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Irrigated rice rotations affect yield and soil organic carbon sequestration in temperate South America

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Abstract

Rice (*Oryza sativa* L.) systems rotated with perennial pastures have intensified in South America to increase annual grain productivity, but the effects on rice yield and soil quality remain poorly understood. We evaluated rice grain yield, crop and pasture biomass production, and soil organic carbon (SOC) and total nitrogen stocks (0–15-cm depth) in three rice-based rotations over 8 yr in Uruguay. Treatments were: (a) rice–pasture [a 5 yr rotation of rice–ryegrass (*Lolium multiflorum* Lam.)–rice, then 3.5 yr of a perennial mixture of tall fescue (*Festuca arundinacea* Schreb.), white clover (*Trifolium repens* L.), and birdsfoot trefoil (*Lotus corniculatus* L.)], (b) rice–soybean [a 2-yr rotation of rice–ryegrass–soybean (*Glycine max* [L.] Merr.)–Egyptian clover (*Trifolium alexandrinum* L.)], and (c) rice–cover crop (an annual rotation of rice–Egyptian clover). Rice after soybean or pasture achieved the highest yield (9.8 Mg ha^{-1}), 9% higher than rice after rice in the rice–pasture and rice–cover crop systems. Estimated belowground biomass under rice–pasture (2.7 Mg ha^{-1}) was 12 and 42% greater than under rice–cover crop and rice–soybean rotations, respectively. Rice–pasture showed an increase of 0.6 Mg ha^{-1} yr^{-1} of SOC; no changes were observed in the intensified rotations replacing pasture with additional rice or soybean. All systems sustained soil total N. These results provide insights for implementing sustainable rice-based rotations, with rice–pasture being the only system that increased SOC while achieving high rice yields and belowground biomass productivity.

1 | INTRODUCTION

Uruguay is a country in South America where agricultural products represent 75% of national exports. Rice (*Oryza sativa* L.) is produced on around 200,000 ha, primarily for export (95%), with average annual yields of around 8.2 Mg ha^{-1}, one of the highest worldwide. The typical crop rotation sequence is alternating rice (1–2 yr) and pasture for beef cattle production (3–4 yr). The inclusion of pasture, either sown or naturally regenerating, in rotation with rice provides sustainability advantages in terms of soil quality and reduced dependence on external inputs compared with other rice systems in the world (Deambrosi, 2003; Pittelkow et al., 2016). For example, average fertilizer nitrogen use in rice in South Asia is around 200 kg ha^{-1} but in South America, it is 120 kg

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Abbreviations: BD, bulk density; SOC, soil organic carbon; TN, total nitrogen.

ha^{-1}, whereas it is only 80 kg ha^{-1} in Uruguay (Chauhan et al., 2017), as a result of biological N fixation by the pasture and recycling of organic N by livestock (Castillo et al., 2021). However, economic pressures are causing farmers to intensify rice–pasture rotations, specifically to reduce the pasture phase of the rotation in favor of more annual grain crops. Hereafter, intensification refers to increased cropping system intensity and the associated external inputs required for annual crop production. For example, in the last 15 yr around one-third (200) of farmers abandoned the integration of pasture and livestock in their rice production systems because of a lack of profit (Oficina de Estadísticas Agropecuarias, 2018; Molina et al., 2019). The annualization of cropping systems, decoupling crops from livestock, has occurred widely over the last 20–30 yr in South America, causing a decrease in pasture area and replacement of historically complex rotations with simplified crop sequences (Carvalho et al., 2021).

Two options for increasing annual grain productivity in Uruguay involve substituting the pasture phase of the rotation with either soybean [Glycine max (L.) Merr.] or rice, both of which are likely to affect yields of the following rice crop (Ribas et al., 2021; Yadvinder-Singh et al., 2008). Soybean production has continued to increase in Uruguay, following trends for much of South America in recent decades, with some farmers rotating soybean and rice in search of economic advantages (Oficina de Estadísticas Agropecuarias, 2018). Short-term revenue might also increase by continuously growing rice, which is common practice in many intensive rice systems worldwide but has not historically been practiced in Uruguay. However, replacing pasture with annual crops may negatively influence crop productivity. Recent research in Uruguay illustrated the positive effects of crop–pasture systems on wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), attributing this benefit to better soil quality or higher soil organic carbon (SOC) content (Ernst et al., 2018; Rubio et al., 2021). However, the positive effects of pasture on yield have been shown to decline over time, meaning that the more years under continuous annual crops instead of pasture, the lower the wheat yield (Ernst et al., 2018).

In the choice between soybean and rice as an intensification option, rotating with soybean is likely to support higher rice yields relative to continuous rice. Crop yield benefits are particularly noteworthy when cereal and legume crops are alternated (Crookston et al., 1991; Stanger et al., 2008). For example, rice yield improvements of 24 to 46% were observed after mungbean [*Vigna radiata* (L.) R.Wilczek] in Vietnam. Similarly, Ribas et al. (2021) found that including soybean in rotations increased rice yield by 26% compared with rice after rice in southern Brazil. Meanwhile, previous research in Uruguay indicated that rice after rice is lower-yielding than rice after pasture (Méndez, 1993). However, crop yields are not only affected by the previous crop and long-term rotation history, but also by the presence of cover crops grown during the winter period. Grass and legume cover crop species are both used in Uruguay, with different C/N ratios strongly influencing decomposition patterns and soil N availability for the subsequent crop.

Core Ideas

- Rice yield was improved after pasture or soybean but decreased for rice after rice.
- Intensified stable rice–pasture rotations with rice or soybean did not decrease SOC.
- The rice–pasture system sequestered 0.6 Mg ha^{-1} yr^{-1} SOC during the 8-yr study.
- Approximately 1.9 Mg ha^{-1} yr^{-1} of belowground biomass was required to sustain SOC.

Soil organic carbon is the foundation of soil quality and future food security (Amelung et al., 2020; Bünemann et al., 2018), helping to regulate nutrient cycles and the soil–plant–water interactions that underpin agricultural productivity (Oldfield et al., 2019). Given the mechanisms controlling SOC storage discussed below, the literature suggests that conversion of rice–pasture to rotations with higher frequency of annual grain crops could have either positive or negative effects on SOC. Briefly, the positive benefits of pasture for SOC are well-documented in rainfed systems (Baethgen et al., 2021), so the loss of pasture could decrease SOC. However, rice is grown under flooded soil conditions, which benefits SOC, thus increasing the frequency of rice in the rotation could offset the loss of pasture, especially considering the high annual rice biomass production (Witt et al., 2000). In contrast, SOC could be reduced when a rainfed crop is included in a continuously flooded rice system (Witt et al., 2000) or sustained when soybean is included (Motschenbacher et al., 2013). The net effects of intensified rotations are therefore uncertain, specifically because the baseline system is composed of two drivers that positively affect the C balance (pasture and flooded rice soils), and the loss of one could potentially by compensated by gains in the other (i.e., pasture being replaced by the increasing frequency of flooded rice under intensification).

