, soil temperature, and internal air temperature of the chamber were simultaneously measured.

The N_2O concentration was analyzed on the sampling day by an Agilent 7890A gas chromatograph (Agilent, USA, 2007) equipped with an electron capture detector (ECD). The carrier gas was argon-methane (5%), the flow rate was 40 mL min^{-1}, and the column temperature was 40 °C. Compressed air was used as a reference gas with a N_2O concentration of 313 ppbv. N_2O concentration was calculated by comparing the peak areas of the samples with that of the reference gas.

N_2O flux was calculated using the following equation:

$$F = \rho \times h \times \frac{dc}{dt} \times \frac{273}{273 + T} \quad (1)$$

where F is the N_2O flux (μg m^{-2} h^{-1}); ρ is the density of N_2O under standard conditions, which is 1.977 g L^{-1}; h is the height of the sampling chamber (m); dc/dt is the rate of N_2O emission (μg h^{-1}); and T is the average temperature in the chamber (°C).

The cumulative N_2O emissions (E_{N2O}, kg ha^{-1}) were calculated as follows:

$$M = \Sigma \frac{F_{i+1} + F_i}{2} \times \left(t_{i+1} - t_i\right) \times 24 \quad (2)$$

where M is the cumulative N_2O emissions (μg m^{-2}); F is the N_2O emission flux (μg m^{-2} h^{-1}); i is the number of samples; and $(t_{i+1} - t_i)$ is the number of days between samplings.

Soil sampling and analysis

Soil samples were collected synchronously with gas sampling at a depth of 0–20 cm in the soil layer with an auger. Five soil samples were taken randomly in each plot and mixed thoroughly. After removing all of the plant roots by sieving (sieve mesh 2 mm), the mixed soil was divided into three subsamples. One subsample was immediately frozen in liquid

nitrogen and kept at − 80 °C for DNA and microbial community composition analysis. The other subsample was maintained fresh at 4 °C for the determination of SWC, NH_4^+-N and NO_3^--N concentrations, as well as soil microbial biomass carbon and nitrogen (MBC and MBN). The SWC was determined by the oven-drying method at 105 °C for 24 h. The soil pH was determined in a soil-water suspension (1:2.5 w/v) using a PH100 ExStick pH meter (Extech Instruments Corp., Nashua, NH, USA). The soil NH_4^+-N and NO_3^--N were analyzed by a flow injection autoanalyzer (Braun and Lübbe, Norderstedt, Germany) after extraction of the soil samples with 0.01 M $CaCl_2$ solution. The soil MBC and MBN were determined using the chloroform fumigation method and were quantified by a multi N/C 2100/2100S TOC Analyzer (Jena, Germany).

DNA extraction and sequencing

DNA extraction was performed from soil samples taken on August 21, 2019, during the key period of nitrogen nutrient regulation, by which time the microorganisms had had the opportunity to adapt to the changes in environmental conditions caused by the application of biochar and straw return (Shi et al. 2019). The total DNA from 1 g of fresh soil was extracted directly from membranes using a Power Soil DNA Isolation Kit (Axygen, USA) according to the manufacturer's instructions. The quality of the DNA was examined by 1.0% agarose gel electrophoresis, and the DNA was stored at − 20 °C for further use. The electrophoresis bands were bright, and the samples were not luminous, which met the requirements for PCR amplification.

The V3-4 hypervariable region of bacterial 16S rRNA gene were amplified with the primers 338F (ACTCCTACGGGAGG CAGCAG) and 806R (GGACTACHVGGGTWTCTAAT) (Munyaka et al. 2015). High-throughput sequencing of the AOA-*amoA*, AOB-*amoA*, *nirK*, *nirS*, and *nosZ* genes were performed for each soil sample, 10-digit barcode sequence was added to the 5′ end of the forward and reverse primers (provided by Allwegene Company, Beijing). The PCR was carried out on a Mastercycler Gradient (Eppendorf, Germany) using 25 μl reaction volumes, containing 12.5 μl KAPA 2G Robust Hot Start Ready Mix, 1 μl forward primer (5 μM), 1 μl reverse primer (5 μM), 5 μl DNA (total template quantity is 30 ng), and 5.5 μl H_2O. Cycling parameters were 95 °C for 5 min, followed by 28 cycles of 95 °C for 45 s, 55 °C for 50 s, and 72 °C for 45 s with a final extension at 72 °C for 10 min. Three PCR products per sample were pooled to mitigate reaction-level PCR biases. The PCR products were purified using a QIAquick Gel Extraction Kit (QIAGEN, Germany), quantified using real-time PCR, the primers and reaction conditions for PCR amplification are shown in Table 1. The PCR products were separated by 2% agarose gel electrophoresis, and the desired fragment was recovered using an AxyPrep DNA Gel Extraction Kit (Axygen, USA). Sequencing was performed using an Illumina MiSeq PE300 sequencing platform (Illumina, Inc., CA, USA) according to the manufacturer's instructions.

Raw sequencing data were processed by Beijing Allwegene Technology Inc. (Beijing, China) using the QIIME pipeline tools (Wood et al. 2015; Chen et al. 2016). The raw data were first screened and sequences were removed from consideration if they were shorter than 200 bp, had a low-quality score (≤ 20), contained ambiguous bases or did not exactly match to primer sequences and barcode tags. Qualified reads were separated using the sample-specific barcode sequences and trimmed with Illumina Analysis Pipeline Version 2.6. And then, the dataset was analyzed using QIIME. The sequences were clustered into operational taxonomic units (OTUs) at a similarity level of 97% (Munyaka et al. 2015), to generate rarefaction curves and to calculate the richness and diversity indices. The Ribosomal Database Project (RDP) Classifier tool was used to classify all sequences into different taxonomic groups (Edgar 2013).

