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Straw and early nitrogen fertilization affect soil properties and upland rice yield[1]

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ABSTRACT

The presence of cover crop straw and early application of total N at sowing may provide significant changes in the microbial population, reflecting on the N dynamics in the soil and in upland rice plants. This study aimed at determining the effect of the early application of nitrogen doses as mineral N and microbial biomass carbon in the soil, as well as in the activity of nitrate reductase, and grain yield of upland rice plants cultivated under no-tillage system (NTS). A randomized blocks design, in a split-plot scheme, with four replications, was used. The treatments consisted of N doses (0 kg ha^{-1}, 40 kg ha^{-1}, 80 kg ha^{-1} and 120 kg ha^{-1}) and the presence or absence of *U. brizantha* cover straw. Maintaining the straw on the soil surface reduces the ammonium levels and increases the microbial biomass carbon content of the soil. The application of increasing doses of N in the soil provides increases in the levels of nitrate and ammonium in the soil up to 28 days after emergence. The activity of the nitrate reductase enzyme in the plants increases and the contents of ammonium and nitrate in the soil decrease with the crop development. The number of panicles and grain yield of upland rice increase with the increase of the nitrogen fertilization, but decrease in the presence of *U. brizantha* straw. Thus, it is recommend the use of early N fertilization in upland rice crop.

KEY-WORDS: *Oryza sativa* L.; Brazilian Savannah; sustainable agriculture.

RESUMO

Palha e fertilização nitrogenada antecipada afetam atributos do solo e produtividade do arroz de terras altas

A presença de palha de plantas de cobertura e a aplicação de N todo na semeadura podem proporcionar alterações significativas na população microbiana, com reflexos na dinâmica de N no solo e na planta de arroz. Objetivou-se determinar o efeito da antecipação da aplicação de doses de N sobre as formas de N mineral e carbono da biomassa microbiana no solo, bem como na atividade da enzima redutase do nitrato, e a produtividade de arroz de terras altas cultivado sob sistema plantio direto (SPD). O delineamento experimental foi em blocos ao acaso, em parcelas subdivididas, com quatro repetições. Os tratamentos foram constituídos por doses de N (0 kg ha^{-1}, 40 kg ha^{-1}, 80 kg ha^{-1} e 120 kg ha^{-1}) e pela presença ou ausência de palha de cobertura de *U. brizantha*. A manutenção da palha na superfície do solo reduz os teores de amônio e aumenta os teores de carbono da biomassa microbiana no solo. A aplicação de doses crescentes de N no solo proporciona incrementos nos teores de nitrato e amônio no solo, até 28 dias após a emergência. A atividade da enzima redutase do nitrato nas plantas aumenta e os teores de amônio e nitrato no solo diminuem com o desenvolvimento da cultura. O número de panículas e a produtividade de grãos do arroz de terras altas aumentam com o incremento na adubação nitrogenada, mas diminuem com a presença de palha de *U. brizantha*. Assim, recomenda-se o uso da adubação antecipada de N na cultura do arroz de terras altas.

PALAVRAS-CHAVE: *Oryza sativa* L.; Cerrado; agricultura sustentável.

INTRODUCTION

The main species used as cover crops for no-tillage farming are mostly grasses with high C/N ratio, which may cause greater mobilization of N in the soil, reflecting the availability of this nutrient for plants grown in this system (Nascente & Crusciol 2013, Nascente et al. 2013). Thus, for the initial deployment of no-tillage farming, it is necessary to apply large amounts of N at sowing, when compared to conventional tillage with soil disturbance (Fageria 2014). It is recommended one application of N at sowing (10-30 kg ha^{-1}) and another topdressing application (20-70 kg ha^{-1}) in the tillers (Nascente et al. 2011a).

In the soil, $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ are the major forms of N available to plants, being the first one predominant in aerated soils, if compared to those under upland rice. However, in this type of aerated system (no-tillage farming), the upland rice does not

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perform well, in relation to the conventional system (plowing and harrowing) (Kluthcouski et al. 2000).

According to D'Andrea et al. (2004), the soil under no-tillage farming has increased availability of nitrate, when compared to conventional tillage, since the soil has higher contents of water, nutrients and organic matter, thus favoring the microbiological activity, particularly the nitrifying bacteria. A soil with large amounts of $N\text{-}NO_3^-$ may decrease the dry matter production and grain yield in plants such as upland rice, which has a low capacity to reduce nitrate (Ali et al. 2007).

