Integral soil quality assessment based on arbuscular mycorrhizal fungi and related microbiological indicators
(AMF-RI)

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Table of contents

List of tables

List of figures

Abbreviations list

AMF	Arbuscular Mycorrhizal Fungi
AMF-RI	Arbuscular Mycorrhizal Fungi and Related Indicators
BD	Bulk Density
CAT	Catalase activity
CFU	Colony-forming units
DEPNA	Dependency Network Analysis
EACTI	Enumeration of culturable actinomycetes
EBACT	Enumeration of culturable mesophilic bacterial
EEGRSP	Easily Extractable Glomalin Related Soil Proteins
EFUNG	Enumeration of culturable fungi
ER-CP	El Retiro - *Crops of Pinus sp.*
ER-PMWF	El Retiro - Pre-montane wet forest
ER-QC	El Retiro - Quarry of clays
ESPO	Enumeration of spores
FISH	Fluorescence in situ hybridization
GRSP	Glomalin Related Soil Proteins
LC-CH	La Ceja - Crops of *Hydrangea sp.*
LC-PMWF	La Ceja - Pre-montane wet forest
LC-QC	La Ceja - Quarry of clays
LRR	Log response ratio
m.a.s.l	Meters above sea level
MBI	Microbial-Based Indicators
MBR	Microbial Basal Respiration
PLFA	Phospholipid fatty acid analysis
REM	Random Effects Models
RN-CF	Rionegro - Crops of *Fragaria ananassa*
RN-CQC	Closed quarry clay
RN-PMWF	Rionegro - Pre-montane wet forest
RP	Phosphate retention

SIR	Substrate induced respiration
SOC	Soil Organic Carbon
SQI	Soil Quality Index
SWC	Soil Water Contents
TEMP	Soil Temperature
TGRS	Total Glomalin Related Soil Proteins
TID	Total Influence Degree
URE	Urease activity

Abstract

Currently, soil quality assessments are based on scoring functions that assign scores to each indicator and combine them to generate an overall soil quality value. Whitin this type of soil quality assessment, microbiological indicators are highly necessary in conjunction with physical, chemical, and other biological indicators since the microbiological component drives a large part of soil functionality in terms of soil nutrient cycling, decomposition, nitrogen fixation, nutrient uptake to plant growth and water retention. However, soil quality assessments face two challenges that scoring functions and the subsequent use of microbiological indicators alone cannot adequately address. First, microbiological indicators are dependent on soil properties and other biological variables, making their interpretation context-dependent. Second, since scoring functions losses transparency during assignation of scores, other methods are necessary to analyze the effects of soil perturbations in response variables, considering local referents to identify the more affected variables. To address these challenges, this research focused on characterizing the responses to soil variations of communities of arbuscular mycorrhizal fungi and related microbiological indicators (AMF-RI). AMF-RI have been considered as generalist and relevant indicators of soil functionality but their implementation in soil quality assessments must be supported by an integral understanding of the relationship between them and the abiotic soil component. This thesis establishes, through a cross-biome meta-analysis, the Total Influence Degree (TID) of soil abiotic changes on AMF-RI. Results suggested a trend of large dependencies between AMF-RI with soil properties such as Pb concentrations, soil structural features, and nutrients stocks, which are explained by the effects of partial correlations among AMF-RI and microbial diversity, microbial biomass carbon and soil microbial respiration. Besides, the responses of AMR-RI facing soil abiotic changes derived from agricultural (AGA) and mining activities (MEA) were described in andosols of the southeast region of Antioquia (Colombia). A confident ANOVA approach was used to determine that populations of culturable mesophilic bacteria and fungi, catalase activity, microbial basal respiration, AMF diversity, AMF spore abundance, and total glomalin related soil proteins, as well as soil abiotic properties like NH4-N, SOC, S, and soil water contents, all suffered significant detriments depending on the degree of pressure exerted by land use. On the contrary, as the deterioration gradient progresses, Ca concentrations, soil temperature, and bulk density rise. Then, we tested a novel approach to integrate a set of soil quality indicators and AMF-RI into a

soil quality measure based on a geometrical analysis of proportions of change. Using Log responses ratios (LRR) in a soil quality assessment to overcome the problems of referent target values and lack of transparency, the perimeter of 2D polygons projected from radius vectors whose length represented a proportion of change in an indicator (LRR) was tested as a soil quality index (SQI_{LRR}). This method allows showing that mining and agricultural activities have a negative but varying degree of impact on abiotic soil features such as soil organic content and water contents, as well as microbiological features such as populations of culturable mesophilic bacteria and fungi, microbial basal respiration and spore density of arbuscular mycorrhizal fungi (AMF), AMF diversity, and the contents total glomalin related soil proteins. The geometrical analysis of proportions of change in soil quality indicators offered a different soil quality measure that, even with little data, accurately distinguishes the effects of various land uses (AGA SQI_{LRR}= 0.163; MEA SQI_{LRR}= 0.296) while also identifying the variables that are more affected. To assess the effect of land use on the fungal community diversity with the LRR, the complete fungal component of the andosols in the research region was examined using meta-barcoding. There were significant correlations (r=0.94) between Shannon and Fisher indices with variations in microbial communities. Thus, fungal diversity enabled the classification of soil samples based on the land use and revealed that the abundances of important orders (Wallemiales and Trichosporonales) changed in the andosols because of variations in temperature and organic matter contents.

I. RESEARCH STATEMENT

The necessity of proposing trustworthy measures of microbiological features for soil quality monitoring has been underlined in favor of constructing sensible systems of possible losses of irremediable portions of soil functionality (Muscolo et al., 2015). By its high sensibility to variations in physicochemical properties and environmental features, microbial parameters are considered as robust soil quality indicators that report on the nature of deleterious soils changes. However, since microbial based indicators (MBI) responses can be highly dependent on context, there is no consensus about microbial responses to alterations of soil properties (L. N. Soucémarianadin et al., 2018; van der Heyde et al., 2017b). As a result, the use of microbial features in a specific soil quality assessment faces not only the lack of a conceptual framework to unravel driving factors of soil microbial activity, but also the lack of well-defined standard methods to do it (Schloter et al., 2018). Previous research about microbial indicators of soil quality in Colombia, for instance, have focused on clarifying the effect of chemical properties such as total nitrogen, total organic carbon and inorganic elements, in the raw values of microbial indicators like abundance of mesophilic bacteria, actinomycetes and fungi, microbial biomass, enzyme activities and microbial diversity estimated by molecular techniques (Tofiño Rivera et al., 2020; Vallejo et al., 2012; Velasquez et al., 2005). Nevertheless, to be helpful for soil management decisions, soil quality assessments based on microbial parameters require an interpretive framework according to soil type and climate region. To consider soil function an MBI equally representative across different soil types is not an approximation to the correct use of MBI because microbiomes may respond differently to changes in soil features while their responses can be highly context dependent. The microorganisms do not interact with abiotic factors in a unique way (Fierer et al., 2021a; L. N. Soucémarianadin et al., 2018). Context factors such as soil management, plant diversity, rainfall regime and topography play an important driving role on microbial metabolic activity as well as microbial community composition (Chen et al., 2020; Cui et al., 2019; Tajik et al., 2020). For example, the strength and direction of relationships between microbiome composition, microbial biomass, basal respiration, dehydrogenase activity, and soil features like pH and bulk density, can be clearly different according to land use and ecosystem sampled (Frąc et al., 2020; Ramírez et al., 2020; Soucémarianadin et al., 2018a).

Microbial-based Indicators (MBI) such as Arbuscular Mycorrhizal Fungi (AMF) and their Glomalin Related Soil Proteins (GRSP) have been proposed to quantify the response of certain microbial phylogenies to soil modifications at a cheap cost. For instance, AMF diversity has been shown to be a good predictor of changes in plant variety, carbon level decline, and rates of mineral fertilization (R. Vasconcellos et al., 2016). Because of the connections between AMF activity and processes of insoluble soil phosphate mobilization, plant disease resistance, and soil structure improvement, AMF are often regarded as reliable markers of soil quality. (Bi et al., 2019; M. Chen et al., 2018), while GRSP are markers of soil structure stability and soil pollution due to their cementing characteristics, important function in fostering the development of water-stable aggregates, and high capacity to bind heavy metals (Kumar et al., 2018; Wang et al., 2020). However, there is uncertainty over the AMF responses to changes in soil characteristics since AMF responses, like other MBI responses, can be very context-dependent (Fierer et al., 2021b; L. Soucémarianadin et al., 2018; van der Heyde et al., 2017a).

Furthermore, a typical technique for determining soil quality indices (SQI) is the scoring additive function, which involves summing the scores of different indicators and weighting them according to their relative value (Maaz et al., 2023; Wander, 2022). Although these functions offer a baseline for monitoring changes in soil quality, it is challenging to grasp their values for two major reasons. First, values computed with scoring additive functions are context-dependent since they may not take into consideration the particular management objectives or environmental circumstances of a certain soil. For instance, while a soil with high amounts of nutrients may be regarded good quality for farming, it may not be ideal for protecting natural ecosystems (Gržinić et al., 2023). Second, there is a lack of transparency because combining multiple soil properties into a single score makes it difficult to identify individual properties that require improvement. Since soil monitoring projects may be costly in terms of both labor and capital, to save expenses and give suitable data for identifying improvements or degradations in soil quality, a soil quality measure must employ an optimal collection of indicators (Stone et al., 2016a).

Finally, the use of raw data during soil parameter assessments as well as the use of a set of variables with large portions of sensitivity attributed to unidentified factors may lead to statistical underestimations of variations on soil quality. For instance, strongly positively skewed distributions of the observed values are the most frequent departures from the assumptions underlying safe inference from an analysis of variance of soil data. There are evidences that show

the necessity of an earlier data transformation analysis to improve the quality of input data together with the representativity of proposed variables (Muñoz, 2018; Tassano et al., 2021). Hence, in certain circumstances, converting data (e.g. logarithmical transformation) produce analyses with reliable conclusions about the sources of soil parameter variations (Webster & Lark, 2019a).

II. Research question and hypothesis

There is a lack of a specific interpretative framework to understand the relationship between MBI such as arbuscular mycorrhizal fungi and related microbiological indicators (AMF-RI) in conjunction with abiotic soil properties in important soil units as Colombian andosols. Thus, the viability in identifying land use effects on soil quality by means of the MBI remain yet uncertain. Likewise, although the AMF are a very important group of soil microorganisms, it is not possible to assert without evidence that variations in their diversity represent a suitable measure of soil quality. Considering the potential utility of AMF-RI to visualize quantitatively deleterious changes in soil quality our research question was: ¿Can a soil quality index based on measurements of responses observed in AMF-RI discriminate the effects of land uses in specific, context-dependent soils? The hypothesis was that the use of a novel approach, which include a geometrical analysis of measures of size effect, a soil quality measure based on responses of AMF as well as relevant abiotic soil properties, can be developed to describe soil degradation processes and identify significant effects caused by land uses in soils. The work is based on the analysis of samples collected for this thesis from andosols of the southeastern region of Antioquia, Colombia.

III. Objectives

The main objective of this thesis is to assess the soil quality of soils with contrasting degrees of alteration caused by land use changes by means of a quality measure based on AMF-RI and soil properties that can discriminate the size effects in single indicators as well as in data set of relevant soil quality features.

IV. Specific objectives

The thesis considers the following goals to fulfill the main objective:

- Identify trending relationships among arbuscular mycorrhizal fungi, culturable microbial populations, enzymes activities and soil properties.
- Characterize the relationships between abiotic soil properties and microbiological indicators related with arbuscular mycorrhizal fungi in the study zone.
- Compare the capability to discriminate variations of soil quality measures performed by means of polygonal projections and a geometric analysis of size effect measures contrasted with conventional scoring functions.
- Describe the variations attributed to land use in fungal diversity across a soil degradation gradient.

V. General structure of the document

This thesis consists of seven chapters. Chapter I discusses the use of AMF-RI in soil quality measurements, along with the need to validate soil quality data using alternative methods such as size effect measurements and geometric analysis. Chapter II presents a theoretical framework to identify potential relationships between microbiological indicators by analyzing a cross-biome data set. Chapters III and IV describe the soils of the study area and characterize the effect of different states of soil degradation on soil properties and AMF-RI. Chapter V describes a soil quality measure performed by integrative approach size effects measurements with a geometrical analysis to compute changes in intercorrelated soil quality indicators. Chapter VI provides a deeper soil microbiological characterization to validate that changes in a relevant biotic soil component as fungal diversity can display gradual variation according to soil degradation stage. Chapter VII provides the general conclusions of the thesis, divided into sections that cover each of the goals reached in the previous chapters. Finally, all references are presented at the end of the document.

1. AN INTRODUCTION TO THE SOIL QUALITY CHALLENGE AND MICROBIAL-BASED INDICATORS AS AN ALTERNATIVE

Abstract

To be helpful for soil management decisions, soil quality assessments performed from Microbial Based Indicators (MBI), require an interpretive framework. Over the last decade there has been a debate around the interpretation of microbial indicators at local scale which cuts across two aspects mainly. First, an increase in a microbial indicator value is considered beneficial, while a decline may be considered detrimental if this leads to a decline in biological function. However, absolute microbial parameter values are more difficult to interpret due to the lack of an interpretive framework. In the following sections of this chapter some MBI will be described along with the advantage or limitations of their implementation on soil quality assessments. Here, the convenience of using a set of MBI called by us as AMF-RI (Arbuscular Mycorrhizal Fungi and Related Microbiological Indicators) will be reviewed. This review allowed us to demonstrate the great advantages in terms of cost and reproducibility that indicators such as AMF-RI can display in soil quality evaluations. Additionally, here we review how effect size measures as well as geometric analyzes of this soil quality data can provide better presentation of variations in a set of variables.

Keywords: Microbial based indicators, interpretation, size effect measures, data quality.

1.1 Introduction

Soil quality is an important consideration when environmental assessments are conducting because soils provide and regulate a complex range of ecosystem services (P. Pereira et al., 2018). Soil ecological services include the physical support to ecosystems and their genetic pool, production of biomass and regulation of biochemical cycles as well as storage and transformation of water, nutrients and pollutants (Baveye et al., 2016). Since soil is a slow-forming resource under constant pressure (Jónsson & Davíðsdóttir, 2016), there is a growing demand for assessments tools with integrative perspectives to interpret the complexity of soil quality. Faced with this scenario, the question to start can be: ¿What is soil quality?

The principal concepts of soil quality place emphasis on defining the innate functional characteristics of soil to characterize the effects of human management later. For example, Carter et al. (1997) proposed that soil quality is the "*capacity of a soil to function, within the ecosystem and land use boundaries, to sustain biological productivity, maintain environmental quality, and promote plant, animal and human health*". Similarly, the Natural Resources Conservation Service, USA, suggests that soil quality is "*the continuous capacity of a soil to operate as a vital living ecosystem that feeds plants, animals, and humans*" (Lehmann et al., 2020).

Whatever the definition of soil quality that a researcher adopts within a measurement tool, there are a couple of conditions to apply before. First of all, the soil functionality may be highly specific and it is driven by its own factors of environmental context. Second, as a consequence, a soil quality assessment requires indicators able to reflect the capacity of soils to function under specific conditions (Bünemann et al., 2018a). The use of Microbial-Based Indicators (MBI) has been useful to supply both requirements, since soil microorganisms are directly linked with different soil quality aspects while their functions are representative of various environmental conditions (Maurya et al., 2020). Notwithstanding, developing a quality metric that employs soil microbial parameters demands a clear understanding of mechanisms whereby soil properties and environmental factors interact (Fierer et al., 2021a).

1.2 MBI of soil quality

The capacity of soils to sustain their functions, within the boundaries of natural or intervened ecosystems, is often inferred from quality indicators that cover a broad range of soil physical, chemical, and biological parameters (Muñoz, 2018). Soil physicochemical properties are relatively stable and the detection of deleterious changes or significant variances in soil functionality can take long periods (Liu et al., 2018). As an alternative, several MBI have been studied to carry out soil quality diagnosis given its biological faculty to display “first light” response facing soil degradation, soil structure transformations, nutrient storage changes, and biological activity. MBIs are highly sensible witnesses of soil functionality because microbial communities are closely related to essential soil processes such as supporting plant growth (nutrient mobilization, pathogen suppression, and stress resistance to abiotic factors) (S. Kumar et al., 2018),

key steps of nitrogen turnover (fixation, nitrification, and denitrification) (Ouyang et al., 2018), and degradation of pollutants (Jeffries et al., 2018).

Abiotic soil characteristics are associated with changes in soil functions, but biological indicators, such as MBI, allow the understanding of variations in soil functionality in terms of biochemical and biophysical transformations, and plant performance (Lehman et al., 2015). However, a suitable indicator needs to be relevant and related to a specific soil threat, soil function, or soil ecosystem service. Figure 1.1 shows the main linkages between soil threats, soil functions, and soil-based ecosystem services.

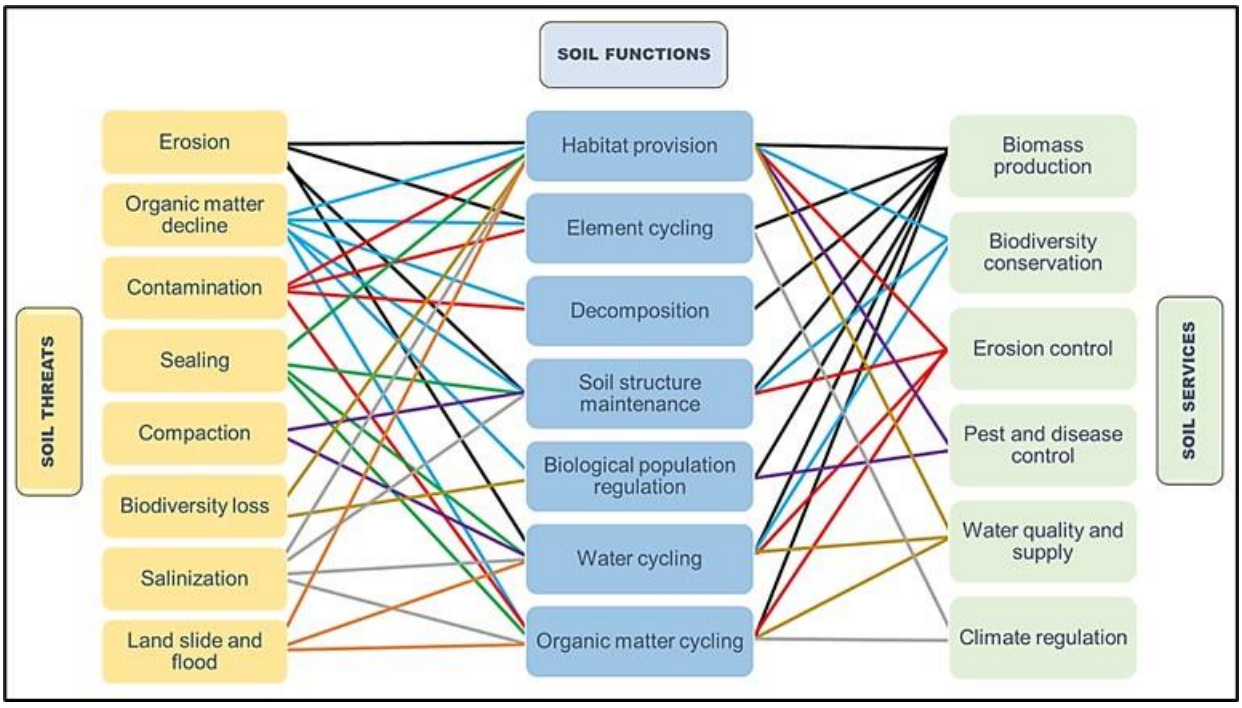


Figure 1.1 *Connections between soil threats, soil functions and the ecosystem services provided by the soil (modified from Bünemann et al., 2018).*

The most of MBI are based on methods to measure the soil microbiome activities as undifferentiated units enable to detect the direction of a change in soil ecosystem functioning (Brookes et al., 2013). For example, as a model of these MBI, so-called black-box indicators, numerous studies have reported the microbial biomass-C rapid response to identify conditions that eventually alter soil organic matter levels (Kaschuk et al., 2010; Y. Li et al., 2018). Alike, enzyme activity in the soil environment is considered to be a major and sensible indicator of overall nutrient cycles reflecting ecosystem disturbance. Enzymes such as beta-glucosidase, dehydrogenase, and urease display significant variations following organic matter inputs and nitrogen mineral

fertilization (Błońska et al., 2017; Irfan et al., 2019). In addition, there are MBIs that measure specific microbial phylogenies responses. Within these high-resolution indicators of soil microbial responses, the current molecular techniques developed to unravel taxa composition in soil microbiomes as sequencing of 16S rRNA (to identify bacteria) and ITS regions (to identify fungi) have been widely employed to represent more specific microbial guilds or functions (Kim et al., 2022; Nannipieri et al., 2012; Victorino et al., 2021a). An overview of methods for studying soil microbiomes is shown in Table 1.1.

1.3 Enzyme activities, culturable microbial population and arbuscular mycorrhizal fungi can be advantageous MBI of soil quality

Soil monitoring projects may be costly in terms of both labor and capital required to collect enough data to draw accurate conclusions. To save expenses and give suitable data for identifying improvements or degradations in soil quality, a soil quality measure must employ an optimal collection of indicators. According to Stone et al. (2016), a suitable indicator for soil quality assessments may have the following features:

- Availability of previous descriptions and reference materials.
- Reproducibility of its quantification methods by different laboratories.
- Short sampling times of less than a week.
- Easiness to explain issues of soil health and biodiversity to the public.
- Low costs.

One of the most useful indicators of the microbiological transformation of soil nutrients is microbial enzyme activity, since these enzymes limit and condition the available forms of nutrients for biogeochemical processes (Yi et al., 2022). For example, catalase, urease, protease, phosphatase, and beta-glucosidase activity respond rapidly to changes in soil matrix components and have been implemented as disturbance indicators (Fei et al., 2020; Ren et al., 2017; Singh, 2016). However, urease and catalase activity have been widely assessed across different soil types due to its large natural distribution, its dependency on several relevant soil factors as well as its easy and cheap quantification (Adetunji et al., 2017; Kaushal et al., 2018).

Table 1. 1 *Methods for studying soil microbiomes*

Method	Specific for (species/community/system)	Results	Advantages (technical)
Microbial biomass-direct microscopy	Community	Overall method. Estimate number of cells for a specific microbial group.	Easy, rapid.
Microbial biomass-dilution plating	Species/community	Estimate of the nature/diversity of a number of strains in the community.	Easy, cheap, and ability to further analyze colonies.
Microbial biomass-chloroform fumigation/extraction	Community	Estimate of microbial biomass.	Cheap and fast. Parameter sensitive to changes in the use of soil.
Microbial biomass-SIR (substrate induced respiration)	Community	Information on active, dominant community responding to substrate.	Easy and cheap.
FISH (fluorescence in situ hybridization)	Species	Information on active, dominant community members.	in situ technique: interactions and location visible.
PCR/qPCR	Species/Community	Proxies of organisms or genes amplified or Quantified.	Routine techniques of high sensitivity; allow detection or quantification.
Microbiome fingerprinting-DGGE/TG-GE	Phylogenetic (species/community)	Information on active, dominant community members.	Well optimized and easy, bands excised for identity check.
Microbial enzyme activities	Functional (species/community)	Proxies of organisms or genes amplified and/or quantified.	Easy, rapid, and cheap.
Microbial activity patterns	Community	Estimate of the structure of dominant microbiota in the community.	Easy, rapid, cheap, and extensive database.
Phospholipid fatty acid analysis (PLFA)	Community	Abundance of functional genes.	Routine technique a sufficient sensitivity; detection or quantification.
Phylochip/geochip microarrays	Species/Community	Targeting functional genes to dissect the microbial community functional structure of environmental samples.	All-in-once analysis in high-throughput. High potential for comparative studies.
High-throughput sequencing	Species/community	Profiles of microbial community composition.	All-in-once analysis in high-throughput. High potential for comparative studies.

Note: modified from Schloter et al., 2018.

Urease is an enzyme generally produced by soil microbes with a crucial role for mineralization of organic nitrogen (Nannipieri et al., 2012). In turn, urease activity responses have been directly correlated with nutrient turnover, decreases in soil moisture, and a rise in soil temperature (Adetunji et al., 2017). Meanwhile, catalase is an enzyme found exclusively in live cells, therefore it is considered as a realistic measure of the strength of soil microbial metabolic activity (Bungau et al., 2021). Catalase is an enzyme that supplies and regulates the content of H_2O_2 to protect microbial cells whose responses have been linked with alterations in soil properties such as pH, nutrient availability, hypoxia, increments on soil pollutants, and microbial populations (del Carmen Cuevas-Díaz et al., 2017; Mencel et al., 2022).

For its part, microbial respiration is defined as the oxidation of organic matter to CO_2 by aerobic microbial communities (Babur et al., 2021). Because of its inextricable link with soil moisture, temperature, microbial activity, and carbon availability, soil microbial respiration is the MBI most often monitored and explained for soil quality (Che et al., 2016). Complementary, although microbial enumeration based on plate-count approaches identify just a small proportion of microorganisms, the plate-count approaches are easily performing methods which remain valuable tools for studying soil microbes. For instance, the contribution of cultivable microbes to nutrient cycling in soil might be significant since cultivable species account for 80 to 90% of microbial biovolume in soil (Blagodatskaya & Kuzyakov, 2013).

Despite the fact that plate-count techniques are only capable of representing about 1% of total soil microorganisms, the number of colony-forming units (CFU) is an indicator positively correlated with enzymes and respiratory activity, and it is still used to characterize the relative abundance of potentially active microbial groups with specific functions or trop hic requirements (Piotrowska-Długosz et al., 2019; Pourreza et al., 2014; Ren et al., 2020). Recently, the enumeration of culturable mesophilic bacteria has been associated with erosion processes, soil moisture and soil organic matter contents (Guida et al., 2022; Tassano et al., 2021). Similarly, the enumeration of culturable fungi seems to be influenced by pesticide concentrations, offer of soil organic nutrients and phosphatase activity (Kataoka et al., 2017; Yao et al., 2022). Further, coexistent correlations have been reported between abundance of culturable actinomycetes and soil factors such as organic carbon contents, temperature, pH, and conductivity (Guillen Ferrari et al., 2019; X. Tan et al., 2021).

On the other side, across a variety of soil types, the characteristics of the Arbuscular Mycorrhizal Fungi communities (AMF) have been recognized as useful indicators of soil quality. Several large-scale studies have found a significant influence of soil pH and organic carbon contents on the community composition as well as a strong influence by anthropogenic actions (Bouffaud et al., 2016; Moora et al., 2014). AMF are extremely important for plants in low-fertility soils because its hyphae enhance the area of nutrient absorption of the plant root system. Despite their importance to ecosystem functioning, AMF communities are infrequently studied in anthropogenic impact studies and soil monitoring programs (Coutinho et al., 2019). AMF and their Glomalin Related Soil Proteins (GRSP) are an example of a set of MBI suggested to link soil quality to microbial phylogeny with reliable, inexpensive measurements and ease of sampling. AMF are a relevant group of soil fungi distributed worldwide whose diversity patterns are strongly correlated with declining carbon levels and mineral fertilization rates (R. L. F. Vasconcellos et al., 2016). GRSP are indicators of soil structure stability as well as soil pollution, considering their cementing properties and high binding capacity for heavy metals during the formation of soil aggregates (Kumar & Verma, 2018; Wang et al., 2020). GRSP are soil glycosylated proteins produced by AMF and released into soil with hyphal turnover able to promote the formation of water-stable aggregates (Liu et al., 2020). The allocation of GRSP in various fractions have been linked to mechanisms of soil organic contents dynamics, especially with soil organic carbon increments (L. Xiao, Zhang, et al., 2019a). Beyond molecular approaches, AMF diversity characterizations remain complemented by descriptions of macro fungal structures such as morphotype characterization of spores, since using molecular techniques to assess the relative abundance of AM fungus may have drawbacks (P. Shi et al., 2012), and ITS universal barcoding primers that are commonly used to characterize the whole fungal community of soil are considered suboptimal for AMF characterization (Berruti et al., 2017a).

1.4 Polygonal projections and size effects measures as alternative to interpret soil quality assessments

Soil quality evaluation needs either the interpretation of soil indicator values related with soil functions that cannot be evaluated directly, or validated target ranges of indicators focused on a soil and land use-specific. For example, a valid Soil Quality Index (SQI) aids in the interpretation of data from various soil measures and indicates if management or land use are having outcomes

for a global view of soil productivity or else environmental protection (Thakur et al., 2022). Besides, there are interpretive frameworks developed through a collaborative process of knowledge co-creation that begin with a survey of a wide mix of experts, prior research or baseline value matches that establishes goal or reasonable values to observed soil quality indicators (Cowie et al., 2018). Nerveless, for anybody outside the soil science field, data interpretation and the structure of the information on soil quality are often complicated and difficult to interpret (Drobnik et al., 2018).

A step ahead, after determining significant differences between a set of values by examining the relative variance of those groups, there are relatively new dimensionless effect size statistics. For instance, odds ratios (which compare the odds of an event between two groups) and log response ratio (which compare mean values between two groups) has been useful estimators of the effect magnitude caused by an intervention action in terms of the degree to which the outcome data varied between two groups (Senior et al., 2020). Measures like these supply outcome values with a simple interpretation. Values higher and lower may indicate either benefit or harm of an experimental intervention (Higgins et al., 2019). Notwithstanding, how can we represent a set of size effects in an integral model of data visualization?

Visualization is a critical component of understanding multi-dimensional data. Visualization has a wide range of applications in industry and academics, including medical imaging, biology, environmetrics, bioinformatics, and pattern recognition (W. Xie et al., 2017). Geometric approaches as polygonal projections based on coordinate systems are capable of representing multidimensional data in two or three dimensions while preserving their inherent overall structure by taking advantage of a polygonal interface bridging high- and low-dimensional spaces (Flexa et al., 2021). Indeed, polygonal projections analysis can discover many features about an object, allowing calculations of a broad spectrum of quantitative shape descriptors that are useful to compare polygons derived from multivariate data (Flexa et al., 2021; Güler et al., 2021). Table 1.2 shows a set of relevant derived parameters employed for morphometric characterization of polygons which are projected onto a horizontal plane.

Table 1. 2 *Morphometric parameters calculated by the PolyMorph-2D plug-in to compare polygonal shapes (modified from Güler et al., 2021)*

Morphometric parameters	
Perimeter length of the polygon boundary which is projected onto a horizontal plane.	Compactness Factor.
Area of the minimum circumscribed circle.	Dispersion Measure.
Perimeter of the minimum circumscribed circle.	Elongation Factor.
Length-to-Width Ratio.	Major axis length.
Width-to-Length Ratio.	Minor axis length.
Ellipticity Factor.	Area of the bounding box.
Circularity Ratio.	Perimeter of the bounding box.

1.5 Conclusions

MBI play a crucial role in determining soil quality. They reflect the activity, abundance, and diversity of microorganisms in the soil, which are essential for nutrient cycling, organic matter decomposition, and plant growth. Microbiological indicators such as microbial biomass, enzyme activity, microbial respiration, culturable microbial population, AMF and GRSP provide valuable information on the status of soil health and the potential for productive agricultural systems. Together, these factors influence soil fertility, water holding capacity, and overall soil health. Monitoring these indicators can help to identify the impact of land use and management practices on soil health. MBI are influenced by a variety of soil characteristics, including pH, texture, organic matter level, and nutrient availability. However, it is crucial to comprehend the link between microbiological indicators and soil characteristics before to their implementation in soil quality indexes.

Presenting clear and accurate information about soil quality assessments is essential for make informed decisions about soil management practices. Methods such as size effect measure and polygonal projections can be helpful to do it. Size effect measures, provide a more comprehensive and informative way to interpret the results of a study, by quantifying the magnitude and direction of the effect rather than simply indicating whether it is statistically significant. Likewise, polygonal projection has several advantages over other visualization methods since polygonal projection display multiple variables simultaneously, allowing for easy identification of patterns and relationships between variables. Besides, polygonal projection can be used to compare multiple datasets or groups, allowing for easy identification of differences or

similarities between groups. Finally, polygonal projection can be easily interpreted by non-experts, making it a useful tool for communicating complex data to a wide audience.

2. MEANINGFUL ASSOCIATIONS BETWEEN MICROBIAL BASED INDICATORS OF ARBUSCULAR MYCORRHIZAL FUNGI AND SOIL PROPERTIES: A META-ANALYSIS

Abstract

Recent studies have tested the sensitivity of Microbial Based Indicators (MBI) like Arbuscular Mycorrhizal Fungi (AMF) for monitoring alterations in soil properties in a wide set of specific environments. However, the direction and strength of AMF responses depend on contextual factors such as land use, vegetation type, geography, and environmental variables, which require a framework to understand meaningful relationships between AMF and soil properties. Likewise, there is a lack in the comprehension of how the interactions among different microbial community aspects can modify the influence of soil properties on tendencies in MBI responses. Based on data compiled over a wide geographic range, in this chapter we analyze the responses observed in several aspects of AMF facing variations in soil properties across different land uses. A Dependency Network Analysis (DEPNA) within a based correlation network sketched by means of average correlation coefficients was performed to check the strength of relationships between soil properties and different MBI, whereas regulating for the effect of another MBI. Average correlation coefficients were estimated through a meta-analysis to account for experimental heterogeneity. Total Influence Degree (TID), computed from partial correlations, suggests large dependencies between MBI (related with AMF diversity, mycorrhizal colonization rate and Glomalin Related Soil Proteins) with soil properties (Pb concentrations, soil structural features, and nutrients stocks). The results suggest that these strong influences may be leaded by the effects of partial correlations with microbial diversity, microbial biomass carbon and soil microbial respiration.

Keywords: Arbuscular Mycorrhizal Fungi, Soil property, Partial correlations, Influence Degree.

2.1 Introduction

Arbuscular Mycorrhizal Fungi (AMF) and their Glomalin Related Soil Proteins (GRSP) have been suggested as Microbial-based Indicators (MBI) to measure the response of particular microbial phylogenies to soil alterations al low cost. For instance, AMF diversity has been documented as an indicator of variations in plant diversity, declination of carbon levels and mineral fertilization rates (R. Vasconcellos et al., 2016). The studies on AMF diversity are advantageous because they are often supported by morphological characterizations developed without complex equipment to identify AMF taxa. Those taxonomical determinations have complemented molecular approaches since fungal universal primers present still limitations to identify and discriminate some AMF taxa deeply (Berruti et al., 2017b; Ontivero et al., 2020).

AMF are also considered as good indicators of soil quality because of the linkages of the AMF activity with processes of insoluble soil phosphates mobilization, plant pathogen resistance, and soil structure enhancement (Bi et al., 2019; M. Chen et al., 2018), whereas GRSP (with their cementing properties, relevant role promoting water-stable aggregates formation and high binding capacity for heavy metals) are considered as indicators of soil structure stability and soil pollution (Kumar et al., 2018; Wang et al., 2020). GRSP are soil glycosylated proteins that are easy to isolate and quantify. GRSP are produced and released into soil by hyphal turnover of AMF (H. Liu et al., 2020b). Their allocation in various fractions have been also linked to mechanisms of soil organic contents dynamics, especially with soil organic carbon pools (L. Xiao, Zhang, et al., 2019b). Nevertheless, since AMF responses can be highly dependent on context as most MBI responses (Fierer et al., 2021b; L. Soucémarianadin et al., 2018; van der Heyde et al., 2017a), there is not a consensus about the AMF responses to soil properties alterations. For example, a large variability in AMF diversity patterns caused by contextual factors such as habitat type, plant diversity and land use intensity, have been documented in experimental designs across a wide spectrum of ecosystems (Albornoz et al., 2022; Faggioli et al., 2019; Sepp et al., 2018).

In addition, some studies reported variations on driver factors of AMF community responses across ecosystems or land uses. For instance, whereas in ecosystems as grasslands, the AMF community composition is guided by plant diversity, in desert areas this factor has no significant effect on AMF community (Adenan et al., 2021). There are also reports about effects at different scales of soil properties such as pH, carbon content, nitrogen content, and soil moisture

on AMF community (Albornoz et al., 2022; Yang et al., 2021). Similarly, environmental factors like forest composition or land use can affect significantly GRSP allocation (Wang et al., 2019; Zhao et al., 2022).

Despite of the MBI dependence on contextual factors, unifying principles guiding microbial responses have been studied by means of cross-biome data compilations and "Random Effect Models" (REM) to find pooled effects (Pathak et al., 2020; Trivedi et al., 2016). REM are able to analyze data compilations following a set of inclusion criteria to estimate average correlations while accounting for experimental heterogeneity among studies, considering the variance within one study together with the variance between studies (Pathak et al., 2020). REM approaches, for instance, serve to prove the strong beneficial effects of N fertilization over AMF symbiosis rates (Hoeksema et al., 2010) as well as the positive relationships between microbial enzyme activity with organic carbon contents (M. Nunes et al., 2020) and the positive relationship of microbial biomass and fungal diversity with C/N inputs and cover crops (Muhammad et al., 2021). Although the REM approach has become a standard methodology for synthesizing correlations (Cheung & Cheung, 2016), to understand the MBI interactions in depth may require post-hoc analysis of the interdependencies observed among physical, biological and biochemical attributes of the soil on MBI (Dahal et al., 2021; Raiesi & Kabiri, 2016). Likewise, evidence supports the important relationships among soil microbial attributes, such as soil microbial enzyme activity, microbial biomass carbon and AMF diversity, to co-regulate the effects of variations in soil features like nutrient supply on soil microbial components (Bai et al., 2021; Xiao, Bi, et al., 2019a).

In view of the effects of contextual factors on MBI responses to variations in soil properties and the importance of the interdependencies observed among microbial attributes within the analysis of MBI interactions, we address the following question about the relationships between MBI related with AMF and soil properties: ¿How to identify response tendencies of MBI related with AMF associated with variations in soil properties? We analyzed peer-reviewed studies performed during the last decade to average a range of divergent responses observed in MBI related with AMF and MBI commonly implemented in soil quality assessments to determine the average correlation and the degree of interactions between soil microbial attributes and variations in soil properties.

Thus, average correlation coefficients were estimated by mean of a REM approach, starting from local MBI correlations documented in contrasting land uses such as forestry areas, mining

sites, crops, pastures and wasteland sites. To study the influence of related microbial aspects on MBI responses, differences between correlations observed and partial correlation were computed from triads of linked variables observed in a model network. We sketch a correlation-based network useful to analyze the structure of set of links, considering the occurrence and weights of these relations. The triads of variables assessed were constituted by a soil property correlated with two MBI correlated at the same time among them. We have estimated an interaction degree between variables called "Total Node Influence" whatever considering the total influence of node *j* on node *i*, as the average influence of node *j* on the correlations over all nodes linked with node *i* (Wang et al., 2018). Through this method, the partial correlations between a given set node-node correlations were measured. The measures computed by our approach allowed to highlight responses of MBI related with AMF, while considering relevant interactions among MBI which came to potentiate the soil properties effects.

2.2 Material and methods

2.2.1 *Study selection and data compilation*

The literature review was carried out in SCOPUS ELSEVIER database using as keywords "soil properties" plus following relevant MBI: "microbial respiration", "catalase activity", "urease activity", "bacterial diversity", "fungal diversity", "actynobacteria diversity", "glomalin soil protein related", "mycorrhizal colonization rate" and "mycorrhizal fungi spore". The selected studies included only those published since 2010 to 2020. With this revision, a set of 564 documents were found and the lists of soil properties (Table 2.1) and MBI (Table 2.2) were developed with relevant measures recorded within this first set of studies. Afterwards, 78 papers were selected from that first set of studies according to the following criteria: 1) The paper showed raw values of at least one MBI and one soil property listed in Table 2.1 and Table 2.2; 2) The soils analyzed were retrieved from mining or agricultural areas; 3) The study assessed at least one control treatment within an area with a different manage system. The information related with land uses, treatments assessed, and raw values of measured indicators was compiled from every study selected.

Table 2. 1 *Physical and chemical parameters commonly measured in soils from mining and agricultural areas.*

PARAMETER	DEFINITION
Bulk Density	Relation between soil weight and its volume.
Total Porosity	Gap, holes or pores between soil particles, which contains water and air in a specific soil portion.
Soil Penetration Resistance	Relation between the force applied to an object against to a soil matrix and the range of distance that this element can move through soil matrix.
Electrical Conductivity	Measure of electrical resistance or ability of a soil to carry an electric current.
Cationic Exchange Capacity	Total capacity of a soil to hold exchangeable positively charged ions on the fine earth fraction.
Soil Moisture	Weight of water stored in the soil.
Soil Water-Stable Aggregates	Mean weight of different soil stable aggregates sizes exposes to humidity.
Water Holding Capacity	The soil moisture that will remain in a soil after water drained off the large pores against a specific force.
Total Potassium	Recoverable amount of the element K.
Total Phosphorous	Recoverable amount of the element P.
Arsenic concentration	Recoverable amount of the element Ar.
Cadmium concentration	Recoverable amount of the element Cd.
Chromium concentration	Recoverable amount of the element Cr.
Cupper concentration	Recoverable amount of the element Cu.
Total Iron recoverable	Recoverable amount of the element Fe.
Nickel concentration	Recoverable amount of the element Ni.
Lead concentration	Recoverable amount of the element Pb.
Zinc concentration	Recoverable amount of the element Zn.
Total nitrogen concentration	Soil total nitrogen concentration.
Nitrates recoverable	Nitrate nitrogen recoverable in a soil (NO_3).
Ammonium nitrogen recoverable	Ammonium nitrogen recoverable in a soil (NH_4).
Total Organic Carbon	Total C stored on soil organic matter.

Table 2. 2 *Definitions of soil quality indicators based on microbial parameter*

PARAMETER	DEFINITION
Acid Phosphatase Activity	Amount of enzyme required to release 1 μmol of p-nitrophenol/ml/min from di-Na p-nitrophenyl phosphate in pH 6.5.
Alkaline Phosphatase Activity	Amount of enzyme required to release 1 μmol of p-nitrophenol/ml/min from di-Na p-nitrophenyl phosphate in pH 11.
Beta-Glucosidase Activity	Formation of p-nitrophenol from a substrate (p-nitrophenyl-β-glucopyranoside) by enzyme present in a specific weight of soil.
Catalase Activity	Formation of H2O2 from a substrate by enzymes stored in a specific weight of soil.
Dehydrogenase Activity	Formation of triphenylformazan-TPF from a substrate by enzyme stored in a specific weight of soil.
Invertase Activity	Reduction of glucose by microbial enzymes present in a specific soil weight.
Urease Activity	Formation of N-NH4 from a substrate by enzyme present in a specific weight of soil.
Microbial Metabolic C Quotient	Relation of microbial respiration per unit of microbial biomass carbon.
Soil Microbial Basal Respiration	CO_2 released by microbial population in a specific weight of soil.
Microbial Biomass Carbon	Carbon mass released from lysed microbial cells.
Mycorrhizal Colonization Rate	Ratio of the AMF body to the plant root area.
Easily Extractable Glomalin-Related Soil Proteins	Glomalin-Related Soil Proteins extracted in an initial round of autoclaving for only 30 min with 20 mmol l−1 sodium citrate, pH 7.0.
Total Glomalin-Related Soil Proteins	Glomalin-Related Soil Proteins extracted in an initial round of autoclaving for only 60 min with 50 mmol l−1 sodium citrate, pH 8.0.
Abundance of AMF	Measure to quantify AMF abundance from a specific soil sample.
Enumeration of Actinomycetes	CFU enumerated by serial dilution method count in a selective culture medium.
Enumeration of Mesophilic Bacteria	CFU enumerated by serial dilution method count in a selective culture medium.
Enumeration of Molds and Yeasts	CFU enumerated by serial dilution method count in a selective culture medium.
Diversity of Actinomycetes	Measure of microbial taxa diversity present in a soil sample (e.g diversity indexes, total richness).
Diversity of Bacteria	Measure of microbial taxa diversity present in a soil sample (e.g diversity indexes, total richness).
Diversity of Fungi	Measure of microbial taxa diversity present in a soil sample (e.g diversity indexes, total richness).
Diversity of AMF	Measure of microbial taxa diversity present in a soil sample (e.g diversity indexes, total richness).

