Biol Fertil Soils (2015) 51:89–98
DOI 10.1007/s00374-014-0953-4

ORIGINAL PAPER

Denitrification and its product composition in typical Chinese paddy soils

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Received: 22 May 2014 / Revised: 20 August 2014 / Accepted: 22 August 2014 / Published online: 6 September 2014

Abstract Denitrification and its products composition were evaluated in four typical Chinese paddy soils with pH (H_2O) ranging from 4.80 to 8.29 after application of 50 or 100 mg kg^{-1} soil $K^{15}NO_3$ and subsequent anaerobic incubation. Denitrification rates, which were indicated by nitric oxide (NO), nitrous oxide (N_2O), and dinitrogen gas (N_2) production, significantly varied among different paddy soils. The denitrification rates of the neutral and alkaline paddy soils were 2.6 to 16.6 times higher, respectively, than those of acidic paddies. Furthermore, denitrification in paddy soils could produce end products other than N_2, and the product composition depended on the paddy soil type. The percentage of total N gases ($NO+N_2O+N_2$) present as N_2O was negatively and linearly correlated with denitrification rate ($P<0.05$). Soil pH and C/N showed positive effects on denitrification rate ($r=0.800$ and $r=0.781$, respectively, $P<0.05$ for both), but negative effects on the percentage of total N gases present as N_2O ($r=-0.976$, $P<0.01$ and $r=-0.781$, $P<0.05$, respectively). Denitrification rate and the percentage of total gases present as N_2O increased as the nitrate (NO_3^-) concentration increased. However, there was no effect of NO_3^- concentration on the percentage of total N gases present as NO. Our results indicate that the potential N loss through denitrification may be higher in alkaline paddies than that in neutral and acidic paddies. Moreover, the variation of the N_2O percentage in denitrification products of different paddy soils should be considered when estimating the denitrification-derived N_2O emission and when calculating the N budget in paddy soils.

Keywords Denitrification · Nitrous oxide · Nitric oxide · Dinitrogen gas · Paddy soil

Introduction

Reactive nitrogen (Nr) levels have increased dramatically worldwide due to anthropogenic activities, particularly N-fertilizer production and use as well as fossil fuel combustion (Galloway et al. 2004). As a consequence, environmental Nr is accumulating at local, regional, and global levels and the excess of Nr can have negative impacts on the environment, such as the greenhouse effect, destruction of the ozone layer, acid rain, nitrate pollution in groundwater, eutrophication of lakes, and offshore water (Vitousek et al. 1997).

Denitrification is the reduction of NO_3^- through the intermediates nitrite (NO_2^-), nitric oxide (NO), and nitrous oxide (N_2O) to form dinitrogen gas (N_2). It is the only pathway by which Nr in terrestrial and aquatic ecosystems are transformed back into inert N_2 gas (Galloway et al. 2004), but denitrification in soil can increase N_2O and NO concentrations in the atmosphere.

The denitrification activity depends on the oxygen partial pressure, pH, NO_3^- concentration, temperature, availability of electron donors, and the quality and quantity of organic materials (Šimek et al. 2000; D'Haene et al. 2003; Amha and Bohne 2011; Rahman et al. 2014). Losses resulting from the complete reduction of NO_3^- to N_2 are rarely measured directly in the field; the large atmospheric N_2 background makes detecting slight increases in N_2 caused by denitrification analytically difficult (Davidson and Seitzinger 2006). Although

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Submitted to: *Biology and Fertility of Soils*

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N_2O is generally regarded as an intermediate of the denitrification pathway to N_2, it can also be a denitrification end product by denitrifying bacteria that lack nitrous oxide reductase (Papen et al. 1989), further complicating the accurate estimation of the denitrification rate. Therefore, knowledge of the soil denitrification and its product composition are essential in determining the soil N budget.

China is one of the major rice growers in the world with approximately 20 % of the world's total area dedicated to rice production (Frolking et al. 2002). The current average N-use efficiency of rice cultivation systems in China (% recovery of applied N in plants) ranges from 30 % to 40 % (Zhu and Chen 2002). A major reason for low N-use efficiency in rice paddy fields is the loss of gaseous N through denitrification (Xing and Zhu 2000; Zhu and Chen 2002; Li and Lang 2014).

Numerous studies have investigated denitrification rate in paddy soils, but mostly by the measurement of the amount of NO_3^- lost (Aulakh et al. 2001; Xing et al. 2002; Zhu et al. 2003; Wang et al. 2011; Ma et al. 2013), detailed knowledge of the total gaseous ($NO+N_2O+N_2$) losses of N via denitrification in rice paddy soils remains inadequate, and the mechanisms underlying N_2O and NO emissions in paddy soil through denitrification remain unclear. As reported by Zhang et al. (2009), the amount of total N gases produced is a better measure of denitrification rate than the amount of NO_3^- lost because NO_3^- may be consumed through immobilization or the dissimilatory reduction of NO_3^- to NH_4^+ (DNRA). Furthermore, the denitrification rate in flooded soils is not controlled by the activity of denitrification enzymes but rather by the rate of NO_3^- production through nitrification process (Zhou et al. 2012), which occurs in aerobic micro zone such as the rhizosphere and the interface between standing water and the soil (Nicolaisen et al. 2004). In other words, denitrification rate is substrate depended. Nevertheless, it is not clear whether NO_3^- concentration would affect denitrification rate and its product composition.

