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Soil N transformation mechanisms can effectively conserve N in soil under saturated conditions compared to unsaturated conditions in subtropical China

Yushu Zhang[1,2,3] · Hong Ding[1] · Xiangzhou Zheng[1] · Zucong Cai[2,4,5] · Tom Misselbrook[3] · Alison Carswell[3] · Christoph Müller[6,7] · Jinbo Zhang[2,4,5]

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Abstract
The connection between moisture and nitrogen (N) transformation in soils is key to understanding N losses, particularly nitrate (NO_3^-) losses, and also provides a theoretical framework for appropriate water management in agricultural systems. Thus, we designed this study to provide a process-based background for management decision. We collected soil samples from the long-term field experiment in subtropical China, which was designed to examine tobacco and rice rotations under a subtropical monsoon climate. The field experiment was established in 2008 with four treatments: (1) no fertilization as control; (2) N, phosphorus (P), and potassium (K) fertilizers applied at recommended rates; (3) N fertilizers applied at rates 50% higher than the recommended amounts and P and K fertilizers applied at recommended rates; and (4) N, P, and K fertilizers applied at recommended rates with straw incorporated (NPKS). Soil samples were collected during the unsaturated tobacco-cropping season and saturated rice-cropping season and were incubated at 60% water holding capacity and under saturated conditions, respectively. Two ^{15}N tracing treatments ($^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$) and a numerical modeling method were used to quantify N transformations and gross N dynamics. Autotrophic nitrification was stimulated by N fertilizer both under unsaturated and saturated conditions. The rate of NO_3^- consumption (via immobilization and denitrification) increased under the NPKS treatment under saturated conditions. Secondly, the rates of processes associated with ammonium (NH_4^+) cycling, including mineralization of organic N, NH_4^+ immobilization, and dissimilatory NO_3^- reduction to NH_4^+, were all increased under saturated conditions relative to unsaturated conditions, except for autotrophic nitrification. Consequently, NO_3^--N and NH_4^+-N concentrations were significantly lower under saturated conditions relative to unsaturated conditions, which resulted in reduced risks of N losses via runoff or leaching. Our results suggest that under saturated conditions, there is a soil N conservation mechanism which alleviates the potential risk of N losses by runoff or leaching.

Keywords ^{15}N · N transformations · Nitrogen retention · Saturated soils

✉ Jinbo Zhang
zhangjinbo@njnu.edu.cn

Yushu Zhang
zys3505@126.com

1 Institute of Soil and Fertilizer, Fujian Academy of Agricultural Sciences, Fuzhou 350013, People's Republic of China

2 School of Geography Sciences, Nanjing Normal University, Nanjing 210023, People's Republic of China

3 Sustainable Agricultural Sciences, Rothamsted Research, North Wyke, Devon EX20 2SB, UK

4 Jiangsu Provincial Key Laboratory of Materials Cycling and Pollution Control, Nanjing 210023, People's Republic of China

5 Jiangsu Center for Collaborative Innovation in Geographical Information Resource Development and Application, Nanjing 210023, People's Republic of China

6 Department of Plant Ecology, Justus-Liebig University Giessen, Heinrich-Buff-Ring 26, 35392 Giessen, Germany

7 School of Biology and Environmental Science, University College Dublin, Belfield, Dublin 4, Ireland

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Introduction

Nitrogen (N) is one of the key elements required for crop growth. However, N fertilizer application to agricultural systems has had multiple negative effects on the environment (Sainju et al. 2008). In some natural ecosystems, reactive N is effectively conserved via the prevailing inherent soil N dynamics (Huygens et al. 2007; Rütting et al. 2008; Rütting and Müller 2007). In subtropical regions, which is characterized by the high precipitation, NO_3^- leaching losses occur readily via leaching or runoff, while NH_4^+ is less mobile (Huygens et al. 2007; Rütting and Müller 2007, 2008). Rates of autotrophic nitrification in native soils in the subtropical regions have been reported as low or absent due to low soil pH (Zhao et al. 2007), while rates of NO_3^- immobilization to organic N reported were relatively high (Zhang et al. 2013a), especially in low NH_4^+-N soils (Rice and Tiedje 1989). As a consequence, inorganic N in the native soils of these regions is dominated by NH_4^+, and available NO_3^- is thought to be conserved efficiently by NO_3^- consumption processes (Zhu et al. 2013b). However, when native land is converted to agricultural use, microbial immobilization of NO_3^- is substantially suppressed and nitrification is stimulated (Schimel and Bennett 2004), leading to NO_3^- dominance in inorganic N in the soil, with associated risks of N loss through runoff, leaching, and denitrification. Therefore, N retention in native subtropical soils is likely to be reduced when converted to agriculture (Han et al. 2012; Yang et al. 2010; Zhang et al. 2013a).

However, N transformations in these agricultural soils may differ under different water regimes. For example, Choi et al. (2003) showed that nitrification was stimulated following ammonium sulfate addition under unsaturated conditions, whereas under saturated conditions, immobilization and subsequent mineralization of organic N to NH_4^+ dominated. Nitrification in saturated soils is assumed to be very low, because of oxygen-limiting conditions. Additionally, dissimilatory NO_3^- reduction to NH_4^+ (DNRA) would become an important NO_3^- removal pathway, particularly under saturated conditions (Yin et al. 2002; Zhang et al. 2015a). During DNRA, N losses via leaching and gaseous emissions can be reduced by decreasing the size of the NO_3^- pool in soil and increasing the NH_4^+ pool, therefore providing additional NH_4^+ for immobilization or for uptake and assimilation by primary producers (Minick et al. 2016; Silver et al. 2005). Thus, comparing with unsaturated condition, low nitrification capacity with high DNRA rate in soils under saturated condition may lead to alleviate the potential risk of N losses by runoff or leaching.

The connection between moisture and nitrogen (N) transformation in soils is key to understanding N losses, particularly nitrate (NO_3^-) losses. The relationship between soil moisture content and net N transformation rates has been extensively studied by adjusting soil moisture content in the laboratory (Di et al. 2014; Guntinas et al. 2012; Wang et al. 2005; Yu and Ehrenfeld 2009). However, net N transformation rates are the outcome of several N cycling processes such as mineralization, immobilization, and nitrification. To quantify the response of inorganic N retention to soil moisture content, the gross rates of processes associated with NH_4^+ and NO_3^- cycling should be determined simultaneously. Additionally, few studies have been conducted with soils that have experienced a long-term difference in moisture status, as compared to an instantaneous adjustment, and this longer term legacy effect may influence soil microbial communities and in turn the N transformation dynamics.

The aim of this study, therefore, was to test the hypothesis that an inherent N retention mechanism exists in saturated, compared with unsaturated soil conditions, which reduces net NO_3^- production helping to keep the reactive N in the system. The study used the long-term field experiment in the subtropical region of China with a tobacco-rice cropping system incorporating an unsaturated tobacco-cropping season and a saturated rice-cropping season. The soil samples collected from tobacco season were incubated at 60% water holding capacity, while the samples collected from saturated rice-cropping season were incubated under saturated conditions, two ^{15}N tracing treatments and a numerical modeling method was used to quantify gross N transformation rates. The ^{15}N tracing model applied in this study has been tested extensively and used in studies on soils from orchard, grassland, paddy, vegetable, peanut, and maize fields, and for forest soils (Huygens et al. 2007; Müller et al. 2004; Rütting et al. 2008; Zhang et al. 2013b, 2015b; Zhu et al. 2011).

