1 Different effects of biochar and a nitrification inhibitor application on paddy soil denitrification: A
2 field experiment over two consecutive rice-growing seasons

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Abstract:

Biochar and nitrification inhibitors are increasingly being proposed as amendments to improve nitrogen use efficiency (NUE). However, their effects on soil denitrification and the major N loss in rice paddies over an entire rice-growing season are not well understood. In this study, using intact soil core incubation combined with N₂/Ar technique, the impacts of biochar and a nitrification inhibitor (Ni), 2-chloro-6-(trichloromethyl)-pyridine, on rice yield and soil denitrification, as well as ammonia (NH₃) volatilization, were investigated over two rice-growing seasons in the Taihu Lake region of China. Field experiments were designed with four treatments: N0 (no N applied), N270 (270 kg N ha⁻¹ applied), N270 + C (25 t ha⁻¹ biochar applied) and N270 + Ni (2-chloro-6- [trichloromethyl] -pyridine, 1.35 kg ha⁻¹ N applied). Compared with single application of N fertilizer alone (N270), biochar (N270 + C) and Ni (N270 + Ni) applications increased rice yields by 4.2–5.2% and 6.2–7.3%, respectively. The cumulative N₂-N and NH₃-N losses in different treatments varied from 11.9 to 21.8% and from 11.5 to 22.0% of the applied N, respectively. Compared with the single application of N fertilizer, the Ni application increased total NH₃ emission by 4.0–20.6% and significantly decreased total N₂-N emission by 9.7–19.4% (*p*<0.05), while the biochar application increased total NH₃ and N₂-N emissions by 8.6–17.9% and 3.3–9.7%, respectively. Overall, the biochar application resulted in an 11–15% higher net gaseous N than the Ni application. Although the biochar application may increase the rice yield and consequently the plant N uptake, it also promoted N loss more than Ni. Therefore biochar may not be good for maintaining soil fertility over a long period. Instead, applying Ni may be an optimal practice to ensure food security, while decreasing gaseous N loss, for rice production in the Taihu Lake region of China.

Keywords: Biochar, Nitrification inhibitors, Rice paddies, N₂/Ar technique, Denitrification, NH₃ volatilization.

1. Introduction

Nitrogen (N) is an important nutrient element for plant growth and is often one of the most limiting nutrient in paddy fields. To encourage high grain yields, high rates, usually over 300 kg N ha⁻¹, of chemical N fertilizers have been applied in rice paddies per growing season, especially in eastern China, leading to high levels of N loss to the environment (Huang and Tang, 2010; Ju et al., 2009). Excessive N fertilizer application has resulted in a cascade of environmental problems, such as surface and ground water pollution, ammonia (NH₃) volatilization and global warming resulted from the emission of nitrous oxide (N₂O) (Chen et al., 2014). Rice paddies with seasonally alternating dry-wet conditions may have high rates of N loss from N fertilizers and low nitrogen use efficiency (NUE). The NUE of rice paddies ranges from 30 to 40% (Xia et al., 2016), or even lower in some cases (Choudhury and Khanif, 2001). During the rice growing season, N fertilizer is mainly lost through NH₃ volatilization, nitrification, and N₂ from denitrification (Xing and Zhu, 2000). Denitrification is a process that converts nitrate (NO₃⁻-N) into N₂, with NO and N₂O as intermediates, and is generally the most important process of N loss after N fertilizers application under flooded water conditions (Verhoeven et al., 2006). Thus, reducing the N loss by reducing the denitrification process is critical important for improving the NUE while mitigating the environmental impacts associated with N fertilizer usage in paddy fields.

For decades, biochar and nitrification inhibitors (Nis) have been used as effective amendments to reduce N losses and increase the NUE (Sohi et al., 2010; Xia et al., 2017). Biochar can alter the microbial activities in the soil (Lehmann et al., 2011), the available N and organic carbon (C) contents (González-Pérez et al., 2004; Prendergast-Miller et al., 2011), the pH (Enders et al., 2012; Van Zwieten et al., 2010) and the level of soil aeration (Kinney et al., 2012), which all exert influences on N loss from denitrification. The effects of biochar on N loss are dependent on the N content of the biochar feedstock, such as poultry manure *versus* wood (Clough et al., 2010; Singh et al., 2010; Spokas and Reicosky, 2009). Nitrification inhibitors can suppress the nitrification process in soil by delaying the oxidation of NH₄⁺ to NO₃⁻, which is an electron acceptor for denitrification, which then may suppresses the denitrification process in flooded soils (Di et al., 2009; Guo et al., 2013). Previously, most of the studies that investigated the effects of biochar or Nis mainly focused on N₂O emissions (Dai et al., 2013; Giltrap et al., 2010; Yanai et al., 2007).

