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Grazing intensity and nitrogen fertilization timing to increase soil organic carbon stock and nitrogen in integrated crop-livestock systems

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ABSTRACT: Integrated crop-livestock systems (ICLS) foster synergistic relationships to increase nitrogen (N) cycling and soil organic carbon (SOC) accrual in agricultural setups. This study evaluated how the grazing intensity and N fertilization (rates and timing) affect both SOC and N fractions, and soil organic matter chemical composition in an ICLS managed under no-tillage in an Oxisol, six years after initiation. The ICLS was compared to a nearby pasture (PA) and a native forest (NF). The treatments consisted of two grazing intensities: Low Sward Height (LH) and High Sward Height (HH) were maintained with high and low stocking rates, respectively. The HH varied between 0.20 and 0.60 m, and LH between 0.10 and 0.30 m according to the plant forage species throughout the experiment. Fertilization using 200 kg ha^{-1} N-urea, not splitting up, was conducted at two timings, either at the winter pasture establishment (autumn), about 35 days after sowing or during the summer cash crop cycle (spring). Total N amount per year, including both phases, pasture and cash crop was the same for all treatments. The SOC and N contents were assessed in soil and particulate organic matter (POM), while carbon (C) and N stocks were specifically determined in the soil. Soil organic matter composition was characterized by FTIR. The combination of HH and N fertilization during the pasture phase increased the content of C from 36.1 to 39.9 ± 0.7 g C kg^{-1} and of N from 2.7 to 3.2 ± 0.1 g N kg^{-1}. The SOC stocks varied from 37.3 to 41.1 ± 0.7 Mg C ha^{-1}, and the N stocks from 2.1 to 3.3 ± 0.1 Mg N ha^{-1} at 0.0-0.10 m soil layer. The SOC content of the POM and the soil organic matter chemical composition determined by FTIR were mainly affected by the grazing intensity. The HH led to an increased in C content within the POM fraction, reaching values of 51.6 ± 1 and 49.2 g C kg^{-1}, respectively to N crop fertilization and N pasture fertilization. Land-use changed how organic functional groups were stored in soil organic matter fractions. The NF had a greater abundance of aliphatic and phenol in the MAOM, while pasture and ICLS systems had greater aliphatic in the POM fraction. In ICLS, SOC accrual was positively associated with more recalcitrant organic functional groups of phenol, aromatic, and carbonyl C-O. The HH increases SOC accrual, while N-fertilization on pasture ensures adequate nutrition of plants and animals during the winter ICLS phase, at the same time as providing greater residual N for subsequent cash crops through enhanced catalyzed by ruminants. Therefore, grazing and fertilization management strategies should be considered to promote sustainable agriculture intensification with ICLS.

Keywords: systems fertilization, decomposition rates, grassland management, nutrients, particulate organic matter.

INTRODUCTION

Integrated crop-livestock systems (ICLS) can improve soil health and crop production, reduce requirements for external inputs, promote environmental and economic resilience, and reduce the environmental footprint of agriculture through ecological intensification of farming systems (Assmann et al., 2018). Agronomic management of ICLS focuses on increasing above-ground biomass production to sustain animal welfare without compromising crop yields (Farias et al., 2020), and provisioning ecosystem services such as carbon sequestration, soil health, and nutrient cycling. The pasture phase has great capacity to accumulate soil organic carbon (SOC) (Soussana and Lemaire, 2014), and SOC accrual can be enhanced by strategies such as fertilization timing and moderate grazing management under no-till systems. The key is to understand how ICLS can be managed to enhance soil-C sequestration without negatively affecting crop and animal yield.

Integrating ruminants into croplands fosters synergistic interactions between the different compartments of the system (soil-plant-animal-atmosphere) that can change the dynamics of SOC and nitrogen (N) in agroecosystems (Cecagno et al., 2018). Understanding how ICLS management of N fertilization and grazing intensity affect SOC and N pools and soil organic matter chemical composition can help elucidate best management practices to increase SOC accrual and nutrient cycling.

New techniques have been sought to enhance the interactions between the components of an agricultural system; among them, a fertilization strategy designed at the system level stands out. System fertilization is based on nutrient cycling between the different phases of a system, that is, in the succession of crops in the same area and their interrelationships. This concept aims to achieve greater efficiency in nutrient cycling, which can reduce fertilization needs, increase efficiency and productivity, reduce losses, and maintain or improve fertility in the long term of rotation seeking maximum nutrient use efficiency, reducing inputs, avoiding losses, and maintaining long-term soil fertility (Assmann et al., 2018; Deiss et al., 2019; Bernardon et al., 2021).

Less stable SOC fractions are more sensitive to management than total SOC, so evaluating the particulate fractions of SOC is an effective tool for determining changes in soil health under contrasting agronomic management (Silveira et al., 2013). According to Christensen (1986), the physically defined fractions, integrate structural and functional properties of SOC, and these fractions have been studied to investigate how soil management affects soil organic matter dynamics (Six et al., 2002). The particulate organic matter (POM) fraction (defined as soil organic matter >53 μm in diameter) is mostly composed of fresh plant litter. It represents a more active or labile soil organic matter pool (Benbi et al., 2014). Carbohydrates and lignin dominate this fraction, and its decomposition is responsive to soil management practices (e.g., tillage, irrigation, straw residue retention) (Christensen, 1986; Six et al., 2002). Moreover, spectroscopic techniques such as Fourier Transform Infrared Spectroscopy (FTIR) are increasingly being used to generate diagnostic information on soil functioning as mediated by mineral and organic functional groups (Demyan et al., 2012; Ge et al., 2014; Mirzaeitalarposhti et al., 2016; Laub et al., 2020; West et al., 2020; Deiss et al., 2021; Hernández et al., 2021). Therefore, the characterization of soil by chemical and physical fractions is particularly important (Cambardella and Elliott, 1993; Boeni et al., 2014; Martins et al., 2015) to better understand the underlying processes governing SOC and N cycles in agricultural systems.

