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Biomass and protein in ruzigrass intercropped with maize subjected to plant densities and nitrogen fertilization

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Abstract – The objective of this work was to evaluate the impacts of nitrogen topdressing fertilization and plant density of second-crop maize on the biomass and crude protein production of ruzigrass (*Urochloa ruziziensis*) grown in intercropping. The experiment was carried out during two growing seasons in a randomized complete block design, in split plots, with four replicates. The treatments consisted of N topdressing rates (0 and 80 kg ha^{-1}) and maize plant densities (40, 60, 80, and 100 thousand plants per hectare). Ruzigrass biomass accumulation was measured at the V14, R1, R3, and R6 stages of maize growth, as well as during ruzigrass desiccation. Ruzigrass crude protein content and production and biomass partitioning to leaves, stems, and senescent tissues were evaluated in the R6 stage of maize. The increase in maize plant density reduced ruzigrass growth. However, nitrogen fertilization and maize plant density did not affect ruzigrass biomass partitioning. During intercropping, N fertilization did not affect ruzigrass yield. After maize harvest, N fertilization resulted in a higher ruzigrass biomass (30.2% in 2019) and crude protein (13.8%) production. Low maize plant densities and N topdress fertilization improve the biomass production of ruzigrass in intercropping.

Index terms: *Urochloa ruziziensis*, *Zea mays*, cover crop, herbage biomass, second-crop maize.

Biomassa e proteína em braquiária consorciada com milho submetido a densidades de plantas e fertilização nitrogenada

Resumo – O objetivo deste trabalho foi avaliar os impactos da adubação nitrogenada de cobertura e da densidade de plantas de milho de segunda safra sobre a produtividade de biomassa e proteína bruta da braquiária (*Urochloa ruziziensis*) cultivada em consórcio. O experimento foi realizado em duas safras, em blocos ao acaso em parcelas subdivididas, com quatro repetições. Os tratamentos consistiram de doses de N em cobertura (0 e 80 kg ha^{-1}) e densidades de plantas de milho (40, 60, 80 e 100 mil plantas por hectare). O acúmulo de biomassa da braquiária foi avaliado nos estádios V14, R1, R3 e R6 do milho, bem como na dessecação da braquiária Avaliaram-se o teor e a produção de proteína bruta e a partição de biomassa em folhas, colmos e tecidos senescentes da braquiária no estágio R6 do milho. O aumento da densidade de plantas de milho reduziu o crescimento da braquiária. No entanto, a adubação nitrogenada e a densidade de plantas de milho não afetaram a partição de biomassa da braquiária. Durante o consórcio, a adubação nitrogenada não impactou a produtividade da braquiária. Após a colheita do milho, a adubação nitrogenada proporcionou maior produção de biomassa (30,2% em 2019) e de proteína bruta (13,8%) da braquiária. As baixas densidades de plantas de milho e a adubação nitrogenada melhoram a produção de biomassa de braquiária em consórcio.

Termos para indexação: *Urochloa ruziziensis*, *Zea mays*, cobertura do solo, biomassa de forragem, milho de segunda safra.

Introduction

In the soybean-maize off-season systems, there is a period without grain cultivation, between maize harvest and soybean sowing, that occurs in the winter in Brazil. During this period, the field is usually left fallow, which produces negative consequences for the soil quality and conservation (Franchini et al., 2012), favoring weed infestation (Severino et al., 2006; Balbinot Jr. et al., 2008; Carvalho et al., 2013). The off-season is characterized by the reduction of temperature and water supply that hamper the production of cover and forage crops. These factors result in low forage availability and poor pasture quality, since pasture crude protein contents commonly decrease to less than 6% (Poppi et al., 2018). The nutritional quality of pasture plays an essential role in animal production, and the assessment of crude protein is an important indicator in this context.

