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EFFECTS OF TILLAGE, FERTILIZATION AND CROP ROTATION ON THE BIOLOGICAL ACTIVITIES OF AN ECUADORIAN SOIL FROM THE ANDEAN HIGHLANDS: TOWARDS THE OPTIMIZATION OF SOIL MANAGEMENT FOR A SUSTAINABLE AGRICULTURE

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"EFFECTS OF TILLAGE, FERTILIZATION AND CROP ROTATION ON THE BIOLOGICAL ACTIVITIES OF AN ECUADORIAN SOIL FROM THE ANDEAN HIGHLANDS: TOWARDS THE OPTIMIZATION OF SOIL MANAGEMENT FOR A SUSTAINABLE AGRICULTURE"

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“En algún lugar,

algo increíble

está esperando

ser descubierto”.

[Carl Sagan]

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Summary and thesis outline

Soil is one of the most important natural resources on the planet where light, water and nutrients contribute to the development of microbes, plants and animals (Gupta *et al.*, 2019). Its quality, however, is threatened by demographic growth, intensive management of arable lands, urbanization and erosion (Blum, 2013). In Ecuador, soil erosion originated by traditional management practices such as conventional (intensive) tillage (CT), is one of the most serious environmental degradation problems that affects about 50% of the cultivated lands (Pimentel and Burgess, 2013). Conventional tillage (CT) is a common agricultural management practice, which facilitates weeds elimination, sowing and seed germination (Ghimire *et al.*, 2012; Nath *et al.*, 2020). When it is used together with monoculture and agrochemicals, CT can affect the soil physical, chemical and biological properties, disturbing the stability of soil organic matter (SOM) thus favoring its mineralization and ultimately affecting the nutrients dynamics and microbial activities (Gajda *et al.*, 2013). Its damage can extend to deeper soil layers, depending on the time and repetition of this practice (Kladivko, 2001). In Ecuadorian soils, it has been estimated that erosion by disc plow tillage, in agricultural soils with a 30% slope, averages 40 t ha^{-1} (Henry *et al.*, 2013).

No-tillage (NT), as a more conservative practice, emerges as an alternative to reduce soil degradation, helping to maintain its physical structure and increasing the carbon (C) storage in soil while providing habitat and quality substrates to the different soil organisms (Young and Ritz, 2000). In a conservative soil management, other procedures as the use of crop residues provides a protective layer to the soil surface and it also acts as a nutrients source and reservoir for plants (Thapa *et al.*, 2018). In the soil system, there is a diversity of microbial communities associated to the plants roots system, playing key roles in the development and health of plants and crops (Southworth, 2013). Among the most beneficial microorganisms highlight the Arbuscular Mycorrhizal Fungi (AMF), well-known for their effectiveness as plant growth promoting microbes and health stimulators (Desai *et al.*, 2016). Therefore, the importance of AMF for a sustainable agriculture makes a need the searching for management tools to improve its presence and activity (Urgiles *et al.*, 2016).

To determine the effects of tillage practices on soil, the study of different characteristics is recommended. Among them, some traits of chemical and biological nature are indicated as the most relevant due to their ability to quickly respond to the soil disturbances (Schloter *et al.*, 2003; Taylor *et al.*, 2010). Some of the main soil determinations are pH, SOM, microbial biomass carbon (Cmic), microbial respiration (BR) and enzymatic activity (Janušauskaite *et al.*, 2013). The evaluation of enzymes is important since they are secreted by the soil microbial organisms and are part of the soil matrix (Aon *et al.*, 2001). One of the most evaluated soil enzymes are phosphatase (Pase) which hydrolyzes various organic and inorganic phosphate esters, and β-glucosidase (β-Gluc) that degrades low molecular weight compounds originated from the cellulose degradation into glucose units (Juma and Tabatabai, 1988; Lu *et al.*, 2013). Also, the determination of AMF propagules quantity, both in the soil and into the roots is a key indicator of soil health and quality. In this context, Bastida *et al.* (2008) mention that the soil quality maintenance is a critical factor to safeguard the environmental and biosphere sustainability.

Integrative information about physical, chemical and biological soil properties in the Andean region of Ecuador is scarce; consequently, evaluating soil quality becomes a subject of great importance and interest in this region for proposing solutions and trends that seek to solve agricultural soil degradation. Therefore, this Thesis Project involves the study of physical, chemical and biological characteristics in a soil from volcanic origin in the Ecuadorian highlands, under bean, maize, and amaranth rotations system including the two most important agricultural management factors: i) fertilization and ii) tillage. This study has been conducted since 2016 in the Experimental Teaching Academic Center "La Tola" (CADET), in the Tumbaco valley in the Andes of Ecuador.

In **Chapter I**, general introduction, hypotheses, and the proposal of general and specific objectives are presented. The general objective of this Doctoral Thesis was "to determine the effects of two contrasting soil management systems: Conventional Tillage (CT) *vs*. no-Tillage (NT), on main biological activities, and arbuscular mycorrhizal symbiosis densities in the bean-maize-bean and bean-amaranth-bean crop rotations, subjected to different fertilization levels in Andean soils from the highlands of Ecuador*"*. A comprehensive description is also given to establish the conceptual and methodological framework of this Thesis.

The **Chapter II** corresponds to a review entitled "*Impacts of tillage on the soil biological activities: The microbial component as key for a sustainable agriculture in the tropics*¨. In this chapter, a compilation of studies based on tillage effects over biological characteristic in tropical soils is presented, highlighting the positive changes in microbial biomass, enzyme activities and AMF propagules which directly influence on soil quality and fertility, thus on crop production, using conservative tillage systems. We also discussed how AMF and other soil microorganism are important contributors of ecosystem services that can be enhanced under reduced or NT managements. Additionally, in this chapter, we systematized accurate information about microbial functions in soil and how they are affected by soil tillage.

The **Chapter III** corresponds to the manuscript entitled "*Soil Biological Properties and Arbuscular Mycorrhizal Fungal Communities of Representative Crops Established in the Andean Region from Ecuadorian Highlands*". In this chapter, soil chemical and biological characteristics in naturalized grasslands, *Zea mays* and *Solanum tuberosum* cropped soils from the highlands of Ecuador were reported, as a baseline to the research project here proposed. In this research, we highlighted the AMF communities and enzyme activities, which were characterized for the first time in these soils. The results have demonstrated the strong effects of the type of soil management over these traits, which allows to consider them as good indicators for soil quality and health in this Andean region. AMF communities richness and diversity indexes were higher under the more conserved soil management and were characterized for the first time in these Andean soils.

In **Chapter IV** the manuscript entitled "*Noticeable shifts in biochemical soil properties after contrasting tillage management in crop rotations of bean, maize and amaranth in Ecuadorian highland soils*¨ is presented. This chapter is associated to the specific objectives, where a) soil microbial activities in Andean soils from the highlands of Ecuador after the bean-maize-bean and bean-amaranth-bean crop rotations under contrasting tillage practices were described, b) the effect of fertilization increasing rates on the rhizosphere´s biological activities occurring in bean-maize-bean and bean-amaranth-bean crop rotations were studied, and c) the effect of soil management practices on arbuscular mycorrhizal fungal structures after bean-amaranth-bean and bean-maize-bean crop rotation, were evaluated. Summarizing, beneficial effects were noticed under the conserved tillage practice (NT) over soil traits. Under NT, an enhanced acid Pase, β-Gluc, BR, total glomalin related soil protein (TGRSP) and AMF spores' number was observed in the bean, maize and amaranth crop rotations. The Cmic was kept stable along time under NT in the crop rotations; we observed that at the end of our study the BR doubled its value under NT, also the AMF spores increased their numbers in 400 % under CT but 530% under NT, while TGRSP increased 25% under NT and decreased in 10% under CT. The results revealed an enhancement of biological activities under conserved soils, which are mostly in agreement with our hypothesis.

Finally, in **Chapter V**, a general discussion, conclusions, and future directions are presented.

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CHAPTER I

General Introduction

1.1 General Introduction

Soil is the consequence of the transformation of geological material or bedrock through time by the influence of various physical, chemical and biological processes, which include natural mechanisms that participate in the renewal of its structure and the accumulation of nutrients in a significant way that allows the development and adaptation of different groups of living organisms (Cottle, 2004; Doula and Sarris, 2016; Kalev and Toor, 2018). Soil is a vital natural resource for humankind because it is where food production, animal husbandry, and water storage in deeper layers are carried out, while it maintains environmental quality at local, regional, and global levels (Bilotta *et al.*, 2007). From a biological point of view, soil is considered a living and dynamic ecosystem that hosts a large number of microorganisms and functions, mainly in the rhizosphere where root exudates and other mechanisms trigger benefits for plants productivity (el Zahar Haichar *et al.*, 2014; Arif *et al.*, 2020). The presence of water in the soil facilitates the osmotic flow from the soil to the plant, the gaseous part or air of the soil contains carbon dioxide (CO_2) and oxygen (O_2), which together develop an ideal environment for the roots of plants to grow and share pore space with organisms and give rise to a close relationship between organic and mineral material (Totsche *et al.*, 2018).

Soil microorganisms are the most abundant biota and represent a particularly important functional group responsible for several processes such as nutrient cycling, organic matter (OM) mineralization, soil fertility, soil restoration and plant health (Cardoso *et al.*, 2013; Sarma and Bhattacharyya, 2016). Their biological activity creates favorable conditions for seed germination and root growth, enhancing crop yields (Duchene *et al.*, 2017). Soils under conserved ecosystems tend to have greater microbial quantity and diversity, with higher populations of nitrogen (N) fixing bacteria, phosphorus (P) solubilizing microorganisms and arbuscular mycorrhizal fungi (AMF), among others (Gougoulias *et al.*, 2014). Into these populations, fungi constitute the largest microbial biomass in soil and their hyphae help in the OM decomposition (Igiehon and Babalola, 2018; Harman and Uphoff, 2019). Detailing, glomalin, which is glycoprotein secreted by AMF hyphae and spores, stabilizes soil structure (Vlček and Pohanka, 2020). However, intensive agricultural management practices have strong impacts on the number, activity, and diversity of microorganisms in the soil, affecting soil structure stability of the microbial communities and their biological functions (Manning *et al.*, 2018; Bongiorno, 2020). Regarding AMF, the study by Patanita *et al.* (2020) highlighted the negative effects of conventional tillage (CT) on the formation of hyphae network. When the removal of topsoil is constant, it impairs their ability to colonize plants.

Current research interest has been focused on soil microbial studies as a sensitive indicators of soil quality and health, showing relationships between microbial diversity, plants, soil and ecosystem sustainability (Naik *et al.*, 2019; Soto *et al.*, 2020). A balance in soil physical, chemical, and biological properties helps to carry out the development of plants and their environment. Although each of its properties are studied separately, they are all well-interconnected to each other, influencing on the soil quality (Webb *et al.*, 2017). Therefore, the degradation of any of the soil´s properties can put its quality at risk and become a threat to crop yields (Lal, 2015). In this sense, it is important to understand soil quality as "The ability of the soil to function within the limits of the natural or managed ecosystem, to sustain plant and animal productivity, to maintain or improve water and air quality, and to sustain human health and habitat" (Bünemann *et al.*, 2018). Since the understanding of such quality is a necessary component for maintaining or improving soil fertility and productivity, it is required to select quality indicators as measurement tools that provide accurate information about the soil´s properties and its current and potential status (Soto *et al.*, 2020). In the last years, biological properties have been widely used as good indicators to monitor soil quality because they can describe the main metabolic processes that take place in the soil and can respond more quickly to soil disturbances due to land use and management (Zornoza *et al.*, 2015). Several are the key biological parameters to determine soil quality; for this reason, Alvear *et al.* (2007) have considered convenient and practical to group them in general and specific parameters. The general ones include all those related to microbial activities such as C and N of microbial biomass, ergosterol content, among others; meanwhile the specific ones include extracellular hydrolytic enzyme activities related to C, N and P cycles.

The OM mineralization is one of the most important processes that occur in the soil, carried out mainly by heterotrophic microorganisms that use dead organic material as primary source of C and energy for their development. In this process, one part remains as soluble forms of nutrients available to plants, and another part of the OM is immobilized in the microbial biomass, so it also becomes a reserve of organic C and nutrients (Lindahl and Tunlid, 2015; Jalal *et al.*, 2020). The microbial biomass (MB) is the living fraction of OM present in the soil at a given time and is part of the energy reserve of the soil and a source of C, N, P and other nutrients required by plants (López-Mondéjar *et al.*, 2018). The MB comprises less than 5% of the soil OM; nevertheless, it performs crucial functions both in the soil and in the environment (Singh and Gupta, 2018). Also, MB is used as an indicator of changes in soil properties due to the management and environmental stress of agricultural systems (Jat *et al.*, 2020). Taking the above into account, Cmic is an effective biological parameter to contrast the effects of management systems in the soil. The Cmic represents 2 to 3% of the total organic C (OC) and can also be evaluated by BR (Cruz and López, 2015). The BR is a metabolic activity of micro- and macro-organisms evidenced through the release of CO_2 or consumption of O_2 (Dacal, 2019). The metabolic coefficient or specific respiration (qCO_2) is an indicator of the CO_2 released to the environment by the Cmic; therefore, it is a good indicator of the efficiency by which the Cmic uses the available C for biosynthesis (Rui *et al.*, 2016). In this sense, it can directly reflect the microbial activity according to the substrate availability.

On the other hand, enzymes are commonly used as indicators of soil quality, since they estimate the rate of processes carried out by microorganisms (Dick, 1994). Microbial enzyme activities play a fundamental role in the transformation and degradation of the SOM, nutrients cycling and intracellular metabolism (Li *et al.*, 2020). Because the great number of studied and known enzymes, it has been necessary to classify them according to their function, being the most studied enzymes the oxidoreductases (catalases, peroxidases, and dehydrogenases) and hydrolases (glucosidases, proteases, phosphatases, urease and arylsulfatase). Moreover, according to their participation in the N nutrient cycle, urease and protease are useful; for P highlight acid and alkaline phosphatases, and also phosphodiesterase; meanwhile for C, is widely used β-Gluc; and for sulfur usually the arylsulfatase is determined (Avellaneda-Torres *et al.*, 2012). Both glucosidases and phosphatases are extracellular enzymes of great importance produced by plant roots, mycorrhizae and other microorganisms. Phosphatase enzymes are responsible for the hydrolysis of several organic and inorganic phosphate esters into the P cycle (Vargas, 2010). Within the group of phosphatases are included the phosphomonoesterases, which are classified into acid and alkaline according to their pH for optimal activity, being an activity that usually increases at the soil surface and decreases with depth (Fraser *et al.*, 2017).The β-glucosidase stands out for its great participation in the degradation of carbohydrates in the soil, and its activity increases with greater amounts of microbial biomass, reflecting the capacity of the soil to break down OM and increase the availability of nutrients for plants (Acosta-Martinez *et al.*, 2007). The use of Fluorescein Diacetate (FDA) as an indicator of microbial activity is another technique widely used to determine enzyme activity in a generalized way, because this method is used to quantify the activity of enzymes such as proteases, lipases and non-specific esterases produced by different decomposing organisms, where the final product of the enzymatic reaction is fluorescein (Wang *et al.*, 2019).

The AMF are important microorganisms belonging to the phylum Glomeromycota, which form mutualistic associations with the roots of higher plants (Mathur *et al.*, 2018). These fungi participate in several plant nutrition process, since their hyphae help in the solutes and water exchange with the host plants and provide structure to the soil due to the glomalin production (da Silva Folli-Pereira *et al.*, 2013). Glomalin is a thermally stable and water insoluble glycoprotein produced by the walls of the AMF hyphae, which acts as a cementing material participating in the stability of aggregates (Gałązka *et al.*, 2017). Soil aggregates in turn, participate in water and nutrient storage, and gas exchange; therefore, it could have a positive impact on crops productivity by improving aeration, drainage, and areas for soil microbial activity (Singh *et al.*, 2013). The amounts of glomalin and the length of mycelium have been found to be significantly higher in undisturbed soils when compared to plowed soils (Borie *et al.*, 2000). Elements such as organic C and N, have been strongly linked to soil proteins related to glomalin, for this reason it is also considered as an important source of these elements for the soil (Wang *et al.*, 2017). When the hyphae of fungi stop fulfilling its nutrients and water transporting functions to the plant roots and die, the glomalin present in their cells is released and stored in the soil (Walley *et al.*, 2014).

According to Lozano (2016), the percentage of total accumulated C and N content in the soil varies according to the type of soil; thus, in tropical soils up to 5% C and N can be found, in mineral soils up to 25%, and in organic soils up to 52% C. As mentioned above, the soil OM plays a critical role in providing and improving soil structure, biological activity, C and N conservation, and erosion minimization (Junior *et al.*, 2020). In this sense, the implementation of diverse types of soil management can drastically modify the quantity and nature of soil OM. For instance, under conservative tillage system Page *et al.* (2013) and Studdert *et al.* (2017) indicate that the use of less aggressive tillage allows an enhanced plant residues incorporation, thus favoring the soil microorganism's quantity and activity due to higher levels of active C. Also, this can be related to an increased C storage ultimately improving the quality and health of soil, and the sustainability of the productive systems. Nevertheless, the effect of conservative tillage system is not immediate and can be appreciated after several years (Janušauskaite *et al.*, 2013). Variations in OM also depend on the quality and quantity of organic waste incorporated into the soil (Huang *et al.*, 2010). Detailing, soil represents an important reservoir of organic C as it contains approximately 55-60% of organic C by mass (Craft *et al.*, 1991). Soil organic carbon (SOC) is vital for biological activities since it is the energy resources to the heterotrophic organisms in the form of labile C, mainly low molecular weight organic compounds (Khatoon *et al.*, 2017).

Land management activities, such as tillage, have some traditional aims which are the improvement of soil structure for crops growing, the incorporation of organic amendments into the soil, and weed's control (Kladivko, 2001). According to the type of soils and cropping situations, the type and degree of tillage also varies, being possible to find some soils with relatively high, medium, or low 'tillage requirement' (Hillel and Hatfield, 2005). Regarding a high degree of tillage management, the CT is a system in which crop residues are incorporated into the soil by the action of the plow or disc harrow, and it generally consists of the inversion and loosening of the surface layer comprising the first 15-30 cm of soil depth by various tools, either manual or mechanical (Dao, 1996). Theoretically, this activity reduces soil compaction and creates favorable conditions for cropping such as aeration, weed control and the formation of the seedbed (Selim, 2019). However, when tillage is carried out crop after crop, it intensifies soil degradation been the arable layer the most affected (Badalíková, 2010). In a long-term basis, tillage can activate intense erosion processes by breaking up the soil aggregates especially in soil with steep slopes, causing the flow of O_2 into the soil which trigger a more intense OM mineralization process and decrease of its content, consequently diminishing crop productivity (Addiscott and Thomas, 2000; Nie *et al.*, 2019). By the contrary, direct sowing or NT is a technology that appears as an alternative to CT and constitutes a conservation tillage system where seeds are placed in the soil with minimal removal of the top layer, including many times the use of cover crops (Zhang *et al.*, 2009). No-tillage has several advantages and benefits for the farmers, in the short and long term; however, as mentioned by Shaxson and Barber (2008) soils that are degraded, susceptible to hardening, with crusted surfaces, low in fertility or have serious weed infestations, may not be immediately suitable for the NT system due to difficulties caused by compaction and poor porosity within the top layer and subsoil. In this case, the establishment of cover crops before the introduction of NT, and the adoption of crop rotations that incorporate large amounts of residues to the soil, will be able to progressively improve the soil physical, chemical and biological conditions for its implementation (Kim *et al.*, 2020).

In a crop rotation system, the remaining resources may be available for the second crop established in the same area (Khanh *et al.*, 2005). The appropriate choice of the species for the rotation largely benefits this practice, i.e. the use of leguminous plants greatly contributes to soil fertility due to its lower C/N relation with higher N contribution. On the other hand, grasses greatly contribute to the SOM for its higher C/N relation, thus enhancing the C accumulation (Rumpel and Kögel-Knabner, 2011). With a good level of coverage, progressive protection against erosion and soil degradation, NT also favors microbial diversity and activity in the soil (Sparovek *et al.*, 2007; Schmidt *et al.*, 2018; Ranaldo *et al.*, 2020).

In Ecuadorian highlands, volcanic ash-derived soils are predominant; there are specific environmental conditions and climatological factors in each altitudinal gradient that could be influencing the formation of the soils, additionally there is also a marked effect of the physical distances to the Andes mountain chain and the pyroclastic sediments that have given rise to the soils. At higher altitude and volcanic slopes, lower temperatures and humid conditions have favored the accumulation of SOM, while in the low zones, higher temperatures and greater rainfall facilitate its rapid decomposition and accelerated pedological development (Cruzatty and Vollmann, 2012).

The type of soils found in the Ecuadorian Andean region are Andisols, Mollisols, Inceptisols and Entisols. These soils are facing accelerated degradation problems due to erosion caused by environmental conditions (wind and rainfall) and by intensive tillage practices such as CT performed in steep slopes causing the loss of OM, soil fertility, acidification, salinization, higher toxicity levels due to chemicals use, which change the physical, chemical and biological properties of these soils (Suquilanda, 2008).

Calvache (2010) mentions the importance of soil conservation measures and efforts to protect top soil layers, such as the use of cover crops and SOM addition, avoid steep slopes plowing, crops diversification, reforestation, efficient water drainage perpendicular to the slopes, among other practices. Economical studies performed by Cruz, *et al.* (2010) have evidenced that maize and bean yields under CT presented higher soil erosion effects compared to grasslands, with the consequent economical affectations due to soil fertility loss. In Ecuadorian highlands, soil degradation has occurred along time due to the use of (intensive) CT practices and to the generalized no addition of OM to the crops in this region. In this sense, Espinosa (2008) suggests following soil conservation practices, as early response measures, successfully applied in other regions, once the effects of soil erosion are still reversible. In Ecuador, these practices, have only targeted the soil physical and chemical properties recovery, and need to include the enhancement of the soil biological characteristics to increase soil fertility and soil microbial activities by continuos SOM incorporation.

Using the previous antecedents as a general framework, it must be highlighted that Ecuador presents scarce studies regarding the effects of crop rotations under CT or NT managements. Therefore, research focused on the improvement of crop production is strongly needed, especially in areas where the erosion and the loss of productivity is increasing as the Andean highlands. Ecuador presents about 26 maize (*Zea mays* L.) landraces in the Andean highlands, with their unique and adaptive capacities to climatic and stress conditions (Tapia *et al.*, 2015). The other crops considered for this project, adapted to grow in the research area, were beans and amaranth. The Andean bean (*Phaseolus vulgaris* L.) is well-known for its chemical compositions and cooking characteristics which contribute to a healthy diet (Kajiwara *et al.*, 2020). The Andean cereal, *Amaranthus caudatus* L., is also a very important crop rich in iron, copper, manganese, and zinc (Nascimento *et al.*, 2014). This cereal has been ultimately preferred by consumers for its health benefits because it is well known for its amino acid profile, having more lysine than other cereals (Kurek *et al.*, 2018).

