Australian Journal of Crop Science AJCS

AJCS 10(2): 176-184 (2016) ISSN:1835-2707

Cover crops can affect soil attributes and yield of upland rice

Adriano Stephan Nascente*, Mábio Chrisley Lacerda, Anna Cristina Lanna, Marta Cristina Corsi de Filippi and Dayanne Medrado Silva

Brazilian Agricultural Research Corporation (EMBRAPA), Rice and Beans Research Center, PO Box 179, 75375-000, Santo Antônio de Goiás, State of Goiás, Brazil

***Corresponding author: adriano.nascente@embrapa.br**

Abstract

Better understanding of the use of cover crops at no-tillage systems in upland rice crop could contribute to increase grain production in this environment and allow moving toward sustainable agriculture. The objective of this research was to determine the effect of pearl millet intercropped with other cover crops on N mineral forms and urease activity in the soil, nitrate reductase enzyme activity in the leaves and yield components and grain yield of upland rice. The experiment was performed in the year 2012/2013 in two locations, in Haplorthox soil under no-tillage system. The experimental design was a complete randomized block with eight replications. The treatments consisted of four types of cover crop [(1) Pearl millet (*Pennisetum glaucum*) – control; (2) Pearl millet + *Crotalaria spectabilis*; (3) Pearl millet + *Brachiaria ruziziensis*; (4) Pearl millet + *C. spectabilis* + *B. ruziziensis*]. The mixture of cover crops pearl millet + *C. spectabilis* allowed the highest levels of nitrate in the soil. The cover crops pearl millet, pearl millet + *C. spectabilis*, pearl millet + *Brachiaria ruziziensis*, and pearl millet + *C. spectabilis* + *Brachiaria ruziziensis* provided similar results for ammonium content and urease activity in the soil, nitrate reductase activity in leaves, yield components and grain yield of upland rice grown under no-tillage. Our results allow inferring that the use of pearl millet, as cover crop, alone or intercropped with *B. ruziziensis* or *C. spectabilis* is a management practice option to provide high rice grain yield.

Keywords: ammonium; *Brachiaria ruziziensis*; *Crotalaria spectabilis*; nitrate; nitrate reductase; *Oryza sativa*; *Pennisetum glaucum*; urease.

Abbreviations: DAE_days after emergence; DAS_days after sowing; FM_fresh material; N-NH_4^+_ammonium; N-NO_3^-_nitrate; NR_nitrate reductase; N_nitrogen; NTS_no-tillage system.

Introduction

Rice is included in the diet of half of the world's population. Mostly, this cereal is grown on irrigated land. However, available water resources have been reduced because of the competing demands of industry and population, and consequently, alternatives are sought that allow greater efficiency of water use (Prasad, 2011). Some alternatives include growing rice under aerobic conditions, such as at a no-tillage system (NTS) (Nascente et al., 2013). Due to the use of cover crops, the NTS provides many benefits, such as increased soil biological activity, reduced soil erosion, conservation of soil moisture and increased nutrient cycling (Nascente et al., 2014; Bassegio et al., 2015). At this system, most of crop N fertilization is done by urea (Fageria, 2014). Urea, when applied to the soil, may suffer action of urease, an enzyme that catalyzes the hydrolysis of urea into ammonia and carbon dioxide. Thus, it is important to analyze its activity in the soil since it provides to the soil the potential to convert organic N into mineral N, initiating the N mineralization process (Lanna et al., 2010). These authors evaluated the urease activity in soil cultivated with common bean (*Phaseolus vulgaris*), after cover crops (*Panicum maximum* cv. Mombasa, *Zea mays* + *Brachiaria brizantha* cv. Marandu, *Sorghum bicolor* and *Stylosanthes guianensis*). The authors concluded that urease activity in the soil was higher in Mombaça straw than *S. bicolor* and *S. guianensis* straws, and did not differ from *Z. mays* + *B. brizantha* straw. They attributed the result to higher biomass production from the cover forage. On the soil, N-NO_3^- and N-NH_4^+ are the main forms of N available to plants, and in aerobic soils nitrate prevails in relation to ammonium (Fageria, 2014). Most plants absorb both nitrate and ammonium (Malavolta, 1980). However, upland rice plants in the early stages of development have reduced capacity to uptake, store and/or metabolize N-NO_3^-. Some researchers brings up the principal hypothesis that rice seedlings present low activity of nitrate reductase (NR) enzyme (Lin et al., 2005; Li et al., 2006; Araújo et al., 2012; Lanna and Carvalho, 2013; Moro et al., 2013). This enzyme is the first of a reactions series that culminates in the production of NH_4^+ in the plant, which is incorporated into amino acids and/or other organic compounds (Ali et al., 2007). The use of cover crops can change the relationship between the mineral forms of N in soils, providing larger amounts of N-NH_4^+, and may enable better development of crops that absorb more and prefer this form of N, such as upland rice (Malavolta, 1980; Holzschuh et al., 2009). An alternative as cover crop is pearl millet (*Pennisetum glaucum*), already widely used during the off-season for straw production in the NTS (Nascente et al., 2013). Another option is the *Brachiaria* species, which provide big production of dry biomass (Nascente and Crusciol, 2012). In addition, the use of legumes species can increase the levels of nutrients in the soil, particularly nitrogen, from biological nitrogen fixation (Fageria, 2014).

In previous studies, Arf et al. (2003), Bordin et al. (2003), Cazetta et al. (2008), Pacheco et al. (2011), Crusciol et al. (2011), Moro et al. (2013) and Nascente et al. (2013) showed that pearl millet was the best cover crop for rice development. For example, Nascente et al. (2013) noticed that pearl millet as a cover crop provided higher rice grain yield than fallow, *B. brizanta*, *B. ruziziensis* and *Panicum maximum*. Therefore, we tried to intercrop pearl millet with other cover crops to observe if its effects would improve more rice grain yield.

This study hypothesized that pearl millet intercropped with other cover crops can change the mineral forms of N in the soil, which could provide better conditions for the development of upland rice in the NTS than pearl millet alone. Thus, this study aimed to determine the effect of pearl millet intercropped with other cover crops on mineral forms of N and urease activity in soil, nitrate reductase activity in the leaves, yield components and productivity of upland rice plants.