67 Currently, relatively little is known about their effects on denitrification in flooded rice paddies.

68 Various methodological approaches, such as the acetylene (C_2H_2) inhibition technique, ^{15}N
69 tracers and mass balance approaches have been used to measure denitrification in terrestrial and
70 aquatic environments. These available approaches are problematic for a variety of reasons, such as
71 changing substrate concentrations, disturbing the physical setting of the process, lacking
72 sensitivity or being prohibitively costly in time, and/or expensive (Groffman et al., 2006). Most
73 fundamentally, it is difficult to directly measure the denitrification rate in flooded rice paddies
74 because of the high atmospheric background level of N_2 (78%) (Groffman et al., 2006). The C_2H_2
75 inhibition method is easy-to-use and cheap, but it also inhibits the nitrification process (Berg et al.,
76 1982; Wrage et al., 2004), which may lead to substrate limitations for denitrification, especially in
77 an environment with low NO_3^- availability (Yan et al., 2011). Nitrifier denitrification generally
78 cannot be studied with C_2H_2 because of the inhibition of NH_3 oxidation, which may cause an
79 underestimation of denitrification rates. ^{15}N -tracer techniques are widely utilized for the direct
80 measurement of denitrification following the addition of $^{15}NO_3^-$ (Cayuela et al., 2013; Dong et al.,
81 2012; Lan et al., 2013). However, excessive $^{15}NO_3^-$ added to soils can increase the availability of
82 N and may overestimate denitrification rates, especially in N-limited systems (Groffman et al.,
83 2006). Detection of the end-product (N_2) is a direct measure of net denitrification, which can be
84 determined by high-precision membrane inlet mass spectrometry (MIMS) and the nitrogen
85 gas/argon (N_2/Ar) technique without the addition of $^{15}NO_3^-$ (Yang and Silver, 2012). The N_2/Ar
86 technique uses an inert gas Ar as a conservative tracer. Thus, measured changes in measured N_2/Ar
87 ratios can be attributed to changes in the abundance of N_2 (Yang and Silver, 2012). Moreover,
88 MIMS provides rapid (< 2 min) measurements on small (< 7 ml) samples with no preprocessing
89 and provides high precision ($\pm 0.03\%$ for N_2/Ar) (Kana et al., 1994; Kana et al., 1998). However,
90 for core incubations, there is no completely adequate control that can account for all solubility flux
91 errors and this generally sets the lower limit of detection (Eyre et al., 2004; Groffman et al., 2006;
92 Kana, 2004). Soil core incubation prevented the competitive consumption of dissolved inorganic N by
93 rice, which could have promoted denitrification to some extent owing to the reduced competition for
94 available N (Nicolaisen et al., 2004). Although this approach is not without problems, it has the
95 advantage of measuring the direct product of denitrification (Groffman et al., 2006). Together with
96 the intact soil/sediment core incubation method, the N_2/Ar technique has been successfully used to

study *in situ* denitrification rates in water bodies and flooded paddy soils (Li et al., 2014; Li et al., 2013; Shan et al., 2016; Zhao et al., 2015). Li et al. (2014) reported that the average cumulative N_2 -N loss measured by the N_2/Ar technique during the rice growth stage (21 d) was 4.7% of the applied N fertilizer.

Rice paddies as a man-managed seasonal wetland ecosystems cover ~20% of the world's total irrigated croplands (Frolking et al., 2002). The current study area is located at the Taihu Lake region, which is a major agricultural intensification region for rice production in China. Currently, rice yield in this region has already stagnated, and the NUE has been low (< 30%), due to poor agricultural practices and irrational fertilization management strategies (Ju et al., 2009; Zhao et al., 2009). In recent years, Nis and biochar have been increasingly proposed as amendments to improve grain yield and NUE (Hu et al., 2013; Major et al., 2010). However, their effects on N loss, especially for N loss as N_2 -N are not well understood. In this study, we conducted a field experiment to investigate the effects of biochar and Ni on rice yield, denitrification and NH_3 volatilization over two rice-growing season in the Taihu Lake region of China. The denitrification rates of different treatments were measured using an intact soil incubation-based N_2/Ar technique. The NH_3 volatilization was measured using a dynamic chamber method. The objectives of this study were to (1) determine the potential rates of denitrification during the different phenological stages of rice growth using the intact soil incubation-based N_2/Ar technique; (2) calculate the cumulative N_2 -N loss through denitrification and the NH_3 -N loss under actual field experimental conditions; and (3) elucidate the impacts of biochar and Ni applications on crop yield, cumulative N loss through denitrification and NH_3 volatilization.

2. Materials and methods

2.1 Experiment site

The experimental field was located in an agro-ecological experimental station in Changshu (31°32'93" N, 120°41'88" E), Chinese Academy of Sciences, Jiangsu Province, China. This region in the Yangtze River Delta has a north subtropical monsoon climate, with a mean precipitation and an average temperature in 2015–2016 of 17.04°C and 1344.8 mm, respectively. The traditional cropping system in this region is winter wheat-rice rotation system. The experimental soil is classified as Gleyi-Stagnic Anthrosol (CRGCST 2001) developed from lacustrine sediments with

a silt clay loam texture (13.3% sand, 54.8% silt and 31.9% clay). The topsoil (0–20 cm) has a 6.99 pH level, containing 26.6 g kg⁻¹ of organic C and 2.83 g kg⁻¹ of total N, resulting in a 9.40 C:N ratio.

