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Cover Crops Affect Soil Mineral Nitrogen and N Fertilizer Use Efficiency of Maize No-Tillage System in the Brazilian Cerrado

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Abstract: Cover crops in no-tillage systems may alter soil mineral N and influence the N fertilizer use efficiency (NFUE) of subsequent maize. The hypothesis of this work is that no-tillage systems with cover crops affect nitrate, ammonium and maize NFUE in the Brazilian Cerrado. The objective was to evaluate the cover crop mineralization effect on soil N mineral and maize NFUE in a no-tillage system, with and without N topdressing. The experiment was arranged in a randomized block split-plot design. The plots were represented by cover crops (*Cajanus cajan*, *Crotalaria juncea*, *Raphanus sativus* and *Mucuna aterrima*). The subplots consisted of the application (WN) or non-application (NN) of N topdressing to maize. The soil was sampled in six layers (up to 60 cm) at the end (April) and at the beginning of the rainy season (November). NH_4^+ was lower for all cover crops and WN and NN management in April. NO_3^- differed between seasons and cover crops in WN and NN. The lignin concentration and N uptake of *M. aterrima* were the highest compared to other species. The highest NFUE was on *R. sativus*, showing higher fertilizer dependency. In a no-tillage system with cover crops, the N topdressing fertilization needs to be improved, considering mineralization.

Keywords: ammonium; annual crops; nitrate; *Zea mays*

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1. Introduction

Nitrogen (N) is a key element to ensure maximum crop yields and food security [1–3]. All around the world, the use of N fertilizers has been estimated to be ~155 million tons and has boosted crop and food production [4]. Nitrogen is the nutrient crops require most [5] and soil net N mineralization is a critical rate for crop development. This process is mainly controlled by the climate and physical and/or biochemical properties [6]. Nitrogen is taken up by plants preferentially in mineral forms (ammonium and nitrate). The absorbed form of N depends on the crop, although these N forms represent only 2% of the total N in the soil [7].

On average, about 50% of N fertilizer is recovered by plants and is susceptible to leaching losses, gaseous emissions and surface runoff [8]. The N fertilizer applied to maize-forage intercropping can be recovered by the forage and maintained in the system. After harvesting, more than 60% of N remains in the system, partially on forage grass and maize residues but mainly on soil. About 12% of the N residual is recovered by soybeans in succession [9].

Therefore, to improve the sustainability of agricultural systems, the dynamics of this nutrient in the systems should be investigated, and the contribution of cover crops to soil mineral N and the N fertilizer use efficiency (NFUE) of a subsequent cash crop should be considered to define the effective need for nitrogen fertilization [10,11]. In this context, the decomposition process of cover crop residues is fundamental because it regulates N mineralization in the soil and should be taken into account in N fertilizer recommendations.

Nitrogen is a nutrient with highly complex dynamics in the soil-plant-atmosphere system [12] and is directly associated with carbon (C) accumulation in the soil [13]. Knowledge of these mineral N dynamics in the soil can facilitate decision-making regarding the most reliable, efficient and sustainable use of crop rotation [14,15] and fertilization [7], and put an end to indiscriminate fertilizer use without concern about avoiding environmental and economic losses.

About half of the N fertilizer applied will become available for plant uptake [2], while the other part will be in the environment [16,17]. In maize, 30–60% of N applied in the form of fertilizer can be recovered by the plants [5], whereas the rest may be lost by leaching in the form of nitrate, ammonia volatilization or even in much smaller quantities during nitrification, especially in the form of N_2O [18–20].

The process of soil degradation and N losses can be minimized by certain techniques, such as no-tillage management with crop rotation and practices such as intercropping. These measures increase the availability of mineral N in relation to monoculture and plowed farming systems [21,22], and, consequently, the efficiency of N fertilizer use [14].

In tropical regions, especially in South America, management practices such as the use of intercropped systems and crop–pasture rotation, including forage or legume species with high biological dinitrogen fixation efficiency as cover crops, are viable alternatives for the recovery of degraded lands and to improve soil carbon accumulation [23]. In Brazil, the double cropping system under no-tillage is mainly based on soybean as the main crop, followed by maize or sorghum as the second commercial crop [9]. In regions with lower rainfall availability, gramineous species are commonly used as cover crops, such as pearl millet (*Pennisetum glaucum*), Brachiaria (*Urochoa* sp.) or *Panicum* sp., normally from March to mid-June [24].

The literature reveals that there is still a lack of information on the use of leguminous species in agricultural systems, especially in the modality of crop succession, intercropping or as a companion crop [25–27]. In no-tillage systems integrated with leguminous species, the recovery efficiency of N-fertilizer applied by annual crops may be higher than that of monocropping or using gramineous species as cover crops. In no-tillage systems combining a more diverse set of cover crop species, the N available in deeper soil layers, which has the potential to be leached, can be absorbed by the roots of the plants before the roots of the annual crops reach this depth. This interaction between species in the integrated system increases nutrient recycling efficiency, especially N. In general, the transfer of N is greater from legume species to non-legumes than the other way around [28].

In this context, we believe that cover crops in no-tillage systems alter soil mineral N levels and influence the nitrogen fertilizer use efficiency (NFUE) of subsequent maize. Thus, we hypothesized that no-tillage systems with different cover crops with different chemical compositions affect the availability of mineral N (nitrate and ammonium) and NFUE of maize in the Brazilian Cerrado differently. To test this hypothesis, ammonium (NH_4^+) and nitrate (NO_3^-) concentrations and NFUE were evaluated in soil under different cover crops grown after maize fertilized with N (WN) and without N (NN) topdressing at the end of the rainy season and the beginning of the following season. The chemical composition of the cover plants was also assessed to help explain N mineralization in this production system.