Results and Discussion

Degradation rate of cover crops straw

During the first weeks of development of upland rice, there was a significant interaction between days after sowing and location for cover crop straw degradation (Table 2 and Fig 2). The amount of cover crop straw in the Palmital Farm at rice sowing time was 690 g m^{-2}; while in the Capivara Farm it was 760 g m^{-2}. At the end of the evaluation period (35 DAS of rice plants), the straw degradation rate was 13.7 g m^{-2} day^{-1} at the Palmital Farm, and 13.1 g m^{-2} day^{-1} at the Capivara Farm. This significant difference may have been caused by the rain and temperature conditions of both locations. Rainfall conditions were 658 and 573 mm at Palmital and Capivara Stations, respectively, during November and December (Fig 1). The maximum and average temperatures were 28.5 and 27.8 °C at the Capivara Farm and 30.2 and 29.8 °C in the Palmital Farm, in this period. Thus, these higher humidity and temperature, which occurred in the Palmital Station, apparently supported a higher straw degradation rate. Temperature and humidity are among the main environmental factors that affect the activity of straw decomposing organisms (Calonego et al., 2012).

Soil levels of $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$

Cover crops affected soil nitrate levels (Table 2). Treatment with pearl millet + *C. spectabilis* provided highest nitrate levels (11.66 mg kg^{-1}) which differed from treatment with pearl millet (9.2 mg kg^{-1}) and pearl millet + *B. ruziziensis* (7.83 mg kg^{-1}). The legume specie *C. spectabilis* is a cover crop that, probably, increased N levels in the soil because of its ability to fix nitrogen (Fageria, 2014). The Palmital Farm soil presented higher nitrate content than the soil of the Capivara Farm. This can be explained by the increased amount of organic matter (47.6 g dm^{-3}) in this environment in relation to the other (11.1 g dm^{-3}) (Table 1). As a result of the higher content of organic matter in the soil, there was probably a larger amount of nitrogen and micro-organisms (Fageria, 2014). It is likely that among the microbial population there were large quantities of nitrifying microorganisms, which quickly turned the ammonium to nitrate from the nitrogen fertilizer applied after rice sowing.

Instead, the ammonium content in the soil did not differ between the cover crops used (Table 2). Ammonium content was higher in the soil of the Capivara Farm (Fig 3A) than in the soil of the Palmital Farm (Fig 3B) in the first seven days after rice sowing. After nitrogen fertilization, there was an increase in ammonium and nitrate amount in the soil, which could be seen in the next evaluation at 0 days after sowing rice for ammonium and 7 days for nitrate. After this period, in the following evaluated weeks of rice sowing, there was a reduction in both ammonium (Figs 3A and 3B) and nitrate (Figs 4A and 4B) levels in the soil of the Capivara and Palmital Farms. Regarding ammonium, it is likely that this reduction was due to the prevailing of nitrate in aerobic soils, and therefore the ammonium was probably converted to nitrate. Nitrate reduction occurred because of the absorption of nutrients by the roots of upland rice plants (Malavolta, 1980; Fageria et al., 2011), and partly due to leaching in function of the great amount of rainfall which occurred in the experimental areas (Fig 1). Nitrate is easily leachable, especially after heavy rainfall (Crusciol et al., 2011; Fageria et al., 2011). Nitrate levels in the soil were much higher in the Palmital that in the Capivara Station (Table 2), and it could be because of the level of organic matter in the soil of the first farm (47.6 g dm^{-3}) was much higher than in the second one (11.1 g dm^{-3}) (Table 1). According to Fageria (2014), soil organic matter can release N and contribute to increase levels of nitrate in the soil.

Urease activity in the soil

Urease activity was, in general, higher in the soil of the Palmital Farm (Figs 5A and 5B) by the time of rice sowing (time 0), compared to the soil of the Capivara Farm. This is due, in part, to a less acidic soil (pH = 6, table 1) as urease is an extracellular enzyme (Reynolds et al., 1987), and its activity is apparently optimal for pH 8.5 to 9.0 (Tabatabai and Bremner, 1972). According to Fageria (2014), ammonia, the main product of urease hydrolysis, can have multiple targets, among them oxidized nitrate. Supporting this statement, we found high nitrate levels in the soil of the Palmital Farm (Figs 4A and 4B) in the first three weeks after rice sowing. In the first week after sowing, urease in the soil of the farm Palmital presented a higher active, probably due to the effect of the soil's pH and to the excellent weather conditions (higher temperatures and humidity, Fig 1).

Nitrate reductase activity

The upland rice cultivation at NTS failed because this crop presents low ability to reduce the $N\text{-}NO_3^-$, in the early stages of growth (Kluthcouski et al., 2000; Araujo et al., 2012; Nascente et al. 2012). This can be related to the low activity of nitrate reductase (NR), a key enzyme for absorption and assimilation of the nitrate pathway, in superior plants. Although this pathway involves a sequential series of reactions, NR is used as a biochemical indicator for measuring N use efficiency at the beginning of crop establishment (Lanna and Carvalho, 2013; Moro et al., 2013). In this study, the activity of NR in rice plants was similar throughout the evaluation period in the Palmital Farm, while NR activity was higher in plants grown in the soil of the Capivara Farm in the first evaluations (10 and 17 DAE), and it decreased after the plants reached the ages of 24 and 31 DAE (Figs 6A and 6B). In the Palmital Farm, plants exhibited NR activity equal to 3.0 μmol $NaNO_2$ h^{-1} FM throughout the evaluation period. In the Capivara Farm, the activity ranged from 4.2 to 2.0 μmol $NaNO_2$ h^{-1} FM, from 7 DAE to 31 DAE (reduction of approximately 50%), respectively. At the same time, at the Palmital Farm, the nitrate content (NR substrate) in the soil ranged from 35 mg kg^{-1}, at 7 DAE, to 0.2 mg kg^{-1} at the end of the evaluation

Table 1. Chemical properties of the soil at 0-0.20 m layer of the Palmital Farm (Goianira) and the Capivara Farm (Santo Antônio de Goiás), August / 2013.

