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ORIGINAL PAPER

Contribution of nitrification happened in rhizospheric soil growing with different rice cultivars to N nutrition

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Abstract A rhizobox with three compartments and soil slicing followed by quick freezing were used to study the spatiotemporal variations of nitrification of rhizospheric soil of Yangdao 6 (Indica) and Nongken 57 (Japonica). The results obtained revealed that ammonium (NH_4^+) was the main N form in flooded paddy soil. A concentration gradient for NH_4^+ was observed with the lowest concentration nearer to the root zone and the concentrations increased with increasing distance from the root zone. No concentration gradient was observed for nitrate (NO_3^-). The nitrification activities of both rice cultivars increased with the development of the incubation time. The nitrification activities were maximal in rhizospheric soil, followed by those in bulk soil and in the root zone. In the rhizosphere, nitrification activities decreased with increasing distance from the root zone. The maximal nitrification activity measured at 44, 51, and 58 days after sowing of Yangdao 6 and Nongken 57 rice cultivars was at a distance of 6 and 2 mm away from the root zone, respectively, and they were 0.88 and 0.73 mg kg^{-1} h^{-1}, respectively. In this experiment, the nitrification activities were significantly and positively correlated with the ammonia-oxidizing bacteria (AOB) abundance (r=0.86, p<0.01). The nitrification activity, NO_3^- concentration, AOB abundance, dry matter and N accumulation and leaf NO_3^- reductase activity associated with Indica were always higher than those with Japonica. Therefore, nitrification in rhizosphere had more important significance for N nutrition, especially for the Indica rice cultivars.

Keywords Paddy soil · Nitrogen use efficiency · Ammonia-oxidizing bacteria

Introduction

Rice is the primary cereal crop in China, and efficient production of rice crop is very important for the development of sustainable agriculture. It is estimated that the rice crop yields in Asia will increase by 64% in 2025 as compared to the 1991 yields, and nitrogen (N) fertilization will increase by 180%. However, the efficiency of rice to utilize the increased N input is expected to decrease from 51.7 to 30.2 kg of rice grain per kilogram of N in 1991 as compared to 2025 (Fischer 1998). At the present time, it is very common for farmers in China to apply 270 kg N fertilizer per hectare for a single rice crop, and N fertilizer recovery by rice plants is usually 30% (Zhu 2000). In some economically developed areas, rice-growing farmers apply more than 300 kg N fertilizer per hectare during the rice growth period. The excessive usage of N fertilizer will not only result in a tremendous waste of the N source but will also increase the costs of agricultural production and lead to serious environmental problems (Vlek and Byrnes 1986). It is important to consequently develop fertilizer practices that utilize the N fertilizer more effectively. This is not only an important issue in China but throughout the world.

In flooded paddy soils, the main N fertilizers are generally NH_4^+-based or urea fertilizers. Because rice plants have aerenchymatous tissue in their shoots and roots that allow atmospheric or photosynthetic oxygen to diffuse down into the rice roots (Armstrong 1971; Justin and Armstrong 1987; Blom and Voesenek 1996) and partial oxygen is released into the soil (Frenzel et al. 1992), thus nitrification occurs immediately in the aerobic niche of the

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rhizosphere or on the surface of the roots. Therefore, even in a flooded paddy soil, rice roots are actually exposed to a mixed N supply ($NH_4^+ + NO_3^-$), although the predominant species of mineral N in bulk soil for paddy rice field is likely to be NH_4^+ (Rubinigg et al. 2002; Briones et al. 2003). The very small amounts of $NO_3^- - N$ from the nitrification on the surface of rice roots, in the rhizosphere and in the oxidized thin surface soil layer, however, might be very important to N nutrition of rice plants (Kronzucker et al. 1999, 2000). Many reports have indicated that rice growth, yield, net N acquisition and N translocation to the shoot are superior when rice plants were fed with a mixture of NH_4^+ and NO_3^- compared with NH_4^+ alone at an identical N concentration (Youngdahl et al. 1982; Raman et al. 1995; Kronzucker et al. 1999). Yield increases of 40–70% have been observed in solution culture (Weissman 1964; Cox and Reisenzuer 1973; Heberer and Below 1989) when rice plants were fed with a mixture of NH_4^+ and NO_3^- compared with either NH_4^+ or NO_3^- as a sole N source.

Chemolithotrophic nitrification is the main nitrification reaction that occurs in terrestrial ecosystems and involves a two-step process (i.e., the conversion of ammonia to nitrite and then to NO_3^-). These steps are carried out by two different groups of organisms, the ammonia-oxidizing bacteria (AOB) and the nitrite-oxidizing bacteria, respectively (Warrington 1878; Winogradsky 1891). There are no known autotrophic bacteria that can catalyze the production of NO_3^- from ammonia (Kowalchuk and Stephen 2001). Therefore, rice plants and AOB are expected to compete for the same substrate, the NH_4^+ formed from mineralization of organic N or from the N fertilizer applied.

