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Rice Cultivars Influence Nitrogen Transformation in Rainfed Rice Ecosystem

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Abstract In a tropical rainfed rice field, soil mineral–N pool, rates of N–mineralization, nitrification and nitrifier population beneath these different rice cultivars were quantified at regular intervals during the cropping season. The experiment was laid out in a completely randomized block design with three replicates. Factors were rice cultivars (Heera, Dhalaheera and Narendra–118) and N–fertilization (0 and 100 kg N ha^{-1}). *In situ* measurements were taken for rates of N–mineralization, nitrification and soil moisture. The mineral–N pool, soil pH , nitrifying bacterial population, plant growth parameters, root air space and grain yield were also estimated. There were intercultivar differences in soil mineral–N pool, rates of N–mineralization, nitrification and nitrifier population. Intercultivar differences in plant biomass production, which indicates the differences in nitrogen utilization potential and indirectly the quantity and quality of litter production may explain in part the differences in N–mineralization processes. The nitrifying bacterial population showed a strong correlation with root biomass and root air space. The rice cultivars differed significantly in aerenchyma tissue differentiation resulting in different degrees of aerobic conditions in their rhizosphere. This explains the differences in nitrifier populations harboured by each of the cultivars in their respective soils and the consequent differences in soil processes. Hence, apart from fertilizer management, choice of rice cultivar also affects nitrifier populations and their processes, which are responsible for supplying nutrients to the rice soil.

Keywords N-mineralization, Nitrification, Nitrifier population, Rainfed rice soil, Rice cultivars.

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Introduction

The roots of rice contain aerenchyma (Justin and Armstrong 1987). The aerenchyma provides a low resistance internal pathway for movement of oxygen (Colmer 2003). Oxygen is essential for nutrient and water uptake (Gibbs et al. 1998). The oxygen also diffuses from root to rhizosphere where it can support aerobic microbial activity (Ueckert et al. 1990). Reddy and Patrick (1986) speculated that root aeration may also influence nutrient dynamics in the rhizosphere.

Substantial differences in the oxidizing power of rice roots, among varieties have been observed (Sanchez 1976). Differences in cultivar rhizosphere oxygenation (per unit area of roots and per plant) or root exudate (per unit weight of root) have been recorded (Armstrong 1969). Aerenchyma formation can differ between genotypes within a species in wheat (Huang et al. 1994). Colmer et al. (1998), showed that rice genotypes differed in their constitutive root porosity. Large variation in the adaptive traits of aerenchyma tissues, among different rice cultivars were observed by Fukao et al. (2006), Hattori et al. (2009). The ability of plants to modify their morphology in response to soil legacies is still poorly understood, (Baxendale et al. 2014), particularly under heterogenous soil conditions (Wubs and Bezemer 2016).

In the present study we examine the differences

in root porosity of three popular rainfed rice cultivars and their consequent influence on rate of N–mineralization, rate of nitrification and nitrifier population size in fertilized and unfertilized plots.

Materials and Methods

Study site

The study site was situated in the farmers rice fields of the Gangetic plains (25°18' N latitude, 80°1' E longitude and 76 meters above mean sea level). The region has a dry tropical climate with typical monsoonal character. The year can be divided into a cold winter (November–February, temperature 10–25°C, a hot summer (April-June, temperature 30–45°C) and a warm rainy season (July–September, with temperature ranging between 24–36°C). The annual rainfall averages 1100 mm, of which 85% falls during the rainy season from the South–West monsoon. The soil is an Inseptisol, deep, flat alluvial, pale brown in color, silty loam in texture (clay 3.6%, sand 30.7% silt 65.7%) and has a neutral reaction. In general the soil is well drained and moderately fertile having 0.68%–0. 76% organic carbon 0.07–0.08% total N and 304–340µg g^{-1} total P. It has a bulk density of 1.38 g cm^{-3} and water holding capacity of 39.2%.

Experimental design

The experimental field consisted of 18 plots each having a dimension of 5 × 3 m. A strip of 0.5 m land was left to separate each plot. The experiment was laid down in completely randomized block design with three replicate. Factors were rice cultivars (Heera, Dhalaheera and Narendra–118) and N-fertilization (0 and 100 kg N ha^{-1}). Following ploughing of all the plots to 20 cm depth and seedbed preparation, a basal dose of KCl +P_2O_5 + farm yard manure, at a rate of 60 kg K : 60 kg P : 1000 kg FYM ha^{-1} respectively was applied. P_2O_5 was added in the form of single super phosphate. Urea was applied to the fertilized plots in three split doses of 40, 30 and 30 kg N ha^{-1} few days after sowing, active tillering and flowering respectively. Seeds of three rice cultivars were sown by dibbling method in their respective plots (4—6 seeds per hill) at a spacing of 15 cm (hill to hill) by 20 cm (row to row). Compared to Heera (cross between CR 404 – 48 × CR289 – 1208) and Dhalaheera (local selection) Narendra–118 (cross between HANSRAJ × IR36) is a taller and late variety. The crop was kept weed free by manual removal of weeds with minimal disturbance in the plots. Rainfall was the only source of irrigation during the cultivation period.

Soil sampling

Soil samples were collected every 10 to 15 days from each replicate plot in triplicate and mixed to form a composite sample. Soil monoliths (10 × 10 × 10 cm) were removed between the rows and stored in polyethylene bags and brought to the laboratory. Each composite soil sample was divided into two parts. One part in the field moist condition was used for determination of pH, soil moisture and mineral–N (NH_4^+–N and NO_3–N). The second part (also in field moist condition) was used for assessing the N-mineralization, nitrification and nitrifier population. Soil samples were taken 18 days after sowing (DAS), during active tillering (38 DAS), panicle initiation (48 DAS), flowering / anthesis (58 DAS), physiological maturity (68 DAS) and pre-harvest (78 DAS). The samples were brought to the laboratory, spread on paper sheets and visible roots and fragments of organic debris were removed and the soil was sieved (2-mm mesh).

Soil analysis

Soil pH (1 : 2, soil : water) was measured using a pH meter equipped with glass electrode. Gravimetric soil moisture content was measured with freshly pulled out soil according to the following equation (Buresh 1991).

$$M = \frac{WCWS - WCDS}{WCDS - WC} \times 100$$

Where, M = Gravimetric soil moisture content (%) ; WCWS = Weight of can plus wet soil (g) ; WCDS = Weight of can plus dry soil (g) ; WC = Weight of moisture can (g).

