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# Soil Dynamics in Carbon, Nitrogen, and Soil Enzymatic Activity Under Maize-Green Manure Cropping Sequences

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**Abstract:** 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 and cellulase activity, 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. 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

## 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 decisive for improving the agronomic performance of the main crop, and mitigating the limitations of continuous cropping [4–6], especially in tropical environments.

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

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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 correlated with soil health [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 improving 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 enzymatic 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 has 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 the 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 climates characterized by dry winter seasons, the succession of crops improves the quality of low fertility soils and that the enzymatic activity is related to this improvement and to crop yields. The objective of this research was to evaluate soil C and N levels, amylase and cellulase activity, and maize yields in a crop succession with sorghum, lablab, or fallow. The relationships among maize yield, enzymatic 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 (21º 15′ S and 48º 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 1,300 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).


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

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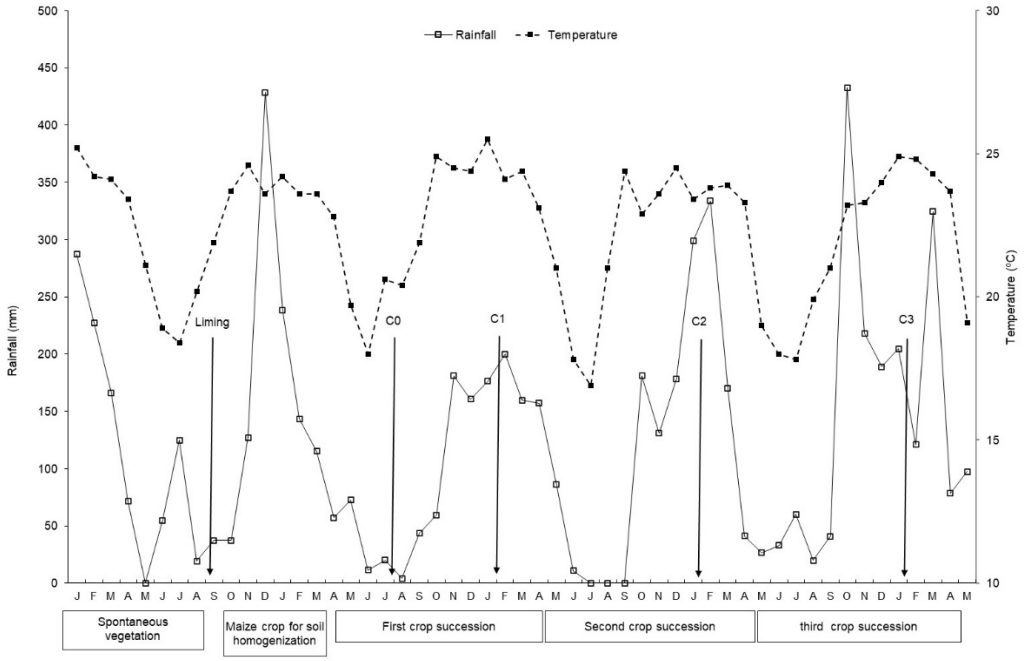


**Figure 2.** Average monthly rainfall and temperature throughout the experiment. C0: reference time for soil sampling during the period of leaf sampling of sorghum and lablab green manure at the first crop succession (4th month after first sorghum and lablab planting); C1, C2 and C3: time of soil sampling 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).

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 | OC | pH | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ | $H^+ + Al^{3+}$ | CEC | V% |
|---|---|---|---|---|---|---|---|---|---|
| | mg $dm^{-3}$ | g $dm^{-3}$ | $CaCl_2$ | ---------- 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 (V%).

*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).

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**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 cropped in entire area was harvested, the experimental plots (18 plots of 5.4 x 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. 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 analyses were conducted.

*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-$NH_4^+$ and N-$NO_3^-$) were determined by

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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 experimental design consisted of randomized blocks with six replicates, performed by the three crop succession (treatments). Data analyses were done with a split plot in time design for crop succession cycles (subtreatments) (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 with the aid of SigmaPlot software. Cluster multivariate analysis was performed by SAS software. 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 (8,713 kg $ha^{-1}$), followed by the succession with lablab (8,480 kg $ha^{-1}$), which was statistically equal to the fallow system (7,732 kg $ha^{-1}$). Between crop cycles, maize yields declined from 8,665 to 7,732 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 | OC | C-Hum | $N\text{-}NO_3^-$ | C/N ratio |
|---|---|---|---|---|---|
| | ---- kg $ha^{-1}$ ---- | ---------- g $kg^{-1}$ ---------- | | ---- mg $kg^{-1}$ ---- | - |
| **Green manure/fallow and maize crop succession** | | | | | |
| Sorghum | 8,713±1,004 A | 13.0±0.99 A | 10.7±1.04 A | 7.74±9.17 A | 12.0±1.59 A |
| Lablab | 8,480±1,036 AB | 12.5±0.89 A | 10.1±0.92 A | 7.76±8.08 A | 11.6±1.67 A |
| Fallow | 7,732±1,003 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 | 8,665±650 a | 13.0±0.66 a | 10.4±0.80 a | 5.8±3.3 b | 12.7±0.54 a |
| C2 | 8,511±923 ab | 12.8±1.44 a | 10.5±1.58 a | 3.1±1.9 bc | 13.4±1.36 a |
| C3 | 7,732±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.