Materials and methods

Study site description

The long-term field experiment was established in 2008 in the Jiangle County, Fujian Province, China (26° 44′ 53″ N, 117° 26′ 48″ E). The site is characterized by a subtropical monsoon climate, with a mean annual temperature of 18.9 °C and mean annual precipitation of 1670 mm (over 30 years). The soil type is defined as an Anthrosol (WRB Soil Taxonomy), which has developed over granite bedrock. The annual cropping sequence was tobacco planted in February or March followed by rice planted in July. During the tobacco-cropping season, irrigation did not occur. However, during the rice-cropping season, the water level was maintained at 5 cm above the soil surface. The four treatments examined were (1) no fertilization applied (CK); (2) N, phosphorus (P), and potassium (K) fertilizers applied at the recommended rate (NPK); (3) N fertilizer applied at 50% above the recommended treatment and P and K at the recommended rate (N_hPK); and (4) NPK with additional straw (NPKS), where rice straw was chopped and incorporated into soil by plowing at a rate of 3600 kg ha^{-1} (dry matter basis) for both crops (see below). Three replicate plots (7 × 4 m) of each treatment were established in a randomized block design, with plots separated by brick frames. Under the NPKS treatment, mean concentrations of N, P, and K in straw were 5.0, 1.6, and 20.5 g kg^{-1}, respectively. Fertilizer rates and timings are given in Table 1. Compound fertilizers, urea, and potassium nitrate were applied as N sources for tobacco; ammonium bicarbonate and urea were applied as N sources for rice every year.

Soil sampling

Soil samples were collected from the plow layer (0–20-cm depth) in July and October 2013, following the tobacco and rice harvest, respectively. Ten soil cores (5 cm in diameter) were taken from each plot and combined providing one composite sample per plot and three replicates for each treatment. Thus, 12 samples were collected following the tobacco harvest when the soil was unsaturated, and 12 additional samples were collected following the rice harvest when the soil was saturated. Soil samples were split into two subsamples, one of which was used to determine soil properties (Table 2) and the other stored at 4 °C in the dark prior to the laboratory incubation experiment.

Laboratory incubation

Soils from each field treatment collected at the end of the tobacco crop season were incubated under unsaturated conditions (at 60% water holding capacity (WHC)), while those collected following the saturated rice-cropping season were incubated under saturated conditions (soil/water = 1:1, *w/w)*. Soils were incubated in 250-ml conical flasks at 25 °C in the dark, with 30 g of dry weight equivalent (DWE) soil in each flask. There were two ^{15}N-labeled NH_4NO_3 treatments for every soil, one labeled with $^{15}NH_4NO_3$ and the other with $NH_4{}^{15}NO_3$, at 9.86 and 9.75 atom% ^{15}N excess, respectively. Labeled ammonium nitrate (NH_4NO_3) fertilizer was added to each flask in solution at a rate of 60 μg N g^{-1} soil, and then further water added as necessary to achieve the required moisture content. The conical flasks were sealed with silicone rubber stoppers and incubated for a total of 144 h. During incubation, rubber stoppers were removed for 1 h every 2 days. Three conical flasks from each treatment were randomly selected at 0.5, 48, 96, and 144 h after ^{15}N fertilizer solution application, and the soils were extracted by a soil/solution ratio of 1:5 with 2 M KCl, to determine exchangeable $NH_4{}^+$-N and $NO_3{}^-$-N concentration and their ^{15}N abundance.

Soil analyses

Soil pH was measured in the ratio of soil to water of 1:5 (*v*/*v*) using a DMP-2 mV/pH detector (Quark Ltd., Nanjing, China;

Table 1 Annual fertilizer application rates in the long-term field experiment over the period 2008–2013 (kg ha^{-1})

Treatments[a]		Tobacco (unsaturated soil)					Rice (saturated soil)					Total amount
		Base fertilizers	Seedling stage	Rosette stage	Vigorous growth stage	Total	Base fertilizers	Green stage	Tillering stage	Heading stage	Total	
CK	N	0	0	0	0	0	0 0	0	0	0	0	0
	P_2O_5	0	0	0	0	0	0 0	0	0	0	0	0
	K_2O	0	0	0	0	0	0 0	0	0	0	0	0
NPK	N	104.4	3.9	7.6	11.5	127.5	66.7	66.2	16.6	16.6	166	293.5
	P_2O_5	97.0	0.0	1.0	1.0	99.0	29.9	0.0	0.0	0.0	29.9	128.9
	K_2O	266.4	13.3	23.3	74.2	377.2	36.0	36.0	0.0	0.0	72.0	449.2
N_hPK	N	156.8	11.5	11.4	11.5	191.2	100	99.4	24.8	24.8	249.1	440.3
	P_2O_5	96.9	0.0	1.0	1.0	99.0	29.9	0.0	0.0	0.0	29.9	128.9
	K_2O	290.9	13.3	36.3	36.7	377.2	36.0	36.0	0.0	0.0	72.0	449.2
NPKS	N	104.4	3.9	7.6	11.5	127.5	66.7	66.2	16.6	16.6	166	293.5
	P_2O_5	97.0	0.0	1.0	1.0	99.0	29.9	0.0	0.0	0.0	29.9	128.9
	K_2O	266.4	13.3	23.3	74.2	377.2	36.0	36.0	0.0	0.0	72.0	449.2
	Straw[b]	3600.0	0.0	0.0	0.0	3600.0	3600.0	0.0	0.0	0.0	3600.0	7200.0

[a] CK = no fertilization; NPK = N, P, and K fertilizers applied at recommended rates; N_hPK = mineral N fertilizers applied at a rate 50% higher than NPK; NPKS = NPK fertilizer and straw residue

[b] The mean concentrations of N, P, and K in straw were 5.0, 1.6, and 20.5 g kg^{-1}, respectively

Table 2 Properties of soils collected from the long-term field experiment after tobacco (unsaturated) and rice (saturated) harvesting in 2013

