

The effect of crop rotation and sheep grazing management on plant production and soil C and N stocks in a long-term integrated crop-livestock system in Southern Brazil

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Abstract

Integrated crop-livestock systems (ICLS) conducted under no-tillage have been shown to favour accumulation of carbon (C) and nitrogen (N) in the soil, but this accumulation may depends on pasture management and crop rotation used, which will affect the amount and the quality of plant residues added to the soil. The objective of this study was to evaluate the impact of two stocking methods (continuous and rotational) and sheep grazing intensities (moderate and low) of winter pasture and the effect of summer crop rotation (soybean and/or maize) on crop yield and C and N stocks in an Acrisol after 14 years of experimentation. The evaluated ICLS was set up for grain yield in the summer crop phase and for sheep meat production in the winter pasture phase. Pasture dry mass production and soybean and corn yield were evaluated throughout the experimental period. After 14 years, soil was sampled at the 0-5, 5-10, 10-20 and 20-30 cm soil layers to evaluate the content and stock of soil C and N. Higher C and N content in soil superficial layers (0-5 and 5-10 cm) were observed under low grazing intensity in the winter and soybean monoculture in the summer. The C and N stocks in 0-30 cm soil layer ranged between 39 and 45 Mg C ha-1 and 4 and 5 Mg N ha-1, respectively. The C and N stocks in the soil were significantly related to the quantity of N present in the pasture and the summer crop residues. This is probably related to the higher efficiency of microbes in using crop residues, and posterior stabilization of microbial residues through organo-mineral association in the soil. The higher content and stocks of C and N associated with low intensity grazing favoured the higher maize yield (40%) compared to moderate grazing intensity in the 2015/2016 season. According to our results, the accumulation of C and N in the soil under the ICLS involving low grazing intensity in the winter and soybean monoculture in the summer was related to the N added/recycled by the soybean and by the hibernal pasture.

Grazing intensities, Stocking methods, Soybean, Maize, Annual ryegrass
Soil Organic C Storage, Soil Organic N Storage, Crop Yield
Soil Management
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Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given: Data will be made available on request



Federal University of Rio Grande do Sul, Brazil Faculty of Agronomy Department of Soil Science

Dear Editor,

The following manuscript "The effect of crop rotation and sheep grazing management on plant production and soil C and N stocks in a long-term integrated crop-livestock system in Southern Brazil" submitted for appreciation by **Soil & Tillage Research** is grounded in a long-term experiment (14-year) that evaluate the adoption of different crop-livestock systems involving the animal effect (sheep grazing) in winter pasture phase, with different stocking methods (continuous and rotational) and grazing intensities (moderate and low), and the cropping system in the summer with monoculture (soybean/soybean) or crop rotation (soybean/maize).

The treatments used for the present study, based on soybean and sheep-meat production, were chosen because it is a viable option for many South American small farmers. In this paper we presented a series of data of soybean and maize grain yield, and ryegrass dry matter production over the years, as well as content and stock of C and N for the 30 cm soil depth after 14 years of adoption of the experiment.

The main results indicated that low grazing intensity contribute to the increase of C stocks in the soil, favouring higher maize yield than moderate grazing intensity. In addition, greater stocks of C and N was observed under monoculture of soybean than crop rotation, due to the low yield and residue quality (high C/N ratio) of maize, which is deeply discussed in the paper.

Therefore, I believe that these data are relevant at a global level and allows the improvement of our knowledge regarding pasture management and crop rotation in integrated crop-livestock systems aiming obtain systems that favour great food production while increasing accumulation of C and N in soil.

I hereby inform that the manuscript was not published previously, and its submission was approved by all authors. If the article is accepted, it won't be published electronically in the same form, in English or in any other language, without the written consent of the copyright-holder.

Yours sincerely,

Porto Alegre, Brazil, 16th September 2019.

Lucas Aquino Alves (corresponding author) PhD student in Soil Science Universidade Federal do Rio Grande do Sul 7712 Bento Gonçalves Avenue, 91540-000, Porto Alegre, Rio Grande do Sul, Brazil Phone: +55 (55) 9 9657 8147 | E-mail: <u>lucasalves.la@hotmail.com</u>



Dear Editor,

We are grateful for the editor's comments, which were of great importance to improve the final quality of the manuscript. All comments were carefully considered in the revision of the manuscript and highlighted in green. When not incorporated, our reasons for disagreeing were respectfully stated bellow.

Sincerely, The Authors

Editor's question: How? What were the fields capacity and PWP limits? And please describe methodology, depth increments and number or replicates per plot.

Author response: The sentence was incorrect, so we changed it for the reader's better understanding (lines 177–181).

Editor's question: How much do these results depend on the time sampling during the season? Can the authors say something about temporal dynamics?

Author response: As our objective was not evaluate soil organic fractions, the stocks of soil organic carbon and total nitrogen have no (or small) variation in the short term (Cecagno et al., 2018; doi:10.5039/agraria.v13i3a5553) through the seasons. Also, we were attempted to sampling soil before sowing for annual crops, because the influence of root exudates or biomass mineralization from the previous crops is the lowest (Schieung et al., 2017; doi: 10.1016/S1002-0160(17)60326-6). We have included this information in Lines 197–200.

Also, our study brings the first evaluation regarding C and N evaluation after 14-y of experiment adoption. Thus, we could not infer about the temporal dynamic of stock of C and N.

Yours sincerely,

Porto Alegre, Brazil, March 04th 2020.

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The effect of crop rotation and sheep grazing management on plant 1

production and soil C and N stocks in a long-term integrated crop-2 livestock system in Southern Brazil

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The effect of crop rotation and sheep grazing management on plant production and soil C and N stocks in a long-term integrated crop-

24 livestock system in Southern Brazil

26 Abstract

25

Integrated crop-livestock systems (ICLS) conducted under **no-till** have been shown to 27 favour the accumulation of carbon (C) and nitrogen (N) in soil. In those systems, however, 28 C and N accumulation in soil might depend on pasture management and the type of crop 29 30 rotation used. The objective of this study was to evaluate the impact of two stocking methods (continuous and rotational) and two sheep grazing intensities (moderate and low) 31 on winter pasture and the effect of summer crop rotation (soybean and maize) on crop 32 33 yield and C and N stocks in an Acrisol after 14-yr under experimental conditions. The evaluated ICLS was set up for grain yield in the summer crop phase and for sheep meat 34 35 production in the winter pasture phase. Pasture production, soybean and corn yield were 36 evaluated throughout the experimental period. After 14-yr, the soil was sampled at the 0-5, 5–10, 10–20 and 20–30 cm layers to evaluate the content and stock of C and N. Higher 37 C and N contents in soil surface layers (0–5 and 5–10 cm) were observed under low 38 grazing intensity in winter and soybean monoculture in summer. The C and N stocks in 39 0-30 cm soil layer ranged between 39 and 45 Mg C ha⁻¹ and 4 and 5 Mg N ha⁻¹, 40 respectively. C and N stocks in the soil were significantly related to N added through the 41 residues of pasture and summer crop. This positive relationship is possibly explained by 42 43 the higher efficiency of microbes in using crop residues enriched in N with posterior stabilization of microbial residues through organo-mineral association in the soil. The 44 higher content and stocks of C and N associated with low intensity grazing favoured a 45 46 higher maize yield (40%) compared to moderate grazing intensity in the 2015/2016 season. According to our results, the accumulation of C and N in the soil under ICLS 47 involving low grazing intensity in the winter and soybean monoculture in the summer 48 49 was related to the N added/recycled by the soybean and by the hibernal pasture.

50

51 Keywords: Grazing intensities, Stocking methods, Soybean, Maize, Annual ryegrass.

52 1. Introduction

53 Soil organic matter (SOM) influences the chemical, physical and biological conditions in the soil as well as determines its productive capacity (Ghosh et al., 2010; 54 55 Lal, 2004). The SOM content is affected by soil management, and crop systems with high input of plant residues such as no-tillage have been shown as strategies to favour SOM 56 57 accumulation (Bayer et al., 2006; Veloso et al., 2018). In addition to quantity, the residues 58 with better quality (higher N content) may result in a greater efficiency of substrate use by the microbiota and contribute to accumulation of C and N in the soil (Cotrufo et al., 59 2013; 2015). 60

61 Integrated crop-livestock systems (ICLS) are defined as systems in which there is temporary alternation or rotation of pastures and crops in the same area over time (Moraes 62 63 et al., 2014), contributing to the accumulation of C and N in the soil (Sá et al., 2017; Luz 64 et al., 2019). C and N accumulation depends on the stocking method, grazing intensity in the pasture phase and on the choice of summer crops (Carvalho et al., 2010). Results have 65 66 highlighted the importance of the intensity and the method of animal grazing during hibernal grazing for the accumulation of SOM, which is largely related to the impact of 67 grazing intensity on root system development (Reeder and Schuman, 2002). However, 68 lack of information exists regarding the impact of ICLS in different production 69 management systems on soil C and N stocks in subtropical environments. 70

Among the commercial crops utilized during summer in the subtropics, soybean stands out for its large cultivated area, currently estimated at 35.8 million hectares in Brazil, of which 11.9 million are cultivated in three southern states (Paraná, Santa Catarina and Rio Grande do Sul) (CONAB, 2019). The soybean residue has low C/N ratio, that in turn, may increase the N availability due to low soil immobilization by microorganisms and, therefore, favour the development of pasture after soybeans (Campos, 2015). On the other hand, the insertion of maize into crop rotation with soybeans propitiate a greater quantity of plant residues, however with a low quality because of a high C/N ratio compared to soybeans alone (Chen et al., 2018). In subtropical Brazil, maize cultivation is less attractive for local farmers due to the frequent occurrence of water deficits that strongly impact maize yield, due to its higher sensitivity to water deficit mainly at the flowering stage compared to soybeans (Steduto et al., 2012).

