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Cover crops and controlled-release urea decrease nitrogen mobility and improve nitrogen stock in a tropical sandy soil with cotton cultivation

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ABSTRACT: Sandy soil often has low nitrogen (N) stock. Thus, crops grown in sandy soil rely on high levels of N fertilization. The use of cover crops and efficient fertilizers can increase N stock in the soil and N availability in the topsoil, and reduce overall fertilizer costs. The objective of this study was to evaluate the effects of cover crops (fallow, a single grass species (ruzigrass), two grass species (ruzigrass + millet), one grass species (millet) with legumes [lime-yellow pea (2018) and velvet bean (2019)], and a mixture of three cover crops [two grass species (ruzigrass + millet) and one legume (lime-yellow pea (2018)) and velvet bean (2019), N sources (conventional urea and controlled-release urea) and N doses (70, 100 and 130 kg ha⁻¹) on N dynamics in an Oxisol (*Latossolo*) with sandy texture in Brazil cultivated with cotton. Systems with the cover crops (average) had 17 % more total N stock in the soil than fallow systems. Inorganic N increased only in systems with legumes. The systems with cover crop mixtures had 70 % more ammonium than fallow systems. Systems only with grass species had low percentages of inorganic N in relation to total N in the soil. The increase in N-fertilizer rates augmented the N stock in the soil (total and inorganic). In the first year, controlled-release urea reduced the availability of inorganic N in cotton flowering, except for the system with mixed cover crops. After the cotton harvest, areas of controlled-release urea application had 12 % more inorganic N than the areas with conventional urea. Our findings show that the combined use of cover crops with high biomass production, moderate dose of N and controlled-release N can increase the availability of inorganic nitrogen in the upper layers of the soil in tropical areas with sandy soil and this can reduce nitrogen fertilizer consumption in the medium and long term in cotton fields.

Keywords: crop rotation, nitrogen sources, nitrogen mobility, Oxisols.

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INTRODUCTION

Nitrogen (N) dynamics in tropical soils are complex, as N loss is often caused by volatilization (Minato et al., 2020) and leaching (Rosolem et al., 2018). Leaching nitrate runoff and denitrification remove up to 95 and 25 Tg of nitrate from the soil each year, respectively. Ammonium volatilization removes up to 37 Tg of ammonium from the soil each year (Billen et al., 2013; Sutton et al., 2013). In sandy soil, the risk of losses from leaching is high due to low cation exchange capacity (CEC) and low soil organic matter content. These factors reduce the soil capacity to store N, as well as increase the need for mineral fertilizers, mainly in non-leguminous crops (Shareef et al., 2019), as for example in cotton crops, which require application of N via nitrogen fertilizer. New techniques are needed to improve N efficiency in tropical climates and sandy soil regions.

Use of cover crops is a common technique to improve N efficiency, and its use has shown benefits for various crop systems (Rosolem et al., 2018; Momesso et al., 2019; Rocha et al., 2019; Rocha et al., 2020; Galdos et al., 2020). In some cases, the use of grass with a high carbon-to-nitrogen ratio (C:N) causes N immobilization due to the use of N available in the soil by microorganisms, which decompose the straw; this momentarily reduces the availability of N in the subsequent crop (Momesso et al., 2019; Rocha et al., 2019; Silva et al., 2020). The low organic matter content in sandy soil results in low N stock (Cordeiro and Echer, 2019; Silva et al., 2020; Cordeiro et al., 2021a). When choosing the cover crop species in these soils, it is important to consider which crop will be grown in the future, as non-legume crops (e.g., cotton) depend on fertilizers and soil N reserve. When compared to using just grass, Chu et al. (2017) and Silva et al. (2020) reported higher N stock and higher soybean and corn yields when using grass and legume cover crop combinations. However, these studies did not evaluate the effect of cover crops on N loss or ammonium and nitrate proportions. This is important because the nitrate fraction is more easily lost in sandy soils, while ammonium can be deposited in the soil CEC (Teutscherova et al., 2018).

Legumes have the capacity to increase N content in the soil via biological nitrogen fixation (BNF) (Cordeiro and Echer, 2019; Cordeiro et al., 2021a). Legumes grown in the off-season increase N absorption and yield of subsequent crops, reducing the necessity for mineral fertilization (Silva et al., 2006). Nitrogen loss from leaching is higher in legume systems than in grass systems, as systems with legumes have lower C:N ratios. Low C:N ratios accelerate N release, decrease root volumes and increase N input in the soil (Rosolem et al., 2017).

Systems with grass have high C:N ratios and nutrient cycling capabilities (principally with nitrates); these factors enhance the soil by enabling large root development (Rosolem et al., 2017) and inhibiting N nitrification (Subbarao et al., 2007; Subbarao et al., 2009). The combination of grass and legumes in the off-season can improve nutrient cycling, increase N stock in the soil, and reduce loss from leaching and nitrification. However, the use of cover crops from different botanical families in the same crop system is not a common practice in tropical climates. Furthermore, there is still no knowledge of cover crops on the dynamics of N in the soil in cotton-growing areas on sandy soils.

Adjustments to N doses are also necessary for these systems, principally for cotton cultivation. Cotton crops require high amounts of mineral fertilizer and are usually grown in areas with high rainfall or irrigation systems. These factors facilitate N movement in the soil. Leaching does not cause significant N loss in applications of less than 100 kg ha⁻¹ of N, but leaching can cause significant N loss (up to 10 %) when high N doses are used (300 kg ha⁻¹ or more) (Wang et al., 2019). This is a problem for cotton cultivation in various countries. In Brazil, average N application is between 100 to 200 kg ha⁻¹ (Echer et al., 2020). Average N application in the United States is around 200 kg ha⁻¹ (Bronson et al., 2019). In China and Australia, N application is often around 300 kg ha⁻¹ (Tian et al., 2018; Rochester and Constable, 2020).

Use of controlled-release fertilizers is a strategy to reduce the applied N dose (Yang et al., 2021), but despite the high efficiency of controlled-release urea (CRU), its use is not common in Brazil and various other countries. Recent studies have suggested that CRU application results in a 9 % increase in cotton yield (Geng et al., 2016a) and 36 % less volatilization loss (Minato et al., 2020). Elemental sulfur-coated urea is a good option for tropical climate environments (Mariano et al., 2019). Applying CRU reduces the amount of the required N dose by up to 50 %. It also reduces inorganic N leaching (Zhang et al., 2018).

