Journal of Soils and Sediments (2021) 21:1089–1098
https://doi.org/10.1007/s11368-020-02811-z

SOILS, SEC 5 • SOIL AND LANDSCAPE ECOLOGY • RESEARCH ARTICLE

Effects of biochar and 3,4-dimethylpyrazole phosphate (DMPP) on soil ammonia-oxidizing bacteria and *nos*Z-N_2O reducers in the mitigation of N_2O emissions from paddy soils

Jie Li[1] · Shuai Wang[1,2] · Jiafa Luo[3] · Lili Zhang[1] · Zhijie Wu[1] · Stuart Lindsey[3]

Received: 21 May 2020 / Accepted: 18 October 2020 / Published online: 24 October 2020

Abstract

Purpose Paddy fields are an important source of nitrous oxide (N_2O) emission. The application of biochar or the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) to paddy soils have been proposed as technologies to mitigate N_2O emissions, but their mechanisms remain poorly understood.

Methods An experiment was undertaken to study the combined and individual effects of biochar and DMPP on N_2O emission from a paddy field. Changes in soil microbial community composition were investigated. Four fertilized treatments were established as follows: fertilizer only, biochar, DMPP, and biochar combined with DMPP; along with an unfertilized control.

Results The application of biochar and/or DMPP decreased N_2O emission by 18.9–39.6% compared with fertilizer only. The combination of biochar and DMPP exhibited higher efficiency at suppressing N_2O emission than biochar alone but not as effective as DMPP alone. Biochar promoted the growth of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB), while DMPP suppressed AOB and increased AOA. Applying biochar with DMPP reduced the impact of DMPP on AOB. The *nir*S-/*nir*K- denitrifiers were decreased and *nos*Z-N_2O reducers were increased by DMPP and the combination of DMPP and biochar. The abundance of the *nir*K gene was increased by biochar at the elongation and heading stages of rice development. Compared with fertilizer only, the application of biochar and/or DMPP promoted the abundance of *nos*Z genes.

Conclusion These results suggest that applying biochar and/or DMPP to rice paddy fields is a promising strategy to reduce N_2O emissions by regulating the dynamics of ammonia oxidizers and N_2O reducers.

Keywords Nitrous oxide · Biochar · Nitrification inhibitor · AOB · AOA · *nir*S-/*nir*K- denitrifiers

1 Introduction

Rice cultivation has been identified as a leading source of anthropogenic nitrous oxide (N_2O) emission, since it provides suitable conditions for the microbial N_2O-forming processes nitrification and denitrification after, sometimes excessive, inputs of chemical or organic fertilizers (Jacobson 2005; Shcherbak et al. 2014). As a greenhouse gas, N_2O is 265 times more potent than carbon dioxide (CO_2) over a time horizon of 100 years, and it is also responsible for stratospheric ozone depletion (Li et al. 2011). Agricultural N_2O emissions account for more than 60% of global anthropogenic N_2O emissions (Pachauri et al. 2014). With a projected increase in global rice consumption of around 45% by 2050 to meet the expected needs of the growing population for food (Rejesus et al. 2012), increasing rice production without increasing the release of N_2O emissions, therefore, presents a major and urgent scientific and societal challenge (Fan et al. 2019; Chen et al. 2019).

Various management strategies have been developed to mitigate N_2O emissions from rice cultivation, including water management modification (Ly et al. 2013), organic material application (Shin et al. 1996; Knoblauch et al.

Jie Li and Shuai Wang contributed to the work equally and should be regarded as co-first authors.

Responsible editor: Yuan Ge

✉ Jiafa Luo
jiafa.luo@agresearch.co.nz

[1] Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110164, China

[2] University of Chinese Academy of Sciences, Beijing 100049, China

[3] AgResearch Limited, Ruakura Research Centre, Hamilton, New Zealand

2011) and fertilizer additives. Over the past few years, amendment of croplands with biochar, produced from pyrolysis of crop straw, seems to be increasing in popularity owing to its wide availability and extensive advantageous characteristics, including improving nutrient retention and reducing indirect gaseous emissions (Steinbeiss et al. 2009; Sarkhot et al. 2012). Although some meta-analyses have indicated that biochar application could reduce N_2O emissions from soils (Cayuela et al. 2014), results are inconsistent among studies (e.g. Fan et al. 2017; He et al. 2018). For instance, some studies report a notable increase in N_2O emissions following biochar application to soils (Wells and Baggs 2014), while other studies have found that the addition of biochar decreased N_2O emissions (Yanai et al. 2007; Wang et al. 2013; Felber et al. 2014), and the reduction is suggested to be due to enhanced soil aeration and pH, reduced availability of inorganic N, and release of polycyclic aromatic hydrocarbons (PAHs). Previous studies have investigated the influence of biochar addition on nitrifier or denitrifier populations, but the results are still being debated. Thus, the underlying possible mechanisms for the impacts of biochar on N_2O emissions require further investigation, both theoretically and experimentally (Spokas et al. 2012; Yu et al. 2013).

One of the most popular agricultural nitrification inhibitors, 3,4-dimethylpyrazole phosphate (DMPP), has been proven to be useful and readily applicable for decreasing N_2O emissions directly, by suppressing the growth of ammonia-oxidizing bacteria (AOB) as well as possibly ammonia-oxidizing archaea (AOA), or indirectly by decreasing denitrifier activity and growth through reducing the presence of nitrate (NO_3^-) (Shi et al. 2017). Chen et al. (2019) conducted an incubation study and suggested that the combination of biochar and DMPP can effectively reduce N_2O emissions and the dynamic changes of AOB and *nos*Z-N_2O reduction caused by the applied biochar and/or DMPP played an important role in controlling emissions. However, to date, only limited experimental evidence exists describing the integrated functions of biochar and DMPP on N_2O emissions, and the relevant mechanisms have not been verified.

Therefore, a year-round observation of N_2O emissions from paddy soil was conducted in Northeast China. Specifically, this work (1) examined and evaluated the effects of application of biochar and/or DMPP on N_2O emissions from paddy soil and (2) systematically explored the soil microbial functional genes in the processes of soil nitrification and denitrification by regulating the effects of biochar and/or DMPP on N_2O emissions. Such information is expected to contribute to progressing the development of appropriate management practices to mitigate the climatic impacts from paddy fields.

2 Materials and methods

2.1 Experimental site

The field experiment was performed at Fangzheng Rice Science and Technology Experimental Station (45° 85′ N, 128° 82′ E) in Heilongjiang province, which has a long history of agriculture in northeastern China. The mean annual air temperature and precipitation are approximately 4.5 °C and 530 mm. The soil is classified as a Udoll (World Reference Base). The rice was irrigated with a layer of water 3 to 5 cm deep from 1 week before planting to 1 month before harvesting, which took place around 7th October.

