



# Article Soil Microbial Activity and Biomass in Semiarid Agroforestry Systems Integrating Forage Cactus and Tree Legumes

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**Abstract:** Vegetation cover affects soil organic matter and activity of soil microorganisms. We investigated the intercropping effect of forage cactus with tree legumes on soil microbial biomass and organic matter in the semi-arid tropic, state of Pernambuco, Brazil. We assessed the following cropping systems: (i) *Gliricidia sepium* intercropped with cactus cv. IPA-Sertânia; (ii) *Leucaena leucocephala* intercropped with cactus cv. IPA-Sertânia; and (iii) Cactus cv. IPA-Sertânia in monoculture. Samples were collected during the dry and rainy seasons in the 0- to 0.10- and 0.10- to 0.20-m soil layers at 0, 1.5, 3.0, and 4.5 m in a perpendicular gradient from tree legume rows. The following responses were determined:  $\delta^{13}$ C and  $\delta^{15}$ N, C and N, microbial activity and biomass, and metabolic, microbial, and mineralization quotient.  $\delta^{13}$ C and  $\delta^{15}$ N varied with the distance from the trees. In the dry season and beginning of the rainy season, the cropping systems showed similar values for C, N, microbial activity, carbon, and nitrogen in the microbial biomass. The presence of tree legumes at the end of the rainy season favored soil microbiota, which showed a reduced loss of C-CO<sub>2</sub>, with no indication of metabolic stress and greater microbial biomass and microbial quotient in relation to forage cactus in monoculture.

**Keywords:** *Gliricidia sepium; Leucaena leucocephala;* microbial biomass; *Nopalea cochenillifera;* soil organic matter

## 1. Introduction

Forage cactus is an important feed source for herds in semiarid regions. It is utilized throughout the year in the tropical semiarid region of Brazil and in other countries, as it adapts to the edaphoclimatic conditions of these regions. Although it is adapted to semiarid regions, the reduced fiber and crude protein concentrations of forage cactus leads to a need to balance the diet of livestock. Thus, one of the alternatives to balance the diet of livestock with proteins and fibers when feeding cactus is intercropping cactus with tree legumes [1]. In addition, tree legumes can enhance N input in the agroecosystem via biological N2 fixation.

Agroforestry systems are recognized for their potential to store large amounts of carbon and nitrogen in the soil [2]. Intercropping legumes with agricultural crops increases N availability to the system; this promotes greater deposition of litter, roots, and nodules of leguminous plants [3], favoring nutrient cycling by adding material with low C:N ratio and,



Citation: Camelo, D.; Dubeux, J.C.B., Jr.; dos Santos, M.V.F.; Lira, M.A., Jr.; Fracetto, G.G.M.; Fracetto, F.J.C.; da Cunha, M.V.; de Freitas, E.V. Soil Microbial Activity and Biomass in Semiarid Agroforestry Systems Integrating Forage Cactus and Tree Legumes. *Agronomy* **2021**, *11*, 1558. https://doi.org/10.3390/ agronomy11081558

Academic Editors: Juan J. Villalba and Jennifer MacAdam

Received: 24 June 2021 Accepted: 3 August 2021 Published: 5 August 2021

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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). therefore, rapid mineralization [4], and improving soil chemical, physical, and biological characteristics. *Gliricidia sepium* and *Leucaena leucocephala* are important tree legume options for animal production in Northeastern Brazil. Both legume species are drought tolerant and nitrogen-fixers, and can be consumed (as a feed or hay) by ruminants, improving the diet of grazing animals and supplying high levels of protein [5,6]. Lira Junior et al. [7] observed that the intercropping between *Urochloa decumbens* and *Gliricidia sepium* led to soil organic matter increases, and of the soil C and N levels by around 37% and 82%, respectively, in comparison to *Urochloa decumbens* in monoculture. Given the importance of soil organic matter, its evaluation using isotopic techniques ( $\delta^{13}$ C and  $\delta^{15}$ N) is a good indicator of the type of existing vegetation and the cycling of organic matter [8].

