The potential of soil conditioners on the recovery of burnt soils in the Montesinho Natural Park

Renata Voitena

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Supervised by

Felícia Maria da Silva Fonseca
Paula Cristina dos Santos Baptista
Paula Fernandes Montanher

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Se mar calmo nunca fez bom marinheiro,
a vida anda me preparando para ser almirante.

ABSTRACT

Fire is a prevalent element in the Mediterranean ecosystem. The recovery of burned soils is a complex and lengthy process that can span several years, with gradual visible improvements occurring over time. Soil conditioners have emerged as a means to mitigate the negative impacts of fires on soil properties. This study investigated the fungal community in burned soil, unburned soil, and soil treated with different conditioners—polyacrylamide, composted olive pomace with almond husk, and technosol—in Montesinho Natural Park. Fungal communities were characterized by a culture-dependent approach, and the isolates were identified by sequencing the internal transcribed spacer region of rDNA. Overall, 31 operational taxonomic units, belonging to seventeen genera, and two phyla were identified. The genera *Aspergillus* and *Penicillium* were the most abundant across treatments, except in superficial technosol. There were no significant differences in fungal richness between the different treatments. However, non-burnt soils exhibited significantly higher fungal diversity indices than burnt soils, whether untreated or treated with polyacrylamide or olive pomace compost, suggesting that fire negatively impacts fungal diversity and these treatments are ineffective in enhancing it. Technosols are likely the most effective in restoring fungal abundance and diversity in the soil after fire. Olive pomace compost was effective in increasing fungal abundance. Nevertheless, all the soil conditioners tested seem to be able to restore fungal communities post-fire. Indeed, burnt soils treated with any of these soil conditioners had a fungal community composition similar to unburnt soils. The saprotrophic - pathotrophic - symbiotrophic trophic mode was the most prevalent across all treatments. Further studies are needed to assess the feasibility of applying the soil conditioners used in this study.

Keywords: polyacrylamide, compost, technosol, fungal community.

RESUMO

O fogo é um elemento predominante no ecossistema mediterrâneo. A recuperação de solos queimados é um processo complexo e demorado que pode durar vários anos, com melhorias visíveis graduais ocorrendo ao longo do tempo. Os condicionadores de solo surgiram como forma de mitigar os impactos negativos dos incêndios nas propriedades do solo. Este estudo investigou a comunidade fúngica em solo queimado, solo não queimado e solo tratado com diferentes condicionadores – poliacrilamida, bagaço de azeitona compostado com casca de amêndoa e tecnosolo – no Parque Natural de Montesinho. As comunidades fúngicas foram caracterizadas por uma abordagem dependente da cultura, e os isolados foram identificados por sequenciamento da região espaçadora transcrita interna do rDNA. No geral, foram identificadas 31 unidades taxonômicas operacionais, pertencentes a dezessete gêneros e dois filos. Os gêneros *Aspergillus* e *Penicillium* foram os mais abundantes entre os tratamentos, exceto no tecnossolo superficial. Não houve diferenças significativas na riqueza fúngica entre os diferentes tratamentos. No entanto, os solos não queimados exibiram índices de diversidade fúngica significativamente mais elevados do que os solos queimados, quer não tratados quer tratados com poliacrilamida ou composto de bagaço de azeitona, sugerindo que o fogo tem um impacto negativo na diversidade fúngica e que estes tratamentos são ineficazes para melhorá-la. Os tecnosolos são provavelmente os mais eficazes na restauração da abundância e diversidade de fungos no solo após o incêndio. O composto de bagaço de azeitona foi eficaz no aumento da abundância de fungos. No entanto, todos os condicionadores de solo testados parecem ser capazes de restaurar as comunidades fúngicas pós-fogo. Na verdade, os solos queimados tratados com qualquer um destes condicionadores de solo tinham uma composição de comunidade fúngica semelhante à dos solos não queimados. O modo trófico saprotrófico - patotrófico - simbiotrófico foi o mais prevalente em todos os tratamentos. Mais estudos são necessários para avaliar a viabilidade da aplicação dos condicionadores de solo utilizados neste estudo.

Palavras chave: poliacrilamida, composto, tecnosolo, comunidade fúngica.