2.2 Experimental design and field management

Five treatments were performed in triplicate in a randomized design, with an area of 7 m × 5 m (35 m^2) for each plot: CK (no fertilizer), synthetic fertilizers (NPK only), NPK with biochar, NPK with DMPP, and the combination of NPK, biochar, and DMPP. Fertilizers applied were urea (46% N), triple superphosphate (19% P), and KCl (50% K). Before transplanting, mixed fertilizer (150 kg N ha^{-1}; 75 kg P_2O ha^{-1}; and 75 kg K_2O ha^{-1}) was applied as a basal fertilizer. The inhibitor DMPP was applied at a rate of 2% of the applied urea nitrogen and the biochar was applied at 24 t ha^{-1}. They were homogenously combined with the fertilizer and then applied into the soil with the soil depth ranging from 0 to 20 cm. Fertilization was carried out 1 day before rice transplanting. Rice seedlings (cv. Meifeng 9) were transplanted into each plot evenly with 3–5 rice seeds per hole at spacings of 15 cm × 30 cm (10 days after wetting of the soil). Field management was consistent with local practices. The top 20 cm of the soil had the following properties: pH of 6.11, soil organic carbon (SOC) of 25.8 g kg^{-1}, total nitrogen (TN) of 2.04 g kg^{-1}, soil available N (AN) of 24.81 mg kg^{-1}, available P (AP) of 23.06 mg kg^{-1}, and available K (AK) of 113.02 mg kg^{-1}.

2.3 Biochar production and characterization

The biochar was produced through pyrolysis of rice straw at 600 °C utilizing a slow-pyrolysis process. The C, H, and N contents of the biochar were measured using a CN analyzer (Leco TruSpec CN, USA). The pH of the biochar was measured at a ratio of 1:2.5 (w/v) biochar-water mixture with a combination electrode. The biochar used in this study had the following properties: pH of 9.40, total nitrogen (TN) of 8.06 g kg^{-1}, and total carbon (TC) of 567.0 g kg^{-1}.

2.4 Gas sampling and auxiliary measurements

A static chamber was utilized to evaluate the in situ fluxes of N_2O from the paddy field (Wu et al. 2019). Static chambers were

constructed of transparent plexiglass and consisted of a chamber base (10 cm in height) and a chamber body (30 cm in diameter and 50 cm in height). The base was inserted into the soil and the body was attached to the base at the time of sampling. A small fan was fixed inside the top of the chamber to homogenize the headspace gases. Gas samples were collected every 2–3 days during the first 2 months, then sampling frequency was reduced to once per week for approximately 16 weeks. During midseason aeration (50 days after planting, 20th June), and the following re-flooding period, gas samples were collected daily. All the measurements were performed at a fixed time between 09:30 and 11:00 am every sampling day. Three samples were taken from the headspace of each chamber, at 0, 45 and 90 min after closing the chamber, using 50 ml syringes. The samples were transferred into tinfoil gas-collecting bags (300 ml) and taken to the laboratory for analysis of N_2O concentration using a gas chromatograph (Agilent 7890B, Delaware, USA). Soil temperature was measured continuously in the chamber with a temperature probe (10 cm soil depth), while the air temperature inside the chamber was measured using a thermocouple 20 cm from the top.

The hourly N_2O fluxes were obtained using the following formula:

$$F = \rho \times h \times {}^{\triangle}c/{}^{\triangle}t \times 273/(273 + T) \times 60$$

In which, F represents N_2O flux (μg m^{-2} h^{-1}); ρ is the density of N_2O gas under standard conditions (1.25 kg m^{-3}); ${}^{\triangle}c/{}^{\triangle}t$ is the change in N_2O concentration in the headspace gas (10^{-9} V V^{-1} min^{-1}); T is the temperature in the chamber enclosure (°C); and h is the height of the chamber (cm).

A linear interpolation method was employed to calculate the cumulative N_2O emission from each treatment over the trial period. Precipitation and air temperature data were obtained from measuring equipment at the experiment site.

2.5 Soil sampling and measurement

Soil samples were collected from each plot (0–20 cm depths) at five stages of rice plant growth (seedling, tillering, elongation, heading, and maturity). Three individual samples were taken from three random points within the plot and then mixed thoroughly. The concentrations of NH_4^+-N and NO_3^--N were measured by sieving fresh soil (< 2 mm) and extracting with 2 M KCl with a KCl-to-soil ratio of 10:1. The MgO-Devarda's alloy distillation method was used to analyze the sample extracts. Soil total N was measured by Kjeldahl digestion while available K was extracted with ammonium acetate and measured using flame photometry. Soil available phosphorus (Olsen P) was measured calorimetrically after extraction with 0.5 M $NaHCO_3$. Soil organic C was determined using the Walkley-Black wet oxidation method (Allen et al. 1987). Soil available N was analyzed through quantification of alkali-hydrolysable N in a Conway diffusion unit with Devarda's alloy in the outer chamber and boric acid-indicator solution in the inner chamber (Shen et al. 2004).

2.6 Soil microbial analysis

The extraction of total DNA contents was carried out using an Omega DNA extraction kit for soil (E.Z.N.A. Soil DNA Kit; Omega Bio-Tek Inc., GA, USA) following the instructions of the manufacturer. The AOB *amo*A, AOA *amo*A, *nir*K, and *nir*S genes in the soil samples were measured by quantitative qPCR (Roche Light Cycler® 96, Switzerland) with the primers shown in Table 1. 10 ml of 2 × SYBR green mix (Takara, Dalian, China), 0.4 ml of each primer (10 mmol), 2 μl of template DNA, and ddH_2O, formed the total volume of 20 ml. A standard curve was applied for the verification of the nitrifier genes, and the denitrifier functional genes were obtained using 10-fold serial dilutions of known copy amounts of the plasmid DNA. For these genes, the PCR amplification efficiency was between 91 and 110%, and R^2 values were greater than 0.98. All qPCR was done by performing melt-curve analysis and agarose gel electrophoresis to amplify the specific products.