Extractable soil ammonium nitrogen was estimated colorimetrically by the phenate method (APHA 1985). Nitrate nitrogen was measured by

Table 1. F-ratios and their significant levels for two way ANOVA with repeated measures for soil pH, mineral–N, N–mineralization, nitrification, ammonium oxidizers, nitrite oxidizers, root biomass, shoot biomass, root air space and soil moisture for three cultivars (Heera, Dhalaheera and Narendra-118) and two fertilization treatments (0 and 100 kg N ha^{-1}) where sampling time was treated as a repeated measure. * p< 0.05, ** p < 0.01, *** p< 0.001, ns = not significant. n=54 and [a]n = 36 respectively.

	Source of variation						
	Between subject			Within subject			
Parameters	Cultivar C	Fertilizer (F)	C×F	Time (T)	T×C	T×F	T×C×F
pH	0.27 ns	1.1 ns	9.1**	49.3***	4.5*	11.4**	9.5**
Mineral–N	188.8***	394.6***	4.5*	74.8***	5.5***	35.9***	7.9***
N–mineralization	1141.6***	169.3***	152.5***	321.8***	19.6***	17.0***	8.5***
Nitrification	645.8***	1049.9***	93.2***	309.5***	9.7***	78.7***	11.5***
A. oxidizers	135.3***	360.1***	9.5**	100.7***	7.7**	16.8***	10.3***
N. oxidizers	159.2***	234.1***	0.42 ns	255.2***	29.8***	14.5***	2.9**
Root biomass	2117.2***	454.7***	61.8***	1725.1***	140.9***	38.8***	16.9***
Shoot biomass	627.3***	281.9***	25.1***	986.7***	101.7***	32.5***	9.4***
Root air space [a]	58.8***	366.4***	6.1*	320.6***	29.6***	118.4***	18.9***
Soil moisture	2.9 ns	4.7*	11.2**	11.7***	3.3*	2.2 ns	1.1 ns

phenol disulphonic acid method (Jackson 1958). *In situ* rates of N–mineralization were measured for thirty days period at the sampling points using the buried bag procedure (Eno 1960). A soil corer (5.0 cm diameter×10 cm depth) was used to obtain an initial sample that was placed in a plastic bag and brought back to the laboratory for analysis. Immediately adjacent to the initial sample further soil cores of the same size were taken. Each intact soil core was wrapped and sealed in a polyethylene bag (after removing coarse roots and large fragments of organic debris in order to avoid any marked immobilization during incubation (Schimel and Parton 1986). The sealed polyethylene bags were replaced into the hole from which they were extracted and retrieved

Table 2. Cropping season averages (±SE) for ammonium N, nitrate—N and mineral–N content / μg g^{-1} dry soil, soil moisture / % and pH of soil in control and fertilized plots of rainfed rice cultivars (n=18). Values in a column with different superscript letters are significantly different from each other at p< 0.05 according to Tukey's HSD test.

Cultivars	Control	Urea
NH_4^+N		
Heera	6.40 ± 1.03[a]	8.78 ± 0.70[a]
Dhalaheera	5.53 ± 0.94[ab]	7.72 ± 0.97[a]
Narendra-118	4.07 ± 0.79[b]	5.48 ± 0.90[b]
NO_3^-N		
Heera	1.51 ± 0.33[a]	2.29 ± 0.35[a]
Dhalaheera	1.02 ± 0.18[b]	2.00 ± 0.33[a]
Narendra-118	0.72 ± 0.18[b]	1.72 ± 0.30[a]
Mineral-N		
Heera	7.92 ± 1.27[a]	11.08 ± 0.55[a]
Dhalaheera	6.56 ± 1.04[ab]	9.73 ± 0.82[a]
Narendra-118	4.80 ± 0.83[b]	7.22 ± 0.85[b]
Soil moisture		
Heera	23.71± 1.59[a]	26.50 ± 0.85[a]
Dhalaheera	24.82± 0.74[a]	21.05 ± 0.69[ab]
Narendra-118	24.39± 1.00[a]	24.00 ± 0.75[b]
pH		
Heera	7.4 ± 0.05[a]	7.6 ± 0.08[a]
Dhalaheera	7.5 ± 0.04[a]	7.5 ± 0.08[a]
Narendra-118	7.5 ± 0.08[a]	7.5 ± 0.09[a]

Table 3. Cropping season averages (±SE) for N-mineralization rates / μg g^{-1} mo^{-1} nitrification rates / μg g^{-1} mo^{-1} , viable population of ammonium oxidizer and nitrite oxidizer/ MPN × 10^5 g^{-1} dry soil, of soil in control and fertilized plots of rainfed rice (n=18). Values in a column with different superscript letters are significantly different from each other at p< 0.05 according to Tukey's HSD test.

Cultivars	Control	Urea
N-mineralization		
Heera	10.47± 1.51[a]	12.31 ± 2.25[a]
Dhalaheera	15.79± 2.28[ab]	18.85 ± 2.33[b]
Narendra-118	19.30± 2.13[b]	25.39 ± 2.37[c]
Nitrification		
Heera	1.22 ± 0.42[a]	2.15 ± 0.60[a]
Dhalaheera	2.04 ± 0.28[b]	3.40 ± 0.60[a]
Narendra-118	2.60 ± 0.28[b]	5.14 ± 0.85[b]
Ammonium oxidizers		
Heera	1.96 ± 0.32[a]	3.80 ± 0.42[a]
Dhalaheera	2.88 ± 0.37[b]	4.26 ± 0.46[a]
Narendra-118	3.66 ± 0.53[b]	6.08 ± 0.67[b]
Nitrite oxidizers		
Heera	1.45 ± 0.22[a]	2.41 ± 0.35[a]
Dhalaheera	2.11 ± 0.38[a]	3.13 ± 0.33[b]
Narendra-118	2.88 ± 0.45[b]	2.88 ± 4.01[b]