Thus, there is a need for results that could guide the impact of the stocking method, 83 grazing intensity in the pasture phase and the crops adopted in the cropping phase on soil 84 C and N stocks and the crop yields. Therefore, this study aimed to evaluate the impact of 85 86 grazing (stocking method and grazing intensity) in hibernal pasture and cash crops (monoculture of soybean, and crop rotation of soybean and maize) in the cropping phase 87 on soil C and N stocks, pasture production, soybean and maize yield in the Brazilian 88 89 subtropics. The study was conducted over 14-yr in an ICLS with grain and sheep-meat production in an Acrisol in the Brazilian subtropics. 90

91

92 2. Material and methods

93 2.1. Description of the experiment

A long-term study was conducted using an ICLS implanted in 2003, in the Experimental Agronomic Station of the Federal University of Rio Grande do Sul, in Eldorado do Sul county, Rio Grande do Sul state, Brazil (latitude 30°05'S, longitude 51°39'O, altitude 46 m). The local climate is classified as subtropical humid (Cfa) according to the Köppen classification (Kottek et al., 2006). The average annual rainfall over the 14-yr experimental period ranged between 1200 and 2000 mm, with an average annual temperature of 19 °C. 101 The soil was classified as a sandy clay loam Acrisol (FAO, 2006). At the 102 beginning of the experiment, the 0-10 cm soil layer presented organic C content of 17 g 103 kg⁻¹, pH-water (1:1 soil/water ratio) of 5.3; exchangeable aluminium (Al), calcium (Ca) 104 and magnesium (Mg) (KCl 1.0 mol L⁻¹) of 0.3, 2.2 and 1.2 cmol_c kg⁻¹, respectively; 105 available phosphorus (P) and potassium (K) (Mehlich 1) of 10 and 135 mg kg⁻¹, 106 respectively; base and Al saturation of 51% and 8%, respectively.

Prior to the experiment, the area was natural grassland of the Pampa Biome,
overseed with exotic species in the winter, such as ryegrass (*Lolium multiflorum*). The
area was desiccated with glyphosate herbicide and limestone was applied (1.0 Mg ha⁻¹)
to raise the soil pH (in water) in the 0-10 cm soil layer to 6.0.

At the beginning of the experimental period, the area was fertilized with 18 kg P ha⁻¹ and 33 kg K ha⁻¹, which is the amount of fertilizer needed for an expected soybean yield of 3.0 Mg ha⁻¹ (CFS-RS/SC, 1995, CQFS-RS/SC, 2004). Almost every year, ryegrass was sown at a density of 32 kg ha⁻¹ in the autumn-winter period and fertilized with nitrogen split in two applications, of 150 kg N ha⁻¹ as urea.

The experimental area of 4.5 ha was divided into 16 experimental units, varying from 0.23 to 0.32 ha per unit. The experiment was designed as randomized blocks, with four repetitions, in a 2×2 factorial system with subdivided plots. The main factors were two different grazing intensities (moderate and low) and two stocking methods (continuous and rotational), with subdivided plots representing two summer growing systems (soybean monoculture - soybean/soybean and crop rotation - soybean/maize).

During the pasture phase, the area was grazed by 11-month-old sheep weighing 35 ± 4 kg. A variable number of sheep were used following the *put-and-take* technique (Mott and Lucas, 1952) to maintain the recommended grazing intensities. Grazing intensities were defined by forage supply, expressed in kg of dry matter (DM) per 100 kg of live weight (LW) per day: (*i*) moderate grazing intensity – forage offer equivalent to 2.5 times
the forage intake potential of sheep; (*ii*) low grazing intensity – forage offer equivalent to
5.0 times the forage of sheep potential intake. The forage intake potential of lambs
according to the National Research Council (1985) is 4.0%, based on DM, low offer
equivalent to 10% of the LW and moderate offer of 20% of the LW.

The two stocking methods differing by the amount of time the sheep were present in each plot. In the continuous stocking method, the sheep remained in the area during the whole grazing period, while in the rotational, the plots were subdivided and the sheep were rotated, remaining approximately two days in each pasture range. This period was calculated in function of live ryegrass leaves and accumulated degree days.

The grass length was monitored every fifteen days using a graduated sward stick 136 (Barthram, 1985) with 30 random points measured per experimental unit during each 137 138 pasture cycle, from 2003 to 2008. After 2008, 150 random points were measured randomly to allow better estimates. Different load adjustment was adopted to maintain a 139 140 forage supply of 2.5 and 5.0 times the consumption potential. At the end of each pasture 141 cycle, the sheep were removed, and the area desiccated with glyphosate herbicide in 142 preparation for the subsequent summer sowing. The average annual fertilization applied during the 14-yr experimental period was 150 kg N ha⁻¹, 30 kg P ha⁻¹ and 50 kg K ha⁻¹, 143 fertilized during the pasture cycle. The season and annual fertilization rates can be found 144 in Alves et al. (2019). The maize was fertilized with rates of 110 and 150 kg N ha⁻¹, 145 146 respectively, only in 2009/2010 and 2011/2012 growing seasons.

In the cropping phase, each plot was divided into two subplots. Each subplot was divided in two crop systems: monoculture - soybean/soybean and crop rotation soybean/maize (50%-50%), both under no-tillage. In the first summer cycle soybean (*Glycine max*) was sown in the whole experimental area. In the second cycle soybean was sown in half the subplot and maize in the other half (subplots monoculture and crop
 rotation, respectively), with 45 cm between lines and a population of 280,000 plants ha⁻¹.
 Soybean and maize were sown from October to November.

154

155 *2.2. Crop yield*

Soybean yield was evaluated over five crop/growing seasons (2003/2004,
2004/2005, 2006/2007, 2010/2011 and 2014/2015). The maize yield was evaluated over
six crop/growing seasons (2005/2006, 2007/2008, 2009/2010, 2011/2012, 2013/2014 and
2015/2016).

160 Yield was evaluated by collecting from five random points in each subplot, each 161 point with two linear meters, totalling 4.5 m². After collection, the samples were dried 162 and the grain yield presented 130 g kg⁻¹ of moisture content.

163

164 2.3. The water requirement satisfaction index (WRSI) of the soybean and maize crops

165 The WRSI was calculated from the ratio between real (ETr) and maximum 166 evapotranspiration (ETm) of the crop (soybean or maize). When water requirement and supply meet, the WRSI is equal to 1 (ETr = ETm). When the ETr < ETm, the crop yield 167 168 may be limited by deficit water stress. The WRSI was calculated for all years from 169 October 15th to March 15th, including the period from sowing to harvesting of the summer crops (soybeans and corn) (Fig. S1). For the correlation study between WRSI and crop 170 171 yield, the reproductive period (stages R1-R6) was used as this period is critical for a good crop yield (Franchini et al. 2012). 172

The values of ETr and ETm were determined for the soybean and maize crop
cycle. The BIPZON model was used to simulate the crop hydric balance (Forest, 1984).
This model allows the input of data from converging variables such as climate (rainfall

and average daily temperature); crop (coefficient of the crop Kc); and soil (available water

177 capacity). The available water capacity was estimated considering the least limiting water

178 range, that means soil water content that do not limit plant growth, taking into account

179 field capacity, wilting point, air-filled porosity, and mechanical resistance (Silva et al.,

- 180 1994). The available water capacity was 60 mm, considering an effective root depth of
- 181 <mark>0.5 m.</mark>
- 182
- 183 *2.4. Determination of forage production*

Above-ground productivity (AGP, Mg ha⁻¹) of the pasture was evaluated in the years 2004, 2005, 2006, 2007, 2010, 2011, 2012, 2015 and 2016. The AGP was calculated by using the accumulated forage mass at the beginning of the grazing period, added to the forage accumulation rate (kg day⁻¹) and multiplied by the number of grazing days, over an average period of 101 ± 9 days.

The residual aboveground forage mass (RFM, Mg ha⁻¹) was calculated at the end of the grazing period in the years 2010, 2011, 2012, 2015 and 2016, using subsamples. Samples were collected randomly using a $0.25m^2$ metal frame (0.5 m × 0.5 m). All the remaining above ground forage, was cut and dried in an oven with air circulation at 65 °C for 72 hours. After drying, the material was weighed and the results expressed as Mg ha⁻¹.

- 195
- 196 2.5. Soil sampling and analyses of the C and N content of the soil

197 The soil was sampled in June, after the soybean harvest and before the ryegrass 198 sowing, because at this time the influence of root exudates or mineralization of biomass 199 from previous crops on the C and N contents in the soil is the lowest (Schieung et al., 200 2017). The soil was sampled with an auger at the layers 0–5, 5–10, 10–20 and 20–30 cm.