Agroforestry systems are also modified by beneficiaries of soil microorganisms. Singh et al. [9] recommended the adoption of agroforestry systems as they provide greater carbon microbial biomass, soil microbial activity, and lower the metabolic quotient compared to monocultures. The soil microbial biomass is the active component of the soil organic pool, responsible for organic matter decomposition, mineralization, and, consequently, primary productivity in most biogeochemical processes in terrestrial ecosystems [10].

Microorganisms are good indicators of organic matter quality and the level of disturbance in edaphic systems [11]. Research shows that root exudates released from the intercropping between grasses and leguminous plants affect the microbial community [12], which is one of the main ways to incorporate microbial biomass carbon [13], especially in tropical environments after rainy seasons. Studies show that varieties of the same species affect the soil microbiota of tropical semiarid regions in different ways [9,14]. This proves that disturbances in vegetation cover and management are among the factors responsible for variations in the soil microbial community [15]. Recently, Lira Junior et al. [7] identified that microbial biomass also changes as it moves away from leguminous trees.

This study hypothesizes that intercropping between Cactus + Gliricidia and Cactus + Leucaena compared with cactus monoculture: (i) increases the estimate of soil microbial biomass; (ii) provides a more efficient degradation of organic matter by the microbiota; (iii) reduces possible microbial metabolic stresses; and (iv) influences seasonal effects, especially related to soil moisture, which have a marked effect on soil microbial responses. Therefore, we determined  $\delta^{13}$ C and  $\delta^{15}$ N levels, total C and N levels, microbial biomass, and respiration, as well as microbial and metabolic quotients, considering the local climate and the distance from leguminous trees in comparison with forage cactus in monoculture.

#### 2. Materials and Methods

#### 2.1. Site Description and Sampling Strategy

The study was conducted at the Caruaru Experimental Station, Agronomic Institute of Pernambuco-IPA (8°14′ S and 35°55′ W). According to FAO [16], the predominant soil in the experimental area is Regosol, and their main characteristics are reported in Table 1. The climate of the site is dry and hot semiarid (BSh) according to the Köppen classification, with average rainfall of 727.1 mm/year. Monthly rainfall data in the experimental period (October 2018 to July 2019) are shown in Figure 1, totaling 793.3 mm.

The following cropping systems were tested: (i) Cactus cv. IPA-Sertânia (*Nopalea cochenillifera* Salm Dyck) intercropped with leucaena (*Leucaena leucocephala* (Lam.) de Wit.); (ii) Cactus cv. IPA-Sertânia intercropped with gliricidia (*Gliricidia sepium* (Jacq.) Steud); (iii) Cactus cv. IPA-Sertânia in monoculture. The experimental design was in randomized blocks with four replications, in a split-plot scheme. The main plot was formed by the different cropping systems (agroforestry and monoculture), and the subplot consisted of four distances from the tree legume row (0.0, 1.5, 3.0, and 4.5 m).

