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Nitrogen fertilization, grazing and timing of phosphorus and potassium replacement in a soybean-ryegrass production system

Abstract – The objective of this work was to investigate nitrogen balance in a soybean-ryegrass system, influenced by off-season N fertilization, sheep grazing, and timing of phosphorus and potassium replacement. A long-term four-year experiment was conducted in a randomized complete block design with four replicates. The present study was carried out in the fourth crop season, when data from three replicates were collected. The split-plot experimental design was adopted. The plots were arranged in a 2x2 factorial arrangement, consisting of two grazing levels (grazed and ungrazed) and two times of P_2O_5 and K_2O replacement (in summer or winter), whereas the subplots received different N rates (0, 50, 100, and 200 kg ha^{-1} N). The analyzed variables were total dry mass yield, N accumulation in ryegrass, and soybean metrics such as biological N fixation (BNF), grain yield, and partial N balance (PNB). The highest rates of N in ryegrass increased grain yield by 14% in grazed areas. The replacement of P_2O_5 and K_2O in winter boosted grain yield by 11%. Off-season N fertilization increased N balance due to the increase in dry mass yield and N cycling, alleviating the negative PNB caused by soybean, regardless of the timing of P_2O_5 e K_2O replacement and grazing or not by sheep.

Index terms: balance, crop-livestock integration, soil fertility, system fertilization.

Adubação nitrogenada, pastejo e época de reposição de fósforo e potássio em sistema de produção de soja-azevém

Resumo – O objetivo deste trabalho foi investigar o balanço de nitrogênio em sistema soja-azevém, influenciado pela fertilização de N na entressafra, pelo pastejo de ovinos e pela época da reposição de fósforo e potássio. Foi realizado experimento de longa duração foi realizado durante quatro anos, no delineamento de blocos ao acaso, com quatro repetições. O presente estudo foi realizado na quarta safra, quando foram coletados dados de três repetições. Adotou-se o delineamento experimental de parcelas subdivididas. As parcelas foram organizadas em delineamento fatorial 2x2, composto por dois níveis de pastejo (com ou sem) e dois momentos de reposição de P_2O_5 e K_2O (no verão ou no inverno), enquanto as subparcelas receberam diferentes doses de N (0, 50, 100 e 200 kg ha^{-1} N). As variáveis analisadas foram produção de massa seca total, acúmulo de N no azevém, e métricas da soja como fixação biológica de nitrogênio (FBN), produtividade de grãos e balanço parcial de N (BPN). As maiores doses de N no azevém aumentaram a produtividade de grãos em 14% nas áreas pastejadas. A reposição de P_2O_5 e K_2O no inverno impulsionou a produtividade de grãos em 11%. A adubação nitrogenada na entressafra

Felipe Dalla-Zen Bertol(✉)
Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.
E-mail: fe_bertol@hotmail.com

Carolina Bremm
Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.
E-mail: carolina.bremm@gmail.com

Luciano Pinzon Brauwers
Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.
E-mail: lucianopinzon@hotmail.com

Lóren Pacheco Duarte
Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.
E-mail: lorenduartee@gmail.com

Gabriela Naibo
Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.
E-mail: naibogabriela@gmail.com

Edicarlos Damacena de Souza
Universidade Federal de Rondonópolis, Rondonópolis, MT, Brazil.
E-mail: edicarlos@ufr.edu.br

Mateus Westerhofer Goulart
Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.
E-mail: mateus.goulart@ufrgs.br

Amanda Posselt Martins
Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.
E-mail: amanda.posselt@ufrgs.br

✉ Corresponding author

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aumentou o balanço de N devido ao aumento da produção de massa seca total e ciclagem de N, aliviando o BPN negativo provocado pela soja, independentemente da época de reposição de P_2O_5 e K_2O e do pastejo ou não de ovinos.

Termos para indexação: balanço, integração lavoura-pecuária, fertilidade do solo, adubação de sistema.

Introduction

Nitrogen fertilization is crucial for achieving high production levels in forage grasses (Mattera et al., 2023). Despite its importance, fertilization is typically neglected in production systems, resulting in insufficient economic returns from cover crops, and low dry matter (DM) production. Furthermore, slow canopy closure, which exposes the soil to raindrop impact, which can cause surface sealing, erosion, and a higher incidence of invasive plants (Serra et al., 2018). These N deficits can be even more pronounced in the soybean [*Glycine max* (L.) Merr.]-ryegrass (*Lolium multiflorum* Lam.) succession system. In high-yielding soybean production, for instance, N export in the soybeans is often higher than the amount supplied by biological N fixation (BNF) (Ciampitti & Salvagiotti, 2018). This can bring long-term consequences including decreases in the N stocks of soil organic matter.

Fertilizing cover crops can help balance the N budget in soybean systems by increasing the soil organic N reserves (Diekow et al., 2005). Nonetheless, this practice is challenging to implement due to the lack of direct economic return from cover crops. Therefore, the integrated agricultural production system (IAPS) presents a viable solution to this problem. In the highlands of the Brazilian subtropics, the IAPS model involves the temporal integration of cattle or sheep grazing during the cool-season, i.e. fall and winter, followed by grain production during spring and summer. Properly managed livestock introduction enhances nutrient cycling (Alves et al., 2019), mitigates soil acidity (Martins et al., 2014), boosts soil microbial activity (Soares et al., 2019), and improves the quality of surface residue (Assmann et al., 2018), all of which affect N dynamics within the system.

Moreover, many areas in the Brazilian subtropics have accumulated soil fertility through years of grain production with liming and fertilization. These soils are often characterized by the absence of acidity problems and high or very high levels of organic matter and essential nutrients, particularly phosphorus and potassium (Manual de calagem…, 2016). These environments support system fertilization, a strategy that targets nutrient replacement, especially P_2O_5 and K_2O, at a stage that maximizes benefits for all crops (Farias et al., 2020). For instance, in soybean production systems with off-season grasses, nutrient replacement of P_2O_5 and K_2O required by soybean during cover cropping can be advantageous for all crop phases (Alves et al., 2022).