Regarding other critical factor in crop production as the fertilization, N is the nutrient which the major influence on crop yields due to the significant amounts required and its deficiency in agricultural soils (Olson and Kurtz, 1982). The SOM is the most important N reserve since it contains more than 90% of the total soil N; however, it is available to the plants after its mineralization, when ammonium (NH_4^+) and nitrate (NO_3^-) are released. In this process, the Cmic uses it first to form proteins and then releases it, becoming available for plant growth (Cassity-Duffey *et al.*, 2020). The N availability under NT is lower during the first years because it is immobilized for a longer time in the Cmic. For this reason, it is necessary to apply additional amounts to maintain production (Yansheng *et al.*, 2020). Another element which frequently affects crop production is the P, and the factors which influence its availability are pH, texture, temperature, and OM content (Richardson and Simpson, 2011; Shaw and Cleveland, 2020). Under NT systems, the reduced mobility of P in the soil results in the stratification of this element in soil, where the highest concentrations are found at the surface due to the accumulation of residues and surface fertilization. Additionally, the considerable amount of residues derived from the agricultural activity itself (between 55 and 75% of all crop biomass) can contribute to the increase in OM level and soil fertility (Coelho *et al.*, 2020). In the study conducted by Alvarado *et al*. (2011), it was determined that adequate waste crop residues incorporation under a NT system allowed storing large amounts of minerals and nutrients in the soil.

Based on all the previous antecedents, we proposed the following hypotheses and objectives for the present Doctoral Thesis.

1.2 Hypotheses

I.- A conservative soil management system such as No-Tillage will increase the main biological activities in the bean-amaranth-bean and bean-maize-bean crop rotations in an Andean soil from the highlands of Ecuador, when compared to Conventional Tillage with plowing.

II.-Higher biological activities and arbuscular mycorrhizal fungal structures densities are expected to occur in an Andean soil from the highlands of Ecuador, under No-tillage and low fertilization rates.

1.3 General objective:

To determine the effect of two contrasting soil management systems: Conventional Tillage *vs*. no-Tillage, on the biological activities, and arbuscular mycorrhizal symbiosis densities in the bean-maize-bean and bean-amaranth-bean crop rotations, subjected to different fertilization levels in Andean soils from the highlands of Ecuador.

1.4 Specific Objectives:

1. To describe soil microbial activities in Andean soils from the highlands of Ecuador after the bean-maize-bean and bean-amaranth-bean crop rotations under contrasting tillage practices.
2. To study the effect of fertilization increasing rates on the rhizosphere´s biological activities occurring in bean-maize-bean and bean-amaranth-bean crop rotations.
3. To evaluate the effect of soil management practices on arbuscular mycorrhizal fungal structures after bean-amaranth-bean and bean-maize-bean crop rotation.

CHAPTER II

Review:

"Impacts of tillage on the soil biological activities: The microbial component as key for sustainable agriculture in the tropics"

Review

¨Impacts of tillage on the soil biological activities: The microbial component as key for a sustainable agriculture in the tropics¨

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Abstract

Tillage practices have shown either beneficial or degrading effects on the physical, chemical, and biological properties of soil, mainly depending on the tillage system used and the origin and characteristics of the soil. Detrimental impacts such as soil erosion are caused by excessive rain, inappropriate irrigation, nutrients depletion and by human actions like intensive use of conventional tillage (CT), which usually deteriorates the soil structure and increases C mineralization with concomitant changes on the soil microbial communities. These effects are enhanced in soils with higher temperatures and organic matter (SOM) mineralization rates such as those of the tropics. Several studies worldwide have demonstrated the effects of CT and no-tillage (NT) systems over soil characteristics. A conservative tillage system like NT, shows physical and chemical advantages over other systems, with an enhanced microbial biomass and biological activities in the soil. This influences directly on soil quality and fertility with beneficial effects on crop production. Adopting NT systems increase the sustainability of soil by increasing soil organic matter (SOM) amounts and stability, microbial biomass, enzymatic activities, soil respiration, and abundance of arbuscular mycorrhizal fungi (AMF) propagules compared to CT. Biological activities are recognized as good soil quality indicators, and their assessment gives important information about soil quality and sustainability. This review shows a summarized compilation of studies that demonstrate the effects of tillage practices over soil quality in tropical soils. Understanding soil dynamics and the effects that conservative tillage practices have on the biological properties of soils from the tropics, denotes a major opportunity for developing research in these fields and will contribute to find new insights towards agricultural sustainability to enhance both, world nutrition and soil conservation.

Keywords: Tropical soils, conventional tillage, no-till, biological activities, AMF.

2.1 Introduction

During the last five decades, world population has multiplied faster than ever before, and it is projected that it will continue growing. The world population in 2005 was 6.5 billion, and it is expected that by 2050 it will rise to more than 9 billion people, increasing the global crop demand in more than 100% (Tilman *et al*., 2011; United Nations, 2014). To satisfy the global food demand, high crop yields must be achieved using intensive cropping practices which include monoculture, continuous cropping, conventional tillage, intensive livestock systems, hillside fragile areas cultivation, increasing use of inorganic fertilizers and pesticides, together with an inadequate water use (Killebrew and Wolff, 2010, Sanaullah *et al.*, 2020).

A **s**ustainable agricultural approach promotes reaching efficient, safe and high-quality food production with environmental protection and, at the same time, improvement of social and economic conditions of farmers, their families and local communities (SAI, 2010). Despite that, intensive farming is practiced in developing countries to supply the food demand although this activity leads to an accelerated soil degradation, desertification, global climate change and loss of biodiversity (Lal *et al*., 2007). Under this context, sustainable agriculture seeks integrated, resource conserving, equitable farming systems that reduce environmental degradation, maintain agricultural productivity, promote economic viability (in the short and the long-term), and maintain stable rural communities with enhanced life quality (Snapp and Pound, 2017). Sustainable or conservation agriculture is based nowadays on three main principles: minimal soil disturbance (reduced and no-tillage), permanent soil cover (mulch), diversified crop rotations, combined with adequate fertilization doses (Mutual *et al.,* 2014).

Traditional tillage intensifies soil degradation exposing subsoil layers poor in nutrient contents. In general, inappropriate amounts of fertilizers are added to soil to replenish nutrients (sometimes with no fertilization at all), resulting in low crop yields with less plant residues and low biomass accumulation on the soil affecting the productivity of the next crops (Liniger *et al*., 2017). Under this situation, new and innovative soil management practices have been suggested to promote soil coverage and eliminate soil removal for cropping activities. In North America, Brazil and Argentina minimum and no-till (NT) practices have been successfully utilized, demonstrating that NT improves soil chemical, physical, and biological properties, thus enhancing soil fertility and crop productivity (Tiecher *et al*., 2017, Mirzavand *et al.*, 2020).

Although the benefits of conservation tillage have been widely demonstrated in developed countries of Europe, Asia, and North America, this concept has been difficult to introduce among small plot owners from developing countries. One reason could be the farmer´s cultural perception towards tillage based on a deep-rooted relationship of the peasant men and women with the ancestral land on the way to obtain their products. Moving the soil for planting purposes is a faster, easier, and traditional way to approach crop production, compared to the more demanding hand labor needed for NT (Espinosa, 2014). Smallholder farmers use intensive (conventional) tillage (CT) to grow their crops, causing long-term degrading effects in their plots, and changing the physical, chemical and biological properties within the soil. A study by Hunke *et al*. (2015), based on a database compilation of near 65 studies in the Brazilian Cerrado, has demonstrated that changes in land use of natural ecosystems, which have been transformed to agricultural productive lands over the last 20–30 years, has degraded soil physical, hydrological, and biogeochemical soil properties, leaving cropland soils more susceptible to surface erosion. This study showed reduction in the infiltration rates near the soil surface, lower soil aggregate stability in croplands than in Cerrado and pastures, topsoil pH and nutrient concentrations were higher in croplands and pasture due to fertilization, showing extremely high extractable P concentrations, while lower N levels than in the natural ecosystem, also higher temperature, oxi-reduction potential, and very low oxygen concentrations were found in the productive areas. Hunke *et al*. (2015) concluded that most soil and water parameters were altered due to land use intensification for crop production, plantations, or pastures. Despite the biological processes that occur in ecosystems, which serve as early warning indicators of environmental stress (Dick, 1994; Obalum *et al*., 2017), at the present NT use is scarce. The biological activities such as microbial respiration, enzyme activities, microbial abundance, soil organic matter, mycorrhizal propagules, among others, are good soil quality indicators which are positively affected by conservational tillage practices such as NT. No-tillage efficiently enhances microbial activity, soil organic carbon accumulation, and arbuscular mycorrhizal fungi (AMF) propagules in the soil, benefiting soil structure (Cornejo *et al*., 2009; Curaqueo *et al*., 2011; Vazquez *et al*., 2019).

Under NT practices, with no residue removal, the soil microbiota is involved in various processes like degradation of crop residues, stimulation of nutrient cycling, and the proliferation of beneficial microorganisms such as mycorrhizal fungi (Mickan *et al*., 2019). The presence of mycorrhizal fungi is associated with soil structural stability, nutrient cycling and transportation to plant, stress alleviation, soil toxicity reduction, and higher crop productivity (Cornejo *et al*., 2008; Borie *et al*., 2010; Barea and Pozo, 2013; Seguel *et al*., 2013, Abdel-Fattah *et al*., 2016).

Considering the beneficial effects evidenced in developed and some developing countries undergoing NT practices, this review discusses the contrasting effects of intensive *vs*. conservative tillage systems over soil properties in tropical areas, and the beneficial implications on the biological activities, including mycorrhizal symbiosis.

2.2 Tropical regions and the main biological indicators of soil quality

2.2.1 Origin and distribution

Soil, defined as the naturally occurring unconsolidated mineral and organic material at the earth's surface, which provides a natural resource for living organisms, has an important role in controlling the earth's environment, and thus affects the sustainability of life on the planet (Paul, 2014; Hou *et al.*, 2020). Soil can be also conceptualized as a nonrenewable resource that interacts with the ecosystems and its agricultural capacity to produce food (Wingeyer *et al*., 2015).

Different types of soils and nutrients within them are main sources for global food production. Tropical soils occur in Tropical Rain Forests and in Tropical Seasonal Forests like Savannas; some of them are located where active and recently extinct volcanoes are. In general, they are very fertile soils that support extensive agricultural development (Lal, 2016; Roberts *et al*., 2017). The principal countries or regions of the world where these types of soils are present are: In the American continent: Costa Rica, Panama, Honduras, Guatemala, El Salvador, Colombia, Ecuador, Peru, Bolivia, Brazil; Asia and the Pacific: Hawaii, Philippines, Fiji, Samoa, New Zealand, Tonga, Indonesia, Solomon Islands, Papua New Guinea; in Africa and the Indian Ocean: Rwanda, Kenya, Tanzania, Cameroon, Reunion, Madagascar, Uganda, Sudan and Democratic Republic of the Congo (Saatchi *et al*., 2011). As it can be seen, most of the tropical soils from the global land surface, are situated in the Northern part of South America, Central Africa, Southeast Asia and Oceania.

In this sense, soils near the equatorial line are highly weathered as those that are found in the flat lands of Brazil, Peru, Ecuador, Colombia, Venezuela and Central America. A set of different soil types are also present in the tropical areas of these regions (Francisco-Nicolas *et al*., 2006; Wood *et al*., 2019). Without the presence of the Andes, soils in all these countries would have been Oxisols or Ultisols as the ones prevailing in the savannas of South and Central America. When the soils get further apart from the Equatorial line and move closer towards the tropical and subtropical limits, soils dominated by clays appear (soils with vertic properties). It is interesting to find Vertisols in Nicaragua, Costa Rica, Panamá, Colombia, Ecuador and Perú, showing the high variety of soils in Tropical America. These soils are formed in the tropics in areas of low precipitation adjacent to the Pacific coast where the climate has been modified by the Humboldt current (Walter and Breckle, 2013). However, most of the soils in countries with the Andes mountain range influence are Entisols, classic Inceptisols and Andisols formed from recent volcanic ash depositions. These soils sustain a high percentage of human population from different countries in this region. The altitude radically modifies the climate, and this process is evident in the highlands of tropical America where it is easy to find a diversity of microclimates that change in relatively short distances. This aspect determines the high diversity of crops, which can be produced all year around due to thermic conditions. In addition to the weather modification, the volcanic ash deposition is an important factor that adds to the soil diversity of the region (Boada and Espinosa, 2016; Yang *et al.*, 2018).

In Tropical South America, these soils cover significant areas. The clay fraction of these soils is dominated by allophane, imogolite and halloysite (short range amorphous minerals) derived from the weathering of pyroclastic materials coming from volcanic deposition. One of the main characteristics of those soils is the capacity to immobilize P on the surface of the amorphous minerals. This is probably the main chemical constraint there. However, it has been demonstrated that the P fixation capacity varies with the dominant clay type, a condition determined by the altitude effect. In these regions, the presence of Aluminum forms strong humus-Al complexes. The trapped Al interacts, in a ligand exchange reaction, with $HPO_4^{=}$ and $H_2PO_4^{-}$ strongly fixing P. In Andisols from

Japan, Colombia and Ecuador this process has been well documented (Zehetner *et al*., 2003; Nishikiori *et al*., 2017) and understood as an important mineral (P) limitation for agricultural production (Neocleous and Savvas, 2019). Phosphorous uptake is limited due to the chemical form in which this mineral is present in the soil, and because it forms mineral complexes, which fixes the available P. The P deficiency in the soil causes reduced yields and may weaken the plant, leading to higher susceptibilities to organisms that usually would not affect them (Naik *et al.*, 2019). Therefore, nutrient availability in the soil helps control plant diseases and have main roles in agricultural sustainability (Gupta *et al*., 2017). In this sense, the living soil organisms, which act as biological indicators of the soil quality and the dynamics occurring there, are also affected by changes in soil properties and nutrient availability (Tajik *et al.*, 2020).

2.2.2 Biological indicators of soil quality

Agricultural management practices strongly affect soil quality. Some physical and chemical indicators of soil quality are bulk density, aggregation, texture, organic matter content, fertility, and salinity components (Tesfahunegn, 2016), but besides them, the biological and biochemical properties constitute more rapid indices to management practices and perturbations, meaning that biological indicators have faster responses to changes than physical and chemical indicators (Gianfreda and Ruggiero, 2006; Paz-Ferreiro *et al*., 2007). Among the most representative biological indicators of soil quality, the microbial biomass, soil enzymatic activities, soil respiration, N mineralization rates, bacteria to fungi ratio, proportions of diverse functional groups of soil fauna such as earthworms and nematodes number, are among the most commonly assessed (Shao *et al*., 2008; Brackin *et al*., 2017; Mahdi *et al*., 2017). Biological or microbial indices, mostly based on soil enzymatic activities, are key indicators of microorganism mediated processes in soil. There is a large diversity of enzymes in soil (near 500 reported enzymes with crucial roles in C, N, P and S cycles) closely correlated to the diversity of soil communities, complexity of the organic matter (SOM) and to the soil physical matrix, as well; but only a few have been identified and assessed (Gianfreda and Ruggiero, 2006, Ozlu *et al.*, 2019). Microorganisms are not the only producers of soil enzymes; plants and soil mesofauna are also responsible for these processes (Tabatabai and Dick, 2002). Although quality indices are presented in many reports (Bastida *et al*., 2008), their applicability to specific climatological, agronomic, among other conditions must be considered.

A study by Suzuki *et al*. (2009), revealed that bacterial communities were more related to soil type (Cumulic Andosol, Lowhumic Andosol, Yellow Soil, Gray Lowland Soil) than to fertilization (chemical fertilizer, rice husk and cow manure, pig manure application) while interestingly, fungal communities were related to fertilization more than soil type, suggesting, that the fungal communities can be strongly affected by fertilization (Baum *et al.*, 2020). This may indicate also that other soil management activities such as tillage, fumigation, cropping and crop rotation, among others, influence on the soil biology as well, especially considering the fungal component. The above is so important since there is scarce information about biological indexes in tropical soils, then, opportunities to understand these processes are abounding in the tropical regions.

2.3 Tillage practices and their effect on the microbial component

According to reports by FAO (2016) and Tamburino *et al.* (2020) the global food productivity has started to decline; tough from a different point of view, this inconvenient situation could be an opportunity to take better agricultural decisions for preserving and protecting tropical soils, as natural non-renewable resources. Although tropical soils have a high potential for agricultural production, it is unfortunate that they are facing gradual degradation processes due to the serious soil erosion caused by the intensive land use (Giam, 2017). Soil erosion is considered worldwide a major environmental and human threat that affects the agricultural capacity of land to produce food in a sustainable way (Wuepper *et al.*, 2020). It has been calculated that more than 10 million hectares are lost to erosion each year, and nearly one-third of the world´s arable soils have been lost during the last 40 years. The global erosion rate recently reported is between 7 to 11 Mg/ha/year for severely eroded soils (Nearing *et al*., 2017). When soil degradation becomes severe, landowners react reluctantly to use soil recovery practices due to the high economical costs that imply soil reclamation. Nevertheless, when soil degradation is gradual, soil erosion can be controlled with conservation and correct land management practices (Fernandez, 2017). Hence, the importance to socialize and promote proper soil management systems within landowners to achieve higher yields that help sustain their family, as for example the type of tillage implemented and the consequences in the agroecosystem.

2.3.1 Tillage, definition and types

As it is understood, intensive agricultural practices, rainfall, wind, water runoff are main factors causing erosion problems in soils, so this becomes an important incentive to think on agricultural practices that can protect tropical soils from erosion, and maintain their quality. Tillage is a very common practice used in the modern agriculture, based on a mechanical manipulation that serves different purposes: soil leveling, seedbed preparation for planting, fertilizers, manures and pesticides distribution, faster decomposition of crop residues through physical breakdown and its incorporation into soil (Chalise *et al.*, 2020). Tillage is also used as a post-emergence weed control and management tool to disrupt or reduce the incidence of diseases and pests (Bailey and Lazarovits, 2003). In agriculture, two main and contrasting tillage practices are used to prepare the soil and produce crops: traditional or conventional tillage (CT), and No-tillage (NT). Conventional tillage includes moldboard plowing, disk harrow or field cultivator, chisel plow, and a combination of newer tools. In CT, little or none crop residue is left on the ground, and soil removal is used to bury weed seeds and control disease-bearing crops. On the contrary, with the conservation tillage alternatives, the main goal is to maintain an effective amount of plant residue covering the soil surface (at least 30 %) to reduce critical erosion due to environmental factors. Conservation tillage alternatives consist of a range of practices that include non-inversion of soil, which helps to retain soil moisture and reduce soil erosion, protects its quality and fertility, and prevents its compaction and degradation (Peigné *et al*., 2007; Shrestha *et al*., 2020). More detailed, some of the conservation tillage systems include NT, strip tillage (ST), shallow tillage, ridge tillage, mulch tillage and reduced tillage (RT). Under NT systems, the soil is left undisturbed; the only soil disturbance comes from seed planting. In this practice, more than 70% of the surface is covered by crop residue. Strip-till is a conservation tillage system that minimally disturbs the soil only for planting or drilling for seeding and leaves the necessary amount of crop residue on the surface after planting. Berms created under this system maintain soil relatively warmer. Under Ridge-till (also known as ridge-plant or till-plant), the soil is left undisturbed from harvest to planting except when fertilization is applied (Simmons and Nafziger, 2014). Crops are planted and grown on ridges formed in the previous growing season, which are annually built and reformed during row cultivation. Tools such as coulters, or horizontal disks are used in most ridge-till systems. These attachments are helpful to remove soil surface residue and weed seeds. To reform the ridges, heavy-duty row cultivators are used. Mulch-till includes any conservation tillage system other than no-till and ridge-till, performed with a chisel or subsoiler plow; tillage before planting include one or more passes with a disk harrow, field cultivator, or combination tool. Under this system herbicides and weed controls are used (Simmons and Nafziger, 2014).

According to Kabir (2005), tillage practices affect chemical and biological properties at microscopic level. This author mentioned that tillage practices negatively affected AMF species richness, infectivity, and survival, while interestingly, conservation tillage increased AMF survival, improved soil aggregate stability and phosphorous uptake by plants. It has also been studied how tillage systems and previous planted crops affect the percentage of residue in the soil surface. There is an effect of characteristics of the crops on the type and amount of residue that they produce, and it influences on its effectiveness to soil protection. In general, with a higher crop yield, more residues will be produced. It has been noticed that crop residues in soil surface, effectively reduce erosion. It´s been reported that a residue cover of 20% to 30% after planting can reduce soil erosion by approximately 50% compared to a bare field, while residue cover of 70% after planting can reduce soil erosion by more than 90% compared to a bare field (Dooley *et al*., 2005).

In this context, soil organic matter (SOM) plays a crucial role protecting the soil surface against erosion among other functions. SOM is defined as diverse organic materials, such as living organisms, plant and animal organic residues, and well-decomposed plant and animal tissues that vary considerably in their stability and in their susceptibility to further degradation into materials known as humus (Mohammadi *et al*., 2011). SOM is rich in nutrients such as nitrogen (N), phosphorus (P), sulfur (S), and near 50% of carbon (C) (Overstreet and DeJong-Huges, 2009).

Crop residues that were once left on the surface to protect soil from wind and rainfall undergo a decomposition process and result in SOM (Medina *et al*., 2015). In tillage practices, SOM is lost mainly through C mineralization due to breakdown of soil aggregates, leaching of organic C, and accelerated rates of erosion, directly affecting availability of soil microorganisms and their biological activities (Kooch *et al.*, 2020).

In general, soils with high OM content have higher microbial activity, which can be assessed by measuring microbial respiration. Generally, microbial respiration is quantified using soil incubation methods designed to measure the final products of the process such as CO_2 evolution. These determinations have brought insights on mineralization and C stability associated with OM quantity and quality related to soil management practices. Mineralized C and N have been reported as excellent indicators of organic C and N which represent an active fraction of the SOM (Cardoso *et al*., 2013; Wang *et al*., 2016; Trivedi *et al*., 2017).

Soil organic matter brings beneficial effects to soil, and it is recognized as the most reliable indicator for soil quality due it is crucial in maintaining soil structure, humidity, and serves as nutritive reserve for microorganisms (Rajan *et al*., 2010; van der Wal and de Boer, 2017). A main role of SOM is the improvement of the soil´s water holding capacity by binding particles together to form aggregates (Hunting *et al*., 2016; Lavelle *et al.*, 2020).

Soil organic matter contributes to the formation and stabilization of soil structure (Di Lonardo *et al*., 2017; Zhao *et al*., 2017), thus maintaining its quality and health. Table 1 shows the effects of tillage on some volcanic soils including tropical soils and on its biological properties.