Farm	pH	SOM	K	Ca	Mg	Al	H+Al	P	Cu	Fe	Mn	Zn
	(H_2O)	g dm^{-3}	------------$cmol_c$ dm^{-3}--------------					----------------mg dm^{-3}---------------				
Capivara	5.5	11.1	0.27	0.8	0.4	0.1	4.9	10.8	1.9	43.1	8.1	5.2
Palmital	6.0	47.6	0.40	2.1	1.2	0.0	3.6	12.8	2.1	34.3	14.5	2.9

pH – hydrogen potential, SOM – soil organic matter, K – potassium, Ca – calcium, Mg – magnesium, Al – aluminum, P – Phosphorus, B – boron, Fe – iron, Mn – manganese, Zn – zinc.

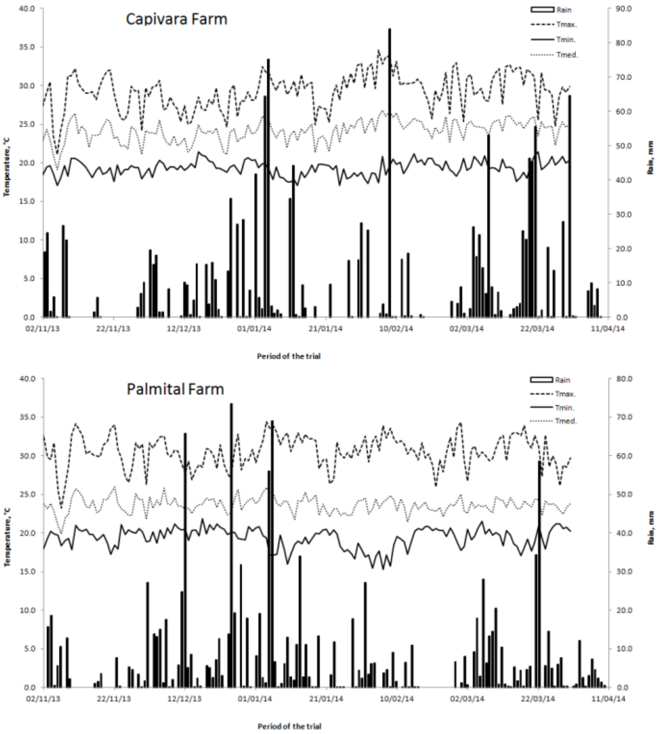

Fig 1. Maximum (Tmax.), minimum (Tmin.) and average (Tmed.) temperatures and rainfall during the trial period of upland rice under no-tillage system in the experimental fields of Palmital farm (Goianira) and Capivara Farm (Santo Antônio de Goiás), growing season 2013/2014.

process. Thus, in the Capivara Farm, the nitrate content ranged from 8 mg kg^{-1} to 0.4 mg kg^{-1}. In the Capivara Farm, NR activity in rice plants followed the nitrate content in the soil during the early stages of rice development. Differently, the NR activity in rice leaves exhibited an anomalous profile in relation to the nitrate content profile in the Palmital Station. It is likely that, in addition to the availability of NO_3^-, other regulators as light and phytohormones (Sivasankar and Oaks, 1995) may also be influencing NR activity, indicating that plants in this environment can uptake and accumulate NO_3^- in the initial stages of development (14 DAE) for use in subsequent periods, and therefore kept a constant level of activity up to 31 DAE.

Upland rice yield components and grain yield

Mixtures of cover crops did not affect plant height, number of tillers, yield components and yield of upland rice. These results may indicate that pearl millet (present in all treatments) is a cover crop that provides changes in soil that favor the development of rice, and its mixture with *C. spectabilis* or *B. ruziziensis* did not affect their performance. Also Arf et al. (2003), Bordin et al. (2003), Cazetta et al. (2008), Pacheco et al. (2011), Crusciol et al. (2011), Moro et al. (2013) and Nascente et al. (2013) achieved better upland rice grain yield with pearl millet as cover crop. They explained that it was because pearl millet has rapid straw degradation, releasing faster nutrients to rice plants. Brazilian national upland rice average is about 2,000 kg ha^{-1} (CONAB, 2015). Using pearl millet, alone or intercropped with other plants as a cover crop previous to upland rice crops, could improve the rice grain yield national average (Table 2). The intercropping pearl millet with *C. spectabilis* also increased the amount of nitrate in the soil. Therefore, introduction of cover crops in the crop systems brings many benefits to the environment and our results allow inferring that these cover crops are important options to be cultivated before upland rice, when aiming to increase grain yield. Regarding the environment, we observed that the Palmital Farm presented higher plant height, number of tillers, number of panicles, number of grains and grain yield than the Capivara Farm. According to Yoshida (1981), the productivity of rice grains is determined by four components: 1) the number of panicles m^{-2}, 2) number of filled grains, and 3) mass of 1000 grains. Therefore, by the results achieved in the components of production we can explain this higher grain yield. The better results obtained in the Palmital Farm may have happened because of higher rate of cover crops straw degradation (Fig 2), higher soil organic matter content (Table 1) and higher urease activity, which caused higher availability of N-NH_4^+ in the soil in the first week after rice sowing (Fig 3). In addition, at the Palmital Farm in November and December it rained 658 mm, while at the Capivara Farm, the number was 573 mm (Fig 1). A lower water availability to rice plants during its development can cause reductions in plant height, leaf area, biomass, tiller abortion, reduction in dry weight of

Table 2. Ammonium ($N\text{-}NH_4^+$) and nitrate ($N\text{-}NO_3^-$) level and urease activity in soil and nitrate reductase activity in upland rice plants at no-tillage system as affected by the local and cover crops. Goianira and Santo Antônio de Goiás, growing season 2013/2014.