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FRAMEWORK AND OBJECTIVES

In the past few years, the rising occurrences of forest fires across the world have become a major environmental problem. Fires can influence forest ecology and functioning, by affecting species composition and regeneration, hydrological and global carbon cycle, nutrient turnovers, and soil properties (Agbeshie *et al.*, 2022). Fire influences forest soils in several ways. It may cause loss of organic matter through combustion, increase water repellency, and reduce nutrient levels through various mechanisms, including volatilization, oxidation, and erosion (Knicker, 2007; Mataix-Solera *et al.*, 2011; Pellegrini *et al.*, 2018). Fires can also alter the biological and physical properties of the soil, making it more vulnerable to erosion (Panico *et al.*, 2020). In the Mediterranean region, the problem of soil erosion due to fires is even worse. Indeed, in this region, the fires usually occur in summer and are followed by heavy autumn rains, resulting in a high potential for surface runoff and erosion (Campo *et al.*, 2006). Restoring burned soil is a challenging and intricate process that may span several years, with visible improvements occurring gradually over time (Inbar, Tamir & Wittenberg, 1998).

Therefore, there is great interest in devising strategies to expedite the recovery timeline for burned soils. One possible strategy is the use of soil conditioners due to their recognized role in restoring the overall health of degraded soils (Baumhardt & Blanco-Canqui, 2014; Leite, 2011.)

Hence, in this study, we will assess the effectiveness of three distinct soil conditioners in the Montesinho Natural Park. The soil conditioners that will be tested include one synthetic conditioner, polyacrylamide, and two organic conditioners, namely olive pomace composted with almond shells and technosol. The fungal community composition of burnt and not burnt soils either treated or not treated with these soil conditioners will be evaluated and used as a bioindicator of the recovery.

1. INTRODUCTION

1.1. THE IMPORTANCE OF THE SOIL

Soils are three-dimensional, dynamic entities that are constantly changing and teeming with life, containing a wide variety of organisms. Fundamentally, soils consist of solid structures composed of organic and mineral compounds (solid phase) and porous spaces that hold water (liquid phase) and air (gaseous phase) (Finkler *et al*., 2018).

According to the United States Natural Resources Conservation Service (NRCS) and the Soil Science Society of America (SSSA), soil quality can be defined as the capacity of a specific type of soil to function within the boundaries of natural or managed ecosystems. This includes sustaining plant and animal productivity, maintaining or enhancing water and air quality, and supporting human health and habitation (Mausbach & Tugel, 1995; Karlen *et al*., 1997)

In the context of sustainable food production, Schulte *et al*. (2014) define soil quality as the ability to support primary production, purify and regulate water, reduce and control greenhouse gas emissions, sustain biodiversity through habitat provision, and support nutrient cycling. They identify five main soil functions that, interacting together, enable soil multifunctionality and its ecosystem services. These functions include biomass production, water filtration and storage, climate and air quality regulation, habitat for organisms, and the cycling of essential nutrients.

One of the primary functions of soil is to support plant productivity. Healthy soils are fundamental for primary production, ensuring nutritious and high-quality food for animals and humans (Montiel, 2021). However, due to the finite nature of Earth's resources, life depends on the natural balance of these ecosystem services. The importance of soils lies in the significant volume of matter and energy moved in their nutrient cycling processes (Aduán, 2004). The natural scarcity of macronutrients such as nitrogen (N) and phosphorus (P) in agroecosystems has traditionally been addressed through the application of manufactured fertilizers. However, some nutrient loss is inevitable, and the efficiency of their cycling is intrinsically linked to the quality of agricultural soils and the soil microbiome. These soils play a crucial role in absorbing and converting nutrients into forms accessible to plants, directly influencing crop productivity and yield, and consequently, the effective retention of nutrients in the soil (Schröder, 2016).

In addition to nutrients, soil is one of the largest carbon sinks, playing numerous roles in carbon dynamics (Silva, 2023). The carbon cycle is one of the most important biogeochemical cycles on the planet due to its relationship with the natural greenhouse effect and climate regulation (Aduán, 2004). Soil contains more carbon than the atmosphere and terrestrial vegetation combined. In many soils, organic matter, which consists of approximately 55-60% carbon by mass, represents most or all of the soil organic carbon stock. In arid and semi-arid regions, there may be a significant presence of inorganic carbon, but changes in its stocks occur slowly and are not easily influenced by traditional soil management practices, thus not playing a relevant role in the management of ecosystem services (FAO and ITPS, 2015).