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

Amylase and cellulase activity 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 not different from lablab. Amylase and cellulase activity declined from C0 to C2. Amylase activity was the highest at C3, while cellulase activity at C3 and C1 were similar.

*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).

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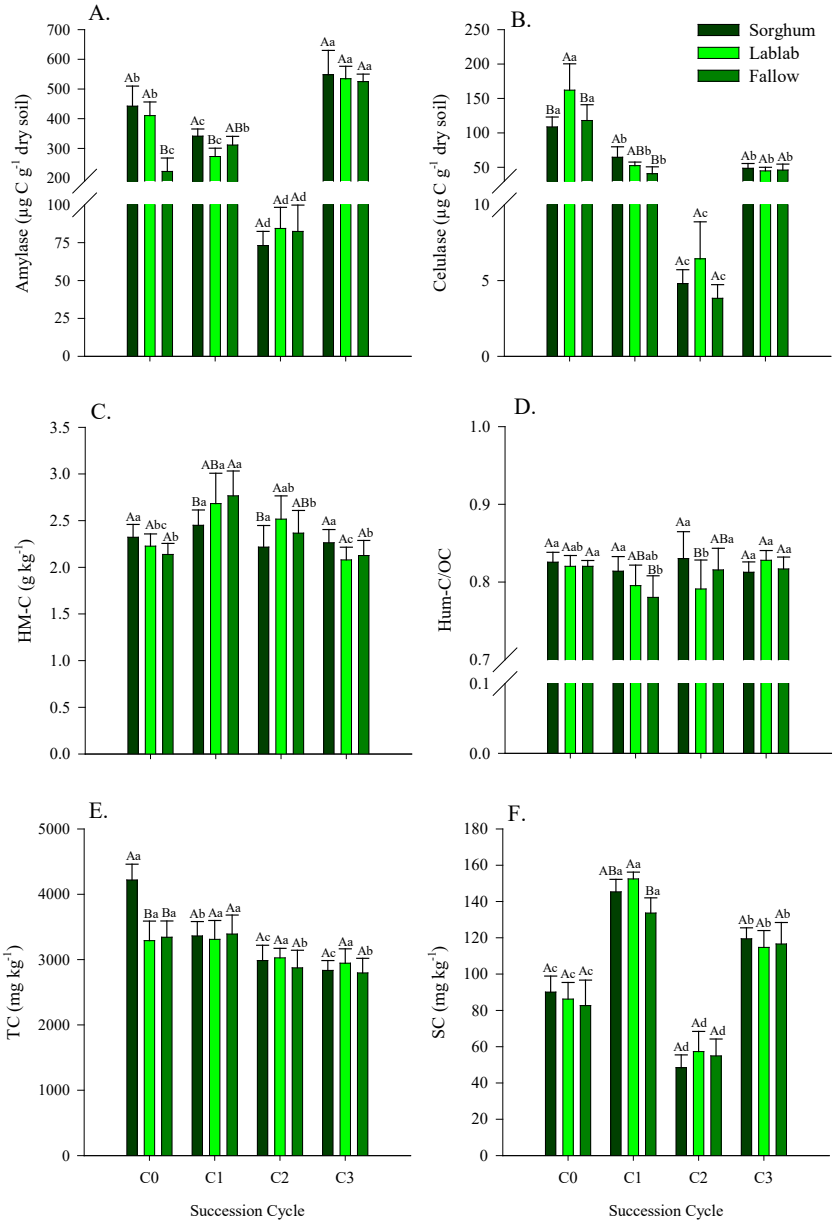


**Figure 3.** Enzymatic activity of amylase (A), cellulase (B), carbon in humic matter (CMH) (C), C-Hum/OC ratio (D) total (TC) (E), and soluble (SC) carbohydrates (F) in soil under maize crop in succession of green manures and fallow biomass being incorporated into the topsoil, during three crop succession 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.

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).

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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, similar to 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).