However, it is unclear if CRU efficiency is dependent on the crop production system, and coating technology. In addition, the effects from the combination of CRU coated with elemental sulfur and polymers and cover crop systems on soil N stock and leaching in tropical regions with sandy soil are not yet fully understood. The objective of this study was to evaluate the interactive effects of cover crops, N sources, and N doses on N stock and N movement in tropical sandy soil with cotton cultivation.

MATERIALS AND METHODS

Characterization of the study area

The present study was conducted in the 2018/2019 and 2019/2020 crop seasons in the of Presidente Bernardes, São Paulo State, Brazil (22° 11' 53" S, 51° 40' 30" W, at an altitude of 401 m). The region has a tropical climate with dry winters (Aw – Köppen Classification System). Rainfall amounts during the cover crop cycles were 292 and 242 mm in 2018/2019 and 2019/2020, respectively. Rainfall amounts during the cotton cycles were 642 mm in 2018/2019 and 944 mm in 2019/2020. The soil of the area is classified as Oxisols (Soil Survey Staff, 2014), which corresponds to a *Latossolo* (Santos et al., 2013), with a sandy texture (14 % clay, 0.00-0.20 m). Before implementation of crop rotation systems, we recorded the following chemical properties (0.00-0.20 m) (2015): pH: 5.0, organic carbon: 8.9 g dm⁻³, total nitrogen: 210 mg kg⁻¹, inorganic nitrogen: 12 mg kg⁻¹, phosphorus: 2.1 mg dm⁻³, potassium, calcium, magnesium, and CEC of 0.6, 7.5, 3.8, and 27.5 mmol_c dm⁻³, respectively. At layers of 0.00-0.20, 0.20-0.40, 0.40-0.60, 0.60-0.80 and 0.80-1.00 m, we recorded the following physical properties: clay contents of 143, 146, 174, 191 and 184 g kg⁻¹; sand contents of 835, 815, 779, 778 and 776 g kg⁻¹; and silt contents of 22, 39, 47, 31 and 40 g kg⁻¹, respectively. Crop rotation systems were implemented in 2015.

Experimental design

Randomized blocks with five replications were used in a split-split plot scheme for the experimental design. Plots were composed of the following rotation systems: fallow (spontaneous vegetation), a single grass species (SG), two grass species (G+G), one grass species and legumes (G+L), and a mixture of three cover crops (two grass species and one legume) (MIX). Subplots were divided by nitrogen (N) doses: 70, 100 and 130 kg ha⁻¹. Sub-subplots were divided by N sources: conventional urea (45 % N) and controlled-release urea (CRU). Controlled-release urea was coated with elemental sulfur (40 % N + 8 % S). Nitrogen fertilizers were applied 25 and 45 days after cotton emergence. Plots had dimensions of 15 × 9 m. Subplots had dimensions of 4.5 × 9 m. Sub-subplots were then divided into 4.5 × 4.5 m areas.

Cover crop management

Cover crops were sown in April 2018 and May 2019. In 2018, basic fertilization was used in all treatments with 20, 30 and 26 kg ha⁻¹ of N, P and K, respectively. In 2019, the cover crops were sown without fertilizers because of the absence of crops of economic interest. Sowing density was 6 kg ha⁻¹ for ruzigrass (*Urochloa ruziziensis*), 2.2 kg ha⁻¹ for

lime-yellow pea (*Macrotyloma axillara* cv. Java), 90 kg ha⁻¹ for black velvet bean (*Mucuna pruriens*), and 18 kg ha⁻¹ for millet (*Pennisetum mericanum*). The same quantities were used in single and intercropped sowings. In September 2018, 1.7 t ha⁻¹ of dolomitic limestone was applied. In September 2019, 1 ton ha⁻¹ of phosphogypsum was applied. Desiccations of cover crops were performed in September 2018 and October 2019 using the non-selective herbicide glyphosate (1.44 L ha⁻¹ of a.i.).

Cotton management

Cotton sowing (FM 983GLT, late cultivar) was performed on November 22, 2018, and November 28, 2019, with 0.90 m spacing between lines and eight seeds per linear meter. For seeding fertilization, 25 and 55 kg ha⁻¹ of N and P were used, respectively (monoammonium phosphate). Thirty days after emergence (DAE), 1.8 kg ha⁻¹ of boron (Ulexita) was applied. Potassium fertilizer was applied 25 and 60 DAE, with 50 kg ha⁻¹ of K (potassium chloride) for each application. Nitrogen fertilization was applied 25 and 45 DAE. In this period, elemental sulfur was applied in the conventional urea plots to balance the sulfur supplied via CRU. The management of pests, diseases, and growth regulator was performed according to the needs of the crop, with the same management practices for all treatments.

Cover crop evaluations

After the desiccation of the cover crops, shoots and roots were collected from three subsamples per plot in an area of 0.2 m² for the shoot (straw) and 2000 cm³ for the root (0.10 × 0.10 × 0.20 m). The root samples were washed in running water, and the shoot samples were submerged in distilled water. They were then dried in an oven for 72 h at 65 °C and weighed on a precision scale (0.01 g). In the fallow, SG, G+G, G+L, and MIX systems, shoot dry weights and ratio (carbon:nitrogen) were 1.4 (44), 4.0 (45), 4.6 (50), 1.6 (42), and 3.6 (43) t ha⁻¹ (2018); and 0.90 (25), 4.5 (30), 4.9 (36), 3.7 (24), and 5.1 (28) t ha⁻¹ (2019), respectively. For roots, we observed 0.94 (65), 2.7 (70), 2.8 (69), 0.95 (64), 3.2 (65) t ha⁻¹ (2018); and 0.68 (44), 1.7 (49), 1.0 (50), 0.78 (42), and 1.1 (40) t ha⁻¹ (2019).