Rice paddy soils are reported to have greater SOC sequestration and content than nonflooded (i.e., the upland, aerobic, rainfed) soils as a result of the flooded periods during irrigation (lower redox potential) that decrease residue and SOC decomposition rates (Chen et al., 2021; Pan et al., 2010; Sahrawat, 2012). As a result, continuous rice tends to have higher SOC than rice-based cropping systems that include rainfed crops such as maize (*Zea mays* L.) in rotation

(Dobermann & Witt, 2000; Witt et al., 2000; Yadvinder-Singh et al., 2008). Although perennial pastures supporting livestock production are also grown under aerobic conditions, it is widely accepted that the integration of livestock and crops in rotation enhances SOC sequestration and tightens nutrient cycles (Brewer & Gaudin, 2020). This is because perennial pastures are often associated with greater C inputs and reduced soil disturbance than annual crops (Ernst & Siri-Prieto, 2009; Franzluebbers et al., 2014; Terra et al., 2006). Previous work has suggested that rice–pasture systems could increase soil quality compared with continuous rice, despite pastures being grown under rainfed conditions. For example, integrated rice–pasture systems improved rice yield and nutrient use efficiency compared with a monocropping rice system with a winter fallow in the south of Brazil (Denardin et al., 2020).

Another important driver of SOC is above- and belowground biomass production (Fujisaki et al., 2018). The crop rotation sequence determines the amount of biomass produced in the system, thus affecting C inputs and soil N supply (Cassman et al., 1996; Witt et al., 2000). A study in rice paddy soils in subtropical China showed a positive relationship between changes in SOC and C inputs (Chen et al., 2016), whereas for double-cropped rice in a subtropical climate, approximately 4% of residue C inputs were transformed to stabilized SOC (Mandal et al., 2008). Recent literature has indicated the higher efficiency of belowground biomass's contribution to SOC (Mazzilli et al., 2015; Sokol & Bradford, 2019). To identify biomass thresholds for maintaining soil quality, several studies in the U.S. Corn Belt have quantified the C inputs needed in a maize–soybean rotation to sustain SOC. Using simulation models, Huggins et al. (1998) and Gollany et al. (2019) estimated that 5.6 C ha^{-1} yr^{-1} (from aboveground biomass and roots under tillage) or 3.8 Mg C ha^{-1} yr^{-1} (aboveground biomass under no-tillage), respectively, was required to sustain SOC in the 0- to 30-cm soil depth. However, the effect of biomass on SOC sequestration and the required C inputs under different rice-based rotations including pasture and annual crops in temperate regions has not been quantified.

In this study, we addressed this important knowledge gap for integrated crop–livestock systems with long pasture phases, which are facing pressure to intensify worldwide (Carvalho et al., 2021; Lemaire et al., 2014). Three contrasting rice-based rotations were evaluated after 34 yr of previous soil use under a rice–pasture rotation: rice-pasture as the current paradigm in Uruguay; rice–soybean as the first step of intensification, which is already practiced in Uruguay and a common system in southern Brazil; and rice–cover crop as an extreme intensification system closer to what could be continuous rice. We hypothesized that rice–soybean and rice–cover crop systems would have a negative effect on SOC caused by the loss of perennial pastures and lower belowground biomass inputs, consequently reducing rice yield. The objectives of this study were to: (a) evaluate rice grain yield during the first 8 yr of a long-term experiment, (b) quantify the evolution of SOC stocks and total N (TN), and (c) assess biomass production and its relationship with SOC changes across rotation systems. Insights from this research can provide information about the implementation of rice-based rotations that can sustain high productivity and soil quality through SOC storage.

2 | MATERIALS AND METHODS

2.1 | Site description and the long-term experiment

The study site is in the East of Uruguay (33˚16′22.21″S; 54˚10′23.10″W, 21 m asl) (Figure 1), located in the Temperate Grassland terrestrial ecoregion (Olson et al., 2001). The climate is mesothermic humid with a mean temperature of 22.3 ± 0.85 ˚C during summer and 11.5 ± 0.82 ˚C in winter. Annual average rainfall is 1,360 ± 315 mm, with high variation within and between years. Annual total potential evapotranspiration was 1,138 ± 177 mm for the period of 1971 to 2016.

The dominant soils at the site are classified as Argialbolls according to the USDA Soil Taxonomy with 0.5% slopes. Soil properties at the beginning of the experiment are presented in Table 1.

The long-term experiment was initiated in 2012 on a field previously under a rice–pasture rotation for 34 yr. One disk harrow and two landplane operations were made before the beginning of the experiment. The experiment was laid out in a randomized complete block design with all phases of the rotations present in time and space. It included three replications with plot sizes of 1,200 m^2 (60 by 20 m). Although the full experiment was composed of six different rice rotation systems, for this study, three systems were evaluated, which represented the extremes in the length of pasture vs. the frequency of rice (rice–pasture, rice–soybean, and rice–cover crop, with winter cover crops grown in all systems), because the other treatments fell in between these ones. The treatments were: (a) rice–ryegrass (*Lolium multiflorum* Lam.) in winter–rice, followed by 3 yr of a perennial pasture mixture of tall fescue (*Festuca arundinacea* Schreb.), white clover (*Trifolium repens* L.), and birdsfoot trefoil (*Lotus corniculatus* L.) (rice–pasture, 5 yr); (b) rice–ryegrass in winter–soybean [*Glycine max* (L.) Merr.]–Egyptian clover (*Trifolium alexandrinum* L.) in winter (rice–soybean, 2 yr); and (c) rice–Egyptian clover in winter (rice–cover crop, 1 yr.) (Figure 2). Rice–pasture, rice–soybean, and rice–cover crop included 15, 6 and 3 experimental units, respectively. One replicate as an example is included in Supplemental Table S1.

