



Potential interaction of soybean-grass intercropping with residual nitrogen for a no-tillage system implementation

Karina Batista^{*✉}, Alessandra Aparecida Giacomini, Luciana Gerdes, Waldssimiler Teixeira de Mattos and Ivani Pozar Otsuk

Instituto de Zootecnia, Agência Paulista de Tecnologia dos Agronegócios, Rua Heitor Pentead, 56, Centro, 13380-011, Nova Odessa, São Paulo, Brazil.

*Author for correspondence. E-mail: batistakarim@gmail.com

ABSTRACT. Combinations between crop intercropping and forage production in a no-tillage system are not well established for environments under low water retention and soil fertility conditions. Our study aimed to evaluate the potential interactions between soybean-grass intercropping with residual nitrogen in a no-tillage system. The experimental design was a randomized block with four replications in a subdivided plot scheme. The main plots in the summer season were: 1) soybean monoculture; 2) soybean - Aruana Guinea grass (*Megathyrsus maximus* cv. Aruana) intercropping, and 3) soybean - Congo grass (*Urochloa ruziziensis* cv. Comum) intercropping. The subplots were the nitrogen rates of 0, 50, 100, and 150 kg ha⁻¹, applied as side-dressing in maize and grasses during the autumn-winter season. Here, the results of the summer seasons are shown. To do so, the parameters evaluated were soybean agronomic traits, dry biomass production, and macronutrient concentrations of plants intercropped. The main effects and interactions were studied. Our findings showed that soybean-Aruana Guinea grass intercropping is an alternative to no-tillage system implementation. Moreover, residual nitrogen from the autumn-winter season directly interfered with the dry biomass production of grasses intercropped with soybeans in the summer season. In short, the systems studied seem suitable for implementing a no-tillage system, which aims to neutralize degraded pastures and produce forage for ensilage. Despite the changes in macronutrient concentrations within the intercropping system and residual nitrogen, and among intercropped crops over the years, Congo grass intercropped with soybeans in the summer season showed great capacity for phosphorus, potassium, and magnesium cycling.

Keywords: macronutrients in an intercropping system; *Megathyrsus*; soybean crop system; sustainable agricultural systems; *Urochloa*.

Received on March 18, 2022.

Accepted on July 12, 2022.

Introduction

Grasslands are a major part of the global ecosystem, covering 37% of the land area and corresponding to 69% of the world's agricultural area. For a variety of reasons, mainly related to soil erosion and weed invasion, many of these grassland areas are showing signs of degradation (O'Mara, 2012). In recent years, Brazil has sought to neutralize the degradation of the grasslands by intercropping grain-producing plants with grasses cultivated in a no-tillage system. This practice is part of the new agricultural revolution and will allow the incorporation of millions of hectares of degraded pasture into the production process with a zero-deforestation commitment (CGEE, 2016).

The use of no-tillage principles (crop rotation, cover crop use, and permanent soil cover) reverse the impacts of accelerated soil erosion and soil organic matter degradation, as well as increases soil biodiversity in farming systems (Freitas & Landers, 2014). Combinations of crop rotation and forage production in a no-tillage system are not well established yet for degraded pasture environments, which in general have low water retention capacity, low phosphorus content, low organic matter content, and low nitrogen supply to plants (Cordeiro & Echer, 2019).

Soybean [*Glycine max* (L.) Merrill] is one of the most important crops worldwide and its high-quality proteins make it a major component in animal feed, both for milk and beef production (Sobko et al., 2020). Brazil is the second-largest soybean producer in the world after the United States of America, with an area of 33.9 million hectares (May, 2019). Edaphoclimatic conditions directly affect soybean grain yield, which

requires a large investment in inputs to achieve high production. Intercropping legumes and grasses for silage have shown improvements in total biomass production, as well as in nutrient balance and use (Zhang et al., 2015). In this sense, intercropping soybeans with grasses for silage could be an alternative to recover degraded pasture lands, as soybeans could be used as a system improver, producing quality silage for lean periods, and not requiring as much investment as in grain production.

Choosing a suitable intercropping system for each case is quite complex, as its success depends on interactions between the species used, management practices adopted, and environmental conditions (Iqbal et al., 2019). Intercropping of maize (*Zea mays*) with grasses in a pasture renovation and straw production for a no-tillage system is a recent practice, which has been widely studied in Brazil (Pereira et al., 2016; Batista, Giacomini, Gerdes, Mattos, & Otsuk, 2019; Rocha et al., 2020; Sapucay et al., 2020). However, intercropping grasses with soybeans is still a challenge due to the small size of soybean plants and low competition capacity (Machado, Cecato, Comunello, Concenço, & Ceccon, 2017). Nonetheless, areas under this practice can yield better quality and nutritional value forage (Gobetti, Neumann, Oliveira, & Oliboni, 2011). Moreover, compared to soybean monoculture, such an intercropping scheme can improve soil fertility through biological nitrogen fixation and increase soil conservation through greater soil coverage (Iqbal et al., 2019).

The literature is scarce regarding the nutritional status of crops under intercropped conditions. Given the impact of nutritional status on plant development and the prevalence of intercropping in tropical and subtropical regions, new knowledge can improve crop management and enlighten the actual effects of this practice on nutrient concentration in plants. Furthermore, most studies on maize intercropped with grasses have focused on nitrogen fertilization effects only on maize (Barzan et al., 2021) and not on the succession crop after grass desiccation, as done here.

Under the hypothesis that soybean-grass intercropping for silage production could be an alternative for no-tillage implementation, our objective was to evaluate the interaction between soybean-grass intercropping and nitrogen rates applied in the autumn-winter season.

Material and methods

Experimental plots were installed on a Red-Yellow Argisol - Ultisol (United States Department of Agriculture [USDA], 2014) located in southeastern Brazil (22°42' S, 47°18' W, and 570-m altitude). According to Köppen's classification, the local climate is Aw type, which stands for a rainy tropical forest with rains in the summer and drought in the winter (Alvares, Stape, Sentelhas, Gonçalves, & Sparovek, 2013). Figure 1 shows the data on temperature, rainfall, and hydric balance data during the experimental period. Before the experiment, the study area had high weed infestation, and soil with high acidity and low base saturation.

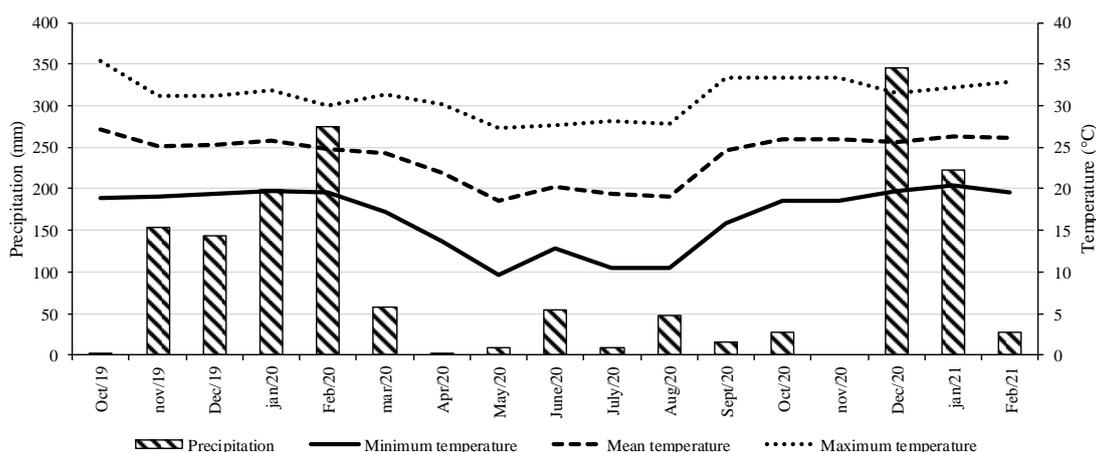


Figure 1. Total monthly rainfall and monthly means temperature from Oct. 31st, 2019 to Feb. 8th, 2021.

