

## Article

# Root and Shoot Biomass Contributions to Soil Carbon and Nitrogen Under Grazing Intensity and Crop Rotation in an Integrated Crop–Livestock System

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**Abstract:** In integrated crop–livestock systems (ICLSs), grazing intensity and crop rotation influence residue dynamics, making it essential to assess shoot and root contributions to soil carbon (C) and nitrogen (N) inputs. This study aimed to assess the shoot and root biomass of Italian ryegrass, soybean, and maize; the distribution of roots within the soil profile; and the contributions of shoot and root biomass to soil C and N under varying winter grazing intensities and summer crop rotations. The experiment was conducted within a long-term (12-year) field protocol, arranged in a randomized complete block design with split plots and four replicates. Grazing intensity was defined as the following: (i) moderate grazing— forage allowance equivalent to 2.5 times the potential dry matter intake of sheep, and (ii) low grazing— forage allowance equivalent to 5.0 times the intake potential. Grazing intensities (moderate and low) were allocated to the main plots, while cropping systems— monoculture (soybean/soybean) and crop rotation (soybean/maize)— were assigned to the subplots. Soil depth layers (0–10, 10–20, 20–30, and 30–40 cm) were treated as sub-subplots. Root samples of Italian ryegrass, soybean, and maize were collected using the soil monolith method. Low grazing intensity (8.6 Mg ha<sup>-1</sup>) promoted greater aboveground biomass production of Italian ryegrass compared to moderate intensity (6.6 Mg ha<sup>-1</sup>). Maize exhibited a higher capacity for both root and shoot biomass accumulation, with average increases of 85% and 120%, respectively, compared to soybean. Root biomass was primarily concentrated in the surface soil layer, with over 70% located within the top 10 cm. Italian ryegrass showed a more uniform root distribution throughout the soil profile compared to soybean and maize. Carbon inputs were higher under crop rotation (17.2 Mg ha<sup>-1</sup>) than under monoculture (15.0 Mg ha<sup>-1</sup>), whereas nitrogen inputs were greater in soybean monoculture (0.23 Mg ha<sup>-1</sup>) than in crop rotation (0.16 Mg ha<sup>-1</sup>). Low grazing intensity in winter and summer crop rotation with high-residue and quality species enhance the balance between productivity and soil C and N inputs, promoting the sustainability of ICLSs.

**Keywords:** Italian ryegrass; maize; root biomass; shoot biomass; soybean



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## 1. Introduction

Integrated crop–livestock systems (ICLSs) involve the temporal alternation or rotation of pastures and cash crops within the same area over time [1,2]. These systems are widely recognized for their positive impacts on soil quality, particularly through the accumulation of soil carbon (C) and nitrogen (N), contributing to enhanced nutrient cycling, improved soil structure, and greater agroecosystem sustainability [3,4].

In the subtropical regions of Brazil, where soybean is the dominant summer crop, often followed by maize, increases in soil C stocks are primarily linked to the input of plant residues (shoots and roots). However, low crop diversification frequently limits these inputs. To sustain soil C stocks under these conditions, an annual plant residue input of approximately  $3.92 \pm 1.30 \text{ Mg C ha}^{-1}$  is required [5]. In ICLSs, winter pastures of Italian ryegrass and black oat (*Lolium multiflorum* Lam. + *Avena strigosa* Schreb)—the main forage grasses used—followed by summer soybean (*Glycine max* (L.) Merrill) can contribute about  $4.80 \pm 0.26 \text{ Mg C ha}^{-1} \text{ year}^{-1}$  to the soil [6]. Nonetheless, the effectiveness of ICLSs in enhancing residue input can be significantly influenced by grazing intensity during the pasture phase [7] and by implementing crop rotation in the summer phase [8].

Grazing intensity, defined by the amount of forage consumed over time, directly influences the quantity of pasture residue returned to the soil. The literature reports conflicting results: while intensive grazing can reduce soil C stocks due to high biomass removal and reduced carbon allocation to roots [9], other studies found increased root biomass of ryegrass and oats under high grazing intensity [10]. Such discrepancies may reflect variations in grazing systems, livestock management, and environmental conditions, underscoring the need for further investigation.

