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NITROGEN ANTICIPATION IN TROPICAL GRASSES AND ^{15}N RECOVERY BY UPLAND RICE

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TÍTULO DA TESE: NITROGEN ANTICIPATION IN TROPICAL GRASSES AND ^{15}N RECOVERY BY UPLAND RICE

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ABSTRACT

Rice is one of the most consumed cereals globally and upland rice production has gained prominence due to its low demand for water resources. Nitrogen is one of the main nutrients influencing rice yield, but despite extensive study, the N soil dynamics in different crop systems are still poorly understood. In this work, the technical and economic feasibility of the early application of nitrogen fertilizer, $(^{15}NH_4)_2SO_4$, for upland rice in grass-rice succession under a no-till system were evaluated. Upland rice was sown in areas previously occupied by *Urochloa brizantha* or *Urochloa ruziziensis*. The experimental design was split-plot in a completely randomized block design with four replications in a 4 x 2 factorial scheme: four nitrogen fertilization management strategies and two grass species. The four nitrogen fertilization management strategies were N fertilization (90 kg N ha^{-1}) applied to the living cover crop 10 days before chemical desiccation (A1), N fertilization (90 kg N ha^{-1}) applied 1 day before rice sowing (A2), N fertilization (90 kg N ha^{-1}) applied at rice tillering (C), and no extra N applied (0 kg N ha^{-1}) (T). Soil physical and chemical characteristics, nitrate and ammonium ratios, soil mineral and organic N, soil total N and C, N in microbial biomass, straw decomposition and N release, rice productivity, N content in grains and aerial part of rice and the *Urochloa* straw, and ^{15}N recovery in the system were analyzed for the two years of the experiment. For these analyses, soil samples were collected at five different times each year: before the beginning of the experiment, at the time of grass desiccation, at rice sowing, at rice tillering, and at rice harvest. Rice yield did not differ among treatments A1, A2, and C. Rice yield was higher in succession to *U. brizantha*. N fertilization in advance resulted in higher N availability to rice during its initial development. Nitrate and ammonium accumulation in the soil were higher when upland rice was sown over *U. brizantha*. N immobilization by the soil microbiota was not consistently increased by N fertilization before rice sowing, but it was influenced by *Urochloa* species. ^{15}N recovery was not affected by *Urochloa* species but was higher in rice plants in treatment C than in treatments A1 and A2.

Keywords: Ammonium. Nitrate. *Oryza sativa. Urochloa brizantha. Urochloa ruziziensis.*

RESUMO

O arroz é um dos cereais mais consumidos no mundo e a sua produção em sistema de terras altas tem ganhado destaque pela baixa demanda hídrica. Nitrogênio é um dos principais nutrientes que influenciam a produtividade do arroz e sua dinâmica no solo em sistemas de produção, embora tenha sido largamente estudada, ainda é pouco compreendida. Neste trabalho foi avaliada a viabilidade técnica e econômica da aplicação antecipada de nitrogênio, $(^{15}NH_4)_2SO_4$, em arroz de terras altas semeado em sistema plantio direto em áreas previamente ocupadas com *Urochloa brizantha* ou *Urochloa ruziziensis.* O delineamento experimental foi de parcelas subdivididas em blocos casualizados, com quatro repetições, em um esquema fatorial (4 x 2): quatro épocas de aplicação do fertilizante nitrogenado e duas espécies de forrageiras. As quatro épocas de aplicação do fertilizante foram: N (90 kg N ha^{-1}) aplicado nas forrageiras 10 dias antes da dessecação (A1); N (90 kg N ha^{-1}) aplicado 1 dia antes da semeadura do arroz (A2); N (90 kg N ha^{-1}) aplicado no perfilhamento do arroz (C); e sem aplicação de N (0 kg N ha^{-1}) (T). Foram analisadas a química e física do solo, nitrato e amônio no solo, N mineral e orgânico no solo, N e C total no solo, N na biomassa microbiana, decomposição da palhada das forrageiras e liberação de N, produtividade do arroz, acúmulo de N nos grãos, na parte vegetativa do arroz e na palhada de *Urochloa* e recuperação de ^{15}N no sistema. As amostras de solo foram coletadas em 5 épocas em cada ano: antes do início do experimento, no momento da na dessecação das forrageiras e na semeadura, no perfilhamento e na colheita do arroz. A produtividade de arroz não diferiu entre os tratamentos A1, A2 e C. A produtividade de arroz foi maior quando semeado em sucessão à *U. brizantha*. A adução antecipada resultou em maior disponibilidade de N para o arroz nos estágios iniciais de desenvolvimento. O acúmulo de nitrato e amônio no solo foi maior quando o arroz foi semeado sobre *U. brizantha*. A imobilização microbiana de N não aumentou com a antecipação da adubação nitrogenada, mas foi influenciada pela espécie de *Urochloa* escolhida. A recuperação de ^{15}N não foi influenciada pela espécie de *Urochloa* mas foi mais alta em plantas de arroz adubadas no perfilhamento.

Palavras-chave: Amônio. Nitrato. *Oryza sativa. Urochloa brizantha. Urochloa ruziziensis.*

SUMÁRIO

GENERAL INTRODUCTION

Rice (*Oryza sativa* L.) is among the most consumed cereals globally and is an essential component of food security for most of the world's population (LOPES et al., 2013; LIU et al., 2016; ZHANG; CHU, 2020). According to the Food and Agriculture Organization (FAO), 2.4 billion people depend on rice as their daily base food, and this number is predicted to double by 2050 (FAO 2017).

Rice can be cultivated in all regions and various environments (BARRETO et al., 2012). Lowland rice, which is produced on flooded lands, accounts for the majority of rice production (PRASAD, 2011; NAYAR, 2014; AMIN et al., 2016; PERON et al., 2019) and requires 15,000 m³ per hectare (BRUINSMA, 2009; SUÁREZ; SÁNCHEZ-ROMÁN, 2016). According to FAO data (FAO, 2020), in 2017 alone, lowland rice production consumed 3 trillion m³ of water globally. In the same year, Brazilian rice production used approximately 22 billion m³ of water (CONAB, 2019). Since part of this water does not return to its original course, the source's availability is reduced (BACK; JUST, 2018).

Upland rice, which is cultivated in aerated soils (DOSSOU-YOVO et al., 2016), is an alternative for sustainable agriculture due to its potential for water savings and superior water use efficiency compared with lowland rice (FENG et al., 2007; PRASAD, 2011; PORTUGAL et al., 2015; NASCENTE; STONE, 2018). Upland rice is grown mainly in Asia, Africa, and Latin America, and Brazil is the largest producer (FAGERIA et al., 2010; NASCENTE; CRUSCIOL; COBUCCI, 2013; CONAB, 2014). Despite its growing importance, upland rice's yield and profit are still lower than those of lowland rice (SANTOS; RABELO, 2008; OLIVEIRA NETO, 2015), resulting in a decrease in upland rice cultivation in recent years (OLIVEIRA NETO, 2015).

One reason for the lower productivity of upland rice compared to lowland rice is drought stress. Water deficits during the flowering and filling phases can significantly reduce productivity (OLIVEIRA NETO, 2015). Water deficits are more pronounced when there is no straw on the soil, which increases the rate of evaporation from the soil surface. Straw can also reduce soil erosion and, consequently, nutrient losses (PACHECO et al., 2011; CARVALHO et al., 2014; HWANG et al. 2017). No-tillage systems (NTS) can be adopted to reduce drought stress and help increase and stabilize upland rice yields (CRUSCIOL; SORATTO; MATEUS, 2006; HEINEMANN; STONE; FAGERIA, 2011; NASCENTE; CRUSCIOL;

COBUCCI, 2013) while also improving the chemical and physical properties of soil (CARNEIRO et al., 2008; CARVALHO et al., 2011; SILVA et al., 2011; PACHECO et al., 2013; ARF et al., 2018; GUIMARÃES et al., 2018).

NTS's key components for improving agricultural sustainability are the maintenance of straw and non-disturbance of the soil (CAIRES et al., 2015). Straw is produced by cover crops planted in winter, and the successive rotation of grain and cover crops provides multiple environmental and ecological benefits over time (HILIMIRE, 2011; RUIS; BLANCO-CANQUI, 2017), such as improved soil quality via nutrient cycling (TORRES; PEREIRA; FABIAN, 2008; MURUNGU et al., 2010; RUIS; BLANCO-CANQUI, 2017) and increased microbial and invertebrate diversity (BALOTA et al. 2014, BOWLES et al. 2017, ISBELL et al. 2017).

The species chosen as the cover crop must have high biomass production capacity and nutrient cycling ability, both inherent characteristics of the genus *Urochloa* (CRUSCIOL et al., 2005; ASSMANN et al., 2017). This genus's grasses are typically used for pasture in Brazil due to their low demand for specific soil and weather conditions (COSTA; OLIVEIRA; FAQUIN, 2006; RODRIGUES et al., 2017). *Urochloa* exhibits great resistance to drought, has higher biomass production and lower seed costs than other cover crops, and can act as a soil conditioner (SILVA et al. 2006, 2008; MORETI et al., 2007; BOER et al., 2008; TORRES; PEREIRA; FABIAN, 2008; TEIXEIRA et al., 2009; FONSECA; MARTUSCELLO, 2010; PACHECO et al., 2011; NASCENTE; CRUSCIOL, 2012; GUIMARÃES et al., 2018). Furthermore, the higher C/N ratio of grasses reduces the decomposition rate, thus prolonging the presence of the residues on the soil (NASCENTE; CRUSCIOL, 2012; PACHECO et al., 2013) and improving soil organic matter (PACHECO et al., 2011; FRANZLUEBBERS; SAWCHIK; TABOADA, 2014; CRUSCIOL et al., 2015).

Urochloa’s vigorous root system, which can explore more than 1 m deep, also contributes to nutrient cycling and increases organic matter by reaching residual fertilizer and increasing soil biological activity (PACHECO et al., 2011; NASCENTE; CRUSCIOL, 2012; GICHANGI; NJARUI; GATHERU, 2017). Some species of the genus *Urochloa* are known to influence soil microorganisms involved in the N cycle, which can lead to changes in N dynamics (REIS et al., 2001; BAPTISTELLA et al., 2020; ROCHA et al., 2020).

According to Pinheiro et al. (2016), NTS production of rice is possible provided that the farmer considers several factors, such as nitrogen application time. Like in many soil-plant systems, nitrogen is one of the most critical nutrients for rice (YOSHIDA, 1981; DUAN et al., 2007; RODRIGUES et al., 2017; LEE; GARLAND; ROSSEL, 2018) and can be the major limiting factor in agricultural practice (ZHANG; CHU, 2020). Nitrogen participates in cell structures, functions, and enzymatic reactions and is part of the chlorophyll molecule involved in photosynthesis (FAGERIA, 2014). In rice, N fertilization enhances plant growth, improves the number of tillers and panicles per area, and increases panicle and grain size (LOPES et al., 2013; LACERDA; NASCENTE, 2016).

However, soil N content can be uncertain and highly variable due to its multiple reaction pathways (BUTTERBACH-BAHL et al., 2013). Ammonification, nitrification, denitrification, and microbial immobilization are some of the processes that affect the availability of inorganic forms of N in the soil and whether N is taken up by plants or lost to the environment (SAVANT; DATTA, 1982; REDDY; PATRICK, 1986; KATYAL; CARTER; VLEK, 1988; REDDY; PATRICK; LINDAU, 1989; LEE; GARLAND; ROSSEL, 2018).

To reduce N losses, the recommendation for upland rice is to apply a small amount of N at sowing and a more significant amount at tillering, when the absorption rate is greater (ALVAREZ et al., 2005; TADESSE; TADESSE, 2019). However, for upland rice in NTS, the high biomass apport over the years could reduce the importance of N losses compared with rice cultivated in plowed and organic matter depleted soils (PINHEIRO et al., 2016), making N anticipation possible. Moreover, soil N is highly influenced by the straw (CRUSCIOL et al., 2005, 2015; NASCENTE; LANNA, 2016). When there is a considerable input of biomass over the soil surface, the extra C and N act as an impulse for microbial activity and, consequently, increase N uptake by microbial cells (KUZYAKOV, 2010; NASCENTE; STONE, 2018).

Soil inorganic N is mostly composed of nitrate and ammonium (NASCENTE; LANNA, 2016; HACHIYA; SAKAKIBARA, 2017; BEECKMAN; MOTTE; BEECKMAN, 2018). In lowland flooded environments, ammonium is the dominant form, while nitrate is frequently present in aerated upland environments (MILLER; CRAMER, 2004). Several studies have demonstrated that rice growth and production are improved when both inorganic forms of N (nitrate and ammonium) are available to plants (BRITTO; KRONZUCKER, 2002; LARK et al., 2004; MILLER; CRAMER, 2004; GUO et al., 2007; HACHIYA; SAKAKIBARA, 2017). When only one form of nitrogen is present, rice tends to grow more slowly and present N deficiency symptoms (MALAVOLTA, 1980; ARAÚJO et al., 2012).

Ammonium is used to synthesize glutamine and other amino acids without first requiring transformation (BEECKMAN; MOTTE; BEECKMAN, 2018). By contrast, nitrate must be transformed to ammonium before it can be used (SALSAC et al., 1987), which requires eight moles of electrons per mole of nitrate (HACHIYA; SAKAKIBARA, 2017). As such, ammonium is more efficient, results in higher chlorophyll content, and improves all crop parameters (WILLIAMS et al., 1987; SATO; YANAGISAWA, 2014), including root branching, which increases the surface area of nutrient uptake (LIMA et al., 2010; PATTERSON et al., 2010; XUAN; BEECKMAN; XU, 2017). However, nitrate also increases rice yield (HU et al., 2015; FAN et al., 2016; WANG et al., 2018) by stimulating leaf expansion (WALCH-LIU et al., 2000; RAHAYU et al., 2005), floral induction (CASTRO MARÍN et al., 2011), and root morphology (ZHANG et al., 1999; REMANS et al., 2006). Thus, nitrate not only facilitates nutrient uptake but also affects different signaling pathways (O'BRIEN et al., 2016). The advantages of both N forms for plant growth reflect not only the sum of their benefits but also the compensation of their detrimental effects.

Although both forms of N are essential for growth and production, rice initially uses ammonium as the primary source, while nitrate is absorbed and metabolized in small amounts in the initial stages (LIN et al., 2005; LI et al., 2006) or stored in root and leaf cells until later (NASCENTE; LANNA, 2016) given the low activity of the enzyme nitrate reductase (NR) before the tillering stage (ALI; SIVAKAMI; RAGHURAM, 2007; ARAÚJO et al., 2012).

N uptake by upland rice is affected by several factors, such as nitrate and ammonium ratios, soil and weather conditions, and water and light availability (NASCENTE; LANNA, 2016). All these factors are directly influenced by straw, the cover crop, the time of N application, and soil N dynamics. So, studies that focus on the system and not only the crop are essential for expanding available knowledge. Accordingly, this work aimed to evaluate the technical and economic feasibility of early application of nitrogen fertilizer, $(^{15}NH_4)_2SO_4$, for upland rice in succession to *Urochloa brizantha* or *Urochloa ruziziensis* in an NTS.

CHAPTER 1 - THE EFFECTS OF PREPLANTING N SUPPLY AND TROPICAL GRASSES ON ^{15}N RECOVERY BY UPLAND RICE

ABSTRACT – The importance of upland rice for sustainable agriculture is growing, underscoring the need for guidance on appropriate nitrogen management in no-tillage systems (NTS). To provide a reference on N dynamics in upland rice plants and soil, the objective of this work was to study the technical feasibility of N fertilization before upland rice sowing in an NTS and N fate in the system. Split plots arranged in a completely randomized block design with four replicates were employed in two consecutive years. The treatments consisted of upland rice grown over *Urochloa brizantha* or *Urochloa ruziziensis* straw with N fertilization (90 kg N ha^{-1}) applied to the living cover crop 10 days before desiccation (A1), N fertilization (90 kg N ha^{-1}) applied 1 day before rice sowing (A2), N fertilization (90 kg N ha^{-1}) applied at rice tillering (C), or no extra N applied (0 kg N ha^{-1}) (T). All treatments received 30 kg ha^{-1} of N at rice sowing. The followed variables were measured: rice yield; N in the rice shoot tissue at harvest; N in the rice grains; total N accumulated at harvest; total ^{15}N absorbed from the soil; N in the *Urochloa* straw at rice harvest; mineral N, organic N and total N in the soil; ^{15}N recovery in soil microbial biomass, in rice grain and shoot tissues, in *Urochloa* straw, and in the soil; and total ^{15}N recovery. Rice yield did not differ among treatments A1, A2, and C. Rice yield was higher in succession to *Urochloa brizantha* than in succession to *U. ruziziensis* in the first year. N immobilization by the soil microbiota was not consistently increased by N fertilization before rice sowing but was influenced by *Urochloa* species. ^{15}N recovery was not affected by *Urochloa* species but was higher in rice plants in treatment C than in treatments A1 and A2.

Keywords: Nitrogen. No-tillage system. *Urochloa brizantha*. *Urochloa ruziziensis*.

1.1 INTRODUCTION

Nitrogen (N) is the most yield-limiting nutrient for upland rice (FAGERIA; MOREIRA; COELHO, 2011; TAYLARAN; ELMUNDO; GONZAGA, 2018), and correct N fertilization can be as important as choosing the right rice genotype (SIKUKU et al., 2016). Nitrogen deficiency in upland rice can be attributed to low soil organic matter, improper N management (TAYLARAN; ELMUNDO; GONZAGA, 2018), and losses due to leaching, volatilization, erosion, and microbial immobilization (FAGERIA; BALIGAR, 2005; FAGERIA; MORAIS; SANTOS, 2010).

To reduce N losses, N fertilization is typically applied when the plant most needs the nutrient. The recommendation for upland rice is to apply a small amount of N at sowing and a more generous amount at tillering (ALVAREZ et al., 2005; TADESSE; TADESSE, 2019). This approach is based on the premise that N losses in the soil are significant enough to reduce rice yield if the fertilizer was applied in advance. However, for upland rice grown in no-tillage systems (NTS), the high biomass apport over the years could reduce the importance of N losses compared with rice grown in a plowed and organic matter depleted soil (PINHEIRO et al., 2016). In NTS, microbial immobilization maintain N for a longer time in organic form, preventing it from being lost (ZAMAN et al. 2002, SILVAN et al. 2003, COSER et al. 2007; NASCENTE; LANNA, 2016).

Several studies in NTS have shown that when there is straw over the soil, N fertilization before upland rice sowing is possible and equally practical as split fertilization in guaranteeing upland rice yield (MARZARI et al. 2005; HERNANDES et al., 2010; NASCENTE et al., 2011; LOPES et al., 2013; ARF et al., 2015, NASCENTE; LANNA, 2016; PINHEIRO et al., 2016). N fertilization before sowing is effective in NTS with soils with high percentages of clay and organic matter (NASCENTE et al., 2012; LOPES et al., 2013) because the addition of biomass in an already enriched organic matter environment increases the release of soil-derived C and N compared to soil without biomass additions, a phenomenon called the priming effect (PE) (DALENBERG; JAGER, 1989; KUZYAKOV; FRIEDEL; STAHR, 2000). PE was first described in 1926 when Löhnis observed intense N mineralization from humus when fresh organic residues were added to the soil (LÖHNIS, 1926).