Table 1 Real-time PCR primers and conditions used for amplification of nitrifier and denitrifier genes

Target gene	Primers	Annealing temperature (°C)	Reference
AOB *amoA*	*amo*A-1F: GGGGTTTCTACTGGTGGT *amo*A-2R: CCCCTCKGSAAAGCCTTCTTC	60	Rotthauwe et al. (1997)
AOA *amoA*	*Amo*AF: STAATGGTCTGGCTTAGACG *amo*AR: GCGGCCATCCATCTGTATGT	60	Francis et al. (2005)
*nir*K	*nir*K 1F: GGMATGGTKCCSTGGCA *nir*K 5R: GCCTCGATCAGRTTRTGGTT	55	Braker et al. (1998)
*nir*S	*nir*S cd3AF: GTSAACGTSAAGGARACSGG *nir*S R3cd: GASTTCGGRTGSGTCTTGA	57	Throbäck et al. (2004)
*nos*Z	*nos*Z 2F: CGCRACGGCAASAAGGTSMSSGT *Nos*Z 2R: CAKRTGCAKSGCRTGGCAGAA	60	Henry et al. (2006)

2.7 Statistical analysis

A one-way analysis of variance (ANOVA) was performed on all data. Duncan post hoc tests were used to assess the statistical significance of the biochar and DMPP effects on soil properties, N_2O emission, nitrifiers, and denitrifiers using the SPSS software package for Windows (Version 16.0, SPSS Inc., Chicago, IL, USA). The Tukey multiple-comparison test was performed to further evaluate the significance ($p < 0.05$) of the detected effects within any treatments.

Structural equation modeling (SEM) was used to test how relationships among soil properties, microbial abundance and N2O production respond to the different treatments. The analysis was conducted using AMOS 17.0 (Amos, Development Corporation, Meadville, PA, USA). The hypothetical relationships among the variables in the models were constructed based on results of correlation analyses (Table 2). Prior to the SEM analyses, the distributions of all involved variables were examined for normality. Several tests were used to assess model fit, i.e., the X^2 test, comparative fit index (CFI), adjusted goodness-of-fit index (AGFI), and root mean square error of approximation (RMSEM).

3 Results

3.1 Climatic variables and N_2O emissions

The average daily soil temperature ranged from 15 to 25 °C (10 cm depth), and the precipitation was 410 mm during the experimental period (Fig. 1). In general, N_2O emissions were primarily induced by fertilization, reaching a peak on the first day after fertilization, then decreasing sharply and returning to background levels 80 days after rice transplanting. In addition, substantial N_2O emissions were detected at the mid-season drainage stage. Throughout most of the growing season, N_2O fluxes from the DMPP and biochar treatments had similar patterns to the fertilizer-only treatment (Fig. 2). Among the fertilized treatments, daily mean N_2O fluxes followed the order: NPK > NPK + biochar > NPK + DMPP + biochar > NPK + DMPP. The N_2O emissions were always lower in the DMPP and/or biochar treatment, leading to 39.6%, 18.9%, and 32.1% lower cumulative net N_2O emissions for the NPK + DMPP, NPK + DMPP + biochar and NPK + biochar treatments, respectively, compared with fertilizer-only treatment (Fig. 2).

3.2 Nitrifier and denitrifier gene abundance

In general, the trends in the variation of AOA *amo*A and AOB *amo*A gene abundances were different during the different rice growing stages (Fig. 3). The AOA *amo*A abundance increased gradually and reached a peak at the elongation stage, while AOB *amo*A abundance peaked at the seedling stage and then decreased gradually. The detected abundance of AOB *amo*A, ranging from 4.7×10^8 to 11.3×10^8, was greater than that of AOA *amo*A (3.1×10^7 to 9.37×10^7), and both were much higher under the fertilized treatments compared with the control. Compared to NPK, the abundance of AOA was enhanced by biochar and/or DMPP during all growth stages, especially in the DMPP treatment at the elongation stage ($p < 0.05$). However, compared with the NPK treatment, the application of DMPP decreased AOB *amo*A abundance by 18–27%. The applied biochar increased AOB *amo*A abundance by 7–15% over all the rice growth stages and the applied biochar with DMPP decreased the abundance of AOB, but the reduction was lower than that of the DMPP-only treatment.

Overall, the nitrite reductase genes (*nir*K and *nir*S) in the soil showed similar trends over time, increasing initially after fertilizer application and then decreasing over the rice growth period. The abundances of *nir*K were greater than those of *nir*S. Compared with the control, the application of fertilizers significantly increased *nir*K and *nir*S growth. The application

Table 2 Correlation coefficients of soil physiochemical properties with N_2O emission fluxes and abundance of the ammonia-oxidizing archaea gene (*amo*A-AOA), ammonia-oxidizing bacteria gene (*amo*A-AOB), nitrite reductase (*nir*S and *nir*K) gene, and N_2O reductase (*nos*Z) gene

Variable factors	N_2O emission fluxes	pH (1:2.5 H_2O)	Soil organic carbon	Total nitrogen	Ammonium-N	Nitrate-N	Microbial biomass carbon
N_2O emission fluxes	–	0.247*	0.136	0.674**	0.374**	0.498**	0.012
*amo*A-AOA	− 0.378**	− 0.501**	0.015	0.566**	− 0.012	0.381**	0.406**
*amo*A-AOB	0.384**	− 0.184	0.287*	0.501**	0.489**	0.301**	0.093
*nir*S	0.174	0.013	0.012	0.354**	0.189	0.634**	0.387**
*nir*K	0.168	− 0.107	0.045	0.478**	0.513**	0.451**	0.435**
*nos*Z	− 0.339**	0.462**	0.074	0.145	0.237*	0.483**	0.243*

*$p < 0.05$; **$p < 0.01$

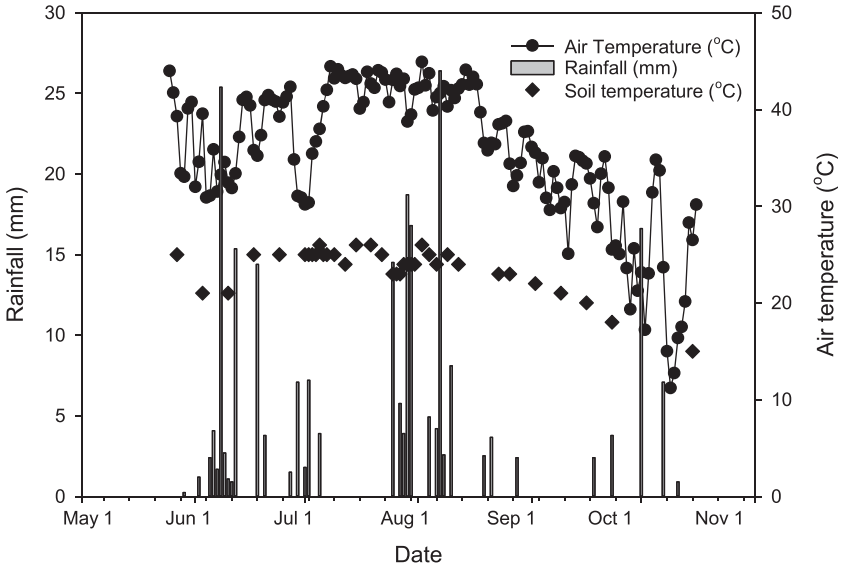

Fig. 1 Daily rainfall, soil (20 cm), and air temperature during field experiments in 2018

of biochar and/or DMPP decreased the abundance of *nir*S at the seedling, tillering, and maturity stages ($p < 0.05$). The abundance of *nir*K was reduced by DMPP and the combination of DMPP and biochar during the whole growth period but was increased by biochar only at the elongation and heading stages ($p < 0.05$).

