European Journal of Agronomy 135 (2022) 126485

Contents lists available at ScienceDirect

European Journal of Agronomy

journal homepage: www.elsevier.com/locate/eja

Feasibility of early fertilization of maize with 15 N application to preceding cover crop

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A R T I C L E I N F O

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

A B S T R A C T

Early nitrogen (N) application on live cover crops or their residues is a potential alternative for supplying N demand while enhancing the yield of subsequent cash crops in tropical regions. The objective of applying N on live forage grasses or their residues to no-till (NT) systems is to promote the gradual release of N via straw decomposition to the subsequent crop. However, the N use efficiency by the subsequent crop under early fertilization has not been determined in the end of growing season. The aim of this study was to evaluate whether the most cultivated tropical forage grasses can supply the N demand and enhance the grain yields of maize via the N recovery when N is applied with different timings than the conventional method. A 3-year field experiment was performed using palisade grass [(*Urochloa brizantha* (syn. *Brachiaria*)] and ruzigrass (*U. ruziziensis*) as cover crops with four N application timings to agricultural system: (i) no-N, zero N application; (ii) CC+N, 120 kg N ha^{-1} applied on live cover crops 35 days before maize seeding; (iii) St+N, 120 kg N ha^{-1} applied on cover crops straw 1 day before seeding; and (iii) Nv4, conventional method of sidedress N application at the maize V_4 (four leaf) growth stage. Except control, all N treatments received 40 kg N ha^{-1}at maize seeding, totalizing 160 kg N ha^{-1}. Straw decomposition and cover crop N accumulation were greater in the treatments in which N fertilizer was applied on palisade grass compared with ruzigrass. High maize yields were achieved with N application on palisade grass or its residues or according to the conventional method, with yields of 13.2, 13.2 and 13.6 Mg ha^{-1}, respectively. Similarly, high maize yields were obtained when N was applied on ruzigrass residues or according to the conventional method (12.1 and 11.8 Mg ha^{-1}, respectively). However, regardless of cover crop species, N recovery was highest when N fertilizer was applied via the conventional method. Additionally, most of the N in maize at harvest came from the soil when N fertilizer was applied to live palisade grass. Thus, best recovery of N fertilizer in the grain occurred in maize fertilized using the conventional method. Our results indicate that agricultural systems characterized by high dry matter from palisade grass have the potential to recycle and supply N to subsequent maize. Although palisade grass combined with early N fertilizer application may enhance maize response and yield, the current conventional method of N fertilizer application on maize allows higher recovery from N fertilizer while increasing the maize yield in tropical food production.

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https://doi.org/10.1016/j.eja.2022.126485
Received 6 April 2021; Received in revised form 7 February 2022; Accepted 15 February 2022
Available online 23 February 2022

1. Introduction

Suitable nitrogen (N) management practices for agriculture have been pursued globally since the recognition of the environmental consequences of inappropriate fertilizer application (Zhang et al., 2015a, 2015b). Excess N from fertilizer in agriculture impacts the environment via reactive N losses through ammonia volatilization and the formation of atmospheric particulate matter, which threatens human health, contamination of groundwater by nitrate leaching, and emissions of nitrous oxide, a greenhouse gas, to the atmosphere (Sanaullah et al., 2020, 2020; Struck et al., 2020; Thakrar et al., 2018; Walker et al., 2006). Conservation practices such as no-tillage (NT) can increase N-use efficiency (NUE) in agricultural systems via nutrient cycling by plant residues (Derpsch et al., 2014; Rosolem et al., 2017). Although the maintenance of plant residues on the soil surface in NT systems alters N dynamics and provides high amounts of N via microbial decomposition processes (Momesso et al., 2022b, 2022a), N recovery from fertilizer applied according to conventional methods is only 15–55%, while unrecovered N from fertilizer in maize production ranges from 50% to 70% (Couto-Vázquez and González-Prieto, 2016; Karwat et al., 2017; Oliveira et al., 2018; Rocha et al., 2019).

Nitrogen management in temperate climates is commonly applied nearest to the time the nutrient is needed by the crop, i.e., sidedressed weeks after maize emergence (Mohammed et al., 2013; Vetsch and Randall, 2004). In tropical climate, rainy summers can promote increased N losses from fertilizer by nitrate leaching and delay in the late N application and at the maize development stage of high N demand. Early application of N in NT systems has been suggested as an alternative to increase the NUE and yield of cash crops in tropical agriculture. In this practice, all or part of the N fertilizer is applied on live cover crops or their desiccated residues prior to maize sowing (Momesso et al., 2020, 2019; Oliveira et al., 2018; Pöttker and Wiethölter, 2004). The rationale for early N fertilization is the ability of cover crops in NT systems to absorb N from soil and fertilizer, which is released back to soil by microbial decomposition of the straw on the soil surface during the growth of the subsequent crop (de Freitas and Landers, 2014; Mueller et al., 2003; Pöttker and Wiethölter, 2004). Furthermore, this practice provides greater flexibility to farmers in the timing of N application. The conventional recommendation is to apply N fertilizer during a specific growth stage of the cash crop, which often occurs during the wet season and favors N losses by NH_3 volatilization and NO_3^- leaching. In addition, post-planting application of N to cash crops typically requires sidedressing equipment and may cause leaf damage and burn, and reduces maize yield (Raun and Schepers, 2015).

The cover crop also influences the effectiveness of N fertilizer uptake by plants. Grasses of the genus *Urochloa* (syn. *Brachiaria* spp.) have proved suitable for use in most tropical agricultural systems and are widely cultivated as fodder or cover crops in intercropping and crop rotation systems for tropical food production, especially maize cultivation (Borghi et al., 2013; Canisares et al., 2021; Karwat et al., 2017; Pariz et al., 2017). These grasses have deep rooting ability, extremely competitive proliferation and aggressiveness, strong stoloniferous growth under rainy or drought conditions (Baruch, 1994; Williams and Baruch, 2000). In addition, these species can increase amounts of N available to subsequent crops based on the decomposition of their residues and are particularly effective at producing high amounts of biomass as a cover crop by recycling of nutrients from deeper soil layers (Fisher et al., 1995; Momesso et al., 2019; Rao, 1998). The large biomass yields of *Urochloa* spp. permit high N accumulation reaching approximately 160 kg ha^{-1} even without additional application of N fertilizer (Momesso et al., 2019; Tanaka et al., 2019). However, even with the high amounts of N accumulated in the cover crops, additional applications of N fertilizer are necessary to supply the subsequent cash crop with enough nutrients, and the effectiveness of N fertilizer recovery remains unclear.