In a subtropical environment, the crop chosen for summer cash cropping can have a great impact on the input of C into the soil [8]. A recent study highlighted that maize in a crop rotation system increases the soil organic C due to the high contribution of residues [11]. However, in water-restricted environments, more sensitive crops such as maize may not express their full residue input potential, and more resistant crops such as soybean may have a better development [12]. In addition to the quantity, the quality of the residue contributed is important. Leguminous plants are more efficient in storing carbon in the soil than grass plants, mainly due to their low C:N ratio and high lability [13,14].

Despite the challenges and high costs associated with sampling and quantifying root biomass, this component is fundamental for a more comprehensive understanding of plant contributions to soil C and N inputs. However, root residues are still often overlooked in ICLSs. In this context, it is essential to investigate how different grazing intensities during the winter pasture phase, combined with distinct summer crop rotations, influence the contribution of plant residues—both from shoots and roots—to C and N inputs in the soil. We hypothesize that ICLSs managed under low grazing intensity, especially when associated with summer crops that produce high biomass or high-quality residues, enhance the input of plant residues and promote greater soil C and N accumulation. This study aimed to assess the shoot and root biomass of Italian ryegrass, soybean, and maize, their vertical distribution in the soil profile, and their respective contributions to soil C and N inputs under low and moderate grazing intensities during the winter pasture phase and different summer crop rotations in a long-term ICLS experiment.

## 2. Material and Methods

### 2.1. Site Description and Characterization of the Experimental Area

This study was conducted as part of a long-term ICLS experiment, established in 2003 at the Agronomic Experimental Station of the Federal University of Rio Grande do Sul (EEA—UFRGS), located in Eldorado do Sul County, Rio Grande do Sul State,

Brazil (30°05' S, 51°39' W, 46 m above sea level). The regional climate is classified as subtropical humid (Cfa) according to the Köppen classification system [15]. Over the 12 yr experimental period, annual rainfall ranged from 1200 and 2000 mm, with an average annual temperature of 19 °C. Climatic data were obtained from the mobile automatic station (model Weather Watch 2000 Station, Campbell Scientific, Inc., Logan, Utah, United States) of the EEA—UFRGS.

The soil at the site is classified as an Acrisol, according to the FAO system [16]. Prior to the experiment, the 0–10 cm soil layer presented the following: organic C content of 17 g kg<sup>-1</sup>; soil pH in water (1:1 soil/water ratio) of 5.3; exchangeable aluminum (Al), calcium (Ca), and magnesium (Mg) (KCl 1.0 mol L<sup>-1</sup>) of 0.3, 2.2, and 1.2 cmol<sub>c</sub> kg<sup>-1</sup>, respectively; available phosphorus (P) and potassium (K) (Mehlich 1) of 10 and 135 mg kg<sup>-1</sup>, respectively; and Ca + Mg + K and Al saturation of 51% and 8%, respectively.

Before the experimental protocol was implemented, the area was covered by natural grassland typical of the Pampa Biome and was occasionally overseeded during the winter with exotic forage species such as Italian ryegrass (*Lolium multiflorum*). In preparation for the experiment in 2003, the existing vegetation was desiccated using glyphosate herbicide, and limestone was applied at a rate of 1.0 Mg ha<sup>-1</sup> to increase the soil pH in water in the 0–10 cm soil layer to 6.0, following regional soil amendment guidelines [17].

## 2.2. Experimental Design and Conduction of the Experiment

The 4.5 ha experimental area was divided into 16 experimental units ranging from 0.23 to 0.32 ha each. The experimental design involved randomized complete block with four repetitions, arranged in a 2 × 2 factorial scheme with split-plots. The main factors were two different grazing intensities (moderate and low) and two stocking methods (continuous and rotational). The subplots corresponded to two summer cropping systems: soybean monoculture (soybean/soybean) and crop rotation (soybean/maize).

The experiment began with direct sowing of ryegrass, at a rate of 32 kg ha<sup>-1</sup>, using 17 cm row spacing. N fertilization totaled 150 kg ha<sup>-1</sup>, applied in two equal splits. During the winter grazing phase, Corriedale and Texel lambs (aged 9–12 months; average weight 35 ± 4 kg) were used. Grazing management employed the *put-and-take* method [18], with a variable number of adjustment animals to maintain targeted forage supply levels.