The measurement of denitrification rates in situ is problematic due to the presence of heterogeneous communities of denitrifers and the physical and chemical complexity of soils (Betlach and Tiedje 1981), thus, elucidating denitrification and its product compositions in paddy soils through laboratory culture is vital to deducing the actual soil N loss through denitrification under field conditions. Therefore, in this study, we first reported the use of total N gases ($NO+N_2O+N_2$) production as an indicator of denitrificaton rate of paddy soils, and the effect of NO_3^- concentration on denitrification rate and its product composition was also investigated by a ^{15}N labeling anaerobic incubation experiment under laboratory conditions. Four typical Chinese paddy soils that differed in pH, clay content, and C/N ratio were chosen, and two NO_3^- concentrations (50 and 100 mg N kg^{-1} soil), which were within the range of observed NO_3^- concentrations in paddy soils after fertilization (Cai and Mosier 2000; Cai 2002), were adopted.

Materials and methods

Site description and soil sampling

Two paddy soils were collected from Yixing (31°17′N, 119°54′E) and Huai'an (33°43′N, 118°86′E), situated in the North and South Jiangsu provinces, respectively, and two from the Ecological Experiment Station of Red Soil, the Chinese Academy of Sciences, Yingtan (28°15′N, 116°55′E), Jiangxi Province. The four paddy soils were developed from different parent materials. The Yixing soil was developed from alluvial deposits and classified as Hydragric Anthrosol (Hy). The Huai'an soil was developed from lacustrine sediment and classified as Anthraquic Cambisol (An). One of the soil samples from Yingtan was derived from red sandstone and classified as Haplic Acrisol (Ha), whereas the other was derived from quaternary red clay and classified as Ferralsol (Fe) according to the World Reference Base for Soil Resources system (IUSS Working Group WRB 2007). Fifteen soil cores from each site were pooled, sieved (<2 mm), and immediately stored at 4 °C until analysis. The key properties of the soils are shown in Table 1.

Anaerobic incubation and sampling

Denitrification rate was determined using the anaerobic incubation method of Xu and Cai (2007) and slightly modified by Zhang et al. (2009). In brief, for each paddy soil sample, a set of 250 mL each Erlenmeyer flask contained 40 g (oven-dried equivalent) of fresh soil and 40 mL of deionized water. The flasks for each soil were then divided into two groups for two levels of NO_3^- concentration treatments; 2 mL of a $K^{15}NO_3$ (20 % ^{15}N atom % excess) solution containing 2.0 mg of NO_3^- (equivalent to 50 mg of NO_3^- kg^{-1} soil (N50)) or 4.0 mg of NO_3^- (equivalent to 100 mg of NO_3^- kg^{-1} soil (N100)) were uniformly added to each flask for groups 1 and 2, respectively, using a 2.5-mL syringe (no carbon addition). The flasks were immediately capped with airtight silicone rubber stopper fitted with butyl rubber septa. A silicone sealant was placed around the stoppers to ensure strictly airtight conditions. The flasks were connected to a multiport vacuum manifold to be vacuumed simultaneously and flushed with highly purified N_2 gas. Such procedure was repeated three times (each for 12 min) to create anaerobic conditions (Zhang et al. 2009). After equilibration at atmospheric pressure, the flasks were incubated in the dark at 25±1 °C. At 3, 6, 12, 24, 48, 96, 168, and 264 h after the addition of $^{15}NO_3^-$ solution, three flasks from each soil and nitrate treatment were randomly selected, and the headspace gases were sampled to determine the N_2O and NO concentrations and N_2O

Table 1 Some properties of the studied soils

Soil	Abbreviation	Total N ($g \cdot kg^{-1}$)	Total C	C/N	pH	Clay (<2 μm; %)	Silt (2 μm to 60 μm)	Sand (60 μm to 2,000 μm)
Hydragric anthrosol	Hy	1.35b[1]	14.9a	11.0a	6.20b	16.4b	82.9a	0.7c
Anthraquic cambisol	An	1.46ab	14.3a	9.79a	8.29a	44.8a	53.5b	1.8c
Haplic acrisol	Ha	1.61a	11.5b	7.03b	4.91c	15.6b	63.6b	20.8b
Ferralsol	Fe	1.19b	8.37c	7.14b	4.80c	35.8a	32.2c	33.0a

[1] The different letters in the columns indicate significant differences between soils at $P<0.05$

and N_2 isotopic compositions using a 25-mL syringe. Immediately after gas sampling, soil in the flask was extracted with 160 mL 2.5 M KCl solution. The mixture (solution plus soil) was shaken at 25 °C for 1 h and filtered through a qualitative filter paper. The filtrates were then stored at 4 °C prior being analyzed for mineral N (NO_3^- and NH_4^+) concentrations and the relative ^{15}N abundance. In order to determine the concentration and isotopic composition of insoluble organic N, the KCl-extracted soil was washed with distilled water to remove residual mineral N and subsequently oven-dried at 55 °C.