In agricultural systems, there are two kinds of organic inputs into the soil (KUZYAKOV, 2010): occasional and continuous inputs. Occasional inputs include decomposition of straw, breakdown of microbial and root cells, and root exudates. Continuous inputs arise from the slow decomposition of straw and roots over longer periods. Because of the availability of soluble organic molecules, occasional inputs create hotspots of microbial activity with much higher turnover rates than in the rest of the soil (KUZYAKOV, 2010). Shortly after, the availability of organics ends, the microbial community is recycled, and the N stored is made available to absorption by plants (HODGE; ROBISON; FITTER, 2000; SCHIMEL; BENNETT, 2004; HARRISON; BOL; BARDGETT, 2007) or consumed by other microorganisms. Continuous inputs, on the other hand, induce the generation of an array of extracellular enzymes that are more efficient at decomposing soil organic matter than the enzymes used to break down occasional inputs (FONTAINE et al., 2004), resulting in higher mineralization of N.

The cover crop root system can alter the ratio between occasional and continuous inputs in the soil and, consequently, the kind of impulse the microbial community will receive. The continuous movement of root tips in species with high root density species, such as those of the genus *Urochloa*, creates a significant number of hotspots that can merge to form large zones of high activity (KUZYAKOV, 2010), accelerating SOM decomposition and nutrient release (BLAGODATSKAYA et al., 2009; CHENG, 2009). Priming effect size is directly related to the amount of organic residues (DUMONTET; PARENTE; COPPOLA, 1985; ASMAR; EILAND; NIELSEN, 1994) or mineral N (JENKINSON; FOX; RAYNER, 1985), but whether the addition of both creates a new whole soil environment that has been poorly studied.

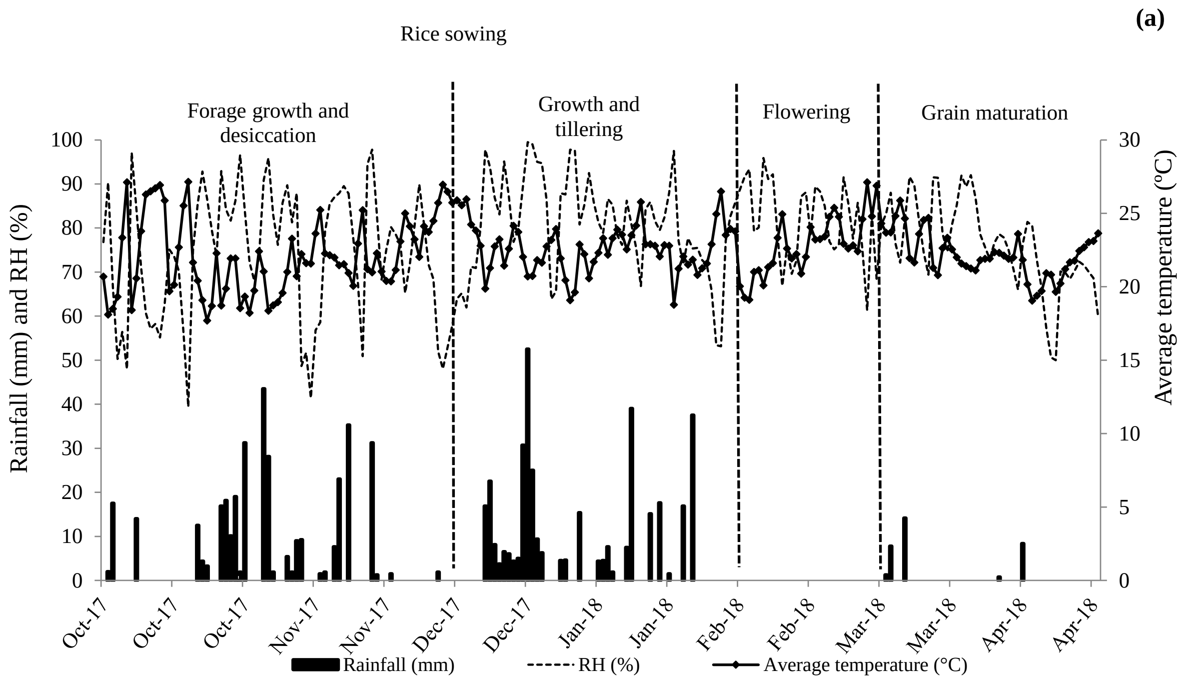
There is a gap in knowledge regarding N's fate when N fertilizer is applied in advance of sowing in NTS. NTS are complex systems that include not only the main crop (upland rice) but also crop rotation, soil microbiota, cover crops, and high amounts of straw on the soil surface. As plants in crop rotation systems share the same space over time, they influence one another through their effects on the soil's chemical, physical, and biological characteristics (CARNEIRO et al., 2008; SILVA et al., 2011; PACHECO et al., 2013). N dynamics depend on many factors, so an overview of the influence of cover crops and N fertilization timing on the system is necessary to understand N’s fate in the soil and rice plants (PACHECO et al., 2013).

To contribute to knowledge in this context, this work aimed to study the technical viability of advance N fertilization of upland rice sown over *U. brizantha* or *U. ruziziensis* straw and N fertilization effects and grass species on soil N and N recovery by rice plants. The following hypotheses guided the study: *(i)* Soil nitrogen availability during upland rice grown varies depending on the *Urochloa* species. *(ii)* Anticipated N fertilization results in higher N microbial immobilization compared with N fertilization at upland rice tillering. *(iii)* Anticipated N fertilization increases rice utilization of other N sources in the soil due to the increase in N microbial immobilization.

1.2 MATERIALS AND METHODS

This work consisted of a field experiment carried out during the 2017–2018 and 2018–2019 growing seasons at Lageado Experimental Farm of São Paulo State University in Botucatu, São Paulo State, Brazil (48º26′W, 22º51′S, 786 m.a.s.l.). The soil is a clayey Ferralsol (FAO, 2014) with 602, 117, and 281 g kg^{-1} of clay, sand, and silt, respectively.

According to Köeppen's classification (ALVARES et al., 2013), the climate is Cwa, a tropical latitude with a dry winter and a hot, wet summer. The average temperature is 20.7ºC, with a maximum of 26.1ºC and a minimum of 15.3ºC (Figure 1). The average annual rainfall is approximately 1360 mm.



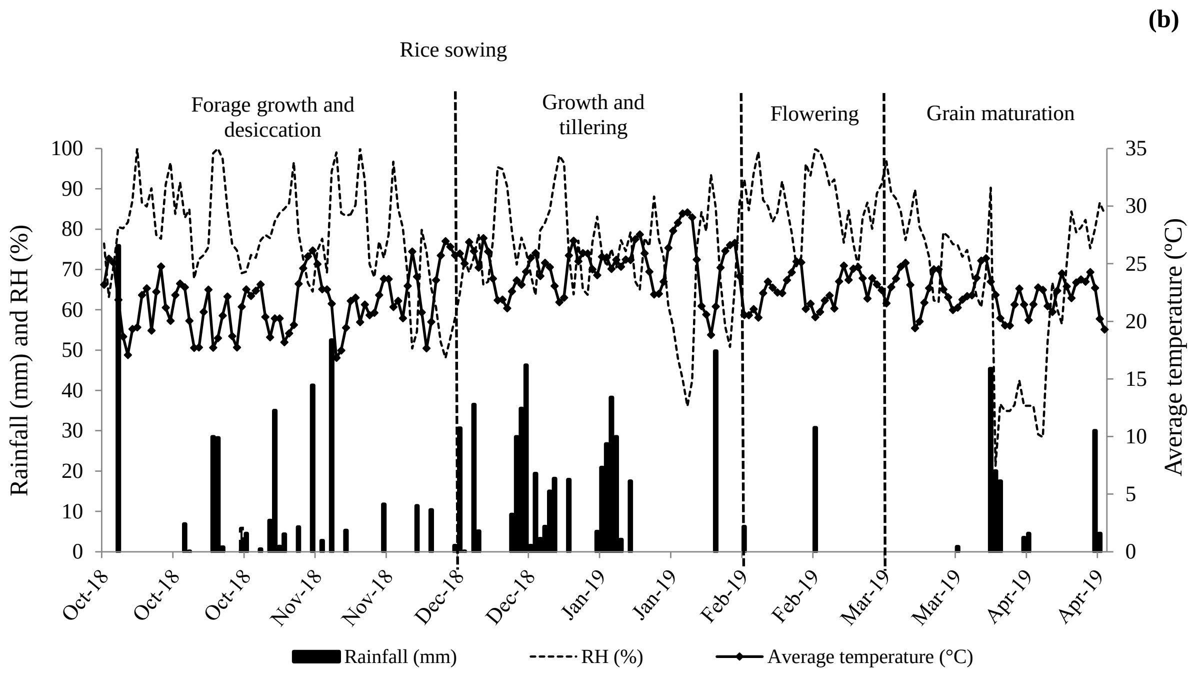


Figure 1. Weather data during the 2017–2018 (a) and 2018–2019 (b) growing seasons.

Treatments and experimental design

Split plots arranged in a completely randomized block design using two cover crops and four N application times with four replicates were employed in both seasons. The experimental plots consisted of 8-m-long rows spaced 0.34 m apart. The treatments were upland rice grown on *Urochloa brizantha* cv. Marandu or *Urochloa ruziziensis* straw with N fertilization *(90 kg N ha^{-1}),* applied in the living cover crop 10 days before desiccation (A1), N fertilization (90 kg N ha^{-1}) applied 1 day before rice sowing (A2), N fertilization (90 kg N ha^{-1}) applied at the rice tillering phase as recommended for rice (RAIJ et al., 1996; ALVAREZ et al., 2005) (C), and a control treatment (no extra N applied) (T). All treatments received 30 kg ha^{-1} of N at seeding, including in treatment T. N fertilization was carried out with ammonium sulfate ($(NH_4)_2SO_4$) in the plot and enriched ammonium sulfate ($(^{15}NH_4)_2SO_4$) in micro plots placed in the center of the plots in order to track N fate.

Field experiment conduction

To reduce differences between seasons and the effect of N applied in 2017/2018 on plants in the 2018/2019 growing season, the experiment was conducted in two adjacent areas occupied by fully grown *U. brizantha* or *U. ruziziensis*.

Before rice sowing, chemical and physical characterizations of the soil were carried out at depths of 0.0 to 0.05, 0.05 to 0.10, 0.10 to 0.20, and 0.20 to 0.40 m. Samples were collected at four random points per plot to characterize soil fertility (RAIJ et al., 2001) and at four random points per block to determine soil density and porosity (KLUTE, 1986). After the rice harvest, the chemical characterization of the soil was repeated following the same methodology. The data are presented in Tables 1, 2, and 3 of the appendix.

We selected two adjacent areas occupied by *U. brizantha* and *U. ruziziensis* for two consecutive growing seasons (2015/2016 and 2016/2017). To reduce differences between the replicates as much as possible, these areas were divided into two equal parts; one was cultivated with rice in the 2017/2018 growing season, and the other was cultivated with rice in the 2018/2019 growing season. The area cultivated with rice in the second year was left with grasses without management during the first year. At the beginning of November, the grasses were desiccated to produce straw for sowing rice under NTS. The rice (IAC 203) was mechanically sown thirty days after grass desiccation using a seeder-fertilizer machine for no-till management.

The seeds were placed at a depth of 3 cm and a sufficient seed density to obtain 150 plants m^{-2}. Fertilization at sowing was conducted according to the recommendation for the crop (RAIJ et al., 1996) using 100 kg ha^{-1} of triple superphosphate (41% P_2O_5), 35 kg ha^{-1} of potassium chloride (60% K_2O), and 140 kg ha^{-1} of ammonium sulfate (21% N). Chemical control with herbicides and fungicides was carried out as for commercial cropping. Ammonium sulfate, enriched or not with ^{15}N, was applied manually in the micro plot and the plot according to the management time of the specific treatment. The rice was harvested manually in April 2018 and 2019, when the upper 2/3 grains of the panicles were hard, and the lower third was semi-hard.

Soil and plant sampling and analysis

The rice yield (kg ha^{-1}) was measured by weighing the husk grains harvested from the plot and adjusted to 13% moisture. Whole aboveground plant samples were collected at harvest by clipping ten plants at ground level. Panicles were removed and measured separately from aboveground vegetative matter. Plant materials were dried at 60ºC until a constant mass was reached.

Plant samples were ground to pass through a 1-mm sieve, and the N concentration in the grain and vegetative material was measured using the methodology of Malavolta, Vitti, and Oliveira (1997). The total N absorbed was calculated from the sum of the N in the grains and shoots. The amount of N from the soil was calculated from the difference between the total amount absorbed and the amount from fertilizer measured by the ^{15}N enrichment technique (TRIVELIN et al., 1994; OLIVEIRA et al., 2008).

To determine the rate of decomposition and the rate of N release from straw, samples were collected with the help of a quadrat (0.25 m^2) at three random points in the plot at 0, 30, 60, 90, and 120 days after desiccation. The straw was dried at 60ºC until reaching constant mass and ground to pass through a 1-mm sieve for dedesiccation of nitrogen levels according to the methodology described by Malavolta, Vitti, and Oliveira (1997).

To determine the amount of mineral N, organic N, and total N in the soil, soil samples were collected in the 0.0–0.40 m layer at four random points per plot in the rice tillering phase, when rice absorbs the highest amount of nitrogen (ALVAREZ et al., 2005). Total N was determined according to the methodology of Camargo et al. (2009). Mineral N was calculated as the sum of the levels of nitrate and ammonium in the soil determined by colorimetry using the sodium salicylate method and reduction with vanadium chloride (MULVANEY, 1996; MIRANDA; ESPEY; WINK, 2001). Organic N was calculated from the difference between total N and mineral N.

The total N content and the abundance of ^{15}N (% of ^{15}N atoms) were determined using a mass spectrometer (Hydra 20-20, Sercon Ltd., Crewe, UK) coupled to an automatic N analyzer (ANCA-GSL, Sercon Ltd., Crewe, UK) at the Laboratory of Stable Isotopes of the Center for Nuclear Energy in Agriculture (CENA/USP) according to the methodology suggested by Trivelin et al. (1994) and Oliveira et al. (2008). The total N content and the abundance of ^{15}N were used to calculate ^{15}N recovery in rice plants, grains, *Urochloa* straw, soil, and soil microbial mass. For this purpose, samples of rice plants and grains, remaining straw, and soil (0.0 to 0.40 m) at harvest and of the microbial mass in the soil in the 0.0–0.10 m layer at rice tillering were collected.

Statistical analysis

Means were compared using Fisher's protected least significant difference (LSD) test. The main factor and interaction effects were considered statistically significant at $P < 0.10$. The straw decomposition and nutrient release data were analyzed by polynomial regression. Statistical analyses were performed using SISVAR® software (FERREIRA, 2014).

1.3 RESULTS AND DISCUSSION

Climatic data

In the 2017/2018 growing season, total rainfall was 762 mm between October 2017 and April 2018. During the rice phases of growth and tillering (between December and February), flowering (between February and March), and maturation (March and April), the accumulated rainfall was 324, 54, and 32 mm, respectively.

In the 2018/2019 growing season, the total rainfall was 970 mm between October 2018 and April 2019, and during the rice growth, flowering, and maturation phases, the accumulated rainfall was 436, 87, and 127 mm, respectively.

Rice yield

In the first year, all treatments that received N presented superior yields (2,689 – 2,816 kg ha^{-1}) compared with the national average for upland rice (2,400 kg ha^{-1}) (CONAB, 2019), except treatment A2 over *U. ruziziensis* straw (Figure 2). When no extra N was applied (T), yields (1,630 kg ha^{-1}) were below the national average in the first year. However, in the second year, all treatments produced (2,686 – 3,272 kg ha^{-1}) more than the national average (2,300 kg ha^{-1}) (CONAB, 2019).

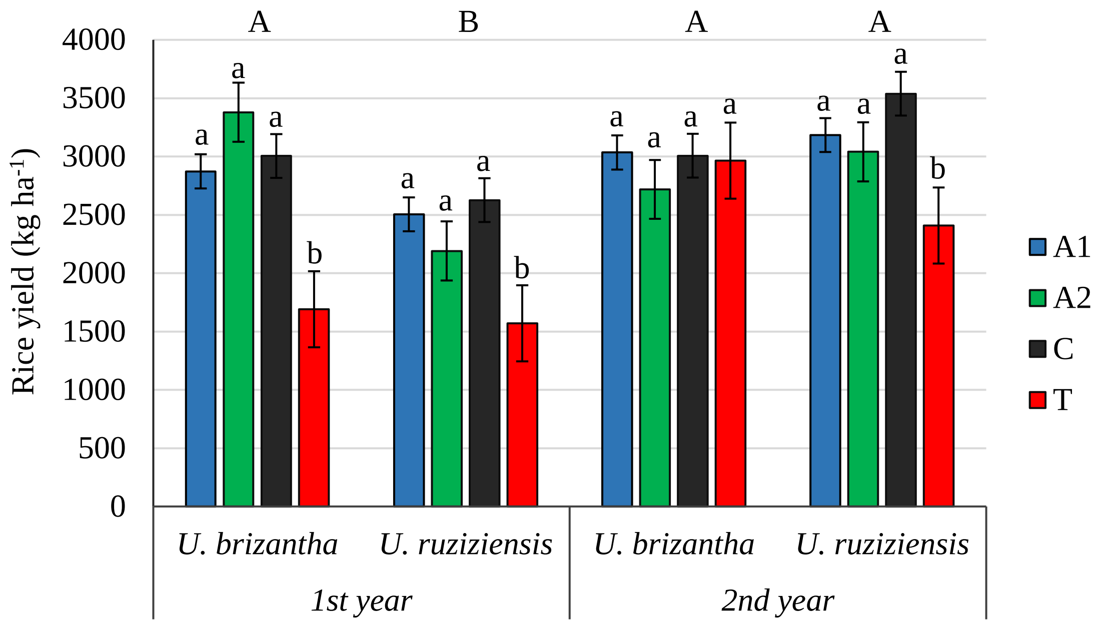


Figure 2. Rice yield under *U. brizantha* and *U. ruziziensis* straw and different timings of N fertilization. Different lowercase letters indicate significant differences between treatments within the same grass, and different uppercase letters indicate significant differences between grasses within the same year by the LSD test at 10% probability ($P < 0.10$). A1: 90 kg N ha^{-1} applied to grass; A2: 90 kg N ha^{-1} applied at rice sowing; C: 90 kg N ha^{-1} applied at rice tillering; T: 0 kg N ha^{-1}.

In the first year, there was no significant difference in yield between the treatments with N fertilization applied in advance of the recommended split application (A1 and A2) and N fertilization at tillering (C) for both grasses (Figure 2). The yield was lower in the treatment without nitrogen fertilization (T). In the second year, the same results were obtained for the area previously occupied by *U.*

ruziziensis. By contrast, in the area formerly occupied by *U. brizantha*, no significant differences in yield were observed between the treatments that received N (A1, A2, and C) and the treatment without N fertilization (T).

This difference in yield between years for rice sown over *U. brizantha* straw can be explained, in part, by differences in climatic conditions. Due to the higher rainfalls during the growth and maturation phases in the second growing season, initial rice development and grain filling were greater in the second year than the previous one, even in treatment T. The increase in soil moisture due to higher rainfalls likely increased water absorption in the superficial layers and reduced the need for deep root growth, which depends on an extra supply of N. In addition, the increase in soil moisture could favore the decomposition of both *U. brizantha* straw and roots, and N mineralization.