Grazing intensity was defined based on forage allowance, expressed as kilograms of dry matter per 100 kg of live weight (LW) per day: (i) moderate grazing intensity—forage allowance equivalent to 2.5 times the forage intake potential of sheep; (ii) low grazing intensity—allowance equivalent to 5.0 times the intake capacity. According to the National Research Council [19], lambs have a potential dry matter intake of approximately 4.0% of their LW. Thus, moderate and low forage allowances corresponded to 10% and 20% of the animal's LW, respectively.

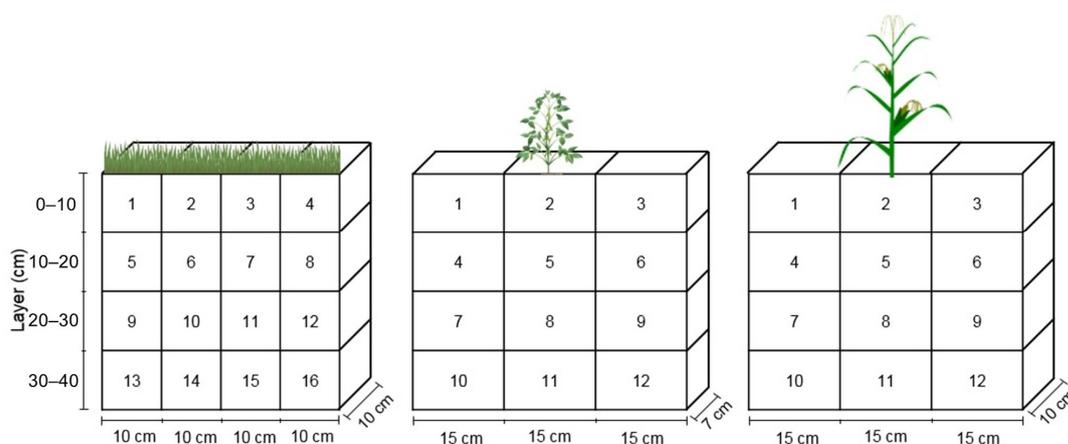
In the present study, only the continuous stocking method was considered. Stocking rate adjustments were made periodically to maintain the predetermined forage allowances (2.5× and 5.0× intake potential). At the end of each winter grazing cycle, animals were removed and the area was desiccated with *glyphosate* herbicide in preparation for the subsequent summer cropping phase. Average annual fertilization input during the 12 yr study period was 150 kg N ha<sup>-1</sup>, 30 kg P ha<sup>-1</sup>, and 50 kg K ha<sup>-1</sup>, all applied during the pasture cycle. Detailed information on seasonal and annual fertilization rates is available in previously published studies conducted in the same experimental area [20].

During the summer cropping phase, each plot was divided into two subplots, each representing one of the two cropping systems: soybean monoculture (soybean/soybean) and crop rotation (soybean/maize), both under no-tillage management. In the first summer season, soybean (*Glycine max*) was sown across the entire experimental area. In the second

season, soybean and maize (*Zea mays*) were sown in separate subplots within each plot to implement the respective systems. Row spacing was 45 cm for both crops, with seeding densities of 280,000 for soybean and 88,000 seeds ha<sup>-1</sup> for maize. Sowing was carried out between October and November each year.

### 2.3. Root and Shoot Biomass Determination and C and N Inputs

Sampling was allocated to the treatments with different grazing intensities in winter and the different cropping systems in summer, excluding the stocking methods, as they were not of interest to this study. Roots and shoots were sampled from three growing seasons: (i) pasture phase with italian ryegrass, (ii) first cropping phase with soybean, and (iii) second cropping phase with soybean (monoculture subplots) or maize (crop rotation subplots). Soybean roots were collected specifically at flowering [21], and maize in the tassel stage, in a narrow trench perpendicular to crop rows. The soil samples containing the roots were collected up to a soil depth of 40 cm using the monolith method [22] (Figure 1).



