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LETUSA MOMESSO MARQUES

IMPACTS OF NITROGEN APPLICATION ON FORAGE GRASSES TO MAIZE IN NO-TILLAGE SYSTEM

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TÍTULO DA TESE: IMPACTS OF NITROGEN APPLICATION ON FORAGE GRASSES TO MAIZE IN NO-TILLAGE SYSTEM

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To all those who work in science with
hope their contributions and discoveries
may change the world.

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“The real voyage of discovery consists not in seeking new landscapes, but in having new eyes”.

Adapted from PROUST, M. Remembrance of Things Past: The Captive. 1923.

ABSTRACT

The success of no-tillage system depends on the knowledge of the agricultural system as a whole. The use of grass *Urochloa* sp. as cover crop in agriculture results in slow organic material decomposition due to high biomass production and changes in soil microbe, in particular in biological processes related to nitrogen (N). Because N is a nutrient present in the main biochemical reactions in plants and microorganisms, N management requires special attention. Therefore, this research aimed to improve N-use efficiency from both agronomic and biological perspectives. The main objectives were to (i) assess the impact of N fertilizer and forage species on maize in the NT system, and (ii) determine the interactions between microbes x N x environmental factors. A field experiment was evaluated, in which palisade grass (*Urochloa brizantha*) and ruzigrass (*U. ruziziensis*) grown with four N management, included: (i) control zero-N (no N application), (ii) N applied on green cover crops at 35 days before maize seeding (35 DBS), (iii) N applied on cover crop residues at 1 day before maize seeding (1 DBS), and (iv) conventional method of N applied at sidedressing in maize growth), at a rate of 120 kg N ha^{-1} as ammonium sulfate. The hypothesis of *Chapter 1* that N applied on alive cover crops or cover crop residues could replace N-sidedressing application (conventional method) for maize was confirmed when: (a) N was applied on palisade grass at 35 DBS or its residues at 1 DBS, and (b) N was applied on ruzigrass residues at 1 DBS. Due to results of first chapter, another experiment was conducted with the objective of assessing whether either the early N application on alive cover crops or on cover crop residues or the conventional method of N application contributed to the recovery of total-N and fertilizer ^{15}N by maize, by cover crop residues, and in the soil over growing season. Although the hypothesis that N applied on palisade grass to achieve high grain yields of maize was previously confirmed, the results *Chapter 2* showed that the best option is applying nitrogen fertilizer as the current fertilizer recommended method (40 kg N ha^{-1} at maize seeding plus 120 kg N ha^{-1} sidedressed in V$_6$ growth stage) for enhance grain yields of maize and N recovery from fertilizer.

Keywords: *Brachiaria*. *Zea mays* L.. Crop residues. ^{15}N. Nitrogen uptake efficiency. Tropical agriculture.

RESUMO

O sucesso do sistema de plantio direto depende do conhecimento do sistema agrícola como um todo. O uso de gramíneas do gênero *Urochloa* como planta de cobertura resulta em lenta decomposição do material orgânico devido à alta produção de matéria seca e alterações nos microrganismos do solo, em particular nos processos biológicos relacionados ao nitrogênio (N). Como o N é um nutriente presente nas principais reações bioquímicas em plantas e microrganismos, o manejo deste nutriente requer atenção especial. Portanto, este trabalho de pesquisa teve como objetivo melhorar a eficiência do uso do manejo do N. O principal objetivo foi avaliar o impacto do adubo nitrogenado aplicado nas duas espécies de gramíneas ou nos seus resíduos para suprir a demanda e aumentar a produtividade de grãos do milho no sistema plantio direto. O experimento de campo foi conduzido durante três anos, no qual *Urochloa brizantha* e *U. ruziziensis* foram cultivadas com 4 manejos da adubação nitrogenada. Os manejos da adubação nitrogenada foram: (i) controle (zero aplicação de N), (ii) N aplicado 35 dias antes da semeadura do milho (35 DAS), (iii) N aplicado 1 dia antes da semeadura do milho (1 DAS), e (iv) método convencional (N aplicado em cobertura no crescimento do milho), com a dose de 120 kg ha^{-1} de N da fonte sulfato de amônio. A hipótese no *Capítulo 1* de que o N aplicado nas plantas de cobertura ou nos resíduos destas plantas de cobertura poderiam ser substituir a aplicação de N em cobertura do atual método convencional para cultura do milho foi confirmada quando o N foi aplicado na *U. brizantha* aos 35 DAS ou em seus resíduos 1 DAS e quando o N foi aplicado nos resíduos da *U. ruziziensis* 1 DAS. Devido aos resultados observados no primeiro capítulo, o *Capítulo 2* objetivou avaliar se a aplicação antecipada de N (nas plantas de cobertura ou nos resíduos das plantas de cobertura) e a aplicação de N no método convencional contribui para o teor total de N e a recuperação do ^{15}N do fertilizante pelo milho, pelos resíduos das plantas de cobertura e no solo ao final da safra. Embora a hipótese de que a aplicação de N na *U. brizantha* tenha sido confirmada anteriormente para atingir altas produtividade de grãos de milho, os resultados do segundo capítulo mostraram que a aplicação do N deve ser realizada como recomendado no método convencional (40 kg ha^{-1} na semeadura e 120 kg ha^{-1} em cobertura) para, além de atingir altas produtividade, recuperar maior quantidade do fertilizante nitrogenado aplicado.

Palavras-chave: *Brachiaria*. Plantas de cobertura. Fertilizante ^{15}N. Eficiência do uso do nitrogênio. Sistema semeadura direta.

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SUMMARY

GENERAL INTRODUCTION

Over time, the plant residues left on the soil surface in a no-tillage (NT) system gradually improve the physical, chemical, and biological characteristics of the soil (SILVA et al., 2014; TIRITAN et al., 2016; MORAES et al., 2019). Used frequently in tropical countries, this practice increases grain yields and improves environmental performance (DERPSCH et al., 2014; GUZMAN; GOLABI et al., 2017). In NT systems, food production costs are lower. It is easier to operate machines in fields, which improves soil sustainability, due to lower soil disturbances. However, the success of a no-tillage system depends on several important factors: growing crops in undisturbed soil, rotating crops and maintaining crop residues on the soil surface (DERPSCH et al., 2014; DUARTE et al., 2018).

Vegetal residues of cover crops are used in integrated livestock farming and grain food production systems (SILVA et al., 2015; MATEUS et al., 2016; MORAES et al., 2019; SCHUSTER et al., 2019). Use of these vegetal residues improves soil properties, inhibits the spread of diseases, reduces pests and weeds as well emissions of greenhouses gasses (FREITAS; LANDERS, 2014; MORAES et al., 2014; Mckenzie et al., 2016; SCHUSTER et al., 2019). The choice of the cover crop is very important and determines the success of the system (PARIZ et al., 2011). This is especially true in tropical soils due to the fact that plant characteristics impact biomass yield and the durability of soil coverage (LEITE et al., 2010; PAVINATO et al., 2017). Cover crop biomass contains nutrients extracted from deeper soil layers and plays an important role in nutrient cycling as a result of the release of nutrients during the decomposition process (CRUSCIOL; SORATTO, 2009; VERAS et al., 2016; ROSOLEM et al., 2017).

Legume and grass cover crops vary widely in their ability to cover the soil (CRUSCIOL et al., 2015; FAGERIA et al., 2016). Although legumes fix atmospheric nitrogen, grasses are better at scavenging nutrients. Among the cover crops, grasses are most widely cultivated and used for livestock as well as agricultural activities by farmers (COSTA et al., 2017; PARIZ et al., 2017; CATUCHI et al., 2019). The most commonly used grasses are from the *Urochloa* genus. These grasses produce a large amount of biomass, which has high soil protection properties and nutrient-cycling efficiency (BORGHI et al., 2013; PACHECO et al., 2017; TANAKA et al., 2019). When cultivated as a cover crop, forage grass is managed as an annual crop in the system to produce biomass (BORGHI et al., 2013). The high dry matter production potential and the high C:N ratio of *Urochloa* result in slow decomposition and increase the possibility of cultivation in warmer regions, even in regions were other cover crops have accelerated rates of decomposition (TIMOSSI et al., 2007; ROSOLEM et al., 2017). Other characteristics, such as vigorous and deep root systems, favor water deficiency tolerance and absorption of nutrients in deeper soil layers, aiding nutrient cycling (CRUSCIOL; SORATTO, 2009; ALMEIDA et al., 2018). Thus, grasses perform well in drought conditions where most of the grain crops or other cover crops do not grow well (CASTRO et al., 2015; CRUSCIOL et al., 2015).

Urochloa species are less demanding when it comes to soil fertility. The roots tolerate aluminum toxicity and low P availability in the soil, which occurs often in acidic tropical soils (ARROYAVE et al., 2018). In addition, the roots of these grasses suppress soil nitrification, which is one of the key microbial processes (SUBBARAO et al., 2009, 2015). *Urochloa* species release brachialactone, a biological nitrification inhibitor (BNI), that blocks ammonia monooxygenase (AMO) and hydroxylamino oxidoreductase (HAO) ammonia oxidizing enzymatic pathways (SUBBARAO et al., 2007, 2009). In the complex soil-plant-atmosphere system, the major processes in the soil are nitrogen fixation, soil organic matter (SOM) mineralization, ammonification, nitrification, and denitrification (WILCKE; LILIENFEIN, 2005; SUBBARAO et al., 2015; KUYPERS et al., 2018). Nitrogen plays a minor role in undisturbed temperate and tropical ecosystems, such as no-tillage systems, where nitrogen leakage is minimized and a large amount of nitrogen is retained in the soil (SUBBARAO et al., 2015). There are mechanisms of nitrogen conservation that involve short-circuiting mineralization, which microorganisms absorb nitrogen and return it to the soil, facilitating nitrogen accumulation in the soil when plants suppress nitrification and directly absorb organic nitrogen (SUBBARAO et al., 2015; KARWAT et al., 2017).

Nitrogen losses occur mainly through critical pathways of nitrification and denitrification (VAN GROENIGEN et al., 2015; ZHANG et al., 2015). *Urochloa* species cultivated in the no-tillage systems are expected to reduce nitrogen losses due to nitrate leaching and nitrous oxide emissions. One key role of tropical forage grasses has been suppressing leached nitrate and mitigating nitrous oxide emissions in pasture soils (BYRNES et al., 2017; KARWAT et al., 2017). However, there is a lack of information about forage grasses as cover crops in systems of grain food production. The nitrogen use efficiency (NUE) of fertilizer can be enhanced for grain crops when it is combined with nitrogen management on cover crops. Nevertheless, cover crop species affect the subsequent crops differently. This becomes important when investigating the influence of cover crops on the grass-maize system.

The organic nitrogen from the biomass of cover crops is degraded through mineralization and subsequently, nitrification in ammonium and nitrate in the soil (KUYPERS et al., 2018). The biomass of cover crops can drive shifts in soil microbial activity, resulting in mineral nitrogen. Since the plant roots take up nitrogen in the inorganic forms ammonium and nitrate (BOSCHIERO et al., 2018), the decomposition rates of nitrogen released in the soil determine synchrony/de-synchrony between soil nitrogen mineralization and plant nitrogen demand (PERVEEN et al. 2014; ROSOLEM et al., 2017). There is a huge potential to reduce nitrogen fertilizer use and nitrogen loss in agricultural system composed of *Urochloa* (KARWAT et al. 2017; MOMESSO et al., 2019; ROCHA et al., 2019).

The current recommended method of applying nitrogen fertilizer to annual crops is to divide the application over two periods. The first application occurs when seeding a crop and the second occurs when the crop is in its growing stage in a manner called ‘sidedressing’. However, an alternative method has been proposed which would actually supply the nitrogen fertilizer needed for annual crops, such as maize, during the cultivation of cover crops like *Urochloa*. The farmers would apply all the nitrogen either to cover crops or during pre-seeding of maize (BASSO; CERETTA, 2000; LARA CABEZAS et al., 2004, 2005; PÖTTKER; WIETHÖLTER, 2004), when the systems have high straw production (CERETTA et al., 2002). The early application of nitrogen could facilitate the main crop seeding, providing flexibility in the operational schedules of farmers. However, the application of all of the nitrogen on *Urochloa* cover crops does not supply the nitrogen to the subsequent maize crops due to the temporarily nitrogen immobilization. In systems composed of grasses grown in succession, microorganisms compete with plants for nitrogen in the soil during crop seeding due to increasing biological activity and consequent plant-microbe competition (MOMESSO et al., 2019).

An alternative and sustainable way to use nitrogen fertilizers could be to follow the initial recommended application of nitrogen fertilizer during maize seeding. However, instead of following the second half of the recommended application method, which says that nitrogen should be applied to growing crops (sidedressing), the nitrogen should actually be applied earlier to cover crops or on cover crop residues. Applying nitrogen directly to maize during seeding aims to reduce competition between the maize crop and microbes for nitrogen immobilization in the soil. The application of nitrogen during maize seeding minimizes the competition between plants and microbes and thus avoids nitrogen immobilization. Applying nitrogen to the cover crops or cover crop residues may be a good substitute for sidedressing when growing forage species. The use of fertilized *Urochloa* can be effective to gradually provide nitrogen to subsequent maize crops during residue decomposition due to the great potential of *Urochloa* to produce biomass and tighten nitrogen cycling.

There has been a growing interest in manipulating plant growth in order to increase NUE and grain yields in tropical food production (BOWATTE et al., 2015). The nitrogen fertilizer used in grass systems enhances grain yields of annual crops by improving biomass production in agricultural systems. In a no tillage system, this biomass gradually releases nutrients to subsequent crops. Keeping this in mind, the current thesis starts by assessing how the forage grass and the maize crop are affected by the timing of nitrogen fertilizer application. In *Chapter 1*, the effects of the timing of nitrogen application on decomposition rates of cover crops was monitored for 3 years during a field experiment. In this experiment, biomass production, nitrogen released by cover crops and the availability of mineral nitrogen in the soil was evaluated in order to enhance grain yields of maize. In *Chapter 2*, the fate of nitrogen fertilizers $[(^{15}NH_4)_2SO_4]$ applied at different times to maize and forage grasses was examined. This thesis presents potential strategies to optimize the sustainable use of nitrogen fertilizer in maize-forage systems and examines how grasses can supply nitrogen to subsequent maize crops in tropical soils during decomposition.

CHAPTER 1

CAN NITROGEN APPLICATION ON *Urochloa* COVER CROPS REPLACE THE NITROGEN RECOMMENDATIONS FOR MAIZE?

ABSTRACT

Nitrogen (N) management is highly dependent on the cropping system. Optimizing N-use efficiency (NUE) and enhancing cash crop yields are challenges for no-tillage (NT) systems. In NT systems, the biomass of cover crops such as *Urochloa* spp. releases nutrients for use by the subsequent crop. Consequently, applying N fertilizer to cover crops can increase plant biomass and enhance N cycling to the soil along with plant residue mineralization. The aim of this study was to (i) investigate N application on *Urochloa* spp. cover crops or cover crop residues as a substitute for N sidedressing (conventional method) for maize and (ii) investigate the supply of mineral N in the soil and the rates of biomass decomposition and N release. The treatments comprised two species, i.e., palisade grass (*Urochloa brizantha*) and ruzigrass (*Urochloa ruziziensis*), and four N management strategies: (i) control zero-N (no N application), (ii) N applied on green cover crops 35 days before maize seeding (35 DBS), (iii) N applied on cover crop residues 1 day before maize seeding (1 DBS), and (iv) conventional method (N sidedressing of maize). The maximum rates of biomass decomposition and N release were observed in the treatments with palisade grass. The biomass of palisade grass and ruzigrass increased with N application, and mineral N in the soil increased with N application regardless of cover crop species. Grain yields and maize NUE were not affected when N was applied on palisade grass 35 DBS or on its residues 1 DBS. However, N applied on ruzigrass 35 DBS decreased maize grain yields. Our results indicate that N fertilizer can be applied on palisade grass 35 DBS or its residues 1 DBS as a substitute for conventional sidedressing application for maize.

Keywords: *Zea mays*. Food production. Cover crop. N-use efficiency. Tropical systems.