Treatments[a]	Unsaturated				Saturated			
	CK	NPK	N_hPK	NPKS	CK	NPK	N_hPK	NPKS
pH	5.61 ± 0.05 a	4.92 ± 0.16 bc	4.81 ± 0.09 c	5.11 ± 0.12 b	5.73 ± 0.04 a	5.64 ± 0.03 b	5.36 ± 0.05 d	5.44 ± 0.02 c
Total N (g N kg^{-1} DWE)	1.12 ± 0.09 b	1.25 ± 0.06 ab	1.32 ± 0.02 a	1.33 ± 0.09 a	1.20 ± 0.04 b	1.33 ± 0.07 a	1.39 ± 0.04 a	1.40 ± 0.03 a
Soil organic C (g C kg^{-1} DWE)	11.01 ± 0.15 b	12.39 ± 0.67 ab	11.18 ± 0.47 b	13.05 ± 1.17 a	10.56 ± 0.65 b	11.95 ± 0.51 ab	11.31 ± 0.61 b	13.34 ± 1.26 a
C/N	9.87 ± 0.95 a	9.94 ± 0.14 a	8.48 ± 0.42 a	9.85 ± 0.97 a	8.80 ± 0.30 a	9.02 ± 0.29 a	8.16 ± 0.57 a	9.52 ± 1.05 a
NH_4^+ (mg N kg^{-1} DWE)	11.99 ± 1.11 a	12.23 ± 0.16 a	12.02 ± 0.82 a	11.82 ± 0.72 a	5.99 ± 0.64 a	5.73 ± 0.27 a	6.39 ± 0.78 a	5.91 ± 0.36 a
NO_3^- (mg N kg^{-1} DWE)	10.49 ± 1.79 c	19.72 ± 2.33 b	45.48 ± 4.46 a	18.90 ± 5.51 b	4.17 ± 0.60 a	4.64 ± 0.49 a	4.21 ± 0.18 a	4.52 ± 0.17 a
NO_3^- ratio[b]	0.47 ± 0.06 c	0.62 ± 0.02 b	0.79 ± 0.01 a	0.61 ± 0.06 b	0.41 ± 0.05 a	0.45 ± 0.02 a	0.40 ± 0.03 a	0.43 ± 0.01 a

Data in the table are shown as mean ± standard deviation. Different lowercase letters indicate significant differences at $p < 0.05$ between different fertilization treatments under the same soil water conditions

DWE dry weight equivalent

[a] CK = no fertilization; NPK = mineral N, P, and K fertilizers applied at recommended rates; N_hPK = mineral N fertilizers applied at rates 50% higher than NPK; NPKS = mineral NPK fertilizer applied and straw residue

[b] Nitrate ratio calculated as $NO_3^-/(NO_3^- + NH_4^+)$

Thomas 1996; Zhang et al. 2013a, b). Soil organic C (SOC) was analyzed by wet digestion with H_2SO_4-$K_2Cr_2O_7$, and total N was analyzed using a semi-micro Kjeldahl digestion with Se, $CuSO_4$, and K_2SO_4 as catalysts (Nelson and Sommers 1996; Zhang et al. 2013a, b). Exchangeable NH_4^+-N and NO_3^--N were extracted at a soil/solution ratio of 1:5 with 2 M KCl, shaking at 250 rpm for 60 min at 25 °C with a mechanical shaker. The extract was filtered, and concentrations of NH_4^+-N and NO_3^--N determined using a segmented-continuous flow analyzer (Skalar, Breda, The Netherlands; Zhang et al. 2013a, b).

Ammonium and NO_3^- were separated by distillation with magnesium oxide and Devarda's alloy for ^{15}N measurements (Bremner and Keeney 1965). In brief, the extract was steam-distilled with MgO to separate NH_4^+; thereafter, NO_3^- was separated by Devarda's alloy. The liberated NH_3 was trapped in a conical flask with boric acid solution. The trapped N was acidified and converted to $(NH_4)_2SO_4$ using 0.02 M H_2SO_4 solution. The H_2SO_4 solution containing NH_4^+ was evaporated to dryness at 65 °C in an oven. The ^{15}N isotopic composition of NH_4^+ and NO_3^- was analyzed using a Sercon SL Elemental Analyzer coupled to a 20-20 isotope ratio mass spectrometer (IRMS, Sercon Ltd., Crewe, UK).

^{15}N tracing model

The ^{15}N tracing model was used to quantify the simultaneously occurring gross N transformation rates (Müller et al. 2007). The model includes the following processes: immobilization of NH_4^+ to recalcitrant organic N (I_{NH4_Nrec}) and labile organic N (I_{NH4_Nlab}), mineralization of labile organic N (M_{Nlab}) and recalcitrant organic N (M_{Nrec}) to NH_4^+, adsorption of NH_4^+ on cation exchange sites (A_{NH4}) and release of adsorbed NH_4^+ (R_{NH4}), oxidation of NH_4^+ to NO_3^- (O_{NH4}) and oxidation of recalcitrant organic N to NO_3^- (O_{Nrec}), dissimilatory NO_3^- reduction to NH_4^+ (D_{NO3}), and immobilization and denitrification of NO_3^- (I_{NO3}).

The variables required by the model are the concentrations and ^{15}N enrichments of NH_4^+ and NO_3^-. Further detail on this ^{15}N transformation model is given by Müller et al. (2007). However, in brief, gross N transformation rates were calculated by zero-order or first-order kinetics by minimizing the difference between modeled and measured contents of exchangeable NH_4^+-N and NO_3^--N and their ^{15}N abundances. Several model modifications, kinetic settings, and variations in the number of considered N transformations and considered N pools were tested to identify the most appropriate model (in accordance with Rütting and Müller 2008). The final model used was selected according to Akaike's information criterion (AIC). Optimization parameters were carried out by Markov Chain Monte Carlo Metropolis algorithm (MCMC-MA). The steps in the optimization algorithm and the model development are described in detail by Müller et al. (2007). The variance of the individual observations was accounted for by the misfit function *f*(*m*) between observations and simulation output. The MCMC-MA routine was carried out using MatLab (Version 7.2, The MathWorks Inc.).

Calculation and statistical analyses

The parameter averages and standard deviations of the model were calculated from the probability density function of each parameter, which was gained from the optimization procedure (Müller et al. 2007). To identify adequate iteration numbers, three parallel sequences were carried out in each analysis. Based on the kinetic settings and parameters, average N transformation rates were calculated and expressed in units of milligrams of N per kilogram soil per day (DWE). The differences in soil properties and N transformation rates between different treatments, within soil moisture groups, were examined by one-way ANOVA. When the difference was significant, Duncan's test was used to compare the differences between treatments. The differences in soil properties and N transformation rates between different soil moisture conditions were examined by paired-samples *t* test for the grouped fertilizer treatments (omitting the CK treatment).

Competition between nitrification and immobilization of NH_4^+ is used as an index of NO_3^- loss (*N/I*), which is calculated using Eq. 1:

$$N/I = O_{NH4}/TI \tag{1}$$

where *TI* is the total rate of NH_4^+ immobilization (I_{NH4_Nrec} + I_{NH4_Nlab}).

The nitrification capacity (*NC*) is calculated using Eq. 2:

$$NC = O_{NH4}/TM \tag{2}$$

where *TM* is the total rate of organic N mineralization (M_{Nlab} + M_{Nrec}).

Results

Properties of soils sampled from the field experiment

When compared to the CK treatment, the soil pH was significantly lower for the fertilized treatments, following both the unsaturated and saturated conditions ($p < 0.01$, Table 2), with lowest pH in the N_hPK treatment. Total N (TN) content was greater in fertilizer treatments than in the CK treatment ($p < 0.05$, Table 2), while SOC content and C/N ratio were relatively stable (although SOC was numerically greater for the treatment where straw residue was added). Within the soil moisture grouping, there were no significant differences between fertilizer treatments in exchangeable NH_4^+-N content. However, for the unsaturated soil, NO_3^--N content of in the N_hPK treatment was 45.48 mg kg^{-1} DWE, which was significantly higher than the other treatments (Table 2). Thus, the nitrate ratio (defined as the ratio of NO_3^--N to total inorganic N) in the N_hPK treatment was significantly higher than for other treatments.