Factors	$N\text{-}NH_4^+$	NNO_3^-	Urease	Nitrate reductase###
Cover crops	mg kg^{-1}	mg kg^{-1}	$N\text{-}NH_4^+$ mg g^{-1} $2h^{-1}$	µmol $NaNO_2$ h^{-1} g^{-1}FM
Pearl Millet (M)	4.33 a#	9.02 b	65.85 a	2.88 a
M + *C. spectabilis* (C)	3.20 a	11.66 a	72.65 a	2.83 a
M + *B. ruziziensis* (R)	3.86 a	7.83 b	71.06 a	2.89 a
M+C+R	2.37 a	9.32 ab	75.18 a	2.84 a
Local				
Capivara	4.22 a	5.53 b	71.43 a	2.81 a
Palmital	2.60 b	13.73 a	71.06 a	2.91 a
Factors	ANOVA – F Probability			
Cover crops (COB)	0.1439	0.0440	0.4812	0.9478
Local (L)	0.0188	<0.001	0.8352	0.2401
DAE	<0.001	<0.001	<0.001	<0.001
L*COB	0.2166	0.5680	0.1447	0.7207
COB*DAE	0.2045	0.3573	0.3958	0.3669
L*DAE	<0.001	<0.001	<0.001	<0.001
L * COB*DAE	0.1066	0.5391	0.4332	0.7947
CV## (%)	33.24	26.50	30.50	14.91

#Means followed by the same letter vertically, do not differ significantly by Tukey's test at p <0.05. ##Coefficient of variation. ###This evaluation was done at 3 days after emergence.

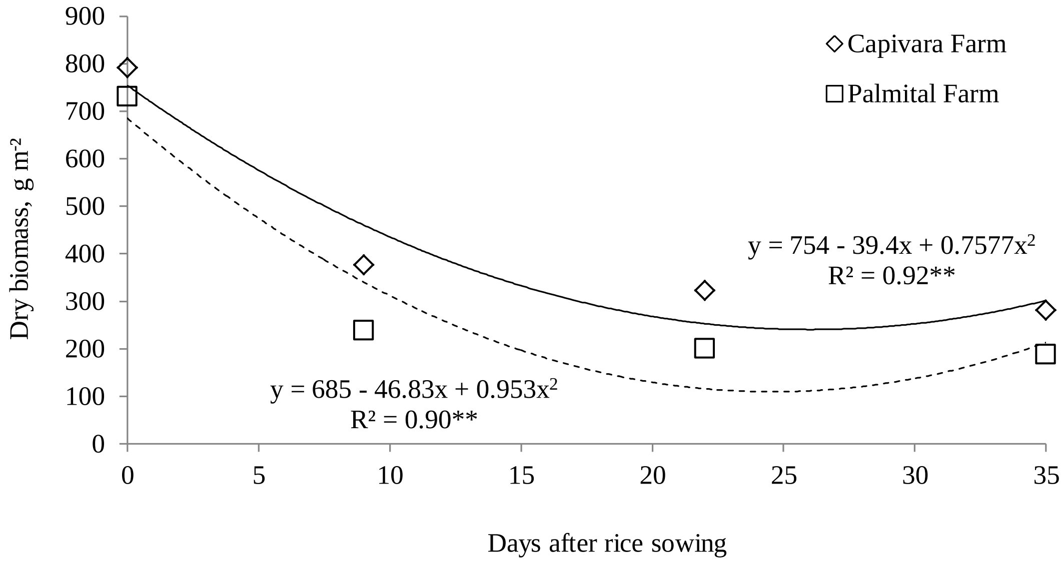

Fig 2. Average of cover crop [Pearl millet (*Pennisetum glaucum*); Pearl millet + (*Crotalaria spectabilis*); Pearl millet + *Brachiaria ruziziensis*; millet + *B. spectabilis*; and pearl millet + *B. ruziziensis* + *C. spectabilis*] degradation in the experimental fields of the Palmital Farm (Goianira) and the Capivara Farm (Santo Antônio de Goiás). Cover crop straws were sampled at 0, 9, 22 and 35 days after sowing (DAS) rice.

roots and rooting depth and delayed reproductive development with reflection in grain yield (Guimarães et al., 2011). In addition, soil fertility at the Palmital Farm was well above that of the Capivara Farm (Table 1). According to Fageria (2009), soil fertility directly affects grain yield of agricultural crops.

Materials and Methods

Site description

The experiments were located in the Capivara and Palmital Farms. The Capivara Farm is located in Santo Antônio de Goiás, GO, Brazil, at 16º28'00" S and 49º17'00" W, and 823 m of altitude. The Palmital Farm is located in the municipality of Goianira, GO, Brazil, at 16°26'14" S, 49°23'50" W, and 720 m of altitude. The climate is tropical savanna, considered Aw according to the Köppen classification. There are two distinct seasons, usually dry from May to September (fall/winter) and rainy from October to April (spring/summer). During the experiment, the temperature and amount of rainfall data were recorded (Fig 1).

At the Capivara Farm, the trial was irrigated by center pivot in soil managed under NTS for three years, which was cultivated with common bean in the last growing season. At the Palmital Farm, trail was rainfed in soil under NTS for five years, with rotation pasture/crop with pasture of *Brachiaria brizantha* in the last two years.

The soil in both locations was classified as a sandy clay loam (kaolinitic, thermic Typic Haplorthox). Before the application of treatments, the chemical characteristics of the soil were determined according to the methods described by Claessen (1997) (Table 1).

Table 3. Plant height, number of tillers, number of panicles, number of grains, mass of 100 grains and grain yield (Yield) of upland rice plants cultivated under no-tillage system (sowing at November / 2013) as affected by local and cover crops. Goianiara (Palmital Farm) and Santo Antônio de Goiás (Capivara Farm), growing season 2013/2014.

Factors	Plant height	Tiller	Panicles	Grains	Mass	Yield
Local	cm	number	n. m^{-2}	number	grams	kg ha^{-1}
Capivara	75.0 b[+]	219.9 b	212.8 b	48.5 b	26.3 a	3085 b
Palmital	86.3 a	281.0 a	275.6 a	86.1 a	21.8 b	4129 a
Cover crops						
Pearl Millet (M)	79 a	256 a	246 a	71.5 a	24.27 a	3646 a
M + C. spectabilis (C)	82 a	258 a	244 a	65.0 a	23.76 a	3548 a
M + B. ruziziensis (R)	80 a	236 a	225 a	68.0 a	24.16 a	3625 a
M+C+R	81 a	253 a	261 a	64.5 a	24.09 a	3610 a
Factors			ANOVA – Probability of F test			
Cover crops (COB)	0.4078	0.8398	0.5953	0.6514	0.6792	0.6745
Local (L)	<0.001	0.0025	0.0015	<0.001	<0.001	<0.001
L*COB	0.4480	0.3732	0.3805	0.3713	0.8792	0.8791
CV (%)[++]	5.98	21.69	20.76	26.11	5.16	6.49

[+]Means followed by the same letter vertically, do not differ significantly by Tukey's test at p <0.05. [++]Coefficient of variation.