The intercropping of second-crop (also known as "safrinha") maize with cover crops in the autumn/winter is a strategy to increase straw production and nutrient cycling in no-till system, thereby enhancing economic returns (Ceccon et al., 2013; Mendonça et al., 2015; Sapucay et al., 2020). Intercropped plants can be used as forage for integration crop-livestock, increasing the profitability of the agricultural system. In Brazil, the usual intercropping is the second-crop maize with *Urochloa* (Syn. *Brachiaria*) species, such as *U. ruziziensis*, known as ruzigrass (Concenço et al., 2012; Baldé et al., 2020). Following the maize harvest, intercropped plants continue to grow, contributing to increase the amount of biomass into the soil and providing benefits such as the erosion prevention and suppression of weed infestation.

The adjustment of maize plant density and nitrogen (N) rates are important to the yield increase of the intercropped cover crop (Sangoi et al., 2019; Coelho et al., 2022). However, a greater understanding of the impact of these cultural practices is needed to maximize the yield and quality of intercropped ruzigrass, since a high density of maize plants reduces the impact of intercropped forage grasses on grain production and decreases the forage production (Youngerman et al., 2018).

Nitrogen fertilization can modify the morphogenic, structural, and biochemical characteristics of forage grasses, altering the tiller density, leaf senescence dynamics, and protein accumulation (Santos et al., 2012, 2019). Although tropical forages have lower nutritional quality than temperate grasses, the N fertilization can improve their quality and biomass production potential, which ultimately translates to an increased animal productivity (Lima et al., 2023). Thus, the reduced production of ruzigrass biomass, caused by high maize plant densities, can be mitigated by increasing the N supply to the system. However, due to the low response of second-crop maize to N fertilization, coupled with its high cost, many farmers do not use N topdressing in Brazil (Fuentes et al., 2018).

The adjustments of maize plant density and N fertilization in intercropping systems are crucial management practices to maximize the maize yield. However, it is also necessary to assess the impact of these practices on the production and quality of forage crops. Understanding these strategies can help technicians and farmers to use maize-ruzigrass intercrop systems in periods of low water supply in tropical climate regions, avoiding the occurrence of forage shortage.

The objective of this work was to evaluate the impacts of nitrogen topdressing fertilization and plant density of second-crop maize on the biomass and crude protein production of ruzigrass grown in intercropping.

Materials and Methods

A field experiment was set in the municipality of Londrina, in the state of Paraná, Brazil (23°11'57"S and 51°10'40"W, at 585 m altitude), during the 2018 and 2019 growing seasons. The soil of the experimental site is classified as Latossolo Vermelho distroférrico (Santos et al., 2018), i.e., an Oxisol, with a very clayey texture, 710 g clay kg^{-1} soil, 82 g silt kg^{-1} soil, and 208 g sand kg^{-1} soil. Soil chemical properties at the depth of 0–20 cm at the beginning of the experiment were: 18.1 g dm^{-3} total organic carbon; 5.1, pH in $CaCl_2$; 3.7 $cmol_c$ dm^{-3} Ca; 1.9 $cmol_c$ dm^{-3} Mg; 0.0 $cmol_c$ dm^{-3}

Al; 0.39 $cmol_c$ dm^{-3} K; 28.8 mg dm^{-3} P (Mehlich-1); 11.1 $cmol_c$ dm^{-3} cation-exchange capacity (T); and 53.7% base saturation. The climate is Cfa, described as humid subtropical mesothermal with hot summers and infrequent frosts, according to the Köppen-Geiger's classification. The cumulative rainfall and monthly average temperature data were recorded throughout the experimental period (Table 1). Meteorological data were collected at the meteorological experimental station of Embrapa Soja, located 400 m from the experimental area.

A randomized complete block experimental design was carried out in split plots, with four replicates. Effect evaluations were performed in the plots for two topdressing N rates (0 and 80 kg ha^{-1}), and in the subplots, for maize plant densities (40, 60, 80 and 100 thousand plants per hectare) intercropped with ruzigrass. The N fertilizer was broadcast on the soil surface when maize crops were at the V5 growth stage (Ritchie et al., 1986). Each split plot measured 5×8 m (40 m^2) and had a net area of 3.2×6 m (19.2 m^2). The maize hybrid was AG9050 PRO3, a super early maturing hybrid with a compact plant architecture.