In the first year, the yield of rice sown over *U. brizantha* straw was higher (2,737 kg ha^{-1}) than that of rice sown over *U. ruziziensis* straw (2,223 kg ha^{-1}). In the second year, no significant difference in rice yield was observed between the grasses. Nascente, Crusciol, and Cobucci (2012) also observed that rice yield did not differ between sowing over *U. brizantha* or *U. ruziziensis* straw. The difference in rice yield between grasses in the first year may reflect changes in the physical, chemical, and biological characteristics of the soil caused by the different species. Tanaka et al. (2019) found that *U. brizantha* exhibited higher biomass production and accumulated more nutrients in its straw than *U. ruziziensis*, even when N was not supplied. When N is added, *U. brizantha* is more N-use efficient than *U. ruziziensis* (ALVIM et al. 1990).

As the yield of rice was lower in the control treatment (T) than in the other treatments, we can assume that the available N present in the soil was not sufficient to supply the rice demand for this nutrient. In all treatments, 30 kg N ha^{-1} was added at sowing, indicating that the difference in yield between the control (T) and the other treatments (A1, A2, and C) was due to the application of N in advance or during tillering.

According to Fageria (2014), high amounts of available N at sowing enable early closure of the canopy by stimulating initial growth and reducing weed infestation, which is one of the most important challenges in upland rice production. An increase in initial N levels in rice significantly enhances net photosynthesis (SIKUKU et al., 2016) by increasing chlorophyll content and Rubisco activity (TOTH et al., 2002). Greater net photosynthesis stimulates tillering and shoot and root growth. Rice yield is influenced by the number of panicles per m² (MILLER; HILL; ROBERTS, 1991; FAGERIA, 2007), which is higher when tillering during initial growth is increased (POLETTO et al., 2011), and 60% of panicles per m² are from the first five tillers (WU; WILSON; McCLUNG, 1998). In addition, enhancing root growth in the initial stages helps prevent the rice from mild drought stress that could occur. Even in well-watered conditions, N leaf increases as rice roots are deeper (ARAI-SANOH et al., 2014).

Combining the high biomass production of *Urochloa* straw with the addition of 90 kg N ha^{-1} may have reduced the potential yield of treatment C by immobilizing part of the fertilizer in microbial cells. As PE can be initiated by an extra supply of mineral N (JENKINSON; FOX; RAYNER, 1985; LEON et al., 1995; KUZYAKOV; FRIEDEL; STAHR, 2000), N fertilization causes an increase in microbial activity, which can ultimately increase the microbial immobilization ratio. Since PE takes time to occur (FONTAINE et al., 2004; BLAGODATSKY et al., 2010), N added at tillering in NTS might be immediately unavailable for plants, as the microorganisms use this N to degrade the straw (FAGERIA; MOREIRA; COELHO, 2011; FAGERIA, 2014; PINHEIRO et al., 2016).

Nitrogen fate

The nitrogen accumulated in shoots (N_1) in the first year was influenced neither by treatment nor by grass species (Table 1). In the second year, there were differences between N treatments and grasses; N_1 was lowest in the *U. ruziziensis* area in the control treatment (T) (27 kg N ha^{-1}) but had an average value of 42 kg N ha^{-1} in the other plots. A large fraction of the N in shoot tissue is conserved and cycled for use in growing tissues or grain filling (ISHIZUKA, 1964; MAE; HOSHINO; OHIRA, 1985). Consequently, the N in rice shoot tissue at harvest represents the amount of N that was not translocated during the grain filling stage.

Table 1. N in rice shoot tissue at harvest (N_1), N in rice grains (N_2), total N accumulated at harvest ($Nt = N_1 + N_2$), total N absorbed from the soil (Ns), and remaining N in the straw (Nr), in kg ha^{-1}.

	N_1		N_2		Nt		Ns		Nr	
	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
Treatment (N)										
A1	10	39a	35a	48a	44a	87a	34a	77	120a	141a
A2	12	40a	33a	46a	45a	87a	31a	71	80c	137ab
C	12	47a	35a	53a	47a	100a	13c	66	93b	131b
T	9	27b	18b	34b	27b	61b	27b	61	87bc	119c
Grass (U)										
U. brizantha	11	45a	34a	46	45a	91a	32a	79a	87b	181a
U. ruziziensis	11	32b	26b	44	37b	76b	20b	59b	103a	83b
						P>F				
N	0.5977	0.0647	0.0046	0.0104	0.0127	0.0164	0.0001	0.2785	0.0005	0.0062
U	0.9122	0.0102	0.0013	0.3221	0.0586	0.0500	0.0001	0.0066	0.0009	0.0001
U x F	0.4820	0.2465	0.0629	0.0843	0.7224	0.2742	0.0043	0.2537	0.0006	0.5681

Means followed by different letters are significantly different by the LSD test at 10% probability. A1: 90 kg N ha^{-1} applied to grass; A2: 90 kg N ha^{-1} applied at rice sowing; C: 90 kg N ha^{-1} applied at rice tillering; T: 0 kg N ha^{-1}.

In both years, N accumulation in grains (N_2) was lowest in the control (T) and did not differ significantly among the other treatments. In the first year, N_2 was higher in the plots sown over *U. brizantha* straw (34 kg N ha^{-1}) than in those sown over *U. ruziziensis* straw (26 kg N ha^{-1}). In the second year, no difference in N_2 was observed between the grasses. According to Sheehy et al. (2004a), the grains receive approximately 32% of all N absorbed by the plants in the early stages. Consequently, a higher N absorption by rice in the initial phase could result in the higher N in the grains of rice sown over *U. brizantha*.

Total accumulated N (Nt) and N absorbed from soil (Ns) were respectively 17% and 31% higher in the *U. brizantha* areas than in the *U. ruziziensis* areas in the first and second years. Ns indicates the amount of N absorbed from other soil sources than the fertilizer. In the first year, Ns was highest in the treatments with advanced N fertilization (A1 and A2) (33 kg N ha^{-1}, on average) and lowest in treatment C (13 kg N ha^{-1}), indicating that rice absorbed the greatest amount of N from fertilizer when N was applied at tillering. In treatments A1 and A2, rice yield was not significantly different from that in treatment C treatment, even though the amount of N absorbed from fertilizer differed. This result can be explained by PE, as microbes immobilized the N from the fertilizer and made N available from other sources (BLAGODATSKAYA et al., 2009; CHENG, 2009).

The higher values of Nt in the second year are due to the greater amount of N in the vegetative part of the rice, which can be explained by the greater precipitation in the second year. The increased rainfall during the rice cycle allowed greater N absorption and, in turn, more generous storage of this nutrient and biomass by the plants. Teronpi and Bharali (2019) observed reductions by up to 32% in the total N uptake and N use efficiency in rice under water stress compared with irrigated rice. The vegetative and reproductive development of rice can also be harmed by water stress (TERRA et al., 2015, ALOU et al., 2018) due to both the lack of water and lower N acquisition. The resulting morphological and physiological changes lead to more insufficient energetic reserves (AWASTHI et al., 2014; ZHOU et al., 2017). The greater Nt in the second year might explain the higher yield compared with the first year.

Nr represents the amount of N still in the straw at harvest. Nr was higher in treatment A1, the only treatment in which N was applied to living grass, in the first year, and in treatments A1 and A2 in the second year. Tanaka et al. (2019) observed a higher growth rate and shoot biomass production in *Urochloa* when N was applied before desiccation. N availability in the soil during grass growth plus higher biomass production guarantees higher N in the straw. Moreover, Nr was higher in *U. ruziziensis* straw than *U. brizantha* straw, which explains the higher yield of rice sown over *U. brizantha* straw in the first year. Tanaka et al. (2019) also observed that *U. brizantha* produced more biomass than *U. ruziziensis*, but *U. ruziziensis* presented higher remaining biomass at the end of the subsequent crop cycle.

Although both grasses exhibited similar straw decomposition (Figure 1, Appendix), in the first year N release was higher in *U. brizantha* straw than in *U. ruziziensis* straw (Figure 2, appendix), resulting in more significant N mineralization and availability for rice plants. In the second year, Nr was higher in *U. brizantha* straw than in *U. ruziziensis* straw. The decomposition ratio and N release rate were equal for both grasses (Figures 1 and 2, appendix), indicating that *U. brizantha* absorbed more N than *U. ruziziensis* in the second year.

The high values of Nr, which ranged between 80 and 140 kg ha^{-1}, illustrate the remarkable capacity of grasses of the genus *Urochloa* for retaining N in the system. Since N is quickly lost to the environment, adding *Urochloa* as a cover crop can increase the nitrogen use efficiency (NUE) by changing the timing of N availability (ASSMANN et al., 2017). *Urochloa*'s root system can explore more than 1 m deep, reaching residual fertilizer and increasing soil biological activity (PACHECO et al., 2011). In addition, the presence of straw with high N content contributes to PE. According to Kuzyakov (2010), even when the substrate is exhausted, some microorganisms remain active, and extracellular enzymes remaining in the soil can contribute to soil organic matter decomposition. The slow release of C from straw and roots decomposition over time can help maximize these benefits by maintaining nutrients in organic form (ASSMANN et al., 2017).

Even in the absence of applied N, *U. brizantha* and *U. ruziziensis* can produce up to 6.3–11.4 Mg ha^{-1} and 6.7–8.6 Mg ha^{-1} of straw, respectively (CRUSCIOL; SORATTO 2007; MENEZES et al., 2009; SIMIDU et al., 2010; PACHECO et al., 2011, 2013; TANAKA et al., 2019). This high straw production and the associated N content provide highs inputs of N to the soil during the rice cycle (D'ANDRÉA et al., 2004; BOER et al., 2007; PACHECO et al., 2011; NASCENTE; CRUSCIOL; COBUCCI, 2012). N mineralization from straw starts with N-containing soil polymers being depolymerized into organic N monomers such as amino acids and followed by ammonification (SCHIMEL; BENNETT, 2004). According to Nascente, Crusciol, and Cobucci (2012), upland rice can benefit from cover crops that provide more massive amounts of ammonium during early development. In this case, because of the slow decomposition of *Urochloa* straw, ammonium is continuously available to rice.

Soil nitrogen pools

There were no differences between the treatments in any of the N pools evaluated in the soil regardless of the year (Table 2). However, in all years, there were differences between grasses. In both years, the values of mineral N, organic N, and total N were highest in the *U. brizantha* areas and lowest in the *U. ruziziensis* areas. Overall, the differences in N pools between the grasses ranged from 16 kg N ha^{-1} for mineral N to 644 kg N ha^{-1} for total N. These results confirm our first hypothesis: the N available in the soil for the subsequent crop depends on the *Urochloa* species. As noted previously, the higher shoot and root biomass production of *U. brizantha* (TANAKA et al., 2019, GALDOS et al., 2020) allow this species to explore the soil further and absorb more N. In addition, the decomposition of this increased biomass leads to more hotspots in the soil in which PE can occur.

Table 2. Mineral N, organic N, and total N in the soil at rice tillering (0.0–0.4 m layer) in kg ha^{-1}.

	Mineral N		Organic N		Total N	
	1st year	2nd year	1st year	2nd year	1st year	2nd year
Treatment (N)						
A1	57	57	6,997	6,273	7,054	6,331
A2	53	56	7,013	6,366	7,067	6,421
C	60	61	6,921	6,480	6,981	6,540
T	48	61	7,066	6,305	7,113	6,367
Grass (U)						
U. brizantha	65a	67a	7,311a	6,534a	7,376a	6,601a
U. ruziziensis	45b	51b	6,687b	6,178b	6,732b	6,229b
			P>F			
N	0.4069	0.5206	0.9109	0.7731	0.9274	0.7650
U	0.0028	0.0004	0.0018	0.0403	0.0014	0.0326
U x F	0.5224	0.9952	0.9713	0.5096	0.9726	0.5033

Means followed by different letters are significantly different according to the LSD test at 10% probability. A1: 90 kg N ha^{-1} applied to grass; A2: 90 kg N ha^{-1} applied at rice sowing; C: 90 kg N ha^{-1} applied at rice tillering; T: 0 kg N ha^{-1}.

Interestingly, rice yield varied among the treatments even though soil mineral N at tillering did not differ. Although the demand for N by rice is most significant at tillering (ALVAREZ et al., 2005; TADESSE; TADESSE, 2019), the requirement of the developing panicle for N is higher than the rate at which roots acquire it during the grain filling period (SHEEHY et al., 1998; ALVAREZ et al., 2005). According to Sheehy et al. (1998, 2004b), 50% of N in the crop at maturity is acquired by the plant before reaching 25% of its final biomass. Consequently, mineral N availability in the soil should be considered at all rice phases, especially the early stages.

The lack of difference in soil mineral N between treatment T, which did not receive broadcast N fertilizer, and the other treatments can be explained by microbial immobilization of N. Mineral N from fertilizer can be consumed by soil microorganisms and transformed into organic form (NASCENTE; LANNA, 2016) during the interval between N fertilization and soil sampling at tillering. Consequently, the amount of N added as fertilizer would be diluted by the much larger pool of organic N. Nascente et al. (2011, 2013) recommend applying a more considerable amount of N at sowing of rice in NTS to avoid a lack of this nutrient due to bacterial N fixation.

^{15}N-fertilizer fate

In both years, the amount of N in the microbial biomass was highest in treatment A1, although treatments C and A1 were equal in the second year (Figure 30). In the first year, the amount of N immobilized in microbial biomass was higher in the *U. brizantha* than in the *U. ruziziensis* area. In the second year, the opposite pattern was observed.

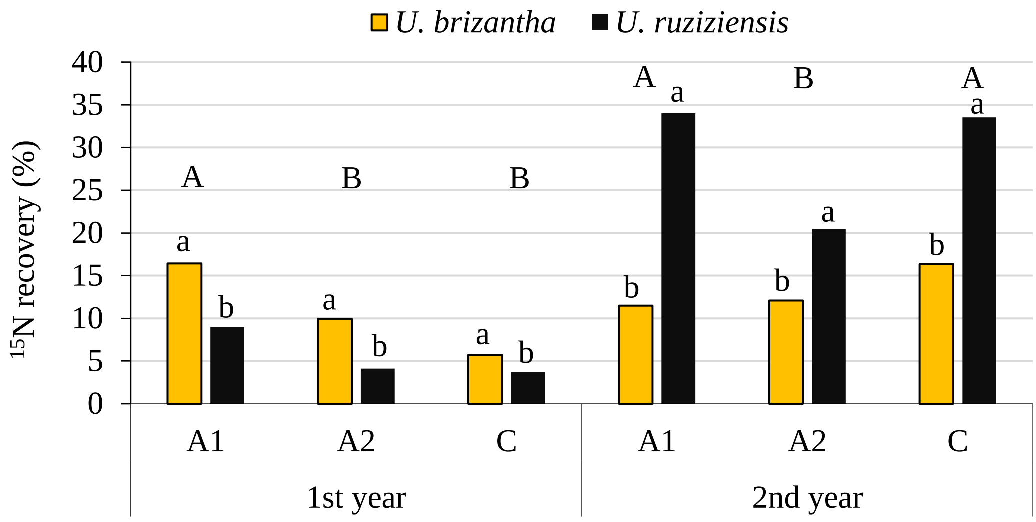


Figure 3. ^{15}N recovery in soil microbial biomass in the 0.0–0.10 m layer at rice tillering. Different lowercase letters indicate significant differences between grasses within the same treatment, and different uppercase letters indicate significant differences between treatments within the same year by the LSD test at 10% probability ($P < 0.10$). A1: 90 kg N ha^{-1} applied to grass; A2: 90 kg N ha^{-1} applied at rice sowing; C: 90 kg N ha^{-1} applied at rice tillering; T: 0 kg N ha^{-1}.

^{15}N recovery in the *U. brizantha* area remained constant in the two years, whereas in the *U. ruziziensis* area, ^{15}N recovery in microbial biomass was much higher in the second year than in the first one. One possible explanation is more significant root growth in the second year due to higher rainfall. As discussed previously, root decomposition creates hotspots in soil (KUZYAKOV, 2010) that increase the microbial community and SOM decomposition. *U. brizantha* produces higher root and shoots biomass than *U. ruziziensis* even when there is less water available (CRUSCIOL; SORATTO, 2007; MENEZES et al., 2009; SIMIDU et al., 2010; PACHECO et al., 2011, 2013; ROSOLEM et al., 2017; TANAKA et al., 2019), so its influence on N microbial immobilization did not differ between years.

Climatic conditions can also explain the differences in treatment C between the two years. Water is an essential element for microbial community growth, and since rainfall during tillering was higher in the second year (Figure 1), a more significant proportion of the N added at tillering was consumed than in the first year. Consequently, the hypothesis that nitrogen fertilization in advance results in higher N microbial immobilization is not always supported and depends on several factors, such as the number of PE hotspots and climatic conditions.

Moreover, as discussed previously, in NTS, N is always changing from mineral to organic form because of the active soil microbiota and organic matter deposition (COSER et al., 2007; HARRISON; BOL; BARDGETT, 2007). Initially, the soil microbiota assimilates more ^{15}N than plants (ROSSWALL, 1982; JACKSON; SCHIMEL; FIRESTONE, 1989; HODGE; ROBISON; FITTER, 2000; INSELSBACHER et al., 2010), but after several days, the microbial community reaches a steady-state, and organic N returns to mineral N and is available to plants (HODGE; ROBISON; FITTER, 2000; SPEDDING et al. 2004, HARRISON; BOL; BARDGETT, 2008; FERREIRA et al. 2010; FERREIRA; MARTIN-DIDONET, 2012). As microbes have faster turnover than plant biomass (INSELSBACHER et al., 2010), the straw in NTS may contribute to N availability less to its ability to cycle N (NASCENTE; CRUSCIOL 2013) and more to its ability to enhance microbial growth, which potentiates PE.

In addition to enhancing microbial growth, plants can alter the soil microbiology soil in two other ways: by supplying energy via rhizodeposition below ground (RAAIJMAKERS et al., 2009; HAICHAR et al., 2014; YORK et al., 2016; ROSOLEM et al., 2017; POIRIER; ROUMET; MUNSON, 2018), which modulates overall biotic activity, and by sending signals that affect specific microbial communities (SUBBARAO et al., 2007; HAICHAR et al., 2014; BAPTISTELLA et al., 2020). So, different species can be more or less effective in releasing N from organic matter (ZHU et al., 2014) because of the different influences each one has on the soil microorganisms. In addition, microbial communities and root exudates are also influenced by the addition of mineral N as fertilizer (ROSOLEM et al., 2017).

In the grains, ^{15}N recovery was highest in treatment C in both years (Figure 4). In the first year, there was no difference between the grasses, but in the second year, recovery was greater in the grains grown in the *U. ruziziensis* area. The differences in ^{15}N recovery in both rice grains and shoot tissue between treatment C and treatments A1 and A2 and the absence of differences in rice yield (Figure 2) and total N accumulated (Table 1) indicate that in treatments A1 and A2 rice assimilated N from sources other than from fertilizer. This confirms our third hypothesis: when N fertilization is applied in advance, upland rice reaches for N from other sources in the soil.