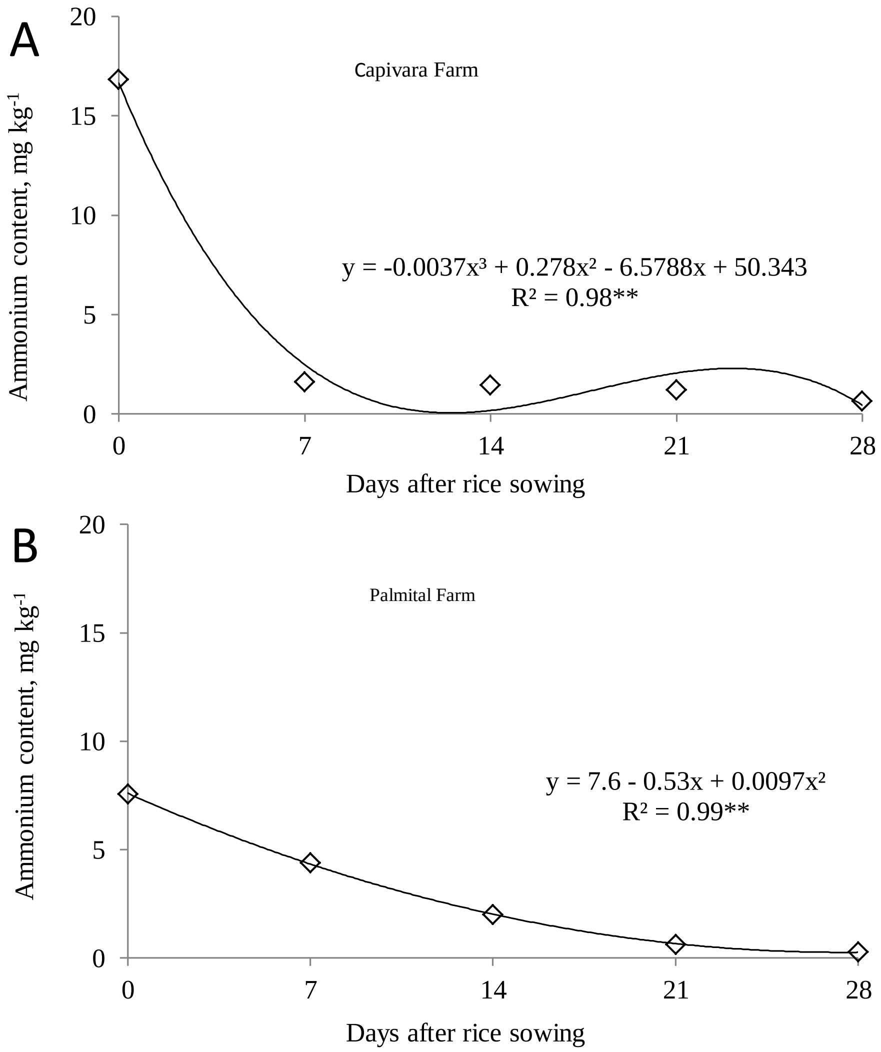

Fig 3. Ammonium content in the soil in the layer 0-0.10 m in the experimental fields of the Palmital Farm (Goianira) and the Capivara Farm (Santo Antônio de Goiás), growing season 2013/2014. The soil samples were collected to 0, 7, 14, 21, 28 and 35 DAS rice.

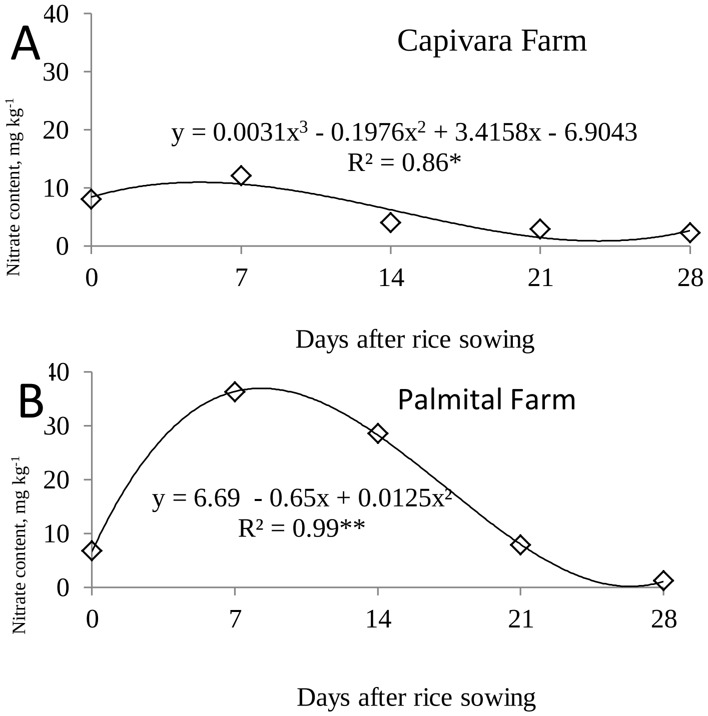

Fig 4. Nitrate content in the soil in the layer 0-0.10 m in the experimental fields of the Palmital Farm (Goianira) and the Capivara Farm (Santo Antônio de Goiás), growing season 2013/2014. The soil samples were collected to 0, 7, 14, 21, 28 and 35 DAS rice. Level of nitrate at the Capivara farm at day 0 was 3.84 mg kg^{-1}