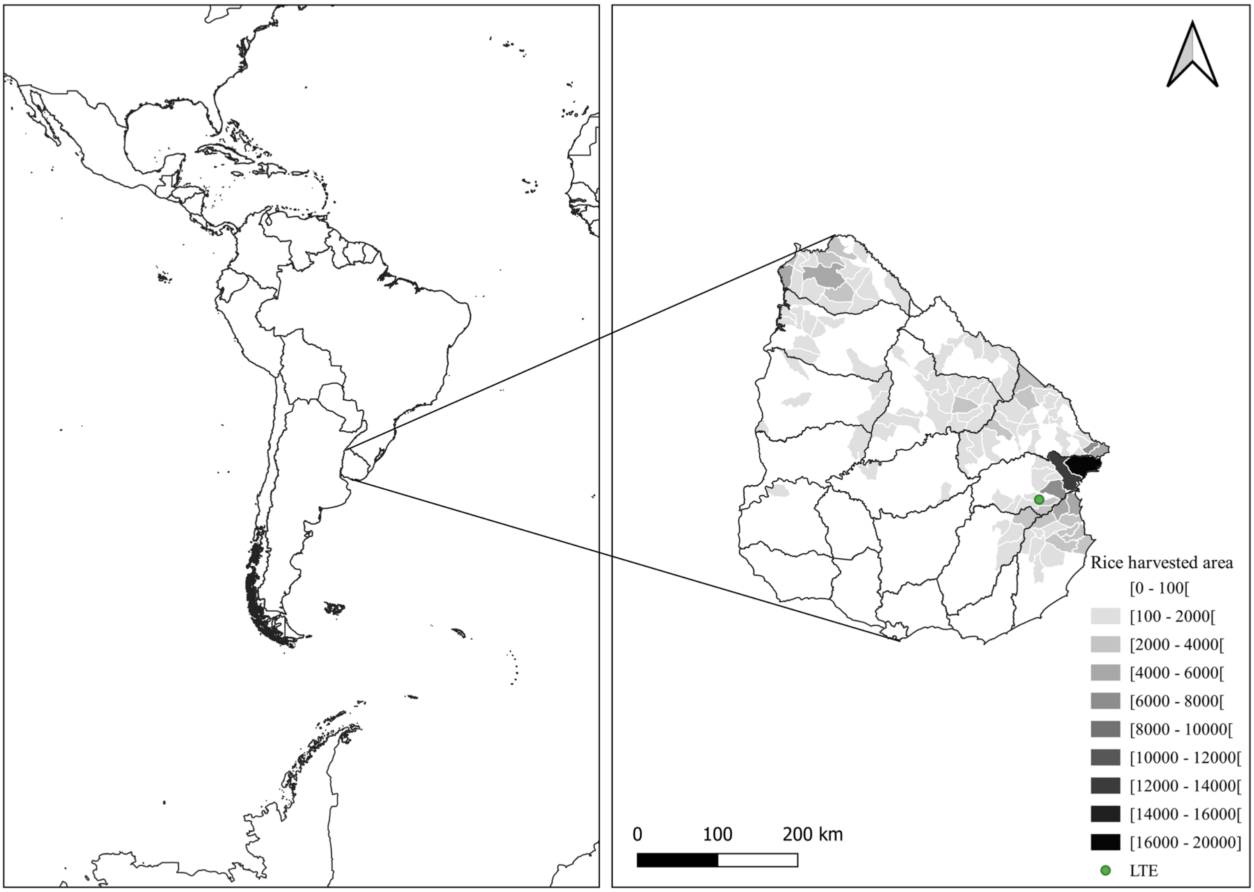

FIGURE 1 Map of South America and Uruguay, with the spatial distribution of rice harvested area (ha) (MGAP, 2011). The location of the long-term experiment is shown in green

2.2 | Agronomic management

All crops in the field experiment were produced under no-till management. For all rotations, rice was planted in October and harvested in March to April. Irrigation was under continuous flooding from 25 to 30 d after crop emergence to 25 d after flowering. For the rice–soybean rotation, soybean (Maturity Groups V–VI) was planted in November and harvested in April to May. Perennial pasture in the rice–pasture rotation, and the cover crops in all rotations, were broadcast immediately after harvest of the row crops. The seeding rates of each species were: rice, 130 to 150 kg ha^{-1}; soybean, 70 to 80 kg ha^{-1}; tall fescue, 15 to 17 kg ha^{-1}; white clover, 2 to 3 kg ha^{-1}; birdsfoot trefoil, 6 to 8 kg ha^{-1}; ryegrass, 18 to 20 kg ha^{-1}; and *T. alexandrinum*, 18 to 20 kg ha^{-1}. The management of N–P–K fertilization followed guidelines developed nationally (Castillo et al., 2015; Deambrosi et al., 2015; Hernández et al., 2013). Rice N fertilization was urea split in two applications, the first one at mid-tillering (V4–V6) immediately before flooding, and the second one at panicle initiation (R0) (Counce et al., 2000). Phosphorus (P_2O_5) and potash (K_2O) fertilization for row crops and perennial pasture was performed at planting. Pasture also received phosphorus fertilizer at the end of the first and second year. Cover crops were not fertilized. The total annual average N–P–K fertilization applied per rotation is presented in Table 2. Crop and pasture management for weeds, diseases, and pests followed recommendations from the local Rice Research Program at the Instituto Nacional de Investigación Agropecuaria. All operations (seeding, fertilization, pesticide application, and harvesting) were managed with machinery, similar to that used by farmers, and perennial pastures of rice–pasture were under direct rotational grazing by sheep during the 3 yr. Detailed crop management information and input use can be found in the study by Macedo et al. (2021).

2.3 | Soil and plant sampling

Rice grain yield was obtained from the whole plot with a combine harvester (New Holland TC 5070). Grain was weighed in a wagon with a digital balance (10 kg precision; Magris MTV 206). Moisture content was measured at

TABLE 1 Characteristics of the initial surface soil (0–15 cm) of the experimental site where rice-based systems were evaluated in Treinta y Tres, Uruguay, starting in 2012

Characteristic	Value
Classification	Argialboll
Texture	Silty clay loam
Clay, g kg^{-1}	300
Silt, g kg^{-1}	510
Sand, g kg^{-1}	190
Bulk density, g cm^{-3}	1.25
Soil organic carbon, g kg^{-1}	14.2
Total nitrogen, g kg^{-1}	1.4
pH	5.7
P content, mg kg^{-1}[a]	10.3
Ca content, cmol kg^{-1}	7
Mg content, cmol kg^{-1}	2.8
K content, cmol kg^{-1}	0.25
Na content, cmol kg^{-1}	0.35

[a] Method of extraction: Bray 1.

TABLE 2 Average annual fertilizer nutrient additions by rotation

Treatment	Nutrient N	P_2O_5	K_2O
	kg ha^{-1} yr^{-1}		
Rice–pasture	39	36	18
Rice–soybean	40	61	65
Rice–cover crop	148	70	51

harvest, and rice yield is reported at a standard moisture content of 13%.

For the baseline soil analysis conducted in 2012 (Table 1), five composite samples (12 cores per sample) were collected in each replication at 0- to 15-cm depth. For soil chemical analysis in subsequent years, surface samples were collected manually from each plot via the same methodology in 2016, 2017, 2018, 2019, and 2020 before planting the rice crop. The samples were air-dried and sieved through a 2-mm mesh, then dried at 45 ˚C for 48 h. The SOC and TN content were measured by dry combustion at 900 ˚C (LECO Truespec; Wright and Bailey, 2001).

For bulk density (BD), samples were collected with a hydraulic jig with a core diameter of 38 mm and a volume of 170.1 cm^3 (0–15 cm depth). Samples were composited and dried at 105 ˚C to a constant weight. Bulk density measurements were made in 2016 and 2019. For 2017, 2018, and 2020, BD values from 2016, 2019, and 2019 were used, respectively. Because of a lack of BD measurements at the beginning of the experiment, recent measurements were made from a field with a similar rotation history and soil conditions (one disk harrow and two landplanes) directly next to the long-term experiment. As this field had the same rice–pasture sequence for many decades, this value was used as an estimate of initial BD for all rotations. We acknowledge this is a limitation in our study, as only SOC and TN concentrations were measured directly at the start of the experiment. It should be noted that the starting BD would have been the same for all rotations; thus, our assumption might affect absolute changes in SOC and TN, but not relative differences among treatments. To explore the implications of this assumption, all regressions below for SOC and TN were also performed with the concentrations alone, and the results and conclusions did not change.