2.2 Field treatments and management practices

The field experiment was conducted during the rice-growing seasons of 2015 and 2016. In this experiment, four treatments were designed to study the effects of biochar and Ni applications on denitrification and NH₃ volatilization. These treatments were arranged in a fully randomized block design with four replicates. Each plot, with an area of 6 m × 7 m, was separated by a 30-cm wide earthen ridge and lined with polyvinyl chloride (PVC) boards which were placed into the soil at a depth of 30 cm to prevent runoff and seepage. The following four treatments were designed: (1) N0 (no N applied); (2) N270 (270 kg N ha⁻¹ applied); (3) N270 + C (270 kg N ha⁻¹ and 25 t ha⁻¹ biochar applied); (4) N270 + Ni (270 kg N ha⁻¹ plus the Ni 2-chloro-6-(trichloromethyl)-pyridine (CP), the application rate of CP was 0.5% of urea-N). For the N270, N270 + C and N270 + Ni treatments, split applications of fertilizers were adopted with four splits, one for each different phenological stage of rice growth, and urea-N was applied as the basal fertilization (50%), tillering topdressing (10%), anthesis topdressing (20%) and heading topdressing (20%). Diphosphorus pentoxide (P₂O₅), as a superphosphate, was only applied as a basal fertilizer and potassium oxide (K₂O), as KCl, was applied as a basal fertilizer (50%) and anthesis topdressing (50%). These three treatments were applied at the same dose levels of urea (N, 46%), superphosphate (P₂O₅, 13.5%) and potassium chloride (K₂O, 60%), resulting in 270 kg N ha⁻¹, 90 kg P₂O₅ ha⁻¹ and 120 kg K₂O ha⁻¹, in total, respectively (Table 1). In addition to the above fertilizers, the treatments of N270 + C and N270 + Ni were also amended with 25 t biochar ha⁻¹ and CP at 1.35 kg N ha⁻¹, respectively. For the treatment with N270 + C, the biochar was produced by thermal decomposition of crop straw under a maximum temperature of 500°C with a furnace residence time of 8 h in a biogas–energy pyrolysis system (Wang et al., 2013), and it was applied together with the basal fertilizer. The Ni CP was applied with urea in four splits forming the N270 + Ni treatment. For the N0 treatment, only P₂O₅ and K₂O were applied as basal fertilizers at the rate of 90 kg P₂O₅ ha⁻¹ and 120 kg K₂O ha⁻¹, respectively. The basal fertilizer was manually broadcast onto the surface soil and integrated by plowing and harrowing before rice seedling transplantation, and topdressings were directly broadcast homogeneously onto the surface

water. All of the treatments were applied to the same rice cultivar ('Changyou 5') and field management practices. During the two rice-growing seasons, the field water regime was managed under an alternating flooding and drainage cycle of flooding – drainage – reflooding – moist mode, during the tillering, panicing, spiking and ripening stages, respectively). In this field experiment, flood water was maintained at a depth of 3–5 cm from 15 June to 24 July, with a subsequent drainage lasted for ~ 10 days before reflooding from 4 August till 20 September, which was followed by a final intermittent irrigation period until harvesting.

To calculate denitrification rates and cumulative N₂-N losses at different phenological stages, the start date of each phenological stage was consistent with the date of N fertilizer application. The end date was the date of the next fertilizer application because the application date is determined by the phenological stage of rice growth. The ripening stage began on September 26 and ended on October 31.

2.3 Measurement of denitrification rates

Soil denitrification rates were measured using a *semi-in situ* incubation experiment, which was performed using an intact core soil incubation system similar to that was described by Li *et al.* (2014). The physical integrity of the soil could be retained in the intact soil core incubation and permitted the control of environmental factors affecting denitrification. The time span between sampling in the field and indoor cultivation was shortened to no more than 2 h in the current study to mitigate the effects of these factors and to best preserve the authenticity of the measured denitrification rate. In brief, the system consisted of a carousel housing four magnets and a PVC intact core sampler, with inner diameters of 60 cm and 7 cm, and heights of 50 cm and 25 cm, respectively. Undisturbed intact soil cores (10-cm depth) were collected from each plot containing a different treatment using the PVC intact core sampler and then were sealed at the bottom with rubber stoppers. Approximately 5l of floodwater were simultaneously collected from each plot for incubation. Before incubation, the PVC intact soil core samples (10-cm depth of soil core) were filled with the corresponding plot floodwater without headspace (15-cm water column), and then the PVC intact soil core samplers were placed around the carousel housing's four magnets and then immediately incubated at less than 20°C; running water surrounded the PVC intact soil core sampler to adjust the temperature and isolate air. An attached stir bar was placed in the PVC intact soil core sampler 6-cm from the top. The stir bars were rotated at 14 rpm under the carousel

housing's four magnets to mix the overlying water without disturbing the intact soil core. Time course incubations were carried out in quadruplicate (time points 0, 2, 4, 6, and 8 h). Water samples (7 ml) for N₂/Ar analyses from each core were collected five times, at 2 h intervals during the 8-h incubation and placed in Exetainer vials (Labco Limited, Buckinghamshire, UK), and 20 µl of saturated HgCl₂ solution was added to the samples at each time point to stop the microbial activity (Li et al., 2014).

The samples that had been collected and placed in Exetainer vials were analyzed with dissolved N₂ and Ar through MIMS (Bay Instruments, Easton, MD, USA) according to the procedure as described by Kana et al. (1994) and Li et al. (2014). In the N₂/Ar method, the change in N₂ concentration was determined based on the change in the measured N₂/Ar multiplied by the predicted water Ar concentration at air saturation at the water incubation temperature. The N₂-N flux (µmol N₂-N h⁻¹) for each plot was calculated by the linear regression of the change in the dissolved N₂ concentration during incubation. The N₂-N fluxes were generally measured five times every other day after each N fertilizer application, thereafter, once a week or more. In the current study, the dissolved N₂ produced through denitrification and anaerobic ammonium oxidation processes were not distinguished (Kana et al., 1994) and was referred to as "denitrification" for simplicity. The mean denitrification rates of the four treatments were determined each day during the sampling period (over 8 h). The cumulative N₂-N losses and denitrification rates for the different phenological stages of rice growth were calculated by averaging the rates throughout the period weighted by the interval of two measurements (Cai et al., 1997; Dong et al., 2012).

2.4 NH₃ volatilization measurement

In this study, we used the same method to measure NH₃ volatilization as Tian et al. (1998) and Zhao et al. (2015), with minor modifications. Briefly, a vacuum pump, dynamic chambers (cylindrical, inner diameter 20 cm and height 15 cm), pipes and porous bottles together compose this NH₃ volatilization system. When the system was working, the dynamic chamber was inserted into surface water or soil to an 8–10-cm depth, and the NH₃ in the chamber could be absorbed by dilute sulfuric acid in the glass bottle (60 ml, 0.03 mol L⁻¹ dilute sulfuric acid) by an air-powered vacuum pump. After fertilization, the NH₃ gas was collected twice per day from 7:00 to 9:00 and from 15:00 to 17:00. The average of the 4 h of NH₃ gas flux was represented as per h of NH₃ gas

flux per d. After fertilization, the NH_3 volatilization gas flux was continuously measured for 4–5 d until the NH_3 gas flux reached a stable state. The concentration of NH_4^+ -N in the acid trap was determined using an automatic chemical analyzer (Smartchem140, AMS, Rome, Italy). We calculated the NH_3 gas flux according to the formula in this literature (Cao et al., 2013).