The samples were dried in a forced-air oven at 45 °C. The larger lumps broken up, ground 201 202 and sieved with a 2.0 mm sieve. A 2.0 g subsample was ground and sieved to <0.100 mm. The soil C and N contents were analysed by dry combustion in a Fisher Scientific 203 204 FlashEA®. Bulk density was measured using the volumetric ring method (Blake and Hartge, 1986), in the 0-10, 10-20 and 20-30 cm layers. Then, the C and N stocks in the 205 0-30 cm layer were calculated by the soil mass method (Ellert and Bettany, 1995). The 206 207 soil used as the reference was sampled from the treatment with moderate grazing intensity under the continuous stocking method and soybean monoculture. The soil density in the 208 0-10 cm layer was used in the calculation of the C and N stocks in the 0-5 and 5-10 cm 209 210 layers.

Soil C accumulation rates were calculated with the ratio between the C stock of the soil and the soil with the lowest C stock (crop rotation, moderate grazing intensity and continuous stocking method) over the duration of the experiment (14-yr).

214

215 2.6. Statistical analyses

Statistical analysis was performed with SAS® 9.4 software and the results were subjected to a normality analysis by the Shapiro-Wilk test and the homogeneity of variances by the Levene test, both at a significance level of 5%. The results were submitted to ANOVA at a 5% significance level (ANOVA, P> 0.05). When significant, the difference between the treatment means was evaluated by the Tukey test, also at 5%.

A factorial model with split plots was used for the analysis of soybean yield and RFM. The effects were two stocking methods and two grazing intensities, with the cropping systems subdivided in plots, considering the effects of the block (B), the stocking method (M), grazing intensity (I), cropping system (S), the interactions M*I, M*S, I*S, M*I*S and the year as a repeated measure of time. A factorial model was used

for the analysis of maize yield and AGP, considering the B, M, I effects and the M*I 226 227 interaction, and the year as a repeated measure of time. The factorial model used subdivided sub-plots for the C and N content in the soil, with the soil layer as the 228 subdivided sub-plot, considering the effects B, M, I, S, soil layer (C) and the interaction 229 between M*I, M*S, I*S, M*I*S, M*C, I*C, S*C, M*I*C, M*S*C, I*S*C and M*I*S*C. 230 For the stocks and the accumulation rates of C and N in the soil, considering the effects 231 232 B, M, I, S and the interaction between M*I, M*S, I*S and M*I*S. In all models, the B effect and its interactions were considered random effects and the other factors were 233 considered fixed effects. 234

The significance of the relationship between C and N content in the soil and C and N inputs by crops and pasture were evaluated using Pearson's correlation coefficients (r) at the 5% level.

238

239 **3. Results**

240 *3.1. Crop and forage production*

Soybean yield was not affected by the cropping system (Tables 1 and 2). In two 241 (2003/2004 and 2006/2007) of the five harvests evaluated, soybean yield was not affected 242 243 by stocking method and grazing intensity. In the 2004/2005 season, the adoption of rotational stocking method, favour greater soybean yield in continuous-moderate (30%) 244 and continuous-low (272%) (Tables 1 and 2). On the other hand, in the 2010/2011 245 season, lower soybean yield was observed in the continuous-moderate treatment 246 compared to the other treatments. In the 2014/2015 season, independent of the grazing 247 intensity, the average sovbean yield observed in rotational stocking method was 48% 248 greater than the continuous stocking method (Table 2). The highest soybean yield during 249

the experimental period was observed in the 2014/2015 season, with an average of 3.0
Mg ha⁻¹.

Maize yield was slightly affected by the stocking methods and grazing intensities. Maize yield was low throughout the evaluation period, ranging from 1.9 Mg ha⁻¹ in the 2007/2008 season to 7.7 Mg ha⁻¹ in the 2015/2016 season (**Fig. 1**). Although maize showed a tendency for higher yields at low grazing intensity, this result was significant only in the 2015/2016 season, which also had the highest maize yields compared to other seasons (**Fig. 1**). In the 2015/2016 season, grain yield in low grazing intensity was 40% higher (2.4 Mg ha⁻¹) than in moderate intensity.

259 Fig. 2A presents the relationship between WRSI and maize yield under different grazing intensities (moderate and low). At low grazing intensity, for every 0.1 unit 260 increase in WRSI, maize yield increased by 0.97 Mg ha⁻¹, versus 0.64 Mg ha⁻¹ at moderate 261 262 grazing intensity. In Fig. 2B, the relationship between WRSI and soybean yield is presented for the combination of stocking method and grazing intensity: continuous-low, 263 264 continuous-moderate, rotational-low and rotational-moderate. Under continuous stocking 265 method with low grazing intensity, the increase of 0.1 unit increase in WRSI favoured the increase of soybean yield by 0.24 Mg ha⁻¹ versus 0.20 Mg ha⁻¹ under continuous stocking 266 267 with moderate grazing intensity. The relationship was not significant under rotational stocking. 268

AGP ranged from 4.8 to 16.8 Mg ha⁻¹ and was affected by grazing intensity (Fig.
3). Over the experimental period, AGP in low intensity grazing (11.1 Mg ha⁻¹) was 32%
higher than in moderate grazing intensity (8.4 Mg ha⁻¹) (Fig. 3). RFM aboveground was
affected by grazing intensity and cropping system over the experimental period (Table
1). RFM mean was 7% higher in soybean monoculture (3.1 Mg ha⁻¹) than in crop rotation

(2.8 Mg ha⁻¹) (Fig. 4). Regarding grazing intensities, low grazing intensity (3.4 Mg ha⁻¹)
resulted in 28% more residue than moderate intensity (2.7 Mg ha⁻¹) (Fig. 4).

- 276
- 277 *3.2. Soil C and N content and stocks*

C and N content in the surface soil layers (0–5 and 5–10 cm) were affected by 278 cropping systems, as well as grazing intensities (Table 1 and Fig. 5). In the 0-10 cm 279 layer, the average C concentration under low grazing intensity was 20% higher (14.1 g 280 kg⁻¹) than under moderate grazing intensity (11.8 g kg⁻¹) (Fig. 5B). Using the average of 281 the two layers (0–5 and 5–10 cm), the highest N concentration was observed under low 282 283 grazing intensity (1.5 g kg⁻¹) compared to moderate grazing intensity (1.2 g kg⁻¹) (Fig. 284 5D). Greater C and N concentration in 0-5 and 5-10 cm soil layers was observed in soybean monoculture, averaging in the 0–10 cm layer 14.3 and 1.6 g kg⁻¹ of C and N, 285 respectively, which is around 22 and 27% greater than under the crop rotation system 286 (Fig. 5A and 5C). 287

C and N stocks in the 0–30 cm soil layer ranged from 39 to 45 Mg ha⁻¹ and 3.9 to 288 4.9 Mg ha⁻¹, respectively, and were affected by cropping system and grazing intensities. 289 290 Higher C and N stock in 0-30 cm soil layer was observed in soybean monoculture (45 291 and 5 Mg ha⁻¹, respectively) compared to crop rotation system (39 and 4 Mg ha⁻¹, respectively) (Table 4). This led to significant accumulation rates of 500 kg C ha⁻¹ yr⁻¹ 292 and 78 kg N ha⁻¹ yr⁻¹ in the soil under soybean monoculture (**Table 4**). In addition, C and 293 N stock was 11 and 9% higher, respectively, under low grazing intensity compared to 294 moderate grazing. Soil accumulation rates were 500 kg C ha⁻¹ yr⁻¹ and 57 kg N ha⁻¹ yr⁻¹ 295 under low intensity and 200 kg C ha⁻¹ yr⁻¹ and 27 kg N ha⁻¹ yr⁻¹ under moderate grazing 296 297 intensity, respectively (Table 4).

298 Significant relationships between the input of C (Fig. 6A) and N (Fig. 6B) via 299 crop residues from each cropping system, monoculture and crop rotation, and the C stock were observed. Each 1000 kg of C annually inputted via above-ground biomass, the C stock increased by 14.5 kg ha⁻¹ in soybean monoculture, versus 4.5 kg ha⁻¹ in the crop rotation system. For N inputs, each 10 kg of N annually inputted via above-ground biomass, the N stock increased by 0.53 Mg ha⁻¹ in soybean monoculture, versus 0.30 Mg ha⁻¹ in the crop rotation system.

305

306 **4. Discussion**

Our study showed that low grazing intensities favour significant increases in soil 307 C and N stocks in subtropical environments, which corroborate to previous studies (Souza 308 309 et al., 2009; Assmann et al., 2014). In our study, considering the 0-30 cm soil layer, the highest rates of C and N accumulation were observed in the systems with low intensity 310 311 grazing and soybean monoculture (Table 4). This higher soil accumulation is associated 312 with higher C and N input via crop residues at low compared to moderate grazing intensity (annual difference 2.7 Mg ha⁻¹), as demonstrated by the higher AGP (Table 3 and Fig. 313 314 3). This higher C and N input in low grazing intensity is due to lower forage intake by the 315 lower number of sheep, leading consequently to higher AGP and RFM (Fig. 3 and 4).