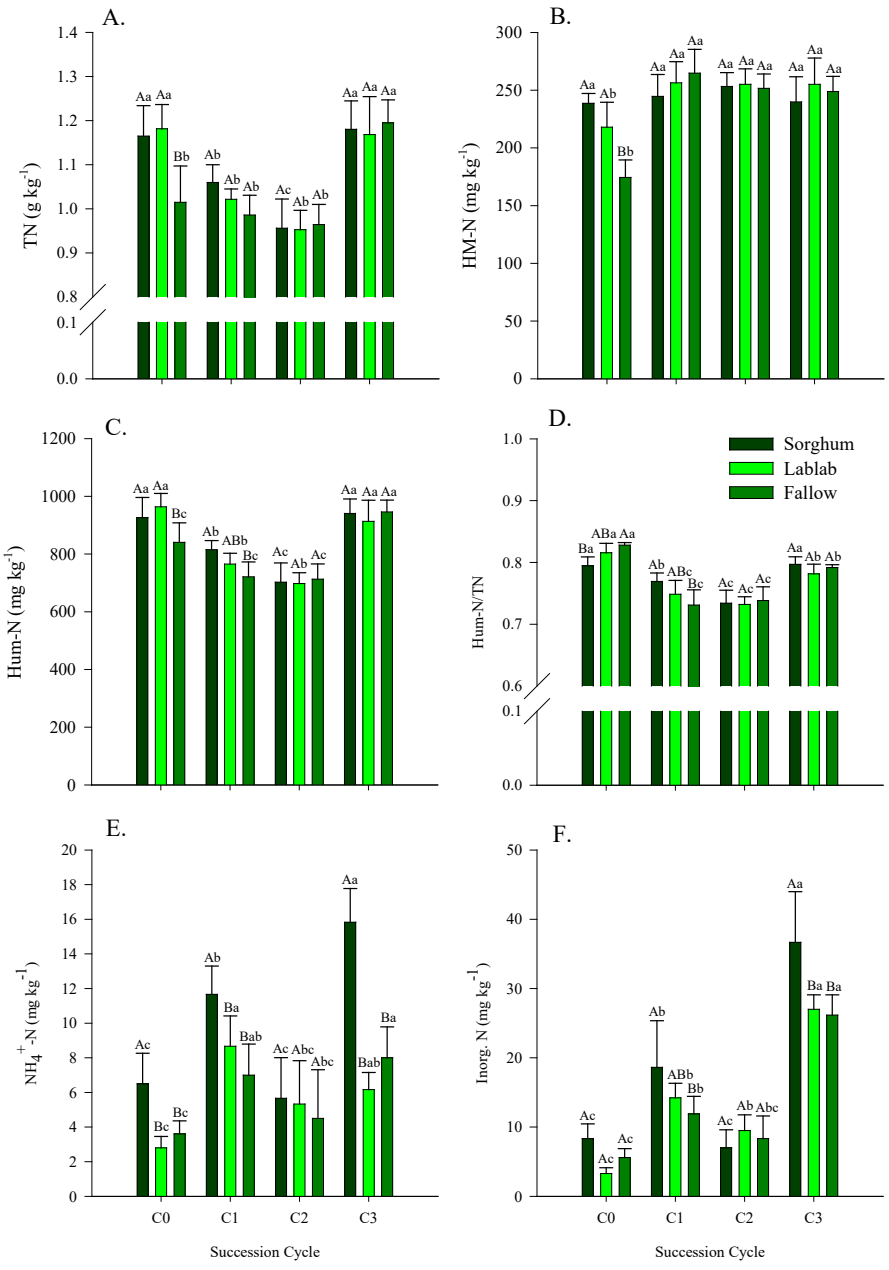


**Figure 4.** Content of total N (TN) (A), N in humic matter (NMH) (B), N in humin (N-Hum) (C), N-Hum/NT ratio (D), ammoniacal N (N-$NH^{4+}$) (E), and inorganic N (inorg N) (F) in soil under maize

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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.

For soil N-$NH_4^+$ and inorganic N (inorg N), there were higher values found for sorghum green manure-maize succession, than 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 occurred (Figure 2), similarities among variables and treatments within the soil sampled at 10 and 22 months after starting the management of crop succession systems were observed. 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).

