Rice genotypes significantly affect nitrification inhibition activity in the rhizosphere

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Abstract

Biological nitrification inhibitor (BNI) can play an important role in inhibiting nitrification and enhancing nitrogen use efficiency (NUE) in agriculture. However, most of current BNI studies have been conducted under hydroponics. Variations in genotype-mediated inhibition of soil nitrification are still unknown. In this study, two rice genotypes, i.e. Wuyujing 3 (WYJ3) and Wuyunjing 7 (WYJ7), which were reported to have weak and strong BNI capacity under hydroponics, respectively, and four soils with different pH (i.e. JX (pH 5.09), FJ (pH 6.00), SC1 (pH 7.96) and SC2 (pH 7.94)) were selected. N uptake rates (esp. NH_4^+ uptake, U_{NH4}), soil N transformation rates and NUE were quantified by ^{15}N tracing experiment to assess the effects of rice genotypes on nitrification inhibition activity. Results showed that rice genotypes with high BNI exudation (i.e. WYJ7) had lower autotrophic nitrification rate (O_{NH4}) and higher U_{NH4} in rhizosphere compared to WYJ3. O_{NH4} in WYJ7 reduced by 0.05, 0.42, 1.14, and 0.48 mg N kg^{-1} d^{-1} compared with WYJ3 for JX, FJ, SC1 and SC2, respectively. Abundance of AOB in soils planted WYJ7 was lower than WYJ3, which was the key factor affecting O_{NH4}. NUE of WYJ7 was higher than WYJ3, although difference between genotypes was not significant. NUE was negatively correlated with O_{NH4} ($P < 0.05$). Our results indicated that some rice genotypes can optimize their N acquisition by regulating soil N transformations (particularly nitrification). Developing rice genotype with strong BNI exudation capacity could be a suitable management practice to increase NUE and yield.

1. Introduction

Agricultural ecosystems compared to undisturbed natural ecosystems are characterised by a more open nitrogen (N) cycle. Autotrophic nitrification is the central process of soil N transformation, which controls N runoff, leaching and denitrification losses by governing the availability of NO_3^- (Wang et al. 2017; Zhang et al. 2018; Zhao et al. 2007). The application of synthetic nitrification inhibitors can effectively reduce soil autotrophic nitrification and increase the NH_4^+ retention time (Qiao et al. 2015), but carries a high economic cost and potential environmental pollution problems. Thus, there is a need to develop plant-derived biological nitrification inhibitor (BNI). Up to now, seven BNIs in plant root exudates have been isolated and identified, namely brachialactone in brachiaria humidicola (Subbarao et al. 2009), sorgoleno, sakuranetin and MHPP in sorghum (Subbarao et al. 2012), 1,9-decanediol in rice (*Oryza sativa* L.) (Sun et al. 2016) and zeanone and HDMBOA in maize (Otaka et al. 2021). To the best of our knowledge, most of the current studies on nitrification inhibition by plant BNI have been conducted under hydroponics, and only a few studies have focused on differences in nitrification inhibition by plant genotypes in soil environments. Mwafulirwa et al. (2021) observed an interactive effect of maize genotype and soil management history on soil nitrification, thus soil may be an important factor influencing plant exudation of BNI. Additionally, it has also been shown that some BNIs are not stable in soil (Subbarao et al. 2012). In conclusion, it is necessary to examine the performance of genotypes with different BNI exudation abilities on soil autotrophic nitrification.

Rice, as a typical NH_4^+-preference crop, its BNI exudation process seems to be more significant for N uptake and NUE. Sun et al. (2016) observed that rice with greater BNI release capacity had higher NH_4^+ uptake. Genotypes with a greater capacity for O_2 release from the root also show a more efficient N uptake rate (Kirk and Kronzucker 2005; Li et al. 2008), these two processes have opposite effects on soil autotrophic nitrification. Soil N transformation processes regulate the concentration and form of inorganic N in the soil, which in turn affects N uptake and NUE in different plants (Wang et al. 2017; Zhang et al. 2016). NH_4^+ availability affects N uptake, utilization and growth of rice (Ishiyama et al. 2004; Ranathunge et al. 2014). There is still a knowledge gap regarding differences in the ability of rice genotypes to regulate soil N transformations (especially autotrophic nitrification), and it remains unclear to what extent such regulation can promote rice NH_4^+ uptake. Therfore, we hypothesized that despite the presence of root O_2 release process, there are variations in the inhibition of soil autotrophic nitrification in rice genotypes and that such variations may cause differences in NH_4^+ uptake and NUE. Considering that high pH and low NH_4^+ concentrations are not in favor for BNI release from plants (Subbarao et al. 2007; Zhang et al. 2019), we also hypothesised that there may be no significant difference in the feedback of rice genotypes to autotrophic nitrification in alkaline soils.

In this study, the two rice genotypes Wuyujing 3 (WYJ3) and Wuyunjing 7 (WYJ7), which had the weakest and strongest BNI exudation capacity, respectively, among 13 *japonica* rice genotypes under hydroponic conditions (Sun et al. 2016), and four soils with different pH were selected. To test our hypothesis, the soil N transformation rates and plant N uptake rates in soil-rice systems were simultaneously quantified using the $Ntrace_{plant}$ tool (He et al. 2020), N use efficiency (NUE) and N loss were determined under a ^{15}N urea labeling experiment. With the two experiments described above, we achieved a direct link between nitrification inhibition capacity and N uptake and NUE in rice.

2. Materials And Methods

2.1 Soil samples

In this study, the two rice genotypes Wuyujing 3 (WYJ3) and Wuyunjing 7 (WYJ7), which had been reported to have a weak and strong BNI exudation capacity, respectively (Sun et al. 2016) were selected. Four paddy soil samples with different pH from three provinces in China, including two acidic soils in Jiangxi (JX) and Fujian (FJ) and two alkaline soils in Sichuan (SC1 and SC2) were collected, after harvesting in October 2021. Soil samples were taken from the cultivated layer at a depth of 0–20 cm at three randomly selected points at each site. After being brought back to the laboratory, impurities such as large gravel particles and dead branches were first removed, then passed through a 2 mm sieve and mixed thoroughly. These samples were stored at 4°C and used for pot experiments as well as soil property analysis. Soil properties are detailed in Table 1.