2.5 Auxiliary measurements

The temperature, precipitation and other meteorological data were collected from the automatic meteorological station (Vaisala, Finland) located at the Changshu Agro-Experimental Station. Surface water (100 ml) of each plot was simultaneously collected at every measurement day and filtered through a 0.45- μm hydrophilic PEPT needle filter (ANPEL, Shanghai, China) for NO_3^- -N, NH_4^+ -N and dissolved organic C (DOC) analyses. Concentrations of NO_3^- -N and NH_4^+ -N were determined using an automatic chemical analyzer (Smartchem140, AMS) and DOC was measured with a C/N 3100 multianalyzer (Analytik Jena AG, Germany). The surface water pH was directly measured with a portable pH meter. Well-mixed soil samples taken during the indoor cultivation of each sample from the 0–10-cm layer were extracted with 2 M KCl at a soil:KCl ratio of 1:10, shaken for 1 h and filtered for NO_3^- -N and NH_4^+ -N analyses with an automatic chemical analyzer (Smartchem140, AMS). The soil pH was determined at a soil:MilliQ water ratio of 1:2.5, shaken homogeneous and analyzed with a portable pH meter. Soil DOC was extracted with distilled water at a soil:MilliQ water ratio of 1:5, shaken for 1 h and filtered through 0.45 μm hydrophilic PEPT needle filter (ANPEL) and measured with a C/N 3100 multianalyzer (Analytik Jena AG). Soil organic C (SOC) and total N (TN) concentrations were analyzed on a CN analyzer (Vario Max CN, Elementar, Hanau, Germany). Soil bulk density (BD) was measured using the intact core method. Soil particle size was determined with a laser particle size analyzer (LS13320, Beckman Coulter, Brea, CA, USA). Soil moisture was measured gravimetrically by drying the soil at 105°C for 16 h. All of the samples were analyzed in triplicate. During rice maturation, the aboveground plant parts from each treatment were harvested to determine the actual yield. Samples of $\sim 5 \text{ m}^2$ areas were simultaneously reserved, air-dried and divided into straw and grain to calculate the dry matter yield.

2.6 Statistical analysis

The statistical analyses and graphical interpretations were carried out using SPSS (version

18.0, Chicago, IL, USA) and Origin (version 8.6, Northampton, MA, USA). The Pearson's correlation analysis was performed to investigate the relationships between denitrification rates, NH_3 volatilization fluxes, pH, inorganic N and DOC of the soil and the surface water content among different treatments. Multiple comparisons of the average N_2 and $\text{NH}_3\text{-N}$ production rates in different treatment's experimental plots were carried out using a one-way analysis of variance followed by Fisher's least significant difference test. All of the tests were carried out at the 0.05 probability level.

3 Results

3.1 Effects of biochar and a Ni application on surface water $\text{NH}_4^+\text{-N}$, pH and rice yield

Chemical properties of surface ($\text{NO}_3^-\text{-N}$, $\text{NH}_4^+\text{-N}$, DOC and pH) and soil (0–10 cm) chemical properties ($\text{NH}_4^+\text{-N}$ and DOC) were significantly affected by N-fertilization application, with peaks appearing on the first day after each N-fertilizer application and sharply decreasing thereafter (Figs. 1a, 1b, 1c, S1, S2). However, the dynamic of soil $\text{NO}_3^-\text{-N}$ was different from those of other soil chemical properties and surface water $\text{NO}_3^-\text{-N}$. The soil $\text{NO}_3^-\text{-N}$ concentrations exhibited an increasing trend and ranged from 0.9 to 15.3 mg kg^{-1} (Fig. 1d). Compared with N270, the addition of biochar significantly increased the surface water pH by the unit of 0.09–0.41, while the application of Ni increased the surface water $\text{NH}_4^+\text{-N}$ concentration by 2.49–9.73 mg L^{-1} and the pH value by 0.03–0.38 units, after each N application over the two rice-growing seasons (Table 2).

The rice yields ranged from 7.34 t ha^{-1} to 10.97 t ha^{-1} among the four treatments over the two growing seasons. Compared with the single application N fertilizer, the additions of biochar or Ni increased rice yields by 4.2–5.2% and 6.2–7.3%, respectively, although the differences were not statistically significant ($p > 0.05$) (Table 3).

3.2 Soil denitrification rates

The daily variations in the dissolved $\text{N}_2\text{-N}$ fluxes during the two rice-growing seasons are shown in Figure 2. There were similar $\text{N}_2\text{-N}$ flux patterns among the different treatments during the entire rice growing season. Among the four treatments, soil denitrification rates ranged from 0.100 to 0.892 $\text{kg N ha}^{-1} \text{ d}^{-1}$ in 2015 and from 0.227 to 0.906 $\text{kg N ha}^{-1} \text{ d}^{-1}$ in 2016 (Fig. 2). N fertilizer application significantly increased denitrification rates, and the peak denitrification rates in the N-fertilized treatments appeared after each N-fertilizer application and sharply decreased

thereafter (Fig. 2). Compared with the N270 treatment, the biochar amendment increased the denitrification rates by 3.3–9.7% (Table 3). In contrast, the Ni application significantly ($p < 0.05$) decreased the denitrification rate by 9.7–19.4% during the two seasons (Table 3).