Nitrogen management directly affects the maize response, and inappropriate timing of N application reduces maize yield (Fernandez et al., 2020; Meisinger et al., 2015; Rocha et al., 2019; Zhang et al., 2020). The agronomic efficiency of early N application on live cover crops or their desiccated residues has not been comprehensively validated, and there is much controversy in the literature regarding this practice. The results reported thus are insufficient to support general recommendations for early application of N fertilization (Momesso et al., 2019; Oliveira et al., 2018; Pöttker and Wiethölter, 2004). One strategy to test the efficacy of this practice is to assess fertilizer N recovery by maize using the ^{15}N labelled fertilizer.

Usually, at least part of the N for maize should be applied at seeding when a mulch of desiccated plant material is present, to avoid N deficiency due to temporary immobilization of N by microorganisms in tropical no-till systems with most cultivated *Urochloa* species (Momesso et al., 2020, 2019), especially following species with high volume of mulch such as palisade grass cultivation. We hypothesized that early application of N recommended for maize following palisade grass as cover crops might be at least as efficient as the conventional split application in which approximately 2/3 of the N applied is sidedressed when the maize is growing (4–6 leaf stage). We, therefore, assessed whether either early N application on live palisade grass and ruzigrass or their straw influences total N content and ^{15}N recovery from N fertilizer by maize, cover crop straw, and soil at maize harvest compared with the conventional method of sidedress N application.

2. Materials and methods

2.1. Site description

A field experiment was conducted during three growing seasons (2015/2016, 2016/2017, 2017/2018). 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 according to the Köppen classification, i.e., tropical with dry winters and warm, wet summers. The mean annual temperature is 20.7 °C, and the mean annual precipitation is 1358 mm. Seasonal precipitation and temperatures during the experiment are shown in Fig. 1A. The site has a clayey soil (630, 90, and 280 g kg^{-1} of clay, silt, and sand, respectively) and is classified as kaolinitic, thermic Typic Haplorthox (Soil Survey Staff, 2014). Soil chemical properties were as follows: pH ($CaCl_2$) 4.8, 4,7 and 4,6; 32, 25 and 28 g dm^{-3} SOM; 19, 18 and 21 mg dm^{-3} P (resin); 4.9, 3,9 and 3,3 $mmol_c$ dm^{-3} exchangeable K^+; 36, 37 and 31 $mmol_c$ dm^{-3} exchangeable Ca^{2+}; 18, 16 and 22 $mmol_c$ dm^{-3} exchangeable Mg^{2+}; 44, 39 and 42 $mmol_c$ dm^{-3} total acidity at pH 7.0 (H+Al); and base saturation of 56%, 60% and 57% in areas cultivated in 2015/2016, 2016/2017 and 2017/2018, respectively (van Raij et al., 2001).

2.2. Experimental design and treatments

The experiment was a randomized complete block with a 2 × 4 factorial scheme with four replicates per treatment. The treatments consisted of two grass cover crops and four treatments, including a control with no-N application and three application times of 120 kg N ha^{-1}. The two cover crops were palisade grass (*U. brizantha* cv. Marandu) and ruzigrass (*U. ruziziensis* cv. Comum)], which are the most cultivated *Urochloa* species in Brazil (Galdos et al., 2020). The three N application timings were (i) CC+N: 120 kg N ha^{-1} applied on the live cover crop 35 days before maize seeding; (ii) St+N: 120 kg N ha^{-1} applied on the cover crop straw 1 day before seeding; and (iii) Nv4: conventional method of N application in maize, 120 kg N ha^{-1} sidedressed at the V_4 (four leaves with visible leaf collars) growth stage (Fig. 1B). The application timing of CC+N was based on previous studies (Momesso et al., 2020, 2019; Tanaka et al., 2019), which showed that early N application at 5 days before cover crop termination, i.e., 35 days before maize seeding, is sufficient for forage response and for adequate biomass production in agricultural systems. For all N applications, N