System fertilization practices, such as the replacement of P_2O_5 and K_2O from soybean during winter, benefit environmental preservation, enhance nutrient recycling and retention in organic forms, e.g. pasture DM, increase yields, forage production, and economic return. System fertilization offers potential advantages allowing producers to capitalize from fertilizer market timing for lower prices and ample time for precise application (Assmann et al., 2018). However, a significant challenge remains in demonstrating N, P_2O_5 and K_2O fertilization impacts on soybean cultivation and evaluating these effects in animal-grazing integrated production systems (Alves et al., 2022).

The objective of this work was to investigate the nitrogen balance in the soybean-ryegrass system, influenced by off-season N fertilization, sheep grazing, and timing of P_2O_5 and K_2O replacement.

Materials and Methods

The experimental area was located at the Estação Experimental Agronômica of the Universidade Federal do Rio Grande do Sul, in the municipality of Eldorado do Sul, Rio Grande do Sul, Brazil (30°5'43"S, 51°41'20"W, at 27 m of altitude).

The soil in the experimental area is classified as a typical Plintossolo Argilúvico Distrófico, according to the Brazilian Soil Classification System (Santos et al., 2018), equivalent to a Dystric Argic Plinthosol. The area features gently undulating relief, and the 0–20 cm layer has a clay content of 14%. Initial soil chemical properties, measured prior to the installation of the N fertilization plots in May 2019, are shown in Table 1. Subsequently, soil acidity was adjusted with 70% neutralizing power dolomitic agricultural limestone applied on the surface at a rate of 7.4 Mg ha^{-1}, in order to raise the pH of the 0–10 cm layer to 6.0.

The climate is classified as humid subtropical (Cfa) according to the Köppen classification (Kottek et al., 2006). Over the past 14 years, the average annual rainfall varied between 1,200–2,000 mm, with an average annual temperature of 19°C (Alves et al., 2019). During the data collection period, from May 2019 to March 2020, the accumulated rainfall was 929 mm, which is below the historical average. Specifically, the rainfall during soybean cycle (November 2019 to April 2020) was 214 mm, characterizing a year of water stress for the crop (Cardoso et al., 2020).

Principal factors were installed in 2017 across 16 main plots, with individual plot areas ranging from 0.23 to 0.41 ha. The study employed a randomized complete block design comprising a 2x2 factorial arrangement and four replicates. The first factor analyzed in the main plots was the use of ryegrass in winter, with sheep grazing in a IAPS, or a cover cropping system without grazing. The second factor analyzed the P_2O_5 and K_2O fertilization timing for replacement of nutrients exported by soybean. The analyzed methods were system fertilization during winter cover cropping in May and June, or the traditional fertilization in summer prior to the soybean sowing in November.

For the study, during the 2019/2020 crop, N rates were established as a subplot factor in the main plots. The subplots were installed in three of the four blocks, encompassing all combinations of grazing and P_2O_5 and K_2O replacement timings. Within each main plot, 80 m^2 areas (8 x 10 m) were excluded and divided into the four different 20 m^2 (8.0 x 2.5 m) sub-plots. These sub-plots received N rates of 0, 50, 100, and 200 kg N ha^{-1} applied using N 46% (common urea) at the V3 stage. The remaining main plot area received the rate of 150 kg N ha^{-1}, used since the experiment inception in 2017.

The data collection period for this study started after the 2018/2019 soybean harvest in April 23, 2019. On May 16, 2019, 'BRS Ponteio' ryegrass was sown with a pure viable seed density of 30 kg ha^{-1}. After ryegrass plants achieved V3 stage on June 17, 2019, N fertilization at 150 kg ha^{-1} in the main plots and different rates in sub-plots was applied alongside the system fertilization at 56 kg ha^{-1} P_2O_5 and 80 kg ha^{-1} K_2O. Grazing begun in July 17, 2019, using 10-month-old Corriedale sheep (*Ovis aries*) under a continuous and moderate put-and-take grazing method. The sheep entered the designated plots when plant height reached 15 cm; this height was maintained through plot rotation and monitored weekly using a sward stick with 150 weekly readings per experimental unit. The grazing period concluded after 113 days with the animals removed from the area on October 26, 2019. Subsequently, on November 18, 2019, the ryegrass was desiccated with 3 L ha^{-1} glyphosate, and 2 L ha^{-1} 2,4-Dichlorophenoxyacetic acid. On November 21, 2019, the designated plots received traditional fertilization at 56 kg ha^{-1} P_2O_5 of and 80 kg ha^{-1} K_2O. Soybean cultivar TMG 7063 IPRO was then sown on November 25, 2019, with a 0.45 m row spacing, and the final harvest was conducted on April 13, 2020.

The total dry mass yield (DMP) of ryegrass in grazed plots was carried out during the grazing period by setting up 0.20 m^2 in area and 50 cm in height grazing exclusion cages within the sub-plots. Samples were taken from a 0.10 m^2 area inside each cage, while samples from random points outside the cage were also collected following the ‘double pairing’ method (Moraes et al., 2014). The plant samples were harvested and then dried to a constant mass in an oven with forced air circulation at 65°C (Tedesco et al., 1995).

Data collection was carried out in five different moments spaced 28 days apart, specifically at 49, 77, 105, 133, and 161 days after sowing (DAS). The amount of DM produced over a given period was determined by subtracting the DM content of the exclusion cage

Table 1. Chemical characterization of the soil in the experiment conducted in May 2019[(1)].