To examine the similarity between different samples, clustering analyses and PCA were used based on the OTU information from each sample using R (Cole et al. 2014). The evolution distances between microbial communities from each sample were calculated using the tayc coefficient and represented as an unweighted pair group method with arithmetic mean (UPGMA) clustering tree describing the dissimilarity between multiple samples (Wang et al. 2012).

Statistical analysis

The N_2O flux and soil physicochemical property data were processed by Microsoft Office Excel 2010 (Microsoft Corporation, USA), and figures were generated by Origin Pro 8.5 (Origin Lab, USA). The least significant difference (LSD) method of one-way analysis of variance (ANOVA) was used to test the differences in N_2O flux, soil physicochemical properties, and the relative abundances of nitrifiers and denitrifiers. Pearson's correlation analysis was used to test the correlation between N_2O flux and soil properties and the abundances of nitrifiers and denitrifiers at the 0.05 level. QIIME1 (v1.8.0) software was used for alpha diversity index analysis. Redundancy discrimination analysis (RDA) was used to explore the correlations between the nitrifiers, denitrifiers and the soil physicochemical properties in R (R, Version 3.6.2).

Results

N_2O flux

The variation in N_2O flux in each treatment was similar in the maize growing season (Fig. 1a). The N_2O flux decreased by

Table 1 Gene primers and reaction conditions used for PCR amplification of the *amoA*, *nirK*, *nirS*, and *nosZ* genes

Target gene	Primers	Sequences (5′–3′)	Product length/bp	Reaction conditions
AOA-*amoA*	Arch-amoA26F	GACT ACATMTTCTAYACWGAYTGGGC	415	95 °C for 5 min; 35 cycles of 95 °C for 45 s, 53 °C for 50 s, 72 °C for 45 s; 72 °C for 10 min (Park et al. 2008)
	Arch-amoA417R	GGKGTCATRTATGGWGGYAAYGTTGG		
AOB-*amoA*	amoA-1F	GGGGTTTCTACTGGTGGT	500	95 °C for 5 min; 35 cycles of 95 °C for 45 s, 50 °C for 50 s, 72 °C for 45 s; 72 °C for 10 min (Park et al. 2008)
	amoA-2R	CCCCTCKGSAAAGCCTTCTTC		
nirK	FlaCu	ATCATGGTSCTGCCGCG	473	95 °C for 5 min; 30 cycles of 95 °C for 45 s, 63 °C for 1 min, 72 °C for 1 min; 72 °C for 10 min (Henry et al. 2004)
	R3Cua	GCCTCGATCAGRTTGTGGTT		
nirS	cd3aF	GTSAACGTSAAGGARACSGG	425	95 °C for 5 min; 30 cycles of 95 °C for 45 s, 57 °C for 1 min, 72 °C for 1 min; 72 °C for 10 min (Throbäck et al. 2004)
	R3cd	GASTTCGGRTGSGTCTTGA		
nosZ	*nosZ*-F	CGYTGTTCMTCGACAGCCAG	300	95 °C for 5 min; 35 cycles of 95 °C for 1 min, 63 °C for 50 min, 72 °C for 45 s; 72 °C for 10 min (Scala and Kerkhof 1998)
	nosZ-R	CGSACCTTSTTGCCSTYGCG		

40.4% in the C treatment, while it increased by 29.0% in the C+SR treatment compared with the CK treatment from June 24 to July 5 ($P < 0.05$). Then, the N_2O flux increased slightly due to rainfall on July 12 and then decreased to a low level. The maximum N_2O emission peak appeared on August 3 after topdressing and irrigation. From July 28 to August 14, the N_2O flux decreased by 11.6% in the C treatment and increased by 53.5% and 123.9% in the SR and C+SR treatments compared with CK, respectively ($P < 0.05$). After August 14, there was no significant difference in N_2O flux among treatments, and N_2O flux remained at a low level.

The cumulative N_2O emissions were between 1.46 and 2.83 kg hm^{-2} in each treatment during the maize growing season (Fig. 1b). The cumulative N_2O emissions in the C treatment decreased by 30.3% compared with the CK treatment and increased by 13.2% and 37.0% in the SR and C+SR treatments, respectively ($P < 0.05$).

Soil physical and chemical properties

The variations in soil temperature at the 5 cm soil layer in each treatment during the maize season are shown in Fig. 2a. There was no significant difference among treatments. Two peaks of soil temperature appeared on July 5 (36.6 °C) and July 22 (35.1 °C). The average soil temperature of each treatment during the maize season was 28.8 °C. The soil pH ranged from 7.4 to 7.9 in all the treatments (Fig. 2b). The pH of the C treatment increased by an average of 0.09 units compared with the CK treatment. The pH values of the SR and C+SR treatments significantly decreased by 0.4 and 0.2 units, respectively. The variations in SWC of all treatments were similar in the same period, and they were not significantly influenced by biochar, straw or combined biochar and straw (Fig. 2c). The average SWC was between 17.6% and 19.2% in all treatments, with the highest value of 26.6% in the SR treatment.

