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Restoring Soil Health with Legume-Based Integrated Farming Systems

Ana Clara Santos Duarte [1], Jaqueline de Cássia de Oliveira [1], Warley Rodrigues de Oliveira [1], Igor Costa de Freitas [1], Álissam de Sá Cardoso [1], Alex José Silva Couto [1], Walter José Rodrigues Matrangolo [2], Karina Toledo da Silva [3], Rodinei Facco Pegoraro [1] and Leidivan Almeida Frazão [1,*]

[1] Instituto de Ciências Agrárias, Universidade Federal de Minas Gerais, Montes Claros 39404-547, Brazil; anaduart07@gmail.com (A.C.S.D.); oliveirajaqueline.c@gmail.com (J.d.C.d.O.); warleyrdo@gmail.com (W.R.d.O.); freitasicde@gmail.com (I.C.d.F.); alissamcardosoeng@outlook.com (Á.d.S.C.); alexcoutoagro@gmail.com (A.J.S.C.); rodinei@ufmg.br (R.F.P.)

[2] Centro Nacional de Pesquisa de Milho e Sorgo, Empresa Brasileira de Pesquisa Agropecuária, Sete Lagoas 35738-000, Brazil; walter.matrangolo@embrapa.br

[3] Empresa de Pesquisa Agropecuária de Minas Gerais, Prudente de Morais 35738-000, Brazil; karinatoledo@epamig.br

* Correspondence: lafrazao@ica.iufmg.br; Tel.: +55-38-21017939

Abstract: Faced with the dual challenge of increasing agricultural production (both intensified and diversified) and improving soil health, this study investigated the capacity of legume-based integrated farming systems to restore soil health in the Brazilian Cerrado. For that, we evaluated two experiments in the Minas Gerais State comparing the following land use systems: native vegetation (NV), conventional tillage with *Zea mays* (CT-8), two pasture systems with *Urochloa decumbens* (PAST-13) and *Urochloa brizantha* (PAST-1), and three integrated production systems arranged with Cratylia argentea + Zea mays (IPS-8A), Gliricidia sepium + Zea mays (IPS-8B) and Cratylia argentea + Urochloa brizantha (IPS-1). To assess seasonal variations in microbial attributes (microbial carbon [Cmic], microbial quotient (qMIC), and enzymatic activity, we collected soil samples during the rainy season (December 2021) and the dry season (July 2022). Soil carbon (C) and nitrogen (N) stocks were also evaluated. The soil C and N stocks in pasture systems were similar to VN, with values of 120 and 8.2 Mg ha^{-1} in PAST-1 at 0–30 cm. Additionally, integrated systems with legume crops promoted an increase in soil C stocks up to 24% (IPS-8B) when compared to monoculture cultivated under conventional tillage (CT-8). We also found that the legume-based integrated farming systems increased Cmic and β-glucosidase activity at the surface layers. Our findings demonstrate that integrated systems utilizing *Cratylia argentea* and *Gliricidia sepium* offer a promising approach to soil health restoration and a potential replacement for annual crop and pasture monocultures in the Brazilian Cerrado.

Keywords: β-glucosidase; *Cratylia argentea*; *Gliricidia sepium*; soil microbial carbon; soil organic matter; sustainable intensification

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1. Introduction

Brazil plays a crucial role in global food, fiber, and agricultural product supply (e.g., grains, meat, sugarcane, coffee, fruits, wood products, cellulose, biofuels) and provides essential ecosystem services, including soil conservation, carbon storage, freshwater provision, and biodiversity maintenance [1,2]. Given that agriculture and land-use change contribute approximately 22% of global greenhouse gas (GHG) emissions [3], the adoption of conservation agriculture in Brazil offers significant potential for climate change mitigation. The Brazilian Agricultural Policy for Climate Adaptation and Low Carbon Emission (ABC+ Plan) aims to reduce CO_2 equivalent emissions by approximately 1.1 billion tons by 2030 [1], aligning with ongoing climate change adaptation efforts [4].

The adoption or maintenance of agroforestry systems, also known as Integrated Crop-Livestock-Forestry Systems, is an agricultural production strategy that combines different species within the same area in rotation, intercropping and/or succession of cultures using sustainable land management as no-tillage, that minimize soil disturbance. That is a target of the ABC+ Plan, as it is a nature-based solution strategy for mitigating GHG emissions and increasing productivity by promoting the intensification of agricultural, livestock, and forestry production [5]. The adoption of integrated farming systems leads to the increase and diversity of production, recovery of degraded pastures, maximization of ecosystem services, and increased profitability [6]. Considering the Brazilian Cerrado's substantial area (2,045,000 km^2, representing approximately 23.3% of the national territory) and its vital role in providing food, energy, and fiber resources, a comprehensive assessment of integrated farming systems (IFS) within this biome is of critical importance [7].

Integrated farming systems have achieved significant adoption and consolidation in the Brazilian Cerrado and are increasingly recognized as a key approach to addressing global challenges related to food, energy, and environmental sustainability. However, a comprehensive understanding of the impacts associated with incorporating diverse components, including leguminous crops, into these conservationist systems remains crucial [8]. In this context, the legume shrub *Cratylia argentea* (cratylia) and the legume tree *Gliricidia sepium* (gliricidia) present considerable potential for agricultural application, stemming from their multifunctional purposes, deep root systems, high protein biomass, reliable seed production, and vigorous regrowth [9,10]. Adapted to conditions of low soil fertility and exhibiting drought tolerance, these species are well-suited for integrated farming systems, promoting biological nitrogen fixation and serving as valuable resources for green manure and animal feed [9,11]. Within integrated farming systems, the integration of these forage shrubs/trees typically involves intercropping with annual crops or pastures within the alleys [12]. Despite this, the impact of these legumes on soil health restoration remains poorly understood.

Several studies have shown that integrated farming systems can improve the soil's physical, chemical, and biological conditions through a constant supply of soil organic matter (SOM) from its productive components (annual crops, forage species and trees). This can be evidenced in silvopastoral and agrosilvopastoral systems by the increase in soil carbon (C) and nitrogen (N) stocks [13,14], levels of labile C [15], microbial C [16], activity of β-glucosidase [17] and urease enzymes [18]. Furthermore, a consensus exists regarding the utility of soil biological indicators, such as microbial C and enzymatic activity, for evaluating integrated farming systems, given the positive influence of appropriate management practices on the soil microbial community [19]. Thus, the evaluation of based-legumes integrated systems becomes important to confirm their use as an efficient strategy for restoring soil health.

In light of the increasing demand for both intensified and diversified agricultural production, coupled with the critical importance of soil health, this study evaluated soil carbon and nitrogen stocks and relevant biological attributes in two experiments in the Cerrado biome. A comparative analysis was conducted, contrasting integrated systems that include *Cratylia argentea* and *Gliricidia sepium* with pasture monoculture and annual crop systems under conventional soil management. We hypothesized that (i) the introduction of integrated farming systems with legume trees or shrubs enhance soil C and N stocks in similar levels to pasture systems and (ii) Integrated farming systems with *Cratylia argentea* and *Gliricidia sepium* can restore soil health when compared to pasture and annual crop monocultures.

2. Materials and Methods

2.1. Location and Characterization of the Study Areas

This study was carried out in two sites in the Cerrado of Minas Gerais, Brazil (Figure 1). The first experiment was implemented at Embrapa Maize and Sorghum Experimental Farm (Figure 1) in the municipality of Sete Lagoas (19°27′15.56″ S 44°10′9.85″ W). The second experiment was carried out at Fazenda Santa Rita at Epamig (Figure 1), in the municipality of Prudente de Morais (19°27′7.45″ S 44°9′39.85″ W). The climate was classified as Cwa (according to the Köppen classification), savannah with dry winter and humid summer with rain, with an average annual temperature of approximately 21.4 °C and the annual precipitation during the studied period (2021–2022) was 1549 mm (Figure 2). The predominant soil in the two study sites is Ferralsol, which has a clayey texture. Soil chemical characteristics for each evaluated land use in both experiments are presented in Table S1.