Compared to the unsaturated soils, soil pH increased significantly following the saturated conditions of the saturated rice-cropping season ($p < 0.01$). Soil exchangeable NH_4^+-N and NO_3^--N contents were greater in the unsaturated soil ($p < 0.01$). For the fertilizer treatments, the soil NO_3^- ratios were greater in the unsaturated soil ($p < 0.01$).

Laboratory incubation experiment

Inorganic N pool concentration and ^{15}N enrichment changes

Soil exchangeable NH_4^+-N concentration decreased, while soil NO_3^--N concentration increased for all treatments over the incubation period (Fig. 1). The rate of increase of soil NO_3^--N concentration (the slopes of the lines in Fig. 1) was greater under unsaturated than saturated soil conditions, indicating a greater net nitrification rate for the unsaturated soil. For the unsaturated soil, the rate of increase of soil NO_3^--N concentration was greater for the NPK, N_hPK, and NPKS treatments than for CK, demonstrating the stimulation of fertilizers on nitrification. A significant dilution was observed in all soils over the incubation period in the ^{15}N enrichment of the NH_4^+-N pool following the addition of the $^{15}NH_4NO_3$ and in the ^{15}N enrichment of the NO_3^--N pool following addition of the $NH_4{}^{15}NO_3$ (Fig. 2). The $^{15}NH_4^+$-N enrichment where the NH_4^+-N pool was labeled was decreased greater under saturated conditions than under unsaturated conditions, indicating that the mineralization rate was greater under saturated conditions than under unsaturated conditions (Fig. 2). While, the ^{15}N enrichment of NO_3^--N following addition of the $NH_4{}^{15}NO_3$ was decreased greater under unsaturated conditions than under saturated conditions, suggesting that nitrification rate was greater under unsaturated conditions than under saturated conditions (Fig. 2).

Effect of fertilizer treatment on N transformation rates

Gross N transformation rates under the two soil moisture conditions are presented in Fig. 3. For the soil samples collected following the rice harvest and incubated under saturated conditions, mineralization and immobilization rates were not significantly different between treatments (Fig. 3a, b). I_{NO3} increased in the order CK < NPK < N_hPK < NPKS and was significantly higher for NPKS than for the other treatments (p < 0.05, Fig. 3c). The rates of O_{NH4} were significantly greater for the fertilization treatments than the CK treatment ($p < 0.05$), but no significant differences were observed between fertilization

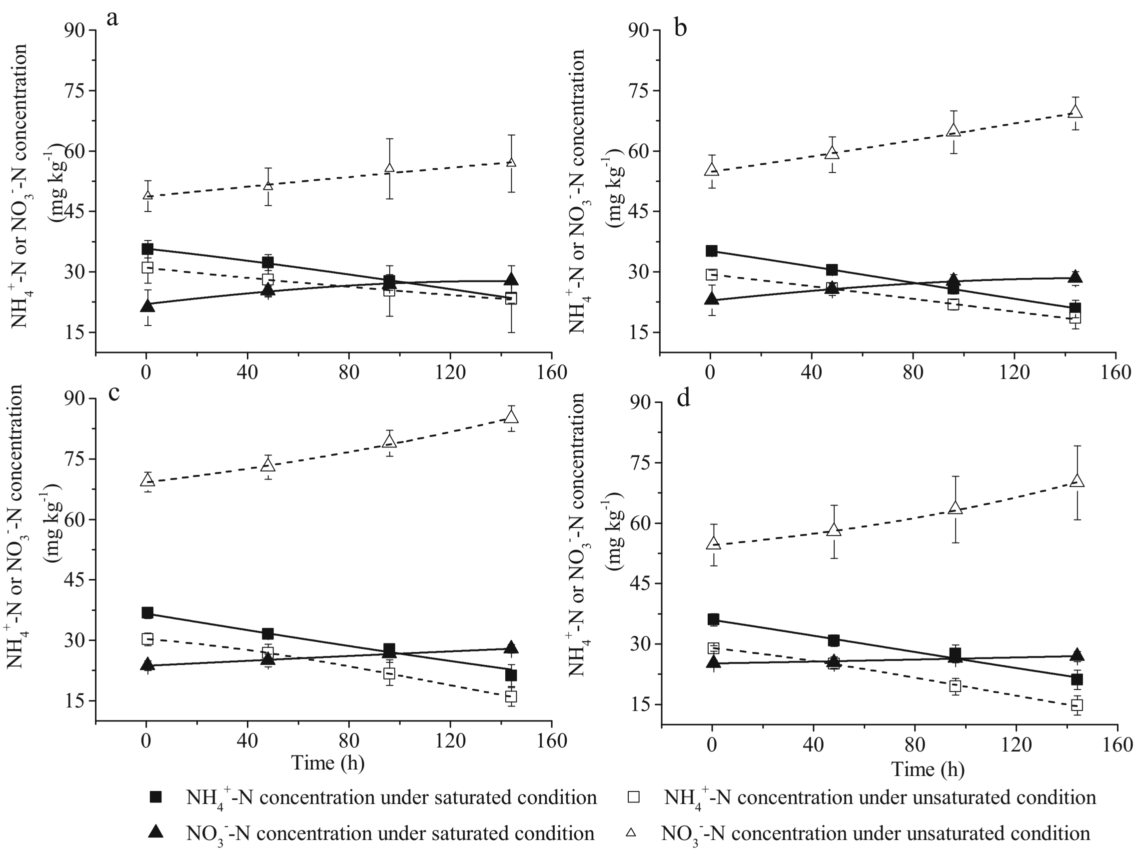


Fig. 1 Measured concentrations of exchangeable NH_4^+ and NO_3^- pools of CK (**a**), NPK (**b**), N_hPK (**c**), and NPKS (**d**) after different incubation time. The concentrations of exchangeable NH_4^+-N or NO_3^--N in soils under saturated or unsaturated conditions were the average of those for the $^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$ treatments ($n = 18$). Treatments were CK, no fertilization; NPK, mineral N, P, and K fertilizers applied at recommended rates; N_hPK, mineral N fertilizers applied at rates 50% higher than NPK; and NPKS, mineral NPK fertilizer applied with straw

treatments (Fig. 3d). Similar trends were observed for the gross rate of D_{NO3}, although the magnitudes of D_{NO3} were much smaller than those of O_{NH4} (Fig. 3e).

For the soil samples collected following the tobacco harvest and incubated under unsaturated conditions, there was no clear trend in the gross rate of organic N mineralization (Fig. 3a); however, the gross rates of NH_4^+-N immobilization were significantly higher for CK and NPK than for N_hPK and NPKS (Fig. 3b). The gross rates of I_{NO3} were very low (less than 0.1 mg N kg^{-1} soil day^{-1} on average) with no significant differences between the treatments (Fig. 3c). The variation of O_{NH4} among the treatments was the same as that observed under saturated condition, but the magnitudes were larger compared to the corresponding treatment (Fig. 3d). The gross rate of DNRA (D_{NO3}) was low under saturated condition with a significantly greater rate observed for NPKS relative to the other fertilizer treatments ($p < 0.05$, Fig. 3e).