**Figure 1.** Sampling of soil and Italian ryegrass, soybean, and maize roots using the monolith method.

After the monoliths were removed, the samples were placed in plastic bags and stored in a refrigerator at 4 °C until ready for root washing. This was performed in order to prevent root decomposition from microbiological activity in the soil [22]. In the laboratory, the monoliths were washed under running water in a 0.5 mm sieve. After impurities were removed with tweezers, the roots were dried at 55 °C in a forced air oven and weighed to quantify dry biomass. Shoot biomass productivity (Mg ha<sup>-1</sup>) was estimated by using the accumulated forage biomass at the beginning of the grazing period, added to the forage accumulation rate (kg day<sup>-1</sup>), and multiplied by the number of grazing days, over an average period of 101 ± 9 days.

The cumulative C and N inputs to the soil were estimated for the 2014/2015 and 2015/2016 harvests. It was considered for the input calculations that 40% of the input residue is C, and the C/N ratios of soybean shoot and root were 17.4 and 33.3, maize 77.5 and 57.3, and ryegrass 40.1 and 55.8, respectively [23,24].

### 2.4. Statistical Analysis

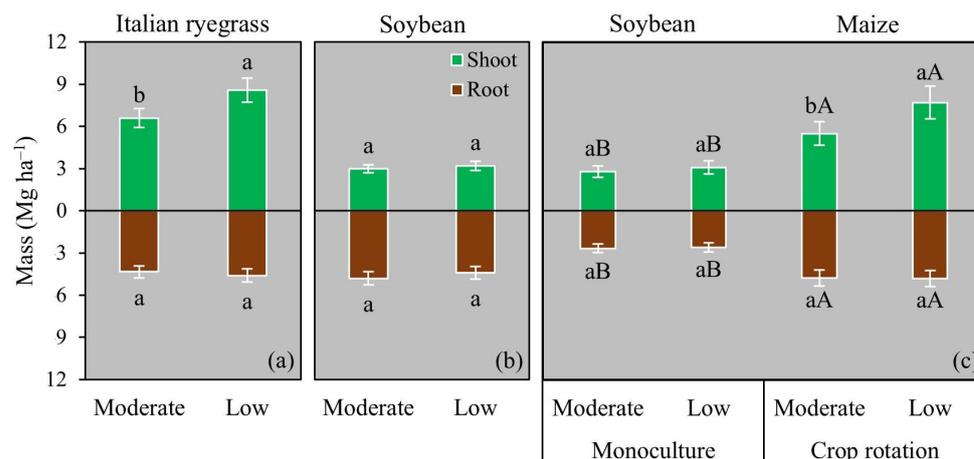
The data were subjected to analysis of variance (ANOVA) using the PROC MIXED procedure of the SAS<sup>®</sup> statistical software, based on a mixed model framework. When the assumptions of homoscedasticity and normality of residuals were not met, data transformation was performed using the Box–Cox method to stabilize variance and normalize the distribution. Means were subsequently compared using Tukey's test at a significance level of  $p < 0.05$ . In all models, block effects and interactions between factors were treated as random, while the main treatment effects were considered fixed. To visualize the spatial

distribution of root parameters in the soil profile, Kriging interpolation was performed using Surfer<sup>®</sup> version 14 (Golden Software) to generate contour plots.

### 3. Results

#### 3.1. Root and Shoot Biomass

In the pasture phase, the root biomass of Italian ryegrass was not changed by different grazing intensities or cropping systems (Figure 2a), and the average root dry biomass up to 40 cm deep was 4.5 Mg ha<sup>-1</sup>. However, the shoot dry biomass changed according to the grazing intensity ( $p > 0.05$ ), increasing 2.0 Mg ha<sup>-1</sup> in the low intensity system (8.6 Mg ha<sup>-1</sup>) as compared to the moderate grazing intensity (6.6 Mg ha<sup>-1</sup>) (Figure 2a).