2. Materials and Methods

2.1. Location and Experimental Design

The experiment was carried out at the Cerrados unit of the Brazilian Agricultural Research Corporation (Embrapa), in Planaltina, Distrito Federal, Brazil (15°35′30″ S, 47°42′30″ W). According to the Köppen–Geiger classification, the regional climate is Aw (tropical savanna), with dry winters and rainy summers, a mean annual air temperature between 22 and 27 °C, and a mean annual rainfall of 1345.8 mm [28]. Rainfall and mean air temperature from the end of one (6 April 2016) to the beginning of the following rainy season (16 November 2016), when the two soil samplings were taken, are shown in Figure 1. The mean temperature from April to November was 22.2 °C (±2.3), and precipitation from April to September was 65.4 mm (dry season), and in October and November it was 255 mm (part of the rainy season).

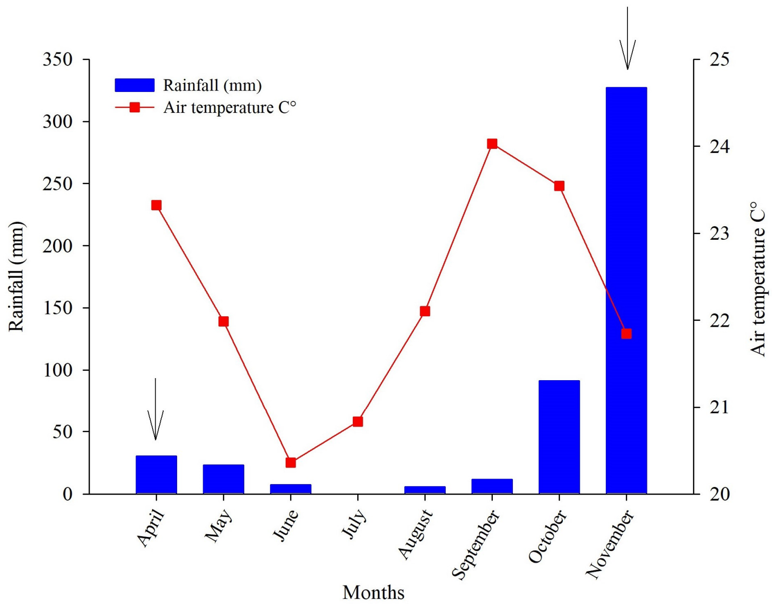


Figure 1. Rainfall and temperature in the period from April to November 2016 in the experimental area. Embrapa Cerrados, Planaltina, DF, Brazil. Arrows indicate the soil sampling dates: 6 April 2016 and 16 November 2016.

The soil was classified as Typical Acrustox and before the experiment, the chemical and physical soil properties (0–20 cm layer) were determined as follows: pH (H_2O) 6.0; organic matter 21.7 g kg^{-1}; P (Mehlich-1) 0.9 mg kg^{-1}; Al^{3+} 0.1 cmol$_c$ kg^{-1}; Ca^{2+} + Mg^{2+} 2.9 cmol$_c$ kg^{-1}; K^+ 0.1 cmol$_c$ kg^{-1}; fine sand 258 g kg^{-1}; coarse sand 76.7 g kg^{-1}; silt 101.8 g kg^{-1}; and clay 563.5 g kg^{-1}.

From 1999 to 2004, alternating soybeans and maize were grown in the area. In the growing seasons from 2004/2005 to 2015/2016, a repeated sequence of maize and cover crops was cultivated under no-tillage management. Every year, from 2004/2005 to 2015/2016, after harvesting the maize hybrid 30F53VYHR (February), the following cover species were sown: pigeon pea (*Cajanus cajan* (L.) Millsp), sunn hemp (*Crotalaria juncea* L.), black Mucuna (*Mucuna aterrima* Merr.) and oilseed radish (*Raphanus sativus* L.).

The experiment was arranged in a randomized block split plot design with three replications. The plots (12 × 8 m) consisted of four cover crops, and the subplots (12 × 4 m) of maize fertilized with N topdressing (WN) or without N topdressing (NN). Two topdressings of 65 kg N ha^{-1} were applied as urea in WN, corresponding to 130 kg total N ha^{-1} in topdressings plus 20 kg N ha^{-1} at sowing, for a total of 150 kg N ha^{-1}, as recommended by Sousa and Lobato [29]. In addition, maintenance fertilization was applied in the planting furrow at maize sowing, consisting of 150 kg ha^{-1} P_2O_5, 80 kg ha^{-1} K_2O, 2 kg ha^{-1} Zn ($ZnSO_4 \cdot 7H_2O$) and 10 kg ha^{-1} FTE BR 12 as a micronutrient source (3.2% S, 1.8% B, 0.8% Cu, 2.0% Mn, 0.1% Mo, 9.0% Zn and 1.8% Ca).

In April 2016, the cover crops *Cajanus cajan* and *Crotalaria juncea* were sown at a density of 20 plants m^{-1}, *M. aterrima* at 10 plants m^{-1} and *R. sativus* at 40 plants m^{-1}. The row spacing for all cover crops was 0.5 m. Cover crops were cut at flowering (between May and August 2016), depending on the species: *Raphanus sativus* was cut in May 2016, *Crotalaria* *juncea* in June 2016 and *Cajanus cajan* (L.) Millsp and *Mucuna aterrima* in August 2016. The crop residues were left on the soil surface. The maize hybrid 30F53VYHR was sown in November 2016 at a row spacing of 0.75 m and five seeds m^{-1}, i.e., to achieve a density of 66,667 plants ha^{-1}.