Kluthcouski et al. (2000), Soares (2004) and Nascente et al. (2011a, 2011b and 2012) suggest that one of the causes for failure of rice crops in no-tillage farming is the fact that the plants, in early growth stages, present low capacity to reduce $N\text{-}NO_3^-$, due to low activity of the nitrate reductase enzyme, which converts nitrate into nitrite (Nambiar et al. 1988, Moro et al. 2014). The use of alternatives that provide increased nitrate reductase enzyme activity, at the beginning of plant development, may enable, in part, the introduction of upland rice in the no-tillage farming system (Cazetta & Villela 2004).

A similar yield was observed when N is applied all at sowing, when compared to the traditional management, in which N fertilization is divided at sowing and topdressing, in a no-tillage system (Nascente et al. 2011a and 2011b). Therefore, despite possible N loses in the soil, that drives its traditional split application, the authors show that the application of total N at sowing can also result in high grain yield. The immobilization of N fertilizer applied all at sowing by the soil microbial biomass, in the presence of straw with high C/N ratio on the surface, could be an explanation of how N is kept in the soil-plant system for a longer time in the organic form (Zaman et al. 2002, Silva et al. 2003, Coser et al. 2007). The soil microbial population is a considerable reservoir of nutrients, as microbes return these nutrients to the environment after the end of their life cycle (Spedding et al. 2004, Ferreira et al. 2010, Ferreira & Martin-Didonet 2012).

Thus, this study aimed at determining the effect of early N application, in the presence or absence of straw, on the forms of mineral N and microbial biomass in the soil, as well as the nitrate reductase enzyme activity and yield of upland rice plants grown under no-tillage farming.

MATERIAL AND METHODS

The experiment was conducted at the Fazenda Capivara (16º28'00"S, 49º17'00"W and 823 m of altitute), in Santo Antônio de Goiás, Goiás State, Brazil, where the climate is characterized as tropical Savannah (Aw), according to the Köppen classification, with two distinct seasons: dry from May to September (autumn/winter) and rainy from October to April (spring/summer). During the experiment, temperature and precipitation were monitored (Figure 1).

The experimental area has been managed under no-tillage farming for three years, with maize and *Urochloa brizantha* (Hochst. ex A. Rich.) R. D. Webster cv. Marandu [syn. *Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf] intercropped in the last harvest. After the maize harvest, the pasture was formed.

The soil is classified as acidic clay loam (kaolinitic, thermic Typic Haplorthox) (FAO 2006).

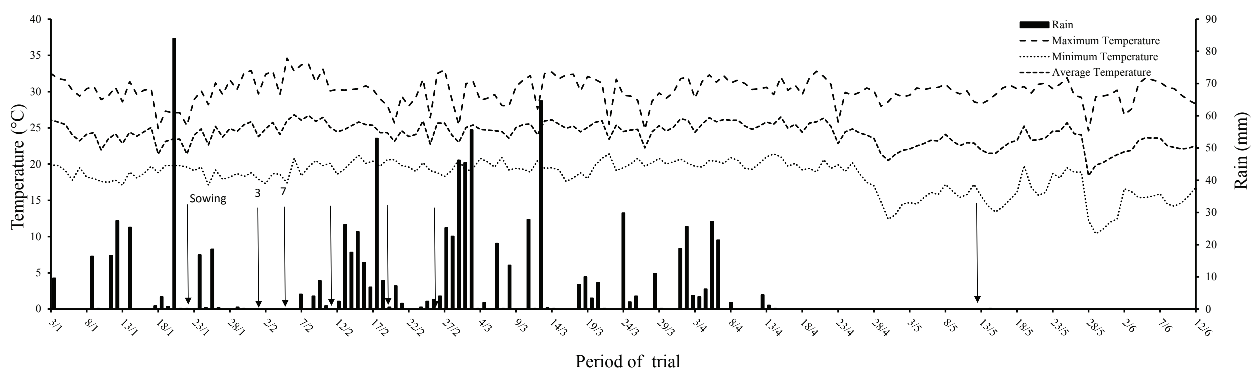

Figure 1. Maximum, minimum and average temperatures and rainfall during the experiment. Numbers on the arrows mean days after the emergence of the crop and when sampling was carried out to evaluate the nitrate reductase enzyme activity in upland rice plants.