Effect of soil moisture condition on N transformation rates

The rates of organic N mineralization (*TM*) and immobilization of NH_4^+ (*TI*) and the ratio of *TI* to *TM* in saturated conditions were significantly higher (15-fold for *TI*) than in unsaturated conditions ($p < 0.05$) (Figs. 4 and 5). There were positive relationships between soil pH and the rates of organic N mineralization ($R^2 = 0.62$; $p < 0.01$, Fig. 6) and immobilization of NH_4^+ ($R^2 = 0.59$; $p < 0.01$, Fig. 6). The rates of I_{NO3} and D_{NO3} were also higher under saturated soil conditions, whereas the rate of autotrophic nitrification decreased under saturated conditions (Fig. 4).

The ratio of autotrophic nitrification to NH_4^+ immobilization (*N/I*) and nitrification capacity (*NC*, defined as the ratio of gross rate of nitrification to total gross rate of mineralization) was lower under the saturated soil conditions than unsaturated soil conditions (Fig. 7). Soil NO_3^--N concentration increased linearly with *NC* ($R^2 = 0.84$; $p < 0.01$).

Discussion

The effects of fertilization on N transformation in soil

It has been reported that N fertilizer application stimulates both gross mineralization and NH_4^+ immobilization in soils

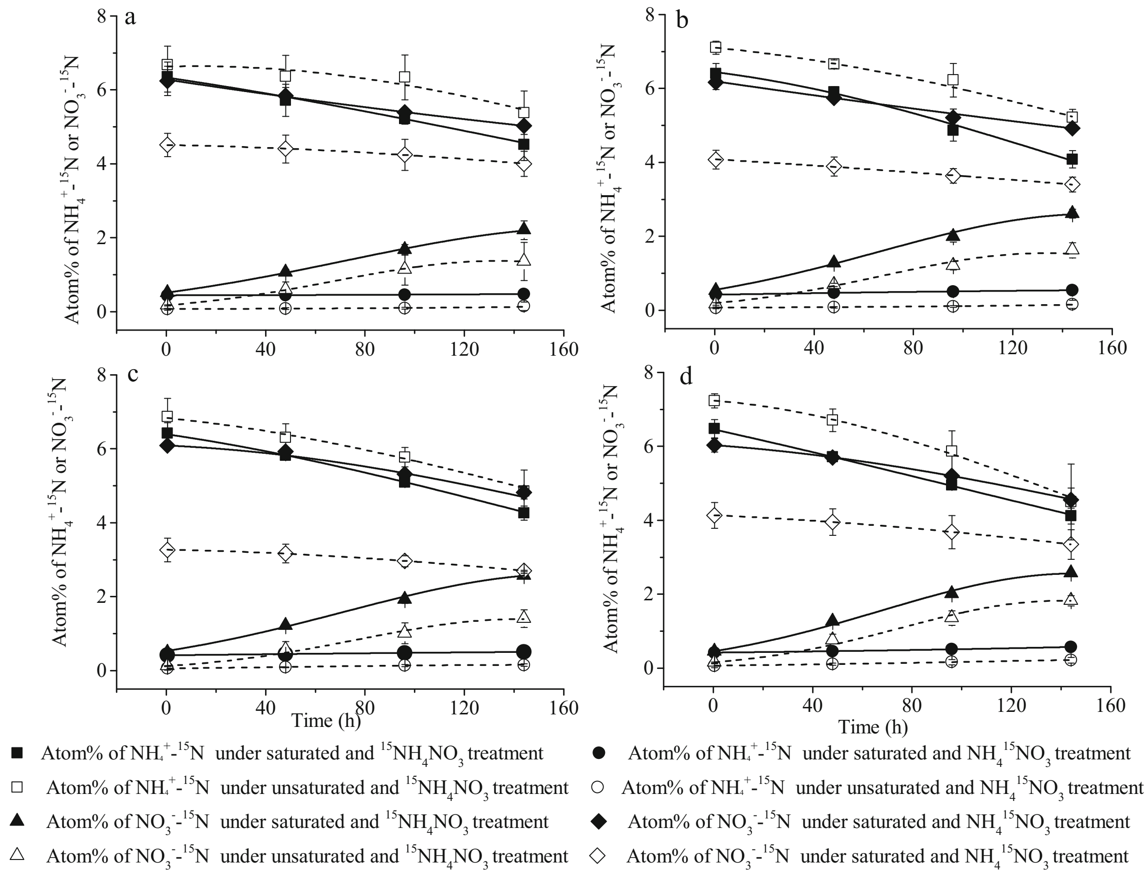


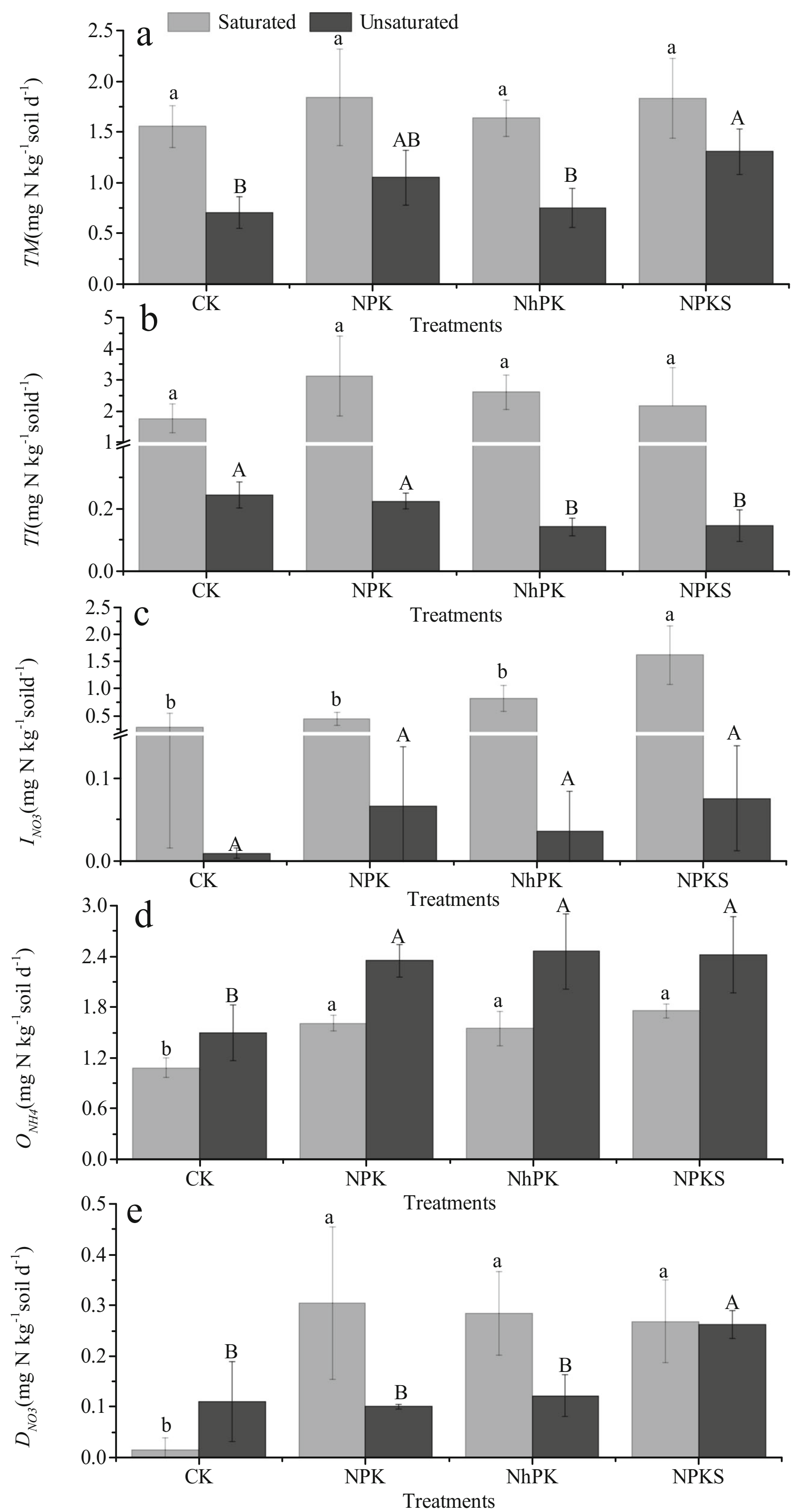
Fig. 2 Measured ^{15}N enrichments of exchangeable NH_4^+ and NO_3^- pools of CK (**a**), NPK (**b**), N_hPK (**c**), and NPKS (**d**) after different incubation times. The ^{15}N enrichment of NH_4^+-^{15}N or NO_3^--^{15}N in soils under saturated or unsaturated conditions was the average of those for the $^{15}NH_4NO_3$ or $NH_4{}^{15}NO_3$ treatments ($n = 9$). Treatments were CK, no fertilization; NPK, mineral N, P, and K fertilizers applied at recommended rates; N_hPK, mineral N fertilizers applied at rates 50% higher than NPK; and NPKS, mineral NPK fertilizer applied with straw