The soil NH_4^+-N content did not significantly change in the C, SR and C+SR treatments compared with the CK treatment (Fig. 2d). However, it decreased by 15.6% in the SR treatment and by 6.9% in the C+SR treatment on August 4 ($P < 0.05$). The soil NO_3^--N content of the C, SR, and C+SR treatments were higher than that of CK and increased by 46.8%, 33.7%, and 21.5% on average, respectively ($P < 0.05$, Fig. 2e). On August 4th, the soil NO_3^--N content reached the highest peak during the growing season; the NO_3^--N content of the C+SR

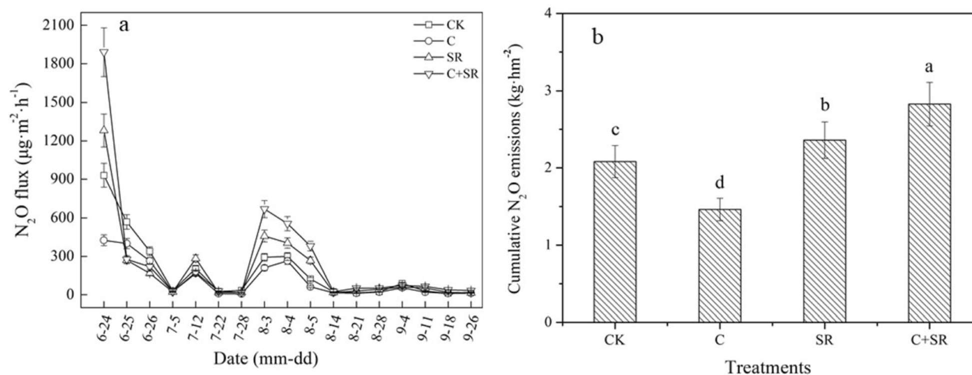

Fig. 1 N_2O flux (a) and the cumulative N_2O emissions (b) of the CK, C, SR, and C+SR treatments during the maize growing season. Data points and error bars represent means and standard errors (n = 3), respectively. Abbreviations: CK, control treatment; C, biochar treatment; SR, straw return treatment; C+SR, biochar plus straw treatment

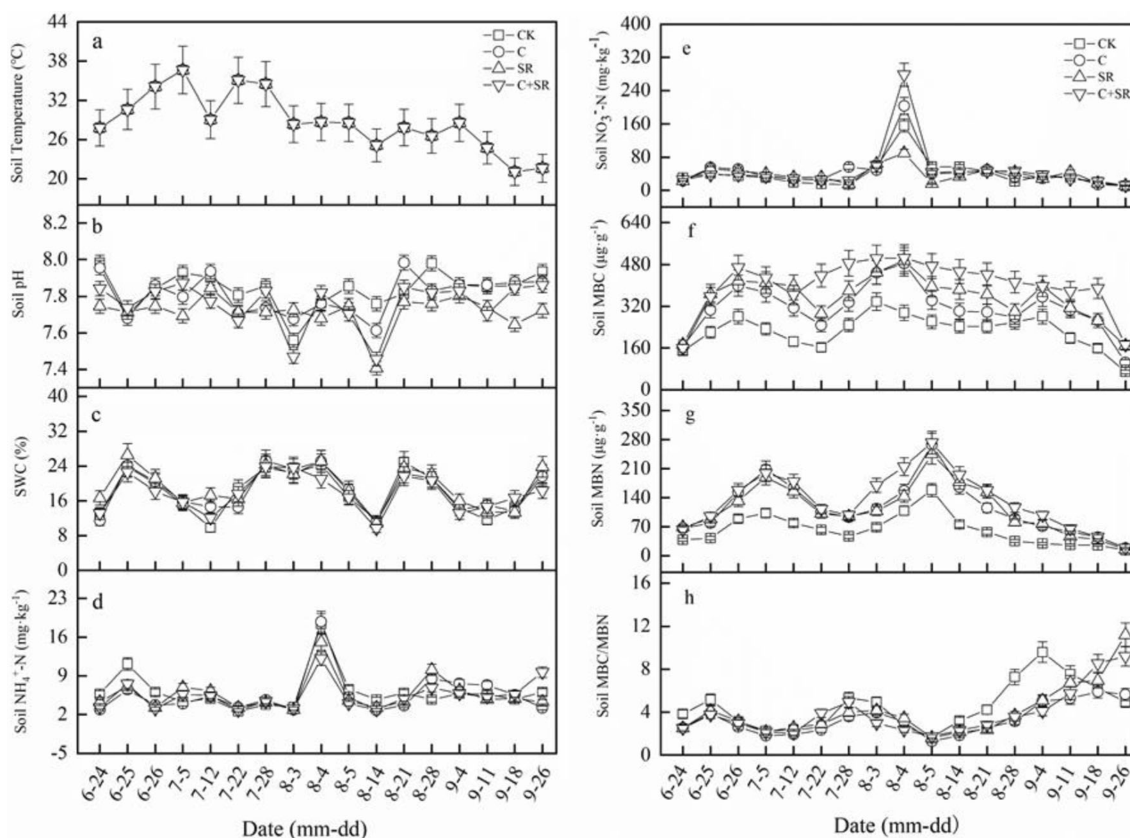

Fig. 2 Soil temperature (a), pH (b), SWC (c), NH_4^+-N (d), NO_3^--N (e), MBC (f), MBN (g), and MBC/MBN(h) of the CK, C, SR, and C+SR treatments during the maize growing season. Data points and error bars represent means and standard errors (n = 3), respectively. Abbreviations: CK, control treatment; C, biochar treatment; SR, straw return treatment; C+SR, biochar plus straw treatment

treatment was significantly higher than that of the other treatments, and NO_3^--N was significantly decreased in the SR treatment compared with the CK treatment.

Soil microbial biomass carbon and nitrogen

The variations in MBC content in the CK, C, SR, and C+SR treatments were basically the same (Fig. 2f). The MBC content increased significantly and reached the peak value on August 4 after fertilization. The MBC contents in each treatment were C+SR (504.2 μg g^{-1}) > SR (495.2 μg g^{-1}) > C (485.1 μg g^{-1}) > CK (295.1 μg g^{-1}), and the MBC contents of the C, SR, and C+SR treatments increased by 64.39%, 67.81% and 70.86%, respectively, compared with CK (P < 0.05). During the maize growing season, the average MBC content of each treatment was C+SR (401.5 μg g^{-1}) > SR (351.7 μg g^{-1}) > C (313.5 μg g^{-1}) > CK (224.9 μg g^{-1}), corresponding to increases of 39.4%, 56.3%, 78.5%, and 0% compared with the CK treatment, respectively (P < 0.05).