Soil depth (cm)	pH (H_2O)	TOC (g kg^{-1})	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	P_2O_5	K_2O	CEC saturation	
			---- ($cmol_c$ dm^{-3}) ----				---- (mg dm^{-3}) ----		Cation (%)	Al (%)
0–10	4.7	12.8	2.4	2.9	0.6	9.6	86	84	36.4	11.7
10–20	4.2	6.4	0.9	2.2	1.1	9.8	38	64	25.0	25.7

[(1)]pH in water solution at a concentration of 1:1 v/v; TOC, total organic carbon; Ca^{2+}, Mg^{2+}, and Al^{3+}, extracted using KCl 1.0 mol L^{-1}; H+Al, determined using a 1.78 mol L^{-1} SMP solution containing triethanolamine, p-nitrophenol, K_2CrO_4, $Ca(CH_3COO)_2$, and $CaCl_2{\cdot}2H_2O$, calibrated at pH 7.5; P_2O_5 and K_2O, extracted using Mehlich-1; CEC, cation exchange capacity saturation covering cation saturation = $(Ca^{2+} + K^+ + Mg^{2+}) / (Ca^{2+} + K^+ + Mg^{2+} + H^+ + Al^{3+}) \times 100$ and Al saturation = $Al^{3+} / (Ca^{2+} + K^+ + Mg^{2+} + Al^{3+}) \times 100$.

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from the samples collected outside the cage in the previous collection period. To calculate the DMP, the production from each period was aggregated.

Nitrogen content was analyzed in every collection period, following the digestion and distillation by steam dragging methods described by Tedesco et al. (1995) using a Kjeldahl distiller. In the grazed plots, the accumulation of N in the dry matter (ANDM) was calculated using a method similar to that used for DMP: the N content was multiplied by the DM produced in each period, then the values were summed across all periods. In the last collection, the sample from outside the cage was used for residue evaluation.

In the ungrazed environment, the average of the 105 and 161 DAS sampling was used to estimate DMP due to the death of tillers and plant lodging. The last cut conducted before the end of the pasture cycle was used to quantify the residue for the subsequent soybean crop; however, ground cover was not considered.

Soybean grain yield and BNF were evaluated throughout the soybean cultivation period, which spans from November to April. To evaluate BNF parameters, seven whole soybean plants were randomly selected from every plot at development stage R6, with a greater accumulation of DM in the plant. Concurrently, the plant stand was measured by counting individual plants in four 1 m rows of harvest area and extrapolated to plants ha^{-1}, thereby determining the DM of the grains, stems, and leaves of soybean plants.

To differentiate the amount of N derived from the soil from that fixed by the soybean, the spontaneous non-N-fixing plants *Conyza bonariensis* (L.) Cronquist, *Amaranthus hybridus* L., and *Brachiaria plantaginea* Hitchc. were collected near the plots. All the aerial parts of both soybean and spontaneous plants were dried in an oven at 65°C until a constant mass was achieved (Tedesco et al., 1995). After drying, the samples were weighed and set aside to determine the $\delta^{15}N$.

The %BNF was calculated using the ^{15}N natural abundance method (Shearer & Kohl, 1986), based on the natural abundance of the collected plants, according to the following equation:

$$\%BNF\left[\frac{\delta^{15}N_{reference}-\delta^{15}N_{soybean}}{\delta^{15}N_{reference}-B_{value}}\right]100$$

where $\delta^{15}N_{reference}$ is the natural abundance of ^{15}N in the reference plant; $\delta^{15}N_{soybean}$ is the natural abundance of ^{15}N in the soybean; and B_{value} is the ^{15}N isotopic discrimination of the leguminous plant under conditions of full dependence on BNF for its development, specifically under greenhouse conditions. Following Guimarães et al. (2008) and Pauferro et al. (2010) examinations on soybean varieties and inoculants *Bradyrhizobium elkanii* and *Bradyrhizobium japonicum* used in Brazil, the adopted B_{value} is -2.62‰.

The percentage of N sourced from the soil was calculated by subtracting the %BNF from the total (100%). To determine the total N accumulation from BNF in kg ha^{-1}, the %BNF was multiplied by the total N accumulated in the plant.

The partial N balance (PNB) offers information on the difference between the total N incorporated via BNF, and N exported from the system by the grains. The value was calculated by the following equation:

$$PNB=\left(\frac{QN_c\times\%BNF}{100}\right)-QN_g$$

where QN_c is the total amount of N accumulated by the soybeans; and QN_g is the total amount of N exported by soybean grain.

Soybean yield was estimated by sampling four 1 m rows spaced 0.45 m apart within the subplots, totaling 1.8 m^2. Each sample was threshed, weighed, and grain moisture was adjusted to 13%. The overall system balance between soybean and the off-season crop was calculated as the difference between the PNB values in the soybean and the N accumulation in the ryegrass DM (PNB-ANDM).

The analysis of variance (ANOVA) was conducted using the R statistical software (R Core Team, 2013), and the data was subjected to Shapiro-Wilk's test for normality and Bartlett's test for homogeneity of variances, both at a 5% significance level, along with a visual inspection of the residuals. When assumptions for ANOVA were not met, outliers were removed following strategies that were limited to 5% of the observations, or data were transformed using the log, square root, or Box-Cox methods. A split-plot factorial model was employed for all studied variables. The fixed factors included the effects of animal grazing and the different replacement times for P_2O and K_2O, the rates of N applied to ryegrass in the subplots, while the blocks and their interactions were considered random factors. The ANOVA results are presented in Table 2. Differences in means between the fixed and qualitative factors and the interaction between them

Table 2. Significance levels of the sources of variation in the experiment of soybean-ryegrass production system and their interactions for the analyzed variables in different phases of the 2019/2020 growing season.