The extent to which grazing intensity and fertilization strategies change SOC and N is poorly understood, particularly in ICLS, since system fertilization considers all cultural phases (pasture and grain crops) in a fertilization scheme across seasons, therefore requiring long-term experiments to generate sufficient data to draw more robust conclusions. We tested the hypothesis that applying N in the pasture phase promotes soil health, improves nutrient input and soil organic matter quality, and can improve soil fertility for subsequent summer crops. This study aimed to determine how soil SOC and N pools and stocks, as well as soil organic matter composition, are affected by contrasting grazing and N-fertilization management in an Oxisol under no-tillage and ICLS grazed by cattle.

MATERIALS AND METHODS

Site description and history of the experimental area

The study was conducted in the municipality of Abelardo Luz, Santa Catarina (southern Brazil), on a 20-ha field experiment that has been under ICLS and no-tillage management since 2012. Since then, the ICLS experimental protocol was maintained with no tillage, for all crops and pastures in the rotation. The crop rotation history of the experimental area since implantation in ICLS is presented in table 1. Species were selected based on adaptation to climate and with the purpose to maximize diversity.

Geographic coordinates of the experimental area are 26° 31' S, 51° 35' W, and 850 m altitude. According to the Köppen Classification system, the climate type is Cfb, humid subtropical. The soil is classified as an Oxisol (Soil Survey Staff, 2014), which corresponds to *Latossolo Bruno distrófico típico* according to the Brazilian Soil Classification System (Santos et al., 2018) with a clayey texture (69.5 % clay). At the beginning of the experiment, soil chemical properties at 0.00-0.20 m layer were: pH($CaCl_2$) = 5.1; organic matter = 54.95 g dm^{-3}; P = 14.2 mg dm^3; K = 187.2 mg dm^{-3}; Ca^{2+} = 5.8 $cmol_c$ dm^{-3}; Mg^{2+} = 1.5 $cmol_c$ dm^{-3}; base saturation = 56.5 %; and CEC = 13.76 $cmol_c$ dm^{-3}.

Total area of the experiment is 14.21 ha, divided into 12 parcels (paddocks) of similar area, plus an area of 10 ha, adjacent to the experiment, for the maintenance of regulatory

Table 1. Agronomic history of the experimental area since ICLS establishment in 2012 in a humid subtropical region of southern Brazil

Season	Crop	System phase	Sward Height[(1)]
Summer 2012/2013	Sorghum (*Sorghum bicolor*)	Pasture	HH 0.60 m LH 0.30 m
Winter 2013	Black oat (*Avena strigosa*)	Pasture	HH 0.30 m LH 0.15 m
Summer 2013/2014	Corn (*Zea mays*)	Crop	- -
Winter 2014	Black oat (*Avena strigosa*) + Ryegrass (*Lolium multiflorum L.*)	Pasture	HH 0.30/0.25 m[(1)] LH 0.15/0.10 m[(2)]
Summer 2014/2015	Soybean (*Glycine max*)	Crop	- -
Winter 2015	Ryegrass (*Lolium multiflorum L.*)	Pasture	HH 0.30 m LH 0.10 m
Early Summer 2015/2016	Corn silage (*Zea mays*)	Crop	- -
Late Summer 2016	Bean (Phaseolus vulgaris)	Crop	- -
Winter 2016	Ryegrass (*Lolium multiflorum L.*)	Pasture	HH 0.20 m LH 0.10 m
Summer 2016/2017	Bean (*Phaseolus vulgaris*)	Crop	- -
Winter 2017	Black oat (*Avena strigosa*)	Pasture	HH 0.25 m LH 0.15 m
Summer 2017/2018	Corn (*Zea mays*)	Crop	- -

(1) The height of 0.30 m was applied for black oats, and the height of 0.25 m was applied for ryegrass in the high-height treatment. (2) The height of 0.15 m was applied for black oats and the height of 0.10 m was applied for ryegrass in the low-height treatment.

animals. The dimensioning of the size of the paddocks was carried out in order to allow the maintenance of at least three test animals in the pasture. The size of the plots was determined according to the treatments, ranging from 10.73 to 12.97 ha, mainly due to the management adopted in the use of N and in the height of the pasture.

The treatments were in a factorial arrangement of 2 × 2, replicated three times. The first factor consisted of Sward Height: High Sward Height (HH) and Low Sward Height (LH). The HH results in low grazing intensity. For the regulation of pasture height, stocking rate of 717 kg of live weight ha^{-1} was used for HH. The LH results in a high grazing intensity, for the regulation of pasture height, stocking rate of 1079 kg of live weight ha^{-1} was used for LH.

The HH and LH varied according to the culture throughout the experiment (Table 1). For black oat, the pasture height was 0.15 m for low height and 0.25 m for HH. The second factor was the N Fertilization Time: (1) N cash crop fertilization (NC) with no N fertilization during the pasture phase and (2) N pasture fertilization (NP) with N fertilization applied during pasture and no N fertilization during the crop phase. We aimed to change N fertilization timing to estimate the carryover effect of N applied to a previous winter cover crop or pasture. Parcel N pasture fertilization received 200 kg of N ha^{-1} during oat cultivation (winter 2017), which was applied in a single time, with the plants in the tilling stage. The N cash crop fertilization, which did not receive N fertilization in the grazing stage, received 200 kg of N ha^{-1} in corn cultivation (summer 2017/2018); when the plants were at stage V5. Since 2012, the ICLS experimental protocol was maintained with no tillage for all crops and pastures in the rotation.

Soil sampling

Soil was sampled after corn harvest in March 2018, six years after the experiment was established. Soil samples were the result of three subsamples per experimental plot in all field replications. Were collected at a layer of 0.00-0.10 m using 0.20 × 0.20 m trenches opened with the aid of a shovel, and later, with the aid of volumetric rings, undisturbed soil samples were collected by the volumetric ring method (Claessen, 1997), at layer from 0.00 to 0.05 and 0.10 m. Nearby areas of native forest (NF) and a conventional pasture (PA) were sampled. Both areas were within a radius of less than 1 km from the experimental unit. In addition, samples from the beginning of the experiment collected in November 2012 were also analyzed. Soil samples were packed in paper bags and taken to the laboratory. Samples were air dried (approximately 25 °C) and sieved using a 0.5 mm sieve. The plant residues and roots >1 mm diameter were removed by hand. For the stock calculation, the soil's density was estimated using volumetric rings, and the soil's density values were 1.10 Mg m^{-3} for the 0.00-0.10 m layer.