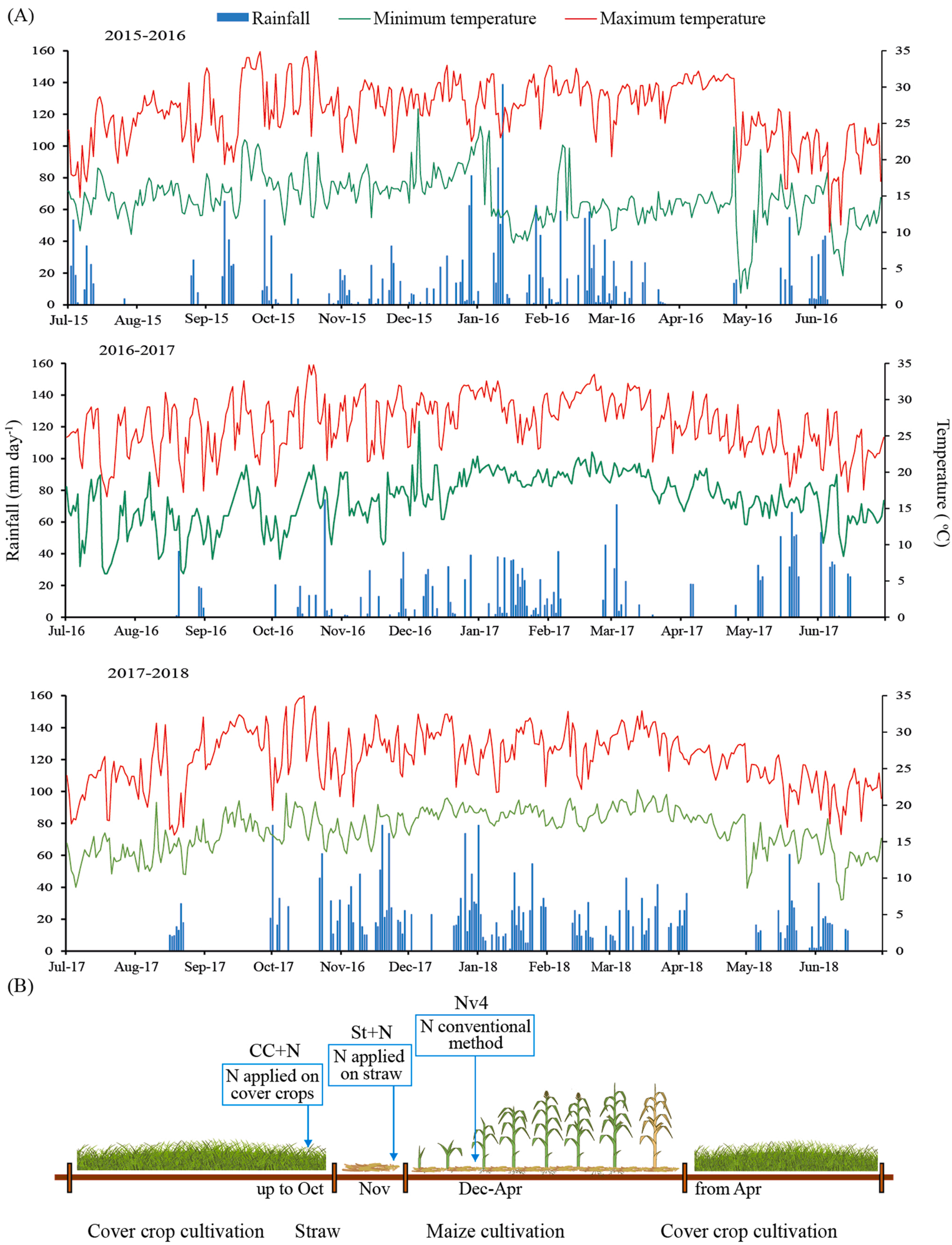

Fig. 1. Daily rainfall, minimum and maximum air temperatures during study period in 2015–2016, 2016–2017 and 2017–2018 growing seasons (A), and times of N application on live cover crops (palisade grass and ruzigrass), on their straw and at sidedressing at in the V_4 growth stage of maize (conventional method) (B).

rate was based on the conventional fertilizer recommendation of the Technical Fertilization and Liming Recommendations (Cantarella et al., 1997), which use the expected grain yield that N rate to maize is 100–170 kg N ha^{-1} (Maize yield = 10–12 Mg ha^{-1} and low–high expected response to N). In addition, 40 kg N ha^{-1} was applied at maize seeding in all N treatments, except the control. Thus, a total of 160 kg N ha^{-1} (Cantarella et al., 1997) was applied as ammonium sulfate: 40 kg N ha^{-1} at maize seeding and 120 kg N ha^{-1} in each treatment according to the N application timing.

The main plots were composed of 10 rows of maize spaced 0.45 m apart with a length of 8 m. In the first growing season (2015/2016), microplots were installed inside the main plots, in which ^{15}N-labelled fertilizer was applied. The microplots were composed of 4 rows and had a width of 1.8 m and length of 2 m (Fig. 2). The ^{15}N fertilizer was applied in the same manner as described previously for the unlabeled fertilizer. For the 40 kg N ha^{-1} rate applied at maize seeding, ammonium sulfate was enriched with 4% ^{15}N atom excess [($^{15}NH_4)_2SO_4$]. For the different timings of N application at 120 kg N ha^{-1}, ammonium sulfate was enriched with 2% ^{15}N atom excess.

2.3. Crop management and sampling

The experimental area has been cultivated using NT practices since 1999. Palisade grass and ruzigrass were seeded at a density of 10 kg seed ha^{-1} (34% viable seed) and cultivated at least eight months before maize seeding. The cover crops did not receive any mineral fertilizer at seeding. Approximately 28 days before desiccation, the grass cover crops were cut 0.30 m above the soil level by mechanical mowers to stimulate growth and N uptake from N fertilizer by cover crops (Fig. 1). The mowed material was left on the soil surface.

The earliest application of N fertilizer was on live cover crops (CC+N) in October 35 days before maize seeding (Fig. 1B). Cover crop desiccation was performed 5 days later in October, i.e., 30 days before maize seeding, by spraying glyphosate at 1.56 kg ha^{-1} (a.i.). The second earliest N application timing was on cover crop straw (St+N) in November at 1 day before maize seeding, that is, 29 days after cover crop desiccation. Maize (hybrid P3456, Pioneer, Sao Paulo) was seeded 1 day later at a depth of 0.03 m using an NT drill at a density of 65,000 seeds ha^{-1}. The basic fertilization in the seeding furrows consisted of 90 kg 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 40 kg N ha^{-1} in all N treatments except in the no-N control. The N fertilizer applied at Nv4 was sidedressed in bands approximately 10 cm from the maize row. The maize was harvested 125 days after seeding from a 10.8 m^2 usable area in each plot with a mechanical harvester, and the grain yield was adjusted to a grain moisture content of 130 g kg^{-1}.

After the maize harvest, *U. brizantha* and *U. ruziziensis* were seeded and grew during the winter-spring. The same mowing, desiccation, and maize cultivation, as well as the N fertilizer treatments, were repeated in the 2016/2017 and 2017/2018 seasons, at approximately the same dates as those of the 2015/2016 season.

2.3.1. Decomposition of cover crop straw and N accumulation

To quantify the mass of straw returned after termination to the subsequent maize crop, we evaluated the decomposition of the cover crop straw during the maize growing in the 2015/2016, 2016/2017, 2017/2018 growing seasons. In each growing season, samples of cover crop straw were collected on the day of cover crop desiccation (0 DAT) and at 30, 60, 90, and 120 DAT. Three samples were collected from each plot and pooled, using a wooden frame with an internal area of 0.25 m^2. For dry weight determination, samples were oven-dried at 65 °C. Subsamples of cover crop residues (0.2 g) were analyzed with an elemental analyzer (LECO-TruSpec® CHNS) to determine the N concentration. The N accumulated in the cover crops was obtained by multiplying the N concentration by the dry mass (DM). The loss of straw and N accumulated in the cover crops (amount released to the agricultural system) was calculated by subtracting the values of DM and accumulated N at each sampling time (30, 60, 90 and 120 DAD) from the values obtained at 0 DAD.

2.3.2. Leaf N concentration and grain yield of maize

In the three growing seasons, the leaf N concentration of maize was determined when 50% of the maize plants were in the full flowering stage. Random sampling was performed by choosing 20 plants per plot and collecting the leaf opposite the ear for N concentration determination (Cantarella et al., 1997). The samples were digested with sulfuric acid, and the N concentration was determined by semi-micro-Kjeldahl distillation. In addition, the grain yield of maize was determined by mechanically harvesting maize from 10.8 m^2 of usable area in each plot

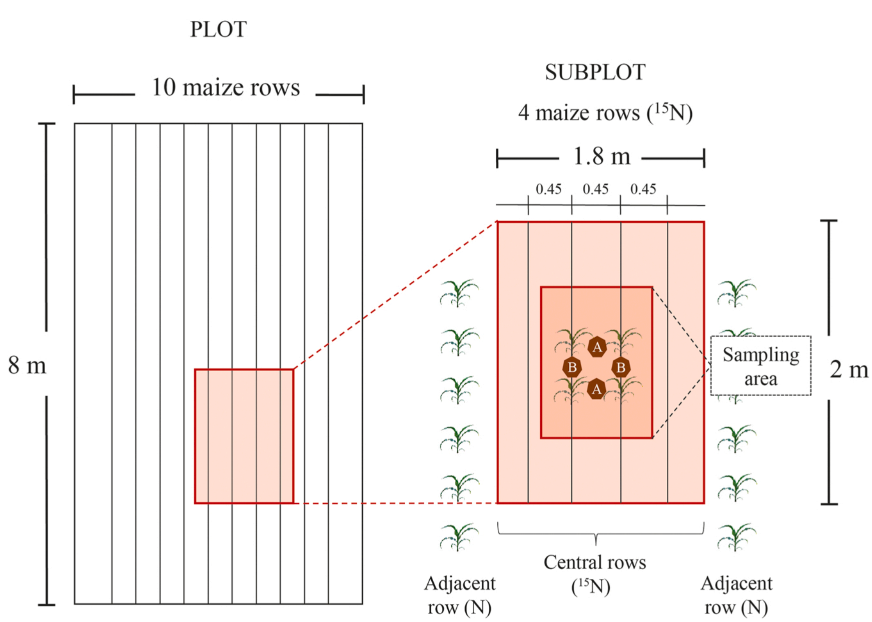

Fig. 2. Schematic representation of the microplot. The ^{15}N-fertilizer was applied in all rows of the microplot. The row central rows were sampled for ^{15}N calculations. The letters A and B represent the locations where soil sampling was performed in each microplot.