2.2.2 *Computing average correlations coefficients*

The averaged values of MBI and soil parameter measured in a treatment within a study were categorized using ranges to estimate Spearman's rank correlation coefficients "r_s1" between pairwise of variables. Then, Zr_s1 values were obtained transforming r_s1 coefficients by Fisher's Z function. We estimated confidence intervals for Zr_s1 "CI1" by the permutation method, using function *perm.ci* from R package RI2by2 (Rigdon & Hudgens, 2015). Then, a REM was performed to weigh Spearman's rank coefficients "r_s1" and estimate average correlation coefficients "r_s2" to pairwise of variables (Kontopantelis & Reeves, 2010).

First, to weigh coefficients rs1, the variances of a null distribution, simulated for each number of treatments observed in pairwise variables by were computed from repeatedly calculating them from two uncorrelated variables permuted 2000 times were considered as Zrs1 variances within a study (Follmann & Proschan, 1999). The null distributions were simulated with R package *infer* (Couch et al., 2021). Second, the inverses of Zr_s1 variances were assigned as the weight of each study to compute Zr_s2 coefficients and C.I 2 as result of weighted average of Zr_s1 and C.I1. Then, we carry out on Zr_s2 coefficients a Fisher's Z inverse transformation to obtain average Spearman's rank correlation coefficients "r_s2" to pairwise of variables (German et al., 2017).

2.2.3 *Dependency Network Analysis (DEPNA) to measure degree of influence of soil properties over MBI*

To sketch the correlation-based network, each MBI and soil parameter was considered as a node. Our model just linked nodes of soil property-MBI and MBI-MBI. The threshold to define a significant edge between *i* and *j* nodes were average correlation coefficients $r_s2_{i,j}$ >0.5 or <-0.5 (Batushansky et al., 2016; Kojaku & Masuda, 2019). Then, a weighed and undirected graphical model was drawn using R package "Igraph" version 1.3.4 (D. Singh & Garg, 2020). The influence degree of a soil property over a MBI was computed considering that a third variable (specifically an MBI) can affect the correlation between two other variables. We used function *all_simple_paths* from R package "Igraph" version 1.3.4 (argument "cutoff=2") to list each triplet of nodes that encompassed intercorrelations between a node of soil property with two MBI nodes which held correlations between them.

Next, partial correlation coefficients between a node *i* (soil property) and a node *k* (MBI), when an influence of a node *j* (MBI) could be present, was computed as a coefficient PC(*i, k|j*) using the equation (1) to describes the measurement of the first order partial correlation (Wu et al., 2020):

$$PC(i,k|\,j) = \frac{C(i,j) - C(i,k)C(j,k)}{\sqrt{\left(1 - C^2(i,k)\right)\left(1 - C^2(j,k)\right)}} \quad (1)$$

where *C(i, j), C(i, k) and C(j, k)* are the correlations (measured by the average rank correlation coefficient rs2) between variables *i - j*, *i - k* and *j – k*, respectively (Figure 2.1 a).

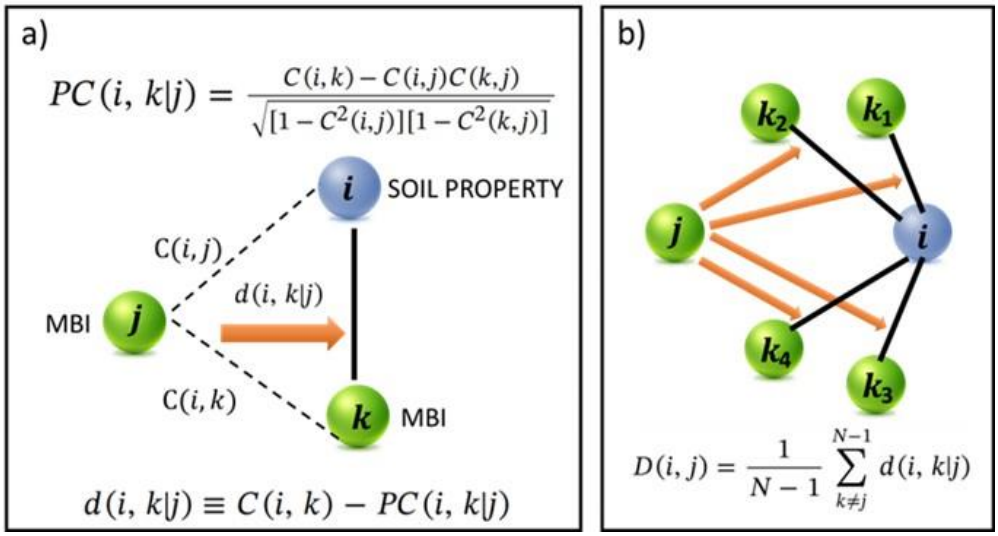
The difference between C(*i, j*) and PC(*i, k|j*) was denoted as dependency effect of variable k on the correlation C(*i, j*), which is expressed by equation (2):

$$d(i,k|j) \equiv \mathrm{C}(i,j) - \mathrm{PC}(i,k|j) \quad (2)$$

When *d*(*i, j* | *k*) equals zero, means that variable *k* has no impacts on the correlation between *i* and *j*. On the contrary, higher values of *d*(*i, j* | *k*), reflects a dependency effect of variable *k* on the correlation between *i* and *j*. The Total Influence Degree (TID) of node *j* on node *i*, D(*i, j*) was defined as the average of the dependency effects of variable *k* on C(*i, j*), over all nodes *k* (Figure 2.1 b). In order to avoid cases with sum over positive and negative influences, we chose to look at the average of absolute values of the influences *d*(*i, j* | *k*) given by equation (3) (Junior et al., 2015):

$$D(i,j) = \frac{1}{N-1}\sum_{k \neq j}^{N-1} d(i,k|j) \quad (3)$$

Figure 2. 1 *Steps to quantify total influence between a pairwise of nodes within a correlation-based network using Dependency Network Analysis (DEPNA)*



Note: modified from Jacob et al., 2020. **Step 1: (a)** To estimate partial correlation of first order between nodes *i* and *k* with respect to a third node *d*(*i, k*| *j*) by mean of partial correlation coefficients (*PC* (*i, k*|*j*)). **Step 2: (b)** The total influence of node *j* on node *i*, *D* (*i, j*) was defined as the average influence of node j on the correlations *C* (*i, k*), over all nodes *k*.

2.3 Results

2.3.1 *Average correlation coefficients*

Selected studies encompassed a revision of MBI relationships observed across a large range of environmental context represented by results documented in 5 continents, 19 countries and 92 geographic regions. Despite the great divergency on correlation coefficients estimated by REM approach, many MBI showed strong positive or negative Spearman's rank correlations (r_s2) with soil properties across land uses and management treatments (Figure 2.2). For example, MBI related with AMF such as Easily Extractable Glomalin Related Soil Proteins (EE-GRSP) and Total Glomalin Related Soil Proteins (T-GRSP) were positively correlated with water-stable aggregates (r_s2=0.67; 0.87), total organic carbon (r_s2=0.98; 0.98) and total N concentrations (r_s2=0.98; 0.95) although negatively correlated with bulk density (r_s2=-0.96; -0.99).

Similarly, the correlations of mycorrhizal abundance were positive with soil nutrients as total organic carbon (r_s2=0.99), total N (r_s2=0.89) and P concentration (r_s2=0.70) but negative with bulk density (r_s2=-0.90). Besides, mycorrhizal colonization rate was positively correlated with

NO3 (r_s2=0.99) and K concentrations (r_s2=0.74) as well as mycorrhizal diversity was positively correlated with NH4 (r_s2=0.86). In contrast, both mycorrhizal colonization rate and mycorrhizal diversity show negative correlations with Pb concentrations (r_s2=-0.99; -0.99). Furthermore, a great number of relationships among MBI was observed. Average correlation coefficients between MBI and their confidence interval are available as supplementary material (Figure S2.1 and Figure S2.2).

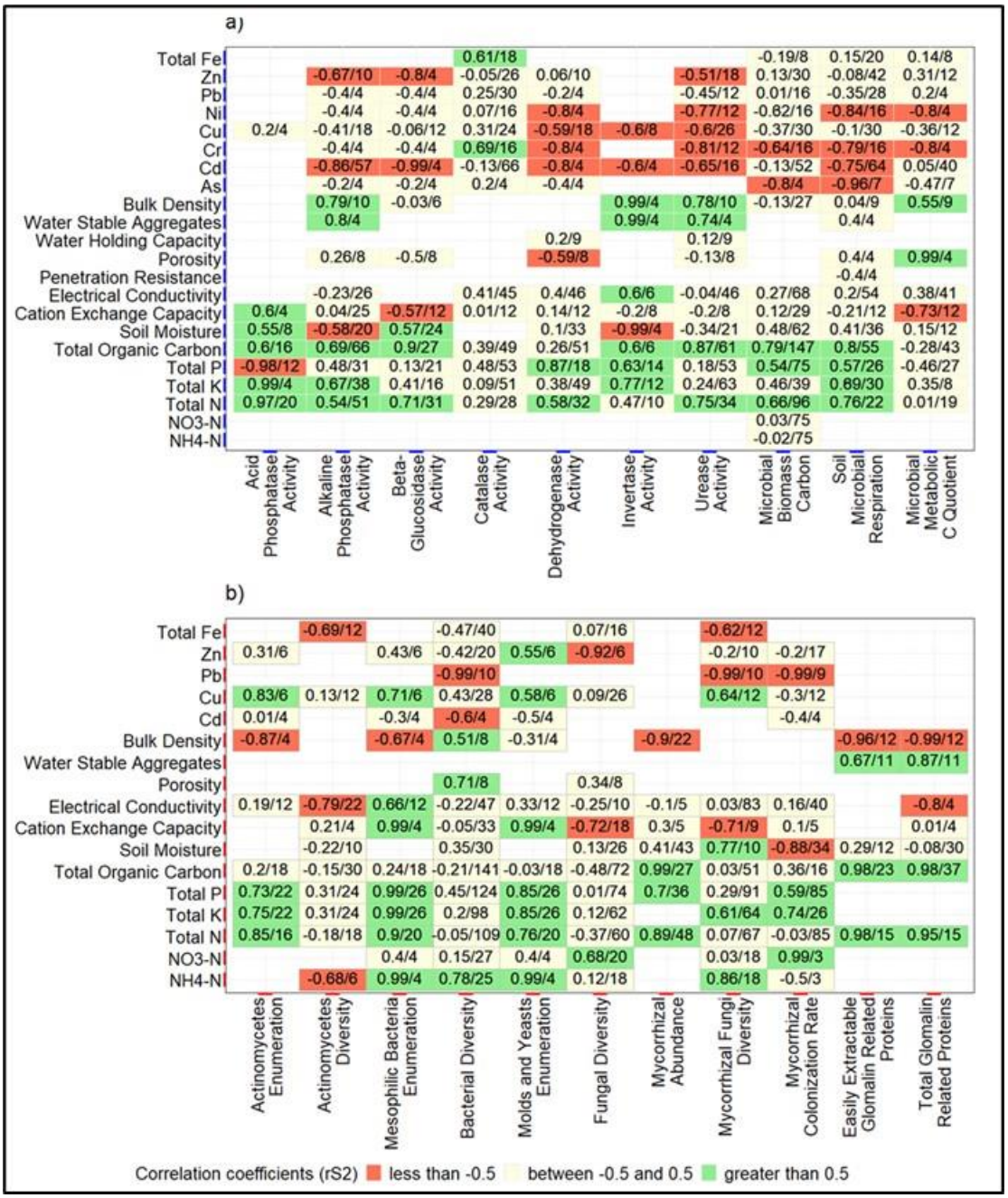
There was also a great number of relationships among MBI. For instance, enzyme activities were generally correlated to each other as well as with soil microbial respiration and microbial biomass carbon (r_s2>0.7). For its part, mycorrhizal colonization rate, mycorrhizal abundance, EEGRSP and TGRSP were positively correlated among them and positively correlated with actinomycetal diversity, microbial biomass carbon and soil microbial respiration (Figure 2.3). In addition, mycorrhizal diversity was positively correlated with mycorrhizal colonization rate (r_s2=0.87) and bacterial diversity (r_s2=0.99). Finally, the high between-study variation contributed to the wide confidence intervals computed for cross-study average correlation values (Figure 2.4 and Figure 2.5). For instance, to MBI as mycorrhizal diversity and MBI which measure the activity of undifferentiated microbial consortia such as alkaline phosphatase activity, Beta-Glucosidase activity, catalase activity, dehydrogenase activity, invertase activity and microbial metabolic C quotient, the r_s2 values spanned almost the entire possible range.

2.3.2 *Total Influence Degree*

The topological analysis of the correlation-based network sketched from average correlation coefficients showed that chemical properties related with soil fertility (such as total organic carbon, total N and K concentration) exerted strong influences on mycorrhizal abundance, mycorrhizal diversity, EEGRSP and TGRSP (Figure 2.6). For instance, total organic carbon has the strongest influences on mycorrhizal abundance (TID=0.44). This influence was driven through dependency effects to microbial biomass carbon (0.51) and soil microbial respiration (0.67). Similarly, influences from K concentration on mycorrhizal colonization rate (TID=0.49) were attributed to their own dependency effects with mycorrhizal diversity (0.52) and soil microbial respiration (0.45). Total N has a high influence on EE-GRSP and T-GRSP (TID=0.53; 0.43) which were guided by dependency effects to microbial biomass carbon and mycorrhizal abundance. Furthermore, highest influences were exerted by soil bulk density on T-GRSP (TID=0.82) followed

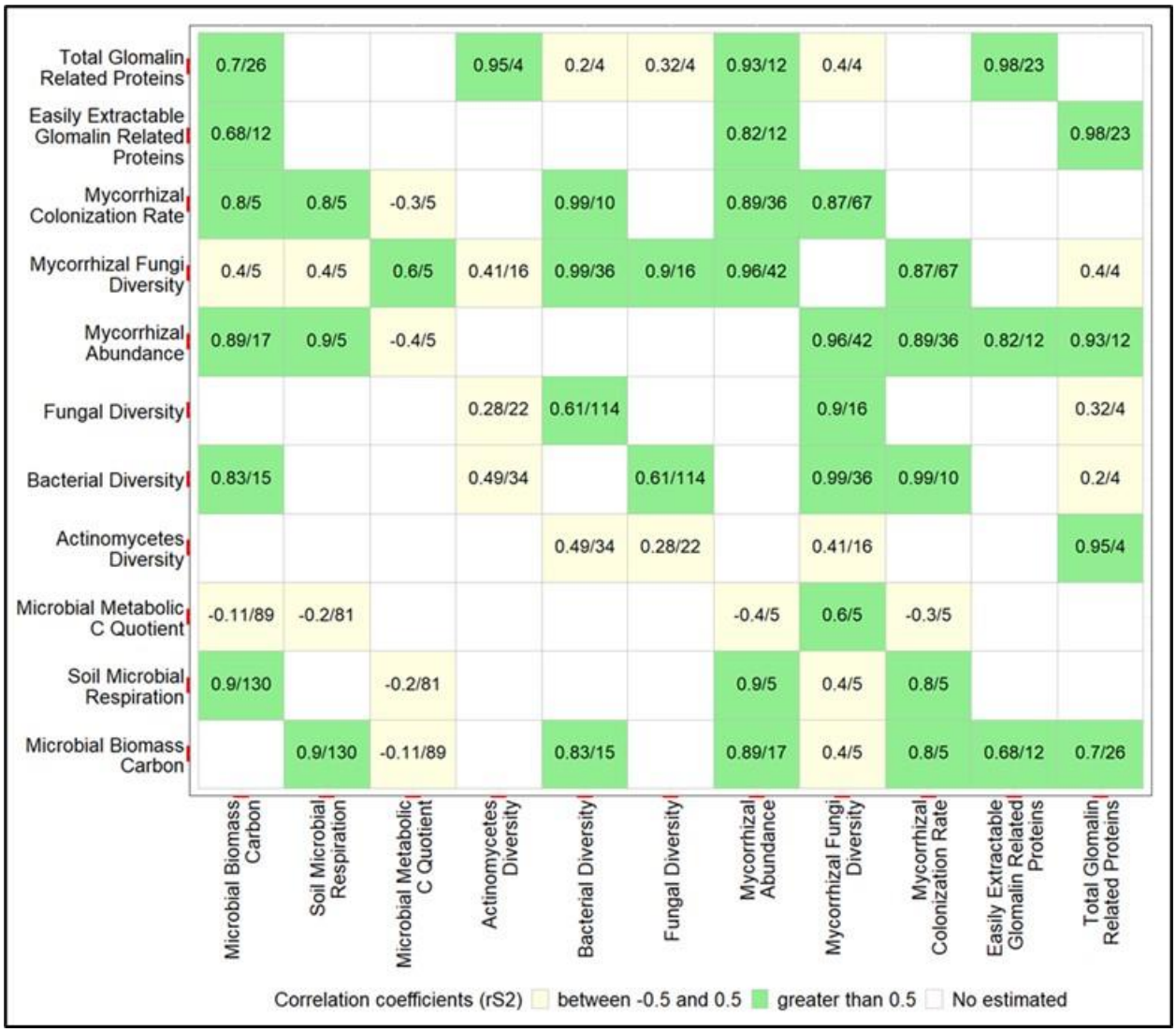
by Pb concentrations on mycorrhizal diversity (TID=0.71) and mycorrhizal colonization rate (TID=0.71). Likewise, T-GRSP and EE-GRSP were strongly influence by water-stable aggregates (TID=0.63; 0.66) and electrical conductivity affected in large manner T-GRSP concentrations (TID=0.64).

Figure 2. 2 *Cross-study averages of the between-treatment Spearman's rank correlation (rs2) for pairs of MBI and soil properties*



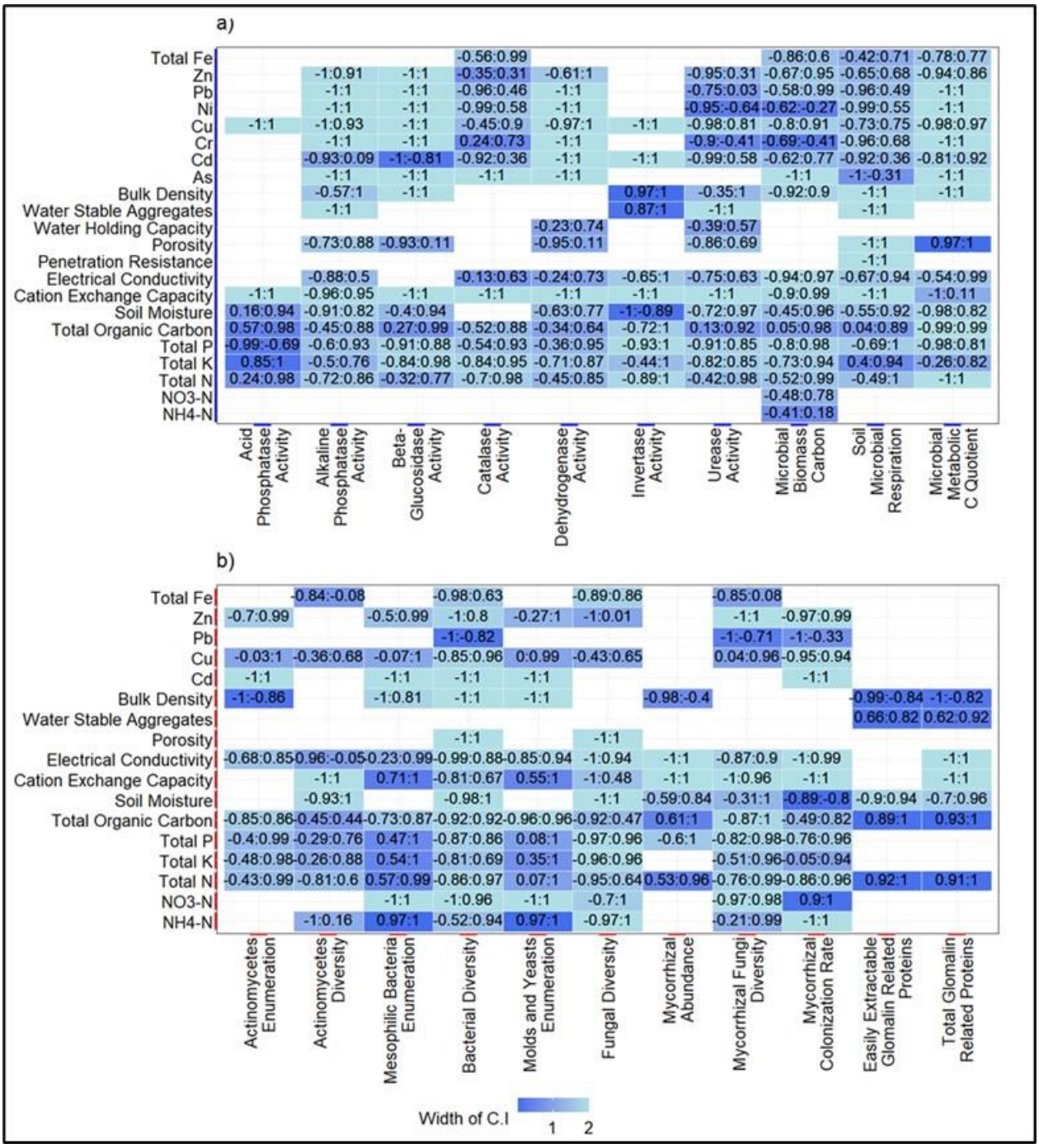
Note: a) r_s2 to MBI which measure the activity of undifferentiated microbial consortia. b) r_s2 to MBI which measures particular microbial phylogenies responses. The number of individual treatment where a pairwise of variables was measured (N).

Figure 2. 3 *Cross-study averages of the between-treatment Spearman's rank correlation (rs2) for pairs of MBI related with AMF and several MBI commonly implemented on soil quality assessments.*



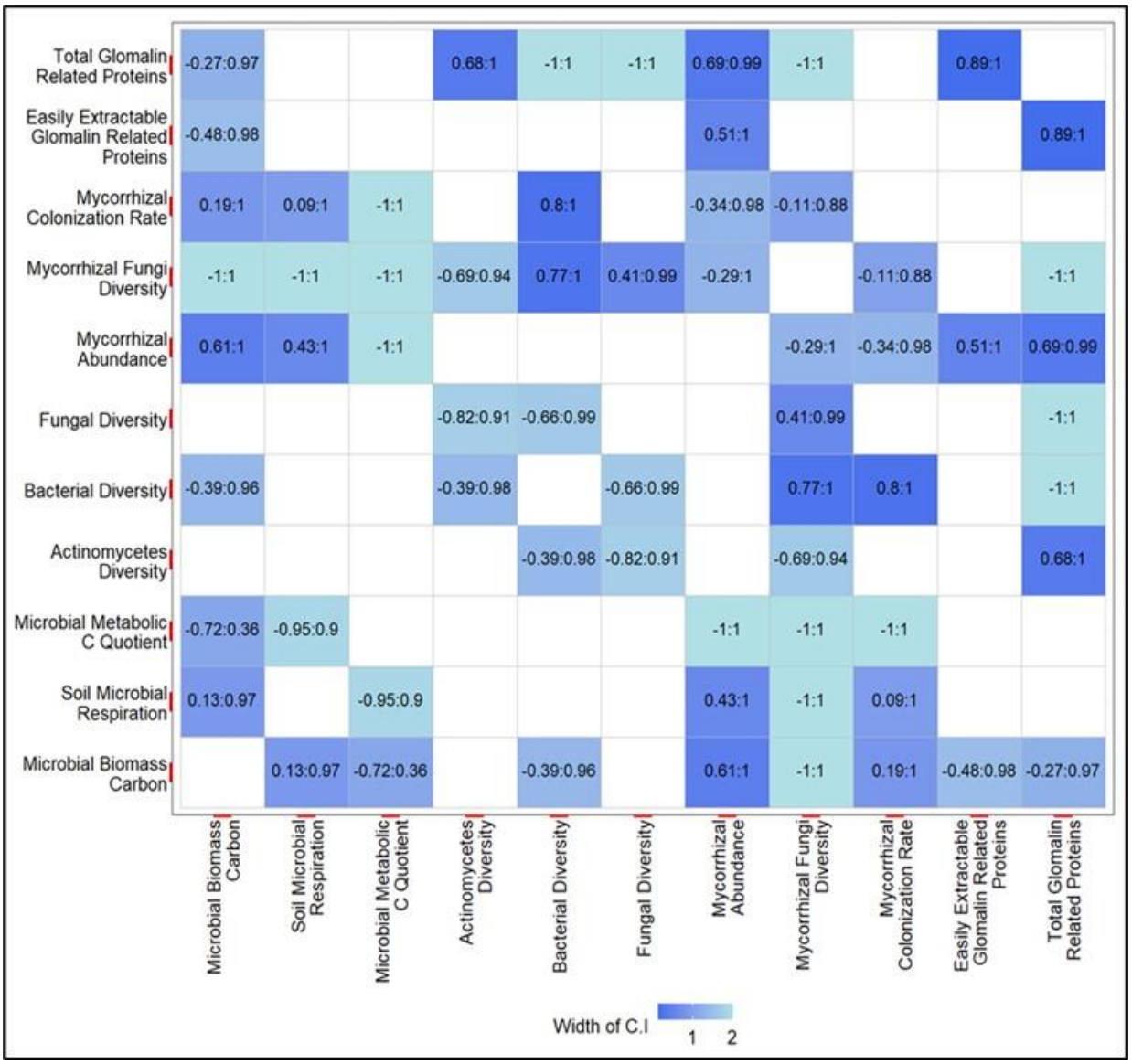
Note: The number of individual treatment where a pairwise of variables was measured (N) is shown after the slash.

Figure 2. 4 *Confidence Intervals (CI2) for the weighted average correlation coefficients (rs2) for pairs of MBI and soil properties*



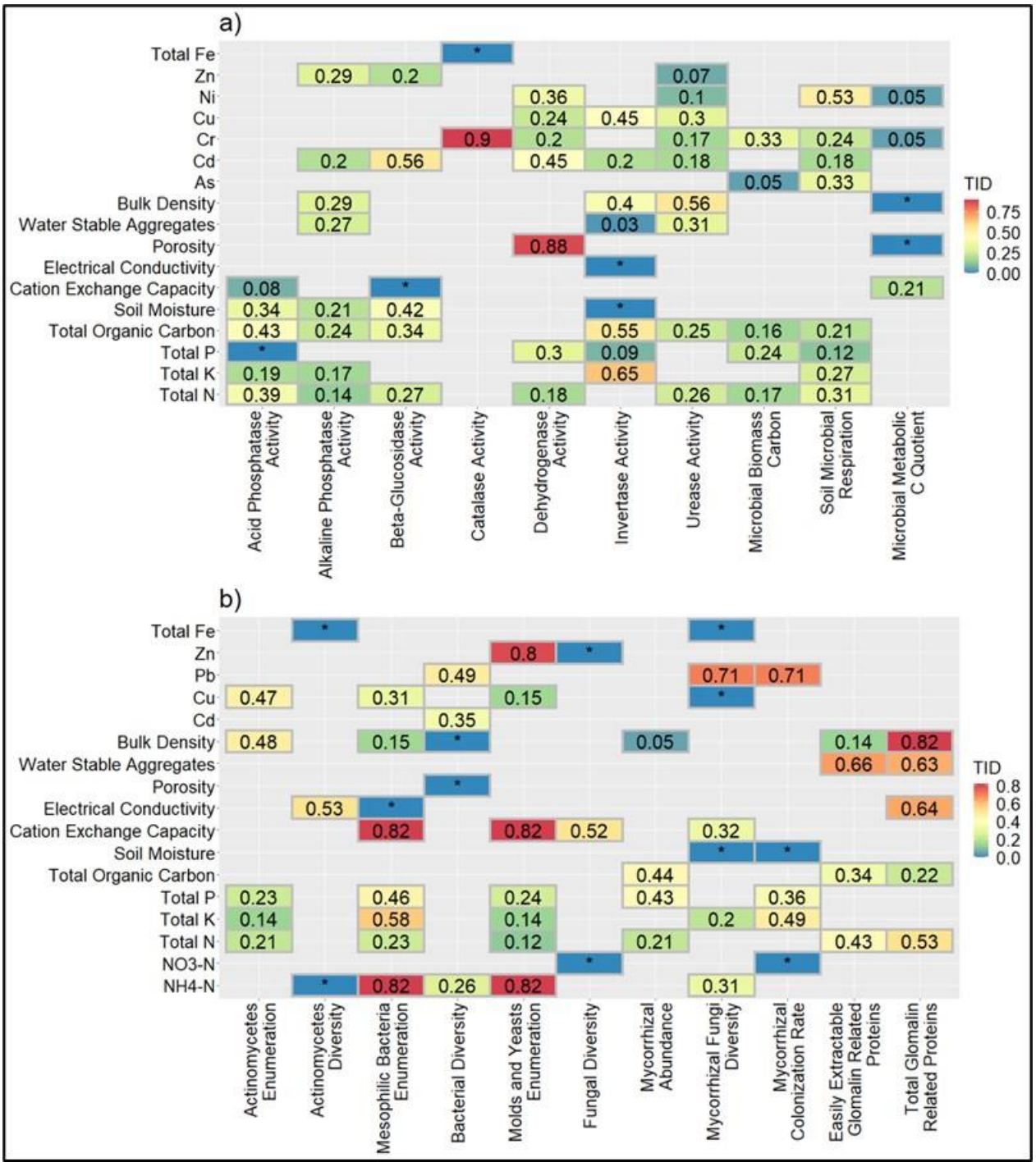
Note: a) C.I 2 to MBI which measure the activity of undifferentiated microbial consortia. b) C.I 2 to which measures particular microbial phylogenies responses. The 2.5% (before colon) and 97.5% (after colon) confidence limits are shown.

Figure 2. 5 *Confidence Intervals (CI2) for the weighted average correlation coefficients (rs2) for pairs of MBI related with AMF and several MBI commonly implemented on soil quality assessments*



Note: The 2.5% (before colon) and 97.5% (after colon) confidence limits are shown.

Figure 2. 6 *Influence between nodes of soil properties and Microbial Based Indicators (MBI) expressed as Total Degree Influence (TID)*



Note: a) TID on MBI which measure the activity of undifferentiated microbial consortia. b) TID on MBI which measures particular microbial phylogenies responses. *There were no partial correlations to compute a TID.

2.4 Discussion

Considering and weighting every observation documented in the cross-biome compilation study, many indicators displayed strong correlation coefficients despite the large confidence interval. The studies that incorporate soil quality variables at large scales are characterized by wide confidence intervals around the cross-study average Spearman's rank correlation (German et al., 2017) because the quality of the confidence intervals depend on the study variance (Field, 2005; Welz et al., 2022). For instance, wide confidence intervals were computed in the relationships between microbial enzyme activities and heavy metal concentrations as well as in relationships between mycorrhizal diversity and NH_4 concentration. The meta-analysis estimated negative effects of heavy metal concentrations against enzyme activities such as alkaline phosphatase, beta-glucosidase, dehydrogenase, invertase and urease. Similar studies have also documented strong and significant negative effects of heavy metal concentration on microbial enzyme activities (Aponte et al., 2020).

However, responses of different soil enzymes fluctuate greatly with heavy metal pressures, since the reduction in soil enzyme activity is caused by reasons such as metal direct inhibition, release reduction or the combined effects of both (Tang et al., 2019). Furthermore, mycorrhizal diversity response varies across studies under different regimes of NH_4 availability because AMF taxa may differ in their uptake of ammonium (Y. Ma et al., 2021; Yoshida & Allen, 2001). Likewise, previous large-scale studies have showed inconsistent findings of specific drivers to context-dependent soil microbial features such as microbial biomass carbon, microbial respiration, and microbial abundance (Smith et al., 2021).

The correlations estimated allowed to corroborate relevant mechanisms of microbial feedback to variations of different soil properties. Thus, relationships among MBI have been considered as important factors to understand events of microbial co-regulation induced by changes in soil properties (X. Bai et al., 2021; Deltedesco et al., 2020). For example, the positive correlations between soil microbial enzymes, microbial biomass carbon and soil microbial respiration have been attributed to shared effects of nutrient limitations or parallel constraints exerted by heavy metals (Carrillo-Saucedo & Gavito, 2020; Raiesi & Sadeghi, 2019). Similarly, positive correlations between microbial biomass carbon and soil microbial respiration may be a consequence of soil temperature effects on microbial biomass carbon and their driver role in CO_2

fluxes on soil (Iqbal et al., 2010). Likewise, the correlations observed among microbial biomass carbon, bacterial diversity and bacterial abundance, have been reported in previous studies as a set of relevant microbial interactions which together conducts and depends on the soil carbon mineralizing (Wang et al., 2018; Yang et al., 2018; Zhang et al., 2017).

Our DEPNA approach showed a considerable influence of total organic carbon, P and K concentrations on AMF abundances, mycorrhizal diversity, and their colonization rate. Supporting our observations, several studies have reported strong influences of nutrient additions and soil fertility state on AMF abundance, for instance (Camenzind et al., 2016; Stevens et al., 2020). The observed influences of soil nutrients on MBI related with AMF may also be explained by relevant partial correlations with soil microbial respiration and microbial biomass carbon. For example, there are reports of a synergistic process between AMF activities with diverse bacterial communities associated with their spores and extraradical mycelium and playing diverse plant growth-promoting roles, from nitrogen fixation to P solubilization (Giovannini et al., 2020). Besides, there are evidence of intensive management and chemical fertilization altered the cumulative soil respiration via increasing AMF abundance and affecting bacterial abundance (Jin et al., 2022). Likewise, the partial correlations observed among the set of MBI {mycorrhizal diversity, mycorrhizal colonization rate and AMF spore density} with the set {microbial biomass carbon and total organic carbon} corroborated that AMF communities can play a crucial role in stimulating the growth of microorganisms by enhancing the stability of the organic carbon pool (Kumar et al., 2018; Zhang et al., 2020).

The TID exerted by Pb concentrations on AMF diversity and mycorrhizal colonization rate was one of the strongest. The effect of Pb pollution have been qualified as extremely harmful, reducing microbial diversity through enzyme inhibition and their consequent decline of microbial transformation of nutrients (X. Sun et al., 2023). Also, since this TID of Pb was estimated considering partial correlations with bacterial diversity, the high degree observed seems to be complementary explained by intense modifications of soil bacterial community under heavy metal stress after AMF inoculations (J. Cao et al., 2020). AMF presence can increase bacterial diversity because fungal spore wall and extraradical mycelium release fungal substances which modify beneficially the soil chemical composition and pH to bacterial community development (Meglouli et al., 2018).

As a result of similar relevant partial correlations, a strong influence exerted by bulk density and total organic carbon was estimated on mycorrhizal abundance, EEGRSP and TGRSP. Soil bulk density acts as relevant limiting factor to GRSP because their increments changes soil electrical conductivity and disperse protein breaker up agents such as Na (Wang et al., 2015; Zhang et al., 2017). Alongside, increments on bulk density significantly affect other promoting factors of the release of GRSP such as mycorrhizal abundance and organic carbon reservoirs (Bonfim et al., 2013; Nautiyal et al., 2019; Šarapatka et al., 2019). Particularly, although electrical conductivity usually correlates weakly with GRSP (Zhang et al., 2017), we found a negative average correlation in conjunction with a strong influence degree between both variables. Supporting our observations, other author has documented that GRSP contents can increases up to a threshold level of salt concentration but decreases when the salinity (a measure derived from electrical conductivity) increased further (Krishnamoorthy et al., 2014) since AMF abundance declines under highest levels of salts like NaCl (Hammer & Rillig, 2011).

In the same way, an upgrade of electrical conductivity influence degree on T-GRSP, as a consequence of partial correlations with actinomycetal diversity was observed. It appeared that firstly, diversity of actinomycetes decreases due to microbial enzymes lost activity by increasing electrical conductivity and their subsequent pH variations (Heng et al., 2022; A. Kumar et al., 2021). Then, there is an impoverishment of plant growth caused mainly by reductions on biochemical process associated with actinomycetes such as C and N aggregation followed by an absence of actinomycetes that alleviate the effects of salinity stress on plants (Buyer et al., 2011; Grover et al., 2016). Other authors have described that vegetal growth depletion not only reduces the AMF spore densities and root colonization but also diminishes the AMF hyphae distributed in rhizosphere soil. Thus, the AMF extrametrical hyphal growth was negatively impacted, which lead to less GRSP secreted (Gong et al., 2022).

Furthermore, the result revealed a large dependency of water-stable aggregates with EEGRSP and TGRSP concentrations. Despite soil aggregates being the result of the interaction of factors like soil carbohydrates, microorganisms and their related secretions, both fractions of GRSP are considered as relevant factors that affect the formation and stability of soil aggregates (R. Zhu et al., 2019). GRSP are soil particle binding agents, insoluble, hydrophobics, heat-resistant, strongly related to the number of water-stable aggregates and aggregate stability (Wu et al., 2014). Moreover, the maintenance of a positive link between GRSP contents and water-stable aggregates

is an indicative of functional unbroken AMF hyphae networks that both extrudes GRSP and functionally cement soil particles (Wilkes et al., 2021).

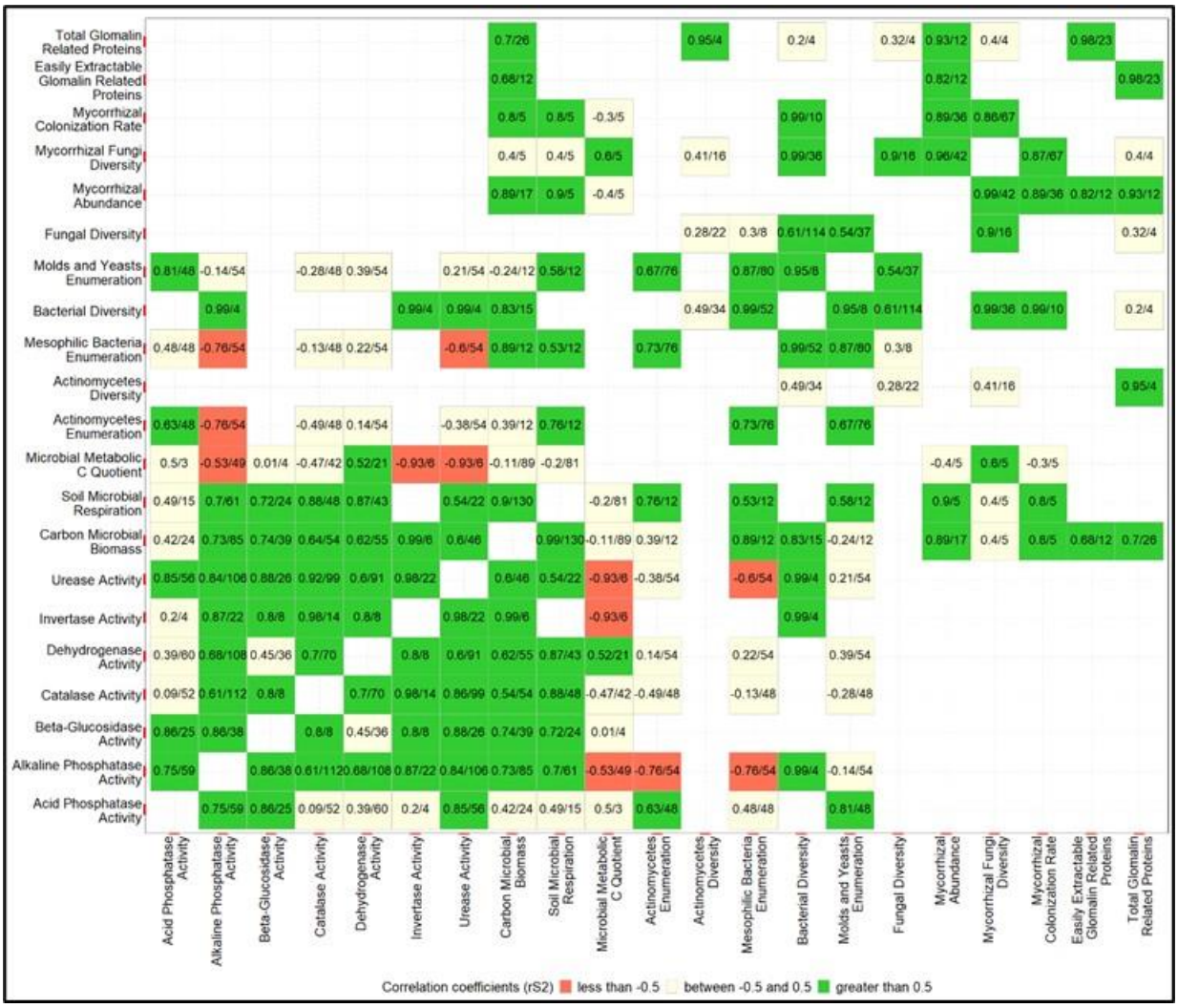
2.5 Conclusions

Despite divergent results across MBI responses observed around different land uses and global geographic regions, there are strong positive relationships between MBI related with AMF and variations in soil properties such as concentrations of soil nutrients, and negative relationships with physical structure and heavy metal pollution. This research highlights the need for context-specific evidence of the effects of different land uses and geographical factors on a whole suite of MBI. Multiple partial correlations among MBI allowed to measure the total degree of influence from soil properties variations driving MBI responses. These first-order partial correlations were useful to explain strong effects of soil properties on MBI related with AMF, and relevant MBI such as microbial biomass carbon, soil microbial respiration, bacterial diversity and actinomycetal diversity.

The partial correlations also corroborated several synergistic effects that increase the sensibility of MBI related with AMF. Consequently, beyond average correlation computed and considering interactions and feedback processes of AMF community with other aspect of soil microbiome, the results suggest that AMF abundance may be a good indicator of total organic carbon and P variations, whereas mycorrhizal colonization rates and mycorrhizal diversity may be good indicators of pollution by heavy metal like Pb. Finally, the high degree of influence suggests that GRSP are largely sensible to variations in soil structural properties such as water-stable aggregates and bulk density.

