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Nitrate Reductase, Micronutrients and Upland Rice Development as Influenced by Soil pH and Nitrogen Sources

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ABSTRACT

The average yield of upland rice under no-tillage system (NTS), a sustainable soil management, is lower than in conventional tillage (one plowing and two disking). One of the reasons given for this drop in crop grain yield would be the low-nitrate assimilation capacity of rice seedlings, due to the low activity of the nitrate reductase (NR) enzyme in the early development phase. A greenhouse experiment was conducted to evaluate the effects of the soil acidic and nitrogen source in the micronutrient concentrations, NR activity and grain yield of upland rice growing under NTS. The soil used in the experiment was an Oxisol. The experimental design was completely randomized in a factorial 3 × 4. Treatments consisted of three levels of soil acidity (high, medium, and low) combined with four nitrogen sources (nitrate, ammonium, ammonium + nitrification inhibitor, and control – without N fertilization). The reduction of soil acidity reduced the concentration of zinc and manganese in rice plants. Generally, the activity of the NR enzyme was higher in plants grown in soils with low acidity and fertilized with calcium nitrate. There was a greater response in growth and yield in rice plants grown in soils with high acidity. Under medium acidity, rice plants grown with ammonium sulfate were more productive (no differences were detected with the addition of the nitrification inhibitor).

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Ammonium; nitrate; *Oryza sativa*; soil acidity; soil fertility; yield

Introduction

Rice is one of the most cultivated cereals in the world, having great social and economic importance for many people. Cultivation in the upland environment is carried out in Asia (Saito et al. 2005), Africa (Oonyu 2011), and Americas (Nascente, Crusciol, and Cobucci 2013), and is increasing in worldwide importance due to water shortages for crop irrigation (Crusciol et al. 2013). In addition, it is important to grow this crop in a sustainable way, such as in no-tillage system (NTS) (Nascente et al. 2011a, 2011b).

However, upland rice grain yield at NTS is lower than in the conventional tillage (Crusciol et al. 2010; Kluthcouski et al. 2000). One explanation given for the decrease in crop grain yield would be the low-nitrate assimilation capacity of rice seedlings, due to the low activity of the nitrate reductase (NR) enzyme in the early development phase, prior to the tillering stage (Araújo et al. 2012). In experiments developed by D'Andréa et al. (2004), Nascente, Crusciol, and Cobucci (2012), and Araújo et al. (2012), it was verified that in NTS there is greater availability of $N{-}NO_3^-$ in the soil

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regarding conventional tillage. Therefore, upland rice failure in NTS may be caused by the predominance of nitrate in the soil. Corroborating this information, Araújo et al. (2012) reported that rice presented better development when there were lower levels of nitrate and a higher amount of ammonium in the soil.

It is noteworthy that NR is an extremely important enzyme in the assimilation of N-NO_3^- by plants (Epstein and Bloom 2006). In this context, because rice came from a hydrophilic environment with a reduced environment, where ammonium prevails, it is likely that it is difficult for rice plants to synthesize NR enzyme in the first month of development, which may explain the low initial development of the crop when in the environment it prevails N- NO_3^-, such as NTS (Fageria et al. 2014).

Moreover, in NTS due to surface correction of acidity, the content of some micronutrients such as copper (Cu), manganese (Mn), zinc (Zn), and iron (Fe) may decrease, the decrease of this last element may compromise the conversion of NO_3^- to NO_2^-, since Fe is essential for NR (Fageria et al. 2014). Therefore, the total nitrate reduction capacity of plants, including additional factors, depends on Fe (Campbell 1999).

One possibility to overcome this problem of low NR enzyme activity in the early rice development phase would be the use of N sources in the ammoniacal form at sowing and/or shortly after emergence. However, as N-ammoniacal changes in N-nitric in the soil occur rapidly, some strategy would be necessary, especially the use of nitrification inhibitors alongside the ammoniacal sources.