The maximum denitrification rates during the two rice-growing seasons occurred at the transplanting stage. The denitrification rate at the tillering stage was the second highest in 2016, even though the least N (27 kg N ha^{-1}) was applied at this stage (Fig. 3). The same amount of N (54 kg N ha^{-1}) was applied at the booting and flowering stages, but the denitrification rate at the latter was lower than that at the former, except N270 + Ni in 2016 (booting *versus* flowering stage, being $0.251 \text{ kg N ha}^{-1} \text{ d}^{-1}$ *versus* $0.359 \text{ kg N ha}^{-1} \text{ d}^{-1}$ in 2015 and $0.373 \text{ kg N ha}^{-1} \text{ d}^{-1}$ *versus* $0.395 \text{ kg N ha}^{-1} \text{ d}^{-1}$ in 2016, respectively) (Fig. 3). The biochar application slightly decreased the soil denitrification rate at the transplanting stage of rice growth in 2015 and at both the transplanting stage and tillering state in 2016, while it increased the denitrification rates at other stages (Fig. 3). The Ni application suppressed the denitrification in all of the stages of rice growth during both seasons, except the tillering stage in 2015 (Fig. 3).

3.3 NH_3 volatilization rates

NH_3 volatilization rates over the two rice-growing seasons are shown in Figure 4. Similar to the denitrification rate, the flux peak of NH_3 emission also appeared primarily after each N-fertilizer application (Fig 4). The daily NH_3 volatilization fluxes in N-fertilized treatments varied from 0.021 to $2.467 \text{ kg N ha}^{-1} \text{ d}^{-1}$ in 2015 and from 0.017 to $3.896 \text{ kg N ha}^{-1} \text{ d}^{-1}$ in 2016. Both biochar and Ni additions increased seasonal total NH_3 volatilization fluxes, compared with the application of N fertilizer alone. The significant difference occurred in the 2016 rice-growing season, when the total NH_3 volatilization was significantly increased by 17.8% for the biochar application and by 20.6% for the Ni application. (Table 3). The enhancement in NH_3 volatilization resulting from biochar and Ni applications mainly occurred at the transplanting stage (Fig. 5).

3.4 Factors influencing soil denitrification and NH_3 volatilization rates

The high peak values of soil denitrification and NH_3 volatilization rate similarly appeared after each N fertilizer application (Figs. 2, 4). Moreover, denitrification rates were positively correlated with ammonia volatilization rates ($r=0.702$, $p<0.01$). Soil denitrification and NH_3 volatilization rates were both significantly affected by floodwater $\text{NH}_4^+\text{-N}$ ($r=0.693$, $p<0.01$; $r=0.814$, $p<0.01$), $\text{NO}_3^-\text{-N}$ ($r=0.687$, $p<0.01$; $r=0.560$, $p<0.01$), DOC ($r=0.739$, $p<0.01$; $r=0.796$,

$p < 0.01$), pH ($r = 0.714$, $p < 0.01$; $r = 0.831$, $p < 0.01$), soil NH_4^+ -N concentration ($r = 0.798$, $p < 0.01$; $r = 0.802$, $p < 0.01$) and DOC ($r = 0.454$, $p < 0.01$; $r = 0.296$, $p < 0.05$) (Table 4).

4 Discussion

Using the intact soil core incubation-based N_2/Ar technique, we evaluated the denitrification rate under flooded paddy soils affected by N fertilization over two entire rice-growing seasons in the Taihu Lake region of China.

4.1 Cumulative N_2 -N losses through denitrification and NH_3 volatilization from paddy soils

The N loss caused by denitrification amounted to 21.9 (2015)–43.1 (2016) kg N ha^{-1} for N_0 , and 35.8–57.0 kg N ha^{-1} for N_{270} , which accounted for 13.3–21.1% of the applied N (Fig. 6). The cumulative NH_3 -N loss for N_{270} was 31.0–49.1 kg N ha^{-1} , which accounted for 11.5–18.2% of the applied N (Fig. 6). The ratio of N loss caused through denitrification was 15.5–16.1% higher than that via NH_3 volatilization for N_{270} (Fig. 6). Compared with N_{270} , Ni application decreased gaseous N losses by 0.9–3.4 kg N ha^{-1} in each rice-growing season through suppressed denitrification. Conversely, biochar addition increased the gaseous N loss by 6.2–10.6 kg N ha^{-1} (Fig. 6). Moreover, studies have reported beneficial effects of biochar as a soil amendment for mitigating N_2O -N emissions (Cayuela et al., 2013; Clough and Condron, 2010; Van Zwieten et al., 2014; Zhang et al., 2010). Total N_2O -N emissions were 0.98–1.99 kg N ha^{-1} over the entire rice growth season (Zhang et al., 2010). When compared to N_2 -N and NH_3 -N emissions, the total N_2O -N emissions were small, and the increased amount of gaseous N released by biochar was more than the amount of N_2O -N emissions mitigated by biochar (Fig. 6). In addition, the Ni amendment also significantly decreased N_2O -N emissions by 48.8% in a field experiment conducted at the same region (Ma et al., 2013). Therefore, Ni addition was more effective in gaseous N mitigation than biochar amendment.