Soil is also home to a large number of organisms and microorganisms. A small soil sample contains an extraordinary number of potential spatial microhabitats, and temporal variations in soil physicochemical factors such as moisture, temperature, and nutrients further increase the diversity of niches available to support microbial populations. These complex communities of organisms play critical roles in sustaining soil and broader ecosystem functions, providing numerous benefits to global cycles and human sustainability. Specifically, soil biodiversity is crucial for the production of food and fiber. It is also an important regulator of other vital soil services, including nutrient cycling, moderation of greenhouse gas emissions, and water purification (Wall *et al.*, 2012).

According to estimates by the Food and Agriculture Organization (FAO and Intergovernmental Technical Panel on Soils (ITPS) (2015), 33% of the world's soils are degraded to varying degrees, with nearly half of them being agricultural soils. The loss, degradation, and desertification of soil compromise the resilience of systems and ecosystem services. One example of degradation occurs in burnt soils, which suffer losses of organic matter, mineral matter, biodiversity, and changes in soil physical properties. Although the issue of burnt soils affects several regions of the globe, the Mediterranean region is strongly impacted due to its hot and dry summers (Lourenço *et al.*, 2012). Portugal is one of the countries with a long history of wildfires (Ferreira Leite *et al.*, 2013).

1.2. BURNT LAND IN PORTUGAL

Fire has been employed in deforestation over the years, whether for agricultural purposes or pasture creation and thus plays a fundamental role as an "ecological factor," influencing both the development and regression of forest systems around the globe (Ferreira Leite *et al.*, 2013). Furthermore, fires are an integral part of many ecosystems, particularly in the Mediterranean ecosystem, where they have exerted significant influence and led to vegetation adaptations, contributing to the current landscape configuration (Fernandes, 2002).

Located on the Iberian Peninsula, Portugal has a long history of forest fires, with the earliest known written references dating back to the late 12th century, already describing changes in forest cover and river silting (Ferreira Leite *et al.*, 2013). In the 15th century, in the year 1464, the author also cited the Royal Charter of King Afonso V, which established zones where setting fires was prohibited to prevent the exacerbation of erosion. Nevertheless, forest fires continued to be documented phenomena in the 19th century. For instance, in the work "O pinhal do Rei" – Pinto (1939), a major forest fire that burned approximately 5,000 hectares in Leiria is mentioned (Ferreira Leite *et al.*, 2013).

In the 20th century, during the 1950s and 1960s, social and economic changes led to rural exodus, and due to the decline of agricultural activities, there were alterations in forest management. Firewood, which was previously collected by the population and used as an energy source, ceased to be utilized, resulting in biomass accumulation. The reduction in sheep grazing led to the accumulation of more fuel in forests and around villages, increasing the amount of combustible material for summer fires (Ferreira Leite *et al.*, 2013).

Since the 1970s, ecosystems have experienced an increase in the frequency and intensity of natural fires (Lourenço *et al.*, 2012). This phenomenon is attributed to a combination of natural and human factors, such as climate change, deforestation, and changes in land use. Most of these fires occur during the summer, coinciding with high temperatures and minimal precipitation, creating dry conditions in the vegetation and soil that facilitate the occurrence and spread of fires (Lourenço *et al.*, 2012).

Despite significant year-to-year variation in the total burned area in Portugal, influenced by the severity of climatic conditions, a rising trend is evident over time. The highest peaks were recorded in 2003 and 2005, when Portugal faced major fires. Since then, controlling fires has become a priority (Ferreira *et al.*, 2008), essential due to the characteristics of land use in the country. However, even with fire prevention policies in place, in 2017 there were 19,104 rural fires, with a total burned area of 537,131 hectares (Fig. 1) (ICNF - SGIF, 2023). These data highlight the ongoing need for effective fire prevention and management strategies to protect ecosystems and affected communities.