The hypothesis of this study was to understand how soil pH and nitrogen fertilizer sources affect NR activity, micronutrient uptake, and upland rice grain yield at NTS. The objective of this work was to evaluate the effects of the acidity of soil and nitrogen sources on micronutrient content, NR enzyme activity, yield components, and grain yield of upland rice growing under NTS.

Material and methods

The experiment was conducted in greenhouse conditions at the Department of Plant Production of the Faculty of Agronomic Sciences/Unesp, Campus de Botucatu-SP, Brazil (22°51′ S, 48°26′ W, 815 m of altitude). The soil (0.00–0.20 m layer) used in the experiment was classified as Oxison (red Latosol according to Brazilian soil classification system), presenting 630, 90 and 280 g kg^{-1} of sand, silt and clay, respectively, from an NTS of 10 years. The soil collected was divided into three equal parts. One portion was maintained at the original pH and in the other two the pH was raised to 5.5 and 6.3. For the elevation of the pH, the dolomitic limestone, composed of 33% CaO and 16% of MgO, was used and the required amount was determined by the incubation method (30 days). The determined amounts of limestone were applied to the soil portions, which remained in incubation for 30 days, with moisture at the maximum water retention capacity of the soil, for corrective reaction. The chemical characteristics of the soil were evaluated before incubation and after 30 days, following the methodology proposed by Bvan et al. (2001) (Table 1).

The soil portions were subjected to a wash, with a goal to force leach NO_3^-, which could possibly be greater in the soil portions that had received liming. The leaching of NO_3^- was carried out in 17 L pots by the addition of water. The volume of water used was twice the water retention capacity of the soil. After the leaching process the nitrate contents removed were 4.5 and 9.45 mg dm^{-3} of soil, for pH 5.5 and 6.3, respectively.

The soil was removed from the pots, dried in the shade and each soil portion was fertilized with 150 mg dm^{-3} of P and 80 mg dm^{-3} of K in the forms of triple superphosphate and potassium chloride, respectively. The micronutrients were applied after the germination of the plants along with the replacement of water in the pots. The amounts applied were: 2.0 mg dm^{-3} B, 3.0 mg dm^{-3} Mn, 10.0 mg dm^{-3} Zn and 1.5 mg dm^{-3} Cu, provided in the forms of H_3BO_3, $MnSO_4$, $ZnSO_4$ and $CuSO_4$, respectively.

The experimental design was completely randomized in the 3 × 4 factorial scheme, with four replications. The treatments were composed of three levels of acidity (pH $CaCl_2$) of the soil (high – pH = 4.5, medium – pH = 5.5 and low – pH = 6.3) with sources of N (1 – nitric – $Ca(NO_3)2.4H_2O$, 2 – ammoniacal – $(NH_4)_2SO_4$, 3 – ammoniacal + nitrification inhibitor – DCD (dicyandiamide) and 4 – control – without N). The amount of N applied, regardless of source, was 80 mg dm^{-3} of soil. The

Table 1. Soil chemical attributes before liming incubation and 30 days after. Botucatu-SP, Brazil.

	pH	SOM*	P (resin)	H+Al	K	Ca	Mg	BS**	CEC***	V
Levels of acidity	$CaCl_2$	g dm^{-3}	mg dm^{-3}	—————— mmol$_c$ dm^{-3} ——————						%
Before liming incubation										
High	4.5	16.9	8.2	55.1	0.46	14	5	19.5	74.5	26
30 days after liming incubation										
Medium	5.5	15.4	7.7	30.7	0.56	25	18	43.6	74.2	59
Low	6.3	15.6	11.2	20.6	0.60	59	29	88.6	109.2	81

	Fe^{2+}	Mn^{2+}	Zn^{2+}	Cu^{2+}	NO_3^-	NH_4^+
Levels of acitidy	——————————mg dm^{-3} ——————————-				———-mg dm^{-3} ———	
Before liming incubation						
High	16.6	41	1.1	8.8	7.0	10.2
30 days after liming incubation						
Medium	11.0	14.4	0.8	7.0	14.0	6.6
Low	7.5	9.7	0.6	6.2	30.0	7.2

*SOM – soil organic matter. ** BS – Base saturation. ***CEC – cation exchange capacity.

experimental units were constituted by plastic pots with 17 kg capacity, completely filled with soil. Twenty seeds of rice were sown per pot (cultivar IAC-47) and, after germination, 10 plants were maintained. The amount of N provided by the nitric, ammonium, and ammonium + DCD source was divided twice, half immediately after the emergence of the rice, and the remainder at 15 days after the first, applied to the soil surface. Soil moisture throughout the experiment was monitored daily by weighing each pot, and the replacement of evapotranspirated water occurred when it reached 85%, increasing to 100% of the field capacity.