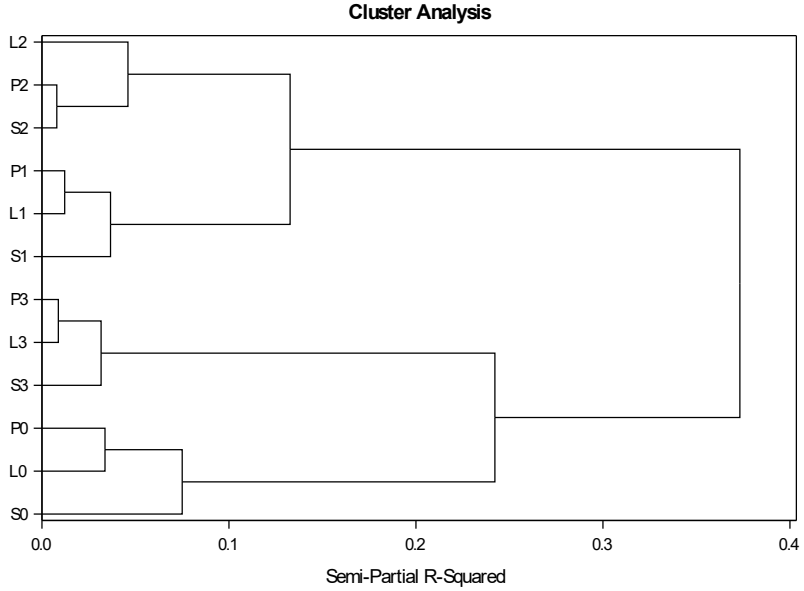


**Figure 5.** Dendrogram of cluster analysis constructed by Ward's method. S – Sorghum-maize crop succession; L – Lablab-maize crop succession; P – No cultivation (fallow-maize crop succession); 0 – Reference time (soil sampling at leaf sampling during the first cultivation of sorghum and lablab), 1, 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.

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## 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 (6,761 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 2,082 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 1,346 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 $mmol_c$ $dm^{-3}$ (data not shown). This was likely due to residual acidity caused by fertilization [37,39]. The export of cations from harvested maize grain in this 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 8,427 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. Enzymatic Activity in Soil in Soil Under Green Manure, Fallow and Maize Crop Succession Cycles*

Enzymatic 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±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 activities [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 activity 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 activity in soil cultivated under rotation systems with and without soil tillage. For cropping systems in which green manures are incorporated, hydrolytic enzyme activity is generally 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 activity (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 water limitations and low temperatures [54].

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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 C [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,

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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). With the exception of 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 similar to 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

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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 mainly linked to 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 changed during succession cycles due to decomposition of incorporated plant residues. There was no variation in humin carbon, which is the most stable form of soil carbon. It is highly resilient, even in a tilled tropical soil. The forms of N in the soil, especially inorg N, increased throughout succession cycles, due to the rapid breakdown and decomposition of buried plant residues by soil tillage and the application of nitrogen fertilizer, especially in the sorghum-maize crop succession. Cellulase activity decreased over time, while amylase activity increased.

The succession of maize with sorghum, as a green manure, stood out in comparison to lablab and fallow, as being the system that presented the highest maize yields, soil C and N levels, and amylase activity, due to better mineral N fertilizer management with a larger amount of buried sorghum and maize residues.

**Supplementary Materials:** The following supporting information can be downloaded at the website of this paper posted on Preprints.org. 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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## References