2.2. Soil Sampling and Analysis

The soil for analyses of ammonium (NH_4^+) and nitrate (NO_3^-) concentrations was sampled on two dates: on 6 April 2016 (after maize harvest, at the end of the rainy season), before cover crop planting and on 16 November 2016 (before maize planting, at the beginning of the rainy season). These two moments were chosen since the dynamic process of N mineralization is strongly related to the moisture level. As the Cerrado region is characterized by two seasons, rainy and dry, soil sampling was performed to represent these two conditions.

Composite samples of five sub-samples were collected per subplot (WN and NN) from the layers 0–5, 5–10, 10–20, 20–40 and 40–60 cm. The samples were ice-cooled in the field and in the laboratory, they were maintained in a freezer for a week until extraction and analysis. The soil ammonium (NH_4^+) and nitrate (NO_3^-) concentrations were analyzed at both samplings for each layer.

The soil mineral nitrogen concentration (mg kg^{-1}) in the forms of N-NO_3^- and N-NH_4^+ was determined through extraction in 50 mL of 2 mol L^{-1} KCl, according to the method proposed by Bremner and Mulvaney [30], and analyzed by colorimetry with a Lachat 228 Quikchem flow injection analyzer (Lachat Instruments, 5600 Lindbergh Drive, Loveland, CO 80539, USA). The soil moisture of each sample was determined using the gravimetric method. The gravimetric soil water content was determined after drying the material at 105 °C for 48 h. The soil mineral N concentrations (NO_3^- and NH_4^+ in mg kg^{-1}) were calculated based on soil dry weight.

2.3. Plant Analysis and Maize Yield

Crop residues of the cover crops were sampled at flowering within two rectangular iron sampling frames per subplot (0.38 × 0.58 m).

The plant biomass samples were dried at 65 °C, and a subsample was weighed to determine plant dry matter, and the value converted to kilograms per hectare. The difference between the cover crop sample weight before and after drying was the evaporated moisture. A 3 g sample of dry biomass was ground and oven-heated at 105 °C for 8 h. The total N content (TN) was analyzed by colorimetry with a Lachat 228 Quikchem flow injection analyzer (Lachat Instruments, 5600 Lindbergh Drive, Loveland, CO 80539, USA). The N concentration in plant tissues was used to calculate N uptake by cover crops, considering dry biomass.

The dry matter, acid detergent fiber (ADF), neutral detergent fiber (NDF) and lignin concentrations of the cover crop residues were analyzed at 105 °C [31]. Analyses of neutral detergent (NDF), fiber and acid detergent (FDA) and fiber were analyzed using the ANKON system [32]. Lignin was analyzed by digestion of the FDA residue with 72% sulfuric acid, which extracts cellulose and hemicellulose, generating lignin and inorganic matter as residues. Cellulose and hemicellulose were calculated as the differences between FDN and FDA residues and between FDA and lignin residues, respectively. The lignin concentration was given by the difference between the acid digestion residue and the ash after burning at 600 °C for 4 h. In November 2016, 4 m rows of each subplot were harvested to determine maize grain yield, and grain moisture was corrected to 13% (w.b.).

2.4. Nitrogen Fertilizer Use Efficiency (NFUE)

Nitrogen fertilizer use efficiency was calculated using the following equation, according to Dobermann [33]:

$$NFUE\left(\frac{kg\ grain}{kg\ N\ applied}\right) = \frac{(Grain\ yield\ in\ WN - Grain\ yield\ in\ NN)}{N\ rate}$$

N rate is the N topdressing rate applied (130 kg ha^{-1} N).

2.5. Statistical Analysis

Before statistical analysis, the normality of the data was checked. Then, analysis of variance with data repeated over time (beginning and end of the rainy season) and space (0–5; 5–10; 10–20; 20–40; 40–60 cm) was performed to assess the effects of the plant species, N application and sampling times, in addition to the interactions between these factors. The data were analyzed using a two-way ANOVA, followed by Tukey's test, considering $p < 0.05$ for total variables, as a post hoc method to detect statistically significant differences among the treatments. This analysis of variance was performed using R version 3.5.0 software [34].

Principal component analysis (PCA) [34] was applied to a dataset with 16 rows comprising cover crops with and without N topdressing of maize (WN and NN), NH_4^+ and NO_3^- concentrations down to a soil depth of 60 cm, maize yield, total N concentration (TN), hemicellulose, cellulose, lignin and the lignin:N ratio.

3. Results

3.1. Mineral N in the Soil

There were significant effects ($p < 0.05$) of sampling dates and soil layers (Tables 1–5) on mineral N (NH_4^+ and NO_3^-) concentrations. The two periods of mineral N evaluation (the end and the beginning of the rainy season) were chosen because they reflect the N dynamics as a function of soil moisture conditions. The mean NH_4^+ concentrations in the five layers differed between the beginning and end of the rainy season in the NN and WN treatments for all studied cover crops, while the mean NO_3^- concentration of the five soil layers only differed between the beginning and end of the rainy season for *Crotalaria juncea* in the WN treatment and for all cover crops except *Mucuna aterrima* in the NN treatment. In a comparison of the cover crops, there were no differences between the species on NH_4^+ concentrations in WN and NN and in both sampling periods. The NO_3^- concentrations differed only at the end of the rainy season under NN, when concentrations were higher under *Mucuna aterrima* than *Raphanus sativus* (Table 1).