3 of 13

Soil Depth	Management	Distance	Sand	Silt	Clay	Textural Class	pН	Р	Ca	Mg	Na	К	Al	Н	NT	TOC	SOM
(m)		(m)		$(g \cdot kg^{-1})^{1}$		(H <sub>2</sub> O) <sup>2</sup>			(cmolc/dm <sup>3</sup> ) <sup>2</sup>					(g·kg <sup>-1</sup> )			
0-0.10	C + L	0	730	170	100	Sandy	5.1	35	2.1	0.9	0.1	0.5	0.2	4.3	0.8	9.2	15.9
		4.5	730	150	120		5.1	79	2.4	0.9	0.1	0.3	0.2	5.1	0.9	10.4	17.9
	C + G	0	760	140	100		5.0	31	2.0	0.9	0.1	0.3	0.2	4.5	0.9	9.7	16.7
		4.5	730	170	100		5.3	66	2.8	0.9	0.1	0.3	0.1	4.2	1.0	11.8	20.3
	С		780	100	120		5.0	105	2.5	0.7	0.7	0.3	0.2	1.4	1.0	10.4	17.9
10–0.20	C + L	0	730	150	120		5.2	34	2.4	0.9	0.1	0.4	0.2	2.8	1.6	9.8	16.9
		4.5	760	120	120		4.9	60	2.0	1.0	0.1	0.2	0.3	3.5	1.6	11.0	19.0
	C + G	0	780	120	100		4.8	32	1.6	0.9	0.1	0.3	0.3	3.3	1.4	8.8	15.2
		4.5	730	150	120		5.1	57	2.2	0.9	0.1	0.2	0.2	3.1	1.8	8.3	14.3
	С		740	140	120		5.1	80	3.1	0.8	0.8	0.2	0.2	1.2	1.6	7.2	12.4

Table 1. Soil chemical and physical characteristics at the beginning of the experiment (Oct/2018).

<sup>1</sup> [17]. <sup>2</sup> [18]. TN: total nitrogen. TOC: total organic carbon. SOM: soil organic matter (TOC × 1.724). C+L: cactus + leucaena; C+G: cactus + gliricidia; C: cactus in monoculture.



□ Monthly precipitation (mm) – Minimum temperature (°C) → Maximum temperature (°C) – Average temperature (°C)

**Figure 1.** Monthly rainfall and temperatures (maximum, average, and minimum) at Caruaru Experimental Station (IPA) during the experimental period. Arrows indicate sampling dates. Source: Instituto Agronômico de Pernambuco-IPA, Brazil.

The experiment was installed in March 2011, when the area was covered with typical Caatinga vegetation, a dry forest native to the region. Tree legumes were planted in three double rows per plot, spaced  $9 \times 1 \times 0.5$  m, with IPA-Sertânia cactus planted between the double rows and in monoculture, at a spacing of  $1 \times 0.25$  m [19]. In the agroforestry plots, the density of tree legumes was 4000 plants/ha, and the cactus density was 32,000 plants/ha. In monoculture, the cactus density was 40,000 plants/ha. In June 2018, the experimental area was fertilized with cattle manure (O.M. = 412 g kg<sup>-1</sup>; N = 15 g kg<sup>-1</sup>; P = 4.9 g kg<sup>-1</sup>; K = 14.9 g kg<sup>-1</sup>) to supply 200 kg ha<sup>-1</sup> N. Weeds were removed annually, and the material was used as mulch. The forage cactus was harvested every two years, and the tree legumes were not cut during the experiment.

Soil was collected in October 2018 (dry season) and in April (beginning of the rainy season) and July 2019 (end of the rainy season). In intercropped plots, single samples were collected in the 0- to 0.10- and 0.10- to 0.20-m layers, at 0.0, 1.5, 3.0, and 4.5 m from the central row of tree legumes (Figure 2). Monoculture plots were sampled at just one point and at the same depths mentioned above. Samples were kept in a refrigerator until microbiological analysis.

## 2.2. Soil Analyses

For the analysis of isotopic composition, the samples of the 0- to 0.10-m layer were ground in an MM400 ball mill (Retsch, Newton, PA, USA) and sieved at 100  $\mu$ m. The values of total organic C (TOC) and total N (TN) were determined by the Dumas dry combustion method, using the CHNS analyzer (Vario Micro Cube; Elementar, Hanau, Germany) coupled to an isotopic ratio mass spectrometer (IsoPrime 100, IsoPrime, Manchester, UK) to analyze  $\delta^{13}$ C and  $\delta^{15}$ N. The results of  $\delta^{13}$ C were expressed as delta  $\delta^{13}$ C (‰) relative to the international standard PDB (Belemnitella Americana of the Pee Dee formation). The