Crop	Variable(1)	Sources of variation(2)						
		G	A	N	GxA	GxN	AxN	GxAxN
Ryegrass (winter)	DMP	ns	ns	***	ns	*	ns	ns
	CNDM	***	ns	**	ns	*	ns	ns
	ANDM	*	ns	***	ns	*	ns	ns
	Residual	**	ns	ns	ns	*	ns	ns
	N residual	ns	*	**	ns	***	ns	*
Soybean (summer)	Soybean yield	*	*	ns	ns	ns	ns	ns
	N in DM	ns	ns	ns	ns	ns	ns	ns
	%BNF	ns	ns	*	ns	ns	*	*
	PNB	ns	ns	ns	ns	*	ns	ns
	N-SOIL	*	ns	ns	ns	ns	ns	ns
	N-BNF	ns	ns	ns	ns	ns	ns	ns
	Delta (PNB-ANDM)	ns	ns	*	ns	ns	ns	ns

(1)DMP, total dry mass yield; CNDM, nitrogen content in dry mass; ANDM, nitrogen accumulation in dry mass; Residual, dry mass productivity of the residue; N residual, amount of nitrogen in the dry mass of the residue; %BNF, percentage of nitrogen derived from biological fixation; PNB, partial nitrogen balance; N-Soil, amount of nitrogen in the soybean plant derived from the soil; and N-BNF, amount of nitrogen in the soybean plant derived from biological fixation. (2)G, ryegrass grazing in winter; A, timing of P_2O_5 and K_2O replacement; and N, N rates applied to ryegrass in winter. *, **, and ***, p-value < 0.05, < 0.001, and < 0.0001, respectively. nsNonsignificant.

were assessed using Tukey's test (p<0.05). For the fixed and quantitative factors, linear regression models were adjusted, showing the equation, R^2, and p-value.

Results and Discussion

Applying 46% N urea at the V3 stage linearly increased the ryegrass DMP under grazing conditions, where each added kg ha^{-1} N resulted in an increase of 17.5 kg ha^{-1} DM. Under ungrazed conditions, the response was quadratic, achieving maximum DMP at a rate of 130 kg ha^{-1} N. Beyond this rate, ryegrass DMP performance in grazed areas exceeded that observed in ungrazed areas (Figure 1 A). Grazing enhanced DMP, compared with the ungrazed condition, the latter was superior only at the 50 kg ha^{-1} N rate, and showed similar performance at the 100 kg ha^{-1} N rate.

Nitrogen fertilization in ryegrass crops directly increases DMP by stimulating tiller emissions (Timm et al., 2019). Under grazing conditions, the linear and increasing response of ryegrass DMP to N fertilization suggests that the N rates applied in this study did not reach the maximum productive potential for the crop (Figure 1 A). This consistent response pattern underscores the significance of N fertilization for ryegrass yield under grazing and emphasizes the need to adjust N rates to optimize production outcomes (Pellegrini et al., 2010).

Residual ryegrass DM was higher in the ungrazed environment compared with the grazed environment at all applied N rates (Figure 1 B). In grazed areas, the residual DM increased by 10.3 kg ha^{-1} for each kg ha^{-1} N applied. Conversely, in ungrazed environments, the ryegrass residue DM decreased by 0.4 kg ha^{-1} for every kg ha^{-1} N applied.

The apparent N recovery (ANR) was influenced by both the applied N rate and grazing management, allowing a direct comparison of the responses to fertilization with a theoretical 1:1 line that represents the amount of N the plant absorbed in relation to what was applied via fertilizer (Figure 1 C). Results indicate that soil contributes with ryegrass nutrition regardless of grazing at rates from 0 to 100 kg ha^{-1} N as indicated by the point position in relation to the 1:1 line. Maximum efficiency is attained in grazed areas at 183 kg ha^{-1} N. However, in the ungrazed environment, the efficiency of converting N from mineral fertilizer into ryegrass DM peaks at 113 kg ha^{-1} N.

The linear increase in residual DM and N accumulation in grazed ryegrass DM, as well as N accumulation in ungrazed ryegrass DM, associated with the increased N input via fertilization (Figures 1 B and C). These factors are crucial for protecting the soil surface against erosion and can enhance carbon and N stocks (Diekow et al., 2005). In ungrazed systems, N fertilization aims to maintain DM on the surface to prevent erosion, this can be achieved with low rates of 50 kg ha^{-1} N (Figure 1 B). Conversely, in grazed environments, while increasing N fertilization raises residual dry mass, higher rates such as 200 kg ha^{-1} N are required to achieve the soil surface protection observed in ungrazed settings.

Furthermore, the N content in ryegrass DM was higher in grazed areas than in ungrazed areas across all tested rates (Figure 1 D). Nevertheless, the rate in N content as urea application increased was more pronounced in the ungrazed environment (0.042 g N kg^{-1} DM) compared with the grazed environment (0.024 g N kg^{-1} DM).

The higher N content in ryegrass DM resulting from increased N rates is caused by an increased leaf-to-stem ratio, reduced fiber content, and higher crude protein content (Timm et al., 2019) as shown in Figure 1 D. Consequently, improvements in pasture acceptability and digestibility may enhance animal performance under appropriate management, supporting the achievement of greater economic returns of N fertilization (Mattera et al., 2023). When animals consume ryegrass, they alter the N flow within the system, as a small portion of ingested N is incorporated into the carcass, while 50–70% returns