Table 1
Soil properties of the four soils used in this study (average ± standard deviation)

	pH	C/N	SOC	TN	DOC	NH_4^+	NO_3^-
			g/kg		mg/kg	mg N/kg	
JX	5.09 ± 0.09c	10.01 ± 0.47bc	14.10 ± 0.82c	1.38 ± 0.02b	74.01 ± 0.47b	10.03 ± 0.38a	4.48 ± 0.10c
FJ	6.00 ± 0.12b	20.35 ± 0.24a	23.80 ± 0.41a	1.18 ± 0.01c	81.63 ± 3.68ab	1.18 ± 0.40b	5.33 ± 0.43c
SC1	7.96 ± 0.02a	10.57 ± 0.23b	12.93 ± 0.39c	1.21 ± 0.01c	69.09 ± 3.65bc	1.08 ± 0.16b	9.52 ± 0.83b
SC2	7.94 ± 0.04a	9.67 ± 0.06c	17.91 ± 0.10b	1.84 ± 0.01a	87.39 ± 3.64a	1.02 ± 0.25b	13.95 ± 0.92a

JX is a paddy soil collected from Jiangxi, China; FJ is a paddy soil collected from Fujian, China; and SC1 and SC2 are paddy soils collected from Sichuan, China. C/N, SOC: TN ratio; SOC, soil organic carbon; TN, total nitrogen; DOC, dissolved organic carbon

2.2 Plant materials and growth condition

Rice seeds (WYJ3 and WYJ7) were soaked in 10% H_2O_2 for 30 min, washed with deionized water, and subsequently placed in an incubator with two foam floating nets at 28°C for 4 days in the dark and after germination seedlings were planted in soil. Normal daylight conditions (light 12 h/dark 12 h, 28°C during the day and 22°C at night), prevailed in the growth chamber throughout the experimental period, with the light field open from 6 am to 6 pm. After 30 days, the seedlings were transplanted into a series of pots with one part used for the ^{15}N urea labeling experiment and the other part for the ^{15}N tracing experiment after the seedlings had grown to the tillering stage.

2.3 ^{15}N urea labeling experiment

In this experiment, the pots were divided into eight groups, i.e. WYJ3 and WYJ7 were transplanted into four soils (JX, FJ, SC1, SC2), respectively. After one month of growing rice in seedling pots, seedlings of uniform growth were selected for transplanting, and one seedling was transplanted per pot. Before transplanting, all soils equivalent to 250 g dry weight were weighed into pots and 5 mL of urea solution (5.28% atom of ^{15}N urea) was added to each pot as a base fertilizer at a rate of 40 mg N kg^{-1} dry soil. Each pot should be watered and cleared of aquatic plants every two days. Destructive sampling of each pot was carried out on day 35 after transplanting, the rice plants were collected and rinsed, then the soils in the pots were uniformly mixed and extracted, N content and ^{15}N abundance were measured after soil samples had been dried at 60°C. N content, ^{15}N abundance and biomass were determined after plants had been dried at 80°C.

2.4 ^{15}N tracing experiment

Each fresh soil sample equivalent to 250 g of dry soil weight was added to each pot before transplanting, followed by the application of $(NH_4)_2SO_4$ as a base fertilizer (40 mg N kg^{-1} soil) and about 2 cm of flooded water. Other managements were the same as in 2.3.

Appropriate modifications were made to the ^{15}N tracing experiment designed by (He et al. 2020). Briefly, on day 30 after transplanting, two sets of ^{15}N isotope markers were set, one for $^{15}NH_4{}^{14}NO_3$ (10.20% atom) and the other for $^{14}NH_4{}^{15}NO_3$ (10.12% atom). 40 mg N kg^{-1} soil of $^{15}NH_4{}^{14}NO_3$ or $^{14}NH_4{}^{15}NO_3$ is uniformly added to the soil core by four-hole injection, destructive sampling was then carried out at 0.5, 24, 48, 72 hours to determine the soil NH_4^+ and NO_3^- concentration and ^{15}N abundance of each treatment. Dissolved organic carbon (DOC), N content, ^{15}N abundance and biomass of each dry plant were also measured.

2.5 Analytical methods

The DMP-2mV/pH detector (Quark Ltd, Nanjing, China) was used to determine soil pH at a ratio of 1:2.5 (w/v). Soil organic carbon (SOC) content was measured using high temperature exothermic digestion by H_2SO_4-$K_2Cr_2O_7$. NH_4^+ and NO_3^- were extracted with 1 *M* KCl at a ratio of 1:5 (w/v) in a shaker for 60 min at 250 rpm under 25°C. DOC was extracted by ultrapure water at a ratio of 1:5 (w/v) and measured by Analyzer Multi N/C (Analytik Jena, Jena, Germany). Concentrations of NH_4^+ and NO_3^- were determined using a continuous flow analyser (SA1000, Skalar, Breda, Netherlands). NH_4^+ and NO_3^- were extracted using micro-diffusion and absorbed by filter paper with 1 *M* oxalic acid (Zhang et al. 2017), the ^{15}N abundance of NH_4^+ and NO_3^- were then determined by stable isotope ratio mass spectrometry (IRMS 20–22, SerCon, Grewe, UK) after a thorough drying. N content and ^{15}N abundance in plants and soils from pot experiment were determined using a Delta V advantage isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany).

DNA was extracted from 0.5 g fresh soil after destructive sampling which was according to the manufacturer's instructions (MoBio Laboratories, Inc., Carlsbad, CA, USA). Real-time fluorescent quantitative PCR was performed on biological trios by CFX-96TM Real-Time System (Bio-Rad Laboratories Inc., Hercules, CA, USA) and the abundance of AOA (Arch-*amo*AF/Arch-*amo*AR) and AOB (*amo*A1F/*amo*A2R) determined by primer sets and thermal conditions are listed in Table S1. Reaction mixtures for AOB and AOA *amo*A were set and standard curves were established according to (Huang et al. 2015), with amplification efficiencies of 96.8% and 99.4% for AOA and AOB, respectively. Amplification specificity was calculated using melting curve analysis, while copy number was calculated by oven drying basis.

Gross N transformation rates in paddy soils were determined by ^{15}N tracing experiment in combination with the $Ntrace_{plant}$ tool (He et al. 2020) to quantify gross N transformation and plant N uptake rates. NH_4^+ and NO_3^- concentrations (mean ± standard deviation) and ^{15}N atomic percent excesses (mean ±

standard deviation) measured by the ^{15}N tracing experiment are used as input variables to the tool with N transformation rates calculated by zero-order kinetics, first-order kinetics or Michaelis-Menten kinetics.

2.6 Calculation and statistical analysis

The following equations were used to calculate fertilizer NH_4^+ retention time (NH_4^+ Retention time), net NH_4^+ release rate (Net R_{NH4}), NH_4^+ immobilization rate (I_{NH4}), N use efficiency (NUE) and N loss.

$$NH_4^+\ Retentiontime = \frac{Fertilizer NH_4^+}{O_{NH4} + I_{NH4}}$$

$$NetR_{NH4} = R_{NH4} - A_{NH4}$$

$$I_{NH4} = I_{NH4-Nrec} + I_{NH4-Nlab}$$

where NH_4^+ Retention time refers to fertilizer NH_4^+ retention time (d), *Fertilizer* NH_4^+ is the amount of NH_4^+ applied in the ^{15}N tracing experiment (40 mg N kg^{-1}), O_{NH4} means the rate of oxidation from NH_4^+ to NO_3^- (mg N kg^{-1} d^{-1}), I_{NH4} is the rate of NH_4^+ immobilization (mg N kg^{-1} d^{-1}), R_{NH4} is the release rate of adsorbed NH_4^+, A_{NH4} is the adsorption rate of free NH_4^+, $I_{NH4-Nrec}$ is the immobilization rate of NH_4^+ to recalcitrant organic N, $I_{NH4-Nlab}$ is the immobilization rate of NH_4^+ to labile organic N.