The variations in MBN content in each treatment present an “M”-shaped bimodal curve (Fig. 2g). The MBN contents reached peak values on August 5, in the order C+SR (272.7 μg g^{-1}) > C (267.8 μg g^{-1}) > SR (245.3 μg g^{-1}) > CK (158.3 μg g^{-1}). The MBN contents of the C, SR, and C+SR treatments increased by 69.1%, 55.0%, and 72.2%, respectively, compared with the CK treatment (P < 0.05). The average MBN contents of each treatment followed the order C+SR (132.9 μg g^{-1}) > C (115.7 μg g^{-1}) > SR (112.9 μg g^{-1}) > CK (62.4 μg g^{-1}). The average MBN content of the C, SR, and C+SR treatments increased by 85.4%, 81.1%, and 113.1%, respectively, compared with the CK treatment (P < 0.05).

The variations in MBC/MBN in each treatment were basically the same before August 5 (Fig. 2h). The MBC/MBN of the SR and C+SR treatments decreased by 3.8% and 5.9%, respectively, and that of the C treatment increased by 18.78% compared with the CK treatment (P < 0.05).

Copy numbers of nitrifying and denitrifying genes

The copy numbers of AOA (2.8 × 10^6–5.0 × 10^6 g^{-1} dry soil) were significantly higher than those of AOB (6.2 × 10^5–9.2 × 10^5 g^{-1} dry soil) (P < 0.05, Fig. 3). Compared with the CK treatment, the copy number of AOA in the C treatment decreased by 40.6% (P < 0.05), and that of AOB increased by 33.9% (P < 0.05). There was no significant difference with respect to the CK treatment in either AOA or AOB numbers for the SR and C+SR treatments.

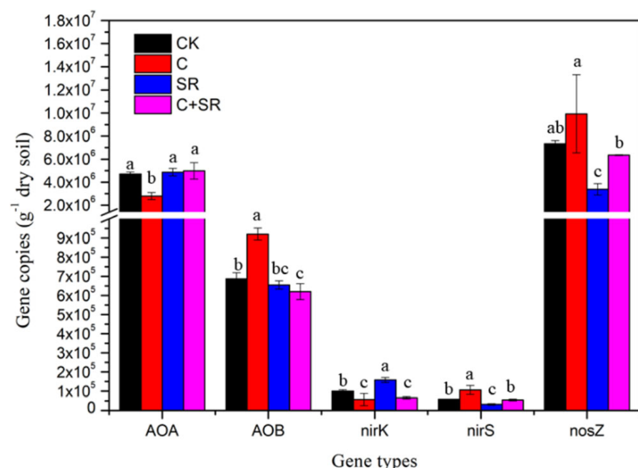

Fig. 3 Copy numbers of nitrification and denitrification functional genes in the CK, C, SR, and C+SR treatments during the experimental period. Data points and error bars represent means and standard errors (n = 3), respectively. Abbreviations: CK, control treatment; C, biochar treatment; SR, straw return treatment; C+SR, biochar plus straw treatment

Regarding denitrifier genes, the copy number of the *nirK* gene decreased by 43.1% in the C treatment, increased by 57.2% in the SR treatment, and decreased by 35.0% in the C+SR treatment ($P < 0.05$). The copy number of the *nirS* gene increased by 42.4% and 31.2% in the C and SR treatments, respectively ($P < 0.05$). The copy number of the *nosZ* gene increased by 35.1% in the C treatment but decreased by 53.9% and 13.6% in the SR and C+SR treatments, respectively ($P < 0.05$).

Correlation analysis of N_2O emissions with soil factors and functional genes

The correlation analysis between N_2O flux and the soil physicochemical properties and the copy number of nitrifiers and denitrifiers are shown in Table 2. N_2O flux was significantly positively correlated with NO_3^--N and negatively correlated with MBC in the CK and C treatments ($P < 0.05$). While N_2O flux was significantly positively correlated with NH_4^+-N in the SR and C+SR treatments ($P < 0.05$). There was no significant correlation between N_2O flux and SWC, pH, MBN, and soil temperature.

N_2O flux was negatively correlated with *nosZ* gene copies in the CK treatment and negatively correlated with AOB, *nirS*, and *nosZ* gene copies in the C treatment ($P < 0.05$). N_2O flux was significantly positively correlated with the *nirK* gene in the SR treatment and positively correlated with the *nosZ* in the C+SR treatment ($P < 0.05$). N_2O flux was significantly negatively correlated with AOB copies ($P < 0.05$). Overall, N_2O flux was negatively correlated with AOB, *nirS*, and *nosZ* gene copies but positively correlated with AOA and *nirK* gene copies.

The correlations between soil environmental factors and the copy numbers of nitrifiers and denitrifiers in each treatment are shown in Table 3. Soil pH was significantly negatively correlated with SWC ($P < 0.01$), MBN content and soil temperature ($P < 0.05$). SWC was significantly positively correlated with soil temperature ($P < 0.01$) and positively correlated with NO_3^--N and NH_4^+-N content ($P < 0.05$). NO_3^--N content was significantly positively correlated with NH_4^+-N and MBC content ($P < 0.05$). NH_4^+-N content was significantly positively correlated with MBN content ($P < 0.05$). There was a significant positive correlation between MBC and MBN content ($P < 0.01$).

There was no significant correlation between gene copy numbers and soil environmental factors in the experiment. There were significant negative correlations between AOA, AOB, and *nirS* copies ($P < 0.01$), but these factors were significantly negatively correlated with *nosZ* ($P < 0.05$). The number of *nirK* copies was negatively correlated with *nosZ* and *nirS* copies ($P < 0.01$). There was a significant positive correlation between *nirS* and *nosZ* copies ($P < 0.05$).

