



# Nutrients released by *Urochloa* cover crops prior to soybean

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Received: 24 May 2018 / Accepted: 17 February 2019 / Published online: 23 February 2019  
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**Abstract** *Urochloa* spp. grow vigorously in the dry-season of the tropics, and have been used successfully to provide abundant surface residue as cover crop for no-till soybean (*Glycine max*). Nitrogen (N) fertilizer application could enhance cover crop biomass production and its ground cover and accelerate residue decomposition, but how these cascading factors affect nutrient availability to the subsequent soybean crop is not known. We evaluated nutrient cycling and soybean nutrition and yield components following different timings of N application to living and desiccated *Urochloa* cover crops. The experiment was conducted in two growing seasons at Botucatu, São Paulo State, Brazil. Treatments consisted of two cover crop grasses

(*Urochloa brizantha* and *Urochloa ruziziensis*) and six N management systems [control (no N application); N application at soybean sowing (40 kg N ha<sup>-1</sup>) plus topdressing (60 kg N ha<sup>-1</sup> at V5 stage); N application 20 days before desiccation (DBD) of cover crops, N application 10 DBD; N application 5 DBD; and N application 1 day before sowing of soybean, using the rate of 100 kg N ha<sup>-1</sup> in the latter four treatments]. Both cover crops produced high amount of shoot biomass (> 9.7 Mg ha<sup>-1</sup>), but *U. brizantha* was 48% more productive than *U. ruziziensis*. Nutrient accumulation in cover crop straw was enhanced due to greater biomass production in treatments with N applied 20 and 10 DBD. Soybean grain yield was 17% greater following *U. brizantha* than following *U. ruziziensis*. Nitrogen application at different times did not affect soybean grain yield. These results suggest that *Urochloa* biomass, macronutrient accumulation, and subsequent release rates can be enhanced with N application, but it had little short-term impact on soybean yield components.

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**Keywords** *Brachiaria* · Nitrogen fertilization ·  
Cropping system · *Glycine max*

## Introduction

Soil degradation is an on-going challenge facing agriculture, and therefore, affects global climate

change by altering the cycling of water, carbon (C), nitrogen (N), sulfur (S), and other elements (Lal and Stewart 1990). According to FAO (2015), 33% of soil in the world is degraded due to agriculture, resulting in serious economic and social instability, deforestation, water pollution, enhanced greenhouse gas emissions, intensive use of marginal lands, and accelerated runoff and erosion (Lal and Stewart 1990). Conservation agricultural systems using cover crops and appropriate fertilizer management could be important practices to enhance the sustainability of agricultural systems.

No-till systems (NTS) maintain crop residue on the soil surface, decrease soil disturbance, and often have rotation of crops over time, resulting in one of the most effective strategies to improve agricultural sustainability (Caires et al. 2015), particularly when combined with crop-livestock integration (Crusciol et al. 2015). *Urochloa* is well-suited for the tropical region with dry winters and low fertility, because these grasses have vigorous and deep roots and high tolerance to water deficit stress (Fisher and Kerridge 1998; Gichangi et al. 2017). These perennial forage grasses have high biomass production potential and efficiency in nutrient cycling. The root system can explore more than 1 m deep, taking advantage of residual fertilization from the previous summer crop, and increasing soil biological activity and organic matter content (Pacheco et al. 2011; Nascente and Crusciol 2012). *Urochloa* straw quality and quantity are considered ideal for maintaining the integrity of NTS (Pariz et al. 2011a) with significant persistence at the soil surface (Kluthouski et al. 2003; Nascente and Crusciol 2012), which could lead to improved soil organic matter and regeneration of long-term productivity (Franzluebbbers et al. 2014; Crusciol et al. 2015).

To keep the soil surface covered, cover crops should have high potential biomass production with low decomposition (Pacheco et al. 2011). However, residues with high C/N ratio may cause temporary immobilization of N in soil (Calonego et al. 2012). Seasonal changes in soil organic C and N fractions are a result of crop residue inputs (quantity and quality) and soil microbial biomass and activity (Franzluebbbers et al. 1995). Fluctuations in soil microbial biomass can cause net N mineralization or immobilization during decomposition of plant residues (Santi et al. 2003). Low quantity of soil and crop residue N could limit subsequent crop growth during decomposition of cover crop straw.

Forage grasses as cover crops may promote constant and more efficient nutrient cycling by changing time of availability and/or release of nutrients (Assmann et al. 2017). A key concept of nutrient cycling in any ecosystem is mass balance. To be sustainable, nutrient outputs should be balanced with nutrient inputs to a system (Wedin and Russelle 2007), and the cycling of nutrients are determined by the soil biotic and abiotic conditions (Ferreira et al. 2011). Therefore, forage grasses as cover crops can maximize benefits to nutrient cycling by supplying sufficient C to keep nutrients in organic forms during non-crop growing periods (Assmann et al. 2017).

Soybean [*Glycine max* (L.) Merr.] is one of the most important crops cultivated in Brazil, grown on 35 million ha (CONAB 2018). This crop grown in succession or rotation with grasses achieves high grain yield (Moraes et al. 2014). Soybean has high quantity of N, mainly as grain protein (50–60 g N kg<sup>-1</sup>). To meet the demand of N, soybean relies primarily on biological nitrogen fixation (BNF), which occurs from symbiosis with the bacterial genus *Bradyrhizobium* supplying 70–200 kg N ha<sup>-1</sup> (Herridge et al. 2008). However, some researchers have reported the necessity to fertilize soybean with N in order to avoid yellowing of leaves during initial growth (Nogueira et al. 2010; Pereira et al. 2010), or at the reproductive stage since BNF may be insufficient to provide enough N to soybean (Petter et al. 2012; Bahry et al. 2013).

One alternative to improve soybean N availability might be to supply N to a cover crop, aiming to both carryover sufficient N to soybean via decomposition of the cover crop and enhance the quantity of cover crop straw. Therefore, our objective was to develop an understanding of how N fertilization timing (i.e. applied to cover crop before desiccation or on cover crop residues) affects cover crop biomass production, nutrient accumulation and release, and subsequent soybean growth and yield components.