During the experimental period, soybean-grass intercropping in the summer and maize-grass intercropping receiving nitrogen rates in the autumn-winter season were studied for no-tillage implementation. The experiment was carried out from September 2019 to September 2021. In this paper, we present the results of the two summer seasons evaluated (2019/2020 and 2020/2021), of which the 2019/2020 season was called the first summer season and the 2020/2021 season was called the second summer season.

The experimental design was randomized blocks with a split-plot scheme and four replications. The main summer plots consisted of the following treatments: 1) soybean monoculture, 2) soybeans intercropped with Aruana Guinea grass (*Megathyrsus maximus* cv. Aruana), and 3) soybeans intercropped with Congo grass (*Urochloa ruziziensis* cv. Comum). The subplots were the nitrogen rates of 0, 50, 100, and 150 kg ha⁻¹, applied manually as side-dressing in maize and grasses rows during the autumn-winter, when maize plants had 5-6 fully expanded leaves.

Before the beginning of the experiment, soil samples were collected at 0-20 cm depth and analysed according to van Raij, Andrade, Cantarella, and Quaggio (2001). Chemical analyses showed the following: pH (CaCl₂) = 4.7; organic matter content (colorimetric method) = 30 g dm⁻³; phosphorus (resin) = 4 mg dm⁻³; potassium (resin) = 1.5 mmol dm⁻³; calcium (resin) = 10.0 mmol dm⁻³; magnesium (resin) = 7.0 mmol dm⁻³; potential acidity (H + Al, SMP buffer solution) = 47 mmol dm⁻³; sulphate (SO₄⁻², turbidimetric) = 9.0 mg dm⁻³; sum of extractable bases = 19.0 mmol dm⁻³; cation exchange capacity = 66.00 mmol_c dm⁻³; and base saturation = 28%. Soil grain size analysis revealed the followings: clay = 239 g kg⁻¹, silt = 91 g kg⁻¹, total sand = 670 g kg⁻¹, coarse sand = 120 g kg⁻¹, and fine sand = 550 g kg⁻¹. According to these results, the soil was classified as medium texture.

The soil was tilled before planting the first soybean crop. It was limed with 2 t ha⁻¹ dolomitic limestone (>12% MgO), and phosphating was performed using 400 kg ha⁻¹ simple superphosphate. The limestone was distributed along crop rows and incorporated into the soil using a disk plough. The simple superphosphate was applied along rows 30 days after liming and incorporated by a disk plough.

A sowing-fertiliser machine for a no-tillage system, with a separate box for differential distribution of large and small seeds, was used to plant soybeans and grasses in the same operation. The soybean cultivar used was M6410IPRO and, at the planting time, seeds were inoculated with *Bradyrhizobium japonicum*. At the planting time, only soybean rows were fertilized with 17 kg ha⁻¹ N, 59 kg ha⁻¹ P₂O₅, and 34 kg ha⁻¹ K₂O. Soybean rows were spaced 0.45 m apart. In the soybean-grass intercropping, soybean and grass rows were spaced 0.225 m apart. The plants were evaluated at soybean flowering and harvesting for ensiling. Soybean plants and grass plants were cut for ensiling at the beginning of soybean maturity when pods had mature colour on the main stem (R7 stage).

Soybean plant and first-pod heights from the soil surface were measured using a graduated ruler. The number of plants per linear meter of each plot was counted for the useful area of the experiment. Dry biomass production of intercropped plants was quantified by collecting plants from a linear meter of each plot, for the useful area of the experiment, excluding the borders. All collected material was weighed and chopped. A subsample of each experimental plot was removed and taken to dry in a forced-air circulation oven at 65°C until constant weight for dry biomass determination. These dried subsamples were also used to measure macronutrients and bromatological characteristics, which are not described herein. Concentrations of nitrogen, phosphorus, potassium, calcium, magnesium, and sulphur were determined according to the method described by Malavolta, Vitti, and Oliveira (1997).

Data from the first summer season were subjected to analysis of variance (ANOVA) using the SAS software (SAS Institute Inc., 2009), and the means were compared by Tukey's test at 5% probability. Data from the second summer season were subjected to analysis of variance by the SASTM GLM procedure at a 5% significance level. Main effects and interactions were studied. Significant interactions were broken down according to the factors involved. Means of each cropping system within each nitrogen rate were compared by Tukey's test, and the effect of nitrogen rates within each cropping system was verified by regression analysis. For the main effects in isolation, means were compared by Tukey's test, and for the effects of nitrogen rates, regression analyses were performed.

Results and discussion

Soybean agronomic traits

Plant height, first-pod height, and plant number per meter showed no significant differences between both systems in the first summer season (Table 1). Although these soybean traits are predominantly controlled by genetic factors (cultivar), they could have been altered in an intercropping system when compared to soybean monoculture (Crusciol et al., 2014). As in our study, Franchini, Balbinot Junior, Debiassi, and Procópio (2014) observed that intercropping of Congo grass with soybeans had no effect on soybean plant and first-pod insertion heights and highlighted that competition for water, light, and nutrients between this grass and soybeans was low.

In the second summer season, only soybean plant height was not significant for the interaction between cropping systems and nitrogen rates applied in the autumn-winter season, and isolation for the cropping systems and nitrogen rates applied in autumn-winter cultivation (Table 1). In the second summer season, soybean first-pod height and plant number per meter showed significance for the interaction soybean-Aruana Guinea grass intercropping and nitrogen rates applied in the autumn-winter season (Table 1). The highest first-pod height of soybeans intercropped with Aruana Guinea grass occurred at a nitrogen rate of 88.79 kg ha⁻¹ (36.58 cm) (Figure 2a). While the nitrogen rate of 71.67 kg ha⁻¹ revealed the highest number of plants per meter for soybeans intercropped with Aruana Guinea grass (1.85 plants per meter) (Figure 2b). These responses may be related to shading by Aruana Guinea grass on soybeans.

Table 1. Plant height and first-pod height (in cm), and plant number per meter of soybeans in the first and second summer seasons.