(Zhang et al. 2012). The ^{15}N tracing results in our study indicated that fertilization did not substantially affect the rates of organic N mineralization (*TM*), except for the NPKS treatment under tobacco-cropping conditions, which was significantly higher than the CK treatment under unsaturated conditions (Fig. 3a). There was a positive relationship between the rates of organic N mineralization and soil pH (Fig. 6a), suggesting that the stimulated organic N mineralization in the NPKS treatment was likely due to the soil pH increase during the tobacco-cropping season (Table 1).

Long-term fertilizer additions stimulated autotrophic nitrification both under saturated and unsaturated conditions (Fig. 3), which agrees with previous reports (Ding et al. 2010; Hao et al. 2003; Meng et al. 2005). The ability of N fertilizers to stimulate O_{NH4} may be due to a positive stimulation of the microbial biomass and activity with the increase in the abundance of ammonia-oxidizing bacteria (AOB) and archaeal nitrifiers (AOA; Chu et al. 2008; Shen et al. 2008; Zhang et al. 2015b). Increased applications of N fertilizers have been shown to result in a significant increase in population size of autotrophic AOB (Chu et al. 2008; Shen et al. 2008), which in turn are responsible for an increased rate of autotrophic nitrification (Zhang et al. 2013b).

The rate of autotrophic nitrification in acid soils increases with increasing soil pH (Jiang et al. 2015; Stempfhuber et al. 2015). We observed a pH increase in the NPKS treatment during the unsaturated tobacco-cropping season (Table 1), but this did not result in an increased rate of autotrophic nitrification (Fig. 3d). It is possible that this was because of the effect of allelochemicals (e.g., ferulic acid and benzoic acid), as products of the straw decomposition process in this treatment which can inhibit the numbers and activity of nitrifiers (e.g., *Nitrosomonas* and *Nitrobacter*) in the soil (Chung et al. 2001; Zhang et al. 2015c).

Generally, SOC content exerts a key control on denitrification and NO_3^--N immobilization (Firestone and Davidson 1989; Greenan et al. 2006; Hayakawa et al. 2012; Recous et al. 1990). The application of organic material increases the content of SOC and thereby may increase the rates of denitrification and NO_3^--N immobilization (Burger and Jackson 2003; Miller et al. 2008; Nishio et al. 2001). In our study, for the saturated soil, rice straw application (which



numerically increased soil SOC; although not statistically significant) increased the I_{NO3} rate (Fig. 3c) and a positive relationship between the rate of I_{NO3} and soil SOC content was observed ($R^2 = 0.524$, $p < 0.05$). However, no such relationship was observed for the unsaturated soil conditions (Table 2), indicating that C substrate was not limiting I_{NO3} under unsaturated conditions. With the ^{15}N tracing model applied in this study (Müller et al. 2004, 2007), removal of

Fig. 3 Gross N transformation rates for different treatments under saturated and unsaturated conditions as estimated using the ^{15}N tracing model (mg N kg^{-1} soil day^{-1}). Different lowercase or uppercase letters indicate significant differences at $p < 0.05$ under saturated and unsaturated conditions, respectively. *TM* total mineralization of organic N to NH_4^+, *TI* total immobilization of NH_4^+ to organic N, O_{NH4} oxidation of NH_4^+ to NO_3^-, O_{Nrec} oxidation of recalcitrant organic N to NO_3^-, I_{NO3} NO_3^- immobilization by the microbial biomass and other NO_3^- consumption rates, such as denitrification, D_{NO3} dissimilatory NO_3^- reduction to NH_4^+. Treatments were CK, no fertilization; NPK, mineral N, P, and K fertilizers applied at recommended rates; N_hPK, mineral N fertilizers applied at rates 50% higher than NPK; and NPKS, mineral NPK fertilizer applied with straw

NO_3^--N through immobilization and denitrification is quantified together as a single rate and cannot be separated into the pathway-specific rates. To achieve this, details on nitrite (NO_2^-) dynamics in soils are also required (Rütting and Müller 2008) which were not determined in this study but would provide valuable additional information if measured in future studies.

Nitrogen conservation mechanisms in saturated soil conditions

Ammonium is the preferred N source for uptake by rice (Luo et al. 1993); organic N mineralization and NH_4^+ immobilization are important processes of NH_4^+ production and consumption in soils. The measured data of ^{15}N enrichment of NH_4^+ pool under $^{15}NH_4NO_3$ treatment and ^{15}N enrichment of NO_3^- under $NH_4^{15}NO_3$ treatment suggested greater