## Materials and methods

### Site description

The experiment was conducted during the 2013–2014 and 2014–2015 growing seasons in Botucatu, State of São Paulo, southeastern Brazil (48°26'W; 22°51'S;

740 m asl). The soil is a clayey, kaolinitic, thermic Typic Haplorthox (USDA Taxonomy). Rainfall and temperature during the experimental period are reported in Table 1.

The experiment was carried out in an area cultivated with *Urochloa brizantha* (Hochst. Ex A. Rich.) R.D. Webster (syn. *Brachiaria brizantha*) cv. Marandu and *U. ruziziensis* (R. Germ. and C.M. Evrard) Morrone and Zuloaga (syn. *B. ruziziensis*). In each growing season, the experiment was conducted in a new area of the field. Management history was NTS for 6 years. Cover crops were sown at a density of 10 kg seed ha<sup>-1</sup> (34% viable seed). In both growing seasons, cover crops were not previously fertilized, i.e., they grew only with the residual fertilization from a previous crop. Cover crops were cultivated approximately 8 months (April to November) before soybean seeding, and managed without weed control.

Before the experiment, soil (0–0.2-m depth) was sampled to evaluate its chemical characteristics (Table 2), according to the method of van Raij et al. (2001). Only in the 2013–2014 growing season, ammonifier and nitrifier bacterial populations were determined (Table 3), according to Sarathchandra (1978), Kowalchuck et al. (1997), and Treusch et al. (2005).

#### Experimental design and treatments

The experimental design was a randomized complete block, arranged in a 2 × 6 factorial scheme, with four replications. Treatment factors consisted of two cover crops (*U. brizantha* and *U. ruziziensis*) and six forms of N management: control (no N application), N application at soybean sowing and topdressing [40 kg N ha<sup>-1</sup> + 60 kg N ha<sup>-1</sup>, respectively (S + T)], N application on cover crop at 20 days

**Table 1** Monthly rainfall and maximum and minimum temperatures during the study period and in the long-term at Botucatu, São Paulo State, Brazil

Climate characteristics	Month											
	June	July	August	September	October	November	December	January	February	March	April	May
2013–2014												
Monthly rainfall (mm)	115	57	0	88	107	45	90	49	119	126	75	73
Mean max. temp. (°C)	21.7	21.8	24.8	25.8	26.4	28.1	30.0	30.9	30.9	28.9	26.7	23.5
Mean min. temp. (°C)	14.0	12.4	14.6	15.6	15.3	17.3	19.1	19.7	20.1	18.8	16.2	13.4
2014–2015												
Monthly rainfall (mm)	1	25	19	96	37	143	264	256	251	265	45	100
Mean max. temp. (°C)	24.5	23.7	26.8	28.0	30.2	28	28.5	31.7	28.4	27.1	26.3	23.1
Mean min. temp. (°C)	13.5	11.7	11.5	12.6	13.4	13.9	15.5	19.1	18.1	17.2	15.2	14.2
Long-term (50-year average)												
Monthly rainfall (mm)	57	43	37	88	113	114	212	267	206	178	70	83
Mean max. temp. (°C)	22.5	22.7	25.2	25.2	26.9	27.4	27.3	27.8	28.3	27.8	26.1	23.5
Mean min. temp. (°C)	13.2	12.9	14.8	14.8	16.2	17.2	18.2	18.9	19.2	18.7	17	14.5

**Table 2** Soil chemical characteristics (0–0.2-m depth) in the experimental area before initiating the experiment

Growing season	pH(CaCl <sub>2</sub> )	SOM <sup>a</sup> (g dm <sup>-3</sup> )	P <sub>(resin)</sub> (mg dm <sup>-3</sup> )	H + Al (mmol <sub>c</sub> dm <sup>-3</sup> )	K <sup>+</sup> (mmol <sub>c</sub> dm <sup>-3</sup> )	Ca <sup>2+</sup> (mmol <sub>c</sub> dm <sup>-3</sup> )	Mg <sup>2+</sup> (mmol <sub>c</sub> dm <sup>-3</sup> )	CEC <sup>b</sup> (mmol <sub>c</sub> dm <sup>-3</sup> )	BS <sup>c</sup> (%)
2013–2014	5.8	14	37	37	3.6	43	34	110	66
2014–2015	5.3	27	36	36	2.3	52	22	112	69

<sup>a</sup>Soil organic matter<sup>b</sup>Cation exchange capacity<sup>c</sup>Base saturation**Table 3** Ammonifier and nitrifier population in soil (0–0.2-m depth) cultivated with *Urochloa brizantha* and *Urochloa ruziziensis* before initiating the experiment in 2013

Cover crop	Ammonifier (CFU <sup>a</sup> g soil <sup>-1</sup> )	Nitrifier population (CFU <sup>a</sup> g soil <sup>-1</sup> )
<i>U. brizantha</i>	13.4	2.5
<i>U. ruziziensis</i>	8.2	2.5

<sup>a</sup>CFU is colony forming unit

before desiccation (DBD), N application on cover crop at 10 DBD, N application on cover crop at 5 DBD, and N application after cover crop desiccation at 1 day before sowing (DBS) of soybean, using the rate of 100 kg N ha<sup>-1</sup> in the last four treatments. The N rate of 100 kg ha<sup>-1</sup> was applied with the intent to increase cover crop biomass production and N uptake, as well as reduce straw C/N ratio when applied before desiccation (20 DBD, 10 DBD, or 5 DBD) as a strategy to minimize N immobilization during early soybean development. Nitrogen application at 1 DBD and at soybean sowing and topdressing was intended to supply N directly to the soybean crop, since in conditions of high grain yield, soybean may demand more N than BNF can supply (Salvagiotti et al. 2008). Each plot consisted of ten 5-m-long rows spaced at 0.45 m, in a total area of 22.5 m<sup>2</sup>. Samples were collected in four central rows, avoiding 0.5 m from the end of each row.