Cropping system	Nitrogen rates (kg ha ⁻¹)				F test for regression			
	0	50	100	150	Means	Linear	Quadratic	
	First season				Second season			
Plants height								
Soybeans in monoculture	77.75 a	62.25 a	55.50 a	74.50 a	52.38 a	61.16 a	ns	ns
Soybeans intercropped with Aruana Guinea grass	78.00 a	62.25 a	56.25 a	62.13 a	64.38 a	57.91 a	ns	ns
Soybeans intercropped with Congo grass	101.75 a	48.88 a	66.00 a	50.00 a	67.63 a	61.47 a	ns	ns
Means		57.79	59.25	62.21	61.46		ns	ns
CV%	10.57°	19.48°						
First-pod height								
Soybeans in monoculture	16.75 a	20.68 a	35.18 a	29.30 a	16.68 a	25.46 a	ns	ns
Soybeans intercropped with Aruana Guinea grass	22.00 a	11.18 a	29.78 a	37.88 a	23.33 a	25.34 a	ns	0.0189
Soybeans intercropped with Congo grass	18.25 a	9.38 a	13.75 a	17.90 a	27.00 a	16.78 a	ns	ns
Means		13.75	26.24	28.36	22.34		ns	ns
CV%	11.52°	27.33°						
Plant number per meter								
Soybeans in monoculture	3.25 a	2.00 a	1.00 a	2.00 a	1.00 a	1.50 a	ns	ns
Soybeans intercropped with Aruana Guinea grass	3.25 a	1.00 a	2.00 a	1.50 a	1.00 a	7.88 a	ns	0.0121
Soybeans intercropped with Congo grass	2.50 a	1.75 a	1.50 a	2.25 a	1.25 a	5.50 a	ns	ns
Means		1.58	1.5	1.92	1.08		ns	ns
CV%	18.23°	16.17°						

Means followed by different capital letters in the columns differ from one another by the F-test ($p < 0.05$). °Coefficient of variation referring to transformed data into \sqrt{x} . ns: not significant ($p > 0.05$).

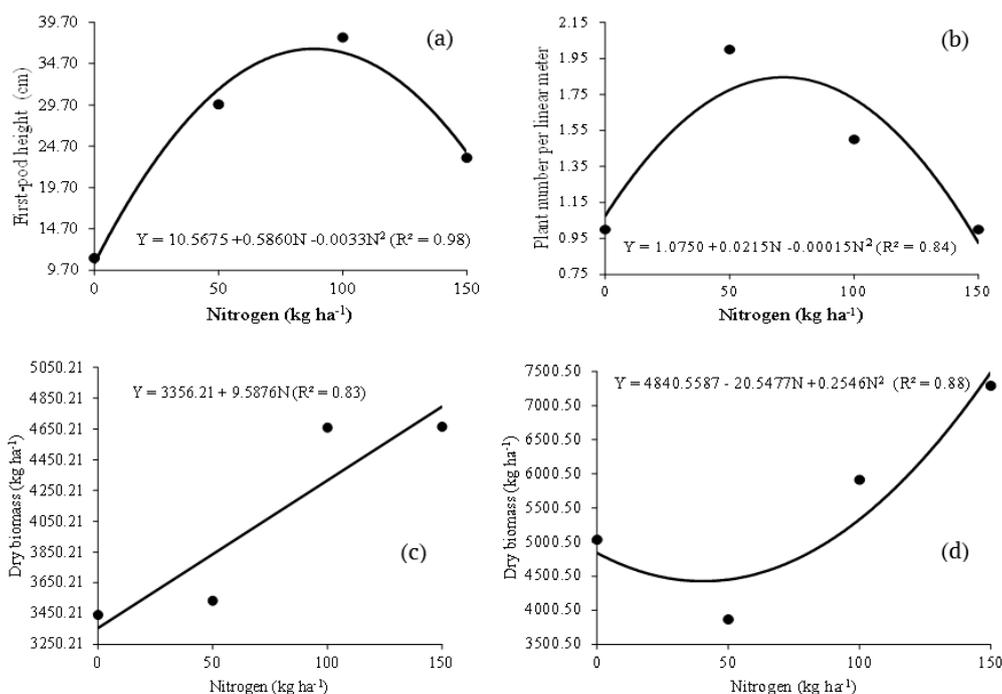


Figure 2. Soybean first-pod height (a), soybean plant number per linear meter (b) in the intercropping of soybeans with Aruana Guinea grass, dry biomass of Congo grass (c) intercropped with soybeans at soybean flowering, and dry biomass of Aruana Guinea grass (d) intercropped with soybeans at harvesting for ensiling in the second summer season.

Dry biomass production of intercropped plants

Soybeans had lower dry biomass production at flowering when intercropped with Congo grass in the first summer season (Table 2). Although the dry biomass production of soybeans intercropped with Aruana Guinea grass did not show significant differences with the soybean monoculture at flowering, the results indicated that soybeans may have benefited from this grass in the first summer season (Table 2). In the first summer season, soybean biomass production at harvesting for ensiling did not show any significant difference between the cropping systems studied (Table 2). However, soybeans had low dry biomass production at flowering and ensiling compared to the results of Bortolon et al. (2018). They reported an average dry biomass production of 6189 kg ha⁻¹ for seventeen evaluated soybean cultivars. Such response may be related to drought stress since it rained only 498.6 mm in the first summer season (Figure 1), and soybeans require about 450–700 mm of water during their growth, consuming about 600–1,000 g of water to produce 1 g of biomass (Xiong et al., 2021; Jha, Kumar, & Ines, 2018). Furthermore, according to Jha et al. (2018), water shortage during soybean growth undermines dry biomass accumulation, leaf area index, and photosynthetic efficiency.

In the second summer season, soybean dry biomass production at flowering and harvesting for ensiling did not show any significant difference in the interaction between cropping systems and nitrogen rates applied in the autumn-winter season (Table 2), as well as for these factors in isolation. In the first and second summer seasons, soybeans had lower dry biomass production when intercropped with grasses, which had high biomass production (Table 2). Therefore, grasses directly interfered with soybean growth due to shading, limiting its development. Furthermore, according to Machado et al. (2017), a microclimate is created when plants intercropped with soybeans have a high biomass production, which prevents moisture dissipation within the canopy, which can negatively interfere with soybean growth.

Table 2. Dry biomass production (kg ha⁻¹) of plants intercropped at soybean flowering and at harvesting for ensiling in the first and second summer seasons.

Cropping system	N rates (kg ha ⁻¹)					F test for regression			
	0	50	100	150	Means	Linear	Quadratic		
Soybean dry biomass production at flowering		First season		Second season					
Soybeans in monoculture	181.60 ab	111.11 a	83.33 a	205.56 a	166.67 a	141.67 a	ns	ns	
Soybeans intercropped with Aruana Guinea grass	322.22 a	61.11 a	55.56 a	127.78 a	44.44 a	72.22 a	ns	ns	
Soybeans intercropped with Congo grass	74.66 b	194.44 a	96.30 a	118.52 a	316.67 a	181.48 a	ns	ns	
Means		122.22	157.41	140.74	175.93		ns	ns	
CV%	22.04*	26.28**							
Soybean dry biomass production at harvesting for ensiling									
Soybeans in monoculture	338.89 a	116.31 a	77.95 a	117.75 a	24.52 a	84.13 a	ns	ns	
Soybeans intercropped with Aruana Guinea grass	368.51 a	40.48 a	46.84 a	53.18 a	74.98 a	53.87 a	ns	ns	
Soybeans intercropped with Congo grass	216.66 a	47.49 a	165.81 a	60.63 a	79.59 a	88.38 a	ns	ns	
Means		68.09	96.87	77.19	59.70		ns	ns	
CV%	14.99*	18.91**							
Grass dry biomass production at flowering									
Soybeans intercropped with Aruana Guinea grass	2382.61 a	4394.43 a	4380.92 a	5433.32 a	4132.13 a	4585.20 a	ns	ns	
Soybeans intercropped with Congo grass	4466.67 a	3441.76 a	3535.10 a	4659.28 a	4664.95 a	4075.27 a	ns	ns	
Means		3482.75	3523.79	4496.71	3926.48		0.0287	ns	
CV%	31.44*	2.13**							
Grass dry biomass production at harvesting for ensiling									
Soybeans intercropped with Aruana Guinea grass	4292.45 a	5034.37 a	3868.26 a	5913.28 a	7293.23 a	5527.28 a	ns	0.0011	
Soybeans intercropped with Congo grass	5699.96 a	4401.15 a	5847.59 a	5396.64 a	5113.07 a	5189.61 a	ns	ns	
Means		4193.56	4323.71	5037.74	5530.58		ns	ns	
CV%	16.66*	2.60**							

Means followed by different capital letters in the columns differ from one another by the F-test ($p < 0.05$). *Coefficient of variation referring to transformed data into \sqrt{x} and **logX. ns: not significant ($p > 0.05$).