results of  $\delta^{15}$ N were expressed as  $\delta^{15}$ N (‰) relative to the  $\delta^{15}$ N air standard (0.3663%). The following formula was used:

$$\delta = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} * 1000 \tag{1}$$

where  $\delta$ : difference between the isotopic ratio of the sample and standard (‰), R sample: Isotopic ratio of the sample (<sup>13</sup>C:<sup>12</sup>C and <sup>15</sup>N:<sup>14</sup>N), R standard (C): Standard isotopic ratio (PDB), and R standard (N): Atmospheric air.





Figure 2. Layout of the soil sampling sites in the agroforestry systems using tree legumes and cactus; Caruaru-PE, Brazil.

Soil basal respiration (SBR) was assessed by quantifying the C-CO<sub>2</sub> released by microbial respiration [20]. Microbial biomass C and N levels (C-mic and N-mic) were estimated by the irradiation-extraction method [21]. The dynamics of the microbial biomass C and N was discussed based on the ratio between C-mic and N-mic (Cmic: Nmic). The metabolic quotient (qCO<sub>2</sub>) was calculated by the ratio between SBR and C-mic [22], and the microbial quotient (qMIC) was obtained by the ratio between C-mic and the total organic carbon (TOC). The mineralization quotient (qM) is the ratio between the cumulated respiration and the SOC and is expressed in %. The quotient indicates the efficiency of micro-flora in metabolizing SOC [23].

## 2.3. Statistical Analysis

Data were analyzed using the repeated measures mixed model analysis of variance, separately for each soil layer, and included nine treatments composed by the eight combinations of distance and species (agroforestry systems) and the single forage cactus treatment. The blocks were considered as random effects. Fixed effects included cropping systems, distances, and seasons. Data were checked for normality and homoscedasticity by assessing residual normality and variance. To contrast the cactus monoculture system with the agroforestry systems, the average value of the distances collected was used. To test the effect of distances, only the values of the agroforestry systems were used. When significant differences were found, the treatments were compared by Tukey's test at the 10% significance level.

## 3. Results

## 3.1. Soil $\delta^{13}C$ and $\delta^{15}N$

The values of  $\delta^{13}$ C (p = 0.0001) and  $\delta^{15}$ N (p = 0.0001) changed with the distance from the tree legumes in the 0- to 0.10-m layer. For the two agroforestry systems (leucaena and gliricidia), soil samples collected under tree canopies showed a more depleted value of  $\delta^{13}$ C (Figure 3), ranging from -22.69% (0 m) to -21.36% (4.5 m), while  $\delta^{15}$ N (Figure 3) values ranged from 10.96% (0 m) to 11.84% (4.5 m).



**Figure 3.** Isotopic natural abundance of  $\delta^{13}$ C and  $\delta^{15}$ N at different distances from the tree legumes intercropped with cactus, at the 0- to 10-cm layer. Different capital letters indicate significant difference (*p* < 0.10) by PDIFF adjusted to Tukey. Bars indicate the standard error of the mean.

## 3.2. Total Organic Carbon (TOC) and Total Nitrogen (TN)

The different cropping systems favored an increase in soil TOC content. The average TOC levels in 2013 were 9.57  $g \cdot kg^{-1}$  in the 0- to 0.20-m soil layer [19]. In 2019, these values increased to 10.61 and 18.24  $g \cdot kg^{-1}$  in the 0- to 0.10- and 0.10- to 0.20-m soil layers, respectively.