Prior to N application, forage accumulated as cover crop was cut by mechanical mower at 0.3-m height. Application of N started on 5 October 2013 and 8 October 2014 for the 20 DBD treatment and applications of N at 10 and 5 DBD were made in sequence. Cover crops were desiccated on 25 October 2013 and 28 October 2014 with the herbicide glyphosate (2.8 kg acid-equivalent ha<sup>-1</sup>). Cover crop desiccation was

made 30 DBS of soybean. For the treatment with application of N at 1 DBS, N was applied on the cover crop straw on 26 November 2013 and 30 November 2014. For the treatment with application at time of sowing, 40 kg N ha<sup>-1</sup> was applied at 0.05–0.10 m distance from the sowing furrow and 60 kg N ha<sup>-1</sup> was topdressed at V5 stage of soybean. The N source for all applications was ammonium nitrate.

#### Crop management

Soybean cultivar BMX Potencia RR was sown on 27 November 2013 and 1 December 2014 using a no-till drill at a density of 377,000 seeds ha<sup>-1</sup> and a depth of 0.03 m. Right before sowing, soybean seeds were inoculated with *Bradyrhizobium japonicum*. For all treatments, the basic fertilization in the sowing furrows consisted of 90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as triple superphosphate and 45 kg K<sub>2</sub>O ha<sup>-1</sup> as potassium chloride (Mascarenhas and Tanaka 1997).

Soybean was cultivated according to crop needs with the following: fungicide application of azoxystrobin and cyproconazole, pyraclostrobin and epoxiconazole; and insecticide application of acephate, methomyl and thiamethoxam.

## Sampling and analyses

Shoot dry matter (DM) of cover crops was evaluated at 0 and 90 days after desiccation (DAD). Biomass was cut at ground level (0.25 m<sup>2</sup> each) from three random locations along a diagonal within each plot and composited, according to Crusciol et al. (2005). Biomass was dried by forced-air circulation at 65 °C for 72 h until constant weight and weighed.

Soybean shoot DM was determined by sampling 10 plants at flowering (R2 stage). Additionally, samples were collected for leaf nutrition diagnosis from the upper third leaf from 30 plants per plot (Ambrosano et al. 1997). Leaves were washed, dried in forced air circulation at 65 °C for 72 h, and ground. Concentrations of N, P, K, Ca, Mg, and S in leaves were determined according to Malavolta et al. (1997).

Soybean was harvested at maturity. Plant height, grain yield, and yield components (final population of plants, number of pods per plant, number of grains per pod, and 100-seed weight) were determined at harvest. Final population was determined by counting the number of plants in two central 4-m rows per plot. Plant height, number of pods per plant, and number of grains per pod were evaluated from 15 plants per plot chosen at random. Plants from four central 4-m rows were threshed mechanically, grain weighed, and yield calculated. The 100-grain weight was performed by randomly collecting eight samples per plot.

## Statistical analyses

A combined analysis of variance across the two growing seasons was performed using the statistical package SISVAR (Ferreira 2011). Before analysis, data were tested for normality and homogeneity of variance. Blocks and block interactions were considered random effects. Cover crop, N management, growing season, and their interactions were considered fixed effects. For all variables, an F test was performed and means were separated using Fisher's protected LSD test at 0.05 probability level.

## Results

### Weather conditions

In the first growing season (October 2013–May 2014), rainfall (683 mm) was 45% lower than the long-term average of 1244 mm and mean temperature (23.0 °C) was higher than the long-term average of 22.2 °C (Table 1). In the second growing season (October 2014–May 2015), rainfall (1361 mm) was 9% greater than the long-term average and mean temperature (21.9 °C) was lower than the long-term average.

### Cover crop biomass production and nutrient accumulation

Accumulation of DM and macronutrients in cover crop shoots at 0 and 90 DAD were affected by cover crop species, N management system, growing season, and cover crop × N management system interaction (Table 4). At 0 DAD, *U. brizantha* had greater DM than *U. ruziziensis* in all treatments with N (20 DBD, 10 DBD, 5 DBD, 1 DBS, and S + T) and in the control (no N application), when averaged across growing seasons (Fig. 1a). *U. brizantha* biomass production varied by N management system: 20 DBD > 10 DBD = 5 DBD > 1 DBS = S + T = control. Meanwhile, *U. ruziziensis* had greatest biomass production in the treatment with N applied 20 DBD and the lowest in the treatment with N applied 1 DBS. At 90 DAD, *U. brizantha* straw remaining was greater than *U. ruziziensis* only when N was applied at 20 DBD, at 10 DBD, and at 5 DBD (Fig. 1b). In the S + T and control (no N application) treatments, DM of *U. brizantha* was lower than that of *U. ruziziensis* (0.60 and 0.71 Mg ha<sup>-1</sup>, respectively), whereas at 1 DBS there was no difference between species in cover crop DM remaining.

For all macronutrients (N, P, K, Ca, Mg, and S) at 0 DAD, *U. brizantha* had greater accumulation than *U. ruziziensis* in all N management systems, including the control (Figs. 1c, e, g, 2a, c, e). Nitrogen and K had greatest contents of all elements in both cover crops, leading to large sources of N and K recycled (Fig. 1c, g). For both cover crops, the treatment with N applied at 20 DBD also had greater macronutrient content than all other N management systems (Figs. 1c, e, g, 2a, c, e).

**Table 4** Cover crop biomass and above-ground macronutrient content at 0 and 90 days after desiccation (DAD), as affected by growing season, cover crop species, and N management system in a field study in Botucatu, State of São Paulo, Brazil

Treatment	Shoot biomass (Mg ha <sup>-1</sup> )		N (kg ha <sup>-1</sup> )		P (kg ha <sup>-1</sup> )		K (kg ha <sup>-1</sup> )		
	0 DAD	90 DAD	0 DAD	90 DAD	0 DAD	90 DAD	0 DAD	90 DAD	
Growing season									
2013–2014	14.4a <sup>a</sup>	0.6b	279a	10b	16b	1b	116a	2b	
2014–2015	9.8b	2.6a	121b	37a	19a	3a	88b	5a	
ANOVA (Pr > F)									
Cover crop (CC)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
N management system (NM)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
Growing season (GS)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
CC × NM	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
CC × GS	0.28	0.12	0.16	0.18	0.29	0.18	0.08	0.07	
NM × GS	0.17	0.30	0.09	0.09	0.20	0.10	0.27	0.10	
CC × NM × GS	0.09	0.08	0.01	0.24	0.26	0.08	0.16	0.27	
Ca (kg ha <sup>-1</sup> )									
		Mg (kg ha <sup>-1</sup> )		S (kg ha <sup>-1</sup> )					
		0 DAD	90 DAD	0 DAD	90 DAD	0 DAD	90 DAD		
Growing season									
2013–2014	70a	6b	43a	1b	14b	1b			
2014–2015	46b	17a	29b	7a	24a	6a			
ANOVA (Pr > F)									
Cover crop (CC)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
N management system (NM)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
Growing season (GS)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
CC × NM	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
CC × GS	0.09	0.19	0.26	0.07	0.20	0.18			
NM × GS	0.18	0.08	0.08	0.28	0.07	0.08			
CC × NM × GS	0.07	0.27	0.40	0.06	0.17	0.31			