Evaluations of total N and inorganic N in the soil

Soil sampling was done three times each year. Soil in the 0.00-0.20 m layer was collected and observed in two periods: ten days after desiccation of the cover crops and when the cotton was in full bloom. After the cotton harvest, soil samples were collected in the following layers of 0.00-0.10, 0.10-0.20, 0.20-0.40 and 0.40-0.60 m. All collections were performed with four subsamples in each experimental unit. Soon after collection, the samples were placed in a thermal box with ice. The samples were stored in a freezer until the moment of analysis.

Before the evaluations, the samples thawed for 30 min before weighing. To evaluate the inorganic N in the soil (nitrate and ammonium), 5 g of soil was weighed on a 0.01 g precision scale. Next, 50 mL of KCl 1 mol L⁻¹ was added. It was then stirred for one hour in an orbital shaker. After filtration, a 25 mL aliquot was separated for distillation. Magnesium oxide (0.2 g) was used in the first distillation to extract the ammonium. Lard alloy (0.2 g) was used in the second distillation to extract nitrate. Sulfuric acid was used for titration. Nitrate and ammonium contents were expressed in mg kg⁻¹ (Cantarella and Trivelin, 2001a).

Kjeldahl method was used to evaluate total nitrogen; 1 g of soil was weighed and 1 mg of permanganate was added to the digestion tubes. This was followed by 30 s of stirring. Then, 2 mL of sulfuric acid (1:1) and 500 mg of reduced iron were added, and the tubes were taken to the digestion block for 40 min at 50 °C. Next, the tubes were removed from the block, and 3 mL of concentrated sulfuric acid and 1 g of digesting

mixture were added and stirred again for 30 s. The tubes were then taken to the digestion block for four hours at 360 °C. One day after the samples had cooled, 50 mL of distilled water was added and stirred. A 20 mL aliquot was separated for distillation with sodium hydroxide. Titration was performed with sulfuric acid. Total N content was expressed in mg kg^{-1} (Cantarella and Trivelin, 2001b). Correction of soil moisture was performed in all samples, using 5 g of soil and drying in an oven for 48 h at 105 °C and weighed on a precision scale (0.01 g).

Stock of total and inorganic N (nitrate and ammonium) of the soil was calculated with consideration of the soil N content (mg kg^{-1}) and soil density in the layers of 0.00-0.10, 0.10-0.20, 0.20-0.40 and 0.40-0.60 m. Soil density was determined by collecting five random soil samples from each system, using steel rings of 0.05 m of diameter and 0.05 m of height. The average density was then calculated: 1.71, 1.65, 1.70, 1.70 and 1.69 g dm^{-3} in fallow, SG, G+G, G+L, and MIX systems, respectively. The N stock in the soil was expressed in kg ha^{-1} .

Data analysis

After performing the Shapiro-Wilk normality test, the data were submitted to ANOVA. Statistical analysis was performed from variance analysis and treatment averages that were compared by the t-Test (LSD) at 5 % probability ($p < 0.05$).

RESULTS

Effects of cover crops on soil N content before cotton sowing

Total N stock in fallow was 17 % lower than in systems with cover crops (averages in the two crop seasons) (Figure 1a). Systems with legumes had the highest inorganic N and ammonium stock in the soil (Figure 1b). Despite having high inorganic N stock in the soil, the MIX system had low nitrate stock. The MIX system had the highest ammonium/nitrate ratio in both years. Similar characteristics were observed with the G+L system in 2019/2020 (0.00-0.20 m) (Figure 1). Grass systems (with one or two species) had the lowest percentages of inorganic N in relation to the total soil N (Figure 1f).

Effects of N sources and N doses in different systems on soil N stock at peak cotton flowering

In 2018/2019, CRU application increased total N stock in the soil by 11 % (average of all N doses) in the SG and MIX systems. In 2019/2020, CRU application increased total N stock by an average of 8 % in all systems (Table 1). In the first year, CRU application resulted in less inorganic N stock than conventional urea, except in the MIX system (average from all applied N doses). In the second year, this effect only occurred with high doses of N (130 kg ha^{-1}) in systems without legumes (fallow, SG and G+G) (Table 1).

Nitrogen fertilization increased the nitrate stock in the soil, regardless of the N source, system, or crop season (Table 2). In 2018/2019, the ammonium stock in the soil increased in fallow and G+L systems with CRU application, SG systems with conventional urea, and G+G and MIX systems with both CRU application and conventional urea application. In 2019/2020, ammonium stock only increased in fallow systems; these increases occurred with both CRU and conventional urea (Table 2). In the first crop season, CRU application generally resulted in lower nitrate and ammonium availability than conventional urea (2018/2019). This was observed in the MIX system; CRU application resulted in 14 % less ammonium stock (average from all applied N doses) than with conventional urea. Low ammonium availability from CRU application was also observed in G+G and G+L systems at 70 kg ha^{-1} of N and G+G systems at 130 kg ha^{-1} of N. With the fallow system in the 2018/2019 crop season, CRU application resulted in 26 % less nitrate stock (average from all applied N doses) than with conventional urea (Table 2).

