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Soil Dynamics in Carbon, Nitrogen, and Enzyme Activity Under Maize–Green Manure Cropping Sequences

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Abstract: The diversification of cropping sequences has a positive impact on soil organic carbon, while improving nutrient cycling and crop yields. The objective of this research was to assess amylase, cellulase, C and N dynamics, and maize yield on a low fertility oxisol in the Brazilian Cerrado. The experiment was conducted under field conditions during three maize crop succession cycles. The treatments consisted of cultivating maize during the summer, after sorghum and lablab cropped as green manure and fallow during the winter. Higher maize yields were achieved by sorghum–maize succession compared to monocropping, due to higher N fertilizer and biomass inputs to topsoil. Sorghum–maize succession also provided a higher proportion of stable C and N compared to other successions. Maize yields declined as tropical soil fertility intrinsically decreased along three crops succession cycles. Cellulase activity decreased over time, whereas amylase activity increased as the plant residues were already in advanced stages of decomposition. The sorghum–maize crop succession stood out compared to lablab and fallow as it provided the highest maize yields, while maintaining higher C and N levels, and amylase activity. This better performance was likely due to larger amounts of incorporated biomass and better mineral N fertilizer management.

Keywords: *Sorghum bicolor*; *Lablab purpureus*; amylase; cellulase; soil management

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1. Introduction

The integrated use of green manures and mineral fertilizers can improve nutrient use efficiency, due in part because of their combined impact on soil microbial dynamics [1]. Well-nourished crops, if provided with adequate amounts of nutrients throughout their development cycle, can be highly productive [1–3]. Thus, establishing a crop management system with diversified cultivation practices between main crops is crucial for improving the agronomic performance of the main crop, and mitigating the limitations of continuous cropping [4–6], particularly in tropical regions.

The positive effects of green manures on nutrient availability, crop growth, and yield are associated with improvements in the chemical and biological properties of the soil, such as greater C and N concentrations and biological diversity [7–9]. Cultivating other plant species in succession increases soil organic matter content and enhances nutrient cycling [10–12]. Such effects are positively correlated with soil fertility [13].

Soil microorganisms are essential for the sustainability of cropping systems, as they consume organic residues, stabilize organic carbon, and cycle soil nutrients [14–16]. The compounds and metabolites related to soil microbial activities have been widely used as bioindicators of soil quality [17,18]. Therefore, an efficient way of assessing the effects of crop succession on soil quality is measuring enzymatic activity, which is a fast and sensitive indicator of land-use change [19–21]. Soil enzyme activity has been suggested as an indicator of soil quality because enzymes are crucial for soil biochemical functioning [22]. Soil enzyme activity is related to several biogeochemical processes, such as carbon (C), nitrogen (N), and phosphorus (P) cycling [15,23–26]. Investigators can link soil enzyme activity to the chemical, physical [27,28], and biological [29] conditions in soil. Soil enzymes high sensitivity and quick response to changes in soil conditions make studying them fundamentally important for assessing the impact of land-use change on soil quality [20,25,27,28].

Soil enzyme and microbial activity have been investigated in long-term tillage and crop rotation systems in subtropical climates [11,30,31] and in transitions from forests and grasslands to crop production under tropical climates [29,32]. Amylase and cellulase are among the essential enzymes in soil as they promote decomposition of plant residues [33,34]. However, there is a lack of research focused on how amylase and cellulase activities are related to maintaining the quality of highly weathered, low fertility tropical soils, and how maize yields grown in sequence with green manures can be impacted by their activity. We hypothesize that in tropical regions with dry winter seasons, crop succession improves the quality of low-fertility soils and that enzyme activity correlates with this improvement and with crop yields. This study aimed to evaluate soil C and N levels, amylase and cellulase activities, and maize yields in a crop succession with sorghum, lablab, or fallow. The relationships among maize yield, enzyme activity, and C and N cycling in these crop successions were assessed over three succession cycles.

2. Materials and Methods

2.1. Experimental Area and Soil Characterization

Research was conducted at the School of Agricultural and Veterinary Studies of the São Paulo State University (FCAV–UNESP) in the municipality of Jaboticabal in São Paulo, Brazil (21°15′ S and 48° 19′ W) (Figure 1). The area is located within the Cerrado and has a humid-temperate climate, with dry winters and hot summers (Cwa climate type, Köppen [35]). Annual rainfall is approximately 1300 mm. January is the month with the highest rainfall, while July and August are the driest months. The mean annual temperature is 24.6 ± 5.2 °C (Figure 2).

Soil at the location was classified as a Hapludox [36]. It was sampled (0–0.20 m depth) for chemical analysis before starting the crop succession (one month before liming) and six months after lime application (Table 1) [37]. Soil physical analysis [38] indicated the following results: clay = 230 ± 11 g kg^{-1}, silt = 210 ± 13 g kg^{-1}, and sand = 650 ± 18 g kg^{-1}.

Table 1. Soil chemical attributes before starting the crop succession (one month before liming) and six months after lime application on whole experimental area (n = 3) [#].

Sampling Occasion	Resin-P mg dm^{-3}	OC g dm^{-3}	pH $CaCl_2$	K^+	Ca^{2+}	Mg^{2+}	$H^+ + Al^{3+}$	CEC	BS%
					---------- $mmol_c$ dm^{-3} ----------				- % -
One month before liming	21 ± 3	11.3 ± 1.5	4.6 ± 0.3	1.7 ± 0.2	10.3 ± 0.9	5.7 ± 0.3	47 ± 3	65 ± 5	27 ± 3
Six months after liming	31 ± 5	12.4 ± 1.6	5.4 ± 0.4	1.7 ± 0.3	32.0 ± 2,5	11.0 ± 0.5	28 ± 2	73 ± 6	62 ± 5

[#]—Soil chemical analysis for fertility (0–20 cm depth) as recommended to tropical soils [37]. Organic carbon (OC); total acidity ($H^+ + Al^{3+}$); cation exchange capacity (CEC); base saturation (BS%).