1. Islam M, Akter T, Sohel U, Mohammad R, Alam S. Green manuring effects on crop morpho-physiological characters, rice yield and soil properties. Physiol Mol Biol Plants. 2019;25:303–12. https://doi.org/10.1007/s12298-018-0624 2.
2. Bargaz, A.; Lyamlouli, K.; Chtouki, M.; Zeroual, Y.; Dhiba, D. Soil Microbial Resources for Improving Fertilizers Efficiency in an Integrated Plant Nutrient Management System. *Front. Microbiol.* **2018**, *9*, 1606, https://doi.org/10.3389/fmicb.2018.01606.
3. He, H.-B.; Li, W.-X.; Zhang, Y.-W.; Cheng, J.-K.; Jia, X.-Y.; Li, S.; Yang, H.-R.; Chen, B.-M.; Xin, G.-R. Effects of Italian ryegrass residues as green manure on soil properties and bacterial communities under an Italian ryegrass (Lolium multiflorum L.)-rice (Oryza sativa L.) rotation. *Soil Tillage Res.* **2019**, *196*, 104487, https://doi.org/10.1016/j.still.2019.104487.
4. Ambrosano EJ, Wutke EB, Tanaka RT, Mascarenhas HAA, Braga NR, Muraoka T. Leguminosas para adubação verde: uso apropriado em rotação de culturas. Coordenadoria de Assistência Técnica Integral, Campinas: 1997, p. 24.
5. Yang, T.; Siddique, K.H.; Liu, K. Cropping systems in agriculture and their impact on soil health-A review. *Glob. Ecol. Conserv.* **2020**, *23*, e01118, https://doi.org/10.1016/j.gecco.2020.e01118.
6. Quintarelli, V.; Radicetti, E.; Allevato, E.; Stazi, S.R.; Haider, G.; Abideen, Z.; Bibi, S.; Jamal, A.; Mancinelli, R. Cover Crops for Sustainable Cropping Systems: A Review. *Agriculture* **2022**, *12*, 2076, https://doi.org/10.3390/agriculture12122076.
7. Thorup-Kristensen, K.; Cortasa, M.S.; Loges, R. Winter wheat roots grow twice as deep as spring wheat roots, is this important for N uptake and N leaching losses? *Plant Soil* **2009**, *322*, 101–114, doi:10.1007/s11104-009-9898-z.
8. Yang, L.; Bai, J.; Liu, J.; Zeng, N.; Cao, W. Green Manuring Effect on Changes of Soil Nitrogen Fractions, Maize Growth, and Nutrient Uptake. *Agronomy* **2018**, *8*, 261, https://doi.org/10.3390/agronomy8110261.
9. Scavo, A.; Fontanazza, S.; Restuccia, A.; Pesce, G.R.; Abbate, C.; Mauromicale, G. The role of cover crops in improving soil fertility and plant nutritional status in temperate climates. A review. *Agron. Sustain. Dev.* **2022**, *42*, 1–25, https://doi.org/10.1007/s13593-022-00825-0.
10. Balota EL, Chaves JCD. Atividade enzimática e mineralização do carbono e nitrogênio sob solo cultivado com adubos verdes na cultura do cafeeiro. Rev Bras Cienc Solo. 2010;34:1573–83.
11. Balota, E.L.; Kanashiro, M.; Filho, A.C.; Andrade, D.S.; Dick, R.P. Soil enzyme activities under long-term tillage and crop rotation systems in subtropical agro-ecosystems. *Braz. J. Microbiol.* **2004**, *35*, 300–306, https://doi.org/10.1590/s1517-83822004000300006.