Maize and ruzigrass were sown after the soybean harvest on March 10, 2018, and March 1, 2019. A seeder and fertilizer spreader with a guillotine-type furrowing mechanism were used to open rows 85 cm apart and the fertilizer was applied. Using manual seeders, three maize seed per hole were sown on marked points. The seeding of ruzigrass between maize rows (42.5 cm away from each row), without fertilization, was done through a mechanized system with double discs and a seed grader adjusted to deliver 5 kg ha^{-1} on a viable seed basis, at the same spacing used for maize rows. The basal fertilizer (25 kg ha^{-1} N, 80 kg ha^{-1} P_2O_5, and 80 kg ha^{-1} K_2O) was applied in conformity to the soil chemical properties and in accordance with the recommendations of the Paraná State Nucleus of the Brazilian Society of Soil Science for 10 Mg ha^{-1} maize yield ceiling (Moreira et al., 2017). At the V2 growth stage (Ritchie et al., 1986), maize plant thinning targeted the plant density of each treatment. The weed control was performed with glyphosate (1.5 kg a.i. ha^{-1}) before maize and ruzigrass sowing, and with atrazine (1.75 kg a.i. ha^{-1}) when maize plants were at V3. The pyrethroid insecticide zeta-cypermethrin (105 g a.i. ha^{-1}) was applied to maize plants at V3 and V6 for insect control. Maize harvest was performed on August 16, 2018, and August 20, 2019. Ruzigrass desiccation was performed on October 02, 2018, and October 10, 2019.

Table 1. Monthly cumulative rainfall and monthly average temperature during the 2018 and 2019 growing seasons in the municipality of Londrina, in the state of Paraná, Brazil(1).

Month	Rainfall (mm)		Average temperature (°C)	
	2018	2019	2018	2019
February	96	133	22.5	24.0
March	151	107	24.2	23.4
April	14	26	22.4	22.7
May	39	101	19.7	20.3
June	27	56	18.7	19.4
July	1	89	20.1	17.9
August	189	12	17.3	19.6
September	126	43	20.4	22.7
October	282	103	21.6	24.7

(1)Meteorological data collected at the Experimental Meteorological Station of Embrapa Soja, Londrina, PR.

Ruzigrass plants were collected from one linear meter per split plot to determine dry biomass and crude protein content, when maize crops were at the V14, R1, R3, and R6 growth stages (Ritchie et al., 1986), at the time of ruzigrass desiccation (October 2, 2018, and October 8, 2019). The quantification of ruzigrass crude protein content was determined by the Kjeldahl's method with sulfuric acid digestion (Claessen, 1997), using a crude protein-to-N conversion factor of 6.25 to calculate the results. The crude protein production was estimated by multiplying biomass weight by crude protein content. The ruzigrass morphological components were evaluated on two subsamples collected per split plot when maize crops were at the R6 growth stage. Then, the material was separated into leaves, stems, and senescent organs, which were oven-dried at 60°C until constant weight was attained. Ruzigrass components are all expressed as weight percentages.

The statistical analysis for each growing season was performed using the R statistical software; the normality of residuals was evaluated by applying the Shapiro-Wilk's test; and the variance homogeneity was determined by the Bartlett's test. According to these tests, there was no need for data transformation. After performing the F-test, at 5% probability, and when the analysis of variance resulted in significant p-values, the means of N fertilization levels were compared by the Tukey's test, at 5% probability, and the effects of maize plant densities on variables were compared by polynomial regression, at 5% probability. When the two-way interaction between factors was significant, the comparison was performed among treatment means of one factor within each level of the other.