Soil Organic Matter fractionation

The soil samples were fractionated according to the methodology suggested by Cambardella and Elliott (1992) to obtain two soil fractions: organic matter associated with minerals (MAOM) and POM. Soil subsamples weighing 20 g were mixed with 60 mL of sodium hexametaphosphate (5 g L^{-1}) and left horizontally agitating overnight for 16 h. The soil suspension was then passed through a 53 µm mesh with the aid of ultrapure water (1.5 L). The two fractions obtained, namely, MAOM (the soil that passed through the mesh) and POM (soil that remained in the sieve), were dried to constant weight at 45 °C. The MAOM contents were estimated through the difference in the carbon contents in the total soil mass in relation to the POM.

SOC, Nitrogen, and C/N ratio in soil and POM fraction

Total contents of SOC and N were quantified in the soil samples and in the POM fractions using an elemental analyzer (Flash EA1112, Thermo Electron Corporation, Milan, Italy). The C/N ratio was calculated by dividing the total C by the total N.

Soil carbon and soil nitrogen stocks in soil

Soil C and soil N stocks were calculated using the methods described by Carvalho et al. (2009) with equation 1:

$$\text{Stock C} = \left(\frac{Cs \times Ds \times \left(\frac{Dref}{Ds} \right) \times e}{10} \right) \quad \text{Eq. 1}$$

in which: stock C is SOC stock at a given layer (Mg ha^{-1}); Cs is SOC content at the sampled layer (g kg^{-1}); Ds is the apparent soil density at the sampled layer at 2018 (kg dm^{-1}); Dref is the soil density for sampled layer in the reference area at 2012 (kg dm^{-1}); and e is the thickness of the layer considered (cm). The variation of the carbon and N stock in the soil was calculated in relation to the reference treatment defined as the beginning of the experiment in 2012 (Stock C, t ha^{-1}).

FTIR Spectroscopy of soil and fractions

Spectroscopy characterization of soil and POM and MAOM fractions was conducted using a Perkin Elmer Frontier FTIR spectrophotometer. The spectra were obtained according to Stevenson (1994). The resolution employed was 4 cm^{-1} with 64 scans per spectrum in a range of 4000 to 400 cm^{-1}. The measures were carried out in duplicate. After the acquisition of the spectra, baseline adjustment was performed. The baseline was automatically corrected using the equipment's software. For potting and principal component analysis (PCA) purposes, spectral data were mean-centered and pre-treated with the first derivative mathematical transformation.

The FTIR analysis characterizes soil organic matter composition and allows for inference regarding carbon persistence in soil by measuring absorbance peak areas corresponding to specific functional groups in soil samples. Organic matter functional group characterization was assessed by integrating peak areas using the local baseline technique (Demyan et al., 2012; Deiss et al., 2020) using non-pretreated absorbance spectra. Local baseline is a virtual straight line added to the base of the peak connecting the peak left and right limits. Local peak areas were determined in the absorbance spectra using the triangle method [R package 'geometry'(Sterratt, 2019)]. Data were processed and analyzed using R version 3.3.3 (R Foundation for Statistical Computing, Vienna, Austria).

Four main organic functional groups were selected to evaluate the impact of experimental treatments on soil organic matter composition: aliphatic C-H functional group of methyl and methlylene groups (peak 2930 cm^{-1}, wavenumber 3010-2800 cm^{-1}) (Orlovic et al., 1986); aromatic C=C stretch, and/or asymmetric -COO- stretch (peak 1620 cm^{-1}, wavenumber 1660-1580 cm^{-1}) (Baes and Bloom, 1989), aromatic C=C stretch (peak 1530 cm^{-1}, wavenumber 1546-1520 cm^{-1}) (Baes and Bloom, 1989); C-O in both polyalcohols and ether functional groups (peak 1159 cm^{-1}, wavenumber 1170-1148 cm^{-1}) (Spaccini and Piccolo, 2007); and C-O of polysaccharides or similar substances (peak 1022 cm^{-1}, wavenumber 1080 to 970 cm^{-1}) (Senesi et al., 2003).

Statistical analysis

The mathematical model applied for SOC and N contents and stocks in the soil is presented by equation 2.

$$Y_{ijk} = m + a_i + d_j + (ad)_{ij} + b_k + e_{ijk} \quad \text{Eq. 2}$$

in which: Y_{ijk} is an observation in block k, referring to treatment (level i of factor A (grazing heights) with level j of factor D (times of N application); *m* is the general mean of the experiment; a_i is the effect of level i of factor A (grazing heights); d_j is the effect of level J of factor D (times of N application); $(ad)_{ij}$ is the effect of the interaction of level i of factor A (grazing heights) with level j of factor D (times of N application); b_k is the random effect of block k; e_{ijk} is the random effect of experimental error.

Statistical analyses of SOC and N contents and stocks in the soil and fractions were performed using STATGRAPHICS software. The SOC and N stock data at different grazing intensities were submitted to analysis of variance (alpha= 5 %) and comparison by LSD test, at a 5 % significance level.

The FTIR spectra were plotted using SigmaPlot software, version 12.5, and PCA was done using the software Pirouette version 4.0 (Infometrix, Seattle, Washington, USA). The FTIR spectra were preprocessed through the multiplicative scatter correction (MSC). The peaks area analysis was conducted using R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria).

The relationship between SOC and organic functional groups abundance was evaluated using linear regression models (‘stats’ R package, R Development Core Team, 2016). Mixed-effects ANOVA ('lme4' R package) (Bates et al., 2015) was used separately for each layer (0.00-0.05 and 0.05-0.10 m), including specific organic functional group abundance (peak areas) as continuous dependent variables, grazing intensity and N as categorical fixed effects, and block as a categorical random effect. Mean comparisons were based on least-square estimates using the Tukey method with an alpha = 0.05 (‘emmeans’ R package) (Lenth, 2019).