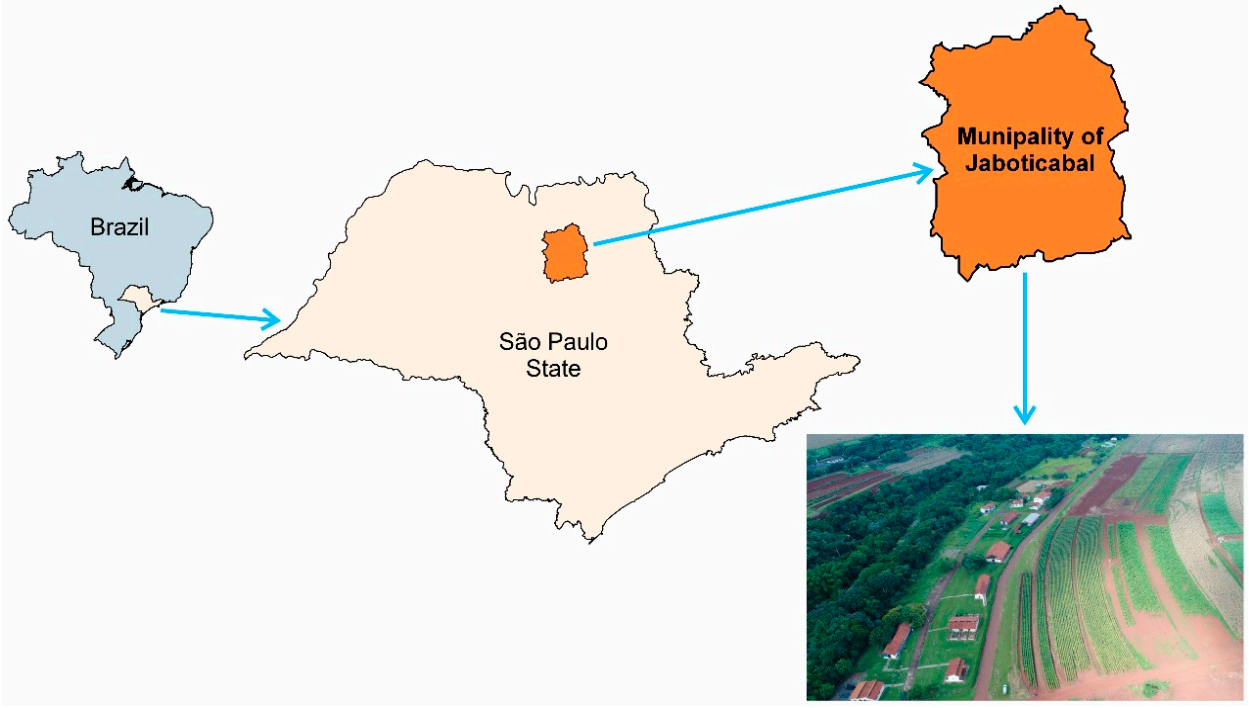

Figure 1. Location of experimental site (not in scale), in the State of São Paulo, Brazil.

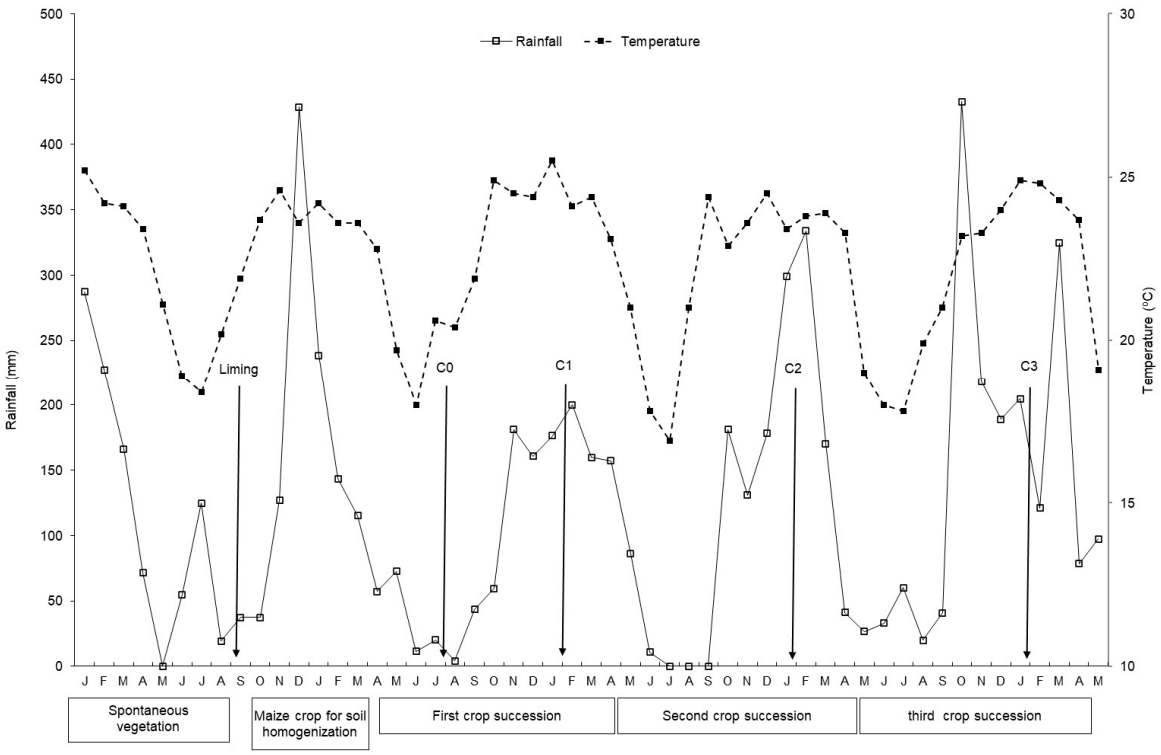

Figure 2. Average monthly rainfall and temperature throughout the experiment. C0: reference time for soil sampling during the period of leaf sampling of sorghum and lablab green manure at the first crop succession (4th month after first sorghum and lablab planting); C1, C2 and C3: time of soil sampling during the period of leaf sampling of maize plants among first, second and third cycles of crop succession (i.e., 10, 22, and 34 months after starting the management of crop rotation systems, with maize cultivation after green manures crops or fallow). The letters of X-axis are the initial of sequential months, starting with January (J) and ending with May (M).

2.2. Field Preparation

One year before establishing the experiment, existing vegetation in the field was incorporated into the soil (surface horizon) by harrowing. At this time, soil fertility was characterized (Table 1). In September, limestone (40% CaO, 10% MgO, 80% PRNT) was applied at 4.2 t ha^{-1} to the entire area to increase base saturation up to 70%. In October, maize was sown and cultivated in the experimental area to homogenize the field. Maize was harvested in March, and residues were then incorporated into the topsoil by plowing and two harrows (Figure 2) (Table 2).

Table 2. Summary of the activities developed in the experimental area before and after the experiment setup.

Management	Before the Experiment	First Green Manure Crop (C0)	First Maize Cycle (C1)	Second Maize Cycle (C2)	Third Maize Cycle (C3)
Weed incorporation	August		-	-	-
Soil preparation	August		-	-	-
Manure seeding	-	April		April	April
Manure harvest and dry matter incorporation	-	September		August	September
Maize seeding	October		November	November	November
Leaf diagnosis and soil sampling	-	July	January	January	January
Maize harvest and dry matter incorporation	March		March	April	April

2.3. Experiment Setup and Execution

At the beginning of April of the first year, soon after maize harvest, the experimental plots (18 plots of 5.4 × 10 m) were demarcated, and sorghum and lablab were planted. Harvesting and incorporation of the residues by harrowing occurred in September, which included weeds in fallow plots. In October, maize was sown and cultivated in each respective plot, performing the first succession cycle. The sorghum and lablab rows were 0.60 m apart, while maize rows were 0.90 m apart. Three succession cycles were performed during the experiment (Figure 2) (Table 2).