Results and Discussion

Maize plant density affected ruzigrass biomass at all sampling times (Figure 1). The increase of maize plant density reduced the ruzigrass biomass over time, from V14 to R6. The ruzigrass biomass decreased with increasing maize plant density, and this effect was highest at the R6 growth stage of maize. There was an interactive effect of N fertilization and maize plant density on ruzigrass biomass in 2019. Nitrogen fertilization had the greatest impact on ruzigrass biomass at the lowest maize plant densities, mainly at ruzigrass desiccation. The effect of N fertilization leading to higher ruzigrass biomass accumulation at desiccation at low densities of maize plant was not observed in 2018.

By these results, higher maize plant densities led to lower ruzigrass growth during the period of these plants coexistence. Youngerman et al. (2018) also observed this trend in cover plants intercropped with maize. As the maize cycle progresses and the canopy closes, there is an increase of interspecific competition in systems with high maize plant density, leading to the suppression of ruzigrass growth. With the increase of maize plant density, there is an increase of the leaf area index (LAI) and solar radiation interception by maize (Sangoi et al., 2019).

Ruzigrass showed a satisfactory growth for an adequate establishment in partial shading. Other studies have also reported a greater ruzigrass tolerance to shading in comparison with other grasses of the genus (Bottega et al., 2016; Faria et al., 2018). However, the morpho-physiological traits of maize plants contribute to interspecific competition due to this crop height and fast initial growth compared with forage species. The availability of sunlight to ruzigrass decreases with the maize canopy closure, aggravated by maize's higher ability to intercept photosynthetically active radiation. At higher densities, maize plants use water and nutrients more intensely, depriving ruzigrass of these resources (Youngerman et al., 2018; Makino et al., 2019). Ruzigrass intercropped with maize at low plant densities showed a greater capacity to overcome interspecific competition. At high maize plant densities, there was a lower difference for biomass yields between treatments. Ruzigrass grown with maize at 60 and 80 thousand plants per hectare had similar dry biomass productions (Figure 1).

Nitrogen fertilization did not influence the ruzigrass biomass production in the 2018 growing season due to the lower water supply from April to July (Figure 1), which compromised the use of N. Ruzigrass intercropped with fertilized maize did not perform better in 2019 between V14 and R6. Biomass production of ruzigrass at desiccation was higher in the fertilized maize treatment, then, it may be inferred that the N fertilization did not increase the competitiveness of ruzigrass against maize, during the cereal development cycle. After the maize harvest, when water deficit was not so severe, the N fertilization favored the ruzigrass growth. Although the greater N supply in the soil system can stimulate the ruzigrass growth (Pontes et al., 2016), N fertilization increases maize LAI, and maintains the leaf area during the grain filling (Coelho et al., 2020). Thus, the beneficial effect of N on ruzigrass was possibly reduced by the increased competition for light imposed by maize. However, in a sandy soil with 180 g kg^{-1} clay and 9.3 g dm^{-3} total organic carbon, Batista et al. (2019) observed a biomass increase of ruzigrass intercropped with maize evaluated at the R6, during the desiccation of ruzigrass, with increasing N rates. Therefore, the response of ruziziensis intercropped with maize to N fertilization is also influenced by soil characteristics.

In both growing seasons, the ruzigrass crude protein content, determined at the R6 growth stage of maize and desiccation, increased with increasing maize plant density (Figure 2), which is possibly due to the dilution effect (Belesky et al., 2011; Santos et al., 2018), as ruzigrass had low biomass in the intercrop with maize at high plant density. The higher crude protein content in ruzigrass, as a consequence of shading, is attributable to either a decrease in photosynthates, an increase of mineralization, or N availability to biomass production (Pontes et al., 2016; Santos et al., 2018; Pezzopane et al., 2019). Nitrogen fertilization also caused the increase of ruzigrass crude protein content, except at the maize R6 growth stage in 2018, as observed in sole ruzigrass crops (Faria et al., 2018). In sole ruzigrass, Barreiros et al. (2020) observed a