As shown in Fig. 3, the abundance of the N_2O reductase gene (*nos*Z) increased gradually after rice transplanting (seedling stage), and the peak appeared at the elongation stage for DMPP treatments. Compared with the control, the abundance of the *nos*Z gene increased slightly in the NPK treatment, but the differences were not significant at most growth stages (except the maturity stage). However, the application of biochar and/or DMPP increased the abundance of *nos*Z genes significantly during the whole growth period compared with the fertilizer-only treatment ($p < 0.05$), with the highest values occurring in the biochar and DMPP treatment.

3.3 Relationships between N_2O flux and soil properties or microbial dynamics

The correlations between N_2O emission, soil properties, and N-cycling genes, were calculated to identify the key factors controlling N_2O emission in the paddy soil. The N_2O emission flux was positively correlated with soil ammonium content, soil nitrate content, soil pH, and total N content ($p < 0.01$).

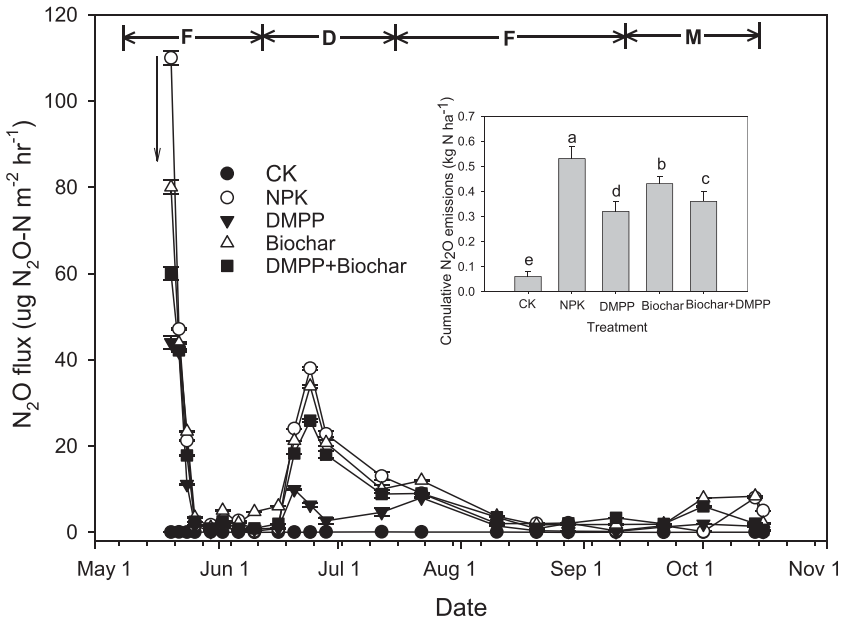

Fig. 2 Effects of different treatments on N_2O fluxes and cumulative N_2O emissions in paddy fields. Error bars represent standard error of the mean ($n = 3$). Different letters indicate significant differences ($p < 0.05$) among treatments. Arrows indicate fertilizer application. F-D-F-M, flooding–mid-season drainage–reflooding–moist irrigation