Table 1. Main effects observed on biological activities under different volcanic soils and tillage systems

Soil	Measurements	Crop	Biological activity parameter and observed effect under NT and CT systems	References
Volcanic Ultisol from Southern Chile	Measurements taken in Summer, after a 3-year rotation cycle	Wheat-lupin-wheat rotation sequence	MBC/MBN: 3,57% higher values under NT than CT	Alvear *et al*. (2005)
			Dehydrogenase activity (µg RF g^{-1}): 11% higher values under NT than CT	
			Acid phosphomonoesterase activity (µmol PNP g^{-1} h^{-1}): 24% higher values under NT than CT	
			Arylsulfatase activity (µmol PNP g^{-1} h^{-1}): No changes between soil management	
			β-Glucosidase (µmol PNP g^{-1} h^{-1}): 13% higher under NT than CT	
			Urease (µmol NH_3 g^{-1} h^{-1}): 312% increase under NT than CT	
Volcanic Mollisol from Andean Ecuador	Soil samples were collected and analyzed after a 10-year continuous NT and CT practice	Comparison between naturalized grasslands, maize, and potatoes	SOM content %: 29.72 and 1.9 % higher values under NT compared to CT (maize and potatoes plots) respectively	Avila-Salem *et al*. (2020)
			Acid phosphomonoesterase activity (µmol PNP g^{-1} h^{-1}): 5% higher values under CT (potato) than NT (naturalized grasslands)	
			Fluorescein diacetate activity: (µg Fluorescein g^{-1} h^{-1}): 46% higher values under NT than CT (potatoes)	

Volcanic soils from French West Indies: Umbric Andosols, Haplic Nitisols, Luvisol, Haplic Ferralsol, Ferralic Cambisol, Vertic Cambisol	Measurements were taken 3 years after last ploughing, at flowering stage	Banana mono-cropping	Soil microbial respiration in 0-10cm layer (µL CO_2 h^{-1} g^{-1}) : 69 % higher values under NT than CT	Clermont-Dauphin *et al.* (2004)
			Earthworm biomass in interrows (g m^{-2}): 313% higher values under NT than CT	
			Earthworm number in interrows (No. m^{-2}): 229% higher under NT than CT	
Inceptisols from Andean Peru	Soil samples taken after 6 years of fallow between cropping phases	Forest, pasture and cropping system	SOM: 2 times higher (63.6 g kg^{-1} in forests than in pasture and cropping systems (30.8 g kg^{-1})	De Valença *et al.*, (2017)
Aquic Inceptisol	Samples taken at harvesting time	Maize and wheat	Alkaline Phosphatase activity (mg. g 24h): 10% higher under NT than CT	Hu, (2015)
Epihumic Wet Andosol	Samples taken and analyzed in autumn after an 8 year of NT, MP (moldboard plow) and RC (rotary	Upland rice (*Oryza sativa*) for 5 years followed by soybean (*Glycine max*) for 3 years.	Nematode density (individuals per 20 g soil): 26.11% higher under NT compared to MP, and 2% higher under NT compared to RC.	Ito, *et al.*, (2015)

	cultivator), cropping.			
Andisol from central Colombia	Measurements taken after a 7-year rotation	Potato based rotations	SOM ($g \cdot g^{-1}$): 29 % higher under RT than CT	Quintero and Comerford (2013)
Pumice temperate Andisol from Japan	41 years of NT (apple orchard) and CT (wheat-soybean) land management.	Apple orchard *vs.* Wheat-soybean rotation	SOM ($g\ kg^{-1}$): 78% higher values under NT than CT	Rahman *et al.* (2008)
Volcanic Ultisol from Southern Chile	Measurements taken for each crop rotation during a 4 year rotation cycle	Oat–wheat and white lupine– wheat rotation	Acid phosphatase (μg PNF g^{-1}): 0,15% lower under NT than CT	Redel *et al.* (2007)
Andisol from central Mexico (Patzcuaro Watershed)	Sampling time was at 5 years after maize was sown	Maize	Dehydrogenase (μg INTF g^{-1}): 139% higher values under NT compared to CT	Roldán *et al.* (2003)
			Urease ($\mu mol\ NH_3\ g^{-1}\ h^{-1}$): 812% higher values under NT compared to CT	
			Protease (μmol NH3 $g^{-1}\ h^{-1}$): 920% higher values under NT compared to CT	
			Phosphatase (μmol PNP $g^{-1}\ h^{-1}$): 214% higher values under NT compared to CT	
			β-Glucosidase (μmol PNP$g^{-1}\ h^{-1}$): 123% higher values under NT compared to CT	

Fine-loamy Typic Fragiudalf soil Northeastern USA	Soil samples were collected and analyzed after rotations ending	Corn-corn, and corn-soybean in a 2-year rotation, and corn, oats, and alfalfa or mixed grass meadow in a 3-year rotation	SOM content %: 13% higher values under NT compared to CT	Sengupta and Dick, (2015)
			Bacterial taxa abundance. Total number of raw sequences reads: 45% higher values under NT than CT	
Ultisol from Australia	Analysis performed after 14 years of continuous tillage	Soybean-oat	SOM content %: 102% higher under NT than CT	So, (2009)
Volcanic Andisol from Japan	Values shown after 4 years of tillage practices	Barly and soybean rotation	Soil respiration (g CO_2-C m^{-2}): 12% decrease under NT compared to CT	Yonemura *et al.* (2014)

Tropical Aquic Cambisol China	Soil samples were collected and analyzed after 5 year rotations	Banana-pineapple (BA), banana-cowpea (BP), banana-rice (BR), and banana monoculture (CK)	Microbial biomass C (MBC), urease (UA), β-glucosidase (GA) and dehydrogenase (DHA) significantly higher under NT compared to CT. Microbial biomass N (MBN), invertase (IA), DHA and acid phosphatase (APA) were on average 42.1%, 47.9% and 36.7% higher in BA, BP and BR compared with CK, respectively. Average MBN and catalase (CA) were 28.1% and 39.6% higher under NT compared to CT.	(Zhong *et al.*, 2021)
NT: no-tillage; CT: conventional tillage				

Conservation tillage (such as minimum tillage or NT, along with crop rotation and residue soil coverage) increases SOM and improves root development, its aggregation and structure (Curaqueo *et al*., 2010) been probably the soil management practice with better long-term impact (Baker *et al*., 2007; Lal, 2011). On the contrary, intensive tillage (CT) and residue burning can diminish SOM, exposing the soil to the negative climatic impacts, and depriving soil organisms from their primary carbon energy source due to soil microorganisms use SOM as food, and break it down by means of mineralization and transforming it into compounds that the plants can take (Lal and Stewart, 2010; Mthimkhulu *et al*., 2016).

In the african region, conservation tillage (NT) increased SOM content enhancing soil aggregation, soil fertility and a reduction in soil erosion, important for a sustaible agriculture in Africa (Mrabet, 2002). On the other hand, Jat *et al.,* (2011) mention that reduced tillage (minimal soil disturbance, surface retention of crop residues & efficient crop rotations) in south Asia, has helped to produce more at less costs, reduce environmental pollution, promote use of organics, improve soil health and enhance crop yields in the region. Moreover, in Australia, the use of NT, has also been adopted, however, ocasional and strategic tillage is performed for pest and weed controls to ensure long term productivity and dryland farming systems based on sustainability, in southern Australia (Conyers *et al.*, 2019).

Based on the above, it is logical in general terms, that NT practices enhance soil quality indices. Nevertheless, the adoption of conservation tillage in a particular site depends on the type of soil, crop, and rotations, and thus it is important that farmers adopt the most sustainable soil and crop management practices that will protect soil resources and guarantee food production for a growing world population (Swinton *et al*., 2015). No-tillage and minimum or reduced tillage are main soil conservation activities that need to be globally practiced and reinforced (Busari *et al*., 2015), to help maintain adequate SOM content and soil microbiota (Frouz, 2020). A more detailed effect played by tillage systems on microbial activities will be deepened in the next paragraphs.

2.3.2 Tillage effects on microbial activities

Soil microorganisms (archaea, bacteria, fungi, algae and protozoa) have important roles and functions in soil, including nutrient cycling, soil structure improvement, and organic matter (OM) transformation and decomposition. According to Green *et al*. (2007), soil microorganisms that participate in nutrient cycling process, soil stabilization, and OM decomposition also create soil microporous channels. This can explain the lower N and P content in the upper layers of soils under conservation tillage due to nutrients leaching (nitrate-N, and P) to deeper soil layers. Even though soil microorganisms respond differently to environmental stresses, it has been widely reported that CT practices have negative effects on soil biomass or organism population abundance (Kabiri *et al*., 2016; Acir *et al.*, 2020). Different functional microbial groups and its interactions are differently affected by CT and NT, and they vary according to the soil type, crop type, residue incorporation, climate (van Capelle *et al*., 2012), among other factors.

Besides SOM, other biological parameters, including microbial biomass, respiration, soil amino acids, enzyme activity, and earthworm's presence have also been suggested as soil quality indicators, along with physical parameters, such as water-filled pore space, which influence biological activity (Datt and Singh, 2019). In detail, soil microbial biomass (SMB) is the mass of living microbial tissues. A higher SMB is found in NT soils in comparison with CT soils as a consequence of a greater storage plant biomass on the undisturbed surface, increased moisture retention, lower soil temperature, and improved microbial activity (Heidari *et al*., 2016; Shen *et al*., 2016). Additionally, NT provides a better aggregate structure and improves soil properties like N content, CEC (Cation exchange capacity), and SOC content, but decreases C/N ratio compared to CT systems (Moussa-Machraoui *et al*., 2010; Celik *et al*., 2012). A greater microbial biomass accumulation is found on the upper layers of non-plowed soils than plowed soils (Sun *et al*., 2020). Table 1 summarizes most recent studies on tillage in volcanic soils and its effects on different microbial activities, showing in general an enhancement in the biological activities when NT is practiced. In this data recompilation, Alvear *et al*. (2005), Avila-Salem (2020), Roldán *et al*. (2003), Hu *et al*. (2015b) and Redel *et al*. (2007) showed that the enzymatic activities studied in soils were in all cases higher under NT that under CT. In the same sense, higher OM content was found in the studies by De Valença *et al* (2017), Quintero and Comerford (2013), Rahman *et al*. (2008), Sengupta and Dick (2015), and So *et al*. (2009).

Another soil quality parameter is microbial respiration, defined as O_2 consumption and CO_2 liberation by microorganisms. CO_2 formation is a last step in the C mineralization. This is one of the oldest parameters used to quantify microbial activity in soils, which permits an estimation of the general biomass activity and allows a prediction on how weather, physical and chemical properties, or agronomical practices such as land management and crop rotations could affect microorganism dynamics (Stagnari *et al*., 2014).

For instance, in Chilean temperate volcanic soils, continuous crop cultivation has been reported to be responsible for soil C loss, especially in those where C has not been transformed into humus, indicating that total C tends to be more mineralized (Aguilera, 1990). In Brazil, two long-term experiments carried out on Oxisols demonstrated that CO_2 respiration, infiltration rates and aggregate stability were effective soil quality indicators of changes occurring due to cropping and tillage systems. Results from this study showed that the adoption of NT and use of cover crops increased CO_2 respiration, suggesting higher biological activity. In NT, enhanced soil aggregate stability, increased infiltration rates, and reduced soil erosion were also observed in comparison with CT (Sithole *et al.*, 2019). In a subtropical Oxisol from Brazil, the increment in crop residue input and biological activity under NT was associated with an improvement in soil fertility and soil quality, compared to CT (Campos *et al.*, 2011). Crop residues are helpful in reducing and preventing soil degradation. Consequently, this information points out that soil quality recovery is possible in soils from the highlands of tropical Latin American where NT practices could be used along with crop rotation, balanced fertilization, and crop residue accumulation (Campos *et al.*, 2011; Calegari *et al.*, 2020).

2.3.3 Tillage effects on the microbial abundance and diversity

As recently mentioned by Lal (2015), tillage practices are critical for sustaining soil quality which is a necessary condition for having successful crops growth and yields. A three-year study conducted in a clay soil from the United States by Leskovar *et al*. (2016) to evaluate the effect of strip tillage-ST (a conservation system that uses a minimum tillage) *vs*. conventional tillage-CT practices, demonstrated an increment in ST of 275% in nematodes, 49% in total bacteria, 27% in active bacteria, and 37% active and total fungi abundance increase in the conservation tillage treatments compared to CT. The lower number of microorganisms (less bacterial and fungi diversity) reported in CT was corroborated by Sengupta and Dick (2015), who reported that CT changes the microbial microenvironment when the soil top layer is homogenized by plowing. This study analyzed the bacterial diversity from NT soils, (continuously maintained under this condition along 52 years in Ohio, USA), and soils from adjacent plots under plow-till

(PT) similarly managed. The bacterial diversity was determined using the 16S rRNA gene pyrosequencing method, finding out that proteobacteria and acidobacteria were predominant in both cases, but in NT these groups had a higher number of bacterial richness, and with five unique phyla reported. In the plowed plots, four unique phyla were found, and 99 % of the community had less than 1% of relative abundance, leading to the conclusion that tillage affects the dominant species in soil that contribute to higher percentages of the total soil communities. In another study, Yang *et al*. (2012c) mentioned that bacteria, fungi, including arbuscular mycorrhizae (AMF), and actinomycetes numbers were significantly larger in the conservation tillage system than those in plowed soils, where more microbial stress was present, indicating that changes in soil chemical or physical properties affect soil biological activity as well. Another interesting study about diversity from Jansa *et al.* (2003), mentions that soil tillage practices (plowing, chiseling, and no-till) have negative effects over the AMF communities colonizing maize roots. With the use of specific PCR markers and techniques, the results showed a dramatic reduction of AMF from the genus *Scutellospora* in the tilled soils, while the species that resisted the soil tillage practices were the ones from the genus *Glomus.* This study concluded that AMF species have different tolerance to tillage activities, and that these intensive practices can strongly reduce the abundance and diversity of AMF. Studies like these mentioned above, lead us to conclude that soil management practices influence on soil microorganisms´ community structure and possibly on its functionality, as well.

2.4 Soil microorganisms and their functionality

Soil is known as the most biologically diverse site on Earth, where microbial interactions take place between them and with various plants and animals in the ecosystem (Braga *et al*., 2016). These interactions are forming a complex web of biological activity. Soil microorganisms allow the sustainability of all ecosystems possible due to a wide range of important services. Their primary functions are nutrient cycling, SOM formation, soil C storage, formation of soil aggregates, water efficient transportation and usage, nutrients acquisition by plants, pests and diseases control, thus enhancing ecosystems health (Souza *et al*., 2015). These important services contribute to the dynamics of natural and agricultural ecosystems (Kihara *et al.*, 2020). Among important microbe specific key roles in soil there are: nitrification, denitrification, sulfur reduction, phosphorous solubilization, minerals fixation, among other processes (Walpola and Yoon, 2012). Due to these microbial processes, the use of microorganisms applied as biofertilizers or bioconverters seems to be a key alternative for sustainable agricultural practices. In this sense, the isolation, identification, and characterization of soil microorganisms is the way to achieve soil sustainability. Among soil microorganisms that benefit ecological processes and that are used as biofertilizers, the AMF stand out. They form beneficial plant-microbe associations that interact in the rhizosphere and contribute to plant health and soil fertility (Rillig *et al.*, 2019). AMF are found in all agricultural soils, accounting for 9–55% of the soil microorganism biomass, and 5–36% of the total soil biomass (Varma, 2008). They are important soil components in a vast diversity of soils, climates, and agricultural systems as beneficial microorganisms that help plant growth (Smith and Read, 2010), plant tolerance to water stress (Chitarra *et al*., 2016), plant health through antagonistic and competitive effects over some pests and pathogens (Thangavel and Sridevi, 2016) and also in the plant reproductive capacity (Marins and Carrenho, 2017). In this sense, as mentioned before, this plant-fungus alliance is of great importance in helping plant growth and health and contributing to the root protection and development. Vast physiological and biochemical processes occurring in the plants and soil are responsible for the different responses occurring in the ecosystems (Bennett and Meek, 2020).

2.4.1 Soil microbial enzyme activities

In soils, biochemical processes such as enzyme catalysis are responsible for the many different reactions involved in energy transfer, nutrient cycling, crop productivity and environmental quality (Adetunji *et al.*, 2020). Enzyme activity is considered as an early indicator of ecosystem stress and environmental perturbation and is a warning sensor of soil quality degradation (Dick, 1994; Tabatabai, 1994; Mohammadi *et al*., 2011; Yang *et al*., 2012b). Enzyme activity is a measure of soil biological processes involved in the main nutrient cycles such as C, N, P. Glucosidases for instance, are widely distributed in nature, they are found in microorganisms, animals and plants, and their activity reflects soil management effects (Acosta-Martinez *et al*., 2007; Schaller, 2016).

Glucosidases are one group of enzymes involved in C cycle with an important role in the saccharification of cellulose (Tabatabai, 1994). Moreover, phosphatases are involved in the mineralization of organic P compounds in soil leading to the release of phosphate, which can be taken up by plants and other microorganisms (Deng and Tabatabai, 1997; Nannipieri *et al*., 2011). Fluorescein diacetate (FDA) hydrolysis has been used to determine amounts of active fungi and bacteria and is widely accepted as a simple and accurate method for measuring total microbial activity in soil samples (Adam and Duncan, 2001; Srivastava, 2012). Dehydrogenase activity is associated with soil microbial respiration (Margesin *et al*., 2000), while urease is involved in the N-cycle as a useful enzyme to convert urea to ammonia making it available to microorganisms and plants (Margon *et al*., 2015). However, it must be mentioned that the group of enzymes that have been measured are a few in comparison to the number of enzymes that would be calculated to be present in soils. According to Gianfreda and Ruggiero (2006), there would be more than 500 enzymes involved in the cycles of N and C. In addition to the enzymes that have been named above, it is important to mention the oxidoreductases (i.e. the dehydrogenases which are enzymes that have a significant role in the oxidation of SOM), hydrolases (including glucosidases, lipases, phosphatases, ureases and others), and transferases are among some of the important enzymes that have been studied (Skujiņš and Burns, 1976; Luo *et al*., 2017).

2.4.2 Tillage effects on microbial functionality

Soil enzyme activity is strongly related to tillage methods. While dehydrogenase activity is not affected by tillage, acid and alkaline phosphatase, and protease are higher in NT systems compared to CT (Roldán *et al*., 2003; Alvear *et al*., 2005). The activity of urease and dehydrogenase has been reported as similar in NT and minimum tillage (Mohammadi *et al*., 2011). In general, conservation tillage practices have positive effects on soil enzyme activities. It is commonly reported that there is higher enzyme activity in NT soils, which can be associated with larger water availability and microbial biodiversity and stability (Zhang *et al*., 2014).

Besides the above-mentioned enzymes, enzymatic proteins have been analyzed to find correlations with soil quality. Many factors can change physical and chemical conditions of the soil and affect its biodiversity, and microbial and enzymatic activities. Factors that influence enzymatic activities are: plant species composition and root type (Grierson and Adams, 2000), clay minerals, humic substances or organo-mineral complexes (Gianfreda *et al*., 2002), tillage practices (Alvear *et al*., 2005; Gianfreda and Ruggiero, 2006; Munkholm *et al*., 2013) temporal, spatial and topographical influences on soil conformation (Bergstrom *et al*., 2000) bulk or rhizosphere soil enzyme activity

(Gianfreda, 2015), cultivated or forest soils (Lino *et al*., 2016), among other factors. These varied responses show that there is a vast research needed on different types of soils, crops, and land management activities.

It has been found that different tillage systems significantly affect soil properties and the spatial distribution of a variety of enzymes (Wallenius *et al*., 2011). Enzymatic activities are higher on the surface layer of NT soils compared to the same layer of plowed soils. Nevertheless, the opposite results are reported on deeper soil layers (Roscoe *et al*., 2000). This can be explained by structure changes of microbial communities and added organic residues in NT management that favors microbial activity by decomposing the OM (Sarto *et al*., 2020).

Gianfreda and Ruggiero (2006), mention that it is not completely accurate to correlate soil enzymatic activity with a specific environmental or anthropogenic factor, since many interactions can be occurring at the same time. In this sense, soil scientists suggest for future research to combine enzyme activity measurements with different microbiological and biochemical studies to clarify with more exactitude how the mechanisms work. This constitutes a big challenge for science towards a better understanding of soil dynamics and microbiological interactions in tropical soils.

2.4.3 Tillage effects on arbuscular mycorrhizal fungi

Soil management practices affect not only soil properties and microbial characteristics, but also AM fungal activity and propagules density (Castillo *et al*., 2006; Cornejo *et al*., 2009; Borie *et al*., 2010; Curaqueo *et al*., 2011). Plowing disrupts soil aggregates by destroying the fungal mycelia which stabilizes soil aggregates, facilitating the erosion and nutrients losses (Borie *et al*., 2008; Martínez *et al*., 2008). In NT systems, the lack of hyphal network disruption affects the amount and composition of soil propagules

(Schalamuk and Cabello, 2010). However, wide ranges of responses are possible, but, in general, mycorrhizae are very sensitive to tillage. Wortmann *et al*. (2008), reported that the quantity of AM fungi root colonization in the second year after tillage was 22 % lower for tilled soils compared to NT. The reduced AMF density with tillage may be a result of the affected hyphal network, breakdown of soil aggregates, and root channels disruption that normally occur under the CT systems, all of them constituting major binding agents for soil macroaggregates (Wu *et al*., 2014). A study conducted by Alguacil *et al*. (2008) evaluating the impacts of tillage practices on AM fungal diversity in warm subtropical crops, demonstrated highly significant differences among the AM fungal populations for different management tillage systems (moldboard, subsoil-bedding, shred-bedding, and NT) affecting the AMF community structure in maize, bean, and sorghum roots. The NT system had a higher number of fungal types, and also under a determined tillage system, different AM fungal populations could colonize the hosts.

A summary of recent studies on tillage in volcanic soils, including tropical soils and its effects on AMF is presented in Table 2, showing beneficial effects under NT conditions.