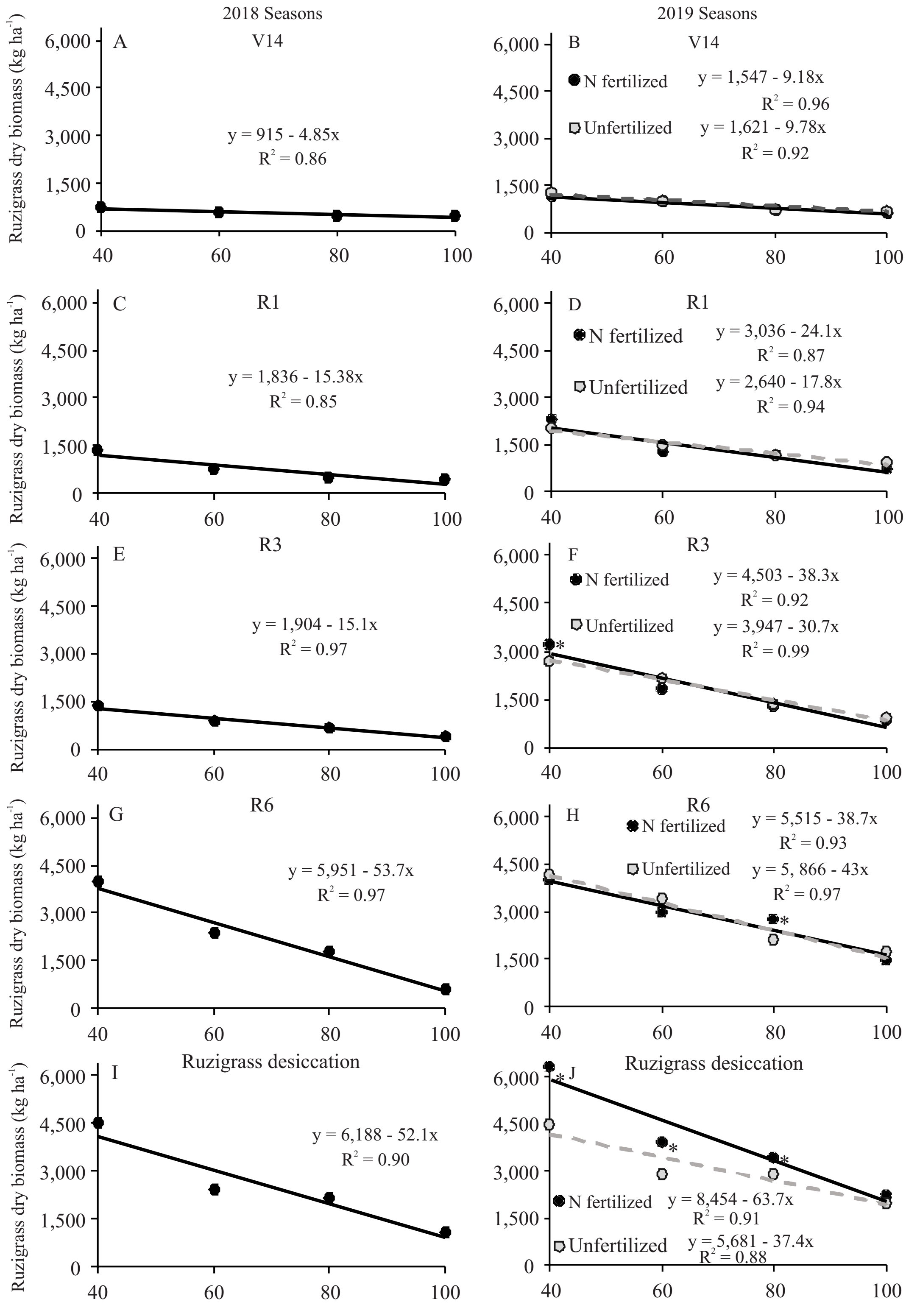

Figure 1. Ruzigrass dry biomass at the V14 (A and B), R1 (C and D), R3 (E and F), and R6 (G and H) stages of intercropped second crop maize, at ruzigrass desiccation (I and J), as a function of maize plant density, in the growing season of 2018, and as a function of the interaction between nitrogen fertilization and maize plant density in the growing season of 2019. *Significant by the Tukey's test, at 5% probability.

crude protein content in the biomass of 12.2% without N fertilization, and crude protein content of 13.4%, with 100 kg ha^{-1} of N fertilization, values that are very similar to those observed in the present study. These results show the ability of ruzigrass to absorb N when this species is intercropped with maize; such ability is essential for the protein synthesis, especially after the maize harvesting.