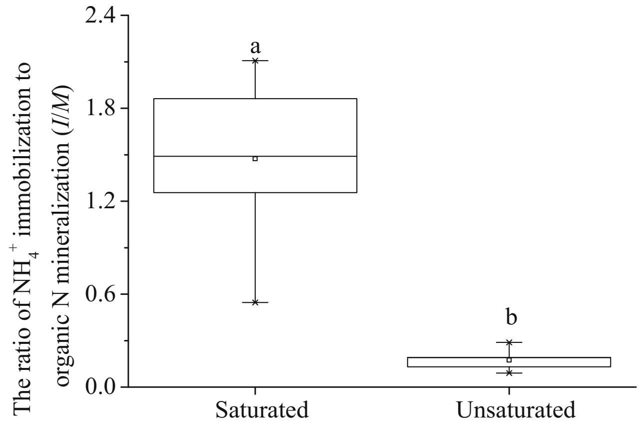
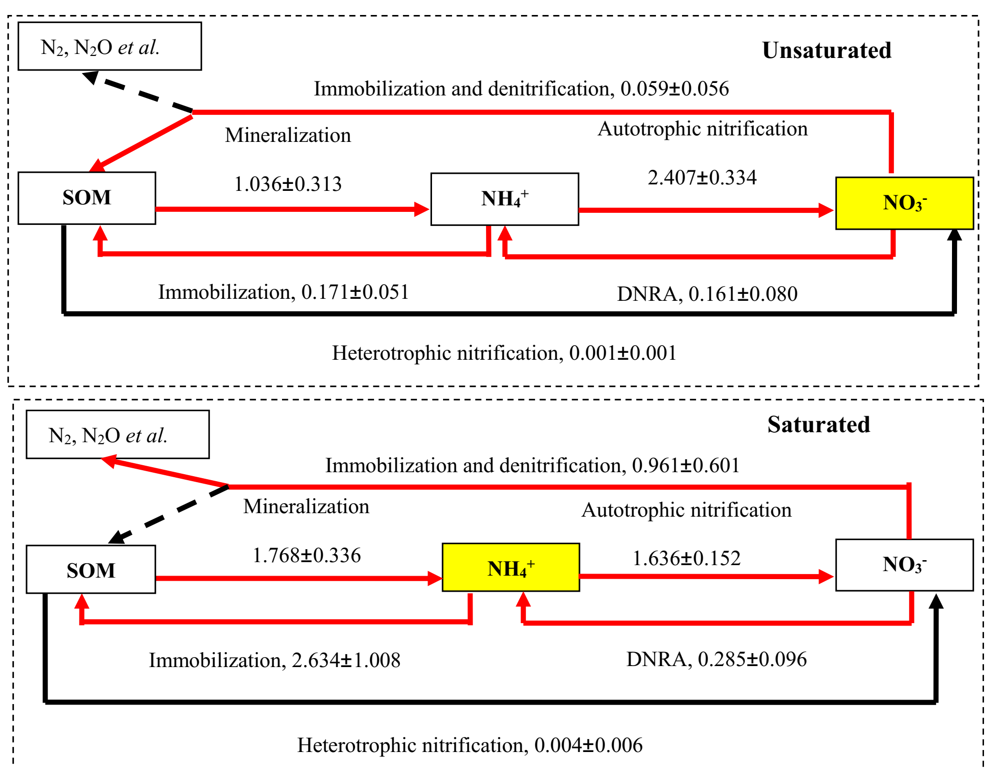


Fig. 5 The ratio of NH_4^+ immobilization to organic N mineralization (*I/M*) for different soil moisture conditions

mineralization rate and lower nitrification rate under saturated conditions than under unsaturated conditions (Fig. 2). However, comparing with unsaturated conditions, there was no increasing of exchangeable NH_4^+-N concentrations under saturated conditions (Fig. 1), indicating that immobilization rate of NH_4^+-N was greater under saturated conditions than under unsaturated conditions.

In line with the qualitative results observed in measured data, the numerical model analysis showed that the rates of organic N mineralization (*TM*) under saturated soil conditions were significantly higher than under aerobic conditions

Fig. 4 Nitrogen transformation pathways in soils under saturated and unsaturated conditions (mg N kg^{-1} soil day^{-1}). The red arrows signify transformation processes where significant differences were found between the unsaturated and saturated soils (paired-sample *t* tests). The yellow highlight signifies the dominant form of inorganic N in the soils. Data presented are the means of gross rates of N transformation for all the fertilizer treatments combined (NPK + N_hPK + NPKS; $n = 9$)



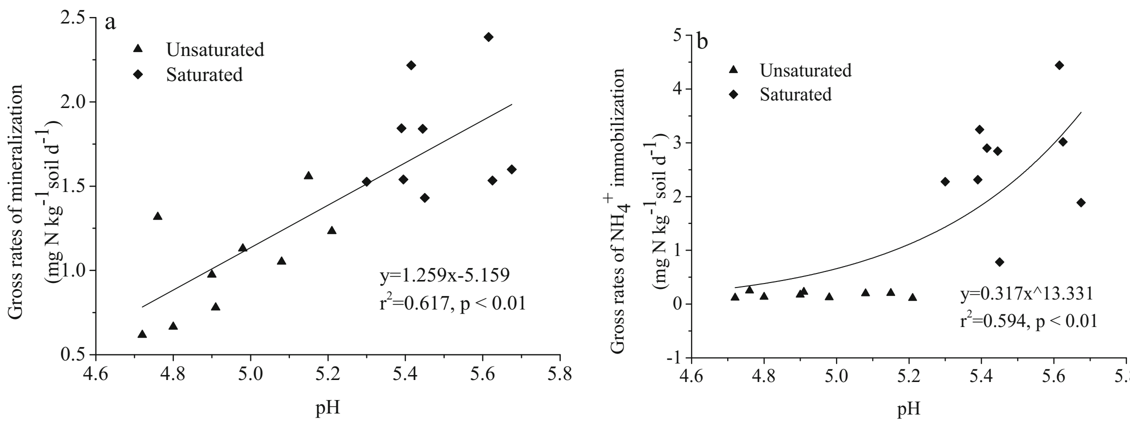


Fig. 6 Relationship between soil pH and **a** gross rate of mineralization and **b** NH_4^+ immobilization. The data from the NPK, N_hPK, and NPKS treatments were analyzed together, $n = 18$

(Fig. 4), which agrees with Mathieu et al. (2006) who made similar observations in the Saône river plain, near Dijon (Eastern France) in an agricultural soil. There was a positive correlation between the rate of organic N mineralization and soil pH (Fig. 6a), which suggest that the increased rate of organic N mineralization in saturated soils may be attributable to soil pH increase (Table 2).

High NH_4^+-N availability in paddy soils may increase N loss through runoff or leaching too, even though NH_4^+-N is less mobile than NO_3^--N, and through NH_4^+ oxidation may result in elevated NO_3^- concentrations (Huygens et al. 2007; Inagaki and Miura 2002; Zhu et al. 2013a). Moreover, NH_4^+ immobilization in soils, controlled by biotic and abiotic processes, may significantly influence N availability for crops as well as affect N losses (Said-Pullicino et al. 2014). Our results showed that the rates of NH_4^+ immobilization (*TI*) in the saturated soil were significantly higher than that under unsaturated conditions (Fig. 4), which corresponds to findings by Compton and Boone (2002) who identified a positive relationship between the rate of inorganic N immobilization and soil moisture content. This indicates that NH_4^+ will not easily accumulate in soil under saturated conditions, which is further supported by the correlation between soil pH and NH_4^+ immobilization rate ($p < 0.01$, Fig. 6b). The ratio of *TI*/*TM* under saturated conditions was significantly higher than that under unsaturated condition, and in more than 75% of all cases higher than 1.2 (Fig. 5). Those results explained the lower soil exchangeable NH_4^+-N concentrations under saturated conditions than that under unsaturated conditions (Table 2). Low soil exchangeable NH_4^+-N concentrations was expected to reduce N loss via anaerobic ammonium oxidation, which was considered as an important N removal pathway in anaerobic agricultural soil (Shen et al. 2016).