Table 1. Ammonium (NH_4^+) and nitrate (NO_3^-) concentrations in soil under different cover crops grown after maize with N fertilizer (WN) and without N (NN) topdressing at the end of the rainy season and the beginning of the following.

Cover crop	NH_4^+		NO_3^-	
	April	November	April	November
	mg kg^{-1}		mg kg^{-1}	
	WN			
Mucuna aterrima	2.58 aB	3.45 aA	4.09 aA	5.64 aA
Raphanus sativus	3.90 aB	4.58 aA	4.08 aA	5.43 aA
Cajanus cajan	3.11 aB	4.06 aA	3.77 aA	3.24 aA
Crotalaria juncea	2.44 aB	4.43 aA	5.34 aA	2.52 aB
CV% [1]	41.16		48.89	
CV% [2]	62.19		74.34	
	NN			
Mucuna aterrima	2.43 aB	3.83 aA	4.16 aA	5.35 aA
Raphanus sativus	3.01 aB	4.44 aA	2.17 bB	3.79 aA
Cajanus cajan	2.72 aB	4.45 aA	2.92 abB	4.09 aA
Crotalaria juncea	2.46 aB	4.47 aA	2.74 abB	3.37 aA
CV% [1]	55.63		49.07	
CV% [2]	55.86		70.20	

Means followed by the same lowercase letters in a column and the same uppercase letters in a row do not differ by Tukey's test ($p < 0.05$). (1) Coefficient of variation related to cover crops. (2) Coefficient of variation related to the sampling period. WN—maize fertilization with N topdressing. NN—no N topdressing on maize.

At the end of the rainy season, the NH_4^+ concentration under WN was highest in the 0–5 cm layer under *Raphanus sativus*, while the NO_3^- concentration did not differ between cover crops in any layer. In all treatments with cover crops, the mean NH_4^+ and NO_3^- concentrations were highest in the 0–5 cm layer, and these values exceeded the means of the other layers (5–10, 10–20, 20- 40 and 40–60 cm), varying from 54–81% for ammonium and 50–64% for nitrate concentrations (Table 2).

Table 2. Ammonium (NH_4^+) and nitrate (NO_3^-) concentrations in soil layers under different cover crops grown after maize with N (WN) topdressing at the end of the rainy season (April).

Depth (cm)	**0–5**	**5–10**	**10–20**	**20–40**	**40–60**
Cover crop			NH_4^+ (mg kg^{-1})		
Mucuna aterrima	4.53 bA	2.21 aB	2.08 aB	2.12 aB	1.96 aB
Raphanus sativus	11.05 aA	2.36 aB	2.17 aB	1.78 aB	2.11 aB
Cajanus cajan	6.50 bA	2.67 aB	2.15 aB	2.41 aB	1.84 aB
Crotalaria juncea	4.50 bA	1.94 aA	1.89 aA	1.98 aA	1.87 aA
CV% [1]			48.50		
CV% [2]			42.24		
Cover crop			NO_3^- (mg kg^{-1})		
Mucuna aterrima	6.78 aA	5.23 aAB	4.44 aB	2.03 aB	1.97 aB
Raphanus sativus	8.37 aA	5.14 aAB	3.31 aB	1.90 aB	1.68 aB
Cajanus cajan	7.72 aA	5.13 aAB	3.26 aB	1.17 aB	1.59 aB
Crotalaria juncea	10.33 aA	5.59 aAB	2.74 aB	3.96 aB	4.08 aB
CV% [1]			55.57		
CV% [2]			67.28		

Means followed by the same lowercase letters in a column and the same uppercase letters in a row do not differ by Tukey's test ($p < 0.05$). (1) Coefficient of variation related to cover crops. (2) Coefficient of variation related to the soil layer. WN—maize fertilization with N topdressing. NN—no N topdressing on maize.

Table 3. Soil ammonium (NH_4^+) and nitrate (NO_3^-)concentrations in five layers under different cover crops grown after maize without N fertilizer (NN) in topdressing at the end of the rainy season (April).

Depth (cm)	**0–5**	**5–10**	**10–20**	**20–40**	**40–60**
Cover crop			NH_4^+ (mg kg^{-1})		
Mucuna aterrima	4.37 aA	2.01 aB	1.76 aB	1.83 aB	2.17 aB
Raphanus sativus	5.59 aA	3.76 aB	1.49 aB	2.33 aB	1.90 aB
Cajanus cajan	5.62 aA	2.49 aB	1.78 aB	1.83 aB	1.86 aB
Crotalaria juncea	3.85 aA	2.11 aB	2.21 aB	1.80 aB	2.33 aB
CV% [1]			27.01		
CV% [2]			49.07		
Cover crop			NO_3^- (mg kg^{-1})		
Mucuna aterrima	7.58 aA	6.98 aA	3.45 aB	1.63 aB	1.18 aB
Raphanus sativus	3.59 bA	3.13 bA	2.19 bB	1.21 bB	0.75 bB
Cajanus cajan	4.87 abA	3.85 abA	3.45 abA	1.08 abB	1.34 abB
Crotalaria juncea	3.78 abA	5.24 abA	1.96 abB	1.42 abB	1.29 abB
CV% [1]			42.61		
CV% [2]			48.40		

Means followed by the same lowercase letters in a column and the same uppercase letters in a row do not differ by Tukey's test ($p < 0.05$). (1) Coefficient of variation related to cover crops. (2) Coefficient of variation related to the soil layer. WN—maize fertilized with N topdressing. NN—maize without N topdressing.