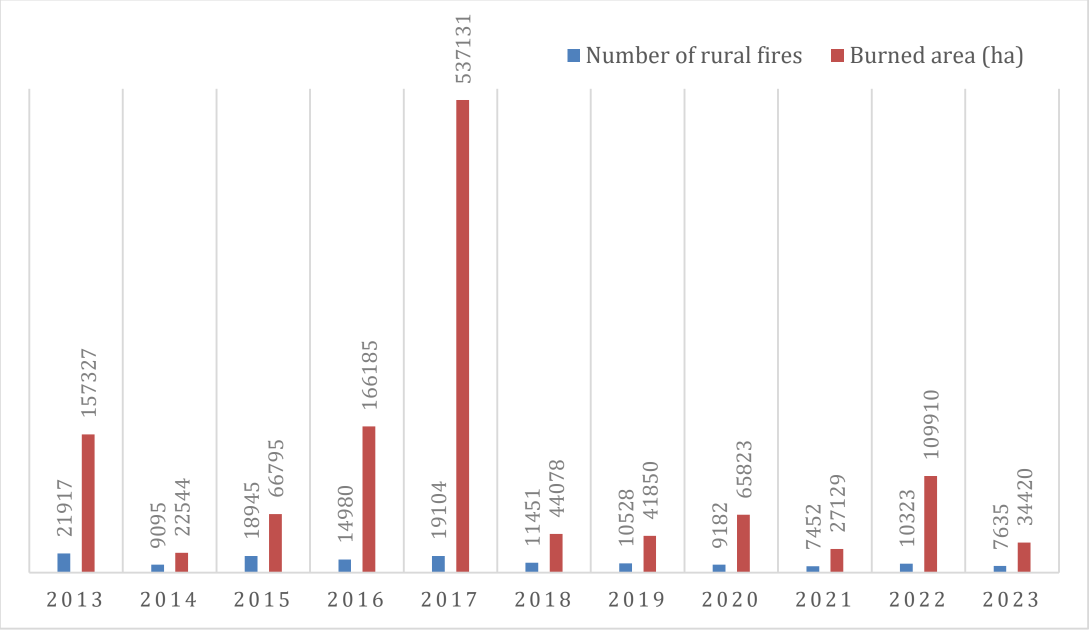

Figure 1: Number of rural fires and corresponding extension of burned area in mainland Portugal (Adapted from ICNF - SGIF, 2023).

As observed in Figure 1, there has been variation in the burned area over the last decade. However, the year 2023 showed the second lowest number of fires and the third lowest burned area since 2013. Despite the decrease in burned area, it remains significant and requires effective management for further reduction.

According to the National Forest Inventory (IFN, 2015), approximately 69.4% of mainland Portugal, equivalent to 6.2 million hectares, is occupied by forested areas, including forests, shrubs, and unproductive lands. Forests, comprising wooded and temporarily deforested areas (cut, burned, and regenerating areas), represent 36% of land use in Portugal, emerging as the primary land use. Given the importance of these areas, proper management is essential to minimize fire risks.

One possible control strategy is the use of prescribed fire, involving the application of low-intensity fire to reduce available fuel load in certain areas, creating a protective discontinuity for forests, inhabited areas, and cultivated lands (Braga, 2023). Although this is an effective strategy for fire management, it is essential to consider the impact of controlled burning and associated environmental losses, particularly soil degradation (Cavalli, 2017).

1.3. IMPACT OF FIRE ON SOIL PROPERTIES

During wildfires, both the vegetative cover and leaf litter layers are consumed, leading to alterations in the physical, chemical, and biological properties of the soil (Agbeshie *et al*., 2022). According to Leite (2011), fire can impact soil properties through heating, nutrient release, and exposure to environmental elements. The extent of these changes depends on various factors, including the intensity, duration, and frequency of the fire, as well as the quantity and type of vegetation present and soil characteristics such as organic matter content, moisture content, and mineral composition (Agbeshie *et al*., 2022).

As illustrated in Figure 2, the main changes at lower temperatures (below 200°C) primarily affect biological properties, such as reduced microbial biomass and the destruction of the seed bank and fine roots. However, physical properties, such as soil water repellency and aggregate stability, may also be altered (Santín & Doerr, 2016). One of the effects of fire on soil is the evaporation of water from the surface layer, resulting in the formation of a compact layer with alterations in water repellency (Redin *et al*., 2011). This compaction reduces macropore volume and aggregate size, hindering water infiltration and increasing soil density and root penetration resistance (Redin *et al*., 2011). Although the matter is subject to debate, fire can also induce changes in soil particle distribution, thereby modifying its texture (Xofis *et al*., 2023).