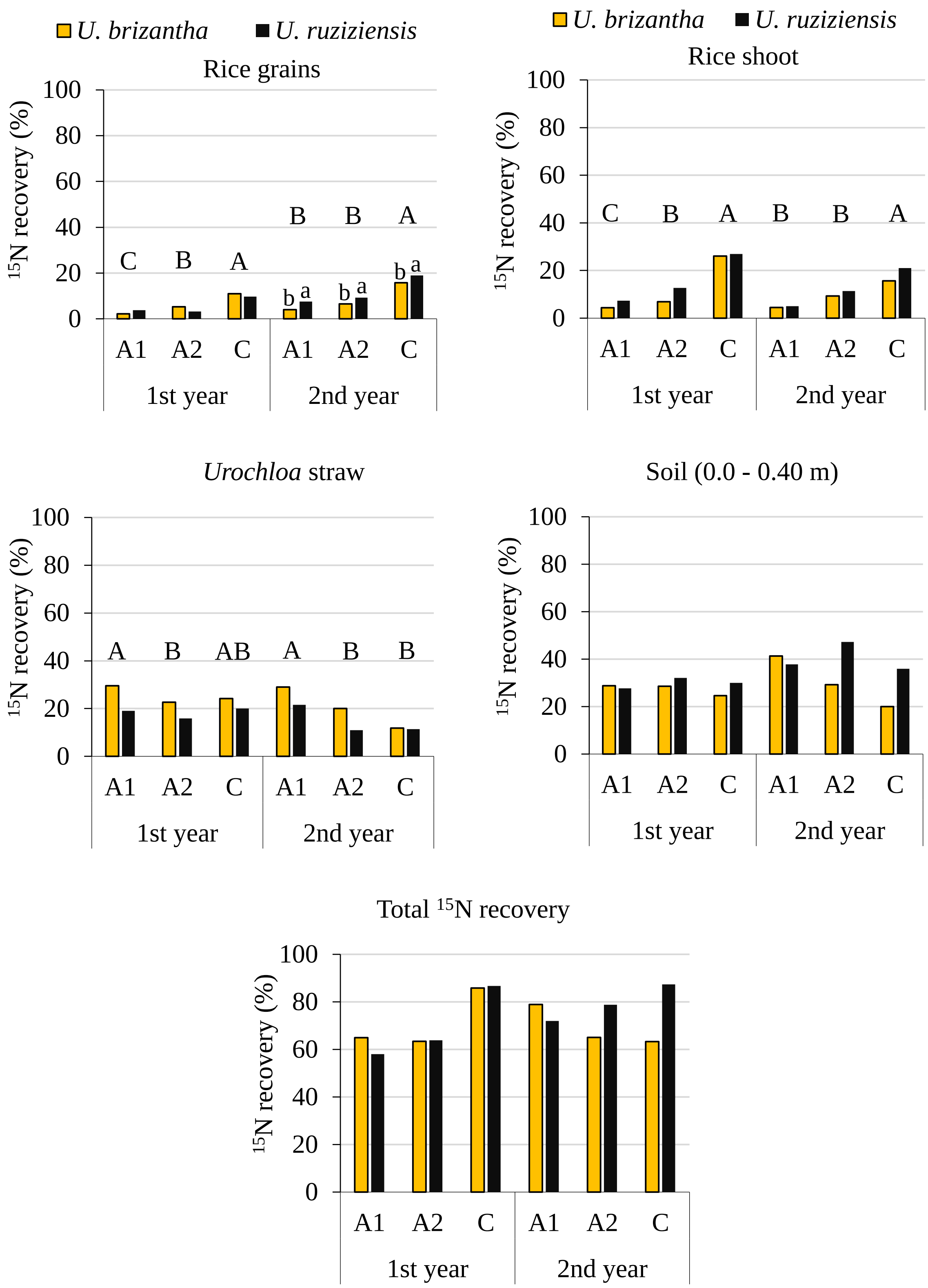


Figure 4. ^{15}N recovery in rice grain and shoot tissues, *Urochloa* straw, and soil (0.0–0.40 m) and total ^{15}N recovery at rice harvest. Different lowercase letters indicate significant differences between grasses within the same treatment, and different uppercase letters indicate significant differences between treatments within the same year by the LSD test at 10% probability ($P < 0.10$). The absence of letters indicates the absence of significant differences. A1: 90 kg N ha^{-1} applied to grass; A2: 90 kg N ha^{-1} applied at rice sowing; C: 90 kg N ha^{-1} applied at rice tillering; T: 0 kg N ha^{-1}.

Chen et al. (2010) and Wang et al. (2011) also found that the majority of N in rice crops came from N mineralized from the soil instead of ^{15}N from fertilizer. According to Jenkinson, Fox, and Rayner (1985), Hart, Rayner, and Jenkinson (1986), and Leon et al. (1995), PE can be measured by the extra soil N that is taken up by plants after the addition of mineral N fertilizers compared to unfertilized plants. As total N accumulated (Table 1) and rice yield (Figure 2) were lower in the control treatment (T), it can be inferred that the lower ^{15}N recovery in rice plants in treatment A1 and A2 is evidence of PE. Although PE begins immediately after substrate addition (or after grass desiccation), some studies have shown that PE may not be apparent for days or even weeks (FONTAINE et al., 2004; BLAGODATSKY et al., 2010). In this case, the N added before grass desiccation or at rice sowing would be available when needed by rice.

No differences in ^{15}N recovery in rice shoots, *Urochloa* straw, and soil (0.0–0.4 m) were observed between the two areas of grasses in either year. ^{15}N recovery in rice shoot tissues was higher in treatment C (24% and 20%, respectively in the first and second year) than in treatments A1 (4% and 5%) and A2 (11% and 9%). ^{15}N recovery in *Urochloa* straw was highest in treatment A1 (27% and 25%) in both years as expected since this treatment was the only one in which N was applied on living grass.

No differences were observed between the treatments in ^{15}N recovery in soil and total ^{15}N recovery. Recovery of ^{15}N in soil ranged between 24 and 34% in the first year and 12 and 46% in the second year. Once again, the differences between years can be explained by climatic conditions, as N transformations in soil depend on water content.

Total ^{15}N recovery was nearly 60% in almost all treatments. When fertilizer was applied at tillering (C treatment), total ^{15}N recovery was above 80% in both years. This exceeds the range of 30 to 65% that some authors (RAUN et al., 2002; TILLMAN et al., 2002; LADHA et al., 2005; HERRERA et al., 2016) rank as usual for ^{15}N recovery by crops in the first year of N fertilization. Kadiyala et al. (2015) noted N losses between 43 and 65% in upland rice systems in a sandier soil. Our lower N losses can be attributed to both soil organic matter and clay content.

1.4 CONCLUSIONS

Advance nitrogen application is a technically viable alternative for upland rice under NTS. *Urochloa brizantha* increases mineral and organic N and the total N of soil compared with *Urochloa ruziziensis*. Advance N application reduces N fertilizer uptake by rice plants but does not reduce rice yield. Microbial immobilization of N fertilizer is not always higher in soils that receive N in advance than in soils where N is applied at rice tillering. The priming effect seems to be key to the feasibility of advance nitrogen application in grass-upland rice systems under no-till management.

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APPENDIX

Table 1. Initial soil chemical characterization.

	pH	SOM[1]	P	K	Ca	Mg	H+Al	CEC[2]	BS[3]	AlS[4]
	$CaCl_2$	g dm^{-3}	mg dm^{-3}	---------- mmol$_c$ dm^{-3} ---------					---- % ----	
1st year										
0.0 – 0.05 m										
U. brizantha	5.01	35	22	6	34	24	40	105	61	39
U. ruziziensis	4.97	31	24	6	32	24	40	103	61	39
0.05 – 0.10 m										
U. brizantha	4.59	23	10	5	17	18	50	90	45	55
U. ruziziensis	4.51	21	13	3	20	16	52	90	42	58
0.10 – 0.20 m										
U. brizantha	4.37	15	6	4	17	11	53	86	38	62
U. ruziziensis	4.40	15	7	2	17	14	52	84	39	61
0.20 – 0.40 m										
U. brizantha	4.57	15	4	3	19	11	41	74	45	55
U. ruziziensis	4.58	14	4	1	17	11	39	69	43	57
2nd year										
0.0 – 0.05 m										
U. brizantha	4.81	39	18	3	29	17	64	113	43	57
U. ruziziensis	4.68	39	22	5	25	16	75	121	38	62
0.05 – 0.10 m										
U. brizantha	4.79	29	16	2	24	11	75	112	34	66
U. ruziziensis	4.40	29	21	3	15	8	91	116	22	78
0.10 – 0.20 m										
U. brizantha	4.82	26	6	1	24	11	62	98	37	63
U. ruziziensis	4.62	26	7	1	14	6	86	107	20	80
0.20 – 0.40 m										
U. brizantha	4.98	23	3	0	19	8	52	79	34	66
U. ruziziensis	4.74	23	4	1	14	6	63	84	25	75

[1] Soil organic matter; [2] Cation exchange capacity; [3] Base saturation; [4] Aluminum saturation.

Table 2. Macroporosity (MA) ($m^3\ m^{-3}$), microporosity (MI) ($m^3\ m^{-3}$), total porosity (PT) ($m^3\ m^{-3}$) and soil density (Ds) ($g\ cm^{-3}$).

	MA	MI	PT	Ds
1st year				
0.0 – 0.05 m				
U. brizantha	0.07	0.40	0.48	1.08
U. ruziziensis	0.07	0.39	0.45	1.07
0.05 – 0.10 m				
U. brizantha	0.08	0.39	0.47	1.11
U. ruziziensis	0.07	0.39	0.45	1.14
0.10 – 0.20 m				
U. brizantha	0.08	0.35	0.43	1.07
U. ruziziensis	0.06	0.39	0.45	1.03
0.20 – 0.40 m				
U. brizantha	0.08	0.38	0.46	1.09
U. ruziziensis	0.06	0.39	0.46	1.00
2nd year				
0.0 – 0.05 m				
U. brizantha	0.06	0.49	0.55	1.35
U. ruziziensis	0.07	0.46	0.53	1.37
0.05 – 0.10 m				
U. brizantha	0.07	0.46	0.53	1.33
U. ruziziensis	0.06	0.44	0.50	1.34
0.10 – 0.20 m				
U. brizantha	0.07	0.45	0.52	1.36
U. ruziziensis	0.06	0.45	0.51	1.36
0.20 – 0.40 m				
U. brizantha	0.07	0.46	0.52	1.33
U. ruziziensis	0.09	0.45	0.54	1.28

Table 3. Soil chemical characterization at harvest.

	pH	SOM[1]	P	K	Ca	Mg	H+Al	CEC[2]	BS[3]	AlS[4]
	$CaCl_2$	g dm^{-3}	mg dm^{-3}	----------		$mmol_c$	dm^{-3}	---------	--- %	----
					1st year					
					0.0 – 0.05 m					
U. brizantha	4.59	35	18	4	30	21	54	109	51	49
U. ruziziensis	4.81	27	22	5	34	25	52	115	55	45
					0.05 – 0.10 m					
U. brizantha	4.48	21	13	5	22	17	69	113	40	60
U. ruziziensis	4.42	21	12	3	21	16	70	111	37	63
					0.10 – 0.20 m					
U. brizantha	4.29	21	6	4	18	12	69	103	32	68
U. ruziziensis	4.36	19	5	2	17	11	70	99	30	70
					0.20 – 0.40 m					
U. brizantha	4.58	12	3	2	18	11	54	85	37	63
U. ruziziensis	4.63	15	3	1	19	9	50	79	36	64
					2nd year					
					0.0 – 0.05 m					
U. brizantha	4.59	33	31	2	34	20	83	139	41	59
U. ruziziensis	4.58	32	30	2	30	18	89	139	36	64
					0.05 – 0.10 m					
U. brizantha	4.59	26	19	1	23	12	86	121	30	70
U. ruziziensis	4.37	25	29	1	15	10	111	137	19	81
					0.10 – 0.20 m					
U. brizantha	4.79	25	11	1	26	13	73	112	35	65
U. ruziziensis	4.44	24	13	1	16	9	102	128	20	80
					0.20 – 0.40 m					
U. brizantha	5.00	21	8	0	21	11	62	94	34	66
U. ruziziensis	4.75	20	6	0	15	7	75	97	23	77

[1] Soil organic matter; [2] Cation exchange capacity; [3] Base saturation; [4] Aluminum saturation.

Table 4. Significant differences in ^{15}N recovery according to the LSD test at 10% probability depending on fertilization timing (N) and grasses (U).

	1st year			2nd year		
	N	F	N x F	N	F	N x F
Rice grains	***	ns	ns	***	*	ns
Rice shoot	***	ns	ns	***	ns	ns
Urochloa straw	*	**	ns	**	ns	ns
Soil (0.0 – 0.40 m)	ns	ns	ns	ns	ns	ns
Microbial biomass	***	***	ns	**	***	ns
Total	ns	ns	ns	ns	ns	ns

***: ($P < 0.01$), **: ($P < 0.05$), *: ($P < 0.10$) and ns: ($P > 0.10$)

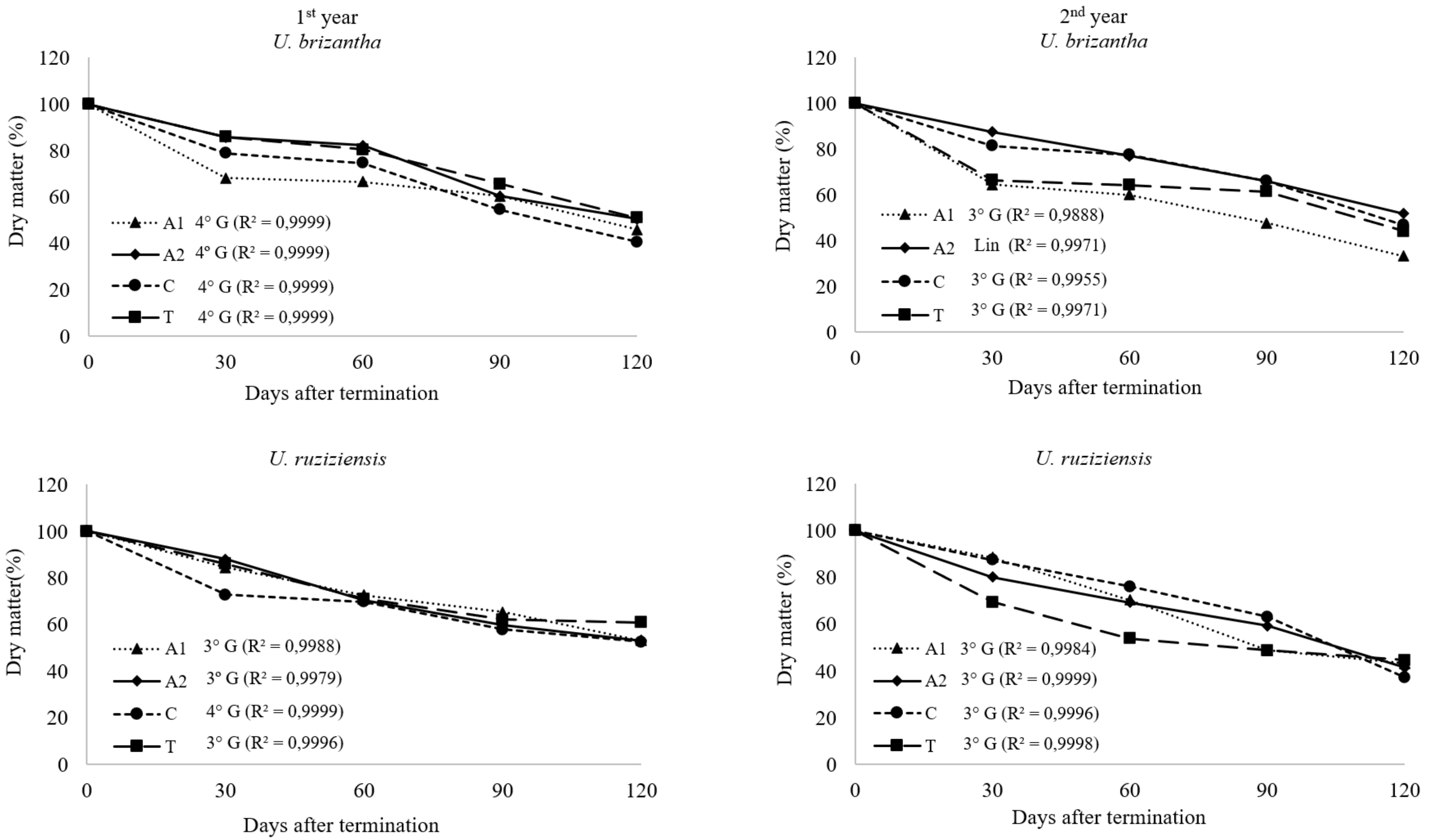

Figure 1. Decomposition ratios of *U. brizantha* and *U. ruziziensis* straw at 0, 30, 60, 90, and 120 days after desiccation. A1: 90 kg N ha^{-1} applied to grass; A2: 90 kg N ha^{-1} applied at rice sowing; C: 90 kg N ha^{-1} applied at rice tillering; T: 0 kg N ha^{-1}.

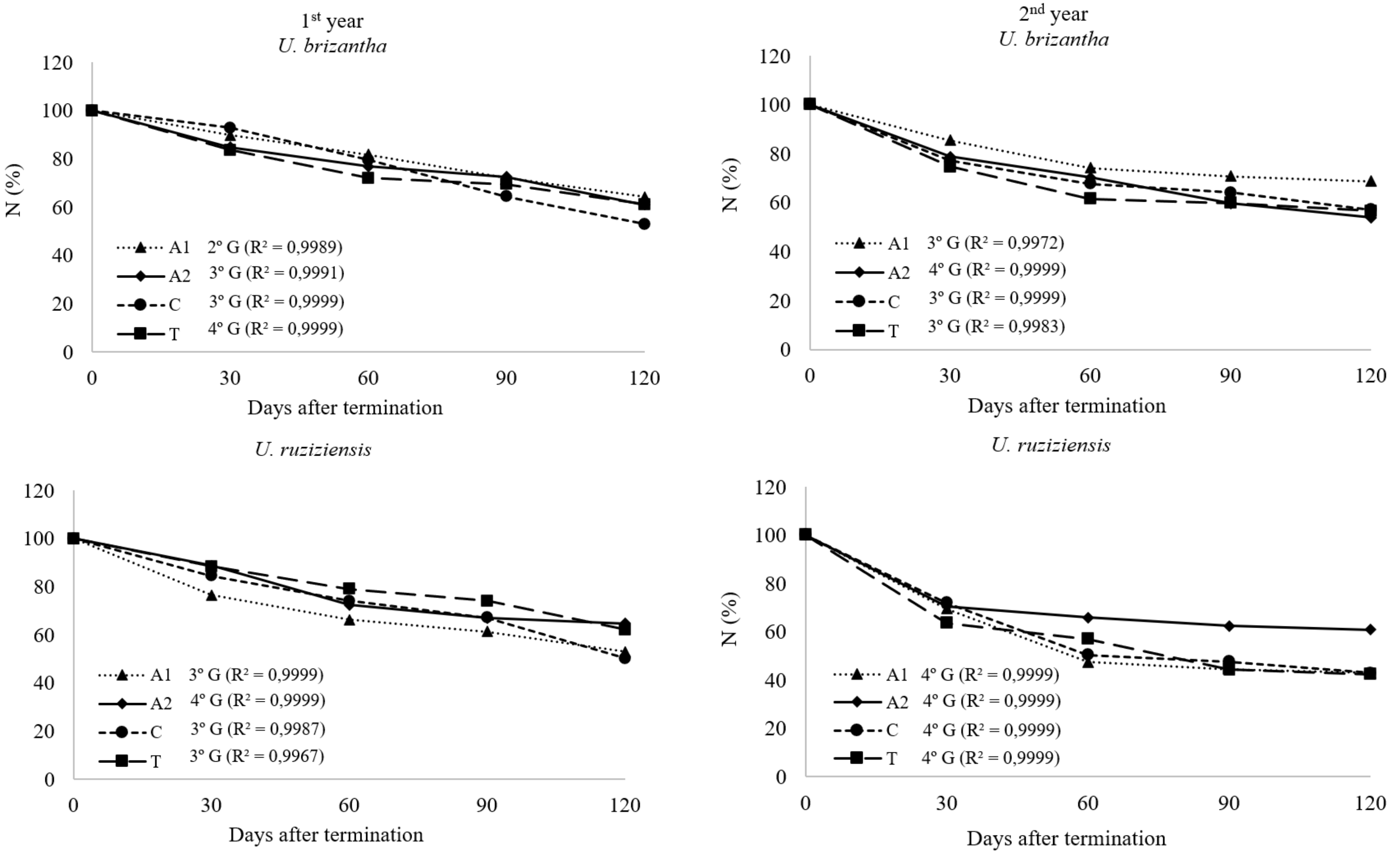

Figure 2. N release rate of *U. brizantha* and *U. ruziziensis* straw at 0, 30, 60, 90, and 120 days after desiccation. A1: 90 kg N ha^{-1} applied to grass; A2: 90 kg N ha^{-1} applied at rice sowing; C: 90 kg N ha^{-1} applied at rice tillering; T: 0 kg N ha^{-1}.