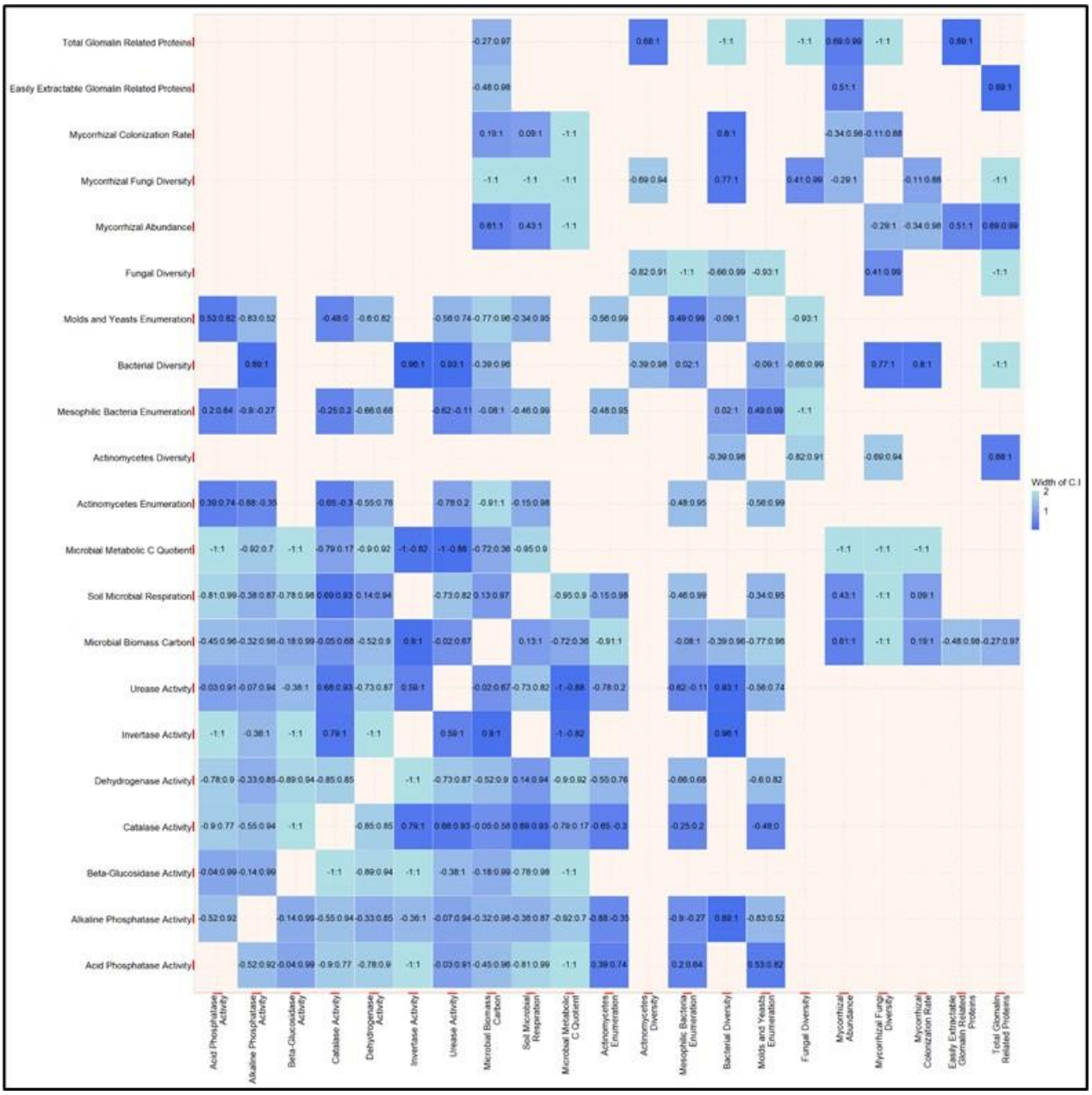
2.6 Supplementary material

Figure 2. 1S *Cross-study averages of the between-treatment Spearman's rank correlation (rs2) for pairs of MBI*



Note: The number of individual treatment where a pairwise of variables was measured (N) is shown after the slash.

Figure 2. 2S *Confidence Intervals for the weighted average correlation coefficients (rs2) for pairs of MBI*



Note: The 2.5% (before colon) and 97.5% (after colon) confidence limits are shown.

3. ANDOSOLS OF THE SOUTHEASTERN REGION OF ANTIOQUIA, COLOMBIA

Abstract

After determining the potential response trends that can be observed in AMF-RI, we set out to assess the effect of different uses of these indicators on andosols of the southeastern region of Antioquia, Colombia. In order to define the environmental context in which the AMF-RI responses were going to be assessed, the geographic locations and specific activities sampled were described. Likewise, each of the soil profiles in the sampled areas were geomorphologically described in order to clearly establish the type of soil where this study was carried out. The field research took place during a rainy season (September – October of 2018) in three municipalities of Antioquia department (La Ceja, El Retiro, and Rionegro) between 2,300 and 2,700 meters above sea level (m.a.s.l). The municipalities are located in the central Andes mountain range. Pre-montane wet forests and mosaics of pasture crops are typical ecosystems in the region. Nine specific activities encompassed the three land uses assessed: Natural forest areas; Agricultural areas; Mining activities areas. All the andosols described share as parental material the Volcanic ash from the Ruíz-Tolima complex. The andosols of the region were classified as Typic Melanudands and Typic Hapludands. To choose 10 sample points in each specific activity, a design-based sampling technique using simple random sampling was used. 90 composite soil samples were collected.

Keywords: Andosols, Antioquia, Land uses, Geomorphological description.

3.1 Introduction

Andosols (or Andisols) have numerous specific features that are uncommon in other types of soil. The prevalence of short-range-ordered minerals (allophane, imogolite, and ferrihydrite) and metal-humus complexes (Al/Fe-humus complexes) in their colloidal fraction are mostly responsible for these features (Takahashi & Dahlgren, 2016). Andosols are a group of soils derived from volcanic ash whit a high porosity and permeability, which present bulk density ≤ 0.9 g cm-3, phosphate retention (RF) ≥ 85%, and Al + ½ Fe extracted with acid oxalate ≥ 2.0% (Alcala de Jesus et al., 2009). Andosols are soils with high cationic and anionic exchange capacity, high buffering power, and a high phosphorus retention. Its acidity has been observed from very strong acid in the superficial horizons (pH: 5.1-5.5) to moderately acid in the deep ones (pH: 6.1-6.5) (Sanchez & Rubiano, 2015). Be sides, andosols have a far larger potential to retain organic carbon than other types of soil (Ordoñez et al., 2022).

Elevated aqueous and exchangeable Al levels in Andosols in conjunction with their intrinsic chemical fertility enhance some types of agricultural productivity (Buytaert et al., 2006; Takahashi & Dahlgren, 2016), seeing that anthropogenic pressure is increasing, and more of these soils are being cultivated. For example, because of their high organic matter content, andosols span a huge region. Andosols cover roughly 124 million hectares globally, equivalent to 0.84% of the world's land surface (Kassaye et al., 2020a). In the Colombian Andes region, andosols occupy 11% of the total area (Roa García et al., 2021). Specifically, the agricultural production of andosols in the Antioquia department is crucial for the supply of other regions of Colombia, with products such as flowers, coffee, beans, potatoes, corn, tomatoes, and vegetables in general. However, the use and abuse of agrochemicals such as herbicides, fungicides, insecticides, and fertilizers is recognized on the andosols of that region (Domínguez et al., 2009).

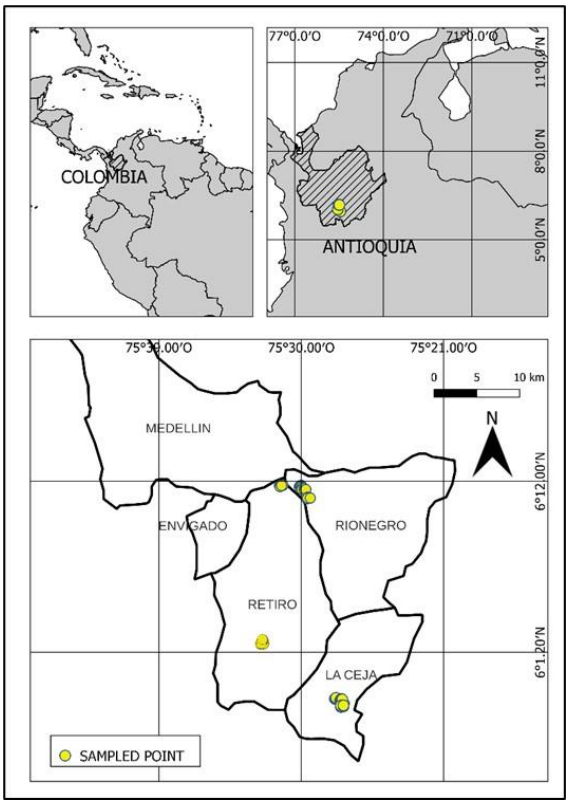
3.2 Fieldwork and soil sampling

This study characterizes the andosols from the southeastern region of Antioquia, Colombia. The field research took place during a rainy season (September – October of 2018) in three municipalities (La Ceja, El Retiro, and Rionegro) (Figure 3.1). The municipalities are located in the central Andes mountain range at altitudes between 2,300 and 2,700 meters above sea level

(m.a.s.l). Pre-montane wet forests and mosaics of pasture crops are typical ecosystems in the region, which exhibit a unimodal temperature cycle with an average temperature of 18°C and an annual bimodal precipitation cycle. Within each municipality, the study collected samples of andosols in three land uses which exhibited different degradation stages. Land uses characterized were: (1) natural forest areas; (2) soils subject to pressure by agriculture activities; and (3) soil subject to considerable pressure by mining activities related to clay extraction. The following are the detailed geomorphological descriptions of andosols retrieved as well as the principal environmental features of each municipality sampled. The degradation stage was defined according to the fraction removed from horizon A. Hence, soils from natural forest areas (NFA) were considered as reference for a non-degraded stage; soils retrieved in agricultural areas (AGA) with a fraction removed from horizon A between 10 and 20%, were cataloged as weakly degraded, and soils of mining extractions areas (MEA), with a removed fraction greater than 60%, were considered highly degraded.

A set of 10 points were randomly selected from the centroid of the zone that encompassed each sampled area. A design-based sampling strategy involving simple random sampling and a stratified simple random sampling was carried out to sample point selection (Brus et al., 2011). Thus, each land use was stratified into two sub-zones. The first sub-zone was defined as the center area of land use. Then, a second sub-zone was considered as the border areas of the land use. Eight sample points were selected randomly within the first sub-zones. Likewise, two sample points were selected randomly within second sub-zones. Five subsamples (soil cores at 20 cm depth) were taken within a 2-m radius of each point to make a composite soil sample, using a soil auger with a diameter of 5 cm (Laboratory Services and Applied Science Division, & U.S Environmental protection Agency, 2011). 90 composite soil samples were collected and stored in plastic bags at 4°C for further analyses.

Figure 3. 1 *Geolocation sampled zones across municipalities of "Rionegro", "La Ceja" and "El Retiro" in Antioquia, Colombia*

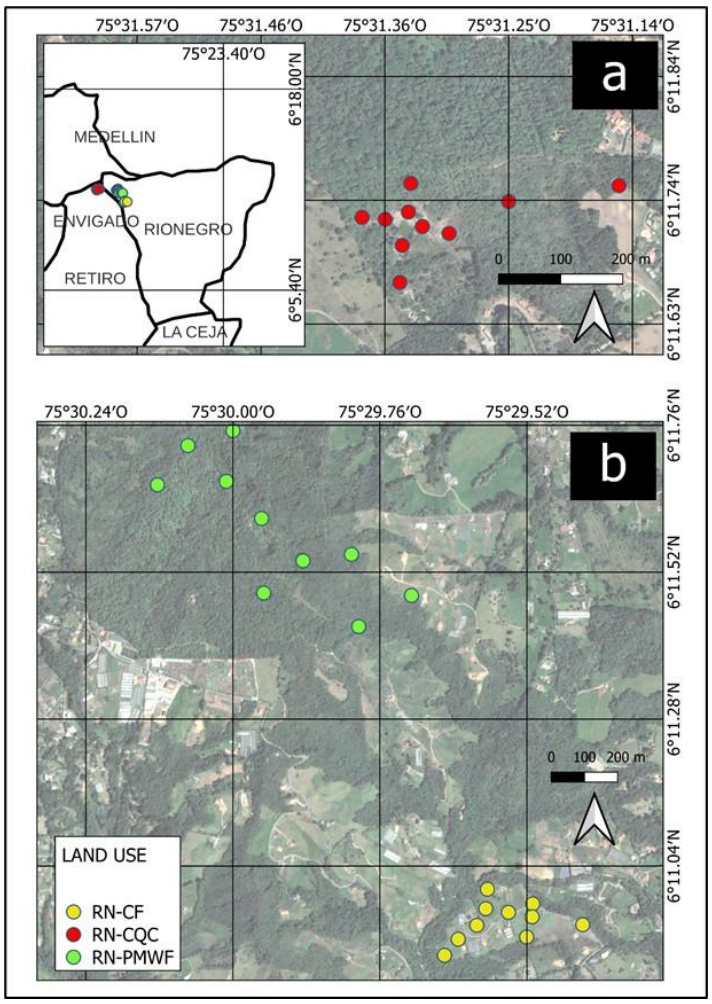


3.3 Municipality of "Rionegro"

Rionegro is located in the northern part of the Central Cordillera of Colombia, about 55 km east of the city of Medellín. Rionegro limits to the north with the municipalities of Guarne and San Vicente, to the east with Marinilla and El Carmen, to the south with La Ceja, and to the west with El Retiro, Envigado, and Medellín. Ecologically and hydrologically, this area is one of the most important since the basin harbors the main water sources of the region. The municipality of Rionegro is delimited by the alluvial plain of the Río Negro and its tributaries; the most important being the Pantanillo River and the Las Palmas, Espíritu Santo, and Fizebad streams, dammed by the La Fe Dam, the Don Diego and Chachafruto streams. The area presents a bimodal rainfall regime, with rainy periods in the months of April and May in the first semester and from October to November in the second. The average annual precipitation varies between 1,800 and 2,500 mm. The municipality has an average altitude of 2,100 m.a.s.l. and an average temperature varying

between 17°C and 21°C (Salazar Suaza & Quijano-Abril, 2020). The predominant life zone corresponds to a premontane wet forest and 1870 Ha have been reported as protected areas by municipal authorities during 2021 (Alcaldía de Rionegro, 2021). Most of the mining activities observed in the zone are associated with materials such as natural clays, sands, and gravels related to the alluvial zones of the Negro River. Figure 3.2 shows the geolocation of natural forest areas, agricultural activities areas, and mining activities that were sampled for our study. Also, figures 3.3 to 3.8 show the soil profile description for every land use sampled.

Figure 3. 2 *Geolocation of sampled points in three land uses across the municipality of Rionegro, Antioquia*



Note: a) Sampled points in an area of mining extraction activities; b) Sampled points in areas of natural forest and agricultural activities. (RN-CF=Crops of *Fragaria ananassa*; RN-CQC=Closed quarry clay; RN-PMWF=Pre-montane wet forest). Imagen from world topographic map of (ESRI, 2013).

Figure 3. 3 Soil profile description of andosols retrieved in a pre-montane wet forest of Rionegro, Antioquia

Land use code: RN-PMWF.
Location: Rionegro, Antioquia.
Altitude: 2,640 m.a.s.l.
Physiographic position: Middle part of low, rounded hills, developed in serpentinized Dunite saprolite, covered with volcanic ash.
Topography: Moderately steep with a slope 30%.
Parent material: Volcanic ash from the Ruíz-Tolima complex.
Natural vegetation: High stubble.
Current use: Pre-montane wet forest with stubble.
Soil moisture regime: Udic.
Soil temperature regime: Isothermal.
Effective depth: Very deep.
Natural drainage: Well drained.
Evidence of erosion: Does not present.
Epipedon: Melanic.
Subsurface horizons: Somber.
Classification: Typic Melanudands.

Soil profile	Horizon	Depth (cm)	Description
	Hi	0 – 7	Fresh organic material, abundant thin, medium, and thick roots; matrix color 2.5YR2.5/1 reddish black; net limit.
	Ah	7 – 46	Color 2.5YR2.5/2 very dark red; clayey; crumby structure, very loose; friable, plastic, non-sticky; medium pores; abundant fine and medium roots; Plane transitional limit.
	Bw_1	54 – 81	Color 5YR6/4 light reddish brown; clayey; with structure in angular, thin, moderate blocks; friable, plastic, very sticky; common fine pores; scarce roots; Net and irregular limit.
	Bw_2g	54 – 81	Color 5YR6/1 gray; clayey; structure in angular blocks, thin, weak, inconsistent; plastic, non-sticky; medium and fine pores; some fine roots; Flat net limit. With evidence of gleyzation.
	LP	81 – 109	A very well defined stone line whose lower limit is marked by a very hard placic 1 cm thick on average; supra lying highly oxidized quartzite rocks of varied sizes up to a fist, underrounded and angular edges supported.
	2Bwb	109 – 118	Palaeosoil. Here horizon A is eroded. Color 7.5YR5/6 strong brown; clayey; with structure in angular, medium, moderate blocks; friable, plastic, non-sticky; common fine pores; scarce roots; Net and irregular limit.
	2C	118 – 130+	Color 2.5Y7/2 pale brown; clay saprolite, unstructured, massive; plastic, non-sticky; abundant fine pores; net limit.

Note: Described by Maria Teresa Florez (Geologist, GAIA research group, Universidad de Antioquia).

Figure 3. 4 *Pre-montane wet forest at 2,640 m.a.s.l in Rionegro, Antioquia*

Figure 3. 5 *Soil profile description of andosols retrieved in F. ananassa crops of Rionegro, Antioquia*

Land use code: RN-CF.
Location: Rionegro, Antioquia.
Altitude: 2,429 m.a.s.l.
Physiographic position: Lower part of low, rounded hills, developed in serpentinized Dunite saprolite, covered with volcanic ash.
Topography: Rounded hills covered with volcanic ash.
Parent material: Volcanic ash from the Ruíz-Tolima complex.
Natural vegetation: Natural vegetation removed.
Current use: *F. ananassa* crops.
Soil moisture regime: Udic.
Soil temperature regime: Isothermal.
Effective depth: Very deep.
Natural drainage: Well drained.
Evidence of erosion: Does not present.
Epipedon: Ochric.
Subsurface horizons: Cambic.
Classification: Typic Hapludands.

Soil profile	Horizon	Depth (cm)	Description
	Ap	0 – 30	Color 2.5YR2.5/3 dark reddish brown; clayey; with crumby, loose, plastic, sticky structure; fine pores; abundant fine roots; Flat abrupt limit.
	B/A	30 – 40	Color 5YR3/4 dark reddish brown by 35% and 7.5YR4/2 brown by 65%; clay silt; with structure in subangular, medium, moderate blocks; friable, plastic, sticky; very fine pores; some fine roots; Very abrupt irregular limit.
	Bw	40 –52	Color 7.5YR5/2 brown; clayey; with structure in angular, thin, moderate blocks; friable, plastic, very sticky; common fine pores; scarce roots; Net and irregular limit.
	$2Bw_g$	55 –68	Color 7.5YR5/1 gray; clayey; structure in angular blocks, thin, weak, inconsistent; plastic, non-sticky; medium and fine pores; some fine roots; Flat net limit. With evidence of gleyzation. Towards the floor plates of hard rock are observed as if they were quartz aligned as the "line of stones".

Note: Described by Maria Teresa Florez (Geologist, GAIA research group, Universidad de Antioquia).

Figure 3. 6 *Crops of F. ananassa at 2,429 m.a.s.l in Rionegro, Antioquia*

Figure 3. 7 *Soil profile description of andosols retrieved in a closed quarry of clays in Rionegro, Antioquia*

Land use code: RN-CQC.
Location: Vereda Pantanillo, municipality of Ronegro, Antioquia.
Altitude: 2,792 m.a.s.l.
Physiographic position: High part of the slope of low, rounded hills, developed in serpentinized Dunite saprolith, covered with volcanic ash.
Topography: Moderately steep with a slope of 37%.
Parent material: Volcanic ash from the Ruíz-Tolima complex.
Natural vegetation: Low stubble.
Current Use: Closed Mining.
Soil moisture regime: Udic.
Soil temperature regime: Isothermal.
Effective depth: Very deep.
Natural drainage: Well drained.
Evidence of erosion: Does not present.
Epipedon: Ochric.
Subsurface horizons: Cambic.
Classification: Typic Hapludands.

Soil profile	Horizon	Depth (cm)	Description
	Hi	0 – 13	Fresh organic material, abundant thin, medium, and thick roots, some highly decomposed; matrix color 5YR2.5/2 Dark reddish brown; net limit.
	Ah_1	13 – 20	Color 5YR2.5/1 black; clay loam; crumby structure, very loose; friable, plastic, non-sticky; common medium and fine pores; abundant fine and medium roots; Plane transitional limit.
	Ah_2	20 – 31	Color 2.5YR2.5/1 reddish black; clayey; structure in angular blocks, thin, weak, inconsistent; plastic, non-sticky; common medium and fine pores; abundant fine and medium roots; Flat net limit.
	A/B	31 – 36	Color 10R2.5/1 reddish black in 70% and 2.5YR5/3 reddish brown in the remaining 30%; clay silt; with a tendency to structure in angular, fine, moderate blocks; friable, plastic, very sticky; common fine pores; scarce roots; Net and irregular limit.
	Bw_{g1}	36 – 46	Color 5YR5/2 reddish gray; clay silt; with structure in subangular, thin, weak blocks; friable, very plastic, very sticky; common fine pores; scarce roots; Net and irregular limit. Evidence of gleyzation.
	Bw_{g2}	46 – 73	Color 5YR6/1 gray; clayey; with structure in angular, thin, moderate blocks; friable, plastic, non-sticky; common fine pores; scarce roots; the presence of mesofauna; Net and irregular limit. Evidence of gleyzation.
	C_g	73 - 90	Color 5YR6/2 pink gray; clay saprolith, unstructured, massive; plastic, non-sticky; abundant fine pores; net limit. Evidence of gleyzation.
	R	90 – 100+	Rock in situ, serpentinized Dunite with chromium, iron and manganese oxides on its surface.

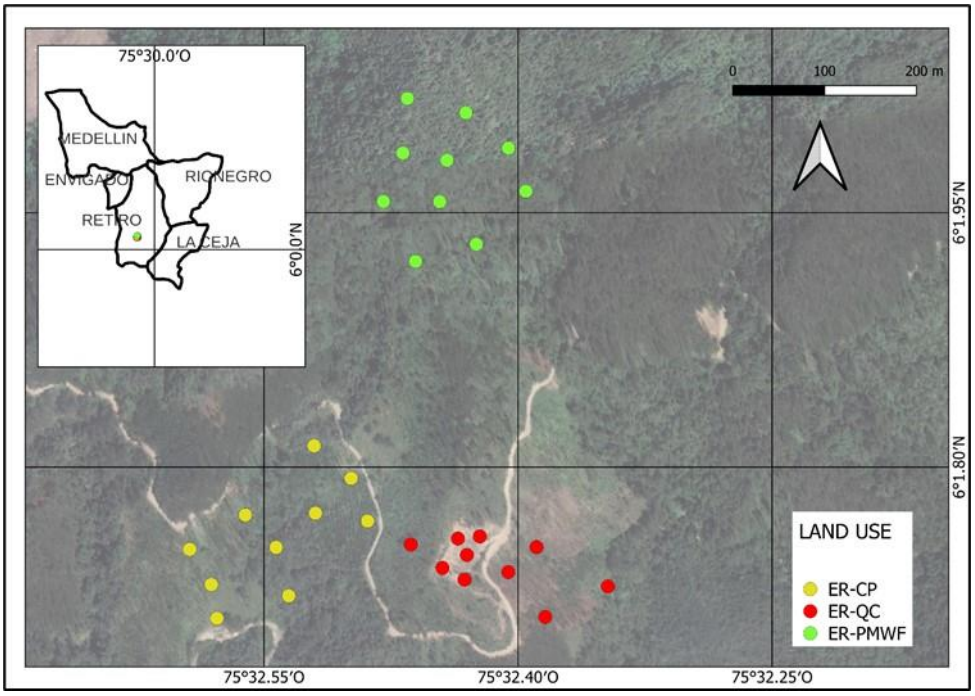
Note: Described by Maria Teresa Florez (Geologist, GAIA research group, Universidad de Antioquia).

Figure 3. 8 *Closed quarry of clays at 2,792 m.a.s.l in Rionegro, Antioquia*

3.4 Municipality of “El Retiro”

El Retiro is a municipality located in the eastern subregion of the department of Antioquia, into a depression of the Central mountain range of the Andes, particularly in the San Nicolás valleys. It borders to the north with the municipalities of Envigado and Rionegro, to the east with the municipalities of Rionegro and La Ceja, to the south with La Ceja and Montebello, and to the west with the municipalities of Santa Bárbara, Caldas, and Envigado. The temperature varies between 16 and 20ºC. It presents a rainfall regime between 1,000 and 4,000 mm per year, with two rainy seasons in April-May and October-November. This municipality is crossed by two main hydrographic basins. The first is that of the Negro River, which occupies an area of 198.8 km2. The second is the Arma river basin that forms it, which occupies 98 km2. Within the municipality, there are life zones such as pre-montane wet forest, very humid lower montane forest, and pre-montane wet forest. Each one covers 18%, 27% and 75% of the total municipal area, respectively. The vegetal coverage with the greatest extension in El Retiro is planted forest, with 31.12%. The most used species belong to the coniferous order. However, the second coverage in extension is a natural forest with 28.87% (Cornare, 2012). The figure 3.9 are shown geolocations of natural forest areas, agricultural activities areas, and mining activities areas which were sampled for our study. Also, figures 3.10 to 3.15 show the soil profile description for every land use sampled.

Figure 3. 9 Geolocation of sampled points in three land uses across the municipality of El Retiro, Antioquia



Note: (ER-CP=Crops of *Pinus sp.*; ER-QC=Quarry of clays; ER-PMWF=Pre-montane wet forest). Imagen from world topographic map of (ESRI, 2013).

Figure 3. 10 *Soil profile description of andosols retrieved in a pre-montane wet forest of El Retiro, Antioquia*

Land use code: ER-PMWF.
Location: Vereda El Carmen, Parte Alta, municipality of El Retiro.
Altitude: 2,416 m.a.s.l.
Physiographic position: High part of the slope of rounded hills, developed in saprolyth from Diorite of the Antioquia Batholith, covered with volcanic ash.
Topography: Moderately steep with a slope of 37%.
Parent material: Volcanic ash from the Ruíz-Tolima complex.
Natural vegetation: High stubble.
Current use: High stubble in pre-montane wet forest.
Soil moisture regime: Udic.
Soil temperature regime: Isothermal.
Effective depth: Very deep.
Natural drainage: Well drained.
Evidence of erosion: Does not present.
Epipedon: Melanic.
Subsurface horizons: Cambic.
Classification: Typic Melanudands.

Soil profile	Horizon	Depth (cm)	Description
	Hi	0 – 6	Fresh organic material, abundant fine, medium, and thick roots, mainly of fern and pine, immersed in a very fine dark reddish brown 5YR2.5/2 color matrix; Abrupt wavy limit.
	Ah	6 – 43	Color 5YR2.5/1 black; clay silt; with crumb structure; friable, slightly plastic, moderately sticky; common fine and very fine pores; common fine roots; some partially decomposed lytic fragments up to 2 mm; Flat abrupt limit.
	B/A	43 – 50	Color 2.5YR2.5/1 reddish black by 30% and 7.5YR4/2 brown by 70%; clay loam to clayey; with structure in subangular, thin, moderate blocks; friable, very plastic, very sticky; common very fine pores; some roots and pedotubules; Very abrupt irregular limit.
	Bw	50 – 67	Color 10R2.5/1 reddish black; clayey; with structure in angular, thin, moderate blocks; friable, very plastic, very sticky; common fine pores; scarce roots; Irregular abrupt limit.
	C	67 – 90	Color 5YR6/3 light reddish brown; clay saprolyte of quartzodiorite from the Antioquia Batholith; massive; moderately plastic, very sticky; Flat clear boundary.
	R	90 – 640+	Rock, quartzidiite from the Antioquia Batholith.

Note: Described by Maria Teresa Florez (Geologist, GAIA research group, Universidad de Antioquia).

Figure 3.11 *Pre-montane wet forest at 2,416 m.a.s.l in El Retiro, Antioquia*

Figure 3. 12 *Soil profile description of andosols retrieved in commercial exploitation of Pinus sp. In El Retiro, Antioquia*

Land use code: ER-CP.
Location: Vereda El Carmen, Parte Alta, municipality of El Retiro.
Altitude: 2,446 m.a.s.l.
Physiographic position: Middle part of the slope of rounded hills, developed in sapromolite from Diorite of the Antioquia Batholith, covered with volcanic ash.
Topography: Moderately steep with a slope 30%.
Parent material: Volcanic ash from the Ruíz-Tolima complex.
Natural vegetation: Pines.
Current use: Commercial exploitation of *Pinus sp.*
Soil moisture regime: Udic.
Soil temperature regime: Isothermal.
Effective depth: Very deep.
Natural drainage: Well drained.
Evidence of erosion: Does not present.
Epipedon: Ochric.
Subsurface horizons: Egric.
Classification: Typic Hapludands

Soil profile	Horizon	Depth (cm)	Description
	Hi	0 – 10	Fresh organic material, abundant thin, medium, and thick roots, some highly decomposed; a dark reddish-brown 2.5YR2.5/3 color matrix; Abrupt wavy limit.
	Ap	10 – 34	Color 5YR2.5/2 dark reddish brown; clayey; with crumby structure, very loose, plastic, sticky; common fine pores; abundant fine roots; some partially decomposed lytic fragments up to 1 mm; Flat abrupt limit.
	Bw	34 – 37	Color 5YR3/4 dark reddish brown; clayey; with structure in angular, thin, moderate blocks; friable, very plastic, very sticky; common fine pores; scarce roots; Irregular abrupt limit.
	2From	37 – 51	Paleosoil; color 5YR2.5/1 black; clay loam; with structure in angular, thin, moderate blocks; friable, plastic, sticky; abundant very fine pores; some roots; Very abrupt irregular limit.
	3C	51– 90+	Color 7.5YR3/4 dark brown; abundant rock fragments of varied forms (preferably angular) and sizes (up to 15 cm in diameter) of amphibole shale some very altered and matrix supported; The matrix has a pseudo-block structure but is very weak and inconsistent; This layer seems to correspond to a very old slope deposit; clay silt, moderately plastic, very sticky; some fine pores; few dead fine roots, translocation of organic matter, abundant mesofauna; Flat clear boundary.

Note: Described by Maria Teresa Florez (Geologist, GAIA research group, Universidad de Antioquia).

Figure 3. 13 *Crops of Pinus sp. at 2,446 m.a.s.l in El Retiro, Antioquia*

Figure 3. 14 *Soil profile description of andosols retrieved in a quarry of clays in El Retiro, Antioquia*

Land use code: ER-QC.
Location: Vereda El Carmen, Parte Alta, municipality of El Retiro.
Altitude: 2,372 m.a.s.l.
Physiographic position: Middle part of the slope of rounded hills, developed from sapromolite of the Diorite of the Antioquia Batholith, covered with volcanic ash.
Topography: Rock escarpment, steep with a slope of 45%.
Parent material: Volcanic ash from the Ruíz-Tolima complex.
Natural vegetation: High stubble located at the top of the escarpment.
Current use: Quarry of clays.
Soil moisture regime: Udic.
Soil temperature regime: Isothermal.
Effective depth: Very deep.
Natural drainage: Well drained.
Evidence of erosion: Does not present.
Epipedon: Melanic.
Subsurface horizons: Cambic.
Classification: Typic Melanudands.

Soil profile	Horizon	Depth Cm	Description
	Hi	0 – 10	Fresh organic material, abundant thin, medium, and thick roots on a matrix of reddish black 2.5YR2.5/1 color; Abrupt wavy limit.
	Ah	10 – 30	Color 2.5YR2.5/2 very dark red; clay loam; with structure in angular blocks, thick, moderate; consistent, plastic, non-sticky; abundant fine pores; abundant fine, medium, and thick roots; Flat abrupt limit.
	A/B	30 – 43	Color 5YR3/4 dark reddish brown in 65% and 5YR4/4 reddish brown in the remaining 35%; silty clay; structure in angular, thin, moderate blocks; friable, very plastic, very sticky; abundant fine pores; some roots; large pedotubules and evidence of translocation of organic matter; to the waxy touch; Very abrupt irregular limit.
	Bw_1	43 –55	Color 5YR4/4 reddish brown; clayey; structure in medium, weak angular blocks; very plastic, not sticky; few fine pores; very oxidized fine and medium roots; waxy and thixotropic touch; Transitional limit.
	Bw_2	55 – 89	Color 7.5YR4/6 Strong brown; clayey; structure in medium, weak angular blocks; very plastic, not sticky; few fine pores; very oxidized fine and medium roots; Thixotropic; Irregular abrupt limit.
	C	89 – 110+	Color 7.5YR5/6 yellowish red; Clay amphibolite saprolyte with abundant heterometric and heterogeneous blocks, very angular at depth These blocks become large and massive, then the rock in sitú emerges.

Note: Described by Maria Teresa Florez (Geologist, GAIA research group, Universidad de Antioquia).

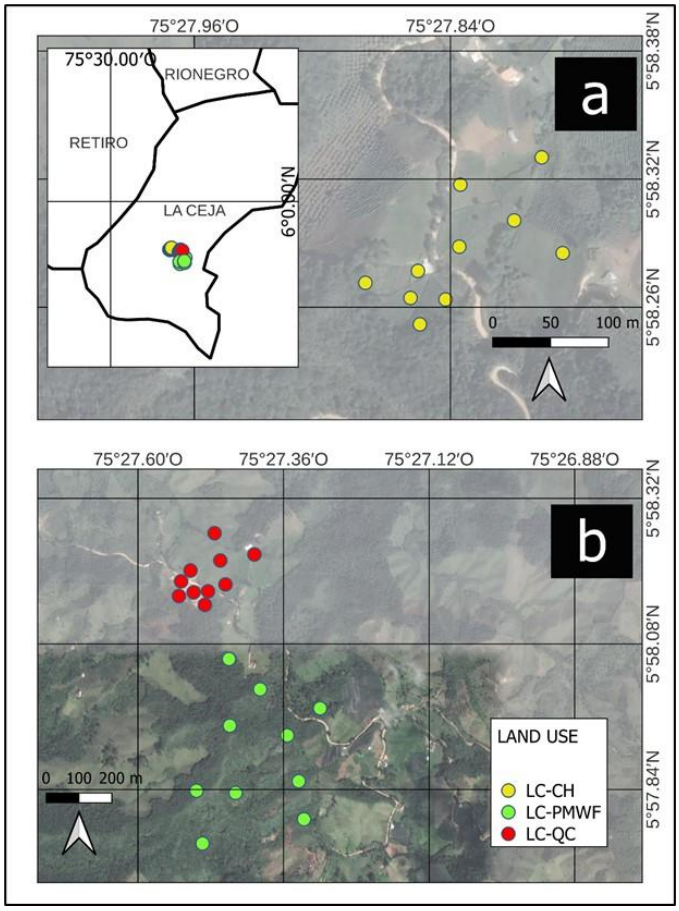
Figure 3. 15 *Quarry of clays at 2,372 m.a.s.l in El Retiro, Antioquia*

3.5 Municipality of "La Ceja del Tambo"

The municipality of La Ceja del Tambo is located in the eastern zone of the Antioquia department, between the valleys of San Nicolás (highlands of the eastern) and the La Ceja valley (valley of the Cimarronas). La Ceja borders on the north with Rionegro; to the northeast with Carmen del Viboral; to the east with La Unión; to the south with Abejorral; to the southwest with Montebello; to the west with El Retiro. The territory of the municipality of La Ceja del Tambo has 17 micro basins, of which the following are mainly mentioned: Pereira Alta, Pereira Media, Pereira

Baja, El Yarumo, Chupadero, El Higuerón, and Payuco. The rivers that run through the territory are La Miel, El Buey, Pantanillo, and Pereira (Cornare, 2006). In the municipal area, the "lower montane very wet forest" predominates. The average temperature presents little variation, with values that fluctuate between 15º C and 17º C, throughout the year. The average annual precipitation is 3,649 mm. The main crops in the municipality are flowers, potatoes, beans, and blackberries. It also has 8,190 hectares of pastures dedicated to livestock (COMGER, 2012). The figure 3.16 are shown geolocations of natural forest areas, agricultural activities areas, and mining activities areas that were sampled for our study. Also, figures 3.17 to 3.22 show the soil profile descriptions for every land use sampled.

Figure 3. 16 *Geolocation of sampled points in three land uses across the municipality of La Ceja, Antioquia*



Note: a) Sampled points in an agricultural activities area; b) Sampled points in areas of natural forest and mining extraction activities and agricultural activities. (LC-CH=Crops of *Hydrangea sp.*; LC-QC=Quarry of clays; LC-PMWF=Pre-montane wet forest). Imagen from world topographic map of (ESRI, 2013).

Figure 3. 17 *Soil profile description of andosols retrieved in a pre-montane wet forest of La Ceja, Antioquia*

Land use code: LC-PMWF.
Location: Vereda Tabacal, Municipio de La Ceja, Antioquia.
Altitude: 2,699 m.a.s.l.
Physiographic position: Middle part of the slope of low, rounded hills, developed from amphibolite saprolyte and covered with volcanic ash.
Topography: Moderately steep with a slope of 37%.
Parent material: Volcanic ash from the Ruíz-Tolima complex.
Natural vegetation: Tall stubble with fern.
Current use: Fallow.
Soil moisture regime: Udic.
Soil temperature regime: Isothermal.
Effective depth: Very deep.
Natural drainage: Well drained.
Evidence of erosion: Does not present.
Epipedon: Melanic.
Subsurface horizons: Cambic.
Classification: Typic Melanudands

Soil profile	Horizon	Depth (cm)	Description
	Hi	0 – 23	Fresh organic material, abundant fine, medium, and thick roots, some highly decomposed mainly of fern and pine, immersed in a very fine dark reddish brown 5YR3/2 color matrix; Abrupt wavy limit.
	Ah	23 – 40	Color 7.5YR2.5/2 very dark brown, although it can vary to 5YR2.5/1 black; clay loam; with structure in subangular, thin, moderate blocks; friable, slightly plastic, moderately sticky; common fine and very fine pores; common fine roots; some partially decomposed lytic fragments up to 2 mm; Flat abrupt limit.
	A/B	40 – 42	Color 7.5YR3/3 dark brown in 70% and 7.5YR4/4 brown in the remaining 30%; clay silt; with structure in subangular blocks, fine, moderate; friable, plastic, very sticky; common fine and very fine pores; some roots and pedotubules; very abrupt irregular limit.
	Bw_1	42 – 47	Color 7.5YR4/4 brown; clayey; with structure in angular, thin, moderate blocks; plastic, non-sticky; fine pores; some roots; the presence of pedotubules filled with organic matter (evidence of translocation) and cracks; irregular net limit.
	Bw_2	47 – 56	Color 7.5YR4/6 strong brown; clay silt; with structure in angular, medium, moderate blocks; friable, plastic, non-sticky; common fine pores; scarce roots; Irregular net limit.
	Bw_3	56 – 66	Color 7.5YR5/6 strong brown; clay silt; with structure in angular, thin, moderate blocks; friable, moderately plastic, very sticky; many fine pores; few dead fine roots; Flat clear boundary.
	BC	66 – 80	Color 7.5YR5/6 strong brown in 60% and 7.5YR6/8 reddish yellowish in the remaining 40%; clay silt; with structure in angular blocks, thin, weak; friable, very plastic, very sticky; many pores; few fine roots; Flat clear boundary.
	C	80 – 128+	Color 7.5YR6/8 yellowish reddish; clay silt; without structure; very plastic, very sticky; many fine pores; few dead and rusty fine roots.

Note: Described by Maria Teresa Florez (Geologist, GAIA research group, Universidad de Antioquia).

Figure 3. 18 *Pre-montane wet forest at 2,699 m.a.s.l in La Ceja, Antioquia*

Figure 3. 19 *Soil profile description of andosols retrieved in commercial crops of Hydrangea sp. in La Ceja, Antioquia*

Land use code: LC-CH.
Location: Vereda Tabacal, Municipio de La Ceja, Antioquia.
Altitude: 2,473 m.a.s.l.
Physiographic position: Middle part of the slope of low, rounded hills, developed from amphibolite saprolyte and covered with volcanic ash.
Topography: Moderately steep with a slope 30%.
Parent material: Volcanic ash from the Ruíz-Tolima complex.
Natural vegetation: High stubble with abundant fern.
Current use: Commercial crops of *Hydrangea sp.*
Soil moisture regime: Udic.
Soil temperature regime: Isothermal.
Effective depth: Very deep.
Natural drainage: Well drained.
Evidence of erosion: Does not present.
Epipedon: Ochric.
Subsurface horizons: Cambic.
Classification: Typic Hapludands

Soil profile	Horizon	Depth (cm)	Description
	Hi	0 – 13	Fresh organic material, abundant thin, medium and thick roots, partially decomposed in a 7.5YR2.5/1 black color matrix; Wavy net limit.
	Ap	13 – 46	Color 7.5YR2.5/3 very dark brown; clay loam; with crumb structure; friable, slightly plastic, moderately sticky; abundant fine and medium pores; abundant thin and thick roots without decomposing; Net and irregular limit.
	A/B	46 - 62	Color 7.5YR2.5/2 dark brown by 80% and 7.5YR4/2 brown by 10%; clay silt; with structure in angular blocks, plastic, slightly sticky; few fine pores; some fine roots; waxy to the touch, thixotropic; Very irregular limit.
	Bw_1	62 – 78	Color 7.5YR4/4 brown; silty clay; structure in angular, medium, strong blocks; slightly plastic, slightly sticky; fine pores; roots; some blocks up to 7 cm lithocromic; waxy to the touch, thixotropic; Very abrupt irregular limit.
	Bw_2	78 – 92+	Color 7.5YR4/3 brown; clayey; structure in angular, medium, strong blocks; plastic, non-sticky; fine pores; roots; waxy to the touch, thixotropic; Very abrupt irregular limit.

Note: Described by Maria Teresa Florez (Geologist, GAIA research group, Universidad de Antioquia).

Figure 3. 20 *Crops of Hydrangea sp. at 2,473 m.a.s.l in La Ceja, Antioquia*

Figure 3. 21 *Soil profile description of andosols retrieved in a quarry of clays and rocks in La Ceja, Antioquia*

Land use code: LC-QC.
Location: Vereda Tabacal, Municipio de La Ceja, Antioquia
Altitude: 2,482 m.a.s.l.
Physiographic position: Mountain escarpment by amphibole rock exploitation.
Topography: Rock escarpment, steep with a slope of 45%.
Parent material: Volcanic ash from the Ruíz-Tolima complex.
Natural vegetation: High stubble located at the top of the escarpment
Current use: Quarry of clays and rocks.
Soil moisture regime: Udic.
Soil temperature regime: Isothermal.
Effective depth: Very deep.
Natural drainage: Well drained.
Evidence of erosion: Does not present.
Epipedon: Melanic.
Subsurface horizons: Cambic.
Classification: Typic Melanudands

Soil profile	Horizon	Depth (cm)	Description
	Hi	0 – 5	Fresh organic material, abundant thin, medium, and thick roots; Abrupt wavy limit.
	Ah	5 –37	Color 7.5YR2.5/1 black; clay loam; with crumb structure; friable, slightly plastic, moderately sticky; common fine pores; common thin, medium, and thick roots; Flat abrupt limit.
	A/B	37 –46	Color 7.5YR3/2 dark brown by 60% and 7.5YR4/2 brown by 40%; clay silt; with structure in angular blocks, plastic, slightly sticky; few fine pores; some fine roots; waxy to the touch, thixotropic; Very irregular limit.
	Bw_1	46 –55	Color 7.5YR4/3 brown; clayey; with structure in angular blocks, plastic, slightly sticky; abundant fine and medium pores; abundant fine and medium roots; waxy to the touch, thixotropic; Net and very irregular limit.
	Bw_2	55 –68	Color 7.5YR3/4 dark brown; clayey; with structure in subangular blocks, plastic, sticky; abundant fine pores; abundant fine and medium roots; waxy to the touch, thixotropic; Net and very irregular limit.
	C	68 –87+	Color 7.5YR6/8 reddish yellow; silty franc; with a heavy structure tending to be massive; abundant blocks of rock from fist-sized to large rocks; slightly plastic, very sticky; some pores; few fine roots; Flat clear boundary.