<sup>a</sup>Means followed by different letters in the same column are significantly different at  $P < 0.05$

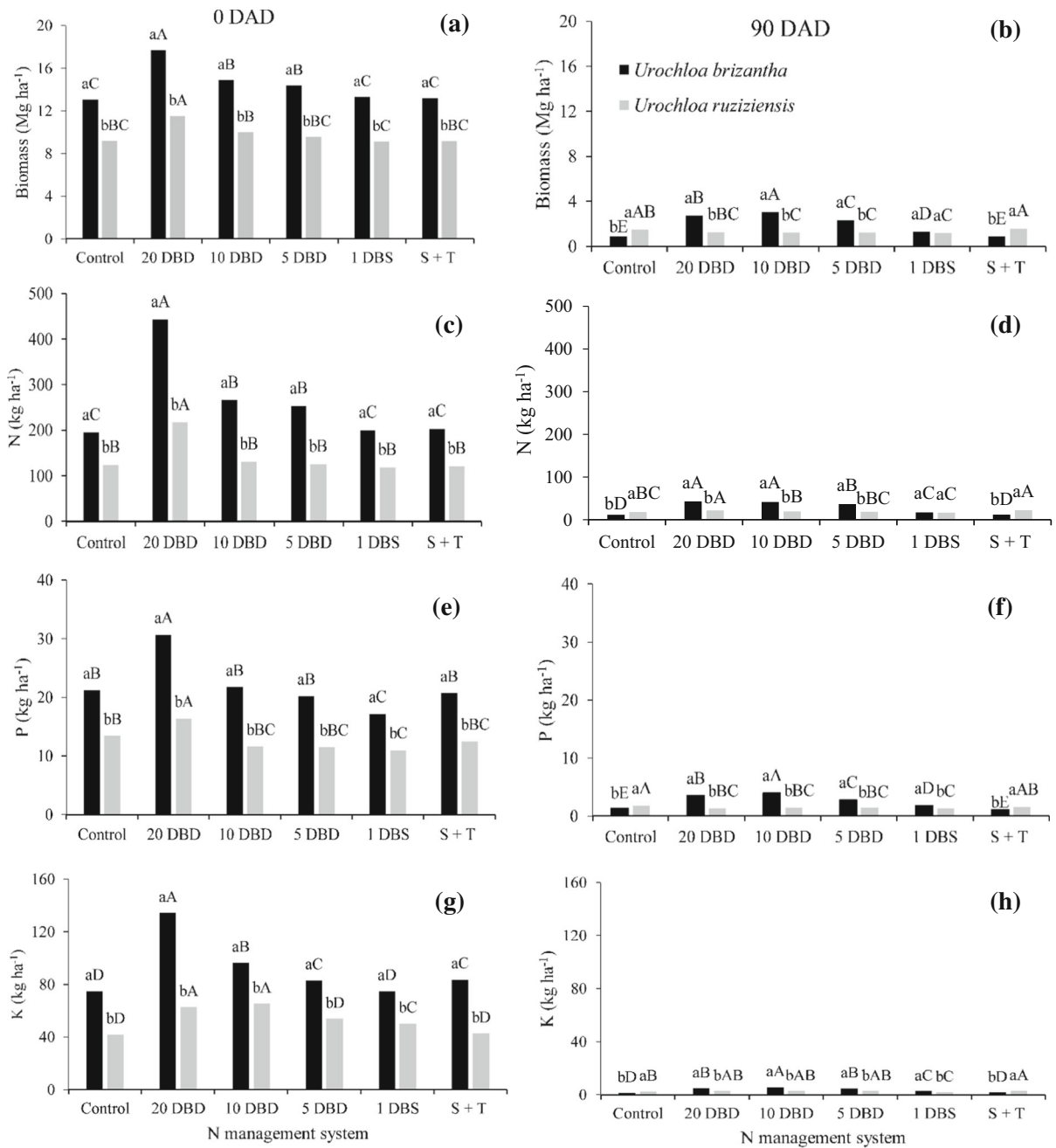
At 90 DAD, N, Ca, and S contents were greater in *U. brizantha* straw than in *U. ruziziensis* straw when N was applied at 20 DBD, 10 DBD and 5 DBD (Figs. 1d, 2b, f). Likewise, *U. brizantha* straw had greater P, K, and Mg contents than *U. ruziziensis* straw at 1 DBS (Figs. 1f, h, 2d). However, N and Ca contents were not different between cover crops when N was applied at 1 DBS (Figs. 1d, 2b). The lowest macronutrient content of *U. brizantha* was when the N management was S + T and without N (control).

On average across cover crops and N management system, the 2013–2014 cover-crop growing season had greater DM biomass and N, K, Ca, and Mg contents than the 2014–2015 at 0 DAD, but the reverse

was true for P and S contents (Table 4). At 90 DAD, the remaining amounts of DM and macronutrients were greater in the 2014–2015 cover-crop growing season than in the 2013–2014 season.