$$NUE(\%) = \frac{C_{NPlant} \times A_{Nplant} \times W_{plant}}{C_{Nf} \times A_{Nf}} \times 100$$

$$SoilNRetention(\%) = \frac{C_{Nsoil} \times A_{Nsoil} \times W_{soil}}{C_{Nf} \times A_{Nf}} \times 100$$

$$Nloss(\%) = 100\% - NUE(\%) - SoilNRetention(\%)$$

where $C_{N\ plant}$ is the N content of the plant (g kg^{-1}), $A_{N\ plant}$ is the ^{15}N atomic percent excess of N in the plant (% atom), W_{plant} is the dry weight of the plant (kg), C_{Nf} is the amount of the applied N fertilizer (g pot^{-1}), and A_{Nf} is the ^{15}N atomic percent excess of applied N fertilizer (% atom). $C_{N\ soil}$ is soil N content (g kg^{-1}), $A_{N\ soil}$ is ^{15}N atomic percent excess of soil N (% atom), W_{soil} is soil dry weight (kg), C_{Nf} refers to the amount of the applied N fertilizer (g pot^{-1}), and A_{Nf} is the ^{15}N atomic percent excess of applied N fertilizer (% atom). N loss is calculated by the ^{15}N mass balance method (Liu et al. 2020).

Statistical analyses on all gross N transformation rates were performed by SigmaStat 3.4 (Systat Software, San Jose, CA, USA). One-way ANOVA combined with LSD test was used to assess differences in the same genotype grown in four soils, and t-test was used to determine the level of variation in the same soil after planting different genotypes. Briefly, the mean and standard deviation of each gross transformation rate, as well as actual experimental replicates, were used to test for differences in gross

soil N transformation rates and plant N uptake rates. The rest of the soil property analyses were carried out in SPSS 25.0 (IBM crop., USA). Pearson correlation and nonlinear analysis were used to test the correlation between N fertilizer fates, soil gross N transformation rates and soil properties.

3. Results

3.1 Soil chemistry and microbial properties

The pH was 5.09, 6.00, 7.96, and 7.94 in JX, FJ, SC1 and SC2, respectively. NH_4^+ concentration was significantly higher in JX than in FJ, SCI and SC2 ($P < 0.001$). Additionally, NO_3^- concentration in two alkaline soils (SC1 and SC2) were significantly higher than in the two acidic soils (JX and FJ) ($P < 0.01$). The initial DOC varied between 69.09 to 87.39 mg kg^{-1} in studied soils, and was elevated after rice plantings. DOC in soils planted with WYJ7 was higher than when planted with WYJ3, with significant differences in the two alkaline soils ($P < 0.05$) (Fig. 1a). The abundance of ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) in acidic soils were significantly lower than in alkaline soils after rice (either WYJ3 or WYJ7) were planted ($P < 0.05$). SC1 under rice (either WYJ3 or WYJ7) planting had the highest AOB abundance (Fig. 1b). SC2 had the highest AOA abundance by WYJ3 planting (Fig. 1c). Moreover, AOA and AOB were always lower in soils planted with WYJ7 than in soils planted with WYJ3. AOB abundance in SC1 with WYJ7 was significantly lower than planting WYJ3 ($P < 0.001$), and AOA abundance in SC2 with WYJ7 was significantly lower than that of WYJ3 planting ($P < 0.05$). AOA abundance increased with increasing pH ($P < 0.01$) (Fig. 4a), this pH-mediated effect on nitrifying microorganisms was also manifested in AOB ($P < 0.05$) (Fig. 4b).

3.2 NUE and N loss

NUE showed large differences among soils (12%-41%, average 25%), with acidic soils showing significantly higher NUE than alkaline soils ($P < 0.001$) (Fig. 2a). Rice planted in JX had the highest NUE among the four soils which was independent of rice genotypes. The second highest was rice planted in FJ, which was still above 30% despite a significantly lower NUEs than JX ($P < 0.05$). NUE of SC1 and SC2 were below 20%, and the high N losses likely contributed to their low NUE. Although the two genotypes did not show statistical differences in NUE, the NUE of WYJ7 was higher than that of WYJ3 (except rice planted in FJ).

N loss did not differ significantly among rice genotypes, but was influenced by soil type. Grouping analyses according to acidity and alkalinity as criteria demonstrated that N losses in acid paddy soils were significantly lower than those in alkaline paddy soils ($P < 0.05$), with N losses in acidic soils ranging from 36% to 58% (average 46%) compared to 70% to 74% (average 72%) in alkaline soils (Fig. 2b). The two acidic soils exhibited large differences, with JX showing significantly lower N losses than FJ (37% *vs.* 56%) ($P < 0.05$). However, two alkaline soils displayed essentially the same N losses. A negative correlation had been found between N loss and NUE ($P < 0.001$) (Fig. 5a). A positive correlation had been

found between N loss and pH ($P < 0.001$) (Fig. 6a). With increasing N loss, soil ammonium retention time ($P < 0.05$) and rice biomass ($P < 0.05$) showed a decrease. ($P < 0.05$) (Fig. 6b-c).

There was also no significant difference in the performance of rice genotypes in soil N retention in each soil. In terms of soil types, JX had the highest soil N retention (average 24%) which showed significant differences with FJ, SC1 and SC2 (average 13%, 12%, and 15% in order) ($P < 0.05$), while soil N retentions of FJ, SC1 and SC2 converged and did not differ statistically (Fig. 2c).

3.3 Gross N transformation rates

The measured and modeled values of soil NH_4^+, NO_3^- and plant N pool concentrations and ^{15}N abundance were consistent (Fig. S1-S4). The autotrophic nitrification rate (O_{NH4}) of rhizosphere were generally low (< 3 mg N kg^{-1} d^{-1}) (Fig. 3a), due to flooded incubation which may limit the availability of O_2. The O_{NH4} in SC1 and SC2 were significantly higher than in JX and FJ for both WYJ3 and WYJ7 treatments ($P < 0.001$), which was consistent with the changes in AOA and AOB. The highest O_{NH4} was observed in SC1-WYJ3 (2.06 mg N kg^{-1} d^{-1}) and the lowest O_{NH4} in FJ-WYJ7 (0.01 mg N kg^{-1} d^{-1}), with a difference of more than 200 times. O_{NH4} in both FJ and SC2 were below 1 mg N kg^{-1} d^{-1}, but the same genotype was significantly different in FJ and SC2 ($P < 0.001$). The O_{NH4} of rice soils increased with increasing pH ($P < 0.05$) (Fig. 4c) and also revealed a positive correlation with the abundance of AOA and AOB ($P < 0.05$, $P < 0.001$) (Fig. 4d-e). From the perspective of genotypes feedback to O_{NH4}, all the O_{NH4} were significantly different after planting two rice genotypes in four soils ($P < 0.05$), which were reflected by the higher O_{NH4} under WYJ3 than WYJ7. More specifically, O_{NH4} in WYJ7 reduced by 0.05, 0.42, 1.14, and 0.48 mg N kg^{-1} d^{-1} compared with WYJ3 for JX, FJ, SC1 and SC2, respectively. It is noteworthy that this difference caused by rice genotypes was also reflected by autotrophic nitrifying microorganisms (AOA and AOB). Furthermore, O_{NH4} also increased with M_{Nrec} (mineralization of recalcitrant organic N to NH_4^+ rate) ($P < 0.05$) (Fig. 4f). Stepwise regressions of AOA, AOB, pH, and M_{Nrec} showed that AOB was the key factor affecting the O_{NH4} ($R^2 = 0.96$, $P < 0.001$). NUE decreased with increasing O_{NH4} ($P < 0.05$) (Fig. 5b), while N loss increased with increasing O_{NH4} ($P < 0.05$) (Fig. 6d).

