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Noticeable Shifts in Soil Physicochemical and Biological Properties after Contrasting Tillage Management in Crop Rotations of Bean, Maize, and Amaranth in Ecuadorian Highland Soils

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Abstract: Soil biological properties are sensitive indicators of soil quality changes due to perturbations occurred under agricultural management. The effects of contrasting tillage, increasing nitrogen fertilization doses, and crop rotations [e.g., bean, maize, bean (BMB) and bean, amaranth, bean (BAB)] on soil physicochemical and biological properties in an Andean soil from Ecuadorian highlands were evaluated in this study. Acid phosphatase, β-Glucosidase, fluorescein diacetate hydrolysis, microbial biomass carbon (Cmic), soil basal respiration (BR), arbuscular mycorrhizal fungi (AMF) spore density, total glomalin content (TGRSP), and soil physicochemical properties were analyzed. Conventional tillage (CT) and crop rotation showed significant effects on soil physicochemical and biological properties. Towards the final crop rotations, no-tillage (NT) promoted BR, TGRSP, and higher AMF spore density in both crop rotations; the Cmic kept stable along time in BMB and BAB, while BR doubled its value when compared to CT. Results indicated that the AMF spore density increased by 308% at the end of the BMB, and 461% at the end of the BAB, while TGRSP increased by 18% and 32% at the end of BMB and BAB, respectively. Biological traits demonstrated to be strongly associated to the organic matter accumulation originated from crop residues under the NT post-harvest which improved soil moisture, biological activity, and AMF interaction. The conservative soil management system has definitively improved general soil properties when compared to soil conditions under the intensive soil management system in this research.

Keywords: phosphatase; arbuscular mycorrhizal fungi; β-glucosidase; enzymes; glomalin

1. Introduction

Soil is a vital resource for food production, carbon (C) sequestration and climate, nutrients and water regulation, and biodiversity enhancement, among other important functions [1,2]. Even though agricultural production is critical for human survival, soil management practices, such as intensive tillage, chemical fertilization, and monocropping, can negatively affect soil physical, chemical, and biological properties, ultimately impacting crop yields and productivity [3]. Intensive conventional tillage (CT) causes loss of organic C and nitrogen (N) due to an accelerated soil organic matter (SOM) breakdown with the subsequent decrease in soil quality and fertility. Mono-cropping, mechanical tillage, chemical fertilization, and residue removal increase soil organic matter (SOM) depletion, disruption of soil structure, and soil moisture loss with negative effects over soil enzyme activity and nutrient availability for plants [4]. As it has been reported by D'Hose et al. [5], the use of (organic) farm compost amendment is a potential supply of plant nutrients in long-term crop rotations of potato, fodder beet, forage maize, and Brussels sprouts. This amendment improved soil quality indexes such as soil organic carbon (SOC), total N, microbial biomass carbon (Cmic), nematodes abundance, and earthworm number, and consequently led to higher crop yields. Mastro et al. [6] mention that the application of nitrogen, phosphorous, and potassium (NPK) fertilizer plus manure (organic fertilization) for 31 years enhanced certain soil physical, chemical, and biological indicators such as pH, electrical conductivity (EC), bulk density, water retention, plant-available nutrients, SOM, Cmic, soil enzyme activities such dehydrogenase activity and phosphatase activity, and crop yield in a crop rotation scheme with maize (*Zea mays*), pearl millet (*Pennisetum americanum*), wheat (*Triticum aestivum*), and cowpea (*Vigna unguiculata*) on an Indian Inceptisol. Additionally, the studies by Sharma et al. [7] have proven that to maintain soil quality and crop yields in a semi-arid tropical Alfisols from southern India under sorghum (*Sorghum vulgare* L.)–castor (*Ricinus communis* L.) bean (*Phaseoulus vulgaris*) rotation, 90 kg N ha^{-1} together with the use of a leguminose organic residue (gliricidia loopings) under CT contributed considerably to higher Cmic, available N, K, S, and hydraulic conductivity (HC). These soils have been reported to suffer from hard setting tendencies and low water infiltration rates due to a compact surface, in need of a primary tillage. On the other hand, Lopes et al. [8], reported in a clayey Oxisols of the Brazilian Cerrado (soils reported with low P availability), an enhanced Cmic, BR and the activity of the β-glucosidase, arylsulfatase, and acid phosphatase, due to P fertilization (granular triple superphosphate) in a long-term experiment (12 and 17 year period). These biological indicators were analyzed and interpreted as a function of historical cumulative grain yields of corn (*Zea mays* L.) and soybean (*Glycine max* L.) yields, with SOC accumulations. The authors mention that the enhanced soil biological properties due to P fertilization using various sources, application rates, and placement methods need to be evaluated under other soil types, regions, and land uses. Moreover, according to a study by Qin et al. [9], inadequate fertilization management showed negative effects on the soil nutrient flows with consequent changes in soil enzyme activities. Specifically in this report, N and P fertilizers negatively influenced the soil enzyme activities such as the β-D-glucosidase (BDG) and phosphatases (PHO), which have been used as indicators for C- and P-cycling, respectively. Since microbial enzyme activities have proven to be sensitive indicators of agricultural intervention, and due to the contrasting information available which includes tillage, chemical or organic fertilization, and diverse crop rotation schemes on different soils or regions, seeking for improved fertilization management strategies and soil quality enhancement reinforces the need to study the close interactions among physical, chemical, and biological activities and their responses.

Crop rotation is an agricultural practice that affects soil biological activities. Wang et al. [10] reported that a more diversified crop rotation improved some soil health indicators such as moisture content, bulk density, and SOM; in addition, the enzyme activities of sucrase, urease and alkaline phosphatase were associated to a more diversified microbial community, reported in the early years the study. Under this context, another suitable practice for sustainable agriculture is no-tillage (NT), where most of the plant residues are left on the topsoil, increasing the SOM and nutrients contents [11]. Under NT practices, 30% or more of plant residues are left on the topsoil after sowing to help maintain the soil moisture for crops development and promote the soil microbial activities. Soil management practices such as NT provide several benefits to agriculture by improving soil quality, reducing the crops establishment time, and minimizing erosion and pollution effects [12].

Additionally, NT practices contribute to a reduction in C loss to the atmosphere and to its storage in soil, thus fighting climate change [13,14]. It has been reported, however, that NT practices may require the careful use of chemical herbicides and/or pesticides to control the growth of weeds and pests [15].