#### Nutrient concentrations in soybean leaves

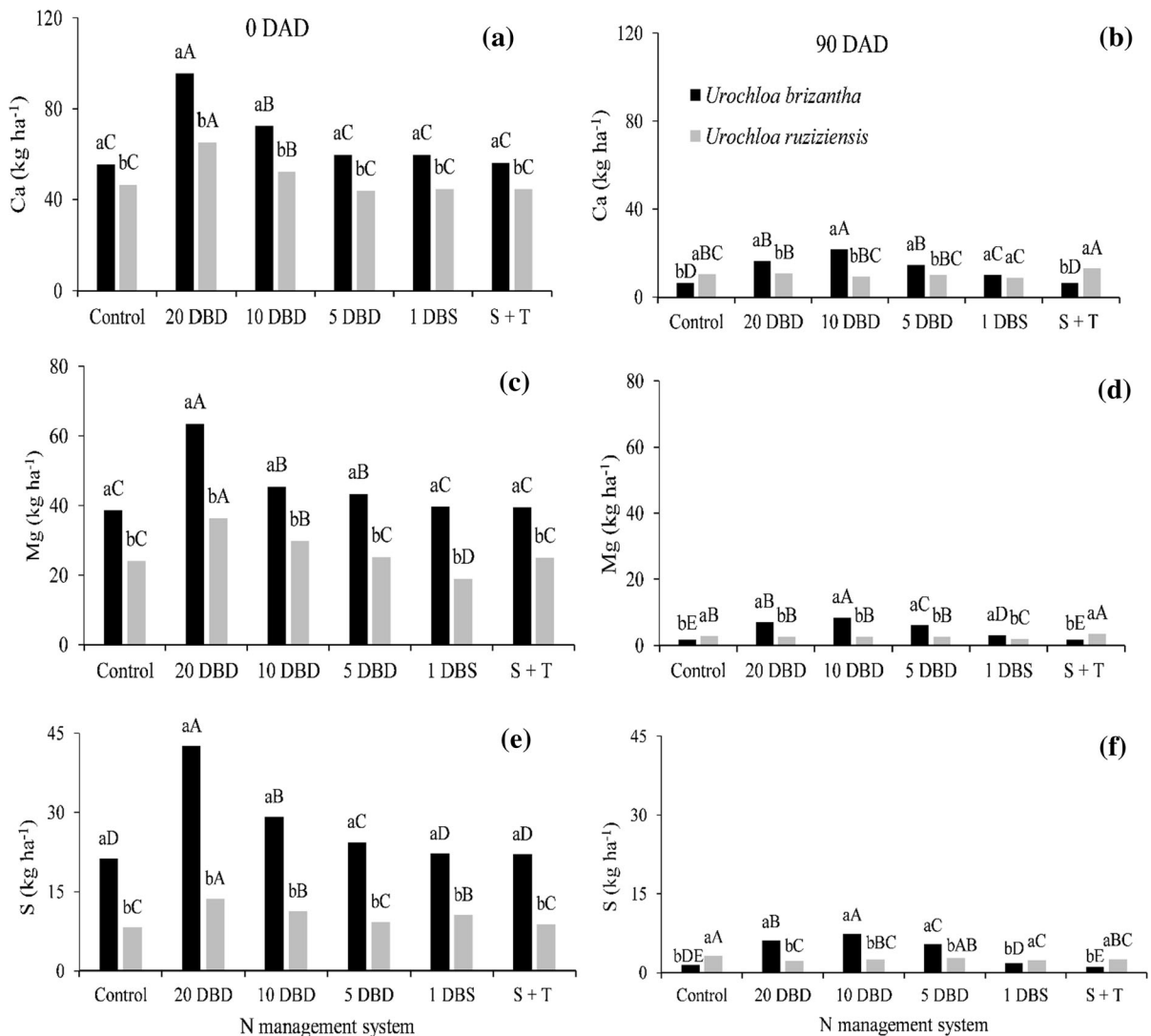
Nutrient concentrations in soybean leaves were affected by growing season, but there was no effect of cover crop species, N management system, and interaction among factors (Table 5), except for Mg concentration. The system without N addition had lower Mg concentration than all other treatments with N addition. Greatest N, K, and Ca concentrations in



**Fig. 1** Shoot biomass (a and b) and N (c and d), P (e and f), and K (g and h) contents of cover crops at 0 (a, c, e, g) and 90 (b, d, f, h) days after desiccation (DAD) as affected by N management system and cover crop species in a field study in Botucatu, State of São Paulo, Brazil. Data are means of two growing seasons. Different lowercase letters denote significant difference between cover crops and different uppercase letters denote significant difference among N management systems (LSD,  $P < 0.05$ ). Control = no N application; 20

DBD = 100 kg N ha<sup>-1</sup> broadcast over cover crop 20 days before desiccation; 10 DBD = 100 kg N ha<sup>-1</sup> broadcast over cover crop 10 days before desiccation; 5 DBD = 100 kg N ha<sup>-1</sup> broadcast over cover crop 5 days before desiccation; 1 DBS = 100 kg N ha<sup>-1</sup> broadcast over desiccated cover crop 1 day before soybean sowing; S + T = 40 kg N ha<sup>-1</sup> at soybean sowing plus 60 kg N ha<sup>-1</sup> topdressing at V5





**Fig. 2** Ca (a and b), Mg (c and d), and S (e and f) contents of cover crops at 0 (a, c, and e) and 90 (b, d, and f) days after desiccation (DAD) as affected by N management system and cover crop species in a field study in Botucatu, State of São Paulo, Brazil. Data are means of two growing seasons. Different lowercase letters denote significant difference between cover crops and different uppercase letters denote significant difference among N management systems (LSD,  $P < 0.05$ ).

soybean leaves were found in the first growing season and greatest P, Mg, and S concentrations were found in the second growing season (Table 5).