The I_{NH4} in all treatments was very low (below 0.5 mg N kg^{-1} d^{-1}), except for WYJ7-SC1 and WYJ7-SC2 (Fig. 3b). The I_{NH4} in the acidic soils after cultivation of the two rice genotypes tended to be uniform, while significant differences were shown in the alkaline soils ($P < 0.05$). The I_{NH4} mediated by WYJ7 was 1.15 mg N kg^{-1} d^{-1} and 0.98 mg N kg^{-1} d^{-1} in SC1 and SC2, respectively, while the I_{NH4} mediated by WYJ3 were both nearly 0.20 mg N kg^{-1} d^{-1} in SC1 and SC2. The I_{NH4} in SC1-WYJ7 was 5.71 times higher than that in SC1-WYJ3, and I_{NH4} in SC2-WYJ7 had 5.01 times higher than in SC2-WYJ3.

The mean value of NH_4^+ retention time in alkaline soils was only 25.91 d (ranging from 17.67 d to 37.40 d), while the average NH_4^+ retention time in acidic soils was as high as 96.46 d (ranging from 55.56 d to 132.59 d), which was 3.72 times higher than alkaline soils (Fig. 3c). A pH-based analysis demonstrated

that the NH_4^+ retention time of acidic soils was significantly higher than the NH_4^+ retention time of alkaline soil ($P < 0.01$). The NH_4^+ retention time was negatively correlated with O_{NH4} ($P < 0.05$) (Fig. 5c). A stepwise linear regression of pH, O_{NH4}, I_{NH4}, TN, SOC and C/N with NH_4^+ retention time as the dependent variable revealed that the O_{NH4} was the key factor affecting the NH_4^+ retention time ($R^2 = 0.69$) ($P < 0.05$). Furthermore, the NH_4^+ retention time was positively correlated with NUE ($P < 0.05$) (Fig. 5d).

3.4 Plant N uptake rates

Both WYJ3 and WYJ7 exhibited the highest NH_4^+ uptake (U_{NH4}) after planting in FJ with 5.15 mg N kg^{-1} d^{-1} and 5.64 mg N kg^{-1} d^{-1}, respectively, while the two rice species planted in SC2 had the lowest U_{NH4} with 3.23 mg N kg^{-1} d^{-1} and 3.65 mg N kg^{-1} d^{-1}, respectively (Fig. 3d). A significant difference had been found in the grouping analysis which, based on acidity and alkalinity ($P < 0.05$), showed that the U_{NH4} in acidic soils was 1.36 times higher than in alkaline soils. The U_{NH4} of rice (either WYJ3 or WYJ7) was higher than U_{NO3} among all treatments. The ratio of U_{NH4} to total N uptake rate ($U_{NH4} + U_{NO3}$) in rice was ranging from 55% to 61%, which was consistent with the nutritional property of its NH_4^+-preference. There was a trend that the U_{NH4} of WYJ7 was higher than that of WYJ3 in all four soils, but statistical differences were not characterized (Fig. 3d). U_{NH4} decreased significantly with increasing O_{NH4} ($P < 0.05$), and U_{NH4} showed a significant positive correlation with NUE ($P < 0.01$) (Fig. 5e-f), except FJ which was characterised by high C/N and low NH_4^+ concentration. Moreover, no correlation was found between U_{NO3} and NUE.

4. Discussion

4.1 pH affects NUE and N loss by regulating autotrophic nitrification

The results of this study showed that soil properties (especially pH) affected rice NUE. Among all soil types, whether planted with WYJ3 or WYJ7, NUE was found to be significantly higher in acidic than in alkaline soils, while N loss was lower in acidic soils than in alkaline soils. NUE increased with NH_4^+ retention time and U_{NH4}, and decreased with O_{NH4}.

In general, NUE is closely related to N uptake by crops (Xu et al. 2012). Rice is an NH_4^+-preference crop with a requirement for NH_4^+. Soil N transformation processes (especially autotrophic nitrification) are important drivers in regulating soil NH_4^+ concentration and NH_4^+ retention time. The lower nitrification of acidic soils than alkaline soils caused a higher NH_4^+ retention time, making NH_4^+ the dominant form of inorganic N. This phenomenon favored rice N uptake, which manifested itself in higher NUE. The coupled nitrification-denitrification process is an important factor contributing to the low NH_4^+ retention time and high N losses in alkaline soils. If large amounts of fertilizer-derived NH_4^+ are rapidly oxidized to NO_3^-, NO_3^- will rapidly diffuse to anaerobic zones by virtue of its strong mobility, thus undergoing a rapid

denitrification and leading to N loss (Yang et al. 2017). Therefore, regulating autotrophic nitrification seems to be an important way to simultaneously optimize NUE and mitigate N losses.

Among the factors affecting autotrophic nitrification, pH and substrate availability are the two key factors (Booth et al. 2005; Li et al. 2020). pH affects autotrophic nitrification in two main ways. Firstly, the release of NH_3 from soil is strongly influenced by pH (pKa (NH_3/NH_4^+) = 9.25), thus autotrophic nitrification rates are generally low in acidic soils (Li et al. 2018; Norton and Stark 2011). Secondly, soil autotrophic nitrification is a microbial-mediated process of NH_3 oxidation. Low pH can suppress the activity of AOB and thus inhibit nitrification (Stempfhuber et al. 2015). The current prevailing view is that AOA dominates the autotrophic nitrification process in acidic soils (Li et al. 2018), and AOB may contribute more in neutral and alkaline soils (Jiang et al. 2015). In this study, AOB was the primary contributor to the nitrification process across all soils. The aerobic status (i.e. moisture condition) is an important reason for the divergence of ecological niches of nitrifying microorganisms between flooded and upland soils. Compared to AOA, AOB is more suitable for survival under flooded, low-oxygen conditions (Dai et al. 2018; Li et al. 2007). Collectively, pH may be critical in influencing autotrophic nitrification in paddy soils. Besides, mineralization can also partially contribute to autotrophic nitrification via NH_4^+ production.

4.2 Rice genotypes significantly affect nitrification rates in rhizosphere

Differences between rice genotypes were shown to be an important factor to explain the observed NUE in several studies. For example, natural divergence of transporters such as *NRT1.1B*, *LHT1*, etc. can lead to differences in uptake of different N forms by *japonica* and *indica* (Guo et al. 2020a; Guo et al. 2020b; Hu et al. 2015). Genotype-mediated NUE also varies considerably among rice subspecies (Chen et al. 2022; Yi et al. 2019). Considering that external NH_4^+ concentration and NH_4^+ retention time can significantly affect rice N uptake, it is reasonable to believe that NUE differences between rice genotypes may be driven by their various feedbacks on soil N transformation. In other words, rice genotype with higher NUE must be more efficient in its feedback on soil N transformation regardless of soil pH. Variations in soil N transformation rates under different rice genotype cultivation were clearly showen in our study. We found significant differences in the autotrophic nitrification rates (all four soils) and NH_4^+ immobilization rates (SC1 and SC2) after rice genotypes cultivation. Autotrophic nitrification rates were significantly lower under WYJ7 than WYJ3 plants. WYJ7 had higher NH_4^+ uptake rates than WYJ3 in all soils, which could be attributed to its stronger nitrification inhibition capacity. These results basically verified our first hypothesis.