Total SOC and N stocks were expressed at a fixed depth (0–15 cm) and calculated as follows:

$$\text{SOC or TN} = c \times d \times \text{BD} \times 10^{-1}$$

where SOC or TN represents the stocks, c is the concentration of SOC or N in g kg^{-1}, d is the depth of soil sampling in cm, and BD is the BD in g cm^{-3}.

Four plant samples of 0.34 m^2 were composited from each plot to determine the rice harvest index (shoot–grain partitioning) each year. With the value of rice grain yield on a dry basis of the whole plot (60 × 20 m), total aboveground biomass was estimated from this harvest index. Aboveground biomass in soybean was estimated with a harvest index of 0.4 (Bolinder et al., 2007) combined with measured grain yield. Three samples of 0.1 m^2 were made for cover crops to calculate aboveground biomass 1 or 2 d before termination with herbicides. To estimate the aboveground biomass in the perennial pasture of the rice–pasture system, three samples of 0.1 m^2 were obtained immediately before and after the grazing periods (7–10 per year). After 3 yr of pasture, biomass was also collected prior to termination with a herbicide application. All plant samples were composited and dried at 60 ˚C for 48 h to a constant weight.

Different shoot/root ratios from the literature were used to estimate belowground biomass. A value of 7 was used for rice based on previous measurements of the shoot/root ratio in Uruguay (Deambrosi and Mendez, personal communication, October 2018), similar to the values reported by Ju et al. (2015) under different N rates and varieties. The shoot/root ratios of cover crops used were those reported by Pinto et al. (2021): 8 and 6 for ryegrass and Egyptian clover, respectively. Shoot/root ratios for soybean (5) as well as the perennial pasture (2) were based on Bolinder et al. (2007).

2.4 | Statistical analysis

Linear regression models were used to evaluate changes in SOC and TN stocks in each treatment. The estimation method

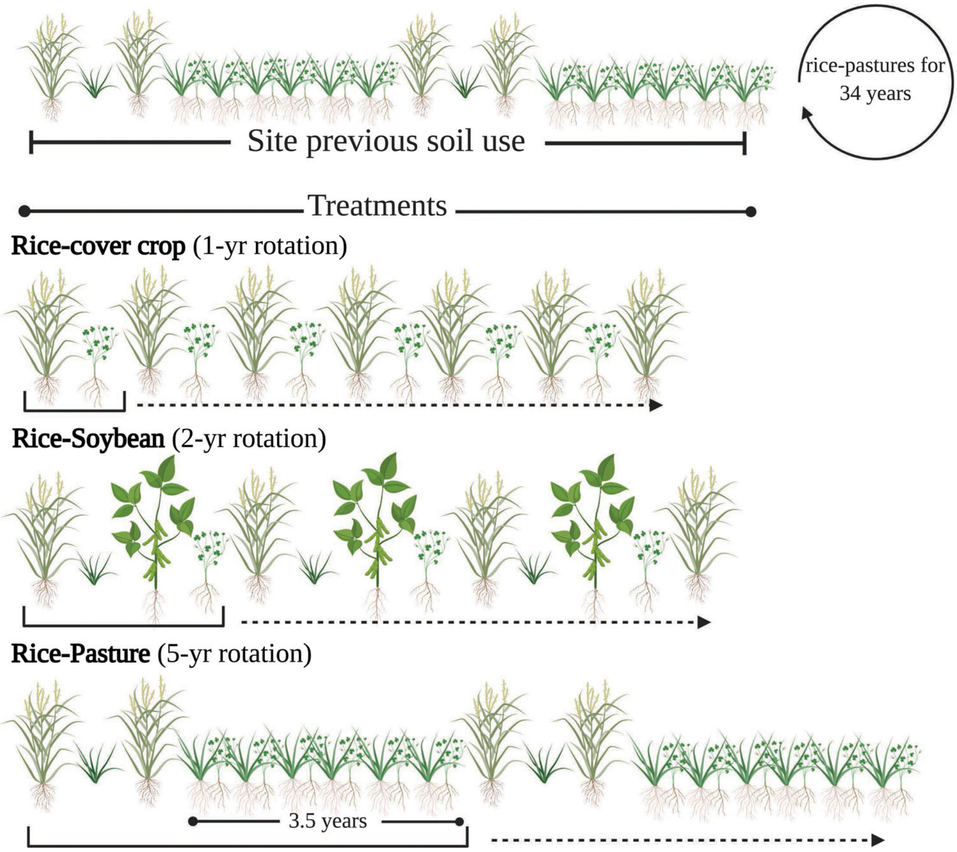

FIGURE 2 Rice-based rotations evaluated and sequence length for each crop during the 8-yr study period. The experiment was initiated in a field that had previously been in a rice–pasture rotation for 34 yr

was restricted maximum likelihood. Both linear and quadratic models were evaluated, and the best fit model was selected based on log-likelihood ratio tests. To evaluate differences between slopes (the rate of SOC change) between treatments, *t*-tests were applied. Biomass variables and rice yield were evaluated by ANOVA with mixed models, where replication and years were considered as random effects and rotation was a fixed effect. Considering that rotation effects on crop productivity tend to accumulate with time, rice yields were also evaluated separately for the most recent 2 yr of the experiment. Pearson correlation analysis among aboveground biomass, belowground biomass, total residues, total biomass and SOC annual changes (slopes) were performed. Additionally, ANOVA was conducted for SOC and N concentrations across all sampling years, and for BD in 2016 and 2019. When appropriate, means were separated by Fisher's LSD at the .05 level. Normality and homogeneity of variance assumptions were tested following standard protocols via the Shapiro–Wilk and Levene tests, respectively. All statistical analysis were conducted with Infostat (Di Rienzo et al., 2017). The ggplot2 package (R statistical software) was used for graphical purposes (Wickham, 2016).

3 | RESULTS

3.1 | Rice yield

The mean rice yield across all treatments and years was 9.4 Mg ha^{-1}. All rice phases of each rotation were present every year (Figure 3). Rice immediately after soybean or pasture (i.e., rice–soybean and the first rice phase in rice–pasture) achieved the highest grain yield (9.8 Mg ha^{-1}), with rice after soybean also showing the lowest variation in yield (7.6% CV). Rice grown in consecutive summers, either after winter legumes or a ryegrass cover crop (i.e., rice–cover crop and the second rice phase in rice–pasture) had the lowest yields, but with differences between them. Rice–cover crop yields were 5.6% greater than that of the second rice phase of rice–pasture (8.7 Mg ha^{-1}), with rice–cover crop also showing the highest variation (11.2% CV). Similar trends were found when only the most recent 2 yr were analyzed (Figure 3), with the exception that yields in the rice–cover crop rotation were not different from the other rice crops.