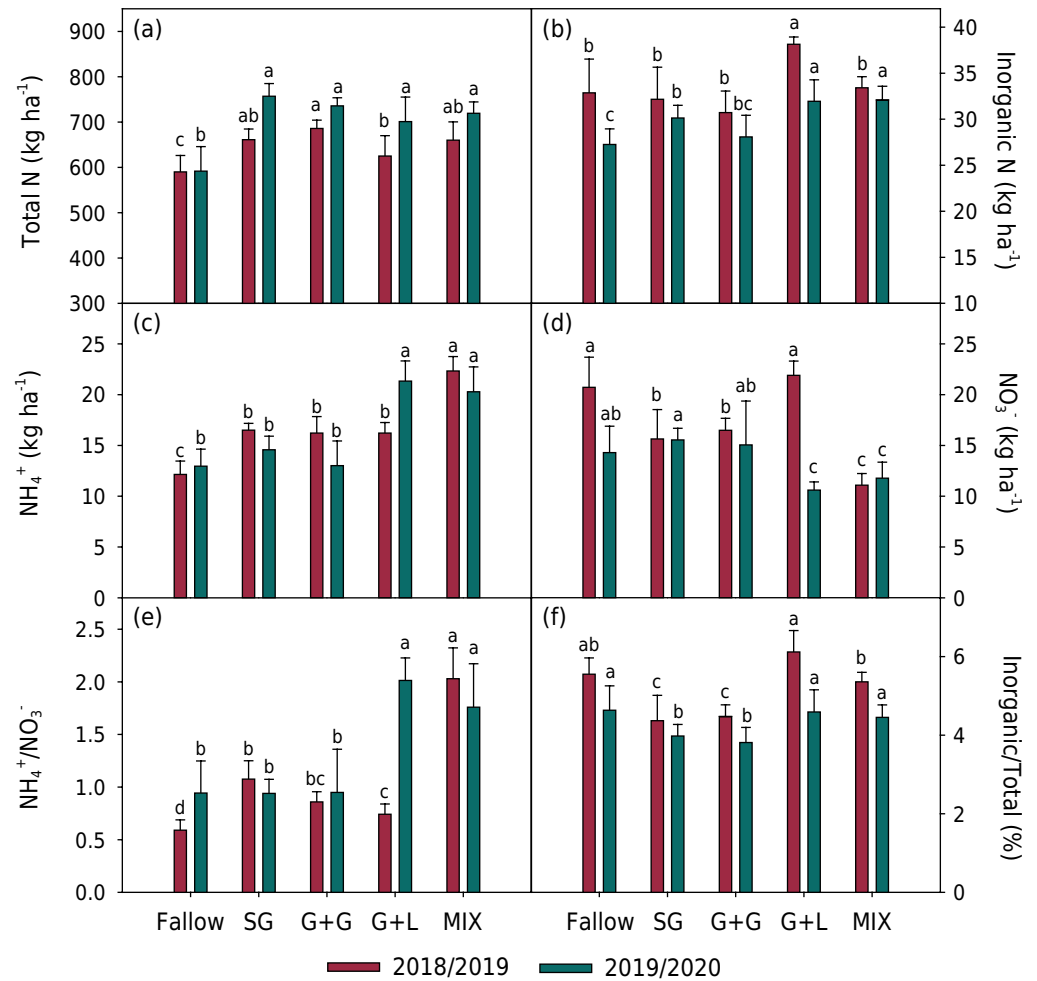


Figure 1. Stock of total nitrogen, inorganic nitrogen, ammonium and nitrate in the soil (0.00-0.20 m); the relationship between ammonium/nitrate; and the relation between the percentage of inorganic nitrogen to total nitrogen in the soil, in cotton pre-sowing. SG: single grass; G+G: grass + grass; G+L: grass + legume; MIX: three species mixtures.

Effect of N sources and N doses in different systems on soil N stock after cotton harvest

Samples collected after the 2018/2019 cotton harvest showed that CRU application increased ammonium stock in the soil by an average of 22 % (14.3 kg ha^{-1}) (average of all applied N doses) in the 0.00-0.60 m layer for the SG, G+G and G+L systems, when compared to conventional urea. In the second crop season (2019/2020), ammonium stock was 13 % higher (9.8 kg ha^{-1}) from CRU application than conventional urea application (average of all systems and applied N doses) (Table 3). In the first crop season (2018/2019), nitrate stock in the MIX system was 14 % higher (11.9 kg ha^{-1}) from CRU application than conventional urea application. In the second crop season (2019/2020), nitrate stock was 17 % higher (14.2 kg ha^{-1}) (average of all applied N doses) from CRU application than conventional urea (Table 3). When compared to conventional urea, CRU application increased inorganic N (nitrate+ammonium) in the soil by 12 % after the cotton harvest at 0.00-0.60 m (average of all applied N doses, systems, and crop seasons) (Table 3).

As expected, increasing N doses ($70\text{-}130 \text{ kg ha}^{-1}$) raised ammonium and nitrate stock in the soil with all systems, N sources, and crop seasons (Table 3). With the application of 130 kg ha^{-1} of N via CRU, the nitrate stock was higher than 120 kg ha^{-1} in the fallow, SG, and G+L systems in 2018/2019. The application of 130 kg ha^{-1} of N with CRU increased ammonium stock to higher than 80 kg ha^{-1} in G+G and MIX systems in 2018/2019 and all systems in 2019/2020 (Table 3).

Table 1. Stock of total nitrogen and inorganic nitrogen in the soil (0.00-0.20 m) during the cotton flowering period

Nitrogen	Total N		Inorganic N		Total N		Inorganic N	
	2018/2019				2019/2020			
kg ha ⁻¹	kg ha ⁻¹							
Fallow								
	Urea	CRU	Urea	CRU	Urea	CRU	Urea	CRU
70	587 Ab	566 Bb	28 Ab	26 Bb	661 Bb	776 Ab	28 Ac	31Ab
100	638 Ba	691 Aa	33 Aab	30 Bb	698 Ba	809 Aa	39 Ab	39 Aab
130	640 Ba	703 Aa	35 Aa	32 Ba	702 Ba	815 Aa	49 Aa	44 Ba
CV (%)	5.2		8.2		7.3		15.2	
Single grass								
70	555 Ba	641 Aa	28 Ac	26 Ba	771 Ba	855 Aa	44 Ab	42 Ab
100	560 Ba	640 Aa	35 Ab	27 Ba	767 Ba	839 Aa	47 Aab	44 Ab
130	564 Ba	660 Aa	49 Aa	29 Ba	790 Ba	846 Aa	54 Aa	50 Ba
CV (%)	5.5		9.4		8.3		11.6	
Grass + grass								
70	655 Ba	701 Aa	27 Ab	21 Bc	793 Ba	819 Aa	44 Ab	41 Ab
100	694 Aa	708 Aa	34 Aa	29 Bb	778 Ba	829 Aa	46 Ab	46 Aa
130	690 Aa	725 Aa	41 Aa	37 Ba	711 Ba	861 Aa	50 Aa	48 Ba
CV (%)	5.5		6.5		5.5		12.5	
Grass + legume								
70	669 Aa	628 Aa	31 Ab	23 Bb	695 Ba	726 Aa	47 Ab	42 Ab
100	701 Aa	666 Aa	32 Ab	32 Ba	691 Ba	744 Aa	52 Aab	48 Aab
130	707 Aa	682 Aa	43 Aa	39 Ba	732 Ba	760 Aa	55 Aa	53 Aa
CV (%)	3.9		7.6		11.4		12.3	
MIX								
70	663 Ba	733 Aa	36 Ab	32 Ab	783 Ba	835 Aa	45 Ab	43 Ab
100	693 Ba	729 Aa	35 Ab	31 Ab	801 Ba	853 Aa	46 Ab	49 Aa
130	702 Ba	730 Aa	42 Aa	40 Aa	808 Ba	867 Aa	55 Aa	53 Aa
CV (%)	2.9		7.2		8.8		16.1	

Averages followed by the same letter did not show statistical difference in the t-Test ($p < 0.05$). Uppercase letters compare the nitrogen source of each dose. Lowercase letters compare the nitrogen doses of each source.