A meta-analysis conducted by Nunes et al. [16] showed that NT management promoted organic C accumulation on topsoil layers, resulting in an increased microbial biomass, soil respiration, soil active C, β-glucosidase activity, and soil protein content. Changes in soil management rapidly affect microbial activity such as basal respiration (BR), microbial C (Cmic), and enzyme activities, which stand out as early indicators of soil management [17,18]. In this context, more than 90% of SOM breakdown is carried out by microbial decomposers such as fungi and bacteria, by which soil biochemical properties such as the FDA could be a good and sensitive microbial indicator for measuring the total microbial activity and assessing the CT impact [19,20]. Nevertheless, according to Aponte et al. [17], soil enzymes by themselves do not reflect all aspects of soil microbial activity and function, and thus other soil biological and physicochemical properties are required additionally to describe soil perturbations as those produced by CT. Plant and arbuscular mycorrhizal fungi (AMF) interactions regulate soil biological activities and are of great interest in agriculture [21]. AMF has been widely studied for its positive effect on soil physicochemical properties due to its glomalin production. Glomalin is a fungal glycoprotein that acts by binding soil particles and increasing the soil's structure stability [22]. It has been demonstrated that diverse tillage systems affect AMF activity and the soil's glomalin content; therefore, they both are considered good soil quality indicators that can complement soil biochemical properties to assess CT effects on soil [23,24]. In the Andean region, there are no studies that integrate physicochemical and biological properties to evaluate the effects of tillage, fertilization, or crop rotation practices; therefore, there is the need to perform these analyses in a research center of an Ecuadorian university, in a mid- and long-term basis. Additionally, Nunes et al. [25] mentioned that latitude together with soil management type, time under NT, soil order, and cropping rotations affect soil chemical and biological properties. Authors reported that conservation tillage increased soil biological activity and SOM labile C and N fractions, implying that it can significantly improve the soil's biological health [26]. Consequently, the objective of this study was to analyze soil physicochemical and biological responses under contrasting tillage management, fertilization, and crop rotation practices in highland soils from a representative site in the Andean region of Ecuador. The hypothesis of this study proposes that soil management such as NT and low fertilization rates will increase soil biological activity induced by enhanced soil physicochemical properties, which will be noticeable at the end of each crop rotation in an Andean soil from the highlands of Ecuador. The main objectives in this study were to understand the effects of contrasting agricultural management and its impact on soil physicochemical and biological properties in the Andean region of South America, by providing new insights about the soil responses mainly to tillage, fertilization, and crop rotation activities.

2. Materials and Methods

2.1. Site Description, Experimental Design, Soil and Plant Sampling

The experiment was carried out in a research field with a total surface of 5.346 m^2 at the Universidad Central del Ecuador Experimental Station (CADET), Tumbaco, Quito, Ecuador (0°13′49″ S, 78°21′18″ W; 2505 m.a.s.l) (Appendix A, Figure A1). The mean annual precipitation in this site is about 870 mm, with an average relative humidity of 75% and annual average temperatures between 10.3 °C and 27.2 °C. Soil samples from two ground test pits within the research area were analyzed for soil taxonomy: test pit "A" was classified as Order Molison, Suborder Ustolls, Great group Durustolls, Subgroup Entic Durustolls, and test pit "B" was classified as Order Mollisol, Suborder Ustolls, Great group Argiustolls, Subgroup Tipic Argiustolls, commonly found in the northern highlands of Ecuador: dark volcanic-ash derived soils, relatively high in organic matter content and allophane material. A soil physicochemical analysis was performed prior to soil treatments, indicating the soil texture, color (according to Munsell color chart), pH, electric conductivity (EC) values, and organic matter (OM) % (Table 1).

Table 1. Soil physicochemical properties before establishment of treatments.

Soil Depth (cm)	Texture	Color	pH	* EC (dS/cm)	** OM (%)
0–20	Sandy loam Clay: 12–15% Sand: 53–58% Silt: 28–33%	10YR2/2 Dark grayish brown	6.94	0.26	3.29

* EC: electrical conductivity; ** OM: organic matter.

The experimental soils were managed under intensive tillage and Nitrogen (N) fertilization for potatoes (*Solanum tuberosum)* harvesting for at least 10 years before 2015. Since April 2015, when this research started, oats (*Avena sativa)* seeds were planted in the whole plot in order to extract chemical residues from previous fertilizations applied to these soils. Oat plants were removed from the plots after 4 months. After this, the following crop rotations schemes were established: (1) bean, maize, bean, and (2) bean, amaranth, bean. The total duration of the study since the first crop establishment was approximately three years (2016–2019). Both crop rotations schemes occurred under contrasting tillage management (conventional tillage: CT, and no-tillage: NT) with increasing N fertilization doses (explained below in Figure 1). The first crop (C0 = initial bean) (*Phaseolus vulgaris*) plot was established and divided in two sections: CT and NT, each with 24 subplots of 12 × 7 m (84 m^2), randomly distributed (Appendix A, Figure A2 and drone-recorded Video S1). C0 corresponds to the starting crop where only beans were cropped. Based on such plot arrangement, the next crops after C0 corresponded to maize (C1 = *Zea mays*) and amaranth (C2 = *Amaranthus caudatus*), which were sowed at the same time, approximately two months after C0 harvesting, both under CT and NT. Half of the total surface was used to crop maize, and the other half was cropped with amaranth, using the same previous treatment of tillage and fertilization The amaranth cycle (C2) subplots' dimensions were 7 × 5.5 m (38.5 m^2) due to the available cropping area. The maize soil samples and roots were collected in January 2018, while the amaranth samples (soil and roots) were collected in February 2018. Finally, the last bean crop after maize (C3) and bean crop after amaranth (C4) were established. The soil samples and roots from the last crop were collected at the end of February 2019. In this third stage, beans were cropped in all the plot. Crop rotations occurred year after year.

In this study, soils under NT received the crop residues from the preceding crop, specifically: bean and amaranth received 100% of crop residue, while maize received approximately 50% of crop residue (due to the more coriaceous nature of its biomass which takes longer degradation time), which was left on the topsoil of the NT plots for its incorporation in the subsoil layers. The climatic conditions for the crops' cycles were recorded as follows: The precipitation and average temperature during the C0 = initial bean (May 2016–September 2016) was 127.2 mm of rainfall and an average temperature of 16.2 °C; during the C1 = maize and C2 = amaranths (October 2017–February 2018), the amount of rainfall was 472.8 mm and the average temperature was 16.2 °C, in the C3 = final bean crop (October 2018–February 2019), the amount of rainfall was 469.7 mm and an average temperature of 16.8 °C. The experiment lasted for 34 months in the field; however, it is important to mention that this is an ongoing soil management research that will continue for the next years under the same tillage, fertilization, and crop rotations scheme, for future analyses.