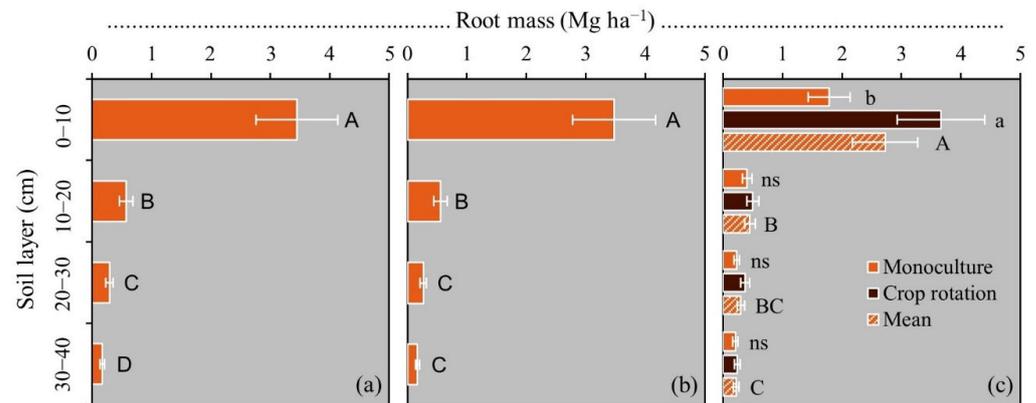
**Figure 2.** Root and shoot biomass of Italian ryegrass in pasture phase (a), soybean in first cropping phase (b), and soybean and maize in second cropping phase (c) as a function of grazing intensities (moderate and low) and cropping system (monoculture and crop rotation) in soil profile in an integrated crop–livestock system in subtropics. Different lowercase letters distinguish means from grazing intensities and different uppercase letters distinguish means from cropping system by Tukey test ( $p < 0.05$ ).

The root and shoot dry biomass of the soybean cropping phase were not changed by different grazing intensities or cropping systems (Figure 2b). In the 2015/2016 cropping phase, the shoot and root dry biomass were changed according to the cropping system, being higher in the system with crop rotation, maize roots (4.8 Mg ha<sup>-1</sup>), and shoots (6.6 Mg ha<sup>-1</sup>), in relation to monoculture, soybean roots (3.0 Mg ha<sup>-1</sup>), and shoots (2.6 Mg ha<sup>-1</sup>), respectively (Figure 2c). In addition, the shoot dry biomass in crop rotation was higher at low grazing intensity than at moderate intensity: 7.7 and 5.5 Mg ha<sup>-1</sup>, respectively (Figure 2c).

#### 3.2. Root Distribution in Soil Profile

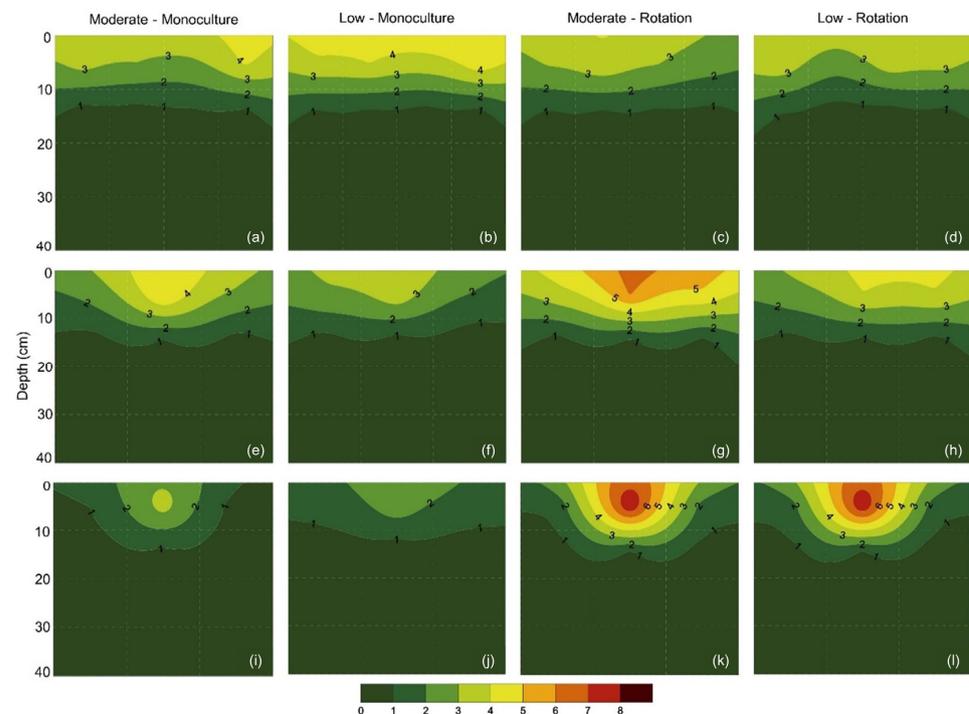
Grazing intensities and cropping systems did not affect Italian ryegrass and soybean root distribution in the soil profile (Figure 3a,b). However, the root dry biomass of Italian ryegrass is higher in the 0–10 cm layer (3.4 Mg ha<sup>-1</sup>), concentrating 76% of the total dry biomass, and drastically decreasing with depth (0.6, 0.3, and 0.2 Mg ha<sup>-1</sup> in the 10–20, 20–30, and 30–40 cm layers) (Figure 3a). The root dry biomass of soybean followed the same trend as ryegrass, with greater concentration in the uppermost soil layer of 0–10 cm (3.5 Mg ha<sup>-1</sup>), concentrating 78% of the total dry biomass, and drastically decreasing with depth (0.6, 0.3, and 0.2 Mg ha<sup>-1</sup> in the 10–20, 20–30, and 30–40 cm layers) (Figure 3b). In the 0–10 cm layer, the root dry biomass was 115% higher in the crop rotation in relation to monoculture (Figure 3c), maize (3.7 mg ha<sup>-1</sup>), and soybean roots (1.8 Mg ha<sup>-1</sup>), respectively. The mean of the root dry biomass of soybean and maize was higher in the upper layer of 0–10 cm