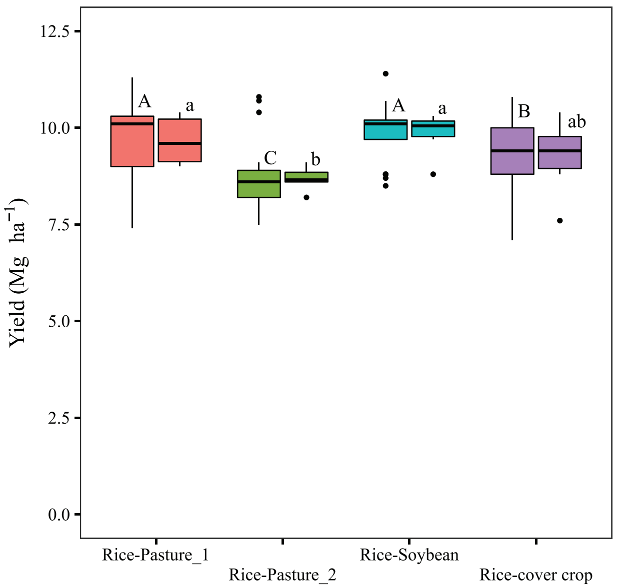

FIGURE 3 Boxplot of rice grain yield (13% moisture content) under different rice rotations. The first and second rice crops in the rice–pasture rotation are reported separately as Rice–pasture_1 and Rice–pasture_2. Horizontal black lines illustrate the median. Black circles are outliers. Different uppercase letters indicate significant differences between treatments for all years and lowercase letters indicate those for the last 2 yr (2017–2018) ($p < .05$)

3.2 | Soil organic C and TN

During the first stage of this long-term experiment, SOC stocks in rice–pasture increased by 0.6 Mg ha^{-1} yr^{-1} ($R^2 = .55$) in topsoil (Figure 4a). Meanwhile, SOC stocks were maintained in the two intensified cropping systems (rice–soybean and rice–cover crop) (Figure 4b,c). Similar to SOC, there was a trend of increasing TN in rice–pasture at a rate of 0.05 Mg ha^{-1} yr^{-1} ($p = .057$) (Figure 4d). In rice–soybean and rice–cover crop, no change in TN was observed (Figure 4e,f). Between the linear and quadratic models, model fit always improved with linear models (except for TN in rice–soybean and rice–cover crop) according to log-likelihood tests. No differences between rice–soybean and rice–cover crop slopes were found for the rate of SOC change; however, the slope for rice–pasture was statistically greater than that of rice–soybean and rice–cover crop.

When considering concentrations instead of stocks, the average SOC concentration was 9.6 and 12.7% greater in rice–pasture than in rice–soybean and rice–cover crop, respectively (Table 3). A similar hierarchy was found in TN concentrations, where rice–pasture achieved the highest value, whereas rice–cover crop the lowest and rice–soybean had an intermediate value. The BD of rice–soybean and rice–cover crop was 4 and 7% greater than that of rice-pasture (1.28 g kg^{-1}), respectively, in 2016 but no differences were found in 2019.

3.3 | Biomass

Rice–cover crop produced 35 and 48% more total biomass (aboveground residues plus belowground biomass and grain yield) on an average annual basis than rice–soybean and rice–pasture, respectively (Table 4). Similarly, aboveground residues were 1.8 Mg ha^{-1} yr^{-1} higher for rice–cover crop than the mean of rice–soybean and rice–pasture (7 Mg ha^{-1} yr^{-1}). In contrast, estimates of belowground biomass in rice–pasture (2.7 Mg ha^{-1} yr^{-1}) were 12 and 42% greater than in rice–cover crop and rice–soybean, respectively. Total residue production (aboveground residues + belowground biomass, without grain) in rice–pasture (9.8 Mg ha^{-1} yr^{-1}) was 0.9 Mg ha^{-1} yr^{-1} greater than in rice–soybean and 1.5 Mg ha^{-1} yr^{-1} lower than in rice–cover crop. The relative contribution of aboveground residues from rice crops was higher than that of the other rotation phases in all treatments. However, although rice contributed to the higher belowground biomass in rice–soybean and rice–cover crop, the pasture phase produced more belowground biomass than the other components in the rice–pasture system (Supplemental Table S2).

Of the different biomass indices, only belowground biomass showed a significative correlation with the annual change in SOC during the study period ($r = .92$, p-value $= .0005$). A quadratic regression was fitted between these two variables. On the basis of the linear relationship of these variables, it was estimated that an annual production of 1.9 Mg ha^{-1} yr^{-1} of belowground biomass is needed to maintain SOC (Figure 5).

4 | DISCUSSION

4.1 | Rice grain yields

We found that rice crops following soybean or pasture the previous summer had a higher grain yield than those following rice, with yields being more stable in the case of rice following soybean (Figure 3). Considering that the soils in this experiment may have accumulated SOC after decades of a rice–pasture rotation in a temperate climate (Deambrosi, 2009), we are unaware of other experimental evidence addressing changes in rice yield and SOC following intensification with annual grain crops. Even though rice was less frequent in the rice–pasture system than in the rice–cover crop or rice–soybean systems, in this study we did not focus on the annualized grain yield of each system because it is not relevant in Uruguay. At the system level, considering that all rice yields ranged from approximately 8 to 10 Mg ha^{-1}, any system with more years of rice would have higher total rice productivity. Instead, we focused on field-level yields of each rice crop under different rotations because the total rice area in Uruguay is limited by irrigation water availability to around 200,000 ha

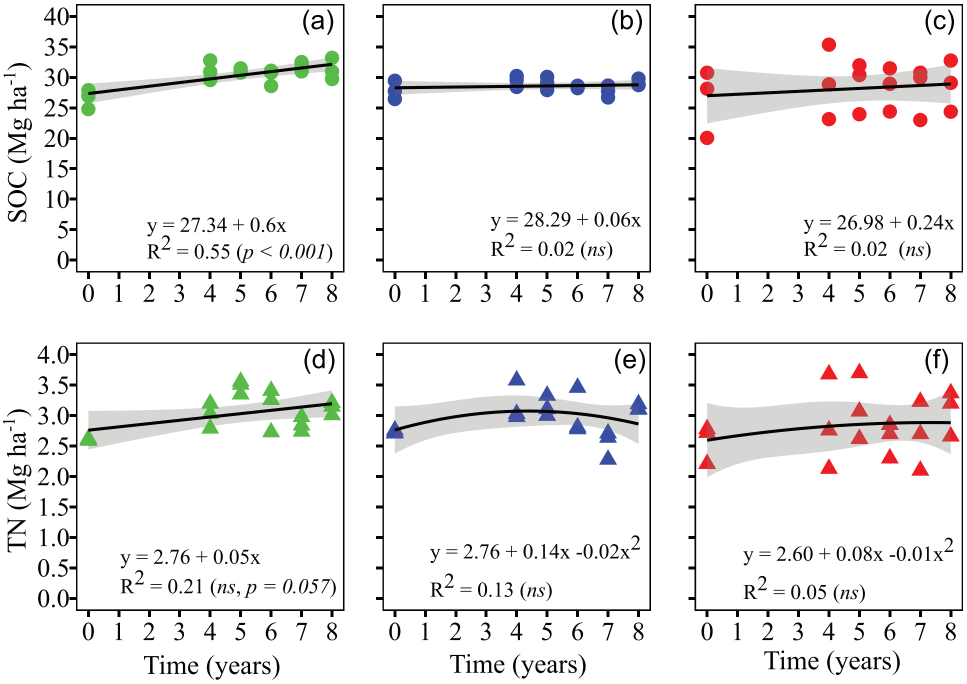

FIGURE 4 Soil organic carbon (SOC) and total nitrogen (TN) stocks in the top 15-cm soil depth over 8 yr in three rice-based systems: rice–pasture (a and d), rice–soybean (b and e), and rice–cover crop (c and f)