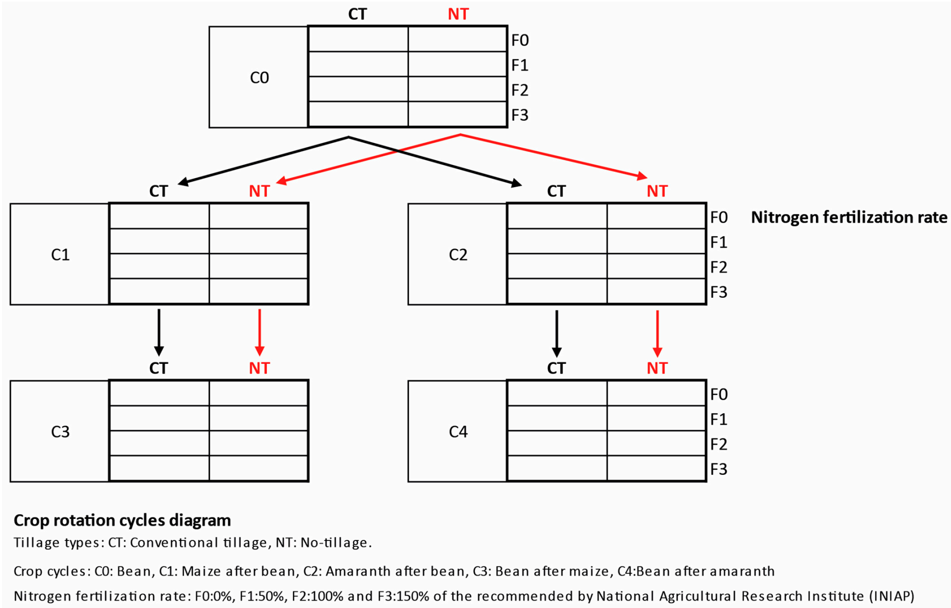

Figure 1. Schematic explanation about the experimental design here performed.

This research followed a split-plot experimental design. The factors under study were tillage (CT and NT), crop rotation (bean, maize, bean and bean, amaranth, bean), and ammonium nitrate (NH_4NO_3) fertilization doses (F0 = 0%, F1 = 50%, F2 = 100% and F3 = 150%) corresponding to F0 = no-N fertilization, F1 = 40 kg N ha^{-1}, F2 = 80 kg N ha^{-1}, and F3 = 120 kg N ha^{-1}. N fertilization was performed in accord with the agronomic recommendation based on soil fertility analysis. For each factor combination (tillage type, fertilization rates and crop rotation), three randomized plot replicates were used (n = 3). Within each plot replicate, five aleatory soil sub-samples (about 500 g soil/soil core) were collected at 0–20 cm depth, then homogenized in sealable plastic bags to complete 1 kg and were stored in coolers to be transported to the laboratory for physicochemical and biological determinations. A separated portion of the 1 kg soil sample was kept frozen at −20 °C until the analysis of enzyme activities. Additionally, three plant individuals (belonging to bean, maize and amaranth crops) from each plot replicate were randomly collected with their intact root system and kept in sealable plastic bags to be analyzed for AMF root colonization only, adding up a total of 24 soil samples and root systems from each crop for analysis.

2.2. Soil Physical and Chemical Determinations

In this study, soil pH and electric conductivity (EC) measurements were made [27,28]. Total porosity was calculated from bulk density assuming a particle density of 2.65 g cm^3 and 98% saturation; total organic carbon (OC) determinations were performed as well [29], as were measurements of the particulate organic matter value (POM) [30]. Soil available P was extracted and determined [31], as was the stability of soil aggregates in water [32].

2.3. Soil Biological Determinations

Microbial biomass carbon (Cmic) and basal respiration (BR) were measured after the chloroform fumigation-incubation procedure [33], with some modifications due to laboratory conditions [33–35]. The Cmic and BR were measured after the frozen samples were kept at RT for 4 h for conditioning before following the fumigation-incubation method. Four replicates of 15 g of soil were sieved (2 mm) and then incubated in 2 oz glass jars for 10 days, at 27 °C and 40% water holding capacity (WHC) [36,37]. For the BR, soil samples were ground to pass by a 4 mm sieve; for this procedure, 16 oz glass jars with pre-moistened filter paper at the bottom were used. Twenty-five g of soil was placed in small aluminum cups with holes to absorb humidity from the moist paper filters. A vial of 9 mL of 0.5 M NaOH as alkali traps was used, following 4 days incubation at 27 °C and back-titration with 0.1 N HCl to determine CO_2-C.

Enzyme activities of the acid phosphatase (Pase) and β–D–Glucosidase (Gluc) were measured [38,39]. These values are expressed in µg ρ–NP g^{-1} dry soil h^{-1}. The fluorescein diacetate hydrolysis (FDA) was also determined [40]. For the three enzymatic determinations, a Perkin Elmer lambda 25 lab UV VIS spectrophotometer, Shelton, CT 06484 USA was used.

2.4. Arbuscular Mycorrhizal Fungal Spores and Root Colonization

Spores of AMF were isolated from soil samples by means of a wet-sieving (250, 106 and 53 µm sieves) and decanting method [41], followed by sucrose gradient centrifugation [42]. After this, the supernatant containing the AMF spores was rinsed for 1 min in the 53 µm sieve and transferred to a Petri dish for sorting and quantification under stereomicroscope. Roots of bean, maize, and amaranth were processed [43]. The method of Koske and Gemma [44] helped with the root clearing and staining with trypan blue. The presence of AMF structures within the roots was observed at 40–100× in microscope slides, according to the line intersection method [45].

2.5. Total Glomalin-Related Soil Protein

The total glomalin related soil protein (TGRSP) was extracted from soil samples [46] and determined spectrophotometrically by means of the Bradford protein assay (Bio Rad Protein Assay; Bio Rad Labs, Hercules, CA, USA) at 595 nm, using bovine serum albumin as standard.

Abbreviations and clarifications for the variables measurements are described as follows: pH in water (1:5, *w*:*v*), EC = Electrical conductivity (mmhos cm^{-1}), Moisture = soil moisture (%), Bulk = bulk density (g cm^{-3}), Porosity = soil porosity (%), WSA = water stable aggregates (%), N = total nitrogen (%), OC = soil organic carbon (%), CN = carbon to nitrogen ratio, p = available P (mg kg^{-1}), POM = particulate organic matter (µm), Pase = acid phosphatase activity (µg pNP/g dry $soil^x$h), Gluc = β-glucosidase activity (µg pNP/g dry $soil^x$h), FDA = Fluorescein diacetate hydrolysis (µg FDA/g dry $soil^x$h), Cmic = microbial biomass carbon (µg C-CO_2 g^{-1} dry soil), BR = Basal respiration (mg CO_2 g^{-1} dry soil), TGRSP = total glomalin-related soil protein (mg g^{-1}), Spores = number of arbuscular mycorrhizal fungi spores in 100 g soil.