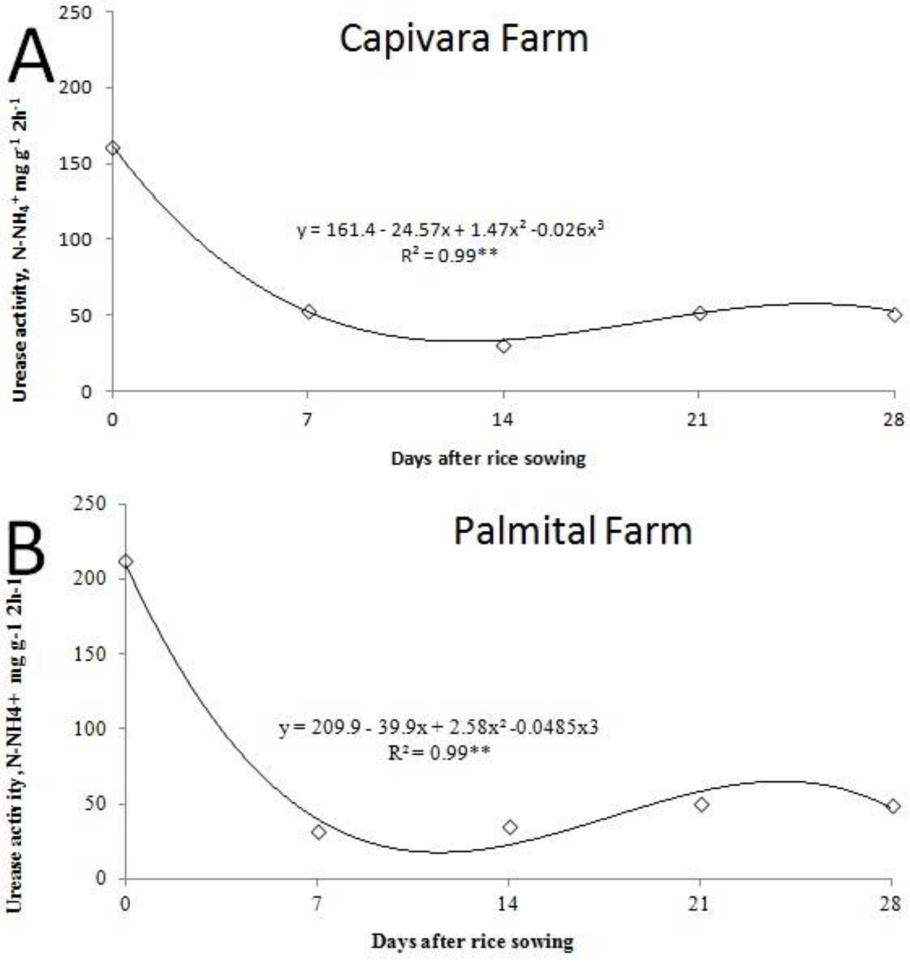

Fig 5. Urease activity in soil layer 0-0.10 m in the experimental fields of the Palmital Farm (Goianira) and the Capivara Farm (Santo Antônio de Goiás), growing season 2013/2014. The soil samples were collected to 0, 7, 14, 21, 28 and 35 DAS rice.

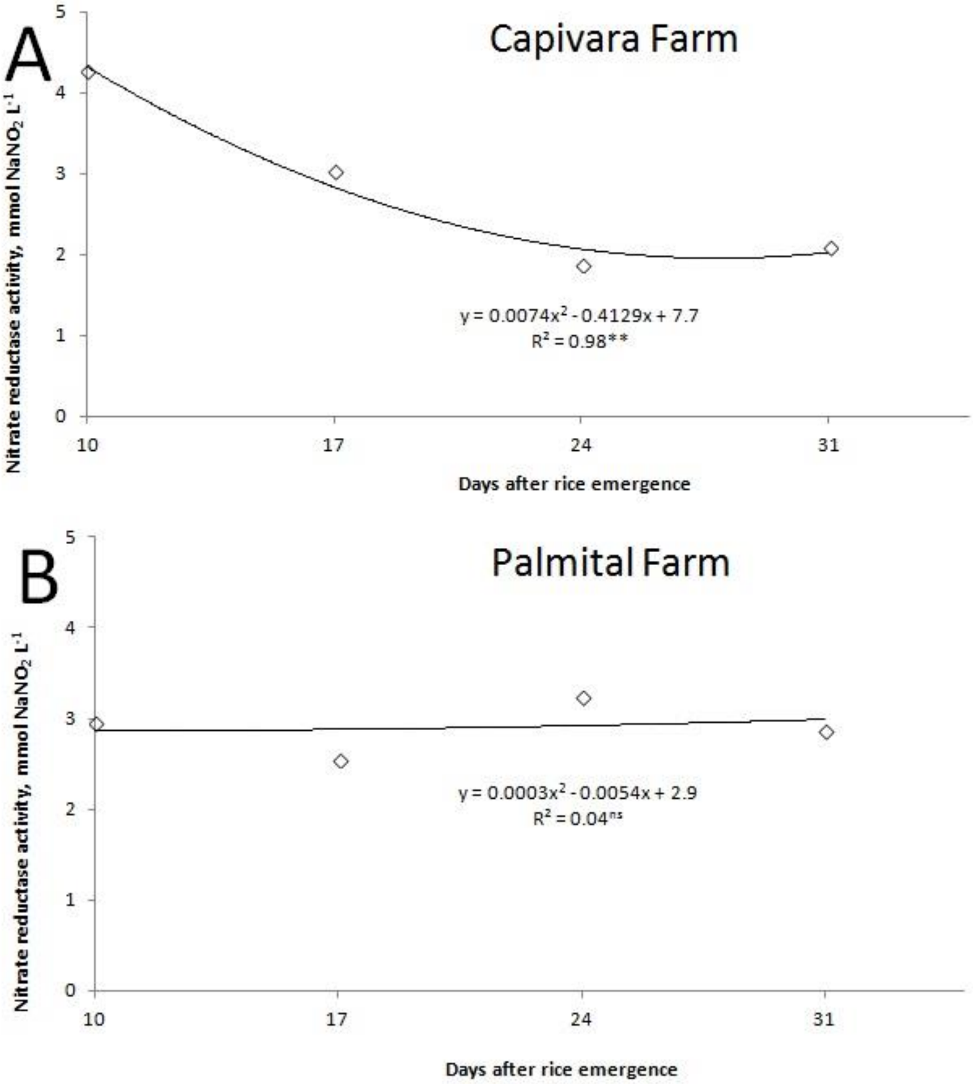

Fig 6. Nitrate reductase activity in the experimental fields of the Palmital Farm (Goianira) and the Capivara Farm (Santo Antônio de Goiás), growing season 2013/2014. Samples of rice leaves were collected at 10, 17, 24 and 31 DAE of rice plants.