Table 2. Main observed effects on AMF under different volcanic soils and tillage systems				
Soil	**Evaluation time**	**Crop**	**Observed effect over AMF**	**References**
Volcanic Mollisol from Andean Ecuador		Comparison between naturalized grasslands (NT), maize and potatoes (both CT**)**	AM spores (No. 100 gds $^{-1}$): 2.31 times higher under NT than CT (potato) and 1.97 times higher under NT than CT (maize).	Avila-Salem *et al.* (2020)
			Total AM hyphae (m g^{-1}): 28% higher under NT than CT (potato and maize plots)	
Volcanic Ultisol from Southern Chile	Measurements were in the sixth year of an on-going tillage-rotation experiment	Before grass sowing	AM spores (No. 100 gds $^{-1}$): 67% higher under NT than CT	Borie *et al.* (2006)
			Total AM hyphae (m g^{-1}): 40% higher under NT than CT	
		After grass sowing	AM spores (No. 100 gds $^{-1}$): 198% higher under NT than CT	
			Total AM hyphae (m g^{-1}): 82 % higher under CT than NT	
			Root colonization (%): 56% higher under NT than CT	
		After wheat harvest	Total glomalin (mg g^{-1}): 24 % higher under NT than CT	
Tropical Ultisol from Indonesia	Samples taken one month after harvest	Tropical tree legume *Gliricidia sepium* and *Peltophorum dasyrachisand,* and a non-leguminous	Density of spores (number g^{-1} soil): 150% higher in undisturbed soil than in disturbed soil	Boddington and Dodd, (2000)
			Extraradical mycelium length of AMF (m g^{-1}): 71% higher in undisturbed soil than in disturbed soil	

		plant, *Zea mays L.*		
Volcanic Ultisol from Southern Chile	Samples taken at Fallow and at harvesting time	Wheat–oat–wheat crop rotation	Proportion of viable hyphae (%): 45% increase under NT, while 216% increase under CT at the end of study	Castillo *et al.* (2006)
			Total hyphae (m/cm^3): 65,6% decrease in NT, while 72% decrease in CT at the end of the study	
			AMF Spores (No./100 cm^3) 106% increase under NT, while 30% increase under CT at the end of study	
			AMF Colonization (%): 9% increase under NT, while 67% increase under CT, at the end of the two-year study	
			Diversity of AMF populations expressed as richness: 17% higher under CT, while 15% higher under NT	
			Diversity of AMF populations expressed as Shannon-Wiener index: 5 % increase under CT, while 7% decrease under NT	

			Diversity of AMF populations expressed as evenness: 1% decrease under CT, while 8% decrease under NT at the end of the study	
Andisol from Southern Chile	Samples taken at the beginning of anthesis	Wheat and oat rotation under greenhouse conditions and fertilization treatments	Mycorrhizal root length (m): 167 % higher with $NO3^-$ (as N source) under NT than under CT, and 400% higher with $NH4^+$ under NT than under CT	Cornejo *et al.* (2009)
			Mycorrhization (%): 140 % higher with $NO3^-$ (as N source) under NT than under CT, and 153% higher with $NH4^+$ (as N source) under NT than under CT	
			AMF extraradical hyphae density (m g^{-1}): 80 % higher with $NO3^-$ (as N source)) under NT than under CT, and 38% higher with $NH4^+$) under NT than under CT	
			Ratio of active AMF hyphae (%): 42 % higher with $NO3^-$ (as N source) under NT than under CT, but 73% higher with $NH4^+$) under NT than under CT	
			AMF spore density (100 g^{-1}): 60 % higher with $NO3^-$ (as N source) under NT than under CT, while 13% higher with $NH4^+$ (as N source) under NT than under CT	
			Extraradical mycelium length of AMF (m g^{-1}): 71% higher in undisturbed soil than in disturbed soil	
Aquic Inceptisol from Northern China	Samples taken at harvesting time	Maize and wheat	External mycelium length (mm g^{-1}): 54 % higher under NT than under CT	Hu, (2015)

Aquic Inceptisol	Samples taken at harvesting time	Wheat and maize	AM fungal species richness and community diversity: Species richness was 31% higher under NT compared to CT, Shannon-Wiener index was 22% higher under NT than under CT, the Evenness index was 11% higher under NT, and the Simpson's index of dominance was 13% higher under NT than CT	Yang, (2012)
NT: no-tillage; CT: conventional tillage				

On the other hand, soil tillage breaks up the AM fungi hyphal network that leads to a noticeable reduction in the mycorrhizal colonization of roots, thus probably interfering in the nutrient's absorption from soil (Guan *et al*., 2014). Despite the above, AMF have the particularity to produce in its extra radical hyphae a glycoprotein known as Glomalin related-soil protein (GRSP), which has a strong cementing capacity that allows the formation of soil aggregates. This glycoprotein also contributes to reduce the SOM degradation through protecting labile compounds inside the soil aggregates surrounded by AMF hyphae (Rillig, 2004; Borie *et al*., 2008). In this sense, AMF constitute key microorganisms that help in soil stabilization and fertility, and that act as biological and natural fertilizers available for a sustainable agriculture (Sindhu and Sharma, 2020). As it can be elucidated, the use of plough disrupts the soil layers, and negatively affects AMF hyphal network, contrarily to what occurs under conservation tillage. As mentioned before, Glomalin is produced by hyphae of AM fungi, and as it has been reported by Borie *et al*. (2006), and Curaqueo *et al*. (2010), the total Glomalin concentration in soils subjected to reduced and NT systems, increased compared with CT, indicating enhanced physical properties of soil under more conservative tillage systems. The study of soil proteins such as Glomalin in tropical soil can be used as an environmental tool to diagnose soil quality and health (Parihar *et al.*, 2020). The studies presented by Hu *et al*. (2015a) and Yang *et al*. (2012a), in soils from China, show higher AMF propagules under NT that CT, as well as the studies in volcanic soils from Avila-Salem (2020), Boddington and Dodd (2000), Borie *et al*. (2006), Castillo *et al* (2006), and Cornejo *et al.* (2009) from Table 2, which were consistent on the fact that NT increases the amounts of AMF propagules compared to plots under CT, corroborating the statement mentioned before that NT enhances quality of soil which are reflected in the increased number of AMF propagules.

2.4.4. AMF and PGPR as main components in ecosystem services

Ecosystem processes occur due to soil microbial activities. The biogeochemical cycles of major plant nutrients are carried out by soil microorganisms (Bardgett, 2017). The role of a group of micro-organisms, known as plant-growth-promoting rhizobacteria (PGPR), as modifiers of soil fertility and productivity has been widely studied in recent years (Chandra *et al.*, 2020). Vast interactions take place in the rhizosphere of plants, and many studies have addressed the functional compatibility of groups of micro-organisms: e.g., AMF, *Rhizobium* and other PGPR, by inoculating them and watching their effect over the plant's growth. Plant–microbe association using microorganisms' inoculations are currently being successfully developed. Considering the need for healthier food production, higher yields and environmental conservation, the inoculation of AMF, bacteria and other PGPR microorganisms used as biofertilizers instead of chemical fertilizers is imperative and worthwhile. Considering the limited sources of phosphate rocks in the world, areas with high P fixing indices, deserve to be targeted. In this sense, as stated by Singh (2016), the use of biofertilizers goes well along with higher P availability. In conservative land management systems, such as NT, microbes play key roles in the soil's ability to provide ecosystem services such as enhancing soil quality, which benefits society, and help with its continual development, being the previous management an alternative to integrate in an "improved" framework of sustainable agriculture.

2.5 Enhancing soil biological activities and sustainability

Soils that have a good quality are nowadays considered as "truly living bodies with biological, chemical, and physical properties and processes performing essential ecosystem services" (Karlen *et al*., 2003). As it has been mentioned before, the increasing global food demand that the world is facing while keeping up an acceptable soil quality, exerts deep pressure over cultivable and productive areas in Latin American countries. The intensive crop production and soil management in Bolivia, Paraguay, Uruguay, Chile, Argentina, part of Brazil Ecuador, Colombia, Peru, and Guatemala indicate that there are more than 200 million of hectares of eroded lands due to deforestation, overgrazing, intensive agricultural activities and soil over exploitation (Wingeyer *et al*., 2015). According to Pimentel and Burgess (2013), the most serious consequence of erosion is the loss of soil fertility and agricultural productivity. Soil degradation occurs due to wind and water erosion, soil compaction, SOM depletion, and nutrient losses (Wingeyer *et al*., 2015). Over the past years, food demand has been partially met by extending the cropping area utilizing land management systems such as CT and NT systems, especially throughout southern Brazil, Paraguay, Uruguay, Argentina and Bolivia. Unfortunately, this increase has been accompanied by a loss of crop diversity due to monoculture (soybean, maize or wheat, mainly), and less crop rotations (Wingeyer *et al.*, 2015; Pervaiz *et al.*, 2020). When crop rotations were considered within NT land practices (soybean/maize rotations), an achievement for increased crops diversity, reduced insect pressure, restored SOM, higher crop residues input and nutrient cycling was observed together with a rapid adoption of glyphosate-tolerant soybean varieties (Benavides *et al.*, 2011). The success of NT in southern Brazil and Argentina was an important reference for its adoption throughout South America. No-tillage practices are being used on 70 to 90% of the grain crop area in Brazil, Argentina Uruguay, Paraguay and Bolivia (Pognante *et al.*, 2011). During the last decade, soil quality indicators were assessed in these countries: SOM decreased by approximately 18% per decade of agricultural use, when soils were undergoing 10–20 years of continuous agriculture, compared to soils with less than 10 years of the same soil management (Sainz Rozas *et al*., 2012). This same author also mentions that the factors that influence on SOM decline in these soils are the elimination of perennial pasture from long-term rotations, increased soil disturbance associated with tillage, limited rooting depth due to machinery traffic and soil compaction, soil erosion, decreased crop residue input, and less crop diversity.

In tropical and subtropical environments from Paraguay and southern Brazil, long term agricultural grain crop rotations have shown that even under NT it is necessary to leave crop residues in the soil to balance the fast SOM decay associated with high temperature and moisture conditions (Eiza *et al*., 2005). The role of minimum soil disturbance, soil acidity adjustment and fertilization seem to be critical factors in soil quality conservation. Garcıa-Préchac *et al*. (2004) stated that long term experiments in Uruguay showed a six-fold erosion reduction under NT compared to CT conditions when incorporating pastures, suggesting their efficacy to reduce soil erosion, even in short-term rotations.

Another report by Santos *et al*. (2006), mentions that in site in the tropical Brazilian savannah plots with *Zea mays* L. and *Phaseolus vulgaris* L. rotations were set for tillage: disk harrow and disk plow, and NT systems studies. The NT management showed greater biological activity than disk harrow and disk plow. Even though intensive tillage has been occurring in the plot before experimental establishment, the NT management treatment during 5 successive years showed improvement in soil biological properties, e.g., higher microbial biomass. These findings suggested that soils can recover from damage due to intensive tillage, and that NT management is important for maintaining high biological activity in soils. Biological activities are key soil quality indicators, sensitive to tillage management in the short-term. Tropical soils, in the Andean region have been traditionally recognized to have a high environmental quality, because of their thickness and high content of organic matter correlated to its ongoing biological activities (Chacón *et al.*, 2016).

No-tillage practices represent an alternative to CT (da Silva *et al.*, 2020). The study on soil benefits conducted by Roldán *et al*. (2003), using NT management in a volcanic Andisol from Mexico, demonstrated that soil quality improved in direct proportion to residue inputs. An NT management with a moderate amount of crop residue (33%) and the use of cover crops such as *Vicia sp.* or *Phaseolus vulgaris* L., rapidly improved soil quality concluding that conservation tillage practices are an alternative technology that contributes to sustainable agriculture in Mexico and can be extrapolated to similar areas or soils elsewhere. The use of a more conservative tillage system is increasing due to a growing interest in sustainable agriculture and demonstrates the efforts to reduce and prevent soil degradation.

2.6 Conclusions and future perspectives

Physical, chemical, and biological properties in soil can be negatively and deeply affected by tillage practices which can lead to long-term effects. Complex soil interactions related to land management practices, influence biological activities specially AMF. Large agricultural areas in tropical Latin America face erosion and degradation due to intensive tillage activities; nevertheless, this condition can be gradually alleviated by changing to conservational land use practices, resulting in soil restoration, productivity increase and more ecosystem services leading towards sustainability, many of them directly provided by microbiota.

According to The Economics of Ecosystems and Biodiversity -TEEB (see http:// www.teebweb.org*), e*cosystem services are the direct and indirect contributions of ecosystems to human well-being. They support our survival and quality of life. Therefore, improving soil quality with an adequate agricultural management, we will be enhancing quality of life, as well. The complex interaction that occurs in soil and the influence of climate, vegetation and human management can make sustainability harder to accomplish. It becomes mandatory to take actions before degradation becomes irreversible or land restoration becomes too costly. Especially, land management in tropical soils, its interactions and the effects on biological properties needs to be studied in depth, with a scientific, social and economic approach. These actions can help with agricultural sustainability and healthier ecosystems in the long run.

Tropical countries face a long-term challenge: having soils with particular characteristics, urges for environmental, economic, social, and political motivations towards the use of conservational tillage. Farmers are willing to adopt agricultural practices once they evidence the benefits of NT on soil properties at the time that management strategies and technology are incorporated to increase crop production. Thus, agronomic, economic, and social benefits of soil conservation (due to a sustainable land management) must be presented to landowners as aims to accomplish agricultural sustainability. In this scenario, the management of the microbiota is a crucial factor to be considered.

It is well known by soil scientists that, in general, CT negatively affects biological activities and AMF; nonetheless, when NT practices are used along with other soil conservational practices, stability and productivity is enhanced. Deeper research in biological or soil quality indices such as enzymatic activity need to be addressed as ecosystem services of soil microbes, leading to a better understanding of the complex interactions and dynamics that occur in soil, and that could be better elucidated in a near future.

Continuous and long-term research in tropical soils must be performed under various conditions, to deeply understand the ecological processes and specific physical, chemical, and biological interactions. A network between soil research institutions devoted to tropical agroecosystems with ongoing projects should share results, but most importantly, must find ways to maintain high productivity of fertile tropical soils, and recover low productive and degraded lands. For this, the use of microorganisms as biotechnological tools towards food production and soil sustainability is what ecosystem services are for, among them, climate regulation, water purification, pests and diseases control, soil biodiversity and cultural services are some of the most important ecosystem services. Research and knowledge transfer will give small farm owners the necessary tools to improve the use of soil and make the best decisions to assure enhanced biological activities in soil, linked to better food quality and production, in a much-needed framework of sustainable agriculture in such habitats.

CHAPTER III

Research paper:

“Soil Biological Properties and Arbuscular Mycorrhizal Fungal Communities of Representative Crops Established in the Andean Region from Ecuadorian Highlands”

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Soil biological properties and arbuscular mycorrhizal fungal communities of representative crops established in the Andean Region from Ecuadorian Highlands

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Abstract

Biological activities determine quality, sustainability, health, and fertility of soils. The purpose of this study was to evaluate chemical and biological characteristics of soils from Ecuadorian highlands subjected to different management practices, as well as the density and diversity of arbuscular mycorrhizal fungi (AMF). Soils from naturalized grasslands and other previously cropped plots with *Zea mays* and *Solanum tuberosum* were analyzed in laboratory for soil biochemical properties, enzyme activity, and AMF colonization to determine the effect of the soil management over its quality. The characterization of AMF propagules associated to spontaneously colonizing plants in the above soils was also performed. Soil previously cropped with *S. tuberosum* showed the highest glomalin content; at the same time, naturalized grassland and *Z. mays* cropped soils showed higher hyphal length. The acid phosphatase activity was higher in naturalized grasslands and *Z. mays* cropped soils compared with that in the *S. tuberosum* cropped soils. Moreover, the highest AMF colonization rates and spore number were found in different spontaneous plant species growing in the naturalized grasslands. This study represents the first characterization of AMF propagules of different cropped and naturalized grassland soils, and also is one of the first reports about changes on biochemical and microbial activities occurring in Andean soils from the highlands of Ecuador, undergoing determinant soil management activities.

Keywords Arbuscular mycorrhizal fungi· Enzyme activities· Grassland· Maize· Potato.

3.1 Introduction

Soil tillage deprives soil microorganisms from their primary carbon (C) energy source and exposes soil to negative climatic impacts (Lal and Stewart 2010; Farooq and Siddique 2015; Kumar *et al.* 2015). Intensive soil management systems as used in the highlands of Ecuador, including continuous tillage aimed to increase crop yields and food production, have contributed to the soil erosion in the region (Espinosa 2014). As an alternative, conservation tillage is considered a good soil management option for crop production. Some conservation tillage alternatives have been widely used in Europe, Asia, North America, and in parts of South America (Derpsch *et al*. 2010). Sometimes the crop residues are left on the top-soil, increasing the organic C on the surface and stimulating the microbial activities, helpful for nutrient cycles which enhance soil fertility and crop yields (Lal 2011; Samal *et al*. 2017). Particularly, the microbial activities are key factors scarcely studied in Andean soils, where soil conservation efforts need to be reinforced in order to attain sustainability.

One of the most important microbes found in soils are the arbuscular mycorrhizal fungi (AMF), which form symbiotic associations with the majority of plant roots in natural and agricultural ecosystems (Cornejo *et al*. 2017; Anwar *et al.* 2018). AMF are well known as helpful microorganisms which increase plant growth, water stress tolerance, and plant health (Santander *et al.* 2017, 2019). Soil management practices affect not only soil properties and microbial responses but also AMF activities and their plant colonization ability (Cornejo *et al.* 2009; Barea and Pozo 2013; Manoharan *et al.* 2017). Therefore, their density, activity, and diversity in soils are main factors in sustainable agriculture. Moreover, soil enzyme activities, particularly those associated to nutrient cycles, are important soil quality indicators that can measure the effects of tillage and are considered warning sensors of soil quality degradation (Gianfreda 2015; Gajda *et al.*

2018; Lin *et al.* 2018). Since the microbial and enzyme activity in soils vary according to soil management such as tillage and cropping, this presents an interesting question about their effect over chemical and biological characteristics of highland soils from Ecuador. Additionally, the knowledge about AMF-plant relations in these particular types of soils can be of great interest for crop production in the region.

There is scarce information regarding biological characterization of highland soils from Ecuador. Therefore, in order to contribute with this lacking information, we aimed to evaluate AMF and biochemical characteristics of some representative agroecosystems in the highlands of Ecuador subjected to different soil managements during the last decades, and to describe the AMF presence and diversity in these soils. Here, we hypothesized that the physicochemical and biological soil properties show a better performance in soils from naturalized grasslands compared with soils under intensive land management. For this, we selected a naturalized grassland plot as representative of conservation tillage management, and soils previously cropped with potato and maize, subjected to intensive tillage. This information will serve as a baseline for further studies in the region, based on the implementation of contrasting tillage systems and fertilization, using AMF characteristics and the soil's biochemical and microbial characteristics as early indicators of shifts in soil quality. This will allow a more sustainable management of soils currently subjected to strong degradation and erosion.

3.2 Materials and Methods

3.2.1 Site Description

We studied three plots previously maintained: (i) a naturalized grassland that has remained unaltered for 7 years, (ii) a plot cropped with maize (*Zea mays*), and (iii) a plot cropped with potato (*Solanum tuberosum*) at Universidad Central del Ecuador Experimental

Station, Tumbaco locality, Quito, Ecuador (0° 13′ 49″ S, 78° 21′ 18″ W; 2505 m.a.s.l.). The plots with maize and potatoes have been used for at least 10 years in crop rotations with intensive conventional soil tillage. The mean annual precipitation in this site is 870 mm, relative humidity is 72%, and annual average temperatures are 10.3 °C min and 23.1 °C max. The dimensions of the plots were 35 × 35 m for the naturalized grassland and 40 × 25 m for the maize and potato plots, respectively. The naturalized grassland plot is located at the south-western side of the maize plot. The distance between both plots is 200 m. The maize plot corresponds to the central-northern plot, while the potato plot is located at the south-eastern side of the maize plot, adjacent to it.

3.2.2 Soil and Plant Sampling

The soils presented typical characteristics from the northern highlands of Ecuador: dark, volcanic ash–derived soils with allophane material. The soil at this site has been preliminarily characterized as a Mollisol. Nine soil samples were taken in September 2017 from each plot, randomly collected at 0– 20 cm depth and homogenized in sealable plastic bags to obtain a total of three composed samples per plot. Soil samples were randomly collected considering a separation of 10– 15 m and avoiding the plot's border. Soil samples were stored in sealable plastic bags and kept in coolers with ice for their transportation. An aliquot of each above-described soil samples was kept frozen (− 20 °C) until the implementation of enzyme activities analysis. Additionally, for descriptive purposes, individuals of herbaceous plants with their intact root system, which were found into the plots, were stored in sealable plastic bags and used for botanical classification (shoots) and AMF colonization (roots).

3.2.3 Soil Chemical Determinations

Soil parameters such as pH (2:5 w/v in water), electrical conductivity (EC, 1:5 w/v in water), available P, total N, and soil organic matter (SOM) were determined according to standard lab protocols (Zamudio *et al.* 2006; Espinosa *et al.* 2014). Available P was extracted and measured according to the Olsen and Sommers (1982) method.

3.2.4 Soil Biochemical Determinations

The acid phosphatase (A-Pase) activity was measured according to Tabatabai (1994). This method is based on the colorimetric determination of the liberated p-nitrophenol, when soil is incubated with a buffer (pH 6.5) of p-nitrophenyl phosphate disodium salt at 37 °C for 1 h, showing an intense yellowish coloration, measured in spectrophotometer at 420 nm. For the fluorescein diacetate activity (FDA) determination, 15 mL of potassium phosphate buffer was added to 2 g of soil in centrifugation tubes, plus 0.2 mL FDA stock solution (1000 μg mL^{-1} FDA: acetone), and incubated for 30 min at 30 °C with shaking. Reaction was stopped by adding 5 mL acetone, followed by 5-min centrifugation at 2000*g*. The amount of FDA hydrolyzed was measured at 490 nm according to the Schnürer and Rosswall (1982) method, using a fluorescein solution as standard.

Total glomalin-related soil protein (TGRSP) was extracted according to Wright and Upadhyaya (1998), with minor modifications. For this, 8 mL of citrate buffer (50 mM, pH 8.0) was added to 1 g of soil and then autoclaved for 1 h at 121 °C. This step was repeated several times on the same sample until the red-brownish color disappeared from the supernatant. The TGRSP content was determined spectrophotometrically by means of the Bradford protein assay (Bio-Rad Protein Assay; Bio-Rad Labs) at 595 nm, using bovine serum albumin as standard.

3.2.5 Arbuscular Mycorrhizal Fungal Structures in Soil and Roots

AMF spores were isolated from soil by means of wet-sieving and decanting (Sieverding, 1991) followed by sucrose gradient centrifugation. After centrifugation, supernatants with the spores were washed for 1 min and transferred to Petri dishes for sorting; then, the total number of spores per 100 g of soil was quantified (× 40–80). The total isolated spores were placed on microscope slides for visualization and identification (see Supplementary Material 1). Additionally, the AMF communities present in these soils were characterized by means of the species richness (S', represented as the total different spores' morphotypes), evenness Shannon-Wiener diversity index (H'), and Simpson's dominance index (D'), according to Marín *et al.* (2016). Detailing, for H', we used the following equation:

$$H' = -\sum_{i=1}^{S} (\mathrm{Di} \times \log_2 \mathrm{Di})$$

were S' is the spores' morphotype richness and Di is the relative density of each spores' morphotype (proportion of each spores' morphotype number with respect to the total spore number in a sample); meanwhile, for D', we used the following equation:

$$D' = \sum_{i=1}^{s} \mathrm{Di}^2$$

The hyphal density in soil was measured by using the method described by Rubio *et al*. (2003) and using Newman's intersection formula (Newman 1966). In plant roots, the AMF root colonization was determined according to each taxonomically identified and randomly collected plant individual (3 to 5 samples per plant species in each plot). Roots were cleared and stained according to the Phillips and Hayman (1970) and Koske and Gemma (1989) methods, but using Parker Quink blue ink (Rodríguez *et al*. 2015) for staining. The presence of AMF structures within the roots was observed at × 40–100 in a gridded Petri dish, according to Giovannetti and Mosse (1980).