2.6. Statistical Analysis

Data were analyzed for each of the soil's physical, chemical, and biological properties. To evaluate normality and homoscedasticity assumptions, the Kolmogorov–Smirnoff and Levene tests were applied. Then, a three-way ANOVA was applied to determine the effect of tillage, fertilization, and crop rotations, considering all interactions over soil variables mentioned. A Tukey's test was undertaken in cases where the ANOVA results were significant ($p < 0.05$). Moreover, a Spearman correlation test between all variables studied in each sampling cycle (C0 to C4) was determined. In addition, a principal component analysis (PCA) was applied to evaluate the grouping of variables and their association with experimental individuals. The analyses were performed in Microsoft R statistical version 3.5.1.

3. Results

3.1. Biological Soil Traits

In our results, the ANOVA for the biological soil traits showed that the contrasting type of tillage here studied (CT and NT) had highly significant effects on certain biological variables such as Pase, β-Gluc, BR, and TGRSP (Table 2). In addition, the crop rotation (Cycle) had highly significant effects on all the variables analyzed, except for FDA. On the contrary, the fertilization factor highly significantly affected only Pase. Moreover, the interaction between Cycle and Tillage produced highly significant effects on almost all the experimental variables, except for FDA. The triple interaction only produced highly significant effects for Pase and slightly influenced the β-Gluc and TGRSP (Table 2 and Figure 2).

Table 2. F-values and significance for the main effects and factor interaction for the soil biological properties analyzed by means of a three-way ANOVA.

Factor	Pase	Gluc	FDA	C_{mic}	BR	TGRSP	AMF Spores
Cycle	1061.2 ***	94.5 ***	2.6 *	5.0 ***	44.2 ***	20.9 ***	53.7 ***
Tillage	17.1 ***	40.8 ***	0.2NS	0.0NS	165.8 ***	56.7 ***	3.8 **
Fertilization	11.7 ***	3.8 *	1.9NS	1.8NS	1.9NS	3.0 *	1.3NS
Cycle × Tillage	115.5 ***	26.9 ***	1.4NS	7.9 ***	37.0 ***	9.7 ***	7.5 ***
Cycle × Fertilization	17.4 ***	3.9 ***	4.3 ***	2.2 *	1.5NS	1.6NS	1.4NS
Tillage × Fertilization	11.3 ***	0.8NS	0.6NS	3.5 *	1.5NS	5.5 **	0.1NS
Cycle × Tillage × Fertilization	16.7 ***	2.3 *	1.2NS	1.0NS	1.2NS	2.2 *	1.5NS

Significance conventions : * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS = non significant. Abbreviations: Pase = acid phosphatase activity; Gluc = b-glucosidase activity; FDA = fluorescein diacetate activity; C_{mic} = microbial biomass carbon; BR = basal respiration; TGRSP = total glomalin related soil protein; AMF = arbuscular mycorrhizal fungi.

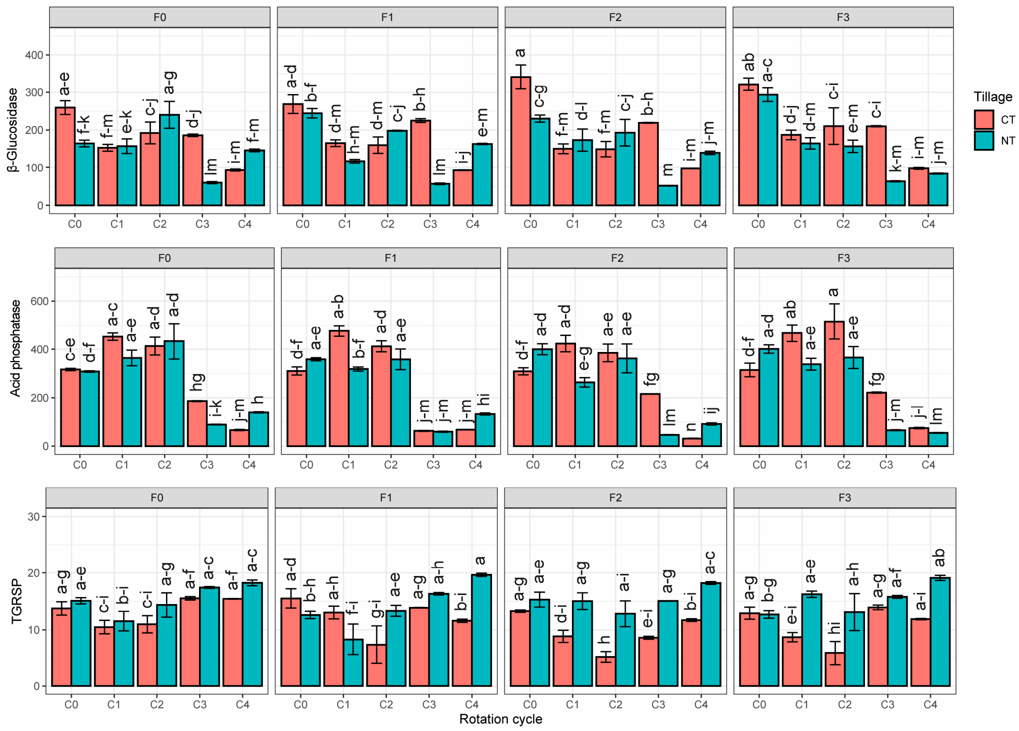

Figure 2. Type of tillage, fertilization rates and crop cycles interaction. Different letters indicate significant differences by the interaction between Tillage, Fertilization and Rotation cycle (Crop) according to the Tukey's multiple range test ($p < 0.05$).

According to our findings in Figure 2, where the interactions between type of tillage, fertilization rates, and crop cycles interactions were statistically analyzed, the data showed that the variables Acid phosphatase activity (µg pNP/g dry soil^{x}h), β-glucosidase activity (µg pNP/g dry soil^{x}h), and TGRSP = total glomalin-related soil protein (mg g^{-1}) were the only ones which showed significative interactions. For the acid phosphatase, there is less microbial activity for this soil enzyme towards the final cycles, both under CT and NT, despite the four different rates of N fertilization (F0 = no-N fertilization, F1 = 40 kg N ha^{-1}, F2 = 80 kg N ha $^{-1}$, and F3 = 120 kg N ha^{-1}). The same behavior occurred for the β-glucosidase activity, where a decrease in this activity was observed although the increasing fertilization rates. For the TGRSP, the C2 crop rotation corresponding to amaranth showed the lowest TGRSP values among the fertilization rates (F1, F2 and F3) under CT. This variable kept relatively constant along the rotations, independently of the fertilizations rates; however, in all the crop cycles, the TGRSP values were always higher under NT, which is the conservative soil management system in this study.