 doi:10.20944/preprints202408.2136.v1




12. Malhi, S.S.; Nyborg, M.; Goddard, T.; Puurveen, D. Long-term tillage, straw management and N fertilization effects on quantity and quality of organic C and N in a Black Chernozem soil. *Nutr. Cycl. Agroecosystems* **2011**, *90*, 227–241, https://doi.org/10.1007/s10705-011-9424-6.
13. Griffiths, B.S.; Ritz, K.; Bardgett, R.D.; Cook, R.; Christensen, S.; Ekelund, F.; Sørensen, S.J.; Bååth, E.; Bloem, J.; De Ruiter, P.C.; et al. Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity–ecosystem function relationship. *Oikos* **2000**, *90*, 279–294, https://doi.org/10.1034/j.1600-0706.2000.900208.x.
14. Zornoza, R.; Acosta, J.; Faz, A.; Bååth, E. Microbial growth and community structure in acid mine soils after addition of different amendments for soil reclamation. *Geoderma* **2016**, *272*, 64–72, https://doi.org/10.1016/j.geoderma.2016.03.007.
15. Matsumoto, L.; Martines, A.; Avanzi, M.; Albino, U.; Brasil, C.; Saridakis, D.; Rampazo, L.; Zangaro, W.; Andrade, G. Interactions among functional groups in the cycling of, carbon, nitrogen and phosphorus in the rhizosphere of three successional species of tropical woody trees. *Appl. Soil Ecol.* **2004**, *28*, 57–65, https://doi.org/10.1016/j.apsoil.2004.06.008.
16. Shamshitov, A.; Kadžienė, G.; Supronienė, S. The Role of Soil Microbial Consortia in Sustainable Cereal Crop Residue Management. *Plants* **2024**, *13*, 766, https://doi.org/10.3390/plants13060766.
17. Mummey, D.L.; Stahl, P.D.; Buyer, J.S. Soil microbiological properties 20 years after surface mine reclamation: spatial analysis of reclaimed and undisturbed sites. *Soil Biol. Biochem.* **2002**, *34*, 1717–1725, https://doi.org/10.1016/s0038-0717(02)00158-x.
18. Gómez-Sagasti, M.T.; Alkorta, I.; Becerril, J.M.; Epelde, L.; Anza, M.; Garbisu, C. Microbial Monitoring of the Recovery of Soil Quality During Heavy Metal Phytoremediation. *Water, Air, Soil Pollut.* **2012**, *223*, 3249–3262, https://doi.org/10.1007/s11270-012-1106-8.
19. Bandick, A.K.; Dick, R.P. Field management effects on soil enzyme activities. *Soil Biol. Biochem.* **1999**, *31*, 1471–1479, https://doi.org/10.1016/s0038-0717(99)00051-6.
20. Daunoras, J.; Kačergius, A.; Gudiukaitė, R. Role of Soil Microbiota Enzymes in Soil Health and Activity Changes Depending on Climate Change and the Type of Soil Ecosystem. *Biology* **2024**, *13*, 85, https://doi.org/10.3390/biology13020085.
21. Bhaduri, D.; Sihi, D.; Bhowmik, A.; Verma, B.C.; Munda, S.; Dari, B. A review on effective soil health bio-indicators for ecosystem restoration and sustainability. *Front. Microbiol.* **2022**, *13*, 938481, https://doi.org/10.3389/fmicb.2022.938481.
22. Liu, C.-A.; Zhou, L.-M. Soil organic carbon sequestration and fertility response to newly-built terraces with organic manure and mineral fertilizer in a semi-arid environment. *Soil Tillage Res.* **2017**, *172*, 39–47, https://doi.org/10.1016/j.still.2017.05.003.
23. Massenssini, A.M.; Bonduki, V.H.A.; Melo, C.A.D.; Tótola, M.R.; Ferreira, F.A.; Costa, M.D. Relative importance of soil physico-chemical characteristics and plant species identity to the determination of soil microbial community structure. *Appl. Soil Ecol.* **2015**, *91*, 8–15, https://doi.org/10.1016/j.apsoil.2015.02.009.
24. Mukhopadhyay, S.; Masto, R.E.; Cerdà, A.; Ram, L.C. Rhizosphere soil indicators for carbon sequestration in a reclaimed coal mine spoil. *CATENA* **2016**, *141*, 100–108, https://doi.org/10.1016/j.catena.2016.02.023.
25. Sobucki, L.; Ramos, R.F.; Meireles, L.A.; Antoniolli, Z.I.; Jacques, R.J.S. Contribution of enzymes to soil quality and the evolution of research in Brazil. *Rev. Bras. De Cienc. Do Solo* **2021**, *45*, https://doi.org/10.36783/18069657rbcs20210109.
26. Uwituze, Y.; Nyiraneza, J.; Fraser, T.D.; Dessureaut-Rompré, J.; Ziadi, N.; Lafond, J. Carbon, Nitrogen, Phosphorus, and Extracellular Soil Enzyme Responses to Different Land Use. *Front. Soil Sci.* **2022**, *2*, https://doi.org/10.3389/fsoil.2022.814554.
27. Brown, S.L.; Chaney, R.L. Use of Amendments to Restore Ecosystem Function to Metal Mining-Impacted Sites: Tools to Evaluate Efficacy. *Curr. Pollut. Rep.* **2016**, *2*, 91–102, https://doi.org/10.1007/s40726-016-0029-1.
28. Rüdisser, J.; Tasser, E.; Peham, T.; Meyer, E.; Tappeiner, U. The dark side of biodiversity: Spatial application of the biological soil quality indicator (BSQ). *Ecol. Indic.* **2015**, *53*, 240–246, https://doi.org/10.1016/j.ecolind.2015.02.006.
29. Marchiori Júnior M, Melo WJ. Carbono, carbono da biomassa microbiana e atividade enzimática em um solo sob mata natural, pastagem e cultura do algodoeiro. Rev Bras Cienc Solo. 1999;23:257–63.
30. Roldán, A.; Salinas-García, J.; Alguacil, M.; Díaz, E.; Caravaca, F. Soil enzyme activities suggest advantages of conservation tillage practices in sorghum cultivation under subtropical conditions. *Geoderma* **2005**, *129*, 178–185, https://doi.org/10.1016/j.geoderma.2004.12.042.
31. Hungria, M.; Franchini, J.C.; Brandão-Junior, O.; Kaschuk, G.; Souza, R.A. Soil microbial activity and crop sustainability in a long-term experiment with three soil-tillage and two crop-rotation systems. *Appl. Soil Ecol.* **2009**, *42*, 288–296, https://doi.org/10.1016/j.apsoil.2009.05.005.
32. dos Santos, J.V.; Bento, L.R.; Bresolin, J.D.; Mitsuyuki, M.C.; Oliveira, P.P.A.; Pezzopane, J.R.M.; Bernardi, A.C.d.C.; Mendes, I.C.; Martin-Neto, L. The long-term effects of intensive grazing and silvopastoral