The denitrification rate in the current study was greater than the value (4.7% of the applied N) reported by Li et al. (2014) using the same method. However, the study conducted by Li et al. (2014) only recorded the cumulative N_2 -N loss for 21 d after fertilization. While in the current study, we measured the denitrification rate over the entire rice growth period. The denitrification rate at the transplanting stage of rice growth was greater than that at the other phenological stages because of the high amount of N-fertilizer (135 kg N ha^{-1}) applied (Fig. 3), resulting in high

inorganic N levels, DOC concentrations and pH values (Figs. 1a, 1b, 1c, S1, S2), which have been closely linked to denitrification (Aulakh et al., 2000; Buresh and De Datta, 1990; Li et al., 2016; Li et al., 2014). Moreover, the rice plants were still young at the transplanting stage, resulting in low N uptake. The second higher fluxes of denitrification rate appeared at the tillering stage in 2016 (Fig. 3), even though the least N (27 kg N ha^{-1}) was applied at this stage. This may result from the interval between the transplanting and tillering stages being only 7 d, resulting in the basal fertilization still being retained in the soil, which affected the denitrification rate at the tillering stage. At the booting and flowering stages, the same amount of N-fertilizer was applied, but the denitrification rate at flowering stage was lower than that at the booting stage (Fig. 3). On the one hand, plant biomass at the flowering stage was more than that at the booting stage, which may increase N uptake by plants. On the other hand, the rice root may secrete more biological Nis, which may suppress the activities of soil-nitrifiers (Subbarao et al., 2015).

The highest of NH_3 volatilization fluxes are generally reported to occur at the transplanting stage of rice growth because of the high amount of urea applied and the high pH (Whitehead and Raistrick, 1991). In contrast to denitrification, the NH_3 flux at the flowering stage was greater than that at the booting stage, even though the same amount of N-fertilizer was applied (Fig. 5). This may be because the biological Ni may also be beneficial to NH_3 volatilization, and a trade-off relationship occurs between NH_3 volatilization and denitrification in flooded rice paddies as a result of different N application methods (De Datta et al., 1989; Freney et al., 1990; Li et al., 2014). Based on our results, the NH_3 volatilization and denitrification at different phenological stages also showed a trade-off relationship under the same amount of applied N. In this study, NH_3 volatilization and denitrification were significantly affected by urea application (Figs. 2, 4). As urea is hydrolyzed into NH_3 (Stevens et al., 1989), the first NH_4^+ -N is lost by NH_3 volatilization, and then later from denitrification. In this study, the high peak values of NH_3 volatilization mostly appeared the first day after N was applied and then sharply decreased, but the high peak values of denitrification occurred 1 day after NH_3 volatilization (Figs. 2, 4). These phenomena were attributed to the differential production processes of NH_3 volatilization and denitrification in soil (Ishii et al., 2011; Tian et al., 1998), and NH_3 volatilization was more affected by NH_4^+ -N concentrations in surface water than denitrification in this study (Table 4).

4.2 Effects of biochar application on paddy soil denitrification rates

Biochar application may be effective in mitigating N_2O emissions from soil (Cayuela et al., 2013; Clough and Condron, 2010; Van Zwieten et al., 2014; Zhang et al., 2010). However, the mechanisms remain unclear. Some studies showed that biochar may decrease soil N_2O emissions by enhancing its further reduction to N_2 (Cayuela et al., 2013; Xu et al., 2014). While this mechanism did not minimize total N losses ($\text{N}_2 + \text{N}_2\text{O}$), it decreased the ratio of $\text{N}_2\text{O}/(\text{N}_2 + \text{N}_2\text{O})$ and the environmental consequences of N_2O . In the current study, we directly measured paddy soil denitrification rates using an intact soil core incubation-based N_2/Ar technique and found that biochar indeed enhances the production of N_2 in flooded rice paddy soil (Fig. 2; Table 3). Different hypotheses have been postulated to interpret the effects of biochar application on denitrification. Biochar can alter the microbial activities in soil (Lehmann et al., 2011), the available N and organic C contents (González-Pérez et al., 2004; Prendergast-Miller et al., 2011), the pH (Enders et al., 2012; Van Zwieten et al., 2010) and the level of soil aeration (Kinney et al., 2012), which are all important influencing factors on denitrification. Another hypothesis proposed that biochar functions as an “electron shuttle” and favors the transport of electrons to microorganisms, and then is in favor of N_2O being reduced to dinitrogen (Cayuela et al., 2013). Surface water pH may be the main factor under anaerobic paddy field conditions that induces the enhancement of denitrification (Peterjohn, 1991). In the current study, peak values of soil denitrification rates occurred on the first or second day after the N applications (Fig. 2), and the surface water pH of biochar-emended treatments ranged from 7.93 to 8.35, which were significantly higher compared with those in N270 (Table 2). Thus, biochar application may increase the surface water’s pH level to an optimal range for denitrification (Šimek and Cooper, 2002), stimulating denitrification enzyme activities (Xu et al., 2014) and increasing the activity of N_2O -reductase enzymes (Peterjohn, 1991), thereby further enhancing N_2 formation from N_2O . When compared with N270, biochar and Ni additions increased the $\text{NH}_3\text{-N}$ volatilization in the two rice-growing seasons owing to increases in surface water pH levels (Keeney and Sahrawat, 1986). However, $\text{NH}_3\text{-N}$ fluxes were not significantly different between N270 + C and N270 + Ni (Table 3), although the pH of the biochar treatment was higher than that of the Ni treatment (Table 2). This may be due to the higher concentration of $\text{NH}_4^+\text{-N}$ in surface water in the Ni treatment than in the biochar treatment, which likely offsets the effects of the relatively lower pH on NH_3

volatilization (Table 2).