Nitrate and ammonium content of the soil after cotton harvest

Crop systems, N sources and doses affected the nitrate and ammonium contents in the two evaluated crop seasons (Table 4). We also observed that cover crop systems and N sources interacted with the nitrate and ammonium contents in all soil depths in 2018/2019 and at depths of 0.00-0.10 and 0.10-0.20 m in 2019/2020 (Table 4).

Controlled-release urea was more efficient in preventing ammonium and nitrate leaching in both crop seasons when used with cover crop systems and low N doses (70 and 100 kg ha⁻¹) (Figures 2 and 3). In our study, we observed more nitrate leaching than ammonium leaching (Figures 2 and 3), especially in the 2019/2020 crop season. In 2019/2020, ammonium contents were highest in the 0.00-0.10 m layer, regardless of the treatment (Figures 3a, 3c, 3e, 3g and 3i). In 2019/2020, nitrate contents were highest in the 0.00-0.10 m layer only with CRU application and in cover crop systems of high biomass input (SG, G+G, and MIX) (Figures 3d, 3f and 3j).

Nitrate leaching was highest with conventional urea and high N doses (130 kg ha⁻¹); we observed 273 % more nitrate in the 0.40-0.60 m layer than in the 0.00-0.10 m. With

Table 2. Stock of ammonium and nitrate in the soil (0.00-0.20 m) during the cotton flowering period

Nitrogen	NH ₄ ⁺		NO ₃ ⁻		NH ₄ ⁺		NO ₃ ⁻	
	2018/2019				2019/2020			
kg ha ⁻¹	kg ha ⁻¹							
	Urea	CRU	Urea	CRU	Urea	CRU	Urea	CRU
70	12 Aa	12 Ab	16 Ab	14 Bb	13 Ac	13 Ab	16 Ab	18 Aab
100	13 Aa	15 Aa	20Aa	15 Bb	18 Ab	16 Ab	22 Aa	21 Aab
130	14 Aa	15 Aa	21 Aa	16 Ba	21 Aa	21 Aa	27 Aa	22 Aa
CV (%)	13.2		10.2		17.2		19.8	
70	12 Ac	11 Aa	16 Ac	16 Ab	23Aa	23 Aa	21 Ab	20 Ab
100	17 Aa	13 Ba	18 Ab	14 Bb	27 Aa	22 Aa	21 Ab	22 Ab
130	24 Aa	12 Ba	26 Aa	17 Ba	28 Aa	25 Aa	26 Aa	25 Aa
CV (%)	13.4		9.7		17.2		11.7	
70	12 Ab	9 Bc	15 Ac	12 Bb	23 Aa	22 Aa	21 Ab	20 Ab
100	12 Ab	12 Ab	22 Aa	17 Aa	22 Aa	23 Aa	24 Aa	23 Aa
130	16 Aa	19 Aa	24 Aa	19 Aa	24 Aa	24 Aa	26 Aa	23 Aa
CV (%)	12.8		7.4		15.1		22.8	
70	15 Aa	11 Bc	17 Ab	12 Bb	27 Aa	24 Aa	21 Ab	18 Ab
100	16 Aa	18 Ab	16 Ab	14 Aa	27 Aa	26 Aa	25 Aab	22 Aa
130	16 Ba	20 Aa	27 Aa	19 Ba	27 Aa	29 Aa	28 Aa	24 Aa
CV (%)	10.9		9.1		14.2		17.5	
70	20 Ab	18 Bb	16 Ab	14 Ab	28 Aa	24 Aa	17 Ab	17 Ab
100	20 Ab	17 Bb	16 Ab	14 Ab	27 Aa	26 Aa	19 Ab	23 Aa
130	23 Aa	20 Ba	20 Aa	19 Aa	28 Aa	30 Aa	27 Aa	23 Aa
CV (%)	8.5		10.2		18.7		15.7	

Averages followed by the same letter did not show statistical difference in the t-Test (LSD) ($p < 0.05$). Uppercase letters compare the nitrogen source of each dose, and lowercase letters compare the nitrogen dose of each source.

CRU application and the same N dose, there was less than a 37 % difference between these layers (Figure 3b). More nitrate leaching occurred in the 2019/2020 crop season. There was 944 mm of rain in 2019/2020; 47 % more than the previous crop season. As previously mentioned, ammonium contents were highest in the 0.00-0.10 m layer in 2019/2020, regardless of the N source, N dose, or system (Figure 3). However, the application of 70 kg ha⁻¹ of N with CRU resulted in higher N content in the topsoil (0.00-0.10 m) when compared to application of 130 kg ha⁻¹ of N via conventional urea.

DISCUSSION

The use of grasses and legumes as cover crops is an effective strategy to increase total N in the soil (Figure 1a). This has been reported in various studies (Mitchell et al., 2017; Yao et al., 2019; Silva et al., 2020). However, the availability of inorganic N is low in grass systems with high C:N ratios in sandy soil with low organic matter (Figure 1b), as soil microorganisms immobilize N in the soil and cause carbon degradation of straw and roots of cover crops. This effect can impair crop yield (Chu et al., 2017; Rocha et al.,

Table 3. Stock of ammonium (NH_4^+) and nitrate (NO_3^-) of the soil (0.00-0.60 m) after the cotton harvest