Before the experiment, in January 2014, chemical analyzes (Claessen 1997) were carried out at a depth of 0-0.20 m to characterize the experimental area. The results were: pH ($CaCl_2$) = 5.1; organic matter = 27 g dm^{-3}; P = 20 mg dm^{-3}; Al = 10 mmol$_c$ dm^{-3}; H + Al = 40 mmol$_c$ dm^{-3}; K = 1.5 mmol$_c$ dm^{-3}; Ca = 19 mmol$_c$ dm^{-3}; Mg = 12 mmol$_c$ dm^{-3}; CTC = 72.5 mmol$_c$ dm^{-3}; V = 44.8 %. P and K were extracted by Mehlich extractant 1, and Ca, Mg and Al with KCl 1 M. In the solution extracted, P was determined by colorimetry and K by flame photometry. Ca and Mg were determined by EDTA titration and Al by NaOH titration. Organic matter was determined using the Walkley & Black method.

The experiment was installed in a complete randomized blocks design, with treatments arranged in split-plots, with four replications. The treatments consisted of N doses (applied 1 day after the rice sowing), with the presence or absence of forage straw (subplots). The plots were 5.0 m (15 rows of rice) x 8.0 m long and the subplots 5.0 m x 4.0 m long. The area considered useful consisted of the five central rows, excluding 0.50 m on each side. Nitrogen doses (0 kg ha^{-1}, 40 kg ha^{-1}, 80 kg ha^{-1} and 120 kg ha^{-1}) were applied as urea. The area was mowed to 5 cm of soil, with a straw chopper (Triton), one week before the rice sowing, with *U. brizantha* straw kept on the soil surface during the treatments with straw (average 8 Mg ha^{-1}) and removed completely in treatments without straw.

Rice (*Oryza sativa* L.) sowing was performed mechanically on January 22, 2014, using the mutant strain 07SEQCL441 CL, derived from the Primavera cultivar. This mutant species is resistant to the Imazapyr + Imazapic (Kifix) herbicide. This material is under registration stage and differs from the Primavera cultivar (one of the most planted in Brazil) only with respect to the herbicide resistance, and was chosen for ease weed management. The emergence occurred on average after 6 days. Spacing of 0.35 m was used between rows, as well as 80 viable seeds per meter. The fertilization in sowing furrows was 360 kg ha^{-1} (5-30-15) (18 kg of N at sowing for all treatments), based on the soil analysis and calculated according to Souza & Lobato (2004). The plant management was performed according to the crop needs.

The soil samples for analyses of microbial biomass carbon, ammonium and nitrate levels were collected in the first five weeks of the crop development (0, 7, 14, 21 and 28 days after emergence - DAE), at the 0-10 cm depth layer. Thus, eight subsamples (four in the row and four between the rows) were collected to form the composite sample in each plot.

The soil solution was extracted with KCl (2 mol L^{-1}), and ammonium and nitrate determinations were carried out by spectrophotometry coupled to the Flow Injection Analysis system (Nascente et al. 2012). The biomass carbon was determined according to Vance et al. (1987). Rice leaves were sampled and analyzed for nitrate reductase enzyme activity at 3, 7, 14, 21 and 28 DAE (Jaworski 1971).

After physiological maturity, at 105 DAE, rice was harvested manually in the useful area of each subplot. The plants were threshed and the grains were dried until reaching moisture of 13 %. Grains were then weighed and data were processed for kg ha^{-1}. To evaluate the production components, 10 panicles were collected at random in each plot and taken to the laboratory for evaluation (number of grains per panicle, number of filled grains, number of empty grains and 100-grain mass).

The data were submitted to analysis of variance and the Tukey test at 5 %, using the SAS statistical package. For quantitative traits, regression analyses were performed. Regarding the data measured during the development of the crop (ammonium, nitrate and microbial biomass carbon in the soil, and nitrate reductase enzyme activity in upland rice leaves), a statistical analysis considering split-plots in time (N doses - plot; with or without straw - subplot; days after rice emergence - sub-subplot) was used. For the productivity components and grain yield, only the split-plot (N doses, with or without straw) was considered. The Pearson's correlation between the contents of ammonium, nitrate and microbial carbon biomass in the soil and the nitrate reductase enzyme activity in rice plants was estimated.