TABLE 3 Bulk density (BD) measured in 2016 and 2019, and soil organic carbon (SOC) and nitrogen concentrations for all years in three rice-based rotation systems (values represent means ± SD)

Treatment	BD 2016	BD 2019	SOC	N
	g cm^{-3}		g kg^{-1}	
Rice–pasture	1.28 ± 0.03 b	1.26 ± 0.02 a	16.1 ± 1.1 a	1.6 ± 0.2 a
Rice–soybean	1.33 ± 0.05 ab	1.32 ± 0.05 a	14.6 ± 0.6 b	1.5 ± 0.1 ab
Rice–cover crop	1.37 ± 0.08 a	1.35 ± 0.1 a	14.1 ± 2.6 b	1.4 ± 0.3 b

Note. Different letters indicate significant differences between treatments ($p < .05$).

TABLE 4 Mean aboveground residues (without grain) and belowground biomass, total residues (aboveground residues + belowground biomass), and total biomass production (Mg ha^{-1} yr^{-1}) ± SD in three rice-based systems across the study period

Treatment	Aboveground residues	Belowground biomass	Total residues	Total biomass
	Mg ha^{-1} yr^{-1}			
Rice–pasture	7.0 ± 0.8 b	2.7 ± 0.4 a	9.8 ± 1.2 b	13 ± 1.3 c
Rice–soybean	7.0 ± 1.2 b	1.9 ± 0.3 c	8.9 ± 1.5 c	14.3 ± 2.0 b
Rice–cover crop	8.8 ± 1.8 a	2.4 ± 0.4 b	11.2 ± 2.2 a	19.3 ± 2.7 a

Note. Different letters indicate significant differences between treatments ($p < .05$).

per year. Thus, even if a certain rotation produces more rice or is more profitable, total rice area is relatively static and cannot expand beyond this limit. Accordingly, the intensification of rice–pasture with annual crops does not necessarily mean an increase in yield per unit of area and time for rice. Rather, intensification with annual crops would replace pasture area, leading to the decoupling of rice and pasture systems, which currently cover around 1 M ha annually. We recognize that in other regions where land is the main limitation, the conversion of continuous rice to a rice–pasture system would represent a reduction in system-level grain yields, causing agricultural expansion to maintain current rice production levels.

We did not find that replacing pasture with annual crops necessarily decreased rice productivity, with both rice–soybean (full 8 yr) and rice–cover crop (last 2 yr) achieving

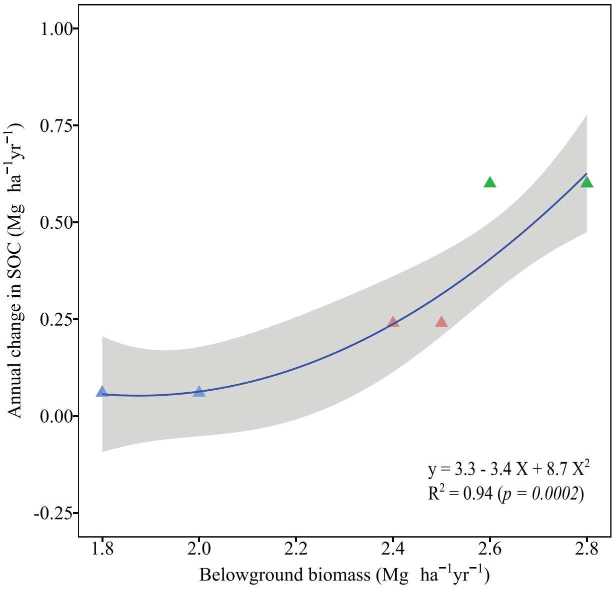

FIGURE 5 Relationship between soil organic carbon (SOC) annual change in the top 15-cm soil depth and the estimated mean annual total belowground biomass in three rice-based systems. Triangle colors represent rotations: green: rice–pasture; blue: rice–soybean; red: rice–cover crop

similar yields to the first year of rice in rice–pasture (Figure 3). This finding does not agree with recent work on rainfed crops in Uruguay, where changes in soil quality or SOC may have a stronger effect on the soil's chemical and physical properties and decreased productivity compared with flooded rice systems (Ernst et al., 2018; Rubio et al., 2021). However, we observed that rice following soybean had higher yields than rice following rice for the two intensification options, as predicted. It is well-known that cereal yields tend to be higher in cereal–legume rotations than in cereal monocultures (Crookston et al., 1991; Stanger et al., 2008). Farmaha et al. (2016) reported that both maize and soybean improved their grain yield when they alternated crops (maize–soy or soy–maize). In rice systems, Xuan et al. (2012) found yield improvements of between 24 and 46% when rice alternated with mungbean, and improvement by 26% were found after soybean in southern Brazil (Ribas et al., 2021).

Although it is logical to expect long-term rotations with pasture will be better for rice yield, our results indicate this only applies to the first year of rice after pasture. In contrast, the second year of rice in the rice–pasture system had lower yields than rice following either rice or soybean in the two intensified continuous cropping systems running for 8 yr, which is opposed to the conclusions of Ernst et al. (2018). These results suggest that the previous crop's effect can be strong, potentially masking the benefit of pasture in the rotation, because the second rice phase of rice–pasture showed the worst performance. However, a closer examination of the two instances of rice following rice indicates other reasons that may explain these yield differences. It was unexpected that rice–cover crop would have a higher yield than the second rice phase of rice–pasture (Figure 3), but this could be the result of several factors. First, the winter cover crop was a legume (*T. alexandrinum*) in the rice–cover crop system, but it was annual ryegrass between the two rice crops of the rice–pasture rotation. Therefore, some combination of biological N fixation associated with the legumes or allelopathy for the grass cover crop preceding rice (Li et al., 2008) could have contributed to the higher rice yields following a legume cover crop. Two other factors could be the higher C/N ratio of ryegrass residue, thus increasing N immobilization in the second rice phase of rice–pasture, and the higher N fertilization rate for rice–cover crop than for the rice–pasture rotation. From a practical perspective, around 40% of national rice area is rice following rice; hence, these results suggest that the inclusion of annual legume cover crops could help maintain high yields in the second year, especially compared with a preceding ryegrass cover crop under no-till. Rice straw management practices that maintain soil cover and contribute to long-term SOC while minimizing negative yield effects on a second rice crop should be included in future research.