At the end of the rainy season, the NH_4^+ concentration under NN did not differ between cover crops in any layer while the NO_3^- concentration was higher under *Mucuna aterrima* than *Raphanus sativus*. In all cover crop treatments, the NH_4^+ concentrations were highest in the 0–5 cm layer and exceeded the means of the 5–10, 10–20, 20–40 and 40–60 cm layers, which ranged from 45–65% (Table 3). For NO_3^-, the concentrations in the first two layers (0–5 and 5–10 cm) under *Mucuna aterrima*, *Raphanus sativus* and *Crotalaria juncea* were higher than in the other layers (10–20, 20–40 and 40–60 cm), while under *Cajanus cajan*, the concentrations were higher in the first three layers than in the others (20–40 and 40–60 cm) (Table 3).

Table 4. Soil ammonium (NH_4^+) and nitrate (NO_3^-) concentrations in five layers under different cover crops grown after maize topdressing with N topdressing (WN) at the beginning of the rainy season (November).

Depth (cm)	**0–5**	**5–10**	**10–20**	**20–40**	**40–60**
Cover crop			NH_4^+ (mg kg^{-1})		
Mucuna aterrima	5.78 aA	3.62 aB	2.82 aB	2.65 aB	2.38 aB
Raphanus sativus	6.56 aA	5.18 aB	2.82 aB	2.78 aB	5.57 aA
Cajanus cajan	7.01 aA	4.04 aAB	2.72 aB	3.22 aB	3.32 aB
Crotalaria juncea	6.54 aA	3.93 aB	3.53 aB	3.48 aB	4.65 aB
CV% [1]			33.84		
CV% [2]			35.06		
Cover crop			NO_3^- (mg kg^{-1})		
Mucuna aterrima	7.74 aA	8.13 aA	1.60 aB	5.18 aA	5.55 aA
Raphanus sativus	6.46 aA	8.61 aA	5.17 aA	3.58 aA	3.31 aA
Cajanus cajan	1.88 aB	5.38 aA	4.04 aA	2.04 aB	2.88 aB
Crotalaria juncea	2.50 aB	0.91 bB	2.01 aB	2.44 aB	4.73 aA
CV% [1]			59.93		
CV% [2]			58.18		

Means followed by the same lowercase letters in a column and the same uppercase letters in a row do not differ by Tukey's test ($p < 0.05$). (1) Coefficient of variation related to cover crops. (2) Coefficient of variation related to the soil layer. WN—maize fertilization with N topdressing. NN—no N topdressing on maize.

Table 5. Soil ammonium (NH_4^+) and nitrate (NO_3-) concentrations in five layers under different cover crops grown after maize without N topdressing (NN) at the beginning of the rainy season (November).

Depth (cm)	**0–5**	**5–10**	**10–20**	**20–40**	**40–60**
Cover crop			NH_4^+ (mg kg^{-1})		
Mucuna aterrima	5.43 aA	4.46 aAB	3.19 aBC	3.40 aC	2.65 aBC
Raphanus sativus	7.27 aA	4.28 aAB	3.57 aBC	2.72 aC	4.36 aBC
Cajanus cajan	7.59 aA	5.62 aAB	3.45 aBC	2.18 aC	3.40 aBC
Crotalaria juncea	5.38 aAB	6.11 aA	5.35 aAB	2.48 aC	3.06 aC
CV% [1]			50.60		
CV% [2]			36.15		
Cover crop			NO_3^- (mg kg^{-1})		
Mucuna aterrima	6.47 aAB	9.65 aA	4.30 aB	3.04 aB	3.30 aB
Raphanus sativus	6.16 aA	2.41 bB	5.50 aA	1.80 aB	3.08 aAB
Cajanus cajan	5.41 aA	6.09 abA	3.01 aA	3.62 aA	2.31 aA
Crotalaria juncea	4.25 aA	1.80 bB	5.21 aA	3.37 aA	2.24 aA
CV% [1]			65.45		
CV% [2]			50.05		

Means followed by the same lowercase letters in a column and the same uppercase letters in a row do not differ by Tukey's test ($p < 0.05$). (1) Coefficient of variation related to cover crops. (2) Coefficient of variation related to the soil layer. WN—maize fertilization with N topdressing. NN—no N topdressing on maize.

At the beginning of the rainy season in the WN treatment, the NH_4^+ concentration did not differ between cover crops in any layer, similar to the NO_3^- concentration, except under *Crotalaria juncea* in the 5–10 cm layer. Ammonium concentrations were highest in the topsoil (0–5 cm) under all cover crops. Nitrate concentrations under *Mucuna aterrima* were significantly lower in the 10–20 cm layer than at the other depths. Under *Raphanus sativus*, there were no differences between layers. Under *Cajanus cajan*, nitrate concentrations were highest in the 5–10 and 10–20 cm layers while for *Crotalaria juncea*, concentrations were highest in the deepest sampled layer (40–60 cm) (Table 4).