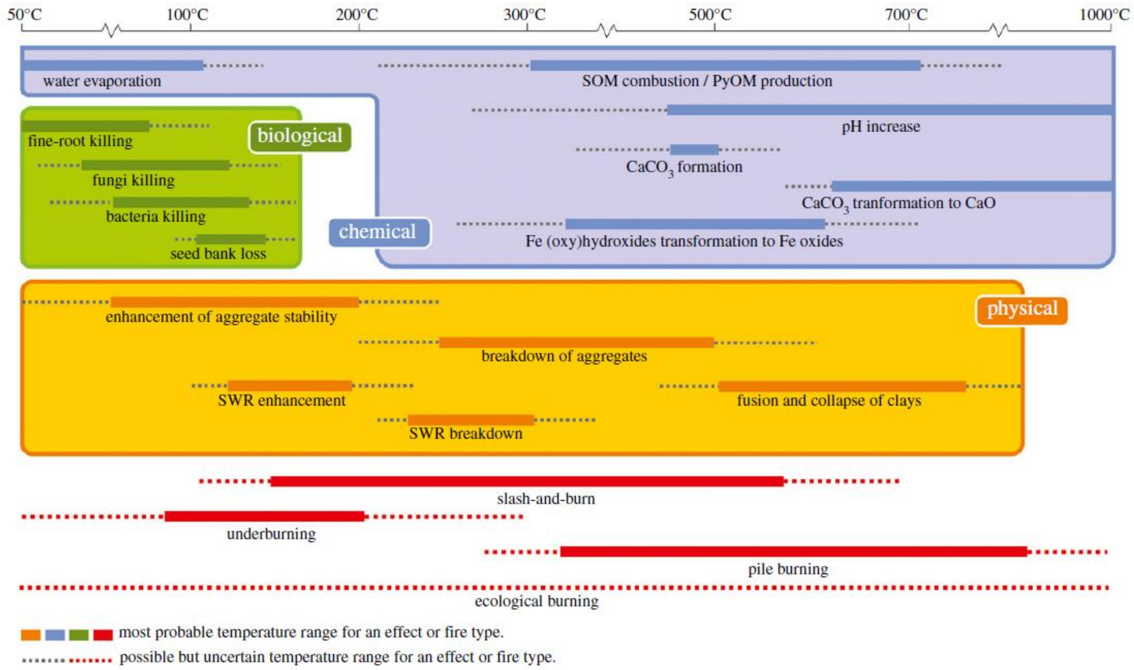

Figure 2: Effects on the biological, chemical and physical properties of soil and associated temperature ranges reached near the mineral soil surface for different types of human-induced fires (slash-and-burn, underburning, pile burning and ecological burning). SOM: soil organic matter; PyOM: pyrogenic organic matter; SWR: Soil water repellency. Adapted from Santín & Doerr, (2016).

At higher temperatures (above 200°C), soil chemical properties undergo changes due to the combustion of soil organic matter, production of pyrogenic organic compounds, and an increase in soil pH (Santín & Doerr, 2016). Organic matter, which is fundamental for soil, comprises microbial biomass and plant material in various stages of decomposition. When vegetative cover is burned, nutrients such as phosphorus, potassium, calcium, and magnesium are released, thereby enhancing their availability and benefiting plant growth in the short term (Redin *et al*., 2011). However, the exposure of soil and rocks significantly contributes to weathering and soil movement (Leite, 2011), amplifying erosion from raindrop impact and leading to increased surface runoff and mineral transport (Leite, 2011).

When higher temperatures (above 350°C) are reached, mineral transformations occur, causing irreversible alterations in soil properties (Alauzis *et al*., 2004), affecting vegetation regeneration and the hydrological, geomorphological, and biogeochemical processes of ecosystems (Vega, De Las Heras & Moya, 2018; Fernández, Fernández-Alonso & Vega, 2020).