CHAPTER 2 - SOIL NITROGEN STATUS AFTER N ANTICIPATION IN A GRASS-RICE UPLAND SYSTEM

ABSTRACT – The importance of upland rice cultivated under no-tillage system is growing due to its favorable water use efficiency. However, nitrogen management differs considerably between aerobic environments such as uplands and lowlands. The rates and proportions of nitrogen transformations and losses vary in oxygen-enriched environments and are influenced by factors such as fertilization timing and cover crop species. This work aimed to study N dynamics in a system of upland rice sown over *Urochloa brizantha* or *U. ruziziensis* straw with different timings of N fertilization. Split plots arranged in a completely randomized block design in a 4 x 2 factorial scheme with four replications were used. The following four treatments were evaluated: 90 kg N ha^{-1} applied over *Urochloa* grass 10 days before desiccation (A1); 90 kg N ha^{-1} applied at rice sowing (A2); 90 kg N ha^{-1} applied at rice tillering (C); and no addition of N (T). The analyzed variables included upland rice yield; soil ammonium, nitrate, and inorganic N accumulation; N in microbial biomass, and ^{15}N soil recovery. No differences in rice yield were observed between the treatments that received N in advance and the treatment in which N was applied at tillering. N fertilization in advance resulted in higher N availability to rice during its initial development. In addition, nitrate and ammonium accumulation were higher when upland rice was sown over *U. brizantha*.

Keywords: ^{15}N recovery. Microbial biomass nitrogen. *Urochloa brizantha. Urochloa ruziziensis*.

2.1 INTRODUCTION

Rice can be grown in both flooded and upland systems. Compared with lowland rice, upland rice requires less water (FAROOQ et al., 2009; CRUSCIOL; SORATTO; MATEUS, 2006; HEINEMANN; STONE; FAGERIA, 2011; PRASAD, 2011), which makes it a sustainable option for guaranteeing food security in a possible water-depleted future. However, upland rice has a lower yield than lowland rice and accounts for a small share of rice cultivation worldwide (SANTOS; RABELO, 2008; PRASAD, 2011; NAYAR, 2014; OLIVEIRA NETO, 2015).

One of the main problems in adopting upland rice is N fertilization (LIN et al., 2005; CARVALHO; SOARES; REIS, 2008). Nitrogen management differs considerably between lowland and upland rice (CHANH; TSUTSUMI; KURIHARA, 1981) since the aerobic conditions in drained soils alter the microbial community and the rate of ammonification and nitrification processes. In upland systems, ammonium added to the soil can be transformed into nitrate and lost by leaching or immobilized by soil microorganisms in a larger proportion than in anaerobic soils.

Understanding N dynamics in the soil is essential not only for reducing N losses but also because rice is affected by nitrate and ammonium proportion in the soil. Several studies have proved that providing both N forms enhances rice growth and yield (QIAN et al., 2004; GUO et al., 2007; LIN et al., 2005; HOLZSCHUH et al., 2009; POLETTO et al., 2011; ARAÚJO et al., 2012; NASCENTE; LANNA, 2016; HACHIYA; SAKAKIBARA, 2017), but there is no consensus in the literature on the ideal nitrate/ammonium ratio.

As rice originated from hydrophilic environments (LIN et al., 2005) in which ammonium is prevalent (SCHJOERRING et al., 2002; LI et al., 2007; FAGERIA et al. 2014; ZHANG; CHU, 2020), it evolved to use ammonium as its main N source (WANG et al., 1993). Consequently, nitrate reductase (NR) activity is low in the first weeks of rice development (NAMBIAR et al., 1988; ARAÚJO et al., 2012; FAGERIA et al., 2014; MORO et al., 2014). However, nitrate is also important to ensure rice yield (HU et al., 2015; FAN et al., 2016; WANG et al., 2018) and can represent as much as 40% of total N taken up by rice plants (ZHANG; CHU, 2020).

Ammonium can improve rice tolerance to drought and osmotic stress compared with nitrate (CABUSLAY; ITO; ALEJAR, 2002; CHAVES et al., 2002; GUO et al., 2007; WASSON et al., 2012; GAO et al., 2017, 2020), which is an important advantage for upland rice. Since water deficits in uplands can range from mild to severe (KAMOSHITA et al., 2008), drought can be one of the most critical stresses limiting upland rice yield (MENGE et al., 2019). Nitrate increases cortical aerenchyma formation by enhancing ethylene production (YANG et al., 2012; GAO et al., 2017, 2020) and, in turn, reduces water transport and uptake (NEGI; IVANCHENKO; MUDAY, 2008; XU et al., 2013; DING et al., 2015). Ammonium, on the other hand, reduces root cortical aerenchyma formation, enhances aquaporin activity and root hydraulic conductivity, and promotes lateral root growth (GUO et al., 2007; LIMA et al., 2010; GAO et al., 2010, 2017; YANG et al., 2012, WASSON et al., 2012; DING et al., 2015, 2016). To be used by the plant, nitrate needs to be reduced to ammonium, which requires eight moles of electrons per mole of nitrate (HACHIYA; SAKAKIBARA, 2017). Consequently, in addition to its morphological advantages, ammonium decreases the energy required to synthesize organic N compounds (WILLIAMS et al., 1987). Agricultural practices that reduce nitrification can, therefore, benefit upland rice.

However, nitrate and ammonium pools in the soil are incredibly dynamic (BUTTERBACH-BAHL et al., 2013) and depend not only on mineral fertilization but also on several other factors (LEE; GARLAN; ROSSEL, 2018), such as the preceding crop. As upland rice is usually sown after pasture in Brazil, especially over *Urochloa* sp. straw (FONSECA; MARTUSCELLO, 2010; MORO et al., 2013; PINHEIRO et al., 2016), it is crucial to evaluate the potential influence of pasture grasses on soil N.

Some species of the genus *Urochloa* are known to influence soil microorganisms involved in the N cycle, which can lead to changes in N dynamics (REIS et al., 2001; BAPTISTELLA et al., 2020; ROCHA et al., 2020). Moreover, soil N is highly influenced by the straw produced by the cover crop (CRUSCIOL et al., 2005, 2015; NASCENTE; LANNA, 2016). When there is a considerable input of biomass over the soil surface, the extra C and N act as an impulse for microbial activity and, consequently, increase N uptake by microbial cells (KUZYAKOV, 2010; NASCENTE; STONE, 2018).

To avoid N losses, split N fertilization is usually recommended for upland rice. According to this practice, a small part of the N is added at rice sowing, and a larger amount is added at rice tillering, when it is most needed by rice (MATSUO; HOSHIKAWA, 1993; FARINELLI et al., 2004; ALVAREZ et al., 2005; TADESSE;

TADESSE, 2019). Several studies have shown that equivalent upland rice production is obtained when N is added in advance in no-tillage systems (NTS) (MARZARI et al. 2005; HERNANDES et al., 2010; LOPES et al., 2013; ARF et al., 2015, NASCENTE; LANNA, 2016; PINHEIRO et al., 2016), but these studies did not follow soil nitrate, ammonium, and N immobilization over time.

To contribute to addressing this gap, this work aimed to study N forms and their distribution in soil under advance N fertilization of upland rice sown over *U. brizantha* or *U. ruziziensis* straw. We hypothesized that (*i*) applying N in advance does not increase N losses compared with N fertilization at tillering, and (*ii*) soil nitrogen dynamics vary as a function of the *Urochloa* species used in the crop system.

2.2 MATERIAL AND METHODS

The experiment was carried out at Lageado Experimental Farm of São Paulo State University in Botucatu, São Paulo State, Brazil (48◦26′W, 22◦51′S, 786 m altitude), during the 2017/2018 and 2018/2019 growing seasons.

The climate is Cwa according to Köeppen's classification (ALVARES et al., 2013), characterized as a tropical latitude with a dry winter and a hot, wet summer (Figure 1).

The soil is classified as a Ferralsol (FAO, 2014) clayey soil with 602, 117, and 281 g kg^{-1} of clay, sand, and silt, respectively. Selected soil chemical and physical properties were determined (0.0–0.40 m) according to Raij et al. (2001) and Klute (1986), respectively, in the first and second years as follows: soil organic matter, 21 and 29 g dm^{-3}; pH ($CaCl_2$), 4.6 and 4.7; P (resin), 11 and 12 g dm^{-3}; K^+, 4 and 2 $mmol_c$ dm^{-3}; Ca^{2+}, 22 and 21 $mmol_c$ dm^{-3}; Mg^{2+}, 16 and 10 $mmol_c$ dm^{-3}; cation exchange capacity (CEC), 88 and 104 mmolc dm^{-3}; base saturation, 47 and 32%; macroporosity, 0.07 and 0.06 m^3 m^{-3}; microporosity, 0.39 and 0.45 m^3 m^{-3}; and soil density, 1.07 and 1.14 g cm^{-3}.

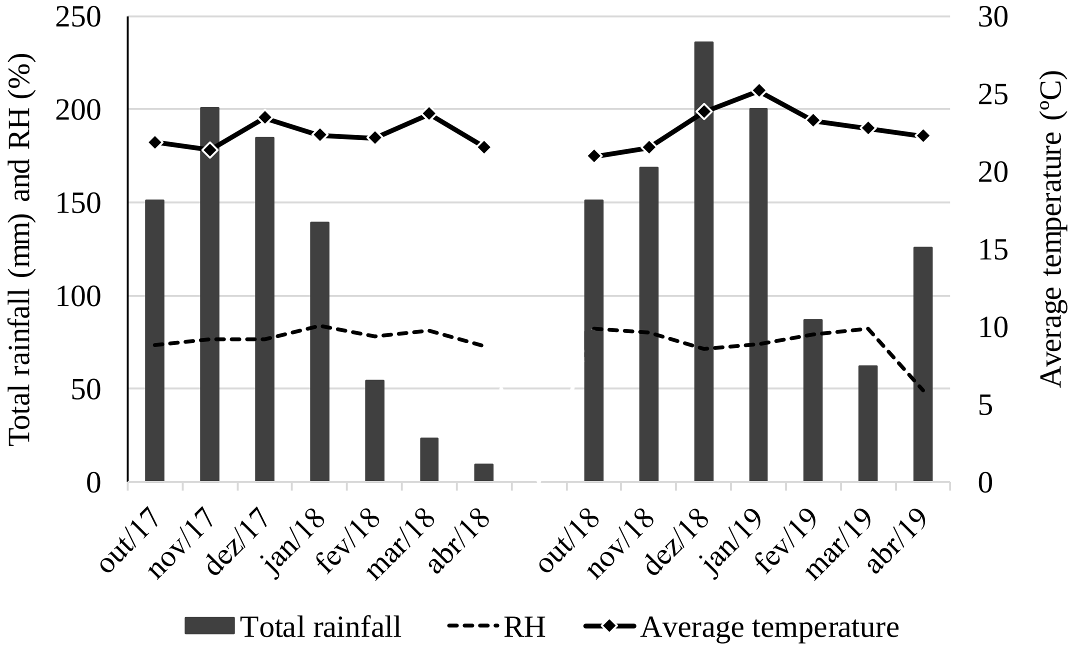

Figure 1. Precipitation, relative humidity (RH), and average air temperatures in the first (2017–2018) and second (2018–2019) years.

Treatments and experimental design

The experiment was conducted in split plots arranged in a completely randomized block design with four replications in a 4 x 2 factorial scheme (four N management treatments and two grasses). The treatments were upland rice sown in areas previously occupied by *Urochloa brizantha* cv. Marandu or *Urochloa ruziziensis* and fertilized with 90 kg N ha^{-1} on live grass 10 days before desiccation (A1), 90 kg N ha^{-1} on grass straw 1 day before rice sowing (A2), 90 kg N ha^{-1} broadcast at rice tillering according to the conventional recommendation (RAIJ et al., 1996; ALVAREZ et al., 2005), (C) and no additional N on the cover crop (T). The experimental plots were 8 m long with rows spaced 0.34 m apart.

All treatments, including treatment T, received 30 kg N ha^{-1} at rice sowing. Nitrogen was applied as ammonium sulfate ($(NH_4)_2SO_4$) in the plot and as ^{15}N-enriched ammonium sulfate ($(^{15}NH_4)_2SO_4$) in micro plots placed in the center of the plots, which were used to follow N fate.

Field experiments

To reduce differences between seasons and the effect of N applied in 2017/2018 on plants in the 2018/2019 growing season, the experiment was conducted in two adjacent areas occupied by fully grown *U. brizantha* or *U. ruziziensis*. The area cultivated in the second year was left with forage without management during the first year.

To produce straw for sowing rice under NTS, the grasses were terminated at the beginning of November. The rice (IAC 203) was mechanically sown 30 days after forage desiccation in both years using a seeder-fertilizer machine for no-till. The seeds were placed at a depth of 3 cm with a sufficient density to obtain 150 plants m^{-2}.

Seeding fertilization was conducted according to the recommendation for the crop (RAIJ et al., 1996) using 40 kg ha^{-1} of P_2O_5 as triple superphosphate, 20 kg ha^{-1} of K_2O as potassium chloride, and 30 kg ha^{-1} of N as ammonium sulfate. Herbicides and fungicides were used to simulate commercial crop operations. The rice was harvested manually in April 2018 and 2019, when the upper 2/3 grains of the panicles were hard, and the lower third was semi-hard.

Soil sampling and analysis

Soil samples were collected at depths of 0.0–0.10 and 0.10–0.20 m to determine the amount of ammonium, nitrate, and inorganic N in the soil, nitrogen in microbial biomass, and recovery of ^{15}N. The samples were collected at grass desiccation, at rice sowing, and at rice tillering. After sampling, the soil samples were placed in a thermal box with ice and subsequently frozen at -15°C to avoid changes in the levels of ammonium and nitrate (MATTOS JÚNIOR; CANTARELLA; RAIJ, 1995). After extraction in the KCl solution, the concentrations of ammonium and nitrate were determined by colorimetry using the sodium salicylate method and reduction with vanadium chloride, respectively (MULVANEY, 1996; MIRANDA et al., 2001) and transformed into ammonium and nitrate accumulation (kg ha^{-1}). Inorganic N was calculated from the sum of ammonium and nitrate. N in microbial biomass was determined according to Embrapa (2007) and transformed into kg ha^{-1}. To estimate soil N accumulation, the soil bulk density was determined by the volumetric ring method (KLUTE, 1986) in the 0.0–0.10 m and 0.10–0.20 m layers.

The total N content and abundance of ^{15}N (% of ^{15}N atoms) were determined in dry soil samples using a mass spectrometer (Hydra 20-20, Sercon Ltd., Crewe, UK) coupled to an automatic N analyzer (ANCA-GSL, Sercon Ltd., Crewe, UK) at the Stable Isotope Laboratory of the Nuclear Energy Center in Agriculture (CENA/USP) according to the methodology suggested by Trivelin et al. (1994) and Oliveira et al. (2008). These parameters were used to calculate ^{15}N recovery in the soil.

The results were subjected to analysis of variance by the F test ($P <0.10$) and compared by the LSD test ($P <0.10$). Statistical analyses were performed using the software SISVAR® (FERREIRA, 2014).

2.3 RESULTS AND DISCUSSION

In both the 0.0–0.10 m and 0.10–0.20 m soil layers, ammonium was higher than nitrate at all sample times, with a few exceptions (Table 1). As expected, ammonium was higher in the treatments that received N fertilizer at the sampling time. At grass desiccation, A1 presented higher ammonium in both layers in both years (13.2 and 19.8 kg ha^{-1} at 0.0–0.10 m and 5.6 and 9.5 kg ha^{-1} at 0.10–0.20 m). At rice sowing, A2 presented higher ammonium in both layers in the first year (17.6 kg ha^{-1} at 0.0–0.10 m and 4.4 kg ha^{-1} at 0.10-0.20 m) and in the 0.0–0.10 m layer in the second year (33.0 kg ha^{-1}). At rice tillering, C presented higher ammonium in both years (17.4 and 23.6 kg ha^{-1} at 0.0–0.10 m and 8.4 and 7.1 kg ha^{-1} at 0.10–0.20 m, in the first and second years, respectively). The addition of N in the ammoniac form (ammonium sulfate) reduced the influence of fertilization timing on nitrate accumulation.

Although nitrate has been reported to be the main N form in agricultural soils (MILLER; CRAMER, 2004; FAGERIA et al., 2014; ROSOLEM et al., 2017; FRIEDL et al., 2017, 2018; LEE; GARLAN; ROSSEL, 2018), low soil nitrate has also been observed (NASCENTE; CRUSCIOL; COBUCCI, 2012; LEE; GARLAND; ROSSEL, 2018). Low soil nitrate can arise from intense root growth and nitrate absorption (FAGERIA; MOREIRA, 2011), nitrate leaching (CARNEIRO et al., 2009; NASCENTE; CRUSCIOL; COBUCCI, 2012), a low nitrification rate, and significant dissimilatory nitrate reduction to ammonium (DNRA) (FRIEDL et al., 2018).

Table 1. Soil ammonium and nitrate accumulation (kg ha^{-1}) in the 0.0–0.10 and 0.10–0.20 m soil layers at grass desiccation, rice sowing, and rice tillering.