Dry biomass production by grasses intercropped with soybeans did not show significant differences in soybean flowering and harvesting for ensilage in the first summer season (Table 2). In absolute values, production differences between these two forages were 2084.06 kg ha⁻¹ at soybean flowering and 1407.51 kg ha⁻¹ at harvesting for ensiling. These results may be related to differences in nutrient demands between these grasses. Congo grass is less demanding on nutrients compared to Aruana Guinea grass and may have adapted better to water-deficit conditions imposed in the first summer season.

Grass dry biomass production at soybean flowering in the second summer season was significant for the interaction between Congo grass intercropped with soybean and nitrogen rates applied in the autumn-winter season (Table 2). At this time, Congo grass dry biomass production increased as nitrogen rates were raised (Figure 2c). Thus, adequate nitrogen management in intercropped systems can increase soil cover. Franchini et al. (2014) observed high dry biomass production for Congo grass intercropped with soybeans (usually above 3 kg ha^{-1}), without significantly influencing soybean yields; these authors also emphasized the high biomass production potential of this grass when used as a cover crop. In the second summer season, dry biomass production of grasses intercropped with soybeans at harvesting for ensiling showed significance for the interaction between Aruana Guinea grass intercropped with soybeans and nitrogen rates applied in the autumn-winter season (Table 2). Aruana Guinea grass intercropped with soybeans showed lower dry biomass production ($4495.98 \text{ kg ha}^{-1}$) at the nitrogen rate of 40.35 kg ha^{-1} (Figure 2d), revealing its high nitrogen requirement when intercropped with soybeans. Therefore, the amount of nutrients to be supplied must be synchronized with a period of greater demand for plants in intercropped systems. It is noteworthy that although dry biomass production of Aruana Guinea grass intercropped with soybeans changes because of nitrogen supply, Machado et al. (2017) revealed that Aruana Guinea grass has a disadvantage in competition with soybeans because it has thin stalks and is susceptible to lodging. Changes in the behaviour of grasses from the first to second summer season may be related to production system improvements due to practices adopted, as Aruana Guinea grass is known to be more demanding on nutrients than Congo grass.

Nutrient concentrations in shoots of intercropped plants

In the first summer season, shoot macronutrient concentrations of soybeans at harvesting for ensiling did not show significant differences between the cropping systems (Table 3). Yet, in the second summer season, these concentrations showed significance for the interaction between cropping systems and nitrogen rates applied in the autumn-winter season (Table 3). The changes in shoot nutrient concentrations of soybeans as a function of the interaction between cropping systems and nitrogen rates applied in the autumn-winter season highlight the scale of intraspecific competition for nutrient uptake (Engbersen, Brooker, Stefan, Studer, & Schöb, 2021). In the second summer season, the nitrogen rate of 28.10 kg ha^{-1} promoted the highest shoot nitrogen concentration (36.90 g kg^{-1}) of soybean monoculture at harvesting for ensiling (Figure 3a). Such response may be related to the absence of the initial inoculum of nitrogen-fixing bacteria and high nitrogen demands for the mineralisation of plant residues (Cordeiro & Echer, 2019). On the other hand, the highest shoot nitrogen concentration (40.77 g kg^{-1}) of soybeans intercropped with Aruana Guinea grass was observed at the nitrogen rate of 65.95 kg ha^{-1} (Figure 3b). According to Chen et al. (2020) in an intercropping system, the crops with stronger root activities have greater nitrogen absorption capacity. Thus, the competitive capacity of intercropped plants to absorb nitrogen from the soil may have altered the response to nitrogen supply in soybeans intercropped with Aruana Guinea grass.

In the second summer season, shoot phosphorus and potassium concentrations decreased for soybean monoculture at harvesting for ensiling as the nitrogen rates applied in the autumn-winter season were raised (Figure 3c and d). For soybeans intercropped with Congo grass, the lowest shoot potassium concentration (18.17 g kg^{-1}) occurred at the nitrogen rate of 73.35 kg ha^{-1} (Figure 3e). Regarding phosphorus, the increasing nitrogen rates reduced biological nitrogen fixation and rhizosphere changes, leading to reductions in phosphorus concentrations in plant shoots (Tian et al., 2020). In a soybean-corn succession, potassium cycling is of paramount importance and must be understood (Rosolem, Mallarino, & Nogueira, 2021) since this nutrient helps both crops in the synthesis of carbohydrates, regulates the opening and closing of stomata, and improves root growth, all of which is required for efficient water use (Singh et al., 2021).

The nitrogen rate of 20 kg ha^{-1} was responsible for the lowest shoot calcium concentration (7.52 g kg^{-1}) of soybean monoculture (Figure 3f). For soybeans intercropped with Aruana Guinea grass, the lowest calcium concentration (7.28 g kg^{-1}) was observed at the nitrogen rate of 90.67 kg ha^{-1} (Figure 4a). While in soybeans intercropped with Congo grass, the highest shoot calcium concentration (8.43 g kg^{-1}) occurred at the nitrogen rate of 73.33 kg ha^{-1} (Figure 4b). Therefore, nitrogen supply should be planned in the autumn-winter season, as the lowest calcium concentration can promote apical meristem necrosis and loss of apical dominance in soybeans (Huber, Römheld, & Weinmann, 2012).

The shoot magnesium concentration of soybean monoculture (Figure 4c) and intercropped with Aruana Guinea grass (Figure 4d) decreased as the nitrogen rates applied in the autumn-winter season increased. Peng

et al. (2020) concluded that, apart from its involvement in metabolic activities as a structural element, magnesium also plays an important role in maintaining nitrogen homeostasis, and hence plant growth. Thus, the lowest shoot magnesium concentration in soybeans receiving the highest nitrogen rate may indicate that nitrogen accelerated the growth of soybeans, with their magnesium content being insufficient to answer that increased demand.

Table 3. Shoot macronutrient concentrations (g kg^{-1}) in soybeans harvested for ensiling in the first and second summer seasons.