Generally, autotrophic nitrification is considered to be a microbial oxidation process under aerobic conditions. A previous study found that the optimum moisture conditions for nitrification under different cropping systems were about 60% water-filled pore space (WFPS) (Linn and Doran 1984). Kiese et al. (2008) found that the relationship between the rates of gross nitrification and soil moisture could be described best by the O'Neill functions, with a soil moisture optimum for nitrification at 65% WFPS. However, Yang et al. (2016) reported

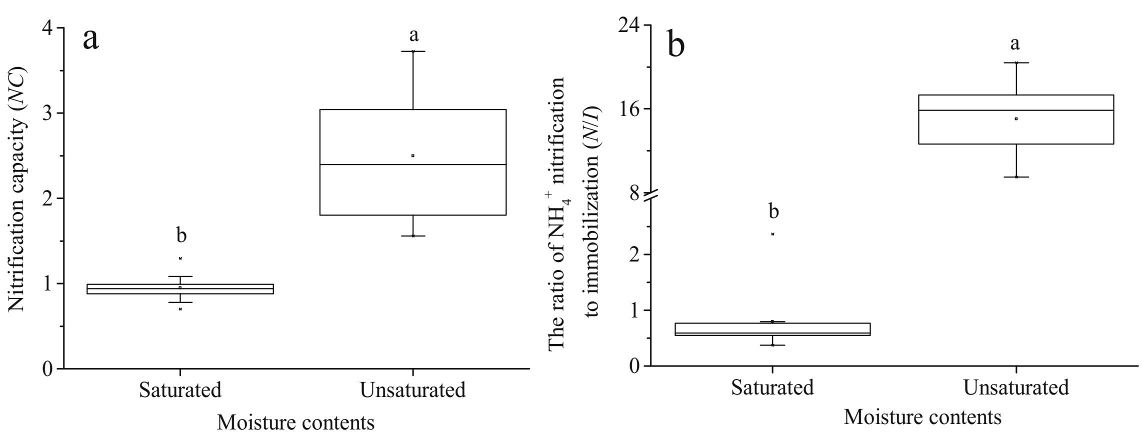


Fig. 7 The nitrification capacity (NC) (**a**) and ratio of nitrification to immobilization (*N*/*I*) (**b**) of soils with different moisture content. The data from the NPK, N_hPK, and NPKS treatments were analyzed together, $n = 18$. *NC* was calculated as [O_{NH4}/TM]. Different lowercase letters indicate significant difference of *N*/*I* and *NC* at $p < 0.05$

that nitrification activity in paddy soils was not suppressed by low oxygen concentrations. Soil nitrifying microorganisms are known to adapt to the local conditions (Mahendrappa et al. 1966; Myers 1975), such as low pH (Wang et al. 2014), low temperature (Wang et al. 2012), or low oxygen concentration (Yang et al. 2016), and O_2 transport to the rhizosphere of rice plants via parenchyma gas transport (Arth et al. 1998; Inubushi et al. 2002) may also facilitate nitrification.

However, the rates of nitrification under saturated conditions were still significantly lower than that under unsaturated conditions (Fig. 4), contributing to a lower accumulation of NO_3^--N and a reduced risk of N loss from the paddy soil. Nitrification capacity (*NC*) is an index of the ability of autotrophic nitrifiers to compete with immobilizing bacteria for NH_4^+ and is a key factor in controlling N losses (Hart et al. 1994). Our study showed that the *NC* of the saturated rice-cropping season soil incubated under saturated conditions was significantly lower than the unsaturated tobacco-cropping soil incubated under unsaturated conditions (Fig. 7a). The positive relationship between NO_3^- contents with *NC* ($R^2 = 0.84$; $p < 0.01$) also suggested that low nitrification rate under water-saturated condition contributed to low NO_3^- content and a lower risk of N loss.

The ratio of autotrophic nitrification to NH_4^+ immobilization (*N/I*) has been suggested as an index to quantify the competitive ability of heterotrophic microbes and nitrobacteria for NH_4^+ and the likelihood for N losses from soils (Stockdale et al. 2002). For the soil samples collected following rice harvest and incubated under saturated condition, *N/I* was lower than 1 (Fig. 7b), suggesting that the substrate of autotrophic nitrification (NH_4^+) would be deficient because of high immobilization rates. This result implies that, if plenty of NH_4^+-N is made available (e.g., through fertilizer application), the rate of nitrification in paddy soil would increase due to increased availability of NH_4^+ to the nitrifying microorganisms. Thus, split applications of N fertilizer are an important management practice to decrease the risk of N loss in paddy soils.

The processes of DNRA reduce the soil NO_3^- content while retaining the N in an available form (Silver et al. 2005). In our study, the rate of DNRA was significantly higher under saturated soil conditions (Fig. 4), which can be explained by the requirement of anoxic soil conditions to promote the DNRA process, and also explains why DNRA plays only a minor or negligible role in aerobic soils (Tiedje 1988). Therefore, under saturated conditions, surplus NO_3^--N can be transformed to NH_4^+-N through the DNRA process thereby potentially conserving N in the system. Accompanied with higher rates of organic N mineralization and NH_4^+ immobilization and lower rates of NH_4^+ autotrophic nitrification in the saturated soils than in unsaturated soils, N can be retained in soils during the saturated rice-cropping season. This observation was supported by the view of Houtermans et al. (2017) that paddy management promotes N sequestration in soils.

It was reported that NO_3^--N immobilization occurs when NH_4^+-N is very low (Rice and Tiedje 1989). In our study, soil exchangeable NH_4^+-N contents were greater in the unsaturated soil than that in the saturated soil. Thereby, the rate of I_{NO3} under saturated conditions was significantly higher than under unsaturated conditions (Fig. 4). Although the processes of NO_3^- immobilization and denitrification could not be considered independently (see above), saturated conditions generally favor the denitrification process (Xing et al. 2002) and N losses via that pathway were therefore likely to have been much higher than N conserved through immobilization. However, because NO_3^- availability is a key factor for denitrification (Greenan et al. 2006), the high rates of NH_4^+ immobilization and DNRA and low nitrification rate resulted in low NO_3^- availability under the saturated soil condition (Table 2), thereby decreasing opportunities for gaseous N losses via denitrification.

Conclusion

Contrary to the general assumption that soils under saturated conditions are prone to N losses, there seems to be an inherent soil N conservation mechanism during the saturated rice-cropping season to effectively conserve N. This is predominantly via reduced NO_3^- production and improved DNRA process. Higher organic N mineralization rates in paddy soils provide sufficient NH_4^+-N for crop uptake, and surplus NH_4^+-N is immobilized rather than nitrified under the saturated soil conditions. Soil inorganic N concentrations under saturated conditions were significantly lower than under unsaturated conditions, decreasing the potential for N loss through runoff or leaching. However, the rates of NO_3^- immobilization and denitrification of the unsaturated rice-cropping soil were significantly higher than in the unsaturated tobacco-cropping soil, and the risk of N loss through denitrification maybe higher in the saturated soil than in the unsaturated soil. The relationship between nitrification, NO_3^- immobilization, and denitrification should be evaluated in more detail in future studies.

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