1.1 INTRODUCTION

Long-term agricultural projections report that the world’s population is growing at a rate of at least one percent per year (USDA, 2019; WORLDOMETERS, 2019). Historically, solutions for meeting the increasing demand for food have emphasized the expansion of cultivated areas, but recently the focus has shifted to increasing yields within existing agricultural systems. No-tillage (NT) has been proposed as an approach to increase yields, N-use efficiency (NUE), and system sustainability by introducing cover crops (ROSOLEM et al., 2017). The maintenance of cover crop residues on the soil surface is more economical than tilling and supports higher yields in the short- and long-term (TURMEL et al., 2015; CAMAROTTO et al., 2018). Crop residues cycle nutrient-replenishing soil organic matter and prevent soil erosion (COLEMAN et al., 2018). The choice of species used as a cover crop determines the dynamics of carbon (C) and nitrogen (N) within the system and thus nutrient cycling (AITA et al., 2004; COLEMAN et al., 2018). Plants of the genus *Urochloa* are commonly used as cover crops in the tropics due to their high biomass and vigorous, deep root systems (PACHECO et al., 2011; SORATTO, 2011; MORO et al., 2013; COSTA et al., 2016). This deep root architecture increases nutrient uptake from soil and enables these species to grow in harsh off-season conditions such as drought (FELISMINO et al., 2012; PACHECO et al., 2017; ROSOLEM et al., 2017). In addition, the residues of *Urochloa* spp. offer other benefits, such as improved soil health, weed suppression and nutrient loss avoidance (CASTRO et al., 2015; BÜCHI et al., 2019).

Nitrogen fertilizer application may maximize the absolute biomass yield of cover crops. Efficient N application improves the sustainability of food-producing systems by preventing loss of excess N through nitrate leaching and nitrous oxide emission. Planning N fertilization by accounting for the decomposition of cover crops and N release can improve the NUE of the subsequent crop (OENEMA et al., 2015; BANI et al., 2018). The N available from cover crops depends on biomass mineralization by microorganisms (GATIBONI et al., 2011, LIU; SUN, 2013). The N quantity, quality and amount of biomass affect the rate of decomposition and consequently the synchronism of N release and N demand by the next crop. Residues with high C/N ratios may reduce N availability through immobilization. Few studies of synchronism of N release from plant residues with crop demand have mainly been performed under tropical climates (CANTARELLA, 2007; ROSOLEM et al., 2017).

The conventional recommendation for N fertilizer in maize is to split the application: up to 30 kg ha^{-1} at planting and sidedressing of the balance when the plant has 5 to 7 leaves (CANTARELLA et al., 1997). NUE and maize grain yield are increased in systems where *Urochloa* spp. are fertilized with N. However, Momesso et al. (2019) observed that despite an increase in cover crop biomass with N application, the early application of N on cover crops was not sufficient to meet the N demand of maize. Since maize demands high amounts of N and grass cover crops can cause temporary N immobilization, it was hypothesized that N application on cover crops or over the cover crop residues could be a substitute for only the second N application used conventionally rather than all N. Moreover, the early application of N could facilitate the management of the main crop, thus providing flexibility in the operational schedules of farmers. This study aimed to measure dry matter (DM) yield, biomass persistence and N release of palisade grass [*Urochloa brizantha* (Hochst. Ex A. Rich.) R.D. Webseter] and ruzigrass [*U. ruziziensis* (R. Germ. and C.M. Evrard) Morrone and Zuloaga] fertilized with N and to assess the effects of N application on live grass or cover crop residues on maize grain yield and NUE.

1.2 MATERIAL AND METHODS

1.2.1 Field experimental characterization

The experiment was conducted over three consecutive seasons from 2015 to 2018, in Botucatu, São Paulo, Brazil (48° 26´ W, 22° 51´ S, 740 m above sea level). The regional climate, according to the Köppen classification, is Cwa, i.e., tropical with dry winter, with a warm and wet summer. Annual rainfall is 1358 mm and the average annual minimum and maximum temperatures are 15.3 and 26.1 ^{o}C, respectively. During the experiment, rainfall and temperature were recorded on a meteorological station located nearby the site (Figure 1). The soil is a clay, kaolinitic, thermic Typic Haplorthox (USDA, 2014), with 630, 90, and 280 g kg^{-1} of clay, silt, and sand, respectively. Selected chemical characteristics of the top soil (0-20 cm) are in Table 1. The experimental area had been cropped under No-till for 9 years before the experiment.

The experiments were conducted in a 2 × 4 factorial design in randomized blocks with four replicates. The treatments consisted of two cover crop species: palisade grass [*Urochloa brizantha* (Hochst. Ex A. Rich.) R.D. Webseter] and ruzigrass [*U. ruziziensis* (R. Germ. and C.M. Evrard) Morrone and Zuloaga], and four N management strategies: (i) control (no N application), (ii) 120 kg N ha^{-1} broadcast over the live grass cover crop 35 days before maize seeding (DBS) (5 days before cover crop termination), (iii) 120 kg N ha^{-1} broadcast over terminated grass cover crop 1 day before seeding of maize (pre-seeding of maize), and (iv) 120 kg N ha^{-1} sidedressed at V_6 growth stage (six expanded leaves) of maize [conventional method, recommended by Cantarella et al. (1997)]. In all treatments, except for the control, 40 kg N ha^{-1} was applied in the seeding furrow, amounting to 160 kg N ha^{-1}, applied as ammonium sulfate. The plots were 4.5 m wide and 8.0 m long and at each end of each plot 1.0 was considered as buffer.

1.2.2 Crop management

The grasses were sown at a density of 10 kg ha^{-1} seed (34% viable seeds) without fertilizer. In all growing seasons the cover crops were cultivated approximately eight months from April to November. The cover crops were cut 30 days before desiccation 0.30 m above soil level by mechanical mowers in order to stimulate growth and N uptake.

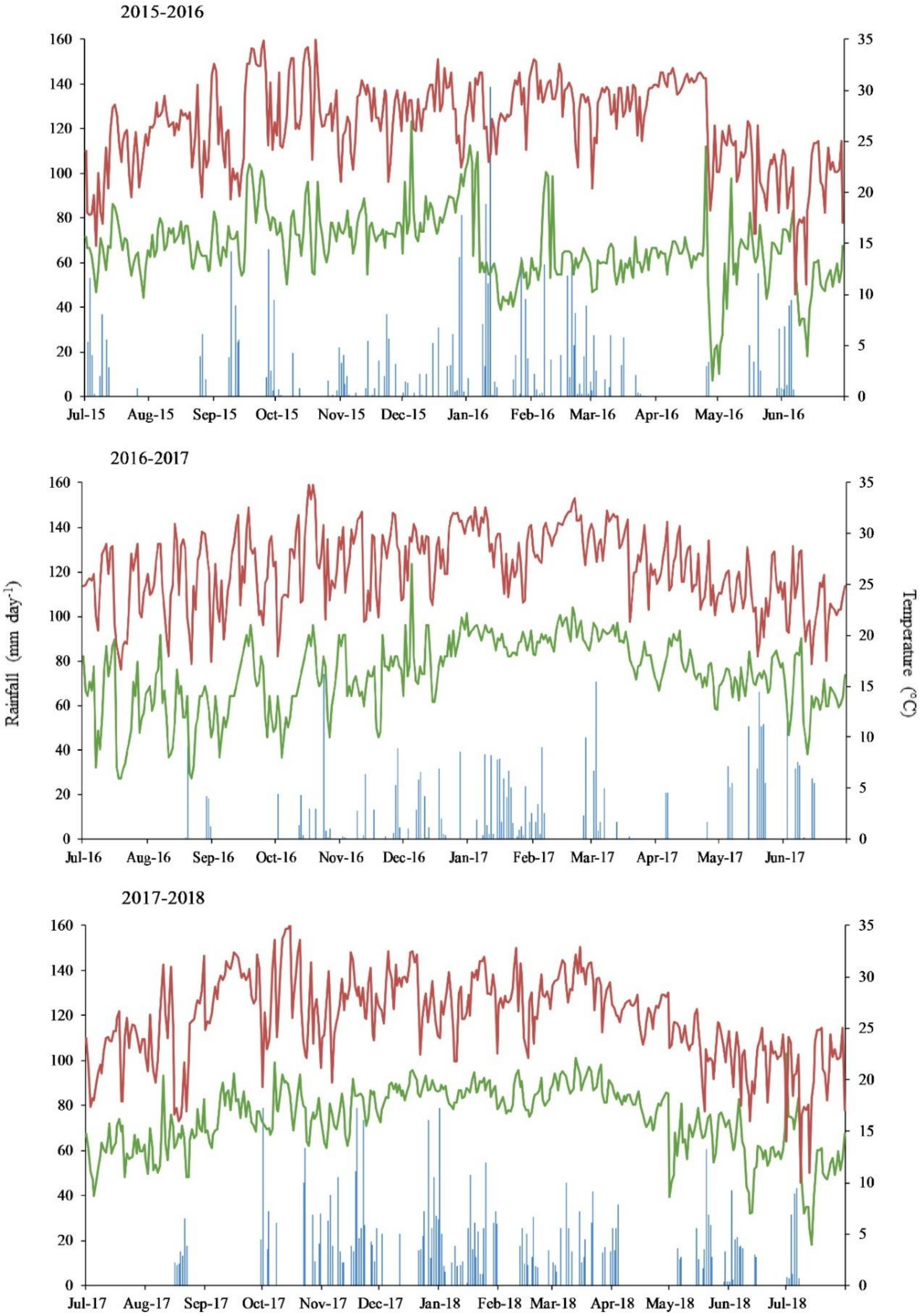

Figure 1. Daily rainfall (— blue bars), maximum (— red lines) and minimum (— green lines) temperatures observed in a meteorological station located near the field site at Botucatu, Sao Paulo State, Brazil, during periods in 2015-2016, 2016-2017, and 2017-2018 growing seasons.

Table 1. Chemical properties of field experiment soil (0-20 cm depth).

Growing season	pH	SOM †	P$_{(resin)}$	H+Al	K$^+$	Ca^{2+}	Mg^{2+}	CEC ‡	BS §
	(CaCl$_2$)	g dm^{-3}	mg dm^{-3}	mmol$_c$ dm^{-3}					(%)
2015/16	4,8	32	19	44	4,9	34	18	101	56
2016/17	4,6	24	18	39	3,8	37	16	96	59
2017/18	4,5	27	20	42	3,3	31	21	98	57

† Soil organic matter.
‡ Cation exchange capacity.
§ Base saturation.

The first N application was carried out on green cover crops at 35 DBS of maize on 24 Oct. 2015, 27 Oct. 2016, and 27 Oct. 2017 (Figure 2). Then 5 days later, the cover crops were desiccated with glyphosate at 1.56 kg ha^{-1} (a.i.) on 29 Oct. 2015, 01 Nov. 2016, and 01 Nov. 2017. The second N application was carried out over the cover crop straws 1 DBS of maize on 25 Nov. 2015, 26 Nov. 2016, and 30 Nov. 2017. The hybrid maize used was P3456 Pioneer, seeded 30 days after cover crop termination at a density of 65,000 seeds ha^{-1}, and all N treatments received 40 kg ha^{-1} of N applied next to the seed line. The balance of the N fertilizer was sidedressed when maize was at the V$_6$ growth stage. Maize was harvest 125 days after planting.

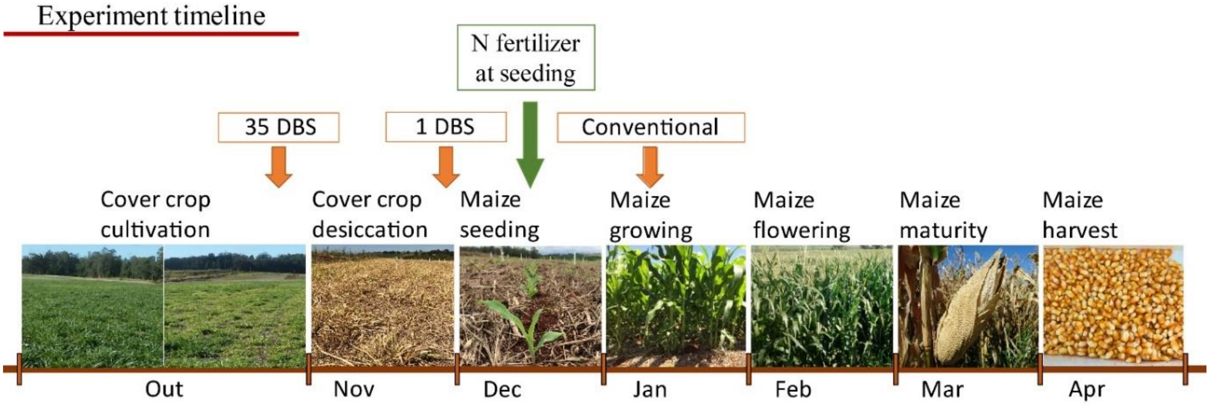

Figure 2. Experiment timeline of crop management and N application timing. Nitrogen application timing treatments in orange boxes are as follows: 35 DBS: 120 kg N ha^{-1} broadcast over cover crop 35 days before maize seeding (5 days before cover crop termination); 1 DBS: 120 kg N ha^{-1} broadcast over crop 1 day before maize seeding; Conventional method: 120 kg N ha^{-1} sidedressed at V$_6$ growth stage of maize. The green box means application of 40 kg N ha^{-1} at maize seeding to all N treatments.

1.2.3 Sampling and analyses

Samples of cover crops dry matter (DM) were taken at termination (DAT), 30, 60, 90 and 120 days later. Two $0.25m^2$ sub-samples were taken per plot using a wood frame and combined. Sampling was performed at random points along diagonal crosswise lines, excluding 1.0 m at either end (border). Fresh samples were oven-dried at 65 ^oC, weighed for dry-weight determination, and ground in a Wiley mill to pass through a 0.5-mm sieve. Subsamples were used to N concentrations determination on elemental analyzer (LECO-TruSpec® CHNS). The N accumulated on dry matter determinations were extrapolated to Mg ha^{-1} of dry matter.

Soil samples (0-10 and 0-20 cm depths) were collected at cover crop termination, and 30, 60, and 90 days later for determination of $N\text{-}NH_4^+$, $N\text{-}NO_3^-$ and total-N. Samples were taken a with 2.5 cm diameter auger. To stop soil N transformations, the samples were put in plastic bags and conditioned in a freezer at -20 ^oC. The $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ were extracted with KCl and distilled (KEENEY; NELSON, 1982). For total-N content, samples were air-dried, ball milled, and analyzed with an elemental analyzer (LECO-TruSpec® CHNS), using 0.2 g of soil.

Maize leaf samples were collected for nutrient concentration analysis when 50% of the plants were at full flowering stage (silking) by taking 20 leaves of the ear (CANTARELLA et al., 1997) per plot. The leaf samples were dried in forced-air circulation at 65 ^oC for 72h and ground. Samples were digested with sulfuric acid for N determination, and with a nitro-perchloric (HNO_3 + $HClO_4$) solution for the other nutrients. Then, concentrations of N, P, K, Ca, Mg, and S in the leaves were determined according to the methods of Malavolta et al. (1997). Shortly, N concentration was determined using the semi-micro-Kjeldahl distillation method, P was determined by colorimetry method, S was determined by turbidimetry method, and K, Ca and Mg were determined using atomic absorption spectrophotometry.