Cropping system	N rates (kg ha^{-1})					F test for regression		
	0	50	100	150	Means	Linear	Quadratic	
	First season	Second season						
Nitrogen								
Soybeans in monoculture	28.00 a	36.24 a	37.20 ab	33.84 a	29.52 a	34.20 a	ns	0.0301
Soybeans intercropped with Aruana	29.75 a				34.08 a	37.55 a	ns	0.0112
Guinea grass		36.00 a	41.76 a	38.34 a				
Soybeans intercropped with Congo grass	28.70 a	37.08 a	28.80 b	37.44 a	33.84 a	34.29 a	ns	ns
Means		36.44	35.92	36.54 a	32.48		ns	ns
CV%	4.28	9.44						
Phosphorus								
Soybeans in monoculture	2.64 a	3.40 a	3.77 a	3.10 a	2.70 a	3.24 a	0.0036	ns
Soybeans intercropped with Aruana	2.71 a					3.33 a	ns	ns
Guinea grass		3.10 a	3.40 a	3.30 a	3.53 a			
Soybeans intercropped with Congo grass	2.73 a	3.13 a	3.07 a	3.46 a	3.23 a	3.22 a	ns	ns
Means		3.21	3.41	3.29	3.16		ns	ns
CV%	3.84	10.37						
Potassium								
Soybeans in monoculture	22.65 a	22.73 a	19.36 a	18.03 a	15.70 b	18.96 a	0.0010	ns
Soybeans intercropped with Aruana	23.98 a					20.43 a	ns	ns
Guinea grass		21.20 a	21.25 a	20.15 a	19.13 ab			
Soybeans intercropped with Congo grass	23.05 a	22.17 a	19.57 a	18.05 a	23.67 a	20.86 a	ns	0.0002
Means		22.03	20.06	18.74	19.50			
CV%	10.24	11.90						
Calcium								
Soybeans in monoculture	7.13 a	7.93 b	7.60 a	8.33 a	10.90 a	8.69 a	ns	0.0052
Soybeans intercropped with Aruana	7.50 a					8.27 ab	ns	0.0001
Guinea grass		9.80 a	7.60 a	7.45 a	8.23 b			
Soybeans intercropped with Congo grass	7.43 a	6.77 b	8.43 a	8.10 a	6.80 c	7.53 b	ns	0.0085
Means		8.17	7.88	7.96	8.64		ns	ns
CV%	6.65 ^e	10.32						
Magnesium								
Soybeans in monoculture	5.05 a	6.15 a	5.24 b	5.82 a	4.71 b	5.48 b	0.0241	ns
Soybeans intercropped with Aruana	5.09 a					6.24 a	0.0412	ns
Guinea grass		6.73 a	6.43 a	5.91 a	5.89 a			
Soybeans intercropped with Congo grass	5.51 a	5.93 a	5.60 ab	5.82 a	5.81 a	5.79 ab	ns	ns
Means		6.27	5.76	5.85	5.47		ns	ns
CV%	9.32	8.15						
Sulfur								
Soybeans in monoculture	1.85 a	2.13 a	2.30 a	1.63 b	1.30 b	1.84 a	0.0003	ns
Soybeans intercropped with Aruana	2.15 a					1.91 a	ns	0.0009
Guinea grass		1.80 b	2.10 a	1.95 a	1.80 a			
Soybeans intercropped with Congo grass	1.90 a	1.83 b	1.80 a	1.90 ab	2.03 a	1.89 a	ns	ns
Means		1.92	2.07	1.83	1.71		ns	ns
CV%	8.48	9.18						

Means followed by different capital letters in the columns differ from one another by the F-test ($p < 0.05$). ^eCoefficient of variation referring to transformed data into \sqrt{x} . ns: not significant ($p > 0.05$).

Shoot sulphur concentration of soybean monoculture decreased as the nitrogen rates applied in the autumn-winter season increased (Figure 4e). While the nitrogen rate of 64.00 kg ha^{-1} promoted the highest shoot sulphur concentration of soybeans intercropped with Aruana Guinea grass (2.03 g kg^{-1}) (Figure 4f). These results may indicate that increasing nitrogen availability promoted changes in nitrogen and sulphur balance in plants and probably decreased shoot sulphur concentrations in soybeans. Thus, excessive nitrogen supply in the autumn-winter season should be avoided so that sulfur deficiency does not occur, as this can increase non-protein nitrogen concentration, decreasing protein nitrogen contents (Sexton, Paek, & Shibles, 1998).

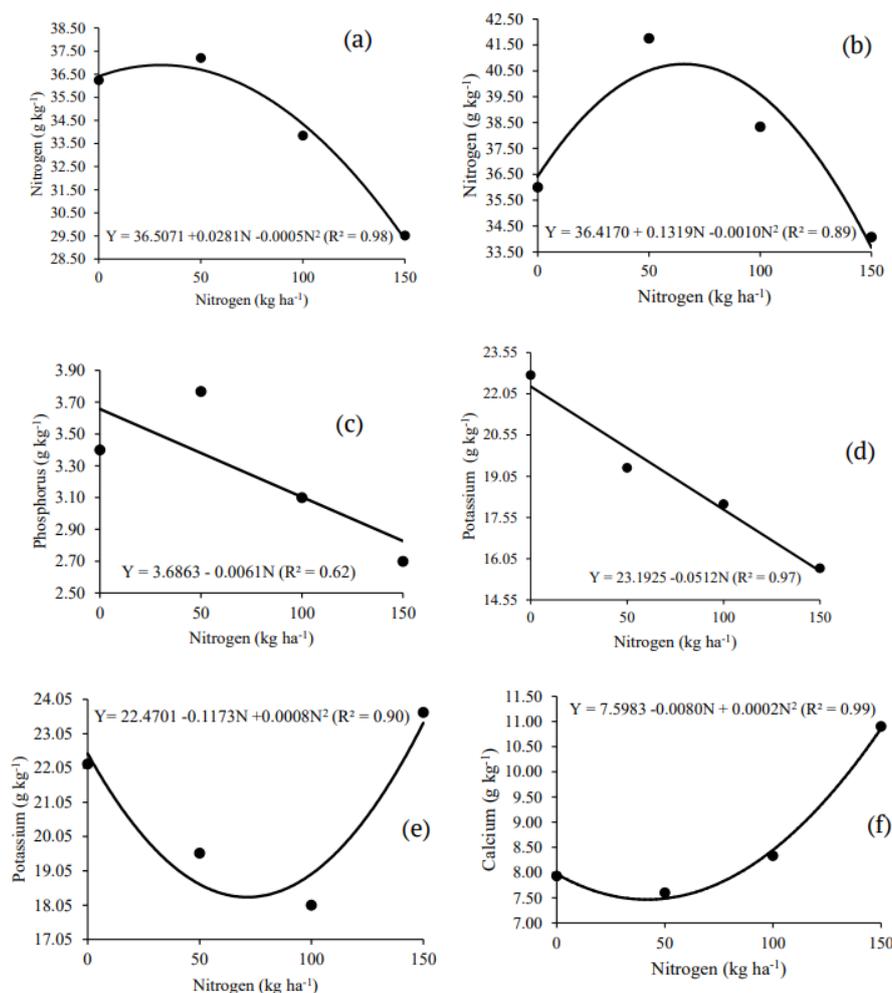


Figure 3. Shoot nitrogen concentrations for soybeans in monoculture (a) and intercropped with Aruana Guinea grass (b), shoot phosphorus (c) and potassium (d) concentrations in soybeans in monoculture, shoot potassium concentrations in soybeans intercropped with Congo grass (e), and shoot calcium concentrations in soybeans in monoculture (f) harvested for ensiling in the second summer season.