3.2.6 Statistics

All composed samples ($n = 3$) were considered and analyzed as independent experimental individuals being checked for the normality (Shapiro-Wilk test) and homoscedasticity (Levene test). For each variable, an ANOVA was performed followed by Tukey's multiple range test. Correlation among variables was evaluated using the R Pearson coefficient. The statistical significance was established at $p < 0.05$. Also, principal component analysis and cluster analysis using both the experimental variables and experimental individuals were performed. Statistical analyses were carried out using the IBM SPSS © software v. 19.0. Additionally, a heat map was elaborated for AMF species abundance and their correlation with soil variables as a visual summary, using the software R v. 3.2.2 (R Development Core Team 2015).

3.3 Results and Discussion

In this study, we expected that the different soils showed contrasting characteristics due to their previous management, being analyzed for chemical and biochemical characteristics, as well as other fungal traits such as AMF spore density, hyphal length, and TGRSP content (Table 1). Our results showed strong differences for pH, EC, available P, total N, SOM, A- Pase, and TGRSP among the three plots. The potato soil presented lower pH values, presumably due to strong previous fertilizations, which also would explain high available P contents. Higher SOM, total N content, and A-Pase values were found in the naturalized grassland and potato soils compared with maize soils (Table 1). These results agree with previous reports by Alvear *et al*. (2007), who found in an Ultisol from

Southern Chile that the N content originated in the SOM mineralization and enzyme activity was narrowly correlated, mainly explained by an increased soil microbial and fungal biomass. Macronutrients such as P and N are continuously being liberated from labile SOM, which also improve the conditions for enzymatic processes, influencing the biodynamics of nutrient cycling (Borie *et al*. 2019).

Strong differences for SOM were found in the three different soils, being evident that the maize cropping is reducing the total amount of SOM. This aspect deserves to be analyzed in depth, since maize is one of the most cropped species in the Ecuadorian highlands. The highest values of SOM in naturalized grasslands strongly agree with the report of Percival *et al*. (2000), who worked with 167 volcanic soils from New Zealand and concluded that in such soils the type of SOM chemical stabilization is a key process for C accumulation. In this sense, the C/N ratio was also assessed as an important trait of SOM stability. Our study reported that the C/N ratio for the three plots was about 10.5 (Table 1), which can be considered an ideal range and suggests a high degree of SOM polymerization (Medina *et al*. 2015). Such values could support bio- chemical processes led by soil microorganisms in an ideal way, by allowing the mineralization of the crop residues or even some recalcitrant SOM constituents (Shahbaz *et al*. 2017).

Several studies show that the TGRSP is a good indicator of stable C in soil, due to its recalcitrance and the increase of the soil aggregate stability (Borie *et al*. 2008; Curaqueo *et al*. 2011; Aguilera *et al*. 2019). Here, we evidenced higher TGRSP content in soils from the potato plot compared with maize and the naturalized grassland plots (Table 1). Despite the underlying mechanisms by which TGRSP contributes to the C sequestration being still largely unknown, it is recognized that TGRSP can act as a regulator of the SOC accumulation, also helping in the P transportation to the plants, maybe as a transient storage. Previous studies by Rillig (2004) and Lovelock *et al*. (2004b) mentioned that the soil type, management, quality, and fertility influence on the TGRSP concentrations. Additionally, studies by Mohan *et al*. (2014) and Soudzilovskaia *et al*. (2015) indicate that the TGRSP and SOC relation was also affected by differences in the crops and AMF community composition.

Table 1. Physicochemical and biological properties of Andean Ecuadorian soils from a naturalized grassland, maize and potato crops

Variable*	Grassland	Maize	Potato	*F*-value
pH	6.54 ± 0.01 b	6.57 ± 0.01 a	6.11 ± 0.1 c	1490***
Available P	37.13 ± 0.42 c	68.5 ± 0.60 b	152.27 ± 0.50 a	13530***
SOM	3.84 ± 0.5 a	2.96 ± 0.5 b	3.77 ± 0.3 a	123***
Total N	0.19 ± 0.01 a	0.15 ± 0.01 b	0.19 ± 0.01 a	915***
C/N ratio	10.50 ± 0.05 a	10.66 ± 0.14 a	10.48 ± 0.08 a	1.13 ns
EC	0.46 ± 0.01 a	0.29 ± 0.01 c	0.36 ± 0.01 b	180***
A-Pase	685 ± 44 a	682 ± 88 a	720 ± 52 a	16.2**
FDA	164 ± 24 a	172 ± 52 a	112 ± 27 a	2.30 ns
TGRSP	2.4 ± 0.01 b	2.4 ± 0.08 b	2.8 ± 0.08 a	46.9***
AM fungal spores	4397 ± 346 a	2227 ± 453 b	1327 ± 128 c	65.8***
Hyphae length	1.13 ± 0.5 a	0.88 ± 0.5 a	0.88 ± 0.2 a	0.33 ns
S'	8.0 ± 0.0 a	8.0 ± 0.0 a	6.0 ± 0.0 b	231***
H'	1.92 ± 0.02 b	2.03 ± 0.02 a	1.70 ± 0.05 c	86.7***
D'	0.12 ± 0.0 b	0.14 ± 0.0 b	0.20 ± 0.0 a	43.6***

*pH in water (2:5 w/v); available P as Olsen (mg kg^{-1}). *SOM*, soil organic matter (%); *total N*, total nitrogen (%); *EC*, electrical conductivity (mmhos cm^{-1}); *A-Pase*, acid phosphatase activity (µg PNP g^{-1} h^{-1}); *FDA*, fluorescein diacetate activity (µg fluorescein g^{-1} h^{-1}); *TGRSP*, total glomalin-related soil protein (mg g^{-1}); AM fungal spores in 100 g of dry soil; hyphae length (m g^{-1}); *S'*, AM fungi species richness; *H'*, evenness Shannon-Wiener's index; *D*, dominance Simpson's index. The values represent the treatment mean ± standard error ($n = 3$). Different letters among treatment for the same variable represent statistical differences according to Tukey's multiple range test ($p < 0.05$). *ns*, non-significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Considering the importance of AMF in agroecosystems, here we included the description of the communities' diversity, being this one of the first studies focused on the AMF communities in agricultural soils in the highlands of Ecuador. Additionally, for descriptive purposes, we analyzed all the different plant species spontaneously growing in the three plots to corroborate their AMF status. Interestingly, our screening evidenced that the AMF colonization occurs practically in all the plants analyzed, even in plant species belonging to widely recognized non-AM host botanical families (Supplementary Material 2). Detailing, four AMF- colonized plant species were present exclusively in the naturalized grassland plots where higher AMF spores and hyphal length were found, while three plant species were present exclusively in the potato plots, and one plant species was found exclusively in the maize plot. Noticeably, we found AMF colonization in roots of plant species that belong to botanical families well-recognized as non-AM-host, such as *Chenopodium paniculata* and *Amaranthus blitum* (Amaranthaceae family), which reinforce the need of deep analysis of AMF-host associations considering species-specific relation (Teixeira-Rios *et al*. 2018; Zeng *et al*. 2018), in particular agroecosystems as the tropical highlands from Ecuador.

The morphological analysis of AMF spores evidenced the presence of a total of 16 AMF species, with seven families and 12 genera into the Glomeromycota phylum (Oehl *et al*. 2011) (Figure 1).

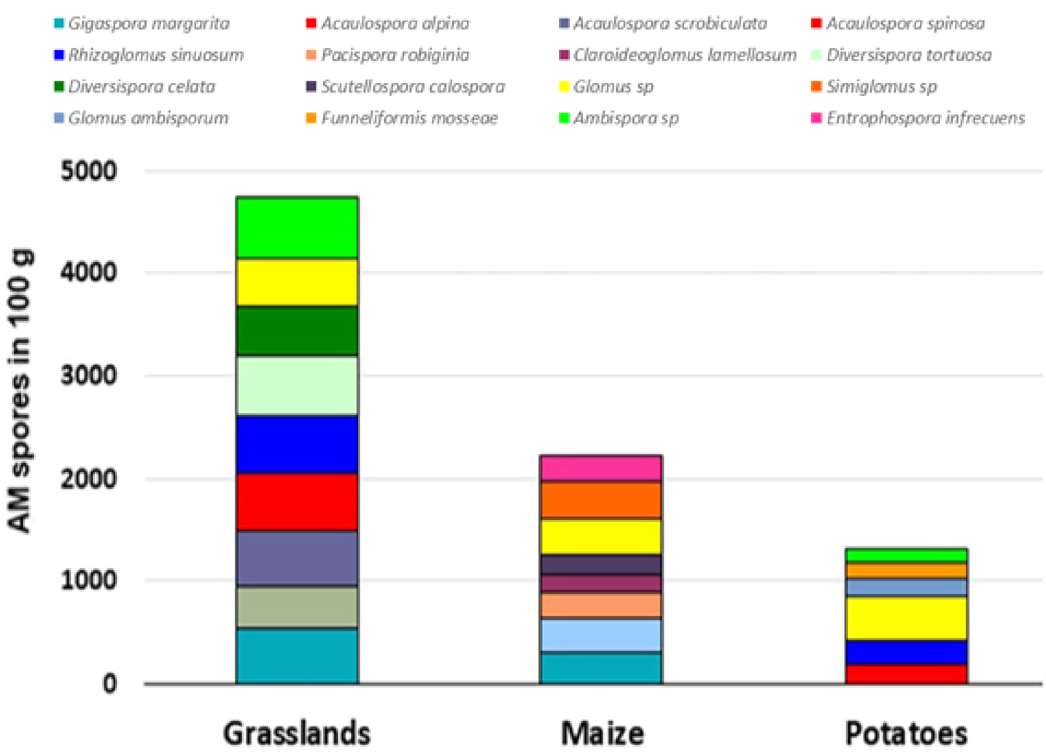

Figure 1. Arbuscular mycorrhizal fungal species according to the number of spores per 100 g of soil for three contrasting crop managements (naturalized grassland, maize crop, and potato crop) in an Andean Ecuadorian soil. The grassland was maintained unaltered for 7 years; meanwhile, the other soils have been used in crop rotations with intensive soil management for 10 years, being maize or potato the last species cropped in the respective plot.

Thirteen species were identified as previously described species, whereas the other three might correspond to undescribed ones. Previous studies by van der Heyde *et al*. (2017) indicate that some effects among crops can be evidenced at different time scales, and therefore, there are time- related responses, especially in plots used for grazing. Thus, the effects of soil management on AMF community composition and fungal structures density could be affected by multiple fungal-specific responses along the time. Particularly, here we found clear differences in both size (density of AMF spores) and presence of AMF species (Table 1; Figure 1), being the naturalized grassland the land management system where the AMF community seems to be more stable according to the different diversity indices obtained (Figure 2). Here, the presence of some AMF species limited to some soils only can suggest a fungus-host selection, which previously described species, whereas the other three might correspond to undescribed ones. Previous studies by van der Heyde *et al*. (2017) indicate that some effects among crops can be evidenced at different time scales, and therefore, there are time- related responses, especially in plots used for grazing. Thus, the effects of soil management on AMF community composition and fungal structures density could be affected by multiple fungal-specific responses along the time. Particularly, here we found clear differences in both size (density of AMF spores) and presence of AMF species (Table 1; Figure 1), being the naturalized grassland the land management system where the AMF community seems to be more stable according to the different diversity indices obtained (Figure 2). Here, the presence of some AMF species limited to some soils only can suggest a fungus-host selection, which previously described species, whereas the other three might correspond to undescribed ones. Previous studies by van der Heyde *et al*. (2017) indicate that some effects among crops can be evidenced at different time scales, and therefore, there are time- related responses, especially in plots used for grazing. Thus, the effects of soil management on AMF community composition and fungal structures density could be affected by multiple fungal-specific responses along the time. Particularly, here we found clear differences in both size (density of AMF spores) and presence of AMF species (Table 1; Figure 1), being the naturalized grassland the land management system where the AMF community seems to be more stable according to the different diversity indices obtained (Figure 2). Here, the presence of some AMF species limited to some soils only can suggest a fungus-host selection, which previously has been described in plant species and even at the genotype level (Aguilera *et al*. 2014, 2017). Moreover, our results suggest that both available P and SOM have a strong effect on the AMF community composition (Figure 3), being also the main experimental variables that allow the complete separation of fungal communities. Detailing, several variables were grouped in a high association degree according to a linear correlation analysis (Table 2) and also considering the PCA (Figure 2a), especially focusing on the SOM and available P (Figure 3). Also, the two first principal components after factorial analysis accounted for more than 90% of experimental variance (Figure 2), where clearly it was possible to associate some soil traits with the presence of particular AMF species, and finally evidencing their influence over the diversity of AMF communities for each soil management (Figure 2b).

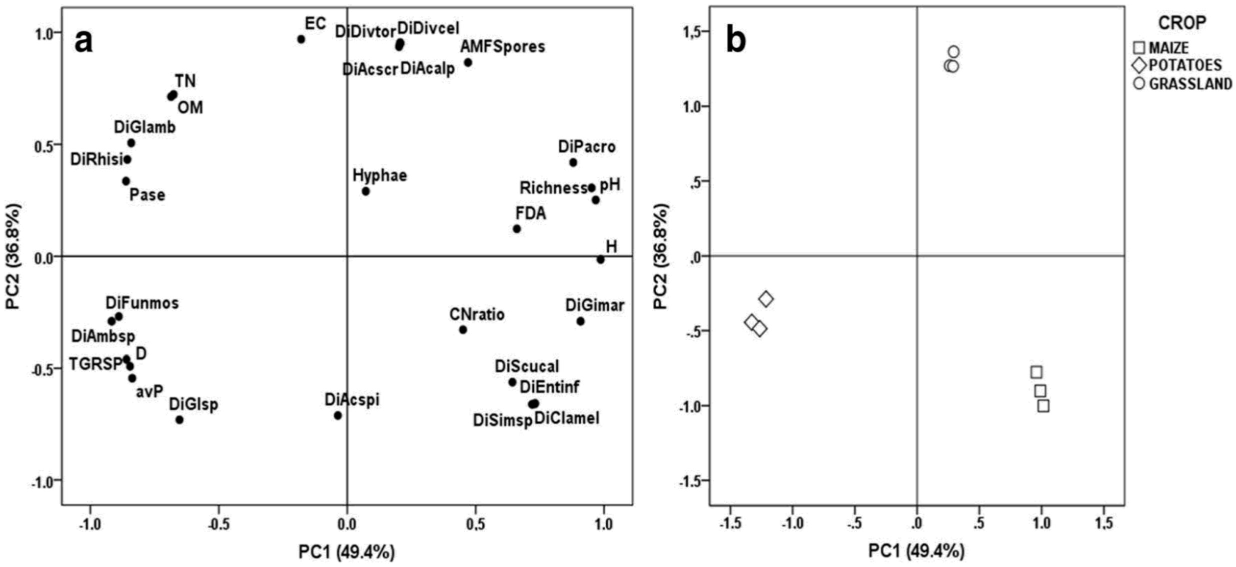

Figure 2. Principal component (PC) analysis for **a:** the studied experimental variables in the rhizosphere of three contrasting soil managements (naturalized grassland, maize crop, and potato crop) and **b**: the grouping of the experimental individuals according to the soil management in an Andean Ecuadorian soil. Percentage values in parentheses indicate the experimental variance explained by each PC. avP, available P; OM, soil organic matter; TN, total nitrogen; EC, electrical conductivity; Pase, acid phosphatase activity; FDA, fluorescein diacetate activity; TGRSP, total glomalin-related soil protein; H, evenness Shannon-Wiener's index; D, Simpson's dominance index. For the arbuscular mycorrhizal (AM) fungal species, we used the relative density (Di) for the PCA analysis. For the detail of all the AM fungal species, please see the legends in Figure 1.

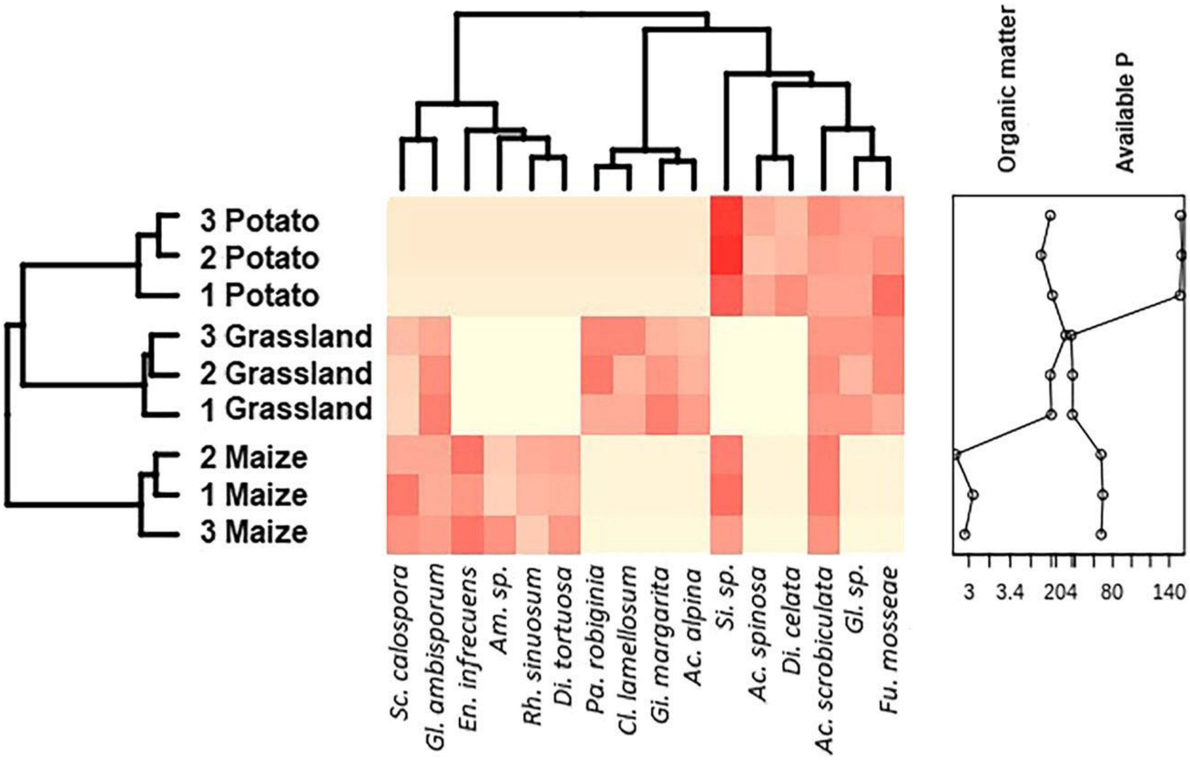

Figure 3. Heat map and clustering classification according to the Bray-Curtis dissimilarity, showing the 16 arbuscular mycorrhizal (AM) fungi species described in the rhizosphere of three contrasting soil managements (naturalized grassland, maize crop, and potato crop) in an Andean Ecuadorian soil and their relationship with the most significant experimental variables (available P and soil organic matter (SOM)) according to canonical correspondence analysis post-backward analysis.

Regarding the multivariate analyses, an inverse significant correlation was observed between pH and TGRSP content ($r = -0.95$, $p < 0.01$). Also, the TGRSP content showed a correlation with the available P content ($r = 0.97$, $p < 0.01$). Although it is recognized that fertile soils with higher P, Ca, or K levels present less glomalin, while those with high C/N ratios such as low-fertility soils present more glomalin, Lovelock *et al*. (2004a) explain that a “recently” produced glomalin can be found in fertile soils from tropical forests. Therefore, our observation could be explained by climate traits that deserve to be analyzed in future research in Ecuadorian highland soils to understand the mechanisms of SOM transformation or turnover of AMF structures that determine high glomalin (as TGRSP) levels associated to fertile soils. Also, we highlight a strong positive correlation between SOM and A-Pase activity ($r = 0.80$, $p < 0.01$), necessary to hydrolyze

P from SOM (Borie *et al*. 2019). This is noticeable, because here the high A-Pase activities were registered in soils with the high amounts of available P. Finally, the direct and strong correlation between the N content with the SOM (r =0.81, p < 0.01) suggests that SOM is mainly condensed, presumably as humins, humic acids, and glomalin (Hayes and Swift 2018). The above correlations can be good indicators of the effects of management practices over the soil biological activities, where microbial-mediated processes are affected by shifts in both physical and chemical traits, and also by changes in AMF community diversity and structure (Aguilera *et al*. 2017).

3. 4 Conclusions

Soil chemical and biochemical characteristics here found showed strong shifts maybe due to the precedent management practices, such as tillage, fertilization, and crop rotations, which are mainly reflected in the arbuscular mycorrhizal fungi (AMF) diversity and community structure. In this sense, assuming that naturalized grasslands are associated to a most conservative soil management, the levels of richness and diversity of AMF communities are concordant. Therefore, we suggest that the shifts in AMF community composition could be considered as a tool to characterize contrasting soil management systems oriented to support soil conservation and sustainability actions in the Ecuadorian highland region, currently characterized by strong soil degradation and erosion. This study represents a baseline for further research and analyses and constitutes a record of changes in soil (bio)chemical properties undergoing different soil managements.