	Grass desiccation				Rice sowing				Rice tillering			
	Ammonium		Nitrate		Ammonium		Nitrate		Ammonium		Nitrate	
	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
					0.0 – 0.10 m							
Treatment (N)												
A1	13.2a	19.8a	2.6	5.3a	9.2b	29.8ab	2.3	38.6a	11.0b	16.1b	5.5b	19.0b
A2	8.9b	9.2c	0.8	1.5b	17.6a	33.0a	1.4	18.5b	10.2b	16.7b	5.3b	23.9a
C	9.0b	10.7b	1.2	1.3b	7.8b	26.1b	1.3	18.3b	17.4a	23.6a	10.2a	20.4b
T	9.0b	8.3c	0.8	1.5b	7.7b	27.7a	1.1	19.7b	10.7b	14.7b	4.1b	15.3c
Grass (U)												
U. brizantha	9.5	11.4b	2.0a	2.4	10.9	29.1	1.6	22.1b	13.8a	19.3a	9.1a	21.5a
U. ruziziensis	8.6	12.6a	0.8b	2.5	10.2	29.2	1.5	25.5a	10.8b	16.3b	3.4b	17.8b
					p-value							
N	0.0755	0.0001	0.1915	0.0001	0.0001	0.0480	0.2467	0.0001	0.0003	0.0012	0.0117	0.0045
U	0.4436	0.0090	0.0817	0.7885	0.3252	0.9500	0.8615	0.0292	0.0029	0.0230	0.0004	0.0133
N x U	0.0994	0.0001	0.3235	0.0006	0.8812	0.6826	0.6365	0.0001	0.0476	0.0058	0.0900	0.0018
					0.10 – 0.20 m							
Treatment (N)												
A1	5.6a	9.5a	0.8	2.2a	4.4a	13.4	0.6a	11.1b	8.1	5.5b	4.3	6.7a
A2	3.8b	9.4a	0.7	0.9b	4.4a	10.3	0.6a	1.6c	7.9	4.8b	3.9	4.6b
C	4.5b	6.7b	0.8	0.5b	3.0b	10.4	0.3b	15.5a	8.4	7.1a	4.0	4.8b
T	4.0b	6.6b	0.8	0.6b	3.0b	10,0	0.4b	16.0a	7.3	4.7b	2.7	2.5c
Grass (U)												
U. brizantha	4.7	7.7	0.7b	1.3a	4.2a	11.8	0.4	16.2a	8.1	6.0a	5.0a	5.9a
U. ruziziensis	4.2	8.4	0.8a	0.8b	3.2b	10.2	0.5	5.9b	7.8	5.1b	2.4b	3.5b
					p-value							
N	0.0256	0.0121	0.8230	0.0003	0.0783	0.1971	0.0523	0.0006	0.4271	0.0032	0.2041	0.0025
U	0.2841	0.2699	0.0216	0.0241	0.0508	0.2003	0.4297	0.0002	0.5929	0.0328	0.0008	0.0014
N x U	0.6406	0.4651	0.8179	0.0275	0.0049	0.0416	0.2129	0.0001	0.0729	0.0556	0.2775	0.0015

Means followed by different letters indicate a significant difference by the LSD test at 10% probability. A1: 90 kg N ha^{-1} applied to grass; A2: 90 kg N ha^{-1} applied at rice sowing; C: 90 kg N ha^{-1} applied at rice tillering; T: 0 kg N ha^{-1}.

Acid soils (pH < 5.0) like those in the present study are detrimental for the nitrifying bacteria population and exhibit a low nitrification rate (HUYGENS et al., 2008; ZHANG et al., 2013; MORO et al., 2017) and reduced ammonium oxidation (WEBER; GAINEY 1962; ZHANG et al. 2011, 2013, 2015). The combination of low nitrification and dissimilatory nitrate reduction to ammonium (DNRA), which can occur simultaneously in microsites with different redox potentials (FRIEDL et al., 2018), results in a low soil nitrate/ammonium ratio. Moreover, *U. brizantha* and *U. ruziziensis* can inhibit soil nitrification (BYRNES et al., 2017; ROCHA et al., 2020).

Higher ammonium, especially at rice sowing, is an advantage for upland rice. A low nitrate/ammonium ratio in the first weeks can permit better initial development of upland rice (POLETTO et al., 2011; ARAÚJO et al., 2012; PACHECO et al., 2013). Higher ammonium favors rice growth not only because ammonium is the preferred N form for rice but also because nitrate reductase is more efficient at low nitrate levels (ARAÚJO et al., 2012). In the initial growth phases, upland rice absorbs and metabolizes higher amounts of ammonium than nitrate (LIN et al., 2005; LI et al., 2006).

As ammonium is the major part of soil inorganic N (Table 2), inorganic N responded to the treatments in the same way as ammonium. Higher values of inorganic N were found in the plots that received N at the sampling time, and ammonium, nitrate, and inorganic N were higher in the second year than in the first one. In the second year, soil inorganic N was highest when sampling was performed at rice sowing. The differences between years can be explained by the higher total rainfall in the second year than in the first. As N is dependent on microbial activity, which is dependent on water content (LEE; GARLAND; ROSSEL, 2018), a higher soil water content would stimulate microorganism growth and consequently N mineralization and transformation.

Table 2. Soil inorganic N (kg ha^{-1}) at depths of 0.0–0.10 and 0.10–0.20 m at grass desiccation, rice sowing, and rice tillering.

	Grass desiccation		Rice sowing		Rice tillering	
	1st year	2nd year	1st year	2nd year	1st year	2nd year
			0.0 – 0.10 m			
Treatment (N)						
A1	15.8a	25.2a	11.4b	68.5a	16.4b	35.0b
A2	9.8b	10.8bc	19.0a	51.5b	15.5b	40.6a
C	10.2b	12.0b	9.2c	44.4c	27.5a	44.0a
T	9.8b	9.7c	8.8c	47.3bc	14.7b	30.1b
Grass (U)						
U. brizantha	12.5	13.9b	12.5	51.2	23.0a	40.7a
U. ruziziensis	10.3	15.0a	11.7	54.7	14.1b	34.1b
			p-value			
N	0.0993	0.0001	0.0001	0.0001	0.0009	0.0047
U	02521	0.0847	0.3378	0.1539	0.0004	0.0104
N x U	0.1259	0.0001	0.7687	0.0009	0.0145	0.0004
			0.10 – 0.20 m			
Treatment (N)						
A1	6.4a	11.7a	4.9a	24.5a	12.4	12.2a
A2	4.5b	10.3a	4.9a	11.9b	11.8	9.5b
C	5.3b	7.2b	3.3b	25.9a	12.4	11.9a
T	4.7b	7.1b	3.4b	26.0a	10.0	7.2c
Grass (U)						
U. brizantha	5.3	9.0	4.6a	28.0a	13.1a	11.8a
U. ruziziensis	5.1	9.2	3.7b	16.1b	10.2b	8.6b
			p-value			
N	0.0248	0.0005	0.0543	0.0018	0.1977	0.0001
U	0.5438	0.6495	0.0708	0.0002	0.0077	0.0001
N x U	0.6387	0.5722	0.0236	0.0002	0.2681	0.0063

Means followed by different letters indicate a significant difference by the LSD test at 10% probability. A1: 90 kg N ha^{-1} applied to grass; A2: 90 kg N ha^{-1} applied at rice sowing; C: 90 kg N ha^{-1} applied at rice tillering; T: 0 kg N ha^{-1}.

The higher N at sowing is probably due to straw decomposition since rice was sown 30 days after desiccation (DAT). *U. brizantha* and *U. ruziziensis* exhibit high release of N as ammonium (TORRES; PEREIRA; FABIAN, 2008; TEIXEIRA et al., 2009) from straw in the first 30 DAT (PACHECO et al., 2011, 2013), and this ammonium can be quickly transformed into nitrate (JACKSON et al., 1989; NASCENTE; CRUSCIOL; COBUCCI, 2012). In addition to straw decomposition, grasses can influence N dynamics in the soil via intense root growth and root exudates (BARDGETT; MOMMER; VRIES, 2014; ALMEIDA; ROSOLEM, 2016; NUÑEZ et al., 2018). These exuded compounds are an energy source for rhizospheric microorganisms and increase their activity and SOM decomposition (POIRIER; ROUMET; MUNSON, 2018). Even after desiccation, grass roots remaining in the soil slowly decompose, altering the forms of soil N. In addition, microbes related to ammonification, nitrification, and N fixation are influenced by the *Urochloa* species and its developmental stage (ROCHA et al., 2020).

Soil ammonium and nitrate were higher in the *U. brizantha* area than in the *U. ruziziensis* area when differences between the grasses were found, with a few exceptions (Table 1). Ammonium and nitrate pools in the soil are extremely dynamic (BUTTERBACH-BAHL et al., 2013), depending on several factors (LEE; GARLAND; ROSSEL, 2018), and can have a turnover time of 1 day (JACKSON et al., 1989). The inability to replicate some results over time, such as the plots in which *U. ruziziensis* exhibited higher ammonium and nitrate, may not be attributable to grass interference. However, the ability of *U. brizantha* to enhance ammonium and nitrate was observed at all three sampling times. Consequently, our hypothesis that soil nitrogen dynamics vary as a function of *Urochloa* species is confirmed.

There are four main potential explanations for the higher mineral N in *U. brizantha* soil. First, Rocha et al. (2020) reported a higher nitrification rate in *U. ruziziensis* soil. The higher conversion rate could lead to higher nitrate loss since nitrate is more leachable than ammonium (CARNEIRO et al., 2009; FAGERIA, 2014; ROSOLEM et al., 2017).

Second, higher dry matter production and higher N accumulation in *U. brizantha* straw (Table 2, appendix) would lead to higher N mineralization (D'ANDRÉA et al., 2004; KLIEMANN; BRAZ; SILVEIRA, 2006; BOER et al., 2007; PACHECO et al., 2011; NASCENT; CRUSCIOL; COBUCCI, 2012).

Third, *U. brizantha* has a denser and more vigorous root system (CRUSCIOL; SORATTO, 2007; MENEZES et al., 2009; SIMIDU et al., 2010; PACHECO et al., 2011, 2013; TANAKA et al., 2019), which can produce more exudates and enhance SOM decomposition and N release. According to Kuzyakov (2010), organic inputs to the soil can be classified as occasional or continuous, and each uniquely alters N dynamics. Straw from grasses represents an occasional input. These inputs provide a larger and faster impulse to the soil microbial community that can end as quickly as it starts (KUZYAKOV, 2010). Continuous inputs, on the other hand, include the slow decomposition of dead roots and shoot residues and influence the soil microbial community for more extended periods.

In the latter case, the extracellular enzymes generated to degrade these organic materials are also more efficient at decomposing SOM compared with the largely intracellular enzymes that break down occasional inputs (FONTAINE et al., 2004). This is important because SOM mineralization provides more than 50% of the total N absorbed by plants (FERNANDES et al., 2011). Consequently, soils occupied by dense root systems can be highly efficient in mineralizing N from SOM and enhancing N availability. According to Bardgett, Mommer, and Vries (2014), root architecture, morphology, physiology, and interactions with soil microorganisms can alter N availability.

Fourth, *U. brizantha* may also fixate N from the air. Baptistella et al. (2020) showed that *Urochloa* can fixate N in substantial amounts through associations with diazotrophic bacteria. In a study of *U. brizantha*, *U. ruziziensis,* and two other *Urochloa* species, Reis et al. (2001) found that N biological fixation can vary from 3 to 26% among species.

Regardless of the underlying mechanism, the ability of *U. brizantha* to improve N accumulation in the soil resulted in a higher rice yield (Table 3). In 2018, rice production was higher in the *U. brizantha* area (2,737 kg ha^{-1}) than in the *U. ruziziensis* area (2,223 kg ha^{-1}). This could be explained by the higher inorganic N found in the *U. brizantha* area at rice sowing and rice tillering. The soil in the *U. brizantha* area also presented higher nitrate levels, which could help enhance rice yield.

Although ammonium supplementation has advantages, several studies have proved that feeding both nitrate and ammonium further increases rice production. This yield increase is due to the stimulation of ammonium uptake by nitrate (COX; REISENAUER, 1973; SARAVITZ; CHAILLOUS; MUSSET, 1994; RAMAN; SPANSWICK; WALKER, 1995; KRONZUCKER et al., 1999; LI et al., 2006; DUAN et al., 2006, 2007), higher shoot and root production (DUAN et al., 2007; HOLZSCHUH et al., 2009; HACHIYA; SAKAKIBARA, 2017), increased N translocation from roots to shoots (KRONZUCKER et al., 1999; LI et al., 2006), stimulation of the tillering process, and synchronism of leaf development between tillers and the main stem (POLETTO et al., 2011).

Table 3. Rice yield (kg ha^{-1}) and ^{15}N soil recovery (%) in the 0.0–0.10 and 0.10–0.20 m soil layers at rice harvest.

	Yield		^{15}N recovery (0.0 – 0.10 m)		^{15}N recovery (0.10 – 0.20 m)	
	1st year	2nd year	1st year	2nd year	1st year	2nd year
Treatment (N)						
A1	2,689a	3,109a	23	31	2.8	6.7
A2	2,785a	2,879ab	26	28	2.9	3.8
C	2,816a	3,272a	23	22	2.1	4.0
T	1,631b	2,686b	-	-	-	-
Grass (U)						
U. brizantha	2,737a	2,931	23	23	2.8	4.8
U. ruziziensis	2,223b	3,042	26	31	2.4	4.9
			p-value			
N	0.0001	0.0886	0.8429	0.3826	0.1331	0.1362
U	0.0026	0.5026	0.5457	0.1424	0.2944	0.9642
U x F	0.0971	0.1319	0.7285	0.7882	0.6715	0.0258

Means followed by different letters indicate a significant difference by the LSD test at 10% probability. A1: 90 kg N ha^{-1} applied to grass; A2: 90 kg N ha^{-1} applied at rice sowing; C: 90 kg N ha^{-1} applied at rice tillering; T: 0 kg N ha^{-1}.

In the second year, rice production did not differ between the two *Urochloa* areas. This discrepancy compared with the first year, is attributable to differences in climatic conditions. In the second year, total rainfall was higher and well distributed over time (Figure 1), and the greater water availability during grass growth allowed *U. ruziziensis* to grow and produce more straw and roots than in the first year. As *U. brizantha* has more outstanding dry mass production and more vigorous growth than *U. ruziziensis* (CRUSCIOL; SORATTO, 2007; MENEZES et al., 2009; SIMIDU et al., 2010; PACHECO et al., 2011, 2013; TANAKA et al., 2019), it is possible that the rainfall differences did not alter the growth rate of this grass.

In both years, rice yield was higher in the treatments that received N (A1, A2, and C) (up to 2,816 kg ha^{-1} in the first year and 3,272 kg ha^{-1} in the second year) than in treatment T (1,631 and 2,686 kg ha^{-1} in the first and second years, respectively). As all treatments received the same amount of N at rice sowing (30 kg N ha^{-1}), the lower yield in treatment T indicates that the soil N content was not sufficient to fulfill the requirements of rice for this nutrient.

The N supply in the initial phases provided by treatments A1 and A2 was sufficient to guarantee greater initial rice development (Tables 1 and 2). As rice production is directly influenced by the number of tillers and panicles per m^2 (MILLER; HILL; ROBERTS, 1991; FAGERIA, 2007; POLETTO et al., 2011) and most panicles are descended from the first five tillers (WU; WILSON; McCLUNG, 1998), N management practices that enhance initial tiller growth would positively impact rice yield. In addition, N stimulates root system growth (HERNANDES et al., 2010) and positively influences root shape and distribution (GAO et al., 2010; YANG et al., 2012; DING et al., 2015; LIANG et al., 2018), which benefits rice plants under possible drought stress at the beginning of plant development. According to Gao et al. (2017), management practices that enhance drought tolerance are the key to future global food security.

Applying most of N at the rice tillering stage (C) is the most widely used N management practice for upland rice, with the aim of reducing N losses (RAIJ et al., 1996). This premise is based on previous work showing significantly high N losses in plowed and/or sandy soils. However, this premise does not consider the complex dynamics of N in clayey soils with high SOM under NTS.

^{15}Nitrogen recovery in the soil at rice harvest was equal for all treatments in both layers (Table 3). ^{15}N recovery ranged from 22 to 31% in the 0.0–0.10 m layer and 2.1 to 6.7% in the 0.10–0.20 m layer. The higher ^{15}N recovery in the 0.0–0.10 m layer indicates that the fertilizer did not move in larger amounts to deeper layers and remained in the rice radicular zone. Therefore, our hypothesis that N anticipation does not increase N losses is confirmed.

However, the N might still be unavailable to plants due to microbial immobilization. The N in microbial biomass (Figures 2 and 3) ranged between 13 and 20 kg ha^{-1} in the 0.0–0.10 m layer and 3.5 and 19 kg ha^{-1} in the 0.10–0.20 m layer. No differences were found among the treatments at grass desiccation, rice sowing, and rice tillering in both layers, with one exception. Moreover, even if N is immobilized by the microbial population, it is returned to the environment after the death and mineralization of microbial cells (HODGE; ROBISON; FITTER, 2000; SPEDDING et al., 2004, FERREIRA et al., 2010; INSELSBACHER et al., 2010; FERREIRA; MARTIN-DIDONET, 2012). According to Hodge, Robison, and Fitter (2000), the heterogeneous nature of SOM, the decomposer population, and the multiple loops and pathways through which N cycles guarantee that N immobilization occurs simultaneously with N mineralization.

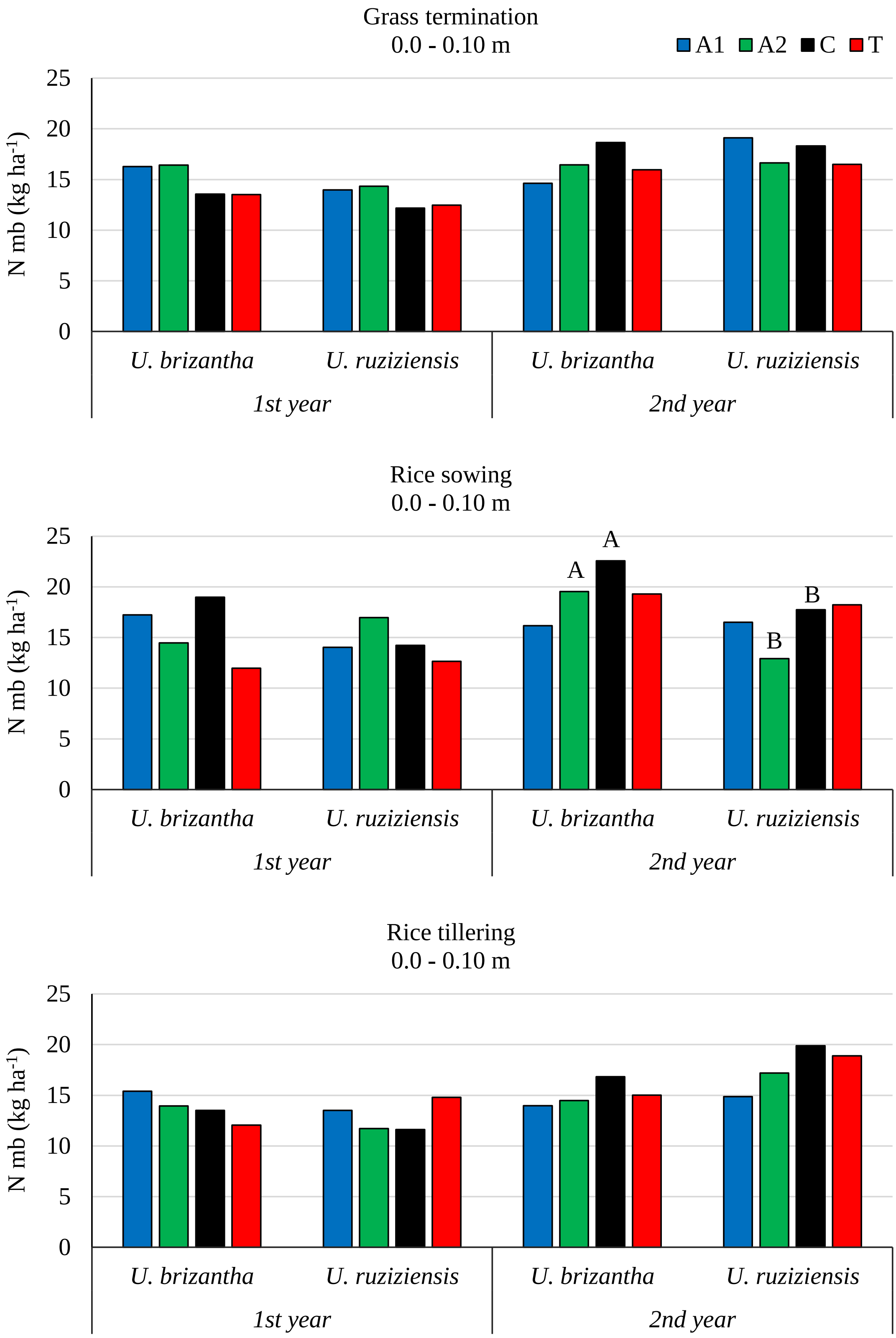

Figure 2. N in the soil microbial biomass (Nmb) (kg ha^{-1}) in the 0.0–0.10 m layer at grass desiccation, rice sowing, and rice tillering. Different lowercase letters indicate differences between treatments within the same grass, and different uppercase letters indicate differences between grasses within the same treatment by the LSD test at 10% probability (P < 0.10). The absence of letters indicates the absence of significance. A1: 90 kg N ha^{-1} applied to grass; A2: 90 kg N ha^{-1} applied at rice sowing; C: 90 kg N ha^{-1} applied at rice tillering; T: 0 kg N ha^{-1}.