316 Soybean monoculture promoted greater increase in soil C when compared to crop 317 rotation. Although the relationship between soil C stock and C input was significant in both soybean monoculture and crop rotation systems (Fig. 6A), for each kg of C added 318 annually via plant residues, there was an increase of 14.5 kg C ha⁻¹ under soybean 319 monoculture and only 4.5 kg C ha⁻¹ under crop rotation. In addition, N input via residues 320 affected soil C stocks under both cultivation systems. Each kg of N added annually via 321 plant residues generated greater increase under soybean monoculture ($530 \text{ kg} \text{ C} \text{ ha}^{-1}$) than 322 under crop rotation (300 kg C ha⁻¹). (Fig. 6B). Thus, our results suggest that residue 323 quality was the main driver for soil C accumulation, as observed by Manzoni et al. 324

(2008), which is possibly associated to greater efficiency of microorganisms in stabilizing
C from labile (higher N content) plant residue into microbial residues that later might be
stabilized in organo-mineral association (Cotrufo et al., 2013, 2015; Veloso et al., 2019,
2020).

The effect of higher N supply for forage production in succession, is probably due 329 to the high biological N fixation which is used to supply the needs of the soybean crop, 330 331 therefore reducing the dependence on N from mineralization of the SOM (Ciampitti and Salvagiotti, 2018). The higher N supply available for the subsequent pasture cultivation 332 in the soybean monoculture system is associated to both lower C/N ratio and higher N 333 334 input through crop residues to the soil, favouring then higher RFM compared to maize. Varvel and Wilhelm (2003) also observed that the soybean crop could contribute larger 335 336 quantities of N to subsequent crops, giving better conditions for the development of 337 pasture in contrast to maize. In addition, the absence of N fertilization under maize 338 (except for the 2009/2010 and 2011/2012 seasons), probably contributed to high N 339 immobilization, negatively affecting the initial development of the successive hibernal pasture (Campos, 2015) when compared to the soybean monoculture system. 340

Mainly due to the frequent droughts (as indicated by the low WRSI observed during the majority of the crop seasons - **Fig. 2**), the average C input over the experimental period was 2.4 Mg ha⁻¹ in the crop rotation system (**Table 3**), very similar to that observed in the soybean monoculture. Although maize adoption in cropping systems in subtropical environments is a viable alternative with a potential for a C input higher than 4 Mg ha⁻¹ (Veloso et al., 2018), the C input by maize in this study was much lower than expected. The greater maize grain yield after low intensity grazing in 2015/2016 season

(Fig. 1) is possibly related to the higher C and N soil content in this system obtained in
the long-term compared to grazing at moderate intensity (Table 4). It can also be

explained by the higher WRSI, which showed a linear relationship with yield (**Fig. 2**). Thus, the system using low grazing intensity, which had a higher capacity to supply the nutritional needs, especially N due to the larger soil stock, was more favoured under superior water supply conditions. (**Fig. 2**).

The soybean grain yield did not respond positively to increases in soil N levels 354 caused by the adoption of low grazing intensities. (Table 2). This is mainly due to the 355 356 fact that the treatments in this study do not affect other soil chemical properties (Alves et 357 al, 2019) and because soybeans obtain much of their N requirement from biological N fixation, depending less on N soil supply (Ciampitti and Salvagiotti, 2018) in contrast to 358 359 maize. However, soybean yield was affected by the different pasture management methods and grazing intensities (Table 2). The rotational method, independent of the 360 361 grazing intensity, resulted in higher soybean yield when compared to the continuous 362 method adopted in the 2004/2005 and 2014/2015 seasons (Table 2). The higher soybean yield in the rotational stocking method can be explained with the fact that continuous 363 364 grazing can affect spatial patterns of the soil attributes, increasing spatial heterogeneity 365 of the pastures and the environment (Laca, 2009, Auerswald et al., 2010). As the animals circulate freely selecting grazing areas, manure is deposited unevenly influencing the 366 367 concentration of nutrients in the soil (McNaughton, 1985; Augustine & Frank, 2001), with the highest concentrations being found near to the rest areas, shade and water troughs 368 (Dennis et al. 2012; Dubeux et al., 2014). This does not happen using the rotational 369 370 stocking method, where the animals are placed in grazing ranges that rotate in space and time, favouring the better distribution of manure within the grazing range (Peterson and 371 Gerrish, 1996), and achieving better control of spatial nutrient cycling and soil fertility. 372

373

374 5. Conclusions

The aboveground productivity of pasture and the maize yield are higher in the system with low grazing intensity during the pasture phase. In contrast, soybean presented higher yields when the rotational pasture method was used in the winter, regardless of the grazing intensity. The residual forage mass in the pasture phase was favoured by the soybean monoculture system in the summer.

Low grazing intensity in the pasture phase favoured the increase in the C and N 380 381 stocks in the soil. The pasture management method, continuous or rotational, did not 382 affect the accumulation of C and N in the soil. The higher quality of soybean crop residues and similar annual input of C determined higher soil C and N stocks, in comparison to 383 384 the rotation soybean/maize. Thus, our results provided field-based evidence that crop residues with greater N content, favoured soil C accumulation. Maize yield was low in 385 386 most of the seasons due to the low water requirement satisfaction index, reducing the 387 potential for the accumulation of C and N in the soil under rotation soybean/maize.

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Highlights

Grazing intensity and stocking method effect on soil C and N stock were evaluated Low grazing intensity resulted in higher soil C and N stocks in long-term Residues quality (C : N ratio) played a key role in soil C accumulation Higher soil N stocks in low grazing intensity favored maize yield



Graphical Abstract. Schematic map of carbon and nitrogen inputs in the soil via crop and pasture residues and the respective carbon and nitrogen stocks in the 0–30 cm soil layer influencing maize yield in Southern Brazil.

1 The effect of crop rotation and sheep grazing management on plant

production and soil C and N stocks in a long-term integrated crop livesteek system in Southern Prezil

- 3 livestock system in Southern Brazil
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The effect of crop rotation and sheep grazing management on plant production and soil C and N stocks in a long-term integrated croplivestock system in Southern Brazil

25

26 Abstract

Integrated crop-livestock systems (ICLS) conducted under no-tillage have been shown to 27 favour the accumulation of carbon (C) and nitrogen (N) in soil. In that systems, however, 28 C and N accumulation in soil might depend on pasture management and the type of crop 29 30 rotation used. The objective of this study was to evaluate the impact of two stocking methods (continuous and rotational) and two sheep grazing intensities (moderate and low) 31 on winter pasture and the effect of summer crop rotation (soybean and/or maize) on crop 32 33 yield and C and N stocks in an Acrisol after 14-yr under experimental conditions. The evaluated ICLS was set up for grain yield in the summer crop phase and for sheep meat 34 35 production in the winter pasture phase. Pasture production, soybean and corn yield were 36 evaluated throughout the experimental period. After 14-yr, the soil was sampled at the 0-5, 5-10, 10-20 and 20-30 cm layers to evaluate the content and stock of C and N. Higher 37 C and N contents in soil superficial layers (0–5 and 5–10 cm) were observed under low 38 grazing intensity in winter and soybean monoculture in summer. The C and N stocks in 39 0-30 cm soil layer ranged between 39 and 45 Mg C ha⁻¹ and 4 and 5 Mg N ha⁻¹, 40 respectively. C and N stocks in the soil were significantly related to N added through the 41 residues of pasture and summer crop. This positive relationship is possibly explained by 42 43 the higher efficiency of microbes in using crop residues enriched in N with posterior stabilization of microbial residues through organo-mineral association in the soil. The 44 higher content and stocks of C and N associated with low intensity grazing favoured a 45 46 higher maize yield (40%) compared to moderate grazing intensity in the 2015/2016 season. According to our results, the accumulation of C and N in the soil under ICLS 47 involving low grazing intensity in the winter and soybean monoculture in the summer 48 49 was related to the N added/recycled by the soybean and by the hibernal pasture.

50

51 Keywords: Grazing intensities, Stocking methods, Soybean, Maize, Annual ryegrass.

52 1. Introduction

53 Soil organic matter (SOM) influences the chemical, physical and biological conditions in the soil as well as determining its productive capacity (Ghosh et al., 2010; 54 Lal, 2004). The SOM content is affected by soil management, especially no-tillage, and 55 crop systems with high input of plant residues have been shown as strategies to favour 56 57 SOM accumulation (Bayer et al., 2006; Veloso et al., 2018). In addition to quantity, the residues with better quality (greater N content) may result in a greater efficiency of 58 substrate use by the microbiota and contributing to accumulation of C and N in the soil 59 (Cotrufo et al., 2013; 2015). 60

61 Integrated crop-livestock systems (ICLS) are defined as systems in which there is temporary alternation or rotation of pastures and crops in the same area over time (Moraes) 62 et al., 2014), contributing to the accumulation of C and N in the soil (Sá et al., 2017; Luz 63 64 et al., 2019). C and N accumulation is dependent on the stocking method, grazing intensity in the pasture phase and on the choice of summer crops (Carvalho et al., 2010). 65 66 Results have highlighted the importance of the intensity and the method of animal grazing during hibernal grazing on the accumulation of SOM, which is largely related to the 67 68 impact of grazing intensity on root system development (Reeder and Schuman, 2002). However, there is a lack of information regarding the impact of ICLS with different 69 production management systems on soil C and N stocks in subtropical environments. 70 Among the commercial crops utilized during summer in the subtropics, soybean 71

stands out for its large cultivated area, currently estimated at 35.8 million hectares in Brazil, of which 11.9 million are cultivated in three southern states (Paraná, Santa Catarina and Rio Grande do Sul) (CONAB, 2019). The soybean residue has low C/N ratio, that in turn, may increase N availability due to lower soil immobilization by microorganisms and, therefore, favour the development of pasture after soybeans (Campos, 2015). On the other hand, the insertion of maize into crop rotation with
soybeans propitiate a greater quantity of plant residues but with a lower quality (higher
C/N ratio) compared to soybeans alone (Chen et al., 2018). In subtropical Brazil, maize
cultivation is less attractive for local farmers due to the frequent occurrence of water
deficits that impact more strongly maize yield, due to its higher sensitivity to water deficit
mainly at the flowering stage compared to soybeans (Steduto et al., 2012).