Soil sampling was performed at 7 and 28 days after emergence (DAE). The soil was collected at four points per pots to form a composite sample, and then the samples were kept in an ice box until the chemical analysis, that was completed on the same day of sampling. The levels of $NO3^-$ and NH_4^+ in the soil were determined by the method proposed by Silva (1999).

Plant sampling was performed at 7 and 28 DAE. Thus, two plants per pot were harvested, and plant shoots were dried in an oven, ground and analyzed for micronutrient content (Fe, Zn, Cu, and Mn) (Malavolta, Vitti, and Oliveira 1997).

The leaves for the determination of the enzyme NR were sampled at random. Four newly expanded leaves were collected in each pot, and the middle third was removed for evaluation. The leaves were collected in the morning between 8:00 am and 10:00 am and analyzed on the same sampling day following the methodology described by Jaworski (1971).

The number of panicles per plant was obtained by counting the panicles of two plants per pot. In these panicles, the total number of spikelets per panicle was counted accordingly and also the fertility of the spikelets and the grain yield of the rice was evaluated. The values presented for these variables correspond to the average of the two plants. The fertility of the spikelets was determined by the ratio of the number of spikelets per panicle to the total number of spikelets per panicle x 100. The mass of 100 grains was obtained by averaging four samples of 100 grains per experimental unit. The water contents of the grains were determined and adjusted to 130 g kg^{-1}. The track of the panicles was completed manually. The material passed through the cleaning process for the separation of the straw and the spikelets, of the spikelets grenades. After completing this process, the grains were weighed and then the productivity in grams per plant (130 g kg^{-1}) was calculated.

The data were submitted for analysis of variance and the means compared by the least significant difference (LSD) test ($p \leq 0.05$).

Results and discussion

In the analysis of the results, it was possible to observe that at 7 DAE the soil ammonium content was not influenced by the soil acidity (Table 2). In the interaction, it was observed that, at each level

Table 2. Ammonium content in the soil at 7 and 28 days after emergence (DAE) of upland rice as a function of levels of soil acidity and source of nitrogen fertilization. Botucatu-SP, Brazil.

Levels of acidity	Sources of nitrogen fertilization: Control	NO_3^-	NH_4^+	NH_4^+ + DCD[1]	Average
	——— mg kg^{-1} ———				
7 DAE					
High	14.6 aB	4.2 aC	28.0 aA	30.0 aA	19.2 a
Medium	18.0 aB	2.2 aC	28.3 aA	28.6 aA	19.3 a
Low	16.0 aC	2.7 aD	26.7 aB	32.0 aA	19.4 a
28 DAE					
High	4.4 bB	5.2 aB	21.4 aA	21.2 aA	13.1 a
Medium	8.8 aB	5.0 aC	22.4 aA	21.9 aA	14.5 a
Low	5.6 bB	6.5 aB	22.0 aA	22.2 aA	14.1a
Average	9.0 B	5.2 C	26.2 A	27.6 A	–

[1]DCD: dicyanodiamide – nitrification inhibitor. Means followed by the same letter, lowercase in the columns and uppercase in the lines do not differ by the LSD test ($p \leq 0.05$).

of soil acidity, the ammoniacal sources exceeded the control and the nitric source. It should be noted that that the soil ammonium content was higher with the ammonia source and with the nitrification inhibitor (DCD) at all acid levels, but at the low acid level the application of the DCD inhibited nitrification, maintaining a higher amount of N in the ammoniacal form for a longer time, in relation to treatment with ammoniacal source only. According to Fageria (2000), nitrification rate in acidic soils is reduced, since it directly affects the population of nitrifying bacteria, and the nitrate levels in the soil fall rapidly at pH values lower than 6.0, becoming quite reduced in soil with pH below 5.0 (Crusciol et al. 2011).