when the above-ground parts of the plants were dry (full maturation).

2.4. Determination of isotopic labeled-N (^{15}N)

The total area of the microplots was 3.6 m^2, and the sampling area was 0.9 m^2 (Fig. 2). To assess ^{15}N recovery, four maize plants were collected from the center of the microplots within each plot at maize harvest at physiological maturity. The maize plants were cut at the ground level and divided into shoot (tassel, leaves, stalk, cob and ear) and grain for ^{15}N determination. At the same time, four samples of cover crop straw per microplot were taken from a wooden frame with an internal area of 0.25 m^2. The samples of maize (stover and grain) and cover crop (straw) were dried for 72 h in a forced air circulation laboratory oven at 60 °C to determine the dry mass, milled in a Wiley mill, and sieved through 0.50-mm mesh. Three soil samples from the 0–40 cm layer were collected using a probe positioned within the center row and between rows (Fig. 2) and combined into one sample per microplot. The soil samples were dried in an oven at 40 °C and ground in a ball mill. The soil bulk density of each soil sample at each position was assessed using the volumetric ring method (Blake and Hartge, 1986) after maize harvest.

For all samples collected, the 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 with an isotope ratio mass spectrometer (PDZ Europa 20–20, Sercon Ltd., Crewe, UK). Nitrogen recovery efficiency (^{15}NR) was used to express the percentage of total N fertilizer recovery by the maize plant. The amount of N derived from fertilizer (NDFF) in maize, cover crop straw or soil was expressed in kg N ha^{-1}. NDFF, ^{15}NR and unaccounted N from fertilizer were calculated 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 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 is the total N ($^{15}N + {}^{14}N$) in the sample (kg ha^{-1});

$$15\text{NR}\ (\%) = \left[\frac{\text{NDFF}}{\text{Fertilizer N rate}}\right] * 100$$

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

$$\text{Unaccounted 15N}\ (\%) = 100 - 15\text{NRt}$$

where unaccounted N is the percentage of fertilizer not recovered at maize harvest and $^{15}NR_t$ is the total N recovery (%) from soil, cover crop straw, and maize stover and grain.

In addition, we calculated the percentage of N recovery in the total N in each compartment in order to determine whether the treatments affected the proportion of N fertilizer uptake in total N in soil, cover crop straw or maize stover and grain.

2.5. Data statistical analyses

Data from cover crops (biomass and N accumulated), ^{15}N fertilizer recovery (soil, cover crop straw, and maize stover and grain) and percentage of the N recovery to total-N of soil, straw of cover crops, and maize stover and grain were initially tested for normality using the Shapiro-Wilk test (Shapiro and Wilk, 1965) and, if necessary, subsequently transformed the data using the log 10 transformation method. However, the Shapiro-Wilk test found that all data tested from variables were normal distributed at the 5% level of significance ($W \geq 0.90$, $p < 0.05$). For variables determined in one year, cover crop and N application timing were considered fixed factors; for variables determined in the three growing seasons, cover crops, N application timing and growing season were considered fixed factors. The growing seasons and their interactions with cover crop and N application timing were not significant at $P < 0.05$ for any of the dependent variables (See Supplementary Material). Thus, the data for the three growing seasons were combined. The block variable was considered a random variable. Analysis of variance (ANOVA) was performed. If the null hypothesis was rejected, a comparison of means was performed with the LSD test ($P \leq 0.05$). Only for loss of straw and N accumulated of cover crops, regressions on the four-sampling time (30, 60, 90 and 120 days after desiccation) were tested across the replications of growing seasons. All data were fit to the non-linear models of quadratic function, and effects were considered significant at $P < 0.05$. The cover crop and N application timing results were subjected to a polynomial regression analysis ($p \leq 0.05$), and a redundancy analysis (RDA) with the Monte Carlo permutation test (999 permutations) was performed to determine whether N recovery in maize stover and grain were correlated with cover crop variables (biomass at 0 and 120 DAT and N release), maize parameters leaf N concentration and grain yields) and N recovery in soil and cover crop straw, and to identify the most important N recovery factor shaping these responses. A one-way PERMANOVA (Anderson 2005) was used to group treatments based on similarity.

3. Results

3.1. Responses of cover crop and maize

The interaction of cover crop and N application timing significantly affected the decomposition of straw and the loss of N accumulated in palisade grass and ruzigrass to the agricultural system after cover crop desiccation (Table 1 and Supplementary Table 1). The greatest amounts of straw decomposition and loss of N accumulated were observed in palisade grass receiving N fertilizer (CC+N, St+N and Nv4), which resulted in losses of approximately 6.4 Mg DM straw ha^{-1} and 216 kg N ha^{-1}, respectively. By contrast, ruzigrass had lower straw decomposition and release of N accumulated to the soil over maize growth; these values were higher in the treatments that received earlier N application on live ruzigrass (CC+N) (6 Mg ha^{-1} and 218 kg ha^{-1}) than in the zero-N treatment (no-N) (1.9 Mg ha^{-1} and 85 kg ha^{-1}).

The leaf N concentration and the grain yield of maize were

Table 1
Cover crops straw at 0 and 120 days after cover crops termination (DAT) and N release from cover crops from 0 to 120 DAT as affected by cover crop and N fertilization. Average of three growing seasons (2015/2016, 2016/2017 and 2017/2018).