Analyses

The N_2O concentrations of samples were determined using an Agilent 7890 gas chromatograph (Agilent, USA). The NO concentrations in the samples were measured using an NO_x analyzer (Model 42i, Thermo Environmental Instruments Inc, Franklin, MA, USA). A segmented flow analyzer (Skalar SAN^{++}, Netherlands) was used to determine the concentrations of NH_4^+ and NO_3^- in soil extracts. The N_2O and N_2 isotopic compositions were determined using a Finnigan MAT 253 isotopic ratio mass spectrometer. A method based on N_2O production from hydroxylamine intermediates, after reduction with Cd/Cu, was used to determine ^{15}N enrichment of NO_3^- in KCl extracts (Stevens and Laughlin 1994), as described in detail by Lan et al. (2013). The ^{15}N enrichment of NH_4^+ in the KCl extracts was determined by distillation with MgO, and by isotopic mass spectrometry (Finnigan MAT 251) after converting NH_4^+ in soil to molecular N_2 using NaBrO. The concentration and ^{15}N composition of insoluble organic N remaining in the soil after KCl extraction were determined by isotopic mass spectrometry (Finnigan MAT 251) after converting NH_4^+ in soil to molecular N_2 by Kjeldahl digestion with NaBrO (Zhang et al. 2009).

Calculations and statistical analyses

N_2 production

The total amount of N_2 evolved in the Erlenmeyer flask was calculated as described by Zhang et al. (2009), by considering the flask headspace volume, N_2 density, and dissolved N_2 in the soil solution, as follows:

$$C = \left[\left(1.15 \times V_g\right) + \left(1.15 \times V_1 \times \alpha\right)\right] \times 10^6 / W \qquad (1)$$

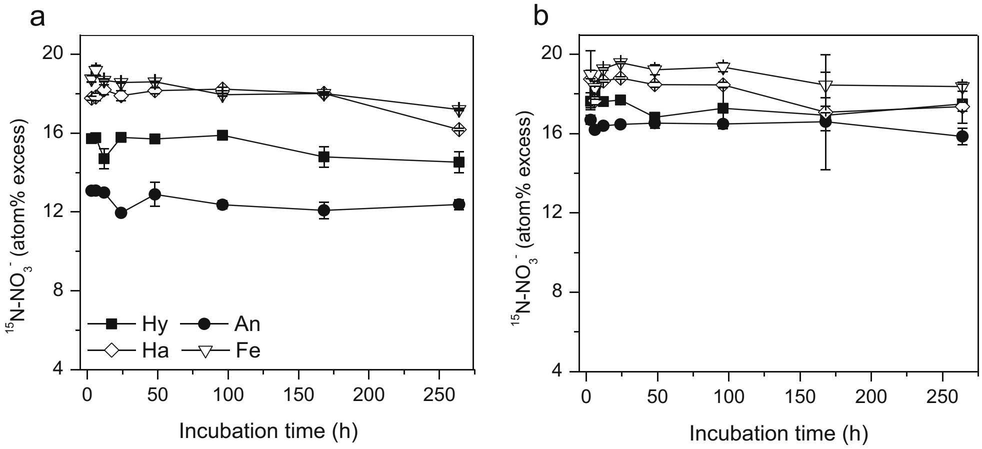
where C is the total amount of N_2 (mg kg^{-1} soil), 1.15 is the density of N_2 at standard pressure and 25 °C (kg m^{-3}), V_g is the headspace volume (m^3), V_1 is the water volume (m^3), α is the Bunsen correction coefficient (0.0143 at 25 °C), and W is the soil weight (kg). The change in air pressure in the flasks was insignificant during incubation and was ignored in the calculation of N_2 production.

The amount of $^{15}N_2$ and $^{14}N_2$ produced from denitrification during the anaerobic incubation period was calculated based on the total amount of N_2 in the flask calculated by Eq. (1), the measured atom % excess of ^{15}N in N_2, and by assuming that the isotopic composition of the produced N_2 was the same as that of the reduced soil NO_3^- (Zhang et al. 2009). To verify whether the atom % excess ^{15}N in NO_3^- could calculate N_2 gas production through denitrification, the temporal variation in the average atom % excess ^{15}N in NO_3^- was evaluated. The preliminary results showed that the atom % excess ^{15}N in NO_3^- slightly decreased in all soils and NO_3^- treatments during incubation (Fig. 1). The lowest atom % excess ^{15}N in NO_3^- was observed in An soil, whereas the highest was obtained in Fe soil. The temporal variations in the atom % excess ^{15}N in NO_3^- ranged from 1.52 % to 3.98 %, with the highest in the An-N50 treatment and the lowest in the Hy-N100 treatment. These results indicate the suitability of this approach.

Denitrification rate, percentage of total evolved N gases ($NO+N_2O+N_2$) present as N_2O or NO, and recovery of added NO_3^--^{15}N

The denitrification rate was expressed as the total N gases ($NO+N_2O+N_2$) production per hour during incubation. We also calculate the percentage of total N gases present as N_2O or NO. The recovery of added $^{15}NO_3^-$ after the anaerobic incubation was calculated using Eq. (2). ^{15}N enrichment in NO and dissolved organic N of KCl extracts were not considered because not determined.

Fig. 1 Temporal variations in the ^{15}N atom % excess of NO_3^- in paddy soils. (**a**, 50 mg N kg^{-1} labeled NO_3^- treatment; **b**, 100 mg N kg^{-1} labeled NO_3^- treatment; Hy, Hydragric Anthrosol, An, Anthraquic Cambisol; Ha, Haplic Acrisol; and Fe, Ferralsol. Data are the mean of three replicates. Bars represent standard deviations)



$$Recovery\,of\,^{15}N(\%) = \frac{(^{15}NH_4{}^{+}+^{15}NO_3{}^{-}+^{15}N_2O+^{15}N_2+insoluble\ organic\,^{15}N)}{added\,^{15}NO_3^-} \times 100\% \qquad (2)$$

Statistical analyses

The differences in the soil properties, denitrification rates, and percentage of total evolved N gases present as NO or N_2O of the different treatments were evaluated by ANOVA and compared by the Tukey's test at $P<0.05$, using SPSS software package 18.0 for Windows. Spearman's rank correlation coefficient analysis was used to determine the edaphic variables correlated with denitrification rate, and the percentage of total N gases present as NO or N_2O using the SPSS software package 18.0 for Windows.

