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ECOSYSTEMS

Long-term effects of cattle rearing on vegetation and soil in riparian forests in southern Brazil

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Abstract: Comprehending the impacts of the presence of cattle on different compartments of riparian forests is essential for the management of these environments, which play a crucial role in biodiversity conservation and human well-being. The objective of this study was to assess the long-term effects of cattle on the structure (tree stratum and natural regeneration) and floristic composition of vegetation, as well as on the litter and soil carbon stock (0-5, 5-15, 15-30, and 30-100 cm) in riparian forests. We compared two forest areas in southern Brazil, one isolated from cattle for 28 years and another used by cattle with a high animal load. Our results indicate that cattle modify the floristic composition and structure of vegetation, mainly affecting litter carbon stock and the abundance, richness, and diversity of natural regeneration. However, we did not observe a significant change in soil carbon stock and its correlation with vegetation variables. Our findings emphasize the need to explore adaptation alternatives of livestock activities with the maintenance of riparian forest ecosystem services. Adequate cattle load management and fencing of riparian forests, with the installation of watering corridors for cattle, are essential actions to reconcile livestock production with forest conservation.

Key words: Cattle exclusion, Fluvisol, forest degradation, livestock, Pampa, soil carbon.

INTRODUCTION

The worldwide degradation of forests associated with watercourses, known as riparian forests, has compromised the maintenance of crucial ecosystem functions and services, including those related to biodiversity and C stocks. Forests play a crucial role in biodiversity conservation (Luby et al. 2022) and human well-being (Meli et al. 2023), providing a wide range of ecosystem services (Acharya et al. 2019, Fearnside 2008). Riparian forests, in particular, play a role in regulating the flow of springs and watercourses and maintaining water quality in watersheds (Ramião et al. 2020). Additionally, riparian forests contribute to the protection of watercourse banks against erosion (Kantharajan et al. 2022), C sequestration (Dybala et al. 2019), and connectivity of different habitats through the formation of ecological corridors (Rosot et al. 2018).

Riparian forests are protected by law in Brazil due to their significance for ecosystems and human well-being. Federal Law 12.561/2012, known as the Brazilian Forest Code, addresses the protection of native vegetation and designates areas along natural watercourses and around springs as Permanent Preservation Areas (PPA). A PPA is defined in the Brazilian Forest Code as: "a protected area, covered or not by native vegetation, with the environmental function of preserving water resources, landscape, geological stability, and biodiversity, facilitating the gene flow of fauna and flora, protecting the soil, and ensuring the well-being of human populations" (Brasil 2012). Despite legal protection, an intense process of degradation of riparian forests has been observed in recent decades in Brazil (Maracahipes-Santos et al. 2020, Milagre et al. 2024, Mendes et al. 2022, Morais Junior et al. 2024), as these forests occupy locations in the landscape suitable for anthropogenic activities (Loebens et al. 2018).

In Brazil, forest degradation is particularly significant in the Pampa biome, which experienced the highest percentage loss of forests among Brazilian biomes in 2021 (Azevedo et al. 2022). Despite being traditionally known for its grasslands, the landscape in the Pampa region consists of vegetation mosaics, with the presence of tree, shrub, and grass formations (Boldrini et al. 2015). Native vegetation covers only 45% of the biome's territory, with approximately 12% of this total occupied by forests, mainly associated with watercourses (riparian forests) and as small islands in grassland areas (MapBiomas 2023). Many of these riparian forests are degraded due to anthropogenic activities (Costella et al. 2013) such as unsustainable livestock farming practices (Stefanello et al. 2021).

Among the ecosystem services provided by the Pampa, soil C storage holds great relevance (Altmann & Berger Filho 2020) due to its role in ecosystem productivity and its potential for climate change mitigation (Lal et al. 2015, Demattê et al. 2022). Many ecosystem benefits depend on soil properties and functions (Dominati et al. 2010). In the Pampa, studies on soil C storage are scarce in both natural and anthropogenic areas and to date have assessed only the shallow soil layers, typically up to a depth of 60 cm (Assmann et al. 2014, Bernoux et al. 2002, Groppo et al. 2015, Morais Junior et al. 2021, Pillar & Bayer 2012, Santos et al. 2020, Fruett et al. 2022). However, more than 75% of the soil's organic C is stored at a depth of up to one meter (Harden et al. 2018), and the Food and Agriculture Organization of the United Nations (FAO) encourages sampling beyond the 0-30 cm layer (FAO 2019).

Factors such as litter quantity and quality, plant species diversity (Li et al. 2020), soil properties (Rasmussen et al. 2018), and land-use history (Mendes et al. 2025) can influence soil organic C stocks. Riparian forest degradation can have negative effects on the physicochemical properties of the soil, including a reduction in soil C stocks (Celentano et al. 2017). Although extensive livestock farming is essential for the conservation of native Pampa grasslands (Overbeck et al. 2007), unsustainable livestock management practices (e.g., high stocking density) can impact forests associated with grazing areas, leading to changes in the structure and functional composition of the forest community (Stefanello et al. 2021). In the soil, the effects of livestock farming can vary, and may or may not lead to compaction (Etchebarne & Brazeiro 2016, Michels et al. 2012) and alteration of C stocks (Li & Jiang 2021, Pineiro et al. 2010, Schulz et al. 2016). In this context, studies providing information for the definition of conservation, management, and restoration strategies for riparian forests of the Pampa and other biomes are of great importance. Therefore, our objective was to assess the effects caused by the presence of cattle with high stocking density on the structure and floristic composition of forest vegetation, as well as on the litter and soil C stocks. We hypothesized that the long-term presence of cattle in riparian forests negatively impacts the structure and floristic composition of the tree stratum and natural regeneration, consequently reducing litter and soil C stocks.

Abbreviations

CF – Conserved riparian forest
DF – Degraded riparian forest

PPA – Permanent Preservation Area
$\delta^{13}C$– Isotopic composition

MATERIAL AND METHODS

Study area

We conducted this study in two riparian forest areas of the Ibirapuitã Biological Reserve (29°55′S and 55°46′W, 93 m altitude above sea level), located in the municipality of Alegrete, state of Rio Grande do Sul, southern Brazil (Figure 1). According to the Köppen classification, the climate in the municipality of Alegrete is of type Cfa - humid subtropical with hot summers. The average annual temperature is 18.6 °C, and the annual precipitation is 1,747 mm, with well-distributed rainfall throughout the year. The monthly average temperature varies from 13.0 °C in the coldest month (July) to 24.8 °C in the hottest month (January) of the year (Alvares et al. 2013). The prevalent soils in the municipality are classified as Entisols by Soil Taxonomy (Soil Survey Staff 2022), usually associated with gently undulating terrain (Streck et al. 2018).