The experimental plots consisted of the cultivation of sorghum, lablab, and the control (fallow) treatments during the winter (April to September), followed by cultivation of maize during the summer (October to March), with six replicates for each treatment (crop successions). The subplots (subtreatments) consisted of four soil sampling periods, represented by one at an initial reference time (C0), at leaf sampling during the first cultivation of sorghum and lablab, followed by three more sampling times, at leaf sampling of maize culture in the 1st (C1), 2nd (C2), and 3rd (C3) cycles (Figure 2) (Table 2).

Based on the results of the soil analysis [39], we applied 230 kg ha^{-1} of 15-30-20 to the first maize crop (C1), and 300 kg ha^{-1} and 230 kg ha^{-1} 4-30-16 fertilizer to the second (C2) and third (C3) maize crops, respectively. These rates were applied at planting. In addition, we applied N twice (i.e., 35 and 60 days after emergence at a total rate of 60 kg ha^{-1}, as ammonium sulphate) in the three maize crops. Lablab, a legume that engages in biological nitrogen fixation, was not fertilized, but sorghum was top-dressed with 70 kg ha^{-1} of N, as ammonium sulphate, in an equal split application 40 and 50 days after emergence, in the three succession cycles.

2.4. Estimated Maize Crop Productivity

Crop productivity was estimated by harvesting two central lines (10 m long), excluding three meters from each end. Grain yields were reported at 13% moisture.

2.5. Soil Sampling

Soil samples were collected at 0–0.2 m depth. In each plot, 20 subsamples were taken on the lines and between lines. Samples were collected during the period of leaf sampling of sorghum and lablab, as a reference time sample (C0, 4th month after first sorghum and lablab sowing) and during the period of leaf sampling of maize crops in the 1st (C1), 2nd (C2), and 3rd (C3) cycle of cultivation (i.e., 12, 24, and 36 months after the management systems with green manure crops or fallow started) (Figure 2) (Table 2). Shortly after sampling, each soil sample was sieved (2 mm mesh), air-dried in the shade, and subsequently stored in sterilized plastic bags under refrigeration until the chemical and enzyme analyses.

2.6. Chemical and Enzymatic Analysis

To evaluate the chemical properties of the soil, organic carbon (OC) was determined by the wet digestion method [40], while total N (TN) and mineral N ($N\text{-}NH_4^+$ and $N\text{-}NO_3^-$) were determined by the Kjeldahl method [40]. With C and N results, the C/N ratio was calculated. The fractionation of soil organic matter (SOM) [41] was performed, and then the C and N in the humic material (MH) and humin (Hum) were determined [42].

Total carbohydrates (CT) were extracted by soil hydrolyses using 1.5 mol L^{-1} H_2SO_4 in a water bath set at 80 °C for 24 h. Soluble carbohydrates (SC) in soil were extracted with 2 mol L^{-1} KCl. Released carbohydrates were determined by the antrone method [43].

The amylase activity in the soil was evaluated using an air-dried soil sample and a starch solution as substrate [33]. The cellulase activity in the soil samples were evaluated using carboxymethylcellulose as substrate [34]. The reducing sugar released in both methods was determined by the Nelson-Somogyi method described by [44]. A detailed description of enzyme analyses and of calculations are shown in Table S1.

2.7. Statistical Analysis

The experiment was set up in a split plot in time design. The main plots, consisted of the maize-sorghum, maize-lablab and maize-fallow crop successions, were set up in a randomized blocks, with six replicates. Data analyses were done considering the three years of crop succession cycles as subplot factor (Figure 2) (Table 2). The data obtained were subjected to analysis of variance (ANOVA) at a 5% probability level (F test), with the mean values being compared by the Tukey test, considering the same probability level. The graphics were generated using of SigmaPlot software (Version 12.0). Cluster multivariate analysis was performed by SAS software (Version 9.3). Cluster analysis organizes variables/treatments into groups (called "clusters") based on how closely associated they are. Outcomes from clustering consist of showing similarity or differences between each pair of treatments. The goal is to partition them into homogeneous groups, meaning that the within-group similarities are larger compared to the between-group similarities.

3. Results

3.1. Maize Grain Yields Under Green Manure, Fallow and Maize Crop Succession Cycles

Maize yields varied as a function of green manure and succession cycles, but there was no interaction between those factors (Table 3). The cultivation of maize after sorghum resulted in the highest yield (8713 kg ha^{-1}), followed by the succession with lablab (8480 kg ha^{-1}), which was statistically equal to the fallow system (7732 kg ha^{-1}). Along crop cycles, maize yields declined from 8665 to 7732 kg ha^{-1}.

Table 3. Maize grain yield, organic C (OC), carbon in humin (C-Hum), and soil nitrate ($N\text{-}NO_3^-$) C/N ratio in different crop succession and succession cycles.

Treatments	Yield ---- kg ha^{-1} ----	OC ---------- g kg^{-1} ----------	C-Hum	$N\text{-}NO_3^-$ ---- mg kg^{-1} ----	C/N Ratio -
Green manure/fallow and maize crop succession					
Sorghum	8713 ± 1004 A	13.0 ± 0.99 A	10.7 ± 1.04 A	7.74 ± 9.17 A	12.0 ± 1.59 A
Lablab	8480 ± 1036 AB	12.5 ± 0.89 A	10.1 ± 0.92 A	7.76 ± 8.08 A	11.6 ± 1.67 A
Fallow	7732 ± 1003 B	12.3 ± 1.08 A	9.9 ± 1.03 A	7.22 ± 6.75 A	12.0 ± 1.75 A
Succession cycle					
C0	-	12.6 ± 0.94 ab	10.3 ± 0.86 a	1.4 ± 1.0 c	11.3 ± 1.18 b
C1	8665 ± 650 a	13.0 ± 0.66 a	10.4 ± 0.80 a	5.8 ± 3.3 b	12.7 ± 0.54 a
C2	8511 ± 923 ab	12.8 ± 1.44 a	10.5 ± 1.58 a	3.1 ± 1.9 bc	13.4 ± 1.36 a
C3	7732 ± 446 b	11.9 ± 0.50 b	9.8 ± 0.50 a	20.0 ± 4.7 a	10.0 ± 0.86 c

C0 (reference time), C1 (first cycle), C2 (second cycle), and C3 (third cycle). OC—organic carbon. Means followed by the same letter, uppercase for green manures and lowercase for succession cycles, are not statistically different at 0.05 probability by the Tukey test.