Alpha diversity of AOA, AOB, nirK, nirS, and nosZ

Biochar addition significantly increased the OTUs and Chao1 index of AOA by an average of 62.1% and 73.3%, respectively ($P < 0.05$, Table 4). Biochar addition and straw return

Table 2 Correlation analysis between N_2O flux and the soil physicochemical property and the copy number of nitrifiers and denitrifiers in the CK, C, SR, and C+SR treatments

Treatment	SWC/ %	Soil pH	NO_3^--N/ mg kg^{-1}	NH_4^+-N/ mg kg^{-1}	MBC/μg g^{-1}	MBN/ μg g^{-1}	T_{5cm}/ °C	AOA	AOB	*nirK*	*nirS*	*nosZ*
CK	− 0.191	− 0.065	0.567**	0.364	− 0.627**	− 0.318	− 0.113	0.241	−0.362	0.37	0.212	− 0.542*
C	− 0.411	0.208	0.574**	0.282	− 0.535**	− 0.240	0.275	0.382	−0.591*	0.423	− 0.601*	− 0.575*
SR	− 0.435	0.233	− 0.463	0.617**	0.323	0.192	0.675	0.183	−0.374	0.614*	− 0.328	− 0.667**
C+SR	0.260	0.371	− 0.479	0.632**	− 0.382	− 0.535	0.143	0.087	−0.542*	0.339	− 0.036	− 0.168

* and ** represent statistical significance at the 0.01 and 0.05 levels

Table 3 Correlations between soil environmental factors and copy numbers of nitrifiers and denitrifiers in the CK, C, SR, and C+SR treatments

	pH	SWC	NO_3^--N	NH_4^+-N	MBC	MBN	T_{5cm}	AOA	AOB	*nirK*	*nirS*	*nosZ*
pH	1											
SWC	− 0.782**	1										
NO_3^--N	− 0.040	0.664*	1									
NH_4^+-N	− 0.359	0.545*	0.690*	1								
MBC	− 0.448	0.224	0.536*	0.264	1							
MBN	− 0.705*	0.528	0.428	0.555*	0.796**	1						
T_{5cm}	− 0.541*	0.750**	0.290	0.010	0.013	0.084	1					
AOA	− 0.183	0.041	0.189	− 0.121	0.428	0.136	− 0.241	1				
AOB	0.237	0.033	− 0.005	− 0.154	− 0.446	− 0.019	− 0.160	− 0.830**	1			
nirK	− 0.196	0.109	0.102	− 0.040	− 0.035	0.037	− 0.209	0.540	− 0.379	1		
nirS	0.348	− 0.008	0.086	− 0.304	− 0.289	− 0.010	− 0.171	− 0.743**	− 0.887**	− 0.589*	1	
nosZ	0.229	− 0.031	− 0.205	0.066	− 0.360	− 0.218	0.144	− 0.691*	− 0.622*	− 0.829**	0.616*	1

* and ** represent statistical significance at the 0.01 and 0.05 levels

increased both the OTUs and Chao1 index of AOB ($P < 0.05$); OTUs increased by 91.8 and 90.0% in the C and SR treatments, respectively, and the Chao1 index increased by 99.6% and 95.2%. Straw return significantly increased the OTUs and Chao1 index of the *nirK* gene compared with CK, with average increases of 58.5% and 32.5%, respectively ($P < 0.05$). The C+SR treatment significantly decreased the OTUs and Chao1 index of the *nosZ* gene compared with CK ($P < 0.05$), with average decreases of 18.1% and 18.4%, respectively. However, there was no significant difference among the OTUs and Chao1 index of the *nirS* gene in each treatment.

Community compositions of nitrifiers and denitrifiers

The community compositions of nitrifiers and denitrifiers at the genus level are shown in Fig. 4. The relative abundance of unidentified genera of AOA reached 99% in the CK, C, SR, and C+SR treatments, so AOA are not further discussed. Among the AOB sequences, *Nitrosospira* and *Nitrosovibrio* were the dominant genera, accounting for more than 74% of the total bacterial community, and *Nitrosospira* was the dominant genus in each treatment. The relative abundance of *Nitrosospira* exceeded 60% in all treatments, with the order of C+SR > CK > C > SR. Therefore, the application of biochar and straw return had a significant effect on the community composition of AOB but not AOA at the genus level.

Agrobacterium, *Rhizobium*, *Bradyrhizobium*, *Ensifer*, *Chelatococcus*, *Mesorhizobium*, and *Xanthomonas* were the dominant genera associated with the *nirK* gene, with relative abundances of more than 30%. The relative abundance of *Agrobacterium* increased significantly in the C and C+SR treatments ($P < 0.05$) but decreased in the SR treatment compared with CK. The relative abundance of *Rhizobium* decreased significantly in the C treatment but increased in the SR and C+SR treatments, which was consistent with the effect of different treatments on N_2O flux. The relative abundance of *Xanthomonas* increased significantly in the SR and C+SR treatments ($P < 0.05$), while there were no significant changes in the C treatment.

There were 4 dominant genera with a relative abundance of ≥1% associated with the *nirS* gene: *Rhodanobacter*, *Magnetospirillum*, *Azospirillum*, and *Sulfurifustis*. Compared with CK, the genus *Rhodanobacter* increased significantly in the SR and C+SR treatments ($P < 0.05$). The relative abundances of the genus *Azospirillum* in the C and C+SR treatments were significantly higher than that in the SR treatment ($P < 0.05$).