Soil Cmic, BR, AMF spore density, Pase, Gluc and TGRSP presented significant differences according to the crop rotation. In this sense, Cmic diminished about 20% under CT along the crop rotation cycles; however, Cmic remained relatively stable under NT along the cycles (Figure 3). Non-significant variations were observed for BR under CT (Figure 3). On the contrary, this variable increased two-fold its value under NT at the end of the crop rotation cycles. Detailing, the BR was higher under NT in the amaranth plots (C2), with similar trends in C3 and C4. The activities of Pase and Gluc presented an unexpected diminishment along the crop cycles for NT. At the end of the study, Pase decreased by 80% under CT and 70% under NT, while Gluc decreased by 65% under CT and 40% under NT. Detailing, in C3 (bean after maize), higher values for both enzymes under CT were found. On the contrary, in C4 (bean after amaranth), higher activities of Pase and Gluc were observed under NT.

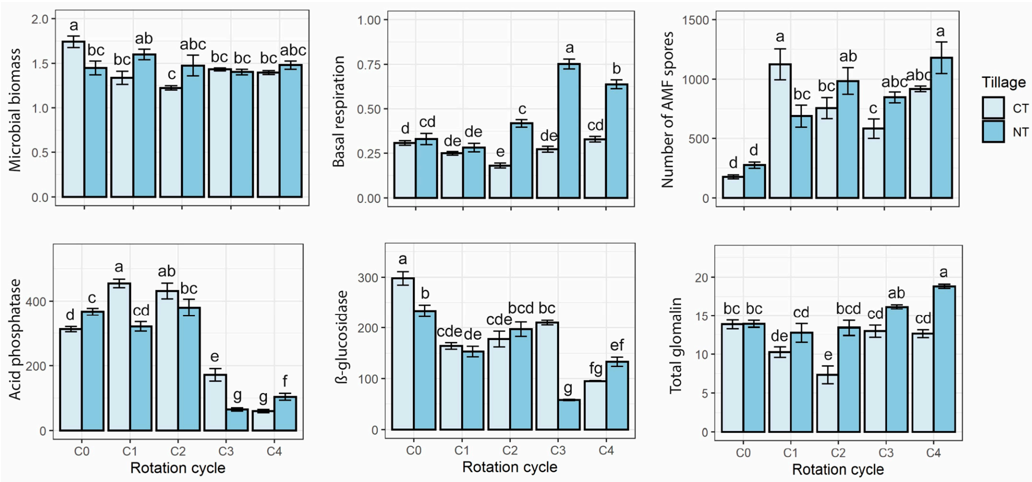

Figure 3. Biological traits of an Ecuadorian highland soil subjected to different crop rotations and tillage management. Different letters indicate significant differences by the interaction between Tillage and Rotation cycle (Crop) according to the Tukey's multiple range test ($p < 0.05$).

3.2. AMF Spores Density

Under CT, the AMF spore density (or number of AMF spores) increased more than 400% at the end of the C4, and it increased nearly 530% under NT (Figure 3). In our study, the AMF density for C0 did not show differences under CT or NT, but in general showed increased AMF spore density in the consecutive cycles under NT; reinforcing the idea, the effects of contrasting tillage managements on AMF spore densities were more evident in the final cycles (C2, C3 or C4) under NT when compared with C0.

Additionally, the glomalin content (here determined as TGRSP) under NT showed increased values towards the last two crop cycles (C3 and C4) when compared to CT (Figure 3). Here, TGRSP increased by 25% throughout time under NT, but decreased about 10% under CT management. The TGRSP showed consistent significant differences under tillage and crop rotation, with higher contents in the NT soils towards the last cycles (C2, C3 and C4) compared to CT, which reinforce its use as feasible indicator of the tillage effects in the Andean soils studied. Finally, we found some significative differences between the initial values in C0 under CT and NT, compared to the final values in C3 and C4 under CT and NT, respectively. In addition, towards the final cycles (C3 and C4), the TGRSP and AMF spores number showed positive correlations with N content and OC (Figure 4D,F).

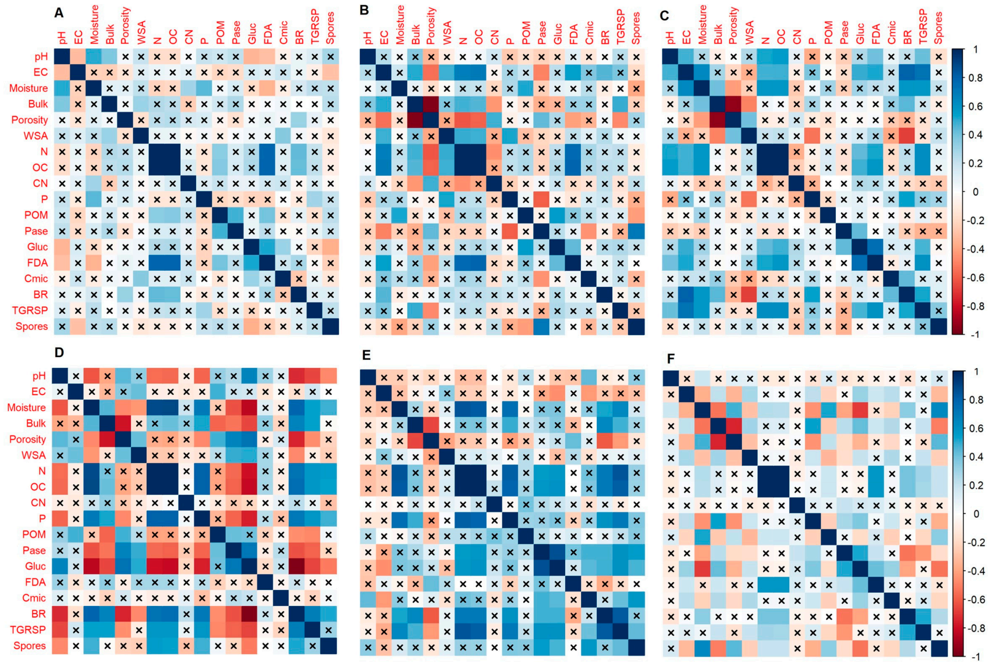

Figure 4. Correlation matrices between biological and physicochemical properties of an Ecuadorian highland soil subjected to different crop rotations and tillage management. Crossed boxes (×) represent non-significant correlations. Red boxes show a negative correlation, while blue boxes show a positive correlation between the variables. (**A**): corresponds to C0 = initial bean, (**B**): C1 = maize, (**C**): C2 = amaranths, (**D**): C3 = bean after maize, (**E**): C4 = bean after amaranth, (**F**): all the variables' correlations along the crop rotations (cycles).

3.3. Physicochemical Soil Traits

For the soil physicochemical variables, the ANOVA (Table 3) showed that both the type of tillage and crop rotation (cycle) had highly significant effects over the soil moisture bulk density, soil porosity, and P content, while when only taking into consideration the type of tillage, it highly significantly affected the soil moisture, bulk density, soil porosity, N, and P content. On the other hand, the interaction between crop rotation (cycle) with tillage highly significantly affected the soil pH, N, OC, and P.