Note: Described by Maria Teresa Florez (Geologist, GAIA research group, Universidad de Antioquia).

Figure 3. 22 *Quarry of clays at 2,482 m.a.s.l in La Ceja, Antioquia*

4. DRIVING FACTORS OF NATIVE ARBUSCULAR MYCORRHIZAL FUNGI AND ASSOCIATED MICROBIAL FEATURES ALONG A SOIL DEGRADATION GRADIENT IN COLOMBIAN ANDOSOLS

Abstract

Arbuscular mycorrhizal fungi (AMF) play crucial roles in nutrient acquisition and ecosystem functioning, enhancing plant growth and productivity, as well as contributing to soil structure and water retention by mean of releasing a set of binding glomalin-related soil proteins (GRSP). Land use practices have a significant impact on AMF communities and their function in soil ecosystems reflecting the quality and diversity of a soil. However, the responses of AMF populations to land use influences in abiotic soil properties may be possibly complemented by other soil microbiological aspects. This study analyzed 90 soil samples of andosols collected from locations devoted to conservation areas of natural forest, agriculture and mining activities in the southeastern region of Antioquia, Colombia, to assess the relationships between soil microbiological features such as populations of culturable soil microorganisms, enzyme activity, and microbial basal respiration and native AMF with soil properties modified in proportion to different soil degradation stages. Our study determined by a confident ANOVA approach that populations of culturable mesophilic bacterial and fungi, catalase activity, microbial basal respiration, AMF diversity, AMF spore abundance and GRSP as well as soil abiotic properties such as NH4-N, SOC, S and SWC suffered significant detriments depending on the degree of pressure exerted by land use. On the contrary, Ca contents, soil temperature and bulk density increase following the degradation gradient. Our explanatory models support the fact that differences observed in AMF communities among land uses were mainly explained by interactions with urease activity and populations of culturable fungi and mesophilic bacteria in conjunction with soil temperature, Ca and S concentrations.

Keywords: Associated microbiological indicators, Arbuscular mycorrhizal fungi, Soil abiotic properties, Degradation gradient, Gradual effects.

4.1 Introduction

The physical characteristics of soils change in response to external forcing, either by conversion of natural forest areas or by poor soil management in areas under anthropogenic pressure, leading to increased soil erosion, nutrient losses, and decreased soil productivity (Lizaga et al., 2019). Next to abiotic soil changes, among the most relevant effects of land use practices there are changes in the soil microbiological component (Santos et al., 2020; Y. Sun, Luo, et al., 2020). Microorganisms are essential to the sustainability and overall functionality of soil ecosystems. Fertility and health of the soil are influenced by microbial communities that live there, both in terms of quantity and composition (Nkongolo & Narendrula-Kotha, 2020). Since soils are ecosystems with intricate relationships between microbiological communities and physicochemical factors, a better understanding of the role of abiotic properties on soil microbial components has emerged as a global priority (Hermans et al., 2020).

Arbuscular mycorrhizal fungi (AMF) are microbes essential for sustaining soil ecosystem processes, encouraging ecological restoration, and tracking variations in soil health caused by land use. Land use have an impact on AMF communities as well as on the quantity of glomalin-related soil proteins (GRSP) that AMF released. The variations in soil nutrients and soil physical structure caused by land use activities can weaken the symbiosis established between AMF and plant roots, reducing AMF colonization intensity and subsequently the GRSP production (J. Chen et al., 2022; Matos et al., 2022). Basically, AMF are important indicators of soil functionality in virtue of its mandatory symbiotic relationship with host plants. It allow vegetal establishment facing soil stress conditions by means of boosting of the radical absorption surface of plants to transfer soil nutrients and water (Mónica et al., 2020). Parallelly, GRSP lead the formation of water-stable aggregates because it aids in the adhesion of soil particles functioning as a binding agent (H. Liu et al., 2020c). The driver role of soil primary nutrients such as phosphorous (P), nitrogen (N) and carbon (C) on AMF communities have been useful to understand the positive correlation between AMF communities with the main edaphic abiotic attributes used for soil quality assessments (Matos et al., 2022). However, as we reviewed in the previous chapter, the potential complementarity of AMF responses with another soil microbiological characteristics raises the important issue of how AMF diversity and their GRSP production are related with another soil microbiological components. For example, among the soil microorganisms, the culturable portion include a good

representation of fungi and bacteria related with plant growth, N fixation capacities, plant protection antagonism and nutrient transformation (de Mastro et al., 2020). Nerveless, little is known about the coexistence of AMF and culturable microbes across soil degradation gradients induced by land use. Likewise, there are reports of effects on soil microbial basal respiration rates associated with changes in microbial biomass after inoculation with AMF (Sales et al., 2021). Even though there are validations of positive effects in soil microbial enzyme activities and microbial biomass in highly degraded soils after inoculations with specific AMF taxa (L. Xiao, Bi, et al., 2019a). However, the relationship between these microbial indicators with native AMF and their GRSP production is not elucidated yet. As to understand soil quality variations is required to describe how the various soil constituents and features interact in a web of processes (Vogel et al., 2018), the study of relationships between soil microbiological features and AMF would allow to establish the scope of soil abiotic changes in a relevant and intercorrelated soil microbiological component, it considering that AMF is a microbiological soil component that is widely recognized as key to monitor relevant soil quality aspects, along with populations of culturable soil microorganisms, enzyme activity, and microbial basal respiration (da Silva et al., 2020; Komplikevych et al., 2023; Riva et al., 2022).

Andosols from Antioquia department (Colombia) have been drastically modified in short periods by land use changes, mainly in their contents of organic matter, iron (Fe), Calcium (Ca) and porosity (Ramírez et al., 2022). Andosols has been considered to be vital soil resources because they are intensively cultivated in virtue of their high productivity, (Kassaye et al., 2020b; Y. Sun, Amelung, et al., 2020). Notwithstanding, in relevant soil units as andosols from the southeastern region of Antioquia, currently there is a lack of detailed descriptions of native AMF communities and the effect of different land uses in soil microbiological component potentially associated with them. In the present study, three municipalities of the southeastern region of Antioquia department were sampled to describe the distribution of AMF spores and soil contents of GRSP across three soils in different degradation stages: (1) conserved natural pre-montane wet forest areas; (2) Areas under weak pressure by agricultural activities; and (3) areas subject to considerable pressure from mining activities. The aim of our study is identifying significant changes on soil abiotic properties caused by land uses and describe the role of these abiotic changes in native AMF diversity, GRSP contents and soil microbiological features associated with AMF communities. Firstly, we assessed the hypotheses that several soil abiotic properties, AMF communities as well as microbiological

features associated with them may change according to soil degradation stage. This hypothesis allows us to validate the correspondence between different soil degradation stages with significant changes in abiotic and biotic soil features either deleterious or cumulative variations. Secondly, we tested the hypothesis that AMF diversity, contents of GRSP and microbiological features may be positively related to another microbiological soil features whose responses are conjunctly explained by soil abiotic properties. Here, the role of soil abiotic characteristics in changes of basal respiration, culturable mesophilic bacteria, fungi, and actinomycetes, and soil microbial enzyme activities such as urease and catalase was investigated.

Our research is the first description of AMF communities in the area and it offers evidence of linkages between abiotic soil properties and microbiological features as explanation of gradual but significant changes on a set of relevant and intercorrelated soil quality indicators. Here are presents two major technical factors. Firstly, we assess the assumption that land uses modify soil abiotic properties and its microbiological features through a confident parametric analysis of variance (ANOVA) approach whose mean squares were calculated using the customary formulas, but secure conclusions about the variance ratios were drawn from a comparison with the F-distribution after data transformation to reach in our experimental design conditions suggested to separate land use effects from other sources of variation. Secondly, the combinations of variables that explained the greatest amount of variance in a microbiological feature were selected using a recently developed dropping column algorithm (DCA) to resolve the best subset regression problem which tests all possible combination of the predictor variables, and then selects the best model (Hofmann et al., 2020).

4.2 Material and methods

4.2.1 Land uses sampled

In the previous chapter the description of 9 soil profiles across the municipalities of Rionegro, La Ceja and El Retiro was presented. The methods to collect soil samples were illustrated in the numeral 3.2. Geomorphological descriptions of each soil profile were detailed starting from the numeral 3.3 to 3.5. Within each municipality, the study collected samples of andosols in three land uses which exhibited different degradation stages according to the fraction removed from

horizon A (J. S. Nunes et al., 2012). Thus, soils from natural forest areas (NFA) were considered as reference for an not degraded stage; soils retrieved in agricultural areas (AGA) with a fraction removed from horizon A between 10 and 20% were cataloged as weakly degraded, and soils of mining extractions areas (MEA), with a removed fraction greater than 60%, were considered as highly degraded. Below is presented a summary of the land uses sampled and their location (Table 4.1). 10 points were sampled in each area and a total of 90 compound soil samples was retrieved and stored in plastic bags at 4°C to posterior laboratory analysis. All the physicochemical and microbiological analyzes of the soil samples were carried out in the laboratories of the GAIA group (Research Group in Environmental Management and Modeling).

Table 4. 1 *Geolocation of areas designated for conservation of native forest, agricultural and mining activities in three municipalities of southeast region of Antioquia-Colombia*

Municipality	Activity	Altitude (m.a.s.l)	Activity code	Land use	Location
La Ceja	Conservation zones of pre-montane wet forest	2.457	LC-PMWF	NFA	05° 57' 48" N; 75° 27' 27" W
	Crops *Hydrangea sp*.	2.427	LC-CH	AGA	05° 58' 15" N; 75° 27' 51" W
	Quarry clays	2.476	LC-QC	MEA	05° 58' 08" N; 75° 27' 31" W
El Retiro	Conservation zones of pre-montane wet forest	2.523	ER-PMWF	NFA	06° 01' 58" N; 75° 32' 24" W
	Forestry crops of *Pinus sp*.	2.370	ER-CP	AGA	06° 01' 46" N; 75° 32' 31" W
	Quarry clays	2.413	ER-QC	MEA	06° 01' 43" N; 75° 32' 26" W
Rionegro	Conservation zones of pre-montane wet forest	2.610	RN-PMWF	NFA	06° 11' 34" N; 75° 29' 59" W
	Crops of *Fragaria ananassa.*	2.549	RN-CF	AGA	06° 10' 55" N; 75° 29' 31" W
	Closed quarry clays	2.774	RN-CQC	MEA	06° 11' 43" N; 75° 31' 21" W

Abbreviations: NFA=Natural Forest Areas; AGA=Agricultural Activities Areas; MEA=Mining Extraction Areas.

4.2.2 Physicochemical characterization of soils

Soil temperature (TEMP) measurements were taken at a depth of 10 cm using a precision, digital thermometer Lamotte 5-0095 (Crotty et al., 2019). In the laboratory soil pH were measured

from soil: water extracts (1:5) using a conductivity meter Lamotte Tracer 1766 (Ziegler-Rivera et al., 2020). To prepare soil extracts, 100 mL of distilled water was mixed with 20 g of air-dried soil. Then, the mixture was agitated in a mechanical shaker for 1 h and filtered by means of vacuum filtration system (Gharaibeh et al., 2021). Bulk density (BD) was calculated by volumetric cylinder method (Al-Shammary et al., 2018). At each sample point, two metal sampling cylinder (2" of height by 2" of diameter) was pressed into the soil. After, the cylinders were excavated and the soil cores had been cut flush with the ends of the cylinders. The total volume of the soil cores was estimated as the internal volume of the cylinder. Soil cores were then empty into a labelled sampling bag. Soil cores were dried at 105 °C for 48 h, and the mass of the dry soil cores was measured. BD (mg/cm^3) was expressed as the average of quotients between the mass of dry soil cores and their total volume. Soil water contents (SWC) were determined by gravimetric method in 200 g of wet soil. Wet soil samples were dried for 48 h at 105° and SWC was expressed as the ratio of the mass of water present to the weight of the dry soil sample (W. Tan et al., 2019).

For chemical characterization, soil samples were dried at environment temperature, then crushed and passed through a 2 mm sieve. The primary soil nutrient quantified were: soil organic carbon (SOC) by Walkley-Black method (Walkley & Black, 1934) adjusted by Enang et al., 2018; ammonia nitrogen (NH_4-N) by colorimetric method of Berthelot reaction with sodium hypochlorite (NaOCl) and 2-Phenylphenol sodium salt (PPS) (Rhine et al., 1998); nitrate nitrogen (NO_3-N) by cadmium reduction method (Vendrell & Zupancic, 1990); potassium (K) by precipitation induced method with addition of a known excess of sodium tetraphenylborate (A. E. Cox et al., 1999).

The secondary soil elements quantified were: calcium (Ca) and magnesium (Mg) by titration in Schwarzenbach EDTA method (Loeppert & Suarez, 1996); copper (Cu) by spectrophotometric method with diethyldithiocarbamate (DDTC) in ammonia media (Uddin et al., 2013); total iron expressed as a sum of Fe(II) plus Fe(III) by spectrophotometric analysis of bipyridyl complex in chlorohydric acid (HCl) and oxalate extracts (Dominik, 2000); manganese (Mn) by spectrophotometric analysis of Mn^{2+} oxidation with sodium paraperiodate ($H_2INa_3O_6$) in strongly acid solution (Gambrell, 1996); phosphorous by ascorbic acid-molybdenum blue method (Irving & McLaughlin, 1990); and sulphur (S) by chloride barium ($BaCl_2$) method (Senthilkumar et al., 2021).

4.2.3 Microbiological characterization of soils

In first place, microbiological density was determined by seeding of serial dilution on selective culture medium plates to enumerate colony-forming units (CFU). Here, 5 g of dry soil and 95 mL of a 0.9% sodium chloride (NaCl) solution were mixed. The solution was stirred for 8 h, and then aliquots of 1 ml were taken to prepare serial dilutions. Mesophilic aerobic bacteria enumeration (EBACT) and fungi enumeration (EFUNG) were performed according to indications provided by Werheni Ammeri et al., 2023.

Thus, EBAC was carried out by spreading 100 µl of 10^{-6} dilution on plate count agar (PCA). CFU were enumerated after an incubation period of 48 h at 28 °C. For EFUN, 100 µl of 10^{-5} soil dilution was spread on potato dextrose agar (PDA). After 7 days of incubation at ambient temperature into a dark cabin, the number of developed CFU was recorded. For actinomycetes enumeration (EACTI), 100 µl of 10^{-5} soil dilution was spread on starch casein agar plates (SCA) and then incubated at 28 °C for 6 days (Fatahi et al., 2019). To every enumeration were seeded 4 replicates, and the average microbial enumeration were expressed as number of CFU g^{-1} dry soil.

The alkali absorption technique was used to assess the microbial basal respiration (MBR) in soil samples (Dehsheikh et al., 2020). 30 g of homogenized dry soil was rewetted (to field water holding capacity) and putted in hermetically sealed jars of 1l together a tube containing 20 ml of sodium hydroxide solution (NaOH, 0.25 M).

Control jars were armed without soil sample. The jars were incubated at 25 °C for 48 h. The carbon dioxide (CO_2) released from the soil sample during the incubation period was captured by NaOH solution tubes. NaOH solution was then titrated with HCl (0.25 M) in the presence of $BaCl_2$ (0.5 M) and three drops of phenolphthalein reagent. MBR was estimated using equation 1:

$$MBR = (N \times (V_1 - V_2) \times 22) \,/\, (T \times DW) \qquad (1)$$

where MBR is expressed as mg emitted of CO2 g^{-1} soil day^{-1}, N is the normality of used HCl, V_1 is the volume of used HCl for titration of remained NaOH in control jar, V_2 is the volume of HCl used for titration of NaOH in jars with soil sample, T is the time (day), and DW is the weight of dry soil sample putted into the jars.

Urease activity (URE) was quantified by colorimetric determination of ammonia method (Kandeler & Gerber, 1988) adjusted according to Cordero et al., 2019. Here, 500 µl of urea solution (80 mM) were added to 1g of dry soil. This mixture was incubated at 18 °C for 2 hours without shaking. Next, sedimented soil was extracted adding 10 ml of potassium chloride (KCl, 2 M), then shaking the solution for 30 minutes at 300 rpm and finally centrifuging them for 5 minutes at 2900g. The solution was filtered through Whatman 42 filter paper. Ammonia levels in the filtered solution after incubation period (t1) were compared to those in the urea solution prior to incubation (t0). In order to determine the ammonia content at t0, soils combined with 500 µl of urea solution were instantly extracted as previously mentioned. Ammonia in the extracts was evaluated Berthelot reaction. Each sample was analyzed using 4 replicates. URE average among replicates was expressed as µg NH_{4+} h^{-1} g^{-1} soil. URE was computed using equation 2:

$$URE = \frac{\triangle NH_4\left(\frac{\mu g}{ml}\right)}{Incubation\ time\ (2h) \times g\ wet\ soil\ (1\ g) \times (1 - SWC)} \qquad (2)$$

$\triangle NH_{4+}$ being the concentration of ammonia in the incubated solutions minus the ammonia in urea solutions don't incubate and SWC is soil water content.

Catalase activity (CAT) was estimated according to Maphuhla et al., 2020. In that order, 5 g of dry soil were mixed with 0.5 mL of toluene. The solution was incubated at 4 °C for 30 min.

Then, 5 ml of hydrogen peroxide solution was added (H_2O_2, 3%) and the mixture was stored again at 4 °C for 1 h. After, to stop the microbial activity in the solution, 3 ml of H_2SO_4 (2 M) was added. The final suspension was titrated with a potassium permanganate solution ($KMnO_4$, 0.01 M) until a pink colour appears permanently in the solution. Each sample was analyzed using 4 replicates. CAT average among replicates was expressed as ml of $KMnO_4$ h^{-1} g^{-1} soil.

4.2.4 Isolation, enumeration and taxonomic determination of AMF spores

The method used for the isolation of spores was a combination of the wet sieving method proposed by Gerdemann & Nicolson, 1963 and the sucrose density-gradient centrifugation method described by Bai et al., 2013 since the wet sieving allows a high percentage of spore extraction and the sucrose density-gradient centrifugation improves the separation of spores from soil and organic

material. 20 g of each soil sample was stirred in 1 L of distilled water. Afterwards, the supernatant was decanted through sieves with a pore size of 300, 150 and 45 µm. The content of each sieve was recovered to be centrifuged for 3 min in distilled water at 3,000 rpm. The supernatant was removed and then, the pellet was homogenized with a sucrose solution (50%). The mixture was centrifuged for 2 min at 2,000 rpm. The supernatant obtained was carefully collected (avoiding mixing the phases) using a 50 ml syringe, placed on Whatman 5 filter paper and then washed twice with distilled water. The filter paper was placed in petri dishes and kept refrigerated at 4 °C. This procedure was carried out to 5 replicates until analyzed 100 g to each soil sample. The filter papers were observed with a stereoscope to direct counts of observed spore. The abundance of AMF spores was expressed as the average number of spore g^{-1} soil (Boya et al., 2023).

The spores were separated by morphotype to be mounted on slides with polyvinyllacto-glycerol (PVLG) and Melzer's reagent for taxonomic identification. The spores were examined using a compound microscope and the species determination was based on morphological descriptions compiled by The International Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM) (http://invam.caf.wvu.edu), The Department of plant Pathology, University of Agriculture in Szczecin (http://www.zor.zut.edu.pl/Glomeromycota) and originally published descriptions of AMF species accepted whose list is available on the web site amf-phylogeny.com.

4.2.5 Determination of GRSP contents

The total amount of GRSP (TGRSP) was quantified in citrate-extracts of soil by a Bradford assay (Hou et al., 2022). Firstly, 0.25 g of dry soil were weighed (previously sieved with a mesh size of 2 mm). Then, 2 ml of a sodium citrate solution (50 mM, pH=7.0) were added ant the mixture was placed in an autoclave for 60 min at 121 °C. After, the solution was centrifugated for 6 min at 10,000 rpm and the supernatant was collected and reprocessed until no more visible red-brown color appeared. Solutions were diluted (1:2) in extraction solution. Sample blanks were prepared to Bradford assay with 20 µl of diluted sample, 250 µl of Bradford reagent and 250 µl HCl (1 M) to obtain a final pH of about 1 since the colour spectra of soil extracts are pH-dependent (Cissé et al., 2020). We used a spectrophotometer UV-VIS Pharo 300 Spectroquant (Mark) for colorimetry at 595 nm wavelength. The calibration curve was plotted using bovine serum protein (BSA) solution as standard to calculate the TGRSP content (Reyna & Wall, 2014).

4.2.6 Data analysis

The AMF communities under different land uses were characterized in terms of diversity calculating spore richness (total amount of spores) and Shannon alpha diversity index (H'). Variations in spore richness, alpha diversity index, soil properties and microbiological features between land uses were analyzed with ANOVA followed by a Tukey honestly significant difference test (HSD) at $p < 0.05$ (Melo et al., 2019). Data transformations were implemented to reach two main assumptions in ANOVAs and therefore obtain safe inferences from the effects of land uses in our soil data. They were: (1) normal distribution of residuals; (2) homogeneity of variances among treatments (Webster & Lark, 2019b). If any of the above assumption was rejected, the transformations tested to reach them were: x^2, x^3, x^{-1}, $x^{1/2}$, $x^{1/3}$, $(x+1)^{1/2}$ and $log(x)$, (Ribeiro et al., 2018). We proved the assumption of normality of the residuals and homogeneity of variances by means of Shapiro-Wilk statistic and Fligner-Killeen test, respectively. Just variables with significant differences between each land uses contrast (NFA vs. AGA; NFA vs. MEA; AGA vs. MEA) were selected to posterior analysis.

To test for significant differences between groups of samples across land uses, an analysis of similarity (ANOSIM) was performed. Furthermore, differences in community structure between land uses were visualized by applying non-metric multidimensional scaling plots (NMDS). NMDS were carried out using function *metaMDS*. The function *envfit* allows to see how soil abiotic properties and microbiological features were related to the patterns observed in the community data. Only the most significant ($p < 0.05$) vectors harboring a correlation ≥ 0.20 relative to the NMDS axes were represented. Significance of vectors was assessed by permutation test. ANOSIM and NMDS were performed in R package "vegan" version 2.6.4. adopting Bray dissimilarity index as partitioning of variation with 999 permutations.

By selecting the best model to explain the greatest amount of variation in a microbiological indicator, in this study sought to achieve better accuracy with few predictors using a combination of soil abiotic factors and potentially associated microbial features. The models were ranked using the Akaike Information Criterion (AIC), which assesses how close a model is to an ideal but unobservable model that produced the data. Also, the adjusted R^2 ($adjR^2$) and root mean square error (RMSE) values, which offer an absolute measurement of the explanatory power of predictors and accuracy of a model, respectively, were used to compare the models (Fundisi et al., 2020). The

models were selected according to smallest RMSE, AIC and highest adjR^2. All-possible subsets of predictors were tested in multiple linear regression models using DCA from R package "lmSubsets" (Hofmann et al., 2020).

Goodness of fit statistic of Anderson-Darling was computed by means function *fitdist* from R package "fitdistrplus" version 1.1.8 to determine whether each dependent variable was coming from a normal distribution (Dino et al., 2021). Since all the dependent variables were fitted to a normal distribution, we proceeded to evaluate the performance of the selected predictors within linear mixed models (LMM) (S. Lo & Andrews, 2015). Municipalities was introduced within LMM as a random variable in order to account for their effects on each microbiological feature. Parametric bootstrapping test (PBT) was carried out with "pbnm" R package (Banghart, 2015) to test the significance of this effect. A p-value ≤0.001 provides evidence that the variance added by random effects was significant (Efendi et al., 2017). In addition, a type II Wald Chi-Squared test was used to find out if explanatory continuous variables in each a model add significantly variations (Arango et al., 2023). Finally, Shapiro-Wilk test were performed to validate the assumption of normality distribution to residual of the explanatory models (P. Wu et al., 2012). The modelation of data and Wald Chi-Squared test were carried out with function *lmer* and *Anova* from R package "lme4" version, respectively (Brown, 2021).

4.3 Results

4.3.1 Effect of land use on soil properties and microbiological features

Primary soil nutrients, secondary elements contents and physicochemical soil properties as well as microbiological features were all influenced by land use types at significance level < 0.01 (Table 4.2). Apparent departures from the assumptions in normal distribution of residual and homogeneity of variances were dealt with data transformations (Table 4.1S and Table 4.2S). With land use as direct factor of variation, abiotic soil properties including NH_4-N, SOC, S and SWC showed significant decreases along the degradation gradient (NFA>AGA>MEA) (Table 4.3, Table 4.4 and Table 4.5). The same trend was observed in all microbiological features excepting EACTI (Table 4.6). In a different manner, abiotic soil properties such as Ca, TEMP and BD showed significant increments according to the degradation gradient (NFA<AGA<MEA). Soil contents of

NO_3-N, P, and K were significatively highest in AGA. Meanwhile, values of Cu contents and pH were significatively highest and lowest in MEA, respectively. Complete statistics of ANOVA assumption and Tukey HSD test are presented in from Table 4.1S to Table 4.6S.

Table 4. 2 *One-Way Analyses of Variance (ANOVA) in abiotic and biotic soil properties of Colombian andosols considering as source of variation land use types*

Variable	Transformation	df	Sum of squares	Mean of squares	f	p-value
TEMP	Log(x)	2	0.43	0.22	41.29	<0.01
BD	*	2	0.57	0.28	117.13	<0.01
SWC	*	2	4459.98	2229.99	75.03	<0.01
PH	*	2	3.11	1.56	45.32	<0.01
Ca	*	2	193340.80	96670.39	31.22	<0.01
Cu	*	2	4.98	2.49	8.51	<0.01
Fe	Log(x)	2	1.42	0.71	7.53	<0.01
K	x^{-1}	2	0.00	0.00	26.06	<0.01
Mg	Log(x)	2	1.93	0.96	6.06	<0.01
Mn	x^{-1}	2	0.24	0.12	4.19	<0.01
NH_4-N	Log(x)	2	2.31	1.16	30.80	<0.01
NO_3-N	*	2	97.10	48.55	30.47	<0.01
P	*	2	4.18	2.09	21.80	<0.01
S	Log(x)	2	1.33	0.67	15.42	<0.01
SOC	*	2	492.94	246.47	98.94	<0.01
EBACT	$x^{1/2}$	2	40.71	20.35	40.04	<0.01
EFUNG	*	2	5507.42	2753.71	91.16	<0.01
EACTIN	*	2	744.39	372.20	23.68	<0.01
URE	Log(x)	2	2.87	1.43	18.03	<0.01
CAT	$x^{1/2}$	2	4.42	2.21	80.19	<0.01
MBR	$x^{1/2}$	2	1.13	0.56	54.56	<0.01
ESPO	$x^{1/2}$	2	12.86	6.43	52.20	<0.01
TGRSP	*	2	324.70	162.35	75.38	<0.01
H‘	*	2	5.78	2.89	136.02	<0.01

Abbreviations: TEMP= Soil temperature; BD= Bulk density; SWC= Soil water contents; SOC= Soil organic carbon; EBACT= Enumeration of mesophilic culturable soil bacteria; EFUNG= Enumeration of culturable soil fungi; EACTI= Enumeration of culturable soil actinomycetes; URE= Microbial urease activity; CAT= Microbial catalase activity; MBR= Microbial basal respiration; ESPO= Spore enumeration of arbuscular mycorrhizal fungi; TGRSP= Total glomalin related soil proteins; H'= Shannon index; * No data transformation; df= Degrees of freedom.

Table 4. 3 *Summary statistics of primary soil nutrients for andosols under three land uses in south eastern region of Antioquia department, Colombia*

		NO_3-N (g kg^{-1})		NH_4-N (g kg^{-1})		P (g kg^{-1})		K (mg kg^{-1})		SOC (%)	
Land use/Activity		**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**
NFA	(n=30)	7.03[a]	1.46	34.47 [a]	7.50	2.91 [a]	0.34	210.20 [a]	38.50	15.02 [a]	1.94
ER-PMWF	(n=10)	7.74	1.19	37.19	8.86	3.08	0.26	230.51	48.73	16.26	1.37
LC-PMWF	(n=10)	6.68	1.60	34.84	7.71	2.83	0.37	200.72	37.45	15.34	1.75
RN-PMWF	(n=10)	6.68	1.44	31.40	4.97	2.82	0.36	199.38	17.63	13.48	1.64
MEA	(n=30)	6.10[b]	0.93	23.15 [b]	3.68	2.60 [b]	0.24	183.96 [b]	25.01	9.30 [b]	0.99
ER-QC	(n=10)	5.60	0.55	21.64	4.65	2.55	0.21	185.47	27.25	9.25	1.13
LC-QC	(n=10)	6.00	0.74	22.35	2.75	2.47	0.15	172.84	13.69	8.99	1.13
RN-CQC	(n=10)	6.69	1.11	25.45	2.33	2.77	0.25	193.57	29.04	9.66	0.60
AGA	(n=30)	8.61[c]	1.34	29.61 [c]	6.41	3.12 [c]	0.34	237.05 [c]	29.57	11.94 [c]	1.65
ER-CP	(n=10)	8.74	1.51	32.24	8.07	3.32	0.43	238.47	33.67	13.66	0.81
LC-CH	(n=10)	8.19	1.40	27.31	4.87	2.99	0.15	226.18	19.50	11.09	1.65
RN-CF	(n=10)	8.90	1.11	29.28	5.45	3.05	0.30	246.49	32.72	11.06	0.70

Abbreviations: NFA= Natural forest areas; MEA= Mining extraction areas; AGA= Agricultural areas; ER-PMWF= El Retiro Pre-montane wet forest; LC-PMWF= La Ceja Pre-montane wet forest; RN-PMWF= Rionegro Pre-montane wet forest; ER-QC= El Retiro Quarry clays; LC-QC= La Ceja Quarry clays; RN-QC= Rionegro Closed quarry clays; ER-CP= El Retiro Forestry crops of *Pinus* sp.; LC-CH= La Ceja Crops of *Hydrangea* sp.; RN-CF= Rionegro Crops of *Fragaria ananassa*; SOC= Soil organic carbon. SD= Standard deviation. *Note:* Different letters denote Tukey's HSD test at $p < 0.05$ between mean values in land uses.

Table 4. 4 *Summary statistics of secondary soil chemical elements for andosols under three land uses in south eastern region of Antioquia department, Colombia*

		Ca (mg kg^{-1})		Cu (mg kg^{-1})		Fe (mg kg^{-1})		Mg (mg kg^{-1})		Mn (mg kg^{-1})		S (mg kg^{-1})	
Land use/Activity		**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**
NFA	(n=30)	400.26 [a]	49.80	2.29 [a]	0.44	4.96 [a]	1.65	209.78 [a]	78.92	10.13 [a]	2.11	11.41 [a]	2.03
	ER-PMWF (n=10)	393.12	60.28	2.14	0.45	4.75	1.48	219.64	76.81	10.51	2.02	12.21	1.10
	LC-PMWF (n=10)	389.92	53.04	2.41	0.47	4.89	2.22	192.29	72.05	10.03	2.56	11.63	2.39
	RN-PMWF (n=10)	417.76	32.05	2.31	0.41	5.25	1.26	217.43	92.00	9.85	1.84	10.41	2.12
MEA	(n=30)	512.59 [b]	58.39	3.55 [b]	0.48	6.73 [bc]	1.96	292.05 [b]	106.92	9.28 [ac]	1.14	8.49 [b]	1.59
	ER-QC (n=10)	508.41	50.96	3.84	0.52	6.96	2.07	304.20	128.16	9.47	1.28	8.84	1.87
	LC-QC (n=10)	546.05	59.57	3.71	0.46	6.99	2.00	334.16	106.13	9.24	1.07	8.04	1.72
	RN-CQC (n=10)	483.30	51.23	3.10	0.41	6.24	1.92	237.79	61.16	9.11	1.14	8.59	1.17
AGA	(n=30)	470.73 [c]	58.32	2.44 [a]	0.67	5.76[ac]	1.77	225.20 [a]	99.80	8.88 [bc]	1.47	10.11 [c]	2.55
	ER-CP (n=10)	449.96	38.66	1.99	0.55	5.19	1.22	214.76	102.72	8.75	1.38	10.44	3.01
	LC-CH (n=10)	511.71	39.87	2.94	0.72	5.63	2.14	259.45	129.64	9.84	1.08	9.65	1.69
	RN-CF (n=10)	450.51	71.24	2.41	0.37	6.46	1.74	201.41	51.79	8.05	1.43	10.23	2.95

Abbreviations: NFA= Natural forest areas; MEA= Mining extraction areas; AGA= Agricultural areas; ER-PMWF= El Retiro Pre-montane wet forest; LC-PMWF= La Ceja Pre-montane wet forest; RN-PMWF= Rionegro Pre-montane wet forest; ER-QC= El Retiro Quarry clays; LC-QC= La Ceja Quarry clays; RN-QC= Rionegro Closed quarry clays; ER-CP= El Retiro Forestry crops of *Pinus* sp.; LC-CH= La Ceja Crops of *Hydrangea* sp.; RN-CF= Rionegro Crops of *Fragaria ananassa*; SD= Standard deviation. *Note:* Different letters denote Tukey's HSD test at $p < 0.05$ between mean values in land uses.

Table 4. 5 *Summary statistics of physicochemical properties for andosols under three land uses in south eastern region of Antioquia department, Colombia*

			TEMP (°C)		BD (g cm^{-3})		SWC (%)		pH	
Land use/Activity			**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**
NFA		(n=30)	18.09 [a]	1.42	0.56 [a]	0.05	40.98 [a]	6.07	5.49 [a]	0.12
	ER-PMWF	(n=10)	18.26	1.95	0.54	0.05	41.25	6.41	5.49	0.10
	LC-PMWF	(n=10)	18.36	1.29	0.56	0.06	37.54	4.81	5.46	0.16
	RN-PMWF	(n=10)	17.64	0.82	0.57	0.05	44.14	5.51	5.53	0.10
MEA		(n=30)	21.55 [b]	1.74	0.75 [b]	0.05	24.27 [b]	4.39	5.15 [b]	0.19
	ER-QC	(n=10)	20.84	2.44	0.75	0.05	25	3.32	5.07	0.09
	LC-QC	(n=10)	21.47	0.53	0.75	0.03	24.17	4.94	5.17	0.14
	RN-CQC	(n=10)	22.35	1.49	0.73	0.06	23.65	5.06	5.22	0.27
AGA		(n=30)	19.45 [c]	1.65	0.62 [c]	0.05	36.33 [c]	5.75	5.68 [a]	0.23
	ER-CP	(n=10)	20.39	1.32	0.62	0.04	38.68	5.03	5.40	0.10
	LC-CH	(n=10)	19.55	1.35	0.65	0.04	36.26	6.7	5.88	0.07
	RN-CF	(n=10)	18.43	1.74	0.59	0.05	34.06	4.94	5.78	0.07

Abbreviations: NFA= Natural forest areas; MEA= Mining extraction areas; AGA= Agricultural areas; ER-PMWF= El Retiro Pre-montane wet forest; LC-PMWF= La Ceja Pre-montane wet forest; RN-PMWF= Rionegro Pre-montane wet forest; ER-QC= El Retiro Quarry clays; LC-QC= La Ceja Quarry clays; RN-QC= Rionegro Closed quarry clays; ER-CP= El Retiro Forestry crops of *Pinus* sp.; LC-CH= La Ceja Crops of *Hydrangea* sp.; RN-CF= Rionegro Crops of *Fragaria ananassa*; TEMP= Soil temperature; BD= Bulk density; SWC= Soil water content; SD= Standard deviation.
Note: Different letters denote Tukey's HSD test at $p < 0.05$ between mean values in land uses.

Table 4. 6 *Summary statistics of microbiological features for andosols under three land uses in south eastern region of Antioquia department, Colombia*

		EBACT (CFU 10^{-6})		EACT (CFU 10^{-5})		EFUNG (CFU 10^{-5})		URE (µg NH_{4+} h^{-1} g^{-1})		CAT (ml $KMnO_4$ h^{-1} g^{-1})		MBR (mg CO2 g^{-1} day^{-1})	
Land use/Activity		**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**
NFA	(n=30)	86.16 [a]	15.09	15.70 [a]	4.15	40.05 [a]	6.10	67.45 [a]	19.80	8.47 [a]	1.08	0.92 [a]	0.18
ER-PMWF	(n=10)	93.82	14.10	16.26	3.53	41.72	6.77	65.72	18.12	7.89	0.96	1.02	0.20
LC-PMWF	(n=10)	80.48	16.49	15.30	4.72	38.88	7.04	64.79	24.55	8.13	0.93	0.89	0.17
RN-PMWF	(n=10)	84.18	12.55	15.54	4.50	39.56	4.46	71.83	17.33	9.37	0.77	0.85	0.13
MEA	(n=30)	58.18 [b]	9.48	9.23 [b]	3.15	21.09 [b]	6.22	43.53 [b]	11.69	5.61 [b]	0.59	0.47 [b]	0.14
ER-QC	(n=10)	53.78	5.86	9.48	3.13	19.88	8.63	34.09	5.69	5.28	0.54	0.44	0.12
LC-QC	(n=10)	55.60	8.28	8.76	3.26	21.22	5.24	43.68	10.60	5.51	0.21	0.42	0.12
RN-CQC	(n=10)	65.16	10.17	9.46	3.33	22.18	4.43	52.82	10.03	6.05	0.66	0.56	0.15
AGA	(n=30)	73.18 [c]	11.90	14.89 [a]	4.48	32.97 [c]	3.84	56.83 [c]	12.61	7.32 [c]	0.98	0.69 [c]	0.19
ER-CP	(n=10)	73.80	14.89	16.06	5.19	34.12	4.14	44.72	9.73	7.28	1.35	0.67	0.13
LC-CH	(n=10)	68.54	11.05	12.94	3.76	33.28	3.52	66.09	11.18	7.45	0.71	0.63	0.14
RN-CF	(n=10)	77.20	8.41	15.66	4.13	31.52	3.74	59.68	5.06	7.24	0.86	0.78	0.25

Abbreviations: NFA= Natural forest areas; MEA= Mining extraction areas; AGA= Agricultural areas; ER-PMWF= El Retiro Pre-montane wet forest; LC-PMWF= La Ceja Pre-montane wet forest; RN-PMWF= Rionegro Pre-montane wet forest; ER-QC= El Retiro Quarry clays; LC-QC= La Ceja Quarry clays; RN-QC= Rionegro Closed quarry clays; ER-CP= El Retiro Forestry crops of *Pinus* sp.; LC-CH= La Ceja Crops of *Hydrangea* sp.; RN-CF= Rionegro Crops of *Fragaria ananassa*; EBACT= Enumeration of mesophilic culturable soil bacteria; EFUNG= Enumeration of culturable soil fungi; EACTI= Enumeration of culturable soil actinomycetes; URE= Microbial urease activity; CAT= Microbial catalase activity; MBR= Microbial basal respiration; SD= Standard deviation. *Note:* Different letters denote Tukey's HSD test at $p < 0.05$ between mean values in land uses.

4.3.2 AMF diversity

From the 10,256 spores extracted among the 90 composite soil samples, 42 distinct morphotypes from among 4 orders and 8 families were detected (Figure 4.1S). Also, 10 genera and 37 species were observed (Figure 4.1). There were 5 different undetermined morphotypes belonged to genus *Acaulospora, Ambispora, Archaeospora* and *Funneliformis*. Every morphotype recorded is presented from Figure 4.2S to Figure 4.5S. The NFA was the land use where the most spores were found (4,168 spores and 36 morphotypes) whereas AGA was the land use with highest number of morphotype observed (3,676 spores and 38 morphotypes). MEA was the land use with lowest number of spore and morphotypes observed (2,412 spores and 21 morphotypes). Along the gradient of soil degradation, H', TGRSP and ESPO exhibited significant declines (Table 4.7).