The increase of maize plant density reduced the ruzigrass crude protein production. The interaction effects of N fertilization and maize plant density affected the crude protein production at R6 and desiccation in 2019 (Figure 3). The effect of high maize plant density on ruzigrass crude protein production was less pronounced at desiccation in comparison with the R6 growth stage of maize. The ruzigrass growth after the maize harvest resulted in similar crude protein production at maize intermediate densities (60 and 80 thousand plants per hectare). Although high maize plant densities increased ruzigrass crude protein content, the high biomass production of ruzigrass at low maize plant densities favored the crude protein production. However, the high ruzigrass growth rates after maize harvest, at maize density of 100 thousand plants per hectare, were insufficient to compensate for the reduced growth during maize development.

Ruzigrass biomass partitioning (green leaves, stems, and senescent tissues) was not affected by N fertilization or maize plant density at R6 (Figure 4). Regardless of treatment, there was a higher amount of ruzigrass biomass in stems (58.6%), followed by green leaves (34.5%), and senescent tissues (7%). Theoretically, with the increase of shading, ruzigrass plants would develop elongated stems, exposing younger leaves to radiation (Belesky et al., 2011), which would increase shading over the lower stratum of plants and could promote senescence in older tissues (Gomes et al., 2020). However, regardless of maize plant density or N fertilization, the partitions of leaves, stems, and senescent tissues were similar. The