RESULTS

Soil organic carbon and nitrogen stocks

Residual dry matter contents after each season, during the six years of experiment in ICLS, is presented in table 2. Generally, greater residual biomass (straw) remained in areas with HH, especially after the pasture phase with black oat and/or ryegrass.

Six years after the experiment initiation, we observed differences in SOC stocks in areas with lower grazing intensity (HH) and N fertilization during the pasture phase (Table 3). The combination, lower grazing intensity and N fertilization during the pasture phase promoted the higher SOC stocks, raising 9.2 % of the initial SOC content after six years compared to the initial sampling (Table 3). In contrast, when only the fertilization timing was changed to the crop phase, but lower grazing intensity was maintained (HH), a decrease in SOC stocks (3.3 % less compared to the initial values) were observed. Under high grazing intensity and both N fertilization regimes, intermediary values were found for SOC stocks.

SOC and N in the POM fraction

The SOC content of POM-C fraction ranged from 41.6 ± 0.3 to 51.6 ± 1 g kg^{-1} (Table 4), with no statistical difference between treatments. However, significant differences were observed between the grazing intensities ($p = 0.042$), where the low grazing intensity achieved higher C contents in the POM fraction (50.42 g kg^{-1}) than the higher grazing intensity (43.59 g kg^{-1}). Higher grazing intensity (LH) associated with N fertilization on pasture had lower SOC in the POM fraction (41.6 ± 0.3 g kg^{-1}), while the POM-N was very similar to the other treatments, resulting in smaller C/N ratio in the POM-C fraction (C/N 12.2). The C/N ratio from the POM-C fraction ranged between 10.4 ± 2.9 to 13.5 ± 0.2. Significant effects of cropping systems on the composition of the POM were expected because this pool is directly derived from the decompositions of fresh plant residues (Oades, 1988).

Table 2. Residual straw biomass after crop and pasture phases in ICLS in southern Brazil

Grazing intensity	N input phase	Crops					
		Summer 2012/2013	Winter 2013	Summer 2013/2014	Winter 2014	Summer 2014/2015	Winter 2015
		Sorghum	Black oat	Corn	Black oat + Ryegrass	Soybean	Ryegrass
		kg ha^{-1}					
High height (HH)	Crop (NC)	5049	1557	7195	1792	4591	4259
	Pasture (NP)	5205	1484	6031	3421	3914	4268
Low height (LH)	Crop (NC)	4538	1250	6639	928	3873	2262
	Pasture (NP)	4817	1251	7227	144	2402	2293
Grazing intensity	N input phase	Early Summer 2015/2016	Late Summer 2016	Winter 2016	Summer 2016/2017	Winter 2017	Summer 2017/2018
		Corn silage	Bean	Ryegrass	Bean	Black oat	Corn
		kg ha^{-1}					
High height (HH)	Crop (NC)	1789	2628	3251	7110	1443	8524
	Pasture (NP)	1569	2030	3238	6294	1466	8724
Low height (LH)	Crop (NC)	1704	2540	1284	5033	632	8486
	Pasture (NP)	1367	2461	1390	4439	642	8521

HH: high pasture height (lower grazing intensity); LH: low pasture height (higher grazing intensity); NC: nitrogen N fertilizer on grain crop; NP: nitrogen fertilizer on pasture.

Soil organic matter chemistry in the particulate and mineral-associated fractions

The FTIR spectra showed notable differences in the soil organic matter chemistry (FTIR functional groups) between the NF, PA, and ICLS land-uses. Organic matter characterization was conducted in the soil and the MAOM and POM fractions (Figure 1). In both POM and MAOM fractions, NF had distinct spectral characteristics from the other land-uses (pasture and ICLS). The first and second principal components (PC1 and PC2) explained over 82.4 % of the spectral variability of the POM-C fraction and 93.5 % of the MAOM-C fraction.

To better understand SOC accrual, selected soil organic functional groups were regressed against SOC content in the soil, as well as, POM-C and MAOM-C fractions. Peak areas assigned to phenol (OH), aromatic (C–C) and/or carboxylate (COO), and carbonyl (C-O) were positively associated with SOC in the soil (Figure 3). Phenol (OH) and aromatic (C–C) and/or carboxylate (COO) were also positively associated with MAOM-C. Less Aliphatic (C-H) and more carbonyl (C-O) was found in the POM-C fraction in the shallower layer (0.00-0.05 m).

Peak area abundances were evaluated across treatments to determine how grazing and N fertilization strategies affect soil organic matter composition. Differences were found at the 0.00 to 0.05 m layer. Both phenol (OH) ($p<0.01$) aromatic (C–C) and/or carboxylate (COO) ($p<0.05$) had greater abundance with lower grazing intensity (HH). The other functional groups of aliphatics and carbonyl did not differ between grazing intensity and fertilization strategies.

DISCUSSION

Soil Organic Carbon and Nitrogen Stocks

Six years after the ICLS experiment initiation, we observed significant increases in SOC stocks in areas with lower grazing intensity (HH) and N fertilization during the pasture phase (Table 3). Under this treatment combination, SOC stocks increased by 9.2 %