Control = no N application; 20 DBD = 100 kg N ha<sup>-1</sup> broadcast over cover crop 20 days before desiccation; 10 DBD = 100 kg N ha<sup>-1</sup> broadcast over cover crop 10 days before desiccation; 5 DBD = 100 kg N ha<sup>-1</sup> broadcast over cover crop 5 days before desiccation; 1 DBS = 100 kg N ha<sup>-1</sup> broadcast over desiccated cover crop 1 day before soybean sowing; S + T = 40 kg N ha<sup>-1</sup> at soybean sowing plus 60 kg N ha<sup>-1</sup> topdressing at V5

Plant height, shoot dry matter, yield components and grain yield of soybean

Soybean plant height, shoot DM, plant population, number of pods per plant, and grain yield were lower in 2013–2014 than in 2014–2015 (Table 6), likely due to differences in rainfall with significant drought periods in the vegetative stage of soybean in



**Table 5** Concentration of macronutrients in soybean leaves as affected by cover crop species, N management system, and growing season in a field study in Botucatu, State of São Paulo, Brazil

Treatment	N (g kg <sup>-1</sup> )	P (g kg <sup>-1</sup> )	K (g kg <sup>-1</sup> )	Ca (g kg <sup>-1</sup> )	Mg (g kg <sup>-1</sup> )	S (g kg <sup>-1</sup> )
Cover crop						
<i>U. brizantha</i>	42.0a <sup>a</sup>	2.3a	18.9a	15.5a	3.9a	2.0a
<i>U. ruziziensis</i>	40.1a	2.3a	18.8a	17.7a	4.7a	2.1a
N management system						
Control (no N)	34.8a	2.2a	18.8a	15.5a	3.8b	1.9a
20 DBD <sup>b</sup>	41.2a	2.3a	19.1a	16.8a	4.4a	2.0a
10 DBD <sup>c</sup>	40.8a	2.2a	18.2a	16.7a	4.3a	2.1a
5 DBD <sup>d</sup>	41.1a	2.3a	18.7a	17.9a	4.5a	2.1a
1 DBS <sup>e</sup>	41.1a	2.3a	19.1a	16.4a	4.4a	2.0a
S + T <sup>f</sup>	43.9a	2.4a	19.3a	16.9a	4.5a	2.2a
Growing season						
2013–2014	43.8a	2.0b	23.2a	21.3a	3.8b	1.6b
2014–2015	38.4b	2.5a	14.4b	11.8b	4.8a	2.5a
ANOVA (Pr > F)						
Cover crop (CC)	0.13	0.73	0.93	0.23	0.14	0.47
N management system (NM)	0.21	0.71	0.64	0.19	0.01	0.61
Growing season (GS)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
CC × NM	0.69	0.07	0.26	0.08	0.48	0.61
CC × GS	0.11	0.06	0.23	0.38	0.26	0.11
NM × GS	0.70	0.81	0.70	0.07	0.10	0.27
CC × NM × GS	0.25	0.37	0.07	0.35	0.33	0.44

<sup>a</sup>Means followed by different letters in the same column within a factor are significantly different at  $P < 0.05$

<sup>b</sup>100 kg N ha<sup>-1</sup> broadcast over cover crop 20 days before desiccation (50 days before soybean sowing)

<sup>c</sup>100 kg N ha<sup>-1</sup> broadcast over cover crop 10 days before desiccation (40 days before soybean sowing)

<sup>d</sup>100 kg N ha<sup>-1</sup> broadcast over cover crop 5 days before desiccation (35 days before soybean sowing)

<sup>e</sup>100 kg N ha<sup>-1</sup> broadcast over desiccated cover crop 1 day before soybean sowing

<sup>f</sup>40 kg N ha<sup>-1</sup> at soybean sowing plus 60 kg N ha<sup>-1</sup> topdressing in V5

2013–2014 (Table 1). Plant height was affected by N management system and growing season (Table 6). Plant height was greater with all N-fertilized treatments than the control. Soybean plant height was greater in the wetter 2014–2015 growing season than the drier 2013–2014 growing season. Soybean shoot DM was not affected by cover crop species or N management system (Table 6). Shoot DM was greater in the wetter 2014–2015 growing season than the drier 2013–2014 growing season.

Soybean plant population and number of pods per plant were greater following *U. brizantha* than following *U. ruziziensis* (Table 6). Neither response variable was affected by N management system. Both plant population and number of pods were greater in

the wetter 2014–2015 growing season than the drier 2013–2014 growing season (Table 1). Number of grains per pod was unaffected by any factor. The 100-grain weight was unaffected by cover crop species and N management system, but was lower in the wetter 2014–2015 growing season than the drier 2013–2014 growing season (Table 6). Soybean grain yield was 17% greater following *U. brizantha* than following *U. ruziziensis* (Table 6). Grain yield was unaffected by N management system. Soybean grain yield was 39% greater in the wetter 2014–2015 growing season than the drier 2013–2014 growing season.

**Table 6** Soybean growth and yield components as affected by cover crop species, N management system, and growing season in a field study in Botucatu, State of São Paulo, Brazil

Treatment	Plant height (m)	Dry matter (Mg ha <sup>-1</sup> )	Plant population (thousand plants ha <sup>-1</sup> )	Pods plant <sup>-1</sup>	Grains pod <sup>-1</sup>	100-grain weight (g)	Grain yield (Mg ha <sup>-1</sup> )
Cover crop							
<i>U. brizantha</i>	0.77a <sup>a</sup>	3.62a	339a	33a	1.94a	16.5a	3.27a
<i>U. ruziziensis</i>	0.74a	3.36a	324b	29b	1.93a	16.5a	2.79b
N management system							
Control (no N)	0.69b	3.04a	336a	31a	1.92a	16.6a	3.15a
20 DBD <sup>b</sup>	0.80a	3.37a	334a	31a	1.91a	16.6a	3.04a
10 DBD <sup>c</sup>	0.76a	3.52a	335a	30a	1.94a	16.5a	3.06a
5 DBD <sup>b</sup>	0.76a	3.59a	332a	30a	1.98a	16.1a	3.01a
1 DBS <sup>e</sup>	0.77a	3.97a	327a	30a	1.94a	16.8a	2.98a
S + T <sup>f</sup>	0.76a	3.46a	326a	30a	1.96a	16.4a	2.94a
Growing season							
2013–2014	0.67b	3.31b	309b	22b	1.92a	19.6a	2.53b
2014–2015	0.85a	3.66a	354a	38a	1.96a	13.4b	3.53a
ANOVA (Pr > F)							
Cover crop (CC)	0.09	0.12	0.03	< 0.001	0.75	0.99	< 0.001
N management system (NM)	0.04	0.07	0.92	0.56	0.86	0.49	0.38
Growing season (GS)	< 0.001	0.04	< 0.001	< 0.001	0.23	< 0.001	< 0.001
CC × NM	0.95	0.32	0.49	0.12	0.44	0.86	0.09
CC × GS	0.07	0.34	0.13	0.07	0.08	0.06	0.12
NM × GS	0.27	0.87	0.65	0.23	0.71	0.60	0.14
CC × NM × GS	0.71	0.84	0.36	0.22	0.24	0.23	0.48