Results

NO_3^- dynamics during incubation

Under anaerobic conditions, the added NO_3^- was continuously consumed with increasing incubation time in all tested soils, except the Ha soil in which maintained a high NO_3^- concentration during the incubation (Fig. 2a, b). The NO_3^- level was negligible in the Fe soil when it was treated with 50 mg N kg^{-1} NO_3^- after 168 h, and in the Hy and An soils after 264 h (Fig. 2a). By contrast, large amounts of NO_3^- (>20 mg N kg^{-1}) remained in all soils at the end of the incubation when treated with 100 mg of NO_3^- (Fig. 2b). However, when treated with both 50 and 100 mg of NO_3^-, the amount of consumed NO_3^- varied significantly among the different soils after 264 h, soils could be ranked An>Hy>Fe>Ha (Fig. 2b).

Nitrogen gas production patterns and denitrification rate

The dynamics of N gases production in different soils significantly varied. Both NO and N_2O concentrations were detected in Hy and An soils 3 h after the start of incubation regardless of the added NO_3^- concentration (Fig. 3a–d); NO and N_2O production in Ha and Fe soils were detected only after 12 and 24 h, respectively (Fig. 3a–d). The N_2O concentrations remained at high levels in the two acidic paddy soils (Ha and Fe), whereas the NO concentrations were at considerably lower levels (Fig. 3a–d). The NO production generally peaked earlier than that of N_2O regardless of the NO_3^- treatment (Fig. 3a–d), and NO was no longer detected in Hy and An soils after 96 h (Fig. 3a, b). Nitrous oxide remained detectable in all treatments at the end of incubation except in the An-N50 treatment, where it was undetectable after 168 h (Fig.3c, d). ^{15}N-labeled N_2 was not detected in Hy and An soils treated with both NO_3^- concentrations until after 6 h, and it was until 168 h in the two acidic paddy soils (Ha and Fe; Fig. 3e, f). Dinitrogen gas production in all soils and NO_3^- treatments continuously increased as the incubation proceeded (Fig. 3e, f). However, at the end of the incubation, the total N gases ($NO+N_2O+N_2$) that accumulated in the headspace significantly varied among soils and nitrate treatments ($P<0.05$; Fig. 3g, h). The accumulated total N gases in the N100 treatment were approximately 1.2 to 1.4 times higher than those of the corresponding N50 treatment. The calculated denitrification rates significantly varied among the treatments after 264 h of incubation, ranging from 0.014 mg N kg^{-1} h^{-1} in the Ha-N50 treatment to 0.273 mg N kg^{-1} h^{-1} in the An-N100 treatment (Table 2). The denitrification rates of the neutral and alkaline paddy soils were 2.6 to 16.6 times higher, respectively, than those of acidic paddies (Table 2).

Recovery of added $^{15}NO_3^-$

Labeled ^{15}N was not fully recovered as NO_3^-, NH_4^+, N_2O, N_2, and insoluble organic N. The $^{15}NO_3^-$ recovery rate in all soils

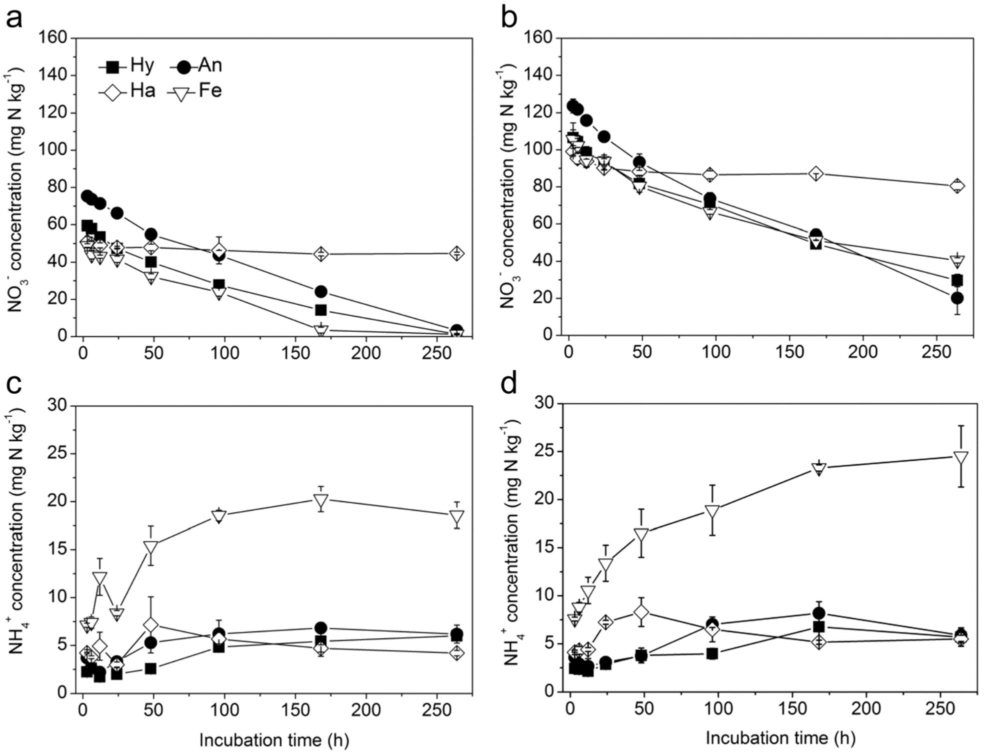


Fig. 2 Changes in the NH_4^+-N and NO_3^--N concentrations in paddy soils during anaerobic incubation. (**a** and **c**, 50 mg N kg^{-1} labeled NO_3^- treatment; b and d, 100 mg N kg^{-1} labeled NO_3^- treatment; Hy, Hydragric Anthrosol; An, Anthraquic Cambisol; Ha, Haplic Acrisol; and Fe, Ferralsol. Data are the mean of three replicates. Bars represent standard deviations)