Kirk and Kronzucker (2005) establish a model to estimate the potential for nitrification, denitrification, and nitrate uptake in the rhizosphere of wetland plants. The model showed that NO_3^- uptake accounted for 34% of total N uptake, and the ratio of N denitrified to total N uptake was 0.20 or 6.6% of the NH_4^+ initially in the soil. The model also showed that rates of denitrification and subsequent loss of N from the soil remain small even where NO_3^- production and uptake were considerable. The calculation showed that wetland plants growing in flooded soil could take up a large part of their N as NO_3^- formed from NH_4^+ in the rhizosphere, without excessive losses of N through denitrification. Researches in our group showed that different rice cultivars responded very differently to NO_3^- added (Zhang et al. 2004; Duan et al. 2005). This raised several questions such as whether there is a relationship between the N use efficiency and the nitrification activity in the rhizosphere of different rice cultivars. What is the contribution of nitrification in the root rhizosphere to N nutrition of rice in soil culture condition? To answer these questions, we have to first detail the knowledge about nitrification in the rhizospheric soil of different rice cultivars. Therefore, we designed this experiment to study the nitrification in the root rhizosphere of two rice cultivars, Yangdao 6 (Indica) and Nongken 57 (Japonica). We selected these two rice cultivars as test cultivars mainly because, in our previous studies, we found that Yangdao 6 had a higher uptake rate of N and responds to a greater extent to NO_3^- than Nongken 57 (Zhang et al. 2004; Duan et al. 2005).

Materials and methods

Paddy soils

A typical paddy soil used in this experiment was collected from the farm of Jiangsu Agricultural Science Academy. The soil collected was completely and homogeneously mixed after being air-dried and grounded to be screened through a 1-mm sieve. The soil had a pH of 6.12 and 26.4% of clay and the contents of organic C, total N, $NH_4^+ - N$ and $NO_3^- - N$ were 28.9 g kg^{-1} soil, 1.2 g kg^{-1} soil, 7.4 mg kg^{-1} soil, and 1.4 mg kg^{-1} soil, respectively.

Rice cultivars

Two rice cultivars (*Oryza sativa* L.), Yangdao 6 and Nongken 57, which are very popular in Jiangsu province, China were used in this experiment. Yangdao 6 (Indica rice cultivar) had a higher N uptake while Nongken 57 (Japonica rice cultivar) had a lower N uptake.

Incubation

A rhizobox made from organic glass was designed to obtain rhizospheric soil samples at different distances away from the root zone. Detail of this rhizobox is shown in Fig. 1 (Li et al. 1994). The rhizobox was divided into three compartments with nylon gauze (30-μm mesh size), serving to separate the rice root soil (root zone soil) from the rhizospheric soil away from the root zone. The rice plants were planted in the middle compartment, and the mesh of the nylon gauze was fine enough to prevent the rice roots from penetrating. The paddy soil (air-dried, 2-mm sieved) was evenly mixed with urea (equivalent to 30 mg N kg^{-1} soil) and KH_2PO_4 (93 mg kg^{-1} soil). Six hundred grams of

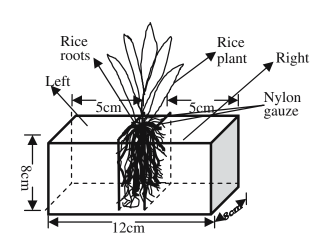

Fig. 1 Specification of the rhizobox

soil–fertilizer mixture were placed into each rhizobox, and deionized water was added immediately. Six rice seeds were directly sown in the center of the middle compartment in each rhizobox. One-centimeter surface water was maintained by adding deionized water every morning and evening throughout the experiment period. The rice plants were grown in a greenhouse and the average temperature was 28°C during the experiment.

Sampling and analysis

Sampling was conducted for three times, i.e., at 44, 51 and 58 days after sowing of rice seeds when the rice seedlings were at the seven-, eight- and nine-leaf stage, respectively. Six rhizoboxes of each rice cultivar were collected at each sampling time (destructive sampling). Three rhizoboxes of each rice cultivar were immediately put into the refrigerator at −20°C for 2 h for later determination of mineral N. The other three rhizoboxes of each rice cultivar were immediately used for the determination of ammonia-oxidizing bacteria. The soil collected in the middle compartment with rice plants was defined as root zone soil and the soils collected on the left and right compartments were divided into rhizospheric soil (0–4 mm away from the root zone) and bulk soil (>4 mm away from the root zone). The soils in the right and left compartments that had been frozen at −20°C for 2 h were carefully cut with a knife to create slices that were 2-mm thick, starting from the nylon gauze and moving outward (Shi et al. 2002). These soil samples were used to measure NH_4^+, NO_3^-, and short-term nitrification activity. The soils in the other three rhizoboxes of each rice cultivar were also cut into 4-mm-thick slices starting from the nylon gauze without being first stored at −20°C as pre-refrigeration will affect the numbers of AOB.

The soil samples were extracted with 2 mol l^{-1} KCl (soil-to-solution ratio 1:10) and the extracts were measured for NH_4^+ and NO_3^- by a continuous-flow auto-analyzer (model Autoanalyzer 3, Bran & Luebbe, Germany).

Short-term estimations are usually used for the assay of nitrification activity. According to the method of Berg and Rosswall (1985), the principle is based on the determination of nitrite after the incubation of soil samples with $NaClO_3$ in the absence of ammonium sulphate for 24 h at 25°C. The procedure is briefly described as follows. A total of 5 g of soil was placed in each of three Erlenmeyer flasks; 2.5 ml of sodium chlorate (75 mmol l^{-1}) were added; the flasks were capped; and two of the flasks were then incubated for 24 h at 25°C. To perform the control, the third flask was immediately placed in a −20°C environment. After incubation, 5 ml of deionized water was added followed by 10 ml of KCl solution (2 mol l^{-1}). The contents were mixed thoroughly and immediately filtered. Five milliliters of the clear filtrate was pipetted into glass test tubes, followed by 3 ml of buffer and 2 ml of the reagent for nitrite determination. The contents were again vigorously shaken and allowed to stand for 15 min at room temperature. Finally, the color intensity was measured at 520 nm.

The numbers of the AOB were measured by most probable number (MPN) counts. This method is based on the presence or absence of bacteria by using an extinction dilution in which replicate tubes of special culture medium are inoculated with 1-ml aliquots of the serial dilution. One prerequisite of the MPN method is that the microorganisms that are to be enumerated selectively must be able to cause some characteristic metabolite or product that can be detected easily by a special regent. Because the nitrite produced by the AOB can be sensitively detected by the Griess–Illosvay reagent, the MPN method was used in this experiment. The culture medium contains $(NH_4)_2SO_4$ 0.5g l^{-1}, NaCl 0.3 g l^{-1}, $FeSO_4 \bullet 7H_2O$ 0.03 g l^{-1}, K_2HPO_4 1 g l^{-1}, $MgSO_4 \bullet 7H_2O$ 0.3 g l^{-1} and $CaCO_3$ 7.5 g l^{-1} and is adjusted to pH 7.8. The test cultures were incubated at a constant temperature of 25°C for 14 days (Li et al. 1996).

The rice plants were oven-dried at 70°C for 48 h till constant weight after they were initially kept in an oven at 105°C for half an hour to kill the enzyme activity and weighed for dry matter accumulation. The dried rice materials were subsequently milled and digested for total N determination by the Kjeldahl method with a VAP50 Kjeldahl meter (Gerhart, Germany).

At the same time, fresh leaves of both rice cultivars were also collected for the measurement of NO_3^- reductase activity (NRA) using the method described by Duan et al. (2005).

Results

KCl extractable $NH_4^+ - N$

The concentrations of NH_4^+ associated with the Yangdao 6 rice cultivar did not change at different sampling days, while those associated with Nongken 57 decreased as time passed. For example, at the site 18 mm away from the root zone, the concentrations of NH_4^+ in Yangdao 6 soil at sampling days 44, 51, and 58 were 10.0, 10.3, and 9.2 mg kg^{-1} soil, respectively, while those of Nongken 57 were 9.7, 6.2, and 4.7 mg kg^{-1} soil, respectively (Fig. 2). In both treatments, the concentrations of NH_4^+ increased with increasing distance from the rice roots at 44, 51, and 58 days after sowing (Fig. 2). For example, among the three sampling dates, the average NH_4^+ concentrations of the soils in the root zone of Yangdao 6 and Nongken 57 were 3.8 and 2.4 mg kg^{-1} soil, respectively. However, in soil 40 mm away from the root zone, they were 12.9 and

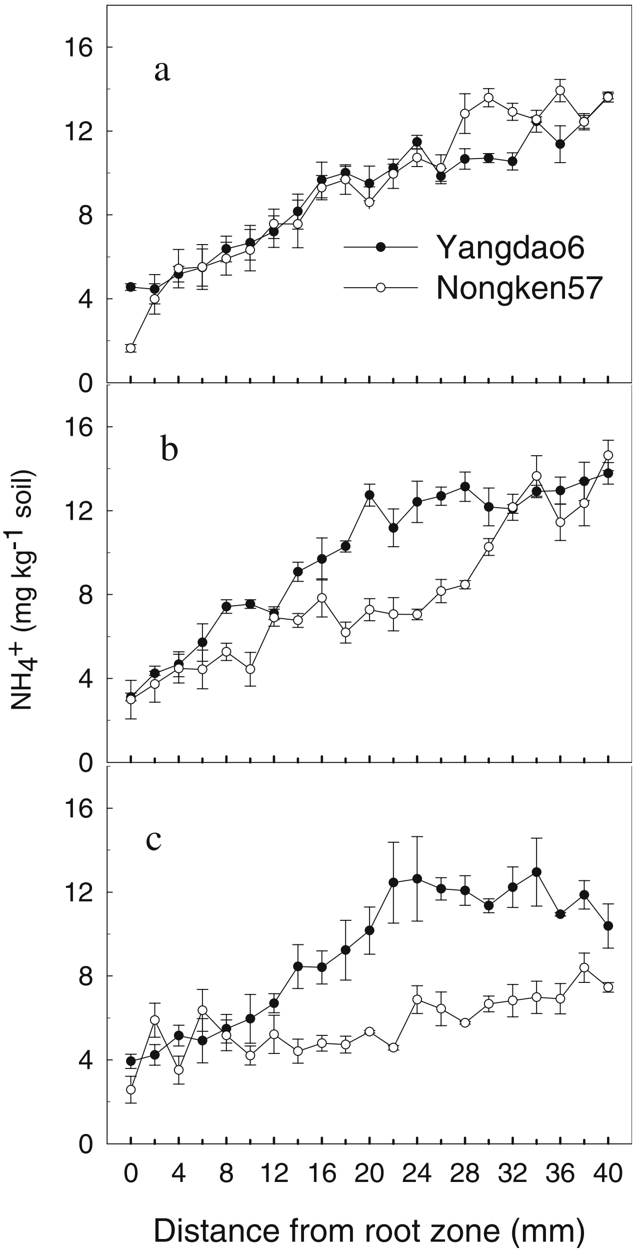

Fig. 2 NH_4^+ concentrations measured at different distances from the rice root in the flooded paddy soil growing Yangdao 6 and Nongken 57 rice cultivars at different sampling dates. **a** At 44 days after sowing; **b** at 51 days after sowing; **c** at 58 days after sowing. *Bars* indicate ±SE

11.6 mg kg^{-1} in the two cultivars, respectively. This illustrates that NH_4^+ was strongly absorbed by the rice roots, thus leading to a very distinct depleted zone of NH_4^+ (Arth and Frenzel 2000).

KCl extractable $NO_3^- - N$

The significant distribution characteristic of the $NO_3^- - N$ concentrations of the soils at different distances from the root zone was that there was almost no concentration gradient, except at the sampling date of 51 days after sowing. In terms of the changes among the different sampling dates, significantly decreased NO_3^- concentration with increased growth period of rice seedlings was found for both rice cultivars (Fig. 3a–c). The average NO_3^- concentrations in soil associated with Yangdao 6 were 1.86, 0.43, and 0.065 mg kg^{-1} soils collected at 44, 51, and 58 days after sowing, respectively. For those of Nongken 57, they were 1.67, 0.25, and 0.031 mg kg^{-1} soil at 44, 51, and 58 days after sowing, respectively. The soil NO_3^- concentrations associated with Yangdao 6 and Nongken 57 decreased by 96.5% and 98.1% from 44 to 58 days after

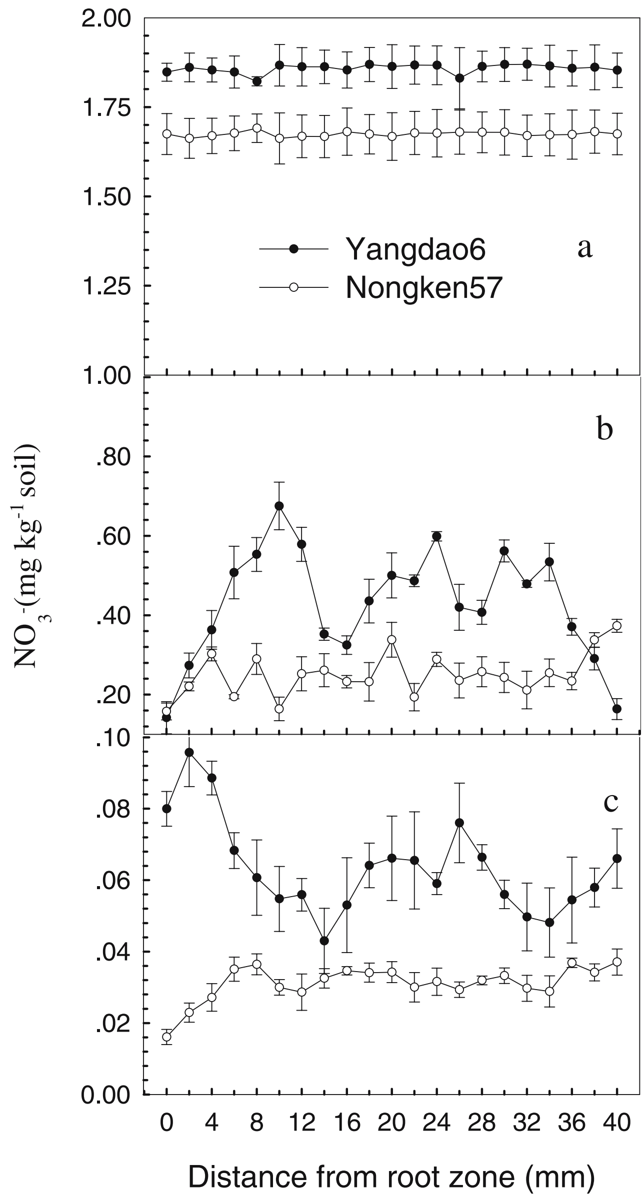

Fig. 3 NO_3^- concentrations measured at different distances from the rice root in the flooded paddy soil growing Yangdao 6 and Nongken 57 rice cultivars at different sampling dates. **a** At 44 days after sowing; **b** at 51 days after sowing; **c** at 58 days after sowing. *Bars* indicate ±SE

sowing, respectively. The NO_3^- was hardly detectable in the last sampling (Fig. 3c), showing that more NO_3^- might be absorbed by the rice plants at later growth stage. The NO_3^- concentrations of the soils growing with Yangdao 6 were always higher than Nongken 57 in the three sampling analysis (Fig. 3). This was due to a higher nitrification of Yangdao 6 growing soil (see below).

Short-term nitrification activity

We conducted further tests to determine the nitrification potential of soils collected at different distances away from the rice root zone of the two cultivars. The nitrification activities of soils growing with both rice cultivars increased with the development of the rice plants (Fig. 4). Maximal nitrification was found in the soil that was 4–6 mm away from the root zone and nitrification activities decreased significantly with increasing distance from the root zone for both rice cultivars. For example, the maximal nitrification activity values measured were 0.88 and 0.73 mg kg^{-1} h^{-1} in Ynagdao 6 and Nongken 57, respectively, while the minimal nitrification activity values measured in the root zone at 44 days after sowing were 0.30 and 0.075 mg kg^{-1} h^{-1}, respectively. An unexpected observation for both rice cultivars and at different sampling dates was that the nitrification activities in the root zone were significantly lower than those in the rhizosphere. This might be attributed to a low pH caused by acidic root exudates and to a lower NH_4^+ concentration in the root zone (Arth and Frenzel 2000; Fig. 4), which resulted in a lower number of AOB (see Table 2). The nitrification activities of Yangdao 6 growing soil were always higher than Nongken 57 growing soil at the different sampling locations and different sampling dates (Fig. 4).

Ammonia-oxidizing bacteria abundance

The AOB abundance in the root zone was always lower than in the rhizospheric soil. The highest numbers of AOB were always found at a distance of 0–4 mm away from the root zone for both rice cultivars (Table 1). Nevertheless, the AOB abundance of the root zone and rhizosphere (0–4 mm away from the root zone) associated with both rice cultivars increased with increasing age of the rice plants. The AOB abundance of Yangdao 6 growing soils was consistently much higher than Nongken 57 growing soils (Table 1). For example, the AOB abundance of Yangdao 6 growing rhizospheric soil was increased by 1.1 times compared with Nongken 57 growing rhizospheric soil at 44 days after sowing and increased by 1.4 and 1.6 times at 51 and 58 days after sowing, respectively. In this experiment, the nitrification activities were significantly and positively correlated with the AOB abundance (r=0.86, p<0.01; data not shown).

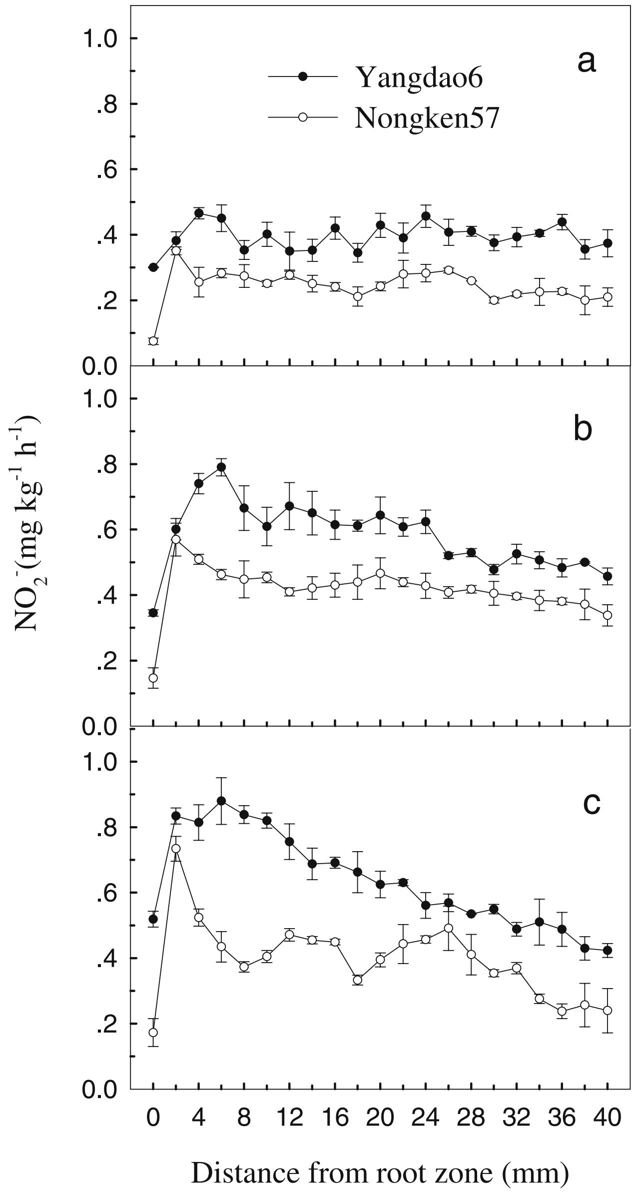

Fig. 4 Nitrification activity (short-term estimation) measured at different distances from the rice root in the flooded paddy soil growing Yangdao 6 and Nongken 57 rice cultivars at different sampling dates. **a** At 44 days after sowing; (**b**) at 51 days after sowing; **c** at 58 days after sowing. *Bars* indicate ±SE

Dry matter accumulation

The dry matter accumulations of both Yangdao 6 and Nongken 57 increased significantly with the development of the rice plants, ranging from 0.22 to 0.77 g $plant^{-1}$ in the whole sampling periods (Fig. 5). In the whole incubation periods, the Yangdao 6 grew always better than Nongken

Table 1 Ammonia-oxidizing bacteria abundance of soils collected at different distances and at different rice growing periods as measured by the method of MPN

Treatment	Incubation time (days)	Root zone (10^5 cells per gram dry soil)	Rhizospheric soil (10^5 cells per gram dry soil)	Bulk soil (10^5 cells per gram dry soil)
Yangdao 6	44	3.46±0.66 (b)	10.6±1.03 (b)	8.85±0.55 (a)
	51	5.90±0.61 (a)	25.1±2.29 (a)	10.5±0.51 (a)
	58	6.13±0.85 (a)	27.4±2.62 (a)	10.7±1.08 (a)
Nongken 57	44	2.09±0.30 (b)	9.77±0.64 (b)	7.23±0.69 (a)
	51	2.55±0.26 (b)	19.4±1.23 (a)	9.48±0.35 (a)
	58	3.31±0.52 (a)	20.5±1.22 (a)	9.86±0.51 (a)

Means followed by different letters in parentheses on the same column indicate significant difference at $p<0.05$ level by least significant difference test

57 did. For example, the dry matter accumulations of Yangdao 6 were 1.49, 1.66, and 1.45 times higher than those of Nongken 57 at 44, 51, and 58 days after sowing.

Total N accumulation

The total N accumulations of both Yangdao 6 and Nongken 57 increased significantly with the development of the rice plants (Fig. 6), ranging from 2.90 to 17.9 mg N plant^{-1} in the whole sampling periods. Compared with the dry matter accumulation, the increases of total N accumulations of both rice cultivars with the development of rice seemed to be more significant. For example, the increases of total N accumulations of Yangdao 6 were 1.69 and 3.94 times at 51 and 58 days than 44 days after sowing, while those of Nongken 57 were 1.56 and 3.84 times. In the whole incubation periods, the total N accumulations of Yangdao 6 were always higher than those of Nongken 57. For example, the total N accumulations of Yangdao 6 were 1.25, 1.32, and 1.28 times higher than those of Nongken 57 at 44, 51, and 58 days after sowing.

Leaf nitrate reductase activity

The leaf NRA values of Yangdao 6 increased significantly with plant growth (Fig. 7). The leaf NRA values of Nongken 57 had no significant differences between 44 and 51 days after sowing, but the leaf NRA value at 58 days after sowing increased significantly compared with the previous samplings. For example, the maximal and minimal leaf NRA values measured were 20.5 and 6.98 mg kg^{-1} FW h^{-1}, respectively. The leaf NRA values of Yangdao 6 were always higher than those of Nongken 57 at 44, 51, and 58 days after sowing.

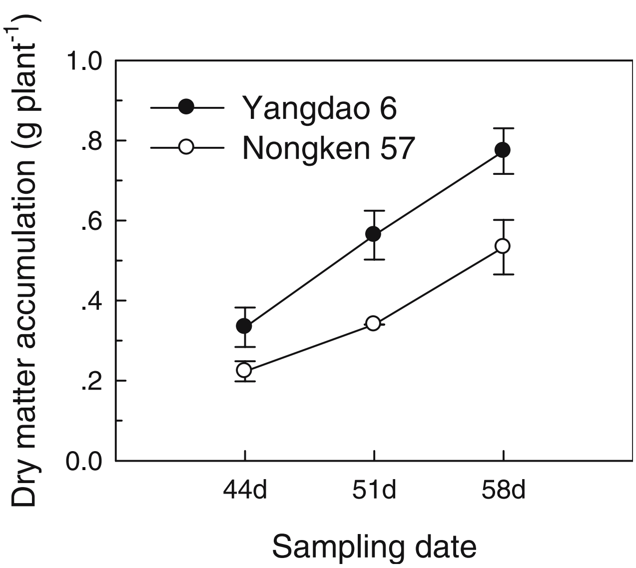

Fig. 5 Dry matter accumulations of Yangdao 6 and Nongken 57 rice cultivars at different sampling dates. *Bars* indicate ±SE

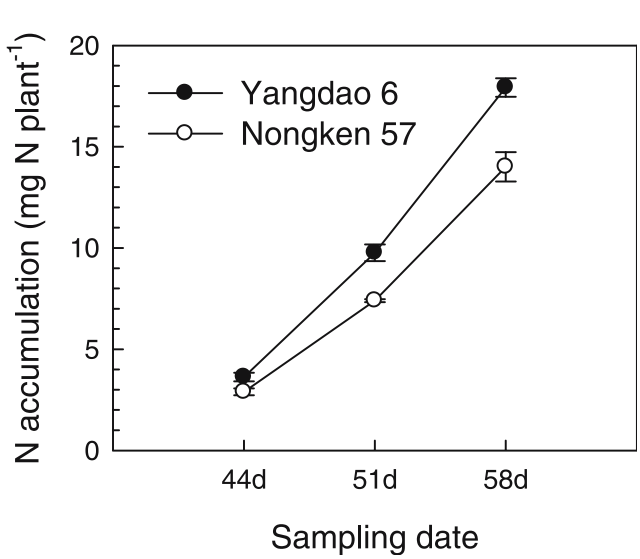

Fig. 6 Total N accumulations of Yangdao 6 and Nongken 57 rice cultivars at different sampling dates. *Bars* indicate ±SE

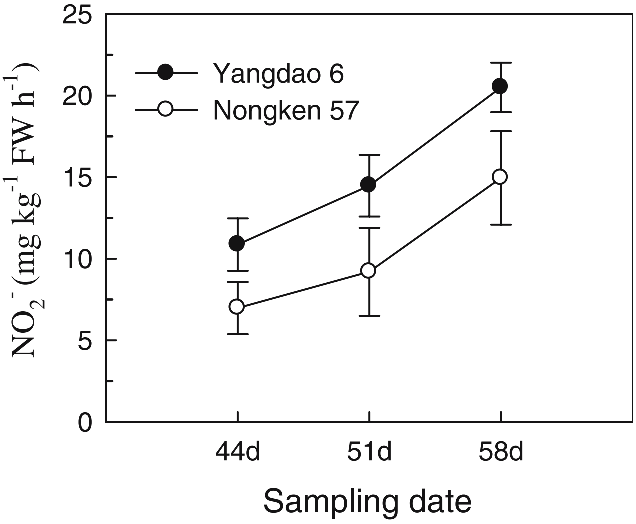

Fig. 7 Leaf nitrate reductase activities measured of Yangdao 6 and Nongken 57 rice cultivars at different sampling dates. *Bars* indicate ±SE

Discussion

Uptake of NH_4^+ and NO_3^- by Indica and Japonica

NH_4^+ is the main form of mineral N in paddy soil (Arth et al. 1998; Briones et al. 2003). In our experiments, the NH_4^+ concentrations were much higher than the NO_3^- in the soils growing with the two rice cultivars. This occurred at all sampling times. A distinctly depleted zone of NH_4^+ was created in the rhizospheric soil of both rice cultivars (Figs. 2 and 3), which is consistent with results reported previously (Arth and Frenzel 2000). This again supports the idea that the transportation way of NH_4^+ in soil is mainly by diffusion.

Although the amounts of NO_3^- are small in lowland paddy soil, this form of N is of importance for growth of rice plants (Kronzucker et al. 1999, 2000). Duan et al. (2005) reported that a ratio of 50/50 $NH_4^+ - N/NO_3^- - N$ could increase the average biomass of rice shoots and roots by about 20% and total N accumulation was increased by 50% when compared with those of 100/0 $NH_4^+ - N/NO_3^- - N$ at the seedling stage. This inferred that some NO_3^- in rhizosphere or on the surface of rice roots can be significantly important for the N nutrition of rice plants (Singh and Singh 1994; Ghosh and Kashya 2003).

During the last 20 years, Chinese farmers have changed to growing Indica and hybrid rice cultivars from Japonica rice because of their abilities to achieve higher yields. This trend has occurred throughout China and is illustrated by the rice cultivation statistics of Jiangsu province, one of the chief rice-producing areas in China. The reason for the high yields of Indica might be attributed to the stronger ability of the Indica, compared to Japonica, to exploit NO_3^- as a N source. Duan et al. (2005) used solution culture experiments to show that the activities of nitrate reductase (NR) and glutamine synthetase in the leaves of Indica were higher than in Japonica, which suggested that Indica could use more NO_3^- than Japonica rice. Fan et al. (2005) discovered that the root cell membrane potential of Yangdao 6 was more responsive to external NO_3^- than those of Nongken 57. In the homogeneous plants, the genotypic difference of NO_3^- absorption has been found in many other crops, such as wheat, barley, and maize (Chanh et al. 1981). The superiority of Indica for NO_3^- uptake was mainly due to the higher V_{max} at the early growth stage (20 days) and the higher affinity of NO_3^- transporters for NO_3^- at the middle growth stage (50 days) (Feng et al. 2003).