Thus, in the medium and long term, fire increases soil susceptibility to wind and water erosion, resulting in a gradual loss of nutrients and soil degradation (Leite, 2011). Given the rapid degradation of soil and its slow formation rate, there is considerable interest in developing new tools and approaches to recover burnt soil. This task is challenging, as the recovery process tends to be slow and may not fully restore the soil to its initial condition. Despite the challenges in accurately estimating the recovery time of burnt soils, some studies suggest that it may take up to 9-10 years (Inbar, Tamir & Wittenberg, 1998; Moody & Martin, 2001).

1.4. STRATEGIES FOR RESTORING FIRE-AFFECTED SOILS: THE USE OF SOIL CONDITIONERS

Several techniques and strategies have been employed to aid in soil recovery after wildfires. Most of these strategies are often applied in severely burned areas, aiming to protect the soil and thus mitigate erosion (Prats *et al.*, 2014). Some of the strategies used include planting native plant species, using vegetative cover, seeding to accelerate vegetation recovery, using erosion control blankets, implementing terraces, and contour plowing, among other water management methods (Prats *et al.*, 2014; Robichaud; Beyers; Neary, 2000; Vieira *et al.*, 2014).

More recently, there has been a growing interest in the use of soil conditioners to recover burned soils, due to their recognized role in improving soil structure, fertility, and overall health (Babla *et al.*, 2022; Baumhardt & Blanco-Canqui, 2014; Leite, 2011). There are three main classes of soil conditioners: natural organic materials, inorganic or mineral materials, and synthetic materials consisting mainly of chemical polymers and surfactants (Sojka *et al.*, 2007). Natural organic materials are known to increase soil water retention capacity and infiltration, improve aggregation, provide a substrate for biological activities, and resist compaction (Sojka *et al.*, 2007), such as compost. On the other hand, inorganic or mineral materials can modify the chemical and physical properties of the soil (Sojka *et al.*, 2007), such as technosols (Silva, 2023). Synthetic materials are highly effective in soil recovery, improving root penetration, infiltration, aeration, erosion resistance, and drainage, producing effects even when applied in small quantities, such as polyacrylamide (Sojka *et al*., 2007).

1.4.1. COMPOST

The category of materials derived essentially from biological matter, originating from plant and/or animal sources, including composts (decomposition of organic waste and by-products by microorganisms), has been shown to increase soil fertility rates (Pereira, 2022). Furthermore, its rich organic matter content improves soil structure, promotes microbial activity, and replenishes essential nutrients, providing a conducive environment for plant growth (Varela, Gobbi & Laos. 2011). Besides, composting represents a sustainable practice within the circular economy framework, where organic waste is recycled to become value-added products. Due to the increasing waste production in increasingly intensive societies, sustainable production approaches that promote waste management in agriculture are necessary. One such approach is the effective and efficient reuse of agricultural residues, transforming them into soil amendments to improve fertility (Babla *et al*., 2022).

Portugal stands out as one of the world's largest producers of olive oil. In 2022, olive oil production reached 1.378 million hectolitres (approximately 126 thousand tons), (INE, 2022). For every 100 kg of olives, about 20 kg of olive oil is produced, and one kilogram of extracted olive pomace contains approximately 400 grams of pulp and 600 grams of pit (Brito, 2016). Olive pomace contains phenolic compounds, lipids, and other organic substances that render it phytotoxic (Pinho *et al*., 2017). Additionally, it exhibits high acidity (Ladhari, *et al*., 2021), making its direct disposal into the environment unfeasible straight from the mills.

Additionally, Portugal excels in almond production. In 2020, Portugal produced 32,000 tons of almonds (FAOSTAT, 2022). Almond production primarily generates three types of waste: green shells, outer shells, and inner skins (Ramalhosa, 2022). These abundant residues can be valorized through composting, yielding agricultural products of interest such as fertilizers or components of horticultural substrates (Coelho *et al*., 2009), as organic plant residues constitute valuable sources of nutrients to enhance soil productivity (Hossain, Niemsdorff & Heb. 2016).

In a recent study, composting olive pomace using almond shells as a structuring agent, was shown to be very efficient in reducing olive pomace high toxicity and also improved the quality of organic matter by forming constituents similar to humic acids (Hernández *et al.*, 2022). However, further studies are needed to assess the impact of using organic amendments in restoring soil biological properties after fires.