At the same time of maize leaf sampling, 5 whole plants were collected per plot to determine the shoot DM of maize. The samples were chopped and dried in a forced-air oven at 65 °C for 72 h and weighed. The time from maize planting to physiological maturity in the first, second, and third years were 130, 129, 130 d after plant emergence, respectively. Maize was harvested 7 days after physiological maturity from a 10.8 m^2 area in each plot using a mechanical harvester. Grain weight was determined, and data were transformed to grain yield per hectare at 130 g kg^{-1} moisture content. Final plant population (the number of plants in the four central rows of the 6-m rows in each plot) was determined by extrapolated data to hectare, and plant height, number of ears per plant, number of grains per ear, and weight of 100 grains were evaluated from 10 plants per plot chosen at random. The N-use efficiency (NUE) was determined when there was an increase in yield compared to the control as in Fageria and Baligar (2005), according to the equation (Eq. [1]):

$$\text{NUE}= \frac{\text{kg ha}^{-1}\text{ yield increased relative to the control}}{\text{kg N ha}^{-1}\text{ applied}} \qquad \text{Eq. [1]}$$

1.2.4 Data Statistical Analyses

All data were initially tested for normality using the Shapiro-Wilk test procedure using the statistical software R (version 3.5.2). All data were distributed normally ($W \geq 0.90$). Data were first submitted to ANOVA to determine the effect of N application timing on palisade grass and ruzigrass. Model fitting was conducted utilizing exponential mathematical model (THOMAS; ASAKAWA, 1993) with the following exponential equation (Eq.[2]):

$$Xt = X_0 . e^{(-k.DAT)} \qquad \text{Eq. [2]}$$

where Xt = DM or N accumulated of cover crop at time t, X_0 = DM or N accumulated at day after cover crop termination, k = constant of residue decomposition or elements release, and DAT = days after cover crop termination.

Data of dry matter and N accumulated of cover crops were first pooled 8 treatments of cover crop under N application (palisade grass + control, palisade grass + 35 DBS, palisade grass + 1 DBS, palisade grass + conventional, ruzigrass + control, ruzigrass + 35 DBS, ruzigrass + 1 DBS, ruzigrass + conventional). The statistical analysis consisted of cover crop under N application over time (days after termination) in three growing seasons. Five sampling times of three growing seasons were used as days after termination: 0, 30, 60, 90 and 120 DAT. Cover crop under N application, sampling time and growing season were set as independent variables. Data were first assessed by ANOVA to determine the presence of any significant effect. Model fitting was conducted utilizing exponential dry matter or N accumulated of cover crop and sampling time using average data of three growing seasons.

For $N\text{-}NH_4^+$, $N\text{-}NO_3^-$ and total-N content in the soil, nutrient concentrations in maize leaves, shoot DM, yield components, grain yield and NUE data were analyzed using the statistical software R (version 3.5.2). Analysis of variance and *F* probability test were performed in these variables. Comparison of means was performed with LSD test ($P \leq 0.05$) when the *F*-test t was significant. Pearson’s correlation analysis was also performed between maize grain yield, maize shoot DM, cover crop DM at 0, 60 and 90 DAT, ammonium in the soil (0-10 cm) at 0, 30, and 60 DAT, nitrate in the soil (0-10 cm) at 0, 60 and 90 DAT.

1.3 RESULTS

1.3.1 Biomass mineralization and N release from plant residues

The decomposition rate of the biomass differed significantly over the 120 days of the experiment, with approximately 52% of shoot biomass remaining for each N application on the grass species (Table 2 and Figure 3). For palisade grass, the DM yield was 18% higher in the treatment with N fertilization 35 DBS than in the control at the beginning of the experiment. From 30 to 60 DAT, the highest DM occurred in the 35 DBS treatment, followed by the 1 DBS treatment, the conventional method and the control. For ruzigrass, 18, 18, 13, and 6% of DM was released in the treatments with N fertilization at 35 DBS or 1 DBS, the conventional method or the control, respectively. All of these values were significantly smaller than the corresponding values for the palisade grass treatments. While the cover crop residues decayed steadily over the 120 days of the experiment, the constant of residue decomposition for biomass mineralization *k* ranged from a minimum of 0.002 in zero-N ruzigrass to a maximum of 0.007 in both palisade grass 35 DBS and ruzigrass 1 DBS (Figure 3).

Nitrogen was released from the cover crop residues following DM mineralization (Table 2 and Figure 3). Nitrogen application on live grass crops resulted in a greater amount of N accumulated in the straw compared with the control. The initial amount of N accumulated was 16% higher, on average, in the palisade grass 35 DBS treatment than in the other N application treatments. For ruzigrass, the initial amount of N accumulated was 92% higher, on average, in the 35 DBS treatment than in the other N applications. The calculated NUE of fertilizer (FAGERIA; BALIGAR, 2005) was 27.6 kg DM kg^{-1} N in the 35 DBS ruzigrass treatment, 52% higher than in the 35 DBS palisade grass treatment. Between 30 and 60 DAT, the amount of N accumulated in DM was higher in the 35 DBS palisade grass treatment than in the control, and the same was observed to the amount of N accumulated in DM in the 35 DBS ruzigrass treatment compared with control. The rate of decomposition of N accumulated in DM *k* differed across the different N treatments of the forage grasses, with a maximum of 0.015 in the 1 DBS palisade grass treatment and a minimum *k* of 0.006 in the ruzigrass control.

Table 2. Dry matter yields and N accumulated in straw residues of palisade grass and ruzigrass under different N application timing in three growing seasons and sampling time (0, 30, 60, 90 and 120 days after desiccation).

Treatment	Dry matter	N
	Mg ha^{-1}	kg ha^{-1}
N application timing		
Palisade grass + 35 DBS†	10.3a††	132a
Palisade grass + 1 DBS‡	9.3b	112b
Palisade grass + Conventional§	9.2b	110b
Palisade grass + Control	8.7c	113b
Ruzigrass + 35 DBS	7.5d	96c
Ruzigrass + 1 DBS	6.5e	83d
Ruzigrass + Conventional	7.1d	74e
Ruzigrass + Control	6.6e	63f
Growing season		
2015-2016	10.1a	124a
2016-2017	7.1b	83b
2017-2018	7.3b	87b
F value		
N application timing (N)	109.3*	45.4*
Sampling time (T)	416.6*	410.9*
Growing season (G)	392.1*	115.2*
N × T	6.1*	7.3*
N × G	5.8^{ns}	3.1^{ns}
T × G	36.2^{ns}	11.4^{ns}
N × T × G	3.8^{ns}	1.2^{ns}

† 120 kg N ha^{-1} broadcast over grass cover crop 35 days before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow.

‡ 120 kg N ha^{-1} broadcast over terminated cover crop 1 day before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow.

§ Conventional N application method: 40 kg N ha^{-1} in the maize seeding furrow plus 120 kg N ha^{-1} sidedressed in V_6 growth stage.

†† Means followed by different letters in the column differ statistically ($P \leq 0.05$) according to LDS test.

* $p < 0.05$; ^{ns} Non-significant.

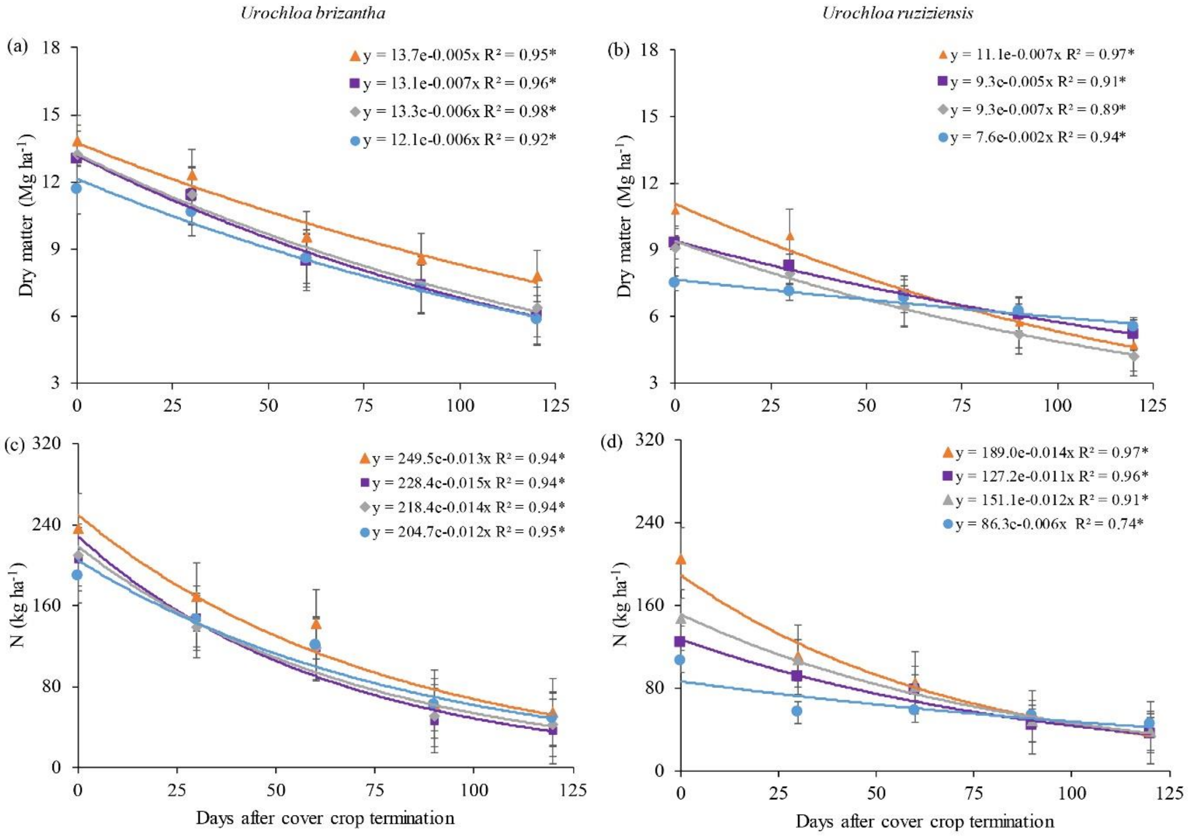

Figure 3. Exponential decomposition of biomass yield of palisade grass (a) and ruzigrass (b), and amount of N in straw of palisade grass (c) and ruzigrass (d) as affected by N application timing [(▲) 35 DBS: 120 kg N ha^{-1} broadcast over grass cover crop 35 days before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow, (◆) 1 DBS: 120 kg N ha^{-1} broadcast over terminated cover crop 1 day before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow, (■) conventional N application method: 40 kg N ha^{-1} in the maize seeding furrow plus 120 kg N ha^{-1} sidedressed in V_6 growth stage, and (●) control: no N application], depending on the days after cover crop termination. *: Significant at 5% by F test. Vertical bars are indicative of the MSD value at 5% probability.

1.3.2 Soil N-NH_4^+, N-NO_3^- and total-N

No interaction effects of cover crop, N application timing and growing season on the nitrogen forms in soil were observed (Tables 3 and 4). In the 0-10 cm soil profile, *Urochloa* spp. did not result in significant changes in N-NH_4^+ content, but N-NO_3^- content decreased at 0 DAT and increased at 60 DAT in the palisade grass treatments (Table 3). The N-NO_3^- content in the soil was higher in the ruzigrass treatments than in the palisade grass treatments at 0 DAT, a time point at which the cover crops were green. However, when residues of the cover crops were present at 60 DAT, the N-NO_3^- content in the soil was lower in the ruzigrass treatments than in the palisade grass treatments. The total-N content in the soil was greater in the palisade grass treatments than in the ruzigrass treatments at 0 DAT.

At cover crop termination, soil N-NH_4^+, N-NO_3^- and total-N were increased by 63, 9, and 24% compared with the control, respectively, in the 0-10 cm soil profile (Table 3). At 30 DAT, high values of N mineral were observed in the 35 DBS treatments, in which total-N was at a maximum, with a value of 1.94 g kg^{-1}. At 60 and 90 DAT, there were no differences in N-NH_4^+, N-NO_3^- and total-N contents between the treatments with different N application times.

At a soil depth of 10-20 cm, the N-NH_4^+ content was highest in the palisade grass treatments at 0 DAT but did not differ between the cover crops at subsequent time points up to 90 DAT (Table 4). A similar trend was observed for total-N at 30 and 60 DAT. There were no differences in N-NO_3^- content between grass species at this soil depth. With respect to the timing of N application, N-NH_4^+ content was highest in the 35 DBS treatment at 0 DAT and in the 35 DBS and 1 DBS treatments at 30 DAT (Table 4). The contents of N-NO_3^- and total-N were highest in the 35 DBS treatments at 30 DAT, with increases of 36 and 11%, respectively, compared to the control.

Table 3. Total N, ammonium (N-NH$_4^+$) and nitrate (N-NO$_3^-$) concentration in the soil (0-10 cm) at 0, 30, 60 and 90 days after cover crop termination (DAT) as affected by cover crops, N application timing and growing season.

Treatment	N-NH$_4$				N-NO$_3^-$				Total N			
	0 DAT	30 DAT	60 DAT	90 DAT	0 DAT	30 DAT	60 DAT	90 DAT	0 DAT	30 DAT	60 DAT	90 DAT
	mg kg^{-1}				mg kg^{-1}				g kg^{-1}			
Cover crop (CC)												
Palisade grass	25.0a††	20.7a	8.4a	7.7a	17.6b	18.1a	9.5a	7.0a	1.93a	1.81a	1.85a	1.51a
Ruzigrass	23.6a	22.4a	7.9a	7.5a	19.6a	18.3a	7.6b	6.6a	1.75b	1.80a	1.79a	1.50a
N application timing (NM)												
35 DBS†	34.1a	23.8ab	8.1a	6.8a	19.8a	20.2a	8.7a	7.3a	2.14a	1.94a	1.83a	1.56a
1 DBS‡	21.0b	28.3a	7.4a	6.0a	17.8b	18.9ab	7.7a	7.5a	1.76b	1.82b	1.78a	1.51a
Conventional§	21.1b	19.6bc	8.5a	6.0a	18.1b	17.1bc	8.7a	6.9a	1.74b	1.78b	1.86a	1.52a
Control (no N application)	20.9b	14.4c	7.3a	7.7a	18.5b	16.5c	8.6a	6.1a	1.72b	1.68c	1.80a	1.49a
Growing season (GS)												
2015-2016	16.6b	18.1b	6.1a	5.1a	17.5b	15.3c	8.9a	7.1a	1.73c	1.69c	1.83a	1.41a
2016-2017	28.7a	20.9ab	8.9a	6.7a	20.4a	18.3b	7.9a	5.1b	1.83b	1.80b	1.87a	1.52a
2017-2018	27.6a	25.6a	8.7a	7.8a	17.8b	20.9a	8.4a	7.9a	1.97a	1.92a	1.75a	1.57a
Source of variation												
CC	0.474	0.403	0.852	0.352	<0.001	0.817	<0.001	0.102	<0.001	0.379	0.365	0.246
NM	<0.001	<0.001	0.136	0.278	0.026	<0.003	0.424	0.069	<0.001	<0.001	0.105	0.215
GS	<0.001	0.012	0.986	0.044	<0.001	<0.001	0.279	<0.001	<0.001	<0.001	0.068	0.198
CC × NM	0.878	0.283	0.063	0.391	0.310	0.912	0.385	0.201	0.613	0.081	0.091	0.141
CC × GS	0.807	0.963	0.965	0.572	0.091	0.647	0.988	0.672	0.224	0.872	0.111	0.081
NM × GS	0.083	0.330	0.902	0.344	0.555	0.086	0.956	0.441	0.852	0.095	0.426	0.525
CC × NM × GS	0.132	0.712	0.841	0.796	0.658	0.726	0.899	0.102	0.245	0.098	0.482	0.681

† 120 kg N ha^{-1} broadcast over grass cover crop 35 days before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow.

‡ 120 kg N ha^{-1} broadcast over terminated cover crop 1 day before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow.

§ Conventional N application method: 40 kg N ha^{-1} in the maize seeding furrow plus 120 kg N ha^{-1} sidedressed in V$_6$ growth stage.

†† Means followed by different letters in the column differ statistically ($P \leq 0.05$) according to LDS test.

Table 4. Total N, ammonium (N-NH$_4^+$) and nitrate (N-NO$_3^-$) concentration in the soil (10-20 cm) at 0, 30, 60 and 90 days after cover crop termination (DAT) as affected by cover crops, N application timing and growing season.