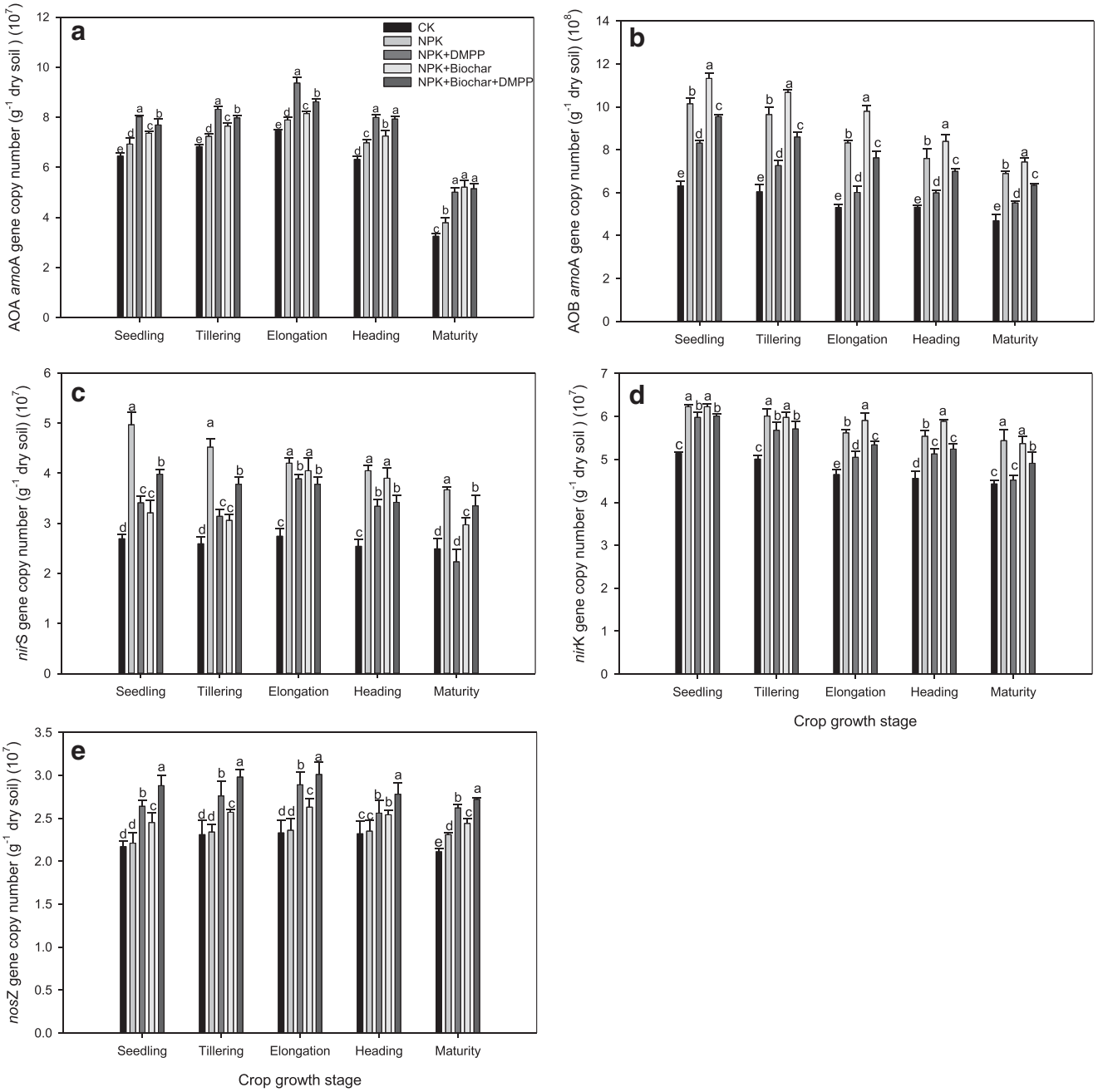

Fig. 3 Effects of different treatments on copy numbers of ammonia-oxidizing bacteria (AOB) *amo*A gene (**a**), ammonia oxidizing archaea (AOA) *amo*A gene (**b**), *nir*S gene (**c**), *nir*K gene (**d**), and nosZ gene (**e**) in paddy soil at different stages of rice growth. Error bars represent standard error of the mean ($n = 3$). Columns with the same letter are not significantly different ($p \geq 0.05$) (Note different *y*-axis unit magnitudes for (**a**) and (**b**))

Among the N_2O emission-related genes, N_2O flux exhibited a significant positively correlation with AOA ($r = 0.378$, $p < 0.01$), but a negative correlation with AOB ($r = -0.384$, $p < 0.01$). Among the denitrifiers, N_2O flux was negatively correlated with *nos*Z ($r = -0.339$, $p < 0.01$). Furthermore, based on the SEM analyses, our data also showed that NH_4^+-N, NO_3^--N, pH, AOA, AOB, *nir*S, and *nos*Z, rather than other soil properties, exerts a direct and dominant effect on N_2O, explaining 79% of variation for N_2O emissions (Fig. 4).

4 Discussion

4.1 N_2O emissions from paddy soil

Consistent with previous studies (Maljanen et al. 2003; Meng et al. 2005; Baggs et al. 2010), N_2O fluxes in all the treatments increased on the first day after fertilization and then declined quickly. The relatively high N availability from fertilizer application and the increase in soil water content from irrigation

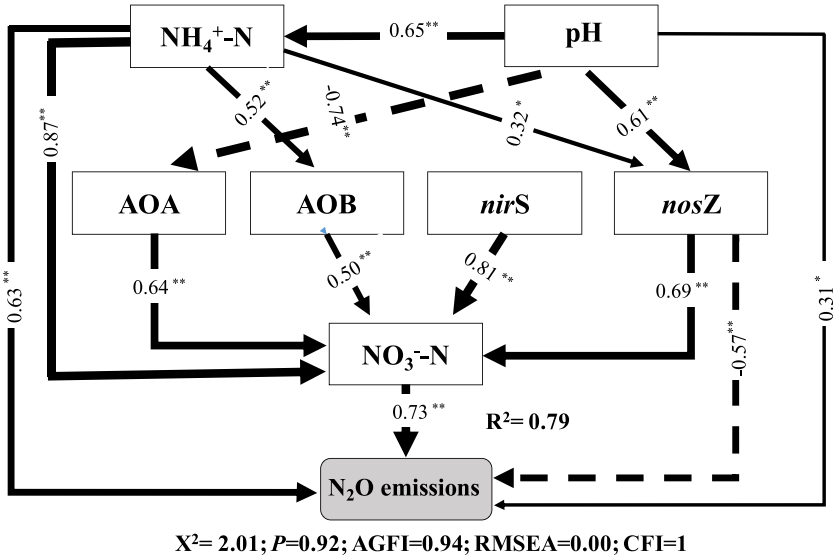

Fig. 4 The structural equation model (SEM) showing the hypothesized relationships between soil properties, microbial abundance, and N_2O production. The arrow thickness indicates the strength of the standardized path coefficients. The solid line arrows represent positive path coefficients, and the dashed lines indicate negative path coefficients. Numbers on the arrow indicate significant standardized path coefficients (*$p < 0.05$; **$p < 0.01$), proportional to the arrow width. R^2 indicates the variance of endogenous variable explained by the model. Goodness-of-fit statistics are shown underneath the modeling frames. NH_4^+, ammonium; NO_3^-, nitrate; CFI, comparative fit index; AGFI, adjusted goodness-of-fit index; RMSEM, root mean square error of approximation

could have directly led to the enhancement of nitrification and/or denitrification and the increase in N_2O emission (Gregorutti and Caviglia 2017). This is also supported by the fact that N_2O flux showed positive correlations with NH_4^+-N and NO_3^--N in the paddy soils (Table 2; Fig. 4). The abundances of AOA and AOB were greatly enhanced by 5.5–14.2% and 29.9–37.6%, respectively, after the application of fertilizer compared to the control treatment (Fig. 3), which confirmed that fertilizer can promote the activity of ammonia-oxidizing microbes by providing substrate for them (Wu et al. 2011), and thus consequently stimulating N_2O emission (Fig. 3). Although a high N rate was applied, it is likely that most of the applied N could be taken up by the plants before being denitrified into N_2O. This could explain why N_2O emission was observed only after the fertilization event and lasted for only a few days. Notably, over the whole growing season, the N_2O emissions were low during the flooding period, and an emission peak appeared at the initial period of the drainage stage, quickly declining and returning to the background by the start of the midseason aeration. These data suggest that soil water status played an important role in N_2O losses. Part of the reason for this peak in N_2O fluxes may be that the drainage process made the soil system aerobic, thus promoting nitrification and N_2O production (Li et al. 2009). In addition, N_2O accumulated in the deep soil could have been released along soil cracks during this period (Cai and Laughlin 2001).

Some studies have shown that denitrification of NO_3^--N in anaerobic soil could be the reason for N_2O losses after irrigation (Aulakh and Singh 1997). However, no N_2O flux peak was found during the re-flooding period in this work. Similarly, Li et al. (2009) and Ma et al. (2009) found negligible N_2O fluxes and NO_3^--N in the soil during the flooding period and suggested that this could probably be attributed to the pressure of standing water preventing N_2O from being released into the atmosphere and the dissolved N_2O then being fully denitrified to N_2 within the soil (Granli and Bøckman 1994). In the present study, the cumulative rates of N_2O emission were between 0.33 and 0.53 kg N ha^{-1} and were within the range of 0.33 to 4.42, reported for rice fields around the world (Akiyama et al. 2005).