The different cropping systems had different TOC levels in the dry and the beginning of the rainy season in the 0- to 0.10-m layer (p < 0.10). Nevertheless, at the end of the rainy season, the cactus monoculture system showed the greatest TOC levels (Figure 4). In the 0.10- to 0.20-m layer (p = 0.021), the soils of the different systems had greater TOC content (23.30 g kg<sup>-1</sup>) at the beginning of the rainy season in relation to the dry and final rainy season (8.91 and 13.15 g kg<sup>-1</sup>, respectively) (Figure 4). The different cropping systems did not differ significantly for N content and C:N ratio in the 0- to 0.10- (p = 0.627; 0.905) and 0.10- to 0.20-m (p = 0.178; 0.492) layers. The soil showed a greater TN content (around 26%) in the dry season than in the other seasons (p = 0.0001). In the dry season, there was also a lower C:N ratio (5.4:1) of the soil compared to the beginning (19.3:1) and end of the rainy season (9.6:1).

### 3.3. Microbiological Characteristics

Microbiological response variables were affected by cropping systems and seasons (Figure 5). Different cropping systems had different SRB in the dry season and the beginning of the rainy season at the 0- to 0.10- (p = 0.045) and 0.10- to 0.20-m (p = 0.007) layers (Figure 5). The soils of the agroforestry systems showed greater microbial activity in the dry season (41.14 and 21.31 µg C-CO<sub>2</sub> g<sup>-1</sup> day<sup>-1</sup>) and at the end of the rainy season (39.23 and 33.18 µg C-CO<sub>2</sub> g<sup>-1</sup> day<sup>-1</sup>) in comparison to the beginning of the rainy season (7.78 and 10.95 µg C-CO<sub>2</sub> g<sup>-1</sup> day<sup>-1</sup>) in the 0- to 0.10- and 0.10- to 0.20-m layers, respectively. However, for cactus monoculture, the greatest SBR occurred at the end of the rainy season (60.81 µg C-CO<sub>2</sub> g<sup>-1</sup> day<sup>-1</sup>) at the 0- to 0.10-m layers.





**Figure 4.** Total organic carbon (TOC) in the soil of different cactus cropping systems at different sampling dates. C + L: cactus + leucaena; C + G: cactus + gliricidia; C: cactus in monoculture. Different capital letters indicate significant difference (p < 0.10) within each period of the year; different small case letters indicate significant difference within each cropping system (p < 0.10) by PDIFF adjusted to Tukey. Bars indicate the standard error of the mean.

C-mic (p = 0.005) and N-mic (p = 0.025) changed depending on the cropping systems and seasons at the 0- to 0.10-m layer (Figure 5). The N-mic contents were statistically similar in the dry season and the beginning of the rainy season for the different cropping systems. In the dry period, the monoculture had higher C-mic (222.55 mg kg<sup>-1</sup>) than the agroforestry systems (158.56 and 153.00 mg kg<sup>-1</sup>). However, at the end of the rainy season, the soils of the agroforestry systems (C + L and C + G) showed greater C-mic (260.32 and 297.95 mg kg<sup>-1</sup>) and N-mic (40.52 and 54.03 mg kg<sup>-1</sup>) in relation to forage cactus in the monoculture (147.41 and 11.18 mg kg<sup>-1</sup>). The microbial biomass showed a lower C-mic:N-mic ratio in the dry season (5.2:1) than in the beginning and end of the rainy season (9.8:1 and 10.4:1, respectively).

N-mic also changed depending on the distance between trees in the 0- to 0.10-(p = 0.002) and 0.10- to 0.20-m (p = 0.002) layers, with a greater content of N-mic (47, 46 and 43.09 mg kg<sup>-1</sup>) under tree canopies, which decreased about 40% for the 0- to 0.10-m layer and 34% for the 0.10- to 0.20-m layer up to 4.5 m away.