At the beginning of the rainy season in the NN treatment, the NH_4^+ concentration did not differ between cover crops in any layer, similar to the NO_3^- concentration, except under *Mucuna aterrima* in the 5–10 cm layer. In a comparison of the layers, NH_4^+ concentrations were highest in the 0–5 and 5–10 cm layers, except under *Crotalaria juncea*, where concentrations decreased from 20–40 cm and deeper. For NO_3^-, concentrations were similar among the cover crops in all layers, except in 5–10 cm, where the nitrate concentration was higher under *Mucuna aterrima* than *Raphanus sativus* and *Crotalaria juncea*. In general, *Mucuna aterrima* and *Cajanus cajan* had the highest nitrate concentrations in the 0–5 and 5–10 cm layers, while *Crotalaria juncea* and *Raphanus sativus* were significantly reduced in the 5–10 cm layer. Under *Raphanus sativus*, the nitrate concentration also decreased at 20–40 cm (Table 5).

3.2. Total Nitrogen, Hemicellulose, Cellulose, Lignin, Lignin:N Concentration and N Uptake

Total nitrogen (TN), hemicellulose, cellulose and lignin concentrations, such as the lignin:N ratio and N uptake of the cover crops, are listed in Table 6. The TN concentrations were higher in the aboveground biomass of *Crotalaria juncea* than of *Mucuna aterrima* and did not differ between *Raphanus sativus* and *Cajanus cajan*.

Table 6. Total nitrogen (TN), hemicellulose, cellulose, lignin, lignin:N ratio and N uptake of the aboveground biomass of cover crops.

Cover Crop	TN	Hemicellulose	Cellulose	Lignin	Lignin:N	N Uptake
	g kg^{-1}					kg ha^{-1}
Mucuna aterrima	17.99 b	131.95 a	253.12 a	70.08 a	3.97 a	482 a
Raphanus sativus	19.03 ab	87.22 b	213.17 b	46.56 b	2.45 b	275 b
Cajanus cajan	20.91 a	124.99 a	195.59 b	85.49 a	4.12 a	418 a
Crotalaria juncea	19.83 ab	135.14 a	274.05 a	47.08 b	2.37 b	287 b
CV %	17.77	5.83	4.53	8.57	19.26	16.46

Means followed by the same letters in a column do not differ by Tukey's test ($p < 0.05$).

The hemicellulose concentration was lower in the aboveground biomass of *Raphanus sativus* than in that of the other cover crops, similarly to the cellulose concentration that was lower in *Cajanus cajan* and *Raphanus sativus*. The cover crops *Raphanus sativus* and *Crotalaria juncea* had the lowest lignin concentration and lignin:N ratio.

Nitrogen uptake in the aboveground biomass of cover crops was higher in *Mucuna aterrima* and *Cajanus cajan* than in *Raphanus sativus* and *Crotalaria juncea*.

3.3. Maize Yield and Nitrogen Fertilizer Use Efficiency (NFUE)

There was an effect of cover crops on maize yield in the treatment without N topdressing on maize ($p < 0.05$). The maize yield after *Raphanus sativus* (5956.19 kg ha^{-1}) was 14% lower than the mean yield of maize grown after the other cover crops (6935.05 kg ha^{-1}). Nitrogen fertilizer use efficiency (NFUE) of maize was greater ($p < 0.05$) after *Raphanus sativus* than *Mucuna aterrima* (Table 7).

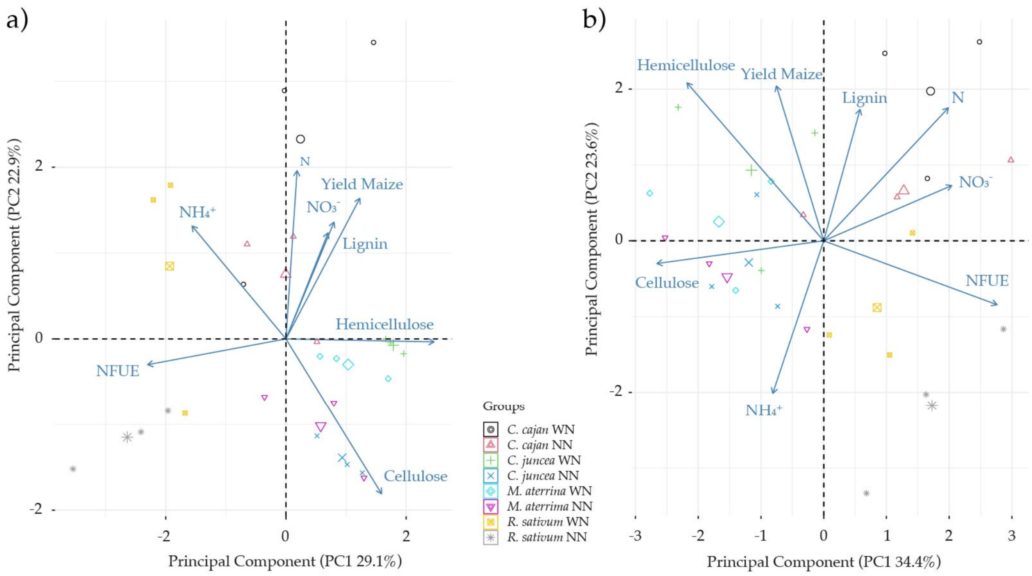
Table 7. Grain yield (kg ha^{-1}) and nitrogen fertilizer use efficiency (NFUE) of maize grown after cover crops with N fertilizer (WN) and without N fertilizer (NN) in topdressing in 2017.