<sup>a</sup>Means followed by different letters in the same column within a factor are significantly different at  $P < 0.05$

<sup>b</sup>100 kg N ha<sup>-1</sup> broadcast over cover crop 20 days before desiccation (50 days before soybean sowing)

<sup>c</sup>100 kg N ha<sup>-1</sup> broadcast over cover crop 10 days before desiccation (40 days before soybean sowing)

<sup>d</sup>100 kg N ha<sup>-1</sup> broadcast over cover crop 5 days before desiccation (35 days before soybean sowing)

<sup>e</sup>100 kg N ha<sup>-1</sup> broadcast over desiccated cover crop 1 day before soybean sowing

<sup>f</sup>40 kg N ha<sup>-1</sup> at soybean sowing plus 60 kg N ha<sup>-1</sup> topdressing in V5

## Discussion

Cover crop biomass production, accumulation, and remaining amounts of nutrients

In the 2013–2014 cover-crop growing season, rainfall distribution was uniform and favored cover-crop growth and development (Table 1). In the 2014–2015 cover-crop growing season, there were periods of drought that limited biomass production. Rainfall differences explained differences in amount

of cover crop biomass production and nutrient accumulation (Figs. 1, 2).

Nitrogen fertilizer application before desiccation stimulated greater growth rate and shoot biomass production (Fig. 1a). Greatest shoot biomass production occurred when N was applied 20 DBD, which could be attributable to longer time between N application and cover crop desiccation. Both cover crops had less biomass production the shorter the time they were supplied with N. Difference in biomass production between species at the same N treatment

was likely due to *U. brizantha* being more N-use efficient than *U. ruziziensis* (Alvim et al. 1990).

Greater shoot biomass production with *U. brizantha* than with *U. ruziziensis* in control (no N application) (Fig. 1a) could be attributable to greater root production and its propensity for high mulching capacity, although self-shading could be an issue in high biomass conditions (EMBRAPA 2002). Pacheco et al. (2011, 2013) observed production of 9.7–11.4 Mg ha<sup>-1</sup> of *U. brizantha* straw and 6.7–7.0 Mg ha<sup>-1</sup> of *U. ruziziensis* straw without N supply. Cover crop shoot biomass production in our study was greater than in previous studies with *U. brizantha* [6.3 Mg ha<sup>-1</sup> (Crusciol and Soratto 2007); 10.3 Mg ha<sup>-1</sup> (Simidu et al. 2010)] and with *U. ruziziensis* [8.6 Mg ha<sup>-1</sup> (Menezes et al. 2009); 7.0 Mg ha<sup>-1</sup> (Pacheco et al. 2011)]. Although *U. ruziziensis* produced lower shoot DM than with *U. brizantha* in the control treatment, the amount of straw was greater than considered necessary to control runoff (4 Mg ha<sup>-1</sup>) (Lopes et al. 1987). Moreover, the amount of straw with both cover crop species in all N treatments, including the control without N, was sufficient to provide full coverage of soil (> 7 Mg ha<sup>-1</sup>) (Kluthcouski et al. 2003).

Although *U. brizantha* produced more biomass than *U. ruziziensis* (0 DAD) regardless of N management, the remaining straw (90 DAD) of *U. ruziziensis* was equal to *U. brizantha* when N was applied 1 DBS, and greater than *U. brizantha* when N was applied as S + T and without N application (control) (Fig. 1b). These results might be linked to the amount of N in the biomass on 0 DAD (Fig. 1c) since straw decomposition is associated with chemical composition of biomass (Xu and Hirata 2005). Nitrogen application to cover crops before desiccation can also alter the chemical composition of biomass (Costa et al 2016) and its decomposition by microorganisms. Bacterial and fungal communities mineralize and release nutrients (N, P, K, Ca, and Mg) of cover crops via decomposition processes (Christensen 1989; Glassman et al. 2018). In addition, rate of straw decomposition is determined by biotic and abiotic factors at the soil surface (Espindola et al. 2006), e.g., water availability and temperature (Parton et al. 2007), inorganic N supply (Mary et al. 1996), and C/N ratio of plant residues (Carvalho et al. 2008; Torres and Pereira 2008). Due to greater remaining biomass of *U. ruziziensis* (90 DAD), macronutrient contents in the

straw of *U. brizantha* were lower than in *U. ruziziensis* in the S + T and control treatments (Figs. 1d, f, h, 2b, d, f).

Application of N on *U. brizantha* before desiccation increased nutrient accumulation with biomass production (Figs. 1, 2). Macronutrient contents (N, P, K, Ca, Mg, and S) at 0 DAD were greater when N was applied 20 DBD in *U. brizantha* than in *U. ruziziensis* due to greater biomass production. This result occurred because when plants are well supplied with N, plants have greater development of root system to support shoot development and uptake of other nutrients (Brouwer 1962). Even when N was not supplied to the system, *U. brizantha* had greater accumulation of nutrients than *U. ruziziensis* because of differences between species and biomass production. Pacheco et al. (2013) also studied the accumulation of nutrients in *U. brizantha* and *U. ruziziensis* and observed no differences in N, P, K, Ca, and Mg accumulation. Authors attributed this to lack of difference in shoot biomass production. Pariz et al. (2011b) also observed greater straw inputs of N, P, and K to the soil surface by *U. brizantha* than with *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs (syn. *Panicum maximum* Jacq.) cv. Tanzânia, *M. maximum* cv. Mombaça and *U. ruziziensis*. They reported that use of these grasses over the winter-spring have a great potential to cover the soil surface in the Brazilian Savanna. In both cover crops, the most extracted nutrients were N and K, similar to that reported by Torres et al. (2008) and Pacheco et al. (2011, 2013). Additionally, cover crops, such as *Urochloa* and other grasses, have deep root systems that can reach 1 m deep, and consequently, can take up nutrients otherwise lost by leaching and return them to the soil surface (Crusciol et al. 2015).