Treatment	N-NH$_4$				N-NO$_3^-$				Total N			
	0 DAT	30 DAT	60 DAT	90 DAT	0 DAT	30 DAT	60 DAT	90 DAT	0 DAT	30 DAT	60 DAT	90 DAT
	mg kg^{-1}				mg kg^{-1}				g kg^{-1}			
Cover crop (CC)												
Palisade grass	20.3a††	17.6a	9.3a	6.9a	15.7a	17.4a	7.9a	6.1a	1.32a	1.50a	1.58a	1.46a
Ruzigrass	17.0b	16.4a	8.8a	6.7a	16.2a	16.4a	7.6a	5.2a	1.28a	1.44b	1.44b	1.47a
N application timing (NM)												
35 DBS†	22.9a	17.5ab	9.2a	6.8a	18.5a	20.1a	7.0a	4.9a	1.32a	1.59a	1.52a	1.56a
1 DBS‡	17.3b	16.6b	9.0a	6.0a	15.0a	16.7b	4.9a	5.0a	1.29a	1.43b	1.57a	1.51a
Conventional§	17.4b	20.8a	9.5a	6.0a	15.2a	16.1b	9.3a	5.1a	1.30a	1.44b	1.50a	1.52a
Control (no N application)	17.0b	12.7c	8.1a	7.7a	15.1a	14.7b	9.6a	4.4a	1.30a	1.43b	1.44a	1.49a
Growing season (GS)												
2015-2016	10.8c	9.3c	10.1a	6.7a	18.6a	19.1a	7.9a	6.7a	1.16c	1.47b	1.52ab	1.39b
2016-2017	25.0a	13.9b	9.2a	6.9a	19.6a	15.9ab	7.7a	4.1a	1.32b	1.28c	1.57a	1.51a
2017-2018	20.2b	25.1a	9.6a	7.1a	9.7b	14.7b	7.5a	5.6a	1.42a	1.59a	1.44b	1.53a
Source of variation	*P > F*											
CC	0.002	0.286	0.301	0.716	0.708	0.225	0.964	0.534	0.098	<0.001	<0.001	0.264
NM	<0.001	0.012	0.195	0.284	0.146	0.002	0.053	0.193	0.852	<0.001	<0.001	0.195
GS	<0.001	<0.001	0.121	0.098	<0.001	<0.001	0.087	0.090	<0.001	<0.001	<0.001	0.047
CC × NM	0.094	0.674	0.755	0.733	0.075	0.781	0.143	0.098	0.570	0.390	0.101	0.699
CC × GS	0.129	0.946	0.097	0.421	0.906	0.826	0.299	0.111	0.330	0.445	0.061	0.419
NM × GS	0.865	0.226	0.286	0.338	0.266	0.091	0.564	0.271	0.211	0.769	0.102	0.524
CC × NM × GS	0.541	0.366	0.793	0.106	0.075	0.768	0.385	0.731	0.340	0.129	0.321	0.598

† 120 kg N ha^{-1} broadcast over grass cover crop 35 days before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow.

‡ 120 kg N ha^{-1} broadcast over terminated cover crop 1 day before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow.

§ Conventional N application method: 40 kg N ha^{-1} in the maize seeding furrow plus 120 kg N ha^{-1} sidedressed in V$_6$ growth stage.

†† Means followed by different letters in the column differ statistically ($P \leq 0.05$) according to LDS test.

1.3.3 Maize crop performance

Nitrogen application over the cover crops resulted in a higher N concentration in maize leaves compared with the control (Figure 4). The N concentration in maize leaves was 30% higher when maize was grown in succession to N-fertilized palisade grass compared with the control, on average. When grown in succession to ruzigrass, the N concentration in maize leaves was 61% higher in the N-fertilized treatments compared with the control, on average.

Table 5. Nutrient (N, P, K, Ca, Mg, and S) concentrations in the leaves of maize crop at 60 d after maize emergence as affected by the cover crop, N application timing, and growing season at Botucatu, Sao Paulo State, Brazil.

Treatment	N	P	K	Ca	Mg	S
			$g\ kg^{-1}$			
Cover crop (CC)						
Palisade grass	28a††	2.8a	26a	4.8a	3.0a	2.1a
Ruzigrass	26b	2.8a	26a	4.9a	3.2a	2.2a
N application timing (NM)						
35 DBS†	28c	2.6c	25bc	5.0a	3.0a	2.2b
1 DBS‡	29b	2.7b	26ab	5.0a	3.2a	2.3b
Conventional§	31a	2.9a	27a	4.7a	3.0a	2.8a
Control (no N application)	20d	3.0a	24c	4.7a	3.2a	1.8c
Growing season (GS)						
2015-2016	24b	2.7b	19c	4.9b	3.2b	1.2c
2016-2017	25b	2.6b	28b	3.6c	2.1c	2.2b
2017-2018	31a	3.2a	30a	6.0a	4.1a	3.2a
Source of variation			*P > F*			
CC	<0.001	0.445	0.570	0.242	0.124	0.198
NM	<0.001	<0.001	<0.001	0.083	0.232	<0.001
GS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CC × NM	0.002	0.326	0.353	0.895	0.238	0.450
CC × GS	0.064	0.211	0.410	0.084	0.283	0.093
NM × GS	0.095	0.089	0.726	0.093	0.205	0.091
CC × NM × GS	0.140	0.086	0.178	0.097	0.276	0.105

† 120 kg N ha^{-1} broadcast over grass cover crop 35 days before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow.

‡ 120 kg N ha^{-1} broadcast over terminated cover crop 1 day before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow.

§ Conventional N application method: 40 kg N ha^{-1} in the maize seeding furrow plus 120 kg N ha^{-1} sidedressed in V_6 growth stage.

†† Means followed by different letters in the column differ statistically ($P \leq 0.05$) according to LDS test.

Although no differences were observed in the maize leaf concentrations of P, K, Ca, Mg, and S between the cover crops and there was no interaction of cover crop and N application time, P and S concentrations were higher than under the conventional method (Table 5), and the K concentration was increased in all N treatments compared with the control.

The plant population was higher in succession to ruzigrass (Table 6); by contrast, shoot DM was 8% higher under succession to palisade grass compared with ruzigrass. Regarding N application time, the shoot DM and plant population were lowest in the zero-N control and did not differ between the treatments with different N application times. N application increased the number of ears per plant in all treatments compared with the control.

There were no differences in maize plant height among the treatments with N application to palisade grass and ruzigrass (Table 6 and Figure 4). By contrast, in the control, plant height was higher in succession to palisade grass than in succession to ruzigrass. Higher numbers of grains per ear were observed in the 1 DBS and conventional palisade grass and ruzigrass treatments. However, for each N application time, the values were lower in the ruzigrass treatments compared to the palisade grass treatments. A higher 100-grain weight was obtained in the 35 DBS and conventional palisade grass treatments and in the 1 DBS and conventional ruzigrass treatments. The ruzigrass control resulted in the lowest 100-grain weight.

Maize grain yield in succession to palisade grass was higher in the 35 DBS, 1 DBS, and conventional treatments than in the zero-N control treatment (Figure 3). However, the grain yield response following ruzigrass varied significantly with N application timing. Higher grain yields were observed in the 1 DBS and conventional treatments, followed by the 35 DBS treatment and the control.

NUE of maize followed the same trend as grain yield (Table 6 and Figure 4). In the 35 DBS treatment, NUE by maize was 47% higher in succession to palisade grass than in succession to ruzigrass than in succession to ruzigrass. For maize in succession to palisade grass, there were no differences in NUE among the different N application treatments, whereas for maize in succession to ruzigrass, NUE was lower in the 35 DBS treatment compared with the 1 DBS and conventional treatments.

The correlations between pairs of factors are shown in Figure 5. Maize grain yield was affected by various factors, including cover crop DM, N accumulated in the cover crops and N-N-NH_4^+ content. Maize grain yield showed significantly positive linear correlations with cover crop DM at 0 DAT (P = 0.0026), cover crop DM at 30 DAT (P = 0.0416), N accumulated in the cover crops at 0 DAT (P = 0.0106), N accumulated in the cover crops at 60 DAT (P = 0.0362), and N-NH_4^+ content in the 0-10 cm soil profile at 30 DAT (P = 0.0008).

Table 6. Shoot dry matter (DM) flowering, plant population, plant height, number of ears per plant, number of grains per ear, 100-grain weight, grain yield, and nitrogen use efficiency of maize crop as affected by cover crop, N application timing, and growing season, and analyses of variance at Botucatu, São Paulo, Brazil. Data reported are means of three harvest, except for the growing season average (GS).

Treatment	Shoot DM	Plant population	Plant height	Ears per plant	Grains per ear	100-grain weight	Grain yield	Nitrogen use efficiency
	Mg ha^{-1}	thousand plants ha^{-1}	m	no. plant^{-1}	no. ear^{-1}	g	Mg ha^{-1}	kg grain kg^{-1} N
Cover crop (CC)								
Palisade grass	13.0a††	61.9b	1.99a	1.06a	611a	28a	11.6a	54a
Ruzigrass	12.0b	63.1a	1.92b	1.04a	528b	26b	9.4b	42b
N application timing (NT)								
35 DBS†	13.7a	62.8a	2.05b	1.11a	624b	27c	11.9b	45b
1 DBS‡	14.2a	62.7a	2.11a	1.11a	655a	28b	12.7a	50a
Conventional§	14.0a	62.8a	2.05b	1.09a	651a	29a	12.7a	50a
Control (no N application)	8.2b	61.8b	1.61c	0.88b	347c	24d	4.7c	-
Growing season (GS)								
2015-2016	11.9b	62.2b	1.81c	1.07a	566a	27a	10.3b	46b
2016-2017	12.6ab	62.6ab	2.11a	1.05a	567a	27a	10.4b	49a
2017-2018	13.1a	62.8a	1.91b	1.03a	575a	27a	10.8a	49a
Source of variation					$P > F$			
CC	<0.001	<0.001	<0.001	0.052	<0.001	<0.001	<0.001	<0.001
NT	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
GS	0.020	0.030	<0.001	0.081	0.213	0.052	0.018	0.006
CC × NT	0.472	0.352	<0.001	0.092	<0.001	<0.001	<0.001	0.001
CC × GS	0.838	0.607	0.057	0.552	0.181	0.077	0.536	0.891
NT × GS	0.817	0.812	0.084	0.102	0.095	0.083	0.105	0.231
CC × NT × GS	0.070	0.340	0.594	0.245	0.068	0.099	0.657	0.112

† 120 kg N ha^{-1} broadcast over grass cover crop 35 days before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow.

‡ 120 kg N ha^{-1} broadcast over terminated cover crop 1 day before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow.

§ Conventional N application method: 40 kg N ha^{-1} in the maize seeding furrow plus 120 kg N ha^{-1} sidedressed in V_6 growth stage.

†† Means followed by different letters in the column differ statistically ($P \leq 0.05$) according to LDS test.

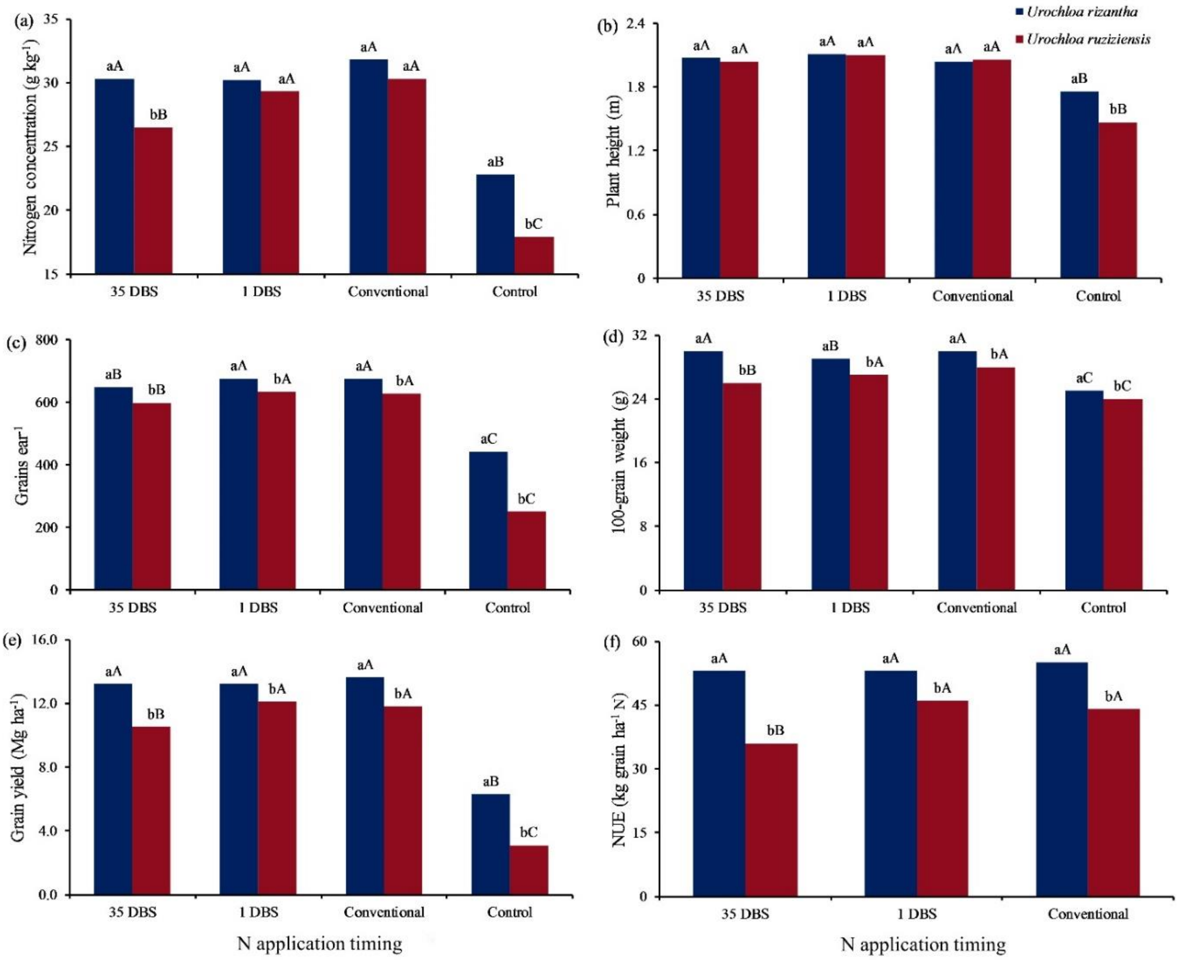

Figure 4. Cover crop × N application timing interaction effect on the N concentration in leaves (a), plant height (b), number of grains per ears (c), 100-grain weight (d), grain yield (e), and N-use efficiency (f) of maize. Data are average of three growing seasons. Nitrogen application timing treatments are as follows: 35 DBS: 120 kg N ha^{-1} broadcast over cover crop 35 days before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow; 1 DBS: 120 kg N ha^{-1} broadcast over crop 1 day before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow; Conventional method: 40 kg N ha^{-1} in the maize seeding furrow plus 120 kg N ha^{-1} sidedressed in V_6 growth stage; and Control: no N application. Different lowercase letters denote significant difference between cover crops and different uppercase letters denote significant difference among N application timing (LSD, $P \leq 0.05$).