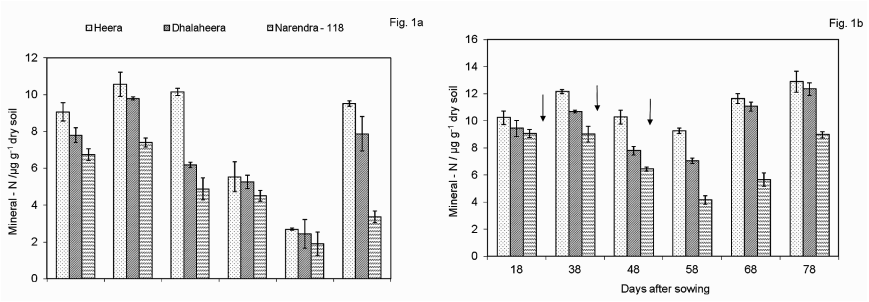

Fig. 1a, 1b. Temporal changes in mineral–N concentration in soil planted to different rice cultivars under (a) control and (b) fertilized condition across the cropping season. Arrows indicate days of fertilization. Bars indicate ±SE.

after thirty days (here in referred to as the incubated sample). Identical laboratory procedures were used for both the initial and the incubated samples. The samples were sieved through a 2 mm mesh to remove fine roots and large stones. Ten gram sub-samples were placed in extraction cups to which 100 ml of 2*M* KCl was added. The supernatant was analyzed for ammonium concentrations using the phenate method (APHA 1985). Similarly, NO_3–N concentration were measured by the phenol disulphonic acid method (Jackson 1958) after extracting the soil in $CaSO_4.2H_2O$. The remaining soil material was dried for 24 h at 105°C to determine soil dry mass. Rate of N–mineralization was calculated as the difference in the concentration of inorganic N (NH_4^+ and NO_3^-) ions in the incubated and initial sample (Hart et al. 1994, Jha et al. 1996). Net nitrification was calculated as the difference in the NO_3 – N concentration in the incubated and initial sample (Hart et al. 1994, Jha et al. 1996). Rate of N-mineralization and nitrification are expressed in units of µg N per gram dry soil per thirty days. Unless otherwise stated, all results were calculated on an oven–dry (105°C) soil weight basis.

Counts of nitrifiers

The viable population of nitrifiers i.e. ammonium oxidizers and nitrite oxidizers was estimated by the most probable number (MPN) technique (Alexander and Clark 1965). Inocula were prepared as follows ; 10 g soil and 90 ml sterile distilled water were placed in a sterile universal bottle (for each composite soil sample) and shaken vigorously on a wrist action shaker for 30 min. Serial ten fold dilutions were made by adding 1 ml of the suspension to 9 ml sterile water. Each successive dilution was shaken by hand for 30 s before a 1 ml portion was withdrawn. For both ammonium and nitrite oxidizers 10^{-4} to 10^{-9} dilutions were used. For each dilution five replicate culture tubes were employed. Ammonium – calcium carbonate medium $(NH_4)_2 SO_4$, 0.5 g ; K_2HPO_4, 1.0 g ; $FeSO_4$, $7H_2O$, 0.03 g ; NaCl, 0.3 g ; $MgSO_4.7H_2O$, 0.3 g ; $CaCO_3$, 7.5 g ; water, 1 liter was used for ammonium oxidizing bacteria and nitrite calcium carbonate me-

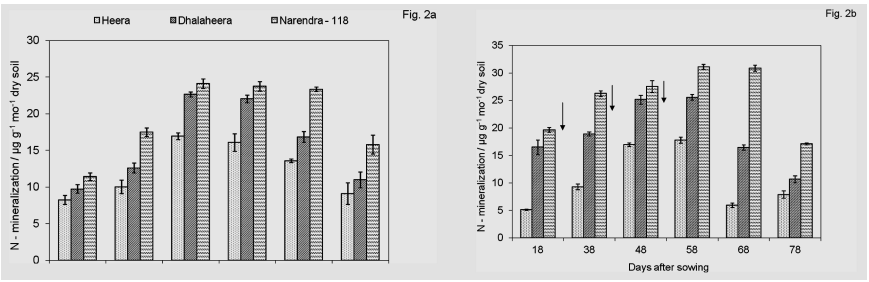

Fig. 2a, 2b. Temporal changes in rate of N-mineralization in soil planted to different rice cultivars under (a) control and (b) fertilized condition across the cropping season. Arrows indicate days of fertilization. Bars indicate±SE.

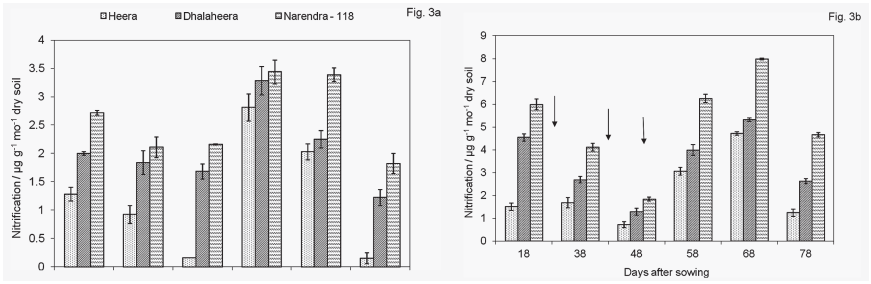

Fig. 3a, 3b. Temporal chenges in rate of nitrification in soil planted to different rice cultivars under (a) control and (b) fertilized condition across the cropping season. Arrows indicate days of fertilization. Bars indicate±SE.

dium (KNO_2, 0.006 g ; K_2HPO_4, 1.0g ; NaCl, 0.3g ; $MgSO_4$. $7H_2O$, 0.1 g ; $FeSO_4$. $7H_2O$, 0.03 g ; $CaCO_3$, 1.0 g ; $CaCl_2$, 0.3 g ; water, 1 liter for nitrite oxidizing bacteria. The inoculated media were incubated in the dark at 28±2°C. Tests for nitrifying activity were made after thirty days by testing each tube for nitrite using Griess-Ilosvay reagent. The number of tubes positive or negative to the test was noted and the most probable number of organisms present was calculated from an MPN Table (Cochran 1950).

Plant growth, grain yield and root porosity (root air space)

Plant growth and grain yield were measured from randomly selected fixed sites in each treatment at 10–15 days interval starting from 18 days after seed germination for both control and fertilized plots. One rice hill was harvested from each plot on each sampling date and roots were collected from a soil block (15 × 20 × 15 cm depth). The soil was carefully washed with tap water on a sieve (0.2 mm). The number of tillers per hill was counted. Subsequently roots and shoots were separated from each other and were dried at 65°C for 48 h, to constant weight, for biomass determination. All estimates described above were conducted in triplicate.