The regulation of soil nitrification by plants is mainly achieved through the BNI exudation process. Many studies on BNI were conducted under hydroponics, and to maximize the release of BNI from plants, a distilled water or NH_4^+ solution was often used by researchers, which may not respond to the quality and quantity of plant-exuded BNI in the plant-soil system (Coskun et al. 2017; Subbarao et al. 2006; Tanaka et al. 2010; Zakir et al. 2008). For example, sakuranetin exuded by sorghum was detected as having the ability to inhibit ammonia monooxygenase and hydroxylamine oxidoreductase, but it was ineffective in

soil assays (Subbarao et al. 2012). According to our pot experiments, autotrophic nitrification rates were significantly lower in soils planted with WYJ7 compared to WYJ3 ($P < 0.05$), both soil AOA and AOB abundances were also lower after WYJ7 planting. This shows that the difference in nitrification inhibition between WYJ3 and WYJ7 persisted across soil types, which, to our knowledge, has not been reported before. The synthesis and release of BNI is an environmentally influenced and plant-regulated mechanism. Higher NH_4^+ concentrations in rhizosphere can significantly stimulate the release of BNI (Otaka et al. 2021; Subbarao et al. 2007; Tanaka et al. 2010; Zakir et al. 2008). The initial ammonium concentration of JX was significantly higher than that of FJ ($P < 0.05$), but its autotrophic nitrification rates were lower after rice cultivation, suggesting that BNI is more capable of regulating autotrophic nitrification than substrate concentration. pH is another key factor affecting BNI exudation process. A study on BNI release from sorghum indicated that optimal BNI release was observed when solution pH = 5-6, while plants did not release BNI at solution pH > 7, even in the presence of NH_4^+ (Subbarao et al. 2012). A work by Zhang et al. (2019) on the mechanism of BNI release from rice showed that at pH = 3.0, rice had the maximum BNI release, but this result appeared to be meaningless in actual agricultural environment. Moreover, they did not investigate the exudation level of plants in an alkaline environment. Our results showed that autotrophic nitrification rates were higher after WYJ3 than WYJ7 planting in the two alkaline soils (SC1 and SC2) ($P < 0.05$). Simultaneously there were also trends in AOA and AOB that were consistent with autotrophic nitrification rates. These findings did not match our second hypothesis. Such differences may imply that rice could also exude BNI and inhibit autotrophic nitrification in alkaline soils, which are not limited to acidic soils.

Differences in autotrophic nitrification inhibition capacity can contribute to differences in soil NH_4^+ availability and thus cause different rice NH_4^+ uptake rates. Our results revealed that WYJ7 had higher NH_4^+ uptake rates than WYJ3 in four soils, unfortunately this difference was not significant. There are two possible reasons for the lack of significant differences. First, the seedlings used in our experiments were still in the tillering stage, when the N demand of seedlings is not strong. A study showed that high N status in plant seedlings leads to low N uptake (Ohlund and Nasholm 2004). The quantity of BNI exuded by plant changes depends on its age (Coskun et al. 2017). Significant differences in NH_4^+ uptake rates may occur when rice reach the jointing stage. Second, the isotopic marker ($^{14}NH_4{}^{15}NO_3$ or $^{15}NH_4{}^{14}NO_3$) added in this experiment was a solution with an NH_4^+-NO_3^- ratio of 1:1. The supply of NO_3^- can enhance plants NO_3^- uptake (Nacry et al. 2013), which may result in NH_4^+ uptake rates only accounting for 55%-61% of the total N uptake rates. Practical paddy soil is an NH_4^+-dominated environment where the applied N fertilizer is usually urea or $(NH_4)_2SO_4$. Thus the NH_4^+ concentration will be much higher than the NO_3^- concentration, leading to NH_4^+ uptake usually accounting for 60%-85% of the total N uptake in the field (Kirk and Kronzucker 2005). Therefore, the rates and the percentages of rice NH_4^+ uptake measured in the pot experiment were somewhat lower than under realistic field conditions. Furthermore, for the accuracy of the ^{15}N tracing experiment, we potted the plants at a density of one plant per pot, while field rice planting is usually 3-4 plants per clutch. Overall, the differences between genotypes in

nitrification inhibition and NH_4^+ uptake may be significantly more pronounced in the field. Subbarao et al. (2021) found a significant increase in yield and NH_4^+ uptake after introducing a chromosome that controlled BNI exudation into wheat. Compared to NO_3^--preference crop, the role of BNI in rice may be more relevant. Future work may need to be carried out in the field in combination with some gene editing techniques to explore the effect of nitrification inhibition ability on NUE and yield.

Interestingly, we observed significantly higher NH_4^+ immobilization rates under WYJ7 than WYJ3 planting in SC1 and SC2 ($P < 0.05$). This could be related to the changes in DOC. Carbon availability can strongly influence the N immobilization process (Ma et al. 2020). Reduced availability of labile carbon leads to a decrease in microbial biomass carbon, thus weakening the microbial N immobilization process (Baldos et al. 2015). Significantly higher DOC contents were found in SC1 and SC2 after WYJ7 compared to WYJ3. As a soluble fraction in rhizosphere, DOC is an accessible carbon source for microorganisms, and root exudates produced during the rice growing period can be rapidly used by microorganisms (Yuan et al. 2016), thus exhibiting higher NH_4^+ immobilization rates. Moreover, the high rates of WYJ7-mediated NH_4^+ immobilization in SC1 and SC2 did not reduce NH_4^+ uptake rates. This may be due to the fact that plants and microorganisms compete for N in different spatial and temporal ecological niches. After inorganic N is immobilized into the microbial biomass which is close to the rhizosphere, it would be gradually acquired by the root system through rapid microbial turnover (Kuzyakov and Xu 2013; Paterson 2003). The coupling of plant and microbial productivity not only contributed to rice NH_4^+ uptake, but also improved N retention in alkaline soils and mitigated N losses from nitrification and denitrification processes. However, we did not experimentally determine the correlation between rhizosphere deposition fluxes and NH_4^+ uptake, and the amount of N obtained by plants through this pathway may not be quantitatively significant.

5. Conclusion

We revealed that the effect of pH (acidic, alkaline soils) on autotrophic nitrification governed rice NUE. Rice genotypes were found to exhibit different regulations on N transformation processes in soils with different pH. Genotypes with stronger nitrification inhibition potentially enhanced NH_4^+ uptake. Moreover, certain genotype can mitigate soil N losses from alkaline soils by stimulating microbial NH_4^+ immobilization processes. More extensive screening of genotypes and field studies are needed to validate the potential of rice-soil interactions in enhancing NUE and yield.

Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

6. Acknowledgements

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Figures

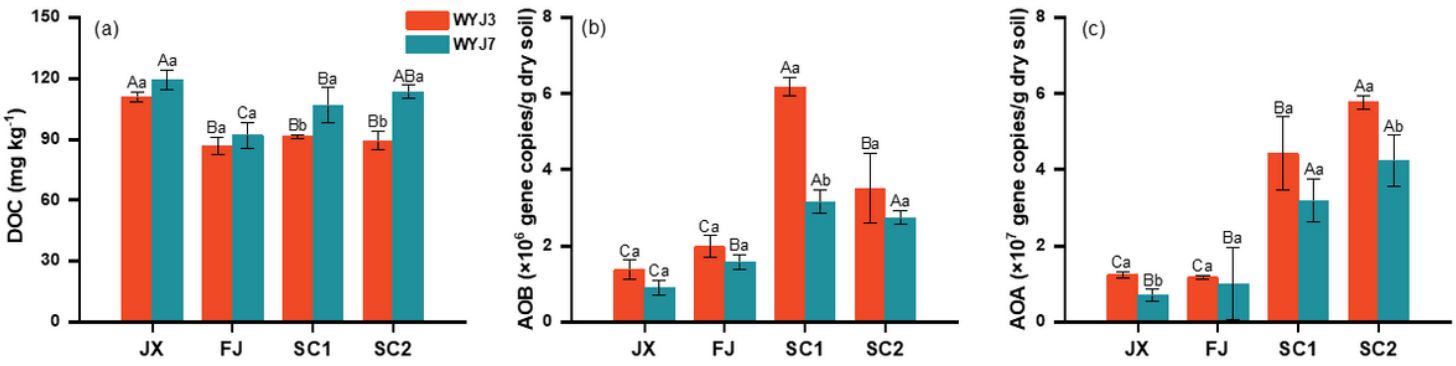

Figure 1

The contents of DOC (a), the abundances of ammonia-oxidizing bacteria (AOB) (b), and the abundances of ammonia-oxidizing archaea (AOA) (c).

WYJ3 is Wuyujing3, *japonica* rice (*Oryza sativa* L.); WYJ7 is Wuyujing7, *japonica* rice. JX is a paddy soil collected from Jiangxi, China; FJ is a paddy soil collected from Fujian, China; and SC1 and SC2 are paddy soils collected from Sichuan, China. Error bars represent standard deviations of the mean values. Different capital letters indicate significant differences in the same rice genotype in different soils ($P < 0.05$), and different lowercase letters indicate significant differences of rice genotypes in the same soil ($P < 0.05$).

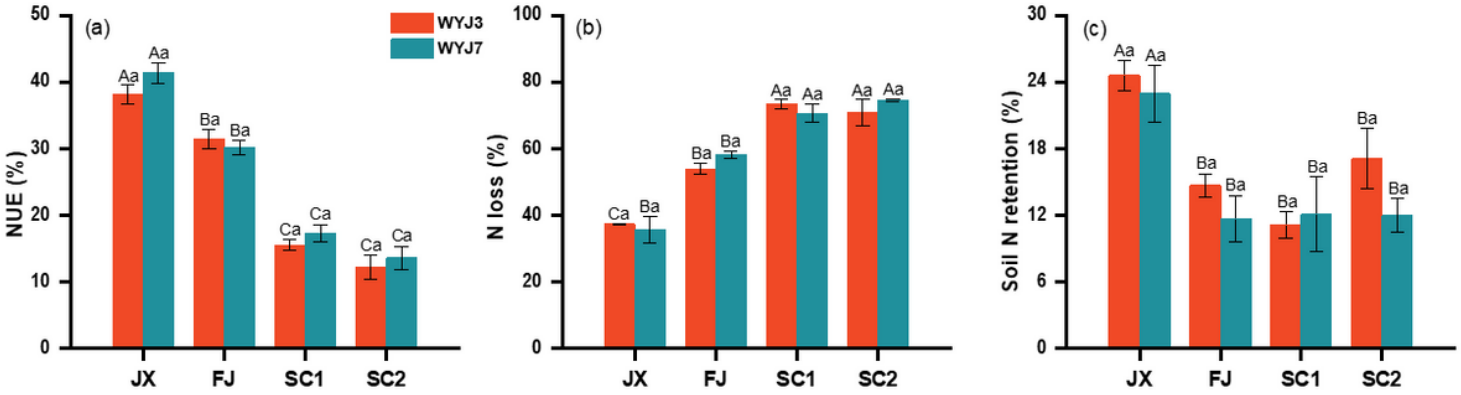

Figure 2

The values of nitrogen use efficiency (NUE) (a), nitrogen loss (N loss) (b), and soil nitrogen retention (Soil N retention) (c).

WYJ3 is Wuyujing3, *japonica* rice (*Oryza sativa* L.); WYJ7 is Wuyujing7, *japonica* rice. JX is a paddy soil collected from Jiangxi, China; FJ is a paddy soil collected from Fujian, China; and SC1 and SC2 are paddy soils collected from Sichuan, China. Error bars represent the standard deviations of the mean values. Different capital letters indicate significant differences in the same rice genotype in different soils ($P < 0.05$), and different lowercase letters indicate significant differences of rice genotypes in the same soil ($P < 0.05$).