Cover crops	N application timing			
	no-N‡	CC+N	St+N	Nv4
Biomass at 0 DAT (Mg ha^{-1})				
Palisade grass	11.6aB†	13.8 aA	12.2aB	12.1aB
Ruzigrass	7.4bB	10.8bA	8.1bB	8.3bB
Biomass at 120 DAT (Mg ha^{-1})				
Palisade grass	5.8 aA	6.0 aA	6.8 aA	7.0 aA
Ruzigrass	1.9 BCE	6.1 aA	4.8bB	4.1bB
N release (kg ha^{-1})				
Palisade grass	211aB	245 aA†	208aB	202aB
Ruzigrass	85 BCE	218bA	105bB	86 BCE

‡Treatments of N application timing are: no-N: no N fertilizer applied (control); CC+N: N applied on live cover crop 35 days before maize seeding; St+N: N applied on cover crop straw 1 day before maize seeding; Nv4: conventional method of sidedress N applied at V4 growth stage of maize.
†Means followed by the different uppercase denote significant differences among N application timing and different lowercase letters denote significant differences between cover crops (LSD, $P \leq 0.05$).

influenced by cover crop and N application timing (Fig. 3 A and 3B). In general, the leaf N concentration was superior in maize with St+N and Nv4 following both cover crops and CC+N following palisade grass; whereas the lowest leaf N concentration was obtained with no-N application on ruzigrass. Grain yield of maize were higher in succession to palisade grass than in succession to ruzigrass. On average, N application on live palisade grass (CC+N) or its straw (St+N) or via the conventional method (Nv4) increased the grain yield (13 Mg ha^{-1}) of maize compared with the control (191 and 5.8 Mg ha^{-1}). In contrast to the results for ruzigrass decomposition, the grain yield of maize in succession to ruzigrass were similar under conventional N application (Nv4) and application of N on straw just before maize seeding (St+N), i.e., 11.7 Mg grain ha^{-1}, and were successively lower in the treatments in which N was applied on live ruzigrass (CC+N) and the control (no-N).

At maize harvest, the interaction between cover crop × N application timing was significant for total N content in the stover and grain of maize (Fig. 3 C and 3D, and Supplementary Table 2). The total N content in stover was greatest for maize in succession to palisade grass and decreased in the following order or treatments: N applied to live palisade grass (CC+N), to palisade grass straw (St+N) and to maize at V4 (Nv4). For maize cultivated after ruzigrass, the stover total N content was greatest in the treatment in which N was applied on ruzigrass straw (St+N). The pattern of total N content in grain differed from that of stover (Fig. 3 C and 3D) and was higher after both cover crops in the treatments receiving N fertilizer according to the conventional method (Nv4); in addition, grain total N content was higher in succession to palisade grass than in succession to ruzigrass. Grain total N content was similar when N was applied on live palisade grass or its straw (CC+N and St+N).

3.2. Labeled fertilizer nitrogen recovery

Although maize grain yields were higher in succession to palisade grass for all N applications (Fig. 3B), N recovery (^{15}NR) in maize grain in the first crop cycle (2015/16) was higher in succession to palisade grass only in treatments St+N and Nv4 (Fig. 4 and Supplementary Table 3). Overall, 26%, 21% and 10% of N in maize grain following palisade grass was derived from fertilizer in Nv4, St+N and CC+N, respectively, and 30%, 14% and 7% of ^{15}N was recovered in maize grain following ruzigrass in Nv4, St+N and CC+N. For both cover crops, ^{15}NR in maize stover was higher in Nv4, with values of 13% in succession to palisade grass and 10% in succession to ruzigrass; this difference between cover crops was statistically significant. The lowest recovery by stover was 4% and 3% in CC+N in succession to palisade grass and ruzigrass, respectively.

^{15}NR in the straw of cover crops was not affected by cover crop and N application timing, but ^{15}NR in the soil was affected by N application timing (Table 2). In the straw of cover crops, only 4% of N was derived from fertilizer. In soil, of the 160 kg ha^{-1} N applied, on average 47% was found in the 0–40 cm layer after both cover crops in treatment Nv4, followed by 38% in St+N and 33% in CC+N. Overall, 18%, 8%, 4% and 39% of the fertilizer applied was recovered in maize grain, stover, cover crop straw, and soil, respectively, whereas 53%, 33% and 8% of the fertilizer applied was uncounted in the plant-litter-soil system in treatments CC+N, St+N and Nv4 (Supplementary Fig. 1).

3.3. N fertilizer recovery as a percentage of the total N content

The contribution of N from fertilizer to the total N content (NC%) in

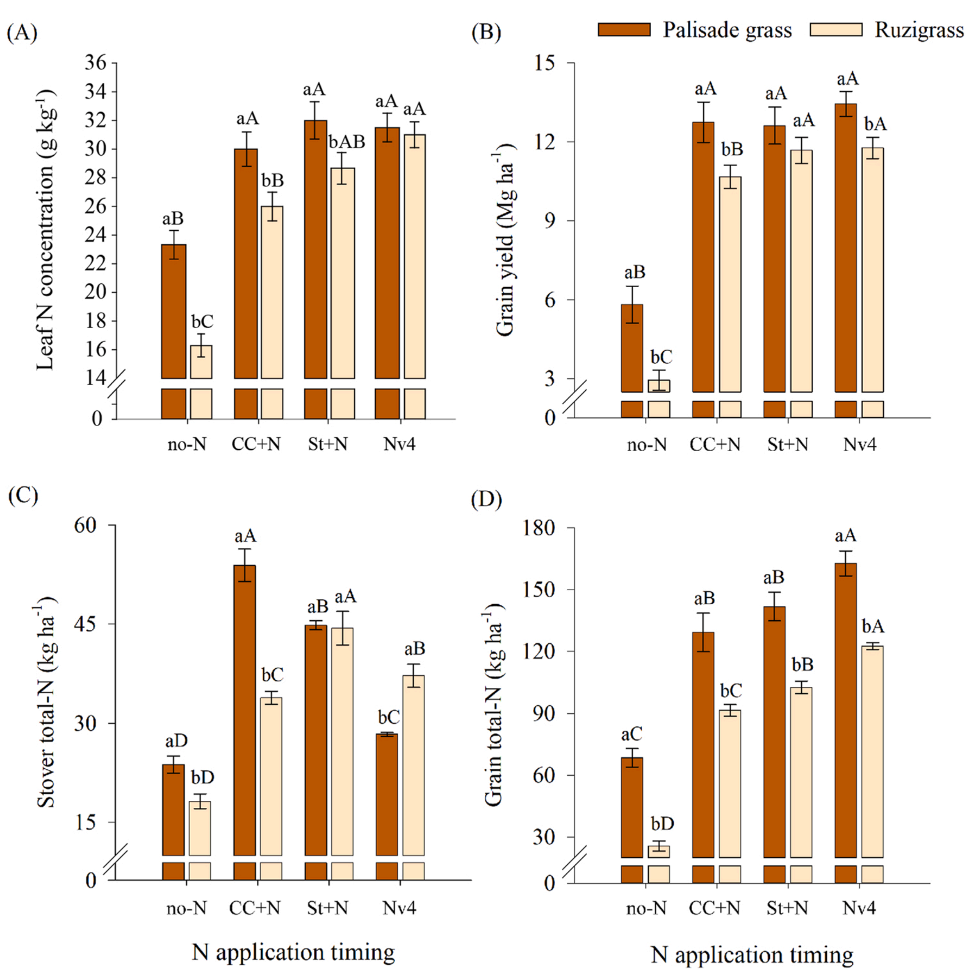

Fig. 3. Cover crop × N application timing interaction effect on leaf N concentration of maize at flowering stage (A), grain yield (B), stover total-N (C) and grain total-N of maize at harvest. Average of three growing seasons (2015/2016, 2016/2017 and 2017/2018). Treatments of N application timing are: no-N: zero N application on grass-maize system; CC+N: N applied on live cover crop 35 days before maize seeding; St+N: N applied on cover crop straw 1 day before maize seeding; Nv4: conventional method of sidedress N applied at V_4 growth stage of maize. 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, $P \leq 0.05$).