3.2. Enzyme Activity in Soil Under Green Manure, Fallow and Maize Crop Succession Cycles

Amylase and cellulase activities were significantly affected by the interaction between green manures and crop sequences (Figure 3A,B). Amylase activity showed higher values in the system with sorghum compared to fallow, at C0 and compared to lablab at C1, respectively. The system cropped with lablab showed higher cellulase activity at C0 and at C2. At C1, higher cellulose activity was observed for sorghum cultivation, compared to the system with fallow, but it was similar to lablab. Amylase and cellulase activities declined from C0 to C2. Amylase activity was the highest at C3, while cellulase activity at C3 and C1 were similar.

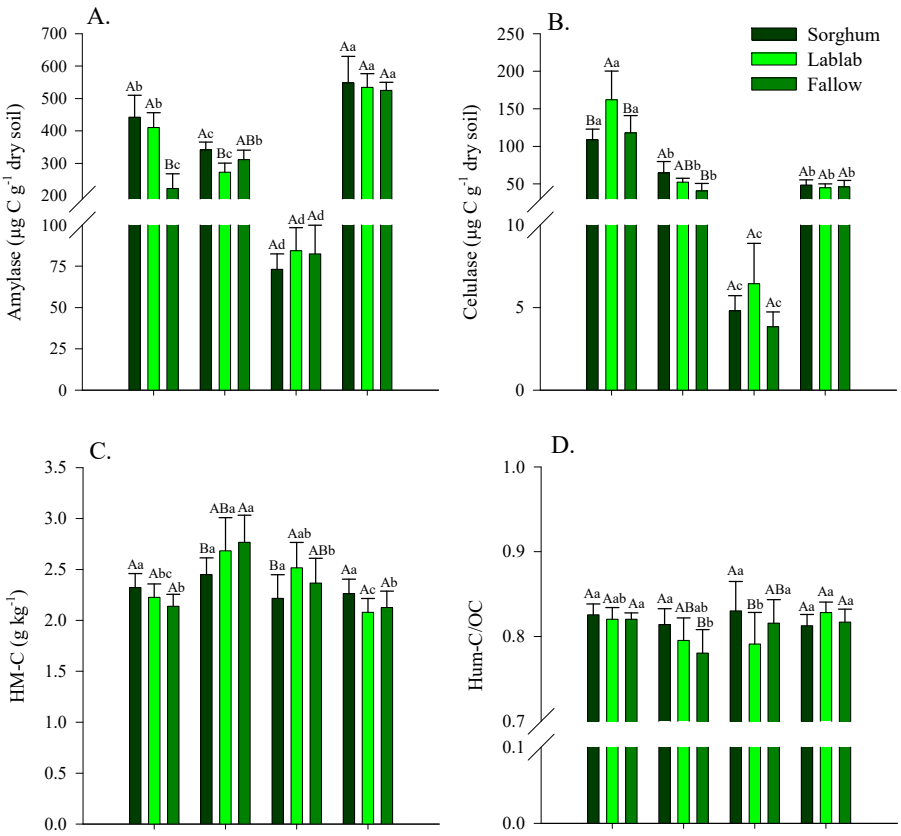

Figure 3. *Cont.*

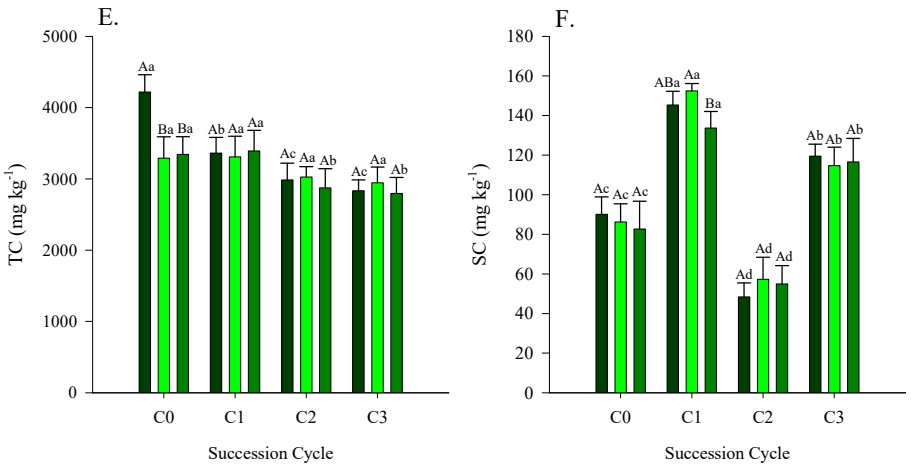

Figure 3. Enzymatic activity of amylase (**A**), cellulase (**B**), carbon in humic matter (CMH) (**C**), C-Hum/OC ratio (**D**) total (TC) (**E**), and soluble (SC) carbohydrates (**F**) in soil under maize crop in succession of green manures and fallow biomass being incorporated into the topsoil, during three crop succession cycles. C0 (reference time), C1 (first cycle), C2 (second cycle), and C3 (third cycle). Distinct uppercase and lowercase letters indicate significant differences ($p < 0.05$) within green manures and succession cycles, respectively.

3.3. Carbon and Nitrogen in Soil Under Green Manure, Fallow and Maize Crop Succession Cycles

Organic C and N-NO_3^- content as well as C/N ratio of the soil varied as a function of succession cycles, while C-Hum was not affected by any factors (Table 3). Higher OC content, varying from 12.6 to 13.0 g kg^{-1}, was observed at C0 to C2, with a slightly lower value of 11.9 g kg^{-1} at C3. The opposite was observed for N-NO_3^-, with lower content (i.e., 1.4 to 5.8 mg kg^{-1}) at C0 to C2, and a higher value of 20.0 mg kg^{-1} at C3. Higher C/N ratios varying from 12.7 to 13.4 were only observed at C1 and C2, with a lower value of 10.0 at C3.

The treatment with sorghum had a lower C concentration in humic matter (CHM) compared to fallow at C1 and lablab at C2, respectively (Figure 3C). When comparing the time within the green manures, no differences were observed for CHM in the system cultivated with sorghum. However, in the presence of lablab, CHM was higher at C1 and C2 than C3. For the fallow treatment, CHM was higher at C1 than C0, C2 and C3 (Figure 3C). In contrast to CHM observations, the treatment with sorghum showed a higher C-Hum/OC ratio than fallow at C1 and lablab at C2, respectively (Figure 3D). When comparing the C-Hum/OC ratio in time within green manures, no differences were observed for sorghum–maize succession. However, in the presence of lablab, the C-Hum/OC ratio was lower at C2 relative to C0, C1 and C3. While for the fallow treatment, the C-Hum/OC ratio was lower at C1 than C0, C2 and C3 (Figure 3D).