was approximately 90 % 3 h after NO_3^- addition to soil. This value gradually decreased to 56 % to 69 % in the N50 treatment and to 61 % to 78 % in the N100 treatment at the end of the incubation. Moreover, ^{15}N was detected in NH_4^+ (particularly in Fe soil; Fig. 2c, d) and soil organic matter (data not shown) after incubation.

Percentage of the total evolved N gases ($NO+N_2O+N_2$) presented as NO and N_2O

The percentage of total N gases present as NO was negligible (<1 %) in the two acidic paddy soils during the entire 264 h of incubation and it was significantly higher in Hy and An soils (Fig. 4a, b), where it decreased as the incubation proceeded and became undetectable after 96 h. The percentage of total N gases present as NO was higher in Hy soil than that in An soil (Fig. 4a, b). However, there was no difference in the percentage of total N gases present as NO when the N50 and N100 treatments were compared (Table 2).

The percentage of total N gases present as N_2O was generally higher than the percentage of total N gases present as NO during the all incubation in all treatments, particularly in the two acidic paddy soils (Fig. 4). The percentage of total N gases present as N_2O increased between 3 and 12 h in Hy and An soils and between 3 and 96 h in Ha and Fe soils but subsequently decreased in all soils and NO_3^- treatments (Figs. 4c, d). The acidic paddy soils (Ha and Fe) maintained higher percentage of total N gases present as N_2O than the neutral (Hy) and alkaline (An) paddy soils. The average percentage of total N gases present as N_2O in Hy and An soils were significantly lower than those in the acidic paddy soils, in which the percentages exceeded 60 % (Table 2). Furthermore, the addition of NO_3^- to soil increased the percentage of total N gases present as N_2O in all tested paddy soils (Table 2).

Discussion

The four paddy soils developed from different parent materials significantly differed in NO_3^- consumption, which ranged from negligible to complete disappearance of NO_3^- at the end of incubation at 25 °C when NO_3^- was added at a rate of 50 mg N kg^{-1} soil (Fig. 2). The obtained denitrification rates of Hy and An soils collected from Jiangsu Province were higher than those of Ha and Fe soils collected from Jiangxi Province when the N50 treatments were compared (Table 2). This confirms the reported positive correlation between denitrification rate and latitude in the forest soils of eastern China by Zhang et al. (2009). However, the denitrification rates of the four paddy soils (from 0.014 mg N kg^{-1} h^{-1} to 0.273 mg N kg^{-1} h^{-1}) were generally higher than those of forest soils (from 0.011 mg N kg^{-1} h^{-1} to 0.127 mg N kg^{-1} h^{-1}) collected from different climatic zones of China and measured using the same method (Zhang et al. 2009). Similar results were reported by Xu and Cai (2007), who found that rice cultivation significantly increases denitrification rate (based