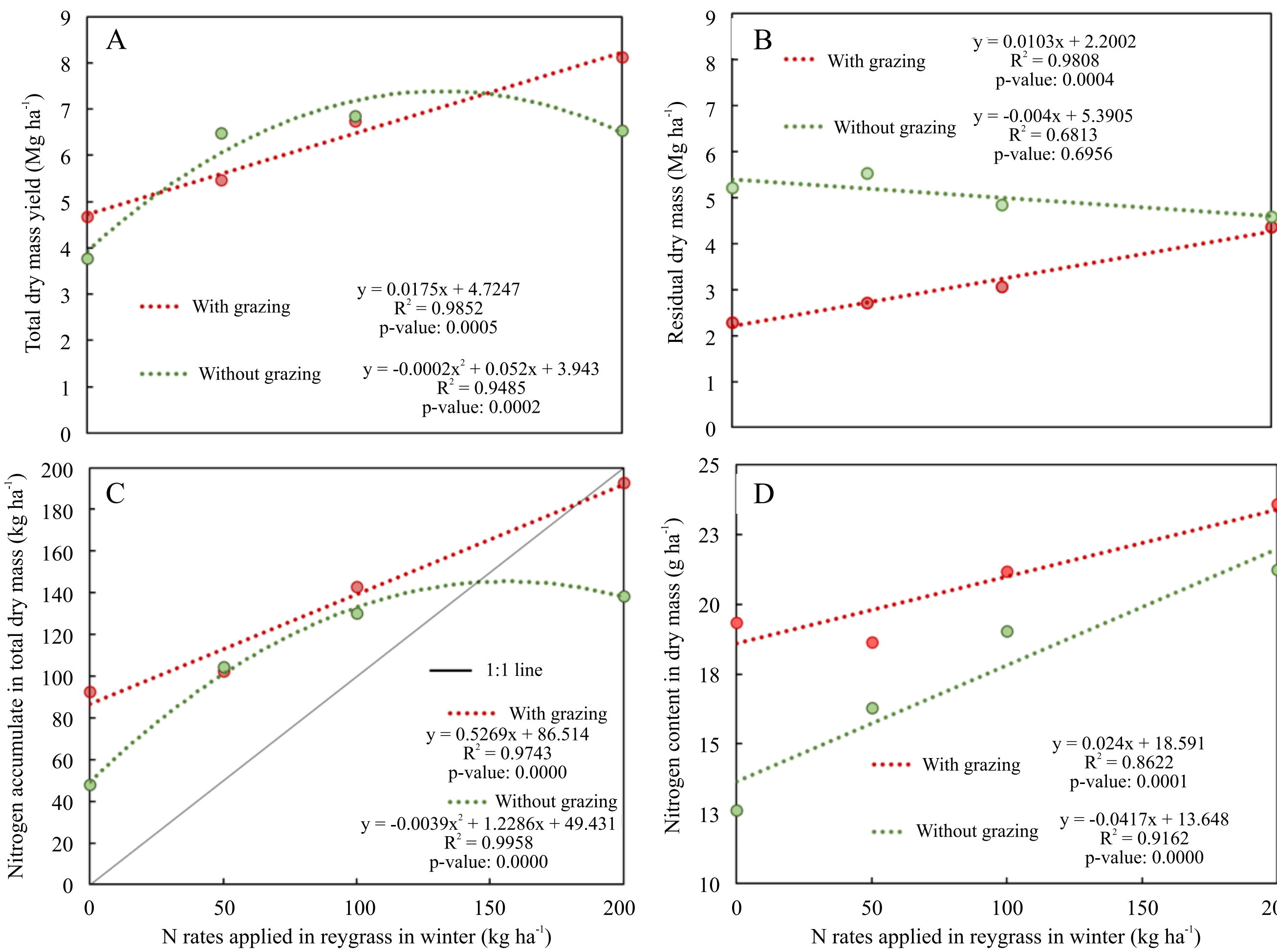

Figure 1. Total dry mass yield (A), residual dry mass (B), nitrogen accumulation in the total dry mass yield of the aerial part (C), and nitrogen content in the dry mass (D) of ryegrass cultivated in winter with or without grazing and different nitrogen rates averaged across different timings of phosphorus and potassium replacement.

to the soil via waste as manure and urine (Whitehead, 2000). Thus, increased N input also enhances nutrient cycling, since minimal N is exported, and tends to be recycled for the next crop under proper pasture management.

The timing of P_2O_5 and K_2O replacement did not influence the response variables measured in ryegrass during the 2019/2020 crop season (Figure 1). However, in the same experimental area, Farias et al. (2020) reported increases in ryegrass DM yield by 19.0% in ungrazed and 16.5% in grazed environments during the 2017/2018 and 2018/2019 crop seasons, when P_2O_5 and K_2O were replaced shortly after soybean harvest, compared with the replacement at soybean sowing. Similarly, Simões et al. (2023) reported a 36% increase in ryegrass DMP during the 2020/2021 crop season, with winter P_2O_5 and K_2O replacement. Notably, these previous studies used a fixed rate of 150 kg ha^{-1} N for ryegrass, leaving questions about potential changes in productive responses to P_2O_5 and K_2O replacement timing and different N rates, which were not observed in this study.

The accumulation of N in the ryegrass residue was influenced by the interaction between ryegrass grazing and the time of P_2O_5 and K_2O replacement (Figure 2). In both P_2O_5 and K_2O replacement seasons, there was less N in the residue DM in the grazed environment than in the ungrazed area. However, in the absence of N fertilization in ryegrass, P_2O_5 and K_2O replacement in winter led to a greater accumulation of N in ryegrass residue compared with summer. Furthermore, in the ungrazed environment, increasing N rates on summer P_2O_5 and K_2O replacement led to N accumulation in the residue similar to the winter replacement.