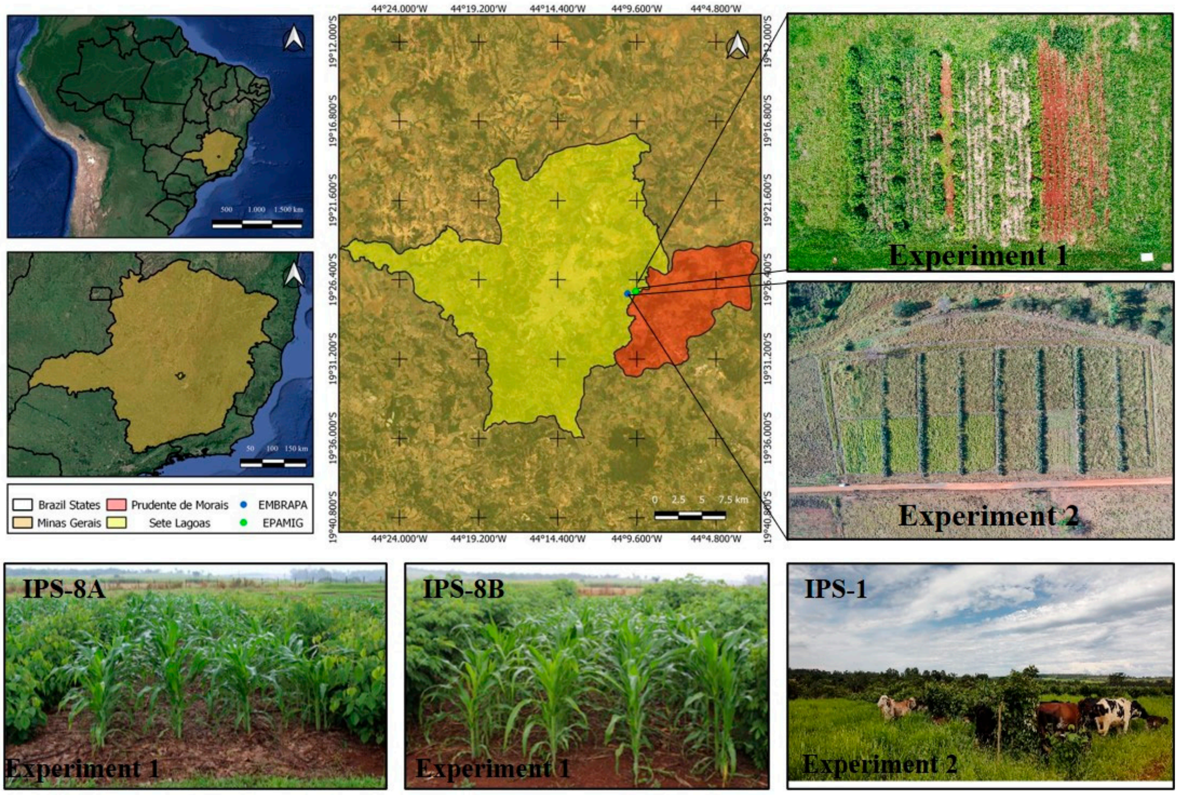

Figure 1. The study areas and the two experiments with integrated production systems (IPS) are located in the municipalities of Sete Lagoas and Prudente de Morais in the Cerrado of Minas Gerais, Brazil.

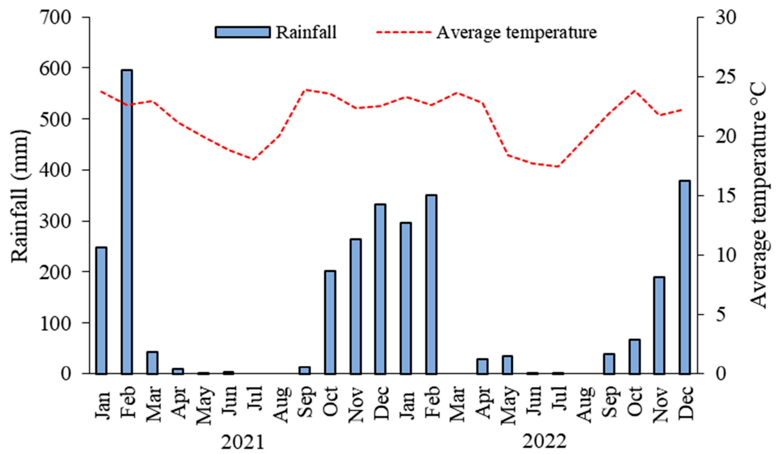

Figure 2. Average annual rainfall and temperature (2021–2022) in the experimental areas located in the municipalities of Sete Lagoas and Prudente de Morais in the Cerrado of Minas Gerais, Brazil.

2.2. Experiment 1: Integrated Farming Systems Using Legumes and Annual Crop

Experiment 1 was carried out at Embrapa Maize and Sorghum Experimental Farm (Figure 1) in a completed randomized design with five treatments (land uses) and four repetitions. The experimental design consisted of paired land uses and each treatment encompassing four plots within a 200 m^2 useful area:

NV: Reference area composed of native vegetation of the Cerrado biome. The area includes both tortuous individuals with a xeromorphic aspect and erect individuals. Species with a maximum height of up to 12 m and an average height of 4.5 m were found in the area, forming well-defined strata in certain places.

PAST-13: Nominal pasture with *Urochloa decumbens* cv. Basilisk. The area was implemented in 2008 with animal grazing and maintenance fertilization of 100 kg N, 40 kg K_2O, and 50 kg P_2O_5 ha^{-1} $year^{-1}$ in 2015/2016. The lack of continuous grazing in the area before soil collection promoted grass regrowth and reestablishment, effectively minimizing exposed soil and weed infestation.

IPS-8A: Integrated farming system composed of *Cratylia argentea* (cratylia) and *Zea mays* cv. BRS Caimbé (maize) is irrigated without external inputs, using green fertilization and mulching with legume residues.

IPS-8B: Integrated farming system composed of *Gliricidia sepium* (gliricidia) and *Zea mays* cv. BRS Caimbé is irrigated without external inputs, using green fertilization and mulching with legume residues.

CT-8: Conventional tillage of *Zea mays*. The area was implemented in 2008 using irrigation, conventional soil tillage and chemical fertilization, with the fertilization rates adjusted annually based on soil analysis results.

The integrated farming systems were set up in 2013 (Figure 3). The two legume-based agroforestry systems were implemented using alleys with *Cratylia argentea* and *Gliricidia sepium* (Figure 1) integrated with maize, using an irrigation system. Each system had an area of 160 m^2, where three rows of each legume were planted with 4 m spacing between rows and 0.5 m between plants. Three to four drastic pruning of the leguminous plants was carried out throughout each year since implementation, with subsequent deposition of leaves and branches on the ground. During the corn-planting season, direct mechanized sowing was carried out on leguminous straw in the cultivation strips between the alleys. The study site has not received external inputs (soluble fertilizers, herbicides, insecticides, or fungicides) since the implementation. Chemical analysis showed the following characteristics: pH in water = 6.6; O.M = 4.13 dag kg^{-1}; P = 70.6 mg dm^{-3}; K = 224.9 mg dm^{-3}; Base Saturation = 86.7%; CEC = 10.8 cmolc dm^{-3}.

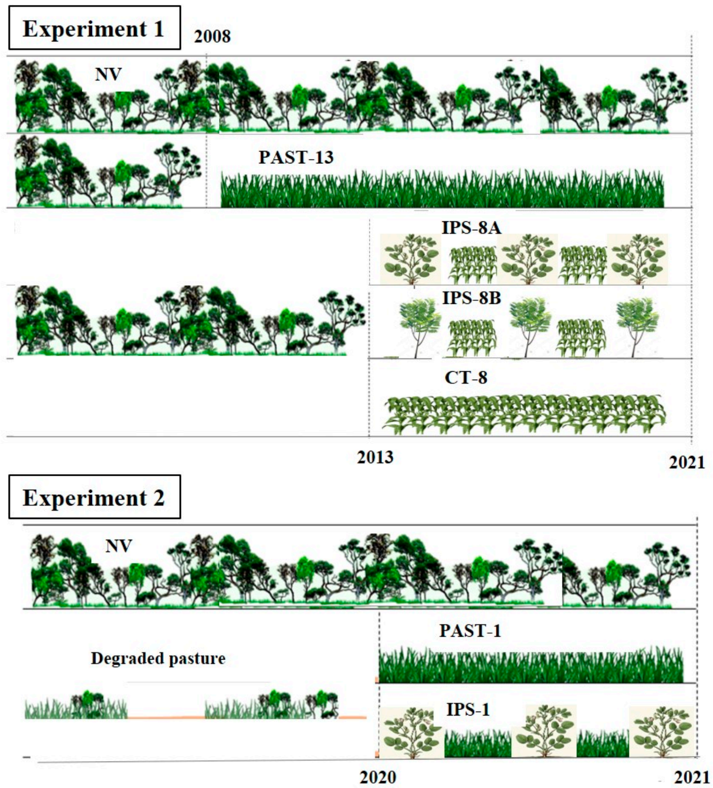

Figure 3. A chronosequence of soil use and management in experimental areas integrating legumes with annual crop (Experiment 1) and pasture (Experiment 2).