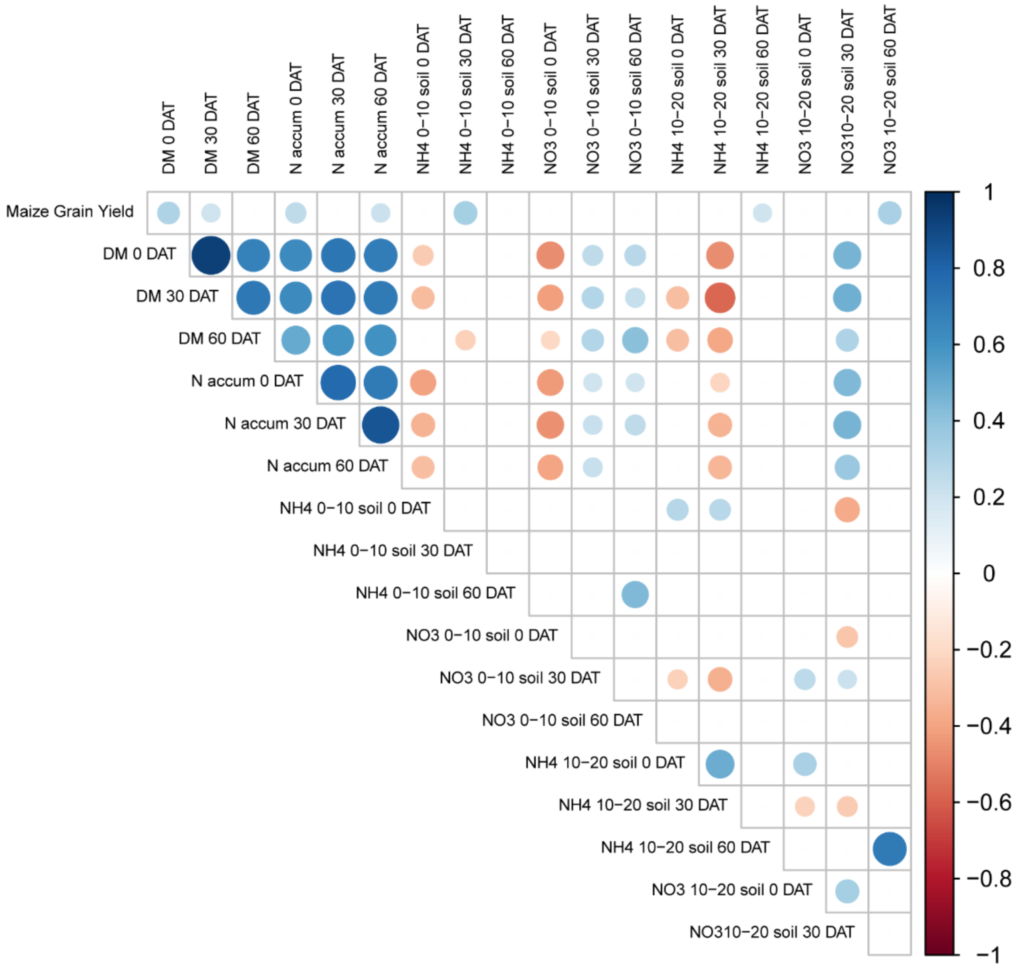

Figure 5. Pearson correlation between maize grain yield, maize dry matter, cover crop biomass at 0, 30, 60, 90 and 120 days after cover crops termination, ammonium in the soil at 0, 30 and 60 days after cover crops termination, and nitrate in the soil at 0, 30 and 60 days after cover crops termination. Pearson correlation coefficients of the blue ellipses are positive, and those of the red ellipses are negative. The darker the color and the smaller the area of the ellipse, the greater the degree of correlation and the larger the absolute value of the Pearson correlation coefficient. The ellipses indicate significant differences of relationship between two variables (*P* > 0.05). Variable names are on the horizontal and vertical. For the interpretation of the references to colors in this figure legend, the reader is referred to the bar.

1.4 DISCUSSION

In this study, N fertilizer applied on cover crops increased biomass yields (Table 2 and Figure 3), consistent with the results of previous field studies (PARIZ et al., 2011, MOMESSO et al., 2019, TANAKA et al., 2019). Nitrogen is directly involved in protein metabolism and chlorophyll biosynthesis and promotes plant growth during the vegetative phase (MARSCHNER, 1995; PARK et al., 2018; YANG; UDVARDI, 2018). Figure 3 indicates the potential of each grass to release N during mineralization of above-ground DM. The higher N accumulation in palisade grass compared with ruzigrass generally accounted for the greater DM of the former cover crop species over time. Grasses of the genus *Urochloa*, especially palisade grass, have high potential for DM yield and nutrient cycling compared with other cover crops such as sun hemp and pearl millet (PACHECO et al., 2013, BORGHI et al., 2013). Several studies have highlighted differences in the DM yields of forage grasses (PACHECO et al., 2011; 2013; ROCHA e al., 2019), and even though high biomass yield and N accumulation were observed in the present study, N management significantly affected the decomposition rates and the subsequent maize crop.

The rates of residue decomposition and N release were estimated from the *k* constant of N management in each species. These estimates indicated high and rapid rates of decomposition and N release from the N-fertilized forage grasses compared with the zero-N application controls, especially for ruzigrass. N-stimulated plant growth can be a fresh source of C and N and is associated with increases in microbial biomass and rapid decomposition (EL-SHARKAWI, 2012; WANG et al., 2015). These residues undergo chemical alteration by fungi and bacteria, with rapid degradation of cellulose and hemicellulose in the first stage (FIORETTO et al., 2005; BANI et al., 2018). Hence, the slow decomposition rates of DM and N release observed in the zero-N application ruzigrass treatment can be explained by the low biomass yield of the cover crop.

There was no difference in N-NH_4^+ content in the soil at a depth of 0-10 cm between the palisade grass and ruzigrass treatments. However, ruzigrass resulted in higher N-NO_3^- content in this layer, possibly as a result of biological nitrification inhibitors (BNIs) (BYRNES et al., 2017; SUBBARAO et al., 2017; NUÑEZ et al., 2018). The exudation of BNIs and DM yield differ among species (SUBBARAO et al., 2007, 2009). At 0 DAT, the N-NH_4^+/N-NO_3^- ratio was 1.4 and 1.2 for palisade grass and ruzigrass in the 0-10 cm soil layer, respectively, and 1.3 and 1.0 in the 10-20 cm layer, respectively. Thus, palisade grass may have led to higher nitrification inhibition than ruzigrass.

The high mineral N and total-N content in the soil cultivated with *Urochloa* spp. were expected because of the high N-cycling potential of these species, as shown by the high DM and N accumulation (Table 2 and Figure 3). Our results are congruent with those found by Moro et al. (2013) for mineral N in soil cultivated with cover crops. Moro et al. observed increased ammonium and nitrate concentrations in soil with cover crops but not with N fertilizer application. Nitrogen fertilizer increased soil ammonium at 0 and 30 DAT, the time points of N application in the 35 DBS and conventional treatments, respectively. Mariano et al. (2015) reported that N fertilization with synthetic and organomineral sources resulted in remarkable increases in ammonium and nitrate content during the sugarcane growth cycle. Application of N fertilizer usually results in a rapid increase in mineral nitrogen availability in the soil solution (INSELSCACHER et al., 2014; MARIANO et al., 2015), which explains the high availability of mineral N in the soil observed in the present study.

Regarding N management, it is clear that fertilizer application on grasses increased N availability in the soil (0 and 30 DAT). The content of N-NH_4^+ and N-NO_3^-

in the soil solution is directly affected by N fertilizer applied during the growing season (INSELSBACHER et al., 2014; MARIANO et al., 2015), which increases mineral N available in the soil. However, variations in N content occurred between sampling times due to the plant response, with green cover crops in the beginning (0 DAT) and subsequent decomposition of the cover crop residues. Mineral N content stabilized 60 days or more after desiccation. Studies have reported depletion of mineral N and subsequent stabilization in tropical soil cultivated with sugarcane (MARIANO et al., 2015; SATTOLO et al., 2017), which explains the low content of N-NH_4^+ and N-NO_3^- in the soil at 90 DAT (Table 4 and 5). The cover crop species recycled N, since the stocks of total-N content in the soil were maintained during the growing season, and N application and the cover crop species were not sufficient to change the total-N content in the soil.

In the three growing seasons, the increase in N fertilizer resulted in an increase in cover crop residue and, in turn, an adequate supply of N to subsequent maize (Fig. 4). Although the nutrient concentrations in maize leaves differed somewhat among the treatments, nutrient cycling by the cover crops and N fertilizer supplied the nutrient requirements of the maize within adequate ranges (CANTARELLA et al., 1997). However, in the control, leaf N concentrations were lower than the range considered adequate (CANTARELLA et al., 1997). N-release by the cover crops and mineral N in the soil were not sufficient to supply the high N demand of maize when there was no N supply. Maize clearly requires N application via mineral or organic fertilizer in systems with grasses. It is well-documented that the presence of grasses in a system can temporarily immobilize N due to the competition between plants and microorganisms for the available N in the soil (SCHIMEL; BENNETT, 2004; KUZYAKOV; XU, 2013; ROSOLEM et al., 2017).

N application increased grain components (Table 6 and Figure 4). Regardless of timing, application of N fertilizer on palisade grass increased the grain components and grain yield of maize, although the values obtained for earlier N application on palisade grass were similar to those obtained with the conventional method. For ruzigrass, only N application at 1 DBS and the conventional method increased the grain components and grain yield of maize. Our results suggest that N fertilization of palisade grass or application of N to palisade grass residues enhances subsequent maize grain yields. These high maize yields were due to increased N uptake, since the decomposition rates (DM and N accumulated) of the residues and the application of N at seeding provided N for the subsequent crop in this agricultural system, as supported by the results of mineral N in the soil. There was a synchronism between N release from palisade grass and maize demand due to mineralization of plant residues (ROSOLEM et al., 2004, 2017). However, the lower grain yield of maize grown after ruzigrass can be explained by the effects of the cover crop. Several studies have observed adverse effects of ruzigrass residues on the succeeding crop (SOUZA et al., 2014; MOMESSO et al., 2019; ROCHA et al., 2019). Rocha et al. (2019) reported that secondary metabolites of forage grasses can act as allelopathic substances that suppress maize growth (ECHER et al., 2012; SOUZA et al., 2014) or that microbial immobilization of mineral N can decrease N availability to maize (ECHER et al., 2012). Despite the high NUE of ruzigrass, the lower DM yield resulted in an insufficient N supply for maize after ruzigrass.

NUE and, in turn, maize grain yields were significantly affected by the timing of N application in the three growing seasons (Figure 4). Several other studies have reported NUE values lower than those observed in the present study (CIAMPITTI; VYN, 2011; MOMESSO et al., 2019). MOMESSO et al. (2019) observed a maximum

NUE of 40 kg kg^{-1} in conventional application, which is low compared with the value of 55 kg kg^{-1} obtained in the present study. As the NUE was highest for maize succeeding palisade grass N-fertilized at 35 DBS, it may be inferred that palisade grass affects the NUE of maize. We observed a grain yield of approximately 13 Mg ha^{-1}, which is a high grain yield for an N application rate of 160 kg ha^{-1}. Importantly, the increase in the grain yield of maize was a consequence of N supply by the N-fertilized cover crops (Fig. 4 and 5); however, the N released by the cover crops may have come (i) directly from the N fertilizer applied on the cover crops or (ii) indirectly from soil N taken up by the cover crops. The correlation study confirmed that DM and N accumulated by the cover crops affected maize grain yield as stated above. In addition, soil ammonium content at 30 DAT (maize seeding) was also positively correlated with maize grain yields. There have been no studies reporting success in increasing maize yields via the application of N to green forage grasses, and thus this study indicates possible strategies for replacing the conventional sidedressing recommendation in *Urochloa*-maize systems. For palisade grass, N fertilizer applied at 35 DBS on green plants or at 1 DBS on plant residues supported high maize grain yields. When ruzigrass is introduced in the system, N fertilizer should be applied on its residues 1 DBS to avoid a yield penalty. The findings of this study have important implications for increasing food production and nutrient use efficiency in agricultural cropping systems. However, more studies are needed to clarify the fate of N fertilizer applied on cover crops to provide a better understanding of the fate of N applied in the system.

1.5 CONCLUSIONS

In this study, we investigated the effects of grass cover crops and N management on grain yield and NUE in maize crops during three seasons. Nitrogen application on palisade grass at 35 DBS and on its residues at 1 DBS resulted in similar maize grain yields as the conventional method of N application. Thus, the use of palisade grass as a cover crop allows for early N application and is an alternative to the recommended sidedressing application. However, when the cover crop is ruzigrass, N fertilization must not be applied while the grass is still growing. Beyond crop nutrition, our results raise questions concerning the impact of the timing of N fertilization on effective N-fertilizer uptake by maize and the environmental consequences of N fertilization for microbial communities in agro-food systems.

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CHAPTER 2

WHAT IS THE BEST TIME FOR N APPLICATION IN *Urochloa*-MAIZE CROP SYSTEM?

Abstract

In food production, residues of *Urochloa* spp. release nitrogen (N) from cover crops to subsequent crop assisting for a sustainable agriculture. Even with high grain yields, managing time of nitrogen application can affect the ^{15}N recovery in tropical grass-cash crop systems. The aim of this study was to verity if *Urochloa* spp. supply N demand and enhance grain yields of maize, and recovery N from fertilizer when N applied in a different time from the conventional method. A field experiment was performed over one growing season using palisade grass (*Urochloa brizantha*) and ruzigrass (*U. ruziziensis*) as cover crops, and three application timings of N [(i) on alive cover crops at 35 days before maize seeding (DBS), (ii) on cover crops residues at 1 DBS, and (iii) conventional sidedressing method of maize]. A control zero-N (no N application) was carried out in both species in order to compare grain yields of maize. Even with zero-N fertilizer, dry matter of palisade grass and ruzigrass were observed around 17 Mg ha^{-1}. High maize yields were reached with N application on palisade grass, its residues and conventional method, resulting in 13.2, 13.2 and 13.6 Mg ha^{-1}, respectively. For ruzigrass, similar maize yields were observed with N application on its residues 1 DBS and N conventional method (12.1 And 11.8 Mg ha^{-1}, respectively). However, the highest recovery of N fertilizer was in maize fertilized at conventional method followed both *Urochloa* spp. There were no differences between cover crops in N recovery by soil and cover crop residues; whereas, the recovery from N fertilizer by maize stover was higher in maize at conventional method of N application following palisade grass. Thus, a better recovery of N fertilizer in the grain was observed in maize fertilized in the current conventional method in both cover crops. Our results indicated that agricultural systems characterized by high dry matter of palisade grass have potential of N supply to subsequent crop. Although N applied on palisade grass increase grain yields of maize, the current conventional method remained higher recovery from N fertilizer by maize following both *Urochloa* spp.

Keywords: *Zea mays. Brachiaria.* Crop residues. ^{15}N fertilizer. Tropical agriculture.

2.1 INTRODUCTION

The use of cover crops in no-tillage (NT) systems has become a sustainable and conservative manner of food production, reducing the negative impacts of agriculture on the environment. This practice results in better soil conservation led by erosion reduction, also enhancing the soil physical, chemical and biological properties. The cover crop plays an important role in nutrient cycling. Since crop residues remain on the soil surface, the decomposing plants slowly release nutrients back to the soil, thus making the nutrients available to the subsequent cash crops. Due to this process, the system requires less fertilizer input. The nitrogen supplied by the decomposing cover crop, along with the biological activity happening in the soil, means that there is little to no need for additional N fertilizer application to subsequent crops. Characteristics of each cover crop species, such as biomass yield and root system architecture help to determine the proper management of fertilizers and crops, thus adding to the success of the cropping system.

There are specific species of cover crops suitable for each environment and main cultivated crop. However, plants from the *Urochloa* genus have shown to be suitable for use in most tropical agricultural systems. These plants have aggressive, strong stoloniferous growth and very competitive proliferation (WILLIAMS; BARUCH, 2000), even under drought conditions and in less fertile soils (BARUCH et al., 1995; CARDOSO et al., 2014). The deep rooting ability of plants from this genus and the high amounts of biomass they produce enhance the recycling of nutrient from deeper soil layers (MOMESSO et al. 2018; TANAKA et al., 2019).

The introduction of the *Urochloa* spp. into an agroecosystem can improve N recycling due to the production of a root exudate that is a biological nitrification inhibitor (BNI) (SUBBARAO et al. 2009). The BNI produced by *Urochloa* spp. suppresses nitrification, which is the microbial oxidation of ammonium (NH_4^+) to nitrate (NO_3^-). This prevents nitrite (NO_3) from leaching into water-bodies and nitrous oxide (N_2O) from being emitted into the atmosphere. In addition, the *Urochloa* spp. grasses are characterized by a deep rooting system that improves N uptake which allows for considerable plant development (FISHER et al. 1994; RAO, 1998; ROSOLEM et al. 2017). The grasses produce about 20 Mg ha^{-1} of dry matter which favors N cycling since the straw has a high C/N ratio and remains on the soil surface for a long time (PACHECO et al. 2013; CRUSCIOL et al. 2015).