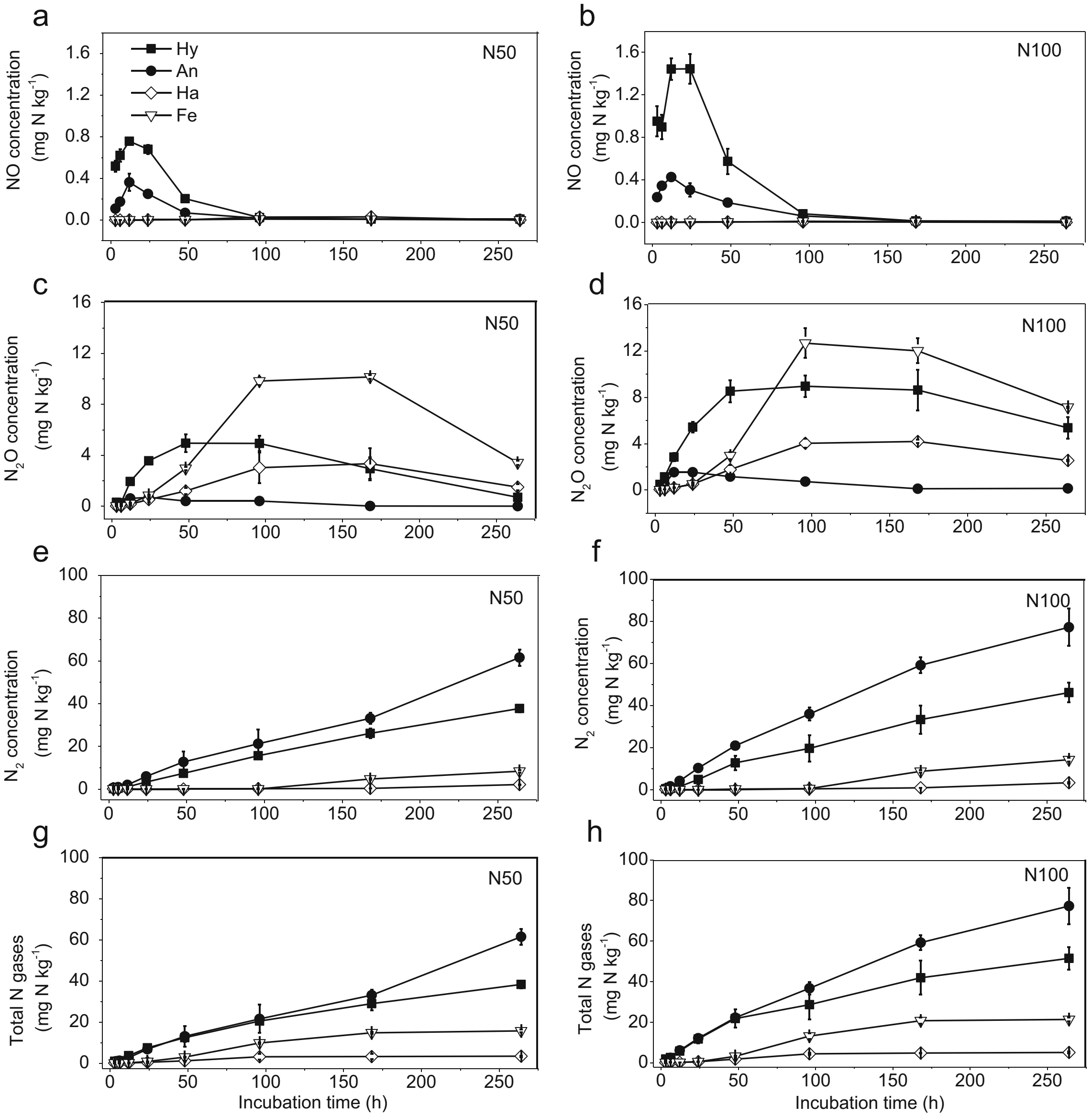


Fig. 3 Production of NO-N, N_2O-N, and N_2-N in paddy soils during anaerobic incubation. (Hy, Hydragric Anthrosol; An, Anthraquic Cambisol; Ha, Haplic Acrisol; and Fe, Ferralsol. N50, 50 mg N kg^{-1} labeled NO_3^- treatment; N100, 100 mg N kg^{-1} labeled NO_3^- treatment. Data are the mean of three replicates. Bars represent standard deviations

on NO_3^- concentration measurements) compared with four other land uses (tea garden land, forestland, brush land, and upland). According to Xu and Cai (2007), this phenomenon is most probably due to increases in the organic C and total N contents in the soil, which promote the population growth and activities of microbial under anaerobic conditions of flooded rice fields.

Parent materials generally affect soil formation processes and lead to differences in soil physicochemical properties, which subsequently affect N transformations (Miller and Donahue 1990). Denitrification rate was reported to be correlated with the amount of easily-decomposable soil organic C, as well as soil pH and C/N (D'Haene et al. 2003; Amha and Bohne 2011). In this study, the four paddy soils developed from different parent materials exhibited different properties such as pH, clay content, and C/N ratio. Spearman's correlation coefficients indicate that the denitrification rates of four Chinese paddy soils were significantly correlated with soil pH (r=0.800, P<0.05) and C/N ratio (r=0.781, P<0.05). Thus, soil pH and C/N may be important regulators of denitrification in paddy soils. The slightly alkaline paddy soil (An) had higher denitrification rate than the neutral (Hy) and acidic (Ha and Fe) paddy soils, which confirming that the optimum pH for denitrifiers is between 7.0 and 8.0 as reported by Wijler and Delwiche (1954) and Davidson et al. (1985). However, Xu and Cai (2007) and Zhang et al. (2009) were unable to

Table 2 Average denitrification rate and percentage of total N gases (NO+N_2O+NO) present as NO and N_2O during anaerobic incubation at 25 °C

Nitrate treatment[1] (mg N kg^{-1})	Soil[2]	Denitrification rate (mg N kg^{-1} h^{-1})	NO percentage (%)[3]	N_2O percentage (%)
N50	Hy	0.146±0.012b	2.12±0.53a	15.2±1.91b
	An	0.233±0.009a	0.66±0.17b	1.86±0.21c
	Ha	0.014±0.009d	0.44±0.12b	67.6±4.86a
	Fe	0.056±0.016c	0.05±0.01c	59.2±4.54a
N100	Hy	0.185±0.011b	2.35±0.22a	22.6±1.09b
	An	0.273±0.031a	0.82±0.14b	2.46±0.35c
	Ha	0.020±0.011d	0.17±0.03c	73.8±8.15a
	Fe	0.081±0.021c	0.06±0.02c	64.5±4.49a

[1] N50, NO_3^--50 mg N kg^{-1} treatment; N100, NO_3^--100 mg N kg^{-1} treatment.

[2] *Hy* hydragric anthrosol, *An* anthraquic cambisol, *Ha* haplic acrisol, *Fe* ferralsol

[3] Data the mean of three replicates±standard deviation. The different letters in the columns indicate significant differences among soils at $P<0.05$ for each nitrate treatment

establish any relationship between pH and denitrification rate. Denitrification is an electron-consuming and heterotrophic process (Ahn 2006), in which the available C provides electrons for NO_3^- reduction, thereby promoting denitrification (Heinen 2006). This phenomenon may explain the positive relationship between denitrification rate and the C/N ratio. Therefore, low denitrification rates in Ha and Fe soils may due to the small amount of easily-decomposable soil organic C, which worth further investigation.