At 28 DAE, the effect of acidity on the soil NH_4^+ contents was observed only for the control, and the highest content was observed for the average acidity (pH 5.5). Furthermore, it was observed, in the average of all the evaluations, that there was no effect of the soil acidity on the soil ammonium contents. As for the sources, it also verified higher levels of ammonium in the soil in the treatments with ammonia and NH_4^+ + DCD. This result was expected, since the application of ammoniacal sources gives rise to soil ammonium content (Fageria 2014).

Soil nitrate contents were influenced by soil acidity (Table 3). In general, in all soil samples, the highest values were observed in conditions of low acidity. According to Flowers and O'callaghan (1983), raising the pH promotes the increase of nitrification. This is because the higher pH provides more suitable conditions for autotrophic nitrification.

Moreover, with the data of each acid level, we observed that at 7 DAE, in all samples nitrate source provided the highest levels of soil nitrate. On the other hand, under conditions of low acidity, the

Table 3. Nitrate content in the soil at 7 and 28 days after emergence (DAE) of upland rice as a function of levels of soil acidity and source of nitrogen fertilization. Botucatu-SP, Brazil.

Levels of acidity	Sources of nitrogen fertilization: Control	NO_3^-	NH_4^+	NH_4^+ + DCD[1]	Average
	——— mg kg^{-1} ———				
7 DAE					
High	20.8 cB	33.2 bA	16.6 aC	19.1 cB	22.4 b
Medium	26.1 aA	27.0 cA	16.8 aC	22.5 bB	23.1 b
Low	23.5 bC	38.6 aA	18.3 aD	28.1 aB	27.0 a
28 DAE					
High	1.9 bC	19.7 bA	7.2 bB	6.6 cB	8.8 b
Medium	4.2 aC	18.8 bA	5.4 cC	9.1 bB	9.4 b
Low	4.8 aC	34.8 aA	18.2 aB	25.5 aA	20.8 a
Average	10.8	33.0	15.7	19.3	–

[1]DCD: dicyanodiamide – nitrification inhibitor. Means followed by the same letter, lowercase in the columns and uppercase in the lines do not differ by the LSD test ($p \leq 0.05$).

application of NH_4^+ and NH_4^+ + DCD reduced the nitrification process, giving lower levels in relation to the treatment with nitrate source. However, at 28 DAE, these same treatments, mainly NH_4^+ + DCD, were not sufficient to prevent nitrification as values similar to the treatment with NO_3^- application.

Regarding micronutrients, it was verified that the copper content (Cu) was influenced by the soil acidity only in the control treatment, in which the contents were lower at low acidity (Table 4). It was verified that Cu contents were higher with the use of ammoniacal sources at all levels of acidity. According to Fageria (2000), ammonium sulfate causes soil pH reduction. In addition, acidification by nitrogen fertilization increases the availability of Cu and Zn in the soil due to the higher solubility of these micronutrients when pH reduction occurs (Lange et al. 2006). This explains the lower content of Cu for the nitric source and higher content for the ammoniacal sources.

Zinc contents were influenced by soil acidity, and the highest values were observed under conditions of high soil acidity (Table 4). The Zn levels in the plant decreased significantly with increasing soil pH, which may be related to the adsorption or precipitation of this micronutrient. There is a decrease in the accumulation of Fe, Mn, and Zn with soil pH elevation above 5.5 (Fageria 2000). Zn availability decreases about 100 times with increasing one pH unit (Tisdale, Nelson, and Beaton 1985). According to Fageria, Slaton, and Baligar (2003), the adequate level of Zn in the rice shoots is 47 mg kg^{-1}. In the present work, the values of Zn were close to this value in conditions of high acidity. It was verified high content of Zn in the rice leaves when using N fertilizer in ammoniacal source without the inhibitor and in conditions of low acidity of the soil. This may have occurred because of the effect of ammonium sulfate on reducing the pH as discussed for Cu. In conditions of medium acidity, the contents of Zn were higher with the application of ammoniac sources of N.