- Thus, there is a need for results that could guide the impact of the stocking method, 83 grazing intensity in the pasture phase and the crops adopted in the cropping phase on soil 84 C and N stocks and the crops yield. Therefore, this study was aimed to evaluate the impact 85 86 of grazing (stocking method and grazing intensity) in hibernal pasture and cash crops (monoculture of soybean, and crop rotation of soybean and maize) in the cropping phase 87 on soil C and N stocks, pasture production, soybean and maize yield in the Brazilian 88 89 subtropics. The study was conducted over 14-yr in an ICLS with grain and sheep-meat production in an Acrisol in the Brazilian subtropics. 90
- 91

92 2. Material and methods

93 2.1. Description of the experiment

A long-term study was conducted using an ICLS implanted in 2003, in the Experimental Agronomic Station of the Federal University of Rio Grande do Sul, in Eldorado do Sul county, Rio Grande do Sul state, Brazil (latitude 30°05'S, longitude 51°39'O, altitude 46 m). The local climate is classified as subtropical humid (Cfa) according to the Köppen classification (Kottek et al., 2006). The average annual rainfall over the 14-yr experimental period ranged between 1200 and 2000 mm, with an average annual temperature of 19 °C. 101 The soil was classified as a sandy clay loam Acrisol (FAO, 2006). At the 102 beginning of the experiment, the 0-10 cm soil layer presented organic C content of 17 g 103 kg⁻¹, pH-water (1:1 soil/water ratio) of 5.3; exchangeable aluminium (Al), calcium (Ca) 104 and magnesium (Mg) (KCl 1.0 mol L⁻¹) of 0.3, 2.2 and 1.2 cmol_c kg⁻¹, respectively; 105 available phosphorus (P) and potassium (K) (Mehlich 1) of 10 and 135 mg kg⁻¹, 106 respectively; base and Al saturation of 51% and 8%, respectively.

Prior to the experiment, the area was natural grassland of the Pampa Biome, with exotic species in the winter, such as ryegrass (*Lolium multiflorum*). The area was desiccated with glyphosate herbicide and limestone was applied (1.0 Mg ha⁻¹) to raise the soil pH (in water) in the 0-10 cm soil layer to 6.0.

At the beginning of the experimental period, the area was fertilized with 18 kg P ha⁻¹ and 33 kg K ha⁻¹, which is the amount of fertilizer needed for an expected soybean yield of 3.0 Mg ha⁻¹ (CFS-RS/SC, 1995, CQFS-RS/SC, 2004). Almost every year, ryegrass was sown at a density of 32 kg ha⁻¹ in the autumn-winter period and fertilized with nitrogen split in two applications, of 150 kg N ha⁻¹ as urea.

The experimental area of 4.5 ha was divided into 16 experimental units, varying from 0.23 to 0.32 ha per unit. The experiment was designed as randomized blocks, with four repetitions, in a 2×2 factorial system with subdivided plots. The main factors were two different grazing intensities (moderate and low) and two stocking methods (continuous and rotational), with subdivided plots representing two summer growing systems (soybean monoculture - soybean/soybean and crop rotation - soybean/maize).

During the pasture phase, the area was grazed by 11 month-old sheep weighing 35 ± 4 kg. A variable number of sheep were used following the *put-and-take* technique (Mott and Lucas, 1952) to maintain the recommended grazing intensities. Grazing intensities were defined by forage supply, expressed in kg of dry matter (DM) per 100 kg of live weight (LW) per day: (*i*) moderate grazing intensity – forage offer equivalent to 2.5 times
the forage intake potential of sheep; (*ii*) low grazing intensity – forage offer equivalent to
5.0 times the forage of sheep potential intake. The forage intake potential of lambs
according to the National Research Council (1985) is 4.0%, based on DM, low offer
equivalent to 10% of the LW and moderate offer of 20% of the LW.

The two stocking methods differed by the amount of time the sheep were present in each plot. In the continuous stocking method, the sheep remained in the area during the whole grazing period, while in the rotational, the plots were subdivided and the sheep were rotated, remaining approximately two days in each pasture range. This period was calculated in function of live ryegrass leaves and accumulated degree days.

The grass length was monitored every fifteen days using a graduated sward stick 136 (Barthram, 1985) with 30 random points measured per experimental unit during each 137 138 pasture cycle, from 2003 to 2008. After 2008, 150 random points were measured randomly to allow better estimates. Different load adjustment was adopted to maintain a 139 140 forage supply of 2.5 and 5.0 times the consumption potential. At the end of each pasture 141 cycle, the sheep were removed, and the area desiccated with glyphosate herbicide in 142 preparation for the subsequent summer sowing. The average annual fertilization applied during the 14-yr experimental period was 150 kg N ha⁻¹, 30 kg P ha⁻¹ and 50 kg K ha⁻¹, 143 fertilized during the pasture cycle. The season and annual fertilization rates can be found 144 in Alves et al. (2019). The maize was fertilized with rates of 110 and 150 kg N ha⁻¹, 145 146 respectively, only in 2009/2010 and 2011/2012 growing seasons.

In the cropping phase, each plot was divided into two subplots. Each subplot was
divided in two crop systems: monoculture - soybean/soybean and crop rotation soybean/maize (50%-50%), both under no-tillage. In the first summer cycle soybean
(*Glycine max*) was sown in the whole experimental area. In the second cycle soybean was

sown in half the subplot and maize in the other half (subplots monoculture and crop
 rotation, respectively), with 45 cm between lines and a population of 280,000 plants ha⁻¹.
 Soybean and maize were sown from October to November.

154

155 *2.2. Crop yield*

Soybean yield was evaluated over five crop/growing seasons (2003/2004,
2004/2005, 2006/2007, 2010/2011 and 2014/2015). The maize yield was evaluated over
six crop/growing seasons (2005/2006, 2007/2008, 2009/2010, 2011/2012, 2013/2014 and
2015/2016).

160 Yield was evaluated by collecting from five random points in each subplot, each 161 point with two linear meters, totalling 4.5 m². After collection, the samples were dried 162 and the grain yield presented 130 g kg⁻¹ of moisture content.

163

164 2.3. The water requirement satisfaction index (WRSI) of the soybean and maize crops

165 The WRSI was calculated from the ratio between real (ETr) and maximum 166 evapotranspiration (ETm) of the crop (soybean or maize). When water requirement and supply meet, the WRSI is equal to 1 (ETr = ETm). When the ETr < ETm, the crop yield 167 may be limited by deficit water stress. The WRSI was calculated for all years from 168 October 15 to March 15, including the period from sowing to harvesting of the summer 169 crops (soybeans and corn) (Fig. S1). For the correlation study between WRSI and crop 170 yield, the reproductive period (stages R1-R6) was used as this period is critical for a good 171 crop yield (Franchini et al. 2012). 172

The values of ETr and ETm were determined for the soybean and maize crop cycle. The BIPZON model was used to simulate the crop hydric balance (Forest, 1984). This model allows the input of data from converging variables such as climate (rainfall and average daily temperature); crop (coefficient of the crop Kc); and soil (available water
capacity). The available water capacity was estimated for the first 50 cm of soil,
considering an effective root depth of 60 mm.

179

180 *2.4. Determination of forage production*

Above-ground productivity (AGP, Mg ha⁻¹) of the pasture was evaluated in the years 2004, 2005, 2006, 2007, 2010, 2011, 2012, 2015 and 2016. The AGP was calculated by using the accumulated forage mass at the beginning of the grazing period, added to the forage accumulation rate (kg day⁻¹) and multiplied by the number of grazing days, over an average period of 101 ± 9 days.

The residual aboveground forage mass (RFM, Mg ha⁻¹) was calculated at the end of the grazing period in the years 2010, 2011, 2012, 2015 and 2016, using subsamples. Samples were collected randomly using a $0.25m^2$ metal frame (0.5×0.5 m). All the remaining above ground forage, was cut and dried in an oven with air circulation at 65 °C for 72 hours. After drying, the material was weighed and the results expressed as Mg ha⁻¹.