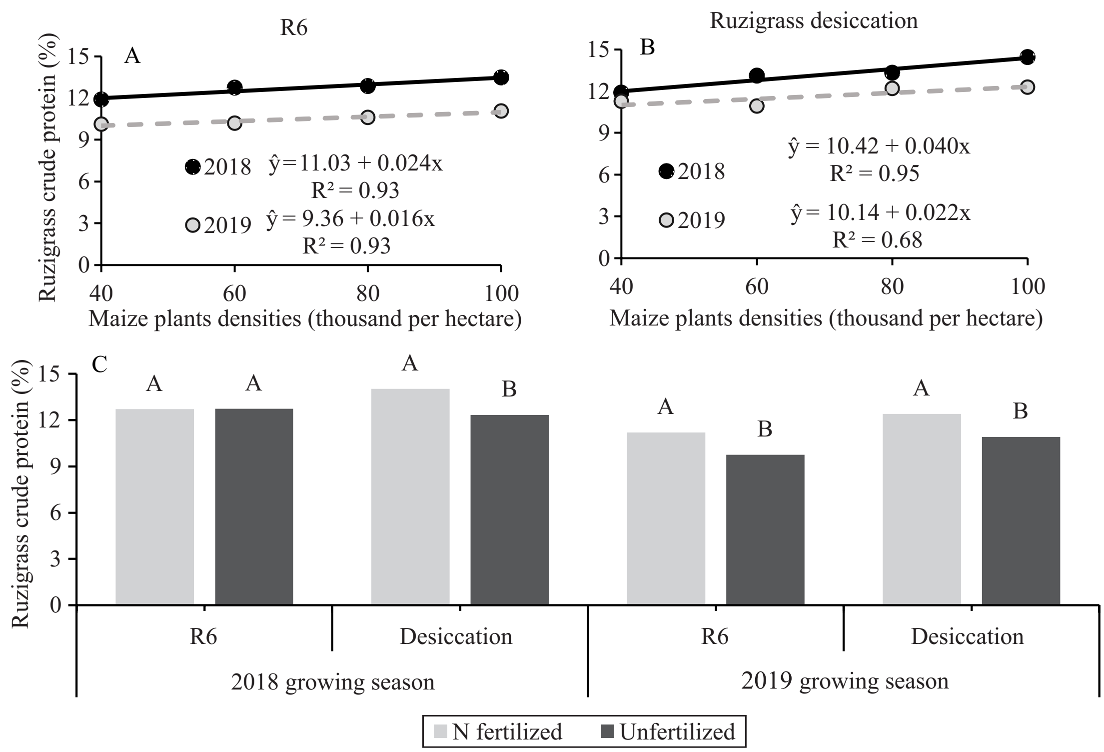

Figure 2. Ruzigrass crude protein content as a function of second-crop maize plant density at the R6 stage of maize (A), and at ruzigrass desiccation (B), in the 2018 and 2019 growing seasons, and as a function of nitrogen fertilization in the R6 stage of maize and at ruzigrass desiccation in the 2018 and 2019 seasons (C). Columns headed by equal letters do not differ by the Tukey's test, at 5% probability.

lack of alteration in the morphological composition of ruzigrass plants with greater shading, resulting from increased maize plant density shows that, despite the reduction of biomass yield, the morphological quality traits (for instance leaf/stem ratio) were not affected.

Considering that a Nellore cattle head weighing 350 kg and reared on pasture without supplementation requires 800.73 g of crude protein, in order to gain and maintain 1 kg live weight (Moraes et al., 2010), and that the grazing efficiency of cattle is 30% for brachiaria (*Urochloa)* species (Cardoso et al., 2020), it can be estimated that the weight gain potential of the studied crops was 200, 118, 106, and 58 kg live weight ha^{-1}, in 2018, for ruzigrass intercropped with maize at 40, 60, 80, and 100 thousand plants per hectare, respectively. In 2019, a growing season with higher water supply, the potential live weight gain was 282, 172, 168, and 110 kg live weight ha^{-1}, for ruzigrass intercropped with fertilized maize at 40, 60, 80, and 100 thousand plants per hectare, respectively. Considering the treatments without N topdressing, in 2019, the potential live weight gain was 179, 111, 122, and 87 kg ha^{-1}, for ruzigrass intercropped with maize at 40, 60, 80, and 100 thousand plants per hectare, respectively.

In accordance with the estimated crude protein production, maize-ruzigrass intercropping have the potential to provide cattle weight gains of 58 to 282 kg ha^{-1}, depending on the N fertilization, maize plant density, and growing season. Although forage supply by the intercropping system has a short time window (about 50 days), it coincides with the forage shortage period in tropical regions (Euclides et al., 2016). In addition of being an option during forage shortage, intercropped ruzigrass shows high protein content (from 10.0 to 14.4% crude protein) which can contribute to overcome the innovation challenge of expanding grain production matrix diversification in Brazil.