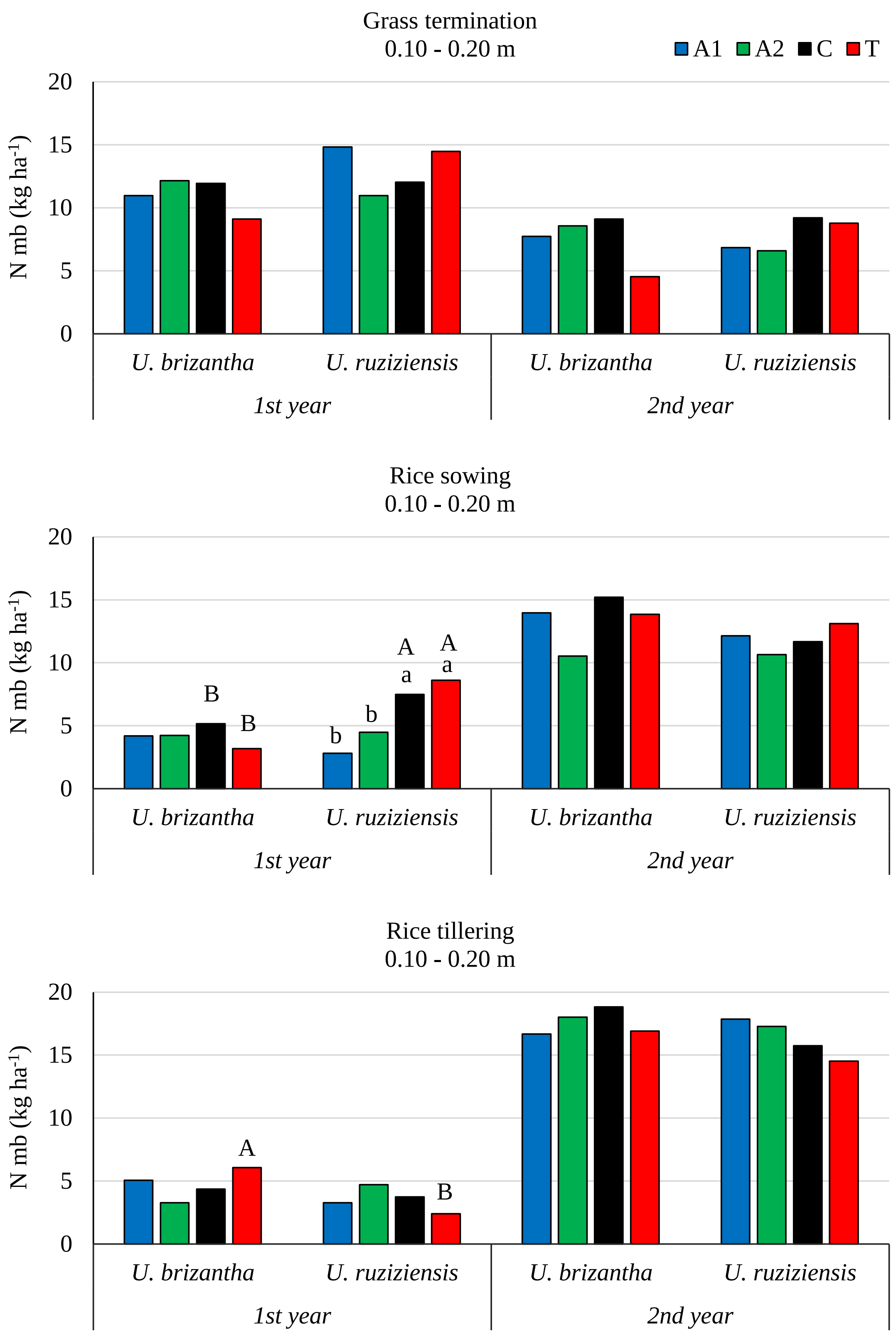

Figure 3. N in the soil microbial biomass (Nmb) (kg ha^{-1}) in the 0.10–0.20 m layer at grass desiccation, rice sowing, and rice tillering. Different lowercase letters indicate differences between treatments within the same grass, and different uppercase letters indicate differences between grasses within the same treatment by the LSD test at 10% probability ($P < 0.10$). The absence of letters indicates the absence of significance. A1: 90 kg N ha^{-1} applied to grass; A2: 90 kg N ha^{-1} applied at rice sowing; C: 90 kg N ha^{-1} applied at rice tillering; T: 0 kg N ha^{-1}.

2.4 CONCLUSIONS

Soil N availability and ammonium and nitrate accumulation were altered by the *Urochloa* species chosen to produce straw for upland rice. At rice tillering, soil nitrate and ammonium accumulation were higher in the *U. brizantha* area than in the *U. ruziziensis* area. In NTS with straw on the soil surface, advance N fertilization did not result in higher N losses nor N microbial immobilization than applying N at rice tillering. Therefore, advance N fertilization under NTS is a viable management strategy for rice farmers.

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APPENDIX

Table 1. pH and soil organic matter (SOM) (g dm^{-3}) in the initial soil characterization.

	pH		SOM	
	2018	2019	2018	2019
0.0 – 0.10 m				
Treatment (N)				
A1	4.7	4.8a	27	34
A2	4.8	4.6b	28	33
C	4.8	4.7b	29	33
T	4.8	4.6b	27	36
Grass (U)				
U. brizantha	4.8	4.8a	29a	34
U. ruziziensis	4.7	4.5b	26b	34
P>F				
N	0.7425	0.0545	0.6914	0.7517
U	0.4588	0.0018	0.0388	0.8952
N x U	0.8295	0.4947	0.5924	0.7559
0.10 – 0.20 m				
Treatment (N)				
A1	4.3	4.7	16	25
A2	4.4	4.6	15	26
C	4.4	4.8	16	26
T	4.4	4.7	15	26
Grass (U)				
U. brizantha	4.4	4.8a	14	26
U. ruziziensis	4.4	4.6b	15	26
P>F				
N	0.7165	0.2113	0.4056	0.7143
U	0.5816	0.0004	0.1239	0.3809
N x U	0.8138	0.2568	0.1014	0.8097

Means followed by different letters indicate a significant difference by the LSD test at 10% probability. A1: 90 kg N ha^{-1} applied to grass; A2: 90 kg N ha^{-1} applied at rice sowing; C: 90 kg N ha^{-1} applied at rice tillering; T: 0 kg N ha^{-1}.

Table 2. *Urochloa* dry matter yield (DMY) (Mg ha^{-1}) and N stock in the *Urochloa* straw (kg ha^{-1}) (NE) at grass desiccation.

	DMY		NE	
	2018	2019	2018	2019
Treatment (N)				
A1	23,3a	20,8	298a	381a
A2	21,0b	18,6	182c	298b
C	24,1a	19,2	209b	301b
T	20,7b	18,9	170c	251c
Grass (U)				
U. brizantha	23,6a	20,7a	223a	353a
U. ruziziensis	20,9b	18,1b	206b	263b
P>F				
N	0,0063	0,4257	0,0001	0,0004
U	0,0011	0,0289	0,0095	0,0001
N x U	0,1041	0,0129	0,0001	0,3743

Means followed by different letters indicate a significant difference by the LSD test at 10% probability. A1: 90 kg N ha^{-1} applied to grass; A2: 90 kg N ha^{-1} applied at rice sowing; C: 90 kg N ha^{-1} applied at rice tillering; T: 0 kg N ha^{-1}.

CHAPTER 3 - NITROGEN MANAGEMENT TIMING AND COVER CROPS ON UPLAND RICE ECONOMIC VIABILITY COMPARED TO FLOODED RICE[1]

ABSTRACT – The objective of this work was to evaluate the economic viability and competitiveness of upland rice compared with flooded rice in a system with different nitrogen fertilization timing and different cover crops. A randomized block design, with four replications, in a 4 × 2 factorial scheme was adopted. The treatments consisted of upland rice grown under *Urochloa brizantha* or *Urochloa ruziziensis* straw without the addition of extra N (0 kg ha-1) (T), with N fertilization (90 kg N ha-1) at rice tillering (C), with N fertilization (90 kg N ha-1) in the cover crop straw one day before rice sowing (A2), with N fertilization (90 kg N ha-1) in the cover crop before desiccation (A1). The total operating cost, gross revenue, operating profit, profitability index, and equilibrium price were determined. Upland rice can result in monetary gain for the farmer as flooded rice when nitrogen is added, regardless the fertilization timing. Under rainfall deficit conditions, rice sown on *U. brizantha* straw shows higher profitability rates than rice sown on *U. ruziziensis* straw. When there is a possibility of a rainfall deficit, *U. brizantha* as cover plant could result in greater economic security.

Keywords: paddy rice, rain-fed rice, *Urochloa brizantha*, *Urochloa ruziziensis*.

[1] Submitted to Pesquisa Agropecuária Tropical

3.1 INTRODUCTION

The search for sustainable agriculture systems that produce more while requiring fewer inputs and water is one of the biggest challenges that agricultural research is facing worldwide. The increase in the average global temperature owing to climate change has led to an increase in the frequency of prolonged periods of drought with changes in the dynamics of precipitation, which may affect the availability of water in the future (BOONWICHAI et al., 2018).

Rice (*Oryza sativa* L.) is the most consumed cereal in the world, and its production requires large amounts of water (MOHANTY et al., 2019), therefore it has received special attention in terms of sustainability. It is estimated that rice grown in flooded soil accounts for most of the rice production (PERON et al., 2019) and for 81% of all Brazilian rice production, on 1.4 million ha (CONAB, 2019).

In 2017, three trillion m^3 of water was used for the global flooded rice production (FAO, 2020), with an average water demand of 15,000 m^3/ha (SUÁREZ; SÁNCHEZ-ROMÁN, 2016). Twenty-two billion m^3 of water was consumed in the 2017/2018 harvest just for the Brazilian production (CONAB, 2019). As part of the water used in irrigation does not return to its original course, there is a reduction in the effective availability of the source (BACK; JUST, 2018). This situation has resulted in upland rice production being viewed as an alternative owing to its water-conserving grain production capacity (PORTUGAL et al., 2015).

In addition to conserving water, upland rice production results in lower greenhouse gas emissions than flooded rice production. As flooded rice is grown in an anaerobic environment, methane (CH_4) and nitrous oxide (N_2O) are formed and emitted (WELLER et al., 2015). As upland rice is cultivated in non-flooded areas, nitrogen loss due to leaching, volatilization, and erosion must be considered, as this is an important contributor to environmental pollution. According to Signor & Cerri (2013), a high amount of N_2O is produced through soil nitrification and denitrification, which are processes associated with the availability and management of nitrogen.

Nitrogen losses due to volatilization and leaching can be reduced by applying nitrogen at the time when the nutrient is absorbed and immobilized by plant tissues, both in growing rice and in advance in growing cover crops. Thus, the study of nitrogen fertilization management is also important. Several studies in no tillage systems have shown that when there is straw over the soil, N fertilization before upland rice sowing is possible and equally practical as split fertilization in guaranteeing upland rice yield (LOPES et al., 2013; ARF et al., 2015; NASCENTE; LANNA, 2016; PINHEIRO et al., 2016). However, none of these studies analyzed the economic aspect of N fertilization in advance.

Decision making must be based not only on technical aspects but also on financial analysis, as the farmer always expects profit, irrespective of which production system will be used (ARAÚJO et al., 2012). Nevertheless, scientific research has to find the balance between monetary profit and environmental protection, which is necessary to preserve the health of the planet; therefore, it needs to ensure that the technologies developed are widely adopted by farmers and result in sustainable food production.

Thus, the objective of this work was to evaluate the economic viability and competitiveness of upland rice compared with flooded rice with the use of different nitrogen fertilization management timing in an area previously occupied by two different cover crops.

3.2 MATERIALS AND METHODS

The experiment was installed in an upland area at the Fazenda Experimental Lageado, which belongs to the Faculdade de Ciências Agronômicas da Universidade Estadual Paulista (FCA/ UNESP), located in the municipality of Botucatu (22º 53' 09"S, 48º 26' 42" W, 840 m altitude), in São Paulo State, Brazil, in the 2017/2018 and 2018/2019 growing seasons.

According to the Köppen-Geiger climate classification, the climatic type of the area is Cwa, which is characterized as high altitude tropical with rainy summers and dry winters. During the two years of the experiment, climatic data (Figure 1) were collected at the Lageado Experimental Farm weather station, which belongs to the Departamento de Recursos Naturais – Setor de Climatologia.

During the 2017/2018 growing season, the total rainfall between October 2017 and April 2018 was 762 mm. During the rice growing and tillering (between December and February), flowering (between February and March), and maturation (March and April) phases, the accumulated rainfall was 324, 54, and 32 mm, respectively. In the 2018/2019 growing season, the total precipitation was 970 mm, and in the growth, flowering, and maturation phases of the rice, the accumulated rainfall was 436, 87, and 127 mm, respectively.

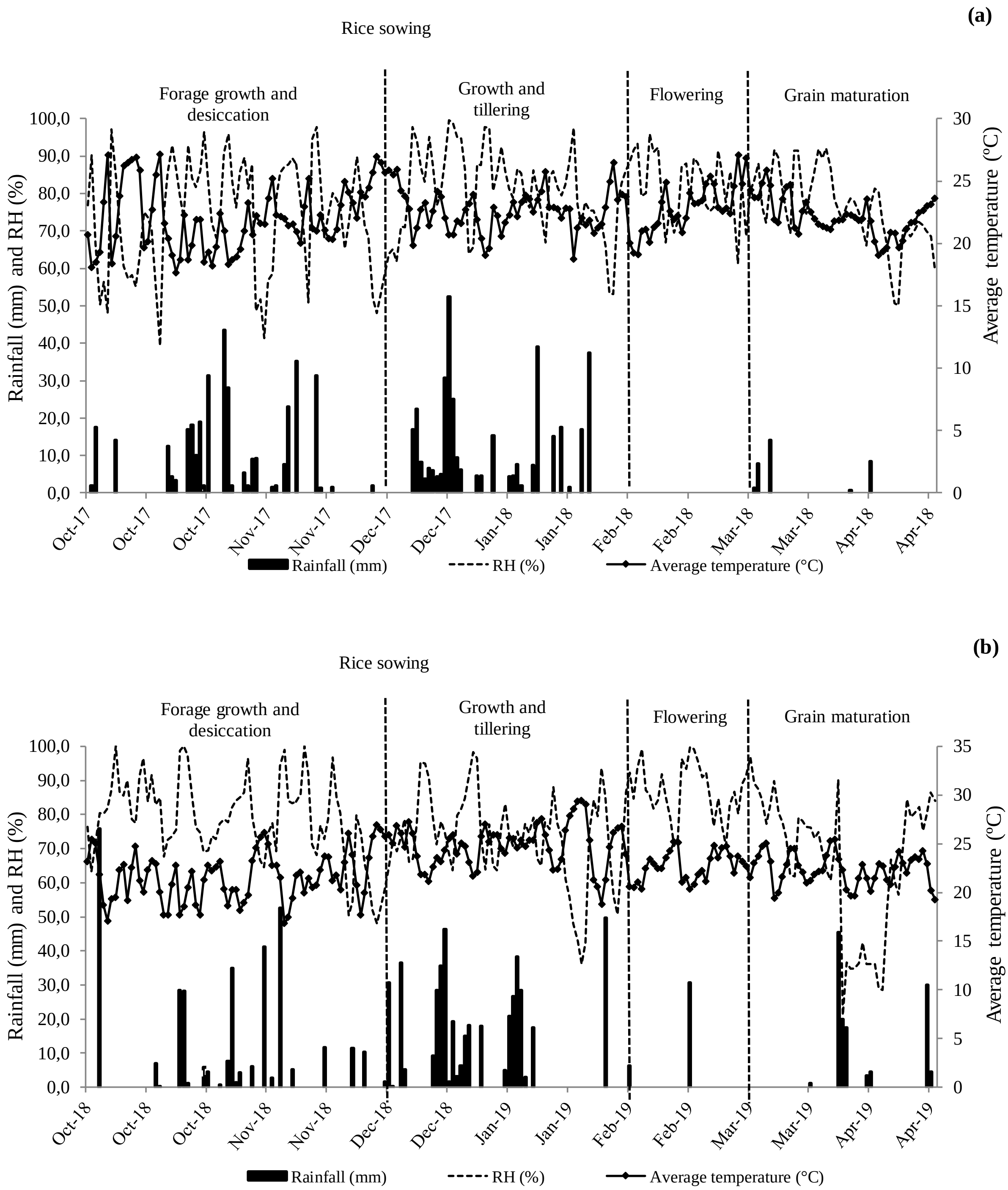

Figure 1. Rainfall, relative humidity of the air (RH) and average temperature for the 2017/2018 (A) and 2018/2019 (B) growing season. Botucatu, São Paulo, Brazil.

The soil is classified as LATOSSOLO VERMELHO Distrófico (SANTOS et al., 2018) or as a Ferralsol (FAO, 2014). Selected soil chemical properties were determined in the 0.0–0.40 m layer in the first and second years as follows: soil organic matter, 21 and 29 g dm^{-3}; pH ($CaCl_2$), 4.6 and 4.7; P (resin), 11 and 12 g dm^{-3}; K^+, 4 and 2 mmol$_c$ dm^{-3}; Ca^{2+}, 22 and 21 mmol$_c$ dm^{-3}; Mg^{2+}, 16 and 10 mmol$_c$ dm^{-3}; cation exchange capacity (CEC), 88 and 104 mmol$_c$ dm^{-3}; base saturation, 47 and 32%.

The experiment consisted of the N fertilization management in upland rice over *Urochloa brizantha* or *Urochloa ruziziensis* straw after the cover crops had been established for two years in the area. A randomized block design was used, with four replications, in a split plot scheme (four N fertilization timing and two cover crops). The treatments consisted of upland rice grown over *U. brizantha* 'Marandu' or *U. ruziziensis* straw without the addition of extra N (0 kg ha^{-1}) (T), with N fertilization (90 kg N ha^{-1}) at rice tillering (C), with N fertilization (90 kg N ha^{-1}) in the cover crop straw one day before rice sowing (A2), with N fertilization (90 kg N ha^{-1}) in the cover crop before desiccation (A1). The treatments description is presented in Table 1. The C treatment consisted of N fertilization at tillering, as recommended by Alvarez et al. (2005).