Experimental design and treatments

The experiment was arranged in a randomized complete block design in factorial scheme with eight replications (blocks). The treatments consisted of the combination of four cover crops [1. Pearl millet (*Pennisetum glaucum*) - control, 2. Pearl millet + *Crotalaria spectabilis*), 3. Pearl millet + *Brachiaria ruziziensis*, and 4. Pearl millet + *C. spectabilis* + *B. ruziziensis*], two locations (the Palmital and Capivara farms) with days after sowing (for the variables cover crops straw degradation, ammonium, nitrate and urease activity in the soil) or days after emergence (for the variable nitrato redutase activity). For upland rice yield components and grain yield the experimental design was in complete block design in factorial scheme 2 (locations) x 4 (cover crops). The plots had the dimension 3.5 m (10 rows of rice) x 8 m size. The usable area of the plot was composed of eight central rows of rice, disregarding 0.50 m on each side.

Cover crop management

Cover crops were sown in August 2013, without the use of fertilizer. For millet, we used the row spacing of 0.20 m and depth of two cm using 20 kg ha^{-1} of pure live seeds. In the intercropping treatments, we used 10 kg ha^{-1} of pearl millet + 10 kg ha^{-1} of seeds of the other cover crop species (*C. spectabilis* or *B. ruziziensis*) with at least 30% of pure live seed, which were mixed in the seed distribution box at the using no-till seeding (Semeato, model Personale Drill 13, Passo Fundo, RS, Brazil). Cover crops were desiccated 15 days before upland rice sowing with glyphosate application (1.8 kg ha^{-1} acid equivalent).

Rice crop management

The sowing was performed mechanically, using 80 kg ha^{-1} of rice seeds from a mutant line 07SEQCL441 CL that was derived from a Primavera variety and was resistant to the Imazapyr + Imazapic herbicide. The seed was sown on November 12th and 14th, 2013 in the Palmital and Capivara Farms, respectively. Rice plants emergency occurred five days after sowing. The row spacing used was 0.35 m, with 80 viable seeds per meter. One day before sowing rice, the early nitrogen topdressing fertilization was done with 100 kg ha^{-1} of N as urea (42% of N). Cultural practices were performed according to standard recommendations for a rice crop to keep the area free from weeds, diseases and insects. Rice harvest was done after physiological maturity (March, 13th, 2014 at the Palmital Farm, and March, 20th, 2014 at the Capivara Farm), by hand in the usable area in each plot. Plots were evaluated regarding: number of panicles m^{-1}, which was determined by counting the number of panicles within 1.0 linear m of one of the rows in the useful area of each plot; mass of 1000 grains, which was evaluated randomly by collecting and weighing two samples of 100 grains from each plot, corrected to 13% of water content; and grain yield, which was determined by weighing the harvested grain of each plot, corrected to 13% of water content and converted to kg ha^{-1}.

Traits Measurements

Cover crop straws were sampled at 0, 9, 22 and 35 days after sowing (DAS) rice. These intervals were chosen once the majority of crops were fertilized with N at the sowing date or at topdressing close to the sowing date (Malavolta, 2006). At each time, samples of straw were collected from a randomly selected 1.0 x 1.0 m area in each plot. The plant material was dried in an oven with forced ventilation at 65 °C until constant weight was achieved.

The soil samples for determination of mineral N levels (N-NH_4^+ and N-NO_3^-) were collected with an auger at 0, 7, 14, 21, 28 and 35 DAS rice. Eight sub-samples (four in row and four inter rows) were collected in each composite sample from each plot at a depth 0-0.10 m, which were homogenized by hand, labeled, wrapped in plastic bags, kept in a cooler with ice and sent to the lab for analysis on the same day. The ammonium and nitrate determinations were performed by spectrophotometry coupled with the Flow Injection Analysis (FIA) system. Samples were also used for determining urease activity, according to the method described by Kandeler and Gerber (1988).

Samples of rice leaves to determine the activity of nitrate reductase (NR) were performed at 10, 17, 24 and 31 days after emergence (DAE) of rice plants, and analyzed in the same day of sampling following the methodology described by Jaworski (1971).

Statistical analysis

For statistical analysis, the SAS Statistical Software, SAS Institute, Cary, NC, USA (SAS, 1999) was used. In qualitative variables (upland rice yield components and grain yield), data were subjected to an analysis of variance and, when the F test proved significant, compared by a Tukey test at $p<0.05$. In this case, factors were cover crops, locations and interactions.

In the quantitative variables, results were submitted to regression analysis at $p< 0.05$. For cover crops straw, mineral N levels and urease activity in the soil, factors were: days after sowing, cover crops, location, and interactions. For nitrate reductase activity, factors were: days after emergence, cover crops, location and interactions.

Conclusions

The use of cover crops allowed many benefits, such as reduction in soil erosion, increase in soil fertility and soil water conservation. The results allowed us to conclude that among the cover crops evaluated (pearl millet alone, pearl millet + *B. ruziziensis*, pearl millet + *C. spectabilis* and pearl millet + *B. ruziziensis* + *C. spectabilis*), intercropping the cover crops pearl millet + *C. spectabilis* provides higher nitrate content in the soil than pearl millet or pearl millet + *B. ruziziensis*. Besides, cover crops pearl millet, pearl millet + *C. spectabilis*, pearl millet + *Brachiaria ruziziensis* and pearl millet + *Brachiaria ruziziensis* + *C. spectabilis* provide similar results for the ammonium content and urease in soil, nitrate reductase activity in leaves, yield components and grain yield of upland rice at a no-tillage system. Therefore, our results allow inferring that the evaluated cover crops are an important option to be considered for upland rice crop when aiming for higher rice grain yield.

Acknowledgements

To Embrapa for the financial support of this research and Antônio da Conceição Teixeira and Santo dos Reis Guizeline for conducing the trials.

References

Ali A, Sivakami S. Raghuram N (2007) Effect of nitrate, nitrite, ammonium, glutamate, glutamine and 2-oxoglutarate on RNA, levels and enzyme activities of nitrate reductase and nitrite reductase in rice. Physiol Mol Biol Plants. 13(1):17-25.