The different cropping systems and seasons also significantly affected  $qCO_2$  (p = 0.0001) and qMIC (p = 0.001) in the 0- to 0.10-m soil layer (Figure 5). In the dry season, the greatest  $qCO_2$  occurred in the cropping systems C + L and C + G (0.35 and 0.36 µg C-CO<sub>2</sub> g<sup>-1</sup> C-mic day<sup>-1</sup>) in relation to cactus in the monoculture (0.18 µg C-CO<sub>2</sub> g<sup>-1</sup> C-mic day<sup>-1</sup>). The  $qCO_2$  was similar between cropping systems at the beginning of the rainy season. However, at the end of the rainy season, the soil of the cactus monoculture had a greater  $qCO_2$  (0.43 µg C-CO<sub>2</sub> g<sup>-1</sup> C-mic day<sup>-1</sup>), and the agroforestry systems had a greater qMIC (3.00% and 3.05% for C+L and C+G, respectively). In the 0.10- to 0.20-m layer, the season affected  $qCO_2$  (p = 0.0001) and qMIC (p = 0.0001). The smallest values of  $qCO_2$  and qMIC (0.05 µg C-CO<sub>2</sub> g<sup>-1</sup> C-mic day<sup>-1</sup> and 1.03%) occurred at the beginning of the rainy season in relation to the dry and final rainy seasons (0.11; 0.12 µg C-CO<sub>2</sub> g<sup>-1</sup> C-micday<sup>-1</sup> and 2.97; 2.18%) in the 0.10- to 0.20-m layer. The qM was significantly (p = 0.0001) affected by the seasons. The qM values were higher in the dry season (3.83; 2.35%) and at the beginning of the rainy season (4.26; 2.35%) compared to the end of the rainy season (0.92; 0.49%) in the 0- to 0.10- (p = 0.001) and 0.10- to 0.20-m (p = 0.001) layers.



C + L C + G C

**Figure 5.** Soil basal respiration (SBR), soil microbial carbon (C-mic), soil microbial nitrogen (N-mic), metabolic quotient (qCO<sub>2</sub>), and microbial quotient (qMIC) in different cactus cropping systems and dates. C + L: cactus + leucaena; C + G: cactus + gliricidia; C: cactus in monoculture. Different capital letters indicate significant difference (p < 0.10) within each period of the year; different small case letters indicate significant difference within each cropping system (p < 0.10) by PDIFF adjusted to Tukey. Bars indicate the standard error of the mean.

## 4. Discussion

## 4.1. Soil $\delta^{13}C$ and $\delta^{15}N$

In the agroforestry systems, organic carbon origin changed as a function of the distance from trees (Figure 3). The lower values of  $\delta^{13}$ C found under the canopies of trees indicate the influence of organic residues from tree legumes, since the  $\delta^{13}$ C of the C<sub>3</sub> plants ranges between -20 and -34% [24]. As the distance from trees increases, the TOC probably originates from the forage cactus, as well as from weeds, since the  $\delta^{13}$ C values of the cactus (-14.43 and -13.22%) and C<sub>4</sub> plants (-13%) are similar [25]. Plants with crassulacean acid metabolism deposit less litter. However, forage cactus contributes to root deposition and root exudates because it is a perennial plant, maintaining its root system as active over the years [26].

The soils under the canopies of the agroforestry systems also accounted for more depleted values of  $\delta^{15}N$  (Figure 3), which can be attributed to biological nitrogen fixation. It also indicates TOC accumulation under the canopy, resulting from the litter deposition of tree legumes. However, as the distance from trees increased, soil  $\delta^{15}N$  also increased (i.e., became more enriched), showing that the TOC originating from cactus and weeds is in an advanced stage of decomposition and that the soil far from tree legumes contains lower levels of N [27]. This is because the transformations from organic N to mineral N result in greater decomposition of the lighter isotope of N (<sup>14</sup>N), leaving the remaining organic matter enriched in <sup>15</sup>N atoms [28].