Table 3. F-values and significance for the main effects and factor interaction for the physicochemical soil properties analyzed by means of a three-way ANOVA.

Factor	pH	EC	Moisture	Bulk	Porosity	WSA	N	OC	CN	P	POM
Cycle	2.87 *	103.34 ***	465.84 ***	56.70 ***	27.93 ***	14.20 ***	1.7	1.98	2.43	76.10 ***	78.77 ***
Tillage	0.0049	6.36 **	22.59 ***	33.67 ***	17.23 ***	0.60	12.82 ***	11.57 **	0.54	27.19 ***	1.02
Fertilization	8.57 ***	3.10 *	6.23 **	0.74	0.81	1.50	0.56	0.50	1.80	2.43	1.90
Cycle × Tillage	11.69 ***	15.09 **	1.97	3.98 **	1.07	3.68 **	8.30 ***	8.48 ***	1.18	14.99 ***	3.25 *
Cycle × Fertilization	2.77 **	1.40	6.05 ***	0.36	0.59	0.89	1.09	0.98	0.55	2.76 *	1.93
Tillage × Fertilization	5.25 **	0.82	4.91 **	0.18	0.23	1.01	0.65	0.64	0.44	6.86 ***	2.11
Cycle × Tillage × Fertilization	1.95	1.21	2.90 **	2.54 **	0.92	2.24 *	0.96	0.75	1.75	3.38 **	1.91

Significance conventions : * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Abbreviations: EC = electric conductivity; WSA= water-stable aggregates; N = nitrogen; OC = organi carbon; CN = carbon-nitrogen ratio; P = phosphorous; POM = particulate organic matter.

Highlighting in our results, the moisture content notoriously increased towards the final crop rotation cycles (C3 and C4), as well as the soil OC both under NT due to the crop residues left on the topsoil, enhancing as expected some of the soil biological properties mentioned in Table 2, and thus becoming an example of good practices for soil conservation and management. The principal component (PC) analysis (Figure 5) showed a total 42% of the variability. In this context, PC1, apparently associated to crop rotation effect, showed to be highly influenced by P and Gluc in C0 compared to other crop rotations, where most of the variability was explained by the number of AMF spores and moisture content. On the other hand, BR, TGRSP, N, OC, and FDA explained most of the variability of PC2 (associated to tillage effect), being all positively correlated in NT, especially from C1 to C4 when distance between NT and CT was more evident compared to C0 (Figure 5). Despite the closer distance between crop rotations (except for C0), a transition from C1 to C4 was observed in PC2.

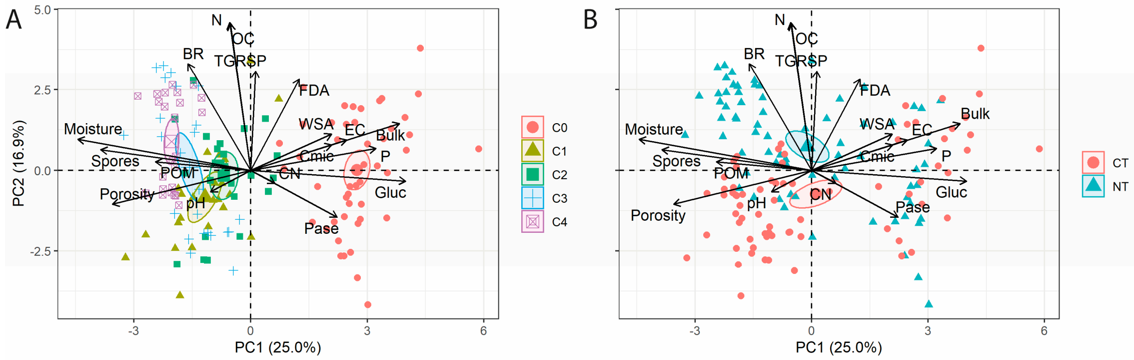

Figure 5. Principal component analysis (PCA) for the experimental variables and treatments used in an Ecuadorian highland soil subjected to different crop rotations (**A**) and tillage management (**B**). The results shown are similar (**A**,**B**) but were stablished the separation between crop cycles and tillage management for visual purposes. The two first PCs extracted accounted for 42% of the total experimental variance.

4. Discussion

It is well-recognized that, in general, the activities of some key soil enzymes are good predictors of the soil's quality [17]. Here, despite the increasing fertilization rates applied to the soil, the activities of Pase and Gluc presented an unexpected diminishment along the time under both CT and NT tillage systems at the final crop cycles. This could be due to a variety of other biotic and abiotic interactions in the soil, or the effect of the species cropped at the final crop cycles. At the end of this study, Pase decreased by 80% under CT and 70% under NT, while Gluc decreased by 65% under CT and 40% under NT. Detailing, in C3 (bean after maize), higher values for both enzymes under CT were found. On the contrary, in C4 (bean after amaranth), higher activities of Pase and Gluc were observed under NT. The above differences suggest a strong influence of the cropped species as well as the rotation sequence on the biochemical soil properties. In this sense, amaranth has been reported to be associated with native microorganisms involved in the SOM breakdown due to the production of exudates that favor the microbial growth, which indirectly can increase the hydrolytic enzyme activity in the rhizosphere [47], especially under NT management. In this context, Bateman et al. [48] emphasized the importance of the "unique rhizosphere environment" from each crop, explaining that newly established crops will be associated to new specific microbial communities, with specific functionalities and enzyme activities, as here reported. Reinforcing this, Zuber and Villamil [49] performed a complete meta-analysis in the topic and concluded that NT represents a more favorable microclimate ultimately associated to a most diverse and greater microbial activity, mainly derived from the increased levels of soil OM. The above could be related with enhanced soil conditions found in soils under NT in the current study, which also allow the increase in the soil OM levels on the topsoil.

Since phosphatases are extracellular enzymes synthesized by the plant roots, fungi, and bacteria in the soil, they have been previously reported with lower activity which is in accord with an increased intensity of soil management [50,51]. In this sense, Pittarello et al. [52] have recognized the crucial role of soil enzymes which, concomitantly with their capability to easily change according to different management systems, supports their use as early indicators of soil quality. In studies such as here performed, Pandey et al. [53] have reported greater Pase activity in soils cultivated with rice under NT and reduced tillage systems, when compared to a continuous tillage system. Moreover, the Pase activity showed contradictory results when compared with our results, which could be due to the non-limiting P conditions as well as the significant increase in AMF populations along time, which suggests a more efficient P supply to the rice crop by means of the mycorrhizal pathway. In this sense, increased Gluc activity in NT could be associated to increases in recalcitrant SOM from the conservative soil management system. Reinforcing this, de Almeida et al. [54] reported that the Gluc activity tends to be higher in soils with high levels of easily decomposable SOM, such as soils with crop rotation or direct sowing, as in our study. In this study, when considering these two enzymes, the fertilization rates did not produce a clear response along the crop cycles besides that at the final steps, when the activity of these two enzymes diminished. This could be attributed to other causes related to a drier season in the final crop rotations, affecting the whole biological soil traits.