In the current study, soil denitrification and NH_3 volatilization rates were also both significantly affected by flood water $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, DOC and soil $\text{NH}_4^+\text{-N}$ concentrations, but the soil $\text{NO}_3^-\text{-N}$ concentration was not the significant factor influencing paddy soil denitrification (Table 4), which was in agreement with a previous study (Li et al., 2014). The dynamics of surface water $\text{NH}_4^+\text{-N}$ and soil $\text{NH}_4^+\text{-N}$ concentrations were similar (Fig. 1a, 1c), exhibited decreasing trends after N fertilizer application as part of denitrification process. However, denitrification did not reduce soil $\text{NO}_3^-\text{-N}$ concentration (Fig. 1d), but can significantly reduce the surface water concentration (Fig. 1b). Based on our results, the substrates of denitrification may mainly come from floodwater (Li et al., 2014; Zhao et al., 2014), and this may explain why the soil $\text{NO}_3^-\text{-N}$ concentrations did not significantly influence the denitrification rates in the current study (Table 4). Many previous studies have reported that the substrates of denitrification mainly result from nitrification processes (Kool et al., 2011) and the rate of denitrification in flooded soils is controlled by the rate of nitrification rather than by the activities of denitrification enzymes (Zhou et al., 2012). Thus, the nitrifier's contribution to the denitrification process may be the main cause of N loss in rice paddy soils (Ishii et al., 2011). In the current study, the surface water $\text{NO}_3^-\text{-N}$ concentration in biochar-amended treatment was slightly higher than that in the Ni-amended treatment (Fig. 1b), because biochar-amended soil stimulated nitrification rates (Xu et al., 2014). Meanwhile, due to rapid denitrification and slow rates of nitrification (Reddy et al., 1989), the surface water $\text{NO}_3^-\text{-N}$ concentrations of biochar- and Ni-supplemented treatments showed little difference and maintained at a relatively low contents especially in 2016 (Fig. 1b).

4.3 Effects of the Ni application on the paddy soil denitrification rate

Unlike biochar, Ni addition significantly decreased the denitrification rate over all of the phenological stages of rice growth by 9.7–19.4% ($p < 0.05$) compared with N270 (Fig. 3). Numerous incubation and field experiments have shown that Ni application may reduce N_2O emissions from soil (Bhatia et al., 2010; Chen et al., 2010; Lan et al., 2013; Majumdar et al., 2000; Malla et al., 2005; Sun et al., 2015), but very few studies have directly measured N_2 production from soil denitrification. Although Ni addition enhanced pH, which is favorable for denitrification (Xu et al., 2014), it significantly decreased the denitrification rate over all of the phenological

stages of rice growth ($p < 0.05$) in this study.

The observed surface water NH_4^+ -N concentrations in the current study were effectively increased when Ni was applied simultaneously with N fertilizer (Table 2). It is most likely that Ni quickly had an inhibitory effect on nitrification, resulting in the high NH_4^+ -N concentrations of surface water after the amendment of N fertilizer to the paddy field. Many studies have shown that nitrification still occurs in flooded rice systems due to O_2 from the roots and rhizosphere activity (Arth et al., 1998; Bodelier et al., 1996; Nicolaisen et al., 2004). Thus, Ni may go on playing a role in inhibiting nitrification in the anaerobic incubation system. And then, the denitrification process could be suppressed owing to the inhibitory effect of Ni on the nitrification process (Aulakh et al., 1984; Di et al., 2009; Guo et al., 2013; Lan et al., 2013). In current study, we found that the N_2 -N fluxes under the Ni-amended treatment were lower than those under other treatments (except N_0 ; Fig. 2). The alternation of drainage and flooding favors nitrification and may induce large amounts of N_2O emissions (Lan et al., 2013), particularly in fertilized systems. This has commonly been reported in previous studies (Sun et al., 2015; Zhang et al., 2016). Thus, Ni also exhibited inhibitor effects on nitrification during drainage period, decreasing nitrification rates and mitigating gaseous N loss.

5 Conclusions

While both biochar and the Ni application increased rice yields, their effects on N loss varied. Biochar addition benefited crop production, but it also increased N loss through both denitrification and NH_3 volatilization though it mitigated N_2O -N emissions. The Ni application promoted NH_3 volatilization, but suppressed denitrification, and significantly decreased total gaseous N loss. Thus, Ni may be an optimal amendment to increase rice yield and decrease gaseous N loss, and its associated environmental cost, for rice production in the Taihu Lake region of China.

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673 Table 1 The rate of fertilizer application over the rice-growing season

Treatments	Fertilizer type	Basal Fertilization	Tillering topdressing	Anthesis topdressing	Heading topdressing
N0	P ₂ O ₅ :K ₂ O (kg ha ⁻¹)	90:120			
N270	N: P ₂ O ₅ :K ₂ O (kg ha ⁻¹)	135:90:60	27:0:0	54:0:60	54:0:0
N270+C	N: P ₂ O ₅ :K ₂ O (kg ha ⁻¹)	135:90:60	27:0:0	54:0:60	54:0:0
	Biochar (t ha ⁻¹)	25			
N270+Ni	N: P ₂ O ₅ :K ₂ O (kg ha ⁻¹)	135:90:60	27:0:0	54:0:60	54:0:0
	Nitrification inhibitor (kg ha ⁻¹)	0.675	0.135	0.27	0.27

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676 Table 2 NH₄⁺-N and pH concentrations of surface water under different treatments after each nitrogen (N) application