Grain yield was determined by harvesting all the hills in lm × lm quadrat area from the middle of each treatment plot. Grain and straw were separated in a rice threshing machine dried in a batch grain drier and weighed. Grain moisture was determined immediately after weighing and sub-samples were dried again in an oven at 65°C for 48 h. Grain weight is expressed on an oven dry (65°C) basis.

Root porosity is one of the most important parameters used to determine cortical oxygen concentration and reflects rhizosphere aerobicity. It was measured thrice (during panicle initiation, flowering and physiological maturity) by the water displacement

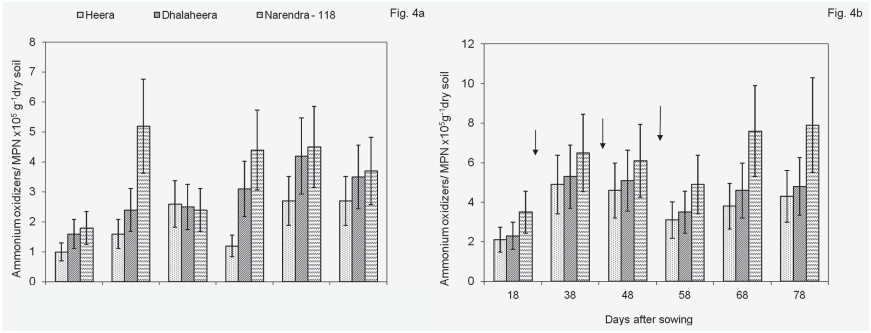

Fig. 4a, 4b. Temporal changes in ammonium oxidizer population in soil planted to different rice cultivars under (a) control and (b) fertilized condition across the cropping season. Arrows indicate days of fertilization. Bars indicate±SE.

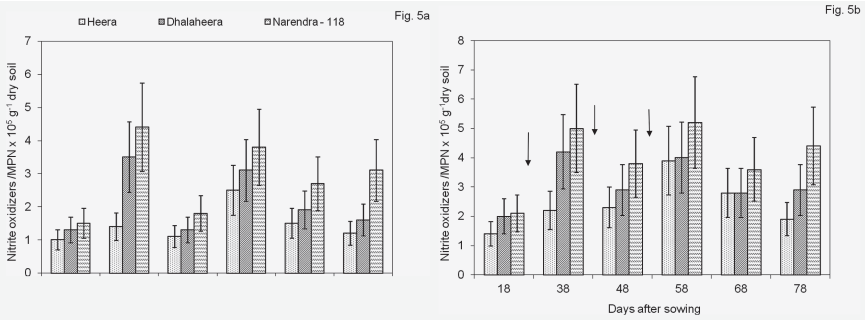

Fig. 5a, 5b. Temporal changes in nitrite oxidizer population in soil planted to different rice cultivars under (a) control and (b) fertilized condition across the cropping season. Arrows indicate days of fertilization. Bars indicate ± SE.

method (Jensen et al. 1969). A 15 ml pycnometer was filled with water and weighed. About 0.2 – 0.5 g of washed fresh roots were cut (2 to 2.5 cm from the root apex) and gently blotted dry. The roots were then carefully inserted into the water–filled pycnometer and reweighed. The roots were retrieved, ground with mortar and pestle and returned quantitatively into the pycnometer for reweighing. The porosity of roots was determined using the following relationships:

$$POR = \frac{(p \,\&\, gr) - (p \,\&\, r)}{(r + p) - (p \,\&\, r)} \times 100$$

Where, POR = Root air space (porosity) in percent (%) ; r=Mass of roots (g) ; p = Mass of water filled pycnometer (g) ; p & r = Mass of pycnometer with roots and water (g) and p & gr = Mass of pycnometer with ground roots and water (g).

Statistical analysis

All data analyses and statistical comparisons were performed using an SPSS package ((SPSS Inc. 2002). A general linear model (GLM) two way analysis of variance with repeated measures was used to analyse the effect of cultivar, fertilizer dose on soil processes, nitrifying bacterial population plant growth parameters and root porosity, where resampling of the same plots on six dates was treated as repeated measures. Tukey's HSD (Honestly significant difference) test was used to determine the significance of differences between cropping season averages. Pearson correlation coefficients for the observed parameters were also calculated. For each set of data analysis, the three replicate plots were considered as independent plots.

Table 4. Cropping season averages (± SE) for root biomass / g hill^{-1}, shoot biomass / g hill^{-1} and number of tillers / tillers hill^{-1} in control and fertilized plots of rainfed rice cultivars (n=18). Values in a column with different superscript letters are significantly different from each other at p<0.05 according to Tukey's HSD test.

Cultivars	Control	Urea
Root biomass		
Heera	0.73 ± 0.16^{a}	1.27 ± 0.35^{a}
Dhalaheera	1.37 ± 0.38^{ab}	1.48 ± 0.39^{a}
Narendra–118	2.08 ± 0.59^{b}	2.47 ± 0.75^{b}
Shoot biomass		
Heera	3.90 ± 1.42^{a}	5.79 ± 1.74^{a}
Dhalaheera	7.29 ± 2.33^{ab}	9.03 ± 3.21^{ab}
Narendra–118	9.45 ± 3.78^{b}	13.62 ± 5.88^{b}
Tiller number		
Heera	6.00 ± 1.06^{a}	7.50 ± 1.11^{a}
Dhalaheera	8.83 ± 1.19^{a}	13.00 ± 2.03^{b}
Narendra–118	14.50 ± 2.12^{b}	17.16 ± 2.68^{c}

Table 5. Cropping season averages (± SE) for root porosity / % and grain yield / kg ha^{-1} at harvest. (n = 9 and 3 respectively). Values in a column with different superscript letters are significantly different from each other at p < 0.05 according to Tukey's HSD test.

Cultivars	Control	Urea
Root porosity		
Heera	13.64 ± 1.24^{a}	19.36 ± 5.91^{a}
Dhalaheera	16.40 ± 1.82^{ab}	20.47 ± 5.72^{a}
Narendra–118	17.03 ± 2.18^{b}	23.46 ± 7.80^{a}
Grain yield		
Heera	1147.15 ± 520.7^{a}	1312.28 ± 183.3^{a}
Dhalaheera	1417.41 ± 516.0^{ab}	1512.22 ± 537.9^{ab}
Narendra–18	2040.50 ± 552.6^{b}	2202.94 ±564.6^{b}

Table 6a. Correlation matrix among mineral–N (Min–N), rate of N–mineralization (N–min), nitrification (Nitrf.), ammonium oxidizers (A. oxi.), nitrite oxidizers (N. oxi.), soil pH (pH), root biomass (Rb), shoot biomass (Sb), root porosity (Rp) and soil moisture (Sm) in control plots (n=54). *Significant at p< 0.05, ** p<0.01, *** p<0.001, NS indicates correlation coefficient are not significant at p<0.05. Data are correlation coefficients (r–values).

Vari-ables	Min-N	N-min	Nitrf.	A. oxi.	N. oxi.	pH	Rb	Sb	Rp	Sm
Min-N	1	– 0.60***	–0.78***	– 0.51***	–0.33*	– 0.84***	–0.76***	– 0.72***	– 0.69***	NS
N-Min			0.56***	0.57***	0.47***	0.55***	0.68***	0.47**	0.93***	0.41**
Nitrf.				0.38*	0.66***	0.67***	0.57***	0.33*	0.97***	NS
A. oxi.					0.63***	0.33*	0.61***	0.62***	0.95***	NS
N. oxi.						NS	0.47***	NS	0.92***	NS
pH							0.78***	0.73***	0.99***	NS
Rb								0.81***	0.92***	NS
Sb									0.97***	NS
Rp										NS
Sm										1

Results

Soil properties beneath different rice cultivars

There were no differences in soil pH due to fertilization, cultivars also had no effect soil pH (Tables 1 and 2). In the control plots there were significant correlation of soil pH with all the parameters except nitrite oxidizer population and soil moisture. There were significant differences in soil moisture due to time, fertilization and the interaction between cultivar × fertilization and time × cultivar (Tables 1 and 2). Soil moisture showed correlation with rate of N–mineralization in control and negative correlation with rate of nitrification in the fertilized plots.

The concentration of extractable NH_4^+ –N and NO_3^- –N were in general higher in the fertilized plots than in control plots with differences amongst the cultivars, Heera and Narendra–118 in the control plots (Table 2). The NH_4 -N/NO_3^- –N ratio was always greater than 1 in the mineral – N pool.

Across all the plots, average values for mineral – N across the cropping season ranged from 1. 91µg g^{-1} dry soil (Narendra–118) to 10.56 µg g^{-1} dry soil (Heera) for control plots (Fig. 1a). Under urea fertilization the average values ranged from 4.17 µg g^{-1} dry soil (Narendra–118) to 12.89 µg g^{-1} dry soil (Heera), Fig. 1b. In general mineral–N left in soil was lowest beneath Narendra–118 and highest beneath Heera, whereas intermediate values were observed for Dhalaheera (Fig. 1). There were significant differences in

Table 6b. Correlation matrix among mineral–N (Min–N), rate of N–mineralization (N–min), nitrification (Nitrf.), ammonium oxidizers (A. oxi.), nitrite oxidizers (N. oxi.), soil pH (pH), root biomass (Rb), shoot biomass (Sb), root porosity (Rp) and soil moisture (Sm) in fertilized plots (n=54). *Significant at p< 0.05, ** p<0.01, *** p<0.001, NS indicates correlation coefficient are not significant at p<0.05. Data are correlation coefficients (r–values).

Vari-ables	Min-N	N-min	Nitrf.	A. oxi.	N. oxi.	pH	Rb	Sb	Rp	Sm
Min-N	1	– 0.86***	–0.50***	– 0.28*	– 0.45**	NS	– 0.54***	NS	– 0.99***	NS
N-Min			0.44**	0.45**	0.62***	NS	0.57***	NS	0.94***	NS
Nitrf.				NS	0.38*	NS	0.51***	0.57***	0.65***	0.45**
A. oxi.					0.67***	NS	0.45**	0.60***	0.73***	NS
N. oxi.						NS	0.41**	0.36*	0.79***	NS
pH							NS	0.32*	– 0.70***	NS
Rb								0.78***	0.99***	NS
Sb									0.75***	NS
Rp										NS
Sm										1

mineral–N due to the major factors, cultivars and fertilization and a weak interaction between cultivar and fertilization (Table 1). The mineral–N concentration decreased with plant growth under both control and fertilized condition (Fig. 1). Soil mineral–N was negatively correlated with all the factors in control plots except soil moisture and except pH shoot biomass and soil moisture in the fertilized condition.

N–mineralization rates were substantially lower in Heera than beneath–118 (Table 3). The rate of N–mineralization ranged from 8.24 μg g^{-1} mo^{-1} (Heera) to 23.72 μg g^{-1} mo^{-1} (Narendra–118) in the control plots (Fig. 2a). In fertilized condition the rate of N–mineralization ranged from 5.11 μg g^{-1} mo^{-1} (Heera) to 31.10 μg g^{-1} mo^{-1} (Narendra–118) Fig. 2b. The cultivars had significant effect on rate of N–mineralization (Tables 1 and 3). Relationships between rates of N–mineralization, nitrification and mineral–N left in soil were significant under both control and fertilized conditions (Tables 6a and b).

The rates of nitrification ranged from 0.15 μg g^{-1} mo^{-1} (Heera) to 3.44 μg g^{-1} mo^{-1} (Narendra–118) in the control plots (Fig. 3a). In the fertilized plots it ranged from 0.72 μg g^{-1} mo^{-1} (Heera) to 7.98 μg g^{-1} mo^{-1} (Narendra–118) Fig. 3b. In the control plots nitrification accounted for 9.9% to 13.5% of N–mineralization where as in the fertilized plots it accounted for 20.2 to 20.5% of N–mineralization (Table 3). The rate of nitrification was correlated with ammonium and nitrite oxidizing population in control plots. The nitrification rates in soil beneath Narendra–118 was double when compared with Heera (Table 3). The rate of nitrification was affected due to cultivars, fertilization and cultivar × fertilization interaction (Table 1).

Nitrifier population beneath
different rice cultivars

The ammonium oxidizer population was largest beneath Narendra–118 and significantly lower in soil beneath Heera, under both control and fertilized conditions (Table 3). The ammonium oxidizer population fluctuated from 1.0×10^5 g^{-1} dry soil beneath Heera to 5.2×10^5 g^{-1} dry soil beneath Narendra–118 under control (Fig. 4a). In the urea fertilized plots the population of ammonium oxidizer fluctuated from 2.1×10^5 g^{-1} dry soil (Heera) to 7.9×10^5 g^{-1} dry soil (Narendra–118) Fig. 4b. The ammonium oxidizer population was strongly correlated with root biomass and root porosity. The variation among cultivars in the population of ammonium oxidizers was similar to the variation among cultivars in nitrite oxidizer population. The nitrite oxidizer population was smallest beneath Heera and it was significantly higher beneath Nrendra–118 under control as well as fertilized condition (Table 3). The nitrite oxidizer population ranged from 1.0 (Heera) to 4.4×10^5 g^{-1} dry soil (Narendra–118) in the control plots (Fig. 5a). The population fluctuated from 1.4 (Heera) to 5.2×10^5 g^{-1} dry soil (Narendra–118) in the fertilized plots (Fig. 5b). The cultivars and fertilization had a significant effect on the ammonium and nitrite oxidizer population. The nitrite oxidizers were strongly correlated with root biomass and root air space. There was a strong correlation of ammonium oxidizers with nitrite oxidizers and both population were strongly correlated with rate of N–mineralization and mineral–N concentration.

Thus, soils beneath the different cultivars differ both in relative amounts of inorganic N, rate of N–mineralization and nitrifier population and there were significant changes in these quantities over time.

Plant growth and grain yield and root air
space in different rice cultivars

The average values for the plant growth parameters measured across the cropping season are presented in Table 4. Narendra–118 produced significantly more tillers in comparison to Heera. Narendra–118 was the most vigorously growing cultivar followed by Dhalaheera and Heera. The root biomass differed significantly due to cultivar, fertilization and cultivar × fertilization interaction (Table 1). Similar results were obtained for shoot biomass.

There were significant differences in root porosity (root air space) due to cultivars and fertilization (Table 1). There was significant effect of cultivar on the growth and grain yield of the rice plants (Tables 4 and 5). The response to fertilization varied among the cultivars. Fertilization enhanced the grain yield by 14.4% over control in Heera, 6.7% in Dhalaheera

and in Narendra–118 the grain yield increased by 7.9% over control (Table 5).

Discussion

Identifying the mechanisms by which different rice cultivars change soil chemistry is necessary to predict the effects of natural and man made modifications on nutrient cycling in tropical rice ecosystems. Plants interact with soil biota and drive specific changes in soil microbial community composition and soil functioning (Schlatter et al. 2015).

Our results clearly show that, there are pronounced differences in nitrogen dynamics and nitrifier population beneath different rice cultivars and these differences are a result of the plants as they can be experimentally induced in fertilized soils. The changes in microbially mediated N transformations support our hypothesis that rice cultivars can induce changes in the rate of N–mineralization, nitrification and nitrifying bacterial population. Differences in soil nutrient concentrations, standing crop biomass and fluctuations in nitrifier population can explain, in part, the differences observed. The three rice cultivars are also different from each other in several key traits, e.g. quantity of aerenchyma tissues and hence root oxidizing capacity, suggesting that they produce changes in soil through different mechanisms. Due to their sessile lifestyle but variable environment plants have evolved remarkable plasticity in their growth and below and above ground morphology (Palmer et al. 2012).

In the present study a correlation between nitrification rates and pH in control plots suggest that nitrifying bacteria are pH sensitive (Ste–Marie and Paré 1999). Changes in soil moisture status, caused by large amounts of evapotranspiration in planted plots might affect rates of nitrification in the soil (Tables 6a and b). Neill et al. (1995) observed that soil moisture may be an important controller of soil inorganic–N pool and N–transformation rates leading to the availability of nitrate–N.

The soil under the different rice cultivars differed both in relative amounts of inorganic N and rate of mineralization and reflected the changes in these quantities over time. Numerous mechanisms have been identified by which plants can alter the physical, chemical and biological properties of soils (Finzi et al. 1998). Many involve changes in the quantity, quality and / or timing of inputs of plant derived substrate. Differences in soil properties can be associated with both natural and anthropogenic changes in plant species composition (Binkley and Resh 1999). Narendra–118 had 65.0% (control) and 53.5% (fertilized) lower mineral-N in soil when compared with mineral-N level in soil planted to Heera. This can be due to differences in their capacity to absorb, translocate and utilize available N from soil. Differences between species and genotypes of plants in their capacity to absorb, translocate and utilize soil and fertilizer N are well known (Mengel 1983). Rice varieties differ in their ability to efficiently use soil nitrogen (deDatta and Broadbent 1988). According to Kundu and Ladha (1997) rice genotypes differ in their ability to increase mineral–N availability in flooded soils and the stronger influences were associated with higher dry matter yield of the plants (shoot plus root). They further proposed that soil N availability to wetland rice, especially in soils with low mineral-N supply may be considerably enhanced by selection of efficient genotypes. In the present investigation, mineral–N concentration decreased during the cropping season. This rapid decline in applied fertilizer N in the inorganic fraction during the early growing season can be ascribed to rapid plant uptake, immobilization into the microbial biomass, loss of nitrogen through nitrification denitrification reactions and possibly some transport across the barrier around the plots (Patrick and Reddy 1976). The increase in mineral-N pool due to fertilization ranged from 50.4% (Narendra–118) to 39.8% (Heera). In a US dryland long term research site 88% increase in inorganic N values were recorded after 67 kg N ha^{-1} fertilizer application (El-Harris et al. 1983). An increase in net formation of mineral-N after N fertilization has also been reported by Priha and Smolander (1995). The NH_4^+–N / NO_{3-}–N ratio was always greater than 1 in the mineral–N pool indicating efficient nitrate uptake by the rice plants. Similar observations were recorded by Jha et al. 1996.

In the present investigation rice cultivars differed in the rate of N–mineralization and nitrification. Plant

species can influence nitrogen cycling through differences in litter quality (Hobbie 1992) and changes in a small fraction of soil organic matter can have large effects on ecosystem N dynamics (Wedin and Tilman 1990). The changes in quantity and quality of litter are one of the likely mechanisms responsible for the changes in N–mineralization and decreasing plant available N. In the control plots Dhalaheera had 28.3% and Narendra–118 had 56.8% higher rate of N-mineralization in comparison to Heera and in the fertilized condition Dhalaheera had 80.0% and Narendra–118 had 142.5% higher rate of N-mineralization in comparison to Heera. Kundu and Ladha (1997) suggested that excretion of organic matter with variable C and N content from the roots of growing plants, sloughing and dying back of roots could result in addition of large quantities of decomposable organic material to the soil. This may stimulate N-mineralization in the soil and probably cause dramatic changes in microbial activities. It is also known that genotypes of various crop plants differ greatly in their capacity to produce dry matter at a given level of N supply and in the amount of dry matter produced per unit of N absorbed (N efficiency ratio), (Reed and Hageman 1980). Genotypes differ not only in the capacity to absorb N but also to translocate and partition N within the plant (Clark 1983). Several other studies have also shown that mineralization rates vary in soils beneath different species (Diekmann and Falkengren–Grerup 1998). Application of fertilizer nitrogen in the form of urea, resulted in enhanced rate of N-mineralization. Application of fertilizer nitrogen has been demonstrated to add to the mineralizable soil nitrogen and in particular to the readily available pool of nitrogen (El-Harris et al. 1983). When fertilizer nitrogen is added to the soil, it interacts with the indigenous soil nitrogen, increasing the mineralization of soil nitrogen, a phenomenon known as priming effect (Dormaar 1975). The ranges of N–mineralization and nitrification are in agreement with a number of other studies (Jha et al. 1996).

The differences in rate of nitrification amongst the cultivars, in the present study may be attributed to the differences in microbial activity of the nitrifier population harboured by each cultivar in their respective soil. Vitousek et al. (1982) had suggested that nitrification is controlled either directly or indirectly by the composition of the vegetation. Nitrate uptake by the soil microflora may be one of the mechanisms responsible for limiting net accumulation of nitrate. In the present study the nitrification rates were higher in fertilized soils under all the three cultivars as compared to control plots. Substrate addition increases potential nitrification rate, because this overcomes competition for mineral nitrogen by nitrifiers (Killham 1990).

There have been few studies of changes in nitrifying bacterial population in soil under different plant species and even fewer under different rice cultivars. There were significant differences in nitrifying bacterial population amongst the rice cultivars. The population of ammonium oxidizers differed under different tree species within a single forest community (Lodhi 1978). In the present investigation the variation in nitrifier population across the three rice cultivars cannot be solely attributed to genotypic variations in addition of organic matter by the rice plants into the soil. A more reasonable explanation would be due to the extent of aerobic conditions created in the soil in response to variation in root porosity of the rice plants and hence variation in the extent of diffusion of oxygen through the roots. Urea fertilization increased the nitrifier population significantly. Several studies have shown that addition of nitrogen fertilizers to soil increases the nitrifying bacterial population (Berg and Rosswall 1985). The ammonium oxidizer population was strongly correlated with rates of N–mineralization under fertilized condition. This supports the contention that enhanced microbial activity is responsible, at least partially, if not entirely for the observed N–mineralization following fertilization. There was a strong correlation of ammonium oxidizers with nitrite oxidizers. This confirms the fact that both processes carried out by these bacteria are coupled (Woldendorp and Laanbroek 1989). The ammonium and nitrite oxidizing bacterial population tended to increase at the end of the season probably due to increased availability of substrate due to reduced plant uptake.

The growth pattern of the individual cultivars and differences in grain yield indicated differential resource utilization potential of the cultivars. Since the plant variables covaried during the crop growth these were interrelated with each other. The significant

differences in root porosity led to different degrees of aerobicity resulting in significantly different number of ammonium and nitrite oxidizers neneath the three cultivars. The presence of well developed root air space was an insurance against periodic soil saturation following heavy rainfall for supplying oxygen to the roots. This ventilation system evidently facilitated the growth of ammonium and nitrite oxidizing bacteria. Apart from substrate addition, fertilization led to further development of aerenchyma tissue and hence fertilized plants supported higher population of nitrifying bacteria.

Thus, the rice cultivars influenced the availability of nutrients in soil by influencing the soil microbial population and their processes. For the present study the ranking was Narendra–118 > Dhalaheera > Heera. The associations between rice cultivars and the quantity and form of N available to plants during the growing season suggest that rice cultivar is one of the likely factors that regulated nitrogen dynamics in tropical rice soil. Further work is needed to elucidate these processes under several rice cultivars.

References

Alexander M, Clark FE (1965) Nitrifying bacteria. In: Black CA (ed). Methods of soil analysis. American Society of Agronomy, Inc, Madison, WI, pp 1477—1486.

American Public Health Association (APHA) (1985) Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC.

Armstrong W (1969) Rhizosphere oxidation in rice: An analysis of intervarietal differences in oxygen flux from the roots. Physiol Planta 22 : 296—303.

Baxendale C, Orwin KH, Poly P, Pommier T, Bardgett RD (2014) Are plant soil feed back responses explained by the plant traits ? New Phytol 204 : 408—423.

Berg P, Rosswall T (1985) Ammonium oxidizer numbers, potential and actual oxidation rates in two Swedish arable soils. Biol & Fertile Soil 1 : 131—140.

Binkley D, Resh SC (1999) Rapid changes in soils following eucalyptus afforestation in Hawaii. Soil Sci Soc Am J 63 : 222—226.

Buresh RJ (1991) Extraction of ammonium, nitrite and nitrate from soil. In: IFDC / IRRI Collaborative project, 1991.

Clark RB (1983) Plant genotype difference in the uptake, translocation accumulation and use of mineral elements required for plant growth. Pl & Soil 72 : 175—196.

Cochran WG (1950) Estimation of bacterial densities by means of the most probable number. Biometrics 6 : 105—116.

Colmer TD (2003) Long distance transport of gases in plants: A perspective on internal aeration and radial oxygen loss from roots. Pl, Cell and Environ 26 : 17—36.

Colmer TD, Gibbered MR, Wiengwera A, Tinh TK (1998) The barrier to radial oxygen loss from roots of rice (*Oryza sativa* L.) is induced by growth in stagnant solution. J Exp Bot 49 : 1431—1436.

deDatta SK, Broadbent FE (1988) Methodology for evaluating nitrogen utilization efficiency by rice genotypes. Agron J 8 : 793—798.