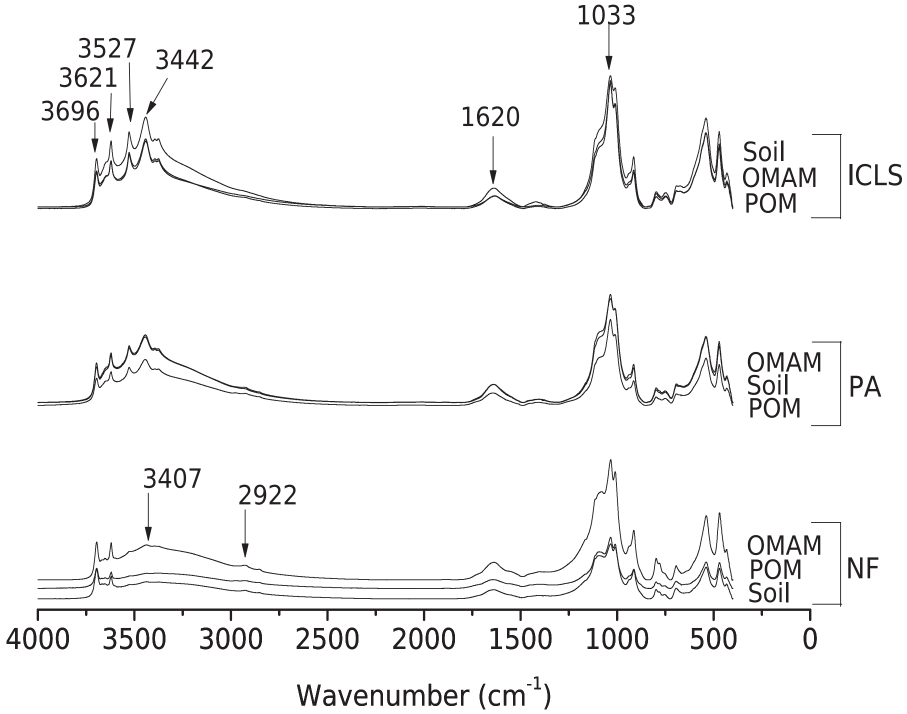

Figure 1. The FTIR spectra of MAOM and POM fractions, and soil, under ICLS, NF, and PA of southern Brazil. The FTIR spectra were plotted using the same scale on the y-axis (absorption intensity). The FTIR coupled with PCA enabled separating distinct land uses (ICLS, NF, and PA) based on spectra characteristics (PCA scores) in both (a) POM and (b) MAOM fractions (Figure 2).

since the initial sampling (Table 3). This result was largely induced by increased vegetal biomass production in pasture managed with lower grazing intensity combined with N-fertilization on the pasture phase. Pastures managed under moderate grazing intensity produce greater biomass due to a stimulatory/compensatory growth mechanism, but lack of N fertilization in that phase can compromise plant growth (McNaughton and Tarrants, 1983) (Table 3). The ICLS can enhance SOC stocks when continuous, adequate amounts of organic matter are added to soils over time (Franzluebbers and Stuedemann, 2014).

Soil N stocks increased over time in all evaluated treatments (38 to 57 % surplus after six years when compared to the initial sampling), but to a lower degree under HH with N fertilization during the crop phase (Table 3). This highlights the importance of N-fertilization on pasture phases to produce more balanced nutrient stoichiometry in soils that helps build up soil organic matter. Moreover, due to intense cycling and lower nutrient exportation by animals when compared to most cash crops, the residual effect of N applied on pastures may reduce or even eliminate the need to apply N for subsequent crops such as corn (Assmann et al., 2018). According to Haynes and Williams (1993), ruminants return most ingested nutrients to the soil as urine and feces (70 to 95 % of ingested plant nutrients).

Nutrient dynamics in ICLS is different than in sole cropping systems due to the catalytic effect of ruminants on processes of decomposition, mineralization, and cycling of nutrients (Assmann et al., 2015). Grazing animals participate in the nutrient cycling process through forage intake, microbial processing of ingested plant biomass in the rumen, temporary nutrient immobilization during digestion, and deposition of urine and feces. Furthermore, the root system of pastures, especially when they receive fertilization and are under moderate grazing intensity, produces root exudates that stimulate microbial rhizospheric activity and promote microbial biomass growth. Microbial biomass and its derived C compounds help building up SOC and N stocks and stimulate further microbial activity (Kuzyakov et al., 2007; Nair and Ngoujio, 2012; Dumontet et al., 2017).

Table 3. Soil total carbon and total nitrogen content (g kg-1) and stock (Mg ha-1) in Oxisol in ICLS under no-tillage after six years of fertilization systems

Grazing intensity	N input phase	Total Carbon		Total Nitrogen		C/N ratio
		g kg-1	Mg ha-1	g kg-1	Mg ha-1	
Initial	-	36.1	37.3	2.7	2.1	13.37
High height (HH)	Crop (NC)	35.1 ± 0.4b	36.1 ± 0.4b	2.8 ± 0b	2.9 ± 0b	12.4 ± 0.3a
	Pasture (NP)	39.9 ± 0.7a	41.1 ± 0.7a	3.2 ± 0.1a	3.3 ± 0.1a	12.5 ± 0.3a
Low height (LH)	Crop (NC)	38.4 ± 0.7ab	39.6 ± 0.7ab	3.2 ± 0.1a	3.3 ± 0.1a	12.3 ± 0.7a
	Pasture (NP)	36.3 ± 0.7ab	37.4 ± 0.7ab	3.1 ± 0.1a	3.2 ± 0.1a	11.8 ± 0.9a

Mean values ± standard deviation (n = 6). a, b: Different lowercase letters in the columns indicate statistical difference by the LSD test ($p<0.05$); Equal letters do not differ significantly from each other. HH: high pasture height (lower grazing intensity); LH: low pasture height (higher grazing intensity); NC: nitrogen N fertilizer on grain crop; NP: nitrogen fertilizer on pasture.

In general, SOC and N dynamics are closely related, and soils with little N limit the buildup of SOC stocks (Lovato et al., 2004). The low grazing intensity (HH) combined with N applied on pasture resulted in the highest SOC and N stocks, totaling 41.1 ± 0.7 and 3.3 ± 0.1 Mg ha^{-1}, respectively. In this context, N fertilization becomes a highly efficient practice for increasing SOC and N stocks, improving soil quality and crop productivity (Teixeira et al., 1994). The treatment under high grazing intensity (LH) and N applied to pasture had the lowest C/N ratio (statistically similar to other treatments), and that is possibly one of the factors governing the depletion in SOC stocks, as the microbial activity can be stimulated by higher soil N availability.