The accumulation of N in soybean at the R6 development stage was not influenced by grazing, P_2O_5 and K_2O replacement timing, or N rates applied during winter, with an average accumulation of 190 kg ha^{-1} N. The contribution of BNF to N nutrition in soybeans was affected by the interaction of ryegrass grazing and

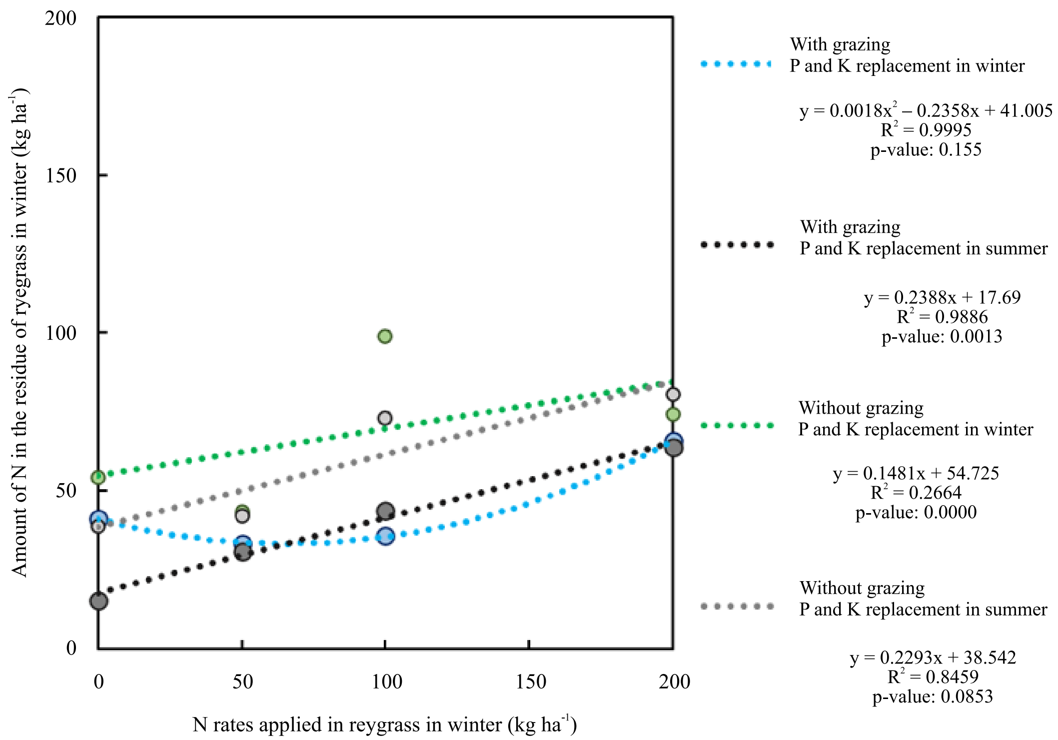

Figure 2. Amount of nitrogen in the residue of ryegrass cultivated during winter with or without grazing, at different nitrogen rates and different timings of phosphorus and potassium replacement.

P_2O_5 and K_2O replacement timing, depending on the 60–72% N rate applied to ryegrass (Figure 3 A).

The only treatment in which %BNF did not decrease as N rates applied to ryegrass increased was the combination of P_2O_5 and K_2O replacement in summer and grazing, yielding an average BNF contribution of 65%. Conversely, increasing the N rate applied to ryegrass led to a reduction in %BNF in soybeans when P_2O_5 and K_2O were replaced in winter, regardless of grazing. Notably, at a rate of 0 kg ha^{-1} N, the grazed environment showed a lower %BNF than the ungrazed environment (Figure 3 A).

While %BNF varied significantly, the amount of kg ha^{-1} N provided by BNF to soybeans remained unaffected by the studied factors. The average accumulation of N from BNF in soybean plant tissue was 121 kg ha^{-1} N. Conversely, soybean soil-derived N was influenced by ryegrass grazing, independently of P_2O_5 and K_2O replacement time or the N rates applied to ryegrass. In grazed conditions, soil-derived N to the soybean was 15 kg ha^{-1} N higher than in ungrazed conditions.

The reduction in soybean BNF after the N application to ryegrass during the winter can be attributed to the enhanced availability of mineral N to soybean plants. Soybean expend less energy absorbing N from the soil solution than via BNF (Kaschuk et al., 2009). Consequently, soybean prioritize N consumption from larger amounts available in the soil, due to greater mineralization from higher-quality residues with a low carbon-to-nitrogen ratio (C/N ratio), which ultimately inhibits BNF (Mapope & Dakota, 2016). The increased mineral N availability is attributed to grazing, which provides higher-quality plant tissue rich in N with a low C/N ratio, allowing microorganisms to degrade the residue faster, thus mineralizing the N that becomes readily available to subsequent soybean crop (Figure 3 B).

Soybean grain yield was not altered by the N rates applied to ryegrass. However, grazing management and fertilization timing independently influenced grain production. Soybean yield was 14% higher when ryegrass was grazed (3.1 Mg ha^{-1}) compared with non-grazed conditions (2.7 Mg ha^{-1}) as shown in Figure 4 A. In addition, grain yield increased 11% when P_2O_5 and K_2O were replaced during winter (3.1 Mg ha^{-1}) in comparison with summer (2.8 Mg ha^{-1}), this result is independent of grazing management (Figure 4 B).

The increase in soybean yield when P_2O_5 and K_2O are replaced in winter rather than summer is