The iron contents (Fe) were influenced by the soil acidity (Table 4); however, they were not by N sources. The difference between N sources for medium and high acidity was small. However, for these levels of acidity all the sources provided higher Fe contents than the control.

The manganese content (Mn) was higher when the soil acidity was high (Table 4). This behavior occurred for all sources of N. The lowest values for Mn were observed when the soil acidity was

Table 4. Copper, zinc, iron and manganese content in the rice shoots at 28 days after emergence (DAE) as a function of levels of soil acidity and source of nitrogen fertilization. Botucatu-SP, Brazil.

Levels of acidity	Source of nitrogen fertilization: Control	NO_3^-	NH_4^+	NH_4^+ + DCD[1]	Average
	———— mg kg^{-1} ————				
Copper					
High	17.8 aB	17.3 aB	22.0 aA	21.5 aA	19.7 a
Medium	16.5 aB	15.0 aB	21.3 aA	19.3 aA	17.3 a
Low	13.5 bB	16.0 aB	21.0 aA	21.8 aA	18.8 a
Average	15.9 B	16.1B	21.4 A	20.9 A	18.6
Zinc					
High	41.8 aA	38.0 aB	45.3 aA	42.3 aA	42.0 a
Medium	22.8 bB	23.8 bB	36.3 bA	32.3 bA	29.0 b
Low	14.3 cC	23.0 bB	32.0 bA	26.5 cB	24.0 c
Average	26.3 A	28.3 A	15.9 B	15.9 B	31.7
Iron					
High	121 aB	127 bAB	124 cAB	133 bA	126 b
Medium	125 aB	143 aA	143 bA	95 cC	127 b
Low	116 aC	110 cC	177 aB	220 aA	156 a
Average	121B	127B	148A	149A	136
Manganese					
High	496 aA	514 aA	457 aB	443 aB	478 a
Medium	43 aB	79 bA	84 bA	85 bA	73 b
Low	35 aB	47 cB	81 bA	57 cB	55 c
Average	191 B	213 A	207 A	195 AB	202

[1]DCD: dicyanodiamide – nitrification inhibitor. Means followed by the same letter, lowercase in the columns and uppercase in the lines do not differ by the LSD test ($p \leq 0.05$).

medium or low. In addition, it was found that there was no standard behavior for the different N sources.

In relation to NR enzyme activity, it was found that in the 7 DAE there was a difference between the level of acidity and the use of the nitrate source (Table 5). For this source, the highest activity was observed under conditions of low and medium acidity. It was also observed that in the three levels of acidity, the enzymatic activity of the control pot was equal to or higher than the N sources. At 28 DAE, it was observed that the low acidity provided higher NR activity in relation to all N sources. Nitric source, in most of the results, provided a higher NR activity regardless of the level of acidity.

Thus, the hypothesis that NR activity would be lower in the early phase of rice development was not confirmed (Table 5). This can be explained by the reduction of nitrate levels in the soil (Table 3). Similar results were obtained by Celestino (2006), who observed a reduction in the NR activity with the development of the rice crop in the absence of nitrogen fertilization, noting that the NR enzyme activity in the leaves and roots can be induced by the presence of the substrate (NO_3^-). The maximum activity of NR was in the period of greatest availability of NO_3^- similar results was achieved by Pacheco et al. (2011).

In addition, higher NR activity (Table 5) also occurred with increasing pH (low acidity) due to the higher nitrate content in the soil (Table 3). The results of the study showed that N-nitric acid levels were higher in the soil than in the N-nitric acid. The availability of nitrate can positively interfere with the increase in NR activity, since it is the substrate of the enzyme (Crawford 1995). The data justify the higher activity of the NR with the application of the nitric source.