The denitrification rates of the four tested paddy soils increased as the NO_3^- concentration increased. The added NO_3^- possibly affected denitrification through two different ways: (a) by directly regulating the availability of the denitrification substrate and (b) by indirectly affecting denitrifying microbes (Drury et al. 1991; Amha and Bohne 2011). The NO_3^- supply in flooded paddy soils is primarily derived from the nitrification of added ammonia or urea fertilizers. Evidence showed that the nitrification rate increases with increasing pH (from 6.0 to 8.5), with the optimum pH at approximately 8.5 (Sahrawat 1982, 2008). Thus, the denitrification-induced loss of the NO_3^- produced through the higher nitrification rates of alkaline paddy soils would

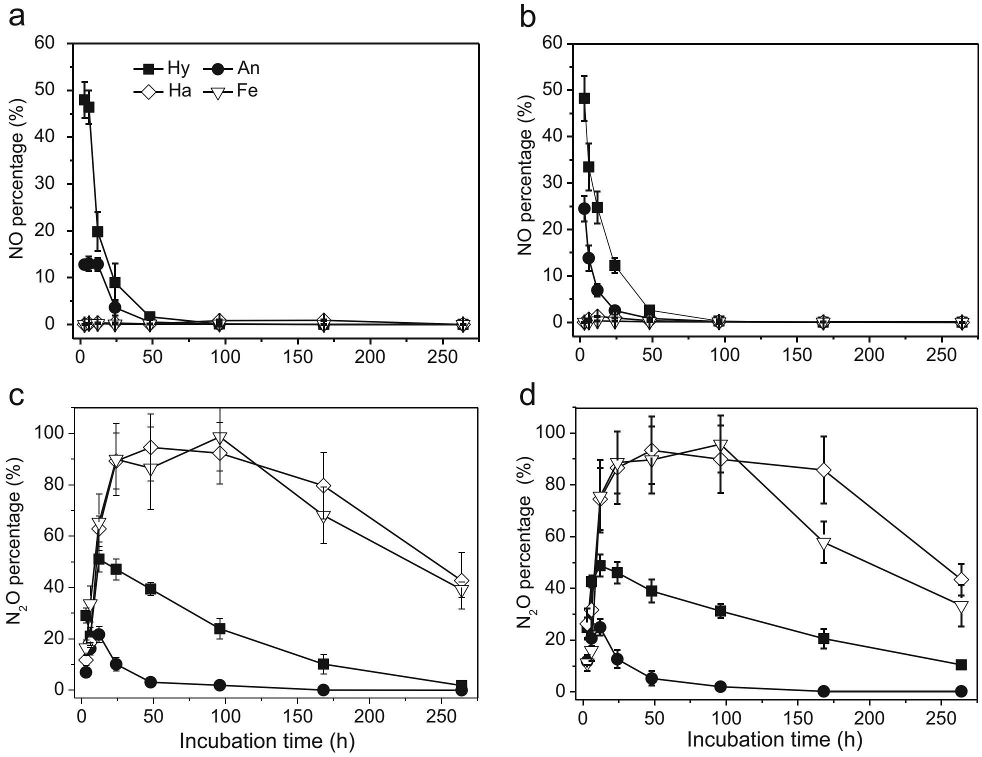


Fig. 4 Changes in the percentage of total evolved N gases present as NO (**a**, **b**) and as N_2O (**c**, **d**) in paddy soils during anaerobic incubation (**a**, **c** 50 mg N kg^{-1} labeled NO_3^- treatment; **b**, **d** 100 mg N kg^{-1} labeled NO_3^- treatment; *Hy* hydragric anthrosol, *An* anthraquic cambisol, *Ha* haplic acrisol, *Fe* ferralsol. Data are the mean of three replicates. *Bars* represent standard deviations)

probably be higher than those in neutral and acidic paddy soils. However, our results also show that when the added NO_3^- concentration was increased from 50 mg N kg^{-1} to 100 mg N kg^{-1}, the denitrification rates in the paddy soils exhibited only an approximately 1.2- to 1.4-fold increase, which indicates that denitrification rate gradually increased when the NO_3^- concentration exceeded 50 mg N kg^{-1}. This is in agreement with many models that consider denitrification to be a function of NO_3^- concentration, as described by Michaelis–Menten relationship (Heinen 2006).

The added of NO_3^--N was not recovered by summing up the N gas products, NH_4^+-N, and organic N at the end of incubation. The amount of N gases produced is a more precise measure of the denitrification rate than the amount of NO_3^- consumed, as also reported by Zhang et al. (2009) and Yu et al. (2014). The greater increase in the NH_4^+ concentration was observed in Fe soil (Fig. 2c, d). Ammonium production through the dissimilatory reduction of NO_3^- to NH_4^+ (DNRA) has been observed in rice paddy soils (reaching 21 % of NO_3^- consumption; Chen et al. 1995a, 1995b; Yin et al. 2002). There is evidence that many soil bacteria and fungi have the ability to perform DNRA. Redox status and ratio of C/NO_3^- have been identified as the most important factors regulating DNRA in soil (Rutting et al. 2011). However, factors affecting DNRA in paddy soils are still poorly known.