1.4.2. TECHNOSOLS

Among soil conditioners, technosols are notable, with properties and pedogenesis dominated by their technical origin. Technosols contain a significant amount of artifacts in their composition, which are materials modified or altered by human action or materials extracted from deeper layers. These can be sealed by materials with properties different from natural rocks or contain geomembranes. The constituent materials of technosols include landfill soils, sludges, mining tailings, and sludges from water and sewage treatment plants (FAO and ITPS, 2015).

Technosols are, therefore, artificially constructed soils or those that spontaneously arise from materials made or exposed by human activity, which would not otherwise occur on the earth's surface. These soils can be an effective alternative in combating soil degradation, recovering degraded areas, and appropriately disposing of certain wastes, thus reducing environmental impacts (Silva, 2023).

A study conducted in coal-fired power plant ash disposal sites showed that these deposits form a unique technosol, developed from the natural transformation of an initially harmful and environmentally problematic surface. The results indicate that, with revegetation and agronomic practices such as pH correction, technosols can accelerate ecosystem recovery by improving microbial activity (Singh *et al.*, 2023).

Another study, conducted in a semiarid limestone quarry in Spain, evaluated the effects of technosols made with organic additives on the restoration of degraded soils. The technosols increased water retention capacity, electrical conductivity, total organic carbon, and nitrogen compared to control soils. Additionally, after application, there was a rapid increase in both microbial activity and soil respiration rates (Soria *et al.*, 2022).

In France, a study with technosols resulting from coking plant landfills and thermally treated industrial soils showed that these soils were dominated by epigeal fauna with intense activity of macro-saprophages and surface soil micro-arthropods, suggesting that the physicochemical properties of the soil influence the communities living there (Colombini, Watteau & Auclerc. 2022).

Another advantage of technosols is their ability to be applied directly to the soil surface, helping to minimize soil loss due to water erosion (Bronick & Lal, 2005). This characteristic, along with other advantages, make technosols an interesting material for application in burned soils.

1.4.3. POLYACRYLAMIDE

Synthetic polymer conditioners such as polyacrylamide (PAM) are recognized as effective options for improving soil stability, infiltration rate, and erosion resistance (Xiong *et al.*, 2018). Polyacrylamide is a polymeric flocculant, with its monomer being acrylamide (Figure 3**Erro! Fonte de referência não encontrada.**), composed of hydrogen, oxygen, nitrogen, and carbon atoms.

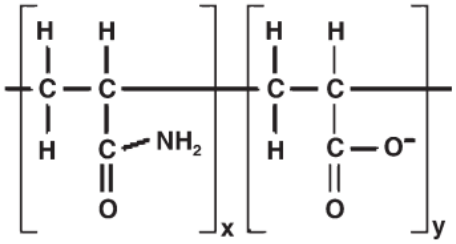

Figure 3: Acrylamide monomer (Adapted from Sojka, 2005).

Polyacrylamide structure varies in terms of polymer chain length, functional group substitutions, and molecular conformation, with notable differences between linear and cross-linked forms (Sojka *et al.*, 2007). Widely used as a soil conditioner, polyacrylamide exists in various forms, such as dry granular spheres, blocks, powders, and liquids (Nascimento, 2017). Application rates vary based on soil geographic features like slope and intended outcomes.

Applied at the soil surface, polyacrylamide plays a crucial role in reducing soil erosion and sediment transport (Baumhardt & Blanco-Canqui, 2014). It binds soil particles through electrostatic interactions, forming aggregates, and enhances soil hydraulic conductivity (Xiong *et al.*, 2018), crucial in preventing further soil degradation in fire-affected areas (Prats *et al.*, 2014). Interaction between polyacrylamide's physicochemical domains and soil chemistry, stabilizes exposed soil aggregates, preserving soil macropore integrity and minimizing surface crust formation (Baumhardt

& Blanco-Canqui, 2014; Sojka *et al.*, 2007). Moreover, polyacrylamide promotes sediment flocculation, contributing to improved soil management.