There were 9 dominant genera with a relative abundance of ≥1% associated with the *nosZ* gene: *Azospirillum*, *Microvirga*, *Chelatococcus*, *Ramibacter*, *Mesorhizobium*, *Paracoccus*, *Sinorhizobium*, *Achromobacter*, and *Pseudomonas*. The relative abundance of *Azospirillum*, the dominant genus in each treatment, was more than 10%, and the order was SR > C > CK > C. The relative abundance of *Sinorhizobium* was significantly increased in the C treatment, while the relative abundance of *Pseudomonas* was significantly increased in the SR treatment compared with CK ($P < 0.05$).

RDA analysis of soil denitrifying bacteria and environmental factors

The RDA analysis results for soil denitrifying bacteria and environmental factors are shown in Fig. 5. Because the number of dominant species of AOA and AOB was less than the number of environmental factors, AOA and AOB are not further discussed. The red arrows represent soil environmental factors, such as MBC, MBN, NH_4^+-N, NO_3^--N, pH, and SWC. The blue arrows represent the dominant genera

Table 4 OTUs and Chao1 index analysis of species harboring AOA, AOB, *nirK*, *nirS*, and *nosZ* genes in the CK, C, SR, and C+SR treatments

Treatment	AOA		AOB		*nirK*		*nirS*		*nosZ*	
	OTUs	Chao1	OTUs	Chao1	OTUs	Chao1	OTUs	Chao1	OTUs	Chao1
CK	154.5 ± 2.1b	159.0 ± 0.8b	256.0 ± 86.3b	268.7 ± 90.8b	1209.3 ± 33.3b	1631.5 ± 418.4b	647.3 ± 45.1ab	738.5 ± 38.8ab	3449.5 ± 44.5a	4567.6 ± 280.8a
C	250.5 ± 48.8a	275.5 ± 49.0a	491.0 ± 141.4a	536.5 ± 127.8a	1344.7 ± 260.6b	1732.7 ± 184.3ab	579.0 ± 56.6b	643.5 ± 43.0b	3068.5 ± 92.6ab	4255.4 ± 197.0ab
SR	205.5 ± 72.8ab	235.9 ± 83.2ab	486.3 ± 81.1a	524.4 ± 81.1a	1917.0 ± 278.6a	2162.1 ± 193.6a	660.0 ± 16.1ab	725.5 ± 36.2ab	2886.7 ± 245.9b	4009.6 ± 137.2ab
C+SR	160.7 ± 11.1b	187.5 ± 23.1b	291.0 ± 39.6b	321.3 ± 43.1b	715.0 ± 68.2c	943.5 ± 109.3c	736.0 ± 86.3a	725.5 ± 36.3a	2824 ± 516.0b	3726.4 ± 587.7b

Different lowercase letters indicate significant differences in a column ($P < 0.05$)

associated with denitrification genes. There were positive correlations between MBC, MBN, and NO_3^--N content. NH_4^+-N content was negatively correlated with MBC, MBN, and NO_3^--N content. Soil pH was positively correlated with SWC. With respect to the *nirK* gene, the genera *Rhizobium*, *Xanthomonas*, and *Mesorhizobium* were positively correlated with MBC, MBN, and NO_3^--N and negatively correlated with NH_4^+-N. The genus *Agrobacterium* was positively correlated with pH and SWC. The genera *Chelatococcus* and *Mesorhizobium* were positively correlated with NH_4^+-N. With respect to the *nirS* gene, the genera *Azospirillum* and *Sulfurifustis* were positively correlated with MBN and NO_3^--N and negatively correlated with NH_4^+-N, while *Rhodonobacter* and *Magnetospirillum* had a negative correlation with pH and SWC. With respect to the *nosZ* gene, the genera *Pseudomonas* and *Sinorhizobium* were positively correlated with pH and SWC and negatively correlated with NO_3^--N.

Discussion

Effects of biochar on N_2O flux

During the whole maize growing season, the addition of biochar significantly reduced the cumulative N_2O emissions by 30.3% (Fig. 1b). Previous studies have also confirmed this conclusion (Harter et al. 2016; Krause et al. 2018; Zaw et al. 2018; Ribas et al. 2019; Song et al. 2019). In this study, soil NO_3^--N and MBC contents were the main factors affecting N_2O emissions in the biochar treatment (Table 2). Biochar addition decreased the soil NO_3^--N content, which was consistent with the results of Song et al. (2019). Biochar can affect the process of nitrogen conversion by adsorbing NO_3^- ions, thus reducing N_2O emissions from soil (Yi et al. 2017; He et al. 2019). The MBC content increased, which might be due to an increase in soil organic carbon content (Song et al. 2019), and promoted the carbon and nitrogen cycle (Huang et al. 2017a, b), thus affecting N_2O emissions. In addition, the addition of biochar increased soil pH (Fig. 2b) and promoted the conversion of N_2O to N_2, therefore reducing N_2O emissions (Zhou et al. 2017a, b; Yuan et al. 2019). In this study, we found that biochar had no significant effect on soil temperature and SWC and the changes in SWC were mainly due to irrigation and rainfall. Therefore, the inhibitory effect of biochar on N_2O emissions could not be attributed to changes in temperature and SWC.