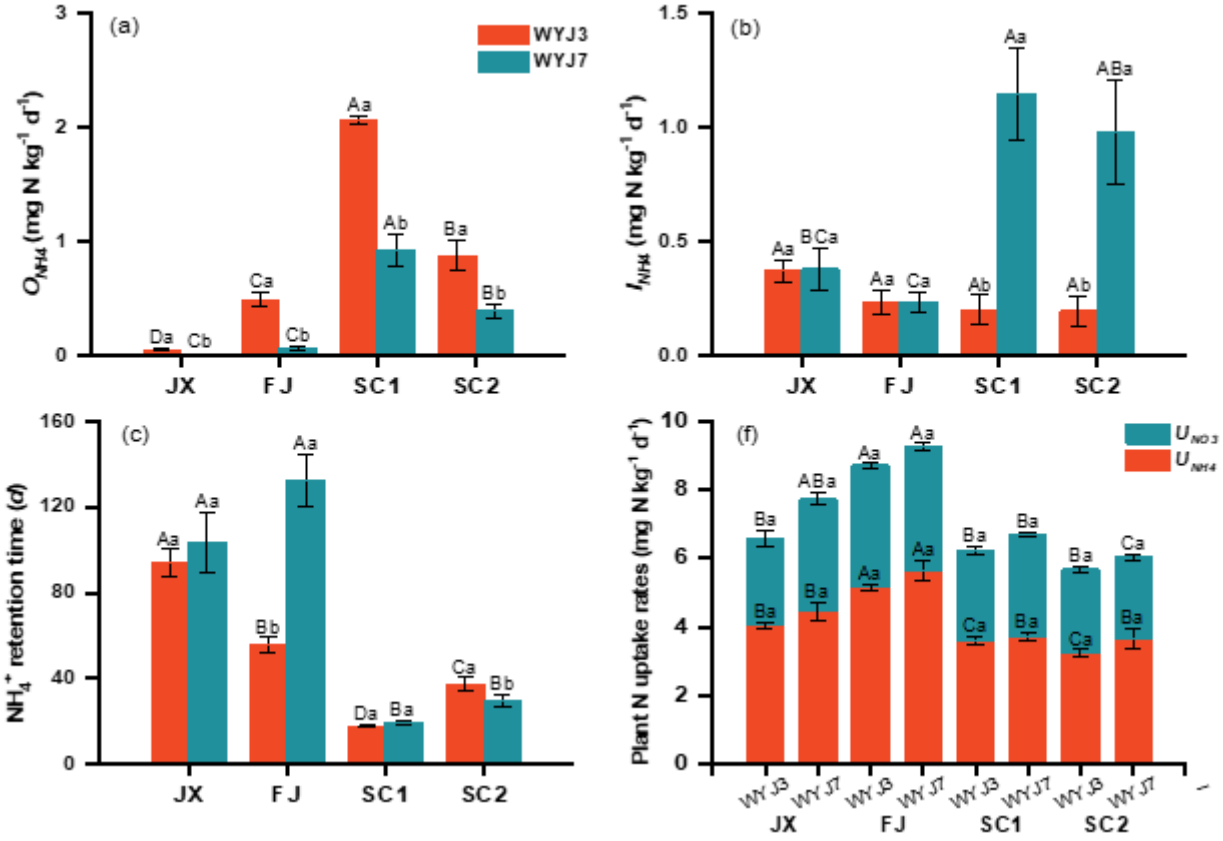

Figure 3

The rates of soil autotrophic nitrification (O_{NH4}) (a), the rates of soil ammonium immobilization (I_{NH4}) (b), the NH_4^+ retention time (c), and the rates of plant nitrogen uptake (U_{NH4} and U_{NO3}).

WYJ3 is Wuyujing3, *japonica* rice (*Oryza sativa* L.); WYJ7 is Wuyujing7, *japonica* rice. U_{NH4} is the rice ammonium uptake rates, U_{NO3} is the rice nitrate uptake rates. JX is a paddy soil collected from Jiangxi, China; FJ is a paddy soil collected from Fujian, China; and SC1 and SC2 are paddy soils collected from Sichuan, China. Error bars are the standard deviations of the mean values. Different capital letters indicate significant differences in the same rice genotype in different soils ($P < 0.05$), and different lowercase letters indicate significant differences of rice genotypes in the same soil ($P < 0.05$).

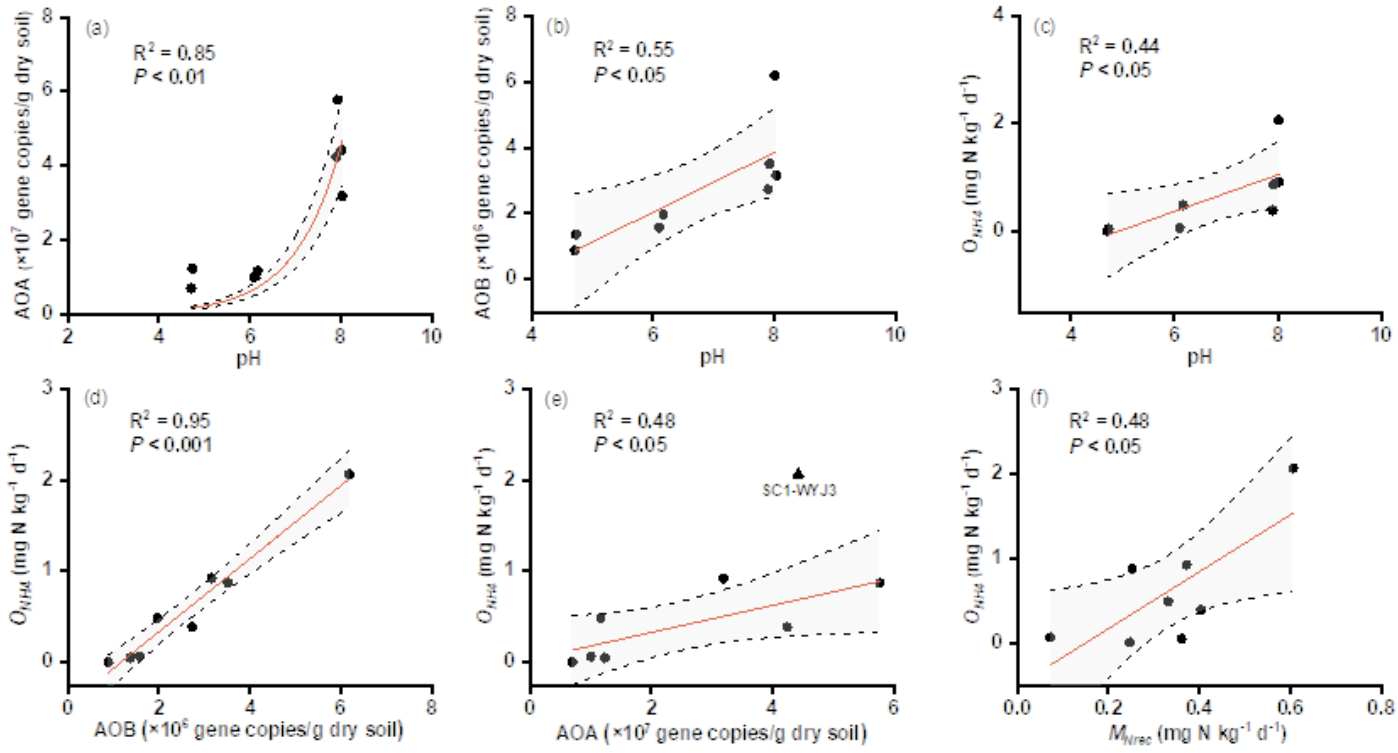

Figure 4

Relationships between soil properties and soil N transformation rates.

AOA, ammonia-oxidizing archaea; AOB, ammonia-oxidizing bacteria; O_{NH4}, autotrophic nitrification rate; M_{Nrec}, recalcitrant organic nitrogen mineralization rate. The solid dots are the mean rates included in the correlation analysis, and the dashed lines are the 95% confidence intervals. Solid triangles were not included in the regression analysis.