The Ibirapuitã Biological Reserve is a 351-hectare state conservation unit located in the Pampa biome. Its landscape comprises tree, shrub, and grass formations, as well as rocky outcrops, and forests predominantly occur along the banks of the Ibirapuitã River, a tributary of the Ibicuí River basin (Moyeed & Clarke 2005). The river terraces of the Ibirapuitã River feature Alluvial Deciduous Seasonal Forest vegetation, characterized by deciduous species adapted to the alluvial environment (IBGE 2012). The main land uses within a 10 km radius of the reserve are agricultural crops, particularly rice

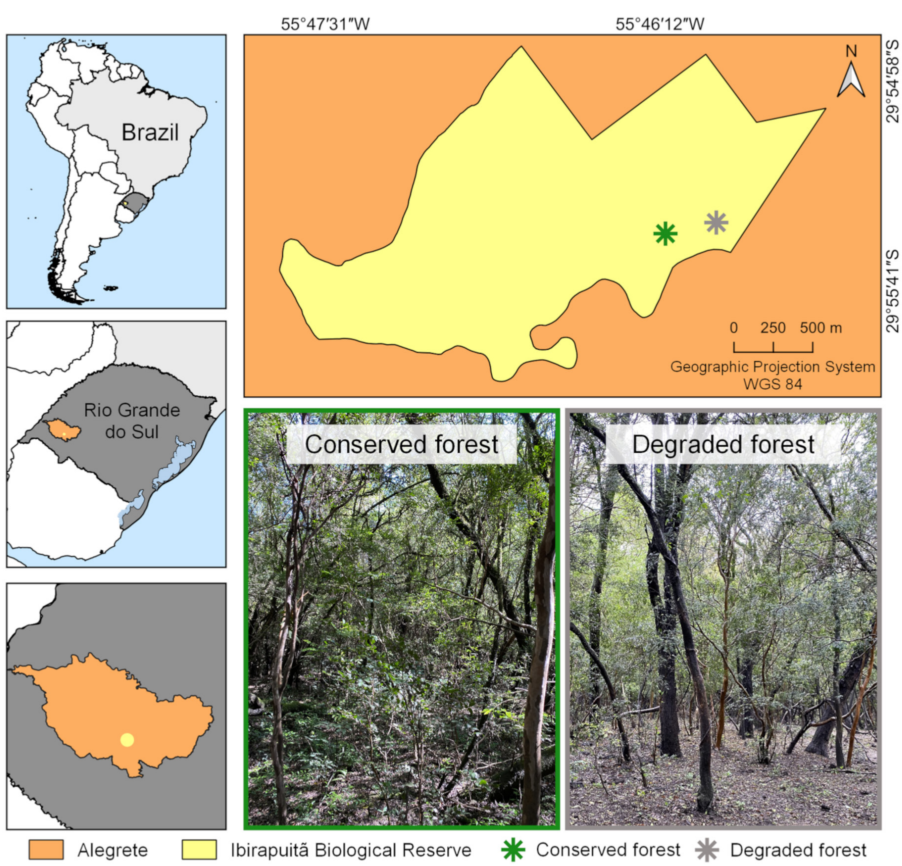

Figure 1. Location and vertical structure of riparian forest areas in the Pampa biome, southern Brazil. Conserved forest: riparian forest isolated from cattle for 28 years. Degraded forest: riparian forest with the presence of cattle.

and soybean cultivation (MapBiomas 2023), and livestock (Dick et al. 2021).

We selected two adjacent riparian forest areas located along the Ibirapuitã River (approximately 30 m wide), partly considered as PPA (50 m marginal strip) (Brasil 2012). The soil in these areas is Fluvisol (IUSS 2022) or Entisol Fluvent (Soil Survey Staff 2022) or "Neossolo Flúvico Ta eutrófico" according to the Brazilian Soil Classification System (Santos et al. 2018), with a clayey texture, high nutrient availability, and very high cation exchange capacity (Sociedade Brasileira de Ciência do Solo 2016). These areas exhibit different conservation levels and are referred to as conserved riparian forest (CF) and degraded riparian forest (DF) (Figure 1). The CF and DF are described below:

Conserved Riparian Forest (CF) – A 16-hectare riparian forest area in good conservation status, with an intermediate to advanced successional stage. The area presents a predominance of tree species from the Myrtaceae and Euphorbiaceae families and has been isolated from cattle for 28 years through the installation of a wire fence. It is characterized by a well-developed regenerating stratum in addition to the tree stratum and has a thick layer of accumulated litter.

Degraded Riparian Forest (DF) – A five-hectare riparian forest area that is legally part of the reserve but is located on a privately owned, not yet expropriated property. The property features native grassland and riparian forest areas and is dedicated to cattle rearing, with a cattle stocking rate of 390 kg of live cattle weight per hectare. It presents a predominance of tree species from the Myrtaceae and Euphorbiaceae families. This area is considered degraded due to the suppression of the regenerating vegetation, caused by the trampling of cattle (Vásquez-Grandón et al. 2018), which use the area for resting and accessing the Ibirapuitã River for watering. The layer of accumulated litter is visibly less thick than in CF.

Data collection

We collected data on vegetation, litter, and soil from the riparian forests in the first quarter of 2022 (summer).

Vegetation

To sample the vegetation in the riparian forest areas, we systematically allocated plots following the positioning of soil and litter sampling points. We recorded the identification (species) and abundance of shrub-tree individuals in two vegetation strata: the tree stratum and natural regeneration. Ten plots per study area were allocated for each stratum, totaling 20 plots of 200 m² (10 m x 20 m) for the tree stratum and 20 plots of 100 m² (10 m x 10 m) for natural regeneration. Each natural regeneration plot was randomly allocated within a tree stratum plot. In the tree stratum, individuals with a diameter at breast height (DBH) ≥ 5 cm were assessed, while in natural regeneration, regenerating individuals with a DBH < 5 cm and height ≥ 30 cm were evaluated. For species that could not be identified on-site, plant material was collected for identification using information from specialized literature, herbaria, and consultation with experts. The taxonomic classification of species was based on the Angiosperm Phylogeny Group IV system (APG IV 2016).

Litter

For each study area, we collected 20 samples of accumulated litter using a square frame of 25 cm x 25 cm. Litter was sampled at points where soil sub-samples were later collected. The samples were dried in an oven at 65 °C until weight stabilization, and dry biomass (g) was determined on a precision scale. Biomass was then extrapolated to hectares (Mg ha^{-1}) from dry biomass values (g) and the collection frame area (625 cm^2). Subsequently, five composite samples (with four sub-samples each) were ground using a knife and roller mill to achieve a particle size of 0.250 mm. C and nitrogen (N) content and the natural isotopic ratio of the macerated material were determined using an elemental analyzer for IRMS (Flash EA 2000, Thermo Scientific) coupled with an isotopic ratio mass spectrometer (Delta V Advantage, Thermo Scientific). The litter C stock (Mg ha^{-1}) was calculated by multiplying the dry biomass (Mg ha^{-1}) by the C content (%).

The isotopic composition ($\delta^{13}C$) was determined from the isotopic ratio using the international Pee Dee Belemnite standard (Faure & Mensing 2005), according to Eq. 1. The results were expressed in δ (‰) units.

$$\delta^{13}C = \left(\frac{R_{sample} - R_{standard}}{R_{standard}}\right) x\ 10^3\ ‰ \qquad (1)$$

Where: $\delta^{13}C$ is the isotopic composition; R_{sample} is the isotopic ratio of $^{13}C/^{12}C$; $R_{standard}$ is the ratio of the international Pee Dee Belemnite standard.

Soil

We collected soil samples at five random points in each study area, spaced at least 150 m apart in CF and 50 m in DF. At each point, four sub-samples spaced approximately 15 m apart were collected to form a composite sample by depth. All samples were collected at least 20 m from the forest edge. The sampled depths were 0-5, 5-15, 15-30, and 30-100 cm. Samples from depths of 0-5, 5-15, and 15-30 cm were collected by opening a trench of 40 cm x 40 cm x 30 cm (length x width x height). Sampling at a depth of 30-100 cm was performed at the center of the trench using a Dutch auger.

After collection, visible roots and other plant residues were manually removed from the soil samples. Subsequently, the material was air-dried, ground in a knife mill, and sieved to 2 mm. Granulometric and chemical analyses of the soil were then performed. In the granulometric analysis, sand, silt, and clay fractions were separated by the pipette method (Teixeira et al. 2017). Chemical analysis followed the methodologies proposed by Tedesco et al. (1995). Percentage of organic matter (%), potential of hydrogen in water - pH_{H2O} (1:1), base saturation (%), cation exchange capacity at pH 7.0 - CEC_{pH7} (cmolc dm^{-3}), and contents of phosphorus - P (mg dm^{-3}), potassium - K (mg dm^{-3}), aluminum - Al (cmolc dm^{-3}), calcium - Ca (cmolc dm^{-3}), and magnesium - Mg (cmolc dm^{-3}) were evaluated (Table I).