Nitrogen	NH_4^+		NO_3^-		NH_4^+		NO_3^-	
	2018/2019				2019/2020			
kg ha ⁻¹	kg ha ⁻¹							
	Fallow							
	Urea	CRU	Urea	CRU	Urea	CRU	Urea	CRU
70	49 Ab	53 Ab	99 Ab	98 Ac	62 Bc	75 Ab	77 Bb	99 Ab
100	56 Aa	57 Ab	106 Ab	109 Ab	71 Bb	80 Aab	90 Ba	111 Aa
130	58 Aa	64 Aa	117 Aa	120 Aa	80 Ba	85 Aa	99 Ba	119 Aa
CV (%)	8.1		7.3		6.5		7.2	
	Single grass							
70	54 Bb	70 Ab	92 Ab	87 Ab	60 Bb	68 Ac	79 Bb	92 Ab
100	57 Bb	75 Aab	107 Aa	113 Aa	63 Bb	75 Ab	89 Ba	100 Aab
130	66 Ba	79 Aa	114 Aa	121 Aa	71 Ba	83 Aa	95 Ba	106 Aa
CV (%)	6.7		7.6		6.7		7.7	
	Grass + grass							
70	60 Bb	67 Ac	79 Ac	81 Ac	62 Bb	68 Ac	81 Bb	89 Ac
100	66 Bb	80 Ab	91 Ab	96 Ab	69 Ba	76Ab	85 Bab	98 Ab
130	73 Ba	92 Aa	100 Aa	109 Aa	72 Ba	86 Aa	93 Ba	108 Aa
CV (%)	6.9		6.1		5.2		6.5	
	Grass + legumes							
70	56 Bb	65 Ab	86 Ac	99 Ac	71 Bb	76 Ab	84 Bb	93 Ac
100	61 Bab	75 Aa	104 Ab	111 Ab	75 Bb	77 Ab	90 Bab	102 Ab
130	67 Ba	80 Aa	115 Aa	126 Aa	85 Aa	90 Aa	93 Ba	115 Aa
CV (%)	6.6		5.3		8.2		5.3	
	MIX							
70	67 Ab	68 Ac	85 Bb	96 Ac	63 Bc	72 Ab	83 Bb	95 Ac
100	72 Aab	75 Ab	88 Bb	103 Ab	68 Bb	74 Ab	87 Bab	102 Ab
130	78 Aa	83 Aa	98 Ba	109 Aa	76 Ba	89 Aa	92 Ba	109 Aa
CV (%)	6.1		3.4		5.0		4.4	

Averages followed by the same letter did not show statistical difference in the t-Test (LSD) ($p < 0.05$). Uppercase letters compare the nitrogen source of each dose, and lowercase letters compare the nitrogen dose of each source.

2019; Momesso et al., 2019) due to the reduced availability of N in the soil (Figure 1). This reduces initial cotton growth (Echer et al., 2012) and cotton yield, especially in sandy soils with low N stock (Cordeiro et al., 2021b). Even with cover crops, total N stock in sandy soil is low in the arable layer (0.00-0.20 m) (between 590 and 760 kg ha⁻¹) (Figure 1a and Table 1), as observed in other studies (Cordeiro and Echer., 2019; Silva et al., 2020).

Combinations of legumes with low C:N ratios and grasses with high biomass production and high C:N ratios are the best options for increasing quantities of total N and inorganic N in the soil (Figure 1). Using a single legume with low dry matter content and a low C:N ratio causes rapid mineralization of N and C (Li et al., 2013). In tropical climates with sandy soil, this undesirable effect leads to lower total C and N stock in the soil (Campos et al., 2020), as soil organic matter mineralization occurs naturally in these environments (Liyanage et al., 2020). Furthermore, in systems with higher doses of nitrogen fertilizers and cover crops with low dry matter production, N's movement in the soil is greater, mostly nitrate (Figures 2 and 3).

The nitrification process transforms ammonium into nitrate; this effect is accelerated in sandy soil in a tropical climate and increases nitrate content in the soil when compared

Table 4. Variance analysis summary of the ammonium and nitrate content in the soil at the layers of 0.00-0.10, 0.10-0.20, 0.20-0.40, and 0.40-0.60 m with different crop rotation systems, nitrogen sources, nitrogen doses, and interactions

Source of variation Depth	NH ₄ ⁺		NO ₃ ⁻	
	2018/2019		2019/2020	
0.00-0.10 m				
Systems (S)	***	***	***	***
Doses of N (DN)	***	***	***	**
Source of N (SN)	***	***	***	***
S × DN	*	NS	NS	NS
S × SN	***	***	*	*
DN × SN	*	NS	NS	**
S × DN × SN	NS	NS	NS	NS
CV%	10.3	11.9	9.1	11.2
0.10-0.20 m				
Systems (S)	***	**	*	*
Doses of N (DN)	***	***	**	***
Source of N (SN)	***	***	***	***
S × DN	NS	*	*	***
S × SN	***	***	*	***
DN × SN	NS	NS	NS	NS
S × DN × SN	NS	NS	NS	NS
CV%	11.2	12.5	12.7	12.5
0.20-0.40 m				
Systems (S)	***	***	***	***
Doses of N (DN)	***	***	***	***
Source of N (SN)	*	*	*	***
S × DN	NS	***	NS	***
S × SN	*	***	NS	NS
DN × SN	NS	NS	NS	NS
S × DN × SN	NS	NS	NS	NS
CV%	16.6	13.2	19.6	11.9
0.40-0.60 m				
Systems (S)	***	***	***	***
Doses of N (DN)	***	***	***	***
Source of N (SN)	***	***	***	***
S × DN	NS	*	*	***
S × SN	**	***	NS	NS
DN × SN	NS	NS	NS	NS
S × DN × SN	NS	NS	NS	NS
CV%	18.4	11.0	16.9	11.2

***: significant at 0.1 %. **: significant at 1 %. *: significant at 5 %. NS: Not significant.

to ammonium content (Campos et al., 2020), which can intensify nitrate leaching by up to 33 kg ha⁻¹ per year (Rosolem et al., 2018). Thus, more nitrate is lost than ammonium (Figures 2 and 3). Ammonium loss can be further reduced by using techniques that elevate organic material, as ammonium is a cation stored in the CEC of the soil (Teutscheroova et al., 2018). Combinations of grass and legumes increased ammonium stock in relation to nitrates in the arable soil layer (0.00-0.20 m) (Figure 1). This suggests