 doi:10.20944/preprints202408.2136.v1



systems on soil physicochemical properties, enzymatic activity, and microbial biomass. *CATENA* **2022**, *219*, https://doi.org/10.1016/j.catena.2022.106619.
33. Ross, D.J. EFFECTS OF AIR-DRY, REFRIGERATED AND FROZEN STORAGE ON ACTIVITIES OF ENZYMES HYDROLYSING SUCROSE AND STARCH IN SOILS. *Eur. J. Soil Sci.* **1965**, *16*, 86–94, https://doi.org/10.1111/j.1365-2389.1965.tb01422.x.
34. Pancholy, S.K.; Rice, E.L. Soil Enzymes in Relation to Old Field Succession: Amylase, Cellulase, Invertase, Dehydrogenase, and Urease. *Soil Sci. Soc. Am. J.* **1973**, *37*, 47–50, https://doi.org/10.2136/sssaj1973.03615995003700010018x.
35. Köppen W. Das Geographische System der Klimatologie 1936:44.
36. Soil Survey Staff. Keys to soil taxonomy. USDA Natural Resources Conservation Service. 2022;13:410.
37. Raij B, Andrade J, Cantarella H, Quaggio JA. Análise Química para Avaliação da Fertilidade de Solos Tropicais. Boletim técnico. 2001:285.
38. Teixeira PC, Fontana GKDA, Teixeira WG. Manual de Métodos de Análise de Solo. 3rd ed. Brasília: Embrapa Solos. 2017.
39. Cantarella H, Quaggio JA, Mattos Jr D, Boaretto RM, Raij B. Boletim 100: recomendações de adubação e calagem para o estado de São Paulo. 2022.
40. Bremner, J.M.; Mulvaney CS. Nitrogen-total. Chemical and microbiological properties, Madison: Methods of soil analysis. Soil Sci Soc Am. 1982:595–624.
41. Dabin B. Méthode d'extraction et de fractionnement des matières humiques du sol Application à quelques études pédologiques et agronomiques dans les sols tropicaux. Cah Orston Ser Pédol. 1976;4:287–97.
42. Stevenson FJ. The Role of Organic Matter in Modern Agriculture. 2nd ed. New York: Developments in Plant and Soil Sciences; 1982.
43. Morse, E.E. Anthrone in Estimating Low Concentrations of Sucrose. *Anal. Chem.* **1947**, *19*, 1012–1013, https://doi.org/10.1021/ac60012a021.
44. Oser BL. Hawk's Physiological Chemistry. New York: McGraw-Hill Book Co. Inc., 1965;14 ed.
45. Abreu, C.H., Jr.; Muraoka, T.; Lavorante, A.F. Relationship between acidity and chemical properties of brazilian soils. *Sci. Agric.* **2003**, *60*, 337–343, https://doi.org/10.1590/s0103-90162003000200019.
46. Anselmo, J.L.; Sul, C.D.S.F.D.A..P.A.D.C.D.; Bossolani, J.W.; Lazarini, E.; Leal, A.J.F.; Alvarez, R.D.C.F.; Arf, M.V. Maize productivity cultivated as first crop in succession to different cover crops. *Oct. 2020* **2018**, *12*, 967–974, https://doi.org/10.21475/ajcs.18.12.06.pne1063.
47. Allison, S.D.; Vitousek, P.M. Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biol. Biochem.* **2005**, *37*, 937–944, https://doi.org/10.1016/j.soilbio.2004.09.014.
48. Sinsabaugh, R.L.; Belnap, J.; Findlay, S.G.; Shah, J.J.F.; Hill, B.H.; Kuehn, K.A.; Kuske, C.R.; Litvak, M.E.; Martinez, N.G.; Moorhead, D.L.; et al. Extracellular enzyme kinetics scale with resource availability. *Biogeochemistry* **2014**, *121*, 287–304, https://doi.org/10.1007/s10533-014-0030-y.
49. Sainju, U.M.; Liptzin, D.; Dangi, S.M. Enzyme activities as soil health indicators in relation to soil characteristics and crop production. *Agrosystems, Geosci. Environ.* **2022**, *5*, https://doi.org/10.1002/agg2.20297.
50. Johnson, A.; Hoyt, G. Changes to the Soil Environment under Conservation Tillage. *HortTechnology* **1999**, *9*, 380–393, https://doi.org/10.21273/horttech.9.3.380.
51. Dotaniya, M.L.; Aparna, K.; Dotaniya, C.K.; Singh, M.; Regar, K.L. Role of Soil Enzymes in Sustainable Crop Production. In *Enzymes in Food Biotechnology*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 569–589, ISBN 978-0-12-813280-7.
52. Lucero, C.T.; Lorda, G.S.; Anzuay, M.S.; Ludueña, L.M.; Taurian, T. Peanut Endophytic Phosphate Solubilizing Bacteria Increase Growth and P Content of Soybean and Maize Plants. *Curr. Microbiol.* **2021**, *78*, 1961–1972, https://doi.org/10.1007/s00284-021-02469-x.
53. Asghar, W.; Kataoka, R. Green manure incorporation accelerates enzyme activity, plant growth, and changes in the fungal community of soil. *Arch. Microbiol.* **2021**, *204*, 1–10, https://doi.org/10.1007/s00203-021-02614-x.
54. Grzyb, A.; Wolna-Maruwka, A.; Niewiadomska, A. Environmental Factors Affecting the Mineralization of Crop Residues. *Agronomy* **2020**, *10*, 1951, https://doi.org/10.3390/agronomy10121951.
55. Junior, C.H.A.; Muraoka, T.; Oliveira, F.C. Carbono, nitrogênio, fósforo e enxofre em solos tratados com composto de lixo urbano. *Rev. Bras. De Cienc. Do Solo* **2002**, *26*, 769–780, https://doi.org/10.1590/s0100-06832002000300022.
56. Stockmann, U.; Adams, M.A.; Crawford, J.W.; Field, D.J.; Henakaarchchi, N.; Jenkins, M.; Minasny, B.; McBratney, A.B.; de Courcelles, V.d.R.; Singh, K.; et al. The knowns, known unknowns and unknowns of sequestration of soil organic carbon. *Agric. Ecosyst. Environ.* **2013**, *164*, 80–99, https://doi.org/10.1016/j.agee.2012.10.001.
57. Fontaine, S.; Mariotti, A.; Abbadie, L. The priming effect of organic matter: a question of microbial competition?. *Soil Biol. Biochem.* **2003**, *35*, 837–843, https://doi.org/10.1016/s0038-0717(03)00123-8.