Cover Crop	Grain Yield		Nitrogen Fertilizer Use Efficiency (NFUE)
	WN	NN	
	kg ha^{-1}		kg Grain kg N Applied^{-1}
Mucuna aterrima	8506.89 a	6978.10 a	11.76 b
Raphanus sativus	8345.66 a	5956.19 b	18.38 a
Cajanus cajan	8317.94 a	6851.36 a	16.35 ab
Crotalaria juncea	8342.74 a	6975.69 a	13.07 ab
CV%	6.96	4.38	15.91

Means followed by the same letters in each column do not differ by Tukey's test ($p < 0.05$). WN—with N topdressing on maize; NN—no N topdressing on maize.

3.4. PCA Analysis

The first and second principal components (PC1 and PC2) were generated to distinguish cover crops after maize in the no tillage system, with and without N fertilization, considering the end (April) and beginning (November) of the rainy season and the soil properties (TN, NO_3^-, NH_4^+), structural components (cellulose, hemicellulose and lignin concentration), maize yield and NFUE (Figure 2a,b). The distribution of selected variables showed a cumulative variance of 52.03 and 58.01% for the sum of the principal components PC1 and PC2 for April and November, respectively. Loadings with an absolute value of >0.5 were considered relevant.



Variables	PC1	PC2	PC1	PC2
	april/16		november/16	
Yield Maize	0.43	0.57	-0.23	0.63
Hemicellulose	0.86	-0.01	-0.67	0.64
Cellulose	0.56	-0.63	-0.82	-0.09
Lignin	0.24	0.43	0.18	0.53
N	0.07	0.69	0.61	0.54
NFUE	0.28	0.48	0.62	0.22
NO_3-	-0.55	0.46	-0.25	-0.62
NH_4+	-0.80	-0.10	0.85	-0.26
% Explained	29.11	22.92	34.41	23.6

Figure 2. PCA biplot of PC1 and PC2 scores. Arrows indicate loadings of the soil properties and structural components, and points indicate the four cover crops preceding maize (*M. aterrima*, *C. cajan*, *R. sativus* and *C. juncea*). (**a**) End of the rainy season (April 2016) and (**b**) Beginning of the rainy season (November 2016).

In Figure 2a (April), the loadings considered for PC1 were hemicellulose, NH_4^+ and NFUE, and for PC2, the variables were maize yield, cellulose and mineral N. The data showed the strongest correlation between maize yield and mineral N. Nitrogen fertilizer use efficiency was strongly correlated with *Raphanus sativus* without N topdressing, indicating that the response to N and NFUE of the commercial crop would be even greater if N were topdressed. *Mucuna aterrima* correlated with hemicellulose and cellulose contents (Table 7), with higher contents of these structural components.

In November (Figure 2b), the loadings considered on PC1 were hemicellulose, cellulose, mineral N, NO_3^- and NFUE, and on PC2, they were maize yield, lignin and NH_4^+. The data showed correlations between maize yield and *Cajanus cajan*, *Crotalaria juncea* and *Mucuna aterrima* because these leguminous cover species have high N contents and consequently provide the soil with high amounts of N from residue decomposition. The hemicellulose contents were positively correlated with maize yield, showing that cover crops with high contents of this component are more readily decomposed and provide plant-available N. Nitrogen fertilizer use efficiency was correlated with *Raphanus sativus* in both treatments (NN and WN), indicating that decomposition and consequent N release of this cover crop contribute less to maize yield, resulting in higher fertilizer dependency for maize.

4. Discussion

4.1. Soil Mineral N, TN and Structural Components (Cellulose, Hemicellulose and Lignin Concentration), Lignin:N in the Aboveground Biomass of Cover Crops

The increase in mean ammonium and nitrate concentrations of 62 and 38%, respectively, from the end of the rainy season (April) to the beginning of the following rainy season (November), reflects the low mineralization of plant residues during the dry season in the Brazilian Cerrado, due to the low (267.5 mm) amount of rainfall (Figure 1). Soil moisture conditions in the dry season were insufficient for the nitrification process, and therefore, there was no increase in the nitrate due to the low microbial activity in this period in the Brazilian Cerrado [35]. After the first rainfalls of this rainy period, the so-called "Birch effect" [36] occurs, which promotes peaks in microbial activity, consequently, N mineralization and an increase in mineral N contents (ammonium and nitrate). The Birch effect occurs in soil under drying–rewetting conditions and increases the mineralization of fresh plant litter and SOM decomposition [37]. It is observed that nitrogen mineralization is more sensitive than carbon during drying-rewetting conditions and appears to be related to historical N input to the soil [38].

The addition of organic matter as a cover crop to the soil may induce a soil priming effect and promote soil carbon and nitrogen turnover, particularly nitrous oxide emission [39], but on the other hand, cover crops increase soil organic matter and increase nutrient availability to plants [40].

The highest nitrate concentrations in the surface layer that decreased with increasing depth were recorded in this study, as also reported by d'Andréa et al. [41], with mean values of 32.11 mg kg^{-1} in the 0–10 layer and a predominance of ammonium throughout nearly the entire profile under native Cerrado and a pasture. The higher concentrations of ammonium recorded by the authors in native Cerrado soil may be attributed to the lower pH, which inhibits the activity of nitrifying bacteria [7], whereas significantly lower values of N in ammonium form were found in agricultural systems, both under no tillage and conventional tillage, to a depth of 20 cm. In the subsurface layers, especially the 40–60 cm layer, differences between the forms of mineral N are less evident, possibly due to the presence of SOM in relatively stable fractions (humic substances), which are not easily decomposed by soil microorganisms [42,43].