192

193 2.5. Soil sampling and analyses of the C and N content of the soil

The soil was sampled with an auger at the layers 0–5, 5–10, 10–20 and 20–30 cm. The samples were dried in a forced-air oven at 45 °C. The larger lumps broken up, ground and sieved with a 2.0 mm sieve. A 2.0 g subsample was ground and sieved to ≤ 0.100 mm. The soil C and N contents were analysed by dry combustion in a Fisher Scientific FlashEA[®]. Bulk density was measured using the volumetric ring method (Blake and Hartge, 1986), in the 0–10, 10–20 and 20–30 cm layers. Then, the C and N stocks in the 0–30 cm layer were calculated by the soil mass method (Ellert and Bettany, 1995). The soil used as the reference was sampled from the treatment with moderate grazing intensity under the continuous stocking method and soybean monoculture. The soil density in the 0-10 cm layer was used in the calculation of the C and N stocks in the 0-5 and 5-10 cm layers.

Soil C accumulation rates were calculated with the ratio between the C stock of the soil and the soil with the lowest C stock (crop rotation, moderate grazing intensity and continuous stocking method) over the duration of the experiment (14-yr).

208

209 *2.6. Statistical analyses*

210 Statistical analysis was performed with SAS® 9.4 software and the results were subjected to a normality analysis by the Shapiro-Wilk test and the homogeneity of 211 variances by the Levene test, both at a significance level of 5%. The results were 212 213 submitted to ANOVA at a 5% significance level (ANOVA, P> 0.05). When significant, the difference between the treatment means was evaluated by the Tukey test, also at 5%. 214 215 A factorial model with split plots was used for the analysis of soybean yield and 216 RFM. The effects were two stocking methods and two grazing intensities, with the cropping systems subdivided in plots, considering the effects of the block (B), the 217 stocking method (M), grazing intensity (I), cropping system (S), the interactions M*I, 218 M*S, I*S, M*I*S and the year as a repeated measure of time. A factorial model was used 219 for the analysis of maize yield and AGP, considering the B, M, I effects and the M*I 220 221 interaction, and the year as a repeated measure of time. The factorial model used subdivided sub-plots for the C and N content in the soil, with the soil layer as the 222 subdivided sub-plot, considering the effects B, M, I, S, soil layer (C) and the interaction 223 between M*I, M*S, I*S, M*I*S, M*C, I*C, S*C, M*I*C, M*S*C, I*S*C and M*I*S*C. 224 For the stocks and the accumulation rates of C and N in the soil, considering the effects 225

B, M, I, S and the interaction between M*I, M*S, I*S and M*I*S. In all models, the B effect and its interactions were considered random effects and the other factors were considered fixed effects.

The significance of the relationship between C and N content in the soil and C and N inputs by crops and/or pasture was evaluated using Pearson's correlation coefficients (r) at the 5% level.

232

233 **3. Results**

234 *3.1. Crop and forage production*

235 Soybean yield was not affected by the cropping system (Tables 1 and 2). In two (2003/2004 and 2006/2007) of the five harvests evaluated, soybean yield was not affected 236 by stocking method and grazing intensity. In the 2004/2005 season, the adoption of 237 238 rotational stocking method, favour greater soybean yield in continuous-moderate (30%) and continuous-low (272%) (Tables 1 and 2). On the other hand, in the 2010/2011 239 240 season, lower soybean yield was observed in the continuous-moderate treatment 241 compared to the other treatments. In the 2014/2015 season, independent of the grazing intensity, the average soybean yield observed in rotational stocking method was 48% 242 243 greater than continuous stocking method (Table 2). The highest soybean yield during the experimental period was observed in the 2014/2015 season, with an average of 3.0 Mg 244 ha ⁻¹. 245

Maize yield was slightly affected by the stocking methods and grazing intensities. Maize yield was low throughout the evaluation period, ranging from 1.9 Mg ha⁻¹ in the 2007/2008 season to 7.7 Mg ha⁻¹ in the 2015/2016 season (**Fig. 1**). Although maize showed a tendency for higher yields at low grazing intensity, this result was significant only in the 2015/2016 season, which also had the highest maize yields compared to other seasons (Fig. 1). In the 2015/2016 season, grain yield in low grazing intensity was 40%
higher (2.4 Mg ha⁻¹) than in moderate intensity.

Fig. 2A presents the relationship between WRSI and maize yield under different 253 grazing intensities (moderate and low). At low grazing intensity, for every 0.1 unit 254 increase in WRSI, maize yield increased by 0.97 Mg ha⁻¹, versus 0.64 Mg ha⁻¹ at moderate 255 256 grazing intensity. In Fig. 2B, the relationship between WRSI and soybean yield is 257 presented under combination of stocking method and grazing intensity: continuous-low, 258 continuous-moderate, rotational-low and rotational-moderate. Under continuous stocking method with low grazing intensity, the increase of 0.1 unit increase in WRSI favoured the 259 increased of soybean yield by 0.24 Mg ha⁻¹ versus 0.20 Mg ha⁻¹ under continuous stocking 260 method with moderate grazing intensity. The relationship was not significant under 261 262 rotational stocking method.

AGP ranged from 4.8 to 16.8 Mg ha⁻¹ and was affected by grazing intensity (Fig.
3). Over the experimental period, AGP in low intensity grazing (11.1 Mg ha⁻¹) was 32%
higher than in moderate grazing intensity (8.4 Mg ha⁻¹) (Fig. 3). RFM aboveground was
affected by grazing intensity and cropping system over the experimental period (Table
1). RFM mean was 7% higher in soybean monoculture (3.1 Mg ha⁻¹) than in crop rotation
(2.8 Mg ha⁻¹) (Fig. 4). Regarding grazing intensities, low grazing intensity (3.4 Mg ha⁻¹)
resulted in 28% more residue than moderate intensity (2.7 Mg ha⁻¹) (Fig. 4).

270

271 *3.2. Soil C and N content and stocks*

C and N content in the superficial soil layers (0-5 and 5-10 cm) were affected by cropping systems, as well as grazing intensities (**Table 1 and Fig. 5**). In the 0–10 cm layer, the average C concentration under low grazing intensity was 20% higher (14.1 g kg⁻¹) than under moderate grazing intensity (11.8 g kg⁻¹) (**Fig. 5B**). Using the average of the two layers (0–5 and 5–10 cm), the highest N concentration were observed under low grazing intensity (1.5 g kg⁻¹) compared to under moderate grazing intensity (1.2 g kg⁻¹)
(Fig. 5D). Greater C and N concentration in 0–5 and 5–10 cm soil layers was observed in
soybean monoculture, averaging in the 0–10 cm layer 14.3 and 1.6 g kg⁻¹ of C and N,
respectively, which is around 22 and 27% greater than crop rotation system (Fig. 5A and
5C).

C and N stocks in the 0–30 cm soil layer ranged from 39 to 45 Mg ha⁻¹ and 3.9 to 282 283 4.9 Mg ha⁻¹, respectively, and were affected by cropping system and grazing intensities. 284 Higher C and N stock in 0–30 cm soil layer was observed in soybean monoculture (45 and 5 Mg ha⁻¹, respectively) compared to crop rotation system (39 and 4 Mg ha⁻¹, 285 respectively) (Table 4). This led to significant accumulation rates of 500 kg C ha⁻¹ yr⁻¹ 286 and 78 kg N ha⁻¹ yr⁻¹ in the soil under soybean monoculture (**Table 4**). In addition, C and 287 N stock was 11 and 9% higher, respectively, under low grazing intensity compared to 288 moderate grazing. Soil accumulation rates was 500 kg C ha⁻¹ yr⁻¹ and 57 kg N ha⁻¹ yr⁻¹ 289 under low intensity and 200 kg C ha⁻¹ yr⁻¹ and 27 kg N ha⁻¹ yr⁻¹ under moderate grazing 290 291 intensity (Table 4).

Significant relationships between the input of C (Fig. 6A) and N (Fig. 6B) via crop residues from each cropping system, monoculture and crop rotation, and the C stock were observed. Each 1000 kg of C annually inputted via above-ground biomass, the C stock increased by 14.5 kg ha⁻¹ in soybean monoculture, versus 4.5 kg ha⁻¹ in the crop rotation system. For N inputs, each 10 kg of N annually inputted via above-ground biomass, the N stock increased by 0.53 Mg ha⁻¹ in soybean monoculture, versus 0.30 Mg ha⁻¹ in the crop rotation system.