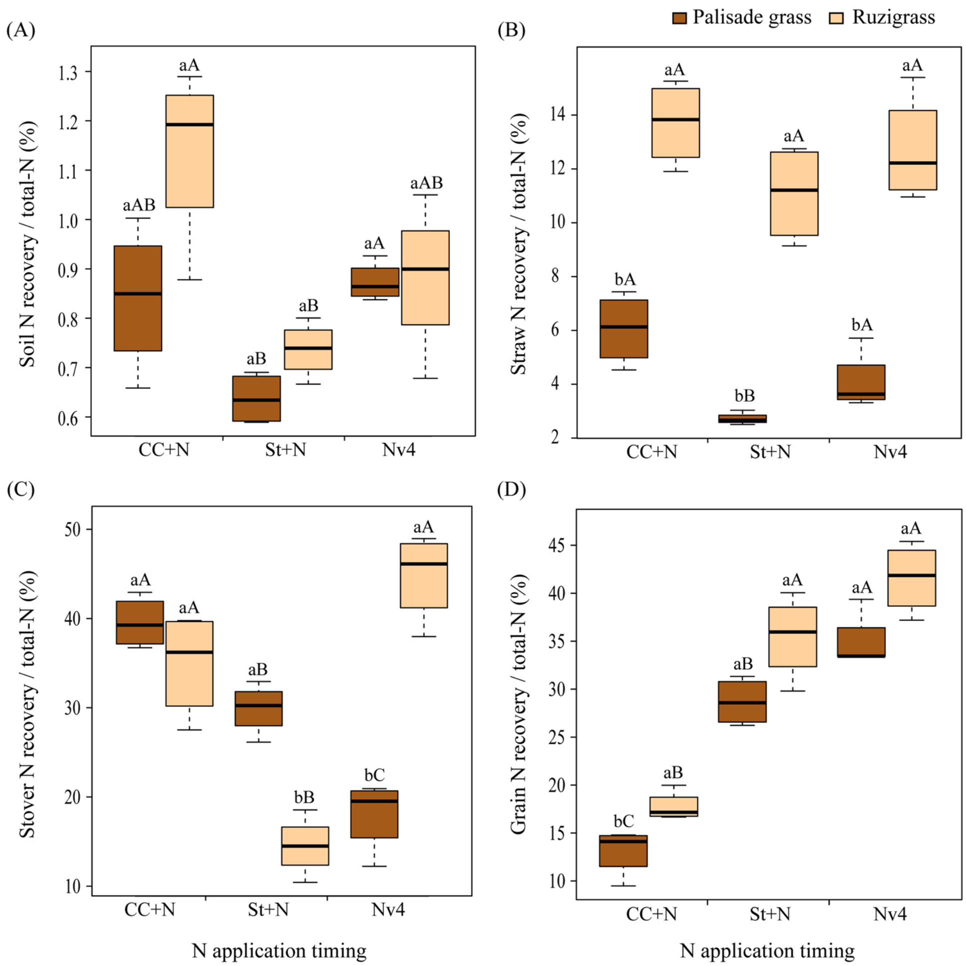

Fig. 4. Percentage of the N recovery to total-N of soil (A), straw of cover crops (B), and stover (C) and grain (D) of maize as affected by cover crop and N application timing in the first (2015–2016) growing season. Nitrogen application timing treatments are as follows: CC+N: N applied on live cover crop 35 days before maize seeding; St+N: N applied on cover crop straw 1 day before maize seeding; Nv4: conventional method of sidedress N applied at V_4 growth stage of maize. Different lowercase letters denote significant differences between cover crops and different uppercase letters denote significant differences between N application timing (LSD, $P \leq 0.05$).

Table 2
^{15}N fertilizer recovery in soil, cover crop straw, and maize stover and grain at harvest as affected by cover crop and N application timing in the 2015/2016 growing season.

Cover crops	N application timing		
	CC+N‡	St+N	Nv4
Soil (%)			
Palisade grass	30.0B†	36.6AB	47.9 A
Ruzigrass	36.2B	38.4AB	46.5 A
Straw (%)			
Palisade grass	4.8	2.1	4.7
Ruzigrass	4.8	4.1	3.9
Stover (%)			
Palisade grass	4.2aC	8.3aB	11.3 aA
Ruzigrass	3.7aC	7.2aB	9.5bA
Grain (%)			
Palisade grass	10aB	21.9 aA	30.1 aA
Ruzigrass	8aB	14.1aB	25.1 aA

‡Treatments of N application timing are: CC+N: N applied on live cover crop 35 days before maize seeding; St+N: N applied on cover crop straw 1 day before maize seeding; Nv4: conventional method of sidedress N applied at V4 growth stage of maize.
† Means followed by the different uppercase denote significant differences among N application timing and different lowercase letters denote significant differences between cover crops (LSD, $P \leq 0.05$).

soil, cover crop straw, and maize stover and grain is shown in Fig. 5. Similar to the pattern of ^{15}NR in the soil, there was no difference in NC% in soil between cover crops (Fig. 5A and Supplementary Table 3). The NC% in straw of ruzigrass was higher than that of palisade grass regardless of N application timing (Fig. 5B). NC% in maize stover was highest in CC+N in succession to palisade grass and decreased in St+N and Nv4; in succession to ruzigrass, NC% in maize stover was again highest in Nv4 (Fig. 5C). For maize grain, NC% was highest in Nv4 in succession to both cover crops and was sharply lower in CC+N in succession to palisade grass (Fig. 5D). Notably, NC% in maize grain was always lower in succession to palisade grass than in succession to ruzigrass.