AOA and AOB play important roles in N_2O production (Caranto et al. 2016). We found that with biochar addition, the AOA copy numbers decreased, and the AOB copy numbers increased (Fig. 3); additionally, N_2O flux was positively correlated with AOA abundance and negatively correlated with AOB abundance. Liu et al. (2014a, b) also found that biochar addition reduced AOA abundance, thus reducing N_2O

Fig. 4 Community compositions of AOA, AOB, *nirK*, *nirS*, and *nosZ* at the genus level in the CK, C, SR, and C+SR treatments. Abbreviations: CK, control treatment; C, biochar treatment; SR, straw return treatment; C+SR, biochar plus straw treatment

emissions caused by nitrification. Harter et al. (2016) found that adding biochar could increase AOB copy numbers in saline-alkali soil, which was consistent with our results. For the biochar treatment, N_2O flux was significantly negatively correlated with AOB abundance but not correlated with AOA abundance. There was a negative correlation between AOA and AOB, indicating that AOA and AOB may have an antagonistic relationship with N_2O emissions, and AOB was more

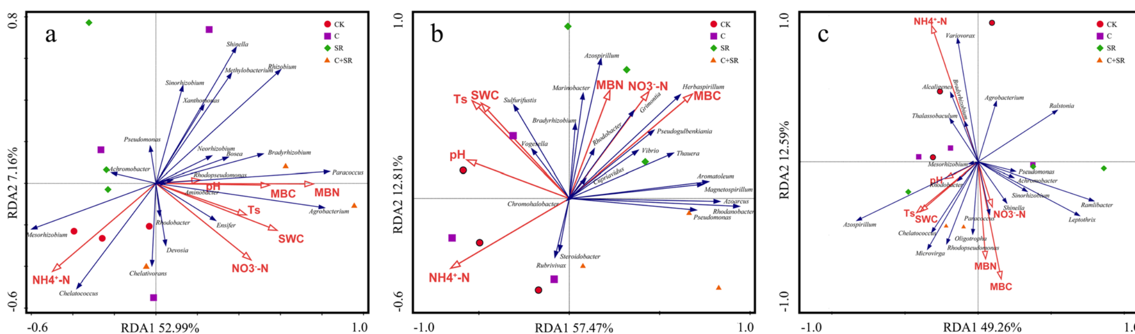

Fig. 5 RDA analysis of denitrifiers and soil environmental factors at the genus level. The different panels show *nirK* (a), *nirS* (b), and *nosZ* (c). Abbreviations: CK, control treatment; C, biochar treatment; SR, straw return treatment; C+SR, biochar plus straw treatment

sensitive to N_2O emissions than AOA (Shi et al. 2019). According to the analysis of community compositions at the genus level (Fig. 4), the AOB genus *Nitrosospira* was the main contributor to N_2O emissions.

For the denitrifiers, we found that N_2O flux was negatively correlated with *nirS* and *nosZ* copies. Biochar addition increased the *nirS* and *nosZ* numbers and accelerated the process of N_2O reduction to N_2, thus reducing N_2O emissions. Ji et al. (2020) found that biochar significantly increased the abundance of the *nosZ* gene and reduced N_2O emissions regardless of soil type, which was in line with our results. For the *nirK* gene, Van Zwieten et al. (2014) found a positive correlation between N_2O flux and *nirK* gene abundance. In this study, we found that the copy number of the *nirK* gene in the C treatment was significantly reduced, and RDA analysis revealed that the NH_4^+-N and NO_3^--N contents were the most important factors affecting the *nirK* gene (Fig. 5). After adding biochar, the NH_4^+-N content increased and the NO_3^--N content decreased in the soil, thus affecting the community richness of the dominant species of the *nirK* gene, which reduced N_2O emissions. This finding was consistent with the results of Krause et al. (2018), who also found that biochar reduced soil N_2O emissions by affecting denitrifier community composition under field conditions. In addition, the relative abundance of *Agrobacterium* (*nirK*) increased significantly in the C and C+SR treatments but decreased slightly in the SR treatment. This result might be due to the promotion effects of biochar on the *Agrobacterium* community. RDA analysis also indicated that the relative abundance of *Agrobacterium* was positively correlated with the MBC and MBN contents (Fig. 5a). The addition of biochar increased the soil organic carbon content; therefore, the promotion effects of biochar on the *Agrobacterium* community are well explained. The genus *Rhizobium* decreased significantly in the C treatment but increased in the SR and C+SR treatments, which was consistent with the effect of different treatments on N_2O flux. Shi et al. (2019) also found a decreased abundance of *Rhizobium* in biochar-amended soil. Hidalgo-García et al. (2019) proved that *Rhizobium* could assimilate nitrate as a substrate and produce N_2O through denitrification. We also found that *Rhizobium* was positively correlated with NO_3^--N in the RDA analysis (Fig. 5a), which helps to elucidate the relationship between N_2O emissions and the abundance of the *nirK* gene.

Effects of straw return on N_2O flux

Numerous studies have shown that straw return can increase N_2O emissions from farmland soils (Liu et al. 2011; Wu et al. 2018; Liu et al. 2019), and the same conclusion was reached in this study. The results showed that NH_4^+-N content was the most important environmental controlling factor of N_2O emissions (Table 2). After straw returning, the contents of NH_4^+-N and NO_3^--N in the soil increased significantly, and they were significantly higher than those in the biochar treatment (Fig. 2d, e). Higher NH_4^+-N and NO_3^--N contents can increase the chemical reaction of substrates for microbial nitrification and denitrification; therefore, soil N_2O emission was promoted (Wu et al. 2017). Mitchell et al. (2013) indicated that substrate carbon concentration was one of the main driving factors affecting N_2O emissions and that straw decomposition provided a carbon source for soil microorganisms and increased the soil MBC content (Zhou et al. 2017a, b). Exogenous organic carbon increases the soil C/N ratio, provides a suitable environment for soil denitrification, and results in a large amount of N_2O emissions (Cui et al. 2016; Huang et al. 2017a, b). In addition, straw return decreases the soil pH to a certain extent, and lower pH inhibits the activity of N_2O reductase (Liu et al. 2011), which might be another reason for the increase in N_2O emissions in straw-amended soil.