The release of N from the decomposing biomass is a dynamic process, mediated by microorganisms that use organic compounds as an energy source and defined mainly by the edaphoclimatic conditions and structural components (cellulose, hemicellulose and lignin concentration), lignin:N in the aboveground biomass of cover crops [44]. Several other factors affect N mineralization from plant residues, and soil chemical and biological analysis can be used to predict N mineralization [45]. In addition, N mineralization is related to soil management and climatic conditions such as temperature, moisture and the water content of the residues [46,47].

The higher correlations observed in PC1 and PC2 for hemicelluloses (0.53) and lignin (0.63) (Table 7) reinforce the importance of these structural components of nitrogen mineralization. The cover crops evaluated in this study accumulated mineral N and its respective forms (ammonium and nitrate) according to the concentration of total N, hemicellulose, cellulose and lignin and the C:N and lignin:N ratios. Plants with low lignin:N (2.27–4.12 mg kg^{-1}, Table 6) and faster decomposition favored N mineralization and soil availability in the form of nitrate [48,49].

4.2. Maize Yield and Nitrogen Fertilizer Use Efficiency (NFUE)

The use of cover crops preceding maize can enhance N availability for the cash crop through N mineralization of the cover residues [8,50]. On the other hand, N immobilized in cover crop residues can reduce soil N availability for maize uptake [51].

Our results confirmed the expected response of maize to N fertilization, but also highlighted the differences in maize response according to the preceding cover crop. In the treatments with leguminous cover crops, maize yield was higher than in the treatments with non-leguminous cover crops. As stated elsewhere, leguminous cover crops have the potential to supplement N fertilization and reduce the demand for this input by providing N through the mineralization of biomass residues [27,52]. Our results indicate that it is possible to select cover crops with higher N content, increase N mineralization and possibly decrease maize N fertilization, and in the long term, it is possible to improve soil quality.

The NFUE of maize after cover crops can be related to N uptake by these species. After the crops with the highest N uptake, *Mucuna aterrima* (482 kg N ha^{-1}) and *Cajanus cajan* (418 kg N ha^{-1}), maize NFUE was the lowest. The reason is that the contribution of N released from decomposed residues was higher, possibly due to the higher lignin concentration and lignin:N ratio and the highest N uptake from the aboveground biomass of these cover crops [48]. In an output-to-input ratio approach (considering all forms of N input and output from the system, including N mineralization), Raimondi et al. [53] calculated maize N use efficiency and claimed that non-leguminous cover crops are less efficient in taking up N derived from the soil, which possibly leads to a higher fertilizer dependency.

The cultivation of *Mucuna aterrima* resulted in lower NFUE in subsequent maize due to the higher lignin concentration, higher lignin:N ratio and highest N uptake in the aboveground biomass of this cover crop (Table 7). This created a favorable synchrony between N release and availability and its uptake by maize [14] and consequently reduced the dependence on N from nitrogen fertilization.

The structural components of cover crops also affected N mineralization and release to the cash crop. *Raphanus sativus* had the lowest lignin:N ratio, which accelerated the decomposition process. The lack of synchronization between cover crop decomposition (*Raphanus sativus*) and N release with cash crop development affected maize N uptake and increased the crop dependence on N fertilization [54].

4.3. Correlation between Maize Yield, NFUE and Soil Properties and Structural Components of Cover Crops

As expected, at the end of the rainy season, the mineral N correlated with maize yield. The soil N reservoir affected maize yield in the subsequent season. As mentioned before, NFUE was most strongly correlated in plots with *Raphanus sativus* due to the greater dependence on N-fertilizer by maize planted subsequently to this cover crop, especially when no N was top-dressed on maize. Higher NFUE can be achieved when soil N availability is low [51], similar to this situation.

The PCA also detected a correlation between *Mucuna aterrima* and the structural components hemicellulose and cellulose. This cover species contains high amounts of these components, as shown in our results. The consequence is a high half-life value and consequently low decomposition and N release rates [48]. In the beginning of the rainy season, treatments with legume species were related to higher maize yields because of the contribution of N input from cover crop residues [27,52].

The hemicellulose content was also correlated with maize yield. This result may have been related to the fact that the decomposition and N release rates met the maize requirement [54].

As observed at the end of the rainy season, maize NFUE was also related to *Raphanus sativus* at the beginning of the rainy season. In this treatment, NFUE was higher due to the lower N input (expressed by the lower N uptake by this cover crop), resulting in a higher N fertilizer dependence [54].

5. Conclusions

Our study showed that nitrogen topdressing in no-tillage maize can be reduced by including preceding cover crops with different chemical compositions and N uptake to favor the supply of mineral N, mainly nitrate. This is the predominant form of N absorbed by maize and contributes to raising yields. Among the cover crops, the highest lignin concentration, highest lignin:N ratio and highest N uptake were observed for *Mucuna aterrima*. This resulted in the highest soil concentration of mineral N (nitrate and ammonium) and the lowest NFUE in maize, indicating a lower dependence on fertilizer N for the cash crop. Therefore, for highly N-demanding crops such as maize, the adoption of a cover crop sequence that favors N contribution is fundamental for the sustainability of the production system and reduces the dependence on nitrogen fertilizers. Our results show that it is possible to select cover crops to reduce nitrogen fertilization in maize. In addition, it is possible in future research to evaluate the benefits of these cover crops on biological and physical soil properties.

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