Grass cover crops can influence the N that is available to subsequent crops based on decomposition rates of the grasses. The high biomass yields of *Urochloa* spp. accumulate large amounts of N, reaching approximately 160 kg ha^{-1} in a system without an additional application of N fertilizer (MOMESSO et al., 2019; TANAKA et al., 2019). However, even with the high amounts of accumulated N in the cover crops, additional applications of N fertilizer are still necessary to supply the subsequent cash crop with enough nutrients.

Maize crops require high quantities of N to increase grain yields and the soils alone do not generally supply the total N that the plants reach that yields. The current recommendation for N fertilizer application requires farmers to apply fertilizer at two different times: the first application of 40 kg N ha^{-1} at seeding and the second application of 140-180 kg N ha^{-1} when maize plants have 4 to 8 leaves. However, N fertilizer is often applied excessively and the excess N has negative affects the environment. One solution to this problem would be to combine the timing of fertilizer application with the efficient use of cover crops to control the N release the subsequent crops and thus improve the fertilizer effectiveness.

The early application of N fertilizer on cover crops has been well studied (SÁ, 1996; PÖTTKER; WIETHÖLTER, 2004; OLIVEIRA et al., 2018). In systems that black oat was used as a cover crop, the early N application to the subsequent maize crop was unprofitable when it was applied on green cover crops or on its residues for maize crop (PÖTTKER; WIETHÖLTER, 2004; OLIVEIRA et al., 2018). Another strategy could be early N application in systems where *Urochloa* is used as a cover crop. Following this rationale, a previous study showed that N fertilizer could be applied on palisade grass crops or on residues of palisade grass and ruzigrass, replacing the conventional N application method to maize crops. We hypothesized that using *Urochloa* spp. as a cover crop could improve N uptake and N fertilizer recovery by maize where N is applied on palisade grass or on residues of palisade grass and ruzigrass. The first objective of this study was to examine the effect of N timing on the amount of accumulated N in the N residues produced by cover crops, as well as on the N supply and grain yield of maize. The second objective was to assess whether either the early N application on green cover crops or cover crop residues or the conventional method of N application contributed to the total-N content and the N recovery from N fertilizer by maize, cover crop residues, and soil at maize harvest.

2.2 MATERIAL AND METHODS

2.2.1 Site description

The N recovery from fertilizer by grass and maize system was evaluated in a field experiment performed in 2015/2016 growing season. The experimental area was located in Botucatu, São Paulo, Brazil (48° 26´ W, 22° 51´ S, 740 m above sea level). The climate is classified as Cwa i.e., tropical with dry winter and warm and wet summer, according to Köppen classification. Mean annual temperature is 20.7 ^{o}C, and mean annual precipitation is 1358 mm. Seasonal precipitation and temperature during the experiment are portrayed in Figure 6. The soil at the site is clay (630, 90, and 280 g kg^{-1} of clay, silt, and sand, respectively), and classified as kaolinitic, thermic Typic Haplorthox (USDA, 2014). Properties were as follows: pH ($CaCl_2$) 4.8; 32 g dm^{-3} SOM; 19 mg dm^{-3} P (resin); 4.9, 34, 18, and 44 mmol$_c$ dm^{-3} exchangeable K, Ca, Mg, and total acidity at pH 7.0 (H+Al), respectively; and a base saturation of 56%.

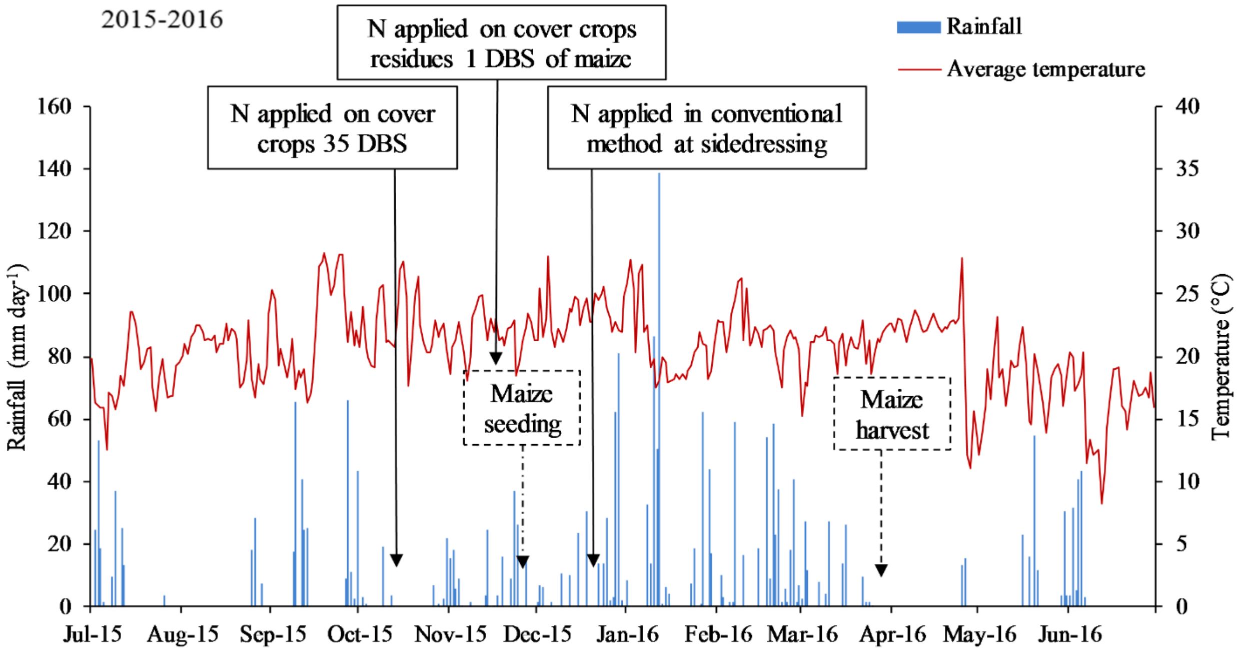

Figure 6. Seasonal precipitation and average air temperature during study period in 2015-2016 growing seasons, and times of N application on cover crops 35 days before seeding (DBS) of maize, on cover crops residues 1 DBS of maize, and conventional method at sidedressing in V_6 growth stage of maize, and maize crop management.

2.2.2 Experimental design and treatments

Field experiment was conducted in a randomized block design with four replications. Treatments consisted of two grass cover crops [palisade grass (*U. brizantha* cv. Marandu) and ruzigrass (*U. ruziziensis* cv. Comum)] and three N application timings: (i) on green cover crops at 35 days before maize seeding (DBS); (ii) on cover crops residues at 1 DBS; and (iii) conventional method at sidedressing in V_6 growth stage (six-leaf) of maize. A control zero-N (no N application) was carried out in both cover crops in order to compare grain yields of maize without N fertilizer in this system.

Except for control (no N added), a total of 160 kg N ha^{-1} (CANTARELLA et al., 1997) were applied as ammonium sulfate: 40 kg N ha^{-1} was applied at maize seeding for all N treatments and 120 kg N ha^{-1} was applied in each treatment according to the

N application timing. The plots were composed by 10 rows of maize spaced 0.45 m apart and 8 m long, and the microplots were 1.8 wide and 2 m long (Figure 7). The N fertilizer was applied in the side band, about 10 cm from the maize row for all treatments.

2.2.3 Crop management and sampling

The experimental area has been cultivated in no-tillage practices since 1999. The *Urochloa* spp. were sown at a density of 10 kg seed ha^{-1} (34% viable seed) during eight months before maize seeding time. Cover crops did not receive any mineral fertilizer. Approximately 28 days before cover crops termination, grasses were cut 0.30 m above soil level by mechanical mowers to stimulate the growth and N uptake.

The earliest fertilizer N application was on alive cover crops at 35 DBS on 24 Oct. 2015 (Figure 2). The desiccation of cover crops was on 29 Oct. 2015 (5 days later 35 DBS application) with glyphosate by spraying with 1.56 kg ha^{-1} (a.i.). The second N application timing was on cover crops residues at 1 DBS on 25 Nov. 2015. The maize (hybrid P3456, Pioneer, Sao Paulo) was sown in December at a depth of 3 cm using a NT drill at a density of 65,000 seeds ha^{-1}. The basic fertilization in the seeding furrows consisted of 90 kg ha P_2O_5 ha^{-1} as triple superphosphate and 45 kg K_2O ha^{-1} as potassium chloride (CANTARELLA et al., 1997). At maize seeding, N fertilizer was applied at the rate 40 kg N ha^{-1} for all N treatments. The conventional method of N application was carried out at sidedressing in V_6 growth stage of maize (six leaves with visible leaf collars). All treatments of N application timing consisted in a rate of 120 kg N ha^{-1}. The maize harvest occurred 125 days after the seeding from 10.8 m^2 usable area in each plot with a mechanical harvest, with grain yield adjusted to 130 g kg^{-1} of grain moisture content.

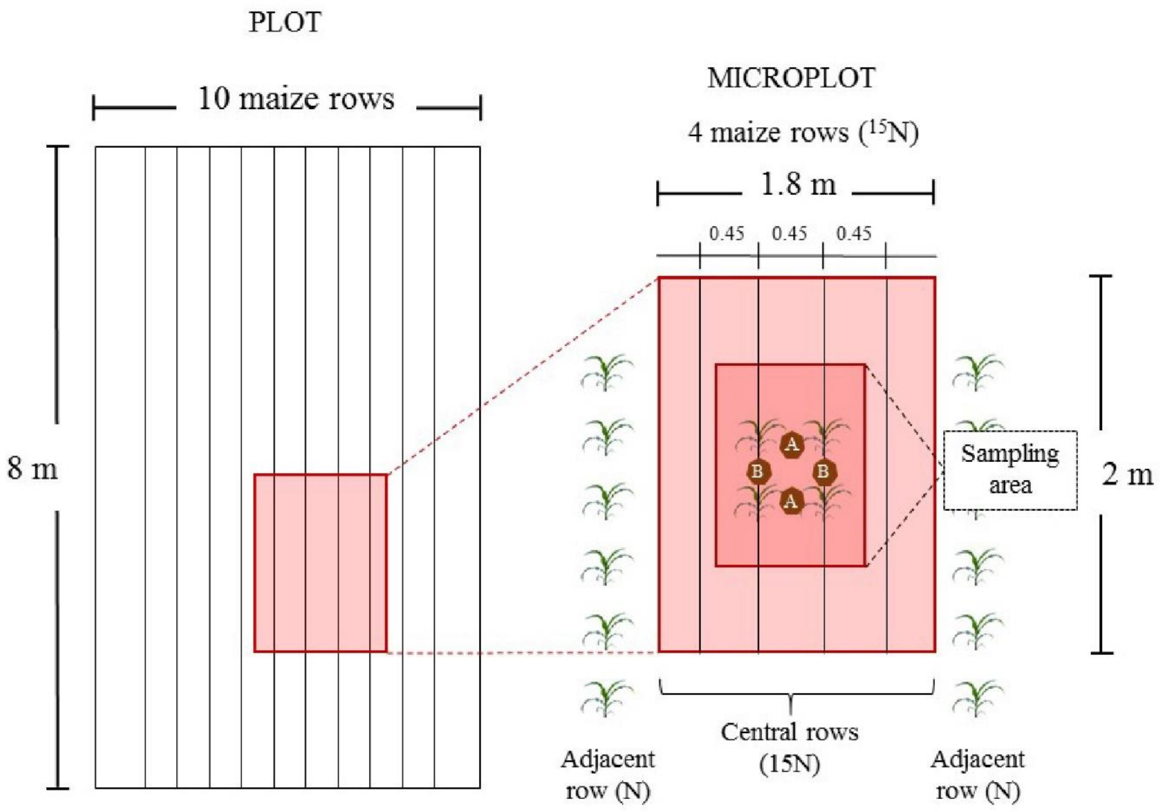

Figure 7. Schematic representation of the microplot. The ^{15}N-fertilizer was applied in the central rows of the microplot, and the central and adjacent rows were sampling to ^{15}N calculations. The letters A and B represent the locations where soil sampling was performed in each microplot.

In addition, the dry matter (DM) of cover crops was collected at day of cover crop termination (0 DAT) and at the end of growing season (120 DAT) in order to investigate the DM amount of cover crop in the system. The sample per plot was collected from an internal area of 0.25 m^2 and was compounded by two samples per plot (CRUSCIOL et al., 2005). For dry-weight determination, samples were oven-dried at 65 ^{o}C. Subsamples from cover crop DM were taken and subjected to wet-digestion procedures with concentrated H_2SO_4 for determination of N concentration by Kjeldahl distillation method (MALAVOLTA et al., 1997).

2.2.4 Isotopic labeled-N determination (^{15}N)

The total area of microplots were 3.6 m^2, consisting of 0.9 m^2 of sampling area (Figure 7). The isotopic ^{15}N labeling was used to determine the amount of N fertilizer resulting in maize, cover crop residues and soil. The ^{15}N fertilizer was applied identical to that described previously for the common fertilizer. The ammonium sulfate was enriched for the rate of 40 kg N ha^{-1} at maize seeding with 4% ^{15}N atoms excess [$(^{15}NH_4)_2SO_4$]. For N application timing, ammonium sulfate was enriched for 120 kg N ha^{-1} with 2% ^{15}N atoms excess. The fertilizer ^{15}N application was to one side of the four center maize rows of each microplot according to the treatments (Figure 7).

To assess ^{15}N recovery, four maize from the designated center were collected in the center of the microplots within each plot at maize harvest. Maize plants were cut at the ground level and divided into shoot (tassel, leaves, stalk, cob and ear) and grain at physiological maturity for ^{15}N determination. At the same time, four samples of cover crop straw per microplot were taken from an internal area of 0.25 m^2 (CRUSCIOL et al., 2005). Maize and cover crop straw samples were dried for 72h in a forced air circulation laboratory oven at 60 ^{o}C to determine dry mass and then milled in a Wiley mill then 0.50-mm mesh sieved. Soil samples in the layers of 0-40 cm were collected using a probe positioned within center row and between rows (Figure 7), which were combined in one sample per microplot. The soil samples were dried in oven at 40 ^{o}C, ground in a ball mill. The soil bulk density at each soil sample and position was assessed using the volumetric ring method (BLAKE; HARTGE, 1986) after maize harvest.

For all samples collected, total N concentration and abundance of ^{15}N atoms were determined in an automatic N analyzer (PDZ Europa ANCA-GSL, Sercon Ltd., Crewe, UK) interfaced to an isotope ratio mass spectrometer (PDZ Europa 20-20, Sercon Ltd., Crewe, UK). Nitrogen recovery efficiency (NRE) was used to express the percentage of the total N fertilizer recovery by maize plant. The amount of N fertilizer in the maize or in the soil was indicated by N derived from fertilizer (NDFF), expressed in kg N ha^{-1}. The calculation of NRE and fertilizer N recovery were as follows:

$$\text{NDFF}\ \left(\text{kg ha}^{-1}\text{of N}\right)= \left[\frac{\alpha - \beta}{\gamma - \beta}\right] .\ \text{total N}$$

where NDFF is the amount of N derived from the fertilizer (kg ha^{-1}), α is the abundance of ^{15}N atoms in the sample (%), β is the natural abundance of ^{15}N atoms (0.366%), γ is the abundance of ^{15}N atoms in the fertilizer (2% of 120 kg N ha^{-1} and 4% of 40 kg N ha^{-1}), and total N in the total of N ($^{15}N+^{14}N$) in the sample (kg ha^{-1}).

$$\text{NRE (\%)} = \left[\frac{\text{NDFF}}{\text{Fertilizer N rate}}\right] .\ 100$$

where NRE is the percentage of ^{15}N recovered by stover, grain, straw of sampled soil layer, NDFF is the amount of N derived from the fertilizer in each these compartments (kg ha^{-1}) and the fertilizer N rate is the rate of enriched fertilizer applied (kg ha^{-1}).