4.2 Effects of biochar and/or DMPP on N_2O emissions

In the present study, N_2O emissions were always lower with biochar than without, indicating that the addition of biochar effectively suppressed N_2O losses. Previous work has also found that biochar addition could significantly increase the yield of rice and reduce N_2O emissions from paddy soils (Zhang et al. 2012; Cayuela et al. 2014). In general, biochar easily combines with minerals in soil to form organic-inorganic complexes, which are difficult for soil microorganisms to utilize, and biochar can also increase soil pH, improve soil aeration, and reduce soil bulk density, inhibiting denitrification (Zhang et al. 2017). However, some researchers reported that biochar had no significant effect on N_2O flux and cumulative emission during the long-term submergence of rice during the growing season. This has been ascribed to a

range of factors, such as the biochar source, application rate and application method, soil type and water management, which could have affected the inhibitive efficiency of the biochar (Li et al. 2015; Lin et al. 2017). Results from the current study also highlighted that the addition of DMPP could significantly reduce N_2O emissions, by 39.6% in the paddy field. It has been reported previously that nitrification inhibitors can fundamentally affect the nitrogen transformation processes in the soil, reducing the supply of substrate for nitrification and denitrification and consequently decreasing the emission of N_2O (Benckiser et al. 2013). In addition, the application of biochar and DMPP showed synergistic effects on N_2O emission, producing a 16% further reduction relative to the biochar-only treatment, indicating that applying the biochar with DMPP could improve the benefit of biochar in decreasing N_2O losses (Fig. 2). Similarly, He et al. (2018) have also confirmed that the addition of biochar with inhibitors could more effectively reduce N_2O emissions compared to biochar only. It is interesting to note that the inhibition efficiency of biochar combined with DMPP on N_2O emissions was lower than that of the DMPP-only treatment (Fig. 2), which indicated that biochar may reduce the mitigating effect of DMPP on N_2O emissions in the paddy field. This is in agreement with previous short-term laboratory incubation studies which have shown that the sorption of DMPP by biochar reduced its effectiveness for nitrification inhibition (Chen et al. 2019).

4.3 Underlying microbial mechanisms of biochar and/or DMPP decreasing N_2O emissions

It has been suggested that ammonia oxidizers play a vital role in the biochemical cycling of nutrients and N_2O emissions from flooded anaerobic soil (Paranychianakis et al. 2013; Zheng et al. 2016). Both AOA *amo*A and AOB *amo*A genes were highly abundant, with AOB numerically dominant over AOA, in this study, which is similar to findings from studies carried out in other paddy soils (Cai and Laughlin 2001). The addition of biochar significantly increased both bacterial and archaeal nitrifiers (Fig. 3). Levicnik-Hofferle et al. (2012) suggested that the abundance of AOA could be enhanced by ammonia produced from biochar or organic matter mineralization. Lin et al. (2017) studied the effects of wheat straw-derived biochar on N_2O emissions and showed that adding biochar to paddy soils increased the abundance of ammonia oxidizers. However, the increase in the abundance of AOA and AOB caused by biochar addition is contrary to its potential for reducing N_2O emission by nitrification inhibition. In view of the higher microbial activity in the biochar treatment (Fig. 3), the potential mechanisms for the mitigation effect may be attributable to its aeration regulation and promotion of complete denitrification (Harter et al. 2014; Padhye 2017).

In the current study, AOB and AOA showed opposite responses to DMPP addition, with decreased AOB abundance and increased AOA abundance, and N_2O emissions from those soils were relatively low. These results further confirm that DMPP inhibits nitrification by reducing AOB abundance, which is in line with previous observations (Kleineidam et al. 2011; Benckiser et al. 2013; Shi et al. 2017; Fan et al. 2019). The contrasting responses of AOB and AOA to DMPP may be related to pH-associated niche differentiation, their differing enzyme systems, and the mixotrophic growth of AOA (Jia and Conard 2009). It has been reported that the bacteria might be more sensitive to different inhibitors than archaea because of their essential metabolic and cellular differences (Shen et al. 2013). It is interesting to note that DMPP could inhibit AOB and N_2O emission, but biochar addition reduced this effect to some extent in the present study (Figs. 1 and 3). According to Hink et al. (2018), in the process of soil aerobic ammoxidation, the amount of N_2O produced by AOA is lower than that by AOB. Therefore, it could be inferred that the DMPP and DMPP plus biochar treatments may have transferred N_2O generation from an AOB-dominant process to the low N_2O-yielding AOA equivalent (Fig. 2), which is evidenced by the positive relationship between N_2O emissions and AOA abundance, but the negative relationship between N_2O and AOB in this study ($r = -0.384$, $p < 0.05$).

It has been shown that under anaerobic conditions, denitrification is the predominant source of N_2O production. As with the inhibition of AOB, the application of biochar and/or DMPP had a significant impact on *nir*K and *nir*S-type denitrifiers in our study, suggesting that the two additives also affected denitrification to influence N_2O emissions (Cayuela et al. 2014; Fan et al. 2019). Di et al. (2009) reported that nitrification inhibitor could reduce *nir*K gene abundance by affecting AOB populations, which also bear the *nir*K gene. For the *nir*S and the *nir*K denitrifiers, their growth was reduced by DMPP, but *nir*K was increased by biochar at the elongation and heading stages. Thus, as reported by Hallin et al. (2018), the different responses of these denitrifiers to biochar and/or DMPP treatment may be linked to their niche differentiation.

In the current study, it was found that the application of biochar or DMPP enhanced the expression of the *nos*Z gene, and the increase in *nos*Z was also negatively correlated with N_2O emissions ($r = -0.339$, $p < 0.01$). Therefore, it appears that biochar and DMPP have an effect on denitrification, inducing the transient expression of *nos*Z, and thus stimulating the complete reduction of N_2O to N_2. Consistent with this result, Hatch et al. (2005) observed that, compared with soil without DMPP treatment, N_2O production decreased and N_2 increased during anaerobic soil incubation with DMPP. These findings support the hypothesis of Anderson et al. (2011) who suggest that the reduction of N_2O emission resulting from biochar application may be due to enhancement of the growth and activity of microorganisms capable of complete denitrification. As such, stimulation of *nos*Z gene

production appears to be the most important factor in reducing N_2O emissions (Conthe et al. 2018). Moreover, the DMPP with biochar treatment had a greater effect on the abundance of *nos*Z than the biochar or DMPP-only treatments (Fig. 3), indicating that the combined use of DMPP and biochar may promote full denitrification to N_2, and further reduce N_2O emissions.