Total (TC) and soluble (SC) carbohydrate content showed a significant interaction between factors (Figure 3E,F). Higher TC content was observed in the treatment cultivated with sorghum at C0, with no additional differences among succession cycles. However, among succession crops, there was a decline in TC in systems that included sorghum and fallow, while no differences were observed for lablab. The SC content differed between green manure treatments only at C1 (Figure 3F), when the SC released in the treatments with sorghum and lablab were higher (around 148 mg kg^{-1}) than the fallow (129 mg kg^{-1}). When comparing succession cycles within all treatments, highest SC occurred at C1 and the lowest SC at C2, while intermediate contents were observed at C0 and C3 (Figure 3F).

Total N, N in humic matter (NHM), N in humin (N-Hum) and N-Hum/NT ratio were affected by the interaction of green manures and succession cycles (Figure 4A–D). Regarding green manures and crop succession, there was only a significant effect for higher NT, N-Hum and NHM content in treatments cultivated with sorghum and lablab compared to fallow at C0 (Figure 4A–C). There was an exception for N-Hum at C1, as sorghum had higher content than fallow (Figure 4C). For the succession cycles, higher NT and N-Hum

content were observed at C0, for both sorghum and lablab. For all treatments, NT and N-Hum content were lowered from C0 to C2; while high content, like those observed at C0 for sorghum and lablab, were observed at C3 for all treatments, even for fallow (Figure 4A,C). NHM content increased from C0 to C3 by 17% for lablab and 43% for fallow, respectively (Figure 4B). The N-Hum/NT ratio for sorghum–maize crop succession was lower at C0 and higher at C1 relative to the fallow treatment, but it did not differ from lablab (Figure 4D). Among succession cycles, for all treatments, the N-Hum/NT ratio was similar to NT and N-Hum content, except for fallow at C0 (Figure 4D).

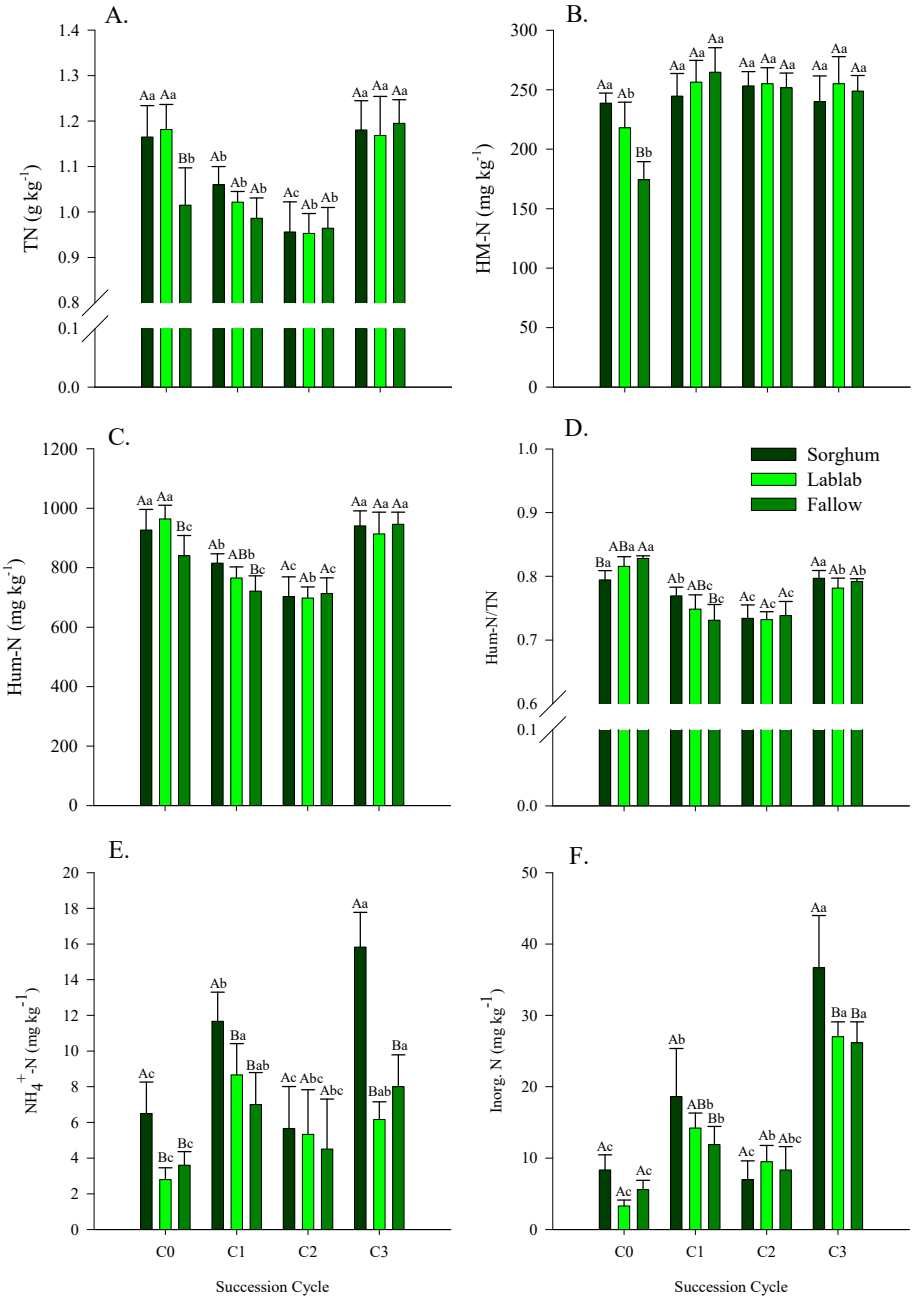

Figure 4. Content of total N (TN) (**A**), N in humic matter (NMH) (**B**), N in humin (N-Hum) (**C**), N-Hum/NT ratio (**D**), ammoniacal N (N-NH^{4+}) (**E**), and inorganic N (inorg N) (**F**) in soil under maize succession systems with green manures and fallow biomass being incorporated into the topsoil, during three crop succession cycles. C0 (reference time), C1 (first cycle), C2 (second cycle), and C3 (third cycle). Distinct uppercase and lowercase letters indicate significant differences ($p < 0.05$) within green manurers and succession cycles, respectively.

There were higher N-NH_4^+ and inorganic N (inorg N)values found in the sorghum green manure-maize succession, than in other treatments, at C0, C1 and C3 (Figure 4E,F). Regarding the variation among succession cycles, from C0 to C3, the N-NH_4^+ content increased by 2.4, 2.2, and 2.2-fold, respectively (Figure 4E). The inorg N content increased by 4.4, 8.2, and 2.9-fold in the sorghum–maize, lablab–maize, and fallow–maize succession, respectively (Figure 4F).