Diekmann M, Falkengren–Grerup U (1998) A new species index for forest vascular plants: Development of functional indices based on mineralization rates of various forms of soil nitrogen. J Ecol 86 : 269—283.

Dormaar JF (1975) Susceptibility of organic matter of chernozemic Ah horizons to biological decomposition. Can J Soil Sci 55 : 473—480.

El-Harris MK, Cochran VL, Elliot LF, Bezdicek DF (1983) Effect of tillage, cropping and fertilizer management on soil nitrogen mineralization potential. Soil Sci Soc Am J 47 : 1157—1161.

Eno C (1960) Nitrate production in the field by incubating the soil in polyethylene bags. Soil Sci Soc Am Proc 24 : 277—279.

Finzi AC, Van Breemen N, Canham CD (1998) Canopy tree–soil interaction within temperate forests: Species effects on soil carbon and nitrogen. Ecol Appl 8 : 440—446.

Fukao T, Xu K, Ronald PC, Bailey-Serres JA (2006) A variable cluster of ethylene response factor like genes regulates metabolic and developmental acclimation responses to submergence in rice. The Pl Cell 18 : 2021—2034.

Gibbs J, Turner DW, Armstrong W, Darwat MJ, Greanway H (1998) Solute transport and water relations of oxygen deficient maize roots I. Development of O_2 deficiency in the stele reduces radial solute transport to the xylem. Aust J Pl Physiol 25 : 745—758.

Hart SS, Stark JM, Davidson EA, Firestone MK (1994) Nitrogen mineralization, immobilization and nitrification. In: Weaver RW, Angle JS, Bottomly PS (eds). Methods of soil analysis, Part 2. Microbial and biochemical properties. Soil Sci Soc Am, Madison, pp 985–1018.

Hattori T, Nagai K, Furukawa S et al. (2009) The ethylene response factors SNORKEL 1 and SNORKEL 2 allow rice to adapt to deep water. Nature 460 : 1026—1030.

Hobbie SE (1992) Effects of plant species on nutrient cycling. Trends Ecol Evol 7 : 336—339.

Huang B, Johnson JW, Ne NeSmith DS, Bridges DC (1994) Growth, physiological and anatomical responses of two wheat genotypes to water logging and nutrient supply. J Exp Bot 45 : 193—202.

Jackson ML (1958) Soil Chemical Analysis. Prentice-Hall Inc. Englewood, Cliffs, New Jersey, pp 197—201.

Jensen CR, Luxmoore RJ, Van Gundy SD, Stolzy LH (1969) Root air–space measurements by a pycnometer method. Agron J 61 : 474—475.

Jha PB, Kashyap AK, Singh JS (1996) Effect of fertilizer and organic matter inputs on nitrifier populations and N–mineralization rates in a dry tropical region, India. Appl Soil Ecol 110 : 1—11.

Justin SHFW, Armstrong W (1987) The anatomical characteristics of roots and plant response to soil flooding. NewPhytol

105 : 465—495.
Killham K (1990) Nitrification in coniferous forest soils. Pl & Soil 128 : 31—44.
Kundu DK, Ladha JK (1997) Effect of growing rice on nitrogen mineralization in flooded soil. Soil Sci Soc Am J 61 : 839—845.
Lodhi MAK (1978) Comparative inhibition of nitrifiers and nitrification in a forest community as a result of the allelopathic nature of various tree species. Am J Bot 65 : 1135—1137.
Mengel K (1983) Responses of various crop species and cultivars to fertilizer application. Pl & Soil 72 : 305—319.
Neill C, Piccolo MA, Steudler PA, Melillo JM, Feigl BJ, Cerri C (1995) Nitrogen dynamics in soils of forests and active pastures in the Western Brazilian Amazon Basin. Soil Biol Biochem 27 : 1167—1175.
Palmer CM, Bush SM, Maloof JN (2012) Phenotypic plasticity in plants. In : Encyclopedia of life sciences, Wiley and Sons Ltd, Chichester, UK.
Patrick Jr WH, Reddy KR (1976) Fate of fertilizer nitrogen in a flooded rice soil. Soil Sci Soc Am J 40 : 678—681.
Priha O, Smolander A (1995) Nitrification, denitrification and microbial biomass N in soil from two N-fertilized and limed Norway spruce forests. Soil Biol Biochem 27 : 305—310.
Reddy KR, Patrick Jr WH (1986) Fate of fertilizer nitrogen in the rice root zone. Soil Sci Soc Am J 50 : 649—651.
Reed AJ, Hageman RH (1980) Relationship between nitrate uptake, flux and reduction and the accumulation of reduced nitrogen in maize (*Zea mays* L.) I. Genotypic variation. Pl Physiol 66 : 1179—1183.
Sanchez PA (1976) Soil management in rice cultivation systems. In: Properties and management of soils in the tropics. N. Carolina State University, pp 413—477.
Schimel DS, Parton WJ (1986) Microclimatic controls of nitrogen mineralization and nitrification in shortgrass steppe soils. Pl & Soil 93 : 347—357.
Schlatter DC, Bakker MG, Bradeen JM, Kinkel LL (2015) Plant community richness and microbial interactions structure bacterial communities in soil. Ecology 96 : 134—142.
SPSS / PC (2002) SPSS for windows. Version 11.5, SPSS Inc, Chicago, Illinois.
Ste–Marie C, Paré D (1999) Soil, pH and N availability effects on net nitrification in the forest floors of a range of boreal forest stands. Soil Biol Biochem 31 : 1579—1589.
Ueckert J Hurek I, Fendrik I, Niemann EC (1990) Radial gas diffusion from roots of rice and kallargrass with *Azospirillum brasilence* Cd. Pl Soil 112 : 59—65.
Vitousek PM, Gosz JR, Grier CC, Melillo JM, Reiners WA (1982) A comparative analysis of potential nitrification and nitrate mobility in forest ecosystems. Ecol Monographs 52 : 155—177.
Wedin DA, Tilman D (1990) Species effects on nitrogen cycling : A test with perennial grasses. Oecologia 84 : 433—441.
Woldendorp JW, Laanbroek HJ (1989) Activity of nitrifiers in relation to nitrogen nutrition of plants in natural ecosystems. Pl & Soil 115 : 217—228.
Wubs ERJ, Bezemer TM (2016) Effects of spatial plant - soil feed back heterogeneity on plant performance in monocultures. J Ecol 104 : 364—376.