Table 1. Treatments description.

Treatment	Description
N fertilization timing	
T	30 kg N ha^{-1} at rice sowing + No extra N supply
C	30 kg N ha^{-1} at rice sowing + 90 kg N ha^{-1} at rice tillering
A2	90 kg ha^{-1} one day before rice sowing + 30 kg ha^{-1} at rice sowing.
A1	90 kg ha^{-1} in the living cover crop + 30 kg ha^{-1} at rice sowing.
Grass	
Urochloa brizantha	Upland rice sown over *U. brizantha* straw
Urochloa ruziziensis	Upland rice sown over *U. ruziziensis* straw

At the beginning of November, the cover crops were terminated to produce straw for sowing rice under the no-tillage system. The rice was sown mechanically with a seeder-fertilizer for direct planting, with 0.34 m spacing between rows and seed density appropriate for obtaining 150 plants m^{-2}. Seeding fertilization was carried out according to the recommendation for the crop (RAIJ et al., 1997) in all treatments, using 100 kg ha^{-1} of triple superphosphate (41% P_2O_5), 35 kg ha^{-1} of potassium chloride (60 % K_2O), and 140 kg ha^{-1} of ammonium sulfate (21% N). Chemical control with herbicides and fungicides was carried out simulating operations performed in commercial crops. The rice was harvested mechanically in April 2018 and 2019, when the top 2/3 panicle grains were hard, and the bottom third was semi-hard. Grain yield was measured by weighing the husk grains harvested from the useful area of the plot and adjusted to 13% moisture.

The economic analysis was carried out according to the methodology proposed by Martin et al. (1998), using the concepts of operational cost developed by Matsunaga et al. (1976). The total operating cost (TOC) was derived from the sum of the effective operating cost (EOC), that is, the expenses resulting from mechanized operations, manual operations, inputs, and other operating costs (depreciation, operating costs, and other operating expenses). Depreciation was calculated using the straight-line method by dividing the difference between the initial value and the final value by the useful life of the equipment. The interest cost was calculated as 5.5% over 50% of the EOC, while the other expenses represented 5% of the EOC.

The gross revenue (GR) was obtained by multiplying yield by the selling price per unit. The operating profit (OP) was calculated as the difference between the GR and the TOC, while the profitability index (PI) represented the percentage of OP over GR. The equilibrium price was obtained by dividing the TOC by yield.

The input and operation values were obtained through consultations with farmers in the region, whose technological level was similar to that used in the present study and in establishments that sold agricultural products in the region. As rice is priced based on the quantity and quality of grains, the grain quality was established, and the data were sent to a rice mill in the region to determine the exact market value of the harvested grain batch. A 50 kg bag was valued at R$41.85 and R$42.45 in 2018 and 2019, respectively.

To determine the competitiveness of upland rice compared with flooded rice, flooded rice production cost data was obtained for 2018 from the Agrianual (2019) and corrected the values by the accumulated inflation of 5.84% from April 2018 to April 2019 (IBGE, 2019), for the 2019 production cost calculation. The average yield values were obtained from the crop monitoring report of the Companhia Nacional de

Abastecimento (CONAB, 2019). The purchase values of a 50 kg bag of flooded rice in April 2018 and 2019 in the same rice mill where upland rice prices were quoted were R$34.50 and R$37.55, respectively.

3.3 RESULTS AND DISCUSSION

The economic indicators to produce 1 ha of upland rice are shown in Table 2. The input costs contributed the most to the overall production cost (R$1,406.65 and R$1,423.80, respectively), accounting for about 57.5% of the total operating cost in both years. The fertilizers accounted for 74% (R$1,034.15 and R$1,049.60 in 2017/2018 and in 2018/2019, respectively) of the input costs.

Among the fertilizers used, N was the costliest in both years (Table 2) and the most necessary, as N is the most limiting nutrient both for rice growth and yield (SANTOS et al., 2017; NIANG et al., 2018). As N is so costly, the farmer must pay attention not only to the dose used but also to the time during which it is applied. As N is better absorbed at tillering (ALVAREZ et al., 2005), the usual recommendation is to apply N fertilization during this phase (TADESSE; TADESSE, 2019). However, several studies report an absence of yield losses in upland rice in rice grown under a no-tillage system when N fertilization is applied in advance (LOPES et al., 2013; ARF et al., 2015; NASCENTE; LANNA, 2016; PINHEIRO et al., 2016). When straw is present on a soil surface and presents a high C/N ratio, as in areas under a no-tillage system, the N fertilizer remains immobilized in the system in organic form, in the microbial biomass of the soil (NASCENTE; LANNA, 2016; ASSMANN et al., 2017), is gradually released during the crop cycle, which can help plants make better use of this nutrient.

Operations accounted on average for only 25% of the total operating cost in both years (Table 2). Given that the rice was sown under a no-tillage system, there was no expense in preparing the soil, which resulted in reduced operating costs. In the first year, the biggest expense arose from the sowing operation (26.5% of the total operations - R$154.78) and in the second year from the spraying operations (28.5% - R$184.20) (Table 2). Owing to the higher relative humidity of the air during the grain ripening phase in the second year compared with the first year (Figure 1), rice was more susceptible to blast (*Pyricularia grisea*), the main fungal disease of rice, requiring extra spraying to control the pathogen.

Table 2. Total operational cost of producing 1 ha of upland rice.

	Unit	Quantity	1st year		2nd year	
		(unit ha^{-1})	UV (R$ $unit^{-1}$)	Total (R$ ha^{-1})	UV (R$ $unit^{-1}$)	Total (R$ ha^{-1})
A- Operations						
Desiccation	HM	0.50	122.80	61.40	122.80	61.40
Pulverization (2017/2018)	HM	1.00	122.80	122.80	-	-
Pulverization (2018/2019)	HM	1.50	-	-	122.80	184.20
Seed treatment	HM	0.10	16.11	1.61	16.11	1.61
Seeding	HM	0.55	281.41	154.78	281.41	154.78
Seeding fertilization	HM	0.50	117.06	58.53	117.06	58.53
Cover fertilization	HM	0.30	121.17	36.35	121.17	36.35
Harvest	HM	0.50	295.43	147.72	295.43	147.72
SUBTOTAL A				**583.18**		**644.58**
B- Inputs						
Triple superphosphate	kg ha^{-1}	100	2.84	284.00	2.82	282.00
Potassium chloride	kg ha^{-1}	35	1.89	66.15	1.90	66.50
Ammonium sulfate	kg ha^{-1}	570	1.20	684.00	1.23	701.10
Rice seeds	kg ha^{-1}	50	84.00	84.00	84.00	84.00
Glyphosate	L ha^{-1}	5	25.00	125.00	27.00	135.00
2,4-D (2017/2018)	L ha^{-1}	1.5	16.60	24.90	-	-
2,4-D (2018/2019)	L ha^{-1}	1	-	-	16.60	16.60
Thiram	L ha^{-1}	0.3	32.00	9.60	32.00	9.60
Thiamethoxam	L ha^{-1}	0.3	310.00	93.00	310.00	93.00
Tricyclazole	g ha^{-1}	300	0.12	36.00	0.12	36.00
SUBTOTAL B				**1,406.65**		**1,423.80**
EOC				**1,989.83**		**2,068.38**
Other expenses				99.49		103.42
Interest cost				54.72		56.88
Linear depreciation				269.05		269.05
TOC				**2,413.09**		**2,497.73**

UV: Unitary value; **HM:** machine hours; **EOC**: effective operating cost**; TOC:** total operating cost.

The data corresponding to the profitability indicators is shown in Table 3. In the first year, the lowest yields were obtained from the T treatment, without the addition of N, both in the area previously occupied by *U. brizantha* and in the area previously occupied by *U. ruziziensis.*

Table 3. Rice yield, gross revenue (GR), total operating cost (TOC), operating profit (OP), and equilibrium price (PriE) of a 50 kg bag (R$) of upland rice grown with different nitrogen fertilization regimes and of flooded rice.

1st year					
Treatment	Yield	GR	TOC	OP	PriE
	kg ha^{-1}	(R$ ha^{-1})	(R$ ha^{-1})	(R$ ha^{-1})	R$
U. brizantha					
A1	2,873	2,405.28	2,373.93	31.35	41.31
A2	3,379	2,828.90	2,373.93	454.97	35.13
C	3,005	2,515.79	2,350.03	165.76	39.10
T	1,691	1,415.71	1,857.10	-441.39	54.91
U. ruziziensis					
A1	2,505	2,097.19	2,373.93	-276.74	47.38
A2	2,191	1,834.31	2,373.93	-539.62	54.17
C	2,625	2,197.65	2,350.03	-152.38	44.76
T	1,570	1,314.40	1,857.10	-542.70	59.14
Flooded rice	7,513	5,188.48	5,336.00	-147.52	35.51
2nd year					
Treatment	Yield	RB	COT	LO	PreE
	(kg ha^{-1})	(R$ ha^{-1})	(R$ ha^{-1})	(R$ ha^{-1})	R$
U. brizantha					
A1	3,035	2,576.11	2,458.56	117.55	40.50
A2	2,718	2,307.04	2,458.56	-151.52	45.23
C	3,007	2,552.34	2,434.67	117.67	40.48
T	2,964	2,515.84	1,927.84	588.00	32.52
U. ruziziensis					
A1	3,184	2,702.58	2,458.56	244.02	38.61
A2	3,040	2,580.35	2,458.56	121.79	40.44
C	3,538	3,303.05	2,434.67	568.38	34.41
T	2,408	2,043.91	1,927.84	116.07	40.03
Flooded rice	7,172	5,386.17	5,481.49	-95.32	38.21

A1: 90 kg N ha^{-1} applied to grass; A2: 90 kg N ha^{-1} applied at rice sowing; C: 90 kg N ha^{-1} applied at rice tillering; T: 0 kg N ha^{-1}.

Apart from lower yield, the T treatments also resulted in lower gross revenues and higher equilibrium prices (Table 3). For the T treatment to be profitable in the first year, sale prices of R$54.91 and R$59.14 would be necessary for the areas cultivated with *U. brizantha* and *U. ruziziensis*, respectively; these prices are R$13.00

and R$17.30 higher, respectively than the estimated sales value in April 2018 (R$41.85).

The other treatments (C, A1, and A2), in which 90 kg ha^{-1} of N was added at different times, resulted in higher yields than the national average for upland rice (Table 3), which was 2,409 kg ha^{-1} in the first year (CONAB, 2019), excepting treatment A2 in an area previously occupied by *U. ruziziensis*. All *U. brizantha* straw treatments resulted in higher yields than the *U. ruziziensis* straw treatments did, irrespective of the N fertilization timing adopted (Table 3).

As the TOC was the same within each treatment for both areas occupied by the two cover crops and yield was lower in all treatments applied under *U. ruziziensis* straw (Table 3), the gross income obtained using this cover crop was lower, and the OP was lower. In the *U. brizantha* area, only the treatment without the addition of N (T) resulted in negative OP values.

The rice produced in the flooded system also resulted in negative OP values. Although the yield of this system was more than 5,000 kg ha^{-1} if compared with all upland rice treatments, the TOC was higher owing to the more intensive mechanized and manual requirements for flooded rice production (Table 3). In this case, upland rice would be more competitive than flooded rice when sown in the *U. brizantha* area with the addition of N in coverage, irrespective of the management season. If using an area occupied by *U. ruziziensis*, flooded rice would be more competitive in the market.

In the second year, upland rice yields, irrespective of treatment, and cover crop were higher than the yields of the first year (Table 3). Such differences can be explained by climatic conditions (Figure 1). In the second year, the precipitation during the rice growing and tillering phases was 436 mm, which was higher than the 324 mm recorded in the first year. This difference of more than 100 mm positively influenced the final rice yield. Similarly, the precipitation during the flowering phase had a great influence on rice yield. In the second year, rainfall during the flowering phase was about 60% higher than in the first year; so, the yield was higher.

According to Niang et al. (2018), the productive capacity of upland rice is mainly influenced by the occurrence, duration, and intensity of rainfall in the region. Rice is a very demanding crop in terms of water availability during its development and responds negatively to water stress (PORTUGAL et al., 2015). Even in regions with high precipitation levels, it is common for drought periods or precipitation levels below those required for the full development of the crop to occur during the rainy season (HEINEMANN; STONE, 2009). These phenomena can lead to low yield (NIANG et al., 2018) and losses reductions by up to 32% in the total N uptake and N use efficiency in rice (TERONPI; BHARALI, 2019).

The impact of water deficiency on grain yield varies within the life cycle of the plant and depends on its growth stages (HEINEMANN; STONE, 2009). The onset of water stress in field conditions without irrigation usually occurs in the second half of the growing season and reaches its maximum during the flowering phase (HEINEMANN; STONE, 2009).

As environmental conditions were favorable in the second year, the grain yields of the areas occupied by *U. brizantha* and *U. ruziziensis* were similar (Table 3); this demonstrates the potential of *U. brizantha* to create favorable conditions for the development of the successive crop, especially under water stress conditions. One possible explanation for this phenomenon is the increase in the population of microorganisms in the soil and the activity of the microbiota in the decomposition of organic matter in areas with the presence of *U. brizantha*, as observed by Freitas et al. (2018) and Rocha et al. (2020). Plants can alter the soil microbiology soil differently by sending signals that affect specific microbial communities (HAICHAR et al., 2014; BAPTISTELLA et al., 2020).

During a water deficit period, there can be substantial reductions in the soil microbial activity and biomass owing to a reduction in the number of soluble nutrients and in water-dependent activities (BLACKWELL et al., 2010). When water was not a limiting factor, all treatments in both areas, except for treatment A2 in the area occupied by *U. brizantha*, resulted in a positive OP, indicating that the farmer had a monetary gain. This was a result of the greater increase in yield than in the TOC between the years evaluated.

Although in the second year there was a reduction in flooded rice yield, there was an increase in the marketing cost of rice sacks, which resulted in a less negative OP compared with the first year. In this case, when rainfall conditions were favorable, upland rice was more competitive than flooded rice.

These same results are confirmed by means of the profitability indices that resulted from all the treatments of the upland and flooded rice (Figure 2). In the first year, when the water deficit limited the productive potential of upland rice, only treatments sown on *U. brizantha* straw and with the addition of N were profitable.

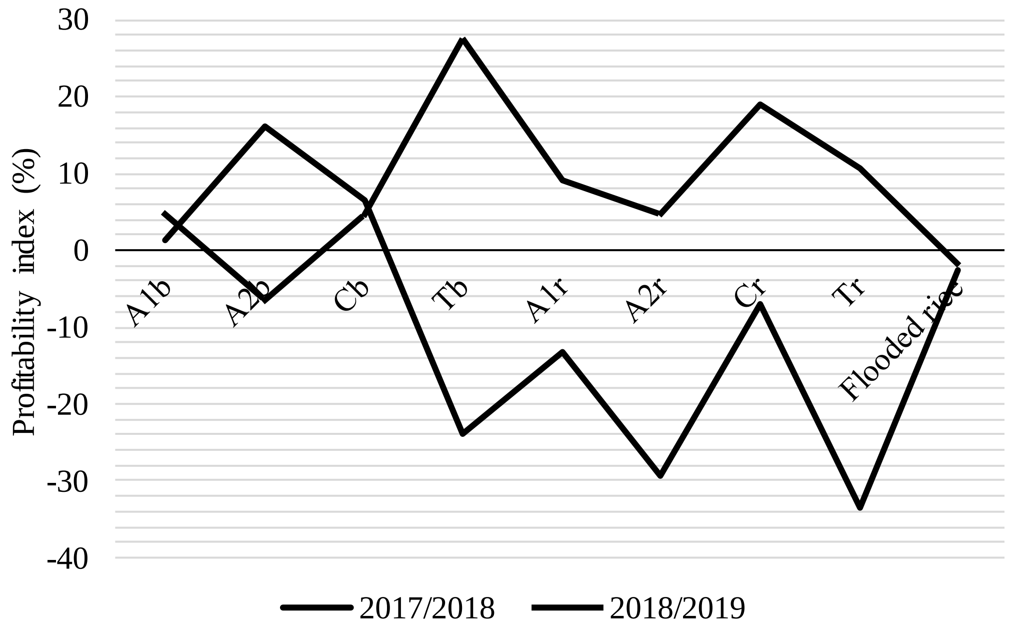

Figure 2. Profitability index (%) of upland rice cultivation under different nitrogen fertilization treatments and of flooded rice in the 2017/2018 and 2018/2019 seasons. A1: 90 kg N ha^{-1} applied to grass; A2: 90 kg N ha^{-1} applied at rice sowing; C: 90 kg N ha^{-1} applied at rice tillering; T: 0 kg N ha^{-1}, b – *U. brizantha* area; r – *U. ruziziensis* area.

As *U. brizantha* has a higher dry matter output than *U. ruziziensis* under free growth conditions (MACHADO; ASSIS, 2010), the straw over the soil after desiccation and during rice growth was thicker, thus reducing the heating of the soil and the loss of water by evaporation. Moreover, the addition of plant material in the form of straw increases the specific surface area of the soil and results in an increase in its water holding capacity (LIBOHOVA et al., 2018). As the soil's water retention capacity represents the amount of water that a given soil can retain to be used by the crop during its development (MOHANTY et al., 2019), the increase in this capacity results in a significant increase in the efficiency of water use.

In the second year, almost all treatments resulted in positive profitability indices (Figure 2), pointing to the competitive capacity of upland rice compared with flooded rice, when water is not a limiting factor.

3.4 CONCLUSIONS

Upland rice (*Oryza sativa*) can result in monetary gain for the farmer as flooded rice when there is no rainfall deficit and when nitrogen is added, regardless of the fertilization timing and the type of cover crop used. Under rainfall deficit conditions, rice sown on *Urochloa brizantha* straw shows higher profitability rates than rice sown on *U. ruziziensis* straw. When there is a possibility of a rainfall deficit, using *U. brizantha* as a cover plant could result in greater economic security.

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GENERAL CONCLUSIONS

In a complex environment like soil under NTS, the behavior of N cannot be assumed to be the same as in plowed soils. Crop rotation and straw maintenance on the soil surface greatly increase the complexity of N dynamics in NTS compared with conventional plowed soils. NTS are characterized by organic matter enrichment over time and the succession of different species, each with different root systems and different influences on the soil microbiota. Consequently, N management needs to be adapted to the specific characteristics of NTS instead of repeating conventional practices that disregard these variations. For example, in NTS, immobilization can have a larger impact than leaching, as shown in this work, and the priming effect can also influence the N cycle and enhance N availability. In this work, upland rice production and N accumulation were the same in all treatments that received extra N supply, even though the plants in the conventional treatment took up more ^{15}N from fertilizer than when N was applied in advance. This indicates the possibility of ^{15}N immobilization when N is applied in advance, but the extra N released by the soil microbial communit was sufficient to assure upland rice yield. In NTS, the species chose as the cover crop also greatly influences the N dynamics of the subsequent crop. Although *U. brizantha* and *U. ruziziensis* belong to the same genus, the soil in the *U. brizantha* area had a higher N content during the subsequent upland rice crop. These discrepancies highlight the need for a renewed and extensive study of N fertilization management to create a new database that considers the greater variability in conservation agriculture and provides improved knowledge of soil N.

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