3.4. *Correlations between 15 N recovery and soil and maize parameters*

The redundancy analysis (RDA) revealed that the soil and maize factors explained 77.29% of the total variability in maize 15 N recovery in stover and soil (Fig. 5). The treatments were segregated into three distinct groups by the PERMANOVA analysis ($p < 0.0001$). Group 1 consisted of Nv4 in succession to palisade grass and ruzigrass. Group 2 contained the CC+N in succession to both cover crops and the Nv4 following ruzigrass. Group 3 contained the St+N in succession to palisade grass and ruzigrass. The monte Carlo permutation analysis showed significant correlations between the soil, maize and 15 N recovery parameters. Cover crop N release ($F = 6.26$; $p = 0.007$), maize grain yield ($F = 5.05$; $p = 0.038$), N-total in maize stover ($F = 3.59$; $p = 0.019$) and grain ($F = 4.18$; $p = 0.016$), 15 N recovery in soil ($F = 4.33$; $p = 0.045$) and cover crop straw ($F = 4.15$; $p = 0.022$) were the main environmental factors responsible for changes in 15 N recovery in maize stover and grain.

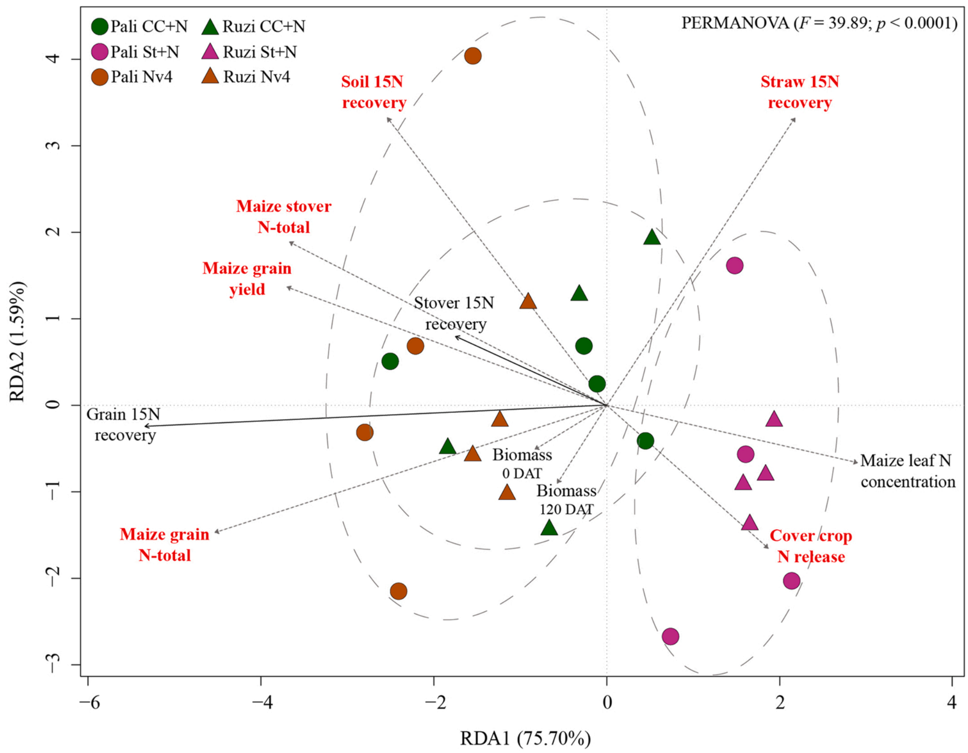

Fig. 5. Redundancy analysis (RDA) of the cover crop, maize, soil total-N and N recovery parameters in the growing season 2015–2016. Arrows indicate correlations between factors. Red color indicates the significance of these correlations ($p \leq 0.05$) by Monte Carlo permutation test (999 permutations) determination. The dashed circles indicate significant clusters according to the permutation analysis (PERMANOVA, $p \leq 0.05$). The description of treatments is in Fig. 3.

4. Discussion

4.1. Effects of N management and cover crop on maize yields

The earlier the N fertilizer was applied to the system (i.e., before grass desiccation) the higher the amounts of biomass were produced, and N was accumulated in the grasses. Because of higher biomass production, larger amounts of N were released upon decomposition of the grasses after desiccation, reaching more than 200 kg N ha^{-1} at 120 DAT, when the 60-day maize crop was intensively growing. Palisade grass produced more biomass and accumulated more N than ruzigrass, which resulted in larger amounts of N released after the grass decomposition. The fine root system of palisade grass, which can reach depths of more than one meter to take up N even from deeper soil layers (Galdos et al., 2020; Pacheco et al., 2011; Tanaka et al., 2019; Tedla et al., 1999) may explain these results. In addition, the decomposed residues of palisade grass showed rapid degradation of the straw. These mulching of cover crops, maintained on soil in no-tillage system, represents a source of nutrients for subsequent crops, especially N.

The increase in palisade grass straw decomposition under early N fertilizer application supplied the N demand of maize and enhanced the leaf N concentration and the yields of maize. Applying N on palisade grass is an alternative to the conventional N fertilization method recommended for maize. The grain yields of maize following palisade grass were approximately 13 Mg ha^{-1} under N fertilization. However, applying all the N fertilizer on cover crops or cover crop residues has been reported to cause lower yields or fertilizer N recovery than the application later, to the main crop (Momesso et al., 2019; Oliveira et al., 2018; Pöttker and Wiethölter, 2004). In most of these studies, black oats were used as the cover crop, which differs from *Urochloa* species in N uptake. In addition, while early N application on straw before cash crop seeding may result in greater grain yield, the risk of N losses remains, especially in regions with high rainfall; thus the conventional method of N application is generally preferred over earlier applications (Pöttker and Wiethölter, 2004).

The enhanced N accumulation by maize confirmed the efficiency of N cycling by palisade grass since the maize plants accumulated similar amounts of N at the flowering stage when cultivated under early N application or the conventional method. However, the differences in total-N content between maize stover and grain indicate N remobilization by maize at the reproductive stage due to the impacts of N available in soil and N released from the cover crops. N remobilization depends on several factors, such as the environment, soil mineral N, N management and maize genotype (Ciampitti and Vyn, 2012, 2013; Fageria and Baligar, 2005). In general, vegetative organs provide 45–65% of grain N at physiological maturity (pre-silking) and 35–55% of grain N thereafter (post-silking) (Hirel et al., 2007; Zhang et al., 2020), but a trade-off can occur between N remobilization at maize physiological maturity and grain N accumulation (Ciampitti and Vyn, 2013; Pommel et al., 2006). In addition, the total N in stover and grain is directly related to shoot dry matter production by maize. Our results indicated that N remobilization was sufficient to enhance maize production regardless of the timing of N application on palisade grass. In addition, the conventional method of N application increased the accumulation of this nutrient in the grains, but this increase was not proportionally converted into grain yield. As a result, the conventional method of application led to lower NUE (Goron et al., 2017; Herrmann and Taube, 2005) and greater N export.