Figure 4. 1 *Species and unidentified morpho-taxa of glomeromycotan spores isolated from andosols under three land uses in south eastern region of Antioquia department, Colombia*

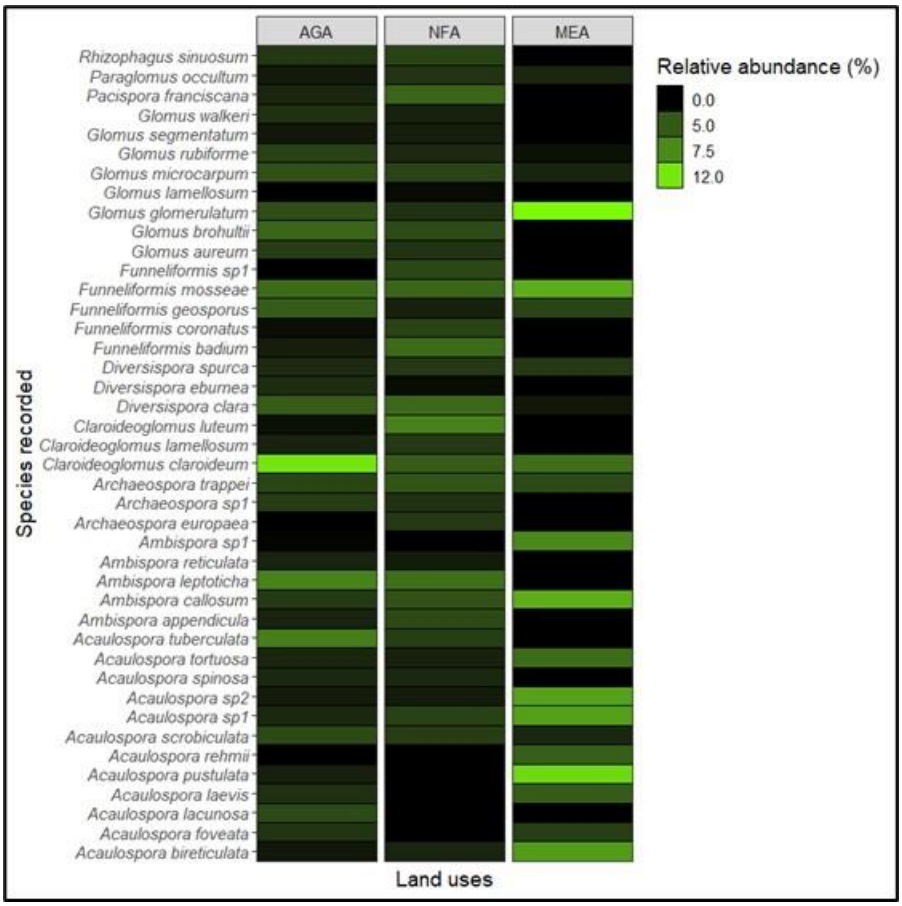


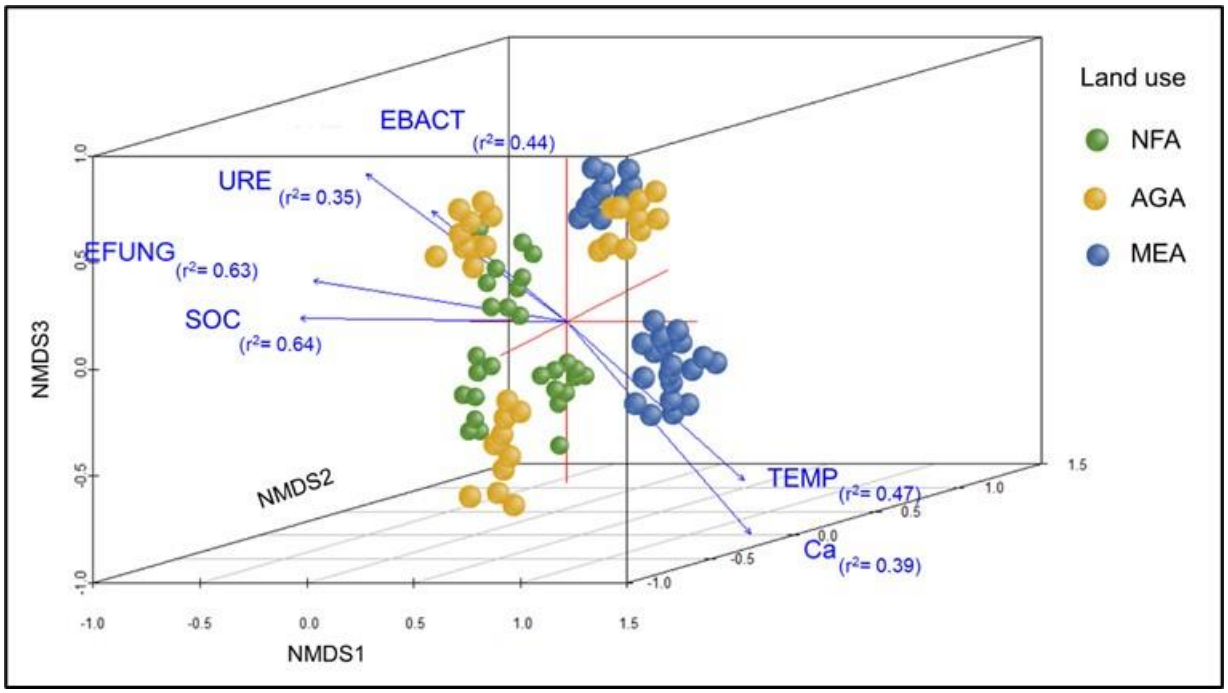
Table 4. 7 *AMF diversity and TGRSP for andosols in three land uses from south eastern region of Antioquia department, Colombia*

			TGRSP (g kg^{-1})	ESPO (spores g^{-1})			H´	
Land use/Activity			**Mean**	**Mean**	**Mean**	**SD.**	**Mean**	**SD.**
NFA		(n=30)	19.48[a]	24.71[a]	24.71[a]	8.22	2.94[a]	0.14
	ER-PMWF	(n=10)	19.33	24.21	24.21	7.22	2.92	0.16
	LC-PMWF	(n=10)	20.63	26.34	26.34	9.93	2.94	0.16
	RN-PMWF	(n=10)	18.48	23.59	23.59	6.95	2.96	0.10
MEA		(n=30)	10.18[b]	10.16[b]	10.16[b]	8.92	2.32[b]	0.14
	ER-QC	(n=10)	9.95	7.02	7.02	6.59	2.24	0.12
	LC-QC	(n=10)	9.38	9.85	9.85	7.60	2.42	0.10
	RN-CQC	(n=10)	11.21	13.62	13.62	7.58	2.30	0.14
AGA		(n=30)	15.05[c]	17.45[c]	17.45[c]	4.84	2.59[c]	0.16
	ER-CP	(n=10)	15.94	16.50	16.50	6.07	2.54	0.10
	LC-CH	(n=10)	13.90	17.35	17.35	3.10	2.49	0.14
	RN-CF	(n=10)	15.32	18.50	18.50	4.48	2.75	0.11

Abbreviations: NFA= Natural forest areas; MEA= Mining extraction areas; AGA= Agricultural areas; ER-PMWF= El Retiro Pre-montane wet forest; LC-PMWF= La Ceja Pre-montane wet forest; RN-PMWF= Rionegro Pre-montane wet forest; ER-QC= El Retiro Quarry clays; LC-QC= La Ceja Quarry clays; RN-QC= Rionegro Closed quarry clays; ER-CP= El Retiro Forestry crops of *Pinus* sp.; LC-CH= La Ceja Crops of *Hydrangea* sp.; RN-CF= Rionegro Crops of *Fragaria ananassa*; ESPO= Spore enumeration of arbuscular mycorrhizal fungi; TGRSP= Total glomalin related soil proteins; H'= Shannon index; SD= Standard deviation. *Note:* Different letters denote Tukey's HSD test at $p < 0.05$ between mean values in land uses.

Three-dimensional non-metric multidimensional scaling (3D-NMDS) ordination based on abundances data and the Bray-Curtis dissimilarity was used to visualize correlations among the factors that influence AMF community composition since it former had a lower and reliable stress values on our data (Figure 4.2). 3D-NMDS plot showed a sample ordination according to land uses. ANOSIM test confirmed that AMF community observed in NFA was significatively different from those observed in AGA ($R=0.25$, $p < 0.01$), but it differed even more from AMF community of MEA ($R=0.90$, $p=0.001$). Likewise, there were verifiable significant differences between AMF communities of AFA and MEA ($R=0.52$, $p= 0.001$). The most important drivers of AMF community assembly were SOC ($r^2=0.64$, $p < 0.01$), EFUNG ($r^2=0.63$, $p= 0.001$), TEMP ($r^2=0.47$, $p= 0.001$), EBACT ($r^2=0.44$, $p= 0.001$), Ca ($r^2=0.39$, $p= 0.001$) and URE ($r^2=0.35$, $p= 0.001$).

Figure 4. 2 *Three-dimensional non-metric multidimensional scaling (3D-NMDS) ordinations of AMF community composition in andosols of south eastern region of Antioquia department, Colombia*



Abbreviations: EBACT= Enumeration of mesophilic culturable soil bacteria; EFUNG= Enumeration of culturable soil fungi; URE= Microbial urease activity; TEMP= Soil temperature; NFA= Natural forest areas; AGA= Agricultural areas; MEA= Mining extraction areas; *Note:* Variables with statistically significant effects on sample ordination ($p < 0.01$) are represented as vectors with their coefficient of determination (r^2); 3D-Stress= 0.124.0 *Note:* Ordination calculated using data for the 42 morphotypes of AMF spores detected in three land uses based on Bray-Curtis dissimilarity measure.

4.3.3 Predictors of microbiological features

Every dependent variables fit with a normal distribution according to non-significant values observed in Anderson-Darling test (Figure 4.6S). Table 4.8 show the distribution of the residuals from the exploratory models as well as the statistical summary of explanatory models proposed after selection of better predictors. Shapiro-Wilk test to check the assumption of normally distributed residuals was not significant to every models. The municipalities considered as a source of random effects did not had significance on explanatory models excluding explanatory model to URE (PBT= 0.001). The predictive power of selected variables in LMM was between 64% (R^2_{URE}=0.64) and 79% (R^2_{EFUNG}=0.79). BD was a relevant predictor into LMM to most of microbiological features (EBACT, CAT, TGRSP, ESPO and H´) followed by NH_4-N contents

(EBACT, EFUNG, URE, CAT) and TEMP (EFUNG, MBR and H´) (Table 4.9). Parallelly, S was a significant predictor to EBACT (Estimated= 3.39; p= < 0.001) and TGRSP (Estimated= 0.18; p= 0.002) as well as Ca was significant to TGRSP (Estimated= -0.01; p= 0.046) and H´ (Estimated= -0.01; p= 0.005) while SOC just was a significant predictor to MBR (Estimated= 0.05; p= < 0.001). Also, according to the results from DCA for feature selection, EBACT, EFUNG and URE were selected for building explanatory models to H´. Likewise, for TGRSP models, EFUNG and MBR were chosen.

Table 4. 8 *Statistical summary of explanatory linear mixed models to microbiological features in Colombian andosols from south eastern region of Antioquia department*

							Shapiro wilk test	
RV	**Predictors**	**R^2**	**RE**	**PBT**	**AIC**	**logLIK**	**w**	**p-value**
EBACT	BD; NH_4-N; S	0.72	4.11	0.033	658.10	-323.10	0.98	0.41
EFUNG	TEMP; SWC; NH_4-N; H´	0.79	0.00	-	529.10	-257.50	0.98	0.55
URE	NH_4-N; EBACT; EFUNG	0.64	32.39	0.001*	701.40	-344.70	0.99	0.82
CAT	BD; SWC; NH_4-N; EBACT	0.72	0.12	0.002	233.10	-109.60	0.99	0.78
MBR	TEMP; SOC; TGRSP	0.71	0.00	-	-89.20	50.60	0.97	0.06
TGRSP	BD; S; Ca; EFUNG	0.70	0.00	-	319.40	-152.7	0.98	0.54
ESPO	TEMP; BD; SOC; EFUNG	0.65	0.77	0.002	399.60	-192.8	0.97	0.16
H‘	TEMP; BD; Ca	0.75	0.00	-	-75.30	43.7	0.98	0.30

Abbreviations: TEMP= Soil temperature; BD= Bulk density; SWC= Soil water contents; SOC= Soil organic carbon; EBACT= Enumeration of mesophilic culturable soil bacteria; EFUNG= Enumeration of culturable soil fungi; URE= Microbial urease activity; CAT= Microbial catalase activity; MBR= Microbial basal respiration; ESPO= Spore enumeration of arbuscular mycorrhizal fungi; TGRSP= Total glomalin related soil proteins; H'= Shannon index; RV= Response variable; RE= Variance explained by random effects; PBT= Parametric bootstrapping test; AIC= Akaike information criterion; logLIK= Log-likelihood; * Significant values to parametric bootstrapping test.

Table 4. 9 *Statistical summary of linear mixed models analyzing fixed effects of predictor variables on soil microbiological features and AMF diversity*

						Type II Wald Chisq. test		
RV	**Predictors**	**Estimated**	**SE**	**CI lower**	**CI Upper**	**Chisq.**	**Df**	**p-value**
EBACT	(Intercept)	66.96	12.27	42.55	91.38			
	BD	-52.55	11.28	-74.99	-30.12	21.69	1	< 0.001
	NH_4-N	0.53	0.18	0.18	0.90	8.81	1	0.003
	S	3.39	0.53	2.33	4.47	39.90	1	< 0.001
EFUNG	(Intercept)	29.04	7.40	14.31	43.78			
	TEMP	-1.42	0.26	-1.95	-0.89	28.72	1	< 0.001
	H´	0.67	0.17	0.32	1.20	14.37	1	< 0.001
	SWC	0.17	0.06	0.05	0.30	7.62	1	0.005
	NH_4-N	0.51	0.07	0.36	0.67	44.02	1	< 0.001
URE	(Intercept)	-0.22	6.17	-12.50	12.06			
	EBACT	0.82	0.10	0.61	1.03	60.95	1	< 0.001
	EFUNG	0.33	0.19	-0.05	0.72	2.95	1	0.082
	NH_4-N	-0.46	0.26	-1.00	-0.07	3.03	1	0.051
CAT	(Intercept)	-5.69	2.76	-11.20	-0.19			
	EBACT	0.04	0.008	0.03	0.06	25.52	1	< 0.001
	NH_4-N	0.04	0.01	0.01	0.08	7.82	1	0.005
	SWC	0.10	0.01	0.06	0.14	27.02	1	< 0.001
	BD	4.80	2.09	0.64	8.97	5.27	1	0.021
MBR	(Intercept)	0.35	0.22	-0.79	-0.09			
	TEMP	-0.01	0.008	-0.04	-0.01	5.41	1	0.022
	TGRSP	0.02	0.01	0.00	0.05	3.84	1	0.048
	SOC	0.05	0.01	0.03	0.07	20.61	1	< 0.001
TGRSP	(Intercept)	13.92	2.76	8.44	19.42			
	EFUNG	0.07	0.02	0.03	0.12	9.43	1	0.002
	BD	-9.30	1.99	-13.28	-5.33	21.67	1	< 0.001
	S	0.18	0.07	0.02	0.33	5.35	1	0.020
	Ca	-0.01	0.002	-0.01	-0.001	3.98	1	0.046
ESPO	(Intercept)	10.34	5.23	0.08	20.76			
	EFUNG	0.13	0.04	0.04	0.22	8.94	1	
	BD	-12.68	3.35	-19.35	-6.01	14.30	1	0.004
	SOC	0.27	0.14	-0.01	0.56	3.59	1	0.062
	TEMP	0.22	0.14	-0.07	0.50	2.33	1	0.131
H‘	(Intercept)	5.12	0.16	4.79	5.45			
	TEMP	-0.02	0.009	-0.04	-0.01	7.28	1	0.006
	BD	-1.98	0.20	-2.39	-1.58	95.05	1	< 0.001
	Ca	-0.01	0.001	0.00	0.1	7.75	1	0.005

Abbreviations: TEMP= Soil temperature; BD= Bulk density; SWC= Soil water contents; SOC= Soil organic carbon; EBACT= Enumeration of mesophilic culturable soil bacteria; EFUNG= Enumeration of culturable soil fungi; URE= Microbial urease activity; CAT= Microbial catalase activity; MBR= Microbial basal respiration; ESPO= Spore enumeration of arbuscular mycorrhizal fungi; TGRSP= Total glomalin related soil proteins; H'= Shannon index; RV= Response variable; ES= Standard error; CI= Confidence intervals; Df= Degrees of freedom.

4.4 Discussion

4.4.1 Land use effects on soil abiotic properties

Land uses has significant effects on soil features according to the fraction of A-horizon soil loss. On the one hand, a consistent trend of detriments was observed to soil nutrients such as NH_4-N, SOC, S and soil water contents along soil degradation gradient. This observation supports the fact that the A-horizon has the largest fraction of SOC within the soil profile and it is a key component of water and nutrient retention (Thaler et al., 2021). On the other hand, bulk density, soil temperature and Ca showed a constant trend of increases following the magnitude of A-horizon soil loss. The decline in the mean values of these variables is mainly due to the degree to which agricultural and mining activities increase tillage, removal of plant cover from the soil, and vehicular traffic. The over-tilling and the use of heavy machinery can lead to soil compaction by breaking down soil aggregates and reducing pore spaces (Puhlick & Fernandez, 2020) whereas the removal of plant cover can have a significant impact on soil temperature and soil moisture since plant cover lowers the quantity of sunlight that reaches the soil's surface and regulate infiltration rates and evapotranspiration (Ni et al., 2019a; Puhlick & Fernandez, 2020). Additionally, soil compaction may impair soil drainage, resulting in waterlogging and the accumulation of dissolved calcium on soil (Eze et al., 2021).

Otherwise, variables such as NO_3-N, P, and K did not follow the A-horizon degradation gradient and they were significatively highest in AGA. The increments of NO_3-N, P, and K in agricultural areas depending mainly in the use of fertilizers which are commonly rich in these nutrients (G. Wang et al., 2021). In turn, MEA had higher Cu concentrations and lower pH values. Previous research has proved that during mineral extractions by open surface operations, Cu can be released into the environment through various pathways, such as the discharge of mine water, the release of tailings and waste rock, and the emission of particulate matter (Corona Sánchez et al., 2021; H. Li et al., 2021). Likewise, the process of soil acidification in mining areas have been widely recognized as a primary consequence of acid mine drainage (Rambabu et al., 2020).

4.4.2 Abiotic factors driving variations in soil microbiological features

Municipalities had no significant influence on the most of suggested LMM, which explained more than 60% of the variance in each soil microbiological feature suggesting that microbiological variations were mostly determined by soil parameters than variables related with localities sampled. Furthermore, the gradual and significant variation of microbiological features coincided with the magnitude of A-horizon soil loss. Notwithstanding, the explanatory model to urease activity was significatively affect by factor municipality. This observation can be explained by the high sensibility of urease enzyme activity to increased and decreased along altitude gradients (R. Cao et al., 2021; Y. Xie et al., 2022) since the municipalities encompassed an altitudinal range from 2,370 to 2,774 m.a.s.l.

LMM suggest that increments of NH_4-N contents were related with large populations of mesophilic culturable bacteria and culturable soil fungi in conjunction with catalase activity. The relationship between high NH_4-N contents and culturable mesophilic bacteria and fungi have been explained as a result of the significant involvement of both culturable bacteria and fungi in microbial decomposers populations, which release N in the form of ammonium after biological processes of mineralization (Amoo & Babalola, 2017; Cocozza et al., 2021). The reported increases in catalase activity related with NH_4-N availability are consistent with prior research that revealed that a drop in soil nutrient content, such as N, would frequently result in a decrease in microbial reproduction as well as a loss of enzyme catalase activity (J. Wu et al., 2020). In addition, into explanatory LMM to mesophilic bacteria, sulfur was a relevant predictor. Sulfur is an essential nutrient for the growth and survival of microorganisms, including culturable bacteria, because various cellular processes of prokaryotic and eukaryotic soil microbial that include electron transport, catalysis of biochemical reactions, respiration and oxidation-reduction reactions depends of iron-sulfur clusters of proteins (Chaudhary et al., 2023; Piutti et al., 2015). Besides, our results indicates that the population of mesophilic bacteria and catalase activity are limited by bulk density. This is because high bulk density can lead to reduced porosity and soil aeration, which can reduce water availability, circulation of gases and nutrient diffusion in soil. As a result, bacterial growth and survival can be inhibited including microbial enzyme activities (Boeddinghaus et al., 2015).

Particularly, our explanatory models of the enumeration of fungi and catalase activity agree with previous research where soil water content was treated as a significant and positive factor to both variables. For instance, culturable soil fungi are particularly sensitive to water stress since

water content regulates sporulation process (Cazarolli et al., 2021). Also, the growth and activity of culturable fraction of soil fungi can be significantly reduced under drought conditions because changes in balance of air-water conditions in the soil resulting in a slower distribution of organic matter which is the vital substrate of fungi (Korniłłowicz-Kowalska et al., 2022; Rutigliano et al., 2013). Also, increase in water availability has a favorable impact on catalase activity because a preceding offer of water molecules is necessary to perform and stimulate the catalase enzyme reaction to breakdown hydrogen peroxide (Eason & Fan, 2014; Karich et al., 2016). To the contrary, our findings imply that soil temperature has detrimental impacts on both the abundances of culturable soil fungi and microbial basal respiration. The results of extensive research in wild areas show that one of the key environmental factors influencing the fungal communities is soil temperature (Brandl et al., 2023; Mittelstrass et al., 2021). In general, fungal growth is favored by warmer soil temperatures, but only up to a certain point and strong variations limits in both terms abundance and diversity fungal communities (Nottingham et al., 2018).

Similarly, microbial respiration increases as soil temperature increases, since highest temperatures can stimulate the metabolic activity of soil microorganisms, allowing them to decompounds organic matter more quickly and release more CO_2. However, strong temperature changes can reduce fungal and bacterial biomass leading subsequent detriments of microbial respiration rates (Ali et al., 2018). Likewise, our results suggest that SOC contents have an impact on soil microbial respiration. In agree with our observations, previous studies have found that soils with higher pools of SOC tend to have higher rates of microbial respiration since there is more organic matter available for decomposition by soil microorganisms (Chen et al., 2019).

4.4.3 AMF communities across land uses

AMF were significantly impacted by land use, as indicated by the percentage of A-horizon soil loss. Starting with NFA and continuing through AGA and MEA, a steady downward trend in AMF diversity, TGRSP, and spore abundance was seen along soil degradation gradient. AMF are expected to be more abundant and diverse in undisturbed forests compared to conversed areas since specially in tropical forest, the high plant diversity and complex soil roots web provide an optimal environment for the establishment and maintenance of AMF populations (C. M. R. Pereira et al., 2022; J. Zhang et al., 2021). Contrary to our expectations, the highest number of morphotypes for

were found in AGA, but not in NFA. The use of various crops appears to have the potential to improve the diversity of AMF in agricultural areas since variations observed in short times such as crops phenology and root architecture influence the distribution and composition of AMF, and hence functionally different plants can associate with diverse AMF communities (Guzman et al., 2021). However, the highest values of diversity were observed in NFA in view of the significant increase of spore abundance.

The ANOSIM confirmed that AMF communities differs significatively between land uses. According to NMDS, differences in AMF diversity are related with microbiological characteristics like urease activity and abundances of culturable fungi and mesophilic bacteria as well as abiotic parameters such as soil temperature, SOC, and Ca concentrations. There is some evidence to suggest that AMF are a source of soil enzyme and they can increase the activity of soil urease, improving also the capacity of the soil to supply N (L. Xiao, Bi, et al., 2019b). Likewise, other authors proposes that AMF symbiosis boost the growth of some microorganisms related to N metabolism in the soil, incrementing the urease activity (J. Ma et al., 2021). Respecting to relationship observed between SOC and AMF, several studies have demonstrated that the effective nutrient uptake by AMF further placed nutritional restriction on soil microbial decomposers and decreased their activities, which can result in an increase in SOC pools (Chowdhury et al., 2022; Ren et al., 2021). Unlike SOC and microbiological features, increments in soil temperature were related with soil samples where AMF diversity was lower, for instance, samples from MEA. In a large-scale study performed by T. Zhang et al., 2016, the diameter of AMF spores and their abundances were shown to be negatively impacted by increases in soil temperature, indicating that soil temperature is a determining factor in the nature of the AMF population. Regarding the impacts of Ca contents, D. Xiao et al., 2022 discovered that soil Ca was the determining factor that caused AMF community compositions since as Ca content increases, microbial communication and motility are reduced, affecting some AMF groups and their activity.

The LMM selected bulk density in addition to populations of culturable fungi, soil temperature, and calcium concentrations as significant predictor of spore abundance, AMF diversity, and TGRSP. Negative estimated values to soil bulk density into LMM agree with observation reported by Faghihinia et al., 2020. This study suggests that the increases in soil bulk density have been linked to subsequent water loss, which has been shown to have detrimental impacts on AMF spore diversity. Likewise, the negative effect of bulk density on TGRSP can be

linked to the previously noted reductions in spore diversity (Gałązka et al., 2020). Besides, sulphur was a relevant predictor into our explanatory model to TGRSP. This result may be attributed to the fact that high glomalin contents improve the uptake activity of sulphur metabolizing enzymes in plants (Bisht & Garg, 2022).

4.5 Conclusions

Our study determined that the use affected significantly and according to the state of soil degradation, abiotic and biotic properties of the soil. All the biotic properties measured in this study (populations of culturable soil microorganisms, enzyme activity, microbial basal respiration, AMF diversity, AMF spore abundance and TGRSP) as well as soil abiotic properties such as NH_4-N, SOC, S and SWC suffered detriments depending on the degree of pressure exerted by land use. That is, the highest values of these variables were observed in forest areas and they decreased as the fraction of the A horizon removed increased. However, soil abiotic parameters such as temperature, bulk density, and Ca concentrations increased significantly as the soil degradation progressed.

The differences observed in AMF communities among land uses along with values of AMF diversity, spore abundances and TGRSP were mainly explained by interactions with microbiological features like urease activity and populations of culturable fungi and mesophilic bacteria in conjunction with soil temperature, Ca and S concentrations. In turn, more than 60% of variation in populations of culturable soil microorganisms, enzyme activities and microbial basal respiration was explained by relationships between soil abiotic and biotic properties that displayed changes in function to soil degradation stage. Finally, we highlight that the municipality factor did not have significant effects in any of the models analyzed to explain the values of soil microbiological features. It confirms that the high sensitivity of AMF communities (including spore abundances and TGRSP), populations of culturable fungi and mesophilic bacteria, catalase activity and microbial basal respiration is focused mainly on responding to changes in land use.

4.6 Supplementary material

Table 4. 1S *Test of normality distribution to residuals of One-Way Analyses of Variance (ANOVA) and test for homogeneity of group variances in primary soil nutrients, secondary elements contents and physicochemical soil properties of Colombian andosols considering as source of variation land use types*

		Shapiro-Wilk Test		Fligner-Killeen Test		
Variable	**Transformation**	**w**	**p-value**	**Chi-squared**	**df**	**p-value**
TEMP	Log(x)	0.98	0.31	0.27	2	0.87
BD	*	0.97	0.06	2.02	2	0.36
SWC	*	0.98	0.11	2.68	2	0.26
pH	*	0.97	0.06	5.44	2	0.07
Ca	*	0.99	0.59	0.90	2	0.64
Cu	*	0.98	0.13	2.94	2	0.23
Fe	Log(x)	0.99	0.75	0.04	2	0.98
K	x^{-1}	0.98	0.09	4.23	2	0.12
Mg	Log(x)	0.97	0.08	2.79	2	0.25
Mn	x^{-1}	0.97	0.05	5.53	2	0.06
NH_4-N	Log(x)	0.99	0.59	1.31	2	0.52
NO_3-N	*	0.99	0.90	5.79	2	0.06
P	*	0.98	0.10	3.74	2	0.15
S	Log(x)	0.99	0.50	1.27	2	0.53
SOC	*	0.99	0.80	11.21	2	0.33

Abbreviations: TEMP= Soil temperature; BD= Bulk density; SWC= Soil water contents; SOC= Soil organic carbon; df= Degrees of freedom; * No data transformation.

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Table 4. 2S *Test of normality distribution to residuals of One-Way Analyses of Variance (ANOVA) and test for homogeneity of group variances in microbiological features of Colombian andosols considering as source of variation land use types*

		Shapiro-Wilk Test		Fligner-Killeen Test		
Variable	**Transformation**	**w**	**p-value**	**Chi-squared**	**df**	**p-value**
EBACT	$x^{1/2}$	0.99	0.45	4.43	2	0.11
EFUNG	*	0.98	0.10	1.36	2	0.51
EACTI	*	0.98	0.10	4.10	2	0.13
URE	Log(x)	0.97	0.06	5.23	2	0.07
CAT	$x^{1/2}$	0.99	0.94	4.88	2	0.09
MBR	$x^{1/2}$	0.98	0.29	3.67	2	0.19
ESPO	$x^{1/2}$	0.98	0.12	0.90	2	0.64
TGRSP	*	0.97	0.06	2.74	2	0.25
H‘	*	0.98	0.31	0.57	2	0.75

Abbreviations: EBACT= Enumeration of mesophilic culturable soil bacteria; EFUNG= Enumeration of culturable soil fungi; EACTI= Enumeration of culturable soil actinomycetes; URE= Microbial urease activity; CAT= Microbial catalase activity; MBR= Microbial basal respiration; ESPO= Spore enumeration of arbuscular mycorrhizal fungi; TGRSP= Total glomalin-related soil proteins; H'= Shannon index; df= Degrees of freedom; * No data transformation.

Table 4. 3S *Multiple-comparison (post hoc Tukey HSD test) for soil primary nutrients and secondary chemical elements by land-use type in andosols of south eastern region of Antioquia department, Colombia*

Variable	**Contrast**	**Mean difference**	**CI Lower**	**CI Upper**	**p-value**
Fe	MEA-AGA	0.16	-0.03	0.35	0.12

Variable	Contrast	Mean difference	CI Lower	CI Upper	p-value
Fe	NFA-AGA	-0.15	-0.34	0.04	0.15
Fe	NFA-MEA	-0.31	-0.50	-0.12	<0.05
S	MEA-AGA	-0.16	-0.29	-0.04	<0.05
S	NFA-AGA	0.13	0.00	0.26	<0.05
S	NFA-MEA	0.30	0.17	0.43	<0.05
P	MEA-AGA	-0.52	-0.72	-0.33	<0.05
P	NFA-AGA	-0.21	-0.40	-0.02	<0.05
P	NFA-MEA	0.32	0.13	0.51	<0.05
K	MEA-AGA	0.00	0.00	0.00	<0.05
K	NFA-AGA	0.00	0.00	0.00	<0.05
K	NFA-MEA	0.00	0.00	0.00	<0.05
SOC	MEA-AGA	-2.64	-3.61	-1.67	<0.05
SOC	NFA-AGA	3.09	2.12	4.06	<0.05
SOC	NFA-MEA	5.73	4.75	6.70	<0.05
NO_3-N	MEA-AGA	-2.52	-3.29	-1.74	<0.05
NO_3-N	NFA-AGA	-1.58	-2.36	-0.80	<0.05
NO_3-N	NFA-MEA	0.94	0.16	1.71	<0.05
NH_4-N	MEA-AGA	-0.24	-0.36	-0.12	<0.05
NH_4-N	NFA-AGA	0.15	0.03	0.27	<0.05
NH_4-N	NFA-MEA	0.39	0.27	0.51	<0.05
Ca	MEA-AGA	41.86	7.60	76.12	<0.05
Ca	NFA-AGA	-70.46	-104.72	-36.20	<0.05
Ca	NFA-MEA	-112.32	-146.59	-78.06	<0.05
Mg	MEA-AGA	0.28	0.03	0.52	<0.05
Mg	NFA-AGA	-0.06	-0.30	0.19	0.84
Mg	NFA-MEA	-0.34	-0.58	-0.09	<0.05
Mn	MEA-AGA	0.05	-0.05	0.15	0.48
Mn	NFA-AGA	0.13	0.02	0.23	<0.05
Mn	NFA-MEA	0.07	-0.03	0.18	0.21
Cu	MEA-AGA	-0.56	-0.89	-0.23	<0.05
Cu	NFA-AGA	-0.16	-0.49	0.18	0.5
Cu	NFA-MEA	0.40	0.07	0.73	<0.05

Abbreviations: NFA= Natural forest areas; MEA= Mining extraction areas; AGA= Agricultural areas; SOC= Soil organic carbon; CI= Confidence interval.

Table 4. 4S *Multiple-comparison (post hoc Tukey HSD test) for soil physicochemical properties land-use type in andosols of south eastern region of Antioquia department, Colombia*

Variable	Contrast	Mean difference	CI Lower	CI Upper	p-value
TEMP	MEA-AGA	-0.10	-0.14	-0.05	<0.05

Variable	Contrast	Mean difference	CI Lower	CI Upper	p-value
TEMP	NFA-AGA	0.07	0.03	0.11	<0.05
TEMP	NFA-MEA	0.17	0.12	0.21	<0.05
SWC	MEA-AGA	-12.06	-15.42	-8.70	<0.05
SWC	NFA-AGA	4.64	1.29	8.00	<0.05
SWC	NFA-MEA	16.70	13.35	20.06	<0.05
pH	MEA-AGA	-0.43	-0.55	-0.32	<0.05
pH	NFA-AGA	-0.09	-0.21	0.02	0.13
pH	NFA-MEA	0.34	0.23	0.45	<0.05
BD	MEA-AGA	0.13	0.10	0.16	<0.05
BD	NFA-AGA	-0.06	-0.09	-0.03	<0.05
BD	NFA-MEA	-0.19	-0.22	-0.16	<0.05

Abbreviations: NFA= Natural forest areas; MEA= Mining extraction areas; AGA= Agricultural areas; TEMP= Soil temperature; BD= Bulk density; SWC= Soil water contents; CI= Confidence interval.

Table 4. 5S *Multiple-comparison (post hoc Tukey HSD test) for microbiological features by land-use type in andosols of south eastern region of Antioquia department, Colombia*

Variable	Contrast	Mean difference	CI Lower	CI Upper	p-value
EBACT	MEA-AGA	-0.92	-1.36	-0.48	<0.05
EBACT	NFA-AGA	0.72	0.28	1.16	<0.05
EBACT	NFA-MEA	1.64	1.20	2.08	<0.05
EFUNG	MEA-AGA	-11.88	-15.26	-8.50	<0.05
EFUNG	NFA-AGA	7.08	3.70	10.46	<0.05
EFUNG	NFA-MEA	18.96	15.58	22.34	<0.05
EACTI	MEA-AGA	-5.65	-8.09	-3.21	<0.05
EACTI	NFA-AGA	0.81	-1.63	3.25	0.71
EACTI	NFA-MEA	6.47	4.03	8.91	<0.05
URE	MEA-AGA	-0.95	-1.57	-0.33	<0.05
URE	NFA-AGA	0.64	0.01	1.26	<0.05
URE	NFA-MEA	1.59	0.96	2.21	<0.05
CAT	MEA-AGA	-0.33	-0.44	-0.23	<0.05
CAT	NFA-AGA	0.20	0.10	0.31	<0.05
CAT	NFA-MEA	0.54	0.44	0.64	<0.05
MBR	MEA-AGA	-0.14	-0.21	-0.08	<0.05
MBR	NFA-AGA	0.13	0.07	0.19	<0.05
MBR	NFA-MEA	0.27	0.21	0.34	<0.05

Abbreviations: NFA= Natural forest areas; MEA= Mining extraction areas; AGA= Agricultural areas; EBACT= Enumeration of mesophilic culturable soil bacteria; EFUNG= Enumeration of culturable soil fungi; EACTI= Enumeration of culturable soil actinomycetes; URE= Microbial urease activity; CAT= Microbial catalase activity; MBR= Microbial basal respiration; CI= Confidence interval.

Table 4. 6S *Multiple-comparison (post hoc Tukey HSD test) for spore abundance of AMF, alpha diversity index and GRSP by land-use type in andosols of south eastern region of Antioquia department, Colombia*

Variable	Contrast	Mean difference	CI Lower	CI Upper	p-value
ESPO	MEA-AGA	-0.66	-0.88	-0.45	<0.05
ESPO	NFA-AGA	0.23	0.01	0.45	<0.05
ESPO	NFA-MEA	0.89	0.68	1.11	<0.05
TGRSP	MEA-AGA	-2.44	-3.34	-1.53	<0.05
TGRSP	NFA-AGA	2.21	1.31	3.12	<0.05
TGRSP	NFA-MEA	4.65	3.75	5.55	<0.05
H’	MEA-AGA	-0.27	-0.36	-0.18	<0.05
H’	NFA-AGA	0.35	0.26	0.44	<0.05
H’	NFA-MEA	0.62	0.53	0.71	<0.05

Abbreviations: NFA= Natural forest areas; MEA= Mining extraction areas; AGA= Agricultural areas; ESPO= Spore enumeration of arbuscular mycorrhizal fungi; TGRSP= Total glomalin-related soil proteins; H’= Shannon index; CI= Confidence interval.

Figure 4.1S *Glomeromycotan taxa from andosols retrieved across three land uses in south eastern region of Antioquia department, Colombia*

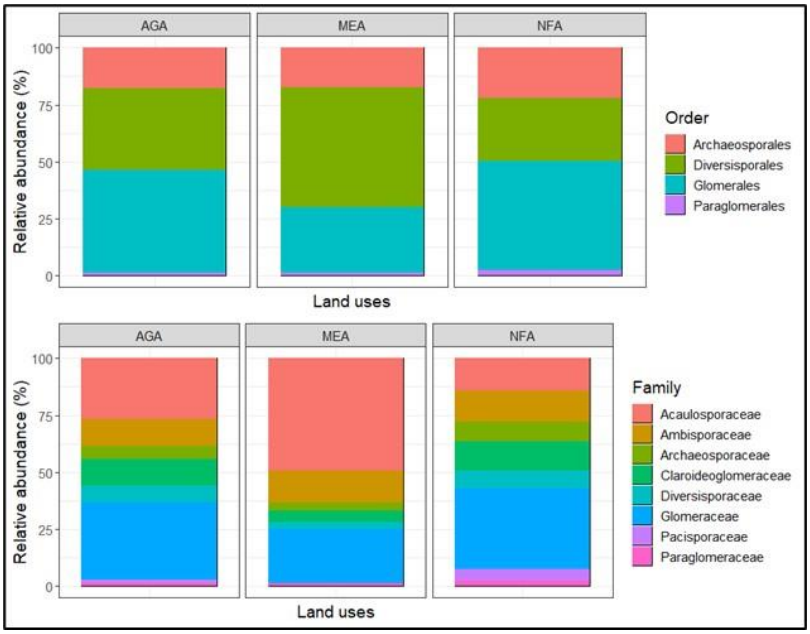
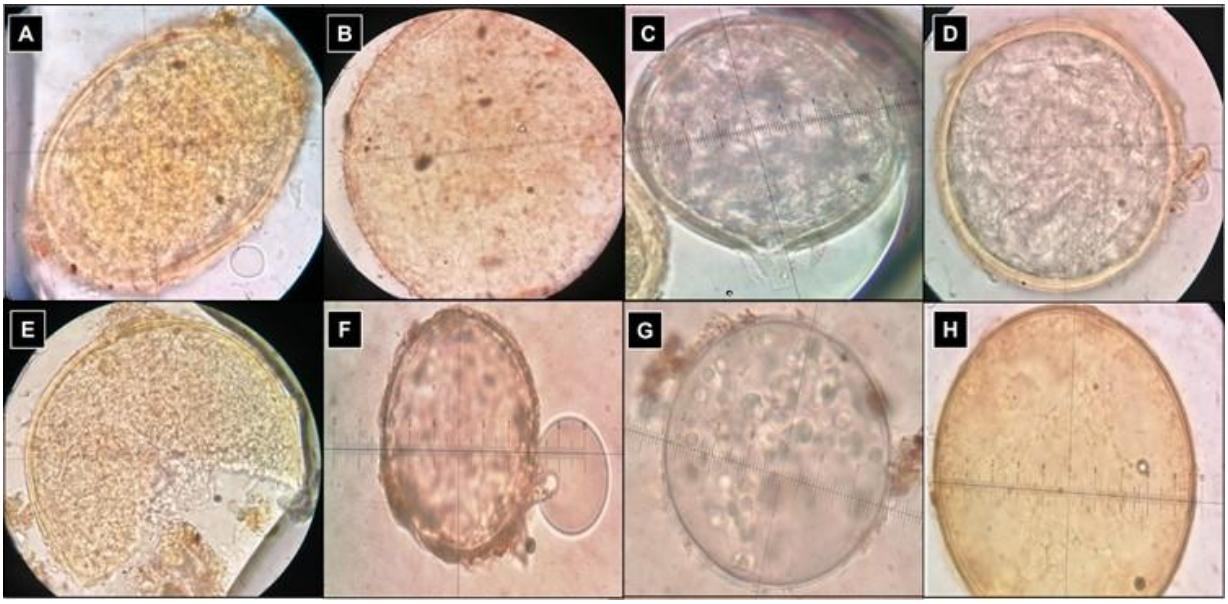
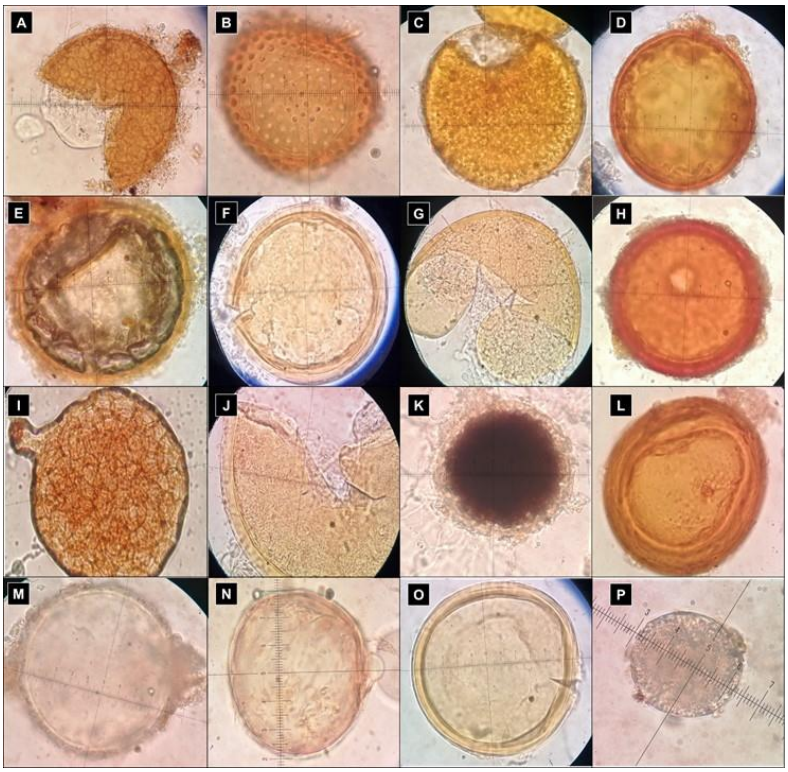


Figure 4.2S *Morphotypes of glomeromycotan spores belonged to order Archaeosporales (families Ambisporaceae and Archaeosporaceae) isolated in andosols of south easternregion of Antioquia department, Colombia*



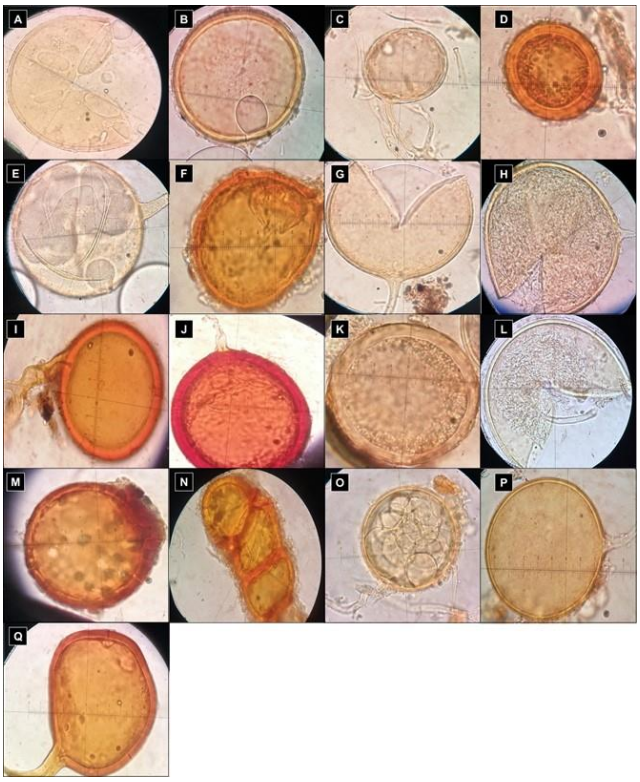
Note: A) Ambispora appendicula; B) Ambispora callosum; C) Ambispora leptoticha; D) Ambispora reticulata; E) Ambispora sp1.; F) Archaeospora europaea; G) Archaeospora trappei; H) Archaeospora sp1.

Figure 4.3S *Morphotypes of glomeromycotan spores belonged to order Diversisporales (families Acaulosporaceae, Diversisporaceae and Pacisporaceae) isolated in andosols of south easternregion of Antioquia department, Colombia*



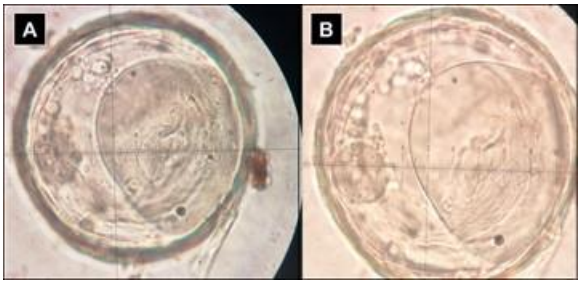
Note: A) Acaulospora bireticulata; B) A. foveata; C) A. lacunosa; D) A. laevis; E) A. pustulata; F) A. rehmii; G) A. scrobiculata; H) A. spinosa; I) A. tortuosa; J) A. tuberculata; K) Acaulospora sp1.; L) Acaulospora sp2.; M) Divesispora clara; N) D. eburnean; O) D. spurca; P) Pacispora franciscana.

Figure 4. 4S *Morphotypes of glomeromycotan spores belonged to order Glomerales (families Claroideoglomeraceae and Glomeraceae) isolated in andosols of south easternregion of Antioquia department, Colombia*



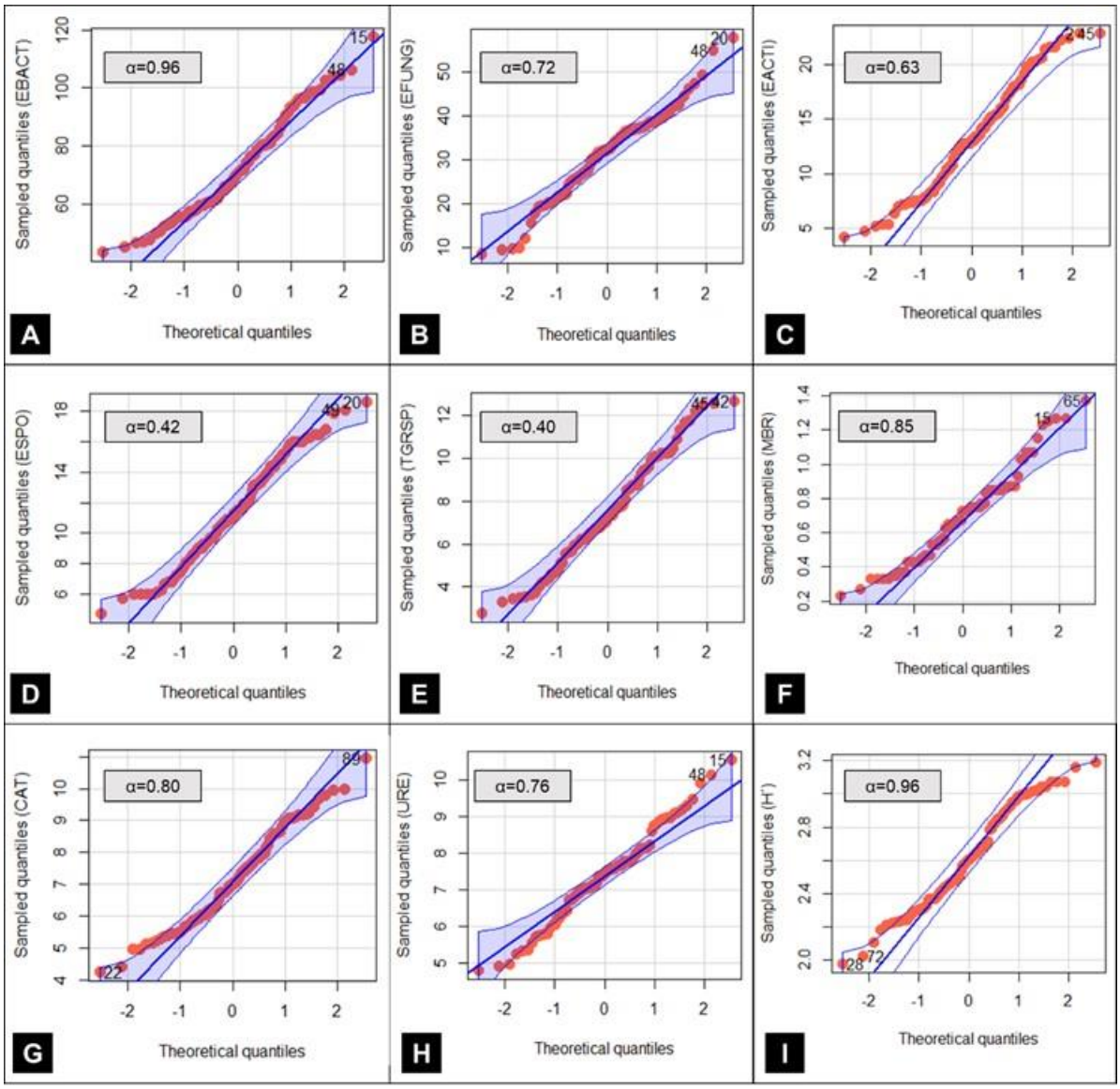
Note: A) Claroideoglomus claroideum; B) C. lamellosum; C) C. luteum; D) Funneliformis badium; E) F. coronatus; F) F. geosporus; G) F. mosseae; H) Funneliformis sp1.; I) Glomus aureum; J) G. brohultii; K) G. glomerulatum; L) G. lamellosum; M) G. microcarpum; N) G. rubiforme; O) G. segmentatum; P) G. walker; Q) Rhizophagus sinuosum.

Figure 4. 5S *Morphotypes of glomeromycotan spores belonged to order Paraglomerales (family Paraglomeraceae) isolated in andosols of south eastern region of Antioquia department, Colombia*



Note: A)Paraglomus occultum; B) P. occultum

Figure 4. 6S *Normal Q-Q plots and Anderson-Darling goodness of fit test to microbiological features in andosols of south eastern region of Antioquia department, Colombia*



Note: Q-Q plot to A) EBACT= Enumeration of mesophilic culturable soil bacteria; B) EFUNG= Enumeration of culturable soil fungi; C) EACTI= Enumeration of culturable soil actinomycetes; D) ESPO= Spore enumeration of arbuscular mycorrhizal fungi; E) TGRSP= Total glomalin-related soil proteins; F) MBR= Microbial basal respiration; G) CAT= Microbial catalase activity; H) URE= Microbial urease activity; I) H´=Shannon index; α= p-value computed to Anderson-Darling goodness of fit to normal distribution test..

5. POLYGONAL PROJECTIONS TO ASSESS VARIATIONS OF SOIL HEALT INDICATORS IN COLOMBIAN ANDOSOLS

Abstract

Scoring additive function serves to determine soil quality, but it has two key limitations: it may not consider specific management objectives or environmental circumstances, and it combines several soil qualities into one score, making it difficult to identify more affected variables. We suggest the use of Log Responses Ratios (LRR) in a soil quality assessment to overcome the problems of referent target values and lack of transparency. To compare observed values as outputs in Colombian andosols, values of soil quality indicators observed in native premontane wet forest were used to compute reference values. The perimeter of 2D polygons projected from radius vectors whose length represented a proportion of change in an indicator was considered as a condensed measure of changes observed. The method allows determining that mining and agricultural activities have a negative but different degree of impact on abiotic soil features like soil organic content and water contents, as well as microbiological features like populations of culturable mesophilic bacteria and fungi, microbial basal respiration, and spore density of arbuscular mycorrhizal fungi (AMF), AMF diversity, and the contents of total glomalin related soil proteins. Our findings imply that the geometrical analysis of proportions of change in soil quality indicators provides an alternative soil quality measure that accurately distinguishes the impacts of different land uses while simultaneously identifying the variables that are more affected, even with a small amount of data. The work includes a graphic representation of how land use affects soil quality as well as an analogous measurement, such as soil quality indices based on scoring additive functions.

Keywords: Arbuscular mychorrizal fungi, Reference values, Log response ratio, Geometrical analysis, Radius vector, Soil quality index.

5.1 Introduction

Soil health is defined as the capacity of a soil to function as a vital living system to sustain all kinds of life within ecosystem or land use boundaries (Monther et al., 2020; L. Silva et al., 2023). Soil health cannot be tested directly but can be deduced from the measurement of soil properties that encompass physical, chemical, and biological factors. Researchers have increased efforts to identify indicators and methods sensitive enough to detect changes in soil health to quantify soil hazards and management repercussions (Karlen et al., 2019). Soil health indicators are soil measurable features involved in at least one soil function with the sensibility to discriminate soil changes on a numerical basis (Stone et al., 2016b). In turn, soil quality indexes (SQI) are proposed to synthesize the soil information gathered from relevant indicators into a numerical value. Scoring additive function is a common method for calculating SQI by adding scores of individual indicators weighted according to their relative importance (Maaz et al., 2023; Wander, 2022). Although these functions provide a baseline for tracking changes in soil quality, they have two main difficulties to interpret their values. First, since scoring additive functions may not account for the specific management goals or environmental conditions of a given soil, values computed with them are context dependent. For example, a soil with high nutrient levels may be considered of high quality for agricultural purposes but may be undesirable for preserving natural habitats (Gržinić et al., 2023). Second, there is a lack of transparency because combining multiple soil properties into a single score makes it difficult to identify individual properties that require improvement.

To face the problem of context dependency in SQI, several researchers have proposed the use of reference criteria to establish the goals of a SQI measure. For instance, undisturbed areas, such as natural ecosystems, provide a reference point for assessing soil quality (Abellán et al., 2019; Gómez et al., 2020; Goyal et al., 2022). The values of a SQI in undisturbed areas can be used to establish a benchmark or target for soil restoration or management in degraded areas since undisturbed areas typically have higher levels of biodiversity, nutrient cycling, and organic matter accumulation, which can result in higher soil quality compared to disturbed areas (Ramos et al., 2022; Z. Zhang et al., 2021). Although scoring functions prioritizes soil properties following expert suggestions or statistical methods such as factor or principal component analysis (Raiesi &

Beheshti, 2022), a numerical expression to comprehend the amount of change either in a relevant soil attribute or the total change in SQI in comparison to reference criterion has not yet been tested.

Size effect measures such as Log Response Ratios (LRR) have been useful in environmental studies to quantify the relative difference between two conditions in terms of outcome variables. LRR are calculated as the natural logarithm of the ratio of the response in one group to the response in the other group (Nakagawa et al., 2023). The advantage of using LRR is that they can be interpreted as the proportional change in response, which is often more meaningful in environmental studies where the absolute scale of the response variable may not be clearly understood. For example, LRR have been used to evaluate the effect of novel tillage systems on microbial communities and soil contents of NH_4 and NO_3 (Yang et al., 2020), and to measure the effect of fertilizer application on soil pH and cation exchange capacity in a degraded soil(Liu et al., 2020).

Since LRR is a powerful tool for evaluating the effects of different management on soil properties, to address the issues of referent target values and lack of transparency in SQI, this study proposes to use LRR in SQI. The method proposed in this study uses LRR to differentiate between overall changes and changes in each soil quality indicator. Here, values of soil quality indicators that have been assessed to be relevant in undisturbed areas were used as referents to compares observed values as outputs and then compute LRR. The LRR were expressed as radius vectors to project 2D polygons. This method is adequate for analyzing variations in a group of vectors since the perimeter of those polygons is determined by the total sum of distances between boundary points projected from radius vectors. Therefore, changes in the lengths of the radius vectors will cause proportional changes in the perimeter (Audet & Ninin, 2013; Jeong, 1994) and the resulting 2D polygon represented a "shadow" of the sum of changes. The aim of our study is to assess a measure of soil quality based on the differences computed between perimeters of polygons conformed from projecting radius vector of LRR. We compare LRR and a SQI computed from our approach of 2D polygonal projection to three land uses in andosols of the southeastern region of Antioquia (Colombia). Natural forest areas along with areas under weak pressure by agriculture activities and areas subject to considerable pressure by mining activities related to clay extraction were sampled to measure relevant soil quality indicators such as populations of culturable mesophilic bacterial and fungi, catalase activity, microbial basal respiration, Arbuscular Mycorrhizal Fungi (AMF) diversity and Glomalin-related soil proteins as well as soil abiotic

properties such as NH_4-N, soil organic carbon, S, Ca, soil water contents, soil temperature and bulk density. To determine which indicators are most impacted, we first hypothesized that the percentage of change for the selected soil quality indicators may indicate varying levels of soil degradation. Our first hypothesis was that the differences between perimeter of 2D polygon project from LRR vectors can be useful to discriminate size effects of land uses in a set soil quality indicator. In addition, since differences between polygon perimeters quantify the departures in values of soil quality indicators compared to values observed in undisturbed soils, our second hypothesis was that a SQI based on the differences between perimeters of projected 2D polygons may have a negative linear relationship with SQI based on scoring additive functions which would measure the individual contribution of indicators to reach a value of "good quality". Hence, bellow we described a methodology to: (1) compute the LRR in two sets of soil quality indicators, considering as reference the values observed in non-degraded areas; (2) draw 2D polygons from radius vectors whose length was the LRR and the estimation of their perimeters; (3) estimate the significance of the difference between perimeters estimated for specific activities across the land uses sampled.

5.2 Material and methods

5.2.1 Study zone: land uses and specific activities

In the chapter 3 were described in detail the 9 soil profiles sampled across the municipalities of Rionegro, La Ceja and El Retiro. Table 5.1 presents the label codes of each specific activity and their geolocation. 10 composite soil samples were collected in each one of the 9 activities, which encompass three land uses: (1) soils from natural forest areas considered as reference for a not degraded stage (NFA); (2) soils retrieved in agricultural areas (AGA) with a fraction removed from horizon A between 10 and 20% cataloged as weakly degraded; and (3) soils of mining extractions areas (MEA), with a removed fraction greater than 60%, considered as highly degraded. The 90 composite soil samples were stored in plastic bags at 4°C to posterior laboratory analysis. All the physicochemical and microbiological analyzes of the soil samples were carried out in the laboratories of the GAIA group (Research Group in Environmental Management and Modeling).

Table 5. 1 *Geolocation of areas designated for conservation of native forest, agricultural and mining activities in three municipalities of southeast region of Antioquia-Colombia*

Municipality	Activity	Altitude (m.a.s.l)	Activity code	Land use	Location
La Ceja	Conservation zones of pre-montane wet forest	2,457	LC-PMWF	NFA	05° 57' 48" N; 75° 27' 27" W
	Crops *Hydrangea sp*.	2,427	LC-CH	AGA	05° 58' 15" N; 75° 27' 51" W
	Quarry clays	2,476	LC-QC	MEA	05° 58' 08" N; 75° 27' 31" W
El Retiro	Conservation zones of pre-montane wet forest	2,523	ER-PMWF	NFA	06° 01' 58" N; 75° 32' 24" W
	Forestry crops of *Pinus sp*.	2,370	ER-CP	AGA	06° 01' 46" N; 75° 32' 31" W
	Quarry clays	2,413	ER-QC	MEA	06° 01' 43" N; 75° 32' 26" W
Rionegro	Conservation zones of pre-montane wet forest	2,610	RN-PMWF	NFA	06° 11' 34" N; 75° 29' 59" W
	Crops of *Fragaria ananassa*.	2,549	RN-CF	AGA	06° 10' 55" N; 75° 29' 31" W
	Closed quarry clays	2,774	RN-CQC	MEA	06° 11' 43" N; 75° 31' 21" W

Abbreviations: NFA=Natural Forest Areas; AGA=Agricultural Activities Areas; MEA=Mining Extraction Areas.

5.2.2 Physicochemical and microbiological characterization

The chapter 4 presents the entire list of physicochemical and microbiological characteristics assessed in soil samples, as well as their statistics. Similarly, the procedures used to perform each metric are discussed in further detail in number 4.2. Only variables whose means varied significantly, following (positively or negatively) the degradation gradient were chosen as indicators of soil quality in this study. These indicators are given with their respective methods of measurement in table 5.2.

Table 5. 2 *Analytical methods for determining soil properties*

Soil property	Abbreviation	Summary method	Unit	Method described in
Soil temperature	TEMP	*In situ* direct measure	°C	Crotty et al., 2019
Bulk density	BD	Volumetric cylinder method	g cm^{-3}	Al-Shammary et al., 2018
Soil water contents	SWC	Gravimetric method	%	Tan et al., 2019
Soil organic carbon	SOC	Wet digestion with dichromate oxidation using Walkley and Black method	%	Enang et al., 2018
Ammonia nitrogen	NH_4-N	Berthelot reaction with sodium hypochlorite	g kg^{-1}	Rhine et al., 1998
Calcium	Ca	Schwarzenbach EDTA method	mg kg^{-1}	Loeppert & Suarez, 1996
Sulfur	S	Chloride barium method	g kg^{-1}	Senthilkumar et al., 2021
Culturable mesophilic bacterial population	EBACT	Enumeration by seeding of serial dilution on PCA culture medium	CFU x 10^{-6}	Werheni Ammeri et al., 2023
Culturable fungi population	EFUNG	Enumeration by seeding of serial dilution on PDA culture medium	CFU x 10^{-5}	Werheni Ammeri et al., 2023
Microbial basal respiration	MBR	Determined in soil incubated for 48 h by measuring CO_2 capture in sodium hydroxide solution	mg CO2 g^{-1} day^{-1}	Dehsheikh et al., 2020
Catalase activity	CAT	Back-titration residual H_2O_2 with $KMnO_4$	ml $KMnO_4$ h^{-1} g^{-1}	Maphuhla et al., 2020
AMF spore density	ESPO	Wet sieving method	Spores g^{-1}	Bai et al., 2013
Total-glomalin related soil proteins	TGRSP	Quantification in citrate-extracts by a Bradford assay	g kg^{-1}	Hou et al., 2022
AMF diversity	H´	Shannon index computed from morphotypes of spores identified		

5.2.3 Reference values and LRR

To obtain reference values for the chemical, physical and microbiological properties from the soils retrieved in NFA, Kernel Density Estimation (KDE) was useful as a nonparametric method that estimated target values. KDE works by smoothing the data using a kernel function, which is a probability density function that is centered at each data point. The resulting smoothed function is an estimate of the underlying probability density function of the data (Kiesse & Corson, 2023). The *x* maximum of the KDE estimate (X_{maxKDE}) is the point where the estimated probability density function takes its maximum value. This point was helpful in determining the variable's most likely value since it is situated in the highest density regions (HDR), which are level sets with relatively dense sample points (Saavedra & Crujeiras, 2022). Thus, $X\text{max}_{\text{KDE}}$ estimated from data observed in NFA were considered as our reference vales to each indicator. KDE functions and their respective $X\text{max}_{\text{KDE}}$ were computed using the function *kdensity* from R package "kdensity" (Moss & Tveten, 2019). The bandwidths were defined by mean of bandwidth selector for Gaussian kernels functions *bw.nrd* from R package "Stats" (Génin et al., 2020).

The natural Log of the Response Ratio (LRR) was calculated using the formula proposed by Pustejovsky (2018):

$$1)\ LRR = ln\frac{x_t}{x_{Ref}}$$

where x_t was the mean value of and indicators in a land use or specific activity considered as the outcome value and x_{Ref} was the $X\text{max}_{\text{KDE}}$ considered as reference value to that indicator. For each LRR, confidence intervals (CI) at 95% were computed. If the 95% CI of LRR did not overlap with 0, then responses were significant at $p < 0.05$. TheLRR and their CI were computed by R package "SingleCasesES" (Swan & Pustejovsky, 2018). Then, LRR were converted into the proportion of change measure, an easy approach to interpret the size of land use or specific activity effect. The exponential function (exp) was used to calculate the proportion and direction of a change in the outcome of an indicator. The absolute value of the proportion of change (C_p) from the baseline to the treatment phase was computed by formula (J. Pustejovsky, 2018) :

$$2)\ Cp = \ |[(\exp\ (LRR) - 1]|$$

5.2.4 Perimeters of projected 2D polygons from radius vectors

Each indicator was considered as a vector whose length (l) is their C_p respect to reference value. The length of vectors to reference values was defined as 1. The 2D polygons were a representation for star-shaped objects which enclosed by the radial area of a path drawn from a set of vertex points (Kang et al., 2004). Vertex points were computed from N angularly equispaced radius vectors projected between the centroid of a Cartesian plane (point x=0, y=0) and the boundary of the vector (defined by the length of a vector) (Jeong, 1994). In the ordered sequence of N vectors, the angular space (Θ) between them (V_n) was expressed as n-1(360°/N); where n denotes the place of a vector v in a specific set of ordered vectors. To computes the Cartesian coordinates of a vertex point (P_n) to a V_n (P_n= x_n, y_n), polar coordinates to V_n were converted in a two-dimensional space by formulas (Borji et al., 2020):

$$3)\ x_n = l_n[\sin(\theta'_n)]$$

$$4)\ y_n = l_n[\cos(\theta'_n)]$$

Where l_n is the length of vector n and Θ´is the angular space between the vector n and v_1 expressed in radians. Thus, a 2D polygon A was delimited by the lines P_n that encompass the ordered path of vertices $||P_1 - P_2|| + ||P_2 - P_3|| + ... + ||P_{n-1} - P_n|| + ||P_n - P_1||$. The Euclidean distance between vertex point P_n and P_{n-1} was computed following Equation 5 (Kang et al., 2004):

$$5)\ d(P_i, P_{i+1}) = \sqrt[2]{\sum_{i=1}^{N}(x_i - y_i)^2}$$

where x_i (i = 1, 2, 3, …, N) and y_i (i = 1, 2, 3, …, N) denote the sorted normalized distances between vertex point P_n and P_{n-1}. N corresponds to the number of vectors or nodes in the 2D polygon. In turn, the perimeter of a polygon A that have N vertices was computed as sum of distances between the ordered set of vertices as shows Equation 6.

$$6)\ A_{p=} \sum_{i=1n}^{N} d(P_i, P_{i+1}) + d(P_n, P_1)$$

Following this process, a 2D polygon of reference values was drawn (*A*) and the SQI based in the differences between the perimeter of polygons drawn from LRR vectors was expressed as:

$$7)\ SQI_{LRR} = \frac{A_p - B_p}{A_p}$$

Where A_p was the perimeter of the polygon drawn from reference values and B_p was the perimeter of a polygon drawn from C_p values.

5.2.5 A minimum data set and SQI based on scoring additive functions

Cp were firstly computed from the total data set of indicators (TDS) for all soil samples. To assess the effect of the number of indicators considered to estimates $SQI_{LRR,}$ there was a selection of indicators to a minimum data set (MDS) by factor analysis using a varimax rotation with the Kaiser normalization criteria for data reduction. The soil variables with factor loadings >0.50 and factors with eigenvalues >1 were defined to be good indicators of soil quality (Raiesi & Pejman, 2021). If multiple abiotic properties or microbiological features were grouped under one factor, correlation coefficients were utilized to determine whether the variables were redundant and should be removed from the MDS analysis. Couples of abiotic variables or microbiological variables highly correlated ($\left| r \right| \geq 0.7,\quad p < 0.01$) within a factor were regarded as redundant, and only the indicator with the highest weighted loading was selected for the MDS (Yu et al., 2018). SQI_{LRR} were also computed with MDS, as well as a SQI based on weighted additive linear functions (SQI_{SLF}). The indicators remaining in the MDS were transformed and normalized (ranging from 0 to 1) using two scoring linear functions, either "more is better" or "less is better", to separate the association between soil indicators and soil quality. The "more is better" scoring function (Equation 8) implies that the higher the value of a soil indicator, such as SOC, NH_4-N, S, SWC, and microbiological features improves the soil quality. The function "less is better" (Equation 9) indicates that soil quality is deteriorated when the value of a soil feature, such as BD, TEMP and Ca rises (F. Li et al., 2023).

$$8)\ S_l = 0.1 + 0.9\left(\frac{x - lw}{hr - lw}\right)$$

$$9)\ S_l = 0.1 - 0.9\left(\frac{x - lw}{hr - lw}\right)$$

where S_l is the liner score of soil indicator between 0 and 1, x is the variable value, and lw and hr are the minimum and maximum value, respectively. SQI_{SLF} was then computed as:

$$10)\ SQI_{SLF} = \sum_{i=1}^{n} W_i S_i$$

where n is the number of soil indicator, and S_i is the score of soil attributes determined by Equations 8 or 9 and W_i is the weighted value of a variable.

5.2.6 Statistical analysis

Pearson correlation analysis was used to examine correlation among soil indicators and identify possible redundant variables to MDS. TDS-SQI_{LRR}, MDS-SQI_{LRR}, and MDS-SQI_{SLF} were compared by means of the Kruskal-Wallis test to ascertain whether there were differences between their values in land use categories. Also, the Wilcoxon rank-sum test was used to compare as two independent samples TDS-SQI_{LRR} and MDS-$SQI_{LRR.}$ If the p-value is less than the significance threshold (0.05), we reject the null hypothesis and infer that the two samples were measured differently, and so the TDS-SQI_{LRR} and MDS-SQI_{LRR} values will not be equal. If the p-value exceeds the significance threshold, we fail to reject the null hypothesis and conclude that there is no evidence to suggest that the two samples were measured differently, and the values of TDS-SQI_{LRR} and MDS-SQI_{LRR} are comparable. Finally, the relationship between TDS-SQI_{LRR} or MDS-SQI_{LRR} to predict the value of MDS-SQI_{SLF} was tested by linear regression analysis. The slope (β1) represented the change in the dependent variable for a one-unit increased in the independent variable. A positive slope indicated a positive relationship between the two variables, while a negative slope indicates a negative relationship. We also used the coefficient of determination (R^2) to measure the proportion of variance in MDS-SQI_{SLF} that can be explained by TDS-SQI_{LRR} or MDS-$SQI_{LRR.}$

5.3 Results

5.3.1 MDS and reference values

The TDS encompassed 14 soil quality indicators were tested across of three land uses to verify the response of soil abiotic and microbiological properties on andosols of southeastern region of Antioquia department in Colombia (Table 5.3 and Table 5.4, respectively). Abiotic soil characteristics as NH4-N, SOC, S, and SWC exhibited substantial declines throughout the degradation gradient (NFA>AGA>MEA) with land use as a direct source of variance (Tukey's HSD test at $p < 0.05$). The similar pattern could be seen in all microbiological characteristics. Abiotic soil characteristics such as Ca, TEMP, and BD, on the other hand, demonstrated substantial increases (Tukey's HSD test at $p < 0.05$) in accordance with the degradation gradient (NFA<AGA<MEA). References values (REF) to interpretates the magnitude of changes caused by specific activities or land uses were computed by KDE as X_{maxKDE} from 30 soil samples retrieved in 3 NFA of premontane wet forest considered as non-degraded areas (Table 5.4).

The MDS of soil indicators was screened using factor analysis, and the first four factors with eigenvalues >1 explained 75.90% of the data variance (Table 5.6). The first factor encompassed Ca, NH4-N, S, CAT and EBACT, accounted for 25.10% of the total variance. However, NH4-N and S were highly correlated ($r = 0.70$, $p < 0.01$), as well as, CAT and EBACT ($r = 0.72$, $p < 0.01$) (Figure 5.1). Therefore, in virtue of their higher loadings Ca, S and EBACT were retained to represent factor 1. Factor 2 comprised SWC, BD and H´ which explained 23.70% of the overall variance, but SWC was no kept into the factor due to their lower loading and high correlation with BD ($r = -0.87$, $p < 0.01$). Factor 3 was dominated by SOC, MBR and TGRSP, accounting for 14.90% of the total variability. As MBR and TGRSP were highly correlated ($r = 0.77$, $p < 0.01$), only TGRSP was selected by their high loading value into the factor. Finally, factor 4 consisted in TEMP and EFUNG explaining for 12.20% of the total variability.

Table 5. 3 *Mean values of soil physicochemical parameters with significant variation between land uses in andosols of southeastern region of Antioquia (Colombia)*

		NH_4-N (g kg^{-1})		SOC (%)		Ca (mg kg^{-1})		S (mg kg^{-1})		TEMP (°C)		BD (g cm^{-3})		SWC (%)	
Land use/Activity		**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**
NFA	(n=30)	34.47 [a]	7.5	15.02 [a]	1.94	400.26 [a]	49.8	11.41 [a]	2.03	18.09 [a]	1.42	0.56 [a]	0.05	40.98 [a]	6.07
ER-PMWF	(n=10)	37.19	8.86	16.26	1.37	393.12	60.28	12.21	1.1	18.26	1.95	0.54	0.05	41.25	6.41
LC-PMWF	(n=10)	34.84	7.71	15.34	1.75	389.92	53.04	11.63	2.39	18.36	1.29	0.56	0.06	37.54	4.81
RN-PMWF	(n=10)	31.4	4.97	13.48	1.64	417.76	32.05	10.41	2.12	17.64	0.82	0.57	0.05	44.14	5.51
MEA	(n=30)	23.15 [b]	3.68	9.30 [b]	0.99	512.59 [b]	58.39	8.49 [b]	1.59	21.55 [b]	1.74	0.75 [b]	0.05	24.27 [b]	4.39
ER-QC	(n=10)	21.64	4.65	9.25	1.13	508.41	50.96	8.84	1.87	20.84	2.44	0.75	0.05	25	3.32
LC-QC	(n=10)	22.35	2.75	8.99	1.13	546.05	59.57	8.04	1.72	21.47	0.53	0.75	0.03	24.17	4.94
RN-CQC	(n=10)	25.45	2.33	9.66	0.6	483.3	51.23	8.59	1.17	22.35	1.49	0.73	0.06	23.65	5.06
AGA	(n=30)	29.61 [c]	6.41	11.94 [c]	1.65	470.73 [c]	58.32	10.11 [c]	2.55	19.45 [c]	1.65	0.62 [c]	0.05	36.33 [c]	5.75
ER-CP	(n=10)	32.24	8.07	13.66	0.81	449.96	38.66	10.44	3.01	20.39	1.32	0.62	0.04	38.68	5.03
LC-CH	(n=10)	27.31	4.87	11.09	1.65	511.71	39.87	9.65	1.69	19.55	1.35	0.65	0.04	36.26	6.7
RN-CF	(n=10)	29.28	5.45	11.06	0.7	450.51	71.24	10.23	2.95	18.43	1.74	0.59	0.05	34.06	4.94

Abbreviations: NFA= Natural forest areas; MEA= Mining extraction areas; AGA= Agricultural areas; ER-PMWF= El Retiro Pre-montane wet forest; LC-PMWF= La Ceja Pre-montane wet forest; RN-PMWF= Rionegro Pre-montane wet forest; ER-QC= El Retiro Quarry clays; LC-QC= La Ceja Quarry clays; RN-QC= Rionegro Closed quarry clays; ER-CP= El Retiro Forestry crops of *Pinus* sp.; LC-CH= La Ceja Crops of *Hydrangea* sp.; RN-CF= Rionegro Crops of *Fragaria ananassa*; TEMP= Soil temperature; BD= Bulk density; SWC= Soil water content; SOC= Soil organic carbon. SD= Standard deviation. *Note: Different letters denote Tukey's HSD test at p <0.05 between mean values in land uses.*

Table 5. 4 *Mean values of soil microbiological parameters with significant variation between land uses in andosols of southeastern region of Antioquia (Colombia)*

		EBACT (CFU 10^{-6})		EFUNG (CFU 10^{-5})		CAT (ml $KMnO_4$ h^{-1} g^{-1})		MBR (mg $CO2$ g^{-1} day^{-1})		TGRSP (g kg^{-1})		ESPO (spores g^{-1})		H´	
Land use/Activity		**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**
NFA	(n=30)	86.16 [a]	15.09	40.05 [a]	6.1	8.47 [a]	1.08	0.92 [a]	0.18	19.48[a]	3.54	24.71[a]	4.11	2.94[a]	0.14
ER-PMWF	(n=10)	93.82	14.1	41.72	6.77	7.89	0.96	1.02	0.2	19.33	3.12	24.21	3.61	2.92	0.16
LC-PMWF	(n=10)	80.48	16.49	38.88	7.04	8.13	0.93	0.89	0.17	20.63	4.48	26.34	4.97	2.94	0.16
RN-PMWF	(n=10)	84.18	12.55	39.56	4.46	9.37	0.77	0.85	0.13	18.48	2.84	23.59	3.48	2.96	0.1
MEA	(n=30)	58.18 [b]	9.48	21.09 [b]	6.22	5.61 [b]	0.59	0.47 [b]	0.14	10.18[b]	2.67	10.16[b]	4.46	2.32[b]	0.14
ER-QC	(n=10)	53.78	5.86	19.88	8.63	5.28	0.54	0.44	0.12	9.95	3.35	7.02	3.29	2.24	0.12
LC-QC	(n=10)	55.6	8.28	21.22	5.24	5.51	0.21	0.42	0.12	9.38	2.13	9.85	3.80	2.42	0.1
RN-CQC	(n=10)	65.16	10.17	22.18	4.43	6.05	0.66	0.56	0.15	11.21	2.3	13.62	3.79	2.3	0.14
AGA	(n=30)	73.18 [c]	11.9	32.97 [c]	3.84	7.32 [c]	0.98	0.69 [c]	0.19	15.05[c]	2.48	17.45[c]	2.42	2.59[c]	0.16
ER-CP	(n=10)	73.8	14.89	34.12	4.14	7.28	1.35	0.67	0.13	15.94	2.86	16.50	3.04	2.54	0.1
LC-CH	(n=10)	68.54	11.05	33.28	3.52	7.45	0.71	0.63	0.14	13.9	1.88	17.35	1.55	2.49	0.14
RN-CF	(n=10)	77.2	8.41	31.52	3.74	7.24	0.86	0.78	0.25	15.32	2.39	18.50	2.24	2.75	0.11

Abbreviations: NFA= Natural forest areas; MEA= Mining extraction areas; AGA= Agricultural areas; ER-PMWF= El Retiro Pre-montane wet forest; LC-PMWF= La Ceja Pre-montane wet forest; RN-PMWF= Rionegro Pre-montane wet forest; ER-QC= El Retiro Quarry clays; LC-QC= La Ceja Quarry clays; RN-QC= Rionegro Closed quarry clays; ER-CP= El Retiro Forestry crops of *Pinus* sp.; LC-CH= La Ceja Crops of *Hydrangea* sp.; RN-CF= Rionegro Crops of *Fragaria ananassa*; EBACT= Enumeration of mesophilic culturable soil bacteria; EFUNG= Enumeration of culturable soil fungi; ESPO= AMF spore density; CAT= Microbial catalase activity; MBR= Microbial basal respiration; H'= Shannon index; TGRSP= Total glomalin related soil proteins; SD= Standard deviation. *Note:* Different letters denote Tukey's HSD test at $p < 0.05$ between mean values in land uses.

Table 5. 5 *Kernel estimation density and reference values to soil properties measured in andosols retrieved in premontane wet forest from southeastern region of Antioquia (Colombia)*

Indicator	Sample size	SD.	Mean	Bandwidth	Max density	X_{maxKDE}
TEMP	30	1.416	18.089	0.637	0.294	18.174
EBACT	30	15.091	86.160	8.102	0.021	94.813
EFUNG	30	6.103	40.053	2.264	0.085	37.815
CAT	30	0.983	8.400	0.528	0.352	8.913
MBR	30	0.180	0.922	0.092	2.287	0.837
TGRSP	30	1.772	9.740	0.806	0.233	9.600
ESPO	30	4.109	24.713	1.643	0.119	25.871
BD	30	0.054	0.755	0.029	6.142	0.746
SWC	30	6.074	40.977	3.261	0.056	41.616
NH4	30	7.505	34.473	3.563	0.051	32.641
Ca	30	49.797	400.263	25.063	0.007	420.103
S	30	2.031	11.413	0.938	0.185	11.448
SOC	30	1.937	15.024	0.982	0.170	15.709
H’	30	0.128	2.932	0.067	3.214	3.006

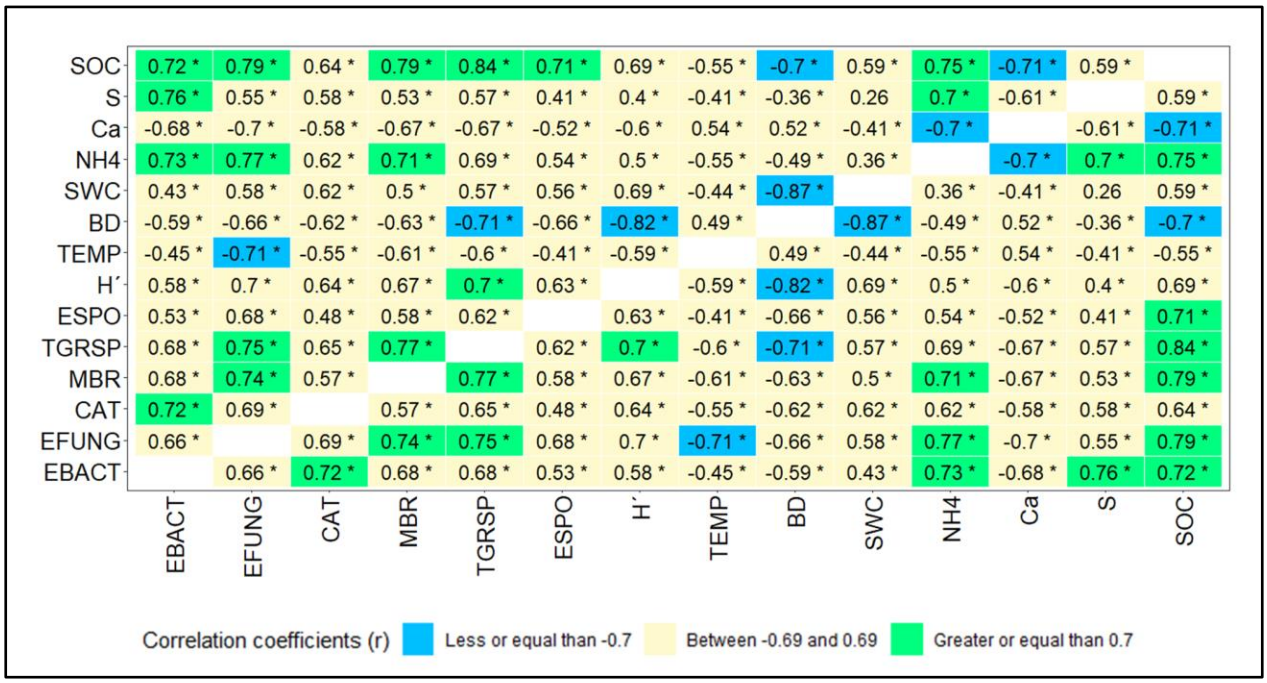
Abbreviations: EBACT= Enumeration of mesophilic culturable soil bacteria; EFUNG= Enumeration of culturable soil fungi; ESPO= AMF spore density; CAT= Microbial catalase activity; MBR= Microbial basal respiration; H’= Shannon index; TEMP= Soil temperature; BD= Bulk density; SWC= Soil water content; SOC= Soil organic carbon; TGRSP= Total glomalin related soil proteins; SD= Standard deviation; X_{maxKDE}= x maximum of the KDE estimated.

Table 5. 6 *Variable loading coefficients with Kaiser normalization to a factors analysis using 14 soil properties in andosols of southeastern region of Antioquia (Colombia)*

Indicator	Factor 1	Factor 2	Factor 3	Factor 4	Communality
BD	-0.248	**-0.881**	-0.290	-0.158	0.948
Ca	**-0.561**	-0.245	-0.394	-0.326	0.643
NH4	**0.651**	0.164	0.419	0.312	0.777
SOC	0.482	0.413	**0.628**	0.242	0.869
S	**0.796**	0.207	0.158	0.000	0.711
SWC	0.130	**0.860**	0.143	0.188	0.822
TEMP	-0.258	-0.273	-0.211	**-0.753**	0.756
CAT	**0.590**	0.468	0.339	0.109	0.702
EBACT	**0.824**	0.332	0.245	0.126	0.866
EFUNG	0.428	0.385	0.396	**0.521**	0.955
MBR	0.450	0.339	**0.547**	0.370	0.755
ESPO	0.274	0.483	0.481	0.115	0.633
H´	0.284	**0.671**	0.311	0.355	0.754
TGRSP	0.450	0.433	**0.559**	0.337	0.817
Eigenvalue	3.519	3.321	2.081	1.708	
Proportion of variance explained	0.251	0.237	0.149	0.122	
Cumulative variance explained	0.251	0.489	0.637	0.759	

Abbreviations: EBACT= Enumeration of mesophilic culturable soil bacteria; EFUNG= Enumeration of culturable soil fungi; ESPO= AMF spore density; CAT= Microbial catalase activity; MBR= Microbial basal respiration; H'= Shannon index; TGRSP= Total glomalin related soil proteins; TEMP= Soil temperature; BD= Bulk density; SWC= Soil water content; SOC= Soil organic carbon. *Note*: Boldface factor loadings (>0.60) are considered highly weighted. Bold-underlined soil properties correspond to the indicators included in the MDS.

Figure 5. 1 *Pearson correlation coefficients between different soil properties*



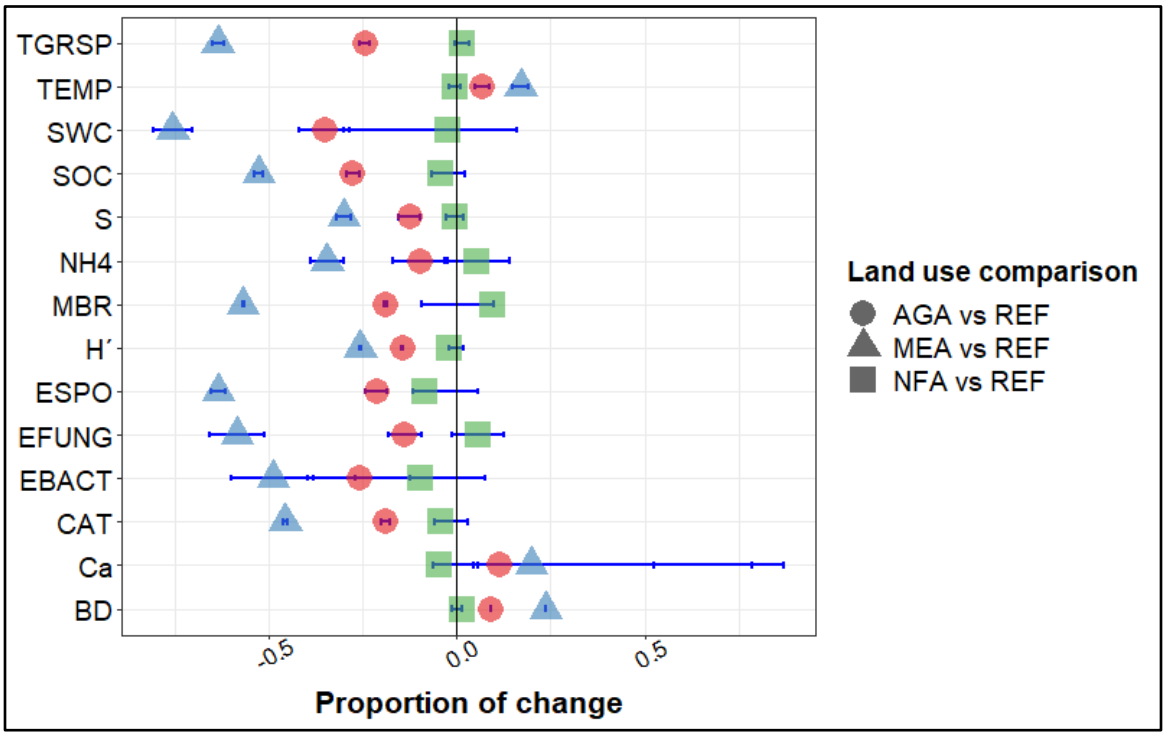
Abbreviations: EBACT= Enumeration of mesophilic culturable soil bacteria; EFUNG= Enumeration of culturable soil fungi; ESPO= AMF spore density; CAT= Microbial catalase activity; MBR= Microbial basal respiration; H'= Shannon index; TEMP= Soil temperature; BD= Bulk density; SWC= Soil water content; SOC= Soil organic carbon; TGRSP= Total glomalin related soil proteins. *Note*: * Significance at $p<0.01$; n=90.

5.3.2 Proportion of change in soil quality indicators

The mean values of each indicator were compared with reference values by using LRR expressed as proportion of change. All indicators measured in NFA did not significatively vary in comparison with reference values. On the contrary, mean values of EBACT, EFUNG, CAT, MBR, TGRSP, ESPO, H´, SWC, NH4 and S were significatively lower ($p<0.05$) in AGA and MEA land uses, respectively. Further, TEMP, Ca and BD increased significatively ($p<0.05$) comparing reference values with AGA and MEA land uses (Figure 5.2). The indicator belonged to TDS more affected in AGA land use were SWC ($C_{p=}$ -0.25), EBACT ($C_{p=}$ -0.22), MBR ($C_{p=}$ -0.20) and ESPO ($C_{p=}$ -0.18) whereas SWC ($C_{p=}$ -0.51), ESPO ($C_{p=}$ -0.50), TGRSP ($C_{p=}$ -0.48) and EFUNG ($C_{p=}$ -0.47) were the indicators more affected in MEA land use. Similarly, the indicators belonged to MDS with stronger C_p values in AGA were EBACT ($C_{p=}$ -0.22), TGRSP ($C_{p=}$ -0.16), H´ ($C_{p=}$ -0.15) and SOC ($C_{p=}$ -0.13). In MEA the indicators from MDS more affected were respectively

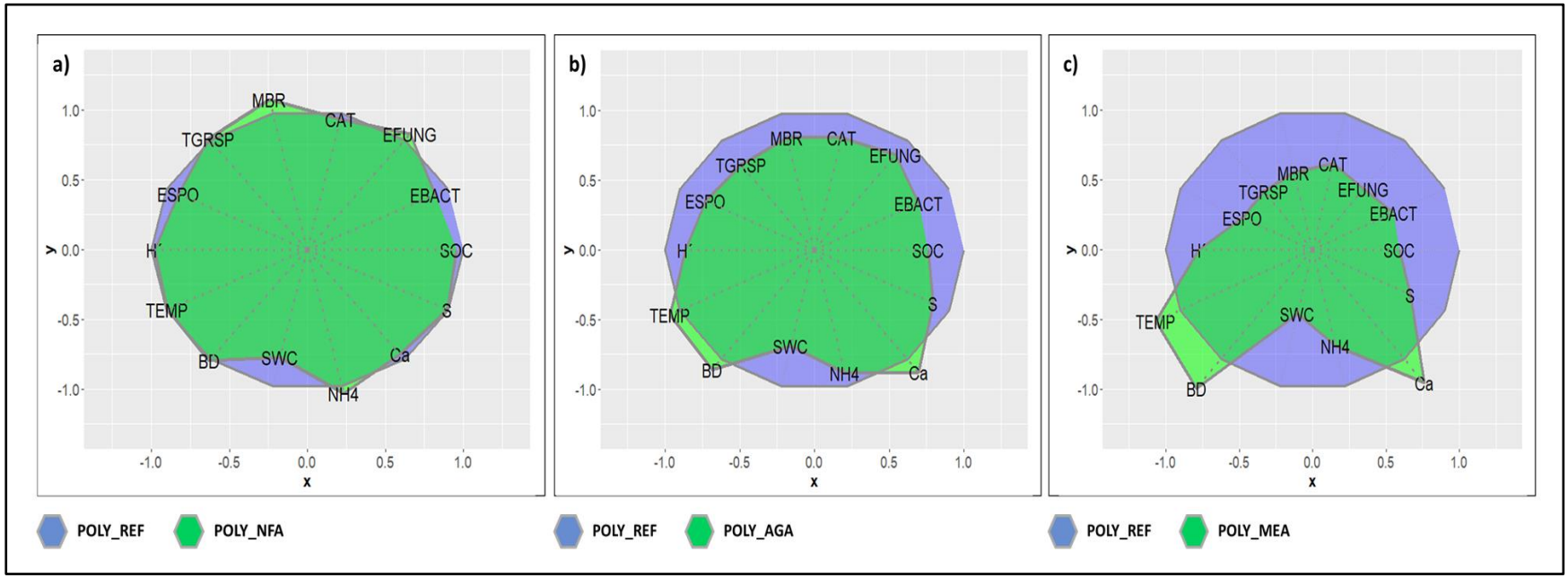
TGRSP ($C_{p=}$ -0.48), EFUNG ($C_{p=}$ -0.47), EBACT ($C_{p=}$ -0.43) and SOC ($C_{p=}$ -0.41). The 2D polygons projected from LRR expressed as C_p values are showed in Figure 5.3.

Figure 5. 2 *Proportion of change computed from Log response ratios and their confidence intervals for soil properties measured in three land uses contrasted with reference values*



Abbreviations: NFA= Natural forest areas; MEA= Mining extraction areas; AGA= Agricultural areas; REF= Reference values; EBACT= Enumeration of mesophilic culturable soil bacteria; EFUNG= Enumeration of culturable soil fungi; CAT= Microbial catalase activity; MBR= Microbial basal respiration; H'= Shannon index; TEMP= Soil temperature; BD= Bulk density; SWC= Soil water content; SOC= Soil organic carbon; TGRSP= Total glomalin related soil proteins.
Note: If the 95% CI of proportion of change did not overlap with 0, then responses were significant at $p < 0.05$ level.

Figure 5. 3 *2D polygons projected from LRR expressed as C_p of soil properties in andosols under three land uses in the south eastern region of Antioquia department (Colombia)*



Abbreviations: POLY_REF= 2D Polygon projected from reference values; EBACT= Enumeration of mesophilic culturable soil bacteria; EFUNG= Enumeration of culturable soil fungi; ESPO= AMF spore density; CAT= Microbial catalase activity; MBR= Microbial basal respiration; H'= Shannon index; TEMP= Soil temperature; BD= Bulk density; SWC= Soil water content; SOC= Soil organic carbon; TGRSP= Total glomalin related soil proteins. *Note:* a) POLY_NFA= 2D Polygon projected from values observed in natural forest areas; b) POLY_AGA= 2D Polygon projected from values observed in agricultural areas; c) POLY_MEA= 2D Polygon projected from values observed in mining extraction areas.

5.3.3 Soil quality indexes

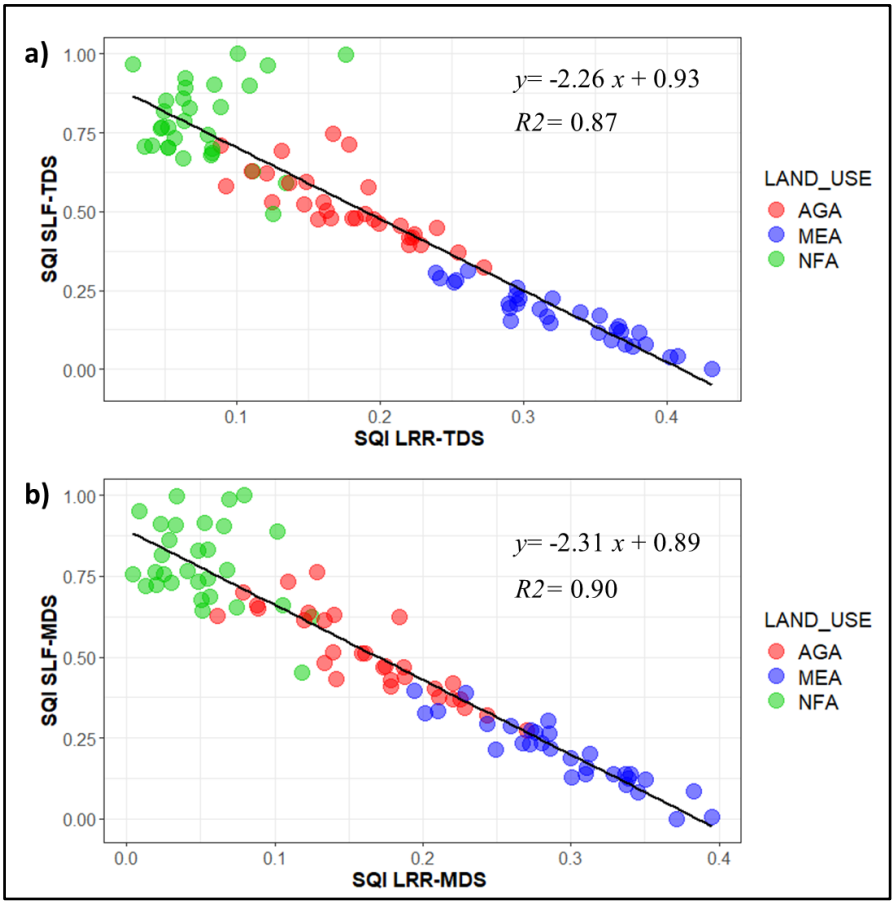
Four soil quality indexes were computed to andosols under three land uses (NFA, AGA and MEA). Indexes denoted as SQI_{LRR}-*TDS* and SQI_{LRR}-*MDS* corresponds to measures computed from LRR expressed like vectors of change using variables of a total dataset and variables the minimum data set, respectively. Similarly, SQI_{SLF}-*TDS* and SQI_{SLF}-*MDS* were the indexes generated from scoring linear functions on both sets of variables. Either by using SQI_{LRR}-*TDS* or SQI_{LRR}-*MDS*, the higher values were estimated to AGA (SQI_{LRR}-TDS= 0.177; SQI_{LRR}-MDS= 0.163) and MEA (SQI_{LRR}-TDS= 0.328; SQI_{LRR}-MDS= 0.296). The lower values were computed to NFA (SQI_{LRR}-TDS= 0.177; SQI_{LRR}-MDS= 0.163). On the contrary, the average value of SQI_{SLF}-*TDS* (0.786) and SQI_{SLF}-*MDS* (0.789) in NFA were significatively higher than AGA (SQI_{SLF}-TDS= 0.518; SQI_{SLF}-MDS=0.509) and MEA (SQI_{SLF}-TDS= 0.168; MEA SQI_{SLF}-TDS= 0.201) (Table 5.7). The Kruskal-Wallis test showed at significance level of $p<0.01$ that mean values to all soil quality index were different in the follows contrast: NFA-AGA, NFA-MEA, AGA-MEA, REF-AGA and REF-MEA. For the comparison between data sets employed to compute SQI, the Wilcoxon rank sum test with continuity correction proved that there were not significant differences between the set of measures performed by SQI_{LRR}-*TDS* in comparison to SQI_{LRR}-*MDS* measures (W= 4565, p= 0.141). Furthermore, between measurements estimated using SQI_{SLF}-*TDS* and SQI_{SLF}-*MDS*, there were no significant differences (W= 3975, p= 0.831). Finally, the linear regression performed on the SQI obtained using the TDS and MDS approaches found that the SQI derived from log responses ratios and scoring linear functions were highly and negatively correlated, either those computed with TDS (R2=0.88) or SQI estimated using MDS (R2=0.90) (Figure 5.4).

Table 5. 7 *Soil quality indexes computed by using of Log responses ratios and scoring linear functions to andosols under three land uses in the southeastern region of Antioquia (Colombia)*

		SQI_{LRR}-TDS		SQI_{LRR}-MDS		SQI_{SLF}-TDS		SQI_{SLF}-MDS	
Land use/Activity	**Sample size**	**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**	**Mean**	**SD.**
AGA	**30**	**0.177[a]**	**0.047**	**0.163[a]**	**0.052**	**0.518[a]**	**0.108**	**0.509[a]**	**0.132**
ER_CP	10	0.166	0.045	0.142	0.051	0.553	0.121	0.540	0.131
LC_CH	10	0.193	0.049	0.192	0.059	0.461	0.083	0.413	0.101
RN_CF	10	0.174	0.047	0.156	0.033	0.540	0.103	0.575	0.110
MEA	**30**	**0.328[b]**	**0.053**	**0.296[b]**	**0.052**	**0.168[b]**	**0.084**	**0.201[b]**	**0.102**
ER_QC	10	0.340	0.059	0.295	0.063	0.145	0.091	0.184	0.103
LC_QC	10	0.350	0.051	0.314	0.058	0.139	0.083	0.167	0.115
RN_CQC	10	0.294	0.030	0.278	0.026	0.222	0.054	0.251	0.072
NFA	**30**	**0.076[c]**	**0.033**	**0.051[c]**	**0.032**	**0.786[c]**	**0.124**	**0.789[c]**	**0.128**
ER_PMWF	10	0.081	0.031	0.047	0.026	0.847	0.133	0.847	0.126
LC_PMWF	10	0.085	0.041	0.067	0.032	0.775	0.112	0.789	0.124
RN_PMWF	10	0.062	0.026	0.039	0.033	0.735	0.111	0.731	0.117

Abbreviations: NFA= Natural forest areas; MEA= Mining extraction areas; AGA= Agricultural areas; ER-PMWF= El Retiro Pre-montane wet forest; LC-PMWF= La Ceja Pre-montane wet forest; RN-PMWF= Rionegro Pre-montane wet forest; ER-QC= El Retiro Quarry clays; LC-QC= La Ceja Quarry clays; RN-QC= Rionegro Closed quarry clays; ER-CP= El Retiro Forestry crops of *Pinus* sp.; LC-CH= La Ceja Crops of *Hydrangea* sp.; RN-CF= Rionegro Crops of *Fragaria ananassa*. *Note:* Different letters denote Kruskal-Wallis test significance at $p < 0.01$ between mean values.

Figure 5. 4 *Linear relationship between soil quality indexes estimated using the total data set (TDS) and the minimum data set (MDS) methods in andosols of southeastern region of Antioquia (Colombia)*



Abbreviations: NFA= Natural forest areas; MEA= Mining extraction areas; AGA= Agricultural areas; SQI LRR= Soil quality index computed from log response ratios; SQI SLF= Soil quality index computed from scoring linear functions. *Note:* a) Soil quality indexes estimated using TDS method; b) Soil quality indexes estimated using MDS method.

5.4 Discussion

5.4.1 Proportion of change in soil quality indicators computed by LRR and reference values

KDE is a method for evaluating a data set's probability density function that performs better than a conventional histogram in analyzing the studied probability distribution. In contrast to histograms, the KDE does not bin the data; as a result, the data's original position is continuous, displaying from a smooth function of probability distribution, an improved estimation of the complete probability along the *x*-axis and identifying accurate values to a *x* variable in the point with higher probability density (Węglarczyk, 2018). Previous studies on

soils have optimally used KDE to estimate in specific environments the threshold or benchmarks values for soil properties such as heavy metals, soil moisture and soil organic carbon (Carranza et al., 2019; Drexler et al., 2022; Miloš & Bensa, 2021). Similarly, since the LRR observed to soil quality indicators in non-degraded areas as NFA did not show any significant departures from these reference values, KDE was found to be a good method for estimating reference values of non-degraded soils to soil quality indicators in our study.

Although, the deleterious effects of land uses were significant to all indicators, the proportion of change determined by comparing our computed reference values with mean values of soil quality indicators allows us to identify that AGA and MEA uses impact negatively in a greater proportion abiotic soil properties such as soil organic content and water contents, as well as, microbiological features like populations of culturable mesophilic bacteria and fungi, microbial basal respiration and AMF spore density, AMF diversity and the contents total glomalin related soil proteins. Likewise, LRR shown that MEA land uses affects mostly soil quality indicators. The proportions of change estimated in our study by using LRR in comparison with reference values agree with several reports which compare values observed in previous non managed soils with values observed after different land uses. For instance, we estimated in a 25% the proportion of change to soil water contents in agricultural areas. J. Liu et al., 2022 found that overwatering in agricultural areas results in waterlogging or runoff which after decline soil moisture by up to 20%. Besides, in our study soil water contents dropped in MEA approximately 51%. Other studies have reported detriments from 32% to 50% of soil water contents caused by coal mining activities (K. Ma et al., 2019; Mason et al., 2021). The detriments observed in soil waiter contents in MEA may be related with the removal of vegetation cover, which result in increased evaporation and reduced infiltration of water into the soil. Likewise, soil compaction and land subsidence caused by excavation performed in quarry clays assessed, reduce the ability of soil to absorb moisture as well as changing the topography of the area altering the natural flow of water (Feng et al., 2019).

The sharp drop of soil organic carbon contents computed by LRR in AGA (13%) and MEA (41%) were also comparable with drawbacks of 15% reported in wetlands and non-tillage soils after being subjected to conventional management systems for the production of crops with an annual cycle like wheat (*Triticum aestivum* L.) (Martín et al., 2019), or even, with the reduction of 50% observed in non-till andosols subjected to plow and rotary crops of soybean (Wulanningtyas et al., 2021). Mainly, the higher losses of soil organic carbon in MEA can be attributed to the total removal of vegetation cover and the subsequent exposure of soil to erosion and compaction (Nascimento et al., 2021; Punia & Bharti, 2023). The loss of soil organic

carbon observed in AGA may be related to the continuous cultivation of crops that did not get enough organic matter inputs since the agricultural activities analyzed in the current study were crops with short life cycles such as *Hydrangea sp.* and *F. ananassa.* (He et al., 2022), with the exception of Pinus crops, which have a long life cycle but generate needles and other litter that degrade slowly, causing an accumulation of organic matter on the litter surface that is difficult to mix into the soil (Kooch et al., 2019).

Although it is not easy to find explicit reports of computed rates of change in AMF spore density, soil glomalin contents, and populations of cultivable microorganisms in comparisons of undisturbed soils with soils under any land use, there is plenty of evidence to support the fact that land use decreases the values of this type of soil quality indicators. In terms of culturable mesophilic bacteria and fungi, several studies have shown much greater numbers of culturable microorganisms in non-degraded regions or areas with vegetal coverings, which is consistent with the deleterious effect computed with LRR in AGA and MEA. For example, agricultural soils have fewer cultivable bacteria and fungi than unmanaged soils because the use of fertilizers causes a nutrient imbalance that negatively impacts the enzymatic activity of soil microorganisms, particularly in the culturable portion that plays a significant role in the soil nutrient cycle. Similar to how pesticides are toxic for soil microorganisms and can significantly reduce their populations (C. Lo, 2010). In addition, to mining areas the density of culturable bacteria and fungi is significantly more reduced by very aggressive soil degradation factors such as heavy metal pollution and a lack of plant cover; in spite of this, microorganism biomass and activity may still be observed in these highly polluted locations (Dos Santos et al., 2013).

In AGA, contents of glomalin related soil proteins and AMF spore density were negatively affected in approximately 15% and 18%, respectively. However, these indicators were more severely impacted in MEA by around 48% and 50%, respectively, following the pattern of progressive affectation seen in all other variables assessed in this study. In different crops types, it have reported that tillage practices can disrupt AMF hyphae networks, leading to a decrease in AMF spore density and glomalin production (Hossain, 2021; Säle et al., 2015). Furthermore, monoculture practices in agriculture reduce AMF diversity since different plant species are necessary to increments AMF associations (Sharmah & Jha, 2014). However, mining activities can have more negative impacts on AMF since the removal of topsoil, exposure of subsoil and the subsequent soil compaction can lead to a loss of AMF hyphae networks and their spores, resulting in a steep decline in AMF abundance and diversity (Caproni et al., 2018; F. Silva et al., 2019).

5.4.2 A soil quality index for assessing the proportion of change in soil quality indicators by using differences between perimeters of 2D polygonal projections and an MDS

In our study, SQI_{LRR} was defined as the difference between the perimeter of a 2D polygon projected from radius vector whose length was equal to 1, and the perimeter of a 2D polygon with vectors whose length was 1 minus the LRR observed in a determined variable. Then, this difference was expressed as a proportion of the perimeter to the initial polygon. The perimeters were computed as the sum of distances between the terminal points of each vector. Specifically, the distance between the terminal points of two radius vectors with equal included angles is equivalent to the difference in their lengths multiplied by the sine of the included angle. This relationship can be derived from the Law of Cosines applied to a triangle formed by the origin and the two terminal points of the radius vectors (Brannan et al., 2012). The equation to computed the distance between terminal points reveals also that the perimeter of a polygon enclosed by terminal points will vary in proportion to changes in the length of the radius vectors (Corral, 2013). Thus, SQI_{LRR} were a proportional measure of the changes observed in our sets of soil quality indicators.

Comparison by using Kruskal-Wallis test proved that there was no significant difference between SQI_{LRR} of NFA and REF. On the contrary, the SQI_{LRR} estimated to NFA and REF were significatively lower than SQI_{LRR} for AGA and MEA indicating in this way that the distances from the reference values and NFA were gradually increased towards AGA and MEA. The total set of variables (TDS=14 indicators) and a minimum data set (MDS=9 indicators) were used to project 2D polygons with 14 and 9 radius vectors, respectively. Both types of polygons were useful to differentiate AGA and MEA land uses from NFA and REF. However, we can state with confidence that the variables included in the MDS provide a collection of indicators sufficient for evaluating significant changes in the andosols under study because the Wilcoxon rank sum test revealed no differences between the measurements conducted taking either the TDS or the MDS into account.

The MDS of soil indicators were screened using factor analysis, which incorporated the loadings of all variables to be indicators that best represented soil quality in a specific soil (Raiesi & Pejman, 2021). Given that our set of variables were highly correlated, factor analysis was a useful statistical technique to reduce the number of variables in our data set by identifying underlying factors that cluster the correlations between them and finding a smaller set of factors that explain most of the variation in the data (Hair, 2010). Our MDS encompassed relevant abiotic soil quality indicators such as Ca, S, bulk density, soil organic carbon, soil temperature,

as well as soil microbiological features like enumeration of mesophilic bacteria and fungi, AMF diversity and contents of glomalin related soil proteins. For example, levels of Ca are monitored widely because their excess reduce soil porosity, affecting nutrient availability, and impacting plant growth (Chuo et al., 2020; Van Wijngaarden et al., 2011). Similarly, since soil compaction reduces water penetration, root development, and nutrient availability, resulting in lower plant growth and production while increasing soil erosion, bulk density, as a measure of soil compaction, is frequently regarded as a meaningful indicator of soil quality (Mubaraka et al., 2022; Zheng et al., 2021). Soil organic carbon is the most common used indicator of soil quality (Bünemann et al., 2018b). It plays a vital role in soil fertility, nutrient cycling, water retention, and soil structure and when their levels in soil decrease, it indicates negative impacts on soil quality (Wander et al., 2019). Likewise, sulfur is an indicator of soil quality due to its essential role in the formation of proteins and enzymes in plants, and its influence on soil pH and microbial activity. Low quantities of available sulfur in a soil are frequently related with an elevated rate of soil erosion or an absence of organic matter (Abera & Wana, 2023). In particular, soil temperature is a sensible environmental factor of soil quality since high soil temperatures can lead to increased loss of soil organic matter and soil moisture which in turn affects the rates of nutrient cycling and plant growth (M. Li et al., 2020; D. Zhu et al., 2019).

The culturable population of bacteria and fungi may not be a total representation of the entire microbial community. However, these populations still provide useful insights into soil microbial load because it plays important roles in soil nutrient cycling, organic matter decomposition, and promoting plant grow and show the soil capacity to support microbial populations (De Mastro et al., 2020; Sui et al., 2023). Complementary, AMF diversity is indicator of resilient soil ecosystems since a diverse population of AMF is capable of performing a wider range of functions such as nutrient absorption and water uptake (Parihar et al., 2019) whereas content of total glomalin related soil proteins is an indicator of soil quality because it reflects the overall activity of AMF in stabilizing soil aggregates, increasing soil water retention, and sequestering carbon (Bertagnoli et al., 2020; Hossain, 2021).

5.4.2 Relationship between SQI_{LRR} and SQI_{SLF}

Both approaches, either SQI_{LRR} or SQI_{SLF}, were suitable to identify significant changes in soil quality among reference values, AGA and MEA land uses. The linear regression analysis carried out on the soil quality indexes obtained using the LRR and SLF approaches shown that the indexes derived from the two approaches were highly correlated, with a R2 coefficient of

0.87 for the TDS and 0.90 for the MDS system, indicating that the results of SQI_{LRR} may explain highly the variance observed in SQI_{SLF}. However, the regression analysis also shows a decreasing line which means that there is a negative relationship between SQI_{LRR} and SQI_{SLF}. Since scoring additive linear functions were measures of the contribution of weighted soil quality indicators to overall score according to "mores is better" or "less is better" criteria (F. Li et al., 2023), the strong and negative relationship observed can prove that the approach to estimated soil quality by using log response ratios allows us to integrate in a measure the departures of a set of soil quality indicator from reference values of good soil quality (Hair, 2010; Pustejovsky, 2018). This tendency was clearly observed across de soil quality indexes computed. For instance, the values computed to NFA were higher using the SQI_{SLF} approach (SQI_{SLF}-*TDS*= 0.786*;* SQI_{SLF}-*MDS*= 0.789) whereas lower values were computed by SQI_{LRR} (SQI_{LRR}-TDS= 0.177; SQI_{LRR}-MDS= 0.163). Likewise, , the values computed to MEA were lower using the SQI_{SLF} approach (SQI_{SLF}-*TDS*= 0.168*;* SQI_{SLF}-*MDS*= 0.201) whereas higher values were computed by SQI_{LRR} (SQI_{LRR}-TDS= 0.328; SQI_{LRR}-MDS= 0.296).

5.5 Conclusions

The varied kinds of land use seen in andosols from the southeastern region of Antioquia (Colombia) seriously harm the soil resources. The percentage of change determined from log responses ratio allows us to identify that agricultural activities primarily affect soil water contents, populations of culturable mesophilic bacteria, and AMF spore density, whereas mining activities mostly affect soil water contents, AMF spore density, total glomalin related soil protein contents, and culturable fungi population. Notwithstanding, next to these soil quality indicators the variables that can best explain and condense the differences between soils from non-degraded native forests and soils subjected to agricultural or mining use were also Ca, S, soil organic carbon, soil temperature and AMF diversity. Our results suggest that the differences between perimeter of 2D polygon projected from log responses ratio expressed as radius vector can be useful to discriminate size effects of land uses in a set soil quality indicator. The soil quality indexes estimated following this novel approach illustrated degrees of change in soil quality indicators across agricultural and mining activities and the reference values computed by kernel density estimator were a good start point to compare different land uses, including soil from native forest. The soil quality index derived from log response ratios demonstrated that soil from farming practices changed soil quality indicators in appreciable amounts, although this change was less pronounced than the alteration on soil quality indicators

shown in areas with mining activities. The geometrical analysis of proportions of change in soil quality indicators offer an alternative measure of soil quality that discriminate land uses effects correctly identifying at the same time the variables more affected even with a minimum data set. Also, display a graphical interpretation of the effect of land use in soil quality and complement with an analogous measure, soil quality indexes based on scoring additive functions. This study improves soil quality assessment research and serves as a crucial reference for improving soil quality in other places.

6. METABARCODING REVEALS IMPACT OF DIFFERENT LAND USES ON FUNGAL DIVERSITY IN ANDOSOLS OF THE SOUTHEASTERN REGION OF ANTIOQUIA, COLOMBIA

Abstract

Changes in soil fungal communities caused by land use have not been sufficiently studied in South-American Andosols, considered as key food production areas. Since fungal communities play an important role in soil functionality, this study analyzed 26 soil samples of Andosols collected from locations devoted to conservation, agriculture and mining activities in Antioquia, Colombia, to establish differences between fungal communities as indicators of soil biodiversity loss using Illumina MiSeq metabarcoding on nuclear ribosomal ITS2 region. A non-metric multidimensional scaling allowed to explore driver factors of fungal communities changes, while the significance of these variations were assessed by PERMANOVA. Furthermore, the effect size of land use over relevant taxa was quantified. Our results suggest a good coverage of fungal diversity with a detection of 353,312 high-quality ITS2 sequences. We found strong correlations of Shannon and Fisher indexes with dissimilarities on fungal communities (r=0.94). These correlations allow to group soil samples according to land use. Variations in temperature, air humidity and organic matter contents leaded changes in abundances of relevant orders (Wallemiales and Trichosporonales). The study highlights specific sensitivities of fungal biodiversity features in tropical Andosols, which may serve as a basis for robust assessments of soil quality in the region.

Keywords: Colombian Andosols, Fungal ITS2 metabarcoding, Response Ratio, Alpha diversity, Relevant fungal taxa.

6.1 Introduction

Changes in the structure of soil fungal communities are important indicators of variation on soil health caused by land use. Furthermore, soil functions, related to nutrient cycling, ecosystem provisioning, and climate regulation, can decrease due to loss of fungal diversity (Brinkmann et al., 2019; J. Li et al., 2019). Although there is information on the effect of different land uses on tropical soil fungal communities (Cai et al., 2018; Lan et al., 2020; McGee et al., 2019), the distribution of fungal taxa, functional groups and biogeographical patterns, at local scale in Colombian soils, is still poorly and discontinuously analyzed (Landinez-Torres et al., 2019). Specifically, Andosols from central Andes mountains range in Colombia are relevant units of agricultural productivity and complex microbial habitats and important water reservoirs, in view of their high spatial variation in phreatic zones, thickness of organic horizons and effective cation exchange capacity (Casamitjana et al., 2020). However, information is lacking to understand how human activities affect the fungal diversity and communities assembly of Colombian Andosols. The assessment of soil fungal communities on a local scale is the basis of studies on their ecological behavior since not only the conditions relating to the bioclimatic regions but also those of a specific site influence the composition of the soil fungal communities (Alzarhani et al., 2019). Therefore, the identification of fungal taxa associated with soils is necessary for understanding these complex communities (Pauvert et al., 2019). In addition, assessing the effect of specific land uses on individual soil microbial taxa is key to propose management practices of the soil microbial diversity to maintain or increase soil quality (Powell & Rillig, 2018; Victorino et al., 2021b).

Metabarcoding based on sequences of the internal transcribed spacer region (ITS) for identification of fungal taxa, have been recognized as a universal barcode able to detect poor sequenced taxa in samples of different nature (Badotti et al., 2017; Forin et al., 2018). Characterization of fungal communities using metabarcoding of ITS region sequences have expanded the capability to identify land use effects that shape these communities across space and time, associated to factors such as leaf litter, soil nutrients, pH and organic matter content (Rosenfeld et al., 2018; Sommermann et al., 2018; Turley et al., 2020). Tropical soils are important to study fungal ecological preferences and their driving fac-tors, considering that diversity of fungal groups can peak in tropical ecosystems (Tedersoo et al., 2022). Previous studies have obtained ITS amplicon pools in soils retrieved from tropical ecosystems such as rainforest, dry forest, littorals, coasts and Andeans forest (Barnes et al., 2016; Ritter et al., 2020; Urbina et al., 2016). Moreover, shifts of fungal dominant taxa has been reported, increases of

facultative fungal abundances in deforested areas (L. Shi et al., 2019), declines of fungal richness and diversity indexes by increments of phosphoric fertilization rates (M. Liu et al., 2018), and positive correlations between pathogenic fungal distribution and soil fertility factors (Castaño et al., 2019).

In this study we focused on the characterization of fungal communities of tropical Andosols, by an Illumina metabarcoding approach targeting the ITS2 region, a globally accepted barcode for fungi (Kolaříková et al., 2021). The impact on fungal communities was assessed on three different types of land use with different stages of degradation, in the south-eastern region of Antioquia (Colombia): (1) natural forest areas; (2) areas under weak pressure by agriculture activities; and (3) areas subject to considerable pressure by mining activities related to clay extraction. We compared features of fungal communities to establish the effect size of land use on soil biodiversity. We characterized fungal communities from Colombian Andosols, testing how features and keystone taxa of soil fungal communities vary with each land use perturbation in this model area. Our hypothesis is that changes in features of fungal communities can be useful to identify and group soils samples according to their degradation stage. In the frame of an increasing demand to understand the factors driving soil fertility and resilience, the results revealed useful information on the key fungal components to be further exploited as indicators in a sustainable ecosystem management perspective.

6.2 Materials and Methods

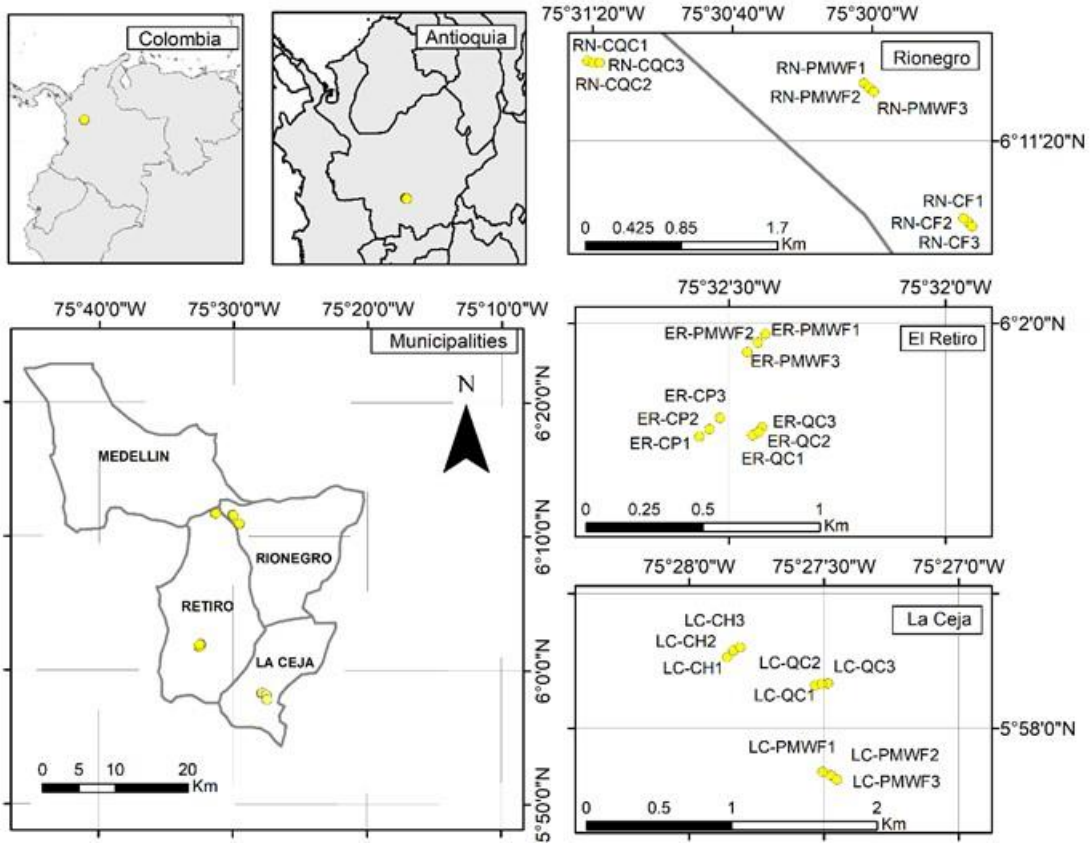
6.2.1 Study site and characterization of land uses

The field research took place in the year 2018 during a rainy season (September and October) in three municipalities (La Ceja, El Retiro and Rionegro), in the southeastern region of Antioquia, Colombia (Figure 6.5). The municipalities are located in the central An-des mountain range at altitudes between 2,300 and 2,700 meters above sea level (m.a.s.l). Pre-montane wet forest and mosaics of pasture-crops are typical ecosystems in the region, which exhibits a unimodal temperature cycle with an average temperature of 21°C and an annual bimodal precipitation cycle with an average monthly precipitation of 387 mm. Within each municipality, samples of Andosols were collected in three land uses characterized by different degradation stages, which were defined according to the fraction removed from horizon A (J. Nunes et al., 2012). Hence, soils from natural forest areas (NFA) were considered as reference of not degraded stage; soils retrieved in agricultural areas (AGA) with a fraction removed from

horizon A between 10 and 20% were cataloged as weakly degraded, and soils of mining extractions areas (MEA), with a removed fraction greater than 60%, were considered as highly degraded.

A point-transect sampling was carried out along 100-m transects delineated from the centroid of the polygon that encompassed each land use. Three equidistant points (at 50 m) were selected along each transects. Five subsamples (soil cores at 20 cm depth) were taken within a 2 m radius of each point to make a composite soil sample, using a soil au-ger with a diameter of 5 cm. Twenty-seven composite soil samples were collected and stored in plastic bags at 4°C for further analyses. Environmental variables (soil temperature, air relative humidity, dew point temperature and barometric pressure) and soil variables (pH, total dissolved solids and electrical conductivity) were measured with a Kestrel 5500 Weather Meter and a Lamotte TRACER 1766, respectively. Organic carbon was quantified after by wet digestion method (Benbi, 2018), and soil moisture following thermo-gravimetric technique (D. N. Singh & Baghini, 2014).

Figure 6. 1 *Geolocation of 27 soil samples retrieved from Colombian Andosols*



Note: Rionegro Closed quarry clays (RN-CQC1, 2, 3), Crops of *Fragaria ananassa* (RN-CF1, 2, 3) and Pre-montane wet forest (RN-PMWF1, 2, 3); El Retiro Quarry clays (ER-QC1, 3), Forestry crops of *Pinus sp.* (ER-CP1, 2, 3) and Pre-montane wet forest (ER-PMWF1, 2, 3); La Ceja Quarry clays (LC-QC1, 2, 3), Crops of *Hydrangea sp.* (LC-CH1, 2, 3) and Pre-montane wet forest (LC-PMWF1, 2, 3).

6.2.2 DNA extraction and PCR amplification

DNA was extracted in triplicate, from 250 mg of soil, previously sieved through 2mm pore size sieve, with DNeasy PowerSoil kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. The ITS2 nuclear ribosomal region was amplified using Invitrogen Platinum HotStart PCR Master Mix (Thermo Fisher Scientific) with primer fITS7 (5′–GTGARTCATCGAATCTTTG–3′) (Ihrmark et al., 2012), and ITS4 (5′–TCCTCCGCTTATTGATATGC–3′)(White et al., 1990). This couple of primers was added to Illumina overhang adapter sequences: forward overhang: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-[locus specific target primer], reverse overhang: 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG- [locus specific target primer]. The cycling conditions were an initial step at 95°C for 5 min, 35 cycles at 95°C for 45 s, 56°C for 45 s, 72°C for 45 s, and a final extension step of 72°C for 7 min. The obtained PCR

products (c.ca 350 bp) were checked on 1 % agarose gel, purified by means of Wizard SV Gel and PCR CleanUp System (Promega), quantified using Qubit 2.0 (Thermo Fisher Scientific, Waltham, MA, USA) and sent to IGA Technology Services S.R.L (Udine, Italy) for Illumina MiSeq platform sequencing (2 x 300bp).

6.2.3 Bioinformatics and statistical analysis

Raw Sequence data were processed using the Quantitative Insights Into Microbial Ecology 2 software package (QIIME2 version 2020.6.0) (Bolyen et al., 2019). To avoid poor quality sequences, reads were truncated (> 280 bp for forward, > 265 bp for reverse reads). The classifier adopted to generate the taxonomic assignment was UNITE community 2020: UNITE QIIME release for Fungi version 20.02.2020. Sequences with ≥ 97 similarity were assigned to the same Operational Taxonomic Unit (OTU). The fungal sequencing data were up-loaded to the NCBI SRA database under accession number PRJNA779046. The generated dataset and metadata were used to create a *phyloseq* object with the R package "phyloseq" version 1.38 (McMurdie & Holmes, 2013). The total number of reads per sample was rarefied at the lowest sequencing depth to allow statistical comparisons (F. Cox et al., 2019). The OTU table was then rarefied at 1,417 sequences per sample by means of the 'rarefy_even_depth' function of the R pack-age "phyloseq" (Shirazi et al., 2021). To identify taxa with a relevant role in the fungal communities, the number of samples in which each species occurs was expressed as average prevalence (aP). The occurrence in more than 30% of total samples was the prevalence thresholds de-fined to identify prevalent species committed to every land use.

With PERMUTEST, the assumption of equal data dispersion among samples was assessed using 999 permutations. A Permutational Multivariate Analysis of Variance (PERMANOVA) was performed using 999 permutations to assess the presence of significant differences between fungal communities compositions (at different taxonomic level), abundances of fungal relevant taxa community and alpha diversity indexes (ACE, Shan-non (H'), Simpson (D'), Inverse Simpson (1/D') and Fisher (S')). To find which taxa were differently abundant contrasting every land use, an exact chi-square test was calculated (Suleiman et al., 2013). Then, non-metric multidimensional scaling plots (NMDS) were carried out to find sample ordination gradients. The NMDS, PERMUTEST and PERMANOVA were per-formed with R package "vegan" version 2.5 (Oksanen et al., 2022), adopting Jaccard's dissimilarity index as partitioning of variation to asses fungal community composition (presence/absence of OTU) (Kujawska et al., 2021). Gower's coefficient was adopted to make comparisons based on

alpha diversity index as a measure of proximity for mixed data types able to homogenize and scale a set of variables (Moschidis et al., 2023; van de Velden et al., 2019). Afterwards, through the function 'envfit', the predictor factors were fitted onto the NMDS ordination plot to identify drive factors of changes in the fungal community (Klavina et al., 2022). Kruskal–Wallis test were implemented to verify significant differences in soil properties and environmental variables between land use (Santorufo et al., 2021).

Log-Response Ratio (LRR) were estimated as a measure of size effect exerted by land uses on parameters regarding and fungal community (Gao et al., 2021). The Mean Response Ratio (MRR) was calculated as the average of LRR to abundances of individual taxa and alpha diversity index (C. Wang et al., 2018). Since sampling variance and standard error of the effect size are not affected by sign changes (J. E. Pustejovsky, 2018), MRR were expressed as an average to positive LRR . The assessed features of the fungal community were abundances of dominant taxa belonging to the taxonomic level which better explains differences between land uses and values of alpha diversity indexes. The taxonomic level was selected according to higher R2 values of PERMANOVA since low R2 values to a factor would explain the differences in community composition less effectively than a factor with grater R2 (Flessa et al., 2021). The results observed in samples retrieved in NFA were considered as control groups. LRR were estimated with the R package SingleCaseES version 0.4.4 (Swan & Pustejovsky, 2018). For each LRR , confidence intervals (CI) of 95% were computed. If the 95% CI of LRR did not overlap with 0, then responses were significant at $p < 0.05$ (C. Wang et al., 2018). Finally, unpaired two-sample Wilcoxon test was used to evaluate differences between MMR contrasting NFA vs AGA and NFA vs MEA.

6.3 Results

6.3.1 Sequencing and soil fungal community composition

In total, 353,312 high-quality ITS2 sequences, averaging 11,330 sequences per sample were retained. One sample retrieved in quarry clays of La Ceja (LC-QC2) was discarded due to the low number of retrieved sequences (less than 1,000 reads). Despite the higher number of valid sequences, Mining Activities Areas (MEA) land use presented a lower number of different OTUs. On the contrary, Natural Forest Areas (NFA) and Agricultural Activities Areas (AGA) showed the highest number of OTUs (Table 6.1S). 494 OTUs, 9 phyla, 29 classes, 53 orders, 117 families, 158 genera and 174 species were identified. Rarefaction curves built on the

rarefied OTU table, suggest that a plateau was reached (Figure 6.1S), indicating that the Illumina metabarcoding approach revealed a good representation of the global fungal composition in every specific activity. Phylum Basidiomycota was dominant in NFA (47.20%) and MEA (69.37%) land uses whereas Ascomycota was dominant in AGA (36.33%) and NFA (44.40%).

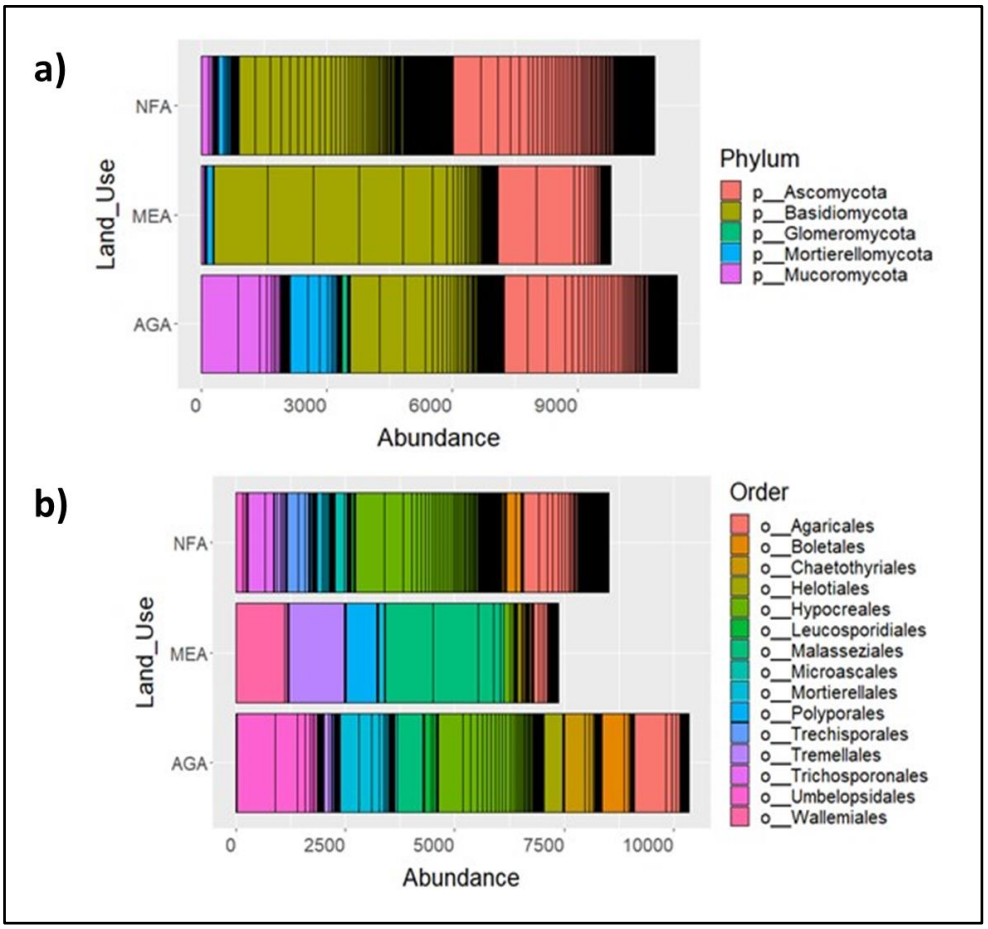
Hypocreales was the most abundant order in NFA (3,303 reads) and AGA (2,412 reads). Malasseziales was the order with higher abundance observed in MEA (2,707 reads). Nevertheless, this order was scarcely observed in NFA (12 reads) (Figure 6.1). The chi-square test showed that the OTU abundance of Basidiomycota was significantly higher in MEA followed by NFA. Besides, we found that OTU abundance of Ascomycota was significantly higher in NFA and AGA land uses (Table 6.1). At order level, chi-square test showed that OTU abundance of Hypocreales was significantly higher in NFA contrasted with AGA ($x2= 268.57$; $p=<0.01$) and MEA ($x2= 2142.47$; $p=<0.01$) as well as in AGA contrasted with MEA ($x2= 1166.44$; $p=<0.01$). Likewise, on the basis of the test the abundance of OTU belonged to order Malasseziales was considered significantly higher in MEA land use contrasted with NFA ($x2= 4173.38$; $p=<0.01$) and AGA ($x2= 579.36$; $p=<0.01$).

Table 6. 1 *Comparisons of OTU relative abundances at phylum level among land uses*

	NFA[1] vs. AGA[2]		NFA vs. MEA[3]		AGA vs. MEA	
PHYLUM	$X^{2\,a}$	$p^{\,b}$	X^2	p	X^2	p
Aphelidiomycota	4.79	0.03	3.91	0.05	0.01	0.92
Ascomycota	150.36	$1.0\times(10^{-3})$	632.69	$1.0\times(10^{-3})$	186.44	$1.0\times(10^{-3})$
Basidiomycota	507.88	$1.0\times(10^{-3})$	1036.27	$1.0\times(10^{-3})$	2874.58	$1.0\times(10^{-3})$
Calcarisporiellomycota	0.01	0.97	0.01	0.94	0.01	0.92
Chytridiomycota	1.66	0.19	0.01	0.94	1.39	0.24
Glomeromycota	41.45	$1.0\times(10^{-3})$	49.16	$1.0\times(10^{-3})$	142.65	$1.0\times(10^{-3})$
Mortierellomycota	419.88	$1.0\times(10^{-3})$	80.19	$1.0\times(10^{-3})$	724.42	$1.0\times(10^{-3})$
Mucoromycota	1212.54	$1.0\times(10^{-3})$	121.40	$1.0\times(10^{-3})$	1653.33	$1.0\times(10^{-3})$
Olpidiomycota	0.01	0.97	0.01	0.94	0.01	0.92
Rozellomycota	6.79	0.01	5.62	0.02	0.01	0.92

Note: Natural forest areas;2 Agricultural activities areas; 3 Mining extraction activities; a chi-square statistic; b p-value (significance level ≤0.01).

Figure 6. 2 *Abundances of fungal dominant phylum and order in soil samples taken from three land uses in Colombian andosols*



Abbreviations: Areas of Agricultural activities (AGA), Mining extraction activities (MEA) and Natural forest areas (NFA). *Note:* a) Abundances at phylum rank; b) Abundances at order rank.

Among the 494 OTU, 223 belonged to the Basidiomycota, 164 to the Ascomycota, 25 to the Mucoromycota, 17 to the Mortierellomycota, 16 to the Glomeromycota, and 41 to unassigned phylum. Regarding different land uses, 121 taxa were observed in NFA, 72 in AGA and 48 in MEA. The land use with higher number of prevalent species was NFA (12 species) followed by AGA (4 species) and by MEA (1 species). *Metarhizium anisopliae* (Metchnikoff) and *Saitozyma* sp. were the most frequent species in NFA, whereas *Trichoderma asperellum* (Samuels, Liechfeldt et Nirenberg) was the most frequent in AGA. *Agrocybe pediades* ((Fr.) Fayod) was a prevalent specie in AGA and MEA land uses both weakly and highly degraded soils (Table 6.2).

Table 6. 2 *Prevalent fungal species identified across different land uses in Colombian Andosols*

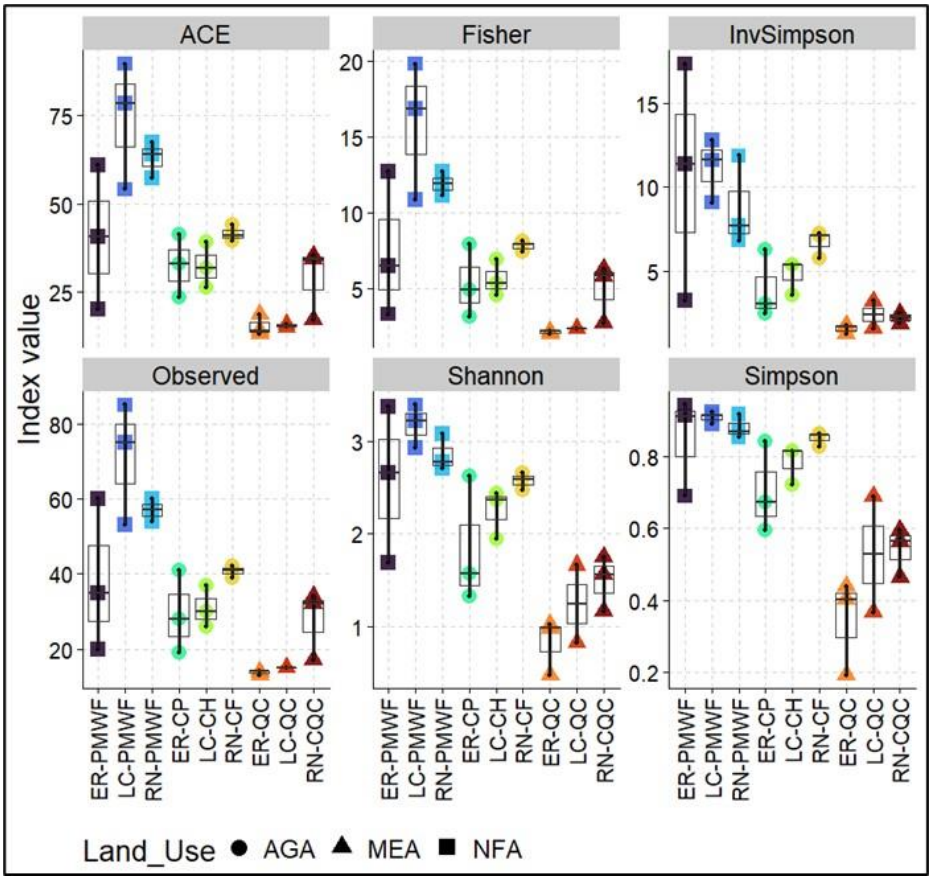
Land use	Specie	Average prevalence (%)	Total reads
Natural forest areas	*Metarhizium anisopliae*	30.76	1167
	Saitozyma sp.	19.23	250
	Fusarium solani	15.38	64
	Trichoderma asperellum	15.38	549
	Apiotrichum scarabaeorum	11.53	603
	Candida sp.	11.53	16
	Clonostachys sp.	11.53	132
	Cryptococcus laurentii	11.53	13
	Cylindrocarpon sp.	11.53	201
	Ganoderma australe	11.53	75
	Ganoderma meredithiae	11.53	18
	Solicoccozyma terricola	11.53	55
Agricultural activities areas	*Trichoderma asperellum*	19.23	441
	Metarhizium anisopliae	15.38	335
	Agrocybe pediades	11.53	13
	Pholiota abieticola	11.53	35
	Suillus cothurnatus	11.53	28
Mining extraction areas	*Agrocybe pediades*	11.53	134

6.3.2 Soil fungal community variations among land uses

The pairwise comparison contrasting land uses ‘NFA vs. AGA’ and ‘NFA vs. MEA’ showed significant differences on soil fungal community composition. However, fungal composition between land uses ‘AGA vs. MEA’ was not significant different among every

taxonomic level analyzed. R2 values were higher and PERMUTEST were not significant considering comparison at level of order followed by species (Table 6.2S). In parallel, land uses show variations in the alpha diversity indexes with significant differences according to PERMANOVA observed between comparison 'NFA vs. AgA' (R2=0.404, p=0.002), 'NFA vs. MeA' (R2=0.746, p=0.001) and 'AGA vs. MeA' (R2=0.580, p=0.001). Higher alpha diversity values occurred in the NFA land use. Inversely, lower values occurred in MEA. The alpha diversity indexes showed consistent patterns among land uses (NFA >AGA > MeA) (Figure 6.2).

Figure 6. 3 *Alpha diversity indexes to fungal communities of Colombian Andosols retrieved from three land uses in Colombian andosols*



Note: Areas of Agricultural activities (AGA), Mining extraction activities (MEA) and Natural forest areas (NFA); Rionegro Closed quarry clays (RN-CQC), Crops of *Fragaria ananassa* (RN-CF) and Pre-montane wet forest (RN-PMWF); El Retiro Quarry clays (ER-QC), Forestry crops of *Pinus sp.* (ER-CP) and Pre-montane wet forest (ER-PMWF); La Ceja Quarry clays (LC-QC), Crops of *Hydrangea sp.* (LC-CH) and Pre-montane wet forest (LC-PMWF).

6.3.3 Fungal community responses to variations in soil properties and environmental factors

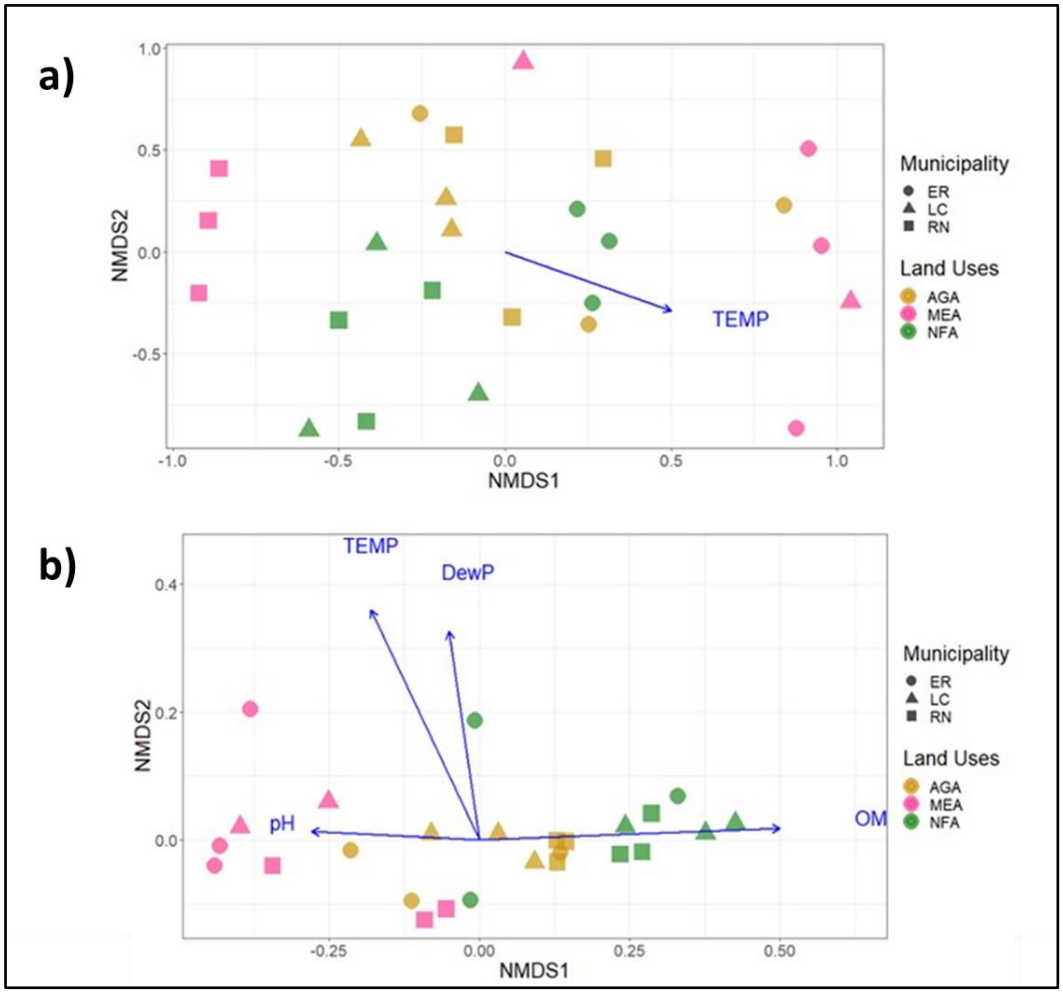
The studied Andosols have an acidic to weakly acidic pH range (from 5.3 to 5.7), with some parameters such as organic matter contents and soil moisture decreasing with the degree of degradation. On the contrary, other parameters such as electrical conductivity and total dissolved solids increase with the degree of degradation. Similarly, environ-mental parameters like relative air humidity and barometric pressure decrease in de-graded areas and other parameters such as soil temperature increase in those areas. In AGA samples, pH and soil temperature decreased. However, significant differences were observed among land uses in soil properties such as electrical conductivity, soil moisture, organic matter contents as well as environmental parameters like relative air humidity and soil temperature (Table 6.3S).

The NMDS dissimilarity distance matrix with Jaccard's coefficient based on fungal composition at order level revealed that there was not a clear gradient of samples according to land use and just temperature was significatively correlated with sample ordination (Figure 6.4a). Notwithstanding, fungal communities from NFA and AGA land uses were closely grouped. Contrary, a gradient of sample position according to land use was detected in the sample ordination based on alpha diversity indexes. Significant positive correlation between sample gradient and organic matter were observed jointly with negative correlations with soil temperature, dew point temperature and pH (Figure 6.4b).

6.3.4 Effect size

Abundances of order Leucosporidiales, Trechisporales and Malasseziales were significatively affected by AGA and MEA land uses. Both land uses exerted a significant effect on alpha diversity indexes (Figure 6.4) which were negatively affected by them. Fisher Index (S') was the index with larger effect size computed in both contrasts (LRR NFA-AGA= -0.637; LRR NFA-MEA= -1.285). Likewise, soil fungal community richness measured by means of alpha diversity indexes decreased with MRR of 0.443 and 1.096 in AGA and MEA land uses respectively. MRR showed effect size to abundances of dominant orders of 1.177 to AGA and 1.539 to MEA. Wilcoxon rank sum test showed significant differences (p-value < 0.05) to MRR contrast between contrast of land uses NFA-AGA and NFA-MEA.

Figure 6. 4 *Non-metric multidimensional scaling of the 26 soil samples considered with driver factors.*



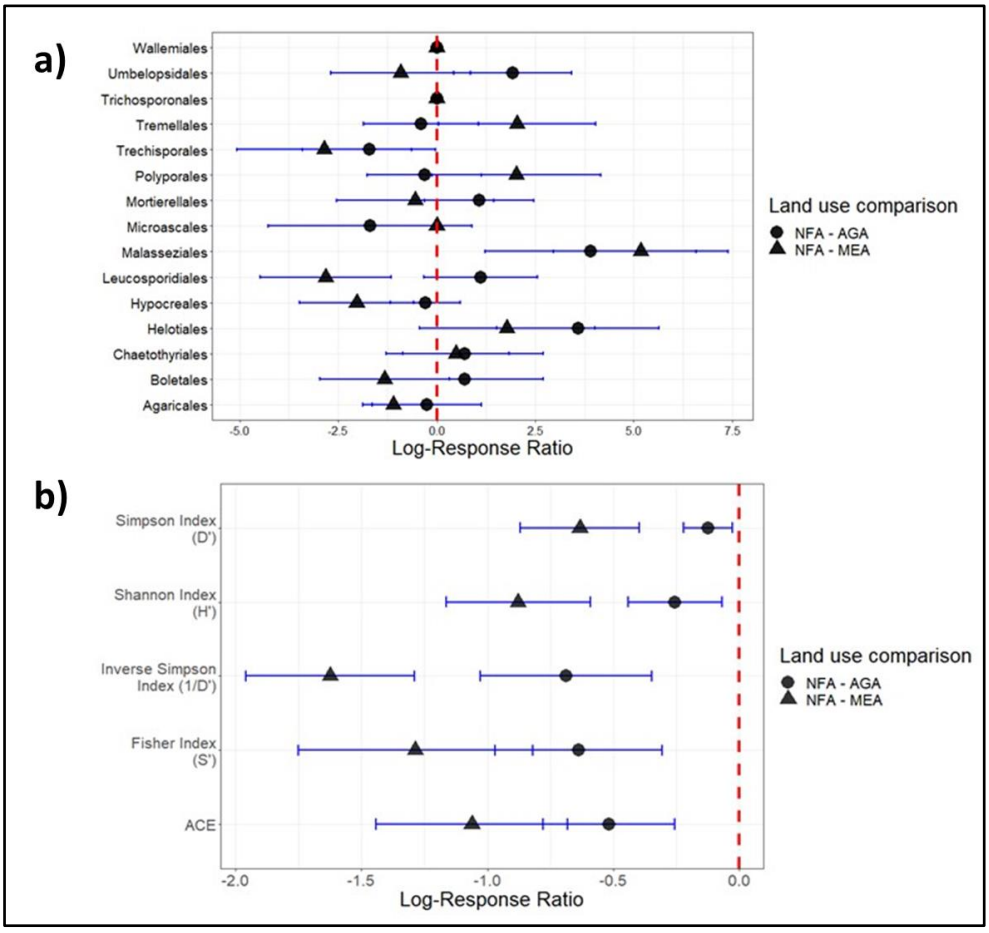
Note: a) Ordination based on order compositions (Stress value=0.243) and b) Ordination based on alpha diversity index values (Stress value=0.027)Areas of Agricultural activities (AGA), Mining extraction activities (MEA) and Natural forest areas (NFA).

6.4 Discussion

The average of high-quality ITS2 sequences retention obtained was comparable with previous sequencing efforts of fungal communities in similar tropical soils. For example, ITS2-ITS4 barcode approach to fungal characterizations of soils from subtropical wet forest got an average of 11,293 high-quality ITS sequences retained (Urbina et al., 2016). The high number of taxa found in our Andosols contrasts with the number detected in similar soils and land uses located at lower altitudes. In Andean agricultural soils of Boyacá, Colombia (located at 2800 m.a.s.l), 143 taxa were identified among 6 phylum by a deep sequencing of the ITS1 barcode (Landinez-Torres et al., 2019), whereas just 30 fungal taxa were identified by a ITS1-ITS2

barcode ap-proach in Andosols from Mexican pine forest, corn fields and rosebush (located at 1300 to 1700 m.a.s.l) (Munguía-Pérez et al., 2011). Studies on fungal communities along altitudinal gradients suggest that altitude and related environmental factors such as air humidity and soil temperature affect fungal community structure (Schiro et al., 2019). However, the influence of soil properties in fungal community assembly observed agrees with previous studies which reported correlation between soil community composition and soil characteristics such as nitrogen content, water retention, pH, and cation exchange capacity (Essene et al., 2017).

Figure 6. 5 *Log-Response Ratios (LRR)*



Note: (a) Abundances of dominant order; (b) Alpha diversity index values. (AGA=Agricultural activities areas, MEA=Mining extraction areas, NFA=Natural forest areas). If the 95% CI of LRR did not overlap with 0, then responses were significant at $p < 0.05$ level.

In our study, Glomeromycota was a phylum rarely observed. However, among the found small set of high-quality ITS2 sequences of this phylum, 10 taxa were identified. Previous fungal community characterizations in comparable environments have reported less AMF diversity values. For example, Landinez-Torres et al. (Landinez-Torres et al., 2019) tested in

Colombian Inceptisols (located at 2,800 m.a.s.l) an ITS1 region amplification by primers BITS and B58S3 identifying for Glomeromycota phylum (among 8 taxa) only 2 orders, 2 families (Glomer-aceae and Acaulosporaceae) and 2 species (*Glomus mosseae* (Nicol. & Gerd.) and *Entrophospora infrequens* (I.R.Hall)). (Berruti et al., 2017c) Parallely, sequences of phylum Basidiomycota were frequently detected, especially in mining areas. This result corroborates the findings of other authors, in which the majority of fungi in tropical Andosols collected from warmer forest-crops mosaics and degraded ecosystems (as forest fragmented and areas where vegetation was totally removed), belonged to phylum Basidiomycota (Gavito et al., 2019; Marín et al., 2017). In the mining areas sampled for instance, *Malassezia* sp. was the most abundant specie. The high abundance of the genus *Malassezia* in degraded areas could be related to low organic mat-ter contents and consequent soil acidification (Zhou et al., 2020).

On the other hand, samples from NFA showed a high abundance of species belonging to order Hypocreales (phylum Ascomycota). In NFA land use, factors such as higher air humidity, lower soil temperature, wild vegetation and the absence of pesticide applications can promote the abundance of this order. Previous studies have reported higher abundances of *Metarhizium* genus in conservated areas positively mediated by temperate environments, wild vegetative cover, high humidity and insect hosts, for instance (McGuire & Northfield, 2020). Likewise, *Fusarium solani* (Mart.) Sacc. abundances are robustly associated with fac-tors such as high soil moisture and presence of entomopathogenic nematodes (S.-Y. Wu et al., 2019). Notwithstanding, conditions observed in NFA like thick layers of litter were favorable to the high prevalence of other taxa such as *Saitozyma* sp. (order Tremellales) since *Saitozyma* genus is a taxa strongly correlated with the edaphic incorporation of carbon in pre-montane wet forest and forestry crops of *Pinus* sp. (Mašínová et al., 2017). In AGA land use, the high abundance of Ascomycota sequences was attributed to the prevalence of *Trichoderma asperellum* Samuels, Lieckf. & Nirenberg, a fungal specie commonly related with soil under mineral fertilizers or agricurltural areas (Argüelles-Moyao & Garibay-Orijel, 2018; Mohammad Golam Dastogeer et al., 2020; Semenov et al., 2022).

The results reported in this study also indicate significant and analogous effects of land use on soil fungal community composition. The NMDS sample ordinations showed a gradient. For instance, samples from not degraded areas clustered near samples from agricultural areas and substantially far from samples of highly degraded mining areas. Furthermore, soils from native not degraded areas separated by distances between 20 km and 25 km (locations RN-PMWF to ER-PMWF; LC-PMWF) were more similar in fungal order composition than those from agricultural and mining extraction areas located in the same municipality. The

PERMANOVA R2 values suggest that the land use factor can explain approximately 10% and 12% of the variation observed in fungal order composition (contrasting NFA-AGA and NFA-MEA respectively). Likewise, land uses explain important variations on alpha diversity index in the same contrast (41% variation in NFA-AGA and 75% variation in NFA-MeA). The observations illustrate the correspondence between dissimilarities of fungal composition in samples with the effect size (LRR) on dominant order abundances.

The land use gradient displayed in NMDS plots based on order abundances and alpha diversity index implied positive correlations between soil fungal diversity with organic matter content and negative correlations with soil temperature. A great part of these effects are induced by clearances of vegetation and partial removals of topsoil horizons from the sampled agricultural areas and mining sites since clearances of vegetation cause subsequently a reduction of soil moisture and nutrients offer as well as a significant increment in temperature (Ni et al., 2019b; Zhao et al., 2019). As a matter of fact, the corresponding variations in soil temperature and organic matter contents observed in anthropogenically impacted areas, determine an enlargement of the effect size observed in the abundances of order Malasseziales (LRR =5.178) and Trichosporonales (LRR =-2.848). Wallemiomycetes (order Malasseziales) for instance have been reported as a xero-tolerant fungi highly abundant in dry environments (Modi et al., 2020). Conversely, populations of representative taxa in temperate wet forest dominated by deciduous trees such as *Apiotrichum scarabaeorum* (Middelhoven, Scorzetti & Fell) Yurkov & Boekhout (order Trichosporonales) de-crease in soils with low organic matter contents (Mašínová et al., 2017).

In particular, the ordination model based on alpha diversity index displayed a sample gradient as a function of the strength of soil degradation. The patterns obtained for every alpha diversity index among land uses indicated that fungal richness was higher in not degraded areas and decreased conforming to the degree of anthropogenic impact. Land use effect size over Fisher Index (S') suggests that the primary fungal community features affected by land use relate to the ratio of the number of taxa and the abundance of those taxa. In the validation of those observations, the sequence abundances of the most relevant taxa (such as prevalent species and dominant order) were negatively affected by land use change, despite high average ITS2 high quality sequences in agricultural (12,486 sequences) and mining areas (15,029 sequences). Furthermore, species richness down-turned from 116 species identified in natural forest areas, to 68 in agricultural areas and 47 species in mining areas.

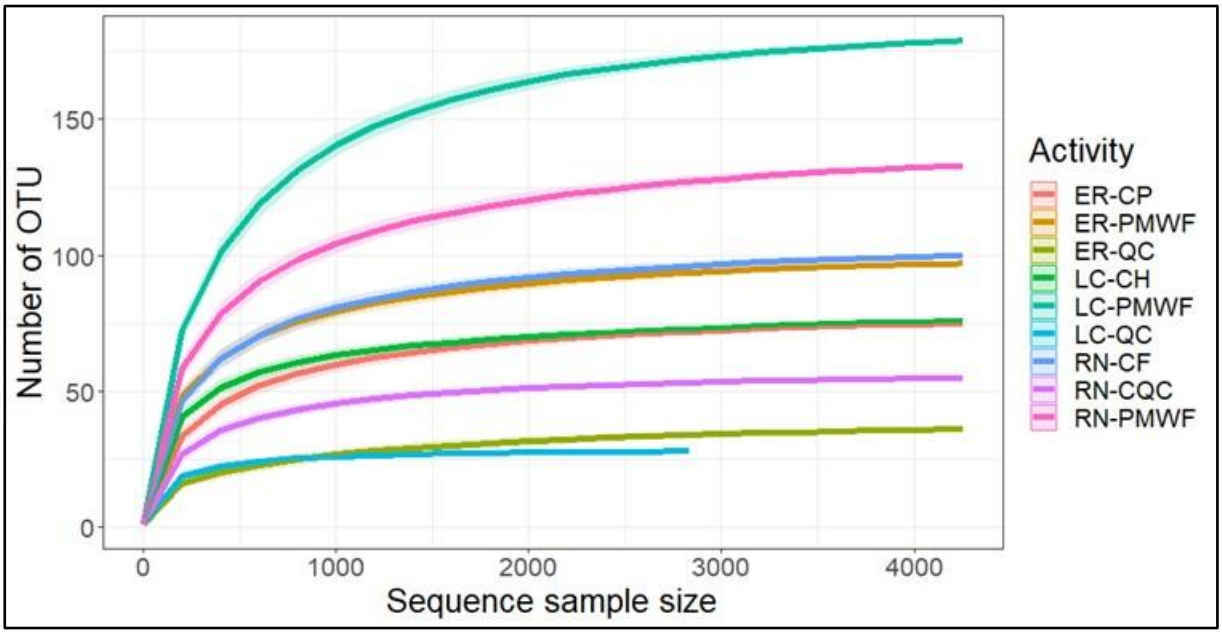
6.5 Conclusions

The comparison of fungal communities features in this study allows establishing the effect size of land use on soil biodiversity, based on the hypothesis that changes in aspects of fungal communities reflect the effect of different degrees of land use. In the framework of an increasing need to understand the factors driving fertility and microbial diversity of soils, the results revealed how environmental factors and soil properties change among different land uses and alter key fungal components.

The species composition and structure of fungal communities in the assessed Colombian Andosols reveal the richness of soil fungal species compared to similar soil types. The relevant taxa such as prevalent species and dominant order Hypocreales, Agaricales, and Trichosporonales were good indicators of the land use effect over soil fungal community. Factors such as soil temperature and soil organic matter contents were more important as drivers than other edaphic factors that determined order composition dissimilarities among land uses or degradation degree patterns. The Response Ratio in abundances of prevalent species belonging to dominant orders allowed the identification of deeper changes on soil fungal communities led by driving factors. However, the stronger response of alpha diversity indexes to the degree of degradation found in this research were better at discriminating changes in soil fungal communities based on the extent of land use.

6.6 Supplementary material

Figure 6. 1S *Rarefaction curves*



Note: Rionegro-Pre-montane wet forest; El Retiro- Pre-montane wet forest; La Ceja- Pre-montane wet forest; Rionegro-Crops of *Fragaria ananassa*; El Retiro-Forestry crops of *Pinus* sp.; La Ceja-Crops of *Hydrangea* sp.; Rionegro-Closed quarry clays; El Retiro-Quarry clays; La Ceja-Quarry clays.

Table 6. 1S *ITS2 Sequencing data obtained from Colombian Andosols retrieved in south-eastern region of Antioquia*

Land use / Specific Activity	Raw sequences	Total valid sequences	Average sequences	Total OTU observed
Natural forest areas	**1,414,082**	**105,682**	**11,743**	**489**
RN- PMWF	497,376	30,478	10,160	167
ER- PMWF	404,467	38,194	12,731	116
LC- PMWF	512,239	37,010	12,337	206
Agricultural activities areas	**1,196,336**	**112,374**	**12,486**	**297**
RN- CF	334,723	25,434	8,478	120
ER- CP	413,927	48,556	16,185	85
LC- CH	447,686	38,384	12,795	92
Mining extraction activities	**914,343**	**135,256**	**15,029**	**159**
RN- CQC	393,152	45,908	15,303	83
ER- QC	262,317	76,191	25,397	45
LC- QC	258,874	13,157	4,386	31

Note: Rionegro-Pre-montane wet forest; El Retiro- Pre-montane wet forest; La Ceja- Pre-montane wet forest; Rionegro-Crops of *Fragaria ananassa*; El Retiro-Forestry crops of *Pinus* sp.; La Ceja-Crops of *Hydrangea* sp.; Rionegro-Closed quarry clays; El Retiro-Quarry clays; La Ceja-Quarry clays.

Table 6. 2S *PERMANOVA and PERMUTEST analysis to compare abundances at different taxonomical level and alpha diversity index values in soil fungal communities characterized in Colombian Andosols*

Variable	CONTRAST	PERMANOVA		PERMUTEST	
		R^2	P-VALUE	Observed	P-VALUE
Phylum abundances	NFA vs. AGA	0.074	0.323	0.523	0.530
	NFA vs. MEA	0.107	0.166	0.780	0.797
	AGA vs. MEA	0.159	0.053	0.672	0.669
Class abundances	NFA vs. AGA	0.112	0.049*	0.094	0.099
	NFA vs. MEA	0.158	0.007*	0.068	0.056
	AGA vs. MEA	0.131	0.020*	0.001	0.001*
Order abundances	NFA vs. AGA	0.106	0.015*	0.695	0.686
	NFA vs. MEA	0.129	0.010*	0.241	0.232
	AGA vs. MEA	0.094	0.089	0.091	0.078
Family abundances	NFA vs. AGA	0.114	0.004*	0.943	0.932
	NFA vs. MEA	0.127	0.004*	0.042	0.035*
	AGA vs. MEA	0.077	0.166	0.002	0.005*
Genus abundances	NFA vs. AGA	0.100	0.007*	0.484	0.455
	NFA vs. MEA	0.113	0.006*	0.145	0.119
	AGA vs. MEA	0.076	0.149	0.020	0.024*
Specie abundances	NFA vs. AGA	0.097	0.005*	0.306	0.312
	NFA vs. MEA	0.110	0.001*	0.437	0.456
	AGA vs. MEA	0.083	0.075	0.122	0.109
Alpha diversity index values	NFA vs. AGA	0.404	0.002*	0.230	0.220
	NFA vs. MEA	0.746	0.001*	0.192	0.186
	AGA vs. MEA	0.580	0.001*	0.883	0.867

Note: * Significance at level ≤0.05

Table 6. 3S *Average values of environmental and physico-chemical parameters in Colombian Andosols retrieved in south-eastern region of Antioquia*

Land use/ Specific Activity	Soil temperature (°C)*	Dew point temperature (°C)	Relative air humidity (%)*	Barometric pressure (hPa)	pH	Electrical conductivity (ds/ms)*	Total dissolved solids (ppm)	Moisture (%)*	Organic matter (%)*
Natural forest areas (N =9)	**18.28**	**14.87**	**80.71**	**763.80**	**5.43**	**667.78**	**332.15**	**41.28**	**10.56**
RN- PMWF (N=3)	18.24	15.20	82.73	743.41	5.37	781.33	407.78	43.20	10.75
ER- PMWF (N=3)	18.96	14.96	77.81	769.42	5.73	818.67	405.56	42.48	9.73
LC- PMWF (N=3)	17.64	14.45	81.60	778.56	5.18	403.33	183.11	38.16	11.21
Agricultural Activities Areas (N =9)	**18.08**	**14.38**	**79.41**	**766.04**	**5.36**	**680.67**	**363.00**	**31.55**	**8.55**
RN- CF (N=3)	17.03	13.49	79.99	755.80	5.07	769.00	396.33	33.87	9.11
ER- CP (N=3)	17.99	14.23	79.36	774.11	5.73	898.00	453.78	34.28	9.11
LC- CH (N=3)	19.21	15.44	78.89	768.22	5.29	375.00	238.89	26.51	7.42
Mining Extraction Activities (N =9)	**22.15**	**15.60**	**67.40**	**753.76**	**5.53**	**853.75**	**420.17**	**22.54**	**4.10**
RN- CQC (N=3)	20.92	13.94	64.48	736.09	5.54	845.00	404.45	17.68	2.92
ER- QC (N=3)	24.48	16.37	61.46	765.91	5.47	876.33	448.89	25.61	5.80
LC- QC (N=3)	20.51	16.92	80.70	762.06	5.61	833.00	400.67	25.21	3.33

Note: Rionegro-Pre-montane wet forest; El Retiro- Pre-montane wet forest; La Ceja- Pre-montane wet forest; Rionegro-Crops of *Fragaria ananassa*; El Retiro-Forestry crops of *Pinus* sp.; La Ceja-Crops of *Hydrangea* sp.; Rionegro-Closed quarry clays; El Retiro-Quarry clays; La Ceja-Quarry clays. * Significant differences observed Kruskal-Wallis test ($p<0.05$) among land uses. (RN=Rionegro; ER=El Retiro; LC=La Ceja). (N=Number of samples analized).

7. GENERAL CONCLUSIONS

This thesis analyzed a set of microbiological based indicators, called AMF-RI, which encompassed AMF spore density, AMF diversity, contents of glomalin related soil proteins, populations of culturable mesophilic bacteria and fungi, urease activity, catalase activity and microbial basal respiration. The main objective of this thesis was to assess context-dependent soil quality (particularly applied for demonstration in samples of andosols collected in the southeastern region of Antioquia, Colombia) by means of a quality measure based on AMF-RI and soil properties able to discriminate the size effects caused by land use changes in single indicators as well as in data set of relevant soil quality features. The primary findings of the thesis are outlined in this section, with an emphasis on how well the proposed objectives were achieved.

7.1 Identification of trending relationships among arbuscular mycorrhizal fungi, culturable microbial populations, enzymes activities and soil properties.

Even though the AMF-RI are extremely sensitive indicators to different changes in soil conditions, as we were able to see from the problem statement and the review conducted in chapter 1, their responses can differ depending on the environment in which they are monitored. However, previous studies on MBI have shown that there may be a response trend to certain variations in context. In this way, we set out to build a database with observations of the parallel variations suffered by the AMF-RI and soil properties altered by agricultural and mining activities through different geographical locations. To assess the strength of the correlations between soil parameters and AMF-RI, Dependency Network Analysis (DEPNA) throughout a based correlation network generated using average correlation coefficients was done on the data collected in Chapter 2. Regardless of divergent AMF-RI responses observed across different land uses and global geographical regions, our findings show strong positive relationships between these indicators and variations in soil properties such as soil nutrient concentrations, as well as negative relationships with physical structure and heavy metal pollution. The partial correlations also supported a variety of synergistic interactions between microbial biomass carbon, soil microbial respiration, bacterial diversity, and actinomycetal diversity that enhance the sensitivity of AMF responses, demonstrating that AMF can more accurately predict soil quality when combined with other microbiological features.

7.2 Characterization of the relationships between abiotic soil properties and microbiological indicators related with arbuscular mycorrhizal fungi in the study zone.

The geographical areas and particular activities sampled were described to identify the environmental context in which the AMF-RI responses were going to be evaluated. Each location was characterized in terms of their life zones, climatic conditions and specific activities assessed. Besides, every soil profile in the studied zone were geomorphologically characterized in chapter 3. The distribution of AMF spores and GRSP soil concentrations across the three primary land uses at various stages of degradation were evaluated in chapter 4. Hare, we examined samples from (1) naturally preserved pre-montane wet forest regions, (2) places little impacted by agricultural operations, and (3) sites under heavy mining activity pressure. To avoid bias in the analysis to determine if AMF-RI and soil abiotic properties were gradually affected by land use, a data transformation was performed to reach several assumptions prior to carry out an ANOVA test.

We found that, depending on the pressure exerted by land use, all AMF-RI, as well as soil abiotic properties like NH4-N, soil organic carbon, S, and soil water contents, suffered negative effects. In addition, we observed that interactions with microbiological features like urease activity and populations of culturable fungi and mesophilic bacteria in conjunction with soil temperature, Ca and S concentrations were the main contributors to the variance observed in AMF communities among land uses, along with values of AMF diversity, spore abundances, and TGRSP. This fact demonstrated that in the study area, the responses of the AMF-RI to variations in soil properties too were explained thanks to its interconnections with other microbiological indicators.

7.3 Comparison of the capability to discriminate variations of soil quality measures performed by means of polygonal projections and a geometric analysis of size effect measures contrasted with conventional scoring functions.

Across chapter 5, we developed a method to compute the perimeter of 2D polygons projected from radius vectors whose length represented a proportion of change in a soil quality indicator as a condensed measure of changes observed in overall soil quality in the analized andosols. The SQI_{LRR},estimated following this approach, have a simple interpretation where values near to 0 indicates a great similarity with reference values of non-degraded soils, whereas

values near to 1 indicates a large departure from reference values. Likewise, the graphical representation of 2D polygons offered a clear visualization of the variations computed to each soil quality indicator. The soil quality index calculated using this innovative methodology showed the degrees of change in soil quality indicators across agricultural and mining activities, and the reference values generated by the kernel density estimator were an effective place to start when comparing various land uses, including soil from native forests.

Either by using SQI_{LRR} with a total data set of variables (SQI_{LRR}-*TDS*) or SQI_{LRR} computed with a minimum data set (SQI_{LRR}-*MDS*), the higher values were estimated to AGA (SQI_{LRR}-TDS= 0.177; SQI_{LRR}-MDS= 0.163) and MEA (SQI_{LRR}-TDS= 0.328; SQI_{LRR}-MDS= 0.296). The lower values were computed to NFA (SQI_{LRR}-TDS= 0.177; SQI_{LRR}-MDS= 0.163). The Kruskal-Wallis test showed that both approaches, either SQI_{LRR} or SQI_{SLF}, were suitable to identify significant changes in soil quality among reference values, AGA and MEA land uses. However, according to the percentage of change calculated from the log responses ratio, we could identify that mining activities mainly impact soil water contents, AMF spore density, populations of culturable mesophilic bacteria, and TGRSP, while agricultural activities primarily affect soil water contents, AMF spore density, and populations of culturable mesophilic bacteria.

7.4 Description of the variations attributed to land use in fungal diversity across a soil degradation gradient

In chapter 6 we tested if the fungal diversity may vary as a function of soil degradation stage, since they are MBI highly correlated with AMF and the changes in the shape of the fungal communities in the soil are crucial markers of variations in soil health. Since the identification of fungal taxa associated with soils is necessary for understanding these complex communities, we performed a deeper soil fungal characterization based on a metabarcoding approach as well as an analysis of the structure of fungal communities to identify relevant variation across land uses. Based on the hypothesis that changes in fungal community characteristics reflect the impact of various levels of land use, the comparison of fungal community features in this study allows determining the amount of the impact of land use on soil biodiversity.

We determined that soil fungal community richness, measured by means of alpha diversity indexes, decreased within order of 0.443 and 1.096 in AGA and MEA land uses respectively. Besides, we observed that parameters like soil temperature and soil organic matter contents had a greater impact on order composition differences between land uses or

degradation degree patterns than other edaphic factors. Furthermore, our results showed that alpha diversity indices responded more strongly to the degree of degradation, which made them more effective at differentiating between changes in soil fungal communities based on the extent of land usage.

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