( $2.7 \text{ Mg ha}^{-1}$ ), concentrating 74% of the total dry biomass, and drastically decreasing with depth (0.4, 0.3, and  $0.2 \text{ Mg ha}^{-1}$  in the 10–20, 20–30, and 30–40 cm layers) (Figure 3c).



**Figure 3.** Root biomass in soil profile of Italian ryegrass in pasture phase (a), soybean in first cropping phase (b), and soybean and maize in second cropping phase (c) as a function of cropping system (monoculture and crop rotation) in soil profile in an integrated crop–livestock system in subtropics. Different lowercase letters distinguish means from cropping systems within each soil layer and different uppercase letters distinguish means of soil layers by Tukey test ( $p < 0.05$ ); and 'ns' indicates no significant difference between comparisons according to Tukey's test ( $p > 0.05$ ).

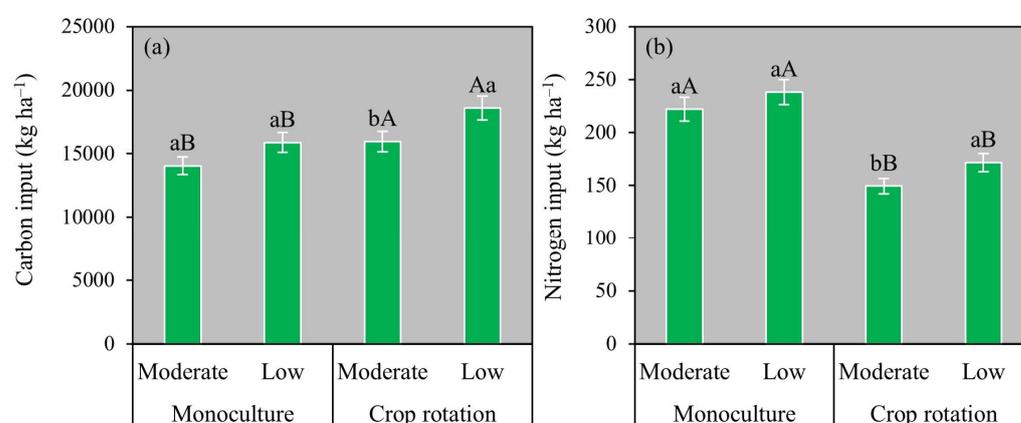
In the pasture phase, the distribution of Italian ryegrass roots (Figure 4a–d) was concentrated practically in the first 10 cm, but with a homogenous distribution laterally. The soybean roots in the first cropping phase (Figure 4e–h) had a higher concentration of roots in the sowing line of the crop, with a concentration in the first 10 cm of the soil. The same happened with the soybean and maize roots of the second cropping phase (Figure 4i,j,l,m).