2.2.5 Data Statistical Analyses

All data were initially tested for normality using the Shapiro-Wilk test (SHAPIRO; WILK, 1965) using the statistical software R (version 3.5.2), and the results indicated that all data were distributed normally ($W \geq 0.90$). For the response variables from cover crops at 0 and 120 DAT and grain yield of maize, control zero-N was considered as N application timing treatments. For the variables related to fertilizer ^{15}N (NDFF, NRE, fertilizer fraction, grain yield of maize, plant total-N content, stover total-N content, and grain total-N content), experimental design was considered without control zero-N (no N application). The analysis of variance (ANOVA) was performed using the SISVAR statistical software package (FERREIRA, 2011). Cover crop and N application timing were considered fixed factors. If the null hypothesis was rejected, a comparison of means was performed with LSD test ($P \leq 0.05$).

2.3 RESULTS

2.3.1 Cover crop and maize performance

Cover crops and N application timing interaction significantly affected DM and N accumulated of cover crops at 0 and 120 DAT (Table 7 and Figure 9). At 0 DAT, there was a pattern of higher DM and N accumulated of cover crops when N was applied at 35 DBS. Nitrogen applied at 35 DBS in palisade grass presented the highest DM (18.4 Mg ha^{-1}) and N accumulated (303 kg ha^{-1}) amounts, followed by numerically similar values in other treatments between N application timing in palisade grass. The DM and N accumulated of ruzigrass followed the same pattern: higher value at 35 DBS N application, followed by other N treatments. However, palisade grass had higher DM and N accumulated in all N application compared to ruzigrass. At the end of growing season (120 DAT), higher DM was observed in palisade grass at 35 DBS and 1 DBS (8.2 and 6.9 Mg ha^{-1}); but there were no differences in DM of ruzigrass between N application timing. At the same time, N accumulated of cover crops in palisade grass resulted in higher amount at 35 DBS followed by 1 DBS = conventional method > control. For ruzigrass, there were no differences between N application, but rather compared to control.

At flowering stage of maize, shoot dry matter (SDM), N concentration in leaves and grain maize yields were influenced by cover crop and N application timing, and it was noted a pattern of these variables results (Table 8 and Figure 10). All N application increased SDM and N concentration in leaves compared to control in palisade grass. Regarding to N application timing, SDM resulted in similar values within each cover crops. However, SDM was higher in palisade grass than in ruzigrass for all N treatments. Regarding to N concentration in leaves, N applied at 35 DBS, 1 DBS and conventional method increased N concentration in palisade grass; while for ruzigrass, higher N concentration were found when N was applied at 1 DBS and conventional method. Besides that, N concentrations in leaves were always lower in both cover crops when there was no N application (control), being higher concentrations in palisade grass than in ruzigrass.

Table 7. Dry matter and N accumulated of cover crops at 0 and 120 days after desiccation as affected by cover crop and N application timing.

Treatment	Dry matter		N accumulated	
	0 DAT	120 DAT	0 DAT	120 DAT
	—— $Mg\ ha^{-1}$ ——		—— $kg\ ha^{-1}$ ——	
Cover crop (C)				
Palisade grass	17.6a[††]	6.8a	278a	48a
Ruzigrass	10.7b	6.1a	165b	46a
N application timing (N)				
35 DBS[†]	15.9a	7.0a	274a	52a
1 DBS[‡]	13.0b	6.3a	223b	49a
Conventional[§]	13.1b	6.3a	204b	49a
Control[¶]	12.3c	6.1a	189b	39b
Source of variation	—— *P* > *F* ——			
C	<0.001	<0.057	<0.001	0.855
N	<0.001	0.386	0.041	0.040
C × N	0.025	0.026	0.042	0.035

[†] 120 kg N ha^{-1} broadcast over grass cover crop 35 days before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow.

[‡] 120 kg N ha^{-1} broadcast over terminated cover crop 1 day before maize seeding plus 40 kg N ha^{-1} at seeding plus 40 kg N ha^{-1} in the maize seeding furrow.

[§] Conventional N application method: 40 kg N ha^{-1} in the maize seeding furrow plus 120 kg N ha^{-1} sidedressed in V_6 growth stage.

[¶] Control: zero-N application.

[††] Means followed by different letters in the column differ statistically ($P \leq 0.05$) according to LDS test.

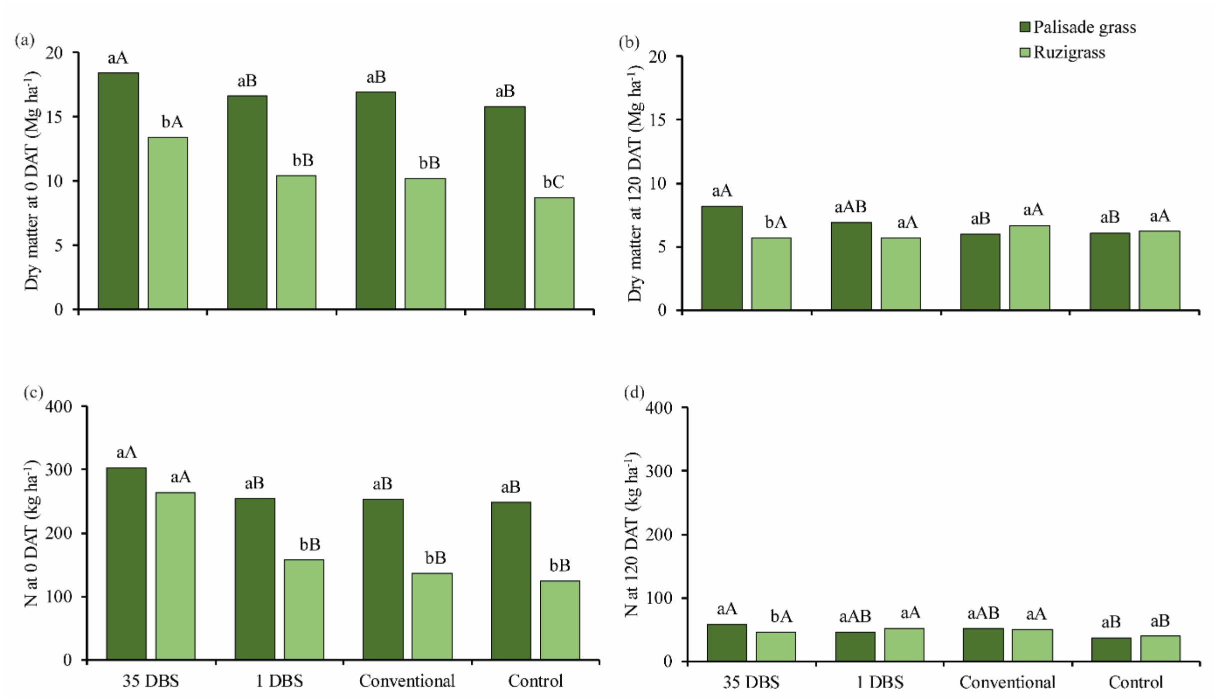

Figure 8. Cover crop × N application timing interaction effect on dry matter of cover crops (a and b) and N (c and d) at 0 (a and c) and 120 (b and d) days after termination (DAT) of cover crops. Nitrogen application timing treatments are as follows: 35 DBS: 120 kg N ha^{-1} broadcast over cover crop 35 days before maize seeding plus 40 kg N ha^{-1} at seeding; 1 DBS: 120 kg N ha^{-1} broadcast over crop 1 day before maize seeding plus 40 kg N ha^{-1} at seeding; Conventional method: 40 kg N ha^{-1} at seeding furrow plus 120 kg N ha^{-1} sidedressed at V_6 growth stage of maize; and Control: no N application. Different lowercase letters denote significant difference between cover corps and different uppercase letters denote significant difference between N application timing (LSD, $P \leq 0.05$).

Grain yield of maize was higher in all N treatments in palisade grass compared to control zero-N (Figure 10), presenting 13.2, 13.2 and 13.6 Mg ha^{-1} of maize grain yield at 35 DBS, 1 DBS and conventional method, respectively. The grain yield of maize following palisade grass zero-N (control) increased 103% compared to ruzigrass zero-N (control), resulting in 6.3 and 3.1 Mg ha^{-1} palisade grass and ruzigrass non-fertilized, respectively.

Table 8. Shoot dry matter, N concentration of leaf at 60 days after maize emergence, grain yields of maize, and grain at maize harvest as affected by cover crop and N application timing.

Treatment	Shoot dry matter	N	Grain yield
	Mg ha^{-1}	g kg^{-1}	Mg ha^{-1}
Cover crop (C)			
Palisade grass	12.4a††	29a	11.6a
Ruzigrass	11.3b	27a	9.3b
N application timing (N)			
35 DBS†	12.7b	30a	11.8b
1 DBS‡	13.6ab	28a	12.2ab
Conventional§	13.9a	34a	12.6a
Control¶	7.3a	20b	5.2c
Source of variation		*P* > *F*	
C	<0.001	0.299	<0.001
N	<0.001	0.003	<0.001
C × N	0.031	0.026	0.013

† 120 kg N ha^{-1} broadcast over grass cover crop 35 days before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow.

‡ 120 kg N ha^{-1} broadcast over terminated cover crop 1 day before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow.

§ Conventional N application method: 40 kg N ha^{-1} in the maize seeding furrow plus 120 kg N ha^{-1} sidedressed in V_6 growth stage.

¶ Control: zero-N application.

†† Means followed by different letters in the column differ statistically ($P \leq 0.05$) according to LDS test.

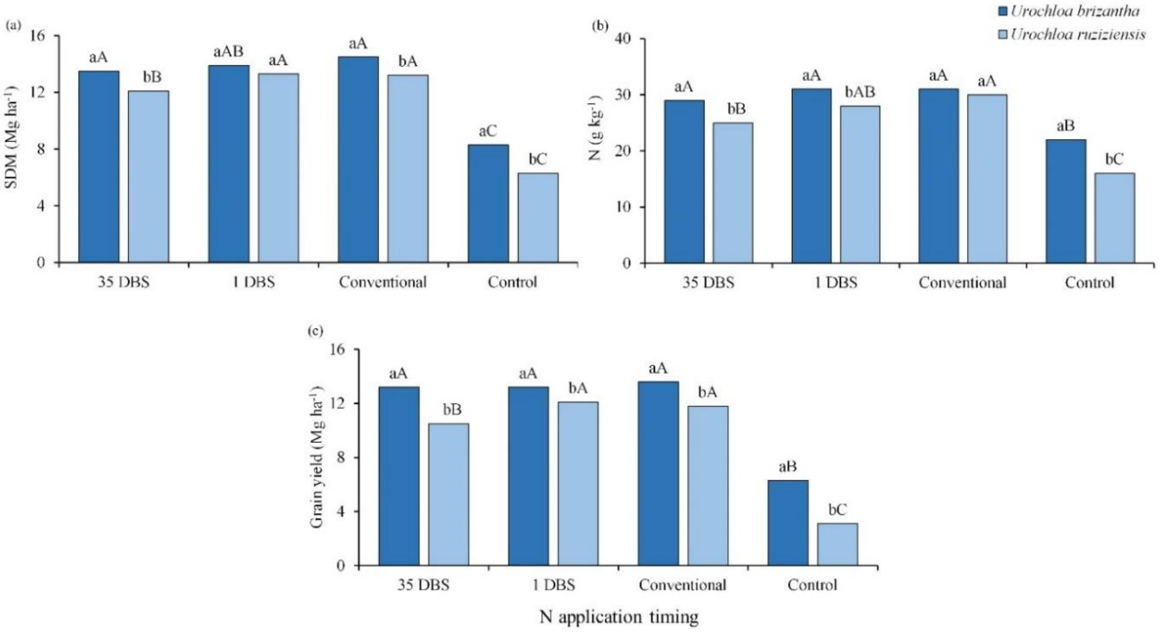

Figure 9. Cover crop × N application timing interaction effect on shoot dry matter (SDM) (a), N concentration in leaf (b), and grain yield (c) of maize. Nitrogen application timing treatments are as follows: 35 DBS: 120 kg N ha^{-1} broadcast over cover crop 35 days before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow; 1 DBS: 120 kg N ha^{-1} broadcast over crop 1 day before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow; Conventional method: 40 kg N ha^{-1} in the maize seeding furrow plus 120 kg N ha^{-1} sidedressed in V$_6$ growth stage; and Control: no N application. Different lowercase letters denote significant difference between cover corps and different uppercase letters denote significant difference between N application timing (LSD, $P \leq 0.05$).

2.3.2 Labeled nitrogen recovery

At maize harvest, plant (stover + grain) N content of maize responded to cover crop × N application timing (Table 9 and Figure 11). The plant N content showed similar trend of N concentration in maize leaf (Figure 10), which all N application timing in palisade grass resulted in similar plant N content. However, maize cultivated following ruzigrass had higher plant N content with N applied at 1 DBS and conventional method.

Table 9. Total-N contents in the plant (stover + grain), distribution of N derived from fertilizer (NDFF) and ^{15}N recovery (NRE) in soil 0-40 cm depth, straw of cover crops, stover and grain of maize at harvest as affected by cover crop and N application timing.

Treatment	Total-N content	NDFF				NRE			
	Stover + grain	Soil	Straw	Stover	Grain	Soil	Straw	Stover	Grain
	kg ha^{-1}	kg ha^{-1}				%			
Cover crop (C)									
Palisade grass	174a††	60.8a	6.8a	13.2a	33.0a	38.4a	4.2a	8.5a	20.4a
Ruzigrass	121b	62.4a	5.1a	11.7b	25.5b	39.0a	3.8a	6.9b	15.4a
N application timing (N)									
35 DBS†	134b	47.5b	7.7a	10.0b	13.7c	30.3b	4.8a	3.6b	8.5c
1 DBS‡	142b	61.5ab	4.8b	10.8b	28.7b	38.4ab	3.0a	7.7b	17.9b
Conventional§	166a	75.9a	5.4b	16.5a	45.4a	47.4a	4.3a	11.7a	27.2a
Source of variation		$P > F$							
C	<0.001	0.814	0.083	0.045	0.046	0.889	0.662	0.048	0.054
N	0.086	0.011	0.042	0.001	<0.001	0.012	0.228	<0.001	<0.001
C × N	0.017	0.835	0.339	<0.001	0.049	0.401	0.380	0.037	0.049

† 120 kg N ha^{-1} broadcast over grass cover crop 35 days before maize seeding (5 days before cover crop termination) plus 40 kg N ha^{-1} in the maize seeding furrow.

‡ 120 kg N ha^{-1} broadcast over terminated cover crop 1 day before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow.

§ Conventional N application method: 40 kg N ha^{-1} in the maize seeding furrow plus 120 kg N ha^{-1} sidedressed in V_6 growth stage.

†† Means followed by different letters in the column differ statistically ($P \leq 0.05$) according to LDS test.

¶ Percentage of fertilizer N on whole plant on plant N content.