RESULTS AND DISCUSSION

The presence or absence of straw as mulching significantly affected the levels of ammonium and carbon biomass in the soil (Table 1). The smallest ammonium (6.97 mg kg^{-1}) and the highest carbon biomass (297 mg kg^{-1}) values were found in the soil with brachiaria straw (*Urochloa brizantha*).

The N levels had a significant effect on ammonium and nitrate concentrations in the soil and on the nitrate reductase enzyme activity in rice plants (Table 1). The levels of ammonium (Figure 2a) and nitrate (Figure 2b) increased with increasing N doses applied to the soil cultivated with upland rice. Pacheco et al. (2011), Nascente et al. (2012) and Nascente & Crusciol (2013) also reported an increase in nitrate and ammonium levels in the soil after the application of urea. Although urea was used as a fertilizer, the ammonium levels were much lower than the nitrate in the soil at all evaluated times, including the soil that had not received topdressing fertilization one day after sowing. In aerobic environments, nitrification is common (Nascente & Crusciol 2013, Moro et al. 2014). Thus, the decrease in ammonium levels and increase in soil nitrate levels may have occurred due to the nitrification process.

Correlation analysis was highly significant and negative for the ammonium content and microbial carbon biomass, while the relationship between nitrate and microbial carbon biomass was significant and positive. This could be an indication of the conversion of N from this microbial carbon biomass, that contains nutrients in its constitution, which returns to the environment after the end of its life cycle (Spedding et al. 2004, Ferreira et al. 2010) as nitrate (Table 2).

According to Coser et al. (2007), soils under no-tillage farming and with high C/N ratio of cover plants, such as brachiaria, have a higher amount of microorganisms and, consequently, a higher N immobilization rate. The correlation was significant and negative between the ammonium and nitrate concentrations. According to Fageria (2014), urea applied to the soil is converted into ammonium and later into nitrate. Thus, it is likely that the ammonium applied to the soil has been absorbed by plants and also converted into nitrate, what would explain this negative correlation. With respect to the nitrate reductase enzyme, it was found that the correlation analysis was not significant for the ammonium and nitrate contents in the soil.

While the ammonium and nitrate concentrations ranged 1.57-17.25 mg kg^{-1} and 35.80-104.19 mg kg^{-1}, respectively (Figures 2a and 2b), the level of nitrate reductase activity in rice plants did not increase with higher N doses (Figure 2c). The N uptake by upland rice plants can be affected by several factors, such as soil and weather conditions, $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ ratios, water availability and light. N uptake is also affected by genotype physiologic conditions: nutrient accumulation in subcellular compartments and efficiency in metabolic pathways involving both absorption of $N\text{-}NO_3^-$ (nitrate reductase) and

Table 2. Pearson's correlation between levels of ammonia, nitrate and microbial carbon biomass (MCB) in the soil and nitrate reductase activity (NR) in rice plants cultivated under no-tillage.

	Ammonium	Nitrate	MCB	NR
Ammonium	1			
Nitrate	-0.1833*	1		
MCB	-0.3481**	0.53636**	1	
NR	0.11837^{ns}	-0.12333^{ns}	-0.04185^{ns}	1

*, ** and ^{ns}: significant at 0.05 and 0.01 and non-significant, respectively.

Table 1. Levels of ammonium, nitrate and microbial carbon biomass in the soil and nitrate reductase enzyme activity in upland rice (*Oryza sativa* L.), and analysis of variance on the basis of N doses applied one day after sowing, presence of *Urochloa brizantha* straw and days after emergence (DAE).

Straw level	Ammonium	Nitrate	Microbial carbon biomass	Nitrate reductase enzyme
	mg kg^{-1}			µmol $NaNO_2$ h^{-1} g^{-1} of fresh matter
With straw	6.97 b*	72.18 a	296.87 a	3.96 a
Without straw	10.32 a	73.16 a	232.21 b	3.84 a
Factor	Analysis of variance (F probability test)			
N doses (N)	< 0.001	< 0.001	0.5487	0.0077
Straw (S)	0.0156	0.8528	0.0443	0.5111
N * S	0.0681	0.1353	0.4180	0.2127
DAE	< 0.001	< 0.001	< 0.001	< 0.001
N * DAE	0.0753	0.0854	0.3349	0.5490
S * DAE	0.0903	0.6062	0.3424	0.2097
S * N * DAE	0.0955	0.4917	0.9798	0.9677
CV (%)	39.32	32.52	46.96	31.23

* Means followed vertically by the same letter do not differ by the Tukey test (p < 0.05).

the assimilation of N-NH_4^+ (glutamine synthetase) (Carelli & Fahl 2006).