A second stage of soil maceration was performed in a roller mill to achieve a particle size of 0.250 mm. A sub-sample of approximately 150 mg was used for determination of C and N contents and natural isotopic ratio on an elemental analyzer for IRMS (Flash EA 2000, Thermo Scientific) coupled to an isotopic ratio mass spectrometer (Delta V Advantage, Thermo Scientific). The soil C stock (Mg ha^{-1}) at different depths was calculated by multiplying the C content (%), soil bulk density (g cm^{-3}), and thickness of the considered soil layer (cm) (Veldkamp 1994). The isotopic composition was determined according to Eq. 1. The proportion of C in the soil from C3 plants was calculated from the isotopic data of each depth using Eq. 2. The $\delta^{13}C$ value for C3 plants was considered as -27.0 ‰ and for C4 plants as -13.0 ‰ (Andriollo et al. 2017). To obtain the proportion of C in the soil from C4 plants, the C3 (%) value was subtracted from 100%.

$$C_3(\%) = \frac{\delta^{13}Csoil - \delta^{13}Cplant\,C_4}{\delta^{13}Cplant\,C_3 - \delta^{13}Cplant\,C_4} x\ 100 \qquad (2)$$

Where: C_3 (%) is the proportion of C_3 plants (%); δ^{13}Csoil is the isotopic composition of C in the soil (‰); $\delta^{13}CplantC_3$ is the isotopic composition of C_3 plants (-27.0 ‰); $\delta^{13}CplantC_4$

Table I. Physicochemical characteristics of the soil at 0-5 cm, 5-15 cm, 15-30 cm, 30-100 cm from conserved riparian forest and degraded riparian forest areas in the Pampa biome, southern Brazil.

Soil characteristics	Conserved Forest				Degraded Forest			
	Soil depths (cm)							
	0-5	5-15	15-30	30-100	0-5	5-15	15-30	30-100
Sand (g kg^{-1})	106	47	79	111	142	93	155	196
Silt (g kg^{-1})	340	320	343	392	314	320	322	342
Clay (g kg^{-1})	554	633	577	497	544	587	523	462
pH in water	5.3	5.0	4.8	4.6	5.1	4.9	4.7	4.7
P (mg dm^{-3})	11.7	6.7	5.2	4.6	12.5	4.1	3.8	3.8
K (mg dm^{-3})	147.0	140.0	112.8	95.2	216.0	133.6	92.0	64.0
Mg ($cmol_c$ dm^{-3})	11.3	15.6	13.6	12.6	8.3	14.1	9.9	7.2
Ca ($cmol_c$ dm^{-3})	31.6	38.0	35.7	32.7	25.4	38.7	22.0	20.3
Al ($cmol_c$ dm^{-3})	0.1	0.1	0.2	1.1	0.2	0.2	0.5	1.1
CEC_{pH7} ($cmol_c$ dm^{-3})	51.5	64.9	62.9	63.6	45.1	65.7	49.2	52.0
Base saturation (%)	83.8	83.1	78.7	70.6	75.8	80.9	67.9	61.1
Organic matter (%)	7.7	4.5	4.3	3.2	7.9	4.8	3.5	2.9
N (%)	0.6	0.3	0.2	0.2	0.7	0.4	0.2	0.2

Where: CEC_{pH7} - cation exchange capacity at pH 7.0.

is the isotopic composition of C_4 plants (-13.0 ‰).

For the determination of soil bulk density at different depths, five trenches of 100 cm x 60 cm x 100 cm (length x width x height) were excavated in each study area. Soil density samples were collected in duplicate using a volumetric ring with a volume of 71.275 cm^3. The soil-filled volumetric rings were then dried in an oven at 105 °C, until reaching a stable weight. Soil bulk density (g cm^{-3}) was calculated by dividing the mass of dry soil (g) by the volume of the volumetric ring (cm^3).

Data analysis

All analyses were conducted using R software, version 4.2.2 (R Development Core Team 2022). Differences in floristic composition between areas were analyzed through permutational multivariate analysis of variance (PERMANOVA) ($p < 0.05$) and non-metric multidimensional scaling (NMDS) in two dimensions, using the Bray-Curtis dissimilarity index. The Shannon Diversity Index (H') and Pielou Evenness Index (J') were calculated, and species rarefaction curves were constructed based on the abundance of individuals from the strata of each study area (Figure 2). The analyses were performed using the vegan (Oksanen et al. 2019) and iNEXT (Hsieh et al. 2016) packages.

Vegetation variables of the tree stratum and natural regeneration (individual abundance, species richness, H', and J'), litter (biomass, C content, C:N ratio, C stock, and $\delta^{13}C$), and soil (density, C content, C:N ratio, C stock, and $\delta^{13}C$) were subjected to one-way analysis of variance (ANOVA) using the F-test at a 5% significance level. To verify the assumptions of ANOVA, the Shapiro-Wilk test for normality of errors and Bartlett's test for homogeneity of variances were applied. Variables that did not

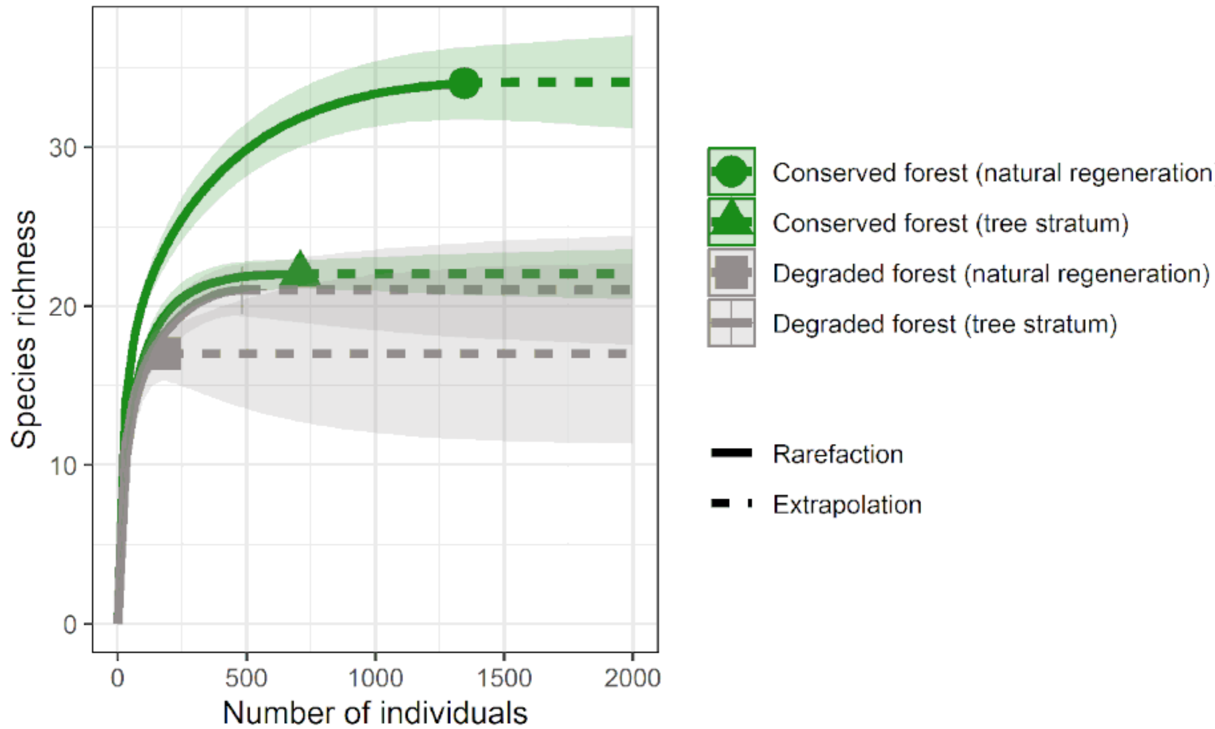

Figure 2. Rarefaction curve based on the abundance of individuals in the tree stratum and natural regeneration from conserved riparian forest and degraded riparian forest areas in the Pampa biome, southern Brazil. The shaded areas depict the 95% confidence interval.

meet the assumptions underwent logarithmic transformation (log x). Analyses were conducted using the ExpDes.pt package (Ferreira et al. 2014).