5 Conclusion

The data obtained from this study showed that in rice paddy fields, application of fertilizer significantly increases N_2O emissions, mainly during the midseason aeration period, which could be due to the relatively high N availability and dramatic changes in soil water content. N_2O emissions were significantly reduced by DMPP and/or biochar amendment. The combination of biochar with DMPP reduced N_2O emission to a greater extent than biochar alone but not as much as DMPP alone, and this was likely to have been due to the sorption of DMPP by biochar when applied together. To some extent, the different impacts of biochar and DMPP on N_2O emission were related to the different response patterns of N-cycling genes. Overall, application of DMPP to paddy fields is an effective strategy to mitigate N_2O emissions by regulating the abundance of ammonia oxidizers and N_2O reducers.

Funding This study was financially supported by the National Natural Science Foundation of China (Nos. 41807107), the National Key Research and Development Program of China (Nos. 2017YFD0200708, 2018YFD0200200).

References

Akiyama H, Yagi K, Yan XY (2005) Direct N_2O emissions from rice paddy fields: summary of available data. Glob Biogeochem Cycles 19

Allen LH, Boote KJ, Jones JW, Jones PH, Valle RR, Acock B et al (1987) Response of vegetation to rising carbon dioxide: photosynthesis, biomass, and seed yield of soybean. Global Biogeochem Cy 1:1–14

Anderson CR, Condron LM, Clough TJ et al (2011) Biochar induced soil microbial community change: implications for biogeochemical cycling of carbon, nitrogen and phosphorus. Pedobiologia 54:309–320

Aulakh MS, Singh B (1997) Nitrogen losses and fertilizer N use efficiency in irrigated porous soils. Nutr Cycl Agroecosyst 7:1–16

Baggs EM, Smales CL, Bateman EJ (2010) Changing pH shifts the microbial source as well as the magnitude of N2O emission from soil. Biol Fertil Soils 46:793–805

Benckiser G, Christ E, Herbert T et al (2013) The nitrification inhibitor 3, 4-dimethylpyrazole-phosphat (DMPP)-quantification and effects on soil metabolism. Plant Soil 371:257–266

Braker G, Fesefeldt A, Witzel KP (1998) Development of PCR primer systems for amplification of nitrite reductase genes (nirK and nirS) to detect denitrifying bacteria in environmental samples. Appl Environ Microbiol 64:3769–3775

Cai ZC, Laughlin J (2001) Nitrous oxide and nitrogen emissions from soil under different water regimes and straw amendment. Chemosphere 42:11–19

Cayuela ML, van Zwieten L, Singh BP et al (2014) Biochar's role in mitigating soil nitrous oxide emissions: a review and meta-analysis. Agric Ecosyst Environ 191:5–16

Chen D, Xing W, Lan Z et al (2019) Direct and indirect effects of nitrogen enrichment on soil organisms and carbon and nitrogen mineralization in a semi-arid grassland. Funct Ecol 33:175–187

Conthe M, Wittorf L, Kuenen JG et al (2018) Growth yield and selection of *nos*Z clade II types in a continuous enrichment culture of N_2O respiring bacteria. Environ Microbiol Rep 10:239–244

Di H, Cameron K, Shen JP et al (2009) Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. Nat Geosci 2:621–624

Fan CH, Chen H, Li B et al (2017) Biochar reduces yield-scaled emissions of reactive nitrogen gases from vegetable soils across China. Biogeosciences 14:2851–2863

Fan X, Yin C, Chen H et al (2019) The efficacy of 3,4-dimethylpyrazole phosphate on N_2O emissions is linked to niche differentiation of ammonia oxidizing archaea and bacteria across four arable soils. Soil Biol Biochem 130:82–93

Felber R, Leifeld J, Horak J et al (2014) Nitrous oxide emission reduction with greenwaste biochar: comparison of laboratory and field experiments. Eur J Soil Sci 65:128–138

Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proceedings of the National Academy of Sciences of the United States of America 102:14683–14688

Granli T, Bøckman OC (1994) Nitrous oxide from agriculture. Norw J Agri Sci 12:1–128

Gregorutti VC, Caviglia OP (2017) Nitrous oxide emission after the addition of organic residues on soil surface. Agric Ecosyst Environ 246:234–242

Hallin S, Philippot L, Loffler FE et al (2018) Genomics and ecology of novel N2O-reducing microorganisms. Trends Microbiol 26:43–55

Harter J, Krause HM, Schuettler S et al (2014) Linking N_2O emissions from biochar-amended soil to the structure and function of the N-cycling microbial community. ISME J 8:660–674

Hatch D, Trindade H, Cardenas L et al (2005) Laboratory study of the effects of two nitrification inhibitors on greenhouse gas emissions from a slurry-treated arable soil: impact of diurnal temperature cycle. Biol Fertil Soils 41:225–232

He T, Liu D, Yuan J, Luo J et al (2018) Effects of application of inhibitors and biochar to fertilizer on gaseous nitrogen emissions from an intensively managed wheat field. Sci Total Environ 628-629:121–130

Henry S, Bru D, Stres B, Hallet S, Philippot L (2006) Quantitative detection of the nosZ gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, narG, nirK, and nosZ genes in soils. Appl Environ Microbiol 72:5181–5189

Hink L, Gubry-Rangin C, Nicol GW et al (2018) The consequences of niche and physiological differentiation of archaeal and bacterial ammonia oxidisers for nitrous oxide emissions. ISME J 12:1084–1093

Jacobson MZ (2005) Atmospheric pollution: history, science & regulation. Cambridge University Press, New York

Jia Z, Conard R (2009) Bacteria rather than Archaea dominate microbial ammoina oxidation in an agricultural soil. Environ Microbiol 11: 1658–1671

Kleineidam K, Košmrlj K, Kublik S et al (2011) Infuence of the nitrification inibitor 3,4-dimethylpyrazolephosphate (DMPP) on ammonia-oxidizing bacteria and archaea in rhizosphere and bulk soil. Chemosphere 84:182–186

Knoblauch C, Maarifat AA, Pfeiffer EM et al (2011) Degradability of black carbon and its impact on trace gas fluxes and carbon turnover in paddy soils. Soil Biol Biochem 43:1768–1778

Levicnik-Hofferle S, Nicol GW, Ausec L et al (2012) Stimulation of thaumarchaeal ammonia oxidation by ammonia derived from organic nitrogen but not added inorganic nitrogen. FEMS Microbiol Ecol 80:114–123