3.4. Similarities of Crop Succession Systems Within Succession Cycles

The cluster analysis (Figure 5) revealed: (1) an arrangement of variables by crop succession associated with the respective succession cycles; (2) similarities among variables and treatments within the soil sampled at reference time 0, which corresponded to the (a) period of leaf sampling of sorghum and lablab at the first succession crop, (b) 4th month after maize biomass was incorporated into the topsoil and green manures were planted for the first time (Figure 2), (c) soil sampled at 34 months after starting the management of crop succession systems, with maize cultivation after green manures crops or fallow. At reference time 3, the period of leaf sampling of maize plants among the third cycle of crop succession (Figure 2), similarities were observed among variables and treatments within the soil sampled at 10 and 22 months after starting the management of crop succession systems. At times 1 and 2, during the periods of leaf sampling of maize plants among first and second cycles of crop succession occurred (Figure 2), and; (3) a remarkable similarity between fallow–maize and lablab–maize crop succession occurred for soil sampled at reference time, 10 and 34 months after the management systems with green manure crops or fallow started, demonstrating a dissimilarity with the sorghum–maize crop succession. However, similarity between sorghum and fallow crop succession and dissimilarity with lablab–maize crop succession was observed for the second succession cycle (i.e., soil sampled at 22 months after the management systems with green manure crops or fallow started) (Figure 2).

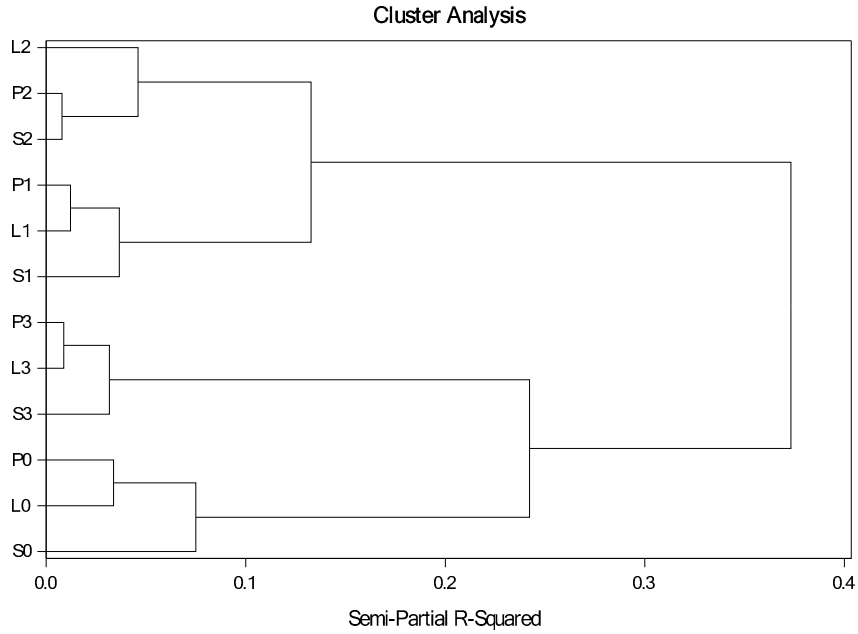

Figure 5. Dendrogram of cluster analysis constructed by Ward's method. S—Sorghum–maize crop succession; L—Lablab–maize crop succession; P—No cultivation (fallow–maize crop succession); 0—Reference time (soil sampling at leaf sampling during the first cultivation of sorghum and lablab), 1, 2, and 3—1st, 2nd, and 3rd cycles of succession with maize crop (i.e., soil sampling at maize leaf sampling 10, 22, and 34 months after the management systems with green manure crops or fallow started), respectively.

4. Discussion

4.1. Maize Grain Yield Under Green Manure, Fallow and Maize Crop Succession Cycles

The higher yield of maize in succession with sorghum, in relation to fallow, was due to the large amount of sorghum biomass (6761 kg ha^{-1}, dry base, annual average of three crop succession cycles) (Table S2) produced and incorporated into the top 20 cm of soil. Sorghum was also fertilized with ammonium sulphate at 70 kg ha^{-1} of N and 77 kg ha^{-1} of S annually, which likely contributed to higher yields. The maize yield of lablab–maize succession, with annual biomass of 2082 kg ha^{-1} (average of 3 crop rotation cycles) (Table S2) being incorporated into the topsoil, did not differ from either sorghum/maize crop succession or fallow, which had annual weed biomass production of 1346 kg ha^{-1} (average of 3 crop rotation cycles) (Table S2) being incorporated. The decrease in maize yields among crop succession cycles (Table 3) was a consequence of the decline in soil fertility, related to the OC (Table 3) and pH, which was lowered from 5.8 to 5.0. Total acidity increased from 21 to 35 $mmol_c$ dm^{-3}n. This was likely due to residual acidity caused by fertilization [37,39]. The export of cations from harvested maize grain, in our study, likely reduced base saturation and increased acidity of the tropical soil [45].

Our results agree with previous findings [46], which reported similar maize yields (from 8427 to 12,770 kg ha^{-1}, average of one crop rotation cycle under no tillage) in succession with fallow, maize, sorghum, crotalaria, or pearl millet. Additionally, maize yields exceeding 14,000 kg ha^{-1} were achieved in that study for one crop rotation cycle, under no tillage, in succession with *Urochloa ruziziensis* or *Raphanus sativus*, due to the long-term presence of straw on the soil surface. These results suggest there was some limiting factor beyond soil fertility restricting maize production potential when comparing crop rotation succession under tillage and no tillage.

4.2. Enzyme Activity in Soil in Soil Under Green Manure, Fallow and Maize Crop Succession Cycles

Enzyme activity is an indicator of the availability of resources in the soil [25,47–49]. At C0, soil amylase activity was higher for both successions with sorghum and lablab (Figure 3A) compared to fallow. As previously reported [29,47], this was due to the interaction among the soil rhizosphere and soil biota adaptation to obtain C, N, P and S from fresh decomposing maize residues (C source). At C1, soil amylase activity was higher for successions with sorghum and fallow. Lablab biomass (2082 $\pm$ 735 kg ha^{-1}, dry base) (Table S2) was incorporated and quickly decomposed, while decreasing enzyme activity [50,51]. At C2 and C3, there was a stabilization among soil maize rhizosphere and soil biota for C, N, P and S acquisition and no differences among treatments were observed (Figure 3A).

Soil cellulase activity was significantly affected by crop succession at C0, when lablab had higher enzyme activity than sorghum and fallow (Figure 3B). As root exudates of plants induced soil bacteria to produce higher cellulase activity [52], there may have been an interaction between N-fixing bacteria in symbiosis with lablab roots increasing cellulase activity for C and nutrient acquisition for nodule formation. Maize roots growing in all treatments, at C1, C2 and C3, interacted less with soil bacteria to induce significant differences in enzyme activity among treatments.

Soil enzyme activity is directly related to the decomposition rate of plant residues, which is an excellent indicator of tillage and green manures on soil quality [3,10,20,29]. This fact was seen in our study when the high rate of residue degradation contributed to decreased activities of amylase and cellulase in the soil after plowing and harrowing the topsoil from C0 up to C2, for all treatments (Figure 3A,B). As reported previously [11], a similar finding was observed for amylase and cellulase activities in soil cultivated under rotation systems with and without soil tillage. For cropping systems in which green manures are incorporated, enzyme hydrolysis is lower than in systems without incorporation [53]. This occurs because of the fragmentation of crop residues, into small particles, which reduces the need for large amounts of enzymes to break down organic residues, while

decomposition and nutrient cycling increases [50,51,53]. However, at C2, both enzymes showed low activities (Figure 3A,B), which could have been due to a dry summer, at C1, followed by a longer dry and cold winter, at C2 (Figure 2). Consequently, lower rates of crop residues decomposed, and enzyme activity was reduced due to the water limitations and low temperatures [54]. Conversely, at C3, an unusual rainy winter, at the beginning of this crop rotation cycle (Figure 2), resulted in the highest amylase activity (Figure 3A) and cellulase activity similar to C1 (Figure 3B). Such events agree with the similarities found in crop succession systems within succession cycles shown by cluster analysis (Figure 5).

4.3. Carbon and Nitrogen in Soil Under Green Manure, Fallow and Maize Crop Succession Cycles

Organic C and C-Hum content, and C/N ratio in the topsoil were not affected by the management of crop rotation systems, even with the larger amounts of sorghum biomass (Table S2) being incorporated into the soil. A previous experiment assessing the effects of the application of organic compost at 60 t ha^{-1} on OC of 26 tropical soils showed there was OC accumulation in soils with initial OC contents higher than 12 g dm^{-3} and TN higher than 1.3 g dm^{-3} [55]. So, for sorghum–maize succession, at C0, the relatively low soil NT content (Figure 4B) limited soil biota growth, and OC stabilization, while C was temporally stored as TC (Figure 3E).

In succession cycles, a slight decrease of 5.2% in OC occurred from C1 and C2 to C3 (Table 3), despite the similarities in OC between C0 and C3. Indeed, the concentration of OC among succession cycles (Table 3) still varied and was slightly higher than the soil OC content observed before the experiment setup (Table 1). This may have occurred due to the greater amount of maize biomass being incorporated into the topsoil in the three crop succession cycles (Table S2). However, the decomposition of organic materials, as a source of readily available C, in the cultivated and turned topsoil as well as the increase in microbial activity [55,56] did not impact new soil C stabilization including the mineralization of native SOM. In fact, OC at C3 (11.9 mg dm^{-3}) (Table 3), 34 months after crop succession cycles started was quite similar to the OC present in topsoil before the experiment was setup (average of 11.8 mg dm^{-3}) (Table 1). The content of C-Hum, the more stable fraction of SOM, was also not affected by the succession cycles (Table 3).

Comparing crop succession, there was a lower CMH content in sorghum–maize crop succession associated with the nitrogen fertilizer applied during the sorghum crop, especially at C1 and C2 (Figure 3C). The added N increased microbial activity and decreased CMH [56,57]. The higher CHM for lablab and fallow treatments on C1 and for lablab on C2 (Figure 3C) was due to the lower fresh biomass of lablab and weeds (Table S2) incorporated into topsoil, which quickly decomposed. Residual fresh C in biomass did not increase microbial activity to obtain C [56,57]. Among succession cycles, CMH content was affected by sorghum–maize crop succession (Figure 3C), due to better N fertilizer management than lablab and fallow treatments.

In the 34 months of management of crop succession systems with maize following green manures or fallow, there was no effect of crop succession or succession cycles on C content in C-Hum (Table 3). As C-Hum represents a very stable fraction of SOM, time and crop management were not enough to affect this fraction [57–60]. Similar results have been reported previously [29,56]. The C-hum/OC ratio (Figure 3D) was affected by the interaction between green manures and succession cycles. This relationship demonstrates the recalcitrance and stability of organic matter due to the lower formation of carboxylic groups that provide strong bonds between C atoms and makes them more stable, increasing their permanence in the soil [58–61]. On that basis, for succession crops, it can be stated that sorghum–maize succession, with sorghum crop receiving adequate N inputs, provided a higher proportion of stable C to the topsoil than the lablab–maize and fallow–maize crop successions, with low N inputs, especially at C1 and C2 (Figure 3D). Following the same pattern of CMH, the C-hum/OC ratio was affected by sorghum–maize crop succession among succession cycles (Figure 3C), due to better N fertilizer management than lablab and fallow treatments.

TC for the cropping systems was higher for sorghum–maize succession only at C0 (Figure 3E). At this occasion, for soil sampling 4 months after maize biomass was incorporated (Figure 2), there was a higher interaction among the sorghum rhizosphere, N application, and soil biota adaptation to obtain C, N, P and S from fresh decomposing maize residues (C source) [48], resulting in higher TC in topsoil than in the lablab and weed rhizosphere. After that, among C1, C2 and C3, it is clear that interactions among the maize rhizosphere, fertilizer application, and soil biota activity to obtain C, N, P and S from fresh decomposing residues of sorghum, lablab and weed (fallow) was not different, thus resulting in similar TC content in topsoil for all crop succession systems (Figure 3E).

Among the succession cycles, the greater reduction in soil TC content (−33%) in the sorghum–maize crop system (Figure 3E) resulted from N fertilizer applied to sorghum and an increase in the decomposition rate of buried plant residues (C source), as higher sorghum biomass was incorporated into topsoil (Table S2) and higher soil inorganic N was available (Figure 4E,F). TC decreased by 19% in the fallow–maize crop system, from C0 to C3 (Figure 3E), as low weed biomass incorporated into the topsoil (Table S2), with high C/N ratio, quickly decomposed to supply C soon after maize was fertilized with N. TC in the lablab–maize system was not affected by succession cycles (Figure 3E), as low lablab biomass incorporated into the topsoil (Table S2), with narrow C/N ratio, was not effective in changing soil TC at all even after maize was fertilized with N.

For crop successions, CS was higher for sorghum–maize and lablab–maize successions (average of 148 mg kg^{-1}) than fallow (129 mg kg^{-1}) only in C1 (Figure 3F), for soil sampling 4 month after the first incorporation of sorghum, lablab and weed biomass (Table S2) (Figure 2). This indicated a soil biota adaptation to the amount and quality of the incorporated new fresh biomass [9], leading to a higher release of SC, respectively, by sorghum and lablab than weed biomass. However, when comparing succession cycles, CS concentrations were higher at C1 > C3 > C0 > C2 for all succession crops (Figure 3F), which shows an inverse correlation with rainfall among 30 days before soil sampling (Figure 1). Except for C0, CS should be associated with the first contribution of fresh maize biomass (Table S2) incorporated into the topsoil.