Additionally, to assess the correlation effects between the evaluated variables and riparian forest areas, a principal component analysis (PCA) was performed using the FactoExtra package (Alboukadel & Mundt 2017). Furthermore, a Pearson correlation at a significance level of 5% was conducted to correlate litter C stock, soil C stock (0-5, 5-15, 15-30, 30-100, and 0-100 cm), abundance, species richness, and H' using the ggcorrplot package (Kassambara 2023).

RESULTS

Vegetation

A total of 2,722 individuals were recorded in the tree stratum and natural regeneration (2,054 individuals in CF and 668 in DF), distributed across 20 botanical families, 35 genera, and 40 species (Table II). The total richness was 37 species in CF (22 in the tree stratum and 34 in natural regeneration) and 26 in DF (21 in the tree stratum and 17 in natural regeneration). In the tree stratum of both riparian forest areas, the most abundant species were *Gymnanthes klotzschiana* Müll.Arg. (CF = 41.6% of individuals and DF = 32.9%) and *Eugenia uniflora* L. (CF = 12.4% and DF = 14.3%). In the natural regeneration, the most expressive species were *Myrciaria tenella* (DC.) (CF = 18.4% of individuals and DF = 44.6%) and *Justicia brasiliana* Roth. (CF = 12.3% and DF = 12.5%).

Unlike the tree stratum (PERMANOVA, p = 0.058), the NMDS analysis of natural regeneration revealed the formation of two distinct groups for species composition (PERMANOVA, p = 0.001) (Figure 3). In the tree stratum, a significant difference between CF and DF was observed

Table II. Species sampled in the tree stratum and natural regeneration from conserved riparian forest and degraded riparian forest areas in the Pampa biome, southern Brazil.

Families / Species	CONSERVED FOREST						DEGRADED FOREST					
	Tree stratum			Natural regeneration			Tree stratum			Natural regeneration		
	N	N/ha	RF	N	N/ha	RF	N	N/ha	RF	N	N/ha	RF
Acanthaceae												
Justicia brasiliana Roth				166	830	5.8				23	115	11.3
Justicia laevilinguis (Nees) Lindau				2	10	0.6						
Ruellia brevifolia (Pohl) C.Ezcurra				60	300	2.6						
Arecaceae												
Syagrus romanzoffiana (Cham.) Glassman				2	10	1.3						
Boraginaceae												
Cordia americana (L.) Gottschling & J.S.Mill.	8	40	3.0									
Celastraceae												
Monteverdia ilicifolia (Mart. ex Reissek) Biral				100	500	5.2				3	15	3.8
Erythroxylaceae												
Erythroxylum myrsinites Mart.							2	10	2.1	3	15	3.8
Euphorbiaceae												
Gymnanthes klotzschiana Müll.Arg.	295	1,475	10.0	23	115	4.5	152	760	10.3	7	35	5.7
Sebastiania brasiliensis Spreng.	22	110	6.0	6	30	1.9	12	60	4.1			
Fabaceae												
Ateleia glazioveana Baill.	14	70	5.0	17	85	2.6						
Calliandra tweedii Benth.	2	10	2.0	64	320	4.5				3	15	1.9
Parapiptadenia rigida (Benth.) Brenan	10	50	4.0				16	80	3.1			
UNF												
UNE				11	55	0.6						
Lauraceae												
Nectandra sp.	4	20	1.0	3	15	1.3						
Ocotea puberula (Rich.) Nees				2	10	1.3						
Malvaceae												
Luehea divaricata Mart.							2	10	1.0			
Meliaceae												
Trichilia elegans A.Juss.				29	145	1.9						
Myrtaceae												
Blepharocalyx salicifolius (Kunth) O.Berg	52	260	7.0	63	315	3.9	27	135	8.2	2	10	1.9
Eugenia pyriformis Cambess.	19	95	3.0	56	280	2.6	25	125	5.2	3	15	5.7
Eugenia uniflora L.	88	440	8.0	28	140	5.2	69	345	9.3	17	85	9.4
Eugenia uruguayensis Cambess.	13	65	2.0	81	405	5.2				2	10	1.9
Myrcia glomerata (Cambess.) G.P.Burton & E.Lucas				6	30	1.3						
Myrcianthes pungens (O.Berg) D.Legrand	6	30	4.0				12	60	4.1	7	35	7.5
Myrciaria tenella (DC.) O.Berg	44	220	9.0	248	1,240	5.8	51	255	8.2	82	410	17.0
Myrrhinium atropurpureum Schott	3	15	3.0	3	15	1.3	5	25	2.1	3	15	3.8

Table II. Continuation.

Families / Species	CONSERVED FOREST						DEGRADED FOREST					
	Tree stratum			Natural regeneration			Tree stratum			Natural regeneration		
	N	N/ha	RF	N	N/ha	RF	N	N/ha	RF	N	N/ha	RF
Phytolaccaceae												
Petiveria alliacea L.				1	5	0.6						
Polygonaceae												
Coccoloba cordata Cham.	35	175	8.0	5	25	1.9	15	75	6.2	4	20	3.8
Ruprechtia laxiflora Meisn.	9	45	3.0	4	20	0.6	7	35	3.1			
Rhamnaceae												
Scutia buxifolia Reissek	10	50	3.0	5	25	1.9	29	145	9.3			
Rubiaceae												
Cephalanthus glabratus (Spreng.) K.Schum.				2	10	1.3						
Guettarda uruguensis Cham. & Schltdl.	16	80	6.0	5	25	3.2	20	100	7.2	8	40	7.5
Psychotria carthagenensis Jacq.				85	425	5.8						
Salicaceae												
Casearia decandra Jacq.	5	25	3.0	2	10	1.3	2	10	2.1			
Sapindaceae												
Allophylus edulis (A.St.-Hil. et al.) Hieron. ex Niederl.	46	230	8.0	42	210	4.5	29	145	10.3			
Cupania vernalis Cambess.				121	605	5.8	2	10	1.0	2	10	3.8
Matayba elaeagnoides Radlk.	4	20	1.0	65	325	5.8	2	10	1.0			
Sapotaceae												
Pouteria salicifolia (Spreng.) Radlk.							2	10	1.0			
Solanaceae												
Solanum pseudoquina A.St.-Hil.	4	20	1.0	3	15	1.3	3	15	1.0	2	10	3.8
Solanum sp.				3	15	1.3						
Thymelaeaceae												
Daphnopsis racemosa Griseb.				32	160	4.5				13	65	7.5
Total	709	3,545	100.0	1,345	6,725	100.0	484	2,420	100.0	184	920	100.0

Where: UNF - unidentified family, UNE - unidentified species, N - number of individuals, N/ha - number of individuals per hectare, RF - relative frequency.

only in abundance (Figure 4). However, use by cattle decreased the abundance, richness, and H' of natural regeneration. The abundance and total species richness of natural regeneration were reduced by 86% and 50%, respectively. The density of individuals in natural regeneration decreased from 6,725 ind/ha to 920 ind/ha, while in the tree stratum, the reduction was from 3,545 ind/ha to 2,420 ind/ha (Table II).