When N was applied before desiccation, *U. brizantha* accumulated greater amounts of N, P, K, Ca, and Mg than *U. ruziziensis* as a consequence of greater biomass production (Figs. 1, 2). However, at 90 DAD, lower quantities of N, P, K, Ca, Mg, and S remained in *U. brizantha* straw than in *U. ruziziensis* straw in the S + T and control treatments. These results can be attributed to the high decomposition rate of substrates, which was dependent on biomass amount, rainfall, temperature, and soil microbial activity (Sinsabaugh et al. 2015; Bell et al. 2018). Nutrient release from cover crop biomass can be altered by growth and decay of environment-specific

microbial communities (Christensen 1989; Varela et al. 2017). Production and decomposition of biomass influence the availability of nutrients in soil (N'Dri et al. 2018), and therefore, our results suggest that well-fertilized *U. brizantha* may release greater quantities of nutrients to the following crop. More detailed studies are needed to determine cover crop-specific decomposition rates and nutrient availability in soil.

#### Nutrient concentration in soybean leaves

All nutrient concentrations were within or near the ranges considered suitable for soybean production (Table 5), according to Ambrosano et al. (1997). Nutrient concentrations in soybean leaves ranged from medium to high (van Raij et al. 1997) (Table 2). Nutrients appeared to cycle effectively through cover crops via decomposition and leaching (Figs. 1, 2), as well as supplied from fertilizer and soil. Silva et al. (2011) showed that inorganic fertilization of 20 kg N ha<sup>-1</sup> reduced nodulation and efficiency of BNF. However, in the present study it is possible that much of the N applied had been temporarily immobilized by soil microorganisms decomposing the large amount of straw on the soil surface, thereby not altering the N supply to soybean. The lack of difference in soybean leaf N concentration among N management systems, even with disparate N timing from 50 DBS to the day of sowing, suggests that inorganic N availability may not have affected BNF. Greater N, K, and Ca concentrations in soybean leaves in the first growing season and greater P, Mg, and S concentrations in the second growing season (Table 5) were related with nutrient contents in cover crop biomass in the respective growing seasons (Figs. 1, 2), except for Mg. Greater Mg concentration with N application may have been due to reduced soil moisture with high biomass yield, C/N ratio, and root-microbial activity, all of which have been shown to increase plant-available Mg release from soils (Mayland and Wilkinson 1989; Senbayram et al. 2015). Soybean leaf Mg concentration without N fertilizer application had a value considered adequate for crop growth, i.e. in the range of 3.0–10.0 g kg<sup>-1</sup> for soybean (Ambrosano et al. 1997).

Plant height, dry matter, yield components, and grain yield of soybean

Low rainfall in 2013–2014 adversely affected soybean growth and nutrient uptake (Table 5). Despite lower grain yield in the 2013–2014 growing season (2.5 Mg ha<sup>-1</sup>), it was close to the national average (2.8 Mg ha<sup>-1</sup>) (CONAB 2015).

Soybean grain yield was a reflection of the results observed in plant population and number of pods per plant, which had greater values when following *U. brizantha* than following *U. ruziziensis* (Table 6). Greater amount of straw left on the soil surface by *U. brizantha* may have favored the establishment of soybean plants and pod formation under adverse weather conditions during critical development stages (Table 1; Figs. 1, 2). Number of pods per plant is one of the main yield components determining grain yield (Carpentieri-Pípolo et al. 2005). Pacheco et al. (2013) did not find a difference in soybean grain yield when comparing the two forages grasses as cover crops, despite slower nutrient release with *U. brizantha* than with *U. ruziziensis*.

Soybean height was the only plant trait affected by N management system, which was 10% lower in the control without N compared with all other N treatments (Table 6). This response reflected N availability to soybean at an early growth stage, and consequently, plant height was not a critical factor for grain yield. Similar results were inferred by Silva et al. (2011) in a fallow area with conventional tillage system, in which N fertilization at sowing (24 kg N ha<sup>-1</sup>) led to greatest plant height of 0.74 m. Pereira et al. (2010) also reported increased plant height with N application than without.

Lack of N management effects on shoot DM, yield components, and grain yield of soybean (Table 6) corroborate previous results. Aratani et al. (2008) found that N application in soybean did not influence yield components and grain yield. Thus, in soils with efficient nodulation, use of N fertilizer has no effect on grain yield, but may decrease nodulation and efficiency of BNF (EMBRAPA 2011). In contrast, Bahry et al. (2013) demonstrated that N application in the reproductive stage was effective to increase soybean grain yield, since from this stage the efficiency of BNF begins to decrease.

This study showed that the use of either *Urochloa* species as a cover crop in the winter dry season of the

Brazilian Savanna would be a viable option for farmers to diversify their operations for more sustainable soybean production (Crusciol et al. 2015). In addition, N application to the forage grass or to the soybean following them did not improve soybean grain yield (Table 6). However, N application to grass cover crops before desiccation could benefit soil fertility and organic matter accumulation in the long-term to promote even greater cycling of nutrients in the system. Economic impacts of such long-term application strategies would also be needed to make effective recommendations.

## Conclusions

Despite large shoot biomass production and nutrient accumulation from both *Urochloa* cover crops, soybean grain yield was greater following *U. brizantha* than following *U. ruziziensis*. Regardless of whether or when N was applied, soybean leaf nutrient concentrations, yield components, and grain yield were unaffected. However, it is noteworthy that application of N to cover crops before desiccation resulted in greater nutrient accumulation in cover crop biomass, and consequently, improved nutrient cycling. However, this greater nutrient accumulation and cycling had little consequence on subsequent soybean grain yield, at least in this short-term evaluation. *U. brizantha* was considered superior to *U. ruziziensis* as a cover crop to promote greater nutrient cycling, increase food production, and allowing farmers to produce in a sustainable way.

**Acknowledgements** The authors would like to thank the Coordination of Improvement of Higher Education Personnel (CAPES) for the financial support, as well as the National Council for Scientific and Technological Development (CNPq) for an award for excellence in research to the second, third, and eighth authors.

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