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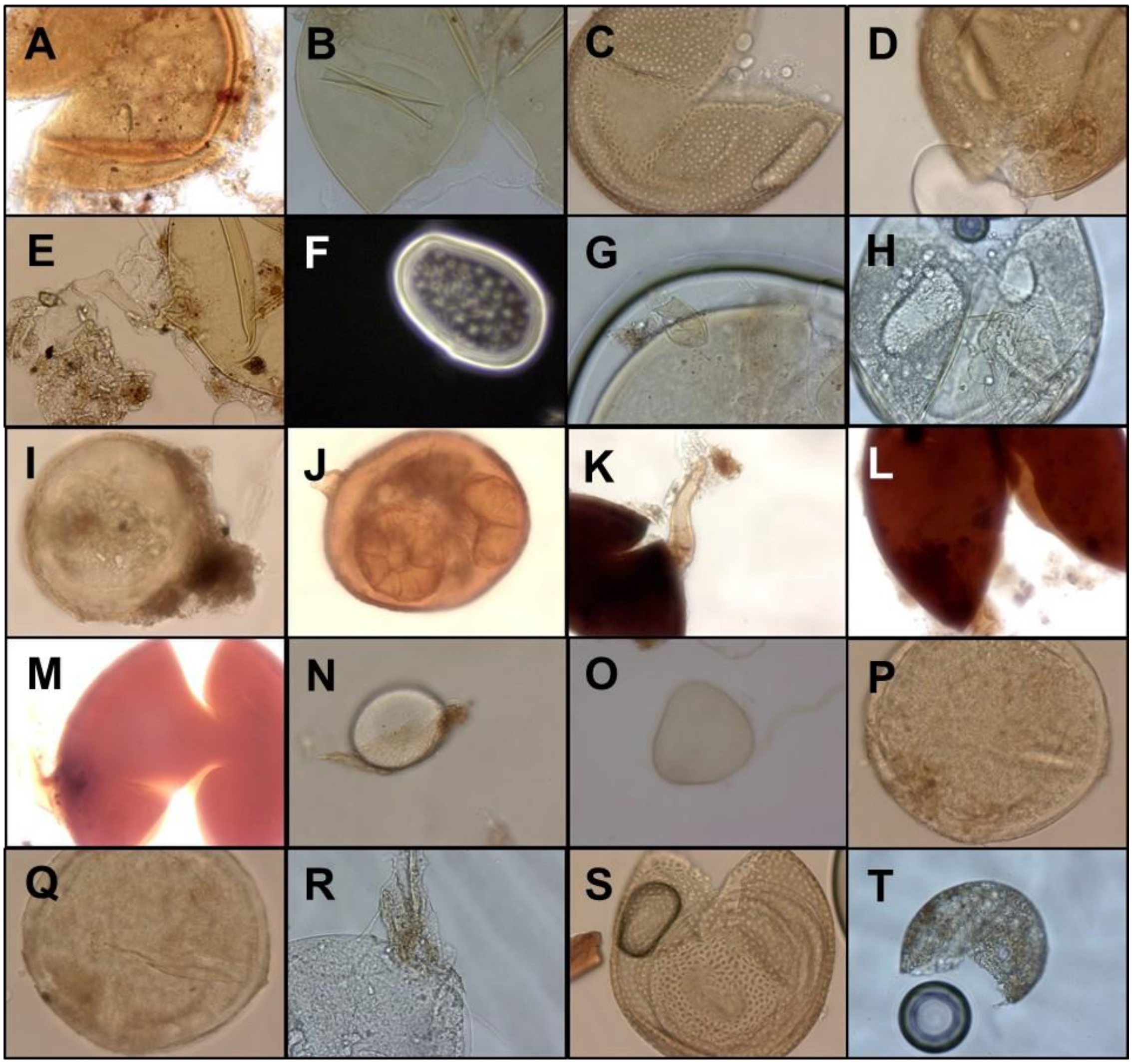
Supplementary Material 1. General view of the most abundant spores of arbuscular mycorrhizal fungi found in the three analyzed plots.



A: Spore with hyphal mantle. B: Single-layered, laminated spore wall. C: Spore with outer layer rounded ornamentation. D: *Entrophospora sp.*, E: Hyphal mantle attached to hyphae. F: Multinucleated spore. G: Hialine and semiflexible layer. H: Spore lipids content. I: Spore´s germination shield. J: Spore with noticeable germination shield. K: Crushed spore and bulbous subtending hyphae. L: *Gigaspora sp*. in Melzer´s reagent. M: Crushed spore in Melzer´s reagent. N: Spore in bright field light. O: Irregular shaped spore. P: Wide laminated spore wall. Q: *Gigaspora sp.* R: Spore´s hyaline outer layer. S: Crushed spore with layer ornamentation. T: Hyaline spore

Supplementary Material 2. AM root colonization (%) of the different plant species present in the analyzed plots subjected to three different managements in an Andean Ecuadorian soil.

Plant species	Soil management		
	Grasslands	Maize	Potato
Pennisetum clandestinum	54	59	55
Verbena litoralis	26		
Cynidon dactylon	43	60	
Solanum nigrum	83		
Conyza floribunda	56		
Avena sativa	70		
Bidens leucanta	56	45	
Trofolium repens	56	44	
Galinsoga parviflora	45	12	62
Galinsoga ciliata		21	18
Taraxacum officinalis		70	
Chenopodium paniculata		18	23
Amaranthus blitum		31	26
Senecio vulgaris		30	28
Malvastrum peruvianum			17
Verbena sp.			45
Physalis peruviana			35

The number of plant species sporadically growing in each plot is similar among them, although the AMF root colonization percentage of those plants varies (9 plant species sporadically found in naturalized grasslands, had an average of 54.3% of AMF root colonization, 10 plant species were found in the maize plot, with an average of 39% of AMF root colonization, and other 9 plant species were found in the potato plots with an average of 34% of AMF root colonization). These results indicate higher AMF root colonization in plants growing sporadically in the naturalized grasslands, representatives of NT plots, compared to a lower AMF root colonization percentage in the CT plots (maize and potato plots).

Chapter IV:

"Noticeable shifts in biochemical soil properties after contrasting tillage management in crop rotations of bean, maize and amaranth in Ecuadorian highland soils"

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Type of contribution: Research article

Noticeable shifts in soil biological properties after contrasting tillage management in crop rotations of bean, maize, and amaranth in Ecuadorian highland soils

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ABSTRACT

Biological properties are sensitive and useful soil quality indicators of changes and perturbations occurring under agricultural management. In the Ecuadorian highlands, these types of studies are scarce. This study aimed to evaluate 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. The enzyme activities of acid phosphatase (Pacid) and β-Glucosidase (Gluc), fluorescein diacetate (FDA) hydrolysis, microbial biomass carbon (C_{mic}), soil basal respiration (BR), AMF spore density, total glomalin content (TGRSP), and soil physicochemical properties were assayed. Conventional tillage (CT) and crop rotation had significant effects on soil physicochemical and biological properties. Towards the final crop rotations, no-tillage (NT) promoted BR, TGRSP, and the AMF spore density in both crop rotations, the C_{mic} kept stable along time in BMB and BAB, while BR doubled its value when compared to CT. 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. A multivariate analysis reported strong and positive relationships among soil organic matter (SOM), soil moisture, and BR under NT. Thus, the biological traits were strongly associated to the accumulation of SOM originated from crop residues from the NT post-harvest system, which improves soil moisture, biological activity, and AMF presence. These results represent the first approach to understand the effects of tillage, fertilization, and crop rotation on the physicochemical and biological soil properties in highlands from Ecuador.

Keywords: Acid phosphatase; Arbuscular mycorrhizal fungi; β-Glucosidase; Enzyme activities; Glomalin.

4.1 Introduction

Soil is a vital resource for food production, carbon (C) sequestration and climate regulation, nutrients and water regulation and biodiversity enhancement, among other important functions (Arshad and Martin, 2002; Panagos *et al*., 2020). Despite this, agricultural management practices, such as tillage, fertilization and crop rotation, often negatively affect soil physical, chemical, and biological properties, ultimately impacting crop yields and productivity (Martínez *et al*., 2008; Saikia and Sharma, 2017). Intensive conventional tillage (CT) causes loss of organic C and nitrogen (N) due to an accelerated soil organic matter (SOM) break down with the subsequent decrease in soil quality and fertility (Roscoe and Buurman, 2003; Cheng and Kuzyakov, 2005; Kabiri *et al*., 2016). Mono-cropping, mechanical tillage, chemical fertilization, and residue removal increase SOM depletion, disruption of soil structure and soil moisture loss with negative effects over soil enzyme activity and nutrient availability for plants (Rao *et al*., 2017). According to Qin *et al.* (2021), fertilization management has negative effects on the soil nutrient flows with consequent changes in soil enzyme activities. Specifically, N and P fertilizers negatively influenced the activities of β-D-glucosidase (BDG) and phosphatases (PHO), which have been used as indicators for C and P-cycling, respectively. Since microbial enzyme activities proved to be sensitive indicators of agricultural intervention, this information can be used to improve fertilization management strategies.

Crop rotation is an agricultural practice that affects soil biological activities. Wang *et al.* (2020) reported that a more diversified crop rotation improved few soil health indicators such as moisture content, bulk density, SOM, as well as the enzyme activities of sucrase, urease and alkaline phosphatase associated to a more diversified microbial community during the early years of establishment of 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 (Rigon and Calonego, 2020). 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 (Davies and Finney, 2002; Mirzavand *et al*., 2020). 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 (Hillel and Hatfield, 2005; Dang *et al*., 2020). Additionally, NT practices contribute to a reduction in C loss to the atmosphere and to its storage in soil, fighting climate change (Derpsch, 2003; Krauss *et al*., 2020; Tahat *et al*., 2020). 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 pest (Nicholls and Altieri, 2013).

A meta-analysis conducted by Nunes *et al*. (2020a) 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 (C_{mic}), and enzyme activities, which stand out as early indicators of soil management (Islam and Weil, 2000; Allison and Jastrow, 2006; Adetunji *et al*., 2020; Mirzavand *et al*., 2020). In this context, more than 90% of SOM breakdown is done 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 (Green *et al*., 2006; Gajda *et al*., 2013; Patle *et al*., 2018). Nevertheless, according to Aponte *et al*. (2020), soil enzymes by themselves do not reflect all aspects of soil microbial activity and function, thus other soil biological and physicochemical properties are required in addition to describe soil perturbations, as those produced by CT.

Plant and AMF interactions regulate soil biological activities and are of great interest in agriculture (Selvaraj and Thangavel, 2021). AMF have been widely studied for their positive effect on soil physicochemical properties due to its glomalin production. Glomalin is a fungal glycoprotein that acts binding soil particles, and increasing the soil's structure stability (Cornejo *et al*., 2017). 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 (Curaqueo *et al*., 2011; Säle *et al*., 2015). 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. Nunes *et al*. (2020b) mentioned that latitude together with soil management type, time under NT, soil order and cropping rotations affect soil chemical and biological properties. They 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. Consequently, the objective of our study was to analyze soil physicochemical and biological responses under contrasting tillage management, fertilization, and crop rotation practices in highland soils from the Andean region of Ecuador. Our hypothesis proposes that NT and low fertilization rates will increase soil biological activity induced by changes in physicochemical properties, which will be noticeable at the end of each crop rotation in an Andean soil from the highlands of Ecuador. In this sense, our results will help to understand the effects of contrasting agricultural management and its impact on soil properties in the Andean region of South America.

4.2. Materials and methods

4.2.1 Site description, experimental design, soil and plant sampling

The experiment was located in a research field with a total surface of 5346 m^2 at the Universidad Central del Ecuador Experimental Station (CADET), Tumbaco, Quito, Ecuador (0º13'49"S, 78º21'18"W; 2505 masl) (Supplementary material 3). The mean annual precipitation is about 870 mm, the relative humidity about 75%, and annual average temperatures between 10.3ºC and 27.2ºC. The soils have been classified as Mollisols, presenting characteristics of Entic Durustolls from the northern highlands of Ecuador: dark volcanic-ash derived soils with allophane material. The whole research surface was previously cropped with oats (due to its extractive properties), in early 2016, in order to prepare the soil before the study. In this study, bean (*Phaseolus vulgaris*)-maize (*Zea mays*)-bean, and bean-amaranth (*Amaranthus caudatus*)-bean crop rotations were established, both under contrasting tillage management (conventional tillage: CT, and no-tillage: NT) and increasing N fertilization doses (explained below). The first crop (C0 = initial bean) plot was divided in two sections: CT and NT, each with 24 subplots of 12 x 7 m^2, randomly distributed. As our focus of study was the analysis of the soil´s physicochemical and biological properties under tillage, fertilization, and crop rotation effect, five rhizospheric soil samples from each subplot were collected and homogenized in the corresponding labeled and sealable plastic bag in order to obtain one composed sample from each subplot. The root systems of individual plants from this crop were collected at the end of September 2016. The following crops 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. The maize cycle (C1) subplots dimensions were 12 x 7 m^2, and the amaranth cycle (C2) subplots dimensions were 7 x 5.5 m^2 due to the available cropping area. The maize rhizospheric soil and the crop's root system were collected in January 2018, while the amaranth samples (rhizospheric soil and the crop's root systems) were collected in March 2018. Finally, the last bean crop after maize (C3), and bean crop after amaranth (C4) were established (Figure 1). The samples from the last crop were collected at the end of September 2019. 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%, which were left on the topsoil of the NT plots.

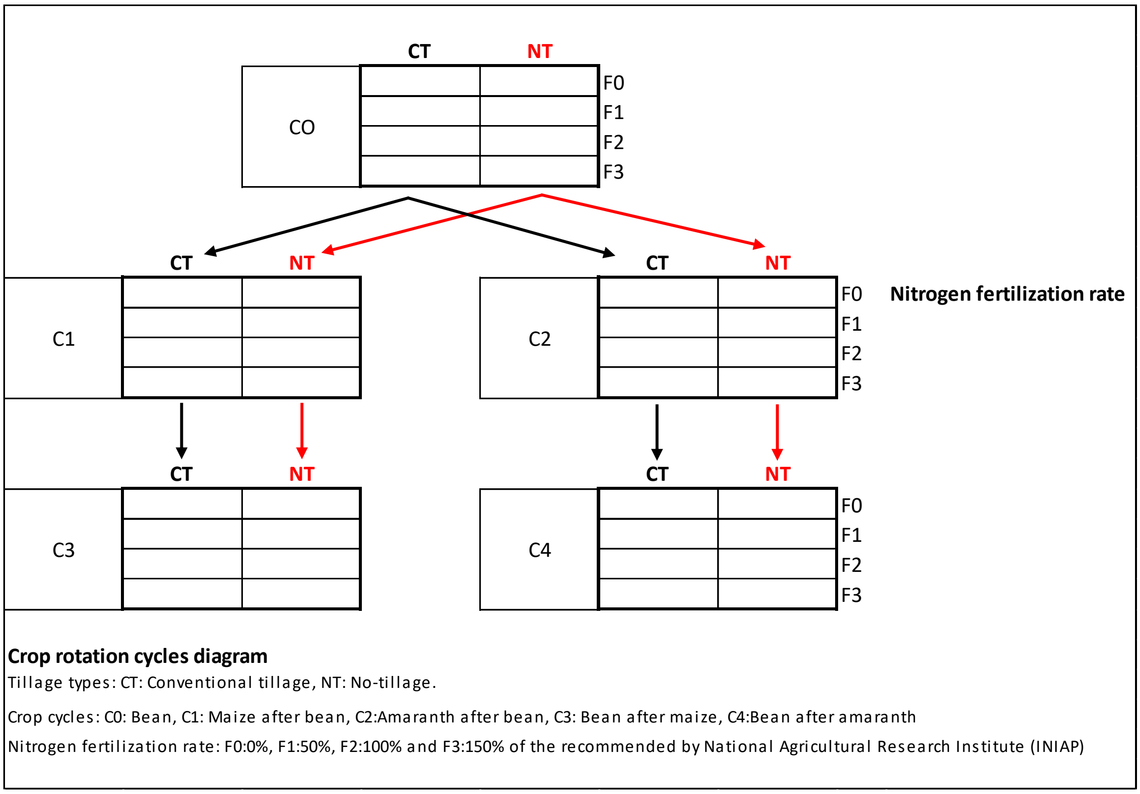

Figure 1. Schematic explanation about the experimental design here performed. C0 corresponds to the starting crop where only beans were cropped. In a second stage, half of the total surface was used to crop maize, and the other half was cropped with amaranth, using the same previous treatment of tillage and fertilization. Finally, in a third stage bean was cropped in all the surface.

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 rhizosferic soil sub-samples 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 -20ºC until the analysis of enzyme activities. Additionally, three randomly collected plant individuals (belonging to bean, maize and amaranth crops) from each plot replicate, with their intact root system, were collected in sealable plastic bags, to be analyzed for AMF root colonization, adding up to 24 soil samples and root systems from each crop for further analysis.

4.2.2 Soil physical and chemical determinations

In this study, determination of pH and EC were made on 1:2 slurries of air-dried soil and water (Smith and Doran, 1996). Soil samples were oven-dried at 105°C for soil moisture and bulk density determinations (Carter, 2007). Total porosity was calculated from bulk density assuming a particle density of 2.65 g cm^3 and 98 % saturation. SOM determinations were performed according to the wet oxidation method in potassium dichromate according to Walkley and Black (1934). Particulate organic matter (POM) was determined in moist soil samples following the colorimetric method described by Magdoff and Weil (2004), and soil available P was extracted with 0.5 M $NaHCO_3$ at pH 8.5 and measured according to Olsen and Sommers (1982). The Kemper and Rosenau (1986) method was used to determine the stability of soil aggregates in water. Four grams of 1-2 mm air-dried soil aggregates were placed in 0.25-mm mesh sieves and slightly pre-moistened, then the sieves were immersed in sieve holders with deionized water and shaken at a rate of 35 rpm at room temperature for 5 mins and the remaining fraction was maintained in the above conditions for other 60 mins. For calculations, the water stability index (WSI) was obtained as: WS1 = 100 (1 -A/B), where A and B are the weights of aggregates passing through the sieve after 5 and 60 min, respectively.

4.2.3 Soil biological determinations

Microbial biomass (C_{mic}) and basal respiration (BR) were measured by the chloroform fumigation-incubation procedure described by Vance *et al.* (1987), with some modifications due to laboratory conditions. For this, we used a degassing chamber and then titration with HCl 0.1 M and CO_2 determination for soil microbial activities and abundance (Vance *et al.*, 1987; Weaver, 1994; Vidal *et al.*, 1997). Enzyme activities of acid phosphatase (Pacid) and β–D–Glucosidase (Gluc) were measured by the colorimetric determination of the released p-nitrophenol (p-NP) when soil is incubated with p-nitrophenyl phosphate disodium salt (Tabatabai, 1994) and p–nitrophenyl–β–D–glucopyranoside (Eivazi and Tabatabai, 1988), respectively. Measured values are expressed in µg p–NP g^{-1} dry soil h^{-1}. The fluorescein diacetate (FDA) hydrolysis was determined by measuring the fluorescein released after soil incubation with FDA, which was measured at 490 nm according to Schnürer and Rosswall (1982). A Perkin Elmer lambda 25 lab UV-VIS spectrophotometer (Shelton, CT 06484 USA) was used for the three enzymatic determinations.

4.2.4 Arbuscular mycorrhizal fungal spores and root colonization

Spores of AMF were isolated from soil samples by means of wet-sieving (250, 106 and 53 µm sieves) and decanting methods followed by sucrose gradient centrifugation (Gerdemann and Nicolson, 1963; Sieverding, 1991). After centrifugation, supernatant containing the AMF spores were 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 according to Phillips and Hayman (1970), using the method of Koske and Gemma (1989) for root clearing and staining with trypan blue. The presence of AMF structures within the roots was observed at 40-100X in microscope slides, according to the line intersection method (Giovannetti and Mosse, 1980).

4.2.5 Total glomalin-related soil protein

The total glomalin related soil protein (TGRSP) was extracted from soil samples according to Wright and Upadhyaya (1998), with minor modifications. Briefly, 8 ml of citrate buffer were added to 1 g of dry soil and then autoclaved for 1 h at 121°C. This step was repeated several times on the same sample until the red-brownish color disappeared from the supernatant. The TGRSP content was determined spectrophotometrically by means of the Bradford protein assay (Bio Rad Protein Assay; Bio Rad Labs, USA) at 595 nm, using bovine serum albumin as standard.

4.2.6 Statistical analysis

Data were analyzed for each of the soil 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, 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. Also, a principal component analysis (PCA) was applied to evaluate the grouping of variables and their association with experimental individuals. The analyses were performed in R statistic version 3.5.1.

4.3 Results and discussion

In our results, the ANOVA showed that the contrasting type of tillage here studied (CT and NT) had highly significant effects over certain variables, such as Pase, β-Gluc, BR, and TGRSP (Table 1). Also, the crop rotation (Cycle) had highly significant effect over 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 β-Gluc and TGRSP.

Table 1. F-values and significance for the main effects and factor interaction for the variables measured and 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 X Tillage	**115.5*** **	**26.9*** **	1.4NS	**7.9*** **	**37.0*** **	**9.7*** **	**7.5*** **
Cycle X Fertilization	**17.4*** **	**3.9*** **	**4.3*** **	2.2*	1.5NS	1.6NS	1.4NS
Tillage X Fertilization	**11.3*** **	0.8NS	0.6NS	3.5*	1.5NS	5.5**	0.1NS
Cycle X Tillage X 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=β-glucosidase activity; FDA=fluorescein diacetate activity; C_{mic}=microbial carbon; BR=basal respiration; TGRSP=total glomalin related soil protein; AMF=arbuscular mycorrhizal fungi.

Soil C_{mic}, BR, AMF spore density, Pacid, Gluc and TGRSP presented significant differences according to the crop rotation (Figure 2). C_{mic} under CT diminished about 20% along the crop rotation cycles; however, under NT the C_{mic} kept relatively stable along the cycles (Figure 2). The unchanging values of C_{mic} under NT could be reflecting a buffered effect in soil, as a response to the accumulation of crop residues on the soil surface as labile SOM, which provides a continuous organic C input that supports the microbial C biomass. According to Espinoza *et al.* (2007), in a 7-year study, the NT favored the OM cycling in the soil, contributing to the presence of higher C_{mic}. 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 microbial diversity (Bertin *et al.*, 2003; De Graaff *et al.*, 2010; Ramirez-Villanueva *et al.*, 2015).