 doi:10.20944/preprints202408.2136.v1




58. Gualberto, A.V.S.; de Souza, H.A.; Sagrilo, E.; Araujo, A.S.F.; Mendes, L.W.; de Medeiros, E.V.; Pereira, A.P.d.A.; da Costa, D.P.; Vogado, R.F.; da Cunha, J.R.; et al. Organic C Fractions in Topsoil under Different Management Systems in Northeastern Brazil. *Soil Syst.* **2023**, *7*, 11, https://doi.org/10.3390/soilsystems7010011.
59. Marschner, B.; Brodowski, S.; Dreves, A.; Gleixner, G.; Gude, A.; Grootes, P.M.; Hamer, U.; Heim, A.; Jandl, G.; Ji, R.; et al. How relevant is recalcitrance for the stabilization of organic matter in soils? *J. Plant Nutr. Soil Sci.* **2008**, *171*, 91–110, https://doi.org/10.1002/jpln.200700049.
60. Cotrufo MF, Lavallee JM. Soil organic matter formation, persistence, and functioning: A synthesis of current understanding to inform its conservation and regeneration. Adv Agron. 2022;172:1–66. https://doi.org/10.1016/bs.agron.2021.11.002.
61. Pfleger P, Cassol PC, Mafra ÁL. Substâncias húmicas em Cambissolo sob vegetação natural e plantios de pinus em diferentes idades. Ciênc Florest. 2017;27:807–17.
62. Souza WJO, Melo WJ. Teores de nitrogênio no solo e nas frações da matéria orgânica sob diferentes sistemas de produção de milho. Rev Bras Cienc Solo. 2000;24:885–96.
63. Balota, E.L.; Yada, I.F.U.; Amaral, H.F.; Nakatani, A.S.; Hungria, M.; Dick, R.P.; Coyne, M.S. SOIL QUALITY IN RELATION TO FOREST CONVERSION TO PERENNIAL OR ANNUAL CROPPING IN SOUTHERN BRAZIL. *Rev. Bras. De Cienc. Do Solo* **2015**, *39*, 1003–1014, https://doi.org/10.1590/01000683rbcs20140675.
64. Zhang, K.; Maltais-Landry, G.; Liao, H.-L. How soil biota regulate C cycling and soil C pools in diversified crop rotations. *Soil Biol. Biochem.* **2021**, *156*, https://doi.org/10.1016/j.soilbio.2021.108219.
65. Villanueva FCA, Boaretto AE, Firme LP, Muraoka T, Franco Filho V do N, Abreu Junior CH. Mudanças químicas e fitodisponibilidade de zinco estimada por método isotópico, em solo tratado com lodo de esgoto. Quim Nova. 2012;35:1348–54.
66. Vargas, L.K.; Scholles, D. Nitrogênio da biomassa microbiana, em solo sob diferentes sistemas de manejo, estimado por métodos de fumigação. *Rev. Bras. De Cienc. Do Solo* **1998**, *22*, 411–417, https://doi.org/10.1590/s0100-06831998000300006.