The unchanging values observed for Cmic under NT seem to be reflecting a buffered effect in soil, which suggests that the accumulation of crop residues on the soil surface produced non-limiting labile SOM; therefore, providing sufficient organic C inputs allowing supports the microbial growth. According to Espinoza et al. [55], in a 7-year study in a similar agroecosystem, the NT favored the OM cycling in the soil, contributing to the presence of higher Cmic. Thus, the accumulation of crop residues on topsoil implies a greater source of C and nutrients for microorganisms, which in the meanwhile increase the biomass and diversity [56–58].

Non-significant variations under CT were observed for BR in the different crop rotations (Figure 3). On the contrary, BR increased 2-fold under NT at the end of the crop rotation cycles. Detailing, the BR was higher under NT in the amaranth plots (C2), with similar trends in C3 and C4. In all cases, the BR showed higher values under NT compared to the CT. The above results support that higher microbial respiration is based in a higher level of labile SOM originated in the crop residues that remained in the NT systems, which 'produced an increased microbial metabolic activity needed for OM turnover. The higher BR values observed under NT, together with significant differences for C2, C3, and C4, agree with recent reports by Bongiorno et al. [59], who have reported a 51% higher BR under reduced tillage, attributed to the higher SOM content in comparison to CT. This reinforces the idea that the microbial community is actively decomposing available SOM as C and energy source, and it is strongly influenced by the soil management occurring under crop rotations, tillage, and fertilization along time, as reported in a previous study in these same soils [60]. On the other hand, differences for BR between CT and NT were higher at the end of the study, while Cmic was very stable. These results suggests that an increased microbial respiration from the constant Cmic can be associated with a higher energy investment in degrading stable SOM, together with an increase in labile C fractions, which determines the prevalence of less-efficient soil microbial communities [61]. In this sense, the study by Ghimire et al. [62] also indicated that reduced tillage management, organic amendments, and legume crops in rotations considerably influenced SOC and microbial biomass concentrations, generating significant increases in relative abundance of bacteria, fungi, and protozoa associated with a higher substrate availability.

Multivariate analysis suggests that the progression of some key traits over time can be well-represented by the separation along the time regarding the starting conditions. The previous is evident in the case of C3 compared with C4, both treatments representing bean crops at the end of the study, where similar conditions of tillage and fertilization regarding the C0 initial crop were used. The positive correlation between soil moisture and AMF spores (Figures 4F and 5A,B) is also in agreement with previous research in the same soils, where a non-disturbing agricultural management, such as the grasslands, presented higher AMF spore density when compared to intensively tilled soils cropped with potatoes and maize [60]. In addition, Curaqueo et al. [23] and Schneider et al. [63] observed a reduction in AMF spore densities under CT in field conditions. Here, the results evidenced a high association between moisture content and AMF spores under NT, which suggests that this management increased not only the soil moisture retention, but also the soil porosity which can favor the presence of AMF populations. Contrarily, the study by Bhardwaj and Chandra [64] reported a negative association between AMF spores and soil moisture in their seasonal study. The above contrasting results evidence the need for a more comprehensive understanding about the complex soil interactions that determine major benefits from the AMF symbiosis. Finally, our results support that some soil components such as SOM, BR, TGRSP, and the AMF spore density can be highlighted as key indicators of the progression of soil resilience status when diverse and contrasting tillage managements are being performed.

5. Conclusions

This research has demonstrated the beneficial effect of no-tillage in comparison with conventional tillage, observed on a series of soil physical, chemical, and biological traits (basal respiration, number of arbuscular mycorrhizal fungi spores, enzyme activities of acid phosphatase, β-glucosidase, and total glomalin related soil protein) which have been reported for the first time in the Andean region. The effect of increasing the Nitrogen fertilization rates was not clear and may require a long-term project to indicate clearer responses in the biological and physicochemical parameters here analyzed. On the other hand, the soil enzyme activities studied showed higher sensitivity to soil disturbance (no-tillage); therefore, they can be considered good indicators of changes in soil management systems in the early- and medium-term. Additionally, the biological traits in this research were strongly associated with the accumulation of organic matter, originated from crop residues from the no-tillage post-harvest activities. This organic matter concomitantly improved the soil moisture, basal respiration, microbial enzyme activities, and arbuscular mycorrhizal fungi, which strongly supports crops establishment and development. The physicochemical and biological changes in these types of soils have been reported in an integrated way for the first time in this region as useful tools to attain sustainability. Reinforcing this, in the crop rotation system where amaranth was included in association with bean (legumes), it demonstrated a contribution to the soil biological activities enhancement by probably increasing the microbiological source of energy (coming from the topsoil organic matter), together with an increased arbuscular mycorrhizal fungi presence. This enhanced soil quality under no-tillage and crop rotations mainly could be leading to promising soil management practices in the volcanic soils of the Andean region of Ecuador focused on soil conservation and land disturbances reduction, as well as improving other soil microbiome ecosystem services. These studies are promissory in agricultural areas of highland zones, which are in deep need of soil conservation and restoration due to the impacts of intensive cropping and heavy rainfall, mainly in areas with steep slopes as those occurring in the Andean region. This will also help to prevent CO_2 loss into the atmosphere and maintain the C stocks under these soils.

Supplementary Materials: The following supporting information can be downloaded at: https://youtu.be/mb70MmlglRo (Video S1).

Author Contributions: Conceptualization: F.B., P.C., M.E.A.-S. and F.M.; methodology: M.E.A.-S., F.M., H.A., N.U.G., S.A.O., F.B. and P.C.; software: H.A.; validation: P.C.; formal analysis: M.E.A.-S. and P.C.; investigation: M.E.A.-S., F.M., D.C., M.O. and K.P.; resources: M.E.A.-S., F.M., P.C. and F.B.; data curation: H.A.; writing—original draft preparation: M.E.A.-S.; writing—review and editing: M.E.A.-S., H.A. and P.C.; visualization: F.B., P.C., J.E. and S.A.O.; supervision: P.C.; project administration: M.E.A.-S. and F.M.; funding acquisition: M.E.A.-S., F.M. and P.C. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study or in the data collection or analysis.