2.3. *Experiment 2: Integrated Farming Systems Using Legumes and Pasture*

Experiment 2 was carried out at the Santa Rita Experimental Farm (Figure 1) in a completed randomized design with tree treatments (land uses) and four repetitions. The experimental design consisted of paired land uses and each treatment encompassing four plots within a 2000 m^2 useful area:

NV: Reference area composed of native vegetation typical of the Cerrado biome, with phytophysiognomy similar to the area of experiment 1.

PAST-1: Nominal pasture area of *Urochloa brizantha* cv. BRS Piatã, implemented in 2020, was established in an area previously occupied by degraded pasture resulting from a lack of management, specifically regarding fertilizer application and stocking rate control.

IPS-1: Integrated farming system composed of *Cratylia argentea* and *Urochloa brizantha* cv. BRS Piatã, with conventional soil preparation and fertilization. This area was also established on an area previously occupied by degraded pasture.

The integrated farming system and pasture were implemented in January 2020 (Figure 3) in an area previously cultivated with degraded pasture with poor soil quality and the presence of invasive plants. The integration farming system was implemented without an irrigation system (Figure 1). In January 2020, the seedlings of Cratylia were planted in an alley system, spaced 25 m between rows and 2 m between plants, with fertilization of 60 g of NPK (0-30-20). In March of the same year, soil preparation was carried out with a plow harrow and leveling harrow and subsequent sowing of Piatã grass. Uniform pruning of Cratylia was carried out in August 2020, and a drastic pruning was carried out in May 2021. A chemical analysis showed the following characteristics: pH in water = 5.5; O.M = 2.8 dag kg^{-1}; P = 8.0 mg dm^{-3}; K = 56 mg dm^{-3}; Base Saturation = 37%; CEC = 9.84 cmolc dm^{-3}. Animals in PAST-1 and IPS-1 systems were managed with a stocking rate of about 1.0 animal units (an animal unit represents 450 kg of body weight of an animal) per hectare (AU ha^{-1}).

2.4. Soil Sampling and Laboratory Analysis

Soil samplings were carried out in December 2021 (rainy season) and July 2022 (dry season) to determine the soil's biological attributes. Four mini-trenches with dimensions of $40 \times 40 \times 40$ cm were opened under each land-use system to take soil samples at 0–5, 5–10, and 10–20 cm depths. In the integrated farming systems, eight mini-trenches were opened, and soil samples were collected in the rows of legume shrubs or trees and between the rows to obtain four composite samples and to ensure representativeness. These composite samples were stored in plastic bags with ventilation to allow gas exchange and then gently packed into coolers with ice. In the lab, soil samples were sieved to 2 mm, and small plant fragments were removed to avoid potential interferences in the analyses. They were immediately stored in a refrigerator at 4 °C for further analysis. Soil samples were also collected to determine the soil C and N content values and stocks in July 2022 at 0–5, 5–10, 10–20 and 20–30 cm depths. Additionally, soil samples were collected to determine soil bulk density using the volumetric ring method [20].

To determine the soil C and N contents, the soil samples were previously air-dried, passed through 2 mm sieves, ground, passed again through 0.150 mm sieves, and subsequently analyzed by combustion dried using an elemental analyzer (Leco CN-2000®, St. Joseph, MI, USA). With the values obtained, the C/N ratios were calculated. Soil C and N stocks (Mg ha^{-1}) were obtained by multiplying these elements (%) by bulk density (g cm^{-3}) and soil layer thickness (cm). Subsequently, soil C and N stock comparisons across land use must be performed with equal soil masses by adjusting the soil depth based on a reference site. For this, the same soil mass for each soil depth was used to accurately determine the soil C and N stocks using the reference area (native vegetation) according to the methodology proposed by Ellert and Bettany [21] and Moraes et al. [22].

Soil samples were air-dried and passed through sieves with a 0.150 mm mesh to determine the content of C oxidized by $KMnO_4$ [and to calculate soil labile carbon (LC)] according to the methodology described by Shang and Tiessen [23]. To determine soil biological attributes, soil samples were previously corrected to 60% of field capacity and pre-incubated for a period of seven days. The determination of soil microbial C (Cmic) was performed using the fumigation-extraction method proposed by Vance et al. [24] and adapted by Silva et al. [25]. The microbial quotient (qMIC) was estimated by the Cmic and soil C content ratio. To quantify the activity of the β-glucosidase enzyme, the methodology proposed by Tabatabai [26] was used, which is based on the colorimetric determination of the p-nitrophenol released by these enzymes as soon as the soil is incubated with a buffered solution of a specific substrate. The activity of the urease enzyme was estimated according to the methodology suggested by Keeney and Nelson [27] and modified by Kandeler and Gerber [28]. The method is based on the principle of determining the N-NH_4^+ quantity released after incubation of the soil with a buffered solution and urea.

2.5. Data Analysis

The Shapiro-Wilk test was applied to verify the occurrence of normal distributions, and the Bartlett test was used to determine the homogeneity of variances. Once the hypotheses of normality and homogeneity were not validated for soil C and N content and stocks, we used the Kruskal–Wallis H test ($p < 0.05$). For soil biological attributes, data normality and homogeneity were validated; therefore, analysis of variance (ANOVA) was performed, and subsequently, the means were compared using the Scott-Knott test ($p < 0.05$). When there was no significant interaction between land use and the sampling period, the direct effects of the land use and/or period were evaluated independently. Additionally, principal component analysis (PCA) was performed to discriminate land use systems according to soil microbial attributes in the two seasons evaluated. Statistical analyses were performed using R software, version 4.2.3 [29].

3. Results

3.1. Soil Content and Stocks of C and N and Bulk Density

In Experiment 1, soil C content was influenced by the land-use systems in all evaluated soil layers (Figure 4A, Table S2). Our results in the 0–5 cm layer showed higher soil C contents in NV (48.58 g kg^{-1}) followed by PAST-13 (43.18 g kg^{-1}), IPS-8A (32.83 g kg^{-1}), IPS-8B (33.55 g kg^{-1}) and CT-8 (27.18 g kg^{-1}). For the subsequent layers (5–10, 10–20 and 20–30 cm), the values ranged from 20.68 g kg^{-1} (CT-8) to 50.10 g kg^{-1} (PAST-13). The soil N content (Figure 4B, Table S2) was higher in NV (5.36 g kg^{-1}) at 0–5 cm layer, and we did not find differences in the deep layers. The C/N ratio ranged from 9.25 to 15.71 (Figure 4C, Table S2) for all the evaluated soil layers, but we did not find differences among the land-use systems.

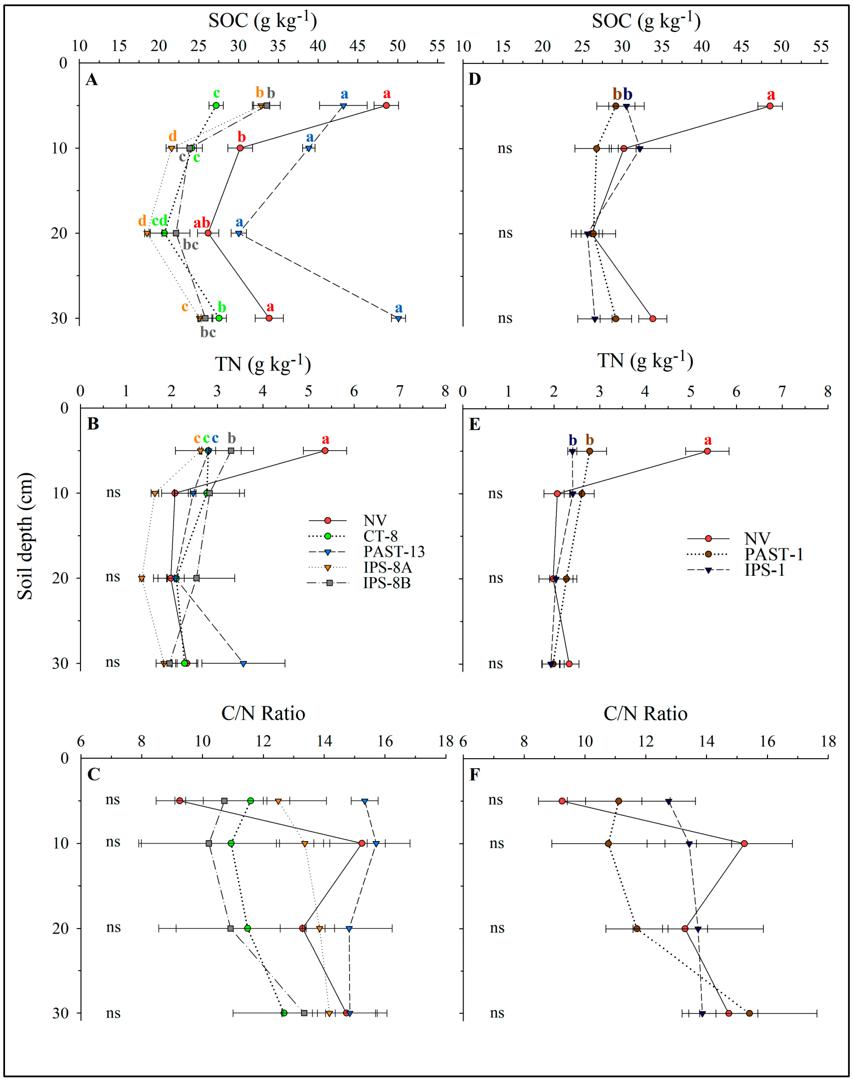

Figure 4. Soil organic carbon (SOC) (**A**,**D**) and total nitrogen (N) contents (**B**,**E**), and C/N ratios (**C**,**F**) under different land-use systems in Experiment 1 located in the municipality of Sete Lagoas, and in Experiment 2 located in Prudente de Morais, Minas Gerais, Brazil. Values represent the average (n = 4). Means followed by "ns" at the same depth, do not show statistical difference between them by the Scott-Knott test at the 5% probability level.