The denitrification of these paddy soils produce end products other than N_2, and product composition depended on the paddy soil type (Table 2). The percentage of total N gases present as NO were smaller than those percentage of total N gases present as N_2O regardless of the soil and NO_3^- treatment (Fig. 4). The acidic paddy soils showed a significantly smaller NO than N_2O percentage than the alkaline and neutral paddy soils (Table 2). This result probably lead to the absence of a significant correlation between the NO and N_2O percentages ($r=0.161$, $P>0.05$). The percentage of total N gases present as NO was correlated with total C content, but not with soil pH and NO_3^- concentration.

In the two acidic paddy soils (Ha and Fe), the percentage of total N gases present as N_2O was approximately 60 % (Table 2); whereas in Hy and An paddy soils, it was below 25 % and N_2 was the main product. The decreased N_2O production at the end of incubation was probably due to the strong reducing conditions, which promoted the complete reduction of NO_3^- to N_2. The percentage of total N gases present as N_2O was negatively and linearly correlated with denitrification rate when the same amount of NO_3^- was added to soil (Fig. 5), which implies that the soil with lower denitrification rate tends to have a higher N_2O proportion in the denitrification products.

The variations in composition of denitrification products of the different paddy soils could be ascribed to their different soil properties. The N gases produced during soil denitrification are reportedly dependent on various edaphic and environmental factors (Zhang et al. 2009; Senbayram et al. 2012). In the present study, a negative Spearman's correlation was established between the percentage of total N gases produced as N_2O and soil pH ($r=-0.976$, $P<0.01$, Table 3), which confirms previous laboratory and field studies, showing that the N_2O/N_2 ratio decreases when the soil pH increases (Goodroad and Keeney 1984; Šimek and Cooper 2002; Liu et al. 2010). Moreover, the percentage of total N gases present as N_2O was also negatively correlated with soil C/N ratio ($r=-0.781$, $P<0.05$, Table 3), probably because higher available organic C substrates in soil promote the complete reduction of low to moderate levels of NO_3 to N_2 gas, thus reducing the evolved N_2O (Ullah et al. 2005). The addition of NO_3^- to soil increased the percentage of total N gases present as N_2O in all tested paddy soils (Table 2), probably because NO_3 reduction is more energy-efficient than that of N_2O (Ullah et al. 2005). Our results suggest that soils that had lower denitrification rates had higher percentage of total N gases present as N_2O in their denitrification products at the same NO_3^- concentration, whereas increased NO_3^-

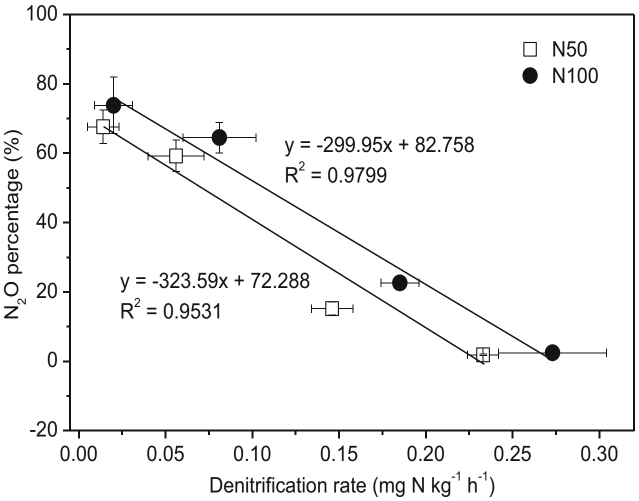


Fig. 5 Relationship between denitrification rate and the percentage of total evolved N gases present as N_2O (N50, 50 mg N kg^{-1} labeled NO_3^- treatment; N100, 100 mg N kg^{-1} labeled NO_3^- treatment. Data are the mean of three replicates. *Bars* represent standard deviations)

Table 3 Spearman's correlation coefficients of denitrification rate and percentage of total evolved N gases (NO+N_2O+NO) present as NO and N_2O with soil properties

Variable	Denitrification rate	NO ratio	N_2O ratio
pH	0.800*	0.586	−0.976**
Clay	0.743	0.095	−0.743
TC	0.586	0.976**	−0.586
C/N	0.781*	0.743	−0.781*

*$P=0.05$ and **$P=0.01$ levels (two-tailed), significant correlations

concentration promoted denitrification and increased the percentage of N_2O in the produced N gases.

Conclusions

Denitrification rate and its product compositions significantly varied among the studied paddy soils. The potential N loss through denitrification was higher in the alkaline paddy soils than that in the neutral and acidic paddy soils, and the N loss was also higher in soils with high C/N ratios. Denitrification in paddy soils could produce end products other than N_2, therefore, the variation of the N_2O proportion in denitrification products of different paddy soils should be considered when estimating the denitrification-derived N_2O emission and when calculating the N budget in paddy soils. However, further studies must be conducted to confirm our results regarding the denitrification and associated N gas production mechanisms in paddy soils. Future work should include an increased number of soil types to establish more definitively the factors affecting denitrification in the paddy soils of China.

Acknowledgments This project was financially supported by the following Sino-German collaborative projects: “Innovative nitrogen management technologies to improve agricultural production and environmental protection in intensive Chinese agriculture” funded by the German Ministry of Education and Research (BMBF FKZ: 0330800C), “Cultivated land conservation and nitrogen fertilizer management technology” funded by the Ministry of Science and Technology of China (MOST grant no. 2007DFA30850), and the DAAD-PPP project (project ID 50751522) with the China Scholarship Council (personal approval no. 2011016097).

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