Litter and soil

The use by cattle altered all assessed litter variables (Table III). There was a decrease in biomass and C stock, while an increase was observed in C content, the C:N ratio, and $\delta^{13}C$. The most significant changes were noted in litter biomass and C stock, which decreased by 53% and 38% in DF, respectively.

No significant differences were observed among the mean values of density, carbon content, C:N ratio, and carbon stock in the

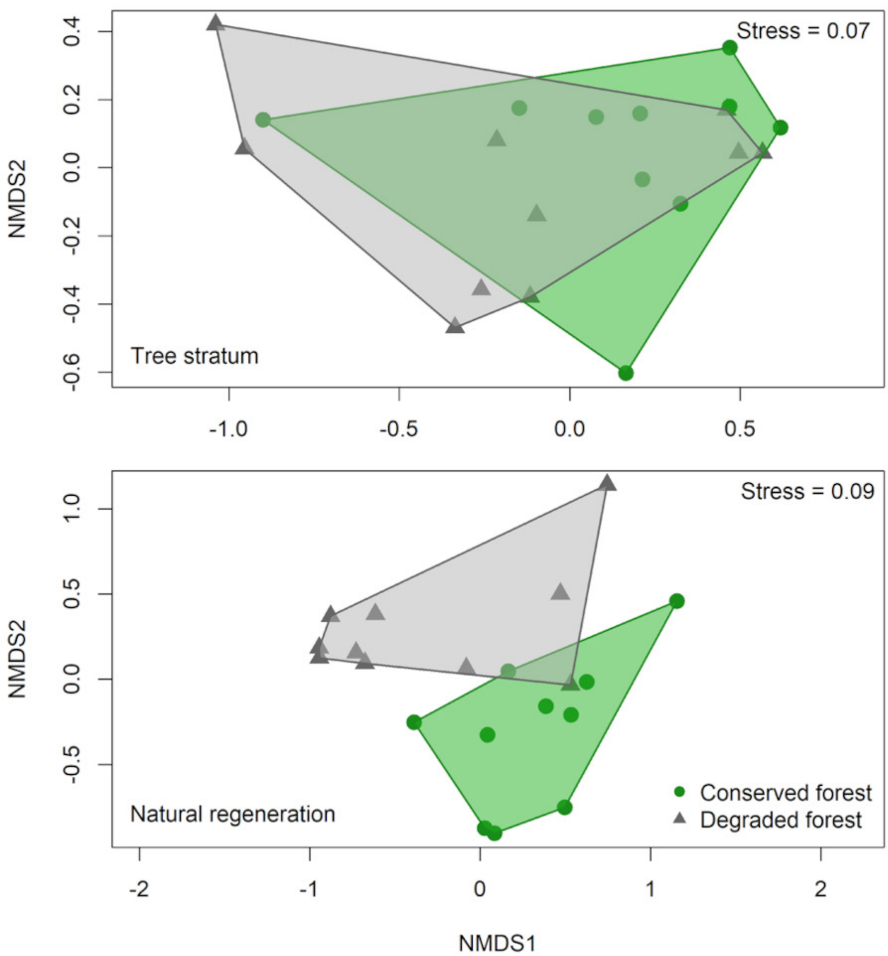

Figure 3. Non-metric multidimensional scaling (NMDS) of the floristic composition of species in the tree stratum and natural regeneration from conserved riparian forest and degraded riparian forest areas in the Pampa biome, southern Brazil.

soil (Table III). However, there was a tendency towards higher density values for DF, especially at intermediate depths (5-15 and 15-30 cm). Although not statistically significant, there was also a tendency for higher C content and stock in the superficial layers of DF (0-5 and 5-15 cm), with an inversion of the trend for deeper layers (15-30 and 30-100 cm). C content decreased with depth, reducing by 69% and 78% from the surface layer to the deepest layer in CF and DF, respectively. Soil $\delta^{13}C$ increased with depth in both areas, with statistically higher values in DF at depths of 15-30 and 30-100 cm.

The proportion of C3 plants in soil organic matter formation decreased with depth, while the proportion of C4 plants increased (Figure 5). In CF, the percentages of C derived from C3 plants in the 0-5 cm and 30-100 cm layers were 78.4% (21.6% of C4) and 44.5% (55.5% of C4), respectively. In DF, the percentages of C derived from C3 plants in the 0-5 cm and 30-100 cm layers were 77.1% (22.9% of C4) and 27.1% (72.9% of C4), respectively.

Vegetation-litter-soil relationship

The first two components of the PCA explained nearly 60% of the original data variability (Figure 6). The PCA highlighted the effects of forest types on vegetation, litter, and soil variables. CF and DF were positioned on opposite sides in spatial distribution, indicating divergent behaviors. CF was positioned to the right on the spatial distribution, with a positive linear relationship with litter biomass, litter C stock,

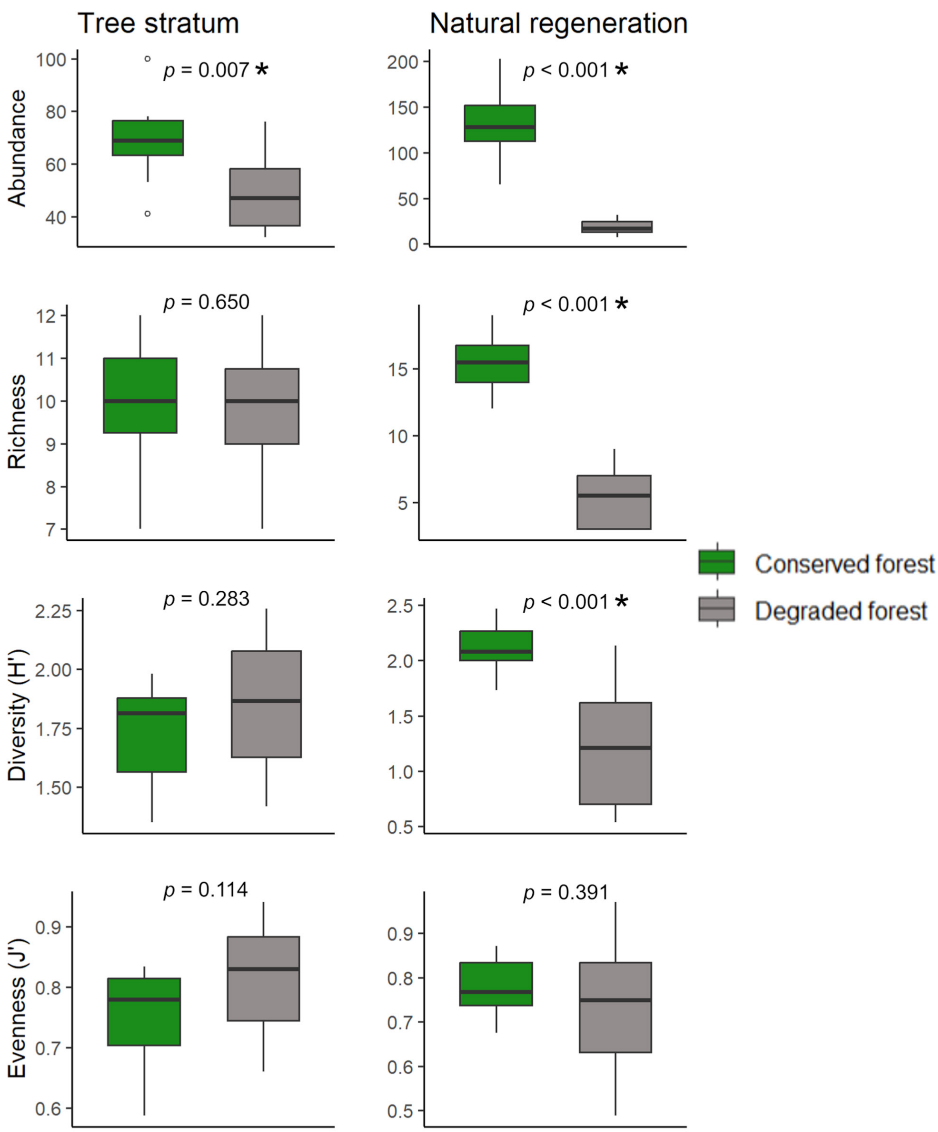

Figure 4. **Abundance, richness, diversity, and evenness of the tree stratum and natural regeneration from conserved riparian forest and degraded riparian forest areas in the Pampa biome, southern Brazil. Asterisks (*) indicate significant differences between the areas evaluated by the ANOVA F-test ($p < 0.05$).**