4.2 | Effect of rotations on SOC and TN stocks

Rice–pasture was the only system with a positive SOC sequestration rate during the study period (Figure 4). Similarly, Bentende et al. (2008) found after 4 yr that SOC concentrations in the first 0- to 15-cm depth in rice–pasture systems were higher (30.6 g kg^{-1}) than in continuous rice systems (26.1 g kg^{-1}). The rice–pasture system combines the benefits of both crops for SOC: pasture was in place for 70% of the rotation sequence (3.5 of 5 yr) and flooded rice soils help slow the microbial respiration of C through the anaerobic conditions (Chen et al., 2021; Sahrawat, 2012). The fact that the other two systems maintained but did not increase SOC suggests that the benefits of pasture cannot be compensated by an increased frequency of flooded rice crops (every year in rice–cover crop and every other year in rice–soybean). The lack of effect for these systems was despite high C inputs through total annual biomass production and winter cover crops (discussed further below). Previous studies focusing on the conversion of continuous rice to rotations including rainfed (non-flooded) crops show that SOC generally decreases because of increased microbial respiration under aerobic soil conditions (Dobermann & Witt, 2000; Witt et al., 2000). However, for rice–soybean versus the rice–cover crop system, we did not observe differences in SOC concentration or sequestration rates (Table 3; Figure 4). This may be a result of rice–pasture being the starting point of the experiment rather than continuous rice.

A new insight from this work is that despite starting with 1.42% SOC, the rice–pasture treatment further increased the SOC by 0.6 Mg ha^{-1} yr^{-1} during the study period (Figure 4). This experiment was initiated in a rice–pasture rotation under conventional management for 34 yr, with an average rice grain yield of 4.3 Mg ha^{-1} during the 1983–1987 period, 5.7 Mg ha^{-1} between 1987 and 1991, and 6.7 Mg ha^{-1} during 1999 and 2009 (Deambrosi, 2009; Méndez, 1993). This positive change in SOC probably occurred for three reasons: higher rice productivity in the current experiment than in the previous period (9.4 vs. 6.7 Mg ha^{-1}), the conversion to no-till in all experimental rotations compared with the conventional tillage practices previously used, and the inclusion of tall fescue mixed with legumes in the pasture phase, which increases belowground C inputs (discussed below) compared with the annual grass in pastures grown during the previous period. The reason for higher rice productivity is because our experimental management was consistent with optimal practices for closing yield gaps in Uruguay, such as planting date and nitrogen fertilization (Tseng et al., 2021). No-till also promotes SOC in surface layers. Given that rice–pasture is the dominant rotation currently practiced in Uruguay, this finding holds broad relevance for improving soil quality through better management and higher cropping system productivity. Importantly, both of these are aligned with farmers' production goals.

Although rotations with pastures can provide important ecosystem services, such as climate regulation, nutrient cycling, and food production (Carvalho et al., 2021; Franzluebbers et al., 2014), there is increasing pressure to intensify systems to help overcome some of the economic challenges related to land leasing arrangements and thin margins for rice in South America (high production costs and low prices). In this context, an important result is that the intensified systems did not decrease SOC in the top 15 -cm soil depth during our midterm experiment. Preserving soil quality can be critical in countries that, like Uruguay, attempt to make sustainability a pillar of an export-oriented agricultural economy. In recent years, national regulations have been enacted to restrict some rainfed crop sequences to prevent soil erosion and degradation (Pérez Bidegain et al., 2018). More broadly, accounting for the potential negative effects on SOC caused by increasing the frequency of annual crops in a rotation is particularly important, given recent declines in pasture-based systems in Argentina, Uruguay, and southern Brazil (Modernel et al., 2016). For Uruguay, recent estimates suggest that 70% of the increase in crop area is from substituting crop–pasture with crop–crop sequences, whereas 30% is by agricultural expansion into natural grasslands (Oficina de Estadísticas Agropecuarias, 2018; Ernst et al., 2018).

Research such as that in the present study is necessary to understand the effects of substituting pasture with annual crops, both in terms of short-term crop productivity and long-term changes in soil quality. It is important to note that SOC gains in rice–pasture will not continue indefinitely; likewise, the potential effects of intensified systems may take more time to appear. Limited studies have evaluated SOC following the conversion of rice–pasture to more intensive alternatives over 8 yr. According to the IPCC (Tier 1), 20 yr is an adequate timeframe for reporting changes in SOC stocks, which assumes that a new equilibrium is reached (IPCC, 2006). However, many reports also show changes in SOC in the midterm (around 3–10 yr) (Ladha et al., 2011), although the rate of change is higher during the initial years (Fujisaki et al., 2018).

Unlike changes in SOC, there was only a trend of increasing TN in rice–pasture (p-value = .057) (Figure 4). Soil C and N biogeochemistry are tightly linked; thus, the benefits for SOC are likely to become evident and translate into higher soil N supplying capacity (Dobermann & Witt, 2000; Sahrawat, 2012). Research has demonstrated that pastures including legumes support biological N fixation (Labandera et al., 1988), thus a rice–pasture rotation not only helps maintain TN but supports lower long-term use of external N fertilizer inputs in rice than in other regions (Castillo et al., 2021; Chauhan et al., 2017). Total N also did not change for rice–cover crop, which has been reported in other studies (Witt et al., 2000), probably because of the submerged soils as well as biological N fixation by free-living microorganisms in floodwater (Ladha & Reddy, 2003). Additionally, the annual legume cover crop included during winter in this treatment, as well as the N fertilization rate (148 kg N ha^{-1}), could be helping to sustain TN (Zhang et al., 2017).

Rice–soybean and rice–cover crop systems sustained TN, which could suggest substantial N contributions through biological N fixation by the annual legume cover crop and the soybean included in these systems. For example, *T. alexandrinum* is reported to be one of the top-ranked species in terms of biological N fixation (Pinto et al., 2021). Additionally, it is possible that the relatively low soybean grain yield (2.5 Mg ha^{-1}), which indicates the low N removal by this crop and the relatively high contribution through biological N fixation, resulted in a slightly negative or neutral apparent N balance (Salvagiotti et al., 2008; Santachiara et al., 2017). In a high grain price scenario, results for rice–soybean and rice–cover crop imply that intensification of the cropping system through an increased frequency of annual grain crops could allow rice farmers to improve profit without sacrificing SOC in the midterm relative to the baseline rice–pasture system of 34 yr. However, we stress that these results were only achieved in combination with other important soil conservation practices in both intensification options, such as no-till and winter cover crops. Future research over the long-term is still needed to understand how the combination of different pasture species, their management, and soil tillage practices can be optimized, in addition to livestock management practices during the pasture phase, to positively affect SOC sequestration and N dynamics in rice-based systems.