The number of panicles per plant was influenced by soil acidity only when N was applied (Table 6). The highest values for this yield component were observed under conditions of high acidity. These values were equal to mean acidity only when the source was the ammoniacal + nitrification inhibitor. With the exception of the control and the nitric source, the lowest number of panicles per plant was observed under conditions of low soil acidity. It was observed that the ammoniacal exceeds the nitrate and the control independently of the acidity of the soil.

The number of spikelets per panicle was not altered by soil acidity (Table 6). Concerning the fertility of the spikelets, the lowest value was observed for the low acidity, when the N source used was the nitrate (Table 6). The other values did not differ significantly. We observed the effect of the sources only concerning low acidity. The values for the ammoniacal sources were superior to the control and the nitric source.

The mass of 100 grains was influenced by the acidity only in the control and nitric source (Table 6). In the control, the greater mass of 100 grains was observed in conditions of high acidity. When the nitrate source was used, in the condition of high and medium acidity, the values were higher than the low acidity of the soil. It was observed that, for the high acidity, the treatments did

Table 5. Nitrate reductase (NR) enzyme activity in rice leaves at rice shoots at 7 and 28 days after emergence (DAE) as a function of levels of soil acidity and source of nitrogen fertilization. Botucatu-SP, Brazil.

	Sources of nitrogen fertilization				
	Control	NO_3^-	NH_4^+	NH_4^+ + DCD[1]	
Levels of acidity	$^2NO_2^-$ (μmol $g^{-1}$of fresh material h^{-1})				Average
7 DAE					
High	12.1 aA	10.7 bAB	11.3 aAB	10.1 aB	11.0 b
Medium	13.3 aA	12.6 aAB	11.4 aB	11.4 aB	12.0 a
Low	12.2 aA	12.9 aA	11.9 aA	11.5 aA	12.0 a
28 DAE					
High	1.6 cB	3.8 cA	2.2 cB	2.3 cB	2.5 c
Medium	2.8 bD	6.6 bA	4.0 bC	5.2 bB	5.0 b
Low	5.4 aB	9.8 aA	5.5 aB	6.3 aB	7.0 a
Average	8.6	10.0	7.9	7.8	–

[1]DCD: dicyandiamide – nitrification inhibitor. The activity of NR is expressed by the amount of nitrite (NO_2^-) formed. Means followed by the same letter, lowercase in the columns and uppercase in the lines do not differ by the LSD test ($p \leq 0.05$).

Table 6. Yield components and yield of upland rice as a function of soil acidity levels and nitrogen fertilization sources. Botucatu-SP, Brazil.

Levels of acidity	Sources of nitrogen fertilization				Average
	Control	NO_3^-	NH_4^+	NH_4^+ + DCD[1]	
			Panicles (n.º plant^{-1})		
High	4 aC	9 aB	11 Aa	10 aAB	8.5 a
Medium	5 aC	6 bC	9 bB	11 aA	7.7 b
Low	4 aC	5 bC	7 cB	8 bA	6.0 c
Average	4.0 D	7.0 C	9.0 B	10.0 A	
			Spikelets (n.º per panicles)		
High	55 aA	64 aA	60 aA	65 aA	61 a
Medium	60 aA	66 aA	62 aA	60 aA	62 a
Low	54 aA	60 aA	63 aA	60 aA	59 a
Average	56 B	63 A	62 A	62 A	
			Spikelets fertility (%)		
High	83 aA	88 aA	79 aA	81 aA	83 a
Medium	78 aA	86 aA	86 aA	86 aA	84 a
Low	72 aB	64 bB	88 aA	86 aA	78 a
Average	78 A	79 A	84 A	84 A	
			Mass of 100 seeds (grams)		
High	3.0 aA	3.1 aA	3.0 aA	3.1 aA	3.0 a
Medium	2.7 bB	2.9 aAB	3.1 aA	3.0 aA	2.9 b
Low	2.7 bAB	2.5 bB	3.0 aA	3.0 aA	2.8 b
Average	2.8 b	2.5 b	3.0 A	3.0 A	
			Grain yield (g plant^{-1})		
High	6 aB	15 aA	15 aA	16 aA	13 a
Medium	6 aC	10 bB	15 aA	17 aA	12 a
Low	5 aB	5 cB	11 bA	13 bA	8.5 b
Average	5.6 C	10.0 B	13.7 A	15.3 A	–

[1]DCD: dicyanodiamide – nitrification inhibitor. Means followed by the same letter, lowercase in the columns and uppercase in the lines do not differ by the LSD test ($p \leq 0.05$).

not influence the results. For the low and medium acidity, the highest values were obtained with the ammoniacal sources.