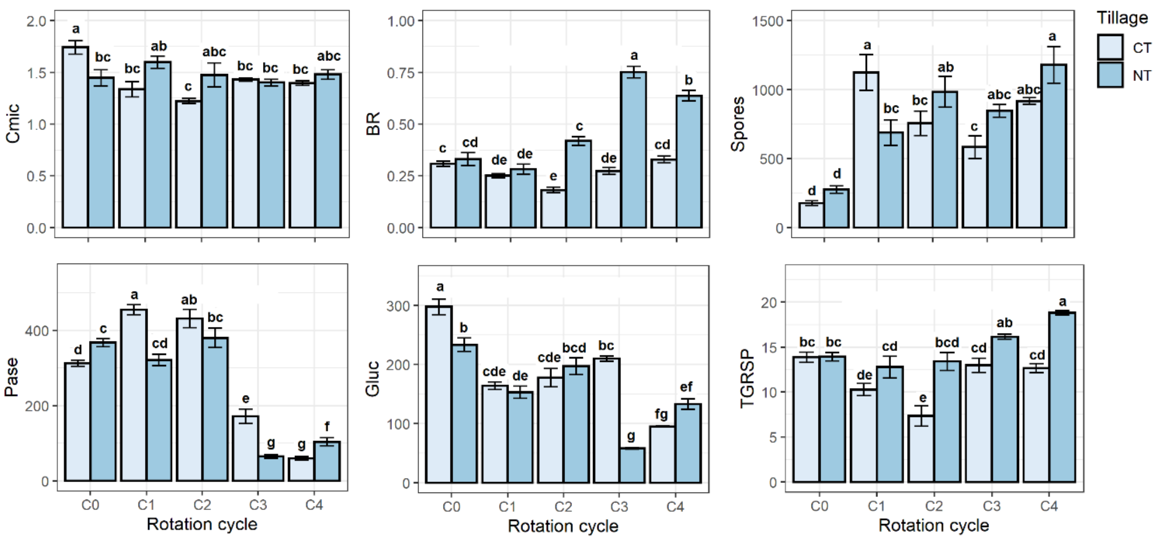

Figure 2. Biological and biochemical traits of an Ecuadorian highland soil subjected to different crop rotations and tillage management. C0=initial bean crop, C1=maize crop after bean crop, C2=amaranth crop after bean crop, C3=bean crop after maize crop, and C4=bean crop after amaranth crop. CT=Conventional tillage, NT=No tillage. Abbreviations: C_{mic}=microbial biomass (mg C-CO_2 g^{-1} dry soil), BR=Basal respiration (mg C-CO_2 g^{-1} dry soil), Spores=number of arbuscular mycorrhizal fungi spores in 100 g soil, Pase=acid phosphatase activity (μg pNP/g dry soil*h), Gluc=β-glucosidase activity (μg pNP/g dry soil*h), FDA=Fluorescein diacetate hydrolysis (μg FDA/g dry soil*h). Different letters indicate significant differences according to the Tukey's multiple range test ($P < 0.05$).

Non-significant variations under CT were observed for BR (Figure 2). On the contrary, this variable increased 2-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. In all cases, the BR showed higher values under NT compared to the CT, which could reflect higher microbial respiration due to higher SOM availability from crop residues and an increased microbial metabolic activity needed for SOM degradation. The higher BR values and significant differences observed under NT for C2, C3, and C4, are in agreement with recent reports by Bongiorno *et al.* (2020), who mentioned 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 are actively decomposing available SOM as C and energy source, and it is strongly influenced by soil management occurring under crop rotations, tillage, and fertilization along time (Avila-Salem *et al.*, 2020). On the other hand, differences for BR between CT and NT were higher at the end of the study, while C_{mic} was stable. These results suggests that an increased microbial respiration from the constant C_{mic} can be associated to a higher energy investment to degrade stable SOM, together with an increase of labile C fractions, and the probable activity of less-efficient soil microbial communities (Dilly *et al*., 2001).

The activities of Pacid and Gluc presented an unespected diminishment along the time for NT. At the end of the study, Pacid 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 Pacid and β-Gluc were observed under NT. 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 increases the potential enzyme activity in the root zone (Moreno-Espíndola *et al.*, 2018), especially under NT management. In this context, Bateman *et al.* (2011) emphasized on the "unique rhizosphere environment" from each crop explaining that with new established crops, specific microbial communities establish as well, with specific functionalities and enzyme activities. Zuber and Villamil (2016), by means of a meta-analysis, concluded that NT represents a more favorable microclimate which could be enhancing diverse and greater microbial activity, derived from the increased OM.

The phosphatases are extracellular enzymes synthesized by the plant roots, fungi, and bacteria in the soil, and previously have been reported with lower activity in accord with an increased intensity of soil management (Roldán *et al.*, 2005; Alvear *et al.*, 2006). Pandey *et al.* (2014), in soils cultivated with rice, reported a greater Pacid activity under NT and reduced tillage systems when compared with a continuous tillage system. In this study, the Pacid showed contradictory results compared to our findings, which could be due to the non-limiting P conditions as well as the significant increase of AMF populations along time, supporting a more efficient P supply to the rice crop by means of the mycorrhizal pathway. Moreover, higher Gluc activity in CT could be associated to increasing recalcitrant SOM. Reinforcing, de Almeida *et al.* (2015) reported that the Gluc activity tends to be higher in soils with high levels of easily decomposable SOM, as such soils that use crop rotation or direct sowing.

According to our results, in the C3 and C4 crop rotations, when comparing CT and NT between crop, there is a positive correlation between Pacid and Gluc (Figure 3D and 3E). Therefore, the effects of previous management could be cumulative throughout the time and produce changes of higher magnitude in soil conditions and microbial activity due to a more stabilized and recalcitrant SOM. Usually, it is considered that the benefits of NT for the soil properties take several years to occur since the soil needs to regain its structure when it has been disrupted before by past intensive tillage practices, and previous crop rotations cycle after cycle. Interestingly, the AMF spore density increased more than 400% under CT at the end of the C4, reaching an increase of near 530% under NT (Figure 2). Castillo *et al*. (2006) reported that tillage and crop rotations modified the AMF colonization and spore density, with increased values under NT management, thus being suggested as an early indicator of soil management changes. 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. The effects of contrasting tillage managements on AMF spore densities were most evident in the final cycles (C2, C3 or C4) under NT when compared with C0, which agrees with other recent reports (Baltruschat *et al*., 2019).

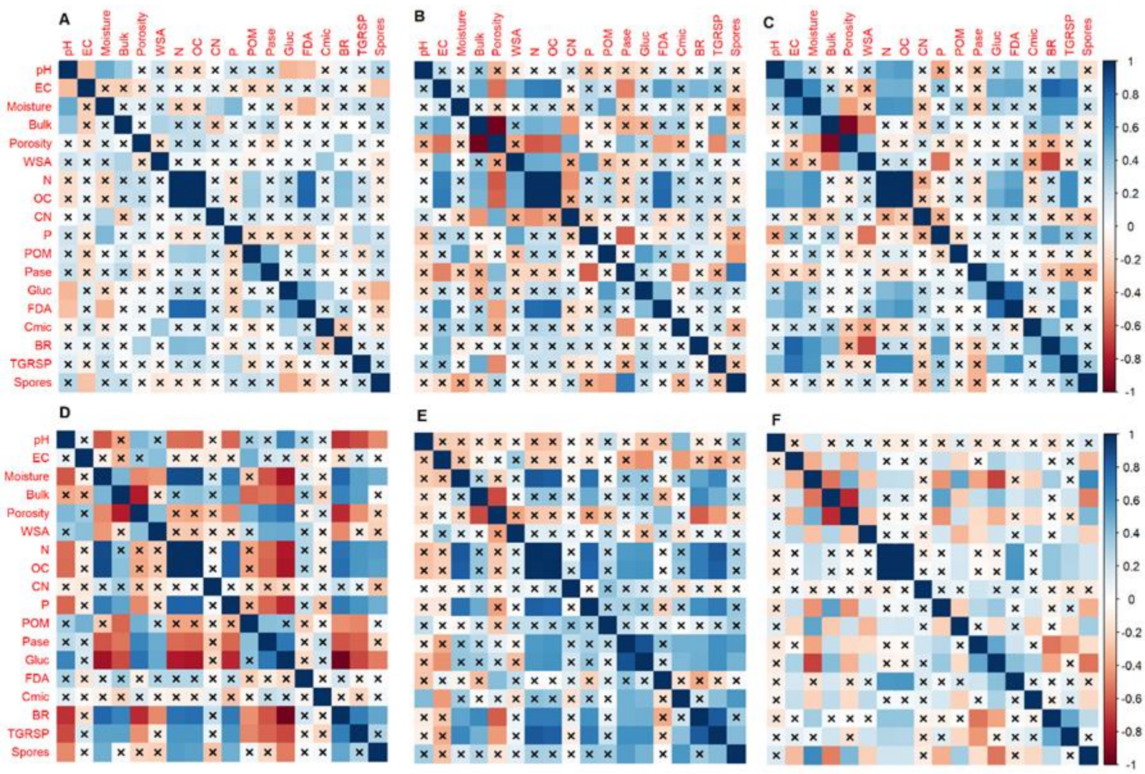

Figure 3. Correlation matrices between biological and biochemical characteristics of an Ecuadorian highland soil subjected to different crop rotations and tillage management. A) C0=initial bean crop. B) C1=maize crop after bean crop. C) C2=amaranth crop after bean crop. D) C3=bean crop after maize crop. E) C4=bean crop after amaranth crop. F) Global correlation considering all the crop cycles. Abbreviations and clarifications: 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*h**)**, Gluc=β-glucosidase activity (μg pNP/g dry soil*h**)**, FDA=Fluorescein diacetate hydrolysis (μg FDA/ g dry soil*h**),** C_{mic}=microbial biomass (mg 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.

Moreover, the glomalin content (here determined as TGRSP) under NT showed incresed values towards the last two crop cycles (C3 and C4), when compared to CT (Figure 2). The TGRSP is well known as a binding agent that improves the soil aggregates formation (Pal and Pandey, 2014). Here, TGRSP increased by 25% throughout time under NT, but decreased about 10% under CT management. This effect could be related to the fact that under CT, the superficial soil layers are destroyed by mechanical tillage, and so are the AMF extraradical hyphal network, fungal microbial biomass, and TGRSP content, as mentioned in a study conducted by Hage-Ahmed *et al*. (2019). In this sense, tillage has a strong negative effect on the binding mechanisms of soil stabilization, while on the contrary, NT positively influences soil aggregate binding agents such as SOM, microbial biomass, and glomalin-related fractions (Zhang *et al*., 2012).

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 including in C0 under CT and NT, which is consistent with the report by Montesdeoca *et al*. (2020). However, at the last cycles (C3 and C4), the effects are most consistent under CT and

NT, suggesting the need for longer timescales to evidence the stabilization effects in soils, which can prevent soil degradation and resilience increase. Towards the final cycles (C3 and C4), the TGRSP and AMF spores number showed positive correlations with N content and OC (Figure 3D and 3F), which suggest that SOM enhance the presence and functionality of the AM symbiosis. As an implication, the AMF play an important role as soil aggregation factor through an increased TGRSP amount, which also result of interest in soil C and N stocking in soil (Fokom *et al.*, 2012).

The principal component (PC) analysis 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 (Figure 4), where most of variability was explained by the number of spores of AMF and moisture content. On the other hand, BR, TGRSP, N, OC, and FDA explained most of variability of PC2 (associated to tillage effect), being all positively related in NT, especially from C1 to C4 when distance between NT and CT were more evident compared to C0 (Figure 4). Although close distance between crop rotations (except for C0), a transition from C1 to C4 was found in PC2. The previous suggests that the progression of some key variables along time, can be well-represented by the separation along the time regarding the starting conditions. The previous is evident if we considered that C3 and C4 represent both bean crops, where similar conditions of tillage and fertilization regarding the C0 initial crop were used.

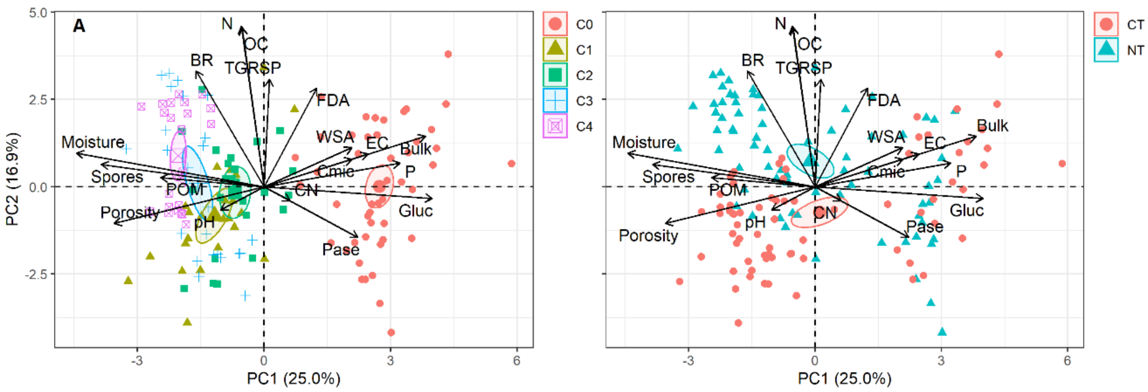

Figure 4. Principal component analysis for the experimental variables and treatments used in an Ecuadorian highland soil subjected to different crop rotations (A) and tillage management (B). The results shown are similar (A and B) but were stablished the separation between crop cycles and tillage management for visual purposes. The two first PCs extracted accounted by a 42% of the total experimental variance. Abbreviations: CO=initial bean crop, C1=maize crop, C2=amaranth crop, C3=bean crop after maize, C4=bean crop after amaranth, CT=conventional tillage, NT=no-tillage, 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 (%), Pase=acid phosphatase activity (μg pNP/g dry soil*h**)**, Gluc=β-glucosidase activity (μg pNP/g dry soil*h**)**, FDA=Fluorescein diacetate hydrolysis (μg FDA/ g dry soil*h**),** C_{mic}=microbial biomass (mg C-CO^2 g^{-1} dry soil**)**, BR=Basal respiration (mg CO^2 g^{-1} dry soil**)**, Spores=number of arbuscular mycorrhizal fungi spores in 100 g soil.

The positive correlation between soil moisture and AMF spores (Figure 3F and Figure 4A) 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 intensive tilled soils cropped with potatoes and maize (Avila-Salem *et al*., 2020). Also, Curaqueo *et al.* (2011) and Schneider *et al.* (2017) observed a reduction in AMF spore density under CT in field conditions. Here, the PCA evidenced 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 (2018) reported a negative association between AMF spores and soil moisture in their seasonal study showing the need for a most comprehensive understanding about the complex soil interactions that determine major benefits from the AMF symbiosis. Finally, our results support that some soil components as SOM, BR, TGRSP, and the AMF spore density can be highlighted as key indicators of the progression of soil resilience status when diverse tillage managements are being performed.

4.4 Conclusions

A clear beneficial effect of no-tillage in comparison with conventional tillage was observed on a series of soil physical, chemical, and biological traits such as basal respiration, number of arbuscular mycorrhizal fungi spores, enzyme activities of acid phosphatase, β-glucosidase and total glomalin related soil protein, suggesting a higher sensitivity to soil disturbance and the feasibility to be used as indicator of tillage effects in the early and medium-time. The biological traits were strongly associated to the accumulation of organic matter originated from crop residues from the no-tillage post-harvest system that concomitantly improves the soil moisture, basal respiration, microbial enzyme activities, and arbuscular mycorrhizal fungi, all of them strongly supporting the crops establishment and development. Moreover, the design of sustainable crop rotations including high value species as amaranth provide new evidence about the possibility to include in the usual crop programs, giving an increased value to the agricultural products and favoring the early expression of desirable soil conditions. In this study, the nitrogen fertilization rates did not show an effect over physical, chemical or all biological traits, except for the phosphatase, probably because of precedent fertilizations from past decades applied to these soils to increase crop yields. This could be masking precise responses. In this sense, the variables measured in this study constitute important indicators for assessing soil quality in the Ecuadorian Andean soils. These are currently subjected to fast rates of degradation due to the very steep slopes and the use of intensive crop rotations and tillage.

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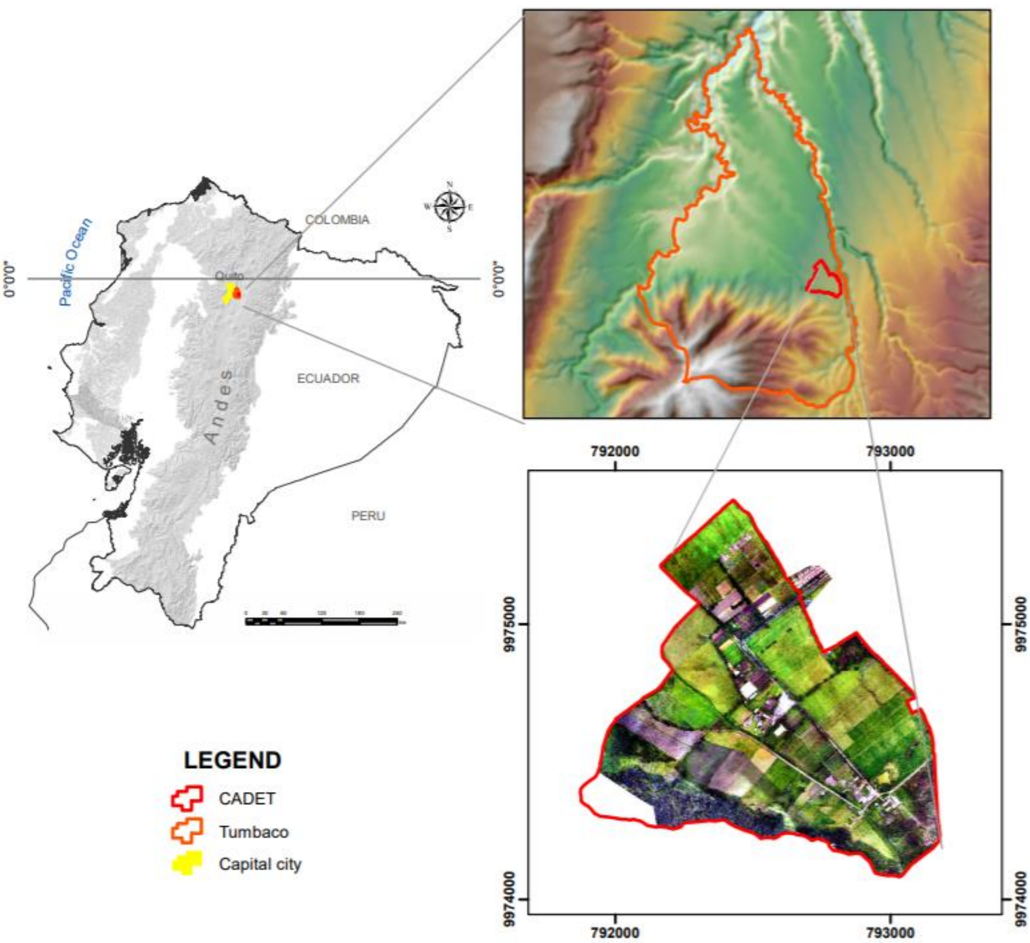

Supplementary material 3. Location of the site of study. The plots were included in a total of about 5,500 m2 into CADET.

Chapter V

General discussion and conclusions

5.1. General discussion

Soil management practices modify crop yield and productivity, but they also modify physical, chemical and biological soil traits (Miner *et al.*, 2020). Worldwide, soil tillage was introduced as an activity to promote seedling establishment, incorporation of fertilizers, herbicides and organic matter concomitantly with the weed control (Köller *et al.*, 2003; Martínez *et al.*, 2008). However, intensive soil management in addition to tillage, as stubble burning and monocultures, lead to soil degradation processes, which results in some physical, chemical and biological characteristic changes that lead to erosion, soil organic matter (SOM) and nutrients depletion, pH changes, loss of edaphic flora and fauna, among others (Gurjar *et al.*, 2017).

Several studies have focused on determining the effects of mechanical tillage, monoculture and residue elimination on enzyme activity and nutrients availability to plants, and how these effects are reflected on crop yields, soil quality and health (Rao *et al.*, 2017). However, there is scarce information of these effects on biological properties in Andean soils from the Ecuadorian highlands. Under this context, this Doctoral Thesis has as general objective to determine the effect of two contrasting soil management systems, Conventional Tillage (CT) *vs*. no-Tillage (NT), on the biological activities, and arbuscular mycorrhizal symbiosis in the bean-maize-bean and bean-amaranth-bean crop rotations, subjected to different fertilization levels in Ecuadorian Andean soils. In the process of our study and undergoing the different crop rotations along our medium to long-term study, we have evidenced and recorded the changes in soil properties due to the contrasting soil management in this initial and transitional phase. At the beginning of our study, we were able to set up a baseline study to record the physical, chemical and biological soil traits of precedent soils (Ávila-Salem *et al.*, 2020). This study evidenced that soils under a conservation tillage (for more than 7 years), as in the case of the naturalized grasslands studied, presented enhanced biological traits (e.i. increased SOM, arbuscular mycorrhizal fungi (AMF) spores' densities and hyphal length) compared to soils with potatoes and maize crops in the same area, which have been managed using intensive tillage under the same time. The presence of an enhanced SOM in the naturalized grasslands has significant effects on the rhizosphere since it is an important source of C and nutrients, which is associated to an increased microbial activity, including the presence of AMF and plant´s health and ultimately growth and productivity (Agnolucci *et al.*, 2020). In our baseline study, AMF density and diversity were morphologically characterized for the first time in Ecuadorian Andean soils. Our data also indicated that soils under pasture had more than four times higher AMF density and diversity, compared to tilled and fertilized soils, as in maize and potato plots. Our results agree with recent studies by Srour *et al.* (2020), where they found that both tillage and the use of fertilizers (with only N or NPK) significantly affect the microbial community structure in terms of diversity, demonstrating that CT and chemical fertilization actions can deplete the soil biological diversity. They reported that tillage significantly decreased bacteria, fungi, fusaria, and oomycete diversity, whereas fertilization only affected bacteria and fungi; AMF, mycoparasites, and nematophagous fungi, fungal saprotrophs and plant pathogens were reported in that study.

Soil management practices influence on microorganism population and soil microbial processes through changes in the quantity and quality of residues in the soil surface and some cm of depth in the profile. Physical, chemical, biological and biochemical properties of the soil can be significantly altered by these actions which in turn lead to changes of the composition, distribution of soil microbial communities and enzyme activities as well (Saikia & Sharma, 2017). Nevertheless, in our baseline study we found that the activity of the acid phosphatase was higher in the potato plots (CT), probably due to the amounts of fertilizers used along the past years to increase the crops' productivity, which are reflected in the microbial effort performed for the mineralization process in these soils, making nutrients available to plants.

Since the main goal in our study was to understand the effects of tillage, fertilization and crop rotations on physical, chemical and biological properties of Andean Ecuadorian soils; for this purpose, we worked with bean-amaranth-bean (BAB), and bean-maize-bean (BMB) crop rotations which are representative Andean crops in the region of study. Soil biological quality indicators in our study were enzyme activities, microbial C biomass, basal respiration, AMF spore's density and total glomalin-related soil protein (TGRSP). Under this framework, our results showed better responses under NT in both BAB and BMB crop rotations, highlighting BAB crop rotation where some soil biological variables responses were increased such as basal respiration, phosphatase, β-glucosidase, arbuscular mycorrhizal fungi spore number, and total glomalin content. This study evidences the early responses to soil management shifts towards soil conservation for improving soil management in Andean region of Ecuador, and the maintenance at least in the medium time. These actions will promote soil sustainability due to higher values found in soil quality indicators included in this doctoral project. Based on our results, tillage had a detrimental effect on the biological properties in the here studied soils, mainly represented by the soil microbial respiration (SBR) in both crop rotations (BMB and BAB). Under NT, SBR measured at the end of our study, was about 70% higher comparted to CT for the BMB crop rotation, while for the BAB crop rotation, SBR showed two-fold increase under NT. These results are in agreement with Clermont-Dauphin *et al.* (2004), where they reported lower SBR under conventional management in the volcanic soils of the uplands of the French West Indies, and associated with a decreased SOM in the 0–10 cm layer, affecting the microbial respiration, earthworm biomass and an increased banana root infestation by nematodes. Moreover, our results were similar to Srour *et al.* (2020), where tillage had a more prominent effect than fertilization on the microbial traits.