Li XL, Zhang XY, Xu H et al (2009) Methane and nitrous oxide emissions from rice paddy soil as influenced by timing of application of hydroquinone and dicyandiamide. Nutr Cycl Agroecosyst 85:31–40

Li XL, Yuan WP, Xu H et al (2011) Effect of timing and duration of midseason aeration on CH_4 and N_2O emissions from irrigated lowland rice paddies in China. Nutr Cycl Agroecosyst 91:293–305

Li B, Fan CH, Xiong ZQ et al (2015) The combined effects of nitrification inhibitor and biochar incorporation on yield-scaled N_2O emissions from an intensively managed vegetable field in southeastern China. Biogeosciences 12:2003–2017

Lin Y, Ding W, Liu D et al (2017) Wheat straw-derived biochar amendment stimulated N_2O emissions from rice paddy soils by regulating the *amo*A genes of ammonia oxidizing bacteria. Soil Biol Biochem 113:89–98

Ly P, Jensen L, Bruun T et al (2013) Methane (CH_4) and nitrous oxide (N_2O) emissions from the system of rice intensification (SRI) under a rain-fed lowland rice ecosystem in Cambodia. Nutr Cycl Agroecosyst 97:13–27

Ma J, Ma ED, Xu H et al (2009) Wheat straw management affects CH_4 and N_2O emissions from rice fields. Soil Biol Biochem 41:1022–1028

Maljanen M, Liikanen A, Silvola J et al (2003) Nitrous oxide emissions from boreal organic soil under different land-use. Soil Biol Biochem 35:689–700

Meng L, Ding W, Cai Z (2005) Long-term application of organic manure and nitrogen fertilizer on N_2O emissions, soil quality and crop production in a sandy loam soil. Soil Biol Biochem 37:2037–2045

Pachauri RK, Allen MR, Barros VR et al (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I. II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, IPCC, Geneva, Switzerland

Padhye LP (2017) Influence of surface chemistry of carbon materials on their interactions with inorganic nitrogen contaminants in soil and water. Chemosphere 184:532–547

Paranychianakis NV, Tsiknia M, Giannakis G et al (2013) Nitrogen cycling and relationships between ammonia oxidizers and denitrifiers in a clay-loam soil. Appl Microbiol Biotechnol 97:5507–5515

Rejesus RM, Mohanty S, Balatas JV (2012) Forecasting global rice consumption. http://www.agecon.purdue.edu/staff/balagtas/rice_timeseries_v6.pdf. Accessed 01 Mar 2014

Rotthauwe JH, Witzel KP, Liesack W (1997) The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine-scale analysis of natural ammonia oxidizing populations. Appl Environ Microbiol 63:4704–4712

Sarkhot DV, Berhe AA, Ghezzehei T (2012) Impact of biochar enriched with dairy manure effluent on carbon and nitrogen dynamics. J Environ Qual 41:1107–1114

Shcherbak I, Millar N, Robertson GP (2014) Global meta-analysis of the nonlinear response of soil nitrous oxide (N_2O) emissions to fertilizer nitrogen. Proc Natl Acad Sci U S A 111:9199–9204

Shen J, Li R, Zhang F, Tang C, Rengel Z et al (2004) Crop yields, soil fertility and phosphorus fractions in response to long-term fertilization under the rice monoculture system on a calcareous soil. Field Crop Res 86:225–238

Shen T, Stieglmeier M, Dai J et al (2013) Responses of the terrestrial ammonia-oxidizing archaeon Ca. Nitrososphaera viennensis and the ammonia-oxidizing bacterium Nitrosospira multiformis to nitrification inhibitors. FEMS Microbiol Lett 344:121–129

Shi XZ, Hu HW, Zhu-Barker X et al (2017) Nitrifier-induced denitrification is an important source of soil nitrous oxide and can be inhibited by a nitrification inhibitor 3,4-dimethylpyrazole phosphate. Environ Microbiol 19:4851–4865

Shin YK, Yun SH, Park ME et al (1996) Mitigation options for methane emission from rice fields in Korea. Ambio 25:289–291

Spokas KA, Cantrell KB, Novak JM et al (2012) Biochar: a synthesis of its agronomic impact beyond carbon sequestration. J Environ Qual 41:973–989

Steinbeiss S, Gleixner G, Antonietti M (2009) Effect of biochar amendment on soil carbon balance and soil microbial activity. Soil Biol Biochem 41:1301–1310

Throbäck IN, Enwall K, Jarvis Å, Hallin S (2004) Reassessing PCR primers targeting nirS, nirK and nosZ genes for community surveys of denitrifying bacteria with DGGE. FEMS Microbiol Ecol 49:401–417

Wang C, Lu HH, Dong D et al (2013) Insight into the effects of biochar on manure composting: evidence supporting the relationship between N_2O emission and denitrifying community. Environ Sci Technol 47:7341–7349

Wells NS, Baggs EM (2014) Char amendments impact soil nitrous oxide production during ammonia oxidation. Soil Sci Soc Am J 78:1656–1660

Wu YC, Lu L, Wang BZ et al (2011) Long-term field fertilization significantly alters community structure of ammonia-oxidizing bacteria rather than archaea in a paddy soil. Soil Sci Soc Am J 75:1431–1439

Wu KK, Gong P, Zhang LL et al (2019) Yield-scaled N_2O and CH_4 emissions as affected by combined application of stabilized nitrogen fertilizer and pig manure in rice fields. Plant Soil Environ 65:497–502

Yanai Y, Toyota K, Okazaki M (2007) Effects of charcoal addition on N_2O emissions from soil resulting from rewetting air-dried soil in short-term laboratory experiments. Soil Sci Plant Nutr 53:181–188

Yu L, Tang J, Zhang R et al (2013) Effects of biochar application on soil methane emission at different soil moisture levels. Biol Fertil Soils 49:119–128

Zhang AF, Bian RJ, Pan GX et al (2012) Effects of biochar amendment on soil quality, crop yield and greenhouse gas emission in a Chinese rice paddy: a field study of 2 consecutive rice growing cycles. Field Crop Res 127:153–160

Zhang K, Chen L, Li Y et al (2017) The effects of combinations of biochar, lime, and organic fertilizer on nitrification and nitrifiers. Biol Fertil Soils 53:77–87

Zheng J, Chen J, Pan G et al (2016) Biochar decreased microbial metabolic quotient and shifted community composition four years after a single incorporation in a slightly acid rice paddy from southwest China. Sci Total Environ 571:206–217