After straw return, the copy number of the AOA gene increased slightly, and that of AOB decreased slightly (Fig. 3), but straw had a small effect on the community composition of AOA and AOB (Fig. 4). For denitrifiers, straw addition increased the copy number of the *nirK* gene, and the OTUs and Chao1 index of the *nirK* gene increased significantly (Table 4), which increased the relative abundance of the genus *Rhizobium*. N_2O flux had a positive correlation with *nirK* gene abundance in this research. Wang et al. (2020) also found that with straw return in paddy soil, a decrease in *nirK* gene abundance inhibited N_2O emission. Therefore, the change in *nirK* gene abundance should be one of the main reasons for stimulating N_2O emission. Straw return also reduced the copy numbers of *nirS* and *nosZ* genes, which was consistent with the results of Liu et al. (2019). Moreover, the abundance of the *nirK* gene was significantly negatively correlated with the abundance of the *nirS* and *nosZ* genes, illustrating that there might be an antagonistic relationship among genes involved in denitrification, which jointly affected denitrification-mediated N_2O emissions. We also found that the relative abundance of *Rhizobium* increased in the SR and C+SR treatments due to the increased MBC, MBN, and NO_3^--N in the straw-amended soil (Fig. 2e). Therefore, *Rhizobium* could assimilate nitrate substrates and produce N_2O through denitrification (Hidalgo-García et al. 2019). The relative abundances of *Azospirillum* (*nosZ*) and *Rhodanobacter* (*nirS*) increased in the SR and C+SR treatments compared with CK, which was in accordance with the results of Shi et al. (2019). Previous studies have shown that the genus *Azospirillum* can drive the reduction of NO_3^- with no accumulation of NO_2^- during aerobic assimilation (Nelson and Knowles 1978). Therefore, we could infer that straw promoted N_2O emissions mainly by affecting the community composition of the *nirK*, *nirS*, and *nosZ* genes.

Effects of the combination of biochar and straw on N_2O flux

Contrary to our expectations, the combination of biochar and straw increased soil N_2O emissions by 37.0% in this experiment (Fig. 1b), resulting in a value even higher than that in the SR treatment. The results showed that the contents of NH_4^+-N and NO_3^--N in the C+SR treatment were higher than those in the C and SR treatments (Fig. 2d, e). We found that NH_4^+-N content was the most important influencing factor of N_2O emissions in the C+SR treatment (Table 2). The microorganisms participating in the N cycle obtained sufficient substrate, improving the nitrification rate, and thus produced a large amount of N_2O (Zhou et al. 2017a, b). Liu et al. (2017) also found that N_2O and NO emissions were strongly affected by soil mineral N and that total N_2O and NO emissions were significantly positively correlated with soil NH_4^+-N and NO_3^--N contents. Biochar addition and straw return provided sufficient carbon sources for soil, increased soil MBC and MBN (Fig. 2f, g), and promoted N_2O emissions caused by denitrification (Zhang et al. 2012; Liu et al. 2014a, b). In addition, the soil pH of the C+SR treatments decreased by 0.2 units compared with CK, which might be one of the reasons for the higher N_2O flux. Overall, the soil NH_4^+-N and NO_3^--N contents were the main environmental factors affecting N_2O emissions from the C+SR treatment.

In this experiment, the copy number of the AOB gene in the C+SR treatment was significantly reduced compared with that in the CK treatment, and N_2O flux was negatively correlated with AOB gene abundance (Table 2), which stimulated N_2O emission from the soil (Harter et al. 2016). The genus *Nitrosospira*, the dominant genus associated with the AOB gene, had the highest relative abundance in the C+SR treatment, which may be one of the main reasons why the AOB gene inhibited N_2O emission. However, for denitrifying genes, the copy numbers of *nirS* and *nosZ* in the C+SR treatment showed an obvious downward trend, while in the C treatment, there was an upward trend (Fig. 3), which illustrated that the influence of straw on the *nirS* and *nosZ* gene communities was greater than that of biochar. The OTUs and Chao1 index of the C+SR treatment were significantly reduced, while there was no significant difference in the *nirS* gene among treatments (Table 4), which indicated that straw mainly affected the denitrification pathway of microorganisms by reducing the abundance of the *nosZ* gene, thus promoting N_2O emission. However, the *nirK* gene copy number in the C+SR and C treatments significantly decreased, while it increased significantly in the SR treatment, and *Agrobacterium* associated with the *nirK* gene increased significantly in the C+SR treatment but had the lowest abundance in the SR treatment (Fig. 4). The results showed that the effect of biochar on the *nirK* gene was greater than that of straw. The increase in the relative abundance of *Rhizobium* might affect N_2O emissions. The abundance of *Rhizobium* was positively correlated with NO_3^--N content and negatively correlated with NH_4^+-N content in the RDA analysis (Fig. 5), which supported the conclusion that *Rhizobium* could use nitrate as a substrate for denitrification (Hidalgo-García et al. 2019). In conclusion, straw inhibited the abundance of AOB and *nosZ* genes, which stimulated N_2O emission.

Conclusions

This study indicated that biochar addition reduced soil N_2O emissions by 30.3%, while straw return and biochar plus straw increased soil N_2O emissions by 13.2% and 37.0%, respectively, during the maize season in the northern China. Biochar addition and straw return shifted the community composition of nitrifiers and denitrifiers. N_2O production was mainly reduced by promoting AOB and inhibiting *nirK* gene abundance in the biochar-amended soil, and it was mainly promoted by inhibiting AOB and *nosZ* gene abundances in the straw and biochar plus straw-amended soil. NO_3^--N, NH_4^+-N, and MBC were the main factors. Based on our results, the application of biochar to cropland is an effective option to mitigate greenhouse gases, whereas direct straw return to the field may not be an effective strategy. We suggest that future work should focus on the effect of straw return with different maturities on N_2O production and reduction.

Author contribution LXR planned and designed research; TZM and ZQW conducted experiments; TZM and KWD conducted chemical analysis; LXR and TZM conducted statistical analysis and wrote the manuscript. All authors read and approved the manuscript.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval and consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare that they have no conflict of interest.

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