The treatment under high grazing intensity and N applied to pasture (LH-NP) had the lowest C/N ratio (statistically similar to other treatments), and that is possibly one of the factors governing the depletion in SOC stocks, as the microbial activity can be stimulated by higher soil N availability.

The C/N ratio is an indicator of the soil organic matter transformation, quality, and its decomposability (Ostrowska and Porebska, 2015; Zinn et al., 2018). In soil, variations in this ratio could be attributed to climate (Yoh et al., 2001; Ostrowska and Porebska, 2015; Zinn et al., 2018), soil type (Cools et al., 2014), microbial biomass, soil quality, and vegetation cover (Ostrowska and Porebska, 2015). Possible reasons that caused lower C/N ratio are that the N-fertilization on pasture increased biomass production, plus the severe canopy removal by high grazing intensity may result in greater deposition of animal excrement (urine and feces), which in turn have a lower C/N ratio than the original plant material.

Soussana and Lemaire (2014) showed that herbivores change the way nutrients cycle, affecting mostly C and N, by returning C in the form of manure and N in the form of urine, thus modifying the C/N ratio and the decomposition of C. In addition, the fertilization of pastures with N fertilizers can change the quality of residue, as observed by Cools et al. (2014). Apolinário et al. (2014), testing N doses and different grazing intensities in *B. decumbens* pasture, found an increase in decomposition rates of residual material with increasing N fertilization doses.

SOC and N in the POM fraction

The POM is directly derived from fresh plant residue inputs (Dieckow et al., 2005), and so this fraction is significantly affected by cropping systems (pasture heights). This explains the variations observed in the SOC content of the POM-C fraction (Table 4).

Low grazing intensity (HH) resulted in higher C contents in the POM fraction (50.42 mg kg^{-1}) compared to the higher grazing intensity (43.59 mg kg^{-1}). Similar results were observed by Silveira et al. (2013), where an increase in POM-C was observed with lower grazing intensity, suggesting that a large proportion of plant and animal derived C inputs in the lowest grazing intensity increased C in the POM fraction.

Table 4. Carbon (POM-C) and nitrogen (POM-N) contents on POM fraction from Oxisol in ICLS under six years in ICLS in a humid subtropical region, two grazing intensities (HH and LH), and two nitrogen fertilization times (NC and NP)

Grazing intensity	N input phase	POM Carbon	POM Nitrogen	C/N ratio
		g kg^{-1}		
High height (HH)	Crop	51.6 ± 1	3.84 ± 0	13.4 ± 0.2
	Pasture	49.2 ± 0.1	3.79 ± 0	13.0 ± 0.1
Low height (LH)	Crop	45.5 ± 1	3.36 ± 0.7	13.5 ± 0.2
	Pasture	41.6 ± 0.3	3.99 ± 0.1	10.4 ± 2.9

Mean values ± standard deviation (n = 2).

Lower grazing (HH) intensities allow for greater litter inputs as well as slower decomposition rates (Liu et al., 2011), mainly due to the higher litter C/N ratio, which enables longer permanence of C in the particulate fraction (Bardgett et al., 1998; Damien et al., 2015).

The C/N ratio of plant residues determines the average residence time of particulate organic C in the soil. In extensive management systems with low plant N nutritional status, the residence time is generally longer (Bardgett et al., 1998) when compared to intensive management with high N nutritional status, which reflects shorter residence times (Klumpp et al., 2009). High grazing intensity and fertilizer N in the pasture tended to reduce C/N ratio, suggesting more efficient nutrient cycling processes.

Carbon and N coupling and decoupling processes is largely determined by the C/N ratio of plant residues (Lemaire et al., 2015). A high C/N ratio generally corresponds to less intensification of soil processes that result in lower net N mineralization (Soussana and Lemaire, 2014). In pasture systems with moderate grazing intensity, C-N coupling is strong enough to prevent net N mineralization from exceeding the plant capacity to absorb nitrate and ammonium. Therefore, the residence time of mineral N (ammonium and nitrate) in the soil is relatively short, reducing the risks of atmospheric emission (nitrous oxide or ammonia) or nitrate leaching, even with relatively large applications of N fertilizers (Ledgard et al., 2011). In addition, losses are reduced due to the rapid absorption of N by microbes and roots (Personeni and Loiseau, 2005).

Soil organic matter chemistry in the particulate and mineral-associated fractions

Our results showed that the organic functional groups are being stored in different soil pools that confer distinct stability and permanence of organic matter in soils. The NF had a greater abundance of aliphatics and phenol in the MAOM-C fraction, while pasture and ICLS systems had greater aliphatics in the POM-C fraction (Figure 1).

Differences in soil mineral and organic functional groups between land-uses were also verified with the PCA scores (Figure 2). The NF had distinct spectral characteristics from the other land-uses (pasture and ICLS). The PCA was carried out to take into account simultaneous co-variations between mineral and organic functional groups, and according to Gomes et al. (2004), it is an appropriate tool to understand differences in soil environments.

The SOC accrual increased with more recalcitrant organic functional groups of phenol, aromatic, and carbonyl (Figure 3). While some studies have shown that soil C accrual increases response to greater microbial processing of organic matter (Demyan et al., 2012; West et al., 2020; Deiss et al., 2021) and subsequent formation of organo-mineral associations (Lehmann, 2007; Lorenz et al., 2007; Schmidt et al., 2011; Cotrufo et al., 2013; Lehmann and Kleber, 2015; Hernandez-Soriano et al., 2018), others have found that plant residue degradation is characterized by a rapid loss of the labile fractions followed by the slow degradation of more recalcitrant plant components (Gu et al., 2004;

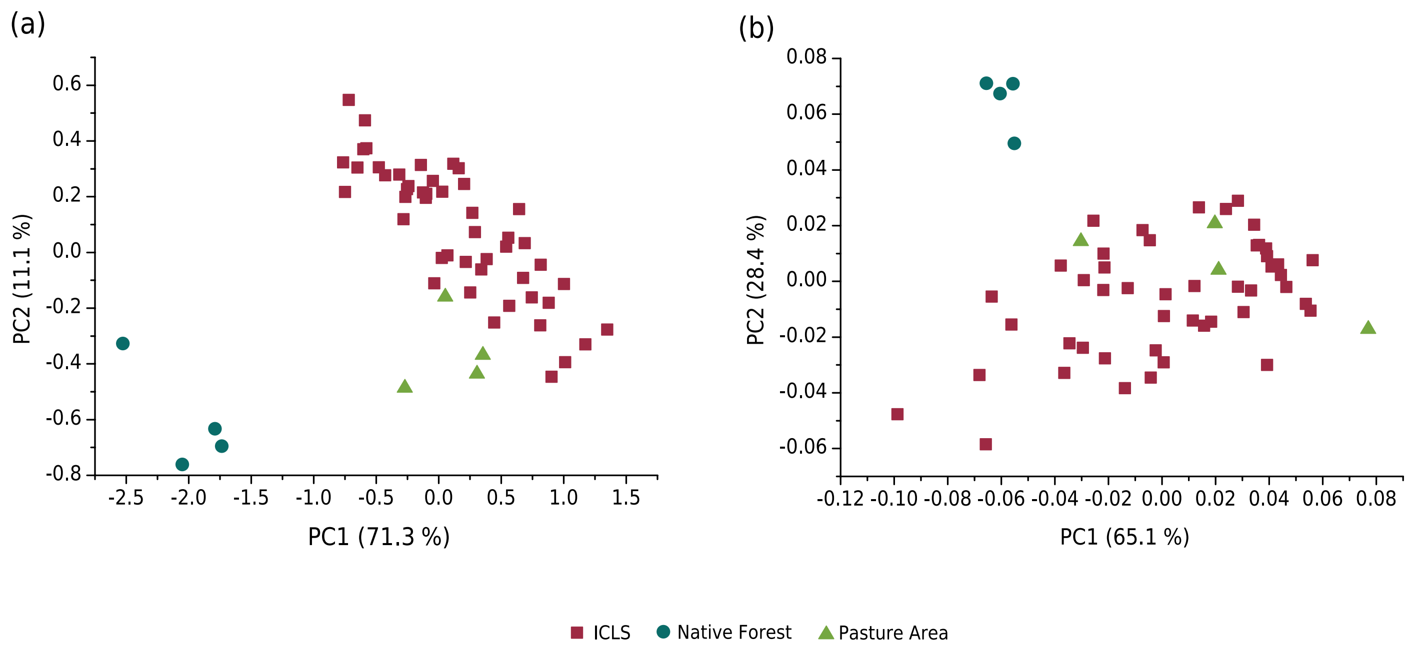

Figure 2. Principal component analysis (PCA) score of NF of (a) PA and (b) MAOM fractions extracted from Oxisol under ICLS, NF, and PA of southern Brazil.

Kelleher and Simpson, 2006; Spence et al., 2011). Our results indicate that organo-mineral associations were occurring with more recalcitrant functional groups of organic matter, characteristic of partially decomposed organic matter (Figure 3).

The negative association between aliphatics and SOC in the POM fraction (Figure 3) could be related to the consumption of those more labile compounds by microorganisms. Liu et al. (2020) attributed to microbial activity the rapid degradation of proteins and aliphatic substances. The aliphatic carbons molecules are largely the result of cutin and suberin biopolymers from higher plants (Augris et al., 1998; Nierop, 1998). While aromatic carbons are predominantly resulting from lignin and tannins (Baldock et al., 1997), and the phenolic group is composed of partially biodegraded lignin subunits (Sanderman et al., 2014).

Greater abundances of phenol and aromatics (0.00-0.05 m) were found in treatments under lower grazing intensity HH (Figure 4). The increase in more recalcitrant functional groups (Figure 3) was possibly resulting from the greater inputs of cover crop biomass when grazed under lower intensity. The SOC increased with more recalcitrant functional groups of phenol and aromatics. The amount of biomass inputs was possibly exceeding the capacity of soil organisms to mineralize organic matter. These more recalcitrant functional groups were contributing to an increased SOC accrual.

Lower grazing intensity increased SOC accrual, while N-fertilization during the cover crop pasture phase ensures adequate nutrition for plants and ruminants during the winter, at the same time as providing greater residual N for subsequent cash crops. Nitrogen-fertilization on the pasture phase reduces the resistance to the decomposition of residual material, and consequently accelerates conversion of residual plant biomass into soil organic matter. Enhanced nutrients catalyzed by ruminants during the grazed cover crop phase ensured a rapid release of N earlier in the subsequent crop growing season.