Araujo JL, Faquin V, Vieira NMB, Oliveira MVC, Soares AA, Rodrigues CR, Mesquita AA (2012) Rice growth and yield at different Nitrate-Ammonium ratios. Rev Bras Cienc Solo. 36(3): 921-930.

Arf O, Rodrigues RAF, Crusciol CAC, Sá ME, Buzetti S (2003). Soil management and nitrogen fertilization for sprinkler-irrigated upland rice cultivars. Sci Agric. 60(2):348-352.

Bassegio D, Santos RF, Secco D, Zanão Junior LA, Werncke I, Sarto MVM (2015). Short-term effects of crop rotations on soil chemical properties under no-tillage condition. Aust J Crop Sci. 9(1):49-54.

Bordin L, Farinelli R, Penariol FG, Fornasieri Filho D (2003) Double crop - commom bean with upland rice, submitted to rates of nitrogen fertilization after green cover under no-tillage system. Bragantia. 62(3): 417-428.

Calonego JC, Gil FC, Santos VF, Santos EA (2012) Persistence and nutrient release from maize, brachiaria and lablab straw. Biosci J. 28:770-781.

Cazetta DA, Arf O, Buzetti S, Sá ME, Rodrigues RAF (2008) Performance of upland rice to the nitrogen rates after different cover crops in no-till system. Bragantia. 67(2): 471-479.

Claessen MEC (1997) Manual for Methods of Soil Analysis, 2nd ed. Rio de Janeiro, Embrapa Solos. 212 p.

CONAB, Brazilian company of food supply (2015). Grains: Available at: < http://www.conab.gov.br/OlalaCMS/uploads/arquivos/15_01_09_09_00_21_boletim_graos_janeiro_2015.pdf>. Acessed in: Feb. 08 2015.

Crusciol CAC, Garcia RA, Castro GSA, Rosolem CA (2011) Nitrate role in basic cation leaching under no-till. Rev Bras Cienc Solo. 35:1975-1984.

Fageria NK (2009) The use of nutrients in crops plants, CRC Press, Boca Raton.

Fageria NK, Moreira A, Coelho AM (2011) Yield and yield components of upland rice as influenced by nitrogen sources. J Plant Nutr. 34:361-370.

Fageria NK (2014) Nitrogen management in crop production. CRC Press, Boca Raton.

Guimarães CM, Stone LF, Oliveira JP, Rangel PHN (2011) Rodrigues, C.A.P. Upland rice root system under water deficit. Pesqui Agropecu Trop. 41(1):126-134.

Holzschuh MJ, Bohnen H, Anghinoni I, Meurer EJ, Carmona FC, Costa SEVGA (2009) Rice growth as affected by combined ammonium and nitrate supply. Rev Bras Cienc Solo. 33:1323-1331.

Jaworski EG (1971) Nitrate reductase assay in intact plant tissues. Biochem Bioph Res Co. 43(6):1274-1279.

Kandeler E, Gerber H (1988) Short-term assay of soil urease activity using colorimetric determination of ammonium. Biol Fert Soils. 6:68-72.

Kluthcouski J, Fancelli AL, Dourado Neto D, Ribeiro CM, Ferraro LA (2000) Yield of soybean, corn, common bean and rice under no-tillage management. Sci Agric. 57(1): 97-104.

Lanna AC, Silveira PM, Silva MB, Ferraresi TM, Kliemann H (2010) Atividade de urease no solo com feijoeiro influenciada pela cobertura vegetal e sistemas de plantio. Rev Bras Cienc Solo. 34:1933-1939.

Lanna AC, Carvalho MAF (2013) Nitrato redutase e sua importância no estabelecimento de plantas de arroz de terras altas. Embrapa Arroz e Feijão, Santo Antônio de Goiás.

Li B, Xin W, Sun S, Shen Q, Xu G (2006) Physiological and molecular responses of nitrogen-starved rice plants to re-supply of different nitrogen sources. Plant Soil. 287:145–159.

Lin S, Li J, Sattelmacher B, Brück H. (2005) Response of lowland and aerobic rice to ammonium and nitrate supply during early growth stages. J Plant Nutr. 28:1495–1510.

Malavolta E (1980) Mineral nutrition elements of plants. Agronômica Ceres, São Paulo.

Malavolta, E (2006) Manual of Plant Nutrition. Agronômica Ceres, São Paulo.

Moro E, Crusciol CAC, Cantarella H, Nascente AS (2013) Upland rice under no-tillage preceded by crops for soil cover and nitrogen fertilization. Rev Bras Cienc Solo. 37(6):1669-1677.

Nascente AS, Crusciol CAC (2012) Cover crops and herbicide timing management on soybean yield under no-tillage system. Pesqui Agropecu Bras. 47:187-192.

Nascente AS, Crusciol CAC, Cobucci T (2012) Ammonium and nitrate in soil and upland rice yield as affected by cover crops and their desiccation time. Pesqui Agropecu Bras. 47:1699-1706.

Nascente AS, Crusciol CAC, Cobucci T (2013) The no-tillage system and cover crops Alternatives to increase upland rice yields. Eur J Agron. 45:124-131.

Nascente AS, Crusciol CAC, Stone LF (2014) Straw degradation and nitrogen release from cover crops under no-tillage. Rev Caatinga. 27:166-175.

Pacheco LP, Barbosa JM, Leandro WM, Machado PLOA, Assis RL, Madari BE, Petter FA (2011) Biomass production and nutrient cycling of cover crops in upland rice and soybean. Rev Bras Cienc Solo. 35:1787-1800.

Prasad R (2011) Aerobic rice systems. Adv Agron. 111:207–236.

Reynolds CM, Wolf DC, Armbruster JA (1987) Factors related to urea hydrolysis in soils. Soil Sci Soc Am Pro. 49:104-108.

SAS Institute (1999) Procedure guide for personal computers. Version 5. SAS Institute Inc., Cary, NC.

Sivasankar S, Oaks A (1995) Regulation of nitrate reductase during early seedling growth. Plant Physiol. 107(4):1225-1231.

Tabatabai MA, Bremner JM (1972) Assay of urease activity in soils. Soil Biol Biochem. 4:479-487.

Yoshida S (1981) Fundamentals of rice crop science. International Rice Research Institute, Manila.