natural regeneration abundance, natural regeneration richness, natural regeneration H', and tree stratum abundance. In contrast, DF was positioned to the left on the spatial distribution, with a positive linear relationship with litter C content, tree stratum H', density of 0-5 cm, density of 5-15 cm, density of 15-30 cm, C content of 0-5 cm, C stock of 0-5 cm, and C stock of 5-15 cm.

There were no significant correlations between vegetation variables and the total soil C stock (0-100 cm) (Figure 7). Litter C stock positively correlated with soil C stock of 30-100 cm, tree stratum abundance, and abundance, richness, and H' of natural regeneration, but negatively correlated with C stock in the 5-15 cm layer. Negative correlations were observed between abundance, richness, and H' of natural regeneration and soil C stocks in the 0-5 and 5-15 cm layers.

Table III. **Litter and soil variables (0-5, 5-15, 15-30, and 30-100 cm) from conserved riparian forest and degraded riparian forest areas in the Pampa biome, southern Brazil. Values are presented as mean ± standard error. Asterisks (*) indicate significant differences between areas assessed by the ANOVA F-test ($p < 0.05$).**

Pools	Variable	Conserved Forest	Degraded Forest	ANOVA (p-value)
Litter	Biomass (Mg ha^{-1})	16.55 ± 1.68	7.82 ± 0.67	0.001*
	C content (%)	27.72 ± 1.35	36.39 ± 0.82	< 0.001*
	C:N ratio	17.81 ± 0.30	21.71 ± 1.17	0.011*
	C stock (Mg C ha^{-1})	4.61 ± 0.59	2.84 ± 0.24	0.023*
	$\delta^{13}C$ (‰)	-28.75 ± 0.10	-27.93 ± 0.17	0.003*
Soil	Density 0-5 (g cm^{-3})	0.89 ± 0.05	0.95 ± 0.06	0.469
	Density 5-15 (g cm^{-3})	1.00 ± 0.03	1.12 ± 0.05	0.077
	Density 15-30 (g cm^{-3})	1.10 ± 0.04	1.18 ± 0.01	0.097
	Density 30-100 (g cm^{-3})	1.16 ± 0.02	1.19 ± 0.02	0.288
	C content 0-5 (%)	7.15 ± 0.65	8.47 ± 1.04	0.311
	C content 5-15 (%)	4.05 ± 0.13	4.47 ± 0.41	0.356
	C content 15-30 (%)	3.13 ± 0.15	2.84 ± 0.24	0.335
	C content 30-100 (%)	2.21 ± 0.12	1.90 ± 0.11	0.100
	C:N ratio 0-5	11.91 ± 0.09	11.91 ± 0.26	0.993
	C:N ratio 5-15	12.44 ± 0.09	12.00 ± 0.21	0.097
	C:N ratio 15-30	13.30 ± 0.41	13.66 ± 0.20	0.455
	C:N ratio 30-100	12.42 ± 0.42	12.12 ± 0.86	0.768
	C stock 0-5 (Mg C ha^{-1})	31.62 ± 2.65	40.01 ± 5.59	0.212
	C stock 5-15 (Mg C ha^{-1})	40.26 ± 1.53	49.93 ± 5.12	0.108
	C stock 15-30 (Mg C ha^{-1})	51.60 ± 2.90	50.34 ± 4.47	0.818
	C stock 30-100 (Mg C ha^{-1})	179.41 ± 10.53	159.13 ± 10.88	0.217
	Total C stock 0-100 (Mg C ha^{-1})	302.89 ± 10.07	299.42 ± 18.18	0.871
	$\delta^{13}C$ 0-5 (‰)	-23.97 ± 0.22	-23.79 ± 0.14	0.516
	$\delta^{13}C$ 5-15 (‰)	-20.86 ± 0.28	-20.77 ± 0.40	0.856
	$\delta^{13}C$ 15-30 (‰)	-19.69 ± 0.18	-17.43 ± 0.55	0.004*
	$\delta^{13}C$ 30-100 (‰)	-19.23 ± 0.75	-16.79 ± 0.44	0.023*

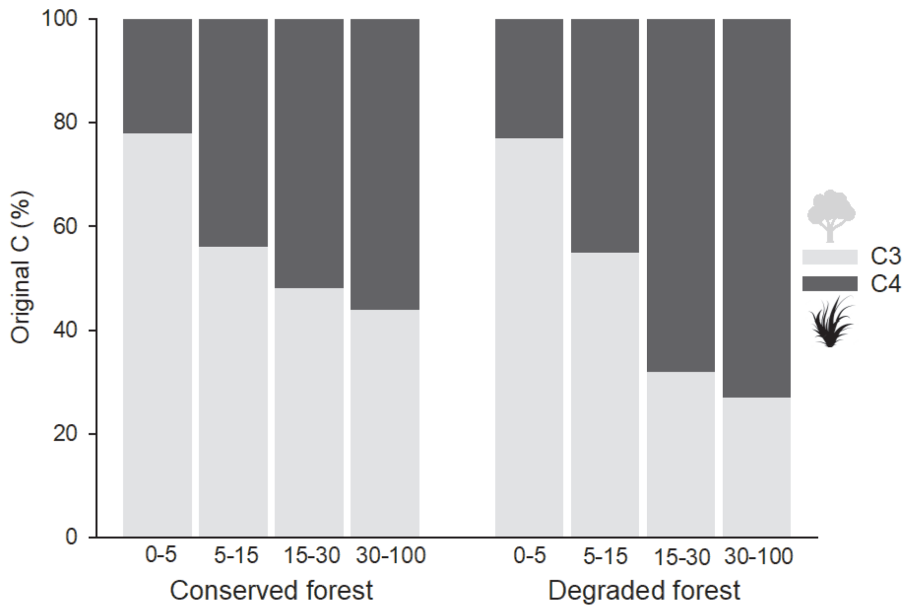

Figure 5. Proportion of carbon from C3 and C4 plants at different soil depths (0-5, 5-15, 15-30, and 30-100 cm) in conserved riparian forest and degraded riparian forest areas in the Pampa biome, southern Brazil.