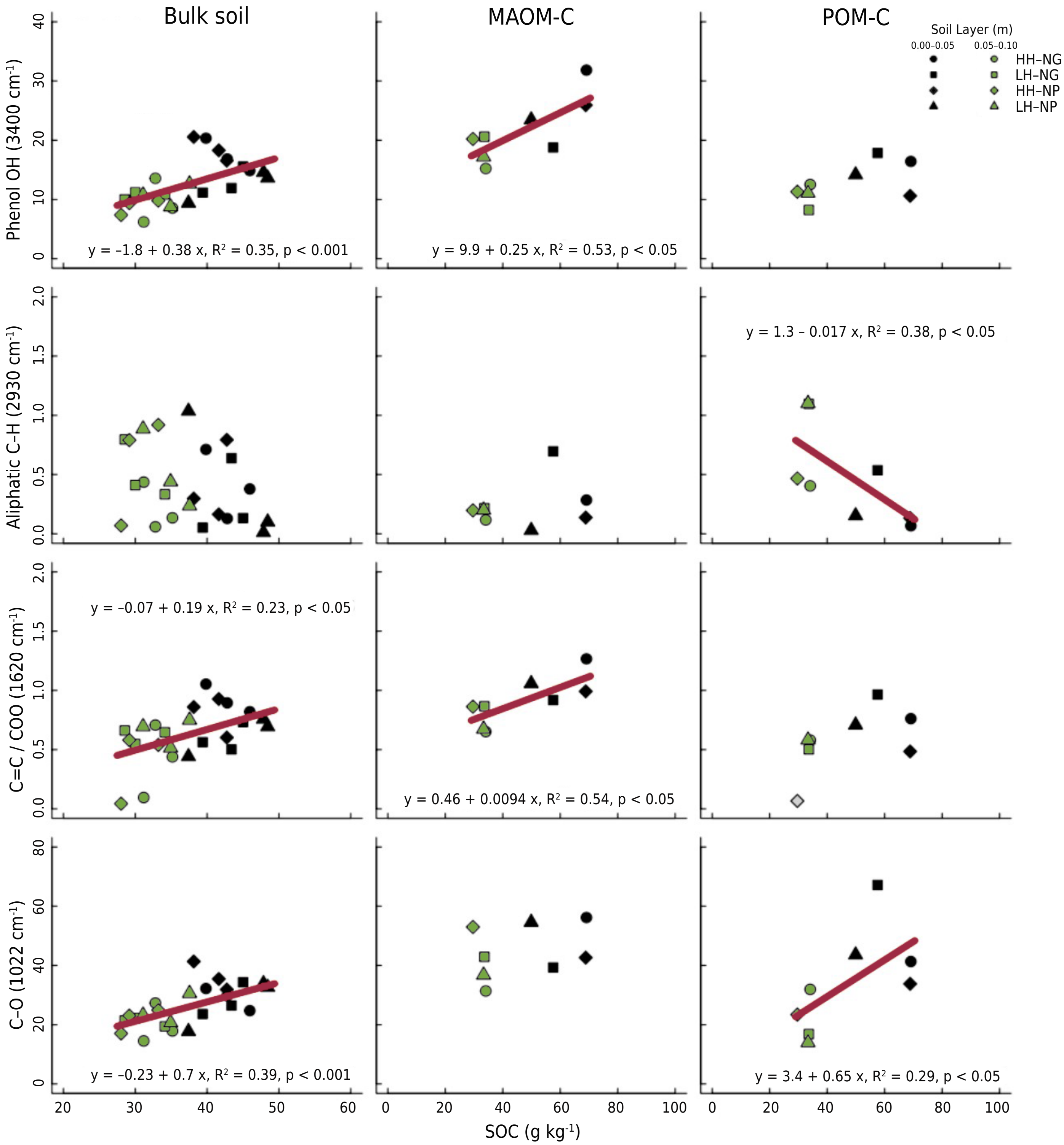

Figure 3. Effect of organic functional groups on soil organic matter accrual in the soil - POM-C and MAOM-C at 0.00-0.05 and 0.05-0.10 m soil layers. Soil organic functional groups were estimated as peak areas in diffuse reflectance FTIR in the mid-infrared region (mid-DRIFTS). Peak areas were assigned to phenol (OH, 3400 cm^{-1}), aliphatics (C-H 2930 cm^{-1}), aromatic (C–C) and/or carboxylate (COO) (1620 cm^{-1}), and carbonyl group (C-O, 1022 cm^{-1}).

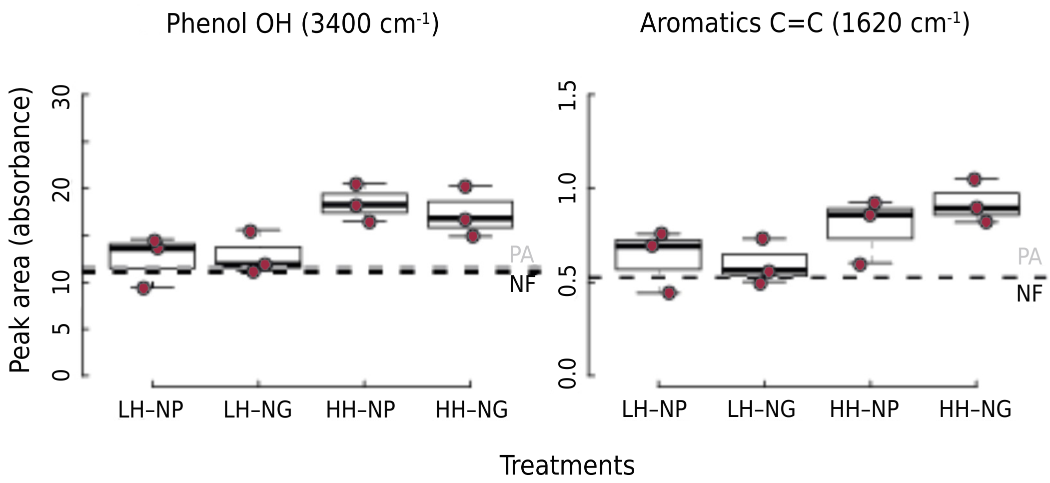

Figure 4. Soil organic matter composition (0.00-0.05 m) as affected by grazing intensity and nitrogen placement. Soil organic functional groups were estimated as peak areas in diffuse reflectance FTIR in the mid-infrared region (mid-DRIFTS). HH: high pasture height (lower grazing intensity); LH: low pasture height (higher grazing intensity); NC: nitrogen N fertilizer on grain crop; NP: nitrogen fertilizer on pasture; NF: native forest; PA: conventional pasture.

CONCLUSIONS

Combination of low grazing intensity and N fertilization during the pasture phase increases SOC and N stocks at 0.00-0.10 m layer in an Oxisol under subtropical climate. Lower grazing intensity accumulates more C in the POM fraction, which is a pool of carbon associated with nutrients that can be more easily cycled between cover crop and cash crop seasons.

Spectroscopy characterization provided qualitative information about soil organic matter, showing that it was affected by the grazing intensity in ICLS under no-tillage conditions grazed by cattle. The low grazing intensity (HH), with sward pasture heights between 0.25 and 0.60 m, after six years of ICLS, promoted the increase of recalcitrant organic functional groups, such as phenol, aromatic, and carbonyl group C-O following greater residual biomass.

Grazing and fertilization management strategies should be considered altogether to promote sustainable intensification of agriculture with ICLS. Pasture fertilization and adequate grazing intensity promote alterations in the N of soil-plant-animal system and increase SOC accrual.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.agsy.2018.01.030.

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