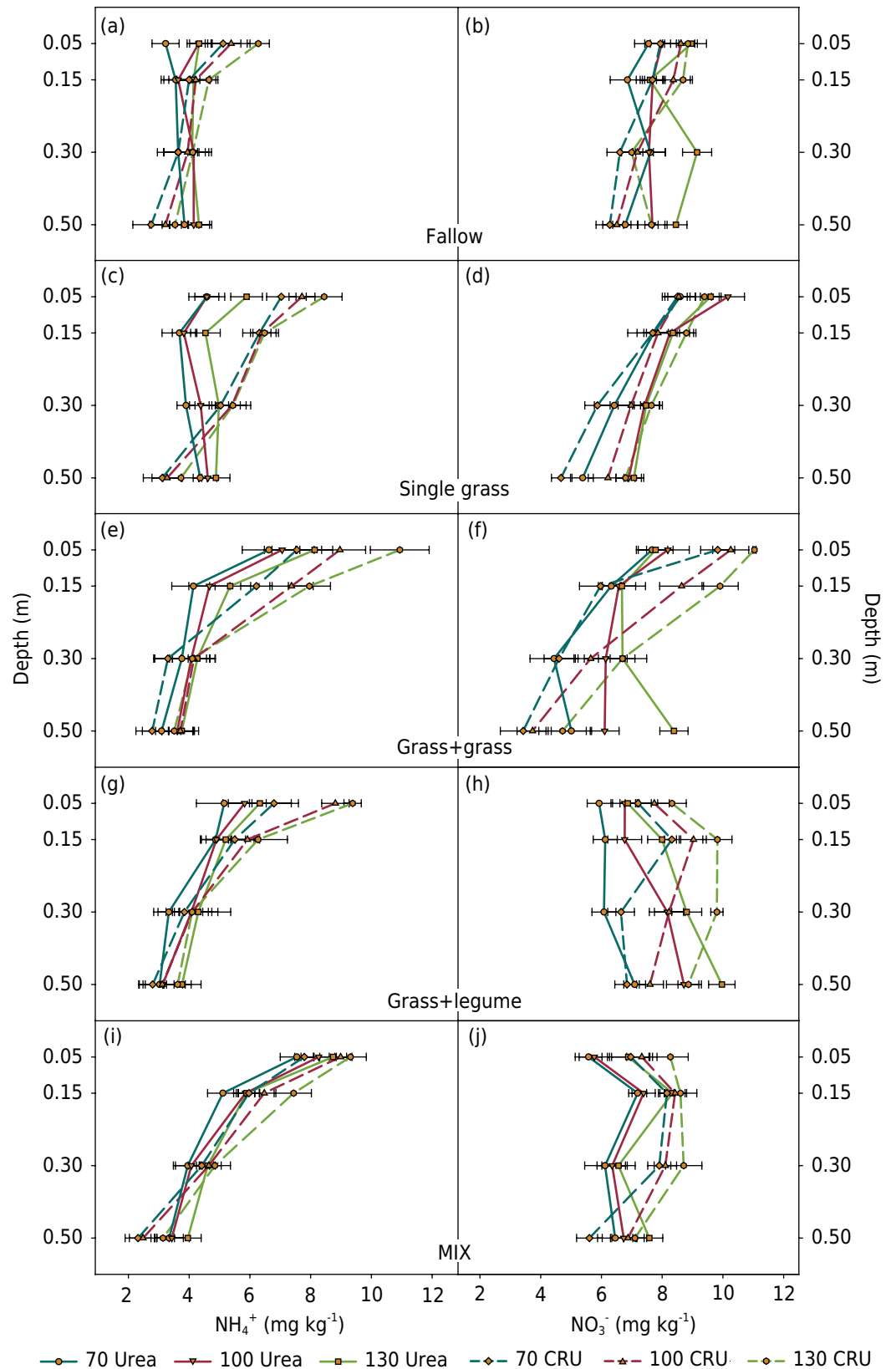


Figure 2. Inorganic nitrogen contents in the soil (NH_4^+ and NO_3^-) after cotton harvest from the 2018/2019 crop season. Horizontal bars represent standard error of the mean.

that using an ample diversity of cover crop species can both reduce N nitrification and increase ammonium content via biological nitrogen fixation (BNF). Tropical grasses are reportedly more efficient in reducing N nitrification in the soil (Subbarao et al., 2007; Subbarao et al., 2009), regardless of the grass species (Rocha et al., 2019).