In Experiment 2, soil C content (Figure 4D, Table S3) was influenced by different land-use systems only at 0–5 cm. Our results were similar between the PAST-1 (29.30 g kg^{-1}) and IPS-1 (30.53 g kg^{-1}) and lower than in NV (48.58 g kg^{-1}). Similarly, the soil N content (Figure 4E, Table S3) showed the same pattern, and differences were found only in the 0–5 cm, and the lowest values were found in PAST-1 (2.78 g kg^{-1}) and IPS-1 (2.39 g kg^{-1}) when compared to NV (5.36 g kg^{-1}). The C/N ratios were similar between the land-use systems, ranging from 9.25 to 15.41 for all the evaluated soil layers (Figure 4F, Table S3).

Soil bulk density was influenced by the land-use systems (Table 1), and the area cultivated with maize (CT-8) showed the lowest values at 5–10 cm (0.81 g cm^{-3}) and 20–30 cm (0.73 g cm^{-3}) in Experiment 1. We also found similar results for soil C stocks between PAST-13 and NV at 0–5 cm and 0–30 cm (Figure 5). Compared to VN, soil C stocks in IPS-8A and IPS-8B decreased respectively 29 and 23% at 0–30 cm, but these systems showed an increase of 24 and 18% in relation to CT-8 at 0–5 cm. Soil N stocks (Figure 5) were influenced by different land-use systems only in the 0–5 cm and 20–30 cm layers, and the results were similar among the cultivated systems and lower than those in NV. Soil N stocks in IPS-8A decreased respectively 43 and 41% in relation to NV at 20–30 cm and 0–30 cm layers.

Table 1. Soil bulk density (g cm^{-3}) under different land-use systems in Experiments 1 and 2, located in the municipalities of Sete Lagoas and Prudente de Morais, Minas Gerais, Brazil.

[a] Land-Use	Soil Depth (cm)			
	0–5	5–10	10–20	20–30
	Experiment 1			
NV	[b] 0.94 ± 0.13 * A	1.04 ± 0.04 A	1.00 ± 0.07 A	1.02 ± 0.01 B
CT-8	0.92 ± 0.08 A	0.81 ± 0.07 B	0.85 ± 0.06 A	0.73 ± 0.08 D
PAST-13	1.07 ± 0.01 A	1.13 ± 0.06 A	1.05 ± 0.06 A	1.07 ± 0.01 A
IPS-8A	1.03 ± 0.02 A	1.04 ± 0.02 A	1.03 ± 0.03 A	1.00 ± 0.01 C
IPS-8B	0.96 ± 0.06 A	1.05 ± 0.05 A	1.03 ± 0.03 A	0.98 ± 0.04 C
	Experiment 2			
NV	[b] 0.93 ± 0.13 A	1.04 ± 0.04 A	1.00 ± 0.07 A	1.02 ± 0.01 A
PAST-1	1.01 ± 0.04 A	0.99 ± 0.07 A	1.00 ± 0.14 A	0.98 ± 0.13 A
IPS-1	1.08 ± 0.02 A	1.04 ± 0.03 A	1.00 ± 0.02 A	0.97 ± 0.01 A

[a] Experiment 1: NV, native vegetation; CT-8, conventional tillage of *Zea mays*; PAST-13, *Urochloa decumbens* cv. Basilisk pasture; IPS-8A, integrated system of *Cratylia argentea* with *Zea mays*; IPS-8B, integrated system of *Gliricidia sepium* with *Zea mays*. Experiment 2: NV: native vegetation; PAST-1: *Urochloa brizantha* cv. BRS Piatã pasture; IPS, an integrated system of *Cratylia argentea* and *U. brizantha* cv. BRS Piatã. [b] Values represent averages (n = 4). Means (n = 4 followed by the same letters in a column do not differ statistically according to the KruskalWallis test ($p < 0.05$). * Mean value standard error of the mean.

In Experiment 2, soil bulk density values ranged from 0.93 to 1.08 g cm^{-3} considering all the evaluated soil depths, and they were not influenced by the land-use systems (Table 1). Similar results ($p < 0.05$) were found for soil C and N stocks at 0–30 cm (Figure 6). However, at 0–5 cm, soil C stocks decreased 35% in PAST-1 and 27% in IPS-1 when compared to VN. The same pattern was observed for soil N stocks, decreasing 44% in PAST-1 and 48% in IPS-1 when compared to VN.

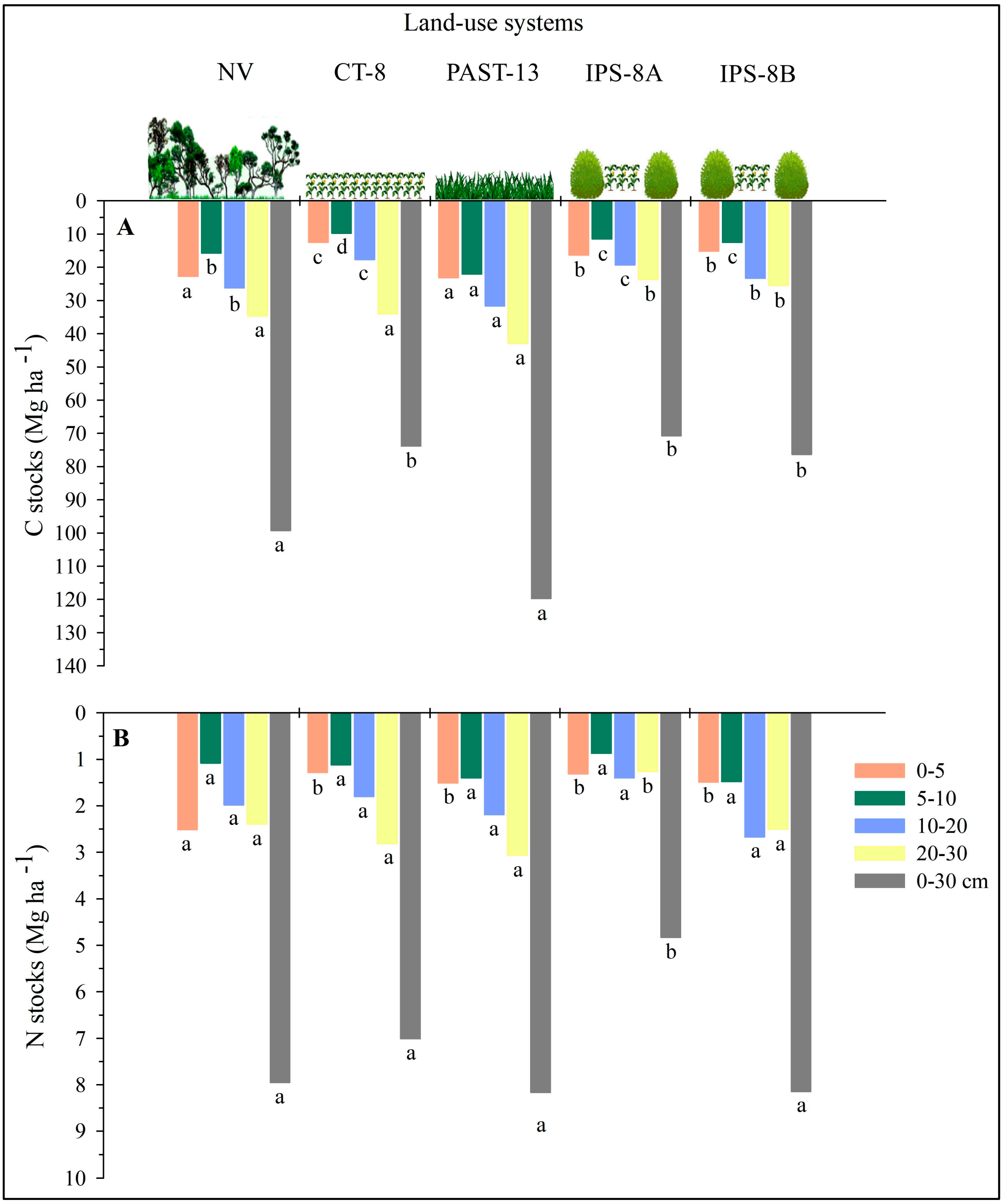

Figure 5. Soil carbon (**A**) and nitrogen (**B**) stocks under different land-use systems in Experiment 1, located in the municipality of Sete Lagoas, Minas Gerais, Brazil. Values represent the average (*n* = 4). Means followed by the same letter at the same depth, do not show statistical difference between them by the Scott-Knott test at the 5% probability level.