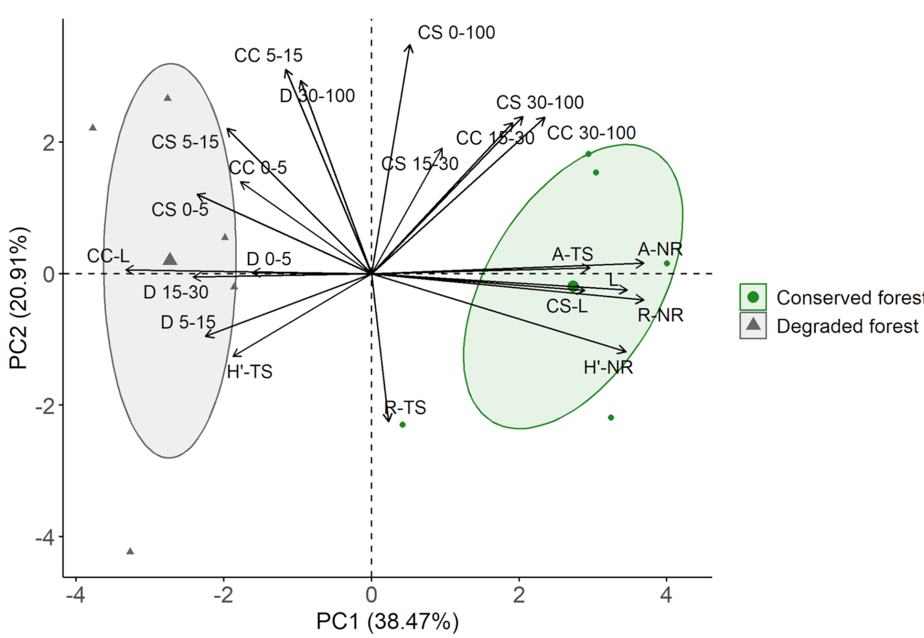

Figure 6. Principal Component Analysis (PCA) for vegetation, litter, and soil parameters at different depths (0-5, 5-15, 15-30, 30-100, and 0-100 cm) in conserved riparian forest and degraded areas in the Pampa biome, southern Brazil. NR - natural regeneration, TS - tree stratum, A - individual abundance, R - species richness, H' - Shannon Diversity Index, L - litter, CC - carbon content, CS - carbon stock, and D - bulk density.

DISCUSSION

Livestock grazing areas in Brazil often extend to the forests along watercourses (Guidotti et al. 2020). Therefore, comprehending the impacts of the presence of cattle on different compartments of riparian forests is essential for the conservation and management of these environments. The current study allowed us to assess the effects of the presence of cattle on vegetation, litter, and soil in riparian forests in southern Brazil. After 28 years of cattle exclusion, we observed that the major impact of the presence of cattle in the forest was on natural regeneration and accumulated litter biomass. Contrary to our hypothesis and previous studies (Galleguillos et al. 2018, Michels et al. 2012, Schulz et al. 2016), no significant alterations were found

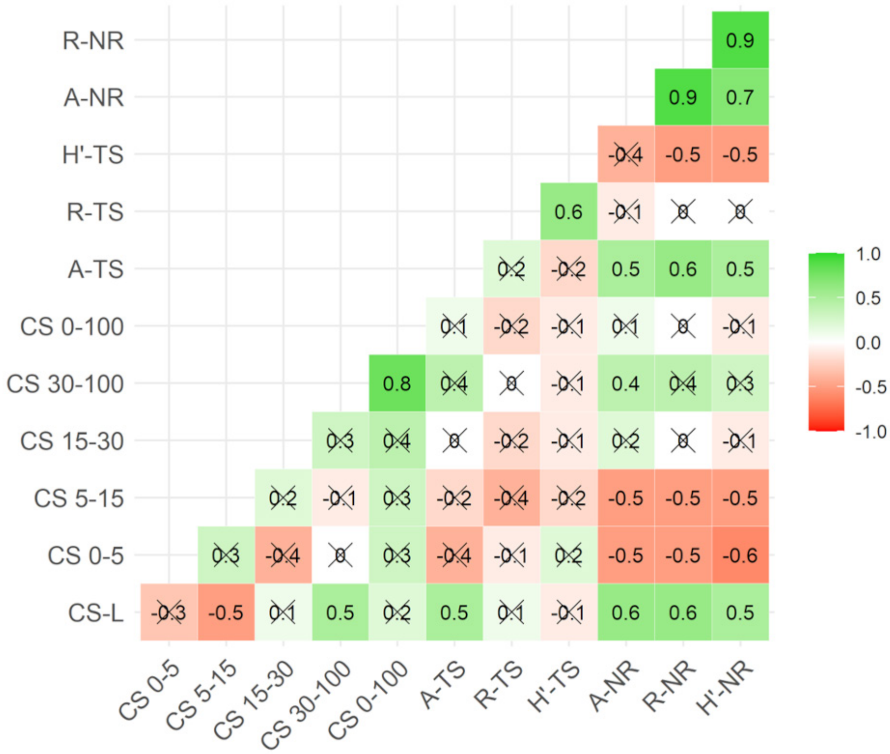

Figure 7. Pearson correlation for vegetation, litter, and soil parameters at different depths (0-5, 5-15, 15-30, 30-100, and 0-100 cm) in conserved riparian forest and degraded riparian forest areas in the Pampa biome, southern Brazil. NR - natural regeneration, TS - tree stratum, A - individual abundance, R - species richness, H' - Shannon Diversity Index, L - litter, and CS - carbon stock.

in the soil C stock between riparian forests with and without cattle.

Most species found in this study are common in riparian forests of southern Brazil (Araujo et al. 2018, Budke et al. 2004, 2010, Gonçalves et al. 2018, Oliveira et al. 2015, Pasdiora et al. 2021, Scipioni et al. 2019, Silva et al. 2020). The high abundance of the pioneer species *G. klotzschiana*, which accounted for over 40% of adults in CF (CF = 1,475 ind/ha and DF = 760 ind/ha), is associated with its wide plasticity and adaptation to alluvial environments, where it can exhibit monodominance (Pasdiora et al. 2021). Similar to *G. klotzschiana*, the most abundant species in natural regeneration (*M. tenella*) (CF = 1,240 ind/ha and DF = 255 ind/ha) is a hydrophilic species commonly found in alluvial forests of southern Brazil (Budke et al. 2004, 2010, Gonçalves et al. 2018, Pasdiora et al. 2021). The dominance of a few species in CF and DF sampling, both in the tree stratum (especially *G. klotzschiana* and *E. uniflora*) and in natural regeneration (especially *M. tenella* and *J. brasiliana*), is common in riparian forests in southern Brazil and is related to specific soil characteristics in alluvial environments (Pasdiora et al. 2021), which are seasonally subjected to floods (Savacinski et al. 2023).

The presence of cattle in the forest altered its structure and floristics, mainly impacting the composition, abundance, richness, and diversity of natural regeneration species. In the tree stratum, besides the decrease in abundance, we also observed a trend in the alteration of floristic composition (PERMANOVA, $p = 0.058$). Compared to the tree stratum, natural regeneration is directly affected by trampling and herbivory due to its lower position in the vertical structure of the forest. Additionally, the recruitment of species in natural regeneration can be influenced by the amount of accumulated litter (Cintra 1997, Hassan et al. 2021), which was reduced in CF. Livestock can impact the structure and species composition in forests (González et al. 2005, Li & Jiang 2021), reducing the abundance of individuals and species richness in the understory and natural regeneration, but not necessarily decreasing diversity (Stefanello et al. 2021). The impact on forest structure and composition depends on the intensity of the disturbance caused (Budke et al. 2010, Stefanello et al. 2021).

Litter production is a crucial indicator of the primary productivity of forest ecosystems and is directly involved in plant-soil interactions (Vitousek 1982). In the current study, cattle grazing halved litter biomass and reduced its C stock by almost 40%, with an increase in C content and the C:N ratio. The lower C content of litter in CF (27.7%) compared to DF (36.4%) and studies in seasonal forests in the south (36.2%) (Sanquetta et al. 2014) and southeast (38,6%) (Zanini et al. 2021) of Brazil may be related to the decomposition state of the collected litter. The chemical composition of litter changes during the decomposition process, and the organic C content decreases over time, as it serves as the primary energy source for decomposers (Giweta 2020). Selected grazing of certain species from natural regeneration may also have contributed to the higher values of C content and the C:N ratio of litter in DF, since cattle select the plants they consume (Stefanello et al. 2021, Schulz et al. 2019), with a preference for more palatable species with higher nutritional quality (lower C:N ratio) (Pauler et al. 2020). Consequently, species with a higher C:N ratio will persist in the forest community and contribute in a greater proportion to litter formation.