#### 4.2. Total Organic Carbon (TOC) and Total Nitrogen (TN)

In a previous study [19], these same agroforestry systems increased TOC levels after eight years of implantation. In the present study, TOC levels were greater than those observed by Iwata et al. [29], who evaluated an agroforestry system with tree and fruit species after thirteen years of implantation (21.9 g kg<sup>-1</sup>) in a Red-Yellow Argisol soil and sub-humid tropical environment. Lira Junior et al. [30] observed an increase of around 37% in TOC levels (41 g kg<sup>-1</sup>) in an agroforestry system with gliricidia and Urochloa decumbens in four years of evaluation in a sub-humid tropical environment, proving the efficiency of this system in increasing C storage. The similarity between cactus agroforestry systems and monoculture in increasing TOC levels (Figure 4) is attributed both to the perennity of forage cactus and to the growth and exudation dynamics of its root system [11]. A study by González et al. [26] proves that soils cultivated with forage cactus have similar TOC levels to native vegetation areas (grass-steppe). However, it is worth mentioning that agroforestry systems allow better soil coverage through the litter of trees. TOC enrichment in the 0.10to 0.20-m layer (23.3 g kg<sup>-1</sup>) in relation to the 0- to 0.10-m layer (9.6 g kg<sup>-1</sup>) during the rainy season indicates that soil carbon is likely to leach during greater rainfall and become trapped in the subsurface layer (0.10–0.20 m). This increased organic C content can then be used as a substrate by soil microorganisms.

The 26% increase in TN in the dry season, on the other hand, can be explained by the greater deposition of litter from leguminous trees, common in this period of the year. As a mechanism to minimize water loss through evapotranspiration, these trees lose their leaves usually at the end of the rainy season and during the dry season [31]. The addition of this material with a low C:N ratio results in its rapid decomposition and mineralization [32], increasing the soil nutrient content, mainly N. However, less litter deposition occurs in the rainy season (beginning and end), which explains the lower TN levels. Another reason for the greater soil N levels is its addition through the symbiosis between leguminous species and N-fixing bacteria. Lira Junior et al. [30] attribute the greater soil N storage in silvopastoral systems with tree legumes, which can triple N stocks compared to *Urochloa decumbens* in monoculture, to the presence of free-living and symbiotic diazotrophic bacteria [33].

The high C:N ratio in the 0.10- to 0.20-m layer at the beginning of the rainy season can be attributed to the increase in TOC, as well as to the fact that the first rains have already occurred. These first rains likely led to mineralization of organic residues in the soil, leaving

only the material with recalcitrant N. The soil C:N ratio tended to decrease at the end of the rainy and dry seasons, showing the effect of the litter of tree legumes (low C:N), which can be deposited mainly at the end of the rainy season and during the dry season [31]. The high C:N ratio in the 0.10- to 0.20-m layer of cactus in the monoculture can be explained by the production of thin, short-lived roots (rain roots) at the beginning of the rainfall period. These roots die at the end of the season and are quickly decomposed [34]. This study shows that regardless of season and cultivation system, mineralization is favored over immobilization (<20:1), indicating greater nutrient availability [35].

## 4.3. Microbiological Characteristics

In agroforestry systems, the dry season accounted for the greatest SBR (41.14 µg C-CO<sub>2</sub> g<sup>-1</sup> day<sup>-1</sup>), the greatest qCO<sub>2</sub> (0.36 µg C-CO<sub>2</sub> g<sup>-1</sup> C-mic day<sup>-1</sup>), the least C-mic:N-mic ratio (5.2:1), and qMIC around 1.43%. The low C-mic:N-mic ratio shows that the microbial biomass was enriched with nitrogen originating both from the organic fertilization that preceded this study [36] and from the litter deposition of leguminous trees, common during the dry season. Due to the lower C:N ratio of this litter, it is rapidly decomposed and mineralized by microorganisms [37], which explains the greater microbial activity. The results found in this study are similar to those found in soils under native vegetation and pasture cultivation in the semiarid region of Pernambuco [38], indicating that agroforestry systems and cactus monoculture are sustainable and beneficial models for microbial biomass. In general, the dry season had a higher qM and lower C-mic:N-mic ratio, indicating a better efficiency of microorganisms in metabolizing organic matter [23].