The upland rice, in its initial growth phase, uses N-NH_4^+ as the main source of N, while the N-NO_3^-, even when available, is only absorbed and metabolized in small amounts (Lin et al. 2005, Li et al. 2006). Additionally, the absorption process may have happened followed by N-NO_3^- storage in root and leaf cells of upland rice plants, until the plants produced nitrate reductase to metabolize this nutrient.

The contents of N-NH_4^+ and N-NO_3^- decreased in the soil with the growth of upland rice plants (Figures 3a and 3b). Similar results were described by Nascente et al. (2012), with upland rice growing under five different plant covers. Reduction in N levels in the soil may be explained by the higher nutrient absorption rate by rice plants, as they

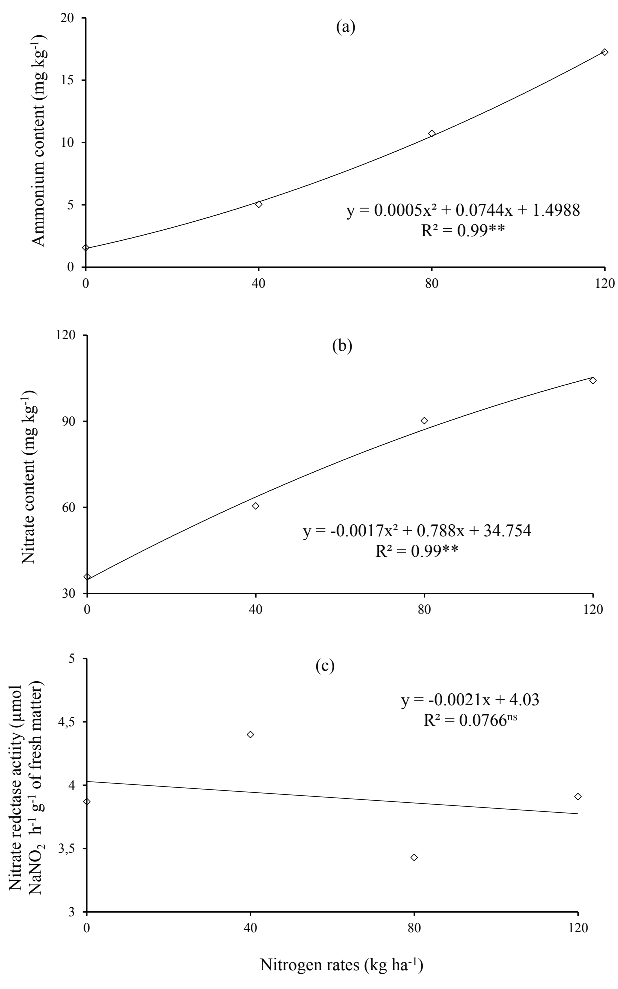

Figure 2. Levels of ammonium (a) and nitrate (b) in the soil and nitrate reductase enzyme activity in rice plants (c) affected by nitrogen levels.

develop, as well as by the leaching of N-NO_3^-, due to the rainfall occurred during this period (Figure 3). N-NO_3^- is easily leachable, and leaching is directly related to rainfall intensity (Carneiro et al. 2009, Fageria 2014).

The microbial biomass carbon content was not significantly affected by the time of evaluation (Figure 3c). Moreover, it was observed an increase in the nitrate reductase activity level with the upland rice growth (Figure 3d). Likewise, Araújo et al. (2012), working with the upland rice BRS Colosso and BRSMG Conai cultivars, observed a lower enzyme activity at the beginning of their development (26 days after transplanting - DAT), with increasing values up to 41 DAT.

One of the reasons attributed to the lower adaptation of rice to no-tillage farming is the low activity of the nitrate reductase enzyme at the beginning of crop development, which would hinder the absorption of N in the nitrate form (N-NO_3^-) (Malavolta 2006, Araújo et al. 2012). In the no-tillage system, there is a greater availability of nitrate, in relation to the conventional tillage (D'Andrea et al. 2004).