Total N (Figure 4A), NHM, (Figure 4B), and N-hum (Figure 3C) in the topsoil demonstrated similar behavior for crop succession showing higher contents of NT, NHM and N-hum than fallow at C0. As with amylase activity (Figure 3A), these results were due to the interaction among soil rhizosphere and soil biota adaptation to obtain C and N from fresh decomposing maize residues (C source); and for fallow, soil biota utilized more soil N. From C1 to C3, there was a stabilization between the soil maize rhizosphere and soil biota for C and N acquisition. No differences among treatments were observed (Figure 4A,B). This also explains the lower N-Hum/NT ratio for sorghum–maize crop system at C0 (Figure 4D). There was an exception for N-hum and N-Hum/NT at C1, when sorghum–maize had higher content (Figure 4C) and ratio (Figure 4D) than fallow treatment, probably due the higher quantity of incorporated sorghum biomass (Table S2) and residual effects of N fertilizer applied during the first sorghum cultivation.

When comparing succession cycles, TN and N-hum decreased from C0 to C2 in the topsoil for sorghum–maize and lablab–maize crop systems, as soil biota required more soil N than the amount supplied by fertilization and by incorporated corn residue mineralization (Figure 4C). High N immobilization of fresh four-month-old maize biomass occurred in topsoil at C0 (Figure 2) (Table S2). There was no difference for the fallow treatment from C0 to C2, as corn N fertilization was sufficient to supply N required by soil biota. However, from C2 to C3, there was an increase in TN (Figure 4A) and N-hum (Figure 4C) for all crop successions. This occurred because at C3, the succession cycle with the highest rainfall intensity (Figure 2), might have had higher mineralization due to a possible higher accumulation of plant residues among C2, the succession cycle with the lowest rainfall and coldest winter (Figure 2). Thus, there was an increase in TN (Figure 4A) and N-hum (Figure 4C) with concentrations like those at C0, except for fallow which showed higher contents than those at C0, C1 and C2. The same pattern was observed

among succession cycles for N-Hum/NT ratio, for all treatments (Figure 4D), for the reasons explained above. However, NHM (Figure 4B) showed an opposite pattern among succession cycles within treatment; there was no effect among cycles for sorghum–maize and lablab–maize crop systems. While for fallow, there was a lower NHM content at C0 than C1, C2 and C3. This clearly shows that N usage by soil biota is dependent on soil tillage [48,62–64], water availability, and temperature to active soil biota during the winter following maize biomass incorporation.

Soil N-NH_4^+ (Figure 4E) and inorg N (Figure 4F) were similar for crop succession cycles. The sorghum–maize system had higher N-NH_4^+ and inorg N than lablab and fallows treatments at C0, C1 and C3 (Figure 4E,F), due to the higher application of ammonium sulphate to provide N for both maize and sorghum crops. This may have also been partially due to N mineralization from higher sorghum–maize biomass incorporated into the topsoil (Table S2). At C2, there were no differences detected, probably due to the low soil water conditions prior to soil sampling (Figure 2), which minimized fertilizer dissolution and decomposition of buried plant residues. For all crop succession, N-NH_4^+ and inorg N followed the following pattern: 0 < C2 < C1 < C3 (Figure 4E,F). The higher content of inorg N at C3, which was the succession cycle with the highest rainfall intensity (Figure 2), was caused by higher mineralization due to a possible higher accumulation of plant residues at C2, (Figure 2). Similar results have been reported previously [61]. Sorghum–maize crop succession had the greatest influence on the release of N-NH_4^+ and inorg N into the topsoil among succession cycles due to the higher application of N fertilizer for both maize and sorghum crops. This also may have been due to the N mineralization from the higher amounts of sorghum–maize biomass annually incorporated into topsoil annually (Table S2), except at C2, in which low rainfall prior soil sampling (Figure 2) caused lower fertilizer dissolution and decomposition of incorporated plant residues.

An increase of 13.8-fold was verified in the N-NO_3^- content in the topsoil, from C0 to C3 succession cycles (Table 3). As for inorg N, the higher N-NO_3^- at C3 was caused by a higher mineralization due to a possible higher accumulation of maize residues at C2, which was the succession cycle with the lowest rainfall and coldest winter (Figure 2). Therefore, the increase in nitrate, at C3, was due to N mineralization from buried maize residues from the previous succession cycles, following rapid conversion of ammonia into nitrate [65].

4.4. Performance of Cycles of Crop Succession Systems for Maize Yield

The best performance of the maize-sorghum crop succession for maize yield (Table 3) was validated by the cluster analysis (Figure 5), which revealed a difference between sorghum–maize, lablab–maize, and fallow–maize successions at reference time, 10 and 34 months after the management systems began. The results for all crop succession systems among succession cycles showed that under tillage, there was an increase in N mineralization from buried plant residues. Specifically, there was high NT, N-Hum and N-inorg at C3 (Figure 4A,C,F). For sorghum treatments, there was less immobilization which was evidenced by the stabilization of the C/N ratio [62,66] over 34 months of succession (Table 3). These results for crop succession systems among succession cycles agree with results from the cluster analysis (Figure 5). However, the best performance of the maize-sorghum sequence for maize yield was due to the best management of mineral N fertilizer for both maize and sorghum growth, nutrition and yield, consequently with larger amounts of incorporated biomass. Nevertheless, our results, as discussed previously, indicated there were some limiting factors beyond soil fertility restricting maize yield potential when comparing crop rotation succession under tillage and no tillage, thus further studies should be conducted under no tillage to unravel such limiting factor and improve benefits to producer, society and soil healthy.

5. Conclusions

Organic carbon, carbon in humic matter, and total and soluble carbohydrates varied over succession cycles as function of the decomposition of incorporated plant residues.

Humin carbon, the most stable form of soil carbon, remained unchanged and demonstrated high resilience even in tilled tropical soils. Soil nitrogen forms, particularly inorganic nitrogen, increased across succession cycles, driven by the rapid breakdown and decomposition of buried plant residues by soil tillage and nitrogen fertilizer application, especially in the sorghum–maize crop succession. While cellulase activity decreased over time, amylase activity increased.

The maize-sorghum succession, utilizing sorghum as a green manure, outperformed lablab and fallow treatments, achieving the highest maize yields, soil C and N levels, and amylase activity. This superior performance resulted from enhanced mineral N fertilizer management and greater incorporation of sorghum and maize residues into the topsoil.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/soilsystems8040115/s1, Table S1: Description of amylase [33] and cellulase [34] evaluation in soil samples and calculation of their activities following the reducing sugar released determination, in both methods, using the colorimetric method of Nelson-Somogyi as described by Oser [44]; Table S2: Average maize, sorghum, lablab and weed dry biomass, excluding grain yield, produced among the three crop succession cycles.

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