Other results also show that Indica rice has stronger assimilation of NO_3^- than Japonica (Kronzucker et al. 2000; Aurelio et al. 2003). In this experiment, NO_3^- concentrations of soils growing with Yangdao 6 and Nongken 57 were decreased by 96.5 and 98.1% at 44 and 58 days after sowing, respectively, and the average NO_3^- concentrations of soils growing with Yangdao 6 and Nongken 57 were 0.065 and 0.031 mg kg^{-1} soil, respectively, in the last sampling (Fig. 3). This indicates a high capacity for NO_3^- acquisition of both rice cultivars. NR is a key enzyme involved in NO_3^- assimilation in crops and its activity is greatly dependent on the NO_3^- concentration. In this experiment, the leaf NRA values of Yangdao 6 were 1.56, 1.57, and 1.37 times higher than those of Nongken 57 at 44, 51, and 58 days after sowing (Fig. 7). The NO_3^-, which is rapidly created from the oxidation of NH_4^+, can be quickly absorbed by rice roots or diffuses to other places in the soil at a rapid rate (Cai 2002). Therefore, the NO_3^- distribution in flooded paddy soil is relatively even, which was also confirmed in our experiment.

The variation of rice nitrification activity

The urea-fertilized rice plants actually received a mixture of NH_4^+ and NO_3^- with ratios depending on root-associated nitrification activity. The results of our experiment showed that the nitrification activities of soils growing with both rice varieties increased with plant age (Fig. 4), which might be linked with an increase in the ability of root-associated oxygen leakage with the growth of rice roots (Ghosh and Kashya 2003). With an increase of oxygen concentration around the rice root, the AOB abundance and activities were increased and the nitrification activity was enhanced, thus producing more NO_3^-. Because the NO_3^- in flooded paddy soil was mostly transformed from NH_4^+ through nitrification that occurred in the rhizosphere and in the interface between paddy soil and flooded water (Adhya et al. 1996; Arth et al. 1998; Arth and Frenzel 2000), the increasing characteristics of nitrification activities with the development of the rice plant might provide relatively sufficient amounts of NO_3^- to improve rice plant growth. Radial oxygen fluxes decreased with distance from the root tip, with the lowest oxygen release at a distance of about 30–60 mm from the root tip (Armstrong 1971; Gilbert and Frenzel 1998). Because of a sharp decrease of pH at the root surface by NH_4^+ uptake by rice roots (Colmer and Bloom 1998) and the depletion of NH_4^+ in the root zone, nitrification activities measured in the root zone were much lower than those measured in the rhizosphere and bulk soil. The maximal nitrification activities measured at 44, 51, and 58 days after sowing of Yangdao 6 and Nongken 57 were at a distance of 6 and 2 mm away from the root zone, respectively (Fig. 4). The difference in the abilities of root-associated oxygen leakage might well explain this result (Ghosh and Kashya 2003). Furthermore, the nitrification activities of soil growing with Yangdao 6 (Indica) were always higher than Nongken 57 (Japonica) at the different sampling places and different sampling times (Fig. 4) and this might explain the reason why the NO_3^- uptake velocity of traditional Indica was faster than that of traditional Japonica (Feng et al. 2003), and the Indica is more able to take up NO_3^- than NH_4^+.

The relation between AOB abundance and nitrification activity

In this experiment, nitrification activities were significantly and positively correlated with AOB abundance (r=0.86, p<0.01). In water-treatment systems, the concentration of AOB is directly proportional to nitrification activity (Chen et al. 1996). Ghosh and Kashya (2003) studied three different rice types growing in an irrigated rice ecosystem and found that the rates of nitrification in fertilized plots were significantly correlated with the AOB population (p<0.05). Thus, ammonia oxidation is thought to be the rate-limiting step for nitrification (Sahrawat 1982; De et al. 1990; Kowalchuk and Stephen 2001). Besides AOB, the methanotrophic bacteria can also oxidize NH_4^+ into NO_2^- by utilizing their methane monooxygenase system and dehydrogenase, and these bacteria potentially account for a significant portion of the total NH_4^+ oxidation in rice microcosms (Bodelier and Frenzel 1999). The methanogens should be further studied to understand their role in the nitrification process in paddy soils.

The contribution of nitrification to plant growth and N accumulation

Among the three sampling dates, the rice plant dry matter and total N accumulations of Yangdao 6 were always higher than those of Nongken 57 (Figs. 5 and 6). Many studies have shown that the rice plant growth and yield response due to a mixed application of NH_4^+ and NO_3^- could be attributed to up-regulation of N uptake and metabolism by NO_3^- (Kronzucker et al. 1999). A higher nitrification activity associated with Yangdao 6 led to a higher NO_3^- production for the rice roots and the subsequent higher absorption and assimilation of NO_3^-, which might be the main reason why Yangdao 6 had a higher dry matter and N accumulations than Nongken 57.

Conclusions

The nitrification activities of soils were as follows: rhizosphere > bulk soil > root zone. Nitrification was increased with the development of the rice plants. Nitrification activity was closely related to the amounts of AOB in the root zone, rhizospheric soil, and bulk soil. The NO_3^- produced by nitrification in the root zone and rhizospheric soil is very important for the N nutrition of rice. Soil growing with Yangdao 6 (Indica) had a greater nitrification ability than Nongken 57 (Japonica) and this might account for the reason why Yangdao 6 had a higher dry matter and N accumulations than Nongken 57.

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