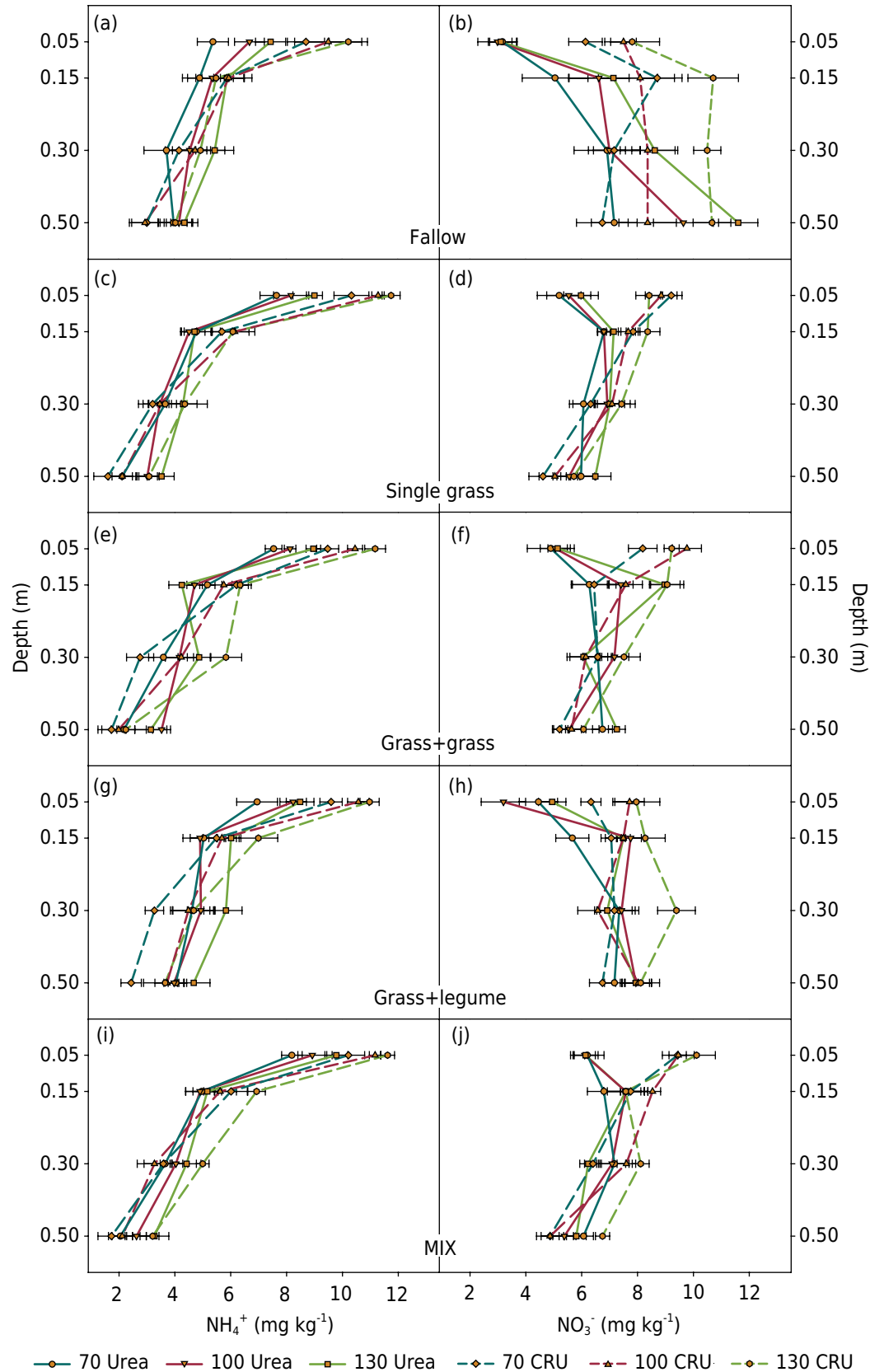


Figure 3. Soil Inorganic nitrogen (NH_4^+ and NO_3^-) after the cotton harvest in the 2019/2020 crop season. Horizontal bars represent the standard error of the mean.

Nitrogen fertilization increases soil N stock (Suzuki et al., 2017; Cordeiro and Echer, 2019; Kumar et al., 2019; Cordeiro et al., 2021a). However, we observed that this technique has a greater impact in systems without cover crops, as N fertilization increased total N

stock in only fallow systems (Table 1). Cover crops can reduce the necessity of N fertilizers because of their capacity to increase soil N stock. Thus, cover crops are more economical than traditional fertilizers. Controlled-release urea application increased total N stock in the soil by 8 % at the end of the second crop season (Table 1). Controlled-release urea application caused less impact on total N stock in the first crop season. A cumulative effect likely increased N stock in the second crop season, as CRU application reduces N loss and can increase total N by up to 12 % (Geng et al., 2016b).

In the year with less precipitation (2018/2019), CRU application reduced the availability of inorganic N in the soil during the bloom period, principally in fallow systems (Tables 1 and 2). Controlled-release urea efficiency is highly dependent on moisture in the surface soil (Zheng et al., 2016) for oxidation of the sulfur that covers urea granules. Which can also be affected by soil pH, but with less importance for this study, since the amount of S contained in fertilizers is low. Therefore, in soils with low levels of inorganic N and years with low water availability, the delayed release of N from CRU application can limit N absorption in the flowering period. The effect on yield depends on the severity of water restriction, as lack of water also limits nutrient absorption and plant growth.

After cotton harvest, we observed that CRU application increased nitrate and ammonium availability by an average of 12 % at depths up to 0.60 m below the surface (Table 4). The N release rate from CRU application can explain differences between N stock in the flowering period and after collection, as the rate release depends on the thickness of the elemental sulfur layer of the granules. Significant N release can occur even after 90 days of CRU application (Zheng et al., 2016). The increase of ammonium and nitrate stock in the soil after harvest, as occurred with CRU application, represents the economic benefit of CRU application. The successive crop will require less fertilizer, as N cycling increases with cover crop use.

Various studies have reported that the use of cover crops (specifically grasses with high biomass input) in the shoot and root areas can reduce N leaching in the soil in tropical climates (Rosolem et al., 2018; Galdos et al., 2020; Rocha et al., 2020). Studies have also shown that CRU application reduces leaching, principally nitrate leaching (Tian et al., 2018; Zhang et al., 2018). However, in environments with tropical climates, high precipitation, and sandy soil, the isolated use of just one of these techniques (cover crops or CRU application) is not enough to efficiently reduce nitrate leaching (Figures 2 and 3). Our results demonstrate that combining cover crops with CRU application is necessary to reduce nitrate movement by using moderate doses of N as recommended for the region.

Ammonium movement can be reduced by using cover crop systems with high biomass input (SG, G+G and MIX), mainly in the second crop season. Increases in organic matter (Raphael et al., 2016), the consequential increase in cation exchange capacity (Sharma et al., 2018), and NH_4^+ retention in the topsoil are all possible explanations of this result (Shaddox et al., 2016). However, with a high dose of 130 kg ha^{-1} of N, the ammonium movement was higher, even with the use of cover crops (Figures 2 and 3).

Even when CRU and cover crops were used, application of N doses above the recommended level increased nitrate movement in soil layers of 0.40-0.60 m in SG and G+L systems in 2018/2019 and G+L and MIX systems in 2019/2020 (Figures 2 and 3). This is a problem because the cotton root system is usually effective up to 0.40 m, so a system without cover crops can result in nitrate losses in the 0.40-0.60 m layer.

CONCLUSION



The use of a diversity of cover crops increases the ratio of ammonium to nitrate in soil. Grass systems reduce the availability of inorganic N. Total N increases in systems with cover crops with high biomass input. Increasing the N dose raises inorganic N stock in the



soil, but the application of 30 % or more of the recommended dose for cotton in the region of our study intensified movement, for nitrate, mainly in systems without cover crops and use of conventional urea. After cotton harvest, we observed that controlled-release urea application resulted in 12 % more inorganic N than from conventional urea application, which can reduce the consumption of N fertilizers in cotton crops.



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