4.2. Effects of early N application on fertilizer recovery in maize

Although the total N content and yield of maize grain differed in succession to palisade grass versus ruzigrass, both cover crops similarly promoted N recovery from fertilizer in different plant parts at maize harvest. Overall, the conventional method of sidedress N application to maize in succession to palisade grass and ruzigrass resulted in high recovery of N fertilizer in the agricultural system by supplying N fertilizer when the maize plants had sufficient root systems to take up the N applied (Marschner, 2012; Yang and Udvardi, 2018). These results are in agreement with those of other studies (Musyoka et al., 2019; Oliveira et al., 2018; Rocha et al., 2019). The amount of unrecovered N reported in this study can be attributed to ammonia volatilization, nitrate leaching and gas emission. Some portion of the uncounted N could be present in the root system of *Urochloa* spp., as a previous report estimated 18 kg N ha^{-1} in the roots of *Urochloa* species cultivated as pasture and demonstrated that well-managed long-term systems can increase the amount of N in roots (Rao, 1998). Thus, *Urochloa* species can transport N to deep soil layers, as their roots can reach more than 1 m deep approximately two months after germination (Huot et al., 2020).

Large amounts of N fertilizer were found in the soil at maize harvest, and the total recovery of fertilizer N in the soil was within the range of 25–45% reported in other studies (Gava et al., 2006; Karwat et al., 2017). The N recovered from fertilizer in soil varies with soil characteristics, fertilizer management, and cropping system (Almeida et al., 2018; Oliveira et al., 2018). Similar patterns of N recovery in the 0–40 cm soil layer as well as changes in N content were observed for palisade grass and ruzigrass. Topsoil layers usually have greater microbial activity in well-established NT systems, and variations in N content in the soil can occur in deeper layers (Zuber and Villamil, 2016). In the present study, conventional N application resulted in 60% greater N recovery in the soil at maize harvest than early N application. The reduction in N fertilizer recovery under early N application is probably related to N losses by NO_3^- leaching, NH_3 volatilization and N_2O emissions over the growing season (Rocha et al., 2020; Rosolem et al., 2017).

The fate of most of the N fertilizer applied on live palisade grass and ruzigrass or its residues in the system at maize harvest could not be determined. Thus, the high straw decomposition of the cover crops over the course of the maize cycle and the high maize yields did not lead to higher recovery of fertilizer N by maize; i.e., early N application on live palisade grass (CC +N) and straw (S +N) did not increase the recovery of N from fertilizer in maize grains. These results were supported by the N recovery analysis (Table 2 and Fig. 4) and the RDA (Fig. 5), reinforcing that the cover crop N release, N-total in maize stover and grain, N recovery in soil and cover crop straw factors were responsible for the increased N recovery in stover and grain. The low recovery in maize of early applied fertilizer N might be due to the dilution effect of the large biomass of palisade grass, release of the N by palisade grass before the peak of demand by maize, and loss of N to the environment by microbial processes of volatilization, nitrification and denitrification (Bani et al., 2018; Costa et al., 2016). Nitrogen fertilizer applied on grasses is susceptible to losses by NH_3 volatilization via stomata (Damin et al., 2010; Smart and Bloom, 2001) and, in the soil, by NO_3^- leaching from microbial oxidation of NH_4^+ to NO_3^- and by NO_2, N_2O, NO emissions from microbial conversion of nitrate and nitrite to gaseous N (Kuypers et al., 2018). Our results suggest that high grain yield and N fertilizer recovery by maize are possible when N is applied according to the conventional method (40 kg N ha^{-1} in the maize seeding furrow plus 120 kg N ha^{-1} sidedressed in the V_4 growth stage).

N application on palisade grass provided similar results in terms of maize grain yield; however, most of the N in maize at harvest came from the soil when N fertilizer was applied to live palisade grass (Figs. 4 and 5). Mulching of palisade grass increases the potential for N cycling for food production, and this *Urochloa* species has proved to be a great option for sustainable production that increases soil organic matter, benefits soil structure and helps to reduce the chemical applications of herbicides to the succeeding cash crop. Our study contributes with the understanding of N uptake and accumulation in cover crop species of the genus *Urochloa* and the dynamics of straw decomposition and N release after termination, thus helping to devise N management strategies that may enhance the sustainability of used in agricultural systems. However, questions about the N dynamics of soil in such systems remain open.

5. Conclusions

Of the two *Urochloa* species used as cover crops in NT system, palisade grass (*Urochloa brizantha*) produced higher dry matter yield and N release in the agricultural system to supply N demand and increase grain yield of maize. Besides that, the timing of N application in agricultural system is an important factor for increasing maize grain yield and forage production. In our study, applying N on live palisade grass and its residues produced high grain yields of maize, similar to those obtained under conventional N fertilization, but the recovery of N from fertilizer by the maize plant and grain was very low. To ensure that both maize grain yields and N recovery from fertilizer are high, N fertilizer should be applied according to the currently recommended method (40 kg N ha^{-1} at maize seeding plus 120 kg N ha^{-1} sidedressed in the V_4 growth stage). Additional studies are needed to better understand soil N dynamics and recovery of N fertilizer under early N application as well as changes in soil microbiology and root composition in short- and long-term experiments.

CRediT authorship contribution statement

Letusa Momesso: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Carlos A.C. Crusciol:** Conceptualization, Funding acquisition, Supervision. **Carlos A.C. Nascimento:** Conceptualization. **Rogério P. Soratto:** Visualization, Methodology. **Lucas P. Canisares:** Data curation. **Luiz G. Moretti**: Data curation. **Ciro A. Rosolem:** Funding acquisition. **Eiko E. Kuramae:** Writing – review & editing. **Paulo C.O. Trivelin:** Methodology. **Heitor Cantarella:** Conceptualization, Writing – review & editing. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Grants from CAPES – Coordination for the Improvement of Higher Level Personnel [PDSE 88881.187743/2018–01] to LM, FAPESP [grant number 2015/17953–6] for partial funding, and FAPESP [2016/12317–7] to CACN. This work was undertaken as part of NUCLEUS, a virtual joint center to deliver enhanced N–use efficiency via an integrated soil–plant systems approach for the United Kingdom and Brazil. Funded in the United Kingdom by the Biotechnology and Biological Sciences Research Council [Grant BB/N013201/1] under the Newton Fund scheme; and in Brazil by FAPESP–São Paulo Research Foundation [Grant 2015/50305–8]; FAPEG–Goiás Research Foundation [Grant 2015–10267001479]; and FAPEMA–Maranhão Research Foundation [Grant RCUK–02771/16]. The authors would like to acknowledge the National Council for Scientific and Technological Development (CNPq) for awards for excellence in research to CACC, RPS, CAR, PCOT and HC. Publication number XXXX of the Netherlands Institute of Ecology (NIOO-KNAW).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eja.2022.126485.

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