The decrease in abundance in the tree stratum and natural regeneration, as well as in the richness and diversity in natural regeneration, contributed to reducing litter biomass and C stock in DF. As observed in our results, studies show that higher individual abundance increases the production of materials that constitute litter (O'Keefe & Naiman 2006), and more diversified subtropical forests produce more litter with higher N concentrations (Huang et al. 2017), contributing to a decrease in the C:N ratio. Cattle herbivory is also a factor that may have contributed to reducing litter production and, consequently, the accumulated biomass in DF (Giweta 2020). Moreover, cattle trampling can increase biomass and C losses from litter by mixing it with the soil (Wei et al. 2021). It is important to highlight that reductions in litter biomass and tree abundance caused by cattle could impair the C storage capacity of DF in the future (Schulz et al. 2018).

Although not statistically significant, the results of soil bulk density, C content, and C stock seem to demonstrate the effects of cattle use in the forest's upper soil layers. As the effects of cattle exclusion depend on the timescale (Li & Jiang 2021), we believe that these effects on soil variables may intensify over time. The trend of higher C stock in DF up to a depth of 15 cm and in CF from 15 to 100 cm reflects the behavior of density and C content with depth. Cattle trampling can lead to soil compaction in forest areas (Galleguillos et al. 2018, Michels et al. 2012, Hernández-Valdez et al. 2023). However, similar to this study, Etchebarne and Brazeiro (2016) found no significant differences in soil density in forest areas with and without cattle exclusion for up to 17 years in the Uruguayan Pampa.

Cattle grazing in grassland and forest areas may or may not alter soil C stock (Pineiro et al. 2010, Li & Jiang 2021, Schulz et al. 2016). The absence of a significant difference in soil C stocks in riparian forests may be related to the positive influence of cattle trampling and feces deposition on the formation and stabilization of soil organic C. Livestock activities can increase soil C by enhancing litter decomposition through physical fragmentation (Chuan et al. 2018) and incorporation into the soil (Wei et al. 2021). Studies also demonstrate that bovine manure increases the organic C content of the soil (Yagüe et al. 2016) and that cattle can be responsible for transporting nutrients from pasture areas to forested areas (Van Uytvanck et al. 2010). The saturation of organic C in the soil, defined as the theoretical maximum of soil organic C that can be associated and stabilized in clay and fine silt (Fujisaki et al. 2018), may also be involved in the absence of a significant difference between the C stocks of CF and DF. These results reinforce that livestock, a traditional economic activity in the Pampa biome, can be conducted sustainably in a grassland-forest mosaic without a negative impact on soil C stocks.

The high total soil C stock in riparian forests (CF = 302.89 Mg ha^{-1} e DF = 299.42 Mg ha^{-1}) is an important finding for Fluvisol in Brazil and emphasizes the potential of these soils for C storage in the Pampa biome. Rio Grande do Sul is one of the Brazilian states with the highest amounts of stored C in soil, mainly due to the colder climate (Bernoux et al. 2002, Demattê et al. 2022). Although approximately 50% of the soil C stock in Brazil is found within the top 30 cm (Gomes et al. 2019), we found 40.8% and 46.9% of the C stock in the 0-30 cm layer in CF and DF, respectively. The significant contribution of the 30-100 cm layer to the total C stock in the study areas demonstrates the importance of sampling the soil to a depth of 100 cm and stratifying this layer (e.g., 30-60 and 60-100 cm) in C quantification studies.

Contrary to our expectations, there was no correlation between the total soil C stock and floristic variables. The higher abundance in the tree stratum and the greater abundance, richness, and diversity in natural regeneration were not determinants for a higher soil C stock in CF. The soil C stock also did not positively correlate with the litter C stock, indicating that other factors may be directly influencing soil C, such as root input (Keller et al. 2021). Soil organic C dynamics are regulated by the interaction of climatic, edaphic, and biotic conditions (Luo et al. 2017). These factors are interconnected, and their effects on soil C stock cannot be easily isolated without considering other factors (Nicolas et al. 2022). Another important aspect is that changes in forest soil C are slower than changes in aboveground C, taking decades to occur (Jandl et al. 2007).

Studies of natural isotopic abundance are an important tool for understanding vegetation dynamics in a specific location in the past. C3 photosynthetic cycle plants have lower $\delta^{13}C$ values (approximately -27.0 ‰), while C4 cycle plants have values close to -13.0 ‰ (Andriollo et al. 2017). In the shallower soil layers (0-5 and 5-15 cm), $\delta^{13}C$ values are directly related to the litter $\delta^{13}C$ values, which are predominantly composed of plant material from C3 plants. The higher contribution of C4 plants (i.e., higher $\delta^{13}C$ values) to the formation of organic matter in the deeper layers, especially in DF, may be linked to changes in vegetation cover. In the past, these areas may have been predominantly occupied by grassland formations, which commonly have a higher proportion of C4 plants, mainly represented by species from the Poaceae and Cyperaceae families (Sage 2016). The greater proportion of C from C4 plants in the deeper layers of DF indicates that this area may have undergone the grassland-forest transition at a later time than CF. Grasslands have naturally been replaced by forests in the Brazilian Pampa (Behling et al. 2024).

Despite the significant economic importance of livestock farming in Brazil and other regions of the world, its practice without sustainable management is associated with adverse impacts on ecosystems (Dick et al. 2021, Li & Jiang 2021). In the current study, the impacts caused by cattle in the forest community demonstrate the need to seek alternatives for proper cattle management in forested areas of rural properties, especially those considered as PPA. The Brazilian Forest Code allows cattle access to PPA for the purpose of watering (Brasil 2012), but Brazilian environmental legislation still lacks specific regulations on this matter. The adjustment of cattle stocking rates and the fencing of riparian forest areas in PPA with the installation of watering corridors for cattle are crucial actions to reconcile livestock production with forest conservation. These actions are essential to avoid the loss of plant biodiversity and impairment of aboveground C storage capacity.

As mentioned earlier, we conducted this study in two adjacent riparian forest areas with the same topographic conditions and soil classification, one isolated from cattle for almost three decades and the other with cattle use. Thus, specific conditions, like those in this study, make it difficult to use other sites in the region as treatment replications (Davies & Gray 2015, Fraser et al. 2020). Additional studies are essential to understand the possible mechanisms associated with livestock (e.g., trampling and feces deposition) that could be linked to the trend of higher C stock in the shallower soil layers of DF. This would also aid understanding whether these potential mechanisms could compensate for the effects of degradation on soil C stock in the forest community.

CONCLUSIONS

We investigated how the presence of a high stocking density of cattle in riparian forest areas influences the long-term composition and structure of vegetation, as well as the litter and soil C stocks. We found that cattle alter the composition and structure of riparian forest vegetation, negatively impacting primarily the abundance, richness, and diversity of natural regeneration. Cattle use reduces the litter C stock but does not significantly alter the soil C stock under the studied conditions. However, by altering the regenerative capacity of the forest, cattle can impact the persistence of species in the forest community over time, jeopardizing the perpetuity of the forest and weakening its future capacity for carbon storage in forest biomass and litter. Our results underscore the importance of proper cattle management in riparian forest areas of rural properties in order to reduce animal load or isolating these areas with the installation of watering corridors. The pursuit of adaptation and coexistence alternatives for livestock activities while maintaining the ecosystem services of natural forests is essential for sustainable livestock management in the grassland-forest mosaic of the Pampa biome.

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