**Figure 4.** Two-dimensional distribution of soil root dry biomass ( $\text{Mg ha}^{-1}$ ) for Italian ryegrass (a–d), soybean (e–h), and maize crops (i–l) as a function of grazing intensities (moderate and low) and cropping system (monoculture and crop rotation) in soil profile in an integrated crop–livestock system in subtropics.

### 3.3. C and N Inputs in the Systems

The total inputs of C and N throughout the harvests in the first and second cropping phases were affected by the interaction between grazing intensity and the cropping system (Figure 5). The highest contributions of C were observed in treatments with crop rotation (17.2 Mg ha<sup>-1</sup>) in relation to monoculture (15.0 Mg ha<sup>-1</sup>), regardless of grazing intensity (Figure 5a). Also, within the crop rotation system, low grazing intensity (18.6 Mg ha<sup>-1</sup>) contributed 16% more C compared to moderate intensity (16.0 Mg ha<sup>-1</sup>) (Figure 5a). Within monoculture, there was no difference in C input between grazing intensities (Figure 5a). Contrary to what was observed for C inputs, N input was higher in monoculture treatments (0.23 Mg ha<sup>-1</sup>) than in crop rotation treatments (0.16 Mg ha<sup>-1</sup>), regardless of grazing intensity (Figure 5b). Furthermore, within the crop rotation system, the low-intensity grazing (0.17 Mg ha<sup>-1</sup>) contributed 15% more N than the moderate intensity (0.15 kg ha<sup>-1</sup>) (Figure 5b). Within monoculture, there was no difference in N input between grazing intensities (Figure 5b).



**Figure 5.** Cumulative carbon (a) and nitrogen (b) inputs over two cropping seasons (first and second cropping phases) as a function of grazing intensities (moderate and low) and cropping system (monoculture and crop rotation) in the soil profile in an integrated crop–livestock system in subtropics. Different lowercase letters distinguish the means from the grazing intensities and different uppercase letters distinguish the means from the cropping system by Tukey test ( $p < 0.05$ ).

## 4. Discussion

### 4.1. Importance of Root Biomass as a Carbon Source

The amount of plant residues produced is directly linked to the inputs and consequent accumulation of C in the soil. However, root residue biomass production is often overlooked as a source of carbon input to the soil system [25], primarily due to the complexity and high cost of sampling and quantifying roots in the field. In our study, the roots of Italian ryegrass had a great contribution to the biomass input, reaching 4.4 Mg ha<sup>-1</sup> of dry biomass (Figure 3a), contributing 37% of the total dry biomass input to the system. This input of root dry biomass is important because it has a longer residence time in soil and is often considered as the main source of soil organic matter, contributing up to three times more to organic matter than shoot biomass [26,27].

### 4.2. Effects of Grazing Intensity and Crop Rotation on Biomass Production and Soil C Sequestration in ICLSs

Although grazing intensity did not change the biomass production of Italian ryegrass roots, as also observed [8] when testing different grazing intensities in white oats in a subtropical environment, the shoot biomass production was 30% higher in the low grazing intensity compared to the moderate intensity (Figure 3a). These results agree with those

obtained in [28], where low grazing intensities increased aboveground biomass inputs. A recent study demonstrated that low grazing intensity resulted in a 10% increase in soil C stocks compared to moderate intensity [12]. These findings underscore the role of low grazing intensity management in enhancing soil organic C stocks and mitigating carbon mineralization, thereby promoting long-term soil C sequestration [7,29]. This agrees with the results observed in our study, where low grazing intensities contributed on average 15% more C to the soil compared to moderate grazing (Figure 5a).