In this study, a mutant strain 07SEQCL441 CL presented nitrate reductase activity values ranging from 3.07 μmol $NaNO_2$ h^{-1} g^{-1} of fresh matter at 3 DAE to 5.27 μmol $NaNO_2$ h^{-1} g^{-1} of fresh matter at 28 DAE, on average, regarding the N doses tested, including the treatment without N topdressing application (only 18 kg ha^{-1} of N at sowing) (Figure 3). This suggests that the upland rice plant may initially store N in nitrate form (N-NO_3^-), and as the synthesis of the nitrate reductase enzyme occurs, it becomes able to process N-NO_3^- and thus produces nitrogenous compounds that can be metabolized.

The straw used as ground cover significantly affected the number of panicles and grain yield of upland rice (Table 3). These data corroborate Nascente et al. (2015), who tested rice development with four kinds of brachiaria managements. These authors found that brachiaria straw, when kept on the soil surface, may lower rice yield.

According to Weih et al. (2008), although there are many advantages in using cover crops, instead of set-aside, some types of topdressing fertilization may have inhibitory effects on seedling emergence, and these effects may be physical, allelopathic or biological (soil microbial population). Rice is adapted to anaerobic environments and, as such, grows better in environments with higher ammonium concentrations (Araújo et al. 2012). Thus, the maintenance of straw on the soil surface increased the microbial population and, therefore, increased the likelihood of N immobilization for a certain period, with direct impact on the number of panicles and grain yield.

Regarding N, it was found that high doses of the nutrient increased the number of panicles per square meter (Figure 4a). The number of grains per panicle increased up to the dose of 75.96 kg ha^{-1} of N (Figure 4b). This increase in production components provided a positive impact on grain yield (Figure 4c).

According to Qian et al. (2004), Nie et al. (2009), Fageria et al. (2011) and Fageria (2014), nitrogen fertilization in rice is directly related to increased crop yield. It is noteworthy that the application of topdressing N all at sowing provided significant increases in crop yield (Table 3 and Figure 4c), what can be an indication that this N was incorporated into the microbial biomass and has not been lost to the environment. Regarding

Table 3. Number of panicles per m² (PAN), number of grains per panicle (NGP), fertility of spikelets (FERT), 100-grain mass (100M) and grain yield (GY) of upland rice, as affected by the presence of straw coverage and topdressing nitrogen levels applied before sowing.

Factor	PAN	NGP	FERT	100M	GY
Straw	———— unit ————		%	g	kg ha^{-1}
With	171.47 b*	2,056 a	52.84 a	24.37 a	3,636 b
Without	199.75 a	2,183 a	57.07 a	22.84 a	4,074 a
Factor	Analysis of variance (F probability test)				
N doses (N)	0.0436	< 0.001	0.5584	0.2498	< 0.001
Straw (S)	0.0219	0.0060	0.1437	0.3376	0.0534
S * N	0.0740	0.9757	0.5858	0.9663	0.7282
CV (%)	15.94	14.79	13.25	25.15	18.44

* Means followed vertically by the same letter do not differ by the Tukey test (p < 0.05).