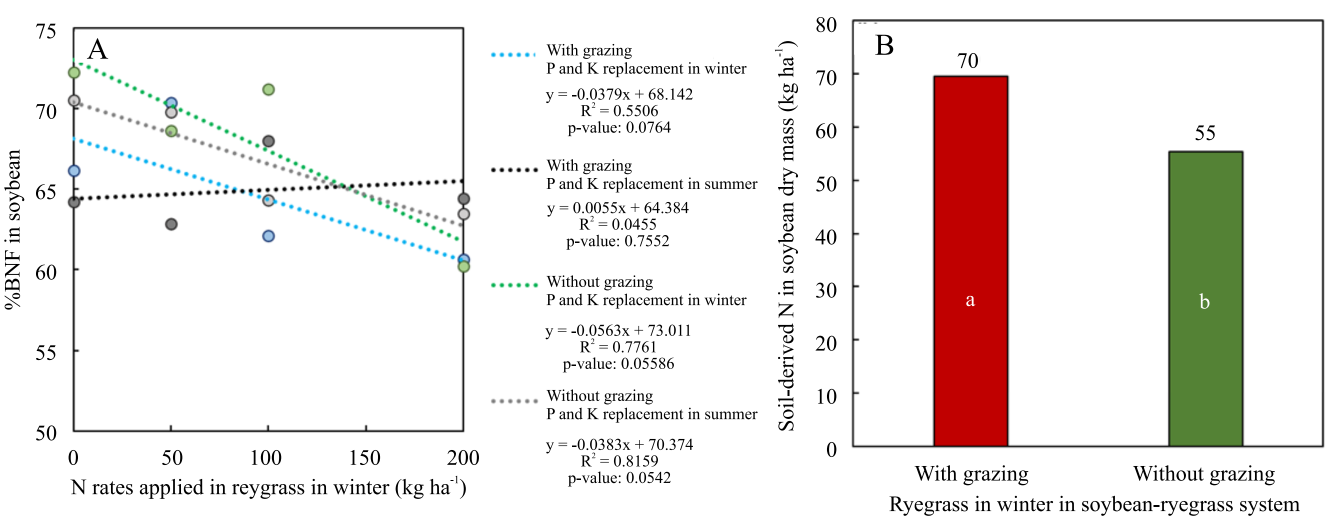

Figure 3. Percentage of biological nitrogen fixation contribution (%BNF) in soybean biomass in response to the use of ryegrass cultivated during winter with or without grazing, at different nitrogen rates applied to ryegrass, and of phosphorus and potassium replacement (A); and quantity of nitrogen derived from the soil in the dry mass of soybean in response to just the grazing of ryegrass cultivated during winter season (B). Different letters in the columns indicate significant differences according to Tukey's test ($p<0.05$).

rare (Figure 4 B). While no response was observed in ryegrass DMP regarding the timing of P_2O_5 and K_2O replacement in this study (Figure 1 A), previous studies in the same experimental area reported greater ryegrass DMP when P_2O_5 and K_2O are replaced in winter (Farias et al., 2020; Simões et al., 2023). Over

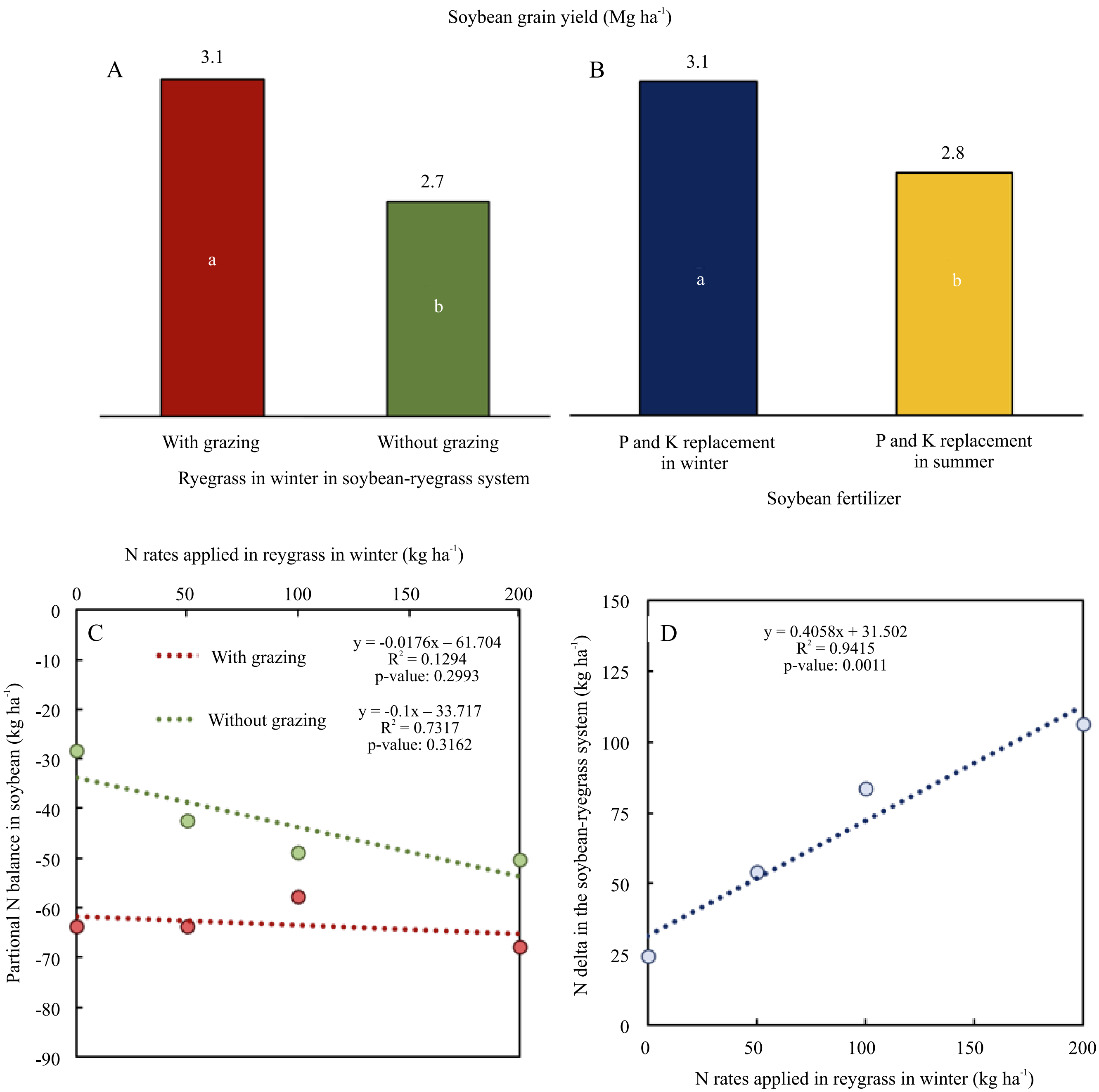

Figure 4. Soybean grain yield after the cultivation of ryegrass during the winter (A) and after phosphorus and potassium application in different periods (B), as well as partial nitrogen balance in soybean in relation to ryegrass cultivated during the winter with or without grazing after fertilization with different nitrogen rates (C), and nitrogen delta in the soybean-ryegrass system (partial nitrogen balance in soybean subtracted from nitrogen accumulation in the aerial part of ryegrass) in relation to different nitrogen rates applied to ryegrass cultivated in winter (D). Different letters in the columns indicate significant differences according to Tukey's test ($p<0.05$).

time, higher DMP is expected to result in greater soil water storage capacity (Nicoloso et al., 2008), which may have been crucial for achieving higher soybean yields in drought years, such as the 2019/2020 crop season (Figure 4 B).