Our results suggest that the cropping system and grazing intensity used in an ICLS do not have a significant impact on the production of soybean root and shoot biomass (Figure 3b). These results reinforce the recent findings in [30], as their study found no effect of grazing intensities on cash crop biomass production in an ICLS. In this study, the authors highlight that moderate grazing does not compromise grain crops in succession. These results may be due to the ICLS's benefits on the soil, such as improved physical [31], chemical [32], and biological soil quality [33], which enhances soil resilience to disturbances [34]. However, the largest maize root and shoot biomass compared to soybeans (Figure 3c) stands out, highlighting the importance of crop rotation in cropping seasons in the ICLS. This result was expected, because maize is C<sub>4</sub> grass and its biomass production, in general, is higher than C<sub>3</sub> plants, such as soybeans [35,36]. In addition, low grazing intensities promoted a greater maize shoot biomass (Figure 3c). This greater production is mainly due to greater N stocks in the soil as promoted by low grazing intensity [12], with N being a nutrient of greater demand for maize growth.

#### 4.3. Nitrogen Inputs and C/N Ratios in Different Cropping Systems

An interesting result worth noting is the higher N input in systems with soybean monoculture compared to crop rotation between soybean and maize (Figure 5b). Despite the higher C input in systems with crop rotation (Figure 5a), the lower C/N ratio of soybean was a determining factor for this higher N input in soybean monocrop. The better quality of legume residues, as in the case of soybean, may be the main driver of soil C accumulation [37], which is possibly associated with higher efficiency of microorganisms in stabilizing C from labile plant residues (higher N content) into microbial residues that could later be stabilized in the organo-mineral association [13,14,38]. This behavior of higher efficiency of legume residues was observed in the experimental area of [12], where C and N stocks were 15 and 25% higher, respectively, in monoculture compared to crop rotation.

#### 4.4. Root Distribution in the Soil Profile and Its Implications

In general, our results demonstrated a higher production of roots on the soil surface, decreasing along the soil profile, both for Italian ryegrass, soybean, and maize (Figure 4). This is known since most studies show that most of the roots are concentrated in the first 10 cm of soil [39,40]. Root distribution in the soil profile demonstrates the importance of the inclusion of annual grasses in crop rotation and succession with different root systems (Figure 5). Maize roots not only penetrated deeper than soybean roots but also occupied a larger volume of the soil, which improves their chemical, physical, and biological properties [23]. The importance of the distribution of Italian ryegrass roots is highlighted, having better lateral distribution and not concentrating only on the sowing line like soybean and maize. This is mainly since the establishment of the Italian ryegrass occurs by natural reseeding; there is no specific sowing line. However, this better root distribution can be important for nutrient absorption, soil aggregation, and consequent soil health [41].

## 5. Conclusions

The results of this study demonstrate that grazing intensity and crop rotation play a key role in regulating the input of plant residues, both shoot and root, and in determining soil C and N stocks in integrated ICLSSs. The adoption of low grazing intensity proved effective in increasing shoot biomass production and carbon inputs to the soil without compromising root biomass. Italian ryegrass roots made a significant contribution, accounting for 37% of the total dry biomass input, and are especially important due to their longer residence time in soil and their role in soil organic matter formation. Crop rotation with high-residue grasses such as maize significantly increased C inputs, while soybean monoculture contributed more N due to its higher residue quality (lower C:N ratio). Including species with distinct root architectures enhanced soil occupancy, root distribution, and, consequently, improved the physical, chemical, and biological processes in the soil. These findings emphasize the importance of adopting management strategies that combine low-intensity grazing with crop rotations involving both high-residue and high-quality species, thereby promoting soil carbon sequestration and enhancing the sustainability of ICLSSs in subtropical environments.

**Author Contributions:** Conceptualization, L.A.A., F.A., I.A. and T.T.; methodology, F.A. and I.A.; software, L.A.A., F.A. and T.T.; validation, L.B.d.O., L.G.d.O.D. and M.T.d.M.; formal analysis, L.A.A. and F.A.; investigation, F.A. and L.G.d.O.D.; resources, I.A. and P.C.d.F.C.; data curation, L.A.A. and F.A.; writing—original draft preparation, L.A.A. and F.A.; writing—review and editing, L.A.A., L.B.d.O. and M.T.d.M.; visualization, L.A.A. and T.T.; supervision, I.A. and P.C.d.F.C.; project administration, I.A.; funding acquisition, I.A. and P.C.d.F.C. All authors have read and agreed to the published version of the manuscript.

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