3.2. Soil Microbial Attributes

In Experiment 1, we observed the effect of seasonality in the soil microbial attributes at 0–5 cm, except for qMIC. At 5–10 cm, we found significative interactions between the seasons and land-use system for qMIC and urease activity, whereas at the 10–20 cm layer, the interaction was significant only for urease activity. PAST-13 system showed the highest Cmic values (708 mg kg^{-1}) at 0–5 cm in December 2021 (Table 2), and also for the average values between the two evaluated seasons at 5–10 cm (362 mg kg^{-1}) and 10–20 cm (303 mg kg^{-1}). In the rainy season (December 2021), NV showed a decrease in qMIC values (0.87%) when compared to the other land-use systems at 5–10 cm. Considering the five land-use systems in Experiment 1, the highest values for Cmic and qMIC were also observed in December 2021. Labile carbon (LC) values were higher in NV (3.85 g kg^{-1}) at 0–5 cm during the dry season (July 2022), and a similar pattern was observed for urease activity, where the lowest activity was found in CT-8, with average values for evaluated soil depths ranging from 28 to 107 µg pNP g^{-1} h^{-1}. In July 2022, the highest β-glucosidase activity was observed at a depth of 0–5 cm, with maximum values in IPS-8B (350 µg pNP g^{-1} h^{-1}), followed by IPS-8A (221 µg pNP g^{-1} h^{-1}).

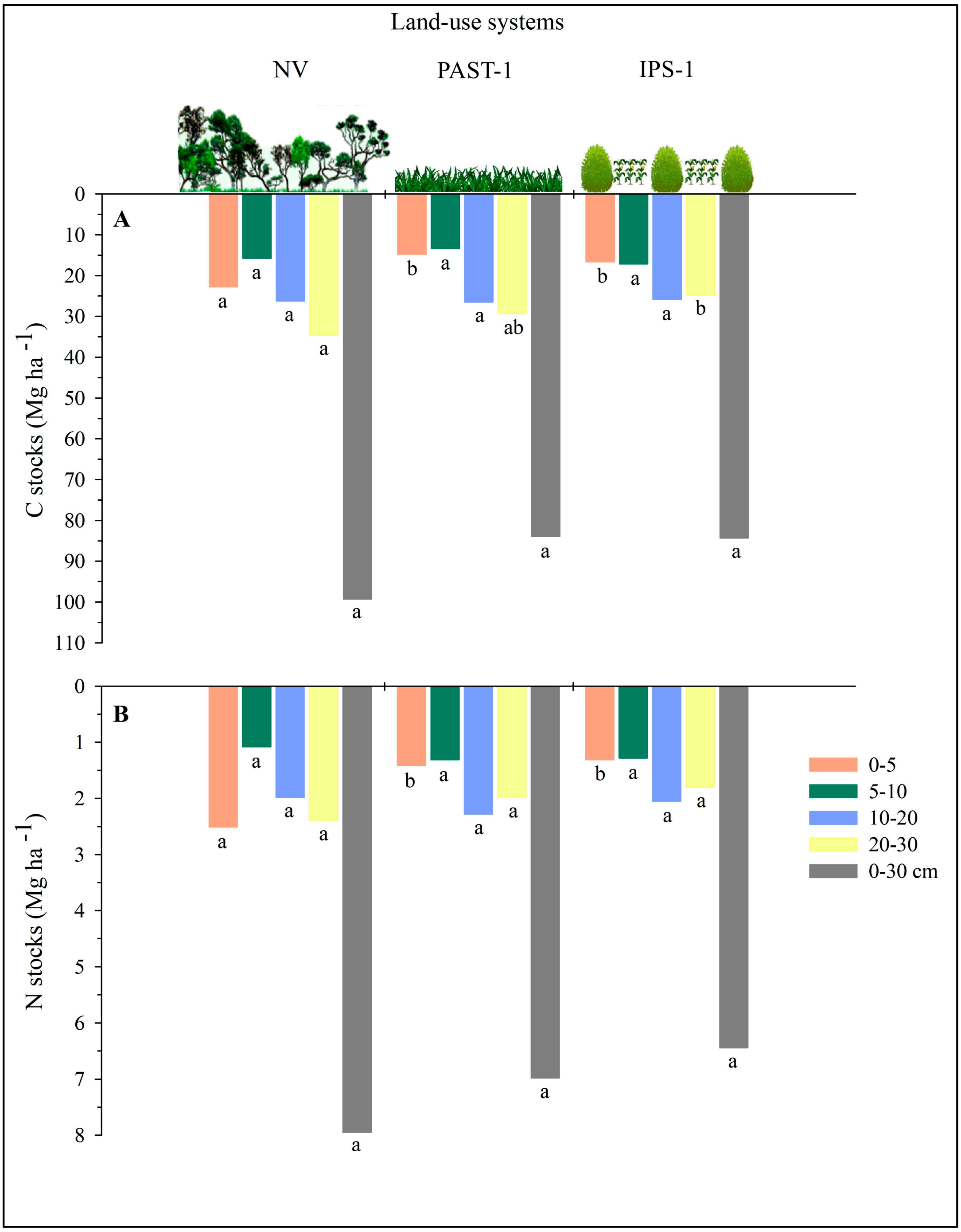

Figure 6. Soil carbon (**A**) and nitrogen (**B**) stocks under different land-use systems in Experiment 2, located in the municipality of Prudente de Morais, Minas Gerais, Brazil. Values represent the average (n = 4). Means followed by the same letter at the same depth, do not show statistical difference between them by the Scott-Knott test at the 5% probability level.

Table 2. Soil biological attributes at different sampling times and under different land use systems in Experiment 1, located in the municipality of Sete Lagoas, Minas Gerais, Brazil.

[a] Land Use	Soil Depth (cm)								
	0–5			5–10			10–20		
	Dec [b]	Jul	Mean [c]	Dec	Jul	Mean	Dec	Jul	Mean
	Cmic (mg kg $^{-1}$)								
NV	398 Ba	269 Aa	333 A	257 Aa	182 Aa	219 B	199 Aa	113 Ab	156 B
CT-8	424 Ba	204 Ab	314 A	340 Aa	115 Ab	227 B	227 Aa	72 Ab	149 B
PAST-13	708 Aa	218 Ab	463 A	479 Aa	246 Ab	362 A	424 Aa	183 Ab	303 A
IPS-8A	481 Ba	110 Ab	296 A	367 Aa	63 Ab	215 B	297 Aa	50 Ab	173 B
IPS-8B	368 Ba	154 Ab	261 A	339 Aa	90 Ab	215 B	297 Aa	66 Ab	182 B
Mean [d]	476 a	191 b		356 a	139 b		289 a	97 b	
	qMIC (%)								
NV	0.82 Aa	0.55 Aa	0.68 A	0.87 Ba	0.62 Aa	0.75 A	0.74 Aa	0.44 Aa	0.59 A
CT-8	1.58 Aa	0.75 Ab	1.17 A	1.42 Aa	0.48 Ab	0.95 A	1.15 Aa	0.35 Ab	0.75 A
PAST-13	1.64 Aa	0.51 Ab	1.07 A	1.24 Aa	0.63 Ab	0.93 A	1.42 Aa	0.61 Ab	1.01 A
IPS-8A	1.49 Aa	0.34 Ab	0.91 A	1.69 Aa	0.29 Ab	0.99 A	1.62 Aa	0.27 Ab	0.95 A
IPS-8B	1.10 Aa	0.46 Ab	0.78 A	1.42 Aa	0.37 Ab	0.89 A	1.37 Aa	0.31 Ab	0.84 A
Mean	1.33 a	0.52 b		1.33 a	0.48 b		1.26 a	0.39 b	
	Labile C (g kg $^{-1}$)								
NV	1.43 Ab	3.85 Aa	2.64 A	0.98 Ab	1.84 Aa	1.41 B	0.79 Ab	1.75 Aa	1.27 A
CT-8	1.21 Ab	2.67 Ba	1.94 A	0.97 Ab	2.08 Aa	1.53 B	0.67 Ab	1.58 Aa	1.13 A
PAST-13	1.60 Ab	3.06 Ba	2.33 A	1.26 Ab	2.62 Aa	1.94 A	0.83 Ab	1.52 Aa	1.17 A
IPS-8A	1.40 Ab	2.80 Ba	2.10 A	0.75 Ab	1.70 Aa	1.22 B	0.46 Ab	1.26 Aa	0.86 A
IPS-8B	1.31 Ab	3.06 Ba	2.18 A	0.88 Ab	1.90 Aa	1.39 B	0.52 Ab	1.55 Aa	1.04 A
Mean	1.39	3.09		0.97 b	2.03 a		0.66 b	1.53 a	
	Urease (µg NH_4^+ g^{-1} 2 h^{-1})								
NV	126 Ab	270 Aa	198 A	101 Ab	172 Ba	136 A	71 Ab	159 Ba	115 A
CT-8	101 Ab	113 Ba	107 A	32 Aa	78 Ca	55 B	18 Ba	38 Ca	28 B
PAST-13	94 Ab	261 2Aa	178 A	59 Ab	242 Aa	150 A	97 Ab	302 Aa	199 A
IPS-8A	99 Ab	271 Aa	185 A	36 Aa	77 Ca	56 B	19 Ba	27 Ca	23 B
IPS-8B	125 Ab	224 Aa	175 A	47 Aa	67 Ca	57 B	20 Ba	21 Ca	21 B
Mean	109 b	228 a		55 b	127 a		45 b	109 a	
	β-glucosidase (µg pNP g^{-1} h^{-1})								
NV	134 Aa	136 Ca	135 A	75 Aa	47 Aa	61 B	47 Aa	36 Aa	41 A
CT-8	140 Aa	198 Ba	169 A	94 Aa	91 Ab	75 B	38 Aa	34 Aa	36 A
PAST-13	176 Aa	153 Ca	164 A	75 Aa	86 Aa	81 B	48 Aa	25 Aa	37 A
IPS-8A	189 Aa	221 Ba	205 A	88 Aa	62 Aa	75 B	59 Aa	26 Aa	42 A
IPS-8B	178 Ab	350 Aa	264 A	104 Ba	124 Aa	114 A	61 Aa	61 Aa	53 A
Mean	163 a	212 a		80 a	82 a		51 a	33 a	

[a] NV: native vegetation; CT-8: conventional tillage of *Zea mays*; PAST-13: *U. decumbens* cv. Basilisk pasture; IPS-8A: an integrated system of *Cratylia argentea* with maize; IPS-8B: an integrated system of *Gliricidia sepium* with maize. Cmic: microbial carbon; qMIC: microbial quotient; LC: labile carbon. [b] Values represent the average (n = 4). Means followed by the same uppercase letter in the column and lowercase letter in the row, at the same depth and for each variable, do not show statistical difference between them by the Scott-Knott test at the 5% probability level. [c] Values represent the average land use for the two sampling times. [d] Values represent the average of two sampling times for all land uses. Means followed by the same capital letter in the column (land uses) and in the line (seasons) do not show statistical difference between them by the Scott-Knott test at the 5% probability level.

In Experiment 2, we also observed the effect of seasonality in the soil microbial attributes at 0–5 cm, except for β-glucosidase activity (Table 3). At 5–10 cm, we found significant interactions between the seasons and land-use systems for Cmic and β-glucosidase. At 10–20 cm, soil microbial attributes were not affected by the land-use systems and evaluated seasons. In the rainy season (December 2021), Cmic values at 5–10 cm were lowest in NV (257 mg kg^{-1}) than in IPS-1 (495 mg kg^{-1}) and PAST-1 (396 mg kg^{-1}) (Table 3). A similar pattern was observed for qMIC at 0–5 cm, where the lowest values were found in NV (0.82%) when compared to PAST-1 (2.09%) and IPS-1 (1.84%). An increase in LC was observed in the dry season (July 2022) at 0–5 cm, where the values ranged from 2.15 (PAST-1) to 3.85 g kg^{-1} (NV). The activity of the urease enzyme also increased in the dry season at 0–5 cm, where the values ranged from 127 (IPS-1) to 270 μg NH_4^+ g^{-1} h^{-1} (NV). The activity of the β-glucosidase enzyme was similar between PAST-1 and IPS-1 systems and higher than NV at 5–10 cm and 10–20 cm depths.

Table 3. Soil quality attributes at different sampling times and under different land-use systems in Experiment 2, located in the municipality of Prudente de Morais, Minas Gerais, Brazil.

Land-Use [a]	**Soil Depth (cm)**								
	0–5			**5–10**			**10–20**		
	Dec [b]	**Jul**	**Mean** [c]	**Dec**	**Jul**	**Mean**	**Dec**	**Jul**	**Mean**
	Cmic (mg kg $^{-1}$)								
NV	398 Aa [b]	269 Ab	333 A	257 Ba	182 Aa	219 A	199 Aa	113 Aa	156 A
PAST-1	596 Aa	144 Ab	370 A	396 Aa	132 Ab	264 A	339 Aa	93 Aa	216 A
IPS-1	566 Aa	182 Ab	374 A	495 Aa	154 Ab	324 A	368 Aa	109 Aa	238 A
average [d]	520 a	198 b		383 a	156 b		302 Aa	105 Ba	
	qMIC (%)								
NV	0.82 Ba	0.55 Aa	0.68 A	0.87Aa	0.62 Aa	0.75 A	0.74 Aa	0.44 Aa	0.59 A
PAST-1	2.09 Aa	0.52 Ab	1.30 A	1.56Aa	0.51 Aa	1.03 A	1.41 Aa	0.37 Aa	0.89 A
IPS-1	1.84 Aa	0.59 Ab	1.22 A	1.65Aa	0.48 Aa	1.06 A	1.47 Aa	0.42 Aa	0.94 A
Mean	1.58Aa	0.55Ab		1.36 Aa	0.54 Ab		1.21 Aa	0.41 Ab	
	LC (g kg $^{-1}$)								
NV	1.43 Ab	3.85 Aa	2.64 A	0.98 Ab	1.84 Aa	1.41 A	0.79 Aa	1.75 Aa	1.27 A
PAST-1	1.05 Ab	2.15 Ba	1.60 A	0.81 Ab	1.80 Aa	1.30 A	0.75 Aa	1.81 Aa	1.28 A
IPS-1	0.90 Ab	2.25 Ba	1.57 A	0.88 Ab	2.29 Aa	1.58 A	0.76 Aa	2.05 Aa	1.41 A
Mean	1.13 a	2.75 a		0.89 b	1.98 a		0.77 b	1.87 a	
	Urease (μg NH_4^+ g^{-1} 2 h^{-1})								
NV	126 Ab	270 Aa	198 A	101 Aa	172 Aa	136 A	71 Ab	159 Aa	115 A
PAST-1	73 Ab	149 Ba	111 A	56 Aa	107 Aa	81 B	43 Aa	89 Aa	66 B
IPS-1	70 Ab	127 Ba	98 A	47 Aa	90 Aa	69 B	38 Aa	69 Aa	53 B
Mean	90 b	182 a		68 b	123 a		51 b	106 a	
	β-glucosidase (μg pNP g^{-1} h^{-1})								
NV	134 Aa	136 Aa	135 A	75 Aa	47 Ba	61 A	47 Aa	36 Aa	41 B
PAST-1	111 Aa	169 Aa	140 A	113 Aa	98 Aa	105 A	68 Aa	82 Aa	75 A
IPS-1	124 Aa	173 Aa	149 A	84 Ab	121 Aa	103 A	79 Aa	82 Aa	80 A
Mean	123 b	159 a		91 a	89 a		65 a	67 a	

[a] NV: native vegetation; PAST: *U. brizantha* cv. BRS Piatã pasture; IPS: integrated system of *Cratylia argentea* with *U. brizantha* cv. BRS Piatã. Cmic: microbial carbon; qMIC: microbial quotient; LC: labile carbon. [b] Values represent the average (*n* = 4). Means followed by the same uppercase letter in the column and lowercase letter in the row, at the same depth and for each variable, do not show statistical difference between them by the Scott-Knott test at the 5% probability level. [c] Values represent the average land use for the two sampling times. [d] Values represent the average of two sampling times for all land uses. Means followed by the same capital letter in the column (land uses) and in the line (seasons) do not show statistical difference between them by the Scott-Knott test at the 5% probability level.

The PCA analysis was performed in order to discriminate the land-use systems according to the soil microbial attributes in the two evaluated seasons at 0–20 cm. The first two principal components explained 84.99% of the data variability for Experiment 1 in the rainy season (Figure 7A) and 89.05% in the dry season (Figure 7B). In the rainy season (December 2021), we observed higher Cmic in PAST-13, higher urease enzyme activity in

NV and qMIC in IPS-8A. The variables qMIC and β-glucosidase were positively correlated, and at the same time, these attributes were correlated negatively with the urease enzyme activity. In the dry season (July 2022), NV and PAST-13 showed higher values of Cmic, qMIC, LC and urease enzyme activity, while IPS-8B enhanced β-glucosidase enzyme activity.

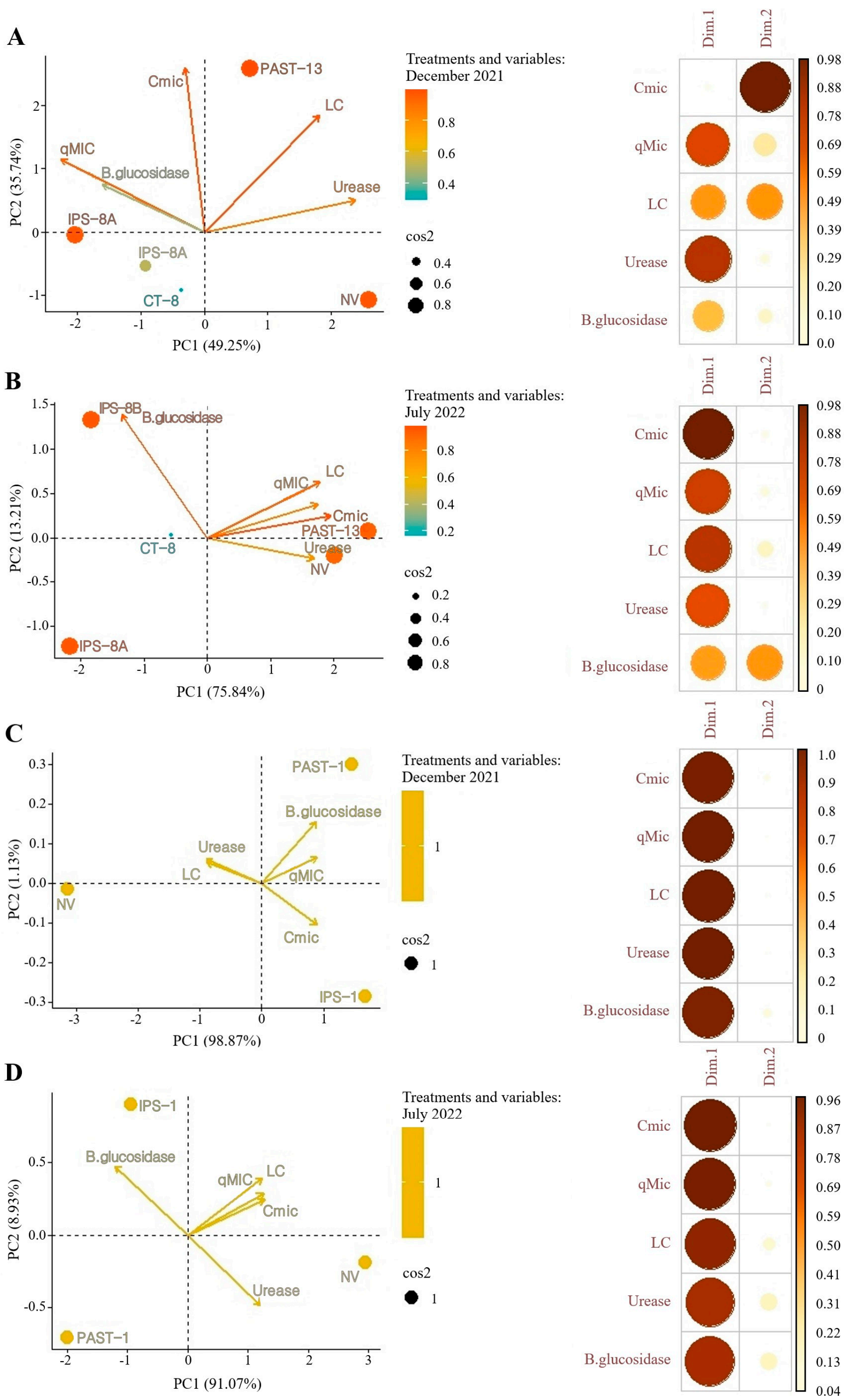

Figure 7. Relationship between principal component 1 (PC1) and principal component 2 (PC2) discriminating different management systems and land use according to the biological attributes of soil quality in the depth 0–20 cm, respectively, in rainy and dry seasons for the Experiment 1 (**A**,**B**) and Experiment 2 (**C**,**D**), located in the municipalities of Sete Lagoas and Prudente de Morais, Minas Gerais, Brazil.

In Experiment 2, the first two principal components explained the total variability of the data in the rainy (Figure 7C) and dry (Figure 7D) seasons. The highest values of Cmic, qMIC and β-glucosidase enzyme activity were observed in PAST-1 and IPS-1 in the rainy season and were positively correlated between them, while NV had higher urease enzyme activity and LC content. In the dry season, the activity of the urease enzyme, Cmic, qMIC and LC were higher in NV, while the activity of the β-glucosidase enzyme was higher in the IPS-1 system in the dry season.

4. Discussion

Overall, our results demonstrate that legume-based integrated agricultural systems effectively restored soil health by enhancing chemical, physical, and biological attributes. Notably, significant improvements in soil quality were observed when comparing integrated systems (IPS-8A and IPS-8B) to the 13-year pasture monoculture (PAST-13). Specifically, IPS-8B significantly increased surface soil nitrogen content, highlighting the crucial role of legumes in improving soil fertility within the Brazilian Cerrado biome. Furthermore, legume-based integrated systems (IPS-8A and IPS-8B) reduced subsurface soil density, likely due to increased system diversification. Furthermore, the integrated systems (IPS-8A and IPS-8B) exhibited higher β-glucosidase activity (0–5 cm) compared to PAST-13, indicating enhanced biological soil quality. This improvement was further supported by a 25% increase in microbial biomass carbon (Cmic) at a 5–10 cm depth in IPS-1 relative to PAST-1.

4.1. Soil C and N Stocks in the Land-Use Systems

The lower soil bulk density observed in CT-8 cultivated with maize (Table 1) may be related to soil management in Experiment 1, which increased soil disaggregation in the surface layers [30]. Residue deposition and continuous soil cover in the other land-use systems (IPS and PAST) can explain their similar values of soil bulk density compared to native vegetation, as these factors contribute to the improvement of soil physical properties [31].

Thirteen years following the conversion of native Cerrado to pasture cultivation (PAST-13), the levels of SOC increased in the soil subsurface layers (Figure 4A). This increase can be attributed to the abundant and extensive root system and exudate production [32,33]. Numerous studies have consistently reported improvements in SOC after the introduction of *Urochloa* spp. [14,16,34]. The greater ground cover and enhanced biomass input from pruned legume residues can explain the superior SOC preservation observed in integrated farming systems (IPS-8A and IPS-8B) at 0–10 cm when compared to conventional maize tillage in experiment 1 (Figure 4A; Table S2). Hazra et al. [35], in a long-term study, also showed a decline in SOC with the adoption of continuous cultivation of cereals. The authors recommended the integration of legumes as a suitable practice for improving SOC stocks, aggregation and biomass supply. The land-use systems showed similar TN and C/N ratios in all evaluated soil layers (Figure 4B; Table S2). However, Barros et al. [36] pointed out that integrated farming systems using legumes as components can promote N input into the soil over time, owing to the symbiosis of diazotrophic bacteria with the roots of these plants.

Soil C and N stocks followed the same patterns observed for SOC since we did not find significant differences in soil bulk density. The land use with PAST-13 increased soil C stocks over time (Figure 5), and the short-time implementation of IPS systems composed of annual crops and legumes was not sufficient to recover soil C stocks in levels similar to PAST-13 and NV. López-Santiago et al. [37], evaluating a silvopastoral system composed of *Leucaena leucocephala* and *Panicum maximum*, found soil C stocks similar to native vegetation. However, variations in C and N storage are directly influenced by local edaphoclimatic conditions, soil texture, land-use history, age of the system, and the inputs and outputs of organic material [37,38].

The soil bulk density in Experiment 2 was similar in the three land-use systems evaluated (Table 1), showing that management with residue input and continuous soil cover can contribute to the maintenance of soil physical properties [31]. A similar pattern was observed regarding SOC and NT contents, and the land use with PAST-1 and IPS-1 just decreased these parameters at 0–5 cm (Figure 4). Once in NV, there is continuous and diversified input of organic residues onto the soil surface [39].