Among the macronutrients in grass shoots harvested for ensiling in the first summer season, only potassium and magnesium had significant differences between the studied cropping systems. Congo grass intercropped with soybeans had the highest concentrations of potassium and magnesium compared to Aruana Guinea grass (Table 4). Based on nutrient demand by intercropped and/or succession crops, the intercropping system will be advantageous depending mainly on its contribution to nutrient absorption, use, and interaction factors (Fan et al., 2020). In this context, Congo grass, when intercropped with soybeans, would be a key source of potassium for maize in succession, as it is readily available for absorption after the mineralisation of crop residues.

In the second summer season, shoot nitrogen, phosphorus, potassium, and magnesium concentrations of grasses were not significant for the interaction between cropping systems and nitrogen rates applied in the autumn-winter season (Table 4). In isolation, nitrogen concentration also did not show significant responses for the tested cropping systems nor nitrogen rates applied in the autumn-winter season (Table 4). While shoot phosphorus, potassium, and magnesium concentrations were higher in Congo grass than in Aruana Guinea grass intercropped with soybeans (Table 4). These findings may indicate that Congo grass is a phosphorus mobilizing species, and therefore it has a greater capacity to use phosphorus in a system concerning Aruana Guinea grass. According to Engbersen et al. (2021) phosphorus-mobilizing species can increase the availability of inorganic phosphorus in the soil for themselves and neighbouring plants, thus facilitating phosphorus uptake by the intercropped plant.

Calcium and sulphur concentrations showed significance for the interaction between cropping systems and nitrogen rates applied in the autumn-winter season (Table 4). The highest shoot calcium concentration (5.29 g kg^{-1}) of Congo grass intercropped with soybeans was observed at the nitrogen rate of 94.75 kg ha^{-1}

(Figure 4g). On the other hand, shoot sulphur concentrations of Aruana Guinea grass intercropped with soybeans reduced as nitrogen rates applied in the autumn-winter season increased (Figure 4h). Batista and Monteiro (2007) emphasized that sulphur supplies should be carefully planned when nitrogen is supplied to grasses, as they observed that nitrogen concentration was closely related to nitrogen supply, while sulphur concentration and N: S ratio depended on nitrogen and sulphur supplies to *Urochloa brizantha* cv. Marandu.

In the first soybean crop, grass shoot macronutrient concentrations before harvesting for ensiling varied in the following order $K > N > Mg > Ca > S > P$, regardless of the grass intercropped. In the second soybean crop, grasses differed from each other, and Aruana Guinea varied in the following order $N > K > Mg > Ca > P > S$, whereas for Congo it was $K > N > Mg > Ca > P > S$. Thus, intercropping soybeans with Aruana Guinea grass may have increased nitrogen uptake throughout the system, while Congo grass promoted potassium uptake (Fan et al., 2020). Thus, with the implementation of an intercropping system, macronutrient concentrations change within the system and between intercropped crops over the years.

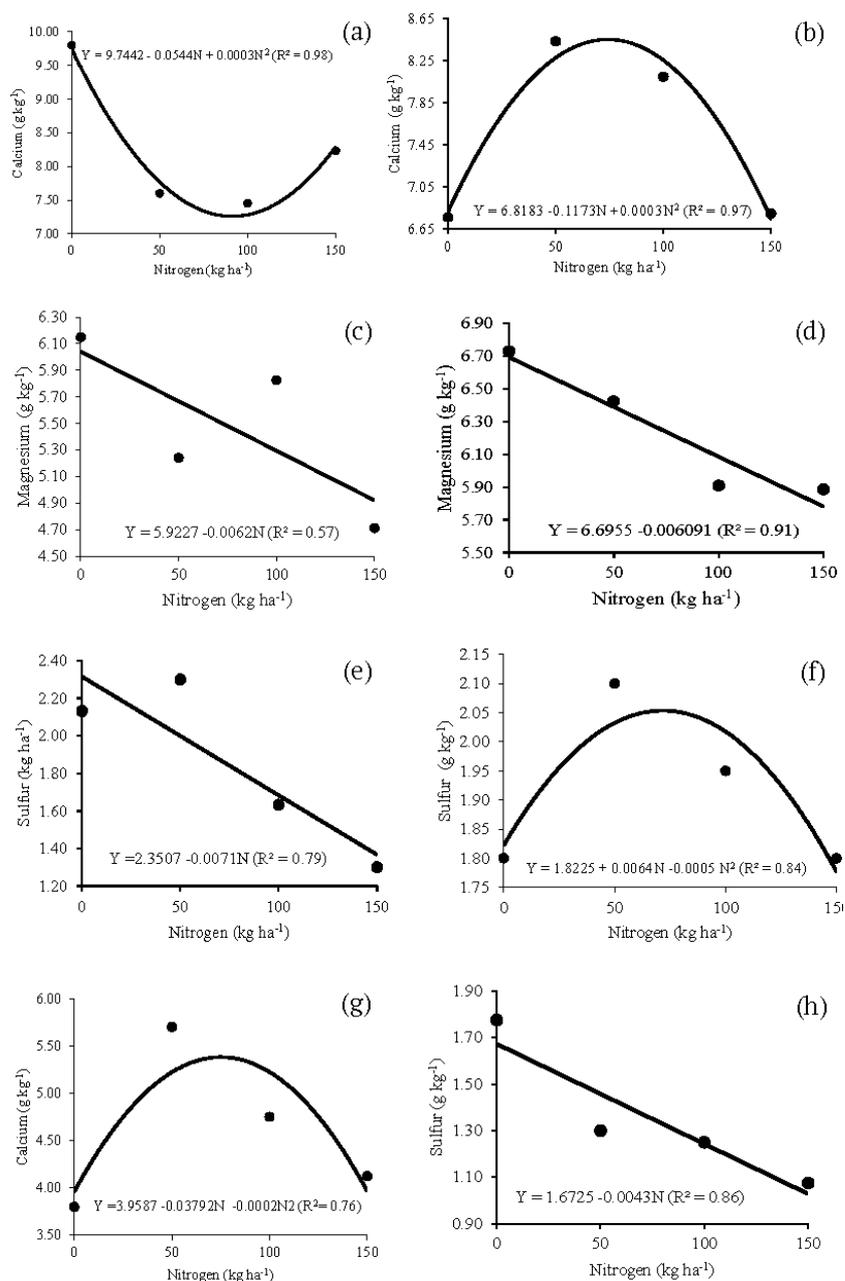


Figure 4. Shoot calcium concentrations of soybeans intercropped with Aruana Guinea grass (a) and with Congo grass (b), shoot magnesium concentrations of soybean monoculture (c) and intercropped with Aruana Guinea grass (d), shoot sulphur concentrations of soybean monoculture (e) and intercropped with Aruana Guinea grass (f), shoot calcium concentrations of Congo grass intercropped with soybeans (g), and shoot sulphur concentrations of Aruana Guinea grass intercropped with soybeans (h) at harvesting for ensiling in the second summer season.

Table 4. Shoot macronutrient concentrations (g kg⁻¹) in grasses harvested for ensiling in the first and second summer seasons.