299

300 **4. Discussion**

Our study showed that low grazing intensities favour significant increases in soil 301 C and N stocks in subtropical environments, which corroborate to previous studies (Souza 302 et al., 2009; Assmann et al., 2014). In our study, considering the 0–30 cm soil layer, the 303 highest rates of C and N accumulation were observed in the systems with low intensity 304 grazing and soybean monoculture (Table 4). This higher soil accumulation is associated 305 with higher C and N input via crop residues at low compared to moderate grazing intensity 306 307 (annual difference 2.7 Mg ha⁻¹), as demonstrated by the higher AGP (Table 3 and Fig. 3). This higher C and N input in low grazing intensity is due to lower forage intake by the 308 lower number of sheep, leading consequently to higher AGP and RFM (Fig. 3 and 4). 309 310 Soybean monoculture promoted greater increase in soil C when compared to crop rotation. Although the relationship between soil C stock and C input was significant in 311 312 both soybean monoculture and crop rotation systems (Fig. 6A), for each kg of C added 313 annually via plant residues, there was an increase of 14.5 kg C ha⁻¹ under soybean monoculture and 4.5 kg C ha⁻¹ under crop rotation. In addition, N input via residues 314 315 caused alterations in the soil C stocks under both cultivation systems. Each kg of N added 316 annually via plant residues generated greater increase under soybean monoculture (0.53 Mg C ha⁻¹) than under crop rotation (0.30 Mg C ha⁻¹). (Fig. 6B). Thus, our results suggests 317 that residue quality was the main driver for soil C accumulation, as observed by Manzoni 318 et al. (2008), which is possibly associated to greater efficiency of microorganims in 319 stabilizing C from labile (higher N content) plant residue into microbial residues that later 320 might be stabilized in organo-mineral association (Cotrufo et al., 2013, 2015, Veloso et 321 al., 2019). 322

The effect of higher N supply for forage production in succession, is probably due to the high biological N fixation which is used to supply the needs of the soybean crop, therefore reducing the dependence on N from mineralization of the SOM (Ciampitti and

Salvagiotti, 2018). The higher N supply available for the subsequent pasture cultivation 326 327 in sovbean monoculture system is associated to both lower C/N ratio and higher N input through crop residues to the soil, favouring then higher RFM compared to maize. Varvel 328 and Wilhelm (2003) also observed that the soybean crop could contribute larger quantities 329 of N to subsequent crops, giving better conditions for the development of pasture in 330 contrast to maize. In addition, the absence of N fertilization under maize (except for the 331 2009/2010 and 2011/2012 seasons), probably contributed to high N immobilization, 332 negatively affecting the initial development of the successive hibernal pasture (Campos, 333 334 2015) when compared to soybean monoculture system.

Mainly due to the frequent droughts (as indicated by the low WRSI observed during the majority of the crop seasons - **Fig. 2**), the average C input over the experimental period was 2.4 Mg ha⁻¹ in the crop rotation system (**Table 3**), very similar to that observed in the soybean monoculture. Although maize adoption in cropping systems in subtropical environments is a viable alternative with a potential for a C input higher than 4 Mg ha⁻¹ (Veloso et al, 2018), the C input by maize in this study was much lower than expected.

The greater maize grain yield after low intensity grazing in 2015/2016 season (Fig. 1) is possibly related to the higher C and N soil content in this system obtained in the long-term compared to moderate intensity grazing (Table 4). It can also be explained by the higher WRSI, which showed a linear relationship with yield (Fig. 2). Thus, the system using low grazing intensity, which had a higher capacity to supply the nutritional needs, especially N due to the larger soil stock, was more favoured under superior water supply conditions. (Fig. 2).

The soybean grain yield did not respond positively to increases in soil N levels caused by the adoption of low grazing intensities. **(Table 2)**. This is mainly due to the fact that the treatments in this study do not affect other chemical attributes of the soil

(Alves et al, 2019) and because soybeans obtain much of their N requirement from 351 biological N fixation, depending less of N soil supply (Ciampitti and Salvagiotti, 2018) 352 in contrast to maize. However, soybean yield was affected by the different pasture 353 management methods and grazing intensities (Table 2). The rotational method, 354 independent of the grazing intensity, resulted in higher soybean yield when compared to 355 the continuous method adopted in the 2004/2005 and 2014/2015 seasons (Table 2). The 356 higher soybean yield in the rotational stocking method can be justified by the fact that 357 continuous grazing can affect spatial patterns of the soil attributes, increasing spatial 358 heterogeneity of the pastures and the environment (Laca, 2009, Auerswald et al., 2010). 359 360 As the animals circulate freely selecting grazing areas, manure is deposited unevenly influencing the concentration of nutrients in the soil (McNaughton, 1985; Augustine & 361 362 Frank, 2001), with the highest concentrations being found near to the rest areas, shade 363 and water troughs (Dennis et al. 2012; Dubeux et al., 2014). This does not happen using the rotational stocking method, where the animals are placed in grazing ranges that rotate 364 365 in space and time, favouring the better distribution of manure within the grazing range 366 (Peterson and Gerrish, 1996), achieving better control of spatial nutrient cycling and soil fertility. 367

368

369 **5.** Conclusions

The aboveground productivity of pasture and the maize yield are higher in the system with low grazing intensity during the pasture phase. In contrast, soybean presented higher yield when the rotational pasture method was used in the winter, regardless of the grazing intensity. The residual forage mass in the pasture phase was favoured by the soybean monoculture system in the summer.

375	Low grazing intensity in the pasture phase favoured the increase in the C and N
376	stocks in the soil. The pasture management method, continuous or rotational, did not
377	affect the accumulation of C and N in the soil. The higher quality of soybean crop residues
378	and similar annual input of C determined higher soil C and N stocks, in comparison to
379	the rotation soybean/maize. Thus, our results provided field-based evidence that crop
380	residues with greater N content, favoured soil C accumulation. Maize yield was low in
381	most of the seasons due to the low water requirement satisfaction index, reducing the
382	potential for the accumulation of C and N in the soil under rotation soybean/maize.

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Fig. 1. Grain yield of maize in an integrated crop-livestock system, submitted different grazing intensities (moderate and low) as mean of the stocking methods, in six years (2005, 2007, 2009, 2011, 2013 and 2015), in Southern Brazil. Different letters distinguish maize yield at each grazing intensity in each year (upper case letters) and each grazing intensity in the different years (lowercase letters) by the Tukey test (p < 0.05).



Fig. 2. Relationship between maize yield (**A**) and water requirement satisfaction index (WRSI) to different grazing intensities (moderate and low) and soybean yield (**B**) and WRSI to interaction between stocking methods (continuous and rotational) and grazing intensities (moderate and low) in 14-yr long-term integrated crop-livestock system experiment in Southern Brazil. ** Significant at P < 0.01. * Significant at P < 0.05. ns Not significant.



Fig. 3. Aboveground productivity (AGP) (winter ryegrass) in an integrated crop-livestock system under two grazing intensities (moderate and low), as mean of the stocking methods in Southern Brazil. Different letters compared the mean of the evaluated years according to the grazing intensities by Tukey test (p < 0.05).



Fig. 4. Residual aboveground forage mass (RFM) (winter ryegrass) in an integrated croplivestock system under different cropping systems (soybean monoculture and soybeanmaize rotation and grazing intensities (moderate and low) for five years in Southern Brazil. Different letters distinguish RPM in the soil within each cropping system (Upper case letters) and grazing intensity (Lower case letters) by Tukey test (p < 0.05).



Fig. 5. Soil carbon (A and B) and nitrogen (C and D) in integrated crop-livestock system under different cropping systems (soybean monoculture and soybean-maize rotation and grazing intensities (moderate and low). When interaction was identified, upper case letters compare the organic carbon contents between the cropping systems within each depth. Lower case letters compare organic carbon contents between depths for each cropping system by Tukey test (p < 0.05).



Figure 6. Relationship between carbon (**A**) and nitrogen (**B**) inputs via crop residues and soil carbon stocks (0–30 cm) in different cropping systems (monoculture and crop rotation) after 14-yr long-term integrated crop-livestock system experiment in Southern Brazil. ** Significant at P < 0.01. * Significant at P < 0.05.

Table 1

Analysis of variance of the stocking methods (S), grazing intensity (G), summer cropping systems (C), years (Y), layer (L) and their effect on crop and forage parameters on [soybean and maize grain yield, net primary production (NPP) and residual forage mass (RFM) of winter pasture] over the years and on soil parameters [content C and N in soil, stock of C and N in soil, and annual C and N accumulation rate (C and N-rate) in an Acrisol subjected for 14 years to different winter pasture methods and intensities and summer cropping systems in a long-term integrated crop-livestock protocol in southern Brazil.

T.ff4	Crop and forage parameters			T ffa a4	Soil parameters						
Effect	Soybean	Maize	NPP	RFM	– Effect	C content	N content	C stock	N stock	C-rate	N-rate
S	**	ns	ns	ns	S	ns	ns	ns	ns	ns	ns
G	***	*	**	**	G	**	*	**	ns	**	**
С	ns	-	-	*	С	*	**	*	**	**	**
Y	***	***	***	***	L	***	***	-	-	-	-
$\mathbf{S} \times \mathbf{G}$	**	ns	ns	ns	$\mathbf{S} \times \mathbf{G}$	ns	ns	ns	ns	ns	ns
$S \times C$	ns	-	-	ns	$\mathbf{S} \times \mathbf{C}$	ns	ns	ns	ns	ns	ns
$\mathbf{S} \times \mathbf{Y}$	***	ns	ns	ns	$S \times L$	ns	ns	-	-	-	-
$\mathbf{G} \times \mathbf{C}$	ns	-	-	ns	G×C	ns	ns	ns	ns	ns	ns
$\mathbf{G} \times \mathbf{Y}$	ns	***	ns	ns	$\mathbf{G} \times \mathbf{L}$	**	*	-	-	-	-
C×Y	ns	-	-	ns	$\mathbf{C} \times \mathbf{L}$	**	*	-	-	-	-
$\mathbf{S} \times \mathbf{G} \times \mathbf{C}$	ns	-	-	ns	$\mathbf{S} \times \mathbf{G} \times \mathbf{C}$	ns	ns	ns	ns	ns	ns
$\mathbf{S} \times \mathbf{G} \times \mathbf{Y}$	**	ns	ns	ns	$\mathbf{S} \times \mathbf{G} \times \mathbf{L}$	ns	ns	-	-	-	-
$\mathbf{S} \times \mathbf{C} \times \mathbf{Y}$	ns	-	-	ns	$\mathbf{S} \times \mathbf{C} \times \mathbf{L}$	ns	ns	-	-	-	-
$\mathbf{G} \times \mathbf{C} \times \mathbf{Y}$	ns	-	-	ns	$\mathbf{G} \times \mathbf{C} \times \mathbf{L}$	ns	ns	-	-	-	-
$\mathbf{S} \times \mathbf{G} \times \mathbf{C} \times \mathbf{Y}$	ns	-	-	ns	$\mathbf{S} \times \mathbf{G} \times \mathbf{C} \times \mathbf{L}$	ns	ns	-	-	-	-

* Significant at P <0.05; ** Significant at P <0.01; *** Significant at P <0.001; ns not significant.