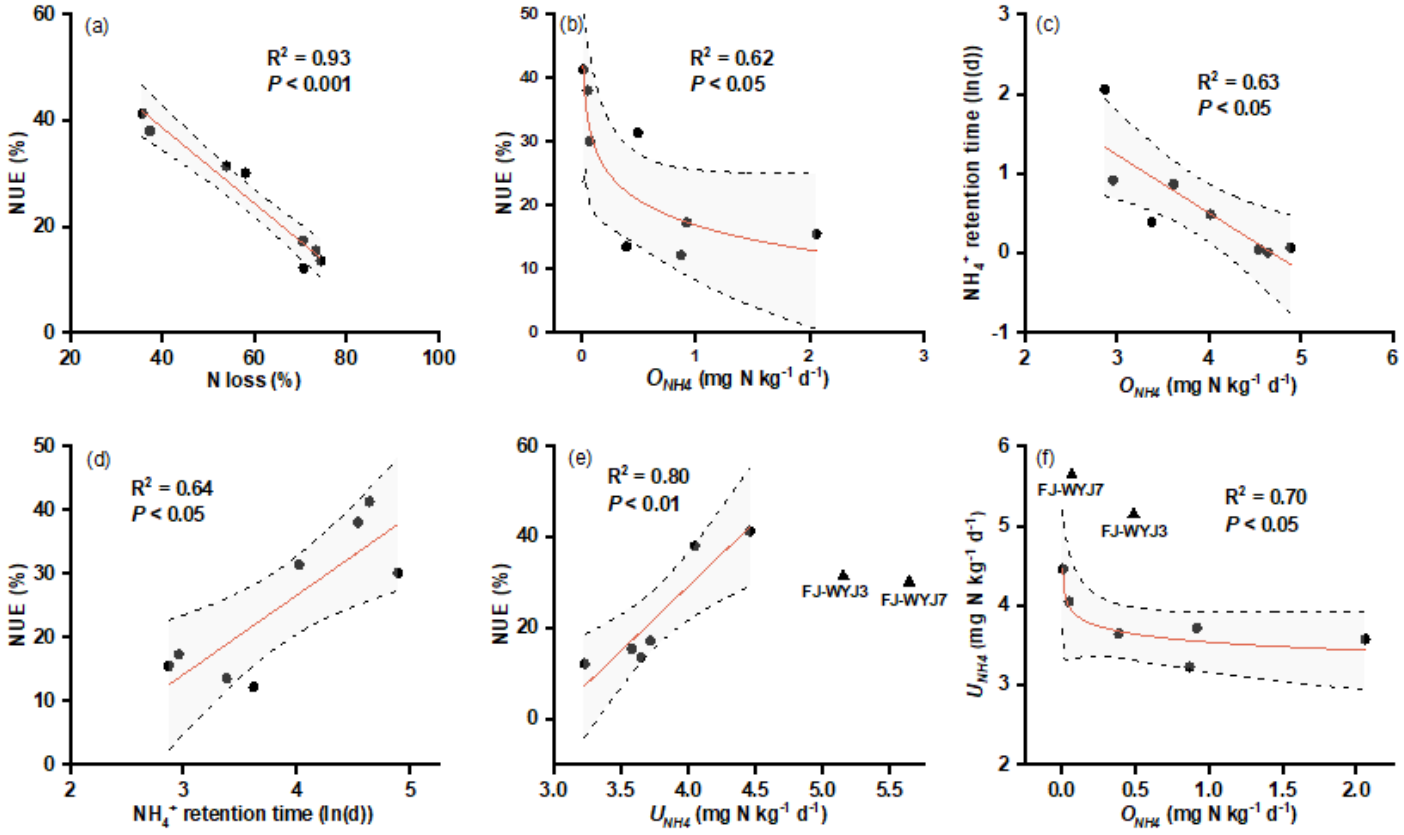

Figure 5

Relationships between soil N transformation and NUE.

NUE, nitrogen use efficiency; N loss, nitrogen loss; O_{NH4}, soil autotrophic nitrification rate; NH_4^+ retention time, soil ammonium retention time; U_{NH4}, rice ammonium uptake rates. The solid dots are the mean rates included in the correlation analysis, and the dashed lines are the 95% confidence intervals. Solid triangles were not included in the regression analysis.

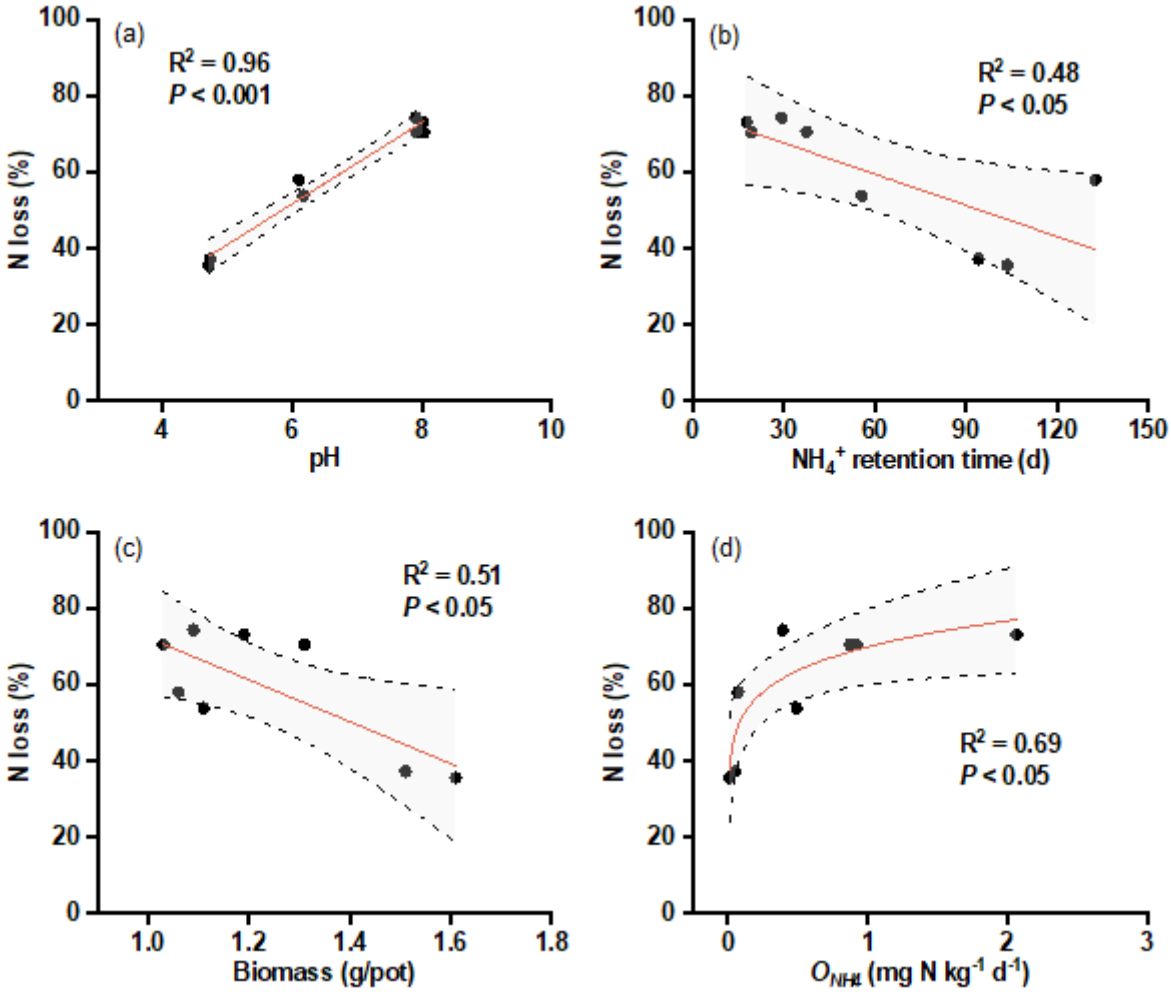

Figure 6

Relationships between soil pH, N loss, soil N transformation and biomass.

N loss, nitrogen loss; NH_4^+ retention time, soil ammonium retention time; O_{NH4}, soil autotrophic nitrification rate. The solid dots are the mean rates included in the correlation analysis, and the dashed lines are the 95% confidence intervals.

Supplementary Files

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