Basal soil respiration (SBR), *q*CO<sub>2</sub>, and N-mic (Figure 5) decreased at the beginning of the rainy season in the different cropping systems. The reduction in *q*CO<sub>2</sub> means less energy expenditure when using the available organic C, which remained constant from the dry season to the beginning of the rainy season. This can be attributed to the climatic conditions of tropical environments, which favor organic matter degradation [14]. The reduction in N-mic had the same trend found by Souza et al. [39] in soils cultivated with black oats and ryegrass. The authors propose that this decrease during periods of greater rainfall correlates with N uptake by plants and possible losses by leaching. In agroforestry systems, the closer the soil is to the canopies of trees, the greater the soil N-mic content. This means greater N immobilization, constituting one of the possible reserves of this nutrient in the soil, although its recycling and release are faster than those of other fractions of soil organic matter [40]. Yadav et al. [41] also identified greater microbial biomass N levels in a land use system based on *Acacia leucophloea* and *Acacia nilotica* in relation to a control treatment (without trees), which improved microbial activity due to the supply of this essential nutrient.

The high C-mic:N-mic ratio at the beginning and end of the rainy season favors the predominance of microorganisms that decompose recalcitrant organic matter, such as fungi and actinomycetes. This likely occurs by the production of extracellular enzymes responsible for degrading substrates with a high C:N ratio [40], although these enzymes have not been measured here. The change in the C-mic:N-mic ratio proves that the composition of the soil microbial community and, consequently, the level of immobilization and mineralization of soil nutrients are affected by climatic factors and cultivation systems [42]. The high C-mic:N-mic ratio associated with low qM and  $qCO_2$  values indicates that the labile SOM pool was used to synthesize stable humic fractions, which are more resistant to microbial decomposition [43].

The monoculture system showed a greater SBR at the end of the rainy season, which may have been caused by the deposition of mowed weeds. This result was also observed by Melloni et al. [44], who found greater basal respiration in soil submitted to manual weeding than in soil without weeding. At the end of the rainy season, agroforestry systems showed lesser SBR, greater C-mic content, and greater *q*MIC. This can be attributed to the greater litter deposition, since leucaena and gliricidia are deciduous [45]. Organic matter supply, along with rainfall, provides better soil microbiological conditions, since *q*MIC

values were greater than 1% [46], favoring organic matter mineralization and resulting in greater carbon incorporation into microbial cells. The values found in agroforestry systems are like those observed by Guimarães et al. [47], who evaluated a fallow area for three years and observed that higher plant complexity favored the development of a more stable microbial population. These results also show the similarity between the leguminous plants used.

#### 5. Conclusions

Although the isotopic composition of soil organic matter near the legume plants indicate a differential effect from these species, both agroforestry systems and cactus monoculture had overall similar effects in increasing soil C and N levels, indicating that all systems might be considered sustainable in this tropical semiarid region. Microbial carbon was greater in the agroforestry systems at the end of the rainy season, but not in other sampling dates. This resulted in greater efficiency of incorporating organic residues into the microbial biomass and that was likely a result of decreasing microbial metabolic stresses due to the supply of N and provision of shade by tree legumes.

Differences between both agroforestry systems and cactus monoculture in regard to microbial activity and biomass were accentuated at the end of the rainy season, likely due to the low C:N ratio of the legumes litter deposited at this time.

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

Funding: This research was partially funded by CAPES (financial code 001).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: The authors D.C., M.V.F.d.S. and M.A.L.J. thank the "Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPq (Brazil), and the author F.J.C.F. thanks the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—CAPES (Brazil) for the assistantship and fellowship. All authors thank CAPES (financial code 001), for the partial financial support. The authors also thank the University of Florida, North Florida Research and Education Center (NFREC) for the laboratory analyses.

Conflicts of Interest: The authors declare no conflict of interest.

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