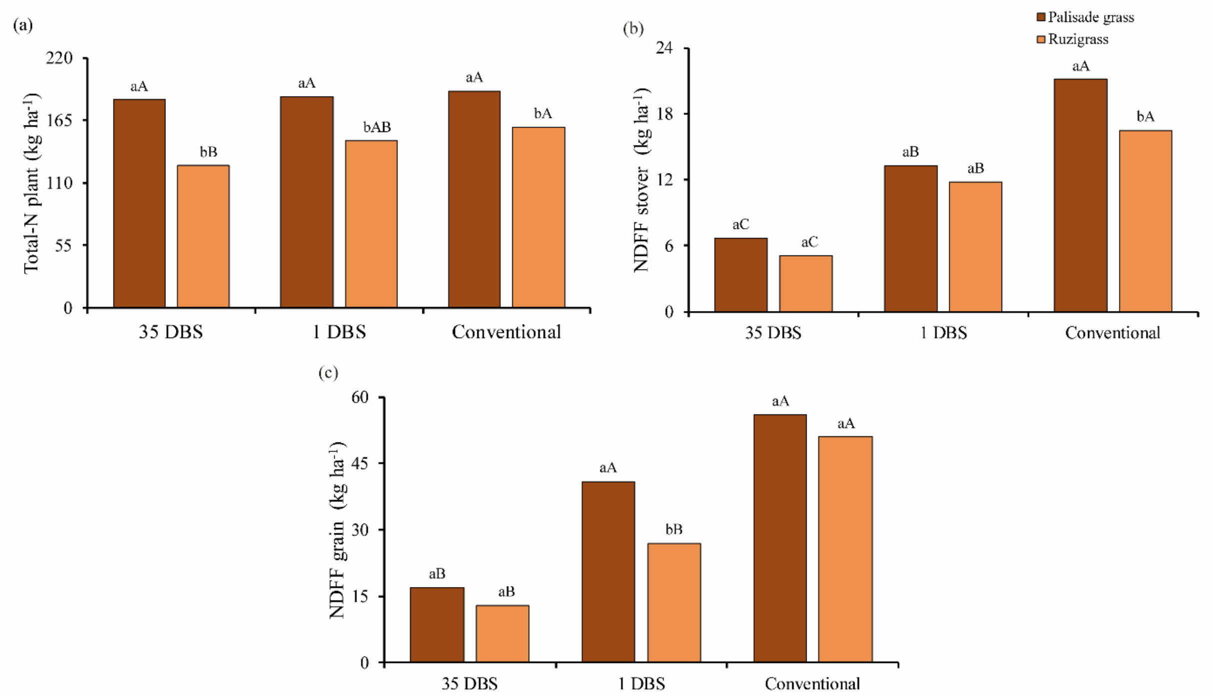

Figure 10. Cover crop × N application timing interaction effect on total-N content in plant (stover + plant) (a), N derived from fertilizer (NDFF) in the stover (b) and grain (c). Nitrogen application timing treatments are as follows: 35 DBS: 120 kg N ha^{-1} broadcast over cover crop 35 days before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow; 1 DBS: 120 kg N ha^{-1} broadcast over crop 1 day before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow; and Conventional method: 40 kg N ha^{-1} in the maize seeding furrow plus 120 kg N ha^{-1} sidedressed in V_6 growth stage. Different lowercase letters denote significant difference between cover corps and different uppercase letters denote significant difference between N application timing (LSD, $P \leq 0.05$).

Distribution of N derived from fertilizer (NDFF) in the soil 0-40 cm layer, straw of cover crops, stover and grain of maize are shown in Table 9 and Figure 11. Cover crop did not affect the NDFF in soil and straw of cover crops, but NDFF in the soil and straw were significantly different between N application timing. For the soil 0-40 cm layer, NDFF was higher when N was applied at 1 DBS and conventional method compared to 35 DBS application. The NDFF in the straw as affected by 35 DBS application was 60 and 42% higher than 1 DBS and conventional method. Regarding maize plant at harvest, NDFF in stover and grain were affected by cover crop × N application timing (Table 9 and Figure 11). The highest NDFF in the stover was observed for N applied in conventional method in palisade grass, resulting in 21 kg ha^{-1} of recovered ^{15}N recovery. For ruzigrass, the highest NDFF in stover was also in conventional N method of application (17 kg ha^{-1}), followed by 12 and 7 kg ha^{-1} at 1 DBS and 35 DBS, respectively. However, NDFF in grain achieve the highest values of 48 and 42 kg ha^{-1} in conventional N method of application following palisade grass and ruzigrass, respectively, and for N applied at 1 DBS in palisade grass (35 kg ha^{-1}).

Although maize reached higher grain yields with all N application in palisade grass (Figure 10), the relation to percentage of ^{15}N recovery (NRE) by maize grain was higher with 1 DBS and conventional method in palisade grass (Figure 12). The NRE by maize grain in followed the order: 26, 21, and 10% for conventional method, 1 DBS, and 35 DBS in palisade grass, respectively; 30, 14, and 7% for conventional method, 1 DBS, and 35 DBS in ruzigrass. For differences between cover crops, NRE by maize grain was similar at 1 DBS and conventional method. Regarding the N found in the system, NRE by maize stover, straw of cover crops and soil were shown in Figure 12. For both cover crops, NRE by maize stover was higher when N applied in conventional method, resulting in 13 and 10% in palisade grass and ruzigrass, respectively; in addition, these results were significant different between cover crops, being higher NRE by stover in palisade grass. The lowest recovery by stover was 4 and 3% at 35 DBS in palisade grass and in ruzigrass, respectively. The NRE in the straw of over crops was not affected by cover crops and N application timing, but NRE in the soil was affected by N application timing (Figure 12). For straw of cover crops, the recovery of fertilizer was around 4% in the all treatments. For soil, higher NRE of 48 and 46% were found in conventional method in palisade grass and ruzigrass, respectively. These values were significantly similar to 36 and 40% of NRE by soil at 1 DBS in palisade grass and ruzigrass.

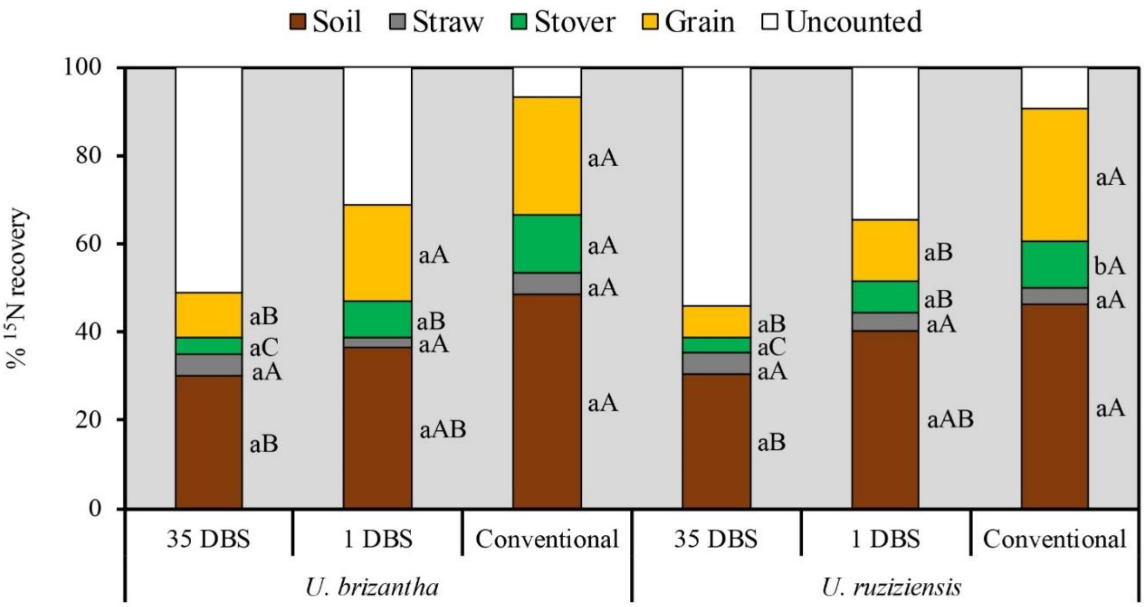

Figure 11. Percentage of ^{15}N recovery (NRE, %) by soil 0-40 cm depth, straw of cover crops, stover and grain of maize as affected the cover crop and N application timing. Nitrogen application timing treatments are as follows: 35 DBS: 120 kg N ha^{-1} broadcast over cover crop 35 days before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow; 1 DBS: 120 kg N ha^{-1} broadcast over crop 1 day before maize seeding plus 40 kg N ha^{-1} in the maize seeding furrow; and Conventional method: 40 kg N ha^{-1} in the maize seeding furrow plus 120 kg N ha^{-1} sidedressed in V$_6$ growth stage. Different lowercase letters denote significant difference between cover corps and different uppercase letters denote significant difference between N application timing for same N fate (LSD, $P \leq 0.05$).

2.4 DISCUSSION

Large amounts of DM were found from *Urochloa* spp. in our results, especially when N was applied on cover crops before termination, which exceeded the recommended of 6 Mg ha^{-1} for NT system in tropical areas, even at the end of growing season (ALVARENGA et al., 2001; COSTA et al., 2016). A higher biomass yield did result in a greater accumulation of N by cover crops when N-fertilized due to ability of *Urochloa* spp. root system to uptake N even from deeper soil layers, which can reach more than one meter in depth (TEDLA et al., 1999; CRUSCIOL et al., 2011, 2012). The potential of dry matter produced has been reported around 13 Mg ha^{-1} (PACHECO et al., 2017; MOMESSO et al., 2019; ROCHA et al., 2019). The cultivation of cover crops for two years in the area before maize seeding, even in control (no N fertilizer), resulted in the establishment of grasses and thus high yield of biomass. These results are comparable with those obtained by Rocha et al. (2019) that observed lower yields of biomass due to the short time of cover crop cultivation. It is clear that cover crop management are extremely important to improve dry matter produced in tropical agriculture system, depending on how cover crops are conducted, different results can be obtained.

Differences between *Urochloa* species also have been stated to dry matter yield and response to N application (PACHECO et al., 2017; MOMESSO et al., 2019; TANAKA et al., 2019). The higher dry matter and N accumulated of palisade grass compared with ruzigrass are congruent with the finds of Tanaka et al. (2019). These observations showed a high potential to growth and coverage the soil surface of palisade grass, even without N fertilizer. However, ruzigrass was more efficient in N fertilizer use, i.e., was higher increase in dry matter with N fertilizer compared to the control per N fertilizer rate (39 kg dry matter per kg N applied), as noted in Chapter 1. Both *Urochloa* species played the role of coverage during the growing season, as observed the dry matter residues at maize harvest (120 DAD). At this time, cover crops reached around 6 Mg ha^{-1}, which still within the DM amount recommended. We could infer that the N input in the system occurred by the high biomass produced and N cycled in both cover crops in the system (ALVARENGA et al., 2001; COSTA et al., 2016).

Urochloa species had positive effect on maize growth and yield compared to

Thomas et al. (2004) and Kawart et al. (2017) when zero-N fertilizer was applied. The authors found grain yields within the range of 3.0 - 4.5 Mg ha^{-1}, lower than our findings that zero-N fertilizer in the system enhanced maize grain yield of 5.2 Mg ha^{-1}. However, the grain yield without N fertilizer was 142% lower than grain yield with N fertilizer application. Even with high N input by cover crops in the system, it is still important the application of N fertilizer to avoid limited maize yield due to high N demand by maize and the critical profitable of maize production (BINDRABAN et al., 2015; MORRIS et al., 2018). The increase of SDM, growth performance and grain yield of maize by N fertilizer observed has been well documented (BORGHI et al., 2013; MORRIS et al., 2018; MOMESSO et al., 2019).

The hypothesis of effect of N application on cover crop was confirmed only for palisade grass: the increase of DM amount and N released by palisade grass supplied N demand by maize and thus increased yields of maize in the system (Figure 10). Nitrogen applied on palisade grass was an alternative to replace conventional N method recommended to maize. The grain yields of maize following palisade grass produced around 13 Mg ha^{-1}. The same was observed for N application on day before sowing. There are no studies about N on cover crops substituting N application at sidedressing and this is the first report of success in replacing the application at sidedressing (conventional method). However, N applied on ruzigrass did not enhance yield of maize in opposite to when palisade grass was cover crop. The failure of total application of N fertilizer on cover crops or cover crop residues has been reported in other studies (SÁ, 1996; PÖTTKER; WIETHÖLTER, 2004; OLIVEIRA et al., 2018; MOMESSO et al., 2019). Pöttker and Wiethölter (2004) showed the possibility of N application before seeding of cash crop, but there is a risk of N losses in regions with high rainfall that suggests the suitable N application is to maintain the conventional method. The majority of studies reported results of black oat as cover crops, which its nitrogen uptake is different from *Urochloa* species.

As a consequence of N released from *Urochloa* spp. in the system, N fertilizer applied on ruzigrass did not supply the total N content in maize plant such as palisade grass. The results of total-N content of maize plant at harvest are congruent with N content obtained in the leaves of maize at flowering in all application timings of N. Although there were differences of total-N content and grain yield of maize following *Urochloa* species, both cover crops enhanced similar N recovery from fertilizer in maize grain. Nitrogen application at sidedressing on residues of palisade grass and ruzigrass resulted in high recovery of N fertilizer due to N fertilizer is readily available during maize growth (MARSCHNER, 2012; YANG; UDVARDI, 2018). These results were in agreement with those found in other studies (OLIVEIRA et al., 2018; MUSYOKA et al., 2019; ROCHA et al., 2019). When N was applied on cover crops, *Urochloa* species grew and absorbed N from soil and fertilizer. Subsequently, residues of *Urochloa* species N-fertilized released gradually these N during maize growth. It is worth noting that high recovery of N from fertilizer in the straw was observed in treatments of N application on cover crops, whereas residues of cover crops maintained similar amount of N recovered from fertilizer in the straw. Even with 60% higher in N application on cover crops compared to application on residues, high amount of N fertilizer was observed in the soil. The recovery of N from fertilizer in the soil was within the range of 25 to 45% reported in other studies (GAVA et al., 2006; KARWAT et al., 2017). The N from fertilized found in the soil has varied depending on soil, management of fertilizer and agricultural system (ALMEIDA et al., 2018; OLIVEIRA et al., 2018).

The N non-recovered from fertilizer obtained in this study can be associated to ammonia volatilization, nitrate leaching and gasses emission. It could be speculated that an amount of N uncounted may be found in the root system of *Urochloa* spp. since authors reported an estimated amount of 18 kg N ha^{-1} in root of *Urochloa* species cultivated as pasture and evidenced the potential to increase the amount of N in the roots in well managed long-term systems (RAO, 1998; OLIVEIRA et al., 2004). Besides that, there are no reports about the influence of microorganisms in tropical systems for food production. During cover crop decomposition, microorganisms involved in N-transforming may be contributing to N losses of N uncounted through nitrification and denitrification when N is applied on cover crops or on cover crops residues. In field conditions, NO_3^- levels in the soil can vary that indicates inhibition of nitrification by residual BNI effect is not consistent of complete (KARWAT et al., 2017). Although is well known that *Urochloa* species inhibit nitrification, there are a gap research regarding to how is mechanism, environmental influence and time of *Urochloa* cultivation on the exudation of biological nitrification inhibitor (SUBBARAO et al., 2015; BYRNES et al., 2017; KARWAT et al., 2017).

The fate of N fertilizer applied on *Urochloa* species or its residues could not be found in the system at maize harvest. However, our study confirmed that the high N uptake and released by cover crops in the system did not mean N recovery from fertilizer by subsequent maize. Based in this study, matching high grain yield and N fertilizer uptake of maize is possible obtaining when N is applied as conventional method (40 kg N ha^{-1} in the maize seeding furrow plus 120 kg N ha^{-1} sidedressed in V_6 growth stage).

2.5 CONCLUSIONS

When *Urochloa* species used as a cover crop in no-tillage system, palisade grass (*Urochloa brizantha*) was a better dry matter producer and N releaser in the agricultural system to supply N demand and raise grain yield of maize. Besides that, the timing of N application in agricultural system is an important factor in increasing the maize grain yield and forage production. This study reported the important knowledge that higher grain yields of maize were achieved when N was applied on palisade grass and its residues or on ruzigrass residues, resulting in similar grain yield obtained in conventional method. However, the fate of N fertilizer in the system was not completely understood. Although the hypothesis of N applied on palisade grass to reach high grain yields of maize was confirmed, the application of nitrogen fertilizer must be applied as current recommended method (40 kg N ha^{-1} at maize seeding plus 120 kg N ha^{-1} sidedressed in V_6 growth stage) to enhance grain yields of maize and high N recovery from fertilizer. The conventional method is still better option to avoid loss of fertilizer. Additional studies should be conducted to better understand the changes that occur in soil microbiology, soil biochemistry, root composition and N losses.

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FINAL CONSIDERATIONS

This study investigated during a period of three years (i.e. 2015-2018) the effects of grass cover crops and N management on grain yield, N-use efficiency and N recovery from fertilizer in maize. In no-tillage system, the N fertilizer applied on palisade grass (*Urochloa brizantha*) at 35 DBS or on its residues at 1 DBS, or on ruzigrass residues at 1 DBS resulted in similar grain yield of maize in conventional method of N application. However, since the earlier applications on cover crops had lower N recovery from fertilizer by maize grain, the application of nitrogen fertilizer must be applied as current recommended method (40 kg N ha^{-1} at maize seeding plus 120 kg N ha^{-1} sidedressed in V_6 growth stage) to enhance grain yields of maize and high N recovery from fertilizer.

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