The results obtained in this study are very interesting, highlighting that these are the first years of the research study. As this study has been designed as a medium to long-term study, the same soil management activities will be continuously monitored throughout the following years under contrasting tillage type, fertilization doses and crop rotations, in order to gain a better knowledge of the soil dynamics occurring in the Andean highland soils from Ecuador over the years. As mentioned by Medina *et al.* (2015), crop residues need a variable time for building stabilized SOM in soil and for the microbial organisms to contribute to the mineralization processes occurring mainly under conserved soils. Therefore, more reliable data will be obtained through out time to perform deep comparisons, and to reach more biologically diverse, active, and healthy soils of agricultural and research importance.

The meta-analysis approach to assess the effects of tillage on microbial biomass and enzyme activities performed by Zuber and Villamil (2016) showed that there were greater microbial communities and also greater enzymatic activities in the globally studied soils. In this context, despite our long-term study is on its initial phase, and our results corresponds to the transition effects from CT to NT, our results strongly suggest that NT allows an increase in crop yields and improves crop rotation performance in the medium-term. In this sense, our prior studies in Montesdeoca *et al.* (2020) found that the levels of SOM, total N, available P, and bulk density showed a trend towards improvements under NT, even in the first stage of this transition. Noticeably, in all our study there were no clear effects of the fertilization doses over physical, chemical or biological soil traits, maybe due to some processes of change subject to multiple variable interactions. However, our results for enzyme activities indicated higher phosphatase and β-Glucosidase activities under NT in the BAB rotation. Enzyme activities are good indicators of soil quality, closely related to the microbial activity and community, since soil enzymes catalyze and increase several biochemical reactions involved in the organic matter transformation, nutrients mineralization and soil aggregation (Nannipieri *et al.*, 2002; Aponte *et al.*, 2020a; 2020b) thus becoming interesting tools for the application in the agricultural research fields.

In our study we included crop rotations with locally produced and commercialized Andean crops with nutritional importance such as bean and maize, and amaranth, characterized by their biomedical properties, to see how these crops behave considering the physical, chemical, and biological soil interactions occurring under tillage, fertilization and crop rotations designed for this Doctoral research. The scientific literature widely reported that the Amaranthacea family, including *Alternanthera, Amaranthus, Celosia, Digeria, Gomphrena, Notorichium,* and *Ptilotus* are non-AM plants (Koske *et al.*, 1992; Brundrett and Abbott, 1995; O'Connor *et al.*, 2001). The studies by Ragupathy and Mahadevan (1993) have interestingly reported in 7 of 17 species analyzed that *Amaranthus* presents AMF structures in their root system in plants studied in tropical plains from India. Under this context, we have observed AMF structures in the bean, maize and amaranth roots colonization, corroborating its presence (see Annexes). We observed that *Phaseolus vulgaris* presented both vesicles and arbuscules in the plant roots, in *Zea mays* arbuscules were mostly observed; however, *Amaranthus caudatus* presented only vesicles, as reported in a study by Rinaudo *et al.* (2010), where they mentioned that only vesicles were observed colonizing *Amaranthus retroflexus* root systems. Furthermore, as mentioned by van der Heijden *et al.* (1998) and Manoharan *et al.* (2017), AMF diversity can promote plant productivity and plant nutrition; therefore, it should be a priority to perform more studies to characterize the AMF communities composition in different soils from our region, within a variety of cultivated crops in order to enhance crop yields in a sustainable way.

An important application of this and further studies would be to deepen in the understanding the benefits of conservation tillage enhancing the diversity of microbial communities, which have important activities in the rhizosphere, as well as their key roles as ecosystem services providers. As mentioned in the study by Giovannini *et al.* (2020), there are multiple beneficial activities of AMF and other microorganisms occurring in soil, in a complex network of diverse interactions, which are playing important functions as plant growth promoting microorganisms and their activities in crops nutrition, and soil health. This beneficial associations and properties need to be furtherly explored and efficiently used by means of designed natural biofertilizers to attain a much-needed sustainable food production in our region, and also globally. There are many plant host-AMF interactions that need to be studied due to the existence of specific benefits, in an effort to design specific inocula for different Andean crops that could enhance nutrients availability to the plant, and therefore its nutraceutical properties. As a forthcoming step, it would be essential to perform innovative studies using "omics" tools to understand AMF diversity and functionality, a research field scarcely supported currently. In this context, Beaudet *et al.* (2018) reported that their studies performed in *Ambispora leptoticha, Acaulospora morrowiae, Claroideoglomus claroideum, Diversispora versiforme, Funneliformis mosseae Paraglomus brasilianum, Racocetra castanea and Scutellospora calospora* could potentially be used to deeply understand the AMF ecosystem functions and evolutionary relationships among this important fungal group. Undoubtedly, in our Andean region, more studies including AMF identification combined with advanced microbial functionality in our soils are required, and there relies the opportunity to enhance soil quality and health to cope with the adverse environmental conditions that we are currently facing such as soil erosion, drought, pests, among other biotic and abiotic stress conditions.

The glomalin (secreted by AMF is an important soil aggregate binding protein, which we found in high amounts especially under NT conditions, contributing to the enhancement of soil stability and structure. Based on the above, we can propose that the use of AMF traits as spores, hyphae and glomalin must be used as soil quality indicators in a complimentary point of view. Overall, this study is of great importance because it represents the first characterization of early responses towards conservation management efforts in soils from Ecuadorian highlands, based on physical, chemical and biological indicators. The findings reported in this study will guide sustainable agricultural practices for an improved and more sustainable soil management to be implemented for small farmers in the highlands of Ecuador, and to preserve soil in the long-term by promoting its resilience.

5.2 Concluding Remarks

As main conclusions from our study, we can mention the following:

1. Intensive tillage practices can negatively affect key soil physical, chemical, and biological properties in Andean soils from the highland regions of Ecuador, and also exert a negative impact on the arbuscular mycorrhizal fungi (AMF) propagules densities associated to the proposed crops in this study. On the contrary, no-tillage (NT) management helps to restore soil health and quality, which are essential for food production. However, NT should not be considered as an isolated action to help mitigate soil loss, and the integration with other soil conservation measures must be done to achieve better soil conservation results, as observed here in the NT plots.

2. Soil biological characteristic, such as AMF presence, glomalin, soil basal respiration and enzymatic activities occurring in these soils, must be studied in more detail as soil fertility enhancers and transporters from soil to plants, and moreover, as soil quality indicators.

3. According to our baseline study, we have compared the soil characteristics among a conservative type of tillage such as NT, as the one occurring in naturalized grasslands (unaltered for the last 7 years), *vs*. the intensive tillage practices applied to adjacent maize and potato plots (occurring for the last 10 years), and we have evidenced that the amounts of soil organic matter, total nitrogen content and enzyme activities (i.e. acid phosphatase) presented higher values than those of the maize plot, suggesting higher microbial biomass and activity.

4. In our initial study from naturalized grasslands, maize and potato plots, we have set the baseline in terms of AMF species diversity for our research project which is aimed to be continued in the research facilities from Universidad Central del Ecuador. In this sense, the following AMF species list, constitute the first report of these important fungal microorganisms isolated and morphologically identified in these soils: *Gigaspora margarita, Acaulospora spinosa, Claroideoglomus lamellosum, Scutellospora calospora, Acaulospora alpina, Rhizoglomus sinuosum, Diversispora tortuosa, Glomus sp., Acaulospora scrobiculata, Pacispora robiginia, Diversispora celata and Simiglomus sp.* A higher AMF community density and composition belonging to these species was evidenced in long-term stabilized soils (i.e. naturalized grasslands), revealing the beneficial effects of a conservative type of tillage system on the diversity of soil microorganisms.

5. We observed enhanced characteristics under NT in the bean-amaranth-bean crop rotation, suggesting a possible synergistic effect of tillage and the plant species used in

the crop rotation, responsible for shaping some physical, chemical, and biological improvements of Andean soils, and as a productive alternative to implement soil restorative actions in this region.

Finally, with this information, the hypothesis of this thesis is partially accepted, in the sense that NT has a strong effect on the improvements of biological activities and AMF densities in the bean-maize-bean and bean-amaranth-bean crop rotations in an Andean soil from the highlands of Ecuador.

5.3. Future directions

As far as our knowledge based on literature research, there is scarce information about studies which report the effects of tillage, fertilization and crop rotation over physical, chemical, and biological traits, including AMF propagules density and diversity in Andean soils. In this sense, our research project constitutes one of the first integrative approach and contribution to soil dynamics knowledge under the effects of anthropic activities in the region, which considers a biotechnological scope for main enzyme activities, mycorrhizal characterization and functionality studies in Andean soils from the highlands of Ecuador. Consequently, deeper studies need to be performed in these research areas, and considering the integrative study of the physical, chemical and biological soil traits under contrasting management activities. The findings presented in this Doctoral Thesis, reveal important information about the benefits of conservation tillage as no-till, and the enhanced soil quality and health in a mid-time scale which must be furtherly communicated among involved stakeholders. As it was reported in our data analysis, no-till promoted higher microbial activity as enzyme activity and biomass, with an enormous microbial potential, which also enhanced symbiotic associations between

AMF and crops. The inclusion of amaranth in the crop rotations, enhanced the biological properties in the studied soils. Even though some important medical and promissory properties have been reported from this crop, there is still scarce information about studies of amaranth under the scientific scope. The findings from this doctoral research need to be furtherly studied and compared among other soils and environmental conditions, due to the enormous potential that AMF and various plant growth promoting microorganisms represent to agriculture and sustainability. Conservation tillage and the enhanced activity of soil microorganisms which favors soil fertility, deserve to be part of a regional and global solution for soil management changes decreasing the dependency on chemical fertilizers, crop losses, water shortages, generalized pollution from greenhouse gases production, soil erosion, among others. This makes us demand stronger soil conservation efforts and sustainability actions in the Ecuadorian Andean region. Consequently, this should be extensively replicated in other soils with agricultural potential and conservation needs to attain higher crop yields and regional resilience. Soil science and integrative research projects of this kind must be designed in our regions to help with the understanding of the agroecological processes occurring in managed soils compared to other more conserved soils. We foresee that soil conservation measures, together with the use of these important soil microorganisms as novel biological tools, would help us to reach higher crop yields and enhanced nutritional properties in Andean crops for an expanding world population. Noticeably, the results in this study have contributed with good soil quality and health indicators, which should be applied and considered in future projects that seek soil quality restoration, enhanced crops productivity and soil sustainability assurance by an enhanced soil microbial diversity. We are aware that positive changes in soil quality and crops productivity take several years to be evidenced when choosing conservative agricultural management principles, but we are also aware of the enormous and unique biodiversity in the Ecuadorian region, which could be present also in the soil microbiology, and that can be considered as a key natural resource to be explored and exploited. The use of enhanced quality and healthy soils with native microbial organisms from our region, for a better local food production is a promising field which can be regionally expanded. In this sense, we reinforce the fact that soil conservation should be a priority for the current and the coming generations, to create and promote environmental conservation policies and actions, offer research funding opportunities to promote the study of the vast natural resources in this region and strengthen its protection while motivating good environmental practices towards soil conservation and productivity.

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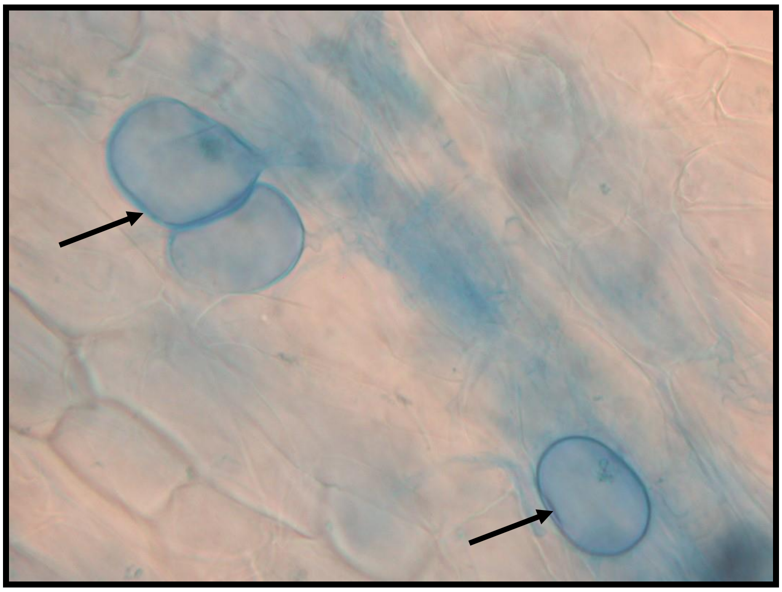
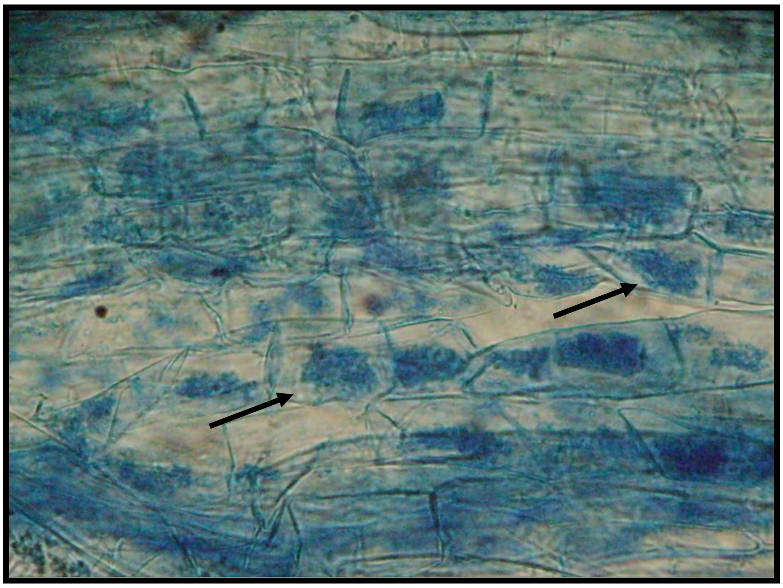


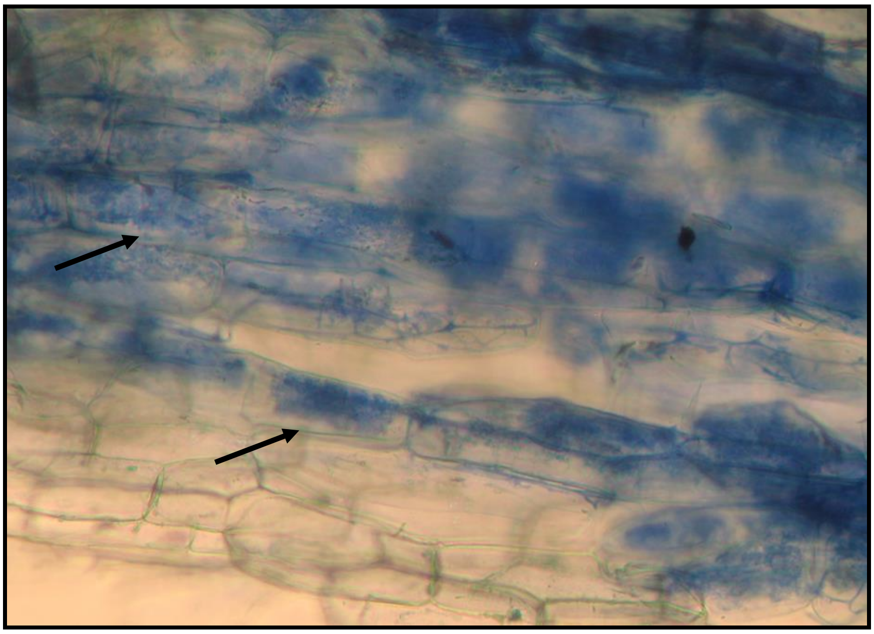
AMF root colonization in *Phaseolus vulgaris.*

Visible vesicules at 1000x microscope magnification



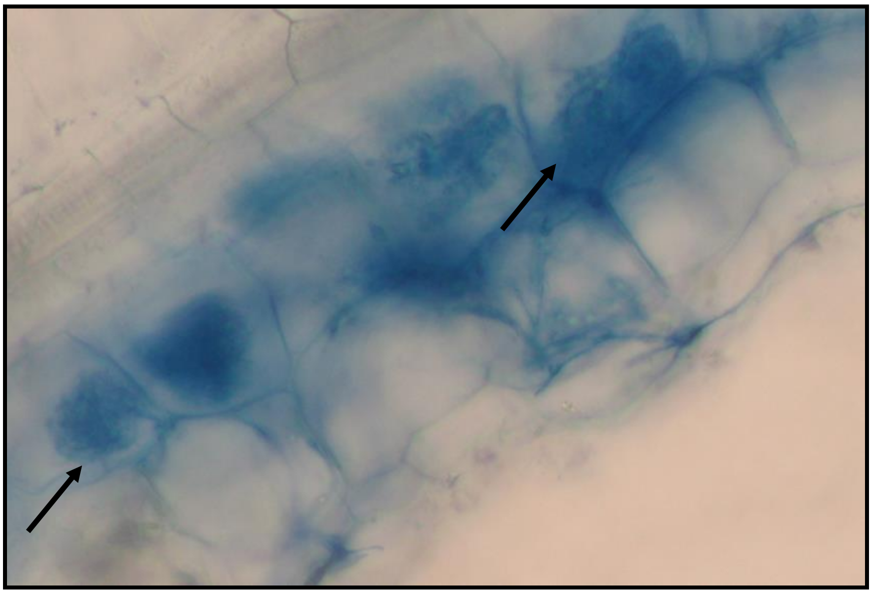
AMF root colonization in *Phaseolus vulgaris.*

Visible arbuscules at 400x microscope magnification



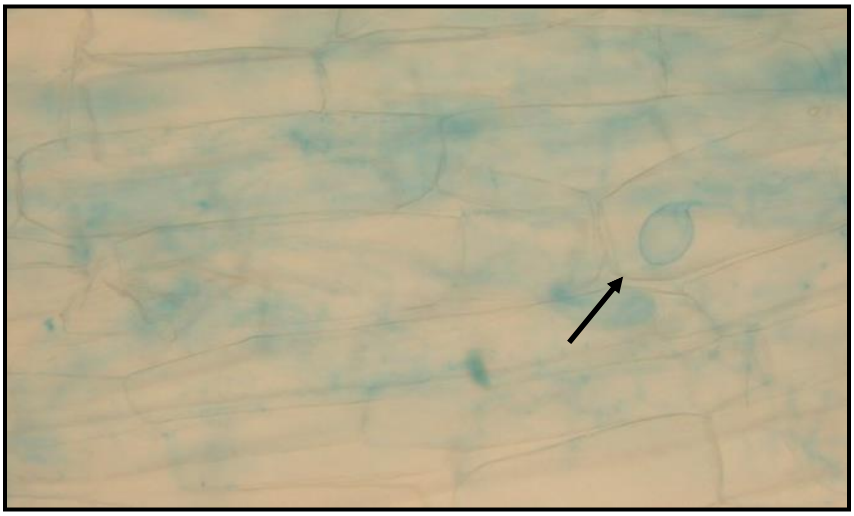
AMF root colonization in *Zea mays.*

Visible arbuscules at 400x microscope magnification



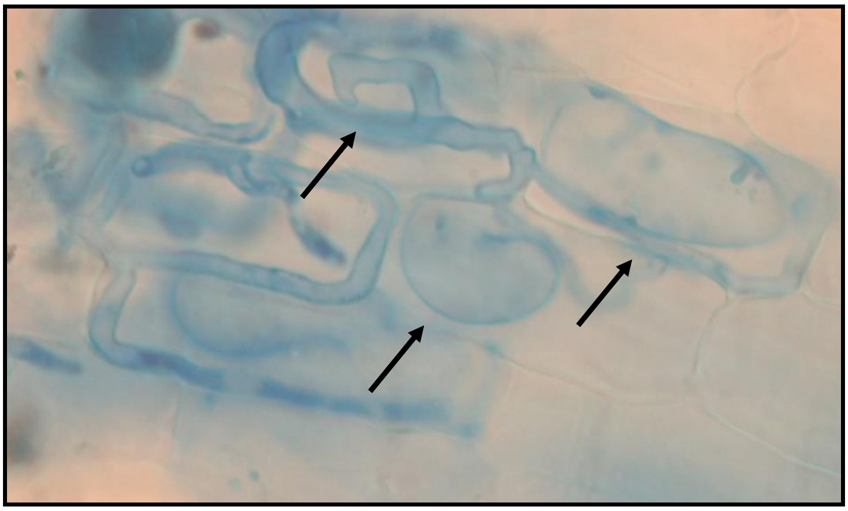
AMF root colonization in *Zea mays.*

Visible arbuscules at 1000x microscope magnification



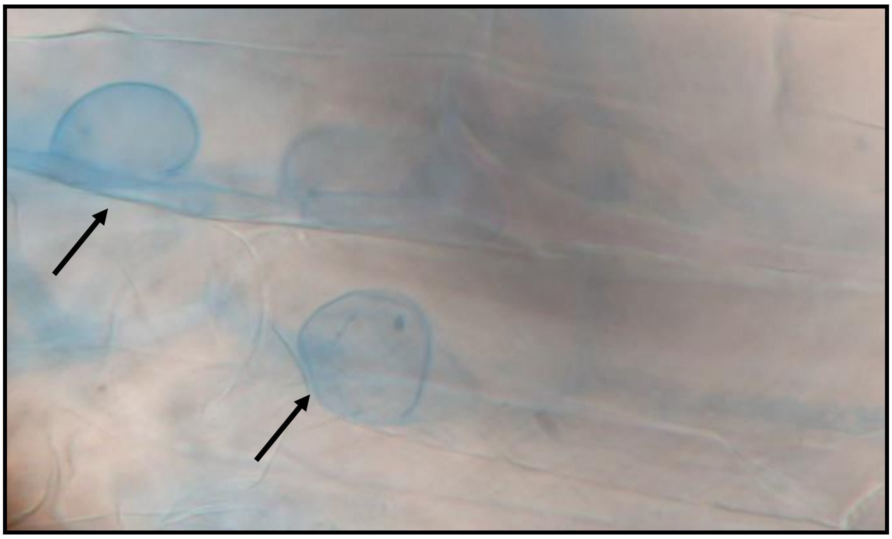
AMF root colonization in *Amaranthus caudatus.*

Visible vesicules at 400x microscope magnification



AMF root colonization in *Amaranthus caudatus.*

Visible vesicules and coils at 1000x microscope magnification



AMF root colonization in *Amaranthus caudatus.*

Visible vesicules at 1000x microscope magnification