Cropping system	N rates (kg ha ⁻¹)					F test for regression		
		0	50	100	150	Means	Linear	Quadratic
	First crop	Second crop						
Nitrogen								
Soybeans intercropped with Aruana Guinea grass	16.80 a	17.46 a	14.58 a	16.56 a	18.00 a	16.65 a	ns	ns
Soybeans intercropped with Congo grass	17.50 a	16.38 a	18.00 a	15.30 a	18.54 a	17.06 a	ns	ns
Means		16.92	16.29	15.93	18.27		ns	ns
CV%	9.72	9.44						
Phosphorus								
Soybeans intercropped with Aruana Guinea grass	1.38 a	1.60 a	1.35 b	1.53 a	1.48 a	1.49 b	ns	ns
Soybeans intercropped with Congo grass	1.78 a	1.75 a	2.10 a	1.88 a	1.83 a	1.89 a	ns	ns
Means		1.68	1.73	1.70	1.65		ns	ns
CV%	9.44	10.37						
Potassium								
Soybeans intercropped with Aruana Guinea grass	17.05 b	16.55 a	16.20 a	15.73 a	14.68 a	15.79 b	ns	ns
Soybeans intercropped with Congo grass	21.98 a	21.275 a	19.45 b	17.23 a	20.45 a	19.60 a	ns	ns
Means		18.91	17.83	16.48	17.56		ns	ns
CV%	8.30	11.90						
Calcium								
Soybeans intercropped with Aruana Guinea grass	3.28 a	4.30 a	4.05 b	3.88 a	3.90 a	4.03 a	ns	ns
Soybeans intercropped with Congo grass	3.80 a	3.80 a	5.70 a	4.75 a	4.13 a	4.59 a	ns	0.0009
Means		4.05	4.88	4.31	4.01		ns	ns
CV%	11.81	10.32						
Magnesium								
Soybeans intercropped with Aruana Guinea grass	4.65 b	5.60 a	5.10 b	4.75 a	5.15 a	5.15 b	ns	ns
Soybeans intercropped with Congo grass	5.59 a	5.39 a	6.42 a	5.95 a	5.56 a	5.83 a	ns	ns
Means		5.49	5.76	5.35	5.36		ns	ns
CV%	6.41	8.15						
Sulfur								
Soybean + Aruana Guinea	1.63 a	1.78 a	1.30 a	1.25 a	1.08 a	1.35 a	0.0099	ns
Soybeans intercropped with Congo grass	2.13 a	1.40 a	2.10 a	1.60 b	1.45 a	1.64 a	ns	ns
Means		1.59	1.70	1.43	1.26		ns	ns
CV%	7.92*	9.18						

Means followed by different capital letters in the columns differ from one another by the F-test ($p < 0.05$). *Coefficient of variation referring to transformed data into \sqrt{x} . ns: not significant ($p > 0.05$).

Conclusion

The study of potential interactions between soybean-grass intercropping and residual nitrogen in summer seasons showed that soybean intercropping with Aruana Guinea grass and with Congo grass can be an alternative for a no-tillage system. Moreover, residual nitrogen from the autumn-winter season directly interfered with the dry biomass production of grasses intercropped with soybeans in the summer season. Thus, the intercropping systems studied seem to be suitable for use in no-tillage system implementation, aiming to neutralize degraded pastures and produce forage for silage. Despite the changes in macronutrient concentrations in the systems (crop intercropping with residual nitrogen), and among crops intercropping over the years, Aruana Guinea grass intercropped with soybeans in the summer season enhanced absorption of calcium and magnesium by soybeans, while Congo grass intercropped with soybeans in the summer season showed the largest capacity for phosphorus, potassium, and magnesium cycling.

Acknowledgements

The authors thank to São Paulo Research Foundation (FAPESP) for financial support (process 2017/50339-5 and process 2019/02387-6).

References

- Alvares, C. A., Stape, J. L., Sentelhas, P. C., Gonçalves, J. L. M., & Sparovek, G. (2013). Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, 22(6), 11-728.
DOI: <https://doi.org/10.1127/0941-2948/2013/0507>

- Barzan, R. R., Jordão, L. T., Firmano, R. F., Secato, T. R., Lima, F., Barzan, L. R., ... Zucareli, C. (2021). Soil chemical attributes and nutritional status of soybean and maize intercropped with *Urochloa* under nitrogen rates. *Agronomy Journal*, *113*(4), 1-11. DOI: <https://doi.org/10.1002/agj2.20744>
- Batista, K., & Monteiro, F. A. (2007). Nitrogen and sulphur in marandu grass: relationship between supply and concentration in leaf tissues. *Scientia Agricola*, *64*(1), 44-51. DOI: <https://doi.org/10.1590/S0103-90162007000100007>
- Batista, K., Giacomini, A. A., Gerdes, L., Mattos, W. T., & Otsuk, I. P. (2019). Nitrogen fertilisation improves the grain production efficiency and sustainability of out-of-crop corn and Congo grass intercropping. *Soil Research*, *57*(4), 397-407. DOI: <https://doi.org/10.1071/SR19002>
- Bortolon, L., Bortolon, E. S. O., Camargo, F. P., Seraglio, N. A., Lima, A. O., Rocha, P., ... Gianello, C. (2018). Yield and nutrient uptake of soybean cultivars under intensive cropping systems. *Journal of Agricultural Science*, *10*(12), 344-357. DOI: <https://doi.org/10.5539/jas.v10n12p344>
- Centro de Gestão e Estudos Estratégicos [CGEE]. (2016). *Land degradation neutrality: implications for Brazil*. Brasília, DF: CGEE.
- Chen, N., Li, X., Šimůnek, J., Shi, H., Hu, Q., & Zhang, Y. (2020). Evaluating soil nitrate dynamics in an intercropping dripped ecosystem using HYDRUS-2D. *Science of The Total Environment*, *718*, 1-13. DOI: <https://doi.org/10.1016/j.scitotenv.2020.137314>
- Cordeiro, C. F. D., & Echer, F. R. (2019). Interactive effects of nitrogen-fixing bacteria inoculation and nitrogen fertilization on soybean yield in unfavorable edaphoclimatic environments. *Scientific Reports*, *9*(1), 1-11. DOI: <https://doi.org/10.1038/s41598-019-52131-7>
- Crusciol, C. A. C., Nascente, A. S., Mateus, G. P., Pariz, C. M., Martins, P. O., & Borghi, E. (2014). Intercropping soybean and palisade grass for enhanced land use efficiency and revenue in a no till system. *European Journal of Agronomy*, *58*, 53-62. DOI: <https://doi.org/10.1016/j.eja.2014.05.001>
- Engbersen, N., Brooker, R. W., Stefan, L., Studer, B., & Schöb, C. (2021). Temporal differentiation of resource capture and biomass accumulation as a driver of yield increase in intercropping. *Frontiers in Plant Science*, *12*, 1-11. DOI: <https://doi.org/10.3389/fpls.2021.668803>
- Fan, Y., Wang, Z., Liao, D., Raza, M. A., Wang, B., Zhang, J., ... Yang, F. (2020). Uptake and utilization of nitrogen, phosphorus and potassium as related to yield advantage in maize-soybean intercropping under different row configurations. *Scientific Reports*, *10*(1), 1-10. DOI: <https://doi.org/10.1038/s41598-020-66459-y>
- Franchini, J. C., Balbinot Junior, A. A., Debiasi, H., & Procópio, S. O. (2014). Intercropping of soybean cultivars with *Urochloa*. *Pesquisa Agropecuária Tropical*, *44*(2), 119-126. DOI: <https://doi.org/10.1590/S1983-40632014000200007>
- Freitas, P. L., & Landers, J. N. (2014). The transformation of agriculture in Brazil through development and adoption of zero tillage conservation agriculture. *International Soil and Water Conservation Research*, *2*(1), 35-46. DOI: [https://doi.org/10.1016/S2095-6339\(15\)30012-5](https://doi.org/10.1016/S2095-6339(15)30012-5)
- Gobetti, S. T. C., Neumann, M., Oliveira, M. R., & Oliboni, R. (2011). Produção e utilização da silagem de planta inteira de soja (*Glycine max*) para ruminantes. *Ambiência*, *7*(3), 603-616. DOI: <https://doi.org/10.5777/ambiencia.2011.03.02rb>
- Huber, D., Römheld, V., & Weinmann, M. (2012). Relationship between nutrition, plant diseases and pests (3rd ed.). In P. Marschner (Ed.), *Marschner's mineral nutrition of higher plants* (p. 283-298). London, UK: Elsevier.
- Iqbal, N., Hussain, S., Ahmed, Z., Yang, F., Wang, X., Liu, ... Liu, J. (2019). Comparative analysis of maize-soybean strip intercropping systems: a review. *Plant Production Science*, *22*(2), 131-142. DOI: <https://doi.org/10.1080/1343943X.2018.1541137>
- Jha, P. K., Kumar, S. N., & Ines, A. V. (2018). Responses of soybean to water stress and supplemental irrigation in upper Indo-Gangetic plain: Field experiment and modeling approach. *Field Crops Research*, *219*, 76-86. DOI: <https://doi.org/10.1016/j.fcr.2018.01.029>
- Machado, L. A. Z., Cecato, U., Comunello, E., Concencço, G., & Ceccon, G. (2017). Establishment of perennial forages intercropped with soybean for integrated crop-livestock systems. *Pesquisa Agropecuária Brasileira*, *52*(7), 521-529. DOI: <https://doi.org/10.1590/S0100-04X2017000700006>
- Malavolta, E., Vitti, G. C., & Oliveira, S. A. (1997). *Avaliação do estado nutricional das plantas: princípios e aplicações* (2. ed.). Piracicaba, SP: Potafos.