4.3 | Biomass production and grazing effects on SOC

It is often thought that increasing C inputs is the most effective way to build SOC (Amelung et al., 2020; Fujisaki et al., 2018), for example through increased biomass production. However, our analysis emphasizes the need to focus on the source of C (e.g., root vs. shoot biomass) and the quality of C inputs from different phases of the cropping system (e.g., rice vs. pasture) to prioritize opportunities for increasing SOC. The three systems produced between 8.8 and 11.2 Mg ha^{-1} yr^{-1} of residues, with different amounts in the above- and belowground fractions (Table 4). Similar values have been reported for other high-intensity rice–rice systems (Witt et al., 2000), which also maintained or gained SOC during the study period.

Although the rice–cover crop system had the highest total annual biomass productivity, which, in theory, would help build SOC, we found that only belowground biomass under the different treatments showed a relationship with the rate of SOC sequestration (Figure 5). Rice–pasture had the greatest belowground biomass production (2.7 Mg ha^{-1} yr^{-1}), representing 28% of total residue, whereas in the other systems, belowground biomass represented 21% of the total residue (Table 4). Root systems and rhizodeposition are increasingly recognized as playing an important role in SOC sequestration (Liang et al., 2002; Mazzilli et al., 2015; Villarino et al., 2021). In a ^{13}C tracer study, Mazzilli et al. (2015) found that the humification coefficient of belowground C inputs into particulate organic C was 24 and 10% for soybean and maize, respectively, whereas that of aboveground C inputs was only 0.5 and 1.0%. Thus, higher belowground biomass as well the sustained contribution of biological N fixation through legumes in rice–pasture could explain why this system showed both the greatest SOC sequestration rate and SOC concentration.

The result that total biomass input was greater in the two intensified systems, but this did not reflect that SOC sequestration could also be related to the animal effect in crop–livestock rotations. Direct grazing on pasture has been shown to improve soil fertility in rice-based systems (Denardin et al., 2020), leading to a greater potential to sequester SOC than continuous cropping systems (Johnson et al., 2007). The integration of livestock in rice systems benefits the subsequent rice crop, improving nutrient use efficiency and rice yield (Castillo et al., 2021; Denardin et al., 2020). The positive effect of rice–pasture in this study suggests that these integrated crop–livestock systems could be improved locally and further adopted in this ecoregion, considering that in Argentina, Paraguay, and southern Brazil, continuous rice cropping systems prevail.

4.4 | Limitations

There are several limitations of this study. One is that we only evaluated the surface soil depth (0–15 cm) when considering changes in SOC or TN, even though some studies suggest including deeper layers, specifically in no-till systems where SOC could decrease in the subsurface layers (Olson et al., 2014). Additionally, detecting SOC changes is most appropriate over long-term timescales (Paustian et al., 2016; Smith et al., 2020), but here, we evaluated SOC and TN over 8 yr during the first stage of this long-term experiment. Nevertheless, many studies have reported short- or midterm differences in SOC and TN with the aim of detecting the initial changes caused by management.

A related limitation is that SOC sequestration rates are not constant over time, and eventually reach a plateau or saturation (Hassink & Whitmore, 1997; Pravia et al., 2019), with the initial changes often being greater than later ones (Fujisaki et al., 2018). For this reason, the reported SOC sequestration rate reported here for rice–pasture should be considered with caution. According to the equations in Hassink and Whitmore (1997), there is still room for SOC retention in the mineral fraction of soil in this experiment (3.2% of C for saturation for a 30% clay soil). Additionally, other research on a rice–rice system after 31 yr found that total SOC increased with greater C inputs at 0- to 15-cm depth, with the stable fraction of SOC showing saturation while the labile soil fraction did not (Sun et al., 2013).

Finally, there is a tradeoff between SOC sequestration, which is often achieved through C inputs such as residue retention and flooded soils, and methane emissions, which represent the primary field greenhouse gas associated with rice production (Bhattacharyya et al., 2014). Currently, SOC is promoted as a climate change mitigation strategy for agriculture, but this does not necessarily apply to flooded rice as it does to crops grown under aerobic soils. Although sequestering SOC in rice soils could partially mitigate the negative effects of high methane emissions, relatively little research has explored this area. Assuming an average global warming potential of 3.8 Mg CO_2 eq ha^{-1} for field greenhouse gas emissions from rice systems (Linquist et al., 2012), the SOC sequestration rate reported here (0.6 Mg C ha^{-1} yr^{-1} for rice–pasture) could offset this by close to 60%. Therefore, future monitoring of this long-term experiment is necessary to account for methane emissions as part of the total C balance and identify tradeoffs associated with different rotations. This could be combined with simulation modeling to understand the potential for SOC saturation in the future, which could result in lower sequestration rates than the present study.

5 | CONCLUSIONS

The design of cropping systems that increase SOC while maintaining or increasing yields is a key step in the transition to sustainable agriculture. With the aim of evaluating potential outcomes of intensifying rice–pasture systems, we assessed three rotations: (a) the business-as-usual system in Uruguay (rice–pasture), (b) an emerging cropping system that is already practiced in southern Brazil and by some farmers in Uruguay (rice–soybean), and (c) one that is not currently practiced in Uruguay but has the highest frequency of rice, similar to most of the rice grown in the world (rice–cover crop). Rice yield, SOC and TN sequestration rates, and biomass production were assessed after 34 yr of rice–pasture prior to the experiment. We found that intensified systems including rice–soybean and rice–cover crop were able to maintain SOC stocks over 8 yr. In contrast, the rice–pasture system (with increased rice productivity compared with before the experiment) showed an opportunity for SOC sequestration. Belowground biomass estimates were the main factor explaining SOC changes in these systems, with approximately 1.9 Mg ha^{-1} yr^{-1} of belowground biomass inputs required to maintain SOC stocks. Our results indicate there is still a challenge to further increase yield in the second year of rice in different rotations, for which the yield was 12% lower than rice after soybean or a perennial pasture, highlighting the short-term agronomic effect of rotations (previous crop, cover crop, and fertilizer management) on crop yields. Although not investigated here, a possible option based on our results could be integrating soybeans into the rice–pasture rotation, for example, by including one soybean crop between two rice crops, allowing for benefits to SOC and simultaneously increasing rice yield for the second rice phase in the rotation.

The findings of this study suggest that for Argialbolls soils in the temperate region of South America under no-till, the inclusion of perennial grass-legume pastures in the rotation and high belowground residue production sequesters SOC and sustains the high productivity of rice. Intensification of the sequence by replacing perennial pastures with soybeans and more rice improves rice yield but does not increase SOC. On the other hand, intensification through growing rice each year with a winter cover crop maintains SOC but tended to slightly reduced productivity compared with the rice–pasture or rice–soybean rotations.

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AUTHOR CONTRIBUTIONS

Ignacio Macedo: conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing—original draft. Alvaro Roel: conceptualization, supervision, writing—review and editing. Walter Ayala: investigation, writing—review and editing. M. Virginia Pravia: writing—review and editing. Jose A. Terra: methodology, project administration, writing—review and editing. Cameron M. Pittelkow: conceptualization, supervision, writing—review and editing

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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