Appendix A

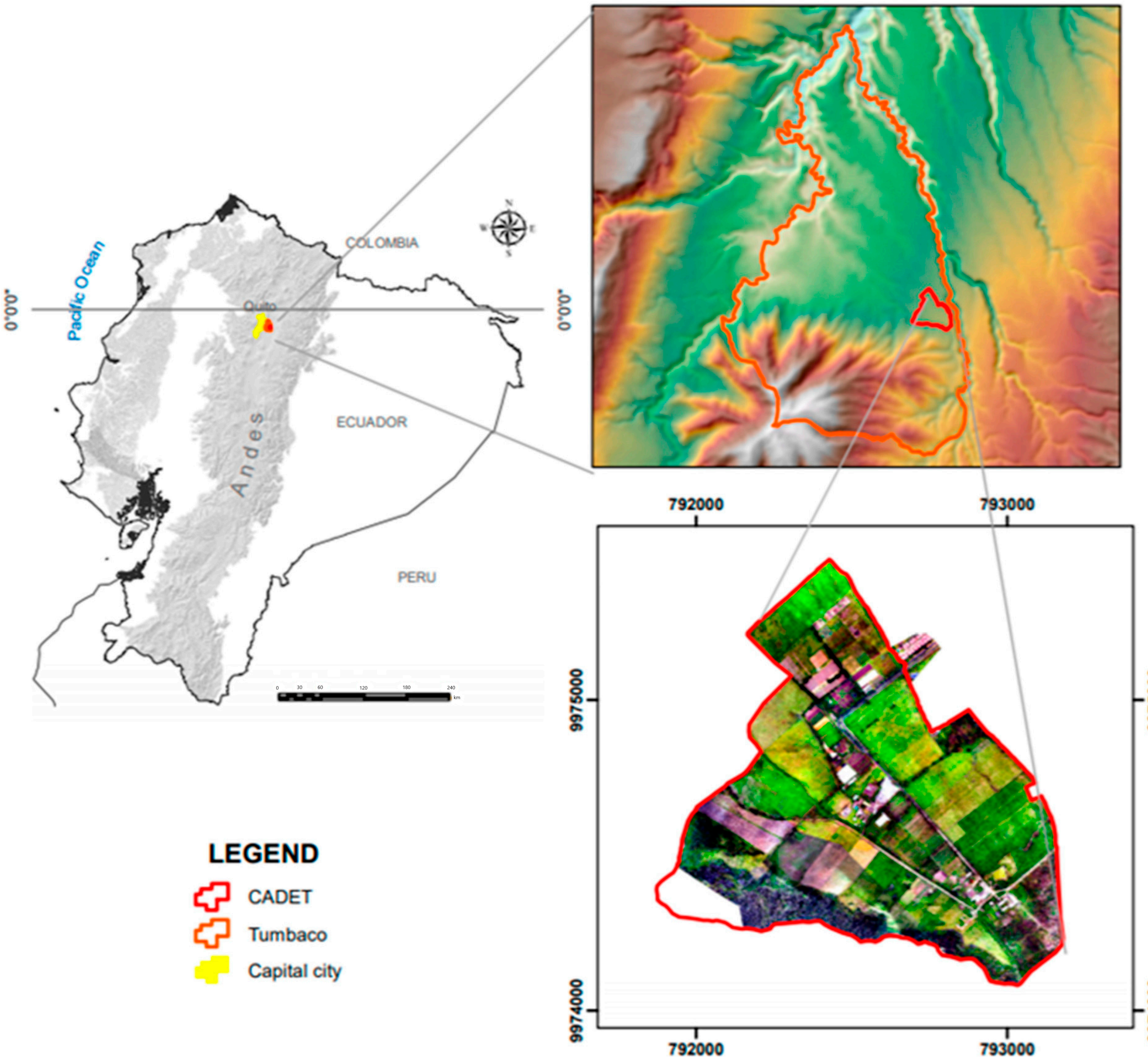

Figure A1. Location of the site of study. The plots were included in a total of about 5500 m^2 into Universidad Central del Ecuador Experimental Station (CADET).

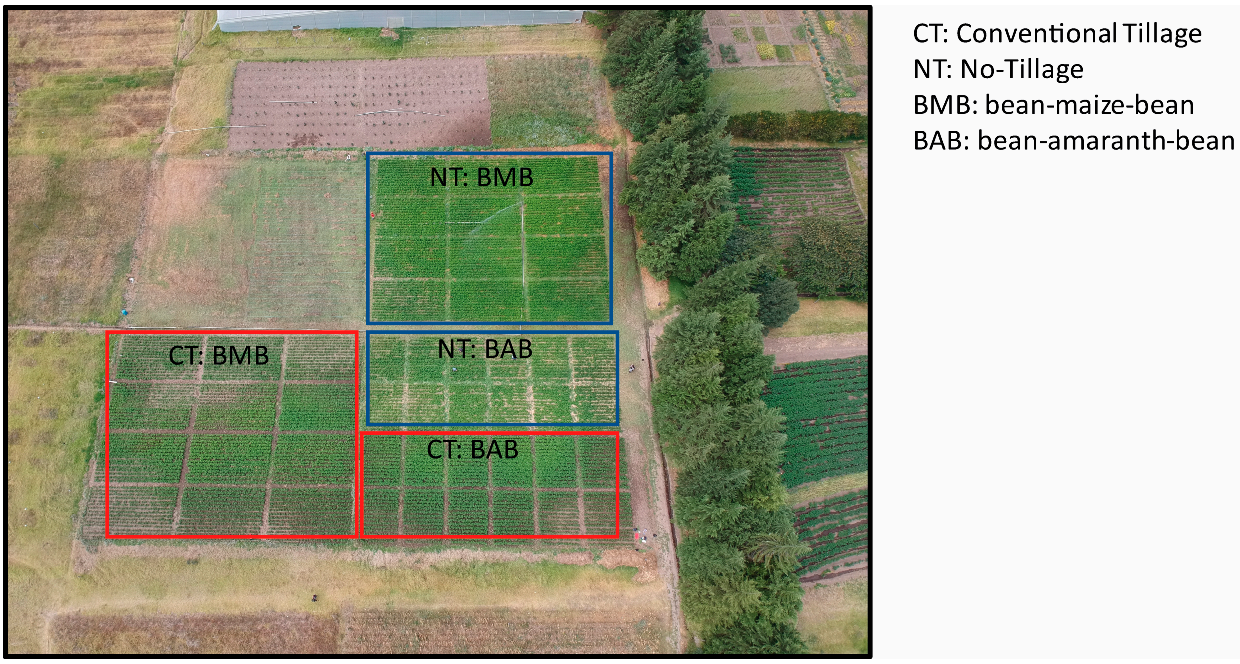

Figure A2. Experimental design at Universidad Central del Ecuador Experimental Station (CADET), Tumbaco, Quito, Ecuador. Video S1: https://youtu.be/mb70MmlglRo.

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