Soil C and N stocks in the IPS-1 and PAST-1 systems were similar (Figure 6). Although these values were lower than those observed in NV ($p < 0.05$) in the soil surface, our results indicate the potential of legume-based integrated farming systems and well-managed pastures to promote soil C accumulation, especially considering the recent conversion from degraded pasture. Coser et al. [40] also reported positive impacts of integrated farming systems with leguminous crops in the Cerrado biome, observing a soil C stock of 66.5 Mg ha^{-1} (0–40 cm) after two years of implementation.

4.2. Soil Microbial Attributes in the Land-Use Systems

In Experiment 1, the Cmic values at 0–5 cm layer were higher in the PAST-13 system in the rainy season (Table 2). According to Alves et al. [41], the abundant and voluminous root system with continuous renewal in *Urochloa* spp. enhance microbial biomass in the rhizosphere. Additionally, the deposition of urine and feces from animal grazing contributes to an increase in Cmic [42]. In Experiment 2, the Cmic values at the 5–10 cm layer were higher in PAST-1 and IPS-1 in the rainy season (Table 3). Lira Júnior et al. [43] also reported increased Cmic in silvopastoral systems with leguminous shrubs, particularly during the rainy season, attributed to intensified litter decomposition. They further reported higher Cmic values (700 mg kg^{-1}) in integrated farming systems composed of *Mimosa caesalpiniifolia*, *Gliridicia sepium* and *U. decumbens*.

During the dry season, qMIC values are expected to decrease due to their direct correlation with Cmic. This index, used to assess organic matter quality, decreases under stressful conditions for soil microorganisms as their ability to utilize carbon is reduced. Conversely, the addition of high-quality soil organic matter (SOM) promotes increased microbial biomass and, consequently, higher qMIC values, even if SOC content remains unchanged [13]. Thus, our findings (Tables 2 and 3) may be attributed to legume-based integrated farming systems, specifically through green manuring, biological nitrogen (N_2) fixation, and the application of mulch in Experiment 1.

Lower labile carbon (LC) levels at the 0–5 cm layer in the land-use systems compared to NV (Table 2, Experiment 1) may be attributed to the SOM in the native vegetation, where there is less anthropogenic interference and constant and diversified deposition of waste on the soil surface [24]. Constant soil cover can also explain the higher LC content observed for PAST-13 in the 5–10 cm layer, corroborating the results found for Cmic. Although higher labile carbon (LC) levels were expected during the rainy season, peak values were observed during the dry season. The atypical rainfall event of April and May 2022 (Figure 2), which immediately preceded the July sampling period, could account for the observed results. Furthermore, the cropping systems in Experiment 1 may have been influenced by irrigation practices. Brito et al. [44] also observed higher values of LC in the dry season for all evaluated management practices and attributed this result to litter accumulated in the soil, resulting from the loss of leaves by the plants during this period, which promoted an increase in organic matter and maintained humidity in the soil. Higher soil moisture provides a greater accumulation of green biomass, which is subsequently converted into plant material and deposited in the soil, thereby influencing the input of

LC [45]. Additionally, these results can be associated with the persistence of residues from the legumes in the integrated farming systems (IPS-8A and IPS-8B). Soil sampling was collected in December 2021, nine months after the last pruning and deposition of residues as mulch, whereas in July 2022, the soil sampling was carried out five months after management. Mulching enables greater deposition of readily accessible C-rich materials on the soil surface, which increases LC levels in these areas [46]. Similar patterns for LC were also observed in Experiment 2 (Table 3). The atypical rainfall that occurred close to the sampling time (Figure 2) in July may have contributed to the increase in LC levels, as observed in Experiment 1. The grazing animals close to the sampling time in July 2022 could also have contributed to the higher LC values observed during this period. Animal waste can provide labile organic matter that is more readily available in addition to grazing, which can stimulate the growth renewal of the root system and root exudates of forage plants [47].

The activity of the urease enzyme (Table 2) was similar among ISP1, ISP2, NV, and PAST-13 in Experiment 1 (0–5 cm), which is related to the presence of legumes in integrated farming systems since the biological fixation of N_2 can influence enzymatic activity as well as the deposition of waste in the form of green manure and mulch. A lower C/N ratio favors the faster decomposition of plant material [48]. Higher activity of urease enzyme in NV than in IPS-1 and PAST-1 systems was observed in Experiment 2 (Table 3). Increased soil organic matter (SOM) quantity and quality, coupled with a favorable soil surface microclimate, enhanced enzymatic activity. In NV, the soil surface microclimate (temperature and humidity), buffered by shading, exhibits less variation, potentially reducing limitations on enzymatic activity compared to managed areas [49]. Similar to labile carbon (LC), urease activity was higher during the dry season. Atypical rainfall (Figure 2) and pruning, as previously mentioned, may have contributed to maintaining soil moisture during this period. Longo and Melo [50] observed higher urea hydrolysis rates during warmer, more humid months. However, urease activity is influenced by urea availability, soil temperature, humidity, and incorporated organic material. Yang et al. [51] further demonstrated that low-intensity watering can enhance urease activity, whereas severe water stress inhibits it.

The increased β-glucosidase activity observed in IPS-8A and IPS-8B (Experiment 1, Table 2) in relation to CT-8 can be attributed to the significant contribution of pruned biomass from *Cratylia argentea* (41.44 Mg ha^{-1}) and *Gliricidia sepium* (74.08 Mg ha^{-1}). Therefore, further studies are required to assess the decomposition rates of leguminous residues. The higher β-glucosidase activity in these systems may be a result of both green legume residue input and the supplemental water provided through irrigation during the dry season [52]. Miguel et al. [53] evaluated different land uses and sampling times and also observed higher β-glucosidase activity in an agroforestry system (80 μg pNP g^{-1} h^{-1}) than in native vegetation (68 μg pNP g^{-1} h^{-1}). In Experiment 2, the higher β-glucosidase activity was observed in PAST-1 and IPS-1 (Table 3). Turner et al. [54], evaluating the soil under 29 pasture sites, reported a correlation between the β-glucosidase activity and qMIC since the enzyme is synthesized by soil microorganisms in response to the abundance of substrate (cellulose). The lower seasonal variability of β-glucosidase compared to urease may stem from its more stable enzyme complex within the soil matrix [55]. This stability renders β-glucosidase less sensitive to temperature, humidity, and pH fluctuations than free enzymes [56]. Furthermore, grazing and animal presence can influence enzyme activity, as demonstrated by Vargas et al. [47].

Our results of PCA identified the seasonality of the soil microbial attributes and the influence of land-use systems in the two experiments (Figure 7). The enhanced microbial attributes observed in pasture areas during both sampling periods highlight the importance of managed grasslands in maintaining soil organic matter. This maintenance is likely due to the continuous soil cover and the abundant and constantly renewing root systems of forage plants [32,33]. Furthermore, the introduction of legume-based integrated farming systems enhanced the Cmic and qMIC as well as stimulated the β-glucosidase enzyme activity. According to Yadav et al. (2011) [57], integrated farming systems can increase soil microbial biomass and enzymatic activity through long-term improvements in soil function and productivity.

5. Conclusions

Even with short-term implementation, an integrated farming system with *Gliricidia sepium* and *Zea mays* increased soil C stocks by up to 24% at the surface layer when compared to an annual crop system under conventional soil management (Experiment 1) and also demonstrated potential for soil C stock recovery in areas previously occupied by degraded pasture, achieving similar levels to well-managed pasture monoculture (Experiment 2). Long-term studies are needed to further evaluate the effect of the adoption of agroforestry systems in terms of increasing soil C and N stocks.

Climate seasonality had a greater influence on soil microbial attributes than on land-use systems. Despite short-term implementation, considering the soil surface, Cmic was similar between the integrated system and pasture in Experiment 2, while integrated farming systems increased the β-glucosidase activity in relation to the pasture system in Experiment 1.

This study suggests that the introduction of leguminous crops into integrated farming systems significantly contributes to soil health restoration, presenting a viable alternative to pasture or annual crop monoculture systems within the Brazilian Cerrado biome.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su17083340/s1, Table S1: Soil chemical characterization for each land use in Experiment 1 and 2, located respectively in the municipalities of Sete Lagoas and Prudente de Morais, Minas Gerais, Brazil; Table S2: Soil C and N contents and C/N ratios under different land-use systems in Experiment 1, located in the municipality of Sete Lagoas, Minas Gerais, Brazil; Table S3: Soil C and N content and C/N ratios under different land-use systems in Experiment 2, located in the municipality of Prudente de Morais, Minas Gerais, Brazil.

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