The grain yield was not influenced by the soil acidity, only in the control treatment (Table 6). With the addition of nitrogen, the average and high acidity provided the highest values, with the exception of the medium acidity for the nitrate source. Ammoniacal sources surpassed the others treatments. Another exception was the nitric source in conditions of high acidity of the soil that provided productivity equal to the ammoniacal sources.

Increasing pH above 5.3 causes a decrease in rice yield (Fageria 2000). Moreover, Fageria, Moreira, and Coelho (2011) showed rice yield reduction when soil pH increased from 4.6 to 6.8. Rice adapted to the upland ecosystem is best developed with the supply of nitrate and ammonium in similar quantities (Ta and Ohira 1981). Therefore, the negative influence of the pH increases on the yield and yield components may be associated with the imbalance of the NO_3^-/NH_4^+ ratio, since the NH_4^+ supply improved crop performance. In addition, the roots are known to be responsible for most of the N uptake; however, not all parts of the roots are efficient to uptake nutrients, like N. The most efficient part of the root is the zone of absorbent hairs, which is only present in new roots (Fageria 2014). Therefore, the high-to-medium pH was not a problem for the root development and allowed growing new roots, which was important to uptake N and allow increasing grain yield. According to Fageria (2014), N is one of the most important nutrients for root growing.

Nitrogen is a nutrient very important for rice development. In this sense, our results showed that the absence of N fertilization resulted in reduction of upland rice grain yield. It should be emphasized that the cultivar used had better yields under conditions of medium and high acidity. Corroborating this information, Fageria, Moreira, and Coelho (2011) cited that rice is an acid-tolerant plant and its growth was linearly increased when Al saturation in the Brazilian Oxisol soil was increased from 0 to 30%. Our data allow infering that in the NTS when pH increases to levels above 5.5 seems to be harmful to the rice development . Besides, this result reinforce the theory that rice develops better in environments with a low nitrate and high ammonium content in the soil, which is found in low soil pH. This increase in pH also resulted in lower absorption of Zn and Mn (Table 4) which are important nutrients for crop development (Fageria 2000). On the other hand, it favored the higher activity of the NR enzyme, which may be related to the higher availability of nitrate. According to Galangau, Daniel-Vedele, and Moureaux (1988) and Vincentz, Moureaux, and Leydecker (1993), the NR enzyme activity in leaves and roots can be induced by the presence of the substrate (NO_3^-), although this higher activity did not translate into higher grain yield.

Furthermore, rice grain yield occurs due to differences in assimilate distribution between the organs during plant growth and development. Dry matter production and photoassimilate translocation correlate positively with the productivity of the crop (Falqueto et al. 2009). Thus, the grain yield of a rice cultivar is determined by four components: (1) number of panicles per square meter, (2) number of spikelets per panicle, (3) spikelet fertility, and (4) mass of 100 grains (Santos, Stone, and Vieira 2006). Thus, based on the results obtained by the production components, the fall in grain yield occurred due to the decrease in the number of panicles and the mass of 100 grains (Table 6).

Conclusions

Our data allow us to infer that the reduction of soil acidity from pH 4.5 to 6.3 decreased zinc and manganese contents in rice plants. We could also see that in general, NR enzyme activity was higher in plants grown on low-acidity soils (pH 6.3) and fertilized with calcium nitrate. In addition, there was a higher development and productivity rate of the plants cultivated in soils with high acidity (pH 4.5). On the other hand, in conditions of medium acidity (pH 5.5), the plants cultivated with ammonium sulfate were more productive, and there were no differences with the addition of the nitrification inhibitor.

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