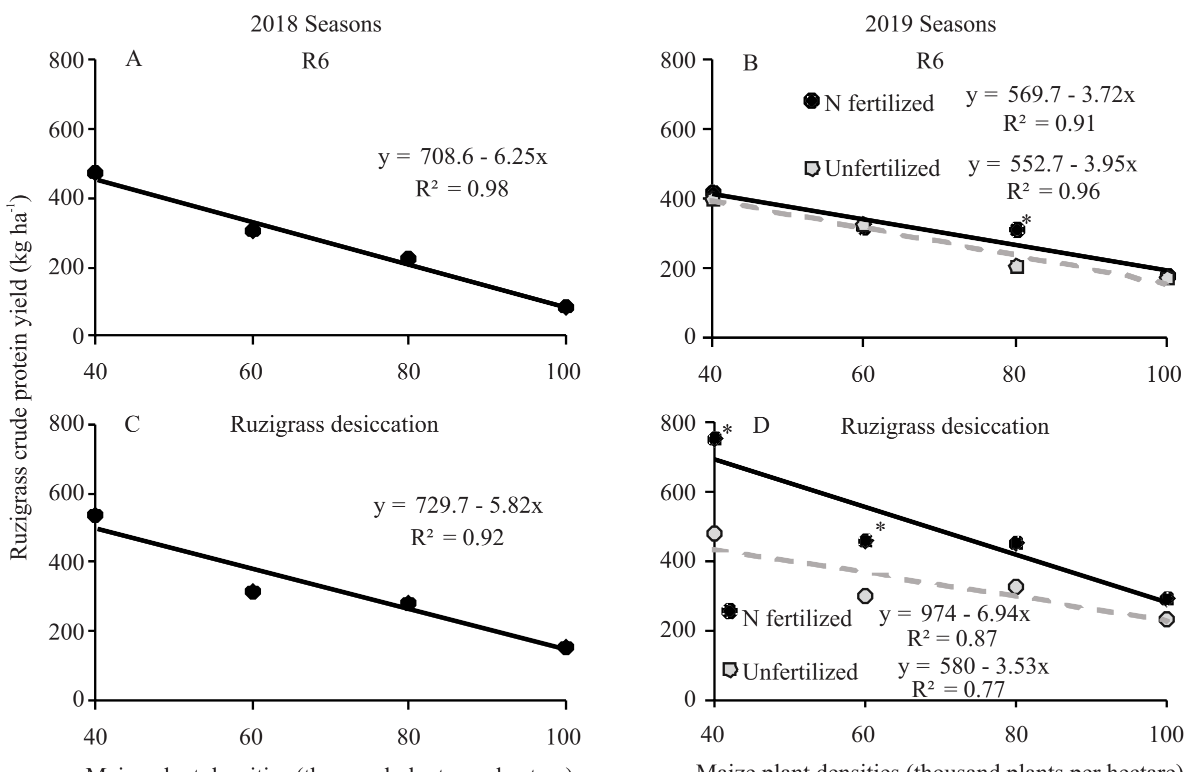

Figure 3. Ruzigrass crude protein yield as a function of second-crop maize plant density at the R6 stage of maize (A), at ruzigrass desiccation (C) in the 2018 season, and as a function of the interaction between nitrogen fertilization and maize plant density in the R6 stage of maize (B) and at ruzigrass desiccation (D) in the 2019 season. *Significant by Tukey's test at 5% probability.

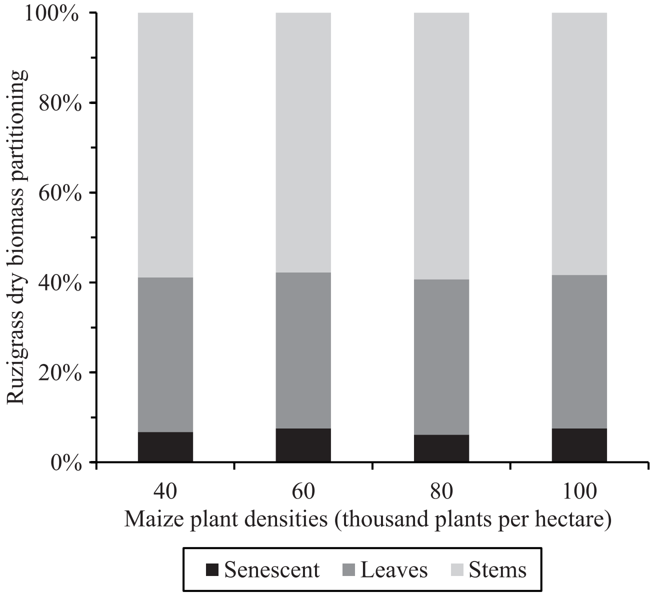

Figure 4. Ruzigrass dry biomass partitioning to leaves, stems, and senescent tissues, at the R6 stage of intercropped second-crop maize, at the 2018 growing season.

Conclusions

1. The increase of second-crop maize (*Zea mays*) plant density decreases the biomass production and enhances the crude protein content of intercropped ruzigrass (*Urochloa ruziziensis*).
2. Nitrogen topdress fertilization does not affect the intercropped ruzigrass biomass production until the harvest of maize.
3. Nitrogen topdress fertilization increases the biomass and crude protein production of ruzigrass after the maize harvest under favorable water conditions.

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