Treatments	Rice cycle	Basal Fertilization		Tillering topdressing		Anthesis topdressing		Heading topdressing	
		NH ₄ ⁺ -N (mg L ⁻¹)	pH	NH ₄ ⁺ -N (mg L ⁻¹)	pH	NH ₄ ⁺ -N (mg L ⁻¹)	pH	NH ₄ ⁺ -N (mg L ⁻¹)	pH
N0	2015	0.88±0.38a	7.69±0.07a	0.26±0.14a	7.76±0.09a	0.85±0.37a	7.45±0.05a	0.14±0.03a	7.57±0.18a
	2016	0.48±0.18A	7.71±0.06A	0.44±0.08A	7.63±0.04A	0.40±0.15A	7.53±0.12A	1.34±0.32A	7.51±0.12A
N270	2015	32.31±2.31b	8.17±0.07b	15.48±3.91b	8.23±0.02b	24.01±3.41b	7.53±0.17a	23.05±3.26b	7.70±0.17a
	2016	31.57±7.46B	8.17±0.07B	24.03±2.10B	7.85±0.02B	18.69±2.77B	7.65±0.15B	15.85±3.48B	7.63±0.15A
N270+C	2015	32.84±3.03b	8.35±0.03c	18.94±1.41c	8.32±0.07b	31.55±0.67c	7.93±0.07b	23.05±4.25b	8.00±0.03b
	2016	35.29±3.79BC	8.35±0.03C	23.88±1.69B	8.09±0.07C	21.87±2.52C	7.93±0.04C	17.58±3.68B	8.04±0.05B
N270+Ni	2015	37.66±2.72c	8.25±0.02b	20.60±1.55c	8.26±0.02b	33.61±1.10c	7.91±0.04b	25.54±2.96b	7.95±0.05b
	2016	41.30±6.70C	8.33±0.08C	27.94±2.21C	8.03±0.07C	24.35±0.32C	7.90±0.06C	19.41±0.63B	8.01±0.03B

677 Different lowercase and capital letters in a single column indicate differences ($p < 0.05$) between the treatments in 2015 and in 2016, respectively.

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681 Table 3 Grain yields (t ha^{-1}), weighted mean N_2 -N fluxes and NH_3 -N fluxes on all of the sampling days ($\text{kg N ha}^{-1} \text{d}^{-1}$) per treatment.

	Year	Treatments			
		N0	N270	N270 + C	N270 + Ni
Grain yields (t ha^{-1})	2015	7.34±0.27a	9.87±0.35b	10.38±0.60b	10.48±0.42b
	2016	7.65±0.17a	10.22±0.94b	10.65±0.96b	10.97±0.62b
N_2 -N fluxes ($\text{kg N ha}^{-1} \text{d}^{-1}$)	2015	0.164±0.040a	0.269±0.103b	0.295±0.080b	0.243±0.050c
	2016	0.319±0.010a	0.422±0.025b	0.436±0.017b	0.340±0.009a
NH_3 -N fluxes ($\text{kg N ha}^{-1} \text{d}^{-1}$)	2015	0.089±0.007a	0.233±0.010b	0.253±0.011b	0.234±0.017b
	2016	0.123±0.009a	0.364±0.020b	0.429±0.041c	0.439±0.019c

682 Different lowercase letters in the same row indicate significant differences between different treatments at $p < 0.05$.

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684 Table 4 Relationships among soil net N_2 -N fluxes and the physiochemical variables of floodwater and soil ($n = 63$)

	N_2^a	NH_3^b	Surface water properties				Soil properties			
			NO_3^- -N	NH_4^+ -N	DOC ^c	pH	NO_3^- -N	NH_4^+ -N	DOC	pH
N_2	1	0.702**	0.687**	0.693**	0.739**	0.714**	0.026	0.798(**)	0.454(**)	0.100
NH_3	0.702**	1	0.560**	0.814**	0.796**	0.831**	-0.039	0.802(**)	0.296(*)	0.067

685 ^a N_2 , soil net N_2 -N fluxes ($\text{kg N ha}^{-1} \text{day}^{-1}$), ^b NH_3 , NH_3 volatilization ($\text{kg N ha}^{-1} \text{day}^{-1}$), ^c DOC, dissolved organic carbon concentration.

686 * $p < 0.05$, ** $p < 0.01$

Figure captions

Fig.1. The dynamics of surface water and soil (0–10 cm) chemical properties (a: surface water $\text{NH}_4^+\text{-N}$; b: surface water $\text{NO}_3^-\text{-N}$; c: soil $\text{NH}_4^+\text{-N}$; d: soil $\text{NO}_3^-\text{-N}$) in different sampling days. The values are the means with their standard deviations ($n = 4$). The down arrow indicates the N application event.

Fig.2. Daily variations of the dissolved $\text{N}_2\text{-N}$ fluxes from the treatment plots over the rice growing seasons in 2015 and 2016. The values are the means with their standard deviations ($n = 4$). The down arrows indicate N applications.

Fig.3. The dissolved $\text{N}_2\text{-N}$ fluxes of N0, N270, N270 + C and N270 + Ni at different phenological stages of rice growth in 2015 and 2016. The values are the means with their standard deviations. The lowercase letters in the parentheses recurrent differences at the same phenological stages among N0, N270, N270 + C and N270 + Ni treatments at $p < 0.05$.

Fig.4. Daily variations of the $\text{NH}_3\text{-N}$ volatilization fluxes from the treatment plots over the rice growing seasons in 2015 and 2016. The values are the means with their standard deviations ($n = 4$). The down arrows indicate N applications.

Fig.5. The $\text{NH}_3\text{-N}$ volatilization fluxes of N0, N270, N270 + C and N270 + Ni treatments at different phenological stages of rice growth in 2015 and 2016. The values are the means with their standard deviations. The lowercase letters in the parentheses recurrent differences at the same phenological stages among N0, N270, N270 + C and N270 + Ni treatments at $p < 0.05$.

Fig.6. Cumulative $\text{N}_2\text{-N}$ losses and $\text{NH}_3\text{-N}$ losses from the treatment plots over the 2015 and 2016 rice growing seasons. The bars represent seasonally cumulative emissions, with the vertical lines indicating the standard deviations ($n = 4$). Different lowercase letters above the bars indicate significant differences among the different treatment plots in the same season, at $p < 0.05$; the values in brackets “[]” indicate the N application (%).

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Fig. 1

Fig. 2

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Fig. 6

Graphical abstract: