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Soil mycobiota in agroecosystem: influence of land use and management

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“There is no doubt that life on the planet has depended and will continue to depend on the soil, and in turn, the life that develops there is a creator of humus and, therefore, productivity, from the fertile furrow springs the spike that is bread of the table of men and peace in his spirit”

Andón Cortés

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Abstract

Abstract

Soil quality is a key factor for sustainable agricultural production and therefore for the conservation of ecosystem biodiversity. Land use and management practices tend to modify the complexity of the native micobiota affecting soil sustainability. The present work investigated the biodiversity of soil fungi of agricultural areas differently managed by means of new generation sequencing technologies – NGS. The main object of this research was the analysis of the fungal biota in the Colombian soils, especially that of the upper Andean agro-environment. The study area was located in the region of Boyacá between 2800 and 3200 m asl. and characterized by different form of soil appropriation (apple and peach orchards, 10-year-old wood, and uncultivated grass field). Moreover, a case study has been analysed in Italy where metagenomic of soil micobiota was pictured in a production farm, in three plots differently managed in terms of fertilization, pesticide and tillage application: conventional, organic, no-tillage**.**

Chapter I represents the general introduction of the thesis. As Colombia was the main context of this research a description of the traditional use of soil in this country has received a special focus, especially the economy and land use in the region of Soracá, Boyacá. The soil management and quality of agriculture in Colombia is discussed and the methods used to remediate polluted soil is reported. Moreover, in the general context, the importance of the soil characteristic for the health of food and agricultural products is considered and the methods to verify the quality of soil is faced up to.

Chapter II (published paper) focuses on the practices of soil appropriation in the indigenous and urban Colombian contexts, both from biologically and socio-culturally point of view. The southern region of Colombian Amazon was taken into consideration, since this region constitutes an important model for the traditional utilization of natural resources and a fundamental basis for the definition of strategies for ecosystem management. In this way, it represents a form of integration that allows the approach of conflicting environmental scenarios in strategic biological zones, in which it arises the importance of facilitating the analysis of the interrelationships of ecosystem components, including the human element, through a look bioethics, that may lead to project biological and social guidelines for sustainable use of biodiversity in Colombia.

In Chapter III (advanced version for submission), the focus is on the mycological knowledge of Colombian soils based on data published in scientific research papers. Published data on diversity of native soil microfungi reported for the different natural regions of Colombia (Andean, Amazonian, Caribbean, Orinoquía, Pacific and Insular) were analysed and compared The analysis shows that most of soil mycological data refer to the Andean Ecoregion picturing the greatest diversity of soil microscopic fungi, while the Pacific region is the least studied, recording very few fungal species. No data are reported on the fungal diversity of the Colombian Insular region. Some of the most relevant aspects of the country's mycological diversity are discussed and the most frequently registered species and genera, as well as the references for each of them, are presented. Also, it is evident the need to continue and complement existing research with taxonomic determinations of metagenomic type, as well as the urgency to deepen the evaluation of the functional potential of fungi, in order to have a better ecological understanding of the populations and microbiological communities of the soil. The above, in order to optimize the utilization, recovery and conservation of the soil in Colombia.

In Chapter IV (advanced version for submission), the soil mycobiota in the Colombian High-Andean agro-environment is assessed through high-throughput sequencing technology. The chapter depicts the entire soil fungal community from qualitative and quantitative point of view in areas with different land use (2 different orchards, 1 uncultivated grass field, and 1 woodland). Beside the evaluation of Colony Forming Units per g of soil dry weight, fungal community was described for its species composition. The bootstrap-based clustering analysis highlighted that different land use strongly influences the soil mycobiota: the uncultivated (grassland and woodland) and cultivated areas are characterized by abundant presence of some exclusive species. Differences in fungal species composition is consistent with the clustering analysis on higher taxonomic hierarchical level composition. The study area, considered as a whole, seems to draw a taxonomic gradient, where the woodland site is the most different from the cultivated sites and the uncultivated grassland in the intermediate position.

Chapter V (published paper) reports data collected in a case study planned in the Italian agro-environment context. The results showed that various agricultural management practices (conventional, organic, non-tillage) and seasonality (spring, autumn) influence the composition of the soil mycological community in agroecosystems, through a metagenomic picture. Metagenomic analyses show that the highest richness indices correspond to soils under organic production systems, while the non-tillage system showed the most divergent communities, with their own composition, prevalence and seasonal trends.

Finally, the Chapter VI (accepted paper), represents a sort of perspective for those areas subjected to conventional management and strongly polluted by pesticides that could be remediated and led towards a sustainable agriculture. The usefulness of soil fungi as key tools for the sustainable bioremediation of chemical pesticides in the soil is discussed as a strategy for the recovery of the quality of degraded agricultural soils. The fungal capacity to transform and degrade these compounds has been especially outstanding for ligninolytic fungi and isolated fungi from bio mixtures. In this way, fungi associated with the biodegradation of chemical pesticides *in situ* are a useful technology for soil bioremediation, however, studies that explore the synergy of fungi and bacteria, the combination of which could constitute a relevant process for the elimination of toxic organic substances and recalcitrant from contaminated agricultural soils are peremptory.

The results of this study provide insight into the complexity of micobiota of managed soils under different farming systems, with the ultimate goal of better understanding the multiple mechanisms governing soil quality and to develop an environmentally sound management that improves production, allowing the maintenance of ecosystem diversity and the wellness of human communities linked.

Resumen

La calidad del suelo es un factor clave para la producción agrícola sostenible y por ende para la conservación de la biodiversidad de los ecosistemas. Las prácticas de uso y manejo del suelo tienden a modificar la complejidad del micobiota nativo afectando la sostenibilidad del suelo. El presente trabajo investigó la biodiversidad de hongos del suelo en áreas agrícolas diversamente gestionadas, mediante la utilización de tecnologías de secuenciamiento de nueva generación-NGS. El principal objetivo de la investigación fue el análisis de la biota fúngica en suelos colombianos, especialmente en el agroambiente alto andino. El área de estudio esta ubicada en el departamento de Boyacá entre 2800 y 3200 msnm y se caracteriza por las diferentes formas de uso del suelo (manzana y durazno, bosque de 10 años y pastizal). Además, se analizó un estudio de caso en Italia donde se representó la metagenómica del micobiota del suelo en una granja de producción, en tres parcelas con manejo diferente en términos de fertilización, aplicación de pesticidas y labranza: convencional, orgánica, sin cultivar.

El Capítulo I, representa la introducción general de esta tesis. Como Colombia fue el contexto principal de esta investigación, una descripción del uso tradicional del suelo en este país ha recibido un enfoque particular, especialmente la economía y el uso de la tierra en el municipio de Soracá, Boyacá. Se discute la gestión del suelo y la calidad de la agricultura en Colombia y se informa sobre los métodos utilizados para remediar el suelo contaminado. Además, en el contexto general, se considera la importancia de las características del suelo para la salud de los alimentos y productos agrícolas y se hace referencia a los métodos para verificar la calidad del suelo.

El capítulo II (documento publicado) se centra en las prácticas de apropiación de suelos en los contextos indígenas y urbanos de Colombia, tanto desde el punto de vista biológico como sociocultural. Para ello, se tuvo en cuenta la región sur de la Amazonía colombiana, ya que esta región constituye un modelo importante para la utilización tradicional de los recursos naturales y una base fundamental para la definición de estrategias para la gestión de los ecosistemas. De esta manera, representa una forma de integración que permite el abordaje de escenarios ambientales conflictivos en zonas biológicas estratégicas, en donde surge la importancia de facilitar el análisis de las interrelaciones de los componentes del ecosistema, incluido el elemento humano, a través de una mirada bioética, que puede conducir a proyectos de directrices biológicas y sociales para el uso sostenible de la biodiversidad en Colombia.

En el Capítulo III (versión avanzada para presentación), el enfoque se centra en el conocimiento micológico de los suelos colombianos a partir de datos publicados en trabajos de investigación científica. Se analizaron y compararon los datos publicados sobre la diversidad de hongos microscópicos nativos presente en el suelo reportados para las diferentes regiones naturales de Colombia (Andina, Amazónica, Caribe, Orinoquía, Pacífica e Insular). El análisis muestra que la mayoría de los datos micológicos del suelo se refieren a la Ecorregión Andina representando la mayor diversidad de hongos microscópicos del suelo, mientras que la región del Pacífica es la menos estudiada, registrando muy pocas especies de hongos. No se informan datos sobre la diversidad fúngica de la región Insular colombiana. Se discuten algunos de los aspectos más relevantes de la diversidad micológica del país y se presentan las especies y géneros más frecuentemente registrados, así como las referencias de cada uno de ellos. Además, se hace evidente la necesidad de continuar y complementar la investigación existente con determinaciones taxonómicas de tipo metagenómico, así como la urgencia de profundizar la evaluación del potencial funcional de los hongos, para tener una mejor comprensión ecológica de las poblaciones y comunidades microbiológicas del suelo. Lo anterior, con el fin de optimizar la utilización, recuperación y conservación del suelo en Colombia.

En el Capítulo IV (versión avanzada para presentación), la micobiota del suelo en el agroambiente alto andino colombiano se evalúa mediante tecnología de secuenciación de alto rendimiento. El capítulo ilustra la comunidad desde el punto de vista cualitativo y cuantitativo en áreas con diferentes usos de la tierra (dos cultivos diferentes, un pastizal sin cultivar y un bosque). Además de la evaluación de Unidades Formadoras de Colonias por gramo de peso seco del suelo, la comunidad fúngica fue descrita a través de la composición de las especies. El análisis de agrupamiento basado en bootstrap destacó que los diferentes usos del suelo influyen en la micobiota del suelo: las áreas no cultivadas (pastizales y bosques) y las áreas cultivadas se caracterizan por la alta presencia de especies exclusivas. Las diferencias en la composición de las especies de hongos son consistentes con el análisis de agrupamiento a nivel jerárquico taxonómico superior. El área de estudio, considerada como un todo, parece ser un gradiente taxonómico, en el cual, el bosque resulta ser más diverso que los sitios cultivados, mientras que el pastizal se encuentra en una posición intermedia.

El Capítulo V (documento publicado) informa los datos recopilados en un estudio de caso planificado en el contexto agroambiental italiano. Los resultados mostraron que diversas prácticas de manejo agrícola (convencional, orgánico, sin cultivar) y estacionalidad (primavera, otoño) influyen en la composición de la comunidad micológica del suelo en los agroecosistemas, a través de una representación metagenómica. Los análisis metagenómicos muestran que los índices de riqueza más altos corresponden a suelos bajo sistemas de producción orgánica, mientras que el sistema sin cultivar mostró las comunidades más divergentes, con su propia composición, prevalencia y tendencias estacionales.

Finalmente, el Capítulo VI (documento aceptado), representa un tipo de perspectiva para aquellas áreas sujetas a manejo convencional y fuertemente contaminadas por pesticidas que podrían ser remediadas y conducidas hacia una agricultura sustentable. La utilidad de los hongos del suelo como herramientas clave para la biorremediación sostenible de plaguicidas químicos en el suelo se analiza como una estrategia para la recuperación de la calidad de los suelos agrícolas degradados. La capacidad de los hongos para transformar y degradar estos compuestos ha sido especialmente sobresaliente para los hongos ligninolíticos y los hongos aislados de las biomezclas. De esta manera, los hongos asociados con la biodegradación de pesticidas químicos *in situ* son una tecnología útil para la biorremediación del suelo, sin embargo, estudios que exploran la sinergia de hongos y bacterias, cuya combinación podría constituir un proceso relevante para la eliminación de sustancias orgánicas tóxicas y recalcitrantes de suelos agrícolas contaminados son perentorios.

Los resultados de este estudio proporcionan información sobre la complejidad del micobiota de suelos gestionados bajo diferentes sistemas agrícolas, con el objetivo final de comprender mejor los múltiples mecanismos que rigen la calidad del suelo y desarrollar un manejo ambientalmente racional que mejore la producción, permitiendo el mantenimiento de la diversidad del ecosistema y el bienestar de las comunidades humanas vinculadas.

I. Introduction

The traditional use of land in Colombia

The Colombian nation is the product of the most varied fusion, where the culture and traditions of the American, European and African peoples interact. In this context, four ethnic sectors differ from western society: indigenous people, Afro-Colombian populations, raizal communities and Rom or Gypsy people. According to the 2005 general census, there are 87 indigenous tribes in Colombia, whose population is mainly located in the rural area of the country, in natural regions such as the forest, the natural savannas of the Orinoco, the Colombian Andes, in the inter-Andean valleys and in the Plain of the Caribbean. The departments with the highest percentage of indigenous people are Guainía, Vaupés, La Guajira, Amazonas, Vichada, Cauca and Nariño (DANE, 2007).

In the Colombian Andes (departments of Boyacá, Cauca, Cundinamarca and Nariño) the main transformation of the ecosystem carried out by pre-hispanic indigenous cultures was undoubtedly agriculture. The cultivation and therefore the relationship with the soil is inherited from ancient indigenous settlers such as Muiscas, Laches and Paeces, among others (Londoño, 1998; Rodríguez, 1999; Mora, 2011). For these reasons, the study on the appropriation of soil resources will be based on the analysis of agricultural activity in the region, since the latter has been deeply documented over time and is directly linked to soil use and management.

The department of Boyacá is located in the Center - East of Colombia, in the central part of the Eastern Andes Mountain Range, reason why the diversity of climates, geographic position, consolidation of its landscapes and access to drinking water, has allowed the inhabitants to enjoy inter-Andean valleys that facilitate the domestication and multiplication of seeds in their extensive and rugged geomorphology, which varies between 300 and 5.490 masl, making the region the area where the mountain range has the largest amplitude in the country. From there, around 1.865.000 tons of food and agricultural products are generated per year (DANE and IGAC, 2005; MADR *et al.*, 2006; Caracol Radio, 2012; López, 2015).

The relief of the department belongs to the Andean system and four morphological units are distinguished at the macro level: the valley of the river Magdalena or region of the west, the Eastern mountain range or region of the north, the plateau or central region and the piedmont of the Eastern Plains or Eastern region. The central region, also called Cundiboyacense plateau occupies 70% of the territory of Boyacá, in which the majority of the population is concentrated and it was the zone in which the greater percentage of the Muiscas indigenous population was concentrated and where the spanish groups were located in the hispanic colonial period (Estupiñan, 2014).

The department is made up of fifteen provinces composed of 123 municipalities with particular historical, social and territorial links. In the central highplateau, the central province is composed of the municipalities of Tunja, Chivatá, Chiquiza, Cómbita, Cucaita, Motavita, Oicatá, Samaca, Siachoque, Sora, Soracá, Sotaquirá, Toca, Tuta and Ventaquemada. The identification of the province as a territorial historical and functional unit of planning in the management of regional development illustrates in a forceful way the correlation between the organization of the territory and the development of a human community (Estupiñan, 2014).

Boyacá is part of a region with a rich indigenous past, settled since the 6th century BC by the Muisca indians, the last pre-hispanic occupiers of the Cundiboyacense plateau, organized in one of the continent's largest confederations at the time, but where there does not seem to be much interest in the majority of the present population to identify or make use of the past (Miñana *et al.*, 2011), which makes it possible to understand why despite its great pre-hispanic indigenous, Muisca legacy, the region does not have the traditional knowledge of agricultural activity today, with its former inhabitants.

The present mainly peasant population is recognized, however, by the protagonism of their individual and collective actions for the processes of knowledge, management and conservation of resources. Such knowledge has a transcendent value to clarify the ways in which peasants perceive and conceptualize the natural resources on which they depend to subsist, even more so in a small-scale economy. In this conception, the cultivated field is not assumed as isolated from the rest of reality, but as the representation of the way the peasants have to relate the environment, culture, experience, production and nature, through polyvalent and multidimensional experiences, in which social and natural contexts cannot be divided (Morales-Hernández, 2004).

Nevertheless, the region has not been oblivious to the loss of traditional knowledge, which implies the affectation of the natural environment and therefore of the related societies. Indeed, when inquiring with the elderly population, that is old people, it is evident to find expressions between silence and broken words, in which they say they feel alone because young people do not like the countryside anymore, so they look for other options of life and migrate to the city, since the work as a farmer is no longer valued (López, 2015).

This process is concerning in agricultural regions with the largest history of cultivation, such as the Cundiboyacense plateau, because the traditional knowledge is removed from its context, taking only isolated elements, which loses its potential for the conservation of both the natural resources that employ as of the cultural base that underpins this knowledge. This happens very often in the case of peasant production systems for use and management (Corrales and Torres, 2002; Casas and Parra, 2007).

In contrast, indigenous populations who are the inhabitants and users of the world's most biologically diverse areas often manage local environments in ways that preserve or increase the diversity of living forms (Casas and Parra, 2007). In fact, when talking about traditional systems and their contribution to sustainability, reference is first made to indigenous systems of agricultural production and management of wilde areas. In agricultural production, chagra systems, the housing orchard and various types of management of orchards and agricultural areas are considered (Corrales and Torres, 2002).

The indigenous Amazonian ethnic groups, for example, present a complex and advanced knowledge on the management of the forest and the agricultural system, which are evidenced in aspects such as: the great number of species and varieties cultivated and managed, the classification systems of the resources and environments and the knowledge about agronomic practices of crops. The agriculture of indigenous Amazonian chagras is a dynamic agroforestry system with long periods of use, involving sustainable and maintainable technological strategies adapted to the conditions of the Amazon forest (Vélez, 1998). In this way, indigenous communities possess knowledge, techniques, practices of use and conservation of the territory as a strategy of survival and adaptation (Niño, 2011), in fact, the greatest use of the chagras on the mainland (itinerant agriculture), is a cultural adaptation to the infertility of the soils that these landscapes present (Pinilla, 2004).

The cultivation of the chagra, as a daily activity for the indigenous Amazonian communities, has adopted derived techniques from a long process of adaptation to the conditions of the forest. The knowledge acquired by the application of this system of agricultural production over many years, makes the chagra the base of the economy in the indigenous communities of the Amazon. This experience manifests itself in the sustainable use of the forest, since it takes the necessary elements for subsistence, but it is given a management that allows the recovery of these resources and even a parallel utilization to the recovery process, as happens in the stubble, which become a collection and hunting site while the regeneration of the old area of cultivation takes place. In spite of the exuberance of the forest, people are aware that the resources it offers are limited, particularly in areas of intensive use (Triana-Moreno *et al.*, 2006).

This allows us to glimpse that, for the indigenous, the agroecosystem chagra is an intrinsic good itself. The biophysical and sociocultural aspects coexist in a dynamic and dialectical harmony. Each component of the chagra recreates each other in a time and space that depends on the cosmovision of each indigenous Amazonian community. In this way, it provides environmental and cultural services, it offers food and spiritual security, and it is a genetic bank based on a solid foundation of traditional knowledge of domesticated, semi-domesticated and native species. In addition, to being a laboratory of experimentation and genetic improvement transmitted from generation to generation (Cabrera, 2004).

Thus, it is evident that the indigenous communities that inhabit the Colombian Amazon have been characterized to build a wide knowledge on the management of these ecosystems, through subsistence practices that have allowed them to adapt to the environment (Palacios, 2007). For indigenous peoples, life has no meaning without territory, because it is the heart of its existence and culture, from the spiritual dimension, territory makes possible the collective and individual development and provides the source of life and security (PNUD, 2011).

The analysis of the changes in the way of life of indigenous groups in tropical forests and their use of natural resources shows a highly complex and dynamic image. In general, the traditional way of life of these peoples has been sustainable for thousands of years and has ensured the functionality of the fragile ecosystems of the tropical moist forest. Practices, such as slash-and-burn agriculture, hunting, fishing, and other forms of traditional extractivism, guarantee subsistence with low impact on nature (Tresierra, 2000).

Ethnic diversity is closely related to biological diversity (ecosystems, soils and waters), whose current situation is the result of a long geological history and processes of evolution, migration and adaptation. Colombia has a multi-ethnic population and a great diversity of landscapes and climates, rocks and soils, as well as an enormous biological diversity. Contrasting two of the country's main natural regions, it can be said that in the Andean zone and the inter-Andean valleys, the most populated region of the country, the destruction of the original vegetation is advanced, therefore, this area in great part is already "anthropized", while in the Amazon there are still large areas in a natural state and is one of the regions with the highest biodiversity in the country, despite the fact that colonization has advanced significantly and increased illegal crops. To this, conflicts in land use (soil use without considering its potential) have are added, since greater "colonization" often corresponds to a conflictive use of land (van der Hammen, 2005).

Changes in soil use by indigenous communities are mainly due to their new, more sedentary structure, which promotes new adaptations that break with the adaptive and sustainable patterns of Amazonia, which probably suggest a loss of traditional knowledge and with it many important aspects of cultural identity. The loss of traditional knowledge has shown that it not only weakens the culture but also the proper management of resources, introducing new market tools that do not favor the good use of resources (Palacios, 2007).

Due to the above, it is essential to analyze the ways of utilization of natural resources, including the soil, of the indigenous Amazonian communities of Colombia, in order to understand the strategies that have allowed these cultures to maintain and conserve biological diversity until today, especially considering that the use of the landscape, is closely related to the forms of appropriation of the territory and the traditional and modern sociocultural factors, therefore, the recent sedentarización and the population growth are definitive factors for the cultural, social and ecological change that have generated greater pressure on ecosystems, causing an accelerated process of transformation of the areas, which in the long term may lead to degradation of ecosystems (Palacios, 2007).

Boyacá, Soracá, Region: economy and land use

In Colombia, all departments of the country are affected by soil erosion. The most significant, ie, over 70% of its area is: Cesar, Caldas, Córdoba, Cundinamarca, Santander, La Guajira, Atlántico, Magdalena, Sucre, Tolima, Quindío, Huila and Boyacá (72%). The departments most affected by the severity of erosion (a practically irreversible process), regarding their area are: La Guajira, Magdalena, Cesar, Huila, Sucre, Santander, Tolima, Boyacá (7%), Atlántico, Norte de Santander, Valle del Cauca and Cundinamarca (IDEAM *et al*., 2015).

In the country, deforestation is one of the main causes soil degradation by erosion. It is estimated that more than 60% of deforested areas between 1990 and 2010 are experiencing some degree of erosion. Socio-economic activities and factors, which cause greater pressure on soils and cause degradation by erosion, are located in irrigation districts (94%), agricultural and livestock territories (93% agricultural, 88% agricultural and livestock, 77% livestock), territories with use conflicts due to overuse of soils (91%), underutilization (80%), and vegetal cover fires (81%) (IDEAM *et al*., 2015).

The department of Boyacá, located between 04°39'10" and 07°03'17" north latitude and 71°57'49" and 74°41'35" west longitude, covers an area of 23.189km^2 corresponding to 2,03% of the country and has 54% rural population and 46% urban. Due to its natural vocation of use, several types of soil are characterized in the department, the most representative are Conservation (41,32%), Agroforestry (35,94%), Agricultural (9,39%) and Livestock (8,83%) the remaining area corresponds to water bodies and urban areas. The criteria used in the determination of soil use refer to the climate, relief, slope, flood and soil characteristics such as their parenting materials, effective depth, natural drainage, stoniness and availability of nutrients expressed in terms of natural fertility. These factors, along with the economic, social and cultural characteristics of man de fine the sustained utilization of soil resources (DANE and IGAC, 2005; Corpoboyacá, 2015).

The Boyacá economy is mainly based on agricultural and livestock production, mineral exploitation, the steel industry, trade and tourism. Agriculture has been developed and technified in recent years, the main crops are potato (55.428 ha), maize (29.127 ha), onion (20.146 ha), wheat (15.540 ha), barley (13.330 ha), cane (13.597 ha) and cassava (3.247 ha). The livestock population is estimated at 1.018.994 bovines, 121.541 porcines, 63.251 equines, 19.084 donkeys, 26.945 mules, 73.197 caprines and 236.563 ovines. In this way, the agricultural sector contributes the Gross Domestic Product (GDP) of the department 69% to the agricultural subsector and 31% to the livestock subsector (MADR *et al*., 2006).

Land devoted to agriculture includes annual crops, semi-perennial crops and perennial crops. Fruit crops occupy a key place for the diversity of microclimates that allow producing a wide variety of fruits in different seasons. For 2004, Boyacá had 11.833 hectares representing 5,3% of the national area with fruit trees, belonging to 36 species, indicating the wide range of thermal floors in which they occur, so it is considered the department with the highest number of cultivation species. The species of major importance for the area under cultivation are guava, orange, pear, curuba and plum. It is estimated that 154.358 hectares form the total of suitable and available soils for the cultivation of fruit trees in Boyacá. In addition, it is meaningful high average propensity to consume fruits, 61 kilos/year per person, which corresponds to the highest average for Colombia (DANE and IGAC, 2005; MADR *et al.*, 2006).

Thus, the department is first in the national production of deciduous trees, which indicates its vocation and tradition over the years in this type of cold climate crops; four species (apple, pear, plum and peach) are cultivated with a large range of varieties that have been adapted to the agro-ecological conditions of the region. In this way, it is possible to notice the importance of the deciduous in this zone and the competition that they represent for the imported products, being preferred internally for their quality and freshness (MADR *et al.*, 2006; Puentes, 2006).

In Boyacá, the main cause of low production and agricultural yield is the land tenure traditionally croft style with a tendency towards something smaller than a croft. In the rural area, there are 28% of crofts (farms of one up to three hectares) and 49% of the smallest crofts (farms of less than one hectare), a reality that has determined a low level of technification and productivity, generating a subsistence economy and a low quality of life for its inhabitants (DANE and IGAC, 2005).

Deciduous fruit trees have not been oblivious to this situation, which have been located in crofting areas, in communities with little or no irrigation infrastructure, with little specialized technical assistance and mixture of different species and varieties. However, despite these indicators the living standards of fruit growers is above the producers of food crops. In addition, this system has had important advances in terms of business management, introduction of new varieties, technological innovations, irrigation infrastructure, associative work mentality, greater extensions, crop planning taking into account market requirements and work under the approach of productive chain (MADR *et al.*, 2006; Puentes, 2006).

As for conflicts of land use in the department, ie, the result of the discrepancy between the use humans now makes the soil and use it should have, according to their natural vocation, these are mainly due to the extension of the agricultural frontier and agricultural activities in hillside lands without the implementation of soil management and conservation practices. In addition, natural vegetation has been significantly affected not only by agriculture but also by livestock, so the few places where it still exists are difficult to reach regions or climates that are not conducive to farming (DANE and IGAC, 2005; Universidad Santo Tomas, 2008; Corpoboyacá, 2015).

Respect to the natural threats, the great tectonic complexity and the geological, geomorphological and climatic variety, together with an intensive productive activity make the department of Boyacá present a high susceptibility to natural risks such as seismic activity, erosion, mass movements and floods (DANE and IGAC, 2005).

Regarding the edaphic element, the measurements of the structure and rate of change or soil degradation are complex given its dynamic condition, which is necessary to identify the limiting factors and estimating the brittleness of the different types of soil, for the purpose of determine the required changes in tillage practices. A property of the soil that is usually altered is the relation mass/volume or apparent density, this property affects the root growth and the emergence of the seedlings. The bulk density is difficult to establish because it also interacts with porosity, moisture, structure and mechanical resistance to penetration, all of which affect plant growth. In this way, man can be considered as one of the main modifying factors soil, it can destroy it with improper handling to slash and burn the forest to implement agricultural and livestock activities that promote erosion as it has happened with the soils of the highplateau landscape or can also contribute to the formation of fertile soils using modern techniques: compost, fertilizers and amendments (DANE and IGAC, 2005).

The territory of the town of Soracá is located in the foothills of the Cundiboyacense plateau and belongs to the Eastern Mountain Range of the Colombian Andes. It is located in the central area of the department of Boyacá at 5º30' north latitude and 73º west longitude of Grewnwich. It has an altitude of 2.942 masl, it is part of the central province and is only 7km from Tunja, the capital city of the department (Alcaldía Municipal de Soracá, 2004; Municipio de Soracá, 2013).

This town has two traditional administrative divisions, the urban sector and the rural sector, the latter consisting of twelve villages: Alto Negro, Centro, Cruz Blanca, Chaine, Faitoque, Otro Lado, Puente Hamaca, Quebrada Grande, Quebrada Vieja, Rominguira, Rosal and Salitre. The extension of the town is 57km^2, of which 0,32 correspond to the urban area and 56,3 to the rural area, meaning that the town is 99% rural. Similarly, most of the population is rural since of 6.407 inhabitants, 5.804 are located in this area (Alcaldía Municipal de Soracá, 2004; Municipio de Soracá, 2013; Alcaldía de Soracá, 2016).

Economic activities of the town are mainly in the primary sector of the economy (productive activities in the extraction and production of raw materials). Soracá is suitable for the production of potato, wheat, fruit trees and pastre for livestock zone, therefore, the use of soils is given by 60% to agriculture, 30% for grazing and 10% to other uses, which means that compared to the total central province and the department have a superior use in the agricultural sector. The system of purely agricultural and livestock production, in most cases has a traditional management (inheritance techniques), but with great contrasts in terms of production and land tenure (Alcaldía Municipal de Soracá, 2004; Alcaldía de Soracá, 2016; Municipio de Soracá, 2013).

Almost all of the natural hedging in Soracá has been gradually destroyed to give way to crops and livestock, the few forest relics or "patches" of natural vegetation are located in hills of difficult agricultural and livestock work, where the agricultural frontier has been expanded, reducing the scarce natural spaces that subsist. The native mount has been transformed and grasses and small stubble predominate as scrub scattered among the grasslands in a single unit of use and tree cover is mainly represented by exotic species of pines and eucalyptus. Human intervention and agricultural development have profoundly altered native forest, the original vegetation and possibly as many local native species disappeared and only a few sporadic bushes and trees which was a dry forest are presented. The lands in the town are intensively exploited which, together with the deforestation of native vegetation, overgrazing and rainfall levels are the factors that have the highest incidence in the trawling and washing of the soil (Alcaldía Municipal de Soracá, 2004; Municipio de Soracá 2013).

The town is characterized by a cold climate which is semi-humid, with an annual average temperature of 12ºC and average annual precipitation of

500 to 800 mm, with variations according to local level. The pattern of rainfall distribution is bimodal type. The wind speed reaches a maximum in June and August between 2,7 and 3,1 m/sec. The average multi-annual solar brightness is between 1.600 and 1.931 hours sun/year. The relative humidity presents minimum values of 71% and gradually increases to a maximum of 91% (Alcaldía Municipal de Soracá, 2004).

As for the fruit trees cropping in the town of Soracá is noteworthy that the University "Fundación Universitaria Juan de Castellanos" (JDC), a Higher Education Institution, linked to the fruit sector through the program Agroecology, was the entity that displayed the Project of Deciduous with which the cultivation of these fruits began as a part of the construction of a new productive meaning for the region (Chico and Villota, 2015). The project is currently being developed at the experimental farm San Isidro Labrador of the JDC, located in the Otro Lado village.

The latter is located in limits with Tunja and is characterized by the association of wastelands and crops with use in traditional agriculture, as well as by the association of herbaceous and natural forest, with use in livestock and protection. The agricultural activity of the village is carried out successfully, although artificial irrigation may be needed in dry period, through reservoirs to maintain water (Municipio of Soracá, 2004).

The JDC Deciduous Project currently has about 3.300 trees planted on an area of 10 hectares at an altitude of 2820 masl and is based on the research and production of species of deciduous fruit trees such as apple tree (*Malus communis*), pear tree (*Pyrus pyrifolia*), peach tree (*Prunus persica*) and plum tree (*Prunus salicina*). In this area, some characteristic elements such as climate, soil and culture have been essential for the development and strengthening of this initiative which has been successfully experimented with varieties such as Apple Anna, Duraznos: Black King, Gold and Rubi Doux, Pear Triumph of Vienna and Plum Horvin (INICIEN, 2017).

These species of deciduous, originating in temperate zones, respond in different ways to seasonal changes inducing physiological responses, allowing them to survive adverse environmental conditions, such as during the winter season characterized by very low temperatures, for which they use as a defense mechanism the cessation of metabolic processes for the visible growth of structures with meristems. However, they have adapted to tropical areas of high altitude that compensates the low latitudes, as in the case of the highplateau, showing good productive behavior at nighttime low temperatures, alternating with warm sunny days for much of the year. These elements are indispensable for the good ripening of fruits and the synthesis of substances or compounds that provide chemical-organoleptic quality to them (INICIEN, 2017).

Within the scope of the JDC's Deciduous Project, four approaches have been considered: 1. Scientific, based on basic research, which may include genetic improvement and molecular characterization; 2. Academic-technical, related to the learning and application of science and technology in agriculture, irrigation systems, energy utilization and environmental management; 3. Industrial, based on planning, control and optimization through production scheduling, establishment and management of the production chain, optimization of resources, marketing and agribusiness and 4. Social, which involves peasant society to the project as a nutritional and economic alternative in its favor through teaching, technical training and good agricultural practices throughout the cycle of the fruit tree (INICIEN, 2017) .

Importance of soil quality for food quality

Soil quality is the capacity of a specific kind of soil to function within natural or managed ecosystem boundaries to sustain plant and animal productivity and maintain or enhance water and air quality, and support human health and habitation (USDA, 2001).

The most widely recognized function of soil is its support for food production. The soil is the basis for agriculture and pasture and soil quality is directly linked to food quality and quantity. It is estimated that 95% of our food is directly or indirectly produced on soils (FAO, 2015a).

Soil is the complex environment where the fungi and bacteria act, converting dead and decaying matter in plant nutrients, ensuring mineral cycling, supply the essential nutrients. A functioning microbiota means functioning soil. Moreover soil represents a buffer to protect plant roots from physicochemical parameter fluctuation such as temprature or pH. A healthy soil contain microbiota that perform many vital functions including controlling plant disease, insect and weed pests. Nutritious and good quality food and animal fodder can only be produced if our soils are healthy living soils. Recently, a holistic production management systems promoting agro-ecosystem health that are socially, ecologically and economically sustainable are necessary in order to protect the soil but without losing the productive capacities. Agroecology, conservation agriculture, organic farming, zero tillage farming and agroforestry are the most important key words for a new and sustainable mangment of soil. The use of beneficial microorganisms represents a promising tools for improving, not only growth and yield, but also the nutrient quality of crops in the modern and sustainable agriculture and horticulture as reported by Baum et al. (2015) and by Goicoechea and Antolın (2017) in their review papers.

Soil quality is strictly linked to security food as well. Soil degradation consists of biological, chemical and physical degradation. The FAO - Food and Agriculture Organization of the United Nations (2015b) reports that about 33 percent of soils in the world are moderately to highly degraded, characterized by poverty and food insecurity. Through soil degradation 12 million hectares of agricultural soils are lost every year. Soils with soil organic matter content lower than 0.8 percent are unproductive and often abandoned. “End hunger, achieve food security and improve nutrition and promote sustainable agriculture” is the Goal 2 of the 2030 Agenda for the Sustainable Development of the United Nations.

Soil quality assesment

Soil quality assesment and its monitoring is a must for a sustainable agro-environment mangment. Soil function describes what the soil does. Soil functions are: sustaining biological activity, diversity, and productivity; regulating and partitioning water and solute flow; filtering and buffering, degrading, immobilizing, and detoxifying organic and inorganic materials, including industrial and municipal by-products and atmospheric deposition; storing and cycling nutrients and other elements within the earth’s biosphere; and providing support of socioeconomic structures and protection for archeological treasures associated with human habitation (Seybold et al, 1998).

Soil quality assessments can be conducted by evaluating indicators (physical, chemical, and biological properties, processes, or characteristics of soils). Quality indicators are selected because of their relationship to specific soil properties. For example, soil organic matter is a widely used indicator, because it can provide information about a wide range of properties such as soil fertility, soil structure, soil stability, and nutrient retention. A complete and useful guide is provided by USDA

(2001) for assessing soil quality in the conservation planning process. For each particular problem (e.g. compaction, crop diseases, soil life) potential management solutions to improve soil quality are suggested.

Beside physical and chemical indicators (e.g. soil structure, water holding capacity, pH, electrical conductivity, N, C, P), biological parameters such as microbial biomass carbon (C) and soil respiration in term of CO^2 production a re particularly important to assess the quality of soil (Doran et al, 1996; Larson and Pierce, 1994; and Seybold et al, 1997). Fungi are listed as one of the most important biological indicators due to their ecological role of decomposer organisms and have the key role for nutrient recycle. Many methods have been developed to measure the quality of soil. A paragraph (Evaluation of bioremediation effectiveness in contaminated matrices by performing ecotoxicological and genotoxic tests) of the chapter 6 in this thesis is partly focused on this subject and presents different methods for soil quality assessment in term of toxicity.

Soil managment and quality of agriculture in Colombia

Soil is a finite natural good and a fundamental component of the environment, consisting of minerals, air, water, organic matter, macro and microorganisms that perform permanent biotic and abiotic processes, fulfilling functions and providing ecosystem services which are vital for society and the planet. The importance of soil for societies has been recognized since prehistoric times as the basis of the subsistence of civilizations for the production of food, fiber, wood, among others, subsequently it has recognized that soil is multifunctional and not only a means for production. Thus, the conservation of the soil or its deterioration depends to a great extent on the use made and the management to which it is submitted. Soils are generated and modified by the climatic, geological and ecological structures and processes of the territory, and their state also depends on the social, economic, technical and political processes to which they are subjected (MADS, 2013).

In Colombia, the most relevant processes of soil degradation are erosion (physical and mechanical loss of soil due to water or wind), sealing of soils with an agricultural vocation (soil occupied by urban constructions and infrastructure), pollution (presence of solid, liquid or gaseous hazardous waste), loss of organic matter, salinisation (presence of salts in the soil), compaction (reduction of soil porosity) and desertification (degradation of arid, semi-arid and dry sub-humid areas); phenomena which seriously affect the Andean, Caribbean and Orinoquia and begin to be felt in the Amazon and the Pacific, particularly in areas of rapid deforestation and mining and oil (MADS, 2013).

Soils located to the east and west of the mountain ranges (Orinoquia, Amazonia and Pacific), make up almost 50% of the national territory, while the area developed the country (Andean and Caribbean regions), which supports 80% socioeconomic and political activity, corresponds to a little less than 40% (Cortés-Lombana, 2004).The Caribbean plains, mountain ranges, plateaus and valleys have the best soils for agriculture, and have been subjected to intense agricultural activities for centuries, causing degradation and pollution (MADS, 2013).

In the country they are carrying out actions to improve this situation by increasing the number of protected areas and initiating watershed management. Also, the diversity of ethnicities and cultures has been recognized, reason why it was necessary to established standards to protect and ensure the life of indigenous and Afro-descendant communities by assigning them large areas of the territory. In the Indigenous Reserves and in areas of collective ownership of Afro-Colombians, land management is decided by community and corresponds to their visions and life plans (MADS, 2013).

However, in most of these positive actions the importance of sustainable soil management and its role in the structure and functioning of ecosystems has not been recognized with sufficient force. In this area and considering the social, political, economic, cultural and environmental context of the country, the existence of an agrarian structure, based on an unequal distribution, use and land tenure is identified. Associated with this, the armed conflict in Colombia is a phenomenon that substantially affects the population in general, because their complex edges account for problems of soil degradation that have led to forced displacement and violation of human rights. In addition, practices surrounding conflicts and socio-environmental such as drug problems, illicit crops, the undervaluing of nature, megaprojects (mining, agriculture, infrastructure, etc.) undertake not only the daily lives of individuals but which also generate adverse effects on the components of nature, such as soil (MADS, 2013).

Among the direct factors that affect soil degradation are the natural ones and the anthropic ones, the latter are related to the forms of use (use and management) of the soil and are those that can be treated more easily, therefore, a strategy aimed at the adequate location of productive activities is fundamental to prevent soil deterioration (MADS, 2013).

Chemical soil degradation refers to the loss of nutrients and their imbalance, undesirable changes in pH (salinization or acidification) and contamination. Nutrient loss or imbalance reduce soil productivity and can even lead to exhaustion when nutrients extracted by crops, particularly monocultures are not replenished. Undesirable changes in soil pH reduce the soil's ability to support plants and have negative effects on soil biota; in the case of acidification (reduction of pH) and elements such as Aluminum (Al) can reach toxic levels; the increase of pH by salts due to excess fertilizers, inadequate irrigation and bad drainage, which occurs particularly in dry and arid areas, means that a considerable number of plants cannot survive (MADS, 2013).

Biological degradation in turn, is related to the loss of organic matter and soil biota, due to inappropriate tillage practices, deforestation, and changes in land use without conservation measures, infrastructure works, and poorly planned urban development, among other aspects. The loss of organic material results in decreased biological activity and populations of micro, meso and macroflora and has negative effects on the physical and chemical properties of the soil; In addition, the mineralization of organic matter (passage from organic forms to minerals) generates CO2 (main greenhouse gas - GHG) and nitrates with pollutant potential of surface and deep waters (MADS, 2013).

The agricultural activities are another important driving force that can generate negative impacts on the soil, its functions and ecosystem services. Unsustainable agricultural production derives from the use of inappropriate technologies, which can lead to degradation of natural capital and threaten the possibility of durable production. The use of such technologies impoverishes and contaminates soil and water sources (due to the increasing use of agrochemicals), causes loss of biodiversity (due to the specialization of crops) and generates GHG. In Colombia, land use in pastures and in livestock farming has largely surpassed the area with a vocation for this use and it is clear that this expansion has been done at the expense of soils that have another vocation, either agricultural or forestry (Corrales and Torres, 2002; MADS, 2013). Underutilization of soils in agriculture and their overuse in livestock are indicative phenomena of inappropriate land use (conflicts of use) that introduce factors of economic and social inefficiency (PNUD, 2011).

Conventional agriculture has caused a significant decline in carbon depositional organic matter and soil quality, as many agricultural systems lose carbon through oxidation and erosion, which rates are higher than fixation and stabilization. The greater oxidation is due to the increase of the aeration of the soil and the greater contact of the residues, in terms of erosion, this increases when the surface of the soil is uncovered and exposed to rain and wind. It is important to note that plant carbon enters the pool of organic soil carbon (COS) in the form of plant residues, roots, root exudates or animal excreta (Burbano, 2010).

This form of agriculture is due to a set of transformations that the Colombian rural society suffered from the middle of the XX century and that altered the forms and relations of production in the field (UNDP, 2011), in fact the farmers agree that from the spread of practices related to conventional or industrialized agriculture intensified agricultural mechanization, the use of chemical products and the monoculture modality. Practices disseminated from the Ministries of Agriculture and commercial houses, which guided the modernization of productive processes to increase productivity and thus their commercial surplus (Clavijo and Pérez, 2014).

Thus, the situation of unsustainability has been the result of not only individual decisions of rural producers and owners, but of the implementation of agricultural development policies that by action or omission have contributed to the country's arrival in this state. They expressly support technologies that are highly dependent on external resources, the use of improved seeds and the adaptation of natural conditions to the needs of these technological packages. On the other hand considering the possibilities or comparative advantages that counts as tropical country, highly biodiverse and enormous potential for producing supported in their own potential it is systematically denied. All of the above produces a very high dependence on external resources, the weakening of the control over the productive processes and an increasing vulnerability of the production systems, in front of the tensions or shocks that press them. In summary, in an–ever lower sustainability (Corrales and Torres, 2002).

Boyacá department has a long history of land use, so over time the ecosystem is degraded and currently there exist traditional practices ever retained soil quality. The soil that is used mainly in the agricultural activity fluctuates in consecutive cycles of potato (*Solanum tuberosum*), to systems of extensive grazing, with the incorporation of rotations with cereals like barley (*Hordeum* sp.), Wheat (*Triticum* sp.), bean (*Vicia faba*) and corn (*Zea mays*). Its use is mainly conditioned by the slope and periods of rain and drought, although mining activity (coal mining) is also evident. The destruction of native forest for the implementation of agricultural works has brought with it the erosion. In addition, despite advances in science and technology, sufficient methodologies have not been developed to take advantage of indigenous resources in order to provide added value (Aguirre-Forero et al., 2012)

Although prehispanic practices such as mixed cropping that allowed nitrogen fixation, cultivation at different altitudes, maintenance of fallow land for the rest of the soil and construction of infrastructure to facilitate irrigation in dry areas were preserved, time should merge with foreign technical or assimilate completely when growing plants was introduced. However, this adoption did not result in an increase in productivity due to the ineffectiveness of foreign technology package. In the cundiboyacense region the use of iron tools was intensified (sickle to reap wheat and barley), which facilitated the deforestation to extend the agrarian border. In addition, the introduction of the ox, a heavy animal that dragged the plow through the soil, contributed to the loss of organic matter and soil structure, compaction and root uptake, contrary to what indigenous digging sticks did. As for the fertilization of the soil, the introduction of livestock did not result into the conscious use of fertilizers of animal origin, but the habit of leaving vegetable waste left on the surface. The practice of slash and burn in dry Andean forests accelerated the loss of vegetation cover, leaving the soil exposed to erosive agents. In short, land use was modified, due to new patterns of settlement, tools, techniques and introduction of foreign species (Mora, 2011).

Currently it is difficult to find in Boyacá potatoes (main agricultural product of the region) produced without agrochemicals. Their crops have intensified even in areas of native and endemic flora, generating in this way multiple conflicts between farmers and environmental authorities. The productive intensity of the soils has generated multiple conflicts in the forms of production and transformation of the landscape. The abuse of "toxic" and chemical agricultural inputs, since the green revolution, accompanied by mining and oil exploitation, have been increasing soil erosion, the decline of genetic material and therefore the loss of considerable diversity that each is further consolidated in the homogeneity of "improved" seeds produced for industrial purposes. Farmers and settlers point to the intensive use of agrochemicals as the main factor in seed loss (López, 2015).

This allows a glimpse than conventional farming negatively impacts the environment and rural society by intensive use of technologies that enhance production and reduce environmental supply, which is expressed in reduced availability of nutrients in the soil, loss of quality and availability of fresh water, less biodiversity, extinction of genetic resources, increased resistance to pesticides in some species and destruction of natural control mechanisms, among other aspects. There is also a complex economic and social problem, such as that faced in rural areas of Colombia, especially in the peasant family farming system, such as that prevailing in the central departments of the country. Boyaca manifested in high levels of poverty, poor schooling, predominance of small farms, increasing migration of rural population to other than agricultural activities and very little added value in agricultural products (Fonseca and Cleves, 2015).

Conventional agriculture set different parameters of production that took away agricultural activity concept from the concept of agroecology and turned it into a business where the deterioration of natural resources is a consequence of the productive process. Thus, productive processes, consumption patterns, social relations of production, technological platform, economic perspectives and relations of society with nature have been transformed, which has generated in local cultures and beliefs the loss of practices and ancestral knowledge, a situation that has led to discouraging the care of the environment and increasing poverty (Plazas et al., 2011).

The continuous depletion of natural resources due to the use of inadequate agricultural practices such as those characterizing conventional agriculture continues to be a major environmental concern worldwide. Land use is one of the most important impacts since it represents the basis of food production and other basic needs, for both rural and urban communities, so it is necessary to implement sustainable forms of agricultural production which preserve the quality of this important resource (Mendez and Viteri, 2007; Beltrán, 2014).

The FAO (2017), raises three tools for sustainable land management, first is conservation agriculture -AC, which aims to achieve sustainable and profitable agriculture and therefore aimed at improving the livelihood of farmers, by applying three principles: minimum soil disturbance (no tillage), permanent land cover and crop rotation. If the two systems, conventional agriculture and Conservation Agriculture, are applied to plots with the same agroecological and fertility conditions, significant differences in productivity may be appreciable after a medium-long term in favor of conservation agriculture. The second principle is the Integrated Management of Plant Nutrition (IMPN) that aims to improve and maintain soil fertility and soil productivity, reducing environmental degradation. The IMPN optimizes soil conditions, ie, the physical, chemical, biological and hydrological to increase agricultural productivity properties while land degradation is minimized. The third strategy corresponds to the Good Agricultural Practices (GAP), standards and regulations that have been developed with the aim of codifying product practices at the farm level and that they ensure compliance with the requirements of trade regulation and state regulatory requirements (food quality and safety).

In this regard, there are forms of agriculture causing minimal impact to the environment and which have developed in communities through participatory research in which ecological, organic or biological agriculture stands out which groups a variety of techniques such as minimum tillage, organic fertilizers, green coverage and biological controls for pests and diseases, among others, leading to a fairer development with the environment (Ramírez-Amaya and Hurtado, 2013). An agriculture with cause, which takes care of plant, microbiological, animal and human nutrition, as well as the proper management of soil, water and the environment, so that diversity is maintained and ultimately sustainable (García, 2005; Plazas et al., 2011).

Thus, agricultural activity with a tendency towards sustainability presents a series of ecological, environmental and cultural qualities that contribute to a better utilization of natural resources within the production system and to establish a less aggressive relationship with the surrounding territories. These characteristics are grouped into the following elements of sustainable production: first, the use of local resources (production that respects the ecosystem conditions, plants and animals of these ecosystems, knowledge of local people, culture, energy sources and the family); second, complementarity, with varying degrees of success, in the use of resources, so that the different cycles can be closed to the maximum, third, it is about keeping the soils permanently covered and emphasizing the use of trees (management of nutrient and energy cycles, strengthening of flows) and, lastly, the tendency towards little or no use of external inputs (Corrales and Torres 2002).

This agricultural activity, which is closer to the current concept of agroecology, is characterized by family participation, collaboration of neighbors (hand returned and treat), management of own seeds, minimum tillage, composting, polyculture, diversity of fauna and flora, little or no pesticide use and conservation of water sources. Agro-ecological processes have been assumed by tradition, practice or need and are recognized by the population as an alternative food production that gives color, smell, flavor and nutritional quality (Plazas *et al*., 2011).

Subsistence agriculture is also a type of natural agriculture, which has been maintained over time in different regions of the world in order to satisfy the demand for natural and nutritious food, so it is developed on the basis of certain values, traditions and different rationalities from those of market agriculture. Subsistence farmers act based on a strong rationale for risk reduction, focusing on food security more than on profit and growing traditional products in their diets. These farmers would be reluctant to change land use if they have to make investments, take credits, change traditional food crops, and put food security at risk (Vieira and Wambeke, 2002).

In peasant production systems and in indigenous and Afro-Colombian communities, it is possible to find elements that provide keys for the design of sustainable production and management systems. In fact, indigenous people are often given the opportunity to participate in conservation, but in the opinion of their organizations they are rarely allowed to demonstrate that their livelihood strategies can be conservation tools. This explains his claim as to obtain autonomy in their management, administrative control of conservation units or ownership rights over the resources of the ecosystem (Tresierra, 2000). Nevertheless, ignoring about the ancestral practices developed by these ethnic groups and peasant communities in relation to the use and sustainable management of the soil could lead to the disappearance of this knowledge, losing a significant part of the cultural heritage and knowledge that can give insights on the management and sustainable management of the soil in the different territories (MADS, 2013).

In this context it is possible to understand that the role of the peasantry in the conservation of natural capital goes beyond being its guardian. Farmers have exercised this function and are able to do so on a larger scale through the development of productive activities that are environmentally and economically viable, which certainly implies important work on the reconversion of their current productive systems, so as to reinforce the elements of sustainability. The recognition of their role should be expressed in better conditions of access to land and the means to produce (Corrales and Torres 2002).

After evaluating some agro-ecosystems using as a methodological reference the Framework for the Evaluation of Natural Resource Management Systems Incorporating Sustainability Indicators - MESMIS, it was found that the sustainability of agro-ecosystems depends on biotic characteristics and production management, organizational status of rural families (dedication of family members to agricultural activities), the best ability to incorporate farming practices, low cost, low risk and a lower level of dependence on external inputs (better use of organic fertilizers and sanitary crop management) (Fonseca and Cleves, 2015).

In this regard, it was possible to learn about the experiences of associations of producers and other organizations that have been reducing the traces in the potato for a little more than a decade, betting on the recovery and production of native seeds with clean practices. It must be noted that the decrease trace not only applies to the seeds, but this involves cleaning, recovery, nutrition and fertilizer soil where they will be planted, this is the reason why farmers have gone to the principle of mutal, joint planting of seeds in the same crop-hole, which grow and feed each other and on allelopathy, technique applied to lower production scale, non-intensive productions, where crop diversity establishes reciprocal relationships between plants, in their chemical and nutritional composition, excreting odors that repel insects and act as ecological barriers. Mutual and allelopathy have been historically transformed due to the arrival of new species, climatic changes and technological innovation (López, 2015).

In this scenario, from the agronomic point of view the solution to recover and maintain the productive capacity of the soils so that the sea can obtain benefits without depleting the resource is in the content of the organic matter of the soil, and alternatives of management that in addition to being effective to adequately maintain this content, are feasible for the farmer's possibilities. For this reason, the interest of the researchers has focused on the exploration of alternatives such as bocashi, a fermented organic fertilizer product of an aerobic decomposition process and thermophilic organic waste, carried out by microorganisms which produce a partially stable material of slow decomposition under favorable conditions. In addition to the bocashi, several biopreparations have been developed, including the rhizosphere broth and the super four broth. The bocashi supplemented with nutritious microbial broths allows not only to add organic matter to the soil, but also to dynamize its biological component and to balance the nutrition of the crops (Restrepo, 1998; Méndez and Viteri, 2007).

Likewise, it is important to search for species that produce green manures with potential as an alternative source of organic matter, especially to manage and rehabilitate acid sulphate soils (SSA) with sustainable projection. In Boyacá, some species that offer good potential as green manures for this type of soils are forage turnip (*Raphanus sativus* var. *oleiferus* Metzg), oats (*Avena sativa*), rye (*Cecale cereale*), sunflower (*Helianthus annus*) and Vetche (*Vicia sativa*). Forage radish (*Raphanus raphanistrum*) proved to be an excellent species in terms of fresh and dry phytomass production up to 75 days after sowing, later its potential decreases by the attack of aphids. The success of these producing species green manure is highly dependent high degree of acidity correction, therefore, calcareous applications such as limes, dolomites or phosphoric rocks should be applied to the soil (Hernández and Viteri, 2006; Viteri et al., 2008).

Soils have an inherent quality, given by their chemical, physical and biological characteristics. Biological properties can show the changes that occur due to the environment, which evidences the effect of certain management practices on the health status of the soil, therefore, these properties can be used as bioindicators. Biological indicators or biomarkers have gained strength because of its sensitivity and speed of response to disturbances and variables introduced in the soil ecosystem and its inclusiveness. Microbial soil populations are good indicators as they can respond rapidly to changes introduced into the system (Murillo et al., 2014).

In fact, the development of bio-inputs involves the study of the microbial diversity associated with each type of soil and the specific vegetable crops, as well as the inherent ecological processes. An interesting possibility is to explore native microbiological reserves because they offer the potential to develop alternative technologies for farmers, who demand accessible methods that do not pose risks to the ecosystem or health. Among the most used microorganisms, there are five morphological groups: filamentous fungi, mycorrhizal fungi, yeasts, Gram positive bacilli and Gram negative bacilli. The effectiveness of the microorganism is the most important for the production of bio-inputs and is the essence of biological products. In Colombia, produced and imported bio-inputs are mainly used for ornamental crops (25%), vegetable crops (16%) and cereals (13%), while the lowest percentage of bio-inputs available on the market is for tuber crops %), cotton (6%) and coffee (4%) (Zambrano-Moreno et al., 2015).

Thus, the use of microbial amendments represents a friendly option from the environmental point of view as an alternative to application of mineral, soluble fertilizers. These amendments are produced from microorganisms with different plant growth promoting capacities and are collectively called plant growth promoting microorganisms - (PGPM) (plant growth promoting microorganisms). PGPM can affect plant growth through a wide range of mechanisms such as inorganic phosphate solubilization, decreased levels of ethylene in plants, fixation of atmospheric nitrogen, biocontrol of plant diseases and production of phytohormones, siderophores and organic acids (Datta et al. al., 2011). Phosphate solubilizing microorganisms are a functional group of PGPM that include not only bacteria but fungi and actinomycetes whose ecological role is essential because they have the ability to solubilize mineral phosphates that have been fixed in the soil and can not be used by plants in their nutrition (Beltrán, 2014).

In spite of this, the development of bio-inputs for agricultural use in the country, from its initial stages to the development of patents, has ignored the processes to promote the use of microbial biodiversity The methods of generating bio-inputs are not promoting the development of new forms of production, culture media, presentations and ways of application, so it is necessary to obtain individual microorganisms or microbial consortia, which allow solving the problems affecting the soil resource(Zambrano-Moreno et al., 2015).

Conventional remediation and bioremediation of polluted soil in Colombia

Colombia is a country with an important agricultural and industrial activity that consumes large volumes of chemicals of varying toxicity. By

2010, it is estimated that a little more than 24,000 tons and 25 million liters of pesticides were produced; about 53,000 tons of this material were imported, as well as the production of drugs, cosmetics and hydrocarbons, among others (ICA, 2011).

The use of pesticides in Colombia has followed the general guidelines of the market of agrochemicals on an international scale. In the 1950s, organochlorines (OCs), mainly dichlorodiphenyltrichloroethane (DDT) and its derivatives, were used together with arsenical pesticides in agricultural, livestock and malaria control. Subsequently, compounds such as organophosphates (OF), phenoxyacetates and dithiocarbamates (maneb, mancozeb and zineb), used in the most important crops of the time: rice, sugar cane, potato and banana began to be used. Phytosanitary problems caused by the resistance of some pests appeared in the late 1960s to the early 1980s, prompting changes to products such as substituted ureas (diuron and linuron), triazines (prometrin), cotoran and paraquat quaternary ammonium). Since the mid-80 to 90 there was a surge in the use of fungicides due to increased flower crops and bananas, as well as an increase in the use of herbicides for weed control. The most commonly used fungicides were mancozeb, sulfur compounds, copper oxychloride, captan and edinfos; the herbicides were based on 2,4-D (phenoxyacetate), propanil, glyphosate, diuron, triazines (atrazine and ametrine), paraquat, picloran, butalla and pendimethalin. Among the insecticides were OF such as chlorpyrifos, methyl-parathion and monocrotophos, as well as carbofuran (insecticide carbamate) (Herrera-Rojas and Polanco-Rodríguez, 1995). In the country, cholinesterase-inhibiting pesticides (OF and carbamates) are the most frequently used insecticide group in crop pest control (Cárdenas et al., 2005; Idrovo, 2000 Diaz et al., 2017).

In general terms, it can be stated that the Colombian population is exposed to non-biodegradable pesticides (OC and base metals or metalloids), exposure to other pesticides is much more limited occupationally exposed groups, especially in agricultural regions, however, the possibility of exposure through ingestion of food among populations residing in areas where the use of these chemicals is common cannot be ruled out. In addition, the model of agricultural development in the country is based mainly on the use of agrochemicals, which are used without sufficient technical research, ignoring the multiplicity of regional characteristics such as the variety of climate, species diversity and the heterogeneity of cultures (Idrovo, 1999; Idrovo, 2000). Thus, the use of pesticides in Colombia presents a disturbing panorama of problems that affect the productive process of crops, contaminate agroecosystems and affect the health and quality of life of human groups (Vergara, 1990).

In the case of agriculture, the risk with permanent crops (long cycle) depends mainly on the use of agrochemicals that can lead to contamination (by salinization, pesticides and heavy metals), while transitional crops (short cycle) represent risk of soil degradation in aspects of loss of organic matter and soil biota, compaction, desertification and erosion, however, it is necessary to consider that the sediments from the latter, can carry toxic substances with potential to contaminate water or other soils (Alcaldía Municipal de Soracá, 2004).

In the center of Boyacá department, pesticides commonly used to control diseases, pests and weeds in crops of potatoes, red beans, corn, and beans are: Dithane, monzate, lorsban, antarcol, fitoras, propineb and malathion, the most used active compound is the Ethylenebidithiocarbamate (Mancozeb), which is used in potato for the control of late blight (*Phytophthora infestans*) and early blight (*Alternaria solani*) and in beans for control of the downy mildew (*Phytophthora phaseoli*) and anthracnose (*Colletotrichum lindemuthianum*) (Gonzalez et al., 2012). The potato that is the most important crop in terms of food and economy in the department, is produced mainly by small farmers who resort to the use of chemical fertilizers and pesticides to increase yields, in fact, it has been perceived that producers are over utilizing pesticides, both in quantity and quality (Ospina et al., 2008).

In this perspective and in view of this wide spectrum of contaminants, solutions have been developed in the country based on biological approaches (bioremediation or bio-adsorption) and physicochemical approaches (advanced oxidation processes). Interdisciplinary research from the biological and chemical base using unconventional techniques has shown promising results to address in a short time the implementation in the field, at a pilot level and to continue development at the industrial level. The development of transversal projects in both basic knowledge and applied in bacteria, fungi and physicochemical processes, will reveal specific metabolic pathways of organisms for bioremediation promising. The results obtained so far are encouraging, the balance between the two technologies (biological and physicochemical) allows to affirm that both have advantages and disadvantages and that each problem must be approached differently. Biological processes are generally cleaner than physicochemical technologies (under some environmental constraints) but, on the other hand, they present catalytic mishaps because they are more sensitive to environmental changes, which leads to a decrease in the conversion or level of mineralization of contaminants. On the other hand, physicochemical processes are easier to control, so higher conversions can be achieved; however, these processes must use catalysts that can generate collateral problems: their treatment (Dussan et al., 2009).

In this way, bioremediation techniques are an important alternative solution to manage/neutralize the effect of the indiscriminate use of fertilizers and chemical pesticides, as they allow eliminating or immobilizing contaminants, transforming them or degrading them to less dangerous or harmless substances. These techniques using microorganisms with the capacity to use the contaminants as an energy source or as acceptors and electron donors within the respiratory chain, improve the quality and physico-chemical conditions of the soil, contributing to the growth, yield and quality of crops (Eweis, 1999).

In Boyacá, Benavidez et al. (2006) investigated bioremediation from an agricultural soil contaminated with nitrogenous fertilizers, using a native consortium of denitrifying bacteria, which allowed to combine and complement the metabolic functions of different microorganisms which allowed combining and complementing the metabolic functions of different microorganisms to collectively denitrify the soil. They concluded that bioremediation of soils contaminated by nitrogenous fertilizers (poultry, urea) can be carried out by the application and / or bioaumentation of indigenous denitrifying microorganisms, using previously reduced forms of inorganic nitrogen or previously reduced ammonia converting it into gaseous substances like N_2O, NO and N_2, which are emitted to the atmosphere (Rich et al., 2003).

The great diversity of microorganisms in terms of richness, abundance and metabolic versatility reveal Colombia's potential to implement bioremediation processes, in order to develop ecologically-friendly practices, without the need to introduce foreign species or to use other techniques currently being discussed such as thermal desorption that would increase the concentration of CO_2 and, therefore, the greenhouse effect that contributes to global warming (Dussan et al., 2009).

On the other hand, the contribution of applied research, besides the implementation of clean and integrated production models where Good Agricultural Practices (GAP) are certified, would allow to reduce the consumption of agrochemicals, for the fruit sector, for example, is equivalent to 30% of total production costs, which would provide an economy of scale beneficent for the producer and thus to the final consumer (MARD, 2006). Regarding clean production, Varona et al. (2005) carried out the study on the use and management of pesticides in flower companies for the control of pests and diseases and found that a high percentage of these companies used alternatives other than the pesticides. The most commonly used alternatives were eradications, traps, the fungus *Trichoderma harzianum*, extracts of garlic-chilli and tobacco extract. Although it is necessary to measure the impact of using these methods, it is positive observe the use of different alternatives to agrochemicals, especially if the introduction of these techniques results in a reduction in the use of these chemical inputs, however, it is also necessary to monitor the potential side effects of so-called biological products.

As management practices, Varona et al. (2012), studied the impact on health and the environment from exposure to pesticides and implementation of GAP in tomato cultivation, the authors found that the most used pesticides were OF, of which Lorsban was the most used during the harvest, followed by Carbamates, dithiocarbamates, pyrethroids and derivatives of N-phosphonomethyl glycine. In the soil analyzes in the conventional plots the presence of pesticides mainly dithiocarbamates was evidenced, whereas in the plots with GAP after the cultivation, only one registered the presence of pesticides. It is clear that the use of GAP helped to reduce pesticide residues in the soil. By comparing the tomato production obtained in the two cropping systems found in plots with GAP increased production, because the fertilization plan successfully met the needs of the soil. The study showed that soils previously treated with OC stored residuals of their metabolites. Finally, the advantages of this production system and the need to strengthen agricultural producers in the implementation of GAP are pointed out.

Outline and scope of the PhD thesis

The scientific literature, partly discussed in the previous paragraph, strongly proves that soil quality in the agro-environment is closely linked to the land use and management. One of the biotic components that influences soil quality is fungal community. Fungi, together with bacteria, ensure the recycling of nutrients, and a balanced and stable fungal community is at the base of the functioning of soil ecosystem and health of the environment and living beings. From mycological point of view, there are areas, on the planet, that have been studied in detail. On the contrary, there are areas where, mycological studies are either at the beginning or completely absent. One of the less studied areas is the Colombian environment, although it is of special importance from economic point of view, as discussed in the introduction.

The main purpose of this thesis is to contribute to the knowledge on the soil fungal community of the Upper Andean Colombian agro-environment. Due to the importance of the soil quality in the agricultural context and the importance of fungal component on the soil quality itself, the mycobiota was analysed with respect to the land use (apple and peach cultivated, woodland, uncultivated grass field).

Moreover, a case study has been identified in an Italian farm to study the effect of soil management on the soil fungal community. This study focused on a cultivated area with different forms of soil management: conventional, organic, no-tillage.

All the mycological analysis was carried out by using next generation technologies.

The following specific objectives were addressed in chapters II to VI of this thesis:

1) To study the soil use and management in the Colombian Amazon, both from biologically and sociocultural level. Colombian Amazon is an important model for the traditional utilization of natural resources in the country (Chapter II).

2) To know by comparative analysis the diversity of native microscopic soil fungi that has been already reported in the

natural regions of Colombia: Andean, Amazonian, Caribbean, Orinoquía, Pacific and Insular (Chapter III).

3) To evaluate, through a metagenomic-based picture, the Mycobiota of a Colombian Upper-Andean agro-environment in order to characterized the soil as a basis for an ecologically sustainable land use (Chapter IV).

4) To evaluate the influence of diverse agricultural management practices (conventional, organic, non-tillage agriculture) and seasonality on the composition of the soil fungal community in agroecosystems, through a metagenomic-based, cross-seasonal picture (Chapter V).

5) To scout, from the literature, soil fungi useful for bioremediation of chemical pesticides, as a sustainable strategy for recovering the quality of degraded agricultural soils (Chapter VI).

This thesis is composed of one published scientific article, one published reflection article, one accepted manuscript under revision procedure, and two advanced stage manuscript. The papers are listed below.

Panelli, S., Capelli, E., Comandatore, F., Landinez-Torres, A., Granata, M., Tosi, S., Picco, A. 2017. A metagenomic-based, cross-seasonal picture of fungal consortia associated with Italian soils subjected to different agricultural managements. Fungal Ecology, 30: 1-9.

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Landinez-Torres, A., Panelli, S., Comandatore, F., Picco, A., Capelli, E., Tosi, S. 2017. Meta-genomic analysis of soil mycobiota in upper Andean Colombian agro-environment. Advanced version to be Submitted to Fungal Diversity

Landinez-Torres, A., Becerra, J., Tosi, S. 2017. Soil fungi biodiversity in Colombia: Microfungi. Advanced version to be submitted to Caldasia.

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II. Paper 1

Soil use and management in the Colombian Amazon

Soil use and management in the colombian amazon

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Abstract

This article reflects and analyzes the problems related to the practices of appropriation of the soil in the southern Colombian Amazon. The proposal considers both the biological and cultural ways of use and management of soil resources in indigenous and urban contexts, since such activities become an essential basis for the definition of strategic guidelines for the management of biodiversity in Colombia. Thus, the study presents a form of integration that allows to approach conflicting environmental scenarios, considering both biological and cultural factors in a number of areas in order to support the decision making process and allow a reasonable treatment of the information to implement mechanisms of environmental regulation, in a strategic biological area such as the Colombian Amazon. Finally, it is stated the importance of facilitating the analysis of the interrelationships of ecosystem components, including the human element, through a bioethical glance, which allow to plan biological and social guidelines for the sustainable use of biological diversity.

Keywords: biological, Colombia, cultural, soil, utilization.

Introduction

The way man takes advantage of natural resources affects directly both the level of social welfare and the existence of biodiversity[10]; in this sense, in the southern region of the Colombian Amazon, which comprises the departments of Caquetá, Putumayo, and Amazon[a], the main productive activities: aquaculture, forestry, mining and energy, livestock, and agriculture, are directly related to the use and management of the soil, and implicitly with biological diversity. It is remarkable to note that, use refers to the general practice, to a habitual and continuous use of resources, whereas management implies their controlling, that is to say, a way to manage them in order to keep them in the time.

The relationship between the main productive activities and biodiversity is conflictive, in this way, it can be evidenced that at biological level, one of the main problems in the south of the Colombian Amazon, especially

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[a] The region is divided into *Northwest Amazon* (Putumayo and Caquetá to the west) whose population is mainly colonist and urban; and *southeastern Amazon* (Amazon dedpartment and Caquetá to the southeast) characterized by the predominantly indigenous population.

in Putumayo and Caquetá to the west, emerges from the unplanned settlement in the region, as well as the inappropriate use and management of the resources through intensive productive activities; this has led to a drastic change of the natural environment and resources, causing a negative chain reaction effect such as the loss of biological diversity, chemical impoverishment of the soil and soil compaction, disturbance of the water balance, and the activation of erosive processes, which, through the time, make the ecological and socio-cultural production systems unsustainable[18].

Therefore, this article discusses the main ways of soil use and management in the southern area of the Colombian Amazon. It also analyzes the impact that these ways of exploitation of resources have had in both biological and socio-cultural aspects; finally, it presents an integrative perspective, from the bioethical view, which allows to approach environmental conflictive situations, considering factors not only biological but also cultural in a number of scenarios in order to facilitate the implementation of mechanisms for environmental regulation.

Soil use

The southern area of the Colombian Amazon corresponds to about 47% of the whole Colombian Amazon area[17]; the main productive activities of the primary sector are based on the use of renewable and non-renewable natural resources. The secondary and tertiary sectors, do not contribute significantly to the economy, due to the limited availability of social equipment and economic infrastructure; thus, livestock, agriculture, and the exploitation of hydrocarbons, stand out as important activities in this subregion[10]. However, it is important to notice that these local production activities are being affected by the impact of crops for illicit use. In fact, for most of the Colombian Amazon area, the latter along with the extraction of valuable timber, fishing, the inappropriate use of biodiversity and forest services, as well as the trafficking of species, constitute the main threats for natural resources at the present time[5].

In relation to agricultural production in the south of the Colombian Amazon, it was estimated that between 1990 and 2003, this production fluctuated between 40 and 60% of the value of the production of goods and services in the area; with regard to the participation by departments in the Gross Domestic Product - GDP, there are differences: in Amazon it ranged between 7 and 30%; in Putumayo between 27 and 69%, and in Caquetá between 44 and 66 per cent[20].

Cattle ranching prevails in terms of livestock activity. In 2003, it was used an area of pastures of 2,331,006 ha of which 2,202,822 are estimated to be in Caquetá and 28,184 ha in Putumayo; with a total of 1,341,116 animals, that is, on average 1.74 head of cattle per hectare. As a matter of fact, in the foothills of Caquetá half of the small farmers (farms of less than 100 ha) deforested annually between 1 and 2 ha of forest reserve, to turn them into grasslands and enhance their properties. This is a grave practice since these farms are located in the mountain range where steep slopes are found; with regard to the farms between 101 and 500 ha, these are located in soils with slopes that are greater than 10%; this makes them susceptible to erosion, even more if there is no management of agroforestry systems. On the other hand, large farms (over 500 ha) that are located on flat land maintain more than 200 ha in introduced pastures[1]. By 2012, it was estimated that the south of the Colombian Amazon comprised a total of 22,268 km^2 of grassland, that is 4.6% of the surface of this subregion, however this corresponds to 61.12% of the total pasture of the whole Colombian Amazon[17].

In addition, other species such as horses are found in this subregion; they are useful in livestock activities, productive and extractive industries (wood); buffaloes are also an alternative to cattle, however, although the effects of their introduction has not been evaluated yet, there is a perception that the ecological impact is significant because their behavior deteriorates the micro-basins[1].

In addition to the above, it has been proved that in this subregion, physiographic units of meson, terraces and floodplains predominate. These are inadequate for agricultural mechanization and highly susceptible to erosion processes as well as the accelerated loss of organic matter, especially when the forest is slashed and burned to be used for agriculture and livestock[19]. It is clear then, that the exuberance of the forest is not due to the good agrological quality of the soil, but to the particular functioning of ecosystems, which is based on a feeding cycle generated by the forest itself and the environmental conditions[5].

With regard to aquaculture production, it was observed that between 1992 and 1998, there was a considerable increase in the destination of the land use for this activity, especially toward the Amazon foothills. In 2003, in terms of area and production in Putumayo there were 609 fish farmers with a mirror of water of 182 ha and close to 2,128 t; in Caquetá there were 1,581 producers with 189 ha and 1,150 t/year and in Amazon 35 t/year[2]. By 2012, in Putumayo there were land covers that had not been detected on the maps in 2002 or 2007 which corresponded to ponds for aquaculture and the exploitation of hydrocarbons[17].

Thus, regarding water and fishery resources it has been evidenced in recent years that the influx of fish has decreased significantly due to the lack of management on the rivers and zoning of fishing areas, overexploitation, lack of technical assistance, and due to the effects caused by mining activity and spills of fuels[5]. In this way, although sustainable development can contribute to the economic welfare, it is clear that conservation challenges must be assumed, especially when considering that the preservation of freshwater ecosystems is directly associated to the conservation of the Amazon forest[2].

Soil Management

According to the proposal by von Hildebrand et al.,[25] about the conservation and sustainable management of biodiversity in the Colombian Amazon, the impact produced by the productive activities can be categorized on a scale of 1 to 10 (1: least impact, and 10: greatest impact). Considering this, alluvial mining was rated 10 because this is a highly destructive practice; the cultivation of coca leaves was rated 5 because, despite its illegal status, it is usually organized under some type of cover which helps to mitigate the effect of physical agents on the ground; finally, the indigenous shifting agriculture was rated 1, since studies show that this does not cause any damage to the Forest. In connection with this comparison, Valencia et al.,[22] also present an analysis of the negative impact of livestock activities, agriculture, mining, and oil exploration on the biodiversity in the south of the Colombian Amazon. This analysis is presented as follow:

With regard to cattle activity, its impact can be seen in the transformation of the landscape and the effect of livestock on the ground; in fact, it is estimated that for each hectare cultivated, 16 ha are used for extensive livestock, a practice that leaves large tracts of land with the minimum number of species possible. Thus, the establishment of a hectare of this type of livestock competes with about 185 tree species, in addition to the remnant of flora and fauna associated to it. In this way, the livestock model that predominates in the region becomes a problem for the conservation and sustainable use of biological diversity; this is the case of Caqueta, where, according to the soil vocation 1,860,263 ha are suitable for the development of this activity, however, close to 2,347,245 ha are used. In 2012 it was estimated that the total area transformed of Caquetá corresponded to

19,556.4 km^2, this means that this is the department with the largest area that has been changed from natural covers to covers of anthropic origin[17]. This has an impact on the compaction of the soil due to the change of its use, the fragmentation and degradation of ecosystems and on the loss of biodiversity; in addition, this creates social conflicts because of the concentration of the ownership of the land, a process that also happens in Putumayo, in a smaller scale, though.

With regard to agricultural activity, the implementation of monocultures like bean in the Andes-Amazon region of Putumayo, has led to serious environmental and health effects, due to the intensive use of agrochemicals and the depletion of soil[19]. Moreover, the cultivation of coffee in Caquetá has affected areas of the District of Soil and Water Conservation, not only of the department, but also of the National Forest Reserves in the Amazon - NFRA. In addition, there are conflicts between the protective vocation of protected areas and the activities that different communities of settlers and farmers develop in these areas.

For its part, the mining activity affects the vegetation cover and fishing activity and generates social and institutional conflicts; therefore, because of this activity hundreds of hectares of primary forest are destroyed each year in the region. In addition, the use of mercury for the separation of gold in the alluvial mining generates environmental pollution and has a negative impact on people’s health. According to Eclac and Natural Heritage[5], mining continues to increase and is one of the main threats to the stability of ecosystems and biodiversity in the Colombian Amazon region and in particular for the Colombian Foothills.

Furthermore, the oil activity developed in Putumayo has impacts related to the destruction of forests and the detriment of soils and water bodies as a result of illegal dumping and the emission of greenhouse gases; however, the strongest damage occurs by the oil spill caused by terrorist attacks.

Impact on biological diversity

The inadequate use and management of resources of biodiversity produces negative impacts such as over- exploitation, deforestation, loss of species of flora and fauna, the contamination of water sources, changes in soil use, among others, which necessarily bring about conflicts at socio-economic and cultural levels.

Specifically, in the south of the Colombian Amazon, Valencia et al.,[22] state that these problems have been motivated from the past mainly because of economic interests. An example of this was the use of rubber (*Ficus elastica*) and quina (*Cinchona officinalis*) as important raw materials, their use exceeded their renewal rate which generated the overuse of the resource as well as significant difficulties of social order. With regard to deforestation in the region, there are approximations in piloting areas that show alarming figures of degradation because of loss and transformation of ecosystems, especially in the ring of settlement whose process is more accelerated in the Amazon foothills with about 0.9% loss a year; this happens due to the population increase, caused by displacement. In this way, by 2001 the area of high-Putumayo was the area with the highest degree of fragmentation and deforestation, since the cover passed from 42% in 1989 to just 28%, represented by grasses that occupy the landscapes of foothills and plains.

In 2007, the departments with the largest losses of forest cover in the whole Colombian Amazon region were Caquetá (43,7%), Meta (16,3%), Putumayo (15,9%) and Guaviare (12,2%)[16]. Despite the fact that the Colombian Amazon is the region with the largest number of deforested hectares, the annual rate has declined in the last twenty years. From 1990 to 2000 the average annual deforestation rate had reached 119,800 ha. Between 2000 and 2005 this rate slightly decreased to 112,565 and dropped to 79,800 ha per year from 2005 to 2010[5]. However, Murcia et al.,[16] estimate that between 2000 and 2007 there have been a greater annual rate of deforestation corresponding to 153,000 ha/year.

The transformation of the natural hedges in the Amazon region is a constant phenomenon; in 2012 in the southern area of the Colombian Amazon the lower conversion of forests was observed to the southeast (Amazonas and Caquetá to the east), whereas the highest was found to the north of the subregion (Putumayo and Caquetá to the west)[17].

With regard to timber resources of the natural forest in the Amazon trapezium, these have been the subject of a persistent illegal exploitation that threatens the environmental goods and services, and cultural offers. In relation to this, table 1 summarizes the areas that are in conflict due to underutilization and overutilization as well as lands without conflict of use or appropriate use.

Table 1. Conflict of land use in the south of the colombian amazon

State of the Land	Type of conflict and degree of intensity	Description
Land with partial or total Anthropic intervention	Without conflicts of use or proper use	Agricultural activity in pastures, stubble and relict forests that are located in areas described for agroforestry. Located in the Colombian Amazon foothills and in the middle and upper terraces of the great rivers of Andean origin: watersheds of the Caquetá, Putumayo and Amazon.
	Conflicts due to sub-utilization (Moderate)	Land with agroforestry vocation, particularly agricultural that are used in extensive animal husbandry activities. Debris and fragmented forests that are located in the alluvial plains of the great rivers of Andean origin, in the northwest of Putumayo and in the foothill of Caquetá.
	Conflicts by overuse (Mild and severe)	Located in the northwest of Caquetá, Putumayo and locally in the Amazon, where lands with forest vocation of protection and protection-production, have been replaced by introduced grasses and low-intensity farming activities; they are also used for the establishment of crops used for illicit purposes.
Land with no or light anthropic intervention	Natural forests, sparse vegetation on rocky outcrops, eroded miscellaneous and rocky outcrops, other natural covers and urban areas.	

Source: Adapted from IGAC and Corpoica[22].

With regard to threatened flora species, the region has a species classified as Critically Endangered (CR) the rosewood or *Aniba rosaeodora*; this is the only species that faces a high risk of extinction in the wild. The ones that are listed as Endangered (EN), or that are facing population decline or extinction risk are as follow: cedar or *Cedrela odorata*, the cinnamon of the Andaquies or *Ocotea quixos* and the *Podocarpus oleifolius* since these are timber species with a strong risk due to harvesting. Among departments, Amazonas and Putumayo have eight threatened species each, whereas Caquetá have four; Most of these threatened species are timber ones, perhaps because of natural population decline caused by the removal and the reduction of habitat. In addition to this, the mentioned above species are not registered in the protected areas of the zone[4].

In relation to protected areas and the strategic ecosystems, according to Camacho[3], the main threats are gold mining, logging and the increase of settlements. Consequently, there exist dredgers even from other nations for gold extraction in Colombian jurisdiction; this causes erosion and low water quality due to the dumping of chemicals; with regard to the illegal extraction of timber for commercial interest, this is accentuated with the establishment of illicit crops and the lack of clarity in the limits of some indigenous reservations, which favors colonists' appropriation of places prohibited by the law; with regard to the progress of the colonization, it can be evidenced some deterioration of the ancestral territories, the loss of resources for traditional medicine, influence of people on natural resources, as well as difficult relations between the state and local institutions. Furthermore, it is necessary to strengthen intercultural dialogue and its dissemination, as well as the designing and implementing management schemes that include, not only the implementation of biological characterizations, but the implementation of sustainable alternatives.

In relation to this, instruments for long-term strategic planning for the management of the Amazon region have been proposed in recent years, specifically in the southern of the Colombian Amazon, in order to guide the management, focus and integrate the actions of all regional actors and agents for the improvement of public policies that ensure the process to progress toward sustainability in the region[7].

Biological diversity has been considered heritage of local communities, therefore, its use and control has been regulated in accordance with the customary law. In this sense, the exchanging terms for both biodiversity and associated knowledge, could be carried out freely, with the exception of those estimated as sacred. Subsequently, this biological diversity was considered as the common heritage of mankind, an approach which caused that transnational agribusiness and biotechnology companies began to take freely the resources of developing countries, being object of intellectual property and *patenting*[23]. Thus, we can distinguish two types of traditional knowledge to be accessed: the processes of transformation and use of biological resources developed by traditional communities and the biological material identified through the generations and that does not involve a process[24].

Later, with the advent of the Convention on Biological Diversity, biodiversity became a world heritage of each nation, this change can be interpreted ambiguously as the existence of a State that on the one hand, organizes access of transnational corporations that industrialize genetic resources; and on the other hand, representing the nation, has to do with public interests, including the rights of future generations, food security, public health, quality of life, and the conservation of natural resources[23]. An effort to protect traditional knowledge that at the end of the day, can paradoxically, corrupt its essence, because this could accelerate the commercialization of resources and the fragmentation of traditional societies[6]. In this way, the main challenge for the Colombian Amazon will be an economic growth and development that does not threaten the livelihood of their cultural and natural riches[15].

Cultural and social impact

In relation to the socio-cultural problems caused by the use and management of natural resources, De La Hoz et al.,[8] have pointed out that the current conflicts are not of Amazonian origin, but are the result of those occurred in distant areas, which affect farmers forcing them to move. This population has settled mainly in the northwest of the Amazon region (Putumayo and Caquetá to the west), where the majority of the migrant population can be found. They are characterized by the loss of food self-sufficiency, in contrast with the southeastern of the Amazon region (Amazon department and the southeast of Caquetá), where a traditional mode of production of subsistence prevails. Two interconnected but contrasting subregions, where agricultural techniques developed by indigenous communities and which allowed the natural balance, are being threatened by intensive agriculture of the settler and the emergence of capitalism; these issues have modified the productive trends and caused changes in the diets affecting health conditions which, combined with a weak production and trading structure, situate the region at a high level of vulnerability, with respect to food sovereignty.

In addition, these authors argue that, despite the fact that there is an estimate of the coefficients of dependency and self-reliance, preliminary studies indicate that in the Amazon households spend 98.5% of their income to cover their needs, of that value 83.3% corresponds merely to food. Moreover, the diet does not reflect the maintenance of indigenous habits. This situation causes nutritional, environmental, and social problems such as the decrease in the hunting and fishing practices, the sale of food produced at home, the contamination of water sources for human consumption, the absence of boiling water, inadequate disposal of excrement and wastes, the late introduction to complementary feeding breast milk, the substitution of foods exclusively by energetic ones, and poor sources of protein[8].

Also for the indigenous communities of the Amazon trapezium, the integration to the market is more related to demand than to supply of products; this has a high impact on food since villagers rely on suppliers to buy products that they used to cultivate or produce, so nowadays it is difficult to find a community that consume only the food that they produce, gather, hunt or fish. In this way, the impacts on food security, which up to now have caused the insertion of indigenous communities to the capitalist system are negative in general[8].

On the other hand, the structural problems of the Colombian society, such as the forced displacement of the population, the crops used for illicit purposes, inequality in the distribution of wealth and the armed conflict, generate social processes with large environmental impacts such as the expansion of the agricultural frontier, the concentration of land and the improper use of the soil. In this respect, Valencia et al.,[22] argue that the forced displacement led to environmental damage due to the extraction of natural resources and mining, the arrival of peasants trying to flee the violence caused by political conflicts, the planting of grasses, the introduction of animals favored by agricultural policies, the establishment of roads, the implantation of settlers and land ownership.

With regard to the crops used for illicit purposes, the authors point out that the dynamics of this problem has a marked tendency toward expansion in strategic ecosystems and rural settlements, an issue discussed over the past several decades, due to its environmental, economic, social, political and bioethical implications[9]. These crops have led to immigration, abandonment of subsistence farming; they also have increased the cost of living, changed patterns of consumption, income of luxury goods, transformed indigenous cultures and increased the use of firearms, violence, and prostitution.

The growth of illicit crops was responsible for half of the deforested area in Colombia in 1998. The largest concentration of these crops are located on the foothills of Caqueta, Cauca and Putumayo[5]. In 2004, the area cultivated in the whole Colombian Amazon was 16.85% of the total land area cultivated; in 2012 it was 21.9%, a representative level, if we take into account that the total area in Colombia declined in the period 2004-2012 in 20%. The departments of Caquetá and Putumayo comprised near 95% of the total coca cultivation in the region. The departments of Guainía and Amazon had only 5%; a decrease of 51% in the period 2004-2010 can be seen. The illicit crops constitute one of the problems with major social, environmental and economic impact[21].

With regard to the armed conflict in the region, there exist the conditions to affect the social and institutional structures, creating and maintaining an environment of insecurity and unrest, affecting the public and private investment and reinvestment and supporting the impoverishment of the regional natural base. This is a problem closely linked to the illicit crops and the extortionate rents, which generate poverty and displacement[22].

For this reason, it is required an analysis in which the complex relations between the armed conflict and the environment can be considered, in order to predict what might happen in the eventual cessation of conflict and disappearance of illicit crops. In this case, it is essential to have a strong environmental policy that in coordination with the agricultural policy, makes the generation of new conflicts impossible[15].

In this way, it can be seen the way environmental problems are a reality of effects that are too important to be left only in the hands of some disciplines, businessmen or politicians, because the preservation of the environment will necessarily impact on the survival of mankind; that is why the interdisciplinary study of the environmental implications are an urgent need in which ethics and bioethics may not be absent[11].

For this reason, there is a need for reflections at bioethical level about life in general and human life in particular, since the impact of the appropriation of natural resources such as soil, inevitably affects the web of life, considered as the system of necessary interrelationships for all living creatures. These reflections seek the construction of attitudes and habits consistent with an ecological awareness, which will make it possible to solve the environmental dilemmas, using the rational ethical decision making based on the application of the traditional principles, assisted by new approaches and concepts such as those of *the ethics of care, the principle of vulnerability* and *the precautionary principle*[b], which are essential elements on the path toward the conservation of biological and cultural diversity[9].

The precautionary principle is conceptually relevant and legally imperative in the Colombian legal system for the policies and interventions in the Amazon. The Colombian State should apply it to protect the environment, public health, cultural diversity, the dignity of local populations and the environmental services of the region against serious risks. In the same way, this principle must be considered in all regional sectoral policies, in the decisions about interventions and projects of development, and it must be inspiring for a comprehensive policy on the Amazon recognizing that there is no formula that can be applied indiscriminately to all the Colombian Amazon territory, since the problems and the social, cultural, environmental and economic conditions vary according to the subregions[5].

Under this scenario, it is indisputable that biological and cultural particularities associated with the use and management of the resource, in the indigenous and urban contexts are contrasting, as they reflect a man-nature interaction that requires an interdisciplinary and plural treatment where sciences and humanities converge[13] in the pursue of a consensual decision-making; this must not be based on the isolated action or intervention, but in correspondence with a profound reflection, planning, design and implementation of appropriate mechanisms and systems of resource appropriation, in order to ensure the persistence of biodiversity through its active management (use-conservation), considering the well-being and the cultural diversity[12].

In this sense, in relation to the environmental ethical problems, it is also proposed the participation of bio-eco-ethical principles[c]: caution,

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[b] Ethics of care and concern for the living aims to provide active and respectful assistance for autonomy and otherness; so this is appropriate for the current complex and pluralized world; on the other hand, the principle of vulnerability has to do dignity, integrity and autonomy, with keeping pluralism and a multicultural society; likewise, the precautionary principle seeks to prevent the risk of serious and irreversible damage to the environment.

[c] Bio-eco-ethical principles recognize that in man-nature relationship, components depend on each other. The natural non-human world is worthy of moral consideration; present and future generations are important; solutions to environmental and social conflicts are built collectively; the non-living world (water resource, air, water, sun, earth, soil, biotopes) is considered as indispensable because without it, life itself would not be possible. Moreover, these principles assign equal consideration for different beings, place intrinsic value on the

exploration, non-discrimination and respect for diversity[11]. These take into account and include the entities and non-human systems, and thus constitute a useful tool in the treatment of the conflict in the use and management practices of resources, such as the land. In the same way, converging ethics proposed by Maliandi and Thuer[14] is considered as a valuable tool in the solution of this type of environmental conflict, due to the fact that the convergence recognizes the difference, diversity, plurality of cultures and otherness.

Conclusions

The environmental dilemma for the use and management of the soil in the Colombian Amazon reflects the interaction between man and nature. In this, there are biological variables involved: ecological and technological relations; and cultural variables: cultural worldview and forms of knowledge. Having knowledge about the rational arguments and counter-arguments about the forms of exploitation of resources evidences the importance of knowledge dialog as a base reflection for the resolution of this kind of conflicts.

Dealing with environmental conflicts urgently requires the considerations to be extended in order to include the entities and non-human systems as it is necessary to consider other living forms as important and worthy of value, recognition and study. To fulfill this purpose, bioethics can be used as this science allows to evidence that some elements for conflict resolution and decision-making in the environmental field coexist to ensure the conservation of biological diversity through the sustainable use and conservation; it also considers the cultural diversity and the well-being of human communities.

In this way, bioethics, as a generator and mediator in the transformation of the interactions between living things and their environment, needs to take part in the approach to environmental conflicts. Thus, its contribution is essential in the analysis of the functional complexity of the connections and interrelationships of ecosystem components, including human communities. In this sense, it is through intercultural dialog, which needs to continue evolving like the ecological and cultural relations, that social

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non-human world, and consider man as part of biodiversity, so their protection is a way to protect cultural diversity. They also consider the knowledge dialogue interdisciplinary and plural, and as an essential element in the construction of knowledge and conservation of biological and cultural diversity.

and biological guidelines can be outlined in order to lead processes to the sustainable management and use of biodiversity.

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III. Paper 2

Soil fungal biodiversity of Colombia: microfungi

Soil fungal biodiversity in colombia: microfungi

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Abstract

The objective of the present study was to know the diversity of native soil microscopic fungi in Colombia, through a comparative analysis of the main research carried out in the different natural regions of the country: Andean, Amazonian, Caribbean, Orinoquía, Pacific and Insular. For this, the literature related to the fungal diversity of the country was compiled and reviewed analytically. The analysis shows that most of soil mycological data refer to the Andean Ecoregion picturing the greatest diversity of soil microscopic fungi, while the Pacific region is the least studied, recording very few fungal species. No data are reported on the fungal diversity of the Colombian Insular region. Some of the most relevant aspects of the mycological diversity for the country are discussed and the most frequently recorded species or genera, as well as the literature references for each of them, are presented. In this reviewed paper 170 different species of soil fungi for Colombia, corresponding to58 genera and 3 phyla are listed. Some recommendations are given regarding the research perspectives on native species of fungi in relation to edaphic environments, considering the great ecosystem heterogeneity of the country. Finally, it is evident the need to continue and complement existing research with taxonomic determinations of molecular and metagenomics type, as well as to deepen the evaluation of the functional potential of fungi, in order to have a better ecological understanding of soil microbiological populations and communities. The above said in order to optimize the use, management and also the recovery and the conservation of the soil in Colombia.

Key words: Colombia, diversity, fungi, natural regions, soil.

1. Introduction

The mycological flora of the tropical zones of the world is less well known than that of the temperate zones. One of the first tropical countries where an effort was made to gather a mycological flora was Colombia. In this respect, the work of Fuhrman and Mayor in 1914, provided information on parasitic fungi and the publication of Chardon and Toro in 1930, was the first which gathered mycological literature for a defined political region and presented the results of comparatively large explorations. Later, with the "Mycological Flora of Colombia" project, a cooperative program in mycology was established in 1974, in which more than 2000 collections of fungi were organized in the departments of Cundinamarca, Antioquia, Valle, Cauca and Boyacá (Dumont *et al.* 1978).

Gradually, the characterization of mycological species for Colombia was enriched through serial publications on the "New or noteworthy fungi from Panama and Colombia", mainly coming from the Sierra Nevada of Santa Marta in the department of Magdalena, at altitudes between 1250 and 2200 m asl, where some new species were identified such as: *Haplosporangium lignicum* (decomposing wood), *Dipodascus albidus* (bromelia exudate), *Hyaloria pilacre* (rotting palm stalk), *Hobsonia gigaspora* (bamboo stems and dead palm), *Entonaema liquescens* (Dead wood), *Myxomycidium flavum* (Trunk decorticated), *Sclerocystis coccogena* (dead twig), *Tulasnella violea* and *Cystobasidium sebaceum* (Martin, 1937; 1938; 1939a; 1939b).

Later, Singer in 1963 described some mycorrhizal species of *Quercus*, while Guzmán in 1964, presented discussions on 95 species of fungi, lichens and myxomicetos from Colombia, including their distribution and comparing them with Mexican mycoflora. Later, Dennis in 1970, briefly analyzed Colombia's fungi and Restrepo in 1972, studied two species of *Panaeolus* of Antioquia (Guzman and Varela, 1978).

In 1971, Rogers within a project between the University of Valparaíso (Chile) and University of Pavia (Italy), verified the adaptive capacity of keratinophilic species to colonize environments under variable adverse conditions, as well as compared the mycobiota of environments in similar conditions but separated by important geographical barriers like the Andes or be on in different latitudes. In the study, the presence of keratinophilic-lithic fungi was demonstrated in an extreme environment and their distribution and density were also reported: *Chrysosporium keratinophilum, Trichophyton ajelloi, Microsporon gypseum = Nannizzia gypseum, Microsporon fulvum, Trichophyton terrestre = Anthroderma quadrifidum*.

In 1976, Llanos and Kjøller presented the second part of a research project carried out by the Danish Esso Company to study soil and associated microorganisms changes after deposition of oil residues, as well as the ability of isolated fungi to decompose crude oil and hydrocarbons. The following species were reported in the study: *Graphium fructicolum, Petriella* sp, *Fusarium oxysporum, Penicillium nigricans, Paecilomyces lilacinus* and *Acremonium sclerotigenum.*

Later, Veerkamp and Gams (1983) describe three new species for Colombia *Trichoderma inhamatum, Rhinocladiella phaeophora* isolated in agricultural soil (corn crop) in Acacias (Meta) and *Mortierella ornata* isolated from the Andean forest soil in the Puracé National Park (Cauca - Huila).

Mycological studies in Colombia have focused mainly on macroscopic fungi and contributions to the taxonomy, diversity and ecology of microfungi are still scarce, which is why it can be ascertained that the mycology of the country is little known. In fact, the attention to microfungi has increased only in the last decades and its research has been directed especially at functional groups, metabolites and functional group in ecosystems.

Therefore, the objective of this work was to present in a general way the state of the art on native soil fungi in Colombia through the analysis of the specialized information obtained from the literature referring to the microfungi. In this review work only investigations on native fungi obtained exclusively from the soil in Colombia were considered and data concerning laboratory or greenhouse experimentations, or those on plant and insect pathogens are excluded.

As well, Urenidiales, although it is the best studied fungal taxon in the country (Pardo-Cardona, 1995, 1997, 2001, 2002, 2003, 2005, 2006; Salazar-Yepes *et al.* 2002; Salazar-Yepes and Buriticá-Céspedes, 2004, 2012; Buriticá-Céspedes and Salazar-Yepes, 2007; Zuluaga, 2008; Vanegas-Berrouet and Salazar-Yepes, 2011; Gómez-Correa *et al.* 2012; Buriticá-Céspedes *et al.* 2014), has not been considered in the present work, since its main substrate are leaves and stems of plants, in addition, many of them correspond to introduced species.

Finally, this analysis aims to provide an overview of the current state of knowledge on soil fungal biodiversity of Colombia, in order to establish a starting point for future investigations of the soil-plant-animal-man system, in relation to the pattern of geographical division of the Colombian territory that includes the five natural regions: Andean, Amazon, Caribbean, Orinoquia and Pacific. The level of study is alpha diversity, which refers to a variety of soil fungus species, which is a significant source of potential wealth to develop strategies for the rational use and management of available soil resources, both biologically and socially, considering agroecosystems and the conservation of biological diversity.

2. Study Area

Colombia is located at the extreme north-west of South America. The country is crossed by the Andes mountain range and the Amazon plain, with coasts on the Atlantic and Pacific Oceans. Its continental portion is between 12°26'46" north latitude and 4°13'30 " south latitude and between 66°50'54" and 79°02'33" west longitude, within the fringe Intertropical. Colombian territory covers an area of 1,141,748 km² continental and 930,000 km² marine. The country has territorial geographic divisions called natural regions differentiated from heterogeneous characteristics of relief, climate, vegetation and soil classes, according to these conditions, six regions are distinguished: Amazonia, Andean, Caribbean, Insular, Orinoquía and Pacific (Fig.1) (IGAC, 1992 ACOPAZOA, 2003).

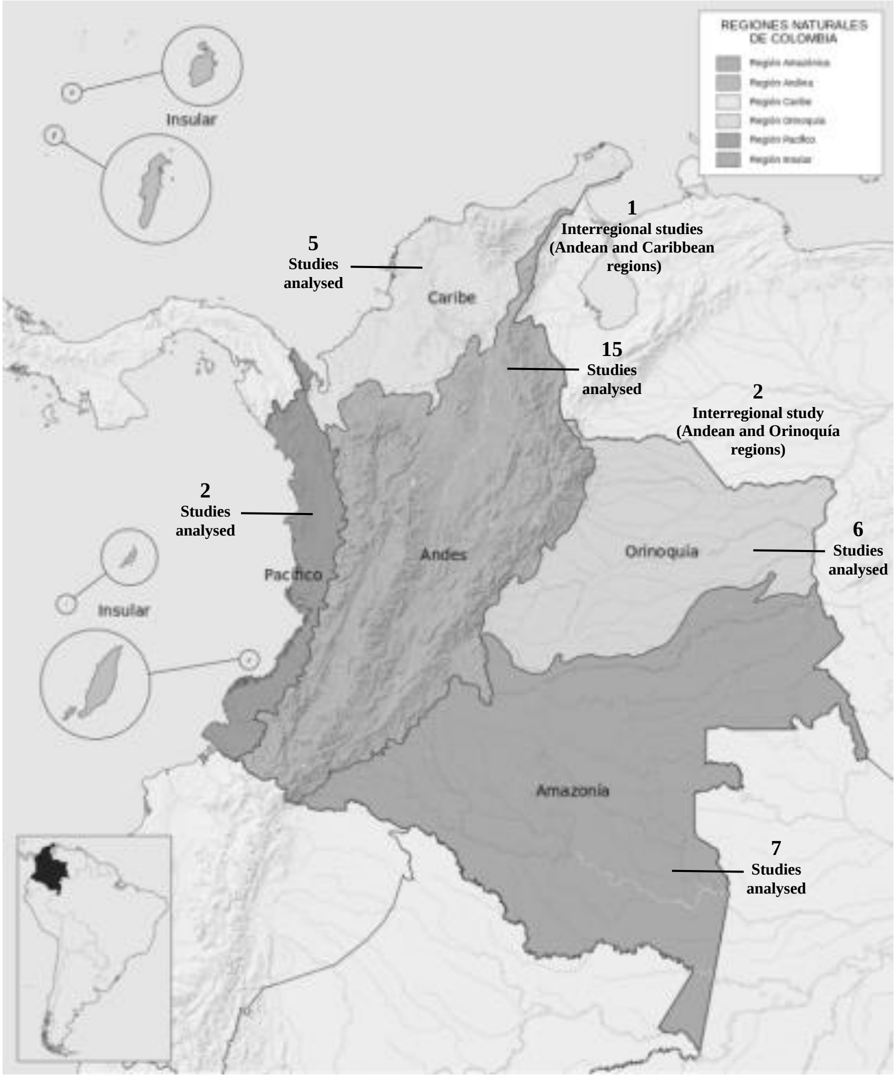

Fig 1. Natural regions of Colombia. Source: Instituto Geográfico Agustín Codazzi. Regiones geográficas de Colombia [mapa], edición 2012, 1:7500000

3. Soil fungi in Colombia

The previous studies on soil fungi of Colombia reported a total of 170 identified species belonging to 58 genera. The list of species is reported in Table 1. Most of the papers deal with endomycorrhizal fungi and *Glomus* is the most recorded genus. In the following paragraph the different papers and taxa are considered in relation to the Colombian natural regions (Table 2). Each recorded taxon is reported in Table 3 with the relative references.

3.1. Colombian Caribbean

The Caribbean region is made up of the departments of Guajira, Magdalena, Atlántico, Bolívar, Sucre, Córdoba, and includes localities of the departments of Antioquia and Cesar, located between 7°56' and 12°25' north latitude and 77°20 '- 71°08' west longitude, covers an area of approximately 142,000 km² and has elevations from 0 to 865 m. Based on floristic composition, communities of mangroves, *Heterostachys ritteriana* and *Philoxerus vermicularis* scrub, cardonal with cactaceae and forests of *Anacardium excelsum* and *Ceiba pentandra* are established. In terms of flora, there are 3429 species corresponding to 1160 genera and 246 families, whose main use is for folk medicine, firewood, food and construction. In terms of fauna, there are 32 species of amphibians, 951 birds, 101 reptiles, 133 arachnids and 434 hymenoptera. The protected area is 1115.1km² and has indigenous populations. The main threats faced by the region are mangrove deforestation, extensive cattle ranching, poor management of garbage, actions of illegal armed groups and drug trafficking, port pollution, sedimentation and water pollution (Rangel-Ch *et al.* 1995c).

3.1.1 Fungi of the Caribbean

The analysis of mycobiota in the Colombian Caribbean has focused on the mycorrhizal component, especially in the last years, perhaps because of its importance in tropical soils and its influence on the composition of plant communities, especially in forest plantations and in crops of agronomic importance. In this regard, Gómez *et al.* (2006) evaluated the incidence of arbuscular mycorrhizal fungi comparing natural ecosystems and banana agroecosystems, by determining the spore diversity and percentage of association, indicating the promising benefit of mycorrhizae present in the natural ecosystem for the recovery of diversity in the banana agroecosystem given, for example, its functional status.

Pérez *et al.* (2012a) compared the diversity of arbuscular mycorrhizae associated with Colosoana (*Bothriochloa pertusa*) and Angletón (*Dichanthium aristatum*) pastures present on cattle farms and found that the percentage of colonization showed a highly significant difference between the two pasture species tudied and the locality. They also point out that there is a high diversity of mycorrhizae associated with these two pastures under different edaphoclimatic conditions, but that there are still no studies to predict the patterns of dispersion and location of these mycorrhizae in specific pasture agroecosystems for the department of Sucre. In the same year, Pérez *et al.* (2012b) studied the fungi present on Colosoana grass rootstocks in cattle farms in the municipality of Sincé, this time considering species of phosphate solubilizers of both the rhizosphere and endophytes. Later, Pérez and Peroza (2013) characterized the colonization percentage and isolated native arbuscular mycorrhizal fungi associated with the Angletón grass, studying the relationship with the different agrological zones. Species *Gigaspora albida, Glomus occultum - Paraglomus occultum* and *Glomus etunicatum* were observed with greater presence, thus 92% of the species found corresponded similar to *Glomus* characteristics, 4% to *Gigaspora* and 4% remaining to *Paraglomus.*

In the dry and rainy season, Pérez *et al.* (1990) evaluated the effect of different types of organic and chemical fertilization on the population density of rhizosphere fungi in the agroecosystem of *Bothriochloa pertusa*. The soil with the treatment of lombricompost showed the greatest diversity of groups of fungi (Dematiaceae, Moniliaceae, *Aspergillus* sp, *Penicillium* sp and *Humicola* sp.). In the dry season, all soils with the respective fertilizers had higher densities. The soil with the treatment of lombricompost presented the best microbiological conditions in the two climatic periods, with greater abundance of microorganisms in the dry season. The highest densities of rhizosphere fungi in the two climatic periods were presented by the soils with treatments of lombricompost and bovine compost.

3.1.2 Biodiversity of fungi in Caribbean soils

For the Caribbean region, 5 investigations related to soil fungi were reviewed, which showed that the studies concentrated mainly on the *phylum* Glomeromycota with the identification of 18 species of *Glomus* and 1 species of *Gigaspora*. Other species of the same *phylum* were reported only as comparable or with similar characteristics (cf) at specific taxon. A report also refers to the genus *Acaulospora*. Among the Ascomycota 4 *Aspergillus* species were identified and strains belonging to *Penicillium*, *Paecilomyces* and *Humicola* were reported. Among the Zigomycota, the genus *Rhizopus* was identified. Yeast strains are also reported among fungi. In summary, 31 species belonging to 9 identified genera and 3 phyla (Table 3).

The studies were conducted almost entirely in the department of Sucre, in the municipalities of Sincé, Sampués, Corozal and Tolu, a study was conducted in the department of Antioquia specifically Chigorodó and Turbo also within interregional studies it marks a Study in the department of Magdalena. There are no records of research on native soil fungi for the departments of Guajira, Atlántico, Bolívar or Córdoba, or for the localities of Cesar that are part of this region. The main uses of the soil considered were for prairies (*Bothriochloa pertusa, Dichanthium aristatium*), agricultural (banana) and natural. The investigations focused on the knowledge of mycorrhizal fungi (3) and functional (2), for the taxonomic determination were mainly taxonomic keys.

3.2. Colombian Amazonia

The Amazon region comprises the departments of Guainía, Guaviare, Vaupés, Putumayo, Caquetá and Amazonas and includes localities in the department of Vichada, located between 4°10' south latitude and 4°05' north latitude and 76°16' and 66°50' west longitude, covers an area of approximately 300,092.7 km² and has elevations between 80 and 500 m. Based on the floristic composition, several types of vegetation have been differentiated: Dense forest of the interfluvios of the rivers Caquetá, Amazonas, Putumayo and Apaporis; Dense forests and savannas of the terraces and erosion surfaces and high hills of the Vaupés river, as well as a mix of forests and savannahs of the Guainía region. There are 5400 species of higher plants belonging to 1620 genera and 240 families, whose main use is for folk medicine, food, and timber exploitation, construction of houses, boats, dyes, poisons and in the manufacture of articles of domestic use. In terms of fauna, there are records of 147 species of reptiles, 868 birds, 95 amphibians and 210 mammals. The protected area is 48,552.9 km² and has indigenous reserves. The main threats facing the region are oil prospecting and exploitation (chemical, physical, biological and cultural disturbance), deforestation by logging and burning, as well as the exploitation of wild animal skins (Rangel-Ch *et al.* 1995d).

3.2.1 Fungi of the Amazon

In the Amazonia region, Vera *et al.* 2002a, evaluated the distribution of phosphate solubilizing fungi in Amazon soils under Arazá cultivation in two soil microhabitats of two different physiographic units (landscapes) and found 21% of colonies with potential solubilizer, a value indicative of the possible reserve of solubilizing fungi present in these soils, also indicate that the main determinant of the composition of the solubilizing fungal community was the rhizospheric effect of the Arazá plants and that there are differential microenvironmental conditions in the soil. These authors (2002b) isolated and characterized 18 fungi with the capacity to solubilize unavailable phosphate from two different sources for the same study area. The main solubilizers of calcium phosphate were *Trichoderma aureoviride, Aspergillus aculeatus, Trichoderma* strain 1 and *Trichoderma* strain 2 and for iron phosphate: *Aspergillus oryzae, Paecilomyces* strain 3, *Gongronella butleri* and *Fusarium oxysporum.*

In the Amazon trapezium Useche *et al.* (2004), evaluated the abundance and distribution of phosphate solubilizing bacteria and fungi under three soil uses: low-intervening forests, stubble and pastures at two depth levels: superficial and deep. In addition, they established relationships between the abundance of the phosphate solubilizing microorganisms and the physicochemical characteristics of the soil to establish their ecological role in the phosphorus cycling in soils of the study area. Among the phosphate solubilizing fungi were: *Penicillium* spp., *P. implicatum, P. cittreo-viridae*, *Paecilomyces* spp., *Aspergillus niger, A. fumigatus, Scopulariopsis* sp., *Moniliella* sp., *Mortierella* sp. and one ascomicete. The relative abundance of phosphate solubilizing fungi is low relative to the total relative abundance in the soils studied. The highest relative abundance was observed in the upper level (0-20 cm) and in the soils under stubble.

In 2005, Cardona *et al*. studied the abundance of actinomycetes and arbuscular mycorrhizae in soils under forest cover and pasture in areas of high, medium and low anthropogenic intervention. Soil fragments located in the high anthropogenic intervention zone presented significant differences between coverings, registering lower values of mycorrhizal abundance in pastures, perhaps due to the high compaction of the soils. In the fragments of the minor intervention area no significant differences were found. Ten morphotypes for arbuscular mycorrhizae were identified. *Glomus* sp. turned out to be the most representative genre. Soils did not present significant differences in their physicochemical characteristics. It was inferred that in areas of high intervention these groups may be possible indicators of processes of alteration of the landscape.

Subsequently, Peña-Venegas *et al.* (2006) made an important contribution to the Illustrated Catalog of Arbuscular Mycorrhizae of the Colombian Amazon, a result of the work of more than three years and more than 400 samples reviewed, in which the morphotypes that appear frequently in soils of the Colombian Amazon are described. The authors note that according to the International Culture Collection of Arbuscular Mycorrhizal Fungi - INVAM, 7 genera are recognized and less than 170 species have been described with a very low representation of new reports for the tropics and the Amazon Basin. The catalog describes 31 morphotypes that, if taken as different species, account for 20% of world diversity. However, the 31 morphotypes in the catalog do not correspond to the total number of species reported for the region. The authors recommend, especially for the genus *Glomales*, the use of molecular techniques to verify the taxonomic determinations and conclude that *Glomus* is the most representative genus of the Colombian Amazon soils, followed by *Acaulospora*. The other genera appear in lesser proportion and diversity of species. Finally, the report of new species for Colombia such as *Scutellospora spinosissima, Glomus tortuosum, Glomus sinuosum, G. microaggregatum, G. viscosum* and *Scutellospora pellucida*, highlights the possibility of using species in research and crop improvement in the region.

In 2007, Peña-Venegas *et al*. Also evaluated the natural presence of mycorrhizal fungi of arbuscular type in acid soils of clay-loamy to clayey texture of southern Colombian Amazonia under forest, stubble and grassland, at two different depths. They studied the richness and abundance of spores related to acidity, cation exchange capacity, organic carbon, total phosphorus, soluble and fixed phosphorus fractions of aluminum, iron and calcium, as well as total soil DNA and detected significant differences in the content of Total DNA and the number of spores with respect to sampling depth. The pH had a significant effect on the DNA content and the number of mycorrhiza spores. The DNA content in the soil was affected by the concentrations of aluminum phosphates, while sporulation was affected by soil phosphate concentrations of iron. Thus, they showed that the number of spores of arbuscular mycorrhizal fungi in Amazonia soils is affected by the depth, pH and type of mineral phosphates present.

Cardona *et al*. (2008) studied arbuscular mycorrhizal fungi associated with the rhizosphere and chili roots (*Capsicum*) and found 9 morphotypes of which 6 belonged to the genus *Glomus* and 3 to the genus *Acaulospora*. These two genera are in their order the most frequently isolated in the Colombian Amazon region. All the seedlings of chilis sampled had associations with arbuscule mycorrhizae. The chemical characteristics of the soil, the presence of other plant species in chagras, the source of collection and the species of the genus *Capsicum*, affected the occurrence of mycorrhizal symbiosis.

3.2.2 Biodiversity of fungi in the Amazon

For the Amazon region, 7 investigations related to the soil fungi were verified, in which the identification of 20 species belonging to the genus *Glomus, Acaulospora, Scutellospora, Archaespora, Entrophospora and Gigaspora* were evidenced for the phylum Glomeromycota. The genus *Glomus* is present with 9 species identified and with more than ten non-identified morphotypes. Ascomycota phylum reports 15 species belonging to the genus *Aspergillus, Penicillium, Trichoderma, Fusarium*, reported as unidentified species for the genera *Paecilomyces, Scopulariopsis* and *Moniliella*. For the phylum Zygomicota 1 species is reported (*Gongronella butleri*) and a report for *Mortierella*. In summary, 34 species belonging to 16 identified genera and 3 phyla (Table 3).

Studies on native soil fungi for all departments in the Amazon region are reported. The main land use that was considered in the studies was the natural one: forest (*Inga* spp.) and low intervened forest, the agricultural one (*Eugenia stipitata*), stubble and grassland (*Brachiaria decumbens*). The investigations focused on the knowledge of mycorrhizal fungi (4) and functional solubilizers of phosphate (3), for taxonomic determination mainly used taxonomic keys and specialists.

3.3. Colombian Andean Region

The region of the Colombian Andes comprises the departments of Boyacá, Caldas, Cundinamarca, Huila, Norte de Santander, Quindio, Risaralda, Santander, Tolima and partially the departments of Antioquia, Cauca, Cesar, Chocó, Nariño and Valle del Cauca. Between 11°10' and 0°30' north latitude and 73°30 'and 77°30' west longitude, covers an area of 287,720 km^2 and has elevations up to 5000 m. According to the floristic composition, forests of the piedmont of the Amazon and the Pacific, paramos and glaciers of the Sierra Nevada del Cocuy are differentiated. There are 11500 species of higher plants belonging to 200 families and 1800 genera. In terms of fauna, there are 974 species of birds, 484 of amphibians, 177 of mammals and 277 of reptiles. It has three independent mountain ranges: the western, central and eastern mountain ranges that exhibit their own climatic, geological and structural characteristics, and it has indigenous reserves. The main threats facing the region are the expansion of the agricultural frontier, the overexploitation of natural resources, pollution and the introduction of foreign species (IAvH, 2004; Rangel, 2005; Rodríguez *et al.* 2006).

3.3.1 Fungi of the Andean region

For the Andean region, Elías *et al*. (1993) studied the antagonism of some *Trichoderma* species in the control of pathogens such as *Fusarium oxysporum* and *Rhizoctonia solani*. Later, Gualdrón-Arenas *et al*. (1997) carried out comparative ecological analyzes, based on qualitative and quantitative aspects of the isolated micoflora in Paramo, and pointed out that the major cause of variation in the composition of the microfung communities is related to the vegetation and soil type.

In 2000, Bolaños *et al.* evaluated the diversity and quantity of arbuscular mycorrhizae associated with the rhizosphere of coffee plants (*Coffea arabica*) cultivated at the National Coffee Research Center - Cenicafé. Some of the species found were *Acaulospora mellea, A. tuberculata A. appendiculum, A. scrobiculata, A. foveata, Entrophospora colombian, Glomus occultum, G. manihotis, G. fistulosum, G. invermaium, G. intraradices, G. macrocarpum, Sclerocystis sinuosa and Scutellospora* sp.

Moratto *et al.* (2005) determined, for the Guerrero Paramo (Cundinamarca), the effect of soil use on phosphate solubilizing fungus and nitrogen fixing bacteria populations under four different conditions of use (Potato plantations, cultivated soils, soils at rest and forest). The authors demonstrate that functional groups of microorganisms such as bacteria and phosphate solubilizing fungi can be susceptible to disturbances that occur with the change in soil use, reducing the number of their populations and their diversity. In addition, they observed that, in relation to crop soils, rest and forest soils present more favorable conditions for the development of fungi and exhibited morphotypes of exclusive fungi, high populations of diazotrophic bacteria and strains of phosphate solubilizing fungi with high solubilization efficiency. In this same study area, Bernal *et al.* (2006) counted cultivable microorganisms (bacteria and fungi), cellulolytic microorganisms and endomycorrhizas present in forest leaf litter, and found eight endomycorrhizal morphotypes mainly of the genus *Glomus* and *Acaulospora.*

Also in the paramo ecosystem, Chitiva-Jaramillo *et al*. (2007) studied the filamentous microfungi and identified as the main genera present *Penicillium, Acremonium, Cladosporium* and *Aspergillus,* also indicated that the isolated species coincide with those reported by Gualdrón-Arenas *et al.* (1997) so they could be considered typical of colombian moorland ecosystems, among them: *Epicoccum purpurascens, Fusarium equiscii, F. sporotichioides, Geotrichum candidum, Gliocadium roseum, Mucor circinelloides, M. hiemalis, Paecilomyces lilacinus, Penicillium rubrum, P. frecuentans, P. verrucosum* and *Trichoderma hamatum.*

On the other hand, Mosquera-Espinosa *et al.* (2010) studied orchid mycorrhizal fungi from different habitats in Colombia, following the methodology of counting nuclei in young hyphae cells and sequencing the ITS region of nuclear ribosomal genes, the isolates corresponded to the genus *Ceratobasidium,* which is associated with a wide variety of orchids of varied habitats, also inquired about the pathogenicity of this genus mycorrhizal on other hosts and its potential as a biocontroller of pathogenic fungi in cultivated plants such as rice (*Oryza sativa*).

García *et al.* (2012), on the other hand, studied the entomopathogenic fungi obtained from the soil, useful to control salivazos (Hemiptera: Cercopidae) of the sugar cane, obtained 26 isolates corresponding to two species *Metarhizium anisopliae* and *Paecilomyces lilacinus* and developed the protocol to conduct bioassays in greenhouses in order to evaluate the pathogenic potential of these fungi.

Posada *et al.* (2012) studied eight coffee plantations in Colombia and Mexico with different intensities of management and differences in their edaphic variables, for which they isolated and evaluated the communities of filamentous microscopic fungi (FMF), iron phosphate solubilizers fungi (PSF-Fe) and phosphate solubilizers fungi of both iron and calcium (PSF- (Fe + Ca)) during 2008 and 2009. They found that organic carbon was positively related to the richness and abundance of FMF, whereas it was variable in relationship with PSF -Fe and PSF - (Fe + Ca). The relationships of available phosphorus, pH and macro-aggregate fractions were highly variable. The different intensities of management were negatively related to PSF -Fe in Colombian coffee plantations. They also analyzed the interactions for each set of variables (chemical, macroaggregate stability and planting management) and explained the resulting relationships. The relationships of each variable are inseparable from the edaphic and geographic context, which imprint marked differences. For these eight coffee plantations in Colombia and Mexico, Posada et al. in 2013 also discussed the potential practical use of four fungal isolates *Cylindrocarpon didymum* and *C. obtusisporum* (obtained in Colombia) and *Penicillium janthinellum* and *Paecilomyces marquandii* (obtained in Mexico), in order to improve the efficiency of the use of phosphorus.

Beltrán-Pineda (2014) also isolated and characterized phosphate solubilizers fungi of rhizosphere from potato crops (*Solanum tuberosum*) in paramo soils, in order to obtain fungal strains with biofertilizer potential to mitigate levels of degradation of the soils in these protected areas, obtained two fungal strains belonging to the genus *Scopuraliopsis* sp. and *Penicillum* sp.

Salazar-Moncada *et al.* (2014) carried out the identification of fungi isolated from different substrates (soils and symphylans) to determine its antagonist capacity, also they verified that capacity for strains from a collection of biocontrol fungi on *Purpureocillium* sp. which was inhibited by *Volutella* sp., *Paecilomyces* sp. and *Peyronella* sp.

Avellaneda-Torres and Torres-Rojas (2015) for their part, made the biodiversity characterization of bacteria and fungi to functional groups soil: nitrogen fixers, solubilizing phosphate and cellulolytic in soils under potato crops, livestock and paramo with little anthropogenic intervention in Risaralda and reported 1060 isolated microbial morphotypes (bacteria and fungi). For each morphotype they presented the identification through molecular markers, the functional group, the georeferenciation and the associated soil use. Lizarazo-Medina and Gómez-Vásquez (2015), also in paramo soil, determined the cultivable microbial populations of functional importance as a nutrient booster in the rhizospheric soil of *Espeletia* spp. of two paramos in Antioquia. The abundance of microorganisms was similar in the two paramos and was reported in both bacteria and fungi phosphate solubilizers and cellulose degrading agents.

Subsequently, Alvarez-Yela *et al.* (2017) compared the functional capacity of a soil without anthropic intervention with one exposed to agricultural activity (potato cultivation) in a paramo ecosystem. In order to do this, it evaluated the behavior of metabolites and metabolic fluxes in these two soil ecosystems and analyzed the microbial community structure, the functional capacities and the dynamism of the biogeochemical cycles through a metagenomic study, in order to understand the impact of agricultural activities, both structural and metabolic in these soils, the results suggested that agricultural activities greatly affect biogeochemical cycles due to the dynamics of carbon, nitrogen and sulfur reactions. In the not intervened soil, an active metabolic activity was obtained, variable and fluctuating in time, where the biogeochemical cycles are highly synergistic. In the intervened soil, the metabolic capacities focused mainly on particular processes of the nitrogen and carbon cycles that occur after fertilization.

3.3.2 Biodiversity of fungi in the Andean region soil

For the Andean region, 15 investigations related to the soil fungi were verified. In summary, 107 species were identified belonging to 73 genera and 3 phyla (Table 3).

The researches were carried out in the following Colombian departments: Risaralda, Cundinamarca, Boyacá, Antioquia, Santander, Huila, Tolima, Caldas, Quindío and Valle del Cauca, besides in the interregional studies it is highlighted the Cauca department. For the Norte de Santander department it is not found data about researches about native fungi of the soil, neither for municipalities of the departments of Cesar, Chocó and Nariño belonging to this Colombian region.

The main uses of the soil that were taken into account during the researches were the agricultural (potato, coffee, cane, flowers), pastureland and the natural (paramo, frailejon plants of *Espeletia barclayana, E. killipii;* scrublands of *Calamagrostis effusa, Calamagrostis* sp., *Cortaderia selloana, Pernettya prostata, Buddleja sp., Lunipus albus, Dentropanax sp.* and forest of *Gynoxys fuliginosa, Weinmannia, Hypericum, Hesperomeles, Diplostephium,* land orchids, epiphytes, hemiepiphytes, lithophytes). The researches were focused on the knowledge of the rhizospheric fungi (3), antagonists (3), mycorrhizal (3) and functional nitrogen solubilizers, phosphorus solubilizers, keratinophiles, ligninolytics and cellulolytics (6). For the taxonomic determination it was mainly used taxonomic keys and molecular techniques.

3.4. Colombian Orinoquía

The region of the Colombian Orinoquía comprises the departments of Arauca, Casanare, Meta and partially the department of Vichada, it is located between 5° and 2° north latitude and 75° and 67° west longitude, it is 154.193.2 km^2 long and presents elevations between 80 and 500m. On the basis of the floristic composition, it has been differentiated several savanna types: dry, wet and flood, likewise forest, including some relictual ones. It was registered 2047 species of superior plants belonging to 180 families and 807 genres, its main use is the traditional medicine, feeding and industry (timber). According to the fauna, it is registered 28 species of amphibians, 644 of birds, 119 of reptilians, 65 of arachnids and 359 of hymenoptera. The protected area is 11,888.8 km² long and presents indigenous reserves. The main threats that are facing the region are the prospection and oil exploitation (chemical, physical, biological and cultural disturb), some illegal armed groups are presented in the area, the intensive livestock and extensive also the area are becoming savanna because of the uncontrolled exploitation of the forests (Rangel-Ch *et al.* 1995a).

3.4.1 Fungi in the Orinoquía

In 1984, Schenck *et al*. described six new species of vesicular-arbuscular mycorrhizal for Colombia: *Acaulospora appendicula, A. longula, A. mellea, A. morrowae*, *Glomus manihotis* and *Entrophospora colombian.* Later, Doot *et al*. (1990) continued studies on fungi arbuscular and vesicle mycorrhizal, but this time to evaluate the population of spores and the effect of phosphate on the host plant. The highest spore densities were found in *Sorghum* sp. and the largest sporulators were *Glomus occultum* and *Acaulospora myriocarpa*. Also they studied the management of vesicular-arbuscular mycorrhizal populations for the benefit of plant growth on infertile acid soils.

Subsequently, Serralde and Ramírez (2004) evaluated in acid soils of the piedmont llanero, during five consecutive years (1997-2001), the native populations of arbuscular mycorrhizal fungi associated with two corn varieties: ICA- Sikuani V-110 and the regional creole variety Clavito, considering their reaction under different treatments: organic fertilizer (gallinaza), green manure (Caupí) and control. 24 morphotypes were isolated and the relationship between soil conditions and the behavior of mycorrhizal populations was determined. The pH and organic material of the soil were selected as predictors. The molecular technique with the use of specific primers allowed the identification of the genre *Glomus*, *Entrophospora* and *Gigaspora*, as well as the individualization of the genus *Glomus* in highly colonized corn roots.

Ortiz and Uribe (2007) carried out native isolates of lignolytic fungi from samples of flooded savanna soil from different agricultural uses. They obtained 25 morphotypes belonging to the genera *Verticillium*, *Penicillium*, *Fusarium*, *Zygorhynchus* and *Mucor* capable of growing in alkaline lignin. If was not found any relation between agricultural use, the number of lignolytic fungi and their enzymatic activity. The results obtained showed peroxidase activity for *Trichoderma viride* and for the morphotypes of *Verticillium*. Regarding laccase activity, the strain 005L was highlighted. The obtained isolates were considered promising for the degradation of organic matter and composting, due to their adaptation to the environmental conditions of the savannas of the Colombian Eastern Plains. The same authors in 2010 identified isolates of ligninolytic and cellulolytic fungi useful for degrading crop residues and improving soil characteristics in the Eastern Plains. They obtained a ligninolytic strain (005L *Verticillium* spp.) and 72 cellulolytic strains. In the comparison of the land uses (grazing savanna and secondary forest) they observed that there was no relationship between the number of genera obtained and the characteristics of the soil. They also found two strains with high exoglucanase activity (055C y 061C *Penicillium* spp.) and a strain with high endoglucanase activity (019C *Trichoderma* spp.). The tests showed that the promising isolates increased their enzymatic activity in the grass substrate and the consortia did not affect the enzymatic capacity of the strains that formed them. The authors recommend using these consortia for the development of biofertilizers soil conditioning, using the consortium of the strains 005L (*Verticillium* spp.) and 055C (*Penicillium* spp.), which showed high ligninolytic and cellulolytic activity.

In 2013, Monroy *et al.* identified native arbuscular mycorrhizal fungi associated with grass and leguminous coverages established in oxisols soils of the piedmont llanero and evaluated their colonization capacity. 26 morphotypes were identified, including *Scutellospora heterogama*, *Acaulospora scrobiculata* and *A. morrowiae*, which accounted for 65% of the population. The coverages with the highest percentage of colonization and diversity of arbuscular mycorrhizal fungi were *Brachiaria brizantha*, *B. dictyoneura* and *Paspalum notatum*. The number of morphotypes found in these soils was similar to that reported by Serralde and Ramírez in 2004. The morphotypes with greater presence of spores were *Glomus*, *Acaulospora*, *Scutellospora* and *Entrophospora*.

3.4.2 Biodiversity of fungi in soils of Orinoquía

For the Orinoquia region, 6 investigations related to the soil fungi were reviewed, in which it was evidenced that the studies concentrated mainly on the phylum Glomeromycota with the identification of 8 species belonging to the genus *Acaulospora*, 10 species to the genus *Glomus*, 2 species to the genus *Entrophospora*, 3 species to the genus *Scutellospora* and 1 is reported for the genus *Gigaspora*. Among the Ascomycota are strains belonging to 10 genera, while 3 genera belong to the phylum Zygomicota. In summary, 23 species belonging to 4 genera of the phylum Glomeromycota and 13 genera without identified species belonging to the phyla Ascomycota and Zygomycota were identified for a general total of 23 species, 17 genera of 3 phyla identified (Table 3).

The studies were carried out entirely in the department of Meta, in the municipalities of Puerto Gaitán, Puerto López and Villavicencio. There are no records of studies on native soil fungi for the departments of Arauca and Casanare, nor for the Vichada towns that are part of this region. The main soil use that was considered in the studies was natural (secondary forest), agricultural (corn, orange, cassava) and prairies (*Brachiaria* spp, *B. brizantha*, *B. dictioneura*, *Arachis pintoi*, *Desmodium ovalifolum*, *Panicum máximum*, *Paspalum notatum*, *Trachypogon vestitus*). The researches focused on the knowledge of mycorrhizal fungi (4) and functional lignolytic and cellulolytic fungi (2). For the taxonomic determination they mainly used taxonomic keys and in one case molecular techniques were used.

3.5. Colombian Pacific

The Pacific region is made by the Chocó department and partly by the Valle, Cauca and Nariño departments. It is located between 7°13'-1°36' north latitude and 77°49'-79°01' west longitude, covers an area of 131,246 km² approx. and has elevations between 0 and 1100 m. It exhibits diverse types of vegetation: mangroves (aquatic communities and marshes, formations of banks or beaches), as well as vegetation of mainland. There are 5474 plant species belonging to 1406 genera and 271 families, whose main commercial use is timber extraction for export, other uses are traditional medicine, woodworking, construction and firewood. In terms of fauna, there are 127 species of amphibians, 577 birds, 104 reptiles, 101 arachnids and 649 hymenoptera. Also, it has indigenous communities. The main threats faced by the region are the extraction of gold and platinum, indiscriminate cutting of trees, shrimp, permanent agriculture and contamination by wastewater discharges (Rangel-Ch *et al.* 1995b).

3.5.1 Fungi of the Pacific

The two studies that are registered for the region are recent and were carried out in the same locality, the rural area of Citronela and Zabaletas, in Buenaventura (Valle del Cauca), in agroecosystems of chontaduro (*Bactris gasipaes*). In 2011, Riascos-Ortiz *et al.* proposed to isolate and morphologically characterize fungi with beneficial potential associated with the rhizosphere of *B. gasipaes* in two different production systems. The genus isolates were *Trichoderma*, *Fusarium* and *Rhizopus*, *Penicillium* and *Thielaviopsis*. Afterwards, Molineros *et al*. (2013) evaluated the colonization levels of arbuscular mycorrhizal fungi in the roots of *B. gasipaes* and determined the influence of rainfall on the colonization of these fungi, demonstrating the existence of temporal variation in mycorrhizal according to the geography of the place. In addition, they found that *Glomus* was the most abundant genus of mycorrhizal fungi and was also detected, the genus *Scutelospora*.

3.5.2 Biodiversity of fungi on Pacific soils

For the Pacific region, two investigations related to soil fungi were reviewed, where 7 genera were identified, two of which belong to the phylum Glomeromycota: *Glomus* with 14 morphotypes and *Acaulospora* with 8 morphotypes. The other reports belong to the phylum Ascomycota with 4 genera, whose species were not identified; in particular anamorphic genera are reported. Among the Zygomicota the genus *Rhizopus* is reported. In summary, 22 morphotypes of 2 genera of the phylum Glomeromycota, 4 genera of the phylum Ascomycota and 1 of the phylum Zygomicota were reported for the latter two phyla species with no identified species, for a general total of 22 morphotypes, 7 genera and 3 phyla (Table 3).

The studies were carried out entirely in the Valle del Cauca departments, in the municipality of Buenaventura. There are no records of studies on native soil fungi for the department of Chocó, nor for the localities of Cauca and Nariño that are part of this region. The soil use considered in the studies was agroecosystem (*Bactris gasipaes*). The researches focused on the knowledge of mycorrhizal fungi (1) and rhizospheric fungi (1). For the taxonomic determination taxonomic keys and literature were used.

Interregional Studies

3.6. Andean and Caribbean Regions

3.6.1 Fungi of the Andean and Caribbean regions

Mahecha-Vásquez *et al.* (2017) evaluated the effect of the management system (monoculture vs. polyculture) and determined edaphic factors that influence the richness and diversity of arbuscular mycorrhizal fungi. They found between 11 and 18 arbuscular mycorrhizal species on average by planting, both in intensive and polyculture systems, and pointed out that pH was the only factor positively correlated with species richness, also they found that monocultures were less acidic and this ratio showed a higher species richness and indicated that the composition of mycorrhizal species in the community exists in heterogeneous patches that are little influenced by crop management practices (mono or polyculture), while height above sea level seems to influence the composition of the mycorrhizal community, so the differences in this composition were the result of multiple edaphic factors.

3.6.2 Biodiversity of soil fungi in the Andean and Caribbean regions

In the Andean and Caribbean regions, a research related to the soil fungi was reviewed, which presented in the identification of 20 species belonging to the genus *Glomus*, *Acaulospora*, *Scutellospora*, *Archaespora*, *Entrophospora* and *Gigaspora* were evidenced for the phylum Glomeromycota. The genus *Glomus* is present with 9 species identified and with more than ten unidentified morphotypes. Ascomycota phylum reports 15 species belonging to the genus *Aspergillus*, *Penicillium*, *Trichoderma*, *Fusarium*, reported as unidentified species for the genera *Paecilomyces*, *Scopulariopsis* and *Moniliella*. For the phylum Zygomicota 1 species is reported (*Gongronella butleri*) and a report for *Mortierella*. In summary, 35 species belonging to 11 identified genera and 5 genera without identified species were identified, for a general total of 35 species, 16 genera of 3 identified phyla (Table 3).

The study on native soil fungi is reported for the departments of Cundinamarca, Antioquia, and Magdalena. The use of soil considered in the study was agricultural (banana, coffee and citrus: lemon, orange and mandarin). The research focused on the information of mycorrhizal fungi. For the taxonomic determination they used taxonomic keys.

3.7. Andean and Orinoquía Regions

3.7.1 Fungi of the Andean and Orinoquía regions

In these regions, Veerkamp and Gams (1983) described three new Colombian fungi: *Trichoderma inhamatum*, *Rhinocladiella phaeophora*, isolated from agricultural soil samples (corn), at 500 masl, near Acacias (Meta) and *Mortierella ornata* isolated from samples of Andean forest floor, at 3100 masl, in the Puracé National Park (Cauca-Huila). Sieverding and Howeler (1985) studied three sites with acid soils in Colombia, cultivated with cassava, in order to know the frequency of mycorrhizal vesicle-arbuscular (VA). They found that the cultures differed in the composition of VA mycorrhizal species and that in all sites the presence of the total mycorrhizal population decreased with increasing fertilization with phosphorus, while at two sites the relative frequency and activity of the species *Glomus manihotis* increased with gradual phosphorus applications.

3.7.2 Biodiversity of soil fungi in the Andean and Orinoquía regions

In the Andean and Orinoquía regions, two researches were carried out in relation to the soil fungi, in which the identification of 20 species belonging to the genus *Glomus*, *Acaulospora*, *Scutellospora*, *Archaespora*, *Entrophospora* and *Gigaspora* were evidenced for the phylum Glomeromycota. The genus *Glomus* is present with 9 species identified and with more than ten unidentified morphotypes. Ascomycota

phylum reports 15 species belonging to the genus *Aspergillus, Penicillium, Trichoderma, Fusarium,* reported as unidentified species for the genre *Paecilomyces*, *Scopulariopsis* and *Moniliella*. For the phylum Zygomicota 1 specie is reported (*Gongronella butleri*) and a report for *Mortierella*. In summary, 35 species belonging to 11 identified genera and 5 genres without identified species were identified, for a general total of 35 species, 16 genres of 3 identified phyla (Table 3).

Studies on native soil fungi are reported for the departments of Santander, Meta, Cauca and Huila. The use of soil considered in the study was agricultural (banana, coffee and citrus: lemon, orange and mandarin) and the natural (Andean forest). The researches focused on the knowledge of mycorrhizal fungi (2). Taxonomic specialists and keys were used for the taxonomic determination.

3.8. Information integrated

In the present study, the data of the Integrated Information System - SiB Colombia (http://catalogo.biodiversidad.co/?pagesize=20&sort=betterMatch&order=asc&page=2&taxonomy=mushrooms), an initiative that aims to provide free access to information on the country's biological diversity was considered, as it facilitates the online publication of data and information on biodiversity, through of the "Catalog of the Biodiversity of Colombia", this disclosure indicates 40 species of fungi, belonging to 14 genera registered for the country (Table 3): *Acaulospora foveata, A. mellea, A. morrowiae, A. rehmii, A. Tuberculata, Archaeospora leptoticha, Aspergillus aculeatus, A. flavus, A. fumigatus, A. niger, Entrophospora colombiana, Fusarium, Gigaspora albida, G. gigantea, G. margarita, Gliocladium catenulatum, Glomus, G. aggregatu, G. brohultii, G. fasciculatum, G. fulvum, G. geosporum, G. glomerulatum,* *G. intraradices, G. invermaium, G. manihotis, G. microaggregatum, G. rubiforme, G. sinuosum, G. tortuosum, G. viscosum, Gongronella butleri, Paecilomyces, Penicillium, P. citreoviride, P. implicatum, Scopulariopsis, Scutellospora pellucida, S. spinosissima, Trichoderma* (MADS *et al.* 2017).

Table 2. Richness of native fungi present in the soil according to the natural regions of Colombia

NATURAL REGION	AVAILABLE PAPERS	SPECIES	GENERA	PHYLA
Caribbean	5	31	9	Ascomycota Zygomycota Glomeromycota
Amazon	7	34	16	Ascomycota Zygomycota Glomeromycota
Andean	15	107	73	Ascomycota Basidiomycota Zygomycota Glomeromycota
Orinoquía	6	23	17	Ascomycota Zygomycota Glomeromycota
Pacific	2	-	7	Ascomycota Zygomycota Glomeromycota
Andean-Caribbean interregional	1	7	5	Glomeromycota
Andean-Orinoquía interregional	2	11	7	Ascomycota Zygomycota Glomeromycota
Information integrated (Catalog SiB)	-	40	14	Ascomycota Glomeromycota

CONCLUSIONS

The distribution of the studies on the native microscopic fungi present in the soil of Colombia, shows that the Andean region is the most favored, since it presents the highest number of species and genera reported, in second place, but with a significant difference in the number of species and genres reported the Amazon region stands out, the Pacific region on the other hand, presents the smallest reports. The insular region has not been considered in the studies analyzed and data on soil fungi are not reported for this region. The high diversity present in the Andean region may be due to its extension, eco-geographical and climatic diversity and to the fact that it is the most populated region, which could favor its study and therefore a deeper knowledge of its mycological diversity.

Interregional studies on native microscopic fungi present in Colombian soil, i.e. those developed in departments that are part of two or more natural regions of the country, often include the Andean region (Andean-Caribbean and Andean-Orinoquía), perhaps because of its strategic geographical position that places it in the center of the country, which makes it close to all other regions with which has borders.

The Amazon, the second region with the greatest fungal diversity of soil reported for the country, is also the best represented since, unlike the other regions, it records information for each of the departments that comprise it, with no doubt is related to the research efforts that, from the universities, the institutions and the government have been made over time in this strategic area of life, both biologically and culturally.

It is evident that the study of microscopic soil fungi has been growing in recent years; however, the need for mycological studies of the soil in the country is still peremptory since, with the exception of the Amazon, all natural regions have departments which there is no general information regarding this important area of knowledge.

It is essential to continue and complement the studies on soil microscopic fungi in Colombia, as well as to compile, structure and systematize existing information and collections through a general tool that allows greater access and understanding of information on biological diversity and ecological aspects of native fungi in the Colombian soil.

This analysis allowed to show that the methodologies for the taxonomic determination should be enriched, since the macroscopic and microscopic characterization through identification keys makes it difficult to classify the soil fungi at the species level, for that reason it is necessary to complement this type of analysis with molecular and metagenomic techniques. In addition, there is an important field of action for mycologists and specialists, which are essential to enrich knowledge in this promising area of knowledge.

Referring to the soil fungi of the country, it has been possible to determine that arbuscular mycorrhizae represent the most widely studied group, perhaps because of the importance of these fungi in the tropics. The genus *Glomus* is dominant in the mycorrhizal composition of Colombia, followed by *Acaulospora* and *Gigaspora*. However, it is necessary to continue studies on this group of fungi to determine the actual diversity and distribution existing, especially in areas of the country where they have not yet been carried out.

The data obtained in this analysis, according to the reported works, allow to affirm that the most representative genus in Colombian soils is *Glomus* with 38 species, followed by *Acaulospora* with 13 species, *Penicillium* with 12 species, *Aspergillus* and *Fusarium* each with 10 species and *Mortierella* and *Trichoderma* each with 7 species. The other genera appear in lesser proportion and with less diversity of species in the soil.

It is essential to initiate studies at the mycological level in some of the country's unexplored departments, so as to contribute to the knowledge of fungal biodiversity within the natural regions of Colombia, in order to conserve the richness of these species, which is urgency, if considered that there are now strong anthropogenic threats that continually change the environmental conditions of ecosystems that have not yet been studied. Likewise, research on native soil fungi in the Insular region becomes crucial.

In order to complement the information about native soil fungi in Colombia, it is also essential to understand the fungal ecological complexity, this is why is necessary to delve into how soil fungi are influenced by aspects such as altitude, latitude and edaphic characteristics such as the PH, especially considering the great ecosystem heterogeneity of the country.

It is important to continue research leading to an understanding of how different uses and management of soil affect mycological diversity, since the data obtained so far are ambiguous, and in some cases contradictory. This idea, could also contribute to the knowledge about the potential of utilization of soil fungi in promising areas such as bioremediation.

Knowledge about the biological diversity of soil fungi as well as the understanding of their ecology in relation to interspecific interactions and with other microbiological entities of the soil, also represents an important area of information that can contribute to a solution in aspects such as optimization of agroecosystems, recovery of highly anthropic areas and conservation of natural ecosystems, especially considering the great functional potential of soil fungi such as arbuscular mycorrhizae, cellulolytic, lignolytic, antagonists, phosphate and calcium solubilizers, among others.

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Supplementary Materials

Soil fungi biodiversity in colombia: microfungi

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Table 1. List of the species recorded in Colombian soil

Absidia corymbifera
Acaulospora appendicula
Acaulospora brasiliensis
Acaulospora cf excavata
Acaulospora cf scrobibulata
Acaulospora denticulate
Acaulospora foveata
Acaulospora longula
Acaulospora mellea
Acaulospora morrowiae
Acaulospora myriocarpa
Acaulospora rehmii
Acaulospora spinosa
Acaulospora tuberculata
Acremonium cerealis
Acremonium killiense
Acremonium strictum
Alternaria alternata
Archaeospora leptoticha
Archaeospora shenckii
Aspergillus aculeatus
Aspergillus candidus
Aspergillus flavoclavatus
Aspergillus flavus
Aspergillus fumligatus
Aspergillus nidulans
Aspergillus níger
Aspergillus oryzae
Aspergillus terreus
Aureobasidium pullulans
Botrytis cinerea
Chaetomium cochlioides
Chrysosporium keratinophilum,
Circinella simplex
Cladosporium cladosporioides
Claroideoglomus etunicatum
Cunninghamella echinulata
Cunninghamella elegans
Curvularia brachyspora.
Curvularia spicifer
Cylindrocarpon destructans
Cylindrocarpon didymum
Cylindrocarpon obtusisporum
Dactylaria fusiformis
Emericella nivea
Emericella rugulosa
Entrophospora colombiana
Entrophospora colombiana
Entrophospora infrequens
Epicoccum purpurascens
Eupenicillium shearii
Fusarium avenaceum
Fusarium equisetii
Fusarium graminearum
Fusarium merismoides
Fusarium moniliforme
Fusarium oxysporum
Fusarium poae
Fusarium redolens
Fusarium solani
Fusarium sporotrichioides
Genasinospora retispora
Geotrichum candidum
Gigaspora albida
Gigaspora heterogama
Gilmaniella humicola
Gliocladium catenulatum
Gliocladium roseum
Gliocladium virens
Glomus fulvum
Glomus aggregatum
Glomus ambisporum
Glomus boreale
Glomus brohultii
Glomus citricola
Glomus claroides
Glomus claroideum
Glomus clarum
Glomus desertícola
Glomus diaphanum
Glomus dimorphicum
Glomus etunicatum
Glomus fasciculatum
Glomus fecundisporum
Glomus fragilistratum
Glomus fulvum
Glomus geosporum
Glomus glomerulatum
Glomus hoi
Glomus intraradices
Glomus invermaium
Glomus leptotichum
Glomus macrocarpum
Glomus maculosum
Glomus manihotis
Glomus microaggregatum
Glomus microcarpum
Glomus multicaule
Glomus occultum
Glomus radiatum
Glomus rubiformis
Glomus sinuosum
Glomus taiwanensis
Glomus tortuosum
Glomus trimulares
Glomus viscosum
Gongronella butleri
Heteroconium chaetospira
Humicola fuscoatra
Humicola grisea
Kuklosospora colombiana
Mariannaea elegans
Metarhizium anisopliae
Microsporon fulvum
Microsporon gypseum =Nannizzia gypseum (perfecto estado)
Mortierella alpina
Mortierella gamsii
Mortierella humilis
Mortierella minutisima
Mortierella ornata
Mortierella ramanniana var autotrophica
Mortierella vinacea
Mucor circinelloides f. circinelloides
Mucor hiemalis
Mucor racemosus
Myrothecium roridum
Nannizia incurvata
Paecilomyces lilacinus
Penicillium brevicompactum
Penicillium cítreo-viridae
Penicillium expansum
Penicillium frecuentans
Penicillium glabrum
Penicillium implicatum
Penicillium italicum
Penicillium janthinellum
Penicillium nigricans
Penicillium rubrum
Penicillium simplicissimum
Penicillium verrucosum
Phialophora cyclaminis
Phoma laveillei
Phoma medicaginis
Pithomyces chartarum
Rhinocladiella phaeophora
Rhizopus oligosporus
Rhizopus oryzae
Sclerocystis sinuossa
Scopulariopsis brevicaulis
Scutellospora heterogama
Scutellospora pellucida
Scutellospora savannicola
Scutellospora spinosissima
Sordaria fimicola
Stachybotrys chartarum
Syncephalastrum racemosum
Trichocladium asperum
Trichocladium canadense
Trichocladium opacum
Trichoderma aureoviride
Trichoderma hamatum
Trichoderma harzianum
Trichoderma inhamatum
Trichoderma koningii
Trichoderma viride
Trichophyton ajelloi
Trichophyton terrestre = Anthroderma quadrifidum (perfecto estado)
Trichosporon beigelii
Verticillium lecanii
Volutella ciliata

Table 3. Native soil fungal records in Colombia and references

REPORTED GENRE	REPORTED SPECIES	REFERENCES
Not specified	Not specified	Álvarez-Yela AC, Alvarez-Silva M, Restrepo S, Husserl J, Zambrano M, Danies G, et al. Influence of agricultural activities in the structure and metabolic functionality of paramo soil samples in Colombia studied using a metagenomics analysis in dynamic state. Ecological Modelling 2017; (351) 63-76.
Aspergillus, *Aureobasidium*, *Beauveria*, *Bionectria*, *Coniothyrium*, *Diplogelasinospora*, *Drechslera*, *Fusarium*, *Geomyces*, *Hypocrea*, *Leptosphaeria*, *Mortierella*, *Mucor*, *Neonectria*, *Paecilomyces*, *Penicillium*, *Preussia*, *Torula*, *Trichoderma*, *Trichosporon*, *Truncatella*, *Umbelopsis*.	Not specified	Avellaneda-Torres LM, Torres-Rojas E. Biodiversidad de grupos funcionales de microorganismos asociados a suelos bajo cultivo de papa, ganadería y páramo en el Parque Nacional Natural de Los Nevados, Colombia. Biota Colombiana 2015; 16(1): 78-87.
Penicillum, Scopuraliopsis	Not specified	Beltrán-Pineda M. Hngos solubilizadores de fosfato en suelo de páramo cultivado con papa (Solanum tuberosum). Revista Ciencia en Desarrollo 2014; 5(2): 145-154.
Glomus, Acaulospora	Not specified	Bernal E, Celis S, Galíndez X, Moratto C, Sánchez J, García D. Microflora cultivable y endomicorrizas obtenidas en hojarasca de bosque (páramo Guerrero - finca Puente de Tierra) Zipaquirá, Colombia. Acta biol. Colomb. 2006; 11(2): 125-130.
Acaulospora, Entrophospora, Gigaspora, Glomus, Sclerocystis, Scutellospora	*Acaulospora appendicula* *Acaulospora mellea* *Entrophospora colombiana* *Glomus intraradices* *Glomus invermaium* *Glomus macrocarpum* *Glomus occultum* *Sclerocystis sinuossa* *Scutellospora* sp.	Bolaños M, Rivillas-Osorio C, Suárez-Vásquez S. Identificación de micorrizas arbusculares en suelos de la zona cafetera colombiana. Cenicafé 2000; 51(4):245-262.
Glomus	*Glomus* sp.	Cardona G, Arcos A, Murcia U. Abundancia de actinomicetes y micorrizas arbusculares en paisajes fragmentados de la Amazonia colombiana. Agronomía Colombiana 2005; 23(2): 317-326.
Acaulospora, *Glomus*	*Acaulospora foveata* *Acaulospora* sp1 *Acaulospora* sp2	Cardona G, Peña-Venegas CP, Arcos A. Ocurrencia de hongos formadores de micorriza arbuscular asociados a ají (Capsicum sp.) en la amazonia colombiana. Agronomía Colombiana 2008; 26(3): 459-470.

	Glomus microaggregatum *Glomus* sp1 *Glomus* sp2 *Glomus* sp3 *Glomus* sp4 *Glomus* sp5	
Absidia, Acremonium, Alternaria, Aspergillus, Cladosporium, Cuninghamella, Curvularia, Emericella,Epicoccum, Fusarium, Geotrichum, Gliocadium, Metarhizium, Monilliella, Mucor, Nannizia, Paecilomyces, Penicillium, Phitomyces, Phoma, Rhizopus, Scopulariopsis, Stachybotris, Syncephalastrum, Trichoderma.	*Absidia corymbifera* *Acremonium killiense* *Acremonium* sp. cepa SG12 *Alternaria alternata* *Aspergillus alutaceus* *Aspergillus flavus* *Aspergillus fumigatus* *Aspergillus nidulans* *Aspergillus niger* *Aspergillus oryzae* *Aspergillus terreus* *Cladosporium* sp. *Cunninghamella elegans* *Cunninghamella echinulata* *Curvularia spicifer* *Emericella nivea* *Epicoccum purpurascens* *Fusarium equisetti* *Fusarium moniliforme* *Fusarium oxysporum* *Geotrichum candidum* *Gliocadium roseum* *Metharrhizium anopsiliae* *Moniliella* sp. SG61 *Mucor hiemalis* *Nannizia incurvata* *Paecilomyces lilacinus* *Paecilomyces* sp. *Penicillium brevicompactum* *Penicillium expansum* *Penicillium frecuentans* *Penicillium italicum*	Chitiva-Jaramillo A, Torrenegra-Guerrero R, Cabrera-Parada C, Díaz-Puentes N, Pineda-Parra V. Contribución al estudio de microhongos filamentosos en los ecosistemas páramo de Guasca y el Tablazo. Estudio preliminar de mohos de páramos colombianos. Grupo de Investigación en Fitoquímica y Biotransformación. Departamento de Química. Pontificia Universidad Javeriana. 2007. [acceso: 03 de marzo de 2017]. URL: http://www.javeriana.edu.co/gifuj/hongos_%20ecosistemas_%20paramo.pdf

	Penicillium rubrum *Penicillium* sp. cepa SG20 *Penicillium* sp. cepa SG64 *Penicillium* sp. cepa SG91 *Penicillium* sp. cepa SG94 *Penicillium verrucosum* *Phoma* sp. cepa SG *Pithomyces chartarum* *Rhizopus oligosporus* *Rhizopus oryzae* *Scopulariopsis brevicaulis* *Stachybotrys chartarum* *Syncephalastrum racemosum* *Trichoderma harzianum* *Trichoderma hamatum*	
Acaulospora *Entrophospora* *Glomus* *Scutellospora*	*Acaulospora appendicular* *Acaulospora foveata=Acaulospora scrobiculata* *Acaulospora mellea=Acaulospora morrowae=Acaulospora longula= Acaulospora spinosa* *Acaulospora myriocarpa* *Entrophospora colombiana* *Glomus fasciculatum* *Glomus glomerulatum* *Glomus manihotis* *Glomus occultum* *Scutellospora heterogama* *Scutellospora savannicola* *Scutellospora* sp.	Dodd JC, Arias I, Koomen I, Hayman DS. The management of populations of vesicular-arbuscular mycorrhizal fungi in acid-infertile soils of a savanna ecosystem. Plant and Soil 1990; (122): 229-240.
Trichoderma	*Trichoderma harzianum* *Trichoderma hamatum* *Trichoderma viride* *Trichoderma aureoviride*	Elías R, Arcos O, Arbeláez G. Estudio del antagonismo de algunas especies de Trichoderma aisladas de suelos colombianos en el control de Fusarium oxysporum y Rhizoctonia solani. Agron. Colomb. 1993; 10(1): 52-61.
Metarhizium, Paecilomyces	*Metarhizium anisopliae* *Paecilomyces lilacinus*	García A, Bustillo A, Castro U, Arenas Y. Selección de hongos entomopatógenos para controlar salivazos (Hemiptera: Cercopidae) de la caña de azúcar en Colombia. Revista Colombiana de Entomología 2012; 38(2): 252-259.

Glomus, Acaulospora	Not specified	Gómez F, Úsuga C, Franco A. Evaluación del recurso micorrizal en ecosistema natural y agroecosistema bananero del Urabá-Antioquia, Colombia. Politécnica 2006; (3): 41-48.
Acremonium, Aureobasidium, Botrytis, Chaetomium, Circinella, Cladosporium, Coniothyrium, Curvularia, Cylindrocarpon, Dactylaria, Diplodia, Emericella, Epicoccum, Eupenicillium, Fusarium, Genasinospora, Geotrichum, Gilmaniella, Gliocadium, Heteroconium, Humicola, Mariannaea, Mortierella, Mucor, Myrothecium, Paecilomyces, Penicillium, Phialophora, Phoma, Sordaria, Trichocladium, Trichoderma, Trichosporon, Verticillium, Volutella, Zygorrhynchus	*Acremonium cerealis* *Acremonium strictum* *Aureobasidium pullulans* *Botrytis cinerea* *Chaetomium cochlioides* *Circinella simplex* *Cladosporium cladosporioides* *Coniothyrium sp.* *Curvularia brachyspora.* *Cylindrocarpon destructans* *Dactylaria fusiformis* *Diplodia sp* *Emericella rugulosa* *Epicoccum purpurascens* *Eupenicillium shearii* *Fusarium avenaceum* *Fusarium equisetii* *Fusarium graminearum* *Fusarium merismoides* *Fusarium oxysporum* *Fusarium poae* *Fusarium redolens* *Fusarium solani* *Fusarium sp.* *Fusarium sporotrichioides* *Genasinospora retispora* *Geotrichum candidum* *Gilmaniella humicola* *Gliocladium roseum* *Gliocladium virens* *Heteroconium chaetospira* *Humicola fuscoatra* *Mariannaea elegans* *Mortierella alpina* *Mortierella gamsii*	Gualdrón-Arenas C, Suárez-Navarro A, Valencia-Zapata H. Hongos del suelo aislados de zonas de vegetación natural del páramo de Chisacá, Colombia. Caldasia 1997; 19(1-2): 235-245.

	Mortierella humilis *Mortierella minutisima* *Mortierella vinacea* *Mucor circinelloides f. circinelloides* *Mucor hiemalis f luteus* *Mucor racemosus f. sphaerosporus* *Mramanniana var autotrophica* *Myrothecium roridum* *Paecilomyces lilacinus* *Penicillium frecuentans* *Penicillium nigricans* *Penicillium rubrum* *Penicillium simplicissimum* *Penicillium verrucosum* *Phialophora cyclaminis* *Phoma laveillei* *Phoma medicaginis* *Sordaria fimicola* *Trichocladium opacum* *Trichoderma hamatum* *Trichoderma koningii* *Trichoderma viride* *Trichosporon beigeiii* *Verticillium lecanii* *Volutella ciliata* *Zygorrhynchus* sp	
Not specified	Not specified	Lizarazo-Medina PX, Gómez-Vásquez D. Microbiota rizosférica de Espeletia spp. de los páramos de Santa Inés y de Frontino-Urrao en Antioquia, Colombia. Acta biol. Colomb. 2015; 20(1): 175-182.
Acremonium, Arthrinium, Aspergillus, Chalara, Chloridium, Chrysosporium, Cladosporium, Cylindrocarpon, Cylindrocladium, Dictyosporium, Doratomyces , Fusarium, Gilmaniella, Gliocladium, Gliomastix, Gnomonia, Graphium, Harposporium, Humicola, Mortierella, Mucor, Myrothecium, Paecilomyces, Penicillium, Periconia,	*Acremonium sclerotigenum* *Fusarium oxysporum* *Graphium fructicolum* *Paecilomyces lilacinus* *Penicillium nigricans* *Petriella* sp.	Llanos C, Kjøller A. Changes in the flora of soil fungi following oil waste application. Oikos 1976; 27(3): 377-382.

Pestalotia, Polyscytalum, Rhinocladiella, Rhizopus, Scopulariopsis, Scytalidium, Sphaerosporium, Stachybotrys, Torula, Trichoderma, Verticillium, Volutella		
Acaulospora, Archaeospora, Claroideoglomulus, Glomus, Kuklospora	*Acaulospora brasiliensis* *Acaulospora cf* excavata *Acaulospora cf scrobibulata* *Acaulospora* sp2 *Acaulospora* sp5 *Acaulospora* sp8 *Acaulospora* sp10 *Acaulospora* sp15 *Acaulospora* sp18 *Acaulospora* sp19 *Acaulospora* sp20 *Acaulospora* sp21 *Acaulospora* sp23 *Archaeospora schenckii* *Claroideoglomus etunicatum* *Claroideoglomulus* sp4 *Claroideoglomulus* sp6 *Glomus broultii* *Glomus* sp5 *Kuklosospora colombiana*	Mahecha-Vásquez G, Sierra S, Posada R. Diversity indices using arbuscular mycorrhizal fungi to evaluate the soil state in banana crops in Colombia. Applied Soil Ecology 2017; (109): 32-39.
Acaulospora, Glomus	Not specified	Molineros FH, Mosquera-Espinosa AT, Gómez-Carabalí A, Tupac J. Temporal variation in arbuscular mycorrhizal fungi colonization of Bactris gasipaes Kunth in Buenaventura, Colombia. Acta Agronómica 2013; 62(4): 344-351.
Acaulospora, Entrophospora, Glomus, Scutellospora	*Acaulospora denticulate* *Acaulospora mellea* *Acaulospora morrowiae* *Acaulospora scrobiculata* *Acaulospora* sp1 *Acaulospora* sp2 *Acaulospora* sp3 *Acaulospora* sp4 *Entrophospora infrequens* *Glomus citricola*	Monroy H, Salamanca C, Cano C, Moreno-Conn L, Orduz-Rodríguez J. Influencia de las coberturas en cultivos de cítricos sobre los hongos formadores de micorrizas arbusculares en Oxisoles del piedemonte llanero colombiano. Corpoica. Ciencia y Tecnología Agropecuaria 2013; 14(1): 53-65.

	Glomus desertícola *Glomus fasciculatum* *Glomus geosporum* *Glomus invermaium* *Glomus macrocarpum* *Glomus microaggregatum* *Glomus occultum o Paraglomus occultum* *Glomus* sp1 *Glomus* sp2 *Glomus* sp3 *Scutellospora heterogama* *Scutellospora pellucid* *Scutellospora savannicola* *Scutellospora* sp1 *Scutellospora* sp2 *Scutellospora* sp3	
Acremonium, Alternaria, Circinella, Cylindrocarpo, Gilmaniella, Gliocladium=Clonostachys, Humicola, Mortierella, Mucor, Penicillium, Rhizoctonia, Trichocladium, Trichoderma, Zygorrhynchus	*Acremonium* sp. *Alternaria* sp. *Circinella* sp. *Cylindrocarpon* sp. *Gilmaniella humícola* *Gilmaniella* sp. *Gliocladium catenulatum* *Gliocladium roseum* *Humicola grisea* *Mortierella* sp. *Mucor* sp1 *Mucor* sp2 *Mucor racemosus* *Penicillium frecuentans* *Penicillium glabrum* *Penicillium* sp1 *Penicillium* sp2 *Penicillium* sp3 *Penicillium* sp4 *Rhizoctonia* sp. *Trichocladium asperum*	Moratto C, Martínez L, Valencia H, Sánchez J. Efecto del uso del suelo sobre hongos solubilizadores de fosfato y bacterias diazotróficas en el páramo de Guerrero (Cundinamarca). Agronomía Colombiana 2005; 23(2): 299-309.

	Trichocladium canadense *Trichoderma koningii* *Trichoderma* sp1 *Trichoderma* sp2 *Trichothecium* sp1 *Zygorrhynchus* sp.	
Ceratobasidium	*Ceratobasidium* spp.	Mosquera-Espinosa A, Bayman P, Túpac J. Ceratobasidium como hongo micorrízico de orquídeas en Colombia. Acta Agronómica 2010; 59(3): 316-326.
Fusarium, Mucor, Penicillium, Verticillium, Zygorhynchus,	Not specified	Ortiz M, Uribe D. Aislamiento de hongos degradadores de lignina a partir de suelos con dos usos agrícolas (sabana de pastoreo y bosque secundario) de sabana inundable, Puerto López (Meta). Sociedad Colombiana de la Ciencia del Suelo. Suelos Ecuatoriales 2007; 37(1): 70-74.
Actinomucor, Cladosporium, Fusarium, Geotrichum, Mucor, Oidiodendron, Phialophora, Penicillium, Trichoderma, Trichosporonoides, Verticillium, Zygorhynchu.	Not specified	Ortiz M, Uribe D. Determinación de la actividad lignocelulolítica en sustrato natural de aislamientos fúngicos obtenidos de sabana de pastoreo y de bosque secundario de sabana inundable tropical. Suelo 2010; 28(2): 169-180.
Acaulospora *Gigaspora* *Glomus* *Scutellospora*	*Acaulospora foveata* *Acaulospora morrowiae* *Acaulospora tuberculata* *Acaulospora* sp *Archaeospora leptoticha* *Gigaspora* sp. *Glomus manihotis* *Glomus viscosum* *Glomus* sp1 *Glomus* sp2 *Glomus* sp3 *Glomus* sp4 *Glomus* sp5 *Glomus* sp6 *Glomus* sp7 *Glomus* sp8 *Glomus* sp9 *Scutellospora pellucida*	Peña-Venegas CP, Cardona GI, Arguelles JH, Arcos AL. Micorrizas arbusculares del sur de la amazonia colombiana y su relación con algunos factores fisicoquímicos y biológicos del suelo. Acta amazónica 2007; 37(3): 327-326.
Acaulospora, Archaeospora, Entrophospora, Gigaspora, Glomus, Scutellospora	*Acaulospora foveata* *Acaulospora mellea* *Acaulospora morrowiae*	Peña-Venegas CP, Cardona GI, Mazorra A, Arguellez J, Arcos A. Micorrizas arbusculares de la amazonia colombiana. Catálogo ilustrado. Instituto Amazónico de Investigaciones Científicas - SINCHI, 1era ed. Colombia; 2006.

	Acaulospora rehmii *Acaulospora tuberculata* *Archaeospora leptoticha* *Entrophospora colombiana* *Gigaspora* sp1 *Glomus brohultii* *Glomus glomerulatum* *Glomus intraradices* *Glomus manihotis* *Glomus microaggregatum* *Glomus rubiformis* *Glomus sinuosum* *Glomus* sp1 *Glomus* sp2 *Glomus* sp3 *Glomus* sp4 *Glomus* sp5 *Glomus* sp6 *Glomus* sp7 *Glomus* sp8 *Glomus* sp9 *Glomus* sp10 *Glomus* sp11 *Glomus tortuosum* *Glomus viscosum* *Scutellospora pellucida* *Scutellospora* sp1 *Scutellospora spinosissima*	
Aspergillus, Paecilomyces, Penicillium	*Aspergillus candidus* *Aspergillus flavus* *Aspergillus níger* *Aspergillus terreus*	Pérez A, De La Ossa J, Montes D. Hongos solubilizadores de fosfatos en fincas ganaderas del departamento de Sucre. Rev. Colombiana Cienc. Anim. 2012b; 4(1): 35-45.
Gigaspora, Glomus	*(cf) Gigaspora albida* *(cf) Glomus aggregatum* *(cf) Glomus boreale* *(cf) Glomus citrícola* *(cf) Glomus claroides* *(cf) Glomus diaphanum*	Pérez A, Espitia F, Martínez E. Diversidad de micorrizas arbusculares en agroecositemas de pastura del departamento de Sucre. Rev. Colombiana Cienc. Anim. 2012a; 4(2): 333-343.

	(cf) Glomus etunicatum *(cf) Glomus fasciculatum* *(cf) Glomus fragilistratum* *(cf) Glomus fulvum* *(cf) Glomus geosporum* *(cf) Glomus hoi* *(cf) Glomus invermayanum* *(cf) Glomus leptotichum* *(cf) Glomus macrocarpum* *(cf) Glomus maculosum* *(cf) Glomus multicaule* *(cf) Glomus occultum* *(cf) Glomus* spp *(cf) Glomus taiwanensis*	
Gigaspora, Glomus, Paraglomus	*(Cf) Gigaspora albida* *(Cf) Glomus aggregatum* *(Cf) Glomus ambisporum* *(Cf) Glomus boreale* *(Cf) Glomus claroideum* *(Cf) Glomus clarum* *(Cf) Glomus diaphanum* *(Cf) Glomus dimorphicum* *(Cf) Glomus etunicatum* *(Cf) Glomus fasciculatum* *(Cf) Glomus fecundisporum* *(Cf) Glomus fragilistratum* *(Cf) Glomus fulvum* *(Cf) Glomus geosporum* *(Cf) Glomus leptotichum* *(Cf) Glomus maculosum* *(Cf) Glomus multicaule* *(Cf) Glomus occultum = Paraglomus occultum* *(Cf) Glomus radiatum* *(Cf) Glomus trimulares* *Glomus sp.* *Glomus sp.* *Sclerocystis sp = Glomus sp*	Pérez A, Peroza V. Micorrizas arbusculares asociadas al pasto angleton (Dichathium aristatum Benth) en fincas ganaderas del municipio de Tolú, Sucre-Colombia. Rev.MVZ Córdoba 2013; 18(1): 3362-3369.

Aspergillus, Humícola, Penicillium, Rhizopus,	Not specified	Pérez R, Vertel M, Pérez A. Efecto de diferentes tipos de abonos sobre hongos edáficos en el agroecosistema de Bothriochloa pertusa, (L) A. Camus, en sabanas sucreñas, Colombia. Revista Livestock Research for Rural Development 2015; 27(1).
Cylindrocarpon	*Aspergillus* sp1 *Chaetomium* sp1 *Cylindrocarpon didymumb* *Cylindrocarpon obtusisporumb* *Cylindrocarpon* sp1 *Cylindrocarpon* sp2 *Cylindrocarpon* sp3 *Cylindrocarpon* sp4 *Cylindrocarpon* sp5 *Cylindrocarpon* sp6 *Cylindrocarpon* sp7 *Cylindrocarpon* sp8 *Cylindrocarpon* sp9 *Cylindrocarpon* sp10 *Cylindrocarpon* sp11 *Fusarium* sp4 *Fusarium* sp5 *Fusarium* sp6 *Fusarium* sp7 *Fusarium* sp8 *Fusarium* sp10	Posada R, Heredia-Abarca G, Sieverding E, Sanchez M. Solubilization of iron and calcium phosphates by soil fungi isolated from coffee plantations. Archives of Agronomy and Soil Science 2013; 59(2): 185-196.
Aspergillus *Chaetomium* *Cladosporium* *Cylindrocarpon* *Fusarium* *Humicola* *Paecilomyces* *Penicillium* *Talaromyces*	*Aspergillus* sp. *Chaetomium* spp. *Cladosporium* sp. *Cylindrocarpon* spp. *Fusarium* spp. *Humicola* sp. *Paecilomyces* sp. *Penicillium* spp. *Talaromyces* sp.	Posada R, Sánchez M, Sieverding E, Aguilar K, Heredia-Abarca G. Relaciones entre los hongos filamentosos y solubilizadores de fosfatos con algunas variables edáficas y el manejo de cafetales. Rev. Biol. Trop. 2012; 60(3): 1075-1096.
Fusarium, Penicillium, Rhizopus, Thielaviopsis, Trichoderma	Not specified	Riascos-Ortiz D, Sarria-Villa GA, Varón F, Gómez-Carabalí A, Mosquera-Espinosa A. Reconocimiento de hongos con potencial benéfico asociados a la rizósfera de chontaduro (Bactris gasipaes H.B.K.) en la región pacifico del Valle del Cauca, Colombia. Acta agronómica 2011; 60(4): 319-327.

Chrysposporium *Microsporum* *Trichophyton* *Arthroderma*	*Chrysosporium keratinophilum* *Microsporon fulvum* *Microsporon gypseum = Nannizzia gypseum* *Trichophyton ajelloi* *Trichophyton terrestre = Arthroderma quadrifidum*	Rogers AL. Isolation of keratinophilic fungi from soil in the vicinity of Bogota, Colombia. Mycopathologia et Mycologia applicate 1971; 44(3): 261-264.
Aspergillus *Cladosporium* *Dendrodochium* *Fusarium* *Myrothecium* *Paecilomyces* *Penicillium* *Peyronellae* *Volutella*	*Aspergillus* sp. *Cladosporium* sp. *Dendrodochium* sp. *Fusarium* sp. *Myrothecium* sp. *Paecilomyces* spp. *Penicillium* spp. *Peyronellae* sp. *Volutella* sp.	Salazar-Moncada DA, Jaramillo-Mazo C, Vásquez-Restrepo J, Ramírez C, Cardona-Bustos NL. Antagonismo de Purpureocillium sp. (cepa UdeA0106) con hongos aislados de cultivos de flores. Actual Biol. 2014; 36(100): 23-31.
Acaulospora, Entrophospora, Glomus	*Acaulospora appendicula* *Acaulospora longula* *Acaulospora mellea* *Acaulospora morrowae* *Glomus manihotis* *Entrophospora colombina*	Schenck NC, Spain JL, Sieverding E, Howeler RH. Several new and unreported vesicular-arbuscular mycorrhizal fungi (endogonaceae) from colombia. Mycologia 1984; 76(4): 685-699.
Acaulospora, Entrophospora, Gigaspora, Glomus, Scutellospora	Not specified	Serralde A, Ramírez M. Análisis de poblaciones de micorrizas en maíz (Zea mays) cultivado en suelos ácidos bajo diferentes tratamientos agronómicos. Revista Corpoica 2004; 5(1).
Acaulospora, Entrophospora, Gigaspora, Glomus	*Acaulospora appendicula* *Acaulospora longula* *Entrophospora colombiana* *Glomus microcarpum* *Glomus fasciculatum* *Glomus manihotis* *Glomus occultum* *Glomus occultum* *Gigaspora heterogama*	Sieverding E, Howeler R. Influence of species of VA mycorrhizal fungi on cassava yield response to phosphorus fertilization. Plant and Soil 1985; (88): 213-221.
Aspergillus, Moniliella, Mortierella, Penicillium, Paecilomyces, Scopulariopsis	*Aspergillus fumigatus* *Aspergillus niger* *Moniliella* sp. *Mortierella* sp	Useche Y, Valencia H, Pérez H. Caracterización de bacterias y hongos solubilizadores de fosfato bajo tres usos de suelo en el sur del trapecio amazónico. Acta Biológica Colombiana 2004; 9(2): 129.

	Paecilomyces spp. *Penicillium cítreo-viridae f.* *Penicillium implicatum* *Penicillium* spp. *Scopulariopsis* sp.	
Aspergillus, Fusarium, Gliocladium, Gongronella, Paecilomyces, Penicillium, Trichoderma	*Aspergillus aculeatus* *Aspergillus flavoclavatus* *Aspergillus oryzae* *Fusarium oxysporum* *Fusarium redolens* *Gliocladium catenulatum* *Gongronella butleri* *Paecilomyces* cepa 1 *Paecilomyces* cepa 2 *Paecilomyces* cepa 3 *Paecilomyces* cepa 4 *Penicillium janthinellum* *Trichoderma* cepa 1 *Trichoderma* cepa 1 agr. *aureoviride* *Trichoderma* cepa 1 agr. *longibrachiatum* *Trichoderma* cepa 1 agr. *viride* *Trichoderma* cepa 2 *Trichoderma* cepa 2 agr. *aureoviride*	Vera D, Pérez H, Valencia H. Aislamiento de hongos solubilizadores de fosfatos de la rizósfera de Arazá (Eugenia stipitata, Myrtaceae). Acta Biológica Colombiana 2002b; 7(1): 33-40.
Aspergillus, Fusarium, Gliocladium, Gongronella, Paecilomyces, Penicillium, Trichoderma	*Aspergillus aculeatus* *Aspergillus flavoclavatus* *Aspergillus oryzae* *Fusarium oxysporum* *Fusarium redolens* *Gliocladium catenulatum* *Gongronella butleri* *Paecilomyces* cepa 1 *Paecilomyces* cepa 2 *Paecilomyces* cepa 3 *Paecilomyces* cepa 4 *Penicillium janthinellum* *Trichoderma* cepa 1 *Trichoderma* cepa 1 agr. *aureoviride*	Vera D, Pérez H, Valencia H. Distribución de hongos solubilizadores de fosfatos en dos microhabitats de suelo de dos unidades fisiográficas de Guaviare, Colombia. Acta Biológica Colombiana 2002a; 7(1): 23-31.

	Trichoderma cepa 1 agr. *longibrachiatum* *Trichoderma* cepa 1 agr. *viride* *Trichoderma* cepa 2 *Trichoderma* cepa 2 agr. *aureoviride*	
Mortierella, Rhinocladiella, Trichoderma	*Mortierella ornata* *Trichoderma inhamatum* *Rhinocladiella phaeophora*	Veerkamp J, Games W. Los hongos de Colombia - VIII. Some new species of soil fungi from Colombia. Caldasia 1983; 12(65): 710-717.

IV. Paper 3

Meta-genomic analysis of soil mycobiota in upper Andean Colombian agro-environment

Meta-genomic analysis of soil mycobiota in upper Andean Colombian agro-environment

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Abstract

Soil mycobiota of the Colombian High-Andean agro-environment is assessed through high-throughput sequencing technology. The entire soil fungal community from qualitative and quantitative point of view in areas with different land use (2 different orchards, 1 uncultivated grass field, and 1 woodland) is depicted. Beside the evaluation of Colony Forming Units per g of soil dry weight, fungal community was described for its species composition. The bootstrap-based clustering analysis highlighted that different land use strongly influences the soil mycobiota: the uncultivated (grassland and woodland) and cultivated areas are characterized by abundant presence of some exclusive species. Differences in fungal species composition is consistent with the clustering analysis on higher taxonomic hierarchical level composition. The study area, considered as a whole, seems to draw a taxonomic gradient, where the woodland site is the most different from the cultivated sites and the uncultivated grassland in the intermediate position.

1. Introduction

Unveiling the global soil fungal diversity is a difficult problem to solve due to the fact that fungi are microscopic and most of them hidden (Wardle & Lindahl, 2014). In the Dictionary of Fungi (Kirk et al., 2008) the entry “number of Fungi” reports a total of 97 330 fungal species. This number reached 99.000 with the addition of 1300 species of Microsporidia by Hibbett et al. (2007). A large gap between known and estimated species numbers (1.5-5.1 million) has been highlighted by Blackwell (2011) but, although many species remain unknown, molecular methodology can drive to discover new species in a shorter time.

Soil is one of the richest reservoir of microorganisms and with the help of new molecular techniques rapid advances for understanding soil fungal biodiversity have been observed in the last decade. In a recent paper Tedersoo et al. (2014) analyzed 365 soil samples from natural locations all over the world (except Antarctica) by pyrosequencing, and fungal biodiversity has been correlated to different environmental factors. Data presented depict a soil fungal biodiversity highly influenced by mean annual precipitation and distance from equator. The highest fungal diversity resulted strongly linked to tropical region (Arnold and Lutzoni, 2007; Tedersoo et al., 2014) and endemism as well (Tedersoo et al., 2014).

The main contributions of the soil fungal community to functioning of all the terrestrial ecosystems are soil stabilization and nutrient cycling and consequently soil quality. In agricultural soils, in particular, the knowledge of the fungal community provides the basis to understand its function and to better manage the agroecosystem. Metagenomics and metabarcoding analysis of agro-environment soil microbioma have been faced by authors in the South America (Carbonetto et al., 2014; Alvarez-Yela et al. 2017) and Europe (Detheridge et al., 2016; Panelli et al., 2017).

In all case, data presented were consistent with the conclusion that the agricultural activities influence the taxonomic composition and metabolic functional diversity. Detailed meta-omics analyses have been particularly pivotal to focus on the effects of agricultural activities on soil ecosystem and they represent a strong tool for future plant protection strategies (Abdelfattah et al. 2017). The present work wants to contribute to the picture of soil mycobiota of Colombia, adding further information to what was already presented by Alvarez-Yela et al. (2017). Colombia is one of the country with one of the highest biodiversity of the world and fungi are expected to be highly represented. In Colombia 35.000 species of plants have been recorded and due to the association between plants and fungi proposed by Hawksworth (1991) it can be conjectured that fungal species could be more than 210.000.

Many researches have been carried out on fungi associated to crops and agricultural soil of Colombia but the mycological data are scattered although this country was one of the first tropical countries where an effort was made to gather a mycological flora. In this respect it must be evaluated the work of Fuhrman and Mayor in 1914, who provided information on parasitic fungi and the publication of Chardon and Toro in 1930, which first gathered mycological literature for a defined political region and presented the results of comparatively large explorations. Later, with the "Mycological Flora of Colombia" project, a cooperative program in mycology was established in 1974, in which more than 2000 collections of fungi were made in the departments of Cundinamarca, Antioquia, Valle, Cauca and Boyacá (Dumont et al., 1978).

Gradually, the characterization of mycological species for Colombia was enriched through serial publications on the "New or noteworthy fungi from Panama and Colombia", mainly coming from the Sierra Nevada of Santa Marta in the department of Magdalena (Martin, 1937; 1938; 1939a; 1939b). Although the literature presents some contributions on micromycetes of

Colombia (Singer, 1963, Guzmán, 1964; Dennis, 1970; Restrepo,1972; Rogers, 1971; Guzman and Varela, 1978; Llanos and Kjøller, 1976; Veerkamp and Gams, 1983).

Throughout history, mycological studies in Colombia have mainly focused on macrofungi and contributions to the taxonomy, diversity and ecology of microfungi are still insufficient, which is why it can be ascertained that the mycology of the country is little known. In fact, the attention to microfungi has increased only in the last 20 years and its research has been directed especially at functional groups, metabolites and dynamics in ecosystems.

Beneficial fungi associated to the rizosphaere of Bactris gasipaes in the valley of the Cauca river in the northwestern of Colombia, were characterized by Riascos-Ortiz et al. (2011).

Abundance and distribution of solubilizing phosphate fungi was investigated by Useche et al. (2004), Moratto et al. (2005), and Posada et al. (2012) in agricultural and semipristine soils.

The present study refers to the Cundiboyacense Region in Colombia, a high plateau located in the Eastern Cordillera of the Colombian Andes covering parts of the departments of Cundinamarca and Boyacá. The area has been rarely analyzed for fungi.

A mycological analysis of this area was carried out by Gualdron et al. (1997), in two natural habitats characterized by *Calamagrostis effusa* at 3600 m asl and *Gynoxys fuliginosa* at 3540 m asl in the paramo of Chisaca (Pasca). At least 62 species of fungi were recorded from soil samples. Sanchez & Romero (2004) analized saprophitic pathogenic fungi associated to Macleania rupestris in the two paramo El Granizo and Guasca.

Fungi different soil managment were studied by Moratto et al. (2005) in the paramo Guerrero (Zipaquirà, Cundinamarca).

Carreño et al. (2006) isolated 525 fungal strains belonging to 21 genera from soil, rizoplane, rizosphaera, and plant tissue of tree apple in cultivated areas in the department of Boyacá. Among them 13 strains were selected for their antagonistic effect against the phytopatogenic oomycete Phytophtora cactorum, attacking apple tree.

Beltrán (2014) studied fungi isolated from the rizosphaere of Solanum tuberosum cultivated in the paramo of Rabanal (Ventaquemada, Boyacá), in order to select fungi able to solubilize phosphate useful as biofertizer strains.

Selection of cellulolitic fungi was carried out by Castillo et al. (2015), analysing soil samples collected from natural woods, cereale crops, and composting farms in Boyacá.

The present research wants to contribute to elucidating soil fungal diversity of the upper Colombian Andean agro-environment in the Boyacá region. This area has been taken into consideration due to its agricultural importance and the knowledge of the microbiological condition is particular important to improve sustainable land use and managment.

Among the new molecular techniques, metabarcoding analysis by next generation sequencing gives new opportunities for studying soil biodiversity. In this research we applied the New Generation Sequencing analysis to obtain a metagenomic picture of culturable and unculturable soil fungi in a Colombian area that has recently been converted to agriculture.

2. Materials and Methods

2.1 Area of Study and sampling sites

The area of Boyacá (fig. 1) belongs to the altitudinal vegetation belt of upper Andean forest (upper mountain cloud forest) between 2800 and 3200 m asl. Since approximately 50 years, due to the human activity, this forest has been transformed in an uncultivated grassland mostly devoted to subsistence agriculture mainly based on potato and onion crops. Since 2012, in the context of improving the economy of the region, the University Juan Castellanos funded a project aimed of introducing economically relevant cultures, such as peaches and apples.

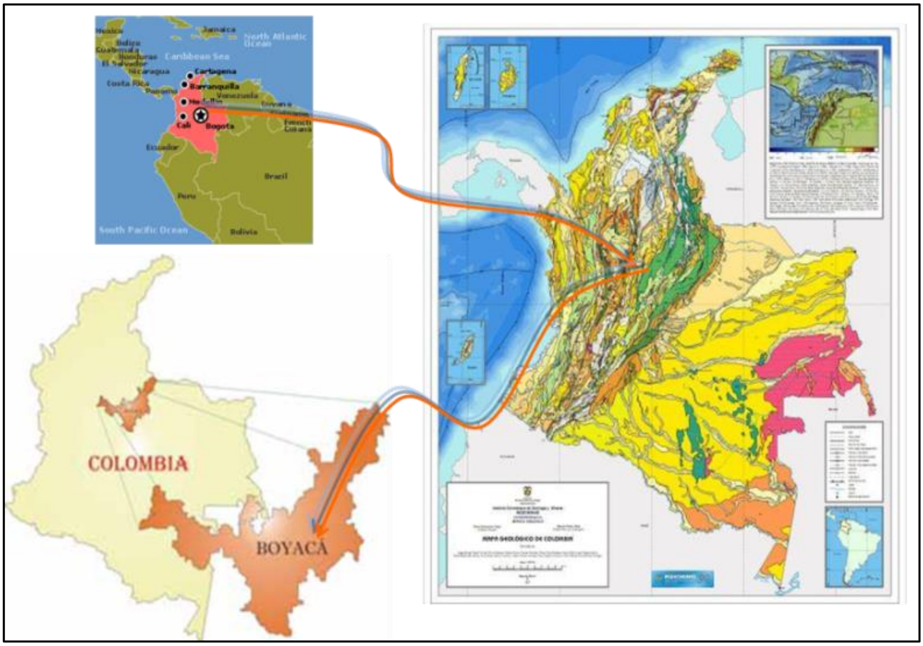


Fig. 1. Region of Boyacá, Colombia, South America.

The study area is at 2900 m asl and the coordinates are: 5º 30’ N y 73º W. Mean annual temperature is 12°C; the climate regime of the region is classified as ‘‘bimodal’’ with two distinct seasons, a wet one from April to June and a dry one. Mean precipitation is 900 mm/y, humidity is 75% and winds are almost constant: 18 Km/h from South-North. The daily temperature can fluctuate between 1ºC and 23ºC depending on the period of year.

The sampling sites in the present research belong to the experimental farm San Isidro Labrador belonging to Fundacion Universitaria Juan de Castellanos (Tunja, Colombia) and is located in the Village of Soracá (fig. 2). The following plots were sampled:

- 1 plot (named peach A) with peach trees var. dorado (Area: 7454.53 mq, Sud oriented).
- 1 plot (named peach B) with peach trees var. dorado (Area: 6690.61 mq, North oriented).
- 1 plot (named apple A and apple B, sampled from points differently exposed to winds) with apple trees var. Ana (area: 4405.95 mq).
- 1 plot (control area named “resting”) represented by an uncultivated

field abandoned for 10 years dominated by graminaceous plants (50 m X 50 m).

- 1 plot (control area named "wood") represented by a 10 year-old woodland (50 m X 10 m) composed by imported (*Eucalyptus globolus*, *Pinus* spp., *Acacia* spp.) and autochtonous (*Vaccinium meridionale*, *Myrtus communis*, local grass) plants.

The orchards were subjected to conventional treatments, fertilized and irrigated as reported in the following table 1.

Table 1. Agronomic management of orchards

ORCHARD	**PESTICIDES**								**FERILIZATION**	**IRRIGATION**	**ACTUAL CONDITION**
	Russet	**vs *Venturia inaequalis***	**vs *Taphrina doformans***	**vs *Phytophtora* spp.**	**vs *Tetranychus* spp.**	**vs leafminer**	**vs thrips**	**vs aphids**			
Peach			Clorotanoil 1cm3/l of water	CuSO4 in soil, 100g /tree	Abamectina, 200cm3/100 l of water alternating Sulphur 1 l/100 l of water	Peretroid Karate 0.5 cm3/l of water			Organic 10 kg per hole at planting; synthetic fertilizer composed by N P and K and minor elements 300 g/plant; foliar fertilizer 1 l/200 l of water	Plot A) 20 liters of water/plant have been applied every 20 days in the dry season	Vegetative growth next to perform first production pruning
Apple	phythoregulators	2g amistar, 5g Mancozeb, 2g benomil/l of water by foliar spray		CuSO4 in soil, 100g /tree			Peretroid Karate 0.5 cm3/l of water	Peretroid Karate 0.8 cm3/l of water	Organic 10 kg per hole at planting; synthetic fertilizer composed by N P and K and minor elements 300 g/plant; foliar fertilizer 1 l/200 l of water. fertilization for production 250g of N, 150g of P, and 400g of K. Increased by 10% for the 2nd production	230 liters of water/plant have been applied every 20 days in the dry season; Plot B) as in plot A but with 30 l/plant	Preparation for first production cycle

Fig. 2. Sampling site, experimental farm San Isidro Labrador, Soracá, Boyacá, Colombia.

2.2 Sample collection

Soil samples were collected during the dry season (January 2016). For quantitative fungal, metagenomic, and physico-chemical analyses three soil samples were collected in each plot to trace a triangle, with vertices placed as far as possible, but at least 10 m away from field edges. Soil samples (500 g each) were aseptically taken at 3 cm depth with sterile spoon, after removing the vegetation cover, other debris, and stones and put in sterile polyethylene bags. All the samples returned to the laboratory in coolers and maintained at low temperature until processed: at -20°C for metagenomics and at 4°C for fungal count. For metagenomics, samples referring to each plot were pooled before freezing.

2.3 Soil chemical analyses

Chemical properties of soils were determined by Labanalysis, Casanova Lonati (Pavia, Italy), according to the Italian standard protocols (DM 13/09/99). The following parameters were evaluated: pH, organic matter, total nitrogen (N_{TOT}), organic carbon (C_{ORG}), C/N ratio, plant-available phosphorous (P), calcium (Ca), magnesium (Mg), potassium (K), soil composition in sand, silt, and clay.

2.4 DNA extraction, ITS1 amplification and Illumina sequencing

Total DNA was extracted from 350 mg of single sample, using the NucleoSpin Soil kit (Macherey-Nagel, Düren, Germany) following the manufacturer's specifications. The extraction buffer SL1 was used, supplemented with 70 µl of SX enhancer. DNA was then quantified on a Qubit fluorometer (ThermoFisher Scientific, Waltham, MA). For amplicon production, the ribosomal ITS1 region was targeted, by using primers BITS and B58S3 (Bokulich and Mills, 2013) linked to Illumina adapters. PCR was performed in a 50-µl volume containing 5 to 10 ng template DNA, 1x HiFi HotStart Ready Mix (Kapa Biosystems, Wilmington, MA), 0.5 µM of each primer. The cycling program, performed on a MJ Mini thermal cycler (Promega corp., Madison, WI), included an initial denaturation (95°C for 3 min), followed by 25 cycles at 94°C for 30 s, 55 or 60°C C for 30s, 72°C for 30 s, and final extension (72°C for 5 min). Amplicons obtained using the two annealing temperatures were pooled as suggested by Schmidt et al. (2013). Clean-up of amplicons was performed using Agencourt AMPure XP SPRI magnetic beads (ThermoFisher Scientific). Illumina sequencing libraries were finally constructed through the link of indexes (Nextera XT Index Kit, Illumina, San Diego, CA), quantified using a Qubit 2.0 Fluorometer (ThermoFisher Scientific), normalized and pooled. Libraries were subjected to paired-end sequencing (2 x 250 bp, nano format) on an Illumina MiSeq sequencer at BMR Genomics (Padova, Italy).

2.5 Data analysis: definition of operational taxonomic units (OTUs) and community analyses

Data analysis was performed using the pipeline PIPITS (Gweon et al., 2015). In brief: raw data were demultiplexed based on the unique barcode assigned to each sample. Barcodes and primers were then trimmed off. Sequencing quality filters were applied, including a minimum length threshold (100 bp) and removal of singletons. High-quality reads were then clustered into operational taxonomic units (OTUs) at 97% similarity using VSEARCH (Rognes et al., 2016) and chimaeras exluded using UCHIME (Edgar et al., 2011). OTUs were finally annotated using the UNITE fungal ITS reference data set within RDP classifier (http://rdp.cme.msu.edu) and the Worcup ITS reference as training dataset.

Species assignation on selected OTUs was manually resolved through BLAST searching against mycobank (mycobank.org), RDP (https://rdp.cme.msu.edu/) and using the specialized fungal pages within GenBank. Relative abundances of microbial taxa in each sample were calculated and compared.

The richness of each sample (α-diversity) was assessed by computing Chao 1, Abundance Coverage Estimator (ACE) and Shannon indexes at the taxonomic rank of orders, using the R library Vegan (Oksanen et al., 2017). Diversity in composition among samples (β-diversity) was compared, always considering orders, through: (i) bootstrap-based clustering analysis, using the R function pvclust (Suzuki and Shimodaira, 2006); (ii) double clustering analysis, through the R function heatmap.2 of the R library Gplots (Warnes et al., 2016), setting the pvclust output as sample dendrogram; (iii) Principal Coordinates Analysis (PCoA) analysis based on Bray-Curtis dissimilarity matrix, performed using the R library Vegan (Oksanen et al., 2017).

2.6 Evaluation of total fungal count

For quantitative mycological analysis the soil samples were processed within 15 days.

Evaluation of fungal count was carried out by Dilution Plate Technique (Gams et al., 1998) which detects culturable microfungi.

As reported in previous studies (Rodolfi et al., 2016; Selbmann et al., 2013; Kostadinova et al., 2009) for each samples10 g of soil was suspended 1:10 (w/v) with sterile water agar (0.15% agar) and shaken on a stirrer for 20 min. The suspensions were further diluted 1:10 and 1ml aliquots of the suspension 10^{-4} were homogeneously distributed with a sterile blent rod onto 16 cm diameter Petri dishes containing MEA medium (Malt Extract Agar – CBS: 20 g malt extract powdered, 1 g peptone, 20 g glucose, 15 g agar, 1000 ml distilled water) and antibiotics (streptomycin 20 ppm, chloramphenicol 100 ppm, penicillin G 30 ppm). Five replicates of each samples were prepared, and incubated at 25°C in the dark Inoculated plates were observed continuously by means of a stereomicroscope for 2 weeks, and the number of developed colonies was expressed as CFU (Colonies Forming Units) per gram of soil (dry weight). Soil dry weight was measured by an infrared moisture balance (AMB Adam Equipment, UK).

3. Results and discussion

3.1. Physicochemical analysis of soil

In the table table 2 the results of physicochemical analysis are reported. The studied areas are similar in their physicochemical characteristic except for the P concentration detected in the Apple A plot. No particular activity has been recorded during the agricultural management that could explains so high concentration of this element (400 mg/kg SS). An error in the measure has been hypnotized by the author.

Table 2. Phsyco-chemical characteristics of the sampling sites

	pH	Organic	NTOT	CORG	C/N ratio	P	Ca	Mg	K	Sand	Silt	Clay
	pH unit	matter (%)	(g/kg SS)	(% SS)		(mg/kg SS)	(meq/100g)	(meq/100g)	(meq/100g)	(%)	(%)	(%)
Peach-A	6,60 ± 0,10	4,90	2,90	2,89	1	3,60	23,4	5,83	5,49	37,5	41,5	16,2
Peach-B	6,07 ± 0,10	4,22	2,71	2,57	0,9	52,0	13,5	3,37	4,60	33,2	44,4	17,9
Apple-A	6,03 ± 0,10	5,14	3,20	3,26	1	-	11,5	3,40	5,14	25,8	46,4	19,6
Apple-B	6,05 ± 0,10	4,63	1,0	2,98	3	9,22	10,9	2,00	5,86	38,8	38,5	13,0
Wood	6,06 ± 0,10	5,43	1,85	3,32	1,8	6,97	9,02	4,35	1,30	34,0	45,8	14,8
Resting	6,41 ± 0,10	4,9	2,12	3,02	1,4	29,6	11,5	4,26	1,75	38,0	39,9	16,5

3.2. Total count of culturable fungi

Quantitative mycological analysis gave a picture of the culturable fungal count. Colony forming Units /g of soil dry weight ranges from $2 \cdot 10^5$ to $8 \cdot 10^5$. Data are plotted in table 3. The peach and apple cultivated areas are richer in fungal propagules compared to the woodland and uncultivated grassland areas. The woodland area has similar fungal charge to the cultivated area and this result can be explained by the presence of litter associated fungi that can influence the fungal charge in the soil.

Table 3. Culturable fungal counts of each study areas. Counts are expressed as colony forming unit per g of soil dry weight

CFU/g of soil dry weight					
appleA	appleB	peachA	peachB	Resting	Woodland
$7X10^5$	$6X10^5$	$5X10^5$	$8X10^5$	$2X10^5$	$5X10^5$

3.3 Taxonomic picture of fungal communities of the agroecosystem in upper Andean Colombian

The mycobiota was evaluated through ITS1 deep sequencing, that produced a total of 856,508 raw reads on the Illumina MiSeq platform; of these, 468,163 passed quality filtering. After removing redundant sequences, the reads were recognized and extracted as ITS1 by the ITSx software against the UNITE ITS reference dataset. The 97% similarity level was finally established for the operational taxonomic units (OTU): PIPITS wrapper (Gweon et al., 2015), after removing chimeras, returned 25,519 OTUs. The amount of unassigned and unidentified OTUs was 0.1% and 8 % respectively.

Taxonomy was assigned to the 25,519 OTUs returned by PIPITS and sequences attributed to five fungal Phyla the relative abundance of each is plotted in the fig. 3. All five areas are dominated by Ascomycota, which is represented by 55% of the clones in the whole area (fig. 3, last column) ranging from 48% (apple A) to 62% (peach A). Basidiomycota are represented by 20 % in the whole area, ranging from 10% (peach A) to the highest value of 29% in the woodland samples. Zygomycota is represented by the 15% of clones in the whole area, ranging from 9 (resting grass land) to 13-14% in the cultivated area. Glomeromycota and Chytridiomycota clones were mostly recorded in the resting grass land, only detected at low frequencies with values less than 0,7% and 0,1% respectively.

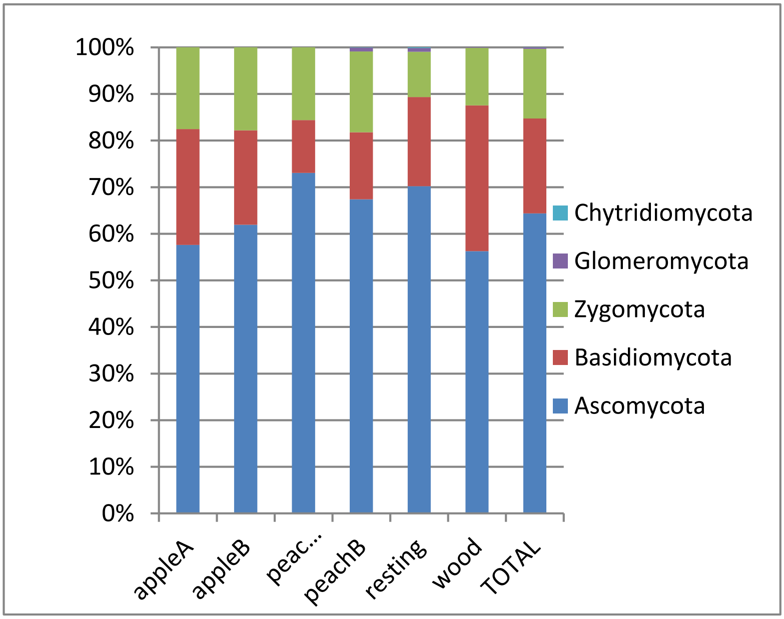


Fig. 3. Relative abundance attributed to of each fungal Phyla in the sampling sites.

The distribution of the orders in the different sample sites is shown in the Fig 4. The total relative abundance of the single order in the whole study area (fig. 4 last column) can be appreciated. Eurotiales (Ascomycota) is the most represented (20%), followed by Mortierellales (Zygomycota) (ca 15%), and by three very well represented Ascomycota orders Hypocreales (ca 13%), Trichisporales (ca 7 %), and Sordariales (ca 5%). The other orders are less represented with values below 5%.

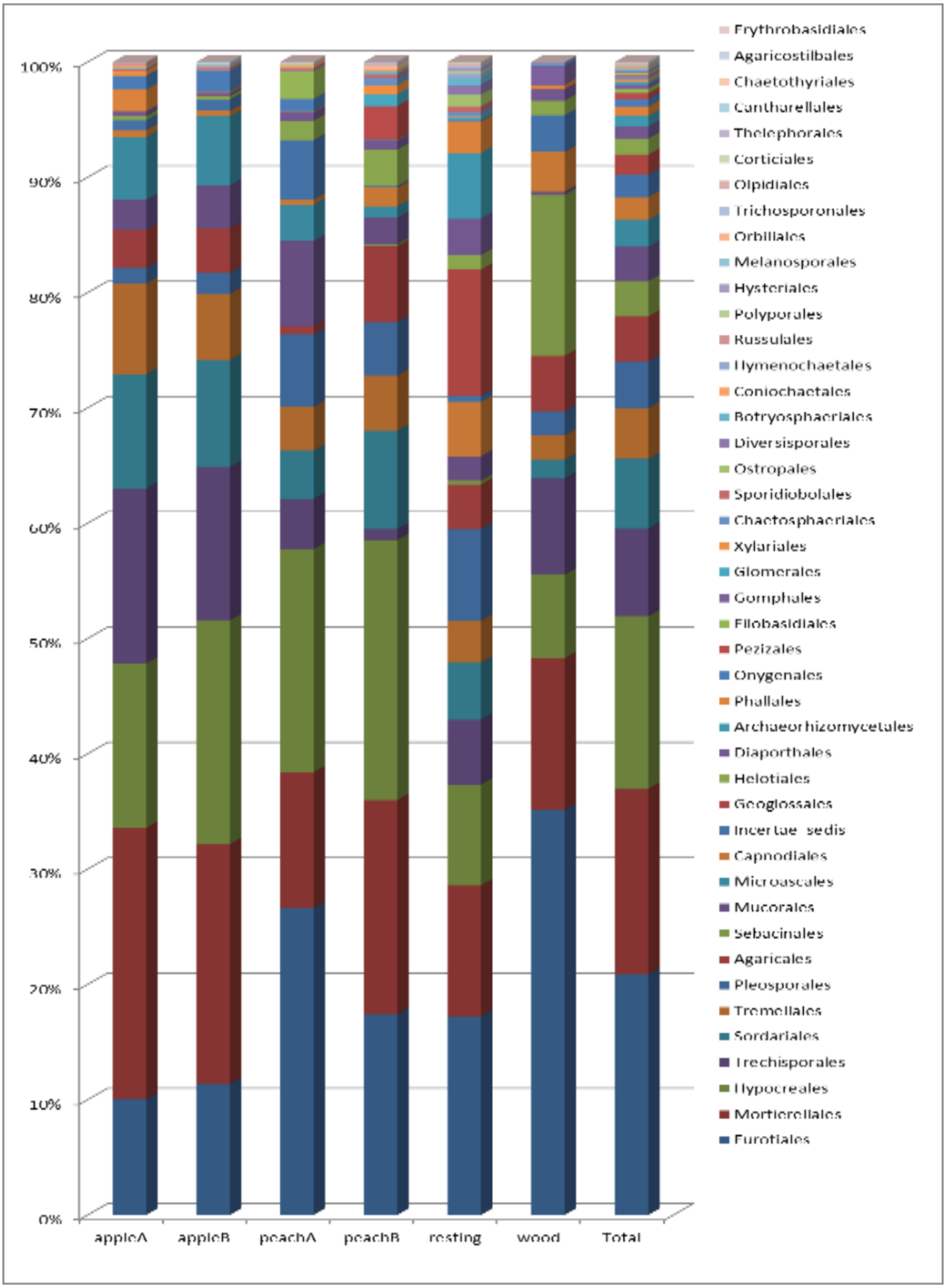


Fig. 4. Distribution of the most common orders in the different sample sites.

In Fig. 5 the distribution of the most common families (with a frequency ≥1%) can be observed. In the whole sampling area Trichocomaceae (Ascomycota, Eurotiales) is the most abundant (14%), although with different weights in the different sampling sites: low in the apple cultivated site (5-6%) and significantly high in the woodland soil. Mortierellaceae (Zygomycota, Mortierellales) is the second most abundant family, with a presence of 10% in the whole sampling area and with the lowest value in the uncultivated grassland site (6%). Netriaceae (Hypocreales) is quite abundant with a value of 3%, mainly recorded in the cultivated sites (4% in average) and with a low presence in the woody site (0,5%).

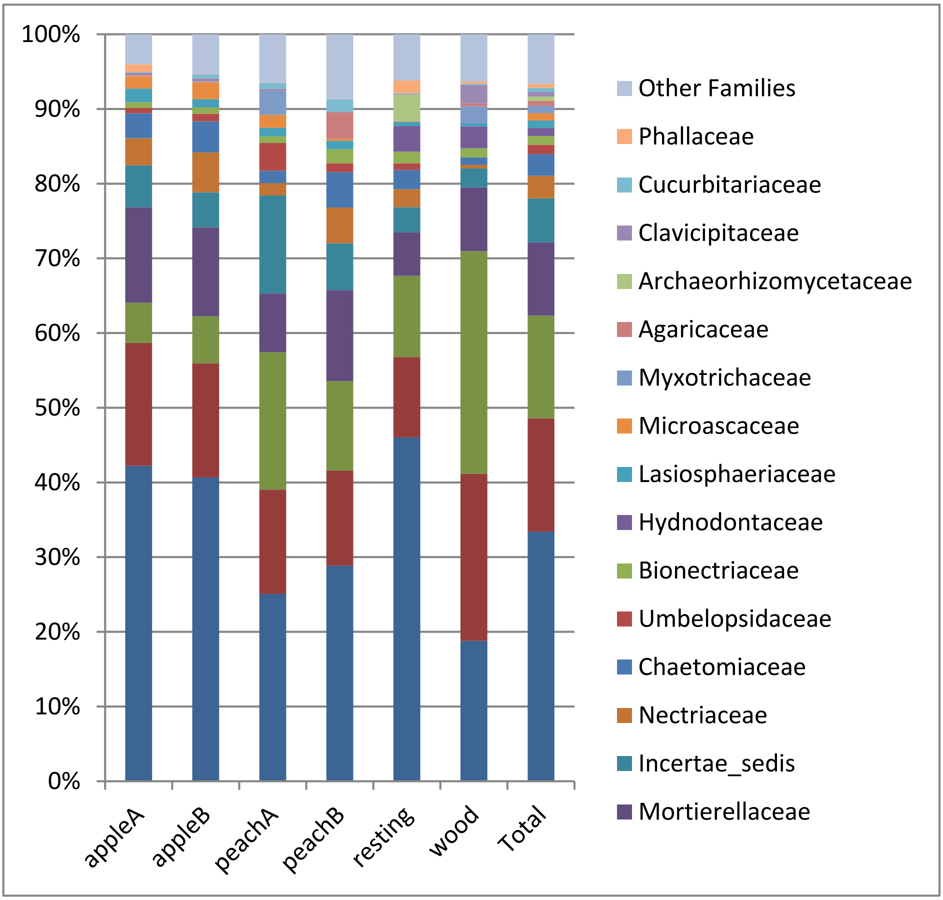


Fig. 5. Distribution of the most common family in the different sample sites.

3.4 Species composition

A finer, manual, bioinformatics analysis of the most abundant OTUs allowed resolution to species level. Due to the fact that Ascomycota and

Basidiomycota are the most frequent phyla in the Fugal Kingdom, the abundance of the OTU belonging to these taxa has been considered when representing ≥1% of total diversity in at least one sample or considered in any case if the OTU is present in only one sampling site at any frequency. In the fungal Kingdom the other phyla, i.e. Zygomycota, Glomeromycota, and Chytridiomycota, represent a number of species lower than 1%. For these phyla, OUT has been considered at any frequency. The tables 4, 5, 6, 7, 8 show the list of the identified species in their taxonomic rank, with their relative abundance in the different sites. The tables 4-8 are reported at the end of the paper.

Species belonging to Ascomycota (table 4) were 77 homogenously distributed for the 65% in the cultivated sites and for 35% in the uncultivated grassland and woody sites. The most represented genus is *Penicillium* with 13 species, *Acremonium* with 6 species, *Sarocladium* with 3 species. The most abundant species is *Penicillium virgatum*, *P. scabrosum*, followed by *Chaetomium udagawae*.

The uncultivated grassland is characterized by the exclusively presence of *Geoglossum fallax* (7%), *Podospora leporina* (0,5%). In the grassland, significant difference in comparison with the other sites can be recorded for *Microsphaeropsis arundinis* (4%), *Phomopsis columnaris* (2%), *Ascochyta medicaginicola* (1%). In the woody site the following ascomycetous species were exclusive: *Oidiodendron tenuissimum* (2%), *Penicillium catenatum* (1%), *Penicillium lilacinoechinulatum* (0,2%). Significant differences are recorded for *Pochonia chlamydosporia* (2,5%), *Penicillium simplicissimum* (ca. 2%), *Preussia africana* (0,5%), *Penicillium madriti* (0,2%).

The sites A and B with apple cultivation are very similar in the distribution of fungal species. They present only two exclusive species: *Petriella setifera* (1%), and *Tetraplosphaeria sasicola* (0,04%). In comparison to the other sites, the presence of the following species are significantly different: *Chaetomium udagawae* (10%), *C. globosum* (3%), *C. leptoderma* (ca. 1%), *Pseudeurotium zonatum* (ca. 1,5%), and *Mycochlamys macrospora* (ca. 1%).

Unlike apple sites, peach A and B sites seem to be significantly different in the distribution of the fungal species. In peach A there are 24% more species than in peach B. Peach A and B harbors the highest number of exclusive species (10) although different in A (5) and B (5).

The exclusive species in peach A are: *Cephalotrichiella penicillata* (3%), *Pyrenochaeta lycopersici* (ca. 1%), *Sarocladium glaucum* (0,3%), *Sarocladium subulatum* (0,04%), *Stephanonectria keithii* (0,04%).

In the Peach A site the following species are recorded with a significant different abundance: *Penicillium scabrosum* (12%), *Stachylidium bicolor* (3%), *Pseudogymnoascus pannorum* (3%), *Penicillum brevicompactum* (2,5%), *Acremonium antarcticum* (*A. furcatum*? 2,5%), *Cladosporium cladosporioides* (1%), *C. fusiforme* (ca. 0,2 %), *Phialocephala humicola* (0,1%), *Acremonium sclerotigenum* (0,1%), *Alternaria infectoria* (0,02%), *Epicoccum nigrum* (ca. 3%).

Peach B harbors the following exclusive species: *Beauveria caledonica* (3%), *Saccobolus globuliferellus* (2%), *Acremonium persicinum* (ca. 0,1%), *A. stromaticum* (ca. 0,1%), *Thielaviopsis basicola* (0,2%).

Peach B presents the following species recorded with a significantly high presence: *Penicillium vancouverense* (5%), *Pyrenochaetopsis leptospora* (1,4%), *Clonostachys rosea* (1,5%), *Nigrospora oryzae* (1%), *Dactylonectria macrodidyma* (1%).

The peach B site is the most different from the others in the ascomycetous species assemblage.

Cultivated sites seem to share the following fungal assemblage which is not recorded in the uncultivated grassland and woody sites: *Stachylidium bicolor, Mycochlamys macrospora, Boeremia exigua, Aspergillus proliferans, Metarhizium marquandii, Fusarium culmorum, F. equiseti, Acremonium antarcticum* (o more probable *A. furcatum*), *A. chrysogenum, Sedecimiella taiwanensis, Kernia nitida, Scedosporium dehoogii*.

Species identified in Basidiomycota were 28 (table 5). The site Peach A is the richest in basidiomycetous species with a double number of species compared to the other sites. Many of them are exclusive of this site: *Agaricus bisporus, Conocybe inopinata, Coprinopsis luteocephala, Coprinopsis radiate, Filobasidium floriforme, Filobasidium magnum, Hannaella oryzae, Naganishia diffluens, Erythrobasidium hasegawianum*.

Peach B must be highlighted for the massive presence of *Agaricus campestre* (3,5%), significantly different compared to the other sites.

The sites Apple and B have the exclusively presence of *Coprinellus verrucispermus*, and *Coprinopsis atramentaria*. *Calvatia cyathiformis* and *Minimedusa polyspora* were recorded exclusively in A and B respectively.

The woodland site harbors the exclusive presence of *Laccaria lateritia* (ca. 2%), *Ramaria abietina* (1%), *Leucoagaricus cinerascens* (0,2%). Although not identified at specific level the highly frequency of an uncultured *Sebacina* (5%) must be underlined. *Sebacina* is a genus strictly associated to plants, comprising orchidaceous plants.

In the phylum of Zygomycota 31 species (table 6) have been identified, mainly belonging to the genera *Mortierella* (15 species) and *Mucor* (8 species). The richest sites were the apple cultivations (A and B) and peach A. The uncultivated grassland and woodland sites harbor half number of species (11 and 10). In the apple sites there is the exclusive presence of *Mortierella exigua*, *M. wolfii*, *Absidia cylindrospora*, and *Actinomucor elegans*. Apple A and B share with the other cultivated sites the exclusive presence of *Backusella lamprospora*, and *Absidia anomala*.

Absidia repens, *Mucor gigaspora*, *Mucor hiemalis*, *Mucor zychae* var. *linnemanniae*, are exclusive of Peach A. The uncultivated grassland and the woody sites share the exclusive presence of *Mortierella humilis*, and *Mucor moelleri*.

Most of the OUTs belonging to Glomeromycota (table 7) have not been referred to species rank. Only two species have been identified: *Glomus mossae*, exclusively recorded in the uncultivated grassland field, and *Entrophospora infrequens*.

And least but not last, *Olpidiaster brassicae* is the only identified Chytridiomycota (table 7), mainly recorded in the uncultivated grassland.

The presence of many unidentified species (es. in the Trechisporales order, or Chytridiomycota and Glomeromycota phyla) reveals that the biodiversity of upper Andean Colombian agro-environment is underestimated.

The fungal community structure in the different areas was studied by diversity and richness indices.

3.5 Structure of fungal communities in relation to different agricultural land use

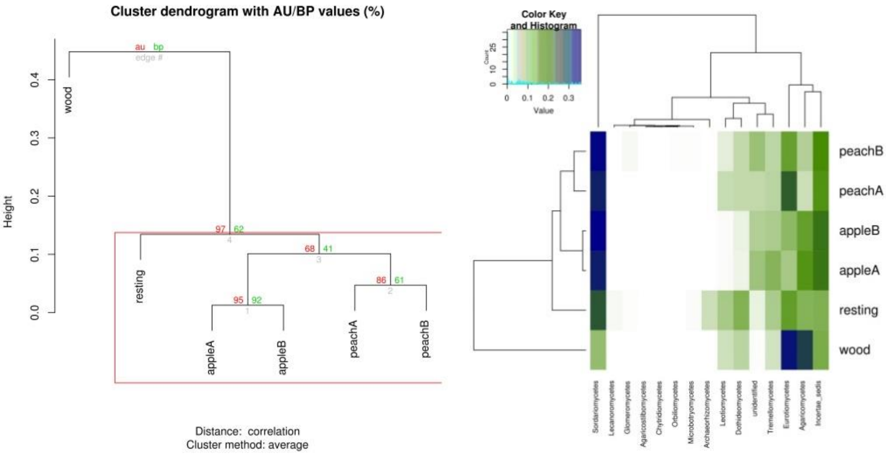
Diversity and richness indexes, investigated at the taxonomic level of species, depicted a quite homogeneous situation among the different soil samples, especially with respect to the Shannon diversity estimator (table 8).

Table 8. Biodiversity and richness estimators calculated at the taxonomy rank of species. Number of observed species (N), Shannon's index of biodiversity (Shannon), Chao1 richness estimator (Chao1) and associated standar error (ES.Chao1), Abundance Coverage Estimator (ACE) and corresponding standard error (SE.ACE)

	N	Shannon	Chao1	SE.Chao1	ACE	SE.ACE
appleA	294	4,16	350,67	16,12	369,44	9,69
appleB	312	4,22	367,88	16,81	372,44	9,26
peachA	388	4,42	445,11	16,15	454,96	10,35
peachB	260	4,50	327,86	20,50	324,33	8,82
resting	222	4,49	238,74	7,32	247,01	7,38
wood	170	4,16	215,54	16,82	215,66	7,24

Woodland and the uncultivated grassland were characterized by the lowest number of observed species, especially the former and the Chao1 estimated richness as well as ACE index. Conversely, the area with highest Chao1 value is Peach A in accordance with the ACE index.

Next, samples were compared and clustered with respect to their composition in fungal classes. From the bootstrap-based clustering analysis (Fig. 6), it clearly emerged that samples were divided into two macro-cluster. The first one included the woodland area supported by highly significant bootstrap value (>95%). The second cluster covered the remaining samples. The uncultivated field (resting) was intermediate between the orchards and the woodlans. Clustering was mainly dictated by the differential abundance of a limited number of fungal classes: Sordariomycetes, Archaeoryzomycetes, Leotiomycetes, Dotideomycetes, Tremellomycetes, Eurotiomycetes, Agaromycetes.



A B

Fig. 6 Clustering of samples with respect to their composition in fungal classes. A, Bootstrap-based clustering analysis. B, Heatmap analysis based on the Euclidean distance of classes and on the dendrogram produced by the clustering analysis. AU: approximately unbiased; BP: bootstrap probability.

Samples were also compared and clustered with respect to their composition in fungal species.

From the bootstrap-based clustering analysis (Fig. 7), it clearly emerged that samples were divided into two macro-cluster accordingly to the class analysis. The first one included the woodland and the uncultivated area supported by highly significant bootstrap value (94%). The second cluster covered the orchards, showing the two subclusters of apple and peach area.

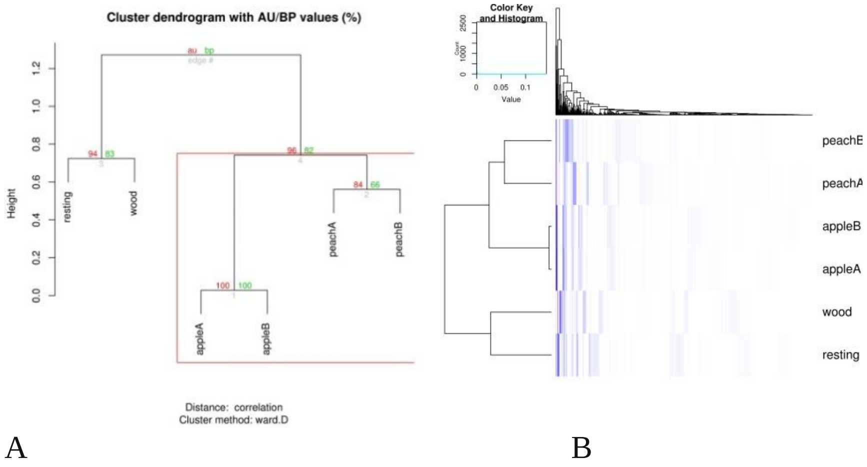


Fig. 7 Clustering of samples with respect to their composition in fungal species. A) Bootstrap-based clustering analysis. B) Heatmap analysis based on the Euclidean distance of classes and on the dendrogram produced by the clustering analysis. AU: approximately unbiased; BP: bootstrap probability.

4. Conclusive remarks

Basing on the species picture composition and structure of fungal community some conclusive remarks can be advanced. Cultivated and not cultivated areas present a dominance of species belonging to the *Ascomycota* phylum, in agreement with the international literature (Tedersoo et al., 2014). Basidiomicetous species are mainly recorded in the woodland area, consistently to the fact that many of them, as saprotrophic or symbiontic (parasitic or mutualistic), show preferences for lignocellulosic material. Zygomycota is homogenously distributed, although with a slight prevalence in the cultivated areas. Among the families, Trichocomaceae is the most abundant followed by Mortierellaceae, and Nectriaceae. The present analysis identified taxa at species level for 77 in Ascomycota, 28 in Basidiomycota, 31 in Zygomycota. Only *Glomus mosseae* and *Entrophospora infrequens* were the specific taxa identified for Glomeromycota; for Chytridiomycota only *Olpidiaster brassicae* was identified. The low resolution obtained in the taxonomic analysis of Glomeromycota, compared to those obtained in the literature (see chapter 3 of this thesis), is due to the specific biomolecular analysis carried out in this research that is based on exclusively on ITS1 deep sequencing. Other gene sequences could be helpful to obtain major resolution of taxon such as Glomeromycota, and Chytridiomycota.

From the bootstrap-based clustering analysis the community is divided in two main macro-clusters with respect to fungal species: the woodland-grassland and the orchards. This result evidences a clear response of microbial community to different use of soil in the agro-environment. The uncultivated areas (grassland and woodland) are characterized by the abundant presence of some exclusive species belonging to Ascomycota (in both) and Basidiomycota (in woodland).

Many basidiomicetous species different from the woodland characterized the peach orchards. This result needs to be deeply investigated with respect to environmental and management condition. The cultivated areas are particularly abundant of *Chaetomium udagawae* and *C. globosum* and this is another interesting aspect that is worth to be investigated in future studies. The differences in fungal species composition is consistent with the bootstrap-based clustering analysis based on class composition. Considering the results obtained, the study area, considered as a whole, seems to draw a taxonomic gradient with the woodland site as the most different from the cultivated sites and the uncultivated grassland in the intermediate position.

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Table 4. Ascomycota OTU analysis and identification: Fungal species identified in the different sampling sites, ordered following their taxonomy. Abundance (%) per site is shown. Identifications have been performed against the GenBank, Mycobank and RDP classifier databases.

		appleA	**appleB**	**peachA**	**peachB**	**resting**	**wood**	**NCBI/Myco.Bank**
	Ascomycota							
1	*Stachylidium bicolor*	0,13%	0,16%	3,17%	0,04%	0,00%	0,08%	98% MB#166350
2	*Mycochlamys macrospora*	0,63%	0,95%	0,11%	0,11%	0,00%	0,00%	100%MB#318058]
	Pseudoeurotiaceae							
3	*Pseudeurotium zonatum*	1,34%	1,83%	0,03%	0,51%	0,07%	0,00%	100% KJ755522.1
	Dothideomycetes							
	Capnodiales							
	Davidiellaceae							
4	*Cladosporium cladosporioides*	0,26%	0,16%	1,13%	0,55%	0,14%	0,31%	100% HM148002.1
5	*Cladosporium fusiforme*	0,05%	0,04%	0,16%	0,04%	0,00%	0,08%	99% KJ596577.1 equipro. *C. cladosporioides* (KY859392.1)
	Pleosporales							
6	*Microsphaeropsis arundinis*	0,05%	0,00%	0,29%	0,11%	3,91%	0,00%	100% KU574704.1
	Cucurbitariaceae							
7	*Pyrenochaeta lycopersici*	0,00%	0,00%	0,86%	0,00%	0,00%	0,00%	100% AM944362.1
8	*Pyrenochaetopsis leptospora*	0,08%	0,53%	0,35%	1,43%	0,07%	0,15%	100% KU158168.1
	Didimellaceae							

9	*Ascochyta medicaginicola*	0,00%	0,00%	0,07%	0,00%	1,36%	0,00%	100% EU167575.1 equipro. *Leptosphaerulina australis* (AY131203.1)
	Pleosporaceae							
10	*Alternaria infectoria*	0,00%	0,00%	0,02%	0,00%	0,00%	0,00%	100% KY496638.1
11	*Epicoccum nigrum*	0,05%	0,02%	2,77%	0,22%	0,32%	0,62%	100% KY412174.1
	Sporormiaceae							
12	*Preussia africana*	0,03%	0,00%	0,03%	0,00%	0,00%	0,46%	100% EU551195.1
	Incertae sedis							
13	*Boeremia exigua*	0,03%	0,16%	0,54%	0,04%	0,00%	0,00%	100% MF039478.1
	Tetraplosphaeriaceae							
14	*Tetraplosphaeria sasicola*	0,03%	0,05%	0,00%	0,00%	0,00%	0,00%	95% KX440178.1
15	*Dendryphion nanum*	0,00%	0,05%	0,04%	0,00%	0,00%	0,00%	100% KC989061.1
	Eurotiomycetes							
	Eurotiales							
	Trichocomaceae							
16	*Aspergillus proliferans*	0,05%	0,05%	0,13%	0,18%	0,00%	0,00%	99% SH034788.06F equipro. *A. niveoglaucus* (KY859366.1) o *A. pseudoglaucus* (KX766180.1)
17	*Penicillium canescens*	0,03%	0,02%	0,01%	0,15%	1,87%	1,85%	100% KY684281.1
18	*Penicillium scabrosum*	3,52%	4,06%	12,23%	2,86%	1,87%	1,08%	100% KM023349.1
19	*Penicillium fluviserpens*	0,08%	0,05%	0,20%	0,00%	0,00%	0,00%	
20	*Penicillium madriti*	0,03%	0,00%	0,02%	0,00%	0,00%	0,23%	100% KU561929.1 equip. *P. castellonense* (KC411683.1)

21	*Penicillium roseopurpureum*	0,18%	0,18%	0,56%	0,40%	0,07%	0,15%	
22	*Penicillium brevicompactum*	0,24%	0,35%	2,56%	0,62%	0,00%	0,54%	100% KY401116.1
23	*Penicillium catenatum*	0,00%	0,00%	0,00%	0,00%	0,00%	1,08%	98% JF911774.1
24	*Penicillium lilacinoechinulatum*	0,00%	0,00%	0,00%	0,00%	0,00%	0,23%	98% KC773837.1
25	*Penicillium malmesburiense*	0,00%	0,00%	0,00%	0,04%	0,18%	0,00%	
26	*Penicillium simplicissimum*	0,03%	0,02%	0,00%	0,00%	0,00%	1,69%	99% KX911715.1
27	*Penicillium vancouverense*	0,55%	0,79%	1,38%	5,13%	0,75%	3,54%	100% NR_121512.1
28	*Penicillium vinaceum*	0,05%	0,05%	0,31%	0,55%	1,33%	1,23%	100% NR_121242.1
29	*Penicillium virgatum*	0,08%	0,22%	0,00%	0,26%	2,98%	14,3%	98% KM458824.1
	Onygenales							
	Onygenaceae							
30	*Auxarthron umbrinum*	0,08%	0,02%	0,01%	0,04%	0,04%	0,00%	100% KP636437.1 equipro. *A. conjugatum* (AM262398.1)
31	*Chrysosporium lobatum*	1,10%	2,03%	1,00%	0,00%	0,72%	0,00%	99% HQ914933.1
	Geoglossales							
	Geoglossaceae							
32	*Geoglossum fallax*	0,00%	0,00%	0,00%	0,00%	6,93%	0,00%	96% HQ222872.1 solo per 50-80 pb
	Leotiomycetes							
	Incertae sedis							
33	*Leohumicola levissima*	0,03%	0,00%	0,01%	0,00%	4,67%	0,69%	99% EU678395.1
	Myxotrichaceae							

34	*Oidiodendron tenuissimum*	0,00%	0,00%	0,00%	0,00%	0,00%	2,00%	99% AF307773.1
	Pseudeurotiaceae							
35	*Pseudogymnoascus pannorum*	0,05%	0,05%	3,25%	0,00%	0,00%	0,15%	100% KX058049.1
	Helotiales							
	Vibrisseaceae							
36	*Phialocephala humicola*	0,00%	0,02%	0,13%	0,00%	0,00%	0,00%	100% AB671503.2
	Sordariomycetes							
	Chaetosphaeriales							
	Chaetosphaeriaceae							
37	*Chaetosphaeria vermicularioides*	0,00%	0,02%	0,02%	0,00%	0,18%	0,08%	100% KM056319.1
	Diaporthales							
38	*Phomopsis columnaris*	0,24%	0,13%	0,50%	0,51%	2,01%	0,85%	100% KX610404.1
	Hypocreales							
	Bionectriaceae							
39	*Clonostachys divergens*	0,18%	0,22%	0,16%	0,22%	0,25%	0,15%	99% NR_137532.1
40	*Clonostachys candelabrum*	0,00%	0,04%	0,00%	0,11%	0,25%	0,23%	100% LT220546.1
41	*Clonostachys rosea*	0,52%	0,57%	0,63%	1,50%	1,04%	0,77%	100% KT007105.1
42	*Stephanonectria keithii*	0,00%	0,00%	0,04%	0,00%	0,00%	0,00%	99% JN116689.1
	Clavicipitaceae							
43	*Pochonia chlamydosporia*	0,10%	0,15%	0,07%	0,07%	0,04%	2,46%	99% KR782313.1

44	*Metarhizium marquandii*	0,05%	0,20%	0,01%	0,04%	0,00%	0,00%	99% KT582066.1
45	*Pochonia bulbillosa*	0,03%	0,02%	0,06%	0,00%	0,04%	0,00%	100% LC010959.1
	Cordycipitaceae							
46	*Beauveria caledonica*	0,00%	0,00%	0,00%	2,67%	0,00%	0,00%	KY471655.1 100%
	Lasiosphaeriaceae							
47	*Podospora leporina*	0,00%	0,00%	0,00%	0,00%	0,54%	0,00%	100% MB 337412
	Nectriaceae							
48	*Fusarium culmorum*	1,57%	1,83%	0,13%	2,02%	0,00%	0,00%	100% KU361545.1
49	*Fusarium equiseti*	7,35%	6,68%	3,38%	0,99%	0,00%	0,00%	100% KU361582.1
50	*Fusarium oxysporum*	5,72%	4,41%	1,57%	3,55%	4,41%	0,23%	100% KX610393.1
51	*Paracremonium inflatum*	0,76%	1,21%	0,37%	0,99%	0,65%	0,15%	96% KM231829.1 equip. *Cosmospora khandalensis* (NR_145058.1)
	Incertae sedis							
52	*Acremonium sclerotigenum*	0,00%	0,00%	0,08%	0,00%	0,00%	0,00%	100% KY039286.1 equip. *A. strictum* (KX674670.1)
53	*Acremonium antarcticum*	0,10%	0,29%	2,47%	0,00%	0,00%	0,00%	100% KT315437.1 equip. *A. furcatum* (LT549083.1)
54	*Acremonium chrysogenum*	0,13%	0,07%	0,20%	0,00%	0,00%	0,00%	99% U57672.1
55	*Acremonium persicinum*	0,00%	0,00%	0,00%	0,07%	0,00%	0,00%	100% KP720661.1
56	*Acremonium psammosporum*	0,05%	0,07%	0,05%	0,00%	0,00%	0,08%	100% KR909178.1 equip. *Collarina aurantiaca* (KJ807177.1)
57	*Acremonium stromaticum*	0,00%	0,00%	0,00%	0,07%	0,00%	0,00%	100% KT809068.1
58	*Dactylonectria macrodidyma*	0,08%	0,04%	0,00%	0,95%	0,04%	0,08%	100% MF567498.1 equip. *D. torresensis* (MF440372.1), *Ilyonectria destructans* (KT722597.1)

59	*Sarocladium glaucum*	0,00%	0,00%	0,35%	0,00%	0,00%	0,00%	99% NR_130686.1
60	*Sarocladium subulatum*	0,00%	0,00%	0,04%	0,00%	0,00%	0,00%	99% NR_145047.1 equip. *S. implicatum* (KJ608090.1)
61	*Sarocladium bactrocephalum*	0,08%	0,05%	1,63%	0,00%	0,00%	0,00%	100% NR_145044.1 equip. *S. strictum* (KU901567.1)
	Niessliaceae							
62	*Sedecimiella taiwanensis*	0,10%	0,29%	2,47%	0,00%	0,00%	0,00%	100% KU935682.1 equip. *Acremonium antarcticum* (KT315437.1)
	Microascales							
	Ceratocystidaceae							
63	*Thielaviopsis basicola*	0,00%	0,00%	0,00%	0,18%	0,00%	0,00%	100% KX826471.1
	Microascaceae							
64	*Kernia nitida*	0,24%	0,53%	1,41%	0,04%	0,00%	0,00%	100% KC485065.1
65	*Petriella setifera*	0,13%	0,09%	0,00%	0,00%	0,00%	0,00%	100% KX449497.1
66	*Scedosporium dehoogii*	0,05%	0,04%	0,03%	0,00%	0,00%	0,00%	99% P132709.1
67	*Lomentosporia prolificans*	0,05%	0,07%	0,01%	0,00%	0,00%	0,00%	100% KP132396.1 equip. *Scedosporium prolificans* (KJ176706.1)
	Incertae sedis							
68	*Cephalotrichiella penicillata*	0,00%	0,00%	0,30%	0,00%	0,00%	0,00%	100% KJ869166.1
	Pezizales							
	Ascobolaceae							
69	*Saccobolus globuliferellus*	0,00%	0,00%	0,00%	2,02%	0,00%	0,00%	99% MB#266137
	Sordariales							
	Chaetomiaceae							

70	*Botryotrichum murorum*	0,45%	0,51%	0,12%	0,07%	0,00%	0,00%	KX901286.1 98%
71	*Chaetomidium leptoderma*	0,58%	1,70%	0,01%	0,26%	0,11%	0,08%	98% JN573176.1, equip. *C. galaicum* (JN573175.1)
72	*Chaetomium globosum*	3,62%	3,02%	1,06%	1,06%	0,00%	0,00%	99% JX241646.1
73	*Chaetomium udagawae*	10,79%	11,07%	2,94%	5,20%	1,94%	0,15%	99% GU183108.1
	Lasiosphaeriaceae							
74	*Cladorrhinum phialophoroides*	0,05%	0,05%	0,00%	0,15%	0,00%	0,00%	100% NR_077198.1
	Sordariaceae							
75	*Apodus oryzae*	0,05%	0,02%	0,05%	0,00%	0,00%	0,00%	100% KY024592.1
76	*Nigrospora oryzae*	0,00%	0,07%	0,48%	1,17%	0,00%	0,08%	100% KY230512.1
	Xylariales							
	Incertae sedis							
77	*Microdochium bolleyi*	0,24%	0,02%	0,00%	0,55%	0,00%	0,00%	100% KY365586.1 equip. *F. oxysporum* (KX058061.1)
	TOTALE 77	**53**	**54**	**57**	**43**	**30**	**33**	

Table 5. Basidiomycota OTU analysis and identification: Fungal species identified in the different sampling sites, ordered following their taxonomy. Abundance (%) per site is shown. Identifications have been performed against the GenBank, Mycobank and RDP classifier databases.

Basidiomycota							
Agaricomycetes							
Agaricales							
Agaricaceae							
Agaricus bisporus	0,00%	0,00%	0,03%	0,00%	0,00%	0,00%	100% KM657921.1
Agaricus campestre	0,13%	0,02%	0,03%	3,52%	0,07%	0,00%	99% KP229419.1 equip. *A. argenteus* (KJ877773.1)
Calvatia cyathiformis	0,05%	0,00%	0,00%	0,00%	0,00%	0,00%	99% KY706183.1
Bolbitiaceae							
Conocybe inopinata	0,00%	0,00%	0,05%	0,00%	0,00%	0,00%	100% JX968165.1
Holocotylon brandegeeanum	0,00%	0,00%	0,00%	0,00%	0,00%	0,15%	??
Leucoagaricus cinerascens	0,00%	0,00%	0,00%	0,00%	0,00%	0,23%	99% AY176410.1
Hydnangiaceae							
Laccaria lateritia	0,00%	0,00%	0,00%	0,00%	0,00%	1,62%	100% KU685668.1 equip. *L. canalicola* (KU685664.1)
Psathyrellaceae							
Coprinellus verrucispermus	0,03%	0,07%	0,00%	0,00%	0,00%	0,00%	98% AY521250.1
Coprinopsis atramentaria	0,03%	0,09%	0,00%	0,00%	0,00%	0,00%	99% AF059286.1
Coprinopsis luteocephala	0,00%	0,00%	0,02%	0,00%	0,00%	0,00%	99% HQ847012.1 equip. *C. xenobia* (KF178382.1), *C. candidolanata* (JF907837.1)

Coprinopsis radiata	0,00%	0,00%	0,01%	0,00%	0,00%	0,00%	100% HQ847024.1
Cantharellales							
Incertae sedis							
Minimedusa polyspora	0,00%	0,07%	0,00%	0,00%	0,00%	0,00%	100% KC176294.1
Tremellomycetes							
Filobasidiales							
Filobasidiaceae							
Cryptococcus stepposus	0,00%	0,00%	0,03%	0,00%	0,04%	0,00%	100% JX188129.1
Filobasidium floriforme	0,00%	0,00%	1,62%	0,00%	0,00%	0,00%	98% KY103419.1
Filobasidium magnum	0,00%	0,00%	0,04%	0,00%	0,00%	0,00%	100% KX229704.1
Piskurozymaceae							
Solicoccozyma aeria	1,47%	0,84%	0,92%	1,83%	0,97%	0,31%	100% KY105430.1, equip. *C. fuscescens* (KT809121.1), *C. aerius* (KP068779.1)
Solicoccozyma terreus	2,62%	2,32%	0,99%	1,39%	1,29%	1,39%	100% KY102961.1
Phallales							
Phallaceae							
Clathrus archeri	1,02%	0,07%	0,05%	0,04%	1,72%	0,31%	100% (KP688381.1)
Gomphales							
Gomphaceae							
Ramaria abietina	0,00%	0,00%	0,00%	0,00%	0,00%	1,39%	99% (AJ408376.1)
Sebacinales							

Sebacinaceae							
Sebacina uncultured	0,00%	0,00%	0,00%	0,00%	0,00%	5,08%	91% (EU909216.1) mycobiont of *Aneura pinguis*
Trechisporales							
Trechisporales uncultured	6,93%	6,73%	0,00%	0,22%	0,00%	0,00%	
Trechisporales sp.	0,00%	0,00%	0,00%	0,00%	1,94%	0,00%	94%
Trechisporales sp.	0,00%	0,00%	0,00%	0,00%	0,00%	3,78%	95%
Hydnodontaceae							
Hydnodontaceae sp	0,00%	0,00%	0,00%	0,00%	0,14%	0,00%	
Hydnodontaceae sp	0,00%	0,00%	0,00%	0,00%	1,36%	2,93%	93%
Trechispora bispora	1,34%	0,95%	2,99%	0,00%	0,00%	0,00%	
Incertae sedis							
Tremellales							
Hannaella oryzae	0,00%	0,00%	0,07%	0,00%	0,00%	0,00%	99% KM246180.1
Tetragoniomycetaceae							
Tetragoniomyces uliginosus	0,03%	0,00%	0,02%	0,00%	0,00%	0,00%	100% JN053514.1
Tremellaceae							
Naganishia diffluens	0,00%	0,00%	0,04%	0,00%	0,00%	0,00%	100% KY104324.1, equip. *Cryptococcus albidus* (KY102588.1), *C. diffluens* (KP780454.1)
Cryptococcus victoriae	0,00%	0,00%	0,14%	0,04%	0,00%	0,08%	100% KX067806.1
Trimorphomycetaceae							
Saitozyma podzolica	3,86%	2,21%	0,92%	1,47%	3,48%	2,16%	100% MF062574.1

Microbotryomycetes							
Sporidiobolales							
Incertae sedis							
Rhodotorula graminis	0,00%	0,00%	0,05%	0,11%	0,00%	0,00%	100% MF102880.1, equip. *R. babjevae* (KX771227.1),
Cystobasidiomycetes							
Erythrobasidiales							
Incertae sedis							
Erythrobasidium hasegawianum	0,00%	0,00%	0,02%	0,00%	0,00%	0,00%	100% KF293978.1, equip. *E. yunnanense* (JF758860.1)
TOTAL 28	**10**	**9**	**19**	**7**	**6**	**9**	

Table 6. Zygomycota OTU analysis and identification: Fungal species identified in the different sampling sites, ordered following their taxonomy. Abundance (%) per site is shown. Identifications have been performed against the GenBank, Mycobank and RDP classifier databases.

	appleA	**appleB**	**peachA**	**peachB**	**resting**	**wood**	**NCBI/Myco.Bank**
Zygomycota	14,46%	14,33%	13,24%	14,35%	8,51%	11,40%	
Incertae sedis							
Mortierellales							
Mortierellaceae							
Mortierella alpina	3,0000%	3,8000%	1,4000%	1,7222%	0,0359%	0,0000%	100% LC125308.1
Mortierella calciphila	0,0000%	0,0000%	0,0106%	0,0366%	0,7179%	0,3852%	95% NR_148088.1
Mortierella capitata	0,0262%	0,0915%	0,1273%	0,0000%	0,0000%	0,0000%	100% AB908052.1

Mortierella elongata	3,3000%	3,1000%	3,3000%	4,0000%	4,0000%	2,5000%	99%MF326586.1
Mortierella exiqua	0,0262%	0,0732%	0,0000%	0,0000%	0,0000%	0,0000%	90% JX976070.1
Mortierella fatshederae	0,0262%	0,0732%	0,1909%	0,4764%	0,2154%	0,0000%	100% JX976003.1
Mortierella gamsii	3,5000%	3,0000%	0,3000%	2,0000%	0,1400%	1,6000%	98% KX610447.1
Mortierella globalpina	1,0000%	0,5000%	3,0000%	2,4000%	0,0000%	2,0000%	100% JX975925.1
Mortierella globulifera	0,0000%	0,0000%	0,0848%	0,0000%	0,1077%	0,0000%	98% NR_077211.1
Mortierella humilis	0,0000%	0,0000%	0,0000%	0,0000%	0,3230%	0,2311%	100% KT896653.1
Mortierella exigua	1,4961%	1,2260%	0,5091%	3,0392%	0,8973%	0,2311%	99% JX975863.1
Mortierella wolfii	0,5249%	0,2928%	0,0000%	0,0000%	0,0000%	0,0000%	99% HQ630344.1
Mortierella zonata	0,0000%	0,0183%	0,0106%	0,0000%	0,1436%	0,0770%	98% JX975958.1
Mortierella indohii	0,1575%	0,1647%	0,2651%	0,0000%	0,0000%	0,0000%	100% JX975878.1 equip. *M. hyalina* (JN943801.1), *M. hygrophila* var. *minuta* (HQ630297.1)
Mortierella sarnyensis	0,1312%	0,0915%	0,0530%	0,0000%	0,0000%	0,1541%	98% JF311973.1
Mucorales							
Backusellaceae							
Backusella lamprospora	0,0787%	0,0183%	0,1000%	0,2000%	0,0000%	0,0000%	99% JN206270.1
Cunninghamellaceae							
Absidia anomala	0,0262%	0,0183%	0,1273%	0,1098%	0,0000%	0,0000%	97% CBS 195.68
Absidia cylindrospora	0,2100%	0,5124%	0,0000%	0,0000%	0,0000%	0,0000%	incide sul conteggio
Absidia glauca	0,0000%	0,0000%	0,4242%	0,0000%	0,0000%	0,0770%	98%KU923829.1

Absidia repens	0,0000%	0,0000%	0,0212%	0,0000%	0,0000%	0,0000%	100%EF030527.1
Mucoraceae							
Actinomucor elegans	0,3150%	0,2013%	0,0000%	0,0000%	0,0000%	0,0000%	100%FJ176396.1
Mucor abundans	0,0000%	0,0183%	0,0212%	0,0000%	0,0000%	0,0000%	98%KM668128.1
Mucor abundans	0,0262%	0,0000%	0,0530%	0,0000%	0,0000%	0,0000%	99% KM668128.1
Mucor circinelloides	0,0000%	0,2928%	0,0000%	0,0000%	0,0000%	0,0000%	100% KX421445.1
Mucor circinelloides var. *griseocyanus*	0,0525%	0,0549%	0,0636%	0,0000%	0,0000%	0,0000%	100% HM999951.1 CBS 198.28
Mucor gigaspora	0,0000%	0,0000%	0,1379%	0,0000%	0,0000%	0,0000%	100% NR_103646.1 CBS 566.91
Mucor hiemalis	0,0000%	0,0000%	0,1167%	0,0000%	0,0000%	0,0000%	100% MF355385.1
Mucor moelleri	0,0000%	0,0000%	0,0000%	0,0000%	0,1795%	0,0770%	100% KU196768.1
Mucor zychae var. *linnemanniae*	0,0000%	0,0000%	0,0424%	0,0000%	0,0000%	0,0000%	99% MB#348506
Rhizopus microsporum	0,0000%	0,0183%	0,0000%	0,0366%	0,0000%	0,0000%	100% MF445290.1
Rhizopus stolonifer	0,0000%	0,0183%	0,0000%	0,0000%	0,0000%	0,0000%	100% KU507201.1, equip. *R. oryzae* (MF491815.1), *Amylomyces rouxii* (KP790015.1)
Umbellopsidaceae							
Umbellopsis ramanniana	0,7087%	1,0064%	3,7013%	1,2092%	0,8973%	0,0000%	100% AB193542.1
TOTAL 31	**18**	**21**	**23**	**11**	**11**	**10**	

Table 7. Glomeromycota OTU analysis and identification: Fungal species identified in the different sampling sites, ordered following their taxonomy. Abundance (%) per site is shown. Identifications have been performed against the GenBank, Mycobank and RDP classifier databases

	appleA	**appleB**	**peachA**	**peachB**	**resting**	**wood**	**NCBI/Myco.Bank**
Glomeromycota							
Glomeromycetes							
Glomerales							
Glomeraceae							
Glomus mosseae	0,000%	0,000%	0,000%	0,000%	0,072%	0,000%	98% AF161056.1
uncultured Glomus	0,000%	0,000%	0,000%	0,147%	0,072%	0,000%	98% KP235575.1
uncultured Glomeraceae	0,000%	0,000%	0,000%	0,073%	0,000%	0,000%	
uncultured glomeraceus AM fungus	0,000%	0,000%	0,000%	0,073%	0,036%	0,000%	
uncultured glomeraceus AM fungus	0,000%	0,000%	0,000%	0,147%	0,000%	0,000%	
uncultured glomeraceus AM fungus	0,000%	0,000%	0,000%	0,257%	0,000%	0,077%	
Diversisporales							
Acaulosporaceae							
Entrophospora infrequens	0,000%	0,000%	0,011%	0,000%	0,538%	0,077%	99% U94714.1
unidentified	0,577%	0,403%	2,970%	3,625%	0,251%	0,000%	

Table 8. Chytridiomycota OTU analysis and identification: Fungal species identified in the different sampling sites, ordered following their taxonomy. Abundance (%) per site is shown. Identifications have been performed against the GenBank, Mycobank and RDP classifier databases

	appleA	**appleB**	**peachA**	**peachB**	**resting**	**wood**	**NCBI/Myco.Bank**
Chytridiomycota							
Chytridiomycetes							
Olpidiales							
Olpidiaceae							
Olpidiaster brassicae	0,0000%	0,0000%	0,0000%	0,0366%	0,1077%	0,0000%	100% AB205207.1

V. Paper 4

A metagenomic-based, cross-seasonal picture of fungal consortia associated with Italian soils subjected to different agricultural managements. A case study.

A metagenomic-based, cross-seasonal picture of fungal consortia associated with Italian soils subjected to different agricultural managements

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Abstract

This work pictures the biodiversity of fungal consortia inhabiting real agroecosystems, sampled in one production farm in two seasons (spring, autumn), coinciding with climate gradients and key moments of the agricultural cycle. Soil was sampled from three plots differently managed in terms of fertilization, pesticide and tillage application: conventional, organic, no-tillage. Metagenomic analyses on ITS1 amplicons depicted the highest indexes of richness for organic. No tillage resulted in inhabitation by the most divergent communities, with their own composition, prevalences and seasonal trends. Ascomycota always predominated, with the exception of conventional, that had high abundance of a single basidiomycete species. Our results showed evidence that agricultural soils under organic and no-tillage systems harbour distinct mycobiota, even in neighbouring fields. From our results, fungal consortia altered even in the first year after the management change.

Keywords: Soil fungi, mycobiota, metagenomics, ITS1, NGS, agricultural managements, organic agriculture, no-tillage, sod-seeding, seasonal gradients

1. Introduction

Fungal communities (mycobiota) fulfil major ecological functions in many environments, among which soils. Here, they seem to dominate the microbial biomass (Joergensen and Witchern, 2008) and participate in nutrient cycling, including natural carbon, nitrogen and phosphorus cycles (Christensen, 1989). Fungi are also involved in a range of other processes, integral for the ecological functioning of all soils: decomposition of organic matter, soil stabilization, plant productivity and protection against pathogens, and composition of the plant community (van der Heijden et al., 2008). Clearly they contribute to soil fertility and quality, and with resident bacterial communities, are considered crucial bioindicators (Schloter et al., 2003; Sharma et al., 2010).

The impact of soil bacteria and fungi is so deep that the ambitious Earth Microbiome Project has been launched, aiming to analyse 200,000 soil samples and construct a Gene Atlas of uncultured microbial diversity for all biomes on Earth (Gilbert et al., 2014). Also, the International Decade of Soils was launched in 2015 by the International Union of Soils Sciences, for addressing the complex relations between the exacerbated exploitation of soils and many compelling health, environmental and social issues (iuss.org).

These concepts apply *a fortiori* at agroecosystems. A solid understanding of microbiota and mycobiota dynamics and diversity is of pivotal importance in agroecosystems, together with a clear elucidation of its responses to natural fluctuations and management decisions. Management considerations include the diverging possibilities offered by conventional fertilization and pesticide application vs. low-input organic farming, or conventional tillage vs. low-disturbance no-tillage practices. The large increase in agricultural productivity has led to ecosystem and soil degradation, accumulation of pesticides, diminished availability and quality of water (Alvarez et al., 1995; Tilman et al., 2002; Foley et al., 2005), and is perceived as a main threat for global biodiversity (Convention on Biological Diversity, 2010). Another important point is that a deep understanding of soil bacterial and fungal communities constitutes the premise for managing them in terms of presence of beneficial species and absence of detrimental ones (Chaparro et al., 2012), and for understanding processes that affect fertility (Carbonetto et al., 2014).

The effects of farming management on soil mycobiota and microbiota are complex and appear variable (Bunemann et al., 2006; Carbonetto et al., 2014). It is usually reported that organic farming favours an higher abundance and diversity of macrobiota, but data on microbial communities, especially on fungi, are less complete (Postma-Blaauw et al., 2010) and often do not refer to “real” fields under production. The adoption of limited tillage systems, less disturbing for soils, is known to augment soil organic matter, water content, and crop yields (Alvarez and Steinbach, 2009), but again effects on mycobiota are still scarcely understood.

This work aims at picturing a broad spectrum of the biodiversity of fungal communities inhabiting different agroecosystems located in the same production farm. To our knowledge, this has been done only a few times so far, in completely different agricultural realities and with other experimental approaches (see for example Moeskops et al., 2010). The farm sampled in our study produces crops and is located in the Padan Plain area (Pavia province, Lombardy, Italy), one of the main European agricultural sites. The sampled plots are subjected to differential management practices that are applied in parallel in an effort to find which one better combines productivity, environmental sustainability and the addition of minor amounts of additives. These management protocols differ for the use of fertilization, pesticides and tillage. A second important objective of this work is to describe the influence of seasonality (e.g., climate fluctuations, but also seasonal agricultural treatments in key moments of the production cycle) on the structure of the mycobiota. These goals will be reached by high throughput deep sequencing using an Illumina MiSeq-based amplicon sequencing of the ribosomal internal transcribed spacer-1 (ITS1) region.

2. Methods

2.1 Management protocols applied to the plots: fertilization, pesticide application and tillage

Samples were collected from three fields belonging to the production farm "La Calvenzana" (Rivanazzano Terme, Pavia, Italy). The first one, "Pomocotogno", spans 5 hectares (ha) and will be hereafter referred to as "conv" because it receives fertilizer and plant protection schemes based on conventional, high-throughput systems. Nitrogen fertilizer (urea, 58 kg ha^{-1}) is applied once a year (normally around mid-April) and plant protection is achieved using herbicides (3 treatments per year) employing the recommendations and thresholds of the EU Regulation (EC) No 1107/2009. Conventional tillage is applied. During recent years, this field has had a rotation of annual crops, with chikpea (harvested in 2015) and grain sorghum (harvested in 2016) most recently.

The "Vallone 2" plot, spanning 4 ha, has been managed since 2010 following the EU Council Regulation No 834/2007 on organic agriculture. After the prescribed 5-year cultivation of alfalfa to enrich soil especially for nitrogen, this field (hereafter "org") yielded its first certified organic production (barley, variety "bio arda") in 2016. Plant protection is achieved here by using mechanical strategies. Conventional tillage is applied and no additional fertilization is given.

The "Valloncino 3" plot (3 ha) has been subjected to conservational no-tillage (or sod-seeding) practices i.e., minimum soil disturbance combined with rotations, since 2015. It will be referred to as "sod". This plot receives two herbicide treatments per year and nitrogen fertilizer (58 kg ha^{-1}) as specified above for conv. The last crop rotation before sampling was sorghum-barley (Manara variety).

2.2 Sample collection

Soils were sampled during autumn (A, end of November 2015) and spring (S, end of April 2016). Three soil samples were collected from each plot to trace a triangle, with vertices placed as far apart as possible, but at least 10 m away from field edges. Soil samples (500 g each) were aseptically taken at 3 cm depth with a sterile spoon, after removal of vegetation cover, stones and other debris, and put in sterile polyethylene bags. Samples were returned to the laboratory in coolers and were kept at -20°C (for the metagenomic analyses) or +4°C (for the other analyses) for 24-48 hours before processing. For metagenomics, samples referring to each plot were pooled before freezing.

2.3 Soil chemical analyses

Chemical properties of soils were determined by Minoprio Analisi e Certificazioni, Como, Italy, according to the Italian standard protocols (DM 13/09/99). The following parameters were evaluated: pH, organic matter, total nitrogen (N_{TOT}), organic carbon (C_{ORG}), C/N ratio, plant-available phosphorous (P), calcium (Ca), magnesium (Mg), potassium (K), soil composition in sand, silt, clay and soil texture.

2.4 DNA extraction, ITS1 amplification and Illumina sequencing

Total DNA was extracted from 350 mg of “A” (autumn) and “S” (spring) samplings of conv, org and sod plots, using the NucleoSpin Soil kit (Macherey-Nagel, Düren, Germany) following the manufacturer's specifications. The extraction buffer SL1 was used, supplemented with 70 µl of SX enhancer. DNA was then quantified on a Qubit fluorometer (ThermoFisher Scientific, Waltham, MA). For amplicon production, the ribosomal ITS1 region was targeted, by using primers BITS and B58S3 (Bokulich and Mills, 2013) linked to Illumina adapters. PCR was performed in a 50-µl volume containing 5 to 10 ng template DNA, 1x HiFi HotStart Ready Mix (Kapa Biosystems, Wilmington, MA), 0.5 µM of each primer. The cycling program, performed on a MJ Mini thermal cycler (Promega corp., Madison, WI), included an initial denaturation (95°C for 3 min), followed by 25 cycles at 94°C for 30 s, 55 or 60°C for 30s, 72°C for 30 s, and final extension (72°C for 5 min). Amplicons obtained using the two annealing temperatures were pooled as suggested by Schmidt et al. (2013). Clean-up of amplicons was performed using Agencourt AMPure XP SPRI magnetic beads (ThermoFisher Scientific). Illumina sequencing libraries were finally constructed through the link of indexes (Nextera XT Index Kit, Illumina, San Diego, CA), quantified using a Qubit 2.0 Fluorometer (ThermoFisher Scientific), normalized and pooled. Libraries were subjected to paired-end sequencing (2 x 250 bp, nano format) on an Illumina MiSeq sequencer at BMR Genomics (Padova, Italy).

2.5 Data analysis: definition of operational taxonomic units (OTUs) and community analyses

Data analysis was performed using the pipeline PIPITS (Gweon et al., 2015). In brief: raw data were demultiplexed based on the unique barcode assigned to each sample. Barcodes and primers were then trimmed off. Sequencing quality filters were applied, including a minimum length threshold (100 bp) and removal of singletons. High-quality reads were then clustered into operational taxonomic units (OTUs) at 97% similarity using VSEARCH (Rognes et al., 2016) and chimaeras exluded using UCHIME (Edgar et al., 2011). OTUs were finally annotated using the UNITE fungal ITS reference data set within RDP classifier (http://rdp.cme.msu.edu) and the Worcup ITS reference as training dataset.

Species assignation on selected OTUs was manually resolved through BLAST searching against mycobank (mycobank.org), RDP (https://rdp.cme.msu.edu/) and using the specialized fungal pages within GenBank. Relative abundances of microbial taxa in each sample were calculated and compared.

The richness of each sample (α-diversity) was assessed by computing Chao 1, Abundance Coverage Estimator (ACE) and Shannon indexes at the taxonomic rank of orders, using the R library Vegan (Oksanen et al., 2017). Diversity in composition among samples (β-diversity) was compared, always considering orders, through: (i) bootstrap-based clustering analysis, using the R function pvclust (Suzuki and Shimodaira, 2006); (ii) double clustering analysis, through the R function heatmap.2 of the R library Gplots (Warnes et al., 2016), setting the pvclust output as sample dendrogram; (iii) Principal Coordinates Analysis (PCoA) analysis based on Bray-Curtis dissimilarity matrix, performed using the R library Vegan (Oksanen et al., 2017).

3. Results

3.1 Structure of fungal communities over seasons and in relation to different agricultural land use and to soil chemical properties

The mycobiota was evaluated through ITS1 deep sequencing, that produced a total of 792,174 raw reads on the Illumina MiSeq platform; of these, 753,419 passed quality filtering. After removing redundant sequences, 27,161 were recognized and extracted as ITS1 by the ITSx software against the UNITE ITS reference dataset. The 97% similarity level was finally established for the operational taxonomic units (OTU): PIPITS wrapper (Gweon et al., 2015), after removing chimeras, returned 1,194 OTUs. The amount of unassigned and unidentified OTUs was within a range of 35% to 70%, with the lowest values in the conv treatment and the highest for sod.

Diversity and richness indexes, investigated at the taxonomic level of orders, depicted a quite homogeneous situation among the different soils and seasons, especially with respect to the Shannon diversity estimator (Table 1). SodA was the sample characterized by the lowest number of observed orders and chao1 estimated richness. Conversely, the org couple resulted, on the whole, the richest.

Table 1. Biodiversity and richness estimators calculated at the taxonomy rank of orders. Number of observed orders (N), Shannon's index of biodiversity (Shannon), Chao 1 richness estimator (Chao1) and associated standard error (SE.Chao1), Abundance Coverage Estimator (ACE) and corresponding standard error (SE.ACE).

	N	**Shannon**	**Chao1**	**SE.Chao1**	**ACE**	**SE.ACE**
convA	22	2.22	23.5	2.22	28.88	2.55
convS	27	2.05	27	0.12	27.35	2.51
orgA	27	2.21	27.2	0.61	28.38	2.38
orgS	26	2.02	26	0	26	2.48
sodA	18	2.04	19.5	2.57	20.41	1.88
sodS	26	2.23	36	10.34	31.95	2.30

Next, samples were compared and clustered with respect to their composition in fungal orders. From the bootstrap-based clustering analysis (Fig. 1A), it clearly emerged that samples were divided into two macro-clusters. The first one included the sod couple, supported by a highly significant bootstrap value (>95%). The second cluster covered the remaining samples. The convS soil was intermediate between the sod couple and the other three samples (the org couple and convA), that clustered together more closely. Clustering was mainly dictated by the differential abundance of a limited number of fungal orders: Pleosporales, Hypocreales, Agaricales, Xylariales, Eurotiales, Helotiales, Capnoidales, Mortierellales, Tremellales, Sordariales. Important peaks corresponded to Pleosporales in sodA, Hypocreales in orgS, and Agaricales in convS (Fig. 1B). The Bray-Curtis dissimilarity index, and associated Principal

Component Analysis (PcoA), confirmed the general picture outlined above with respect to between-soil distances and clustering (Fig. 2A and B).

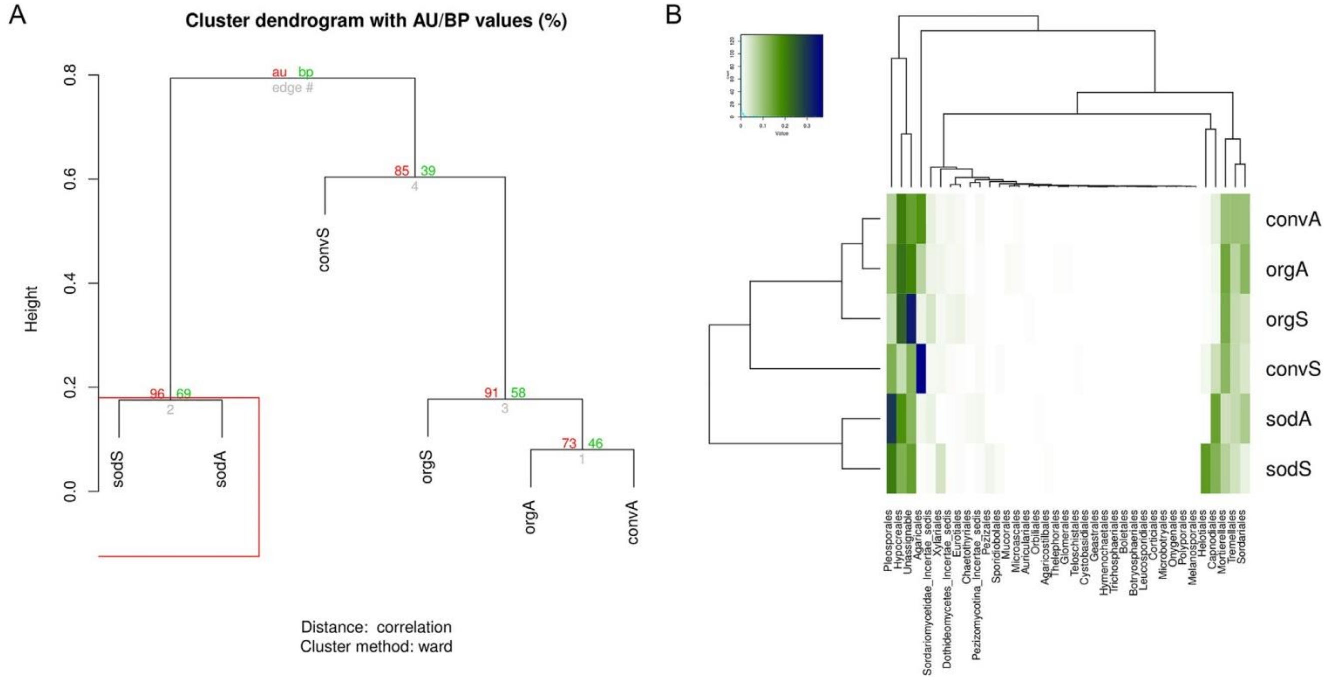

Fig. 1. Clustering of samples with respect to their composition in fungal orders. A. Bootstrap-based clustering analysis. B. Heatmap analysis based on the Euclidean distance of orders and on the dendrogram produced by the clustering analysis. ‘conv’= conventional management; ‘org’= organic; ‘sod’= sod-seeding (no-tillage). ‘A’= autumn; ‘S’= spring, AU: approximately unbiased; BP: bootstrap probability.

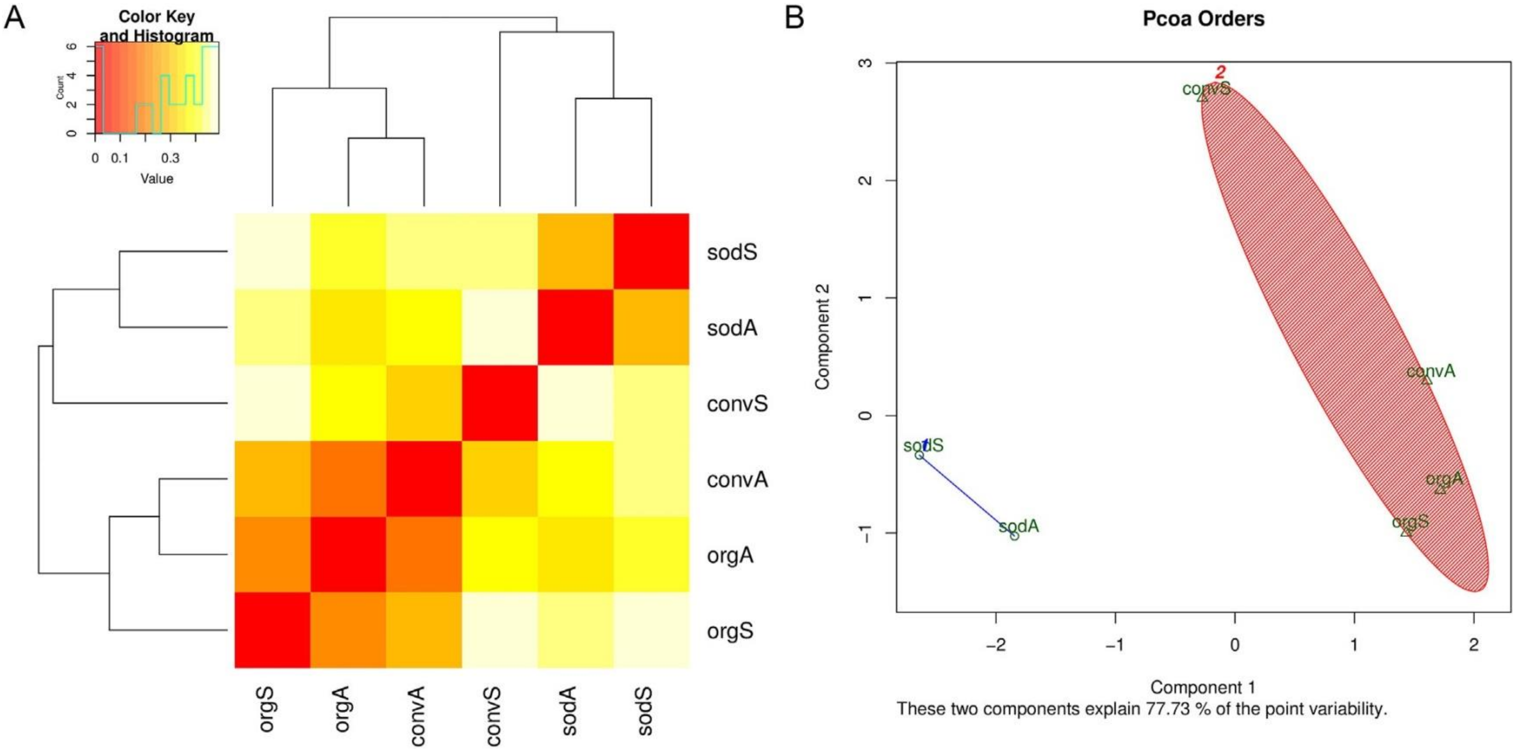

Fig. 2. Bray-Curtis metrics calculated for the taxonomic rank of orders. A. Dissimilarity index among samples B. PcoA of mycobiomes of conv, org and sod. Standard error ellipses show 95% confidence areas.

Soil chemistry, though relatively homogenous among plots, showed the most pronounced differences between the sod plot and the conv/org couple (Table 2).

Table 2. Chemical properties of soils. Abbreviations: N_{TOT}: total nitrogen; C_{ORG}: organic carbon, meq/100 g: milliequivalents in 100 g.

	pH	Organic matter (g/kg)	N_{TOT} (g/kg)	C_{ORG} (g/kg)	C/N ratio	P (mg/kg)	Ca (meq/100 g)	Mg (meq/100 g)	K (meq/100 g)	Sand (g/kg)	Silt (g/kg)	Clay (g/kg)	Texture
conv	7.76	17	1.3	10	7.7	63	21.99	2.57	0.41	128	455	417	Clayish-loamy
org	8.67	19	1.4	11	7.9	23	23.45	1.44	0.38	188	525	287	loamy
sod	8.53	13	1	8	8	57	19.43	1.23	0.39	188	515	297	loamy

3.2 Taxonomic picture of fungal communities

Taxonomy was assigned to the 1,194 OTUs returned by PIPITS and sequences attributed to six fungal phyla. Ascomycota was generally the dominant phylum, with a range between 27.7% (convS) and 59.8 (sodS) and comparable abundances in spring (S) and autumn (A) samples. convS constituted a notable exception, with a strong prevalence of Basidiomycota, accounting for 45.7% of fungal diversity, vs. 31% of the companion convA sample. By comparison, Basidiomycota made up 6.9-9.2% of sod (S and A respectively) and 14.2-14.3% of org (S and A). Zygomycota was also well-represented with around 9% in the two conv samples, 12.5-18.5% in org (A and S respectively), and 4.6-10.2% in sod (A and S). Phyla with abundances <1%, include Chrytridiomycota (exclusively in the org couple), Glomeromycota (in conv and org samples) and Rozellomycota (org and sod).

Among Ascomycota, Sordariomycetes were the most abundant class, especially in org (33% orgS). The Ascomycota class Dothideomycetes was particularly abundant in the sod system, especially in autumn, with about 20% prevalence (vs. 1.8-9% in the other samples). The most abundant Basidiomycota class was Agaricomycetes, reaching 40% in convS, the sample characterized a high abundance of Basidiomycota.

At the rank of orders, the most represented Sordariomycetes were Hypocreales (especially for org, with a peak of 21% in spring) and Pleosporales (around 5% in conv, 8-15% in orgA and -S, 4-8% in sodA and -S). With regard to Basidiomycota, the order Agaricales dominated in convS (37.2%) with less in other soils (17% in convA, 6.6% in orgA and around 1-2% in the others). For Zygomycota, the order Mortierellales accounted for about 9% of fungal diversity in the conv couple, 18% in orgS and 4-10% in the remaining samples.

At the taxonomic level of families, Psathyrellaceae (Basodiomycota: Agaricomycetes) were especially abundant in conv soils (Fig. 3): 37% in convS and 16% in convA, trough only 5% in orgA vs. 0.5% in orgS, and 0.3% in the sod system. Another well-represented family was Mortierellaceae (Zygomycota), with abundances around 10% in conv and org, almost equally distributed among seasons, and less represented (around 4%) in sod. In Ascomycota, Nectriaceae constituted around 2-4% of all samples, without any clear seasonal trends. In contrast, a seasonal gradient characterized another Sordariomycete family, Hypocreaceae in org soil (9% in orgS but only 1.9 in orgA). There was also a marked seasonal trend in the Sordariomycete family Lasiosphaeriaceae, with peaks in autumn for all soils. The opposite pattern was observed in Mycosphaerellaceae (class: Dothideomycetes) and Helotiaceae (class: Leotiomycetes) with peaks in spring for conv and sod systems.

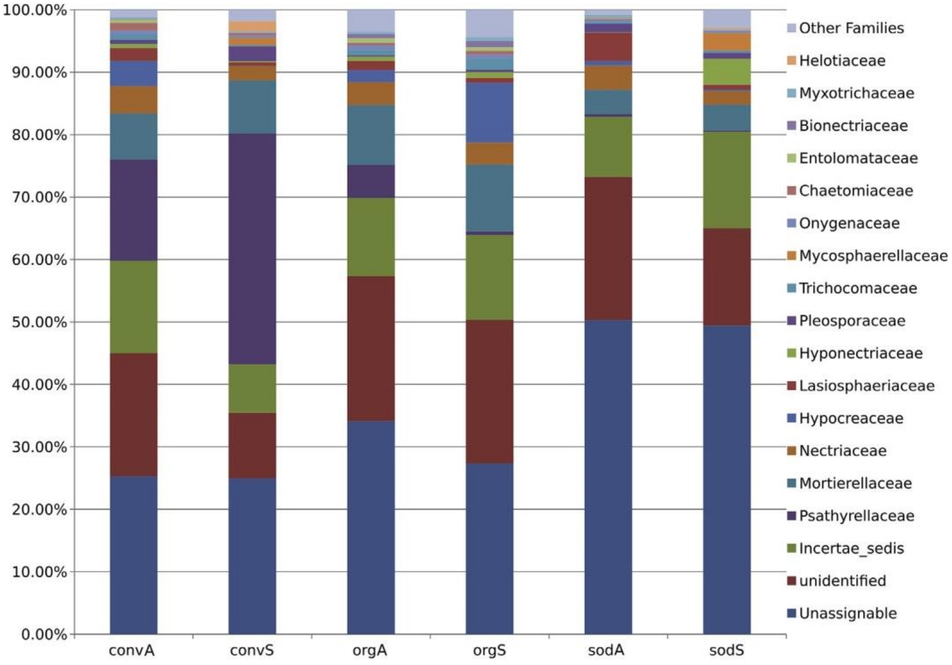

Fig. 3. Structure of soil mycobiota in different agroecosystems, across seasons. The histogram shows the relative distribution of the most abundant families.

At the genus level, *Psathyrella* predominated in convS (36%), together with *Cryptococcus*, to a lesser extent, though it was virtually absent from all other samples (Fig. 4). *Coprinellus*, also in Psathyrellaceae, accounted for 16% of the OTUs in convA, 4% in orgA but was nearly absent from other samples. *Cryptococcus* (Basidiomycota: Tremellomycetes), a polyphyletic genus now largely reclassified as better specified below for the species level, accounted for 10% in convS, but only 4-6% in the other samples, without a clear seasonal trend. In Zygomycota, *Mortierella* had an abundance of 7-10% in conv and org, 3-4% in sod and no clear seasonal trends. In Ascomycota, *Acremonium* (Sordariomycetes) reached similar abundances (around 9.5) in orgS and orgA, while in the other soil systems it was generally more prevalent in autumn. *Mycosphaerella* was retrieved uniquely in sodS (2.8%), while *Aspergillus* was most abundant (1.3%) in orgS.

In general, from the community analyses and taxonomic picture presented above, the sod system was the most different: its taxonomic picture appeared peculiar and tended to have different species composition, abundances and seasonal trends from other systems. Among the other samples, convS stood out due to its high Basidiomycota content, almost exclusively ascribable to the *Psathyrella* genus.

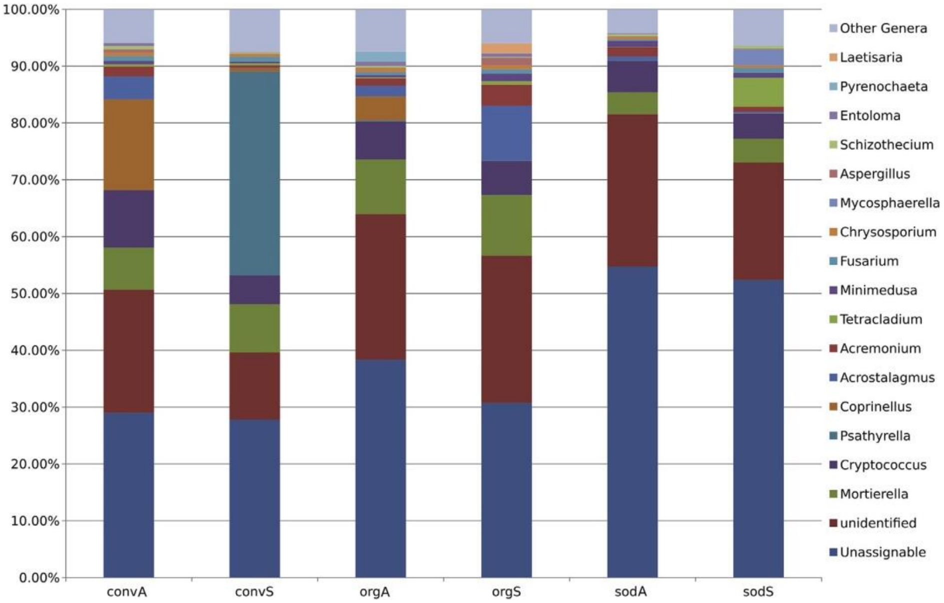

Fig. 4. Structure of soil mycobiota in different agroecosystems, across seasons. The histogram shows the relative distribution of the most abundant fungal genera.

3.3 Species composition

A finer, manual, bioinformatic analysis of the most abundant OTUs (i.e., representing >1% of total diversity in at least one sample) allowed resolution to species level (Table 3). OTUs 100% homologous to *Psathyrella pseudogracilis* had 35% prevalence in convS. Two different Basidiomycota OTUs strictly related to *Coprinellus verrucispermus* together had 16% abundance in convA. Overall, in the conv system, these two species showed opposite seasonal trends. *C. verrucispermus* showed similar trends, though lower abundance, in org. Two species of *Cryptococcus* were recognized: *Cryptococcus stepposus* (current name:

Filobasidum stepposum, Family Filobasidiaceae) and *Cryptococcus aerius* (currently reassigned *as Solicoccozyma aeria*, Family Piskurozymaceae), the former only in the conv soils, the latter in all samples, with highest prevalences and autumn peaks in org and conv. The Zygomycota *Mortierella* genus resolved in several species, the most represented of which were *Mortierella elongata*, especially in conv soils, and *Mortierella alpina*, in org. Several species of Ascomycota, were particularly abundant in sod soils, e.g., *Alternaria alternata*, *Leptosphaerulina chartarum*, *Cladosporium herbarum*. The Sordariomycetes genus *Fusarium* contained various species, including ubiquitous species (e.g., *Fusarium oxysporum*) and others that were mostly restricted to a specific agricultural management (e.g., *Fusarium incarnatum-equiseti* species complex in sod). Similarly, *Chaetomium globosum* was strongly linked to the autumn sampling in the organic soil. Other Ascomycota identifiable to the species level were particularly abundant in the sod-seeding plot: *Aspergillus heyangensis (*Eurotiomycetes), *Idriella lunata* (Leotiomycetes), and *Tetracladium setigerum* (Leotiomycetes), all with strong seasonal specificity.

Table 3. OTU analysis and identification: Fungal species identified in the plots, ordered following their taxonomy. Comparative abundance (%) per season is shown. Identifications have been performed against the GenBank, Mycobank and RDP classifier databases.

Species	**Autumn vs. Spring (%)**			**GenBank**	**%**
	CONV	**ORG**	**SOD**		
Phylum: Ascomycota					
Class: Dothideomycetes; Order: Pleosporales; Family: Pleosporaceae					
Alternaria alternata[a]	2.15 vs 2.74	1.87 vs 0.15	11.35 vs 9.83	KF94448	100
Pyrenophora avenae	0 vs 1			FJ907537	100
Order: Pleosporales; Family: Didymellaceae					
Epicoccum nigrum	0.08 vs 3.44	0.11vs 0.04	2.38 vs 2.32	FJ426997	100
Leptosphaerulina chartarum	1.68 vs 0.03	2.8 vs 0.36	6.8 vs 0.14	EU52999	100
Order: Capnodiales: Family:					
Cladosporium herbarum[b]	0 vs 3.27	0.07 vs 0.56	1.35 vs 4.65	KU85661	100
Family: Mycosphaerellaceae					
Septoria stellariae	0 vs 1			KF21574	
Class: Sordariomycetes; Order: Hypocreales; Family: Stachybotryaceae					
Stachybotrys chartarum	0.98 vs 0.20	1.04 vs 0.56	0.56 vs 0.1	KP26900	100
Family: Nectriaceae					
Fusarium oxysporum	3.43 vs 0.83	2.44 vs 2.59	2.94 vs 0.48	KX61039	100
Fusarium oxysporum[c]	0.62 vs 0.50	0.2 vs 0.09	0.23 vs 0.7	KX61039	100
Fusarium incarnatum-equiseti species	1.13 vs 1.43	0.81 vs 0.55	6.11 vs 7.10	GQ50576	100
Fusarium tricinctum[d]	0 vs 0.07	5 vs 0.24	1 vs 1.34	JX241657	100
Fusarium brachygibbosum	0.47 vs 0.27	0.3 vs 0.07	2.8 vs 0.33	KX55091	100
Cylindrocarpon olidum	0.3 vs 0.51	0.53 vs 0.96	0.24 vs 0.04	KC427019	99
Neocosmospora striata[e]	1.64 vs 0.28	1.39 vs 0.85	0.32 vs 0.23	JQ95488	100
Family: Bionectriaceae					
Clonostachys rosea	0.07 vs 0.03	0.44 vs 0.9	0.08 vs 0.02	KT00710	100
Family: Hypocreaceae					
Acrostalagmus luteoalbus	4.1 vs 0.06	1.92 vs 9.6	0.71 vs 0.23	KC461515	100
Order: Sordariales: Family: Chaetomiaceae					
Chaetomium murorum[f]	0.04 vs 0.25	0.2 vs 0.15	0 vs 0.23	KT34731	98

Chaetomium globosum	2.8 vs 1.20	6.7 vs 0.43	0.16 vs 0.35	JX241646	99
Chaetomium grande[g]	1.91 vs 0.60	0.79 vs 1.41	0.48 vs 0.23	KC461515	100
Cladosporium macrocarpum	0.2 vs 0	0.89 vs 0.54	0.08 vs 0	KX66440[b]	99
Family: Lasiosphaeriaceae					
Schizothecium inaequale	0.59 vs 0.23	0.30 vs 0.17	0.56 vs 0.1	AY999117	99
Order: Amphisphaeriales; Family:					
Microdochium nivale	0 vs 0.93	0.39 vs 0		AM50226	100
Order: Microascales; Family: Microascaceae					
Doratomyces asperulus	0.07 vs 0	0.5 vs 0.09	0.08 vs 0.12	KX85862	100
Order: Incertae sedis; Family:					
Gibellulopsis nigrescens	2.69 vs 0.8	3.7 vs 4	1.83 vs 0.3	EF543857	100
Class: Eurotiomycetes; Order: Eurotiales; Family: Trichocomaceae					
Aspergillus heyangensis	0.04 vs 0		4.8 vs 0	FJ491520	98
Class: Leotiomycetes; Order: Helotiales; Family: Helotiaceae					
Idriella lunata			0 vs 4	[h]	98
Family: Incertae sedis					
Tetracladium furcatum	0.23 vs 0.10	0.11 vs 0.11	0.48 vs 0.10	GU58684	96
Tetracladium setigerum	0.16 vs 0.35	0.14 vs 0.13	0 vs 4.68	KX85862	100
Unclassified Ascomycota					
Tricellula aquatic	0 vs 1.62		0 vs 0.04	[h]	99
Phylum: Basidiomycota					
Class: Agaromycetes; Order: Agaricales; Family: Psathyrellaceae					
Psathyrella pseudogracilis	0 vs 35	-		KC992853	100
Coprinellus verrucispermus (A)	14 vs 0.02	1.8 vs 0		AY521250	98
Coprinellus verrucispermus (B)	1.7 vs 0	2.3 vs 0		AY521250	99
Family: Pluteaceae					
Volvopluteus gloiocephalus voucher LOU18619		0.33 vs 0.06		HM5622	100
Order: Cantharellales; Family: Incertae					
Minimedusa polyspora	0.59 vs 0	0 vs 0.90		KC176294	100
Class: Tremellomycetes; Order: Tremellales; Family: Tremellaceae					
Cryptococcus stepposus[i]	0 vs 1.38	_	_	JX188129	100
Cryptococcus aerius[l]	10.1 vs 2.67	6.68 vs 5.94	3.4 vs 3.94	AB03266	100
Phylum: Zygomycota					
undetermined class; Order: Mortierellales; Family: Mortierellaceae					
Mortierella elongate	5.19 vs 3.87	2.29 vs 1.28	0.79 vs 1.02	LC125298	100
Mortierella elongata voucher MT146	1.52 vs 0.53	2.40 vs 1.58	0.56 vs 2.13	FJ161922	100
Mortierella alpina (A)	0.20 vs 1.94	2 vs 2.90	0.79 vs 0.65	KF38530	100
Mortierella alpina (B)	1.33 vs 1.64	2.87 vs 5.53	2.14 vs 1	KX05806	100
Mortierella globulifera	_	0.42 vs 0	_	NR_0772	100
Mortierella exigua	0.3 vs 0.71	0.6 vs 0.19	0.08 vs 0.19	JX975863	99
Mortierella gamsii	0.27 vs 0.1	1.2 vs 0.64	0 vs 0.49	HQ63030	100

[a] *A. citri* (KU375628.1) is equiprobable.

[b] *C. macrocarpum seminudum* (CBS 779.71) is equiprobable.

[c] *F. solani* (JX435185) is equiprobable.

[d] *F. heterosporum* (KR909451) is equiprobable.

[e] *N. vasinfecta* (JX997932.1) is equiprobable.

[f] *C. piluliferum* (KF915989) is equiprobable.

[g] *C. megalocarpum* (NR144832.1) is equiprobable.

[h] CBS strain database knawl.nl *Idriella lunata*: CBS681.92: *Tricellula aquatica*: CBS383.58)

[i] Current name: *Filobasidium stepposum* (Family: Filobasidiaceae).

[l] Current classification: *Solicoccozyma aeria* (Family: Piskurozymaceae).

4. Discussion

Our work evidences a response of microbial communities to different agricultural management practices, as also seen in other studies (van der Heijden and Hartmann, 2016 for a review). Also, our work considers organic and no-tillage plots that have been recently shifted from the conventional management, allowing to highlight fungal diversity responses at early phases. This complements previous studies on long-established agroecosystems and field experiments (e.g., Klaubauf et al., 2010; Carbonetto et al., 2014; Hartmann et al., 2015).

In agreement with published data, we found that the plot conducted under organic agriculture had the highest ecological indexes of richness and the highest observed number of fungal orders (Hartmann et al., 2015). The same has been extensively reported for the better-characterized bacterial communities (Sugiyama et al., 2010; Chaudhry et al., 2012; Hartmann et al., 2015). The differences in ecological estimators under recently altered management were not dramatic, but it is likely that they will alter further with time. Indeed, for bacteria under organic farming, indexes of α-diversity increase after the first few years, but then tend to decrease in the long term (van Diepeningen et al., 2006).

Clustering analysis of mycobiota composition revealed a situation where the two most divergent consortia, with a significant statistical support, were those inhabiting the conservational no-tillage (sod) plots. The same pattern also emerged from PcoA analysis on Bray-Curtis dissimilarity index. On one hand, these findings could be at least in part related to the soil chemical properties: even in a rather homogeneous context, sod seems to slightly diverge from conv and org in relation to the organic matter and the N_{TOT}. On the other hand, these observations confirm previous data on bacterial communities (Carbonetto et al., 2014), indicating that tillage is a main agent of soil disturbance (Langdale et al., 1992). Finally, another difference emerges when looking at the seasonal responses of fungal communities observed coherently in conv and org soils: they are less pronounced in sod, that often displays unique seasonal trends.

As in all other studies (e.g., Klaubauf et al., 2010; Xu et al., 2012; Urbina et al., 2016), Ascomycota were the predominant fungal phylum in soil, representing around 50% of fungal OTUs in org and nearly 60% in sod. Peculiarly, we observed only a few Glomeromycota, even in the organic management, where they would be expected to be higher (see Oehl et al., 2004). This may be due to a limited coverage and biases exerted by the primers, as reported by other authors (Kohout et al., 2014; Hartmann et al., 2015).

On the whole, the conv system was characterized by the highest Basidiomycota content. Sample convS was dominated by a large increase, in spring, of *Psathyrella pseudogracilis*, absent from all the other samples; clustering and Bray-Curtis analyses placed this sample between the “sod” couple (characterized by the lowest Basidiomycota prevalence) and the remaining three samples (org couple and convA). Psathyrellaceae were also prevalent in the autumn sample, and represented by *Coprinellus verrucispermus*. These genera, together with *Mortierella* (a zygomycete retrieved, in our system, especially in conv and org, with species-dependent distributions) were among the main management-sensitive taxa, i.e., taxa that most significantly responded to the management regimes and were responsible for the observed shifts in the community structure. In a previous study (Hartmann et al., 2015) the interpretation was that these are coprophilous genera that were uniformly responding to the application of organic fertilizers (farmyard manure and slurry). Even if these fertilizers are not used in the farm sampled in the present study, a wider effect of nitrogen (urea) fertilization, performed around mid-April, may partly explain the *Psathyrella pseudogracilis* prevalence in convS. However, the fact that a comparable phenomenon is not observed in sod (subjected to the same fertilization regimen) and that the related genus *Coprinellus* is found (always in conv and not in sod) almost totally in autumn, implies that other factors are probably at work. These saprotrophic genera are able to grow not only on dung, but also on straw, leaf litters and wood (Larsson and Orstadius, 2008). The natural habitat of *Psathyrella pseudogracilis* is along forest borders, parks and lawns, usually on rotten plant debris, while *Coprinellus verrucispermus* is often found in potting compost (Osono 2007). It seems thus reasonable to assume that these two species are acting as litter decomposers in conv, but that the situation is different in sod.

Another point is that both genera have known bioremediation properties linked to the degradation of either lignin or organic pollutants (Ikehata et al., 2004; Tortella et al., 2005). Their strong increase in conv could be, at least in part, related to the stress status of the soil and to the fact that this is the plot receiving the most “intense” herbicide treatment. A final point is the reported antagonistic effect of *Psathyrella* against the phytopathogen *Fusarium oxysporum* (Reinoso et al., 2013). Indeed, the prevalences of the two appear inversely correlated from our results.

There are several factors that could contribute to effects of management on communities. First, the “legacy” effect of the preceding crop in the rotation, leading to divergent soil fungal consortia e.g., through the link to soil N dynamics (Detheridge et al., 2016). Even if the “crop history” of the three soils is very similar, sod is the one with the lowest contents in N and organic matter. This is unexpected for a no-tillage plot, even since only one year, but this is the only plot that did not host nitrogen-fixating crops in the last rotations.

Many reports have addressed the effects of organic farming on bacterial and fungal diversity, with variable conclusions because of differences in experimental systems and management definitions. Reports on the effects of tillage on fungi are scarce (e.g. Mathew et al., 2012; Avio et al., 2013), especially those relying on metagenomics (Clifton et al., 2015). Instead, it is precisely for sod soils that our results pictured the most divergent fungal communities, with peculiar seasonal trends and species composition. The most abundant species in sod are generally ascomycetes belonging to the orders Pleosporales (e.g., *Alternaria alternata* and the grass parasite *Leptosphaerulina chartarum* sp.nov.), Hypocreales (e.g., some species of *Fusarium*), Eurotiales (e.g., *Aspergillus heyangensis*), Helotiales (e.g., the root pathogen *Idriella lunata*, *Tetracladium setigerum*). Among these orders, Hypocreales are of vast economic importance as they include many plant pathogens (e.g., *Fusarium* sp.) as well as potential biocontrol agents (Castella and Cabanez, 2014). In our experiment, species within the genus *Fusarium* are among those that most significantly respond to the changing of seasons and agricultural managements, in accord to Hartmann et al. (2015). *Fusarium incarnatum*, particularly abundant in sod soil, *Fusarium oxysporum* (in conv), *Fusarium tricintum* (in org) are all crop pests and mycotoxin producers (Castella and Cabanez, 2014). On the whole, the *Fusarium* genus appears more abundant in the sod plot, in accord to Miller et al. (1998) that reported an increased seed infection rate linked to no-tillage protocols.

In conclusion, agricultural soils under short-term organic and no-tillage systems harbour distinct and specific fungal communities, here characterized till the species level. These consortia are responding to seasons mainly in a tillage-dependent way. Our data contribute to pose the bases for evaluating the success of individual soil managements, also in terms of promotion/suppression of beneficial or pathogenic taxa and, in perspective, to acquire knowledge for managing their mycobiota content.

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VI. Paper 5

Fungi as a toolbox for sustainable bioremediation of pesticides in soil and water

Fungi as a toolbox for sustainable bioremediation of pesticides in soil and water

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Abstract

Pesticides can help reduce yield losses caused by pests, pathogens, and weeds, but their overuse causes serious environmental pollution. They are persistent in the environment and are biomagnified through the food chain, becoming a serious health hazard for humankind. Bioremediation, where microbes are used to degrade pesticides *in situ*, is a useful technology. This review summarizes data on the fungi involved in the biodegradation of chemical pesticides; and their application in soil and water bioremediation. Indications for future studies in this field are given.

Keywords: Pesticides, Agrochemicals, Antibiotics, Sustainable bioremediation, Fungi, Synthetic microbial community, Environmental risk assessment.

Introduction

Because of their unique functions, fungi are involved in ecosystem services essential to human well-being. Amongst others, fungi also carry out the transformation and detoxification of pollutants. For this reason, learning from nature, they represent an effective toolbox for a sustainable bioremediation of pesticides in soil and water. Many researches have revealed the untapped potential of fungi, and recent years have witnessed very interesting developments regarding the application of fungi not only to improve environmental quality but also human health (e.g. Gargano et al. 2017).

Pesticides are a diverse group of inorganic and organic chemicals that include herbicides, insecticides, nematicides, fungicides, antibiotics and soil fumigants (Verger and Boobis 2013; Verma et al. 2014). They are employed in agriculture to enhance crop yield and quality, and to maximize economic returns by preventing pest or weed attack. They are bioactive, toxic substances, capable of directly or indirectly influencing soil fertility and health as well as agroecosystem quality (Pinto et al. 2012; Verma et al. 2014). Given that belowground biodiversity is closely linked to land management, agricultural intensification exerts many pressures that lead to loss of biodiversity.

Consequently, soil pollution is one of the main factors behind the decline of taxonomic and functional biodiversity, and that of agricultural soil sustainability (Harms et al. 2017). Most pesticide emission (99 %) in Europe is associated with agricultural practices, whereas industrial and urban sources such as the manufacturing of pesticides or the at-home use of insecticides have a minor impact (EEA 2016).

The extensive and massive use of pesticides in agricultural activities has a serious impact on the environment, compromising soil and water quality (Pinto et al. 2012; Zhang et al. 2015; Pinto et al. 2016). In addition to pesticides, large quantities of antibiotics are added to agricultural fields worldwide through the application of wastewater, manures and biosolids, also resulting in antibiotic contamination (Jechalke et al. 2014; Zhang et al. 2015; Pan and Chu 2016). A clear correlation between agriculture and water contamination was observed in Mar Chiquita lake (Argentina), where large amounts of endosulfan residues were detected soon after application and post-application periods (Ballesteros et al. 2014). The presence of the fungicide thifluzamide in the water in rice paddies in China was maximal after application, with variation over time associated with the dilution effect of rainfalls in the area (Wei et al. 2015).

Preventive measures are required, to mitigate the impact of agriculture on the environment. These must take into account both the use of safe pesticides and the optimization of farmer procedures. Aravinna et al. (2017) found that most of the 32 studied pesticides leached off rice paddies following specific pathways. Since direct runoff and erosion from soil were the main vehicles of dispersion, authors suggested alternative strategies (high resident time for pesticides, holding ponds for rice drainage water, delayed filling of paddies after pesticide application, and the use of less mobile compounds) to reduce the movement of the pesticides.

The intensive use of organic agrochemicals (OACs) poses risks to both wild lives and human health. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species through air, water and soil (Miller 2004). Around 30% of pesticides marketed in developing countries do not meet internationally accepted quality standards, posing a serious threat to human health and the environment (Popp et al. 2013). They are persistent in the environment and are biomagnified through the food chain, and it has been estimated that millions of agricultural workers worldwide experience unintentional pesticide poisoning each year. The correlation between long-term exposure to pesticides in occupational settings and illness is known, but recently non-occupational exposures have also been associated with an elevated rate of chronic diseases (Parrón et al. 2014).

Varieties and consumption of pesticides worldwide have increased dramatically, by up to 4 times since 40 years ago (Mnif et al. 2011), but reports on overall consumption (2- 4 million tons per year) are not in agreement. According to De et al. (2014), about 45 % are used in Europe, 25 % in the USA, and 25 % in the rest of the world. The main pesticide consumer is Spain (around 79,000 ton of active ingredients sold between 2011 and 2014), followed by France (~ 75,000), Italy (~ 64,000), Germany (~ 46,000) and United Kingdom (~ 23,000) (Eurostat 2016). The United States applies over 1 billion pounds annually (Alavanja 2009) with dramatic consequences for human beings and environment (Carvalho 2017). According to other authors (Huang McBeath and McBeath 2010), China is the world's largest pesticide user, with a pesticide output of around 3.7 million tons (National Bureau of Statistics of China - http://data.stats.gov.cn), and a consumption of about 1.8 million tons in 2014. More than 350 insecticides, herbicides, microbicides, nematicides and other pesticides are reported to be used. The average amount of pesticides used per hectare in China is roughly 1.5- to 4-fold higher than the world average (Qiu 2011), thus resulting in contamination of water bodies in the receiving areas and disturbance of ecological equilibrium (Hui et al. 2003). Overall, use of pesticides in China breaks down as herbicides 47.5 %, insecticides 29.5 %, fungicides 17.5 % and others 5.5 % (De et al. 2014).

The adverse effects of OAC pollution have been of concern for a long time and many highly toxic and persistent pesticides have been banned worldwide. Although relatively safer pesticides have been developed and replaced the highly toxic ones, environmental pollution resulting from the long-term application of pesticides is far from being solved. Obsolete pesticides still represent a threat to environment, biodiversity, and human health for the region of Southeast Europe and their risk to the environment and to humans needs to be assessed in order to mitigate it. Many organochlorines, organophosphates and pyrethroids have been banned but this has not yet solved the problem (Aravinna et al. 2017). In Argentina, hexachlorocyclohexane pesticides have been limited since the late ’90s and were definitely banned in 2011, but samples taken from a saline lake in 2014 showed levels to be more than five times over the legal limit of 4 ng/l for lindane levels in the environment (Ballesteros et al. 2014). Likewise in China, although the use of organichlorine pesticides has been banned for over 20 years, they can still be found in the water and sediments of main drainage areas (Nakata et al. 2005; Xue et al. 2006; Zhou et al. 2006), due to run-off from aged and weathered agricultural soils and from anaerobic sediments (Zhou et al. 2006). Water bodies and sediments, the water, the soil and even the air in many cities in China are polluted by OACs, in both urban and suburban areas (Gong et al. 2004; Nakata et al. 2005; Yang et al. 2008).

OACs pose pivotal environmental problems, due to their high resistence in the environment and the consequent low natural attenuation. As an example, organochlorine pesticides were poorly affected by photochemical, chemical and biological processes, and more than 95% of them impacted on non-target organisms (Mrema et al. 2013). As a consequence, regulatory and risk assessment procedures have to be adopted against OACs. Driven by the carcinogenicity of pesticides, Directive 91/414/ EEC aimed to regulate the authorization of pesticides marketing within the EU.

The particular attention given to pesticides is because, as confirmed in recent studies, even low doses might trigger adverse effects on wildlife and humans (EEA 2005). As groundwater is our primary source of drinking waters, both the Groundwater Directive 2006/118/EC and the Drinking Water Directive 98/83/EC deal with maximum pesticide exposure concentrations: 0.1 µg/l of a single pesticide and 0.5 µg/l total pesticide load. Risk assessment needs to consider not only the source of contamination, but also the multifaceted direct and indirect pathways of contact with human beings. Kim et al. (2017) reported a number of routes pesticides might follow to meet human beings; the resulting direct and indirect multi-pathway exposure may affect human health.

Experimental evidence of progress in natural restoration processes highlight that time is our ally, since the abandonment of disturbed/polluted agricultural land for long time can reduce contamination (Kardol and Wardle 2010). Studies by Morriën et al. (2017) reported that nature restoration on ex-arable land resulted in increased connettance of soil biota networks, as restoration progresses. Such results confirm that soil biota provide many and varied services, and that detoxification of pollutants and xenobiotics is one of the primary ones.

In this context, innovation involves the search for solutions inspired by nature, with the strategy being to accelerate the natural attenuation processes in contaminated sites. Bioremediation has arisen as a useful technology to degrade OACs (Singh 2008; Velázquez-Fernández et al. 2012), with several benefits over landfill disposal and incineration, such as the formation of non-toxic end products, lower costs of disposal, reduction of effects on health and ecology and on the long-term liabilities associated with destructive treatment methods, and the ability to perform the treatment *in situ* without unduly disturbing native ecosystems (Sarkar et al. 2005). Over the past decade, numerous microorganisms capable of degrading antibiotics and pesticides have been isolated, and detoxification processes for target pollutants have been analyzed. Fungi and especially ligninolytic fungi have been suggested as the most promising group of organisms, as they are able to transform recalcitrant compounds through a unique set of extracellular oxidative enzymes (Anastasi et al. 2013; Harms et al. 2017). Comparative genomic analysis of 49 fungi with different nutritional modes, such as saprotrophic fungi, white-rot fungi (WRF), brown-rot fungi, soft rot fungi and symbiotic fungi indicate that there is a relationship between nutrition models and the enzymes for lignocellulose degradation. Saprotrophic fungi have a greater number of enzymes than symbiotic fungi, and brown-rot fungi have a smaller number than WRF and soft rot fungi (Wu et al. 2015a). This might provide some insight into how to choose fungi in OACs degradation.

Finally yet importantly, the metabolic activity of fungal or microbial consortia could potentially produce unknown reaction products that are more toxic than the parent compounds. García-Carmona et al. (2017) highlighted the importance of carrying out environmental monitoring activities ante- and post-operam phases, using bioassays to determine the success of the bioremediation process. Although it is fundamental to assess the quality of the environment to ensure it remains free of toxic residues, most of the analytical tests available for determining the concentration of toxic chemicals do not give the biological impacts of toxicants. For this reason, biotoxicity testing has grown steadily in recent years and is a useful tool in environmental risk assessment (Shen et al. 2016; Prokop et al. 2016).

Indeed, there is a clear need to develop and define decontamination of hazardous pollutants as a concept that will support sustainable remediation by involving a broader uptake of principles, approaches and tools that integrate environmental, social and economical dimensions into remediation processes (Ridsdale and Noble 2016). Several organizations, academia and standardization committees are currently assessing remediation process and evaluating the complexity of the concept of sustainability. Documents have been developed by many countries across Europe and globally, addressing sustainable indicators for remediation activities (Harclerode et al. 2015).

The present review summarizes the current state of scientific knowledge on research and application of fungi as effective bioresources, considering recent advances in understanding their capacity to face up the pesticide contamination.

Bioremediation of OACs by fungi in the soil system

Large quantities of OACs are being added to agricultural fields worldwide through the application of wastewater, manures and biosolids, resulting in pesticide and antibiotic contamination and elevated environmental risks in terrestrial environments (Jechalke et al. 2014; Zhang et al. 2015; Pan and Chu 2016). A large proportion of the OACs applied to soils with manure or biosolids are retained in surface soil, whereas those added through irrigation with wastewater can seep down to lower levels or be diffused in surface run-off. Once present, OACs interact with the solid phase of soil and are prone to microbial transformation (Hammesfahr et al. 2008; Jechalke et al. 2014). In particular, veterinary antibiotics interact with the soil solid phase in sorption and desorption reactions. Sorption and desorption control not only their mobility and uptake by plants but also their biotransformation and biological effects. OACs, like microorganisms are not distributed homogeneously in soil but are concentrated in hotspots. The multiplicity of surfaces, voids, and pores provided by soil aggregates harbor a vast amount of biological diversity and chemical variability, and cause patchy distribution of natural organic matter, oxides, nutrients, and microorganisms on soil particle surfaces (Hammesfahr et al. 2008; Jones et al. 2012). Sorption, sequestration, and subsequent release of OACs likely also occur at and from hotspots. Little is known about the behavior of OACs at environmentally relevant concentrations in agricultural soil.

Recently, many studies have highlighted the ability of fungi to transform and degrade recalcitrant OACs. In particular, one of promising group is the ligninolytic fungi that possess a unique set of extracellular enzymes suitable to degrade lignin and are able to transform recalcitrant compounds (Čvančarová et al. 2015) (Supplemental data Table I; Table I References). Nguyen et al. (2014) reported the removal of diverse trace organic contaminants (Trichloroethyl chloroformate (TrOC) including phenolic and non-phenolic compounds, pharmaceuticals, pesticides, steroid hormones, industrial precursors and products, and phytoestrogens) by live (biosorption + biodegradation), intracellular, enzyme-inhibited and chemically inactivated (biosorption only) whole-cell preparations and the fungal extracellular enzyme extract (predominantly laccases) from *Trametes versicolor* (strain ATCC 7731). They showed how non-phenolic

TrOC were readily biodegraded while the removal of hydrophilic TrOC was negligible. The whole-cell culture showed considerably higher degradation of the major compounds, indicating the importance of biosorption and subsequent degradation by intracellular and/or mycelium associated enzymes. However, there are too few studies that examine both adsorption and degradation of antibiotics in agricultural soil, with most using unrealistically high concentrations (in mg/kg levels) to overcome limitations in measurement. In addition, no model has been developed to estimate the adsorption and degradation of different types of antibiotics in agricultural soil and the environmental risks they may pose. Pan and Chu (2016) evaluated the adsorption and degradation of five antibiotics (tetracycline, sulfamethazine, norfloxacin, erythromycin, and chloramphenicol) by native microorganisms (bacteria and fungi) in non-sterilized (test) and sterilized (control) agricultural soils under aerobic and anaerobic conditions. They showed that all antibiotics were susceptible to microbial degradation under aerobic conditions, and most antibiotics were degraded by more than 92% in non-sterilized soil after 28 days of incubation. For all the antibiotics, a higher initial concentration was found to slow down degradation and prolong persistence in soil. The degradation pathway of antibiotics varied in relation to their physicochemical properties as well as the microbial activities and aeration of the recipient soil. In their (1996) study, Pan and Chu were the first to develop a model for the prediction of antibiotic persistence in soil.

Given the public concern for environmental pollution by OACs, there is increasing attention towards the development of biopurification systems for reducing the risk from point source contamination of soil resources. Various treatment methods (e.g. land filling, recycling, pyrolysis and incineration) have been used for the removal and remediation of these chemicals from the contaminated sites, but microbial degradation of pesticides is so far the most important and effective way to remove these compounds from the environment (Hai et al. 2012; Verma et al. 2014), (Supplemental data Table I; Table I References).

Microorganisms have the ability to interact both chemically and physically with substances, leading to structural changes or to complete degradation of the target molecule. In particular, fungi may transform pesticides and other xenobiotics by introducing minor structural changes to the molecule, producing nontoxic molecules that can be released into the soil for further degradation by microflora (Hai et al. 2012), (Supplemental data Table I; Table I References). Mir-Tutusaus et al. (2014) investigated the degradation of the insecticides imiprothrin and cypermethrin and the insecticide/nematicide carbofuran using the white-rot fungus *T. versicolor*. Experiments with fungal pellets demonstrated extensive degradation of the tested agrochemicals, while *in vivo* studies with inhibitors of cytochrome P450 revealed that this intracellular system plays an important role in the degradation of imiprothrin and carbofuran, but not of cypermethrin. The simultaneous degradation of the compounds successfully took place with minimal inhibition of fungal activity and resulted in reduction of global toxicity, thus supporting the potential use of *T. versicolor* for the treatment of several OACs.

To date, the number of studies investigating novel treatment techniques for the removal of OACs from contaminated agricultural soils is limited. The bacteria-dominated conventional activated sludge process has been proved to be ineffective for OAC removal. While the importance of a mixed microbial community to initiate and complete OAC removal in the soil environment has been convincingly demonstrated by several researchers, studies concerning the removal of OACs from soils have predominantly focused on selected bacterial or fungal species separately. Few studies have explored the bioaugmentation synergy of fungi together with bacteria (Hai et al. 2012; Zhang et al. 2015; Madrigal-Zúñiga et al. 2016). Combining cultures of bacteria and fungi could be key to the removal of toxic and recalcitrant organic substances from contaminated agricultural soils.

On-farm biopurification systems constitute a biotechnological approach to the mitigation of point source contamination by pesticides. The main component of biopurification systems is the biomixture, which acts as the biologically active core that accelerates the degradation of OACs. Madrigal-Zúñiga et al. (2016) studied the results of employing the ligninolytic fungus *T. versicolor* in the bioaugmentation of compost- (GCS) and peat-based (GTS) biomixtures for the removal of the insecticide-nematicide carbofuran (CFN). The transformation products of CFN were detected at the moment of CFN application, but their concentration decreased continuously until complete removal in both biomixtures. Mineralization of ^{14}C radiolabeled CFN was faster in GTS than in GCS. The authors demonstrated the complete elimination of toxicity in the matrices after 48 days. Overall data suggested that the bioaugmentation improved the performance of the GTS rather than the GCS biomixture.

Pinto et al. (2016) also studied the potential use of different substrates in biomixtures like cork, cork and straw, coat pine and LECA (Light

Expanded Clay Aggregates) in the degradation of terbuthylazine, difenoconazole, diflufenican and pendimethalin pesticides. Bioaugmentation using the WRF *Lentinula edodes* inoculated into the CBX was also assessed. The results obtained from this study clearly demonstrated the relevance of using natural biosorbents such as cork residues to increase the capacity for pesticide dissipation in biomixtures for establishing biobeds. Furthermore, greater degradation of all the pesticides was achieved by the use of bioaugmented biomixtures. Indeed, biomixtures inoculated with *L. edodes* EL1 were able to mineralize the selected xenobiotics, revealing that this WRF might be a suitable fungus to be used as inoculum source to improve the degradation efficiency of sustainable on-farm biopurification systems.

Fungi isolated from biomixtures represent a biological source of potentially active bioremediation agents, and the adaptation skills developed by these microorganisms could make the difference in OAC removal (Supplemental data Table I; Table I References). This strategy was assessed by Pinto et al. (2012), who isolated fungi from a loamy sand soil and a biomixture contaminated with terbuthylazine, difenoconazole and pendimethalin. The ability of autochthonous fungi (*Penicillium brevicompactum* and *Lecanicillium saksenae*) to degrade xenobiotics was compared with that of allochthonous strains taken from a culture collection (*Fusarium oxysporum*, *Aspergillus oryzae* and *L. edodes*). The best biodegradation yield was achieved with *P. brevicompactum*: its higher ability to metabolize terbuthylazine was presumably acquired through chronic exposure to contamination with the herbicide.

Bioremediation of OACs by fungi in aquatic ecosystems

Many OACs are common contaminants of fresh water due to their high water solubility associated with a low soil adsorption, and a high stability that assures them a long half-life. Contamination is heterogeneously distributed along watercourses as evidenced in several studies where pesticides were recurringly found in real water samples. In one accurate survey, more than 160 water samples taken in 23 European countries were assayed for the presence of pharmaceuticals, pesticides and recognised endocrine-disrupting chemicals (Loos et al. 2010). Among the most frequently detected compounds were the insecticide (DEET), and 7 other pesticides (chloridazon-desphenyl, DMS, desethylatrazine, chloridazon-methyldesphenyl, bentazone, desethylterbutylazine, dichlorprop) exceeded the European threshold of 0.1 µg/l. Overall, 29% of the water samples could not be considered safe (Loos et al. 2010). In a similar study in the USA, groundwater in 18 states was screened for 65 organic contaminants: along with plasticizers and detergent metabolites, 66% of the total pollutant load was ascribable to insect repellant (Barnes et al. 2008).

The extent of freshwater contamination and the actual risk to human life depend on several factors concerning the hydrogeological characteristics of the soil, weather conditions and the chemical-physical properties of the OACs. The environmental fate of a given compound is a critical issue in which the water/soil surface is the first barrier. For instance, the sorption kinetics of three widely used pesticides (simazine, imidacloprid, and boscalid) were found to be correlated with soil organic carbon content and the hydrophobicity of the pesticide, which ultimately affected soil retention behavior and bioavailability in waters (Salvestrini et al. 2014). Leaching into surface waters is also a matter of season, and a complex and unpredictable scenario is influenced by a variety of phenomena. A rainy period can cause massive run-off of OACs from the soil, contaminating the receiving basin (Sandin et al. 2018). The detection of high levels of OACs, however, is not exclusively coincident to their recent and massive use, but is ascribable to their persistency, their slow natural degradation and their accumulation in the various diffusion pathways (Aguilar et al. 2017). They could then travel long distances in surface or groundwaters and the contamination can last for several decades (Ballesteros et al. 2014; Aravinna et al. 2017).

The so-called ecological services may help to contain the diffusion of OACs. The adaptation of microflora (fungi, Gram-positive and negative bacteria, actinobacteria, and sulfate-reducing bacteria) to soil environmental conditions may attenuate the pesticides released into groundwater sources (Mattsson et al. 2015). Several factors such as soil composition, temperature, aeration due to soil weaving, and depth influence autochthonous microbial community activity; if this balance fails, OACs are free to move among different ecological niches (i.e. sediments and water), alter their functioning and ultimately directly affect their animal inhabitants. For instance, significant ecological risk was associated with the presence of the insecticide fipronil and its metabolites in water ponds: the lowest concentrations measured (up to 200 ng/l) affected the proper development of larval insects and crustaceans (Wu et al. 2015b). Evidence of the pesticide’s toxicity against fish has already been reported, and it clearly interferes in several metabolic pathways (Odukkathil and Vasudevan 2013; Ballesteros et al. 2014; Guerreño et al. 2016).

The preservation of water quality is a priority, but OAC removal cannot be based only on natural attenuation. Water treatment plants (WTPs) are the major barriers where OACs should be removed. Not being specifically designed for micropollutant removal, however, they are often only partially effective, with a strong impact on the receiving ecosystem. Pesticides such as atrazine, fluconazole, tebuconazole, diazinon and diuron are particularly resistant to commonly used treatments (Köck-Schulmeyer et al. 2013; Luo et al. 2014). There is plenty of evidence confirming the presence of OACs in WTP effluents at toxicologically and estrogenically relevant concentration, making them one of the most impactful sources of contamination (Bicchi et al. 2009; Campo et al. 2013; Jarošová et al. 2014).

Particular attention has been given to advanced biological oxidation. Novel cost-effective and eco-friendly processes based on fungi are an attractive option. Fungi are well-known for their physiological adaption skills, including the natural activation of tolerance mechanisms against pesticides (Talk et al. 2016). Some reports have already demonstrated that in comparison with bacteria, fungi can better tolerate the presence of organic contaminants. Although the insecticide endosulfan inhibited both fungi and bacteria, bacterial community structure significantly changed at concentrations as low as 0.1 mg/kg, while modifications to fungal community structures required 1 mg/kg of pollutant (Zhang et al. 2015). Linuron reduced the bacterial count, and especially total bacteria, N_2-fixing bacteria and nitrifiers, but not fungal numbers (Cycoń et al. 2010).

The provenance of isolated fungi is of unquestionable importance. Strains isolated from contaminated niches indeed seem to develop specific adaptation skills due to chronic exposure. Carles et al. (2017) demonstrated that the aquatic microflora found in association with submerged leaves exposed to nicosulfuron is more efficient in its degradation than are communities that come from a less polluted site. The authors indicated fungi as the main constituents of this active microflora and as being responsible for herbicide degradation. In the literature, several fungi isolated from contaminated areas or WTPs have been identified as degraders of nicosulfuron, diuron, isoproturon, glyphosate, chlorpyrifos, chlorfenvinphos and atrazine (Song et al. 2013; Carranza et al. 2014; Oliveira et al. 2015).

Fungi can thus transform a broad range of recalcitrant organic compounds, including OACs (Gao et al., 2010). A number of fungi that are OAC degraders, mostly belonging to Basydiomycetes, such as *Trametes*, *Pleurotus, Phlebia, Cerrena, Coriolopsis,* etc., have been already investigated (Koroleva et al. 2002; Marco-Urrea et al. 2009; Xiao et al. 2011; Ulčnik et al. 2013; Chan-Cupul et al. 2014; Ceci et al. 2015). Several pesticides as lindane, atrazine, diuron, terbuthylazine, metalaxyl, DDT, gamma-hexachlorocyclohexane (g-HCH), dieldrin, aldrin, heptachlor, chlordane, lindane, mirex, etc. were effectively transformed by fungal treatment based on mycelium or enzymes (Table 2, Supplementary Materials).

A bioremediation approach based on fungi may involve both biosorption and biodegradation processes; the latter combines biosorption, where the molecule binds to the fungal wall, and bioaccumulation with the pollutant being transported inside the cell in contact with intracellular enzymes (Kulshreshtha et al. 2014). Concentrations of the insecticide lindane decreased during time in the presence of two WRFs (*T. versicolor* and *Pleurotus ostreatus*) and one brown-rot fungus (*Gloeophyllum trabeum*), but the lack of any change in the chromatogram profile indicated that a fast adsorption process was mainly involved (Ulčnik et al. 2013). However, this phenomenon is often strain-dependent, and expecially related to metabolic differences between Ascomycetes and Basidiomycetes. Belonging to the brown-rot fungi, *G. trabeum* lacks the ligninolytic enzymes, responsible for lignin degradation and likely for that of OACs as well: adsorption onto fungal mycelium was mainly involved in the removal of endosulfan. On the contrary, the white-rot fungi actively degraded, producing endosulfan sulphate via oxidative pathways (Ulčnik et al. 2013). Although biosorption is a phenomenon that cannot not ignored, it is often secondary or at least negligible compared to biodegradation (Carles et al. 2017). For instance, the removal of clofibric acid found for heat-killed mycelium was less than 10 %, but more than 97 % for active *T. versicolor* (Marco-Urrea et al. 2009).

Fungi have developed a specific mechanism that employs few enzymes and molecules with high oxidizing power, physiologically aimed at transforming lignocellulose structures. The same enzymatic pathway may play a pivotal role in transforming other aromatic molecules. White-rot fungi usually deploy extracellular lignocellulosic enzymes such as peroxidases (EC 1.11.1.x) and laccases (EC 1.10.3.2). The involvement of redox enzymes in fungal-mediated oxidation is confirmed by the direct induction of enzyme production in the presence of OACs. The fungus *T. versicolor* responded to 17 pesticides by increasing laccase production in comparison with the control: particular attention was given to the transformation products of the herbicides diquat and monuron, capable of increasing fungal activity 10- and 17-fold, respectively (Mougin et al. 2002). The laccase production of *Pycnoporus sanguineus*, *Trametes maxima*, *Pleurotus* spp1, *Pleurotus* spp2, *Cymatoderma elegans*, and *Daedalea elegans* was stimulated by the presence of atrazine even at high concentrations of 3750 mg/l. Likewise, the manganese peroxidase activity of *Pleurotus* spp1 and *C. elegans* was positively correlated with the pesticide (Chan-Cupul et al. 2014). Oxidoreductase stimulation was also observed with picloram (Maciel et al. 2013), bentazon (Da Silva Coelho et al. 2010) and carbofuran (Mir-Tutusaus et al. 2014).

Although these oxidoreductases are probably the most-known enzymes for aromatic compound degradation, alternative pathways can be stimulated by the presence of OACs. Two clones (laccase-positive and laccase-negative) of *Mycelia sterilia* were used to treat atrazine (20 µg/ml): even though one clone was defective in laccase production, comparable transformation yields (70-80%) were reached, indicating that the fungus can deploy alternatives to laccase in the degradation process (Vasil'Chenko et al. 2002). This behavior is commonly found in brown-rot fungi, which can trigger both nonenzymatic and enzymatic mechanisms, i.e. the Fenton mechanism or cellobiose dehydrogenase (CDH) reactions (Fan and Song 2014). The degradation of atrazine (20 µg/l) by an unidentified mycelial fungus was associated with the presence in the liquid medium of OH radicals and CDH. Moreover, CDH secretion was induced by the presence of the herbicide itself (Khromonygina et al. 2004). In addition, some fungi may associate extracellular oxidoreductases with intracellular enzymes such as the cytochrome P450 system (cyt450). In an effort to better characterize the degradation skills of *T. versicolor*, cyt450 inhibitors were used: fungal performance against clofibric acid and fipronil decreased (Marco-Urrea et al. 2009; Wolfand et al. 2016). Mori et al. (2017), suggest that in *Phanerochaete sordida*, cyt450 is involved in the initial stage of reduction of the clothianidin N-nitro group, but that the enzymes responsible of the further urea derivatives production are unknown.

Fungal intra- and interspecies variability has long been recognized and has found confirmation in OAC treatment. Literature data about a given species cannot be taken for granted and preliminary screening is often required. Despite *Phanerochaete chrysosporium* often being indicated as the fungal model for organic degradation including pesticides (Wang et al. 2014), it was almost ineffective against clofibric acid (Marco-Urrea et al. 2009). Among five Basidiomycetes, only *T. versicolor* extensively degraded this herbicide (Marco-Urrea et al. 2009). Alvarenga et al. (2014) treated methyl parathion with several fungi, including 3 *Aspergillus sydowii*. Based on ability to grow in the presence of the pesticide, only the isolate *A. sydowii* CBMAI 935 was selected for further studies. It indeed grew almost 4 times more than the other *A. sydowii*. Bioremediation potential is often substrate-targeted, and the choice of fungus cannot be taken for granted. For instance, *A. sydowii* CBMAI 935, which totally converted methyl parathion (Alvarenga et al. 2014) was not the best performing one against the insecticide esfenvalerate. Among 6 fungi, *Microsphaeropsis* sp. *Acremonium* sp. and *Westerdykella* sp. gave better results than the *Aspergillus* strain (Birolli et al. 2016).

Although the majority of these strains are effective in OAC removal in model solutions, only few researchers have taken the next step, and assessed bioremediation potential in contaminated waters. The experimentation with model solutions (single-compound solutions, high concentrations, no interfering molecules, etc.) is the only way to acquire information about degradation pathways (Masaphy et al. 1993; Birolli et al. 2016), but it is less predictive of fungal performance in real environmental water samples. Each type of wastewater has its own critical issues, making it difficult to predict fungal behavior. Some data highlight the robustness of fungal systems, although detailed case-by-case investigation is needed. A partially diluted leachate was shown to disturb the growth of *T. versicolor* and *Stereum hirsutum*, but this did not prevent them totally degrading linuron and dimethoate at 10 mg/l. As regards dimethoate, the presence of adsorbents enhances final yields from 50% to 97%, because the adsorption action combines with and exalts fungal biodegradation processes (Castellana and Loffredo 2014). The immobilization of *Bjerkandera adusta* and *Irpex lacteus* on coffee grounds, almond shells and a biochar favored the removal of the non-phenolic herbicides fenuron and carbaryl from a municipal landfill leachate (Loffredo et al. 2016).

Surface waters, ground waters and municipal wastewaters represent a very unique environment, characterized by extreme chemical and physical conditions, the presence of a heterogeneous and variable mixture of micropollutants and an active autochthonous microflora. When inoculated into real surface water, a fungal consortium (*Aspergillus fumigatus, Aspergillus terreus, Cladosporium tenuissimum, Cladosporium cladosporioides, Fusarium begoniae, Penicillium citrinum, Penicillium melanoconidium* and *Phoma glomerata*) was not stable over time, probably due to the presence of toxic pesticides and interaction with the natural microbial population: *P. citrinum, A. fumigatus* and *A. terreus* were the most robust to the environmental conditions and were found to degrade the spiked chlorfenvinphos (Oliveira et al. 2015).

The set-up of active microbial consortia offers the intriguing possibility of strengthening and combining the bioremediation potential of different organisms: the combination of *Bacillus subtilis* and *A. niger* led to higher degradation rates of nicosulfuron than those obtained by using each strain singly (Lu et al. 2012). The biodegradation of aldicarb, atrazine and alachlor by *Coriolus versicolor* was strongly enhanced by combination with activated sludge. Along with modifications in fungal morphology, when the bacterial-fungal consortium was established, the bio-absorbed fraction of especially atrazine was reduced: over 98% of atrazine was removed by degradation processes in two weeks (Hai et al. 2012).

The fate of the treated OACs must be carefully considered. Residual toxicity is a critical issue. Interestingly fenuron and carbaryl degradation (up to 70%) catalyzed by *B. adusta* and *I. lacteus* led to significant abatement of the phytotoxicity (rapeseed and flax tests) (Loffredo et al. 2016). Mori et al. (2017) monitored the neurotoxicity of clothianidin and the main metabolite it produced during *P. sordida* treatment: following treatment the insecticide still altered the viability of the neuronal cell line, but the metabolite was no longer neurotoxic.

Despite their well-demonstrated properties, the application of whole cell systems has some drawbacks including the fact that a living organism needs controlled growing conditions in terms of nutrients, pH, O_2, etc. (Majeau et al. 2010). The addition of synthetic nutrients can strengthen fungal mycelium activity, but it should be carefully balanced to allow subsequent scale-up of the process. The fact that *T. versicolor* needed 1% of glucose as carbon source to degrade atrazine would ultimately interfere with its potential use in real WTPs (Khromonygina et al. 2004). Likewise several fungi such as *A. niger* and *Dacryopinax elegans*, etc. required both easily available carbon and nitrogen sources to efficiently act against nicosulfuron and diuron, respectively (Lu et al. 2012; Arakaki et al. 2013). Particular attention should be instead given to those fungi, like *A. sydowii* and *Penicillium decaturense*, that maintained the same performance without glucose addition, indicating potential for using methyl parathion or triclosan as sole carbon source (Alvarenga et al. 2014; Tian et al. 2016).

A promising alternative is offered by the direct use of fungal enzymes, capable of catalyzing strong, rapid oxidation reactions, with less technical drawbacks in comparison with fungal cultures. The potential of enzymes-based methods has been worldwide recognized; the Swiss Industrial Biocatalysis Consortium defined oxidative enzymes as the biocatalysts displaying the highest development potential for the next decades (Meyer and Munch 2005). Great importance is given to the discovery of novel enzymes with wide substrate specificity, stable and applicable to industrial uses. A number of articles have reported the ability of fungal enzymes to degrade OACs. The potential of laccase-mediator systems has been assessed for the degradation of isoproturon (Margot et al. 2015), imiprothrin (Mir-Tutusaus et al. 2014), chloroxuron (Palvannan et al. 2014), isoproturon (Zeng et al. 2017), atrazine (Chan-Cupul et al. 2016). Laccases cannot be considered a novelty, unlike a phytase of *A. niger* capable of degrading organophosphorus pesticides (Shah et al. 2017) or a cellulase of *Trichoderma longibrachiatum* active against dicofol (Wang et al. 2015). Particular attention should be given to the use of crude enzyme extracts of ligninolytic enzymes with a lower economic impact on the process than that of purified enzymes (Matute et al. 2012; Kaur et al. 2016). A crude extract of *Trametes pubescens* laccases degraded up to 19 compounds in a model solution and confirmed its potential in a study on real municipal wastewater where the presence of suspended particles, colloids, solvents and xenobiotics as well as autochthonous microorganisms posed strong environmental pressure. The transformation of all the detected compounds determined also a strong reduction of the estrogenicity of the water sample (Spina et al. 2015).

Application of synthetic microbial communities in bioremediation

Bioremediation is a crucial way to eliminate OAC pollution in agricultural ecosystems. However, many factors affect the efficiency of bioremediation in pesticide pollution, such as the microbes applied, treatment sites, rhizosphere effects and soil chemical and physical properties (Zhou and Hua 2004). Bioremediation of soil or water pollution often cannot reach expected results in practice because the target contaminant cannot be degraded completely, and sometimes intermediate products occur that are more toxic than the original pesticides. Long-term application of various pesticides results in pollution with more than one type of chemical compound, which are unlikely to be degraded by a sole microbe. Thus, attention has shifted to synthetic systems based on communication between cells, rather than on individual isolated cell functionality (Biliouris et al. 2012). A promising way to overcome the difficulties is to create artificial synthetic microbial communities that contain several microbes to retain the key features of their natural counterparts (Großkopf and Soyer 2014).

The so-called *synthetic microbial community* is created by a bottom-up approach where two or more defined microbial populations are put together in a well-characterized and controlled environment (De Roy et al. 2014). In synthetic communities, mixed populations can perform complex tasks, although in changing environmental conditions (Brenner et al. 2008). Synthetic communities have several potential advantages over monocultures or natural communityies: 1) the species in a synthetic community are known and the community structure is relatively simple and controllable, while the natural community may contain many microorganisms with unknown functions; 2) synthetic communities can perform more complicated functions than individual organisms because members of microbial consortia communicate and differentiate (Brenner et al. 2008); 3) synthetic communities are often more robust to environmental fluctuations because they can resist invasion by other species and weather periods of nutrient limitation better than monocultures (Brenner et al. 2008); 4) synthetic communities can be described through mathematical models more easily than natural systems, and they can be used to develop and validate models of more complex systems (Liu et al. 2017).

Liu et al. (2017) proposed three design principles to develop a cooperative, steady-state community that is performing a desirable biotechnological function. Firstly, safety should be prioritized by beginning with innocuous or commensal organisms (Brenner et al. 2008). Secondly, the community can convert low-cost and/or recalcitrant waste material into a biotechnologically relevant product, partially or de-novo biosynthesize a compound via heterologous metabolic pathways (Jagmann and Philipp 2014). Thirdly, the bioremediation process should be optimized and regularly monitored on the basis of the knowledge of stability and division of different microorganisms (Liu et al. 2017).

Bioremediation of polluted soils and water is one field of application synthetic microbial communities. Due to the complex structure of some pollutants, such as the diuron pesticides, adding synthetic microbial communities is much more effective than adding single microorganisms. The herbicide diuron is used in the control of broad-leaved weeds on agricultural land. Several fungal-bacterial consortia were investigated by combining three different diuron-degrading bacteria and two fungal strains. The fastest mineralization of diuron was obtained by the three-member consortium (*Mortierella* LEJ702, *Variovorax* SRS16, and *Arthrobacter globiformis* D47). As measured by evolved $14CO_2$ it mineralized about 32 % of the added diuron within 54 days, whereas the single strains or other consortia achieved no more than 10% mineralization. In addition, the production of diuron metabolites by the consortium was minimal. This may be due to cooperative catabolism, where the first organism transforms the pollutant to products that are then used by the other organisms. In addition, fungal hyphae may function as transport vectors for bacteria, thereby facilitating the more effective spreading of degrader organisms in the soil (Ellegaard-Jensen et al. 2014).

Similarly, a fungal-bacterial consortium consisting of Mortierella sp. LEJ702 and the 2,6-dichlorobenzamide (BAM)-degrading Aminobacter sp. MSH1 achieved more rapid mineralisation of BAM than did the bacteria alone, especially at lower moisture contents (Knudsen et al. 2013). Methylotrophic and hydrocarbon-utilizing yeasts and bacteria alone did not degrade PCBs significantly, but PCB degradation reached about 50% when WRFs were applied together (Šašek et al. 1993).

Evaluation of bioremediation effectiveness in contaminated matrices by means of ecotoxicological and genotoxic tests

In order to improve the effectiveness and performance of bioremediation processes it is important to pursue three essential goals at the same time. Focus should be not only on reducing chemical concentrations, but also on reducing chemical mobility between the environmental compartments and eventually lowering toxicity levels while ensuring that contaminants do not get into the natural biological cycle (Loehr and Webster 1997; Chakraborty et al. 2013).

Bioremediation is often monitored by following the concentration of targeted contaminants (Molina-Barahona et al. 2005). Numerous studies in recent years have shown that traditional chemical analyses are insufficient for a full assessment of the contaminated site because, for example, they do not provide any information about the interactions between chemicals and they do not consider the partition and the mobility of pollutants (Frische 2003; Molina-Barahona et al. 2005; Ma et al. 2005; Molnár et al. 2007). An integrated approach that links the various fields and levels of study involving contaminated sites has proven to be an efficient way to evaluate the effectiveness of bioremediation in contaminated sites (Chapman and Anderson 2005; Wernersson et al. 2015; Marziali et al. 2017). Consequently, to achieve the desired goals and implement a successful bioremediation program, given the chemical and biological complexity of the tasks involved, close collaboration between microbiologists, chemists and engineers is required (Van Gestel et al. 2001; Chakraborty et al. 2013).

Additionally, the use of ecotoxicological and genotoxic tests to evaluate the effectiveness of bioremediation may be a valid tool to partially overcome the existing gap between the reported successes of bioremediation on the laboratory scale, and that in the field.

Signals that bioremediation is going on should be monitored. Two important chemical compounds produced by microorganisms during their degradation activity are CO_2 and soluble phosphorus. Both increase notably in soil treated with insecticides and inoculated with fungi (Boyle 1995; Abd El-Ghany and Masmali 2016). However, it must be taken into consideration that during and after a bioremediation process the disappearance of the parent compounds or evidence of metabolic activity (e.g. CO_2 production) may not indicate detoxification. Although the fate of the toxicants may be followed by chemical analyses, many reaction products resulting from the bioremediation process and their potential toxicity are not known. The elimination of mother compounds does not necessarily result in toxicity removal, and evaluating the efficiency of the process is important to assess not only the removal of a specific compound, but also potential ecotoxicity. In fact, biodegradation of pesticides can proceed partially or totally due to the structure of the molecule itself or to unfavourable environmental or test conditions, or to the lack of 'acclimatized' microbial communities (De Henau 1997).

In some instances, it has been shown that an effective process of bioremediation corresponds with a decrease in the toxicity of the analysed matrix (Baud-Grasset et al. 1993; Dorn and Salanitro 2000). To acquire complete and useful information in an ecotoxicological assessment and to determine the effectiveness of bioremediation treatments, it is suggested that a battery of tests be used (Keddy et al. 1995; Van Gestel et al. 2001; Tigini et al. 2011). The battery should include a number of biological reference organisms that are representative of the different trophic levels, in order to select species with different roles in ecosystems, and different exposure conditions (Van Straalen and Van Gestel 1997). Moreover, environmental risk assessment must integrate chemical characterization, ecotoxicity and bioremediation data, in order to accurately assess the ecological hazard.

As emphasized by Shen et al. (2016), an increased level of ecotoxicity within the various bioindicators could either indicate incomplete decomposition of the substance or could result from the formation of intermediate products generated via the bioremediation process. For this reason, chronic tests are sometimes more appropriate in evaluating the toxicity caused by by-products (Lofrano et al. 2014).

In certain circumstances, there is a clear need to monitor the bioremediation process using different bioindicators. In Lizano-Fallas et al. (2017), for example, the ecotoxicity test with *Daphnia magna* showed clear detoxification, whilst the detoxification patterns remain unclear when applying the phytotoxicity test. Ecotoxicological tests can also be used to determine the most suitable bioremediation technique in a given case, as reported in Dudášová et al. (2016).

Without worldwide-recognized guidelines for water quality assessment, literature data are difficult to compare due to the variety of model organisms, end-points, etc. Synthetic indices summarizing the findings can help monitor the effectiveness of biological treatment. Such indices have already been applied for toxicity monitoring of wastewaters (Tigini et al. 2011) but municipal effluents containing AOCs have never been taken into consideration nor has estrogenic activity been included so far.

Several toxicity assays were included in treatability study protocol to measure remediation efficiency. Assessing the toxicity of complex matrixes such as soil could acquire methods from bioassays used to the test toxicity of chemical compounds, reported by the Organization for Economic Co-operation and Development (e.g. OECD 201 2006; OECD 211 2012). The OECD has published a series of standardized tests for determining the biodegradability of a given compound, based on the evaluation of overall parameters (such as COD, TOC and BOD) or metabolic tests, e.g. respirometry (OECD 209 1984) as Polo et al. (2011) used; or that reveal susceptibility of toxic compounds, comprising that of herbicides, to biological treatment._Standardized testing procedures using different organisms have been approved by various environmental organizations, including the US Environmental Protection Agency, American Society for Testing and Materials, International Standardization Organization (Siciliano et al. 2015). Many scientists have explored the effects of polluted soil on the whole organism using various microorganisms, animals, and plants, or by means of cellular, and biochemical biomarkers, or by ecological scale up systems. Here below, tests at some different biological hierarchical levels of analysis are presented and discussed.

Organismal level

Concerning complex matrices such as soil, quality assessments are performed with organisms on extracts of the polluted matrix, generally applying short-term exposure periods (Van Gestel et al. 2001). Experimental models have included aquatic organisms such as *Daphnia magna*, *Raphidocelis subcapitata*, *Danio reri*o, *Myriophyllum aquaticum* and *Lemna minor* (Feiler et al. 2004). The use of freshwater and marine biota may be particularly useful in order to provide a more complete comprehension of the fate of pesticides and the environmental outcomes of agricultural activities (Guida et al. 2008). Terrestrial animals such as nematodes (*Caenorhabditis elegans*) (Traunspurger et al. 1997), oligochaetes (*Lumbriculus variegatus*) (Phipps et al. 1993), springtails such as *Folsomia candida* (Houx et al. 1996), and fish embryos (Hollert et al. 2003; Zielke et al. 2011) are considered amongst the most reliable models.

Amongst the higher plants, important experimental models include *Lepidium sativum*, *Cucumis sativus*, and *Sorghum saccharatum* (germination rate, inhibition of root elongation). Since assays based on animals, plants and algae are considered expensive, time consuming and require large sample volumes, recent studies have emphasized the benefits of rapid, reproducible and cost effective bacterial assays for toxicity screening and assessment. *Arthrobacter globiformi* (Neumann-Hensel and Melbye 2006), *Bacillus cereus* (Rönnpagel et al. 1995; Prokop et al. 2016), *Vibrio proteolyticus* (Ahlf and Heise 2005) and yeasts (*Saccharomyces cerevisiae*) (Weber et al. 2006) are often used. Among the bacterial bioassays, the *Vibrio fischeri* luminescence inhibition test is the most common. The review of Parvez et al. (2006) remarks that the *Vibrio fischeri* inhibition test is the most sensitive, cost effective, easy to operate and requires only 5–30 min for toxicity prediction.

Cellular and biomolecular level

Biomarkers signal the adaptative responses of organisms to xenobiotic exposure. Various studies have highlighted the cytoxic and genotoxic effects on organisms of OACs and their metabolic products. The exposed organisms may exhibit histological, cellular, molecular, biochemical and/or physiological, or even behavioural changes (Depledge et al. 1993) that enable information to be obtained on the biological effects of pollutants or their remains during or after a bioremediation process (Fontanetti et al. 2011).

Genetic endpoints and biomarkers. The most-used biomarkers are mitotic index, chromosome aberrations, micronuclei, sister chromatid exchanges and mutations.

Bacteria have been recommended for bioassays to evaluate genotoxicity in a vaiety of samples (Mortelmans and Zeiger 2000; White and Claxton 2004). The Ames test, one of the most famous and widely-used, is a short term bacterial reverse mutation assay especially designed to evaluate the mutagenic potential of a wide range of chemical substances (Mortelmans and Zeiger 2000). It was found to be very sensitive in tests with a wide range of mutagenic and carcinogenic chemicals, as reported in the review paper of Chahal et al. (2014).

With regards to plant models, higher plants are recognized as excellent genetic models to detect cytogenetic and mutagenic agents and are frequently used in environmental monitoring studies. The main organisms employed are *Allium cepa*, *Vicia faba* and *Tradescantia* spp. as reported in a review by De Souza et al. (2016). Their protocols were standardized // under the International Program on Plant Bioassays (IPPB) conducted by the United Nations Environment Programme (UNEP) (Ma 1999). In addition, the US Environmental Protection Agency (USEPA) and the World Health Organization (WHO) validated // plant bioindicators as an efficient model to detect environmental genotoxicity.

One of the most used higher plant models is *V. faba*. The main advantages are its year-round availability, that it is economical to use, and easy to grow and handle. Its use does not require sterile conditions and rate of cell division is fast. The *V. faba* test, meticulously reported and discussed in the review of Iqbal (2016), enables the assessment of a variety of endpoints i.e., chromosomal aberration, mitotic index, micronuclei and nuclear aberration.

Enzymatic biomarkers. Enzyme activity inhibition has been widely evaluated as a biomarker to measure the toxicity of a matrix. Dehydrogenases, for example, are directly involved in many of the vital anabolic and catabolic processes of living organisms, and their activity is inhibited by chemical toxicants. Recently, many studies have reported the use of terrestrial organisms to obtain enzymatic biomarkers in response to residual pesticides (Henson-Ramsey et al. 2011; Radwan and Mohamed 2013; Stepić et al. 2013), and among these, earthworms' enzymes were widely used to understand the impacts of pesticides. In two earthworm species, *Eisenia fetida* and *Lumbricus terrestris*, multiple esterases, including acetylcholinesterase (AChE), butyrylcholinesterase, and carboxylesterase (CE), were assessed as biomarkers for malathion exposure (Henson-Ramsey et al. 2011). Several studies have also reported AChE, catalase (CAT), and glutathione-S-transferase as biochemical biomarkers in *Eisenia andrei* for the insecticides endosulfan, temephos, malathion, and pirimiphos-methyl (Stepić et al. 2013), and AChE, CAT, CE, and the efflux pump as biomarkers in *E. andrei* and *Octolasion lacteum* for dimethoa. Recently, surface-enhanced laser desorption/ionization-time-of-flight (SELDI-TOF) mass spectrometry (MS) has strongly contributed to the identification of more accurate, precise biomarkers, e.g. specific for human cancers (Silsirivanit et al. 2014), or for endosulfan exposure in Japanese rice fish (*Oryzias latipes*) (Lee et al. 2013). In a recent paper, selective protein biomarkers for 6 pesticides (captan, carbaryl, carbofuran, and α-endosulfan chlorpyrifos, propoxur) were found in *E. fetida*, by means of SELDI-TOF MS technology (Park et al. 2015).

Estrogen and androgen biomarkers. It is well-documented that several chemicals from agricultural, industrial, and household sources possess endocrine-disrupting properties, which provide a potential threat to human and wildlife reproduction (Colborn et al. 1993; Colborn 1995; Jensen et al. 1995). A suggested mechanism is that environmental contaminants alter the normal functioning of the endocrine and reproductive system by mimicking or inhibiting the action of endogenous hormones, by modulating the production of endogenous hormones, or by altering hormone receptor populations (Sonnenschein and Soto 1998). Several pesticides exert estrogenic and antiandrogenic activities through interaction with estrogen and androgen receptors. The risks associated with OAC exposure has been known for decades: many pesticides, such as p,p´-dichlorodiphenyl trichloroethane (DDT) (Welch et al. 1969), methoxychlor (Bulger et al. 1978; Cummings 1997), β-benzene hexachloride (BHC) (Coosen and van Velsen 1989), endosulfan, toxaphene, and dieldrin (Soto et al. 1995), and fenvalerate (Garey and Wolff 1998) were the first to be signaled as estrogenic. Despite increased institutional awareness and more compelling legislation pressure, the most recent literature still reports the occurrence of pesticides in watercourses and in the trophic chains, that show conspicuous estrogen or androgen levels (Saillenfait et al. 2016; Brander et al. 2016; Guo et al. 2017; Khalil et al. 2017; Scott et al. 2017; Miccoli et al. 2017; Marcoccia et al. 2017). Several bioassays have been developed and standardized in order to describe the estrogenic potency of OACs. Andersen et al. (2002) indicated that several currently used OACs, such as methiocarb, fenarimol, chlorpyrifos, deltamethrin, and tolclofos-methyl, possess estrogenic activity on the basis of cell proliferation assays and transactivation assays using MCF-7 human breast cancer cells. Kojima et al. (2004) tested 200 pesticides in vitro for agonism and antagonism to two human estrogen receptor (hER) subtypes, hERα and hERβ, and a human androgen receptor (hAR) by means of highly sensitive transactivation assays, using Chinese hamster ovary cells. The results demonstrated that many pesticides possess in vitro estrogenic and antiandrogenic action through ERs and/or AR. Although it appears that various pesticides exert hormonal effects at concentrations that are orders of magnitude higher than that required for physiologic hormones, wide exposure to large numbers of OACs may have additive and synergistic effects. Bioassay with YES (yeast estrogen screen) and YAS (yeast androgen screen) can determine hormonally active compounds still present in the environment. Since the the first papers on this subject (Purvis et al. 1991), much more sophisticated bioassays have been developed, such as that proposed by Eldridge et al. (2007) in which a bioluminescent strain of *Saccharomyces cerevisiae* was genetically engineered to respond to androgenic chemicals.

Ecological level

The risk to natural systems of pollution with the chemical residues of bioremediation processes is underestimated. The ecological scaling-up experiment illustrated by Rodea-Palomares et al. (2016) underlined how real-world exposure to chemical pollution is often dominated by low-dose complex mixtures combined with other biotic and abiotic stressors. In the paper, a novel screening method (GSA-QHTS) was reported, that coupled the computational power of global sensitivity analysis (GSA) with the experimental efficiency of quantitative high-throughput screening (QHTS). In the study, they reported that GSA-QHTS allowed for the identification of the main pharmaceutical pollutants that were driving the biological effects of low-dose complex mixtures at the microbial population level. The target complex community was a river benthic microbial community inoculum obtained from an unpolluted stream. The effects of the toxic compounds in the mixture was evaluated together with other physico-chemical stressors, on a series of community-level metabolic end points. Photosynthetic parameters, the dark-adapted basal fluorescence, the light-adapted steady-state fluorescence, the maximum photosynthetic efficiency, as well as the extracellular enzymatic activities b-Glu and Phos were considered as both autotrophic and heterotrophic global fitness indicators suited to study the effects of chemical pollution on freshwater benthic microbial communities.

Prospect

Bioremediation is based on the idea that different organisms will work together to remove (biodegrade) the waste substances or pollutants (OACs) from the environment. Although there exist limitations to bioremediation practice, including the nature of organisms, the enzyme involved, the concentration and availability and final survival of microorganisms, as well as the cost/benefit ratio (i.e. cost versus overall environmental impact), these limitations can be solved to some extent by understanding the genetics and biochemistry of the desired microbe. The advent of synthetic communities has shown enormous potential to facilitate the bioremediation process, the degradative fungi appearing to be particularly effective.

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Fungi as a toolbox for a sustainable bioremediation of pesticides in soil and water.

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Supplementary Material 1

Fungal species list for biodegradation of pesticide pollutants

Table I. Fungal species list for biodegradation of pesticide pollutants

Pesticide types	**target pesticide**	**Fungal species**	**Fungal role**	**Origin**	**Literature**
organochlorine	aldrin	*Phanerochete chrysosporium*	white-rot		Kennedy et al 1990
	chlordane	*Phanerochete chrysosporium*	white-rot		Kennedy et al 1990
	DDT	*Phanerochete chrysosporium*	white-rot		Arisoy 1998
	DDT	*Pleurotus sajor-caju*	white-rot		Arisoy 1998
	DDT	*Pleurotus florida*	white-rot		Arisoy 1998
	DDT	*Pleurotus eryngi*	white-rot		Arisoy 1998
	DDT	*Gloeophyllum trabeum*	brown-rot		Purnomo et al 2008
	DDT	*Gloeophyllum sepiarium*	brown-rot		Purnomo et al 2008
	DDT	*Gloeophyllum ungulatum*	brown-rot		Purnomo et al 2008
	DDT	*Gloeophyllum striatum*	brown-rot		Purnomo et al 2008
	DDT	*Daedalea malicola*	brown-rot		Purnomo et al 2008
	DDT	*Daedalea albida*	brown-rot		Purnomo et al 2008
	DDT	*Daedalea serialis*	brown-rot		Purnomo et al 2008
	DDT	*Daedalea dickinsii*	brown-rot		Purnomo et al 2008
	DDT	*Fomitopsis palustris*	brown-rot		Purnomo et al 2008
	DDT	*Fomitopsis annosa*	brown-rot		Purnomo et al 2008
	DDT	*Fomitopsis insularis*	brown-rot		Purnomo et al 2008
	DDT	*Fomitopsis pinicola*	brown-rot		Purnomo et al 2008
	DDT	*Boletus edulis*	ectomycorrhizal		Huang et al 2007
	DDT	*Gomphidius viscidus*	ectomycorrhizal		Huang et al 2007

DDT	*Laccaria bicolor*	ectomycorrhizal		Huang et al 2007
DDT	*Leccinum scabrum*	ectomycorrhizal		Huang et al 2007
DDT	*Trichoderma harzianum*	saprotrophic	field soil	Katayama and Matsumura 1993
DDD	*Trichoderma sp.*	saprotrophic	marine sponges	Ortega et al 2011
DDD	*Penicillium miczynskii*	saprotrophic	marine sponges	Ortega et al 2011
dieldrin	*Trichoderma harzianum*	saprotrophic	field soil	Katayama and Matsumura 1993
dieldrin	*Phanerochete chrysosporium*	white-rot		Kennedy et al 1990
endosulfan	*Trichoderma harzianum*	saprotrophic	field soil	Katayama and Matsumura 1993
endosulfan	*Phanerochaete chrysosporium*	white-rot		Kullman and Matsumura 1996
heptachlor	*Phanerochete chrysosporium*	white-rot		Arisoy 1998
heptachlor	*Pleurotus sajor-caju*	white-rot		Arisoy 1998
heptachlor	*Pleurotus florida*	white-rot		Arisoy 1998
heptachlor	*Pleurotus eryngi*	white-rot		Arisoy 1998
pentachloronitrobenzene	*Trichoderma harzianum*	saprotrophic	field soil	Katayama and Matsumura 1993
pentachlorophenol(PCP)	*Trichoderma harzianum*	saprotrophic	field soil	Katayama and Matsumura 1993
pentachlorophenol(PCP)	*Phanerochaete chrysosporium*	white-rot		Kang and Stevens 1994
pentachlorophenol(PCP)	*Pleurotus ostreatus*	white-rot		Rüttimann-Johnson and Lamar 1997
pentachlorophenol(PCP)	*Irpex lacteus*	white-rot		Rüttimann-Johnson and Lamar 1997
pentachlorophenol(PCP)	*Trametes versicolor*	white-rot		Rüttimann-Johnson and Lamar 1997
pentachlorophenol(PCP)	*Bjerkandera adusta*	white-rot		Rüttimann-Johnson and Lamar 1997
pendimethalin	*Fusarium oxysporum*	saprotrophic	soil	Singh and Kulshreyha 1991

	pendimethalin	*Paecilomyces varioti*	saprotrophic	soil	Singh and Kulshreyha 1991
	pendimethalin	*Rhizoctonia bataticola*	saprotrophic	soil	Singh and Kulshreyha 1991
	lindane	*Rhizopus oryzae*	saprotrophic		Young and Banks 1998
	lindane	*Phanerochete chrysosporium*	white-rot		Arisoy 1998
	lindane	*Pleurotus sajor-caju*	white-rot		Arisoy 1998
	lindane	*Pleurotus florida*	white-rot		Arisoy 1998
	lindane	*Pleurotus eryngi*	white-rot		Arisoy 1998
	mirex	*Phanerochete chrysosporium*	white-rot		Kennedy et al 1990
	PCB 77	*Phanerochaete chrysosporium*	white-rot		Vyas et al 1994
	PCB 77	*Trametes versicolor*	white-rot		Vyas et al 1994
	PCB 77	*Coriolopsis polysona*	white-rot		Vyas et al 1994
	Delor 106 (PCB)	*Phanerochaete chrysosporium*	white-rot		Novotný et al 1997
	Delor 106 (PCB)	*Trametes versicolor*	white-rot		Novotný et al 1997
	Delor 106 (PCB)	*Coriolopsis polyzona*	white-rot		Novotný et al 1997
	Six PCB congeners	*Trametes versicolor*	white-rot		Beaudette et al 2000
	Six PCB congeners	*Bjerkandera adusta*	white-rot		Beaudette et al 2000
	Six PCB congeners	*Phanerochaete chrysosporium*	white-rot		Beaudette et al 2000
organophosphate	chlorpyrifos	*Phanerochaete chrysosporium*	white-rot		Bumpus et al 1993
	chlorpyrifos	*Hypholoma fasciculare*	white-rot		Bending et al 2002
	chlorpyrifos	*Coriolus versicolor*	white-rot		Bending et al 2002
	chlorpyrifos	*Trichoderma harzianum*	saprotrophic	soil	Omar 1998
	chlorpyrifos	*Pencillium brevicompactum*	saprotrophic	soil	Omar 1998
	fonofos	*Phanerochaete chrysosporium*	white-rot		Bumpus et al 1993

	glyphosate	*Penicillium citrium*	saprotrophic		Zboinska et al 1992
	methyl parathion	*Aspergillus sydowii*	saprotrophic	marine	Alvarenga et al 2014
	methyl parathion	*Penicillium decaturense*	saprotrophic	marine	Alvarenga et al 2014
	terbufos	*Phanerochaete chrysosporium*	white-rot		Bumpus et al 1993
herbicide	alachlor	*Phanerochaete chrysosporium*	white-rot		Ferrey et al 1994
	alachlor	*Ceriporiopsis subvermispora*	white-rot		Ferrey et al 1994
	alachlor	*Phlebia tremellosa*	white-rot		Ferrey et al 1994
	alachlor	*Cunninghamella elegans*			Pothuluri et al 1993
	arochlor	*Pleurotus ostreatus*	white-rot		Zeddel et al 1993
	arochlor	*Trametes versicolor*	white-rot		Zeddel et al 1993
	three aroclors	*Phanerochaete chrysosporium*	white-rot		Yadav et al 1995
	atrazine	*Phanerochaete chrysosporium*	white-rot		Mougin et al 1994
	atrazine	*Pleurotus pulmonarius*	white-rot		Masaphy 1993
	atrazine	*Agrocybe semiorbicularis*	white-rot		Bending et al 2002
	atrazine	*Auricularia auricola*	white-rot		Bending et al 2002
	atrazine	*Coriolus versicolor*	white-rot		Bending et al 2002
	atrazine	*Dichotomitus squalens*	white-rot		Bending et al 2002
	atrazine	*Flammulina velupites*	white-rot		Bending et al 2002
	atrazine	*Hypholoma fasciculare*	white-rot		Bending et al 2002
	atrazine	*Phanerochaete velutina*	white-rot		Bending et al 2002
	atrazine	*Pleurotus ostreatus*	white-rot		Bending et al 2002
	atrazine	*Stereum hirsutum*	white-rot		Bending et al 2002
	diuron	*Agrocybe semiorbicularis*	white-rot		Bending et al 2002

	diuron	*Hypholoma fasciculare*	white-rot		Bending et al 2002
	diuron	*Stereum hirsutum*	white-rot		Bending et al 2002
	diuron	*Coriolus versicolor*	white-rot		Bending et al 2002
fungicide	carbendazim	*Trichoderma sp.*	saprotrophic	mutant strain	Tian and Chen 2009
	metalaxyl	*Coriolus versicolor*	white-rot		Bending et al 2002
	metalaxyl	*Stereum hirsutum*	white-rot		Bending et al 2002
	iprodione	*Hypholoma fasciculare*	white-rot		Bending et al 2002
	iprodione	*Stereum hirsutum*	white-rot		Bending et al 2002
	iprodione	*Coriolus versicolor*	white-rot		Bending et al 2002
PAH	five PAHs	*Bjerkandera adusta*	white-rot	soil and lignite	Gramss et al 1995
	five PAHs	*Gymnophilus sapineus*	Wood-degrading	soil and lignite	Gramss et al 1995
	five PAHs	*Hypholoma fasciculare*	Wood-degrading	soil and lignite	Gramss et al 1995
	five PAHs	*Hypholoma frowardii*	Wood-degrading	soil and lignite	Gramss et al 1995
	five PAHs	*Hypholoma sublateritium*	Wood-degrading	soil and lignite	Gramss et al 1995
	five PAHs	*Kuehneromyces mutabilis*	Wood-degrading	soil and lignite	Gramss et al 1995
	five PAHs	*Lenzites betulina*	Wood-degrading	soil and lignite	Gramss et al 1995
	five PAHs	*Pleurotus ostreatus*	white-rot	soil and lignite	Gramss et al 1995
	five PAHs	*Agrocybe praecox*	Wood- and straw-degrading	soil and lignite	Gramss et al 1995
	five PAHs	*Stropharia coronilla*	Wood- and straw-degrading	soil and lignite	Gramss et al 1995

five PAHs	*Stropharia rugoso-annulata*	Wood- and straw-degrading	soil and lignite	Gramss et al 1995
five PAHs	*Agaricus aestivalis*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Agaricus arvensis*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Agaricus bisporus*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Agaricus campestris*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Agaricus porphyrizon*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Agrocybe dura*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Bovisa nigrescens*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Clitocybe odora*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Collybia dyophila*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Collybia maculata*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Coprinus comatus*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Lepista nebularis*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Lepista nuda*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Lepista saeva*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Lycoperdon perlatum*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Marasmius oreades*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Megacollybia platyphylla*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Phallus impudicus*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Psathyrella velutina*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Stropharia aeruginosa*	Terricolous	soil and lignite	Gramss et al 1995
five PAHs	*Amanita muscaria*	Ectomycorrhizal	soil and lignite	Gramss et al 1995

five PAHs	*Amanita rubescens*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Amanita spissa*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Hebeloma crustuliniforme*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Hebeloma hiemale*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Hebeloma sinapizans*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Laccaria amethystina*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Lactarius deliciosus*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Lactarius deterrimus*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Lactarius rufus*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Lactarius torminosus*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Morchella conica*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Morchella elata*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Morchella esculenta*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Paxillus involutus*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Russula aeruginea*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Russula foetens*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Suillus granulatus*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Suillus variegatus*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Tricholoma lascivum*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Tricholoma terreum*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Xerocomus badius*	Ectomycorrhizal	soil and lignite	Gramss et al 1995
five PAHs	*Botrytis cinerea*	Mitosporic	soil and lignite	Gramss et al 1995
five PAHs	*Scytalidium lignicola*	saprotrophic	soil and lignite	Gramss et al 1995

five PAHs	*Trichoderma sp.*	saprotrophic	soil and lignite	Gramss et al 1995

Supplemental Material 1 – Table I: References

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Table II. Fungi and their enzymes capable of transforming OACs; whole-cell and enzymatic treatments are reported.

			Whole-cell treatment
Fungal species	**Pesticide**	**Enzymes involved**	**Literature**
Aspergillus niger	nicosulfuron	laccase, phenol oxidase	Lu et al. 2012
Auricularia fuscosuccinea	endosulfan		Yanez-Montalvo et al. 2016
Aspergillus sydowii, Penicillium decaturense	methyl parathion		Alvarenga et al. 2014
Aspergillus sydowii, Penicillium raistrickii, Cladosporium sp., Microsphaeropsis sp., Acremonium sp., Westerdykella sp, Cladosporium sp.	esfenvalerate		Birolli et al. 2016
Aspergillus fumigatus, Aspergillus terreus, Penicillium citrinum, Trichoderma harzianum	chlorfenvinphos		Oliveira et al. 2015
Aspergillus oryzae	3-phenoxybenzoic acid		Zhu et al. 2016
Aspergillus oryzae, Fusarium oxysporum, Lentinula edodes, Penicillium brevicompactum, Lecanicillium saksenae	terbuthylazine, difenoconazole and pendimethalin		Pinto et al. 2012
Aspergillus sydowii	trichlorfon		Tian et al. 2016
Aspergillus versicolor	triclosan		Taştan and Dönmez 2015
Coriolus versicolor	aldicarb, atrazine, alachlor		Hai et al. 2012
Dacryopinax elegans	diuron	laccase, manganese peroxidase, lignin peroxidase	Arakaki et al. 2013
Ganoderma lucidum	lindane	laccase, manganese peroxidase, lignin peroxidase	Kaur et al. 2016
Ganoderma lucidum	bentazon	laccase, manganese peroxidase	Da Silva Coelho et al. 2010
Ganoderma lucidum, Trametes sp	picloram	laccase	Maciel et al. 2013
Gloeophyllum trabeum, Trametes versicolor, Pleurotus ostreatus	lindane, endosulfan		Ulčnik et al. 2013

Mycelia sterilia	atrazine	laccase	Vasil'chenko et al. 2002
Penicillium citrinum, P.citrinum, Fusarium proliferatum	methylparathion		Rodrigues et al. 2016
Penicillium griseofulvum	b-hexachlorocyclohexane		Ceci et al. 2015
Phanerochaete sordida	clothianidin	cytochrome P450, manganese peroxidase	Mori et al. 2017
Pleurotus pulmonarius	atrazine		Masaphy et al. 1993
Phlebia tremellosa, Phlebia brevispora, Phlebia acanthocystis	Heptachlor, heptachlor epoxide		Xiao et al. 2011
Saccharomyces cerevisiae	diazinon		Ehrampoush et al. 2017
Talaromyces flavus	nicosulfuron		Song et al. 2013
Trametes versicolor	imiprothrin, cypermethrin, carbofuran, oxytetracycline	laccase, cytochrome P450	Mir-Tutusaus et al. 2014
Trametes versicolor	fipronil	cytochrome P450	Wolfand et al. 2016
Trametes versicolor	6 pesticides, 2 phytoestrogens		Nguyen et al. 2014
Trametes versicolor, Stereum hirsutum	linuron, dimethoate		Castellana and Loffredo 2014
nonsporulating mycelial fungus	atrazine	cellobiose dehydrogenase	Khromonygina et al. 2004

Enzymatic treatment

Enzymes involved	**Pesticide**	**Literature**
laccases of *Agaricus blazei*	metsulfuron	González Matute et al. 2012
phytase of *Aspergillus niger*	chlorpyrifos	Shah et al. 2017
extracellular extract of *Auricularia fuscosuccinea*	endosulfan	Yanez-Montalvo et al. 2016
laccase of *Trametes versicolor*	sulfamethoxazole, isoproturon	Margot et al. 2015
laccase of *Trametes versicolor*	chloroxuron	Palvannan et al. 2014
laccase of *Trametes versicolor*	lindane, endosulfan	Ulčnik et al. 2013
cellulose of *Trichoderma longbrachiatum*	dicofol	Wang et al. 2015
laccase of *Trametes versicolor*	isoproturon	Zeng et al. 2017

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VII. Summary and conclusions

In this thesis the biodiversity of soil fungi of agroenvironment was investigated. Two areas were studied subjected to different forms of soil use (the Colombian area with apple and peach cultivated sites, a woodland, and an uncultivated grassland) and management (the Italian area with conventional, organic, no-tillaged sites). The main questions were to know how the soil mycobiota in the agroenvironment is influenced by the different land use and managemen tand to understand its importance in the soil characteristics as a basis for ecologically sustainable agronomic management. The experimental analyses were carried out by mass NGS techniques on soil samples collected in the Upper-Andean Colombian area (Chapter IV) and in an Italian farm (V). Before facing up the experimental aspect of the thesis, chapter II describes the forms of soil use and management in the Colombian Amazon, since this region constitutes an important model for the utilization and conservation of the soil resource, and chapter III analyzes the structure of soil mycological community in Colombia, by the study of the scientific available litterature on soil fungal diversity of each natural region in Colombia. The Chapter VI represents a source of information regarding a sustainable technological outlook for those areas subjected to conventional management and strongly polluted by pesticides that could be remediated and led towards an eco-friendly agriculture. The usefulness of soil fungi as key tools for the sustainable bioremediation of chemical pesticides in the soil is discussed as a strategy for the recovery of the quality of degraded agricultural soils.

CONCLUSIONS

The distribution of the Colombian soil microfungi inferred from the scientific literature shows that the Andean region present the highest number of species and genera reported. High diversity in this region may be due both to its size, strategic location, eco-geographical and climatic diversity as well as the fact that it is the most populated region of the country, which may favor its study and a deeper knowledge on its mycological characteristics. In fact, studies at the interregional level often include this region.

The Amazon region, the second with the highest soil fungal diversity reported, is also the best represented since, unlike the other regions, it records information for each of the departments that comprise it, which may be due to the efforts of research that have been done in this strategic area for life, perhaps one of the most conserved ecological and human level in the country. Indeed, as noted in Chapter II, in this region the environmental conflict of use and soil management reflects the human-nature interaction, which involves both biological and cultural variables, therefore, in order to know the appropriate forms of resource utilization, a dialogue of knowledge is necessary, whose approach requires the inclusion of entities and non-human systems, important living forms and worthy of value, recognition and study. In this regard, bioethics is a key tool for this purpose, since it allows the resolution of problems and decision-making in the transformation of interactions between living beings and the relationship between them and their environment, by contributing in the analysis of the functional complexity of the connections of the ecosystem components, in order to outline guidelines that lead to the sustainable management of biodiversity.

It is evident that the study of soil microfungi has been growing in the recent years, however, the mycological study of the soil in the country is still peremptory since, with the exception of the Amazon, all natural regions have departments for which no general information is available. Likewise, it is essential to systematize existing information and collections through a general tool that allows greater access and understanding of soil fungi diversity in Colombia. In addition, it is necessary to enrich the methodologies for the taxonomic determination of this biological group since the macroscopic and microscopic characterization through keys makes it difficult to classify the soil fungi at the species level, which is why the use of molecular techniques and metagenomic scale are necessary.

The arbuscular mycorrhizae represent the most widely studied group of soil fungi for the country, proving the importance of these fungi in the tropics, among them and the genus *Glomus* turns out to be dominant, followed by *Acaulospora* and *Gigaspora*. At the genus level, according to the reported works, *Glomus* is also the most representative with 38 species, followed by *Acaulospora* with 13 species, *Penicillium* with 12 species, *Aspergillus* and *Fusarium* each with 10 species and *Mortierella* and *Trichoderma* each with 7 species. The other genera appear in a smaller proportion and with less diversity of species.

It is essential to initiate studies in the Insular region, as well as in the unexplored departments of the country, which prevails, considering that today there are strong anthropic threats that continually modify the environmental conditions of ecosystems that have not been studied yet. Knowledge on the biological diversity of soil fungi, as well as the understanding of their ecology in relation to interspecific interactions and other soil microbiological entities, also represents an important area of knowledge that can give solution in different aspects such as the optimization of agroecosystems, the recovery of highly anthropic areas and the conservation of natural ecosystems, especially if one takes into account the great functional potential of soil fungi in promising scope such as bioremediation.

Results reported about the upper Andean Colombian agroenvironment contribute to the knowledge on soil mycobiota. These data represent the first collected by next generation sequence analysis and give a rich picture of the soil mycobiota highlighting cultivable and uncultivable fungi. Cultivated and not cultivated areas present a dominance of species belonging to the *Ascomycota* phylum, in agreement with the international literature (Tedersoo et al., 2014). Many basidiomicetous species are recorded in the woodland area, consistently to the fact that many of them, as saprotrophic or symbiontic (parasitic or mutualistic), show preferences for lignocellulosic material. Zygomycota is homogenously distributed, although with a slight prevalence in the cultivated areas. Among the families, Trichocomaceae is the most abundant followed by Mortierellaceae, and Nectriaceae. The present analysis identified taxa at species level for 77 in Ascomycota, 28 in Basidiomycota, 31 in Zygomycota. Only *Glomus mosseae* and *Entrophospora infrequens* were the specific taxa identified for Glomeromycota and for Chytridiomycota only *Olpidiaster brassicae*. The low resolution obtained in the taxonomic analysis of Glomeromycota, compared to those obtained in the literature (see chapter 3 of this thesis), is due to the specific biomolecular analysis carried out in this research that is based on exclusively on ITS1 deep sequencing. Other gene sequences could be helpful to obtain major resolution of taxon such as Glomeromycota, and Chytridiomycota.

The entire soil fungal community from qualitative and quantitative point of view were analyzed in areas with different land use, cultivated and uncultivated. The bootstrap-based clustering analysis highlighted that different land use strongly influences the soil mycobiota: the uncultivated (grassland and woodland) and cultivated areas are characterized by abundant presence of some exclusive species. Differences in fungal species composition is consistent with the clustering analysis on higher taxonomic hierarchical level composition. In the study area, considered as a whole, it is possible to foresee a taxonomic gradient, where the woodland site is the most divergent from the cultivated sites and the abandoned grassland is in the intermediate position.

The biodiversity of fungal consortia in Italian agroecosystems sampled in the spring and autumn seasons, two key moments of the agricultural cycle, under diverse management practices at the level of fertilization, pesticides and tillage: conventional, organic, non-tillage, indicated that the highest rates of richness are presented in the soil with organic agriculture, while the non-tillage presented the most divergent communities, with their own composition, prevalence and seasonal trend. Ascomycota is the predominant phylum of fungi in the soil with the exception of conventional agriculture, which has a high abundance of a single species of basidiomycetes. Soils under organic system and non-tillage have a distinct mycobiota, even in neighboring fields. Agricultural management practices influence microbial communities, as well as their diversity, presenting high fungal diversity in the early stages, when there are changes in soil management in established agroecosystems.

Psathyrella pseudogracilis, *Coprinellus verrucispermus* and *Mortierella* were among the main management-sensitive taxa, i.e. taxa that most significantly responded to the management regimes and were responsible for the observed shifts in the community structure. *Psathyrella* and *Coprinellus* have been known for their bioremediation properties linked to the degradation, their strong increase in conventional system could be related to the stress status of the soil, since it is the one that receives the most "intense" herbicide treatment. In addition, the antagonistic effect of *Psathyrella* against the phytopathogen *Fusarium oxysporum* (Reinoso et al., 2013) is known and in this study the prevalences of the two appear inversely correlated. The main factor that could contribute to the effects of management on communities is the "legacy" effect of the previous crop in the rotation, leading to divergent soil fungal consortia. Species within the *Fusarium* genus are among those most significantly responding to the changing seasons and agricultural managements.

Agricultural soils under short-term organic and no-tillage systems harbour different fungal communities. Data shown in this study contribute to the baseline for evaluating the success of single soil management, also in terms of promotion/suppression of beneficial or pathogenic taxa, in perspective, to acquire knowledge for managing their mycobiota content.

Microbial degradation is the most important and effective way to eliminate recalcitrant compounds from the environment. Fungi, in particular, can transform pesticides and other xenobiotics by introducing minor structural changes in the molecules, producing non-toxic compounds that could be released into the soil for further degradation. The fungal capacity to transform and degrade these compounds has been especially outstanding for ligninolytic fungi and biomixture isolated fungi. In this way, fungi associated with the biodegradation of chemical pesticides *in situ* are a useful technology for soil bioremediation, however, studies that explore the synergy of fungi and bacteria, whose combination could be a relevant process for the elimination of toxic organic substances and recalcitrant from contaminated agricultural soils are peremptory.

The use of ecotoxicological and genotoxic tests in order to evaluate the bioremediation effectiveness can be a valid tool to partially overcome the existing gap between the reported successes of bioremediation on the laboratory scale and the field scale. In this respect, the effects of contaminated soil on the whole organism have been explored using various microorganisms, animals and plants, through cellular and biochemical biomarkers, or through ecological scale up systems, in different biological hierarchical levels of analyzes: a) Organismal level, through experimental models with aquatic organisms such as *Daphnia magna, Raphidocelis subcapitata, Danio rerio, Myriophyllum aquaticum* or *Lemna minor*, terrestrial animals such as nematodes (*Caenorhabditis elegans*), oligochaetes (*Lumbriculus variegatus*) and springtails (*Folsomia candida*), and embryos of fish and higher plants such as *Lepidium sativum, Cucumis sativus* and *Sorghum saccharatum,* these tests however, are considered expensive, require a lot of time and a large volume of samples. Bacterial assays are fast, reproducible and and profitable, *Saccharomyces cerevisiae, Vibro proteolyticus, Bacillus cereus* are used and the luminescence inhibition test of *Vibrio fischeri* is highlighted, b) At the cellular and biomolecular level, genetic criteria and biomarkers include mitotic index, chromosomal aberrations, micronuclei, sister chromatid exchange and mutations. In bacteria the Ames test is one of the most used and in the models of superior plants *Allium cepa, Vicia faba* and *Tradescantia* spp. Among the enzymatic biomarkers, earthworms (*Eisenia fetida, terrestrial Lumbricus, Eisenia andrei, Octolasion lacteum*) are used and the multiple esterases acetylcholinesterase (AChE), butyrylcholinesterase and carboxylesterase (CE), as well as AChE, catalase (CAT) and glutathione-S-transferase and the efflux pump. Among the estrogenic and androgenic biomarkers, the cell proliferation assay and transactivation assay, the YES bioassay (yeast estrogen) and YAS (androgen yeast) and a bioluminescence strain of genetically modified *Saccharomyces cerevisiae* were indicated. C) At the Ecological Level a new screening method (GSA-QHTS), which coupled the computational power of the global sensitivity analysis (GSA) with the experimental efficiency of quantitative high-throughput screening (QHTS), is used to study effects of chemical contamination on freshwater microbial communities.

Although limitations for bioremediation practice might be occurred, including the nature of organisms, the enzyme involved, the concentration and availability and finally survival of microorganisms, as well as cost/benefit ratio, to some extent, these limitations can be solved by understanding the genetics and biochemistry of desired microbe. The advent of synthetic community showed giant potential ability in facilitating the bioremediation process, especially the effective utility of degradative fungi.

In this reasearch, numerous data were collected on the mycological characteristics of soil in areas of great economic importance from an agricultural point of view: the Upper-Andean Colombian area and the Po Valley in Italy. The data show new records for science mainly those collected from the Colombian area, that was negletted until now from mycological analysis. The picture of soil mycobiota, focused by mass NGS techniques in this work, showed to be strongly influenced by the land use and management. This makes evidence of the highly sensitivity of fungal component to the environmental conditions and quality. The microbiological condition of soil, the characterization of the entire community of fungi, and bacteria as well, is of great importance in order to characterized areas of particular agri-environmental importance. This is true above all when the area wants to focus on the quality of its agricultural products even when it wants to define the products with a Definition of Origin (DO) mark.

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Work Experience

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Tutor, support and assistance to the teaching of Botany and Botánica Systemic and Botany Seminar. Department of Earth and Environmental Sciences. Università degli Studi di Pavia. Pavia - Italy.

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Assistant Professor in the programs of Veterinary Medicine and Agriculture Engineering, Faculty of Agrarian and Environmental Sciences. Juan de Castellanos University. Tunja, Boyacá, Colombia.

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Researcher in the DOTEZ (Research group in Origin Denotation, Terroir and Zonification) and INPANTA (Research group in Animal production, nutrition and Agriculture feeding) groups. Scientific Research Institute of Juan de Castellanos University. Tunja, Boyacá, Colombia.

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Teacher of Medical Plants Seminar, Social Education Higher Institute - ISES. Bogota, Colombia.

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Teacher of Natural Sciences and Environment to the Piapoco y Sikuani Indians teachers in Guainía - Colombia. Pedagogic and Technologic University of Colombia – UPTC. Tunja, Boyacá, Colombia.

Education

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PhD student in Earth and Environmental Sciences. Ciclo XXX. Department of Earth and Environmental Sciences. Università degli Studi di Pavia. Pavia - PV (Italy).

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Master's Degree in Bioethics, course in El Bosque University, Bogotá - Cundinamarca (Colombia). Cum Laude, after the discussion of the thesis entitled "Appropriation of forest resources in the Colombian Amazon: a view from bioethics".

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Specialization in Bioethics, course in El Bosque University, Bogotá - Cundinamarca (Colombia). Thesis entitled "Nature, ethnoknowledge and culture: a view at bioethics".

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Trainning

- Seminar in Institutional Identity and Teaching.
- Seminar in University Teaching.
- Practical and theoretical course: Cell and Molecular Biology.
- Course: Fitogeography and floristry of Neotropical areas with emphasis on Colombia.

- Course: Introduction to Colombians' Amphibian Taxonomy and Systematics.
- Course and Workshop: Bryophytes of Colombia, North-Eastern Zone.
- Course and Workshop: Biostatistics applications in Ecology

Adviser

Adviser Bachelor's Degree Thesis in Science and Technology for Nature "Quantitative analysis of soil mycobiota in agro-environment and assessment of its degrading potential for hydrocarbons" of Alessio Salice. 2017. Department of Earth and Environmental Sciences. University of Pavia.

Publications

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