Table 2

Soybean grain yield in an integrated crop-livestock system under different stocking methods (continuous and rotational) and grazing intensities (moderate and low) in five years in southern Brazil.

Veen	Contir	nuous	Rotational			
rear –	Moderate	Low	Moderate	Low		
		Yield (Mg	, ha ⁻¹)			
2003/2004	1.25 Ab	1.49 Ab	1.32 Ac	1.15 Ac		
2004/2005	0.57 Cc	1.19 Bc	1.50 Ac	1.61 Ab		
2006/2007	0.81 Ac	0.93 Ac	0.87 Ad	0.85 Ad		
2010/2011	1.35 Bb	1.60 ABb	1.81 Ab	1.78 Ab		
2014/2015	2.24 Ba	2.56 Ba	3.67 Aa	3.43 Aa		

Upper case letters compare treatments (continuous - moderate, continuous - low, rotational - moderate and rotational - low) in different years. Lower case letters compare the years within each stocking method and grazing intensity. Different by Tukey test p<0.05.

Table 3

Average of annual C and N input to the soil by shoot crop residues of winter ryegrass and summer crops in an integrated crop-livestock system, under different grazing intensities (moderate and low) in winter and cropping systems (soybean monoculture and soybean/maize rotation) in summer in Southern Brazil.

Innut	Croppin	g systems	Grazing intensity					
Input	Monoculture ^a	Crop rotation ^b	Moderate	Low				
	Winter (livestock season) contribution (kg ha ⁻¹ year ⁻¹)							
С	1260	1168	1066	1364				
Ν	19	18	16	21				
	Summer	(crop season) cont	ribution (kg ha-1	year ⁻¹)				
С	740	1281	955	1073				
Ν	43 31		35	39				
Annual input								
C	2000	2449	2020	2437				
N	62	49	51	60				

^a Soybean residue and ^b Soybean+maize residue in summer; ryegrass residue in winter in both cropping systems.

Table 4

Soil C and N stocks (0–30 cm) in an integrated crop-livestock systems with two grazing intensities (moderate and low) on winter ryegrass and two cropping systems (monoculture and crop rotation) in summer in an Acrisol in Southern Brazil.

Sailnanamatan	Croppin	ng system	Grazing intensity		
Son parameter	Monoculture	Crop rotation	Moderate	Low	
Stocks ^a (Mg ha ⁻¹)					
С	45 A	39 B	40 b	44 a	
Ν	5 A 4 B		4 a	5 a	
Annual accumulation	rates ^b (kg ha ⁻¹)				
С	500 A	200 B	200 b	500 a	
Ν	78 A	6 B	27 b	57 a	

^{*a*} Different letters distinguish carbon and nitrogen stocks in the soil within each cropping systems (Upper case letters) and grazing intensities (Lower case letters) by Tukey test (p < 0.05).

^b The soil C and N accumulation rates were calculated as the difference between C stock in each treatment in relation to the treatment with lower stock (soybean / maize succession, grazing intensity moderate and stocking method continuous), divided by time of the experiment (14 years). Upper case letters compare means of cropping systems, and lower case letters compare means of grazing intensities by Tukey test p<0.05.

Declaration of interests

¹ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

□The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:



Figure S1. Water requirement satisfaction index (WRSI) for all evaluated harvests in the period from October 15 to March 15.

DECLARATION

I, DAVID HENRY MOON (RNE: V113471-I), revised the main body of the text from the article entitled "The effect of crop rotation and sheep grazing management on plant production and soil C and N stocks in a long-term integrated crop-livestock system in Southern Brazil" for the authors Lucas Aquino Alves, Luiz Gustavo de Oliveira Denardin, Amanda Posselt Martins, Cimélio Bayer, Murilo Gomes Veloso Carolina Bremm, Paulo César de Faccio Carvalho, Debora Rubin Machado and Tales Tiecher. I am a British citizen and my mother language is English, received by Degree in Applied Biology from John Moore's University, Liverpool, UK and my PhD in Virology from the University of Reading, UK.

David Henry Moon David Henry Moon

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL COMISSÃO DE ÉTICA NO USO DE ANIMAIS

Formulário de Encaminhamento de Projeto de Pesquisa à CEUA

ANTES DE PREENCHER ESSE FORMULÁRIO:

1. Certifique-se de que o seu projeto está registrado no Sistema de Pesquisa da UFRGS e contém todas as informações necessárias para avaliação dos aspectos éticos de experimentação animal, conforme as orientações para encaminhamento de projetos apresentadas na página da CEUA/UFRGS.

2. Leia o Guia de Severidade de Procedimentos Científicos na página da CEUA/UFRGS.

A aprovação da CEUA/UFRGS tem validade especificada somente para a atividade na forma em que foi submetida. Mudanças substanciais nas atividades devem ser comunicadas previamente, mediante ofício a CEUA/UFRGS. Exemplos: mudança de número e/ou espécie animal, tratamento, categoria de anestésico usado, método de eutanásia, mudança na substância utilizada e/ou testada, processo invasivo, mudança de severidade do procedimento.

Este documento é um comprovante de que a solicitação deu entrada na CEUA/UFRGS.

1. DADOS DO PROJETO:

N° do projeto no Sistema de Pesquisa:

Titulo: Adubação sistêmica como alternativa para intensificação sustentável de sistemas integrados de produção agropecuária

Classificação de severidade de procedimentos: Leve

Departamento ou Programa de Pós-Graduação: Programa de Pós Graduação em Zootecnia

Unidade: Faculdade de Agronomia, UFRGS

Projeto de Tese ou Dissertação? 🛛 Sim 🗌 Não

2. PESQUISADOR:

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3. COLABORADORES:

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- Jean Victor Savian – Zoot., Dr., aluno de pós-doutorado do Programa de Pós-Graduação em Zootecnia, Faculdade de Agronomia/UFRGS.

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- Lucas Aquino Alves – Eng. Agr., aluno de mestrado do Programa de Pós-Graduação em Ciência do Solo, Faculdade de Agronomia/UFRGS.

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- Adão Luis Ramos Dos Santos - Departamento de Solos/UFRGS

4. LOCAL:

Nome e endereço do estabelecimento, laboratório ou do biotério onde serão realizados os procedimentos: Estação Experimental da Universidade Federal do Rio Grande do Sul, Eldorado do Sul – RS, Rodovia BR 290, km 146.

5. TEMPO DE EXECUÇÃO:

Período previsto para desenvolver este projeto:

Início (mês/ano): 05/2018

Término (mês/ano): 05/2021

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6. TERMO DE RESPONSABILIDADE

Estou ciente dos princípios éticos para o uso de animais para fins didáticos e de pesquisa cientifica. Conheço e cumprirei a legislação vigente, que normatiza as formas de conduta e tratamento com os animais (Lei 11.794/08 e Decreto 6899/09 da Presidência da República).

Nesse trabalho considerou-se a possibilidade de utilizar métodos alternativos aos modelos animais. Entretanto, concluiu-se que eles não estão disponíveis ou são inadequados por razões científicas. Considerou-se o princípio dos 3R (replacement, reduction e refinement, isto é, substituição, redução e refinamento). Ou seja:

0 buscou-se a substituição de vertebrados por seres não sencientes,

será utilizado um número mínimo de animais no experimento

0 os procedimentos foram planejados de modo a minimizar o desconforto provocado ao animal durante o experimento.

Este estudo não é desnecessariamente duplicativo, tem mérito científico e a equipe que participa desta atividade foi treinada e é competente para executar os procedimentos descritos nesse protocolo.

Comprometo-me a informar, por ofício, a CEUA/UFRGS eventuais alterações significativas nos experimentos aqui descritos, antes da implementação das mesmas.

Tudo o que foi declarado nesse protocolo é a absoluta expressão da verdade. Estou ciente que o não cumprimento das condições aqui especificadas é de minha total responsabilidade (pesquisador principal) e que estarei sujeito às punições previstas na legislação em vigor.

LOCAL E DATA:

Porto Alegre, 2017 Assinatura do docente ou pesquisador responsável:

X bouding, Mumm Prof.ª Dr.ª Carolina Bremm

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