- May, P. H. (2019). *Valuing externalities of cattle and soy-maize systems in the Brazilian Amazon; Application of the TEEBAgriFood Evaluation Framework*. Rio de Janeiro, RJ: TEEB for Agriculture and Food; UNEP.
- O'Mara, F. P. (2012). The role of grasslands in food security and climate change. *Annals of Botany*, *110*(6), 1263-1270. DOI: <https://doi.org/10.1093/aob/mcs209>
- Peng, W. T., Qi, W. L., Nie, M. M., Xiao, Y. B., Liao, H., & Chen, Z. C. (2020). Magnesium supports nitrogen uptake through regulating NRT2. 1/2.2 in soybean. *Plant and Soil*, *457*(1), 97-111. DOI: <https://doi.org/10.1007/s11104-019-04157-z>
- Pereira, F. C. B. L., Mello, L. M. M., Pariz, C. M., Mendonça, V. Z., Yano, E. H., Miranda, E. E. V., & Crusciol, C. A. C. (2016). Autumn maize intercropped with tropical forages: crop residues, nutrient cycling, subsequent Soybean and soil quality. *Revista Brasileira de Ciência do Solo*, *40*, 1-20. DOI: <https://doi.org/10.1590/18069657rbcS20150003>
- Rocha, K. F., Souza, M. de, Almeida, D. S., Chadwick, D. R., Jones, D. L., Mooney, S. J., & Rosolem, C. A. (2020). Cover crops affect the partial nitrogen balance in a maize-forage cropping system. *Geoderma*, *360*, 1-7. DOI: <https://doi.org/10.1016/j.geoderma.2019.114000>
- Rosolem, C. A., Mallarino, A. P., & Nogueira, T. A. R. (2021). Considerations for unharvested plant potassium. In T. S. Murrell, R. L. Mikkelsen, G. Sulewski, R. Norton, & M. L. Thompson (Eds.), *Improving potassium recommendations for agricultural crops* (p. 147-162). Cham, SW: Springer Nature. DOI: <https://doi.org/10.1007/978-3-030-59197-7>
- Sapucay, M. J. L. C., Coelho, A. E., Bratti, F., Locatelli, J. L., Alvadi, L. S., Balbinot Junior, A., & Zucareli, C. (2020). Nitrogen rates on the agronomic performance of second-crop corn single and intercropped with ruzigrass or showy rattlebox. *Pesquisa Agropecuária Tropical*, *50*, 1-10. DOI: <https://doi.org/10.1590/1983-40632020v5065525>
- SAS Institute Inc. (2009). *SAS/STAT® 9.2 User's guide*. Cary, NC: SAS Institute Inc.
- Sexton, P. J., Paek, N. C., & Shibles, R. (1998). Soybean sulfur and nitrogen balance under varying levels of available sulfur. *Crop Science*, *38*(4), 975-982. DOI: <https://doi.org/10.2135/cropsci1998.0011183X003800040016x>
- Singh, V. K., Dwivedi, B. S., Rathore, S. S., Mishra, R. P., Satyanarayana, T., & Majumdar, K. (2021). Timing potassium applications to synchronize with plant demand. In T. S. Murrell, R. L. Mikkelsen, G. Sulewski, R. Norton, & M. L. Thompson (Eds.), *Improving potassium recommendations for agricultural crops* (p. 363-384). Cham, SW: Springer Nature. DOI: https://doi.org/10.1007/978-3-030-59197-7_13
- Sobko, O., Stahl, A., Hahn, V., Zikeli, S., Claupein, W., & Gruber, S. (2020). Environmental effects on soybean (*Glycine max* (L.) Merr.) production in Central and South Germany. *Agronomy*, *10*(12), 1-14. DOI: <https://doi.org/10.3390/agronomy10121847>
- Tian, J., Tang, M., Xu, X., Luo, S., Condrón, L. M., Lambers, H., ... Wang, J. (2020). Soybean (*Glycine max* (L.) Merrill) intercropping with reduced nitrogen input influences rhizosphere phosphorus dynamics and phosphorus acquisition of sugarcane (*Saccharum officinarum*). *Biology and Fertility of Soils*, *56*, 1063-1075. DOI: <https://doi.org/10.1007/s00374-020-01484-7>
- United States Department of Agriculture [USDA]. (2014). *Keys to soil taxonomy* (12th ed.). Washington, DC: USDA.
- van Raij, B., Andrade, J. C., Cantarella, H., & Quaggio, J. A. (2001). *Análise química para avaliação da fertilidade de solos tropicais*. Campinas, SP: IAC.
- Xiong, R., Liu, S., Considine, M. J., Siddique, K. H. M., Lam, H.-M., & Chen, Y. (2020). Root system architecture, physiological and transcriptional traits of soybean (*Glycine max* L.) in response to water deficit: A review. *Physiologia Plantarum*, *172*(2), 405-418, DOI: <https://doi.org/10.1111/ppl.13201>
- Zhang, J., Yin, B., Xie, Y., Li J., Yang, Z., & Zhang, G. (2015). Legume-cereal intercropping improves forage yield, quality and degradability. *PLoS ONE*, *10*(12), 1-14. DOI: <https://doi.org/10.1371/journal.pone.0144813>