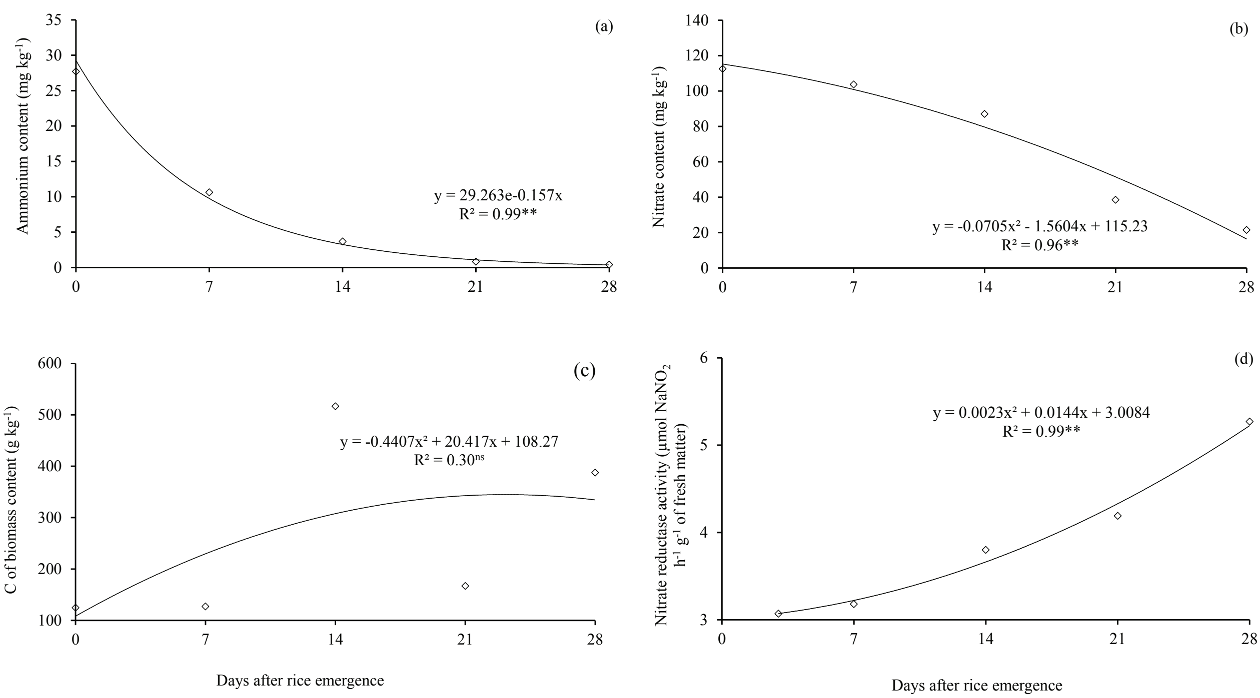

Figure 3. Levels of ammonium (a), nitrate (b) and microbial biomass carbon in the soil (c) and nitrate reductase activity enzyme (d) in the upland rice, as affected by days after emergence.

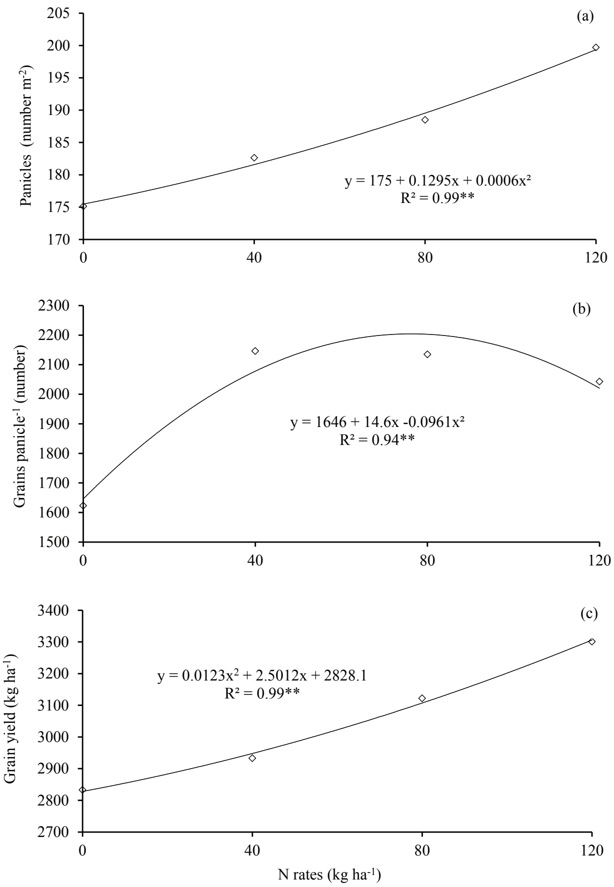

Figure 4. Number of panicles per square meter (a), number of grains per panicle (b) and grain yield (c) of upland rice, as affected by nitrogen doses.

the nitrate reductase profile assessed in this study, it can be inferred that the low activity of this enzyme in the early stages of crop development may be due to its low amount in the cells. However, it was observed an increase in its activity, as the growth/development process of upland rice plants was established. Therefore, the use/remobilization of N assimilated during reproductive stages may have largely determined upland rice plants productivity.

CONCLUSIONS

1. The maintenance of *Urochloa brizantha* straw on the soil surface in upland rice crops reduces ammonia levels and increases the carbon content of the microbial biomass in the soil;
2. The application of increasing doses of N in the soil, in no-tillage farming, provides increases in the levels of nitrate and ammonium in the soil up to 28 days after emergence;
3. During the development of rice cultivation, there is an increase of the nitrate reductase enzyme activity in rice plants and a reduction of ammonium and nitrate levels in the soil;
4. The number of panicles and grain yield of upland rice increase with the increase of nitrogen fertilization, but decrease with the presence of *U. brizantha* straw.

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