Higher soybean yields in grazed compared with ungrazed environments have been consistently observed in studies of the soybean-ryegrass system in the Brazilian subtropics (Soares et al., 2019; Farias et al., 2020; Simões et al., 2023). This trend was also confirmed in this study (Figure 4 A). Overall, grazed environments showed higher above-ground DMP and greater N accumulation in ryegrass DM than ungrazed environments (Figures 1 and 1 C). Alves et al. (2020) noted that grazed environments in this experimental area had higher N stocks in the soil, which likely explains the greater proportion of soil-derived N to soybean, and the subsequent impact on soybean grain yield (Figures 3 B and 4 A). The reduced grain yield in ungrazed environments may be linked to the higher energy cost for soybean symbiosis with N-fixing bacteria (Kaschuk et al., 2009), and the drought conditions during the 2019/2020 crop season, which negatively impacted BNF (Sinclair & Nogueira, 2018). In ungrazed environments, N mineralization is likely slower due to higher residual dry matter and higher C/N ratio (Figures 1 B and D). In contrast, grazed environments likely provide higher amounts of mineral N readily available in the soil solution. This promotes lower soybean %BNF, higher soil-derived N in soybean dry matter, lower soybean %BNF, and energy saving allowing the plant to redirect energy toward grain production (Figures 3 A, B and 4 A).

The PNB in ungrazed environments was less negative than in grazed environments (Figure 4 C). The differences in PNB between environments were 35, 21, 9, and 18 kg ha^{-1} N for the ryegrass N rates of 0, 50, 100, and 200 kg ha^{-1} N, respectively, regardless of P_2O_5 and K_2O replacement timing. Furthermore, each kg ha^{-1} N applied to ryegrass decreased the PNB by 0.1 kg ha^{-1} N in ungrazed environments, compared with a decrease of 0.02 kg ha^{-1} N in grazed environments.

The negative PNB values indicate the extent of N export through soybean production relative to the amount fixed by BNF. More negative PNB in grazed environments compared with ungrazed environments results primarily from higher soybean yields and a greater contribution of soil-derived N to soybean across all N rates applied in the off-season crop (Figures 3 B, 4 A, and 4 C). In ungrazed environments, increasing the N rate applied to ryegrass grown in the off-season caused the PNB to become more negative (Figure 4 D). This is mainly driven by the reduced BNF contributions as the N rate increases (Figure 4 C). Consequently, in soybean-ryegrass systems without winter grazing, strategies to replace negative PNB values, such as N fertilization in pastures, are essential.

The N delta in the system was affected by N application to ryegrass, showing positive values across all rates, regardless of ryegrass grazing and P_2O_5 and K_2O replacement timing (Figure 4 D). For every kg ha^{-1} N applied to the ryegrass, the total N delta increased by 0.4 kg ha^{-1} N.

One strategy to counter negative PNB values is to boost ryegrass DMP through N fertilization, thus elevating the N delta (Figure 4 D). This delta remained positive, even without N fertilization on ryegrass. This can be attributed to the site's history of low soybean grain yields (< 3 Mg ha^{-1}), as seen in Alves et al. (2021) and the drought during the 2019/2020 crop season, which further reduced soybean yield (Cardoso et al., 2020). Nevertheless, the increase in DMP and N accumulation in ryegrass DM due to elevated N rates, independent of grazing, and P_2O_5 and K_2O replacement, contributed to an higher N delta in the soybean-ryegrass system (Figures 1 A and C). This indicates that N fertilization in ryegrass has the potential to offset negative PNB values in soybean cultivation (Figures 4 C and D).

Conclusions

1. Increasing nitrogen fertilization of ryegrass during the winter is a strategy to increase total dry mass yield (DMP) in grazed environments. However, in an ungrazed environment, DMP reaches a limit of 130 kg ha^{-1} N.

2. Soybean grain yield in the summer is not affected by the N rates applied to ryegrass. However, higher N rates reduce the %BNF and increase soil-derived N.

3. P_2O_5 and K_2O replacement during winter is the most recommended strategy because it did not affect ryegrass yield and increased soybean yield.

4. Increasing the rate of N applied to ryegrass is a strategy to mitigate the negative PNB from N export in soybean grains, and increase the amount of N in

the system, regardless of grazing management and the period of P_2O_5 and K_2O replacement.

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Author contributions

Felipe Dalla-Zen Bertol: conceptualization, data curation, formal analysis, investigation, methodology, project administration, writing – original draft, writing – review & editing; **Carolina Bremm**: data curation, formal analysis, funding acquisition, methodology, project administration, writing – original draft, writing – review & editing; **Luciano Pinzon Brauwers**: data curation, writing – review & editing; **Lóren Pacheco Duarte**: data curation, writing – review & editing; **Gabriela Naibo**: data curation, writing – review & editing; **Edicarlos Damacena de Souza**: writing – review & editing; **Mateus Westerhofer Goulart**: data curation, writing – review & editing; **Amanda Posselt Martins**: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, writing – original draft, writing – review & editing.

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No generative artificial intelligence (AI) was used in this study.

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The authors declare no conflicts of interest.

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