Davidson, Davidson & Roa-Espinosa (2009), investigated the effects of polyacrylamide and straw application on soil erosion and vegetation recovery post-fire, over three years. While soil erosion reduction with polyacrylamide was not significant, noteworthy vegetation recovery was observed in treated areas during the first two years post-fire.

Another study evaluated the application of synthetic gypsum produced from flue gas desulfurization (FGD) and polyacrylamide on soil surfaces, confirming the effectiveness of the combination in reducing soil erosion and suggesting its practicality for field-scale use (Acuña-Guzman, Norton, 2023). However, further research is needed to understand soil solution chemical interactions and their effects on the rhizosphere.

Although effective as a soil conditioner (Xiong *et al.*, 2018), polyacrylamide's efficacy in soil recovery after fires is not always observed (Macdonald & Larsen, 2009; Prats *et al.*, 2014). More studies are necessary to elucidate its true effect. Photolytic degradation of polyacrylamide exposed to sunlight is also a concern, although some studies suggest microbial degradation into non-toxic products over time (Xiong *et al.*, 2018; Labahn *et al.*, 2010).

1.5. MICROBES AS BIO-INDICATORS OF BURNT SOIL RESTORATION

Soil microorganisms constitute the most diverse community in the biosphere, representing at least one-quarter of the Earth's total biodiversity (Sokol *et al.*, 2022). One gram of soil can contain hundreds of millions of microorganisms (Roesch *et al.*, 2007). Among these, bacteria and, to a lesser extent, fungi are the most abundant groups, followed by archaea, protists, nematodes, and viruses (Ling, Wang & Kuzyakav, 2022). The presence of these microorganisms is crucial for maintaining soil health and ecosystem functions (Hirsch, 2018).

Soil microorganisms are responsible for nutrient cycling and the decomposition of organic matter (Suman *et al.*, 2022). Furthermore, according to the same author, microorganisms contribute to improving soil structure through the production of substances such as polysaccharides, which bind soil particles together. Some microorganisms establish symbiotic associations with plants, enhancing plant fitness, suppressing diseases, and fixing nitrogen (Hirsch, 2018; Fierer, Wood & Mesquita, 2021). Moreover, soil microorganisms have the ability to degrade and detoxify pollutants and contaminants, contributing to carbon sequestration by incorporating organic carbon into microbial biomass and soil organic matter (Tang, Yang & Antonietti, 2022). This process is crucial for mitigating climate change by promoting carbon storage in the soil.

Due to their importance in regulating essential processes in ecosystems, soil microorganisms are often considered indicators of soil health (Fierer, Wood & Mesquita, 2021). Monitoring microbial communities can provide valuable information on the effects of different methods used in the recovery of burned soils. Previous studies have shown that soil microorganisms respond to various types of soil conditioners. For instance, the application of soil conditioners derived from food waste and rice husk altered the microbial community structure, especially those involved in carbon and nitrogen fixation (Hou *et al*., 2017). Similarly, the application of commercial soil conditioners in loamy, sandy-loam, and silty-loam soils affected the bacterial population of these soils (Ozturk, 2005). These studies highlight the potential of using microbial data to evaluate the recovery of burned soils.

Several microbial indicators of soil health have been proposed. Generally, these indicators are based on (i) the abundance of specific microbial taxa; (ii) the abundance and composition of specific microbial groups, such as nitrifiers, arbuscular mycorrhizal fungi, and plant growth-promoting rhizobacteria; (iii) microbial biomass; (iv) microbial activities, including carbon dioxide release (microbial respiration) or enzymatic activities associated with the C, N, and P cycles; (v) microbial functional genetic composition; (vi) C and N mineralization rates; (vii) nitrification rates; and (viii) nitrogen fixation (Fierer, Wood & Mesquita, 2021).

These microbe-based metrics provide valuable insights into soil health, but their application requires careful consideration of contextual factors. Interpreting these metrics requires a deep understanding of the context, including management practices (Fierer, Wood & Mesquita, 2021).

2. MATERIAL AND METHODS

2.1. STUDY AREA AND COLLECTION OF SOIL SAMPLES

In the Montesinho Natural Park (PNM), near to the village of Aveleda (41°53'57.06"N, 6°40'55.39"W), was previously established an assay (Figure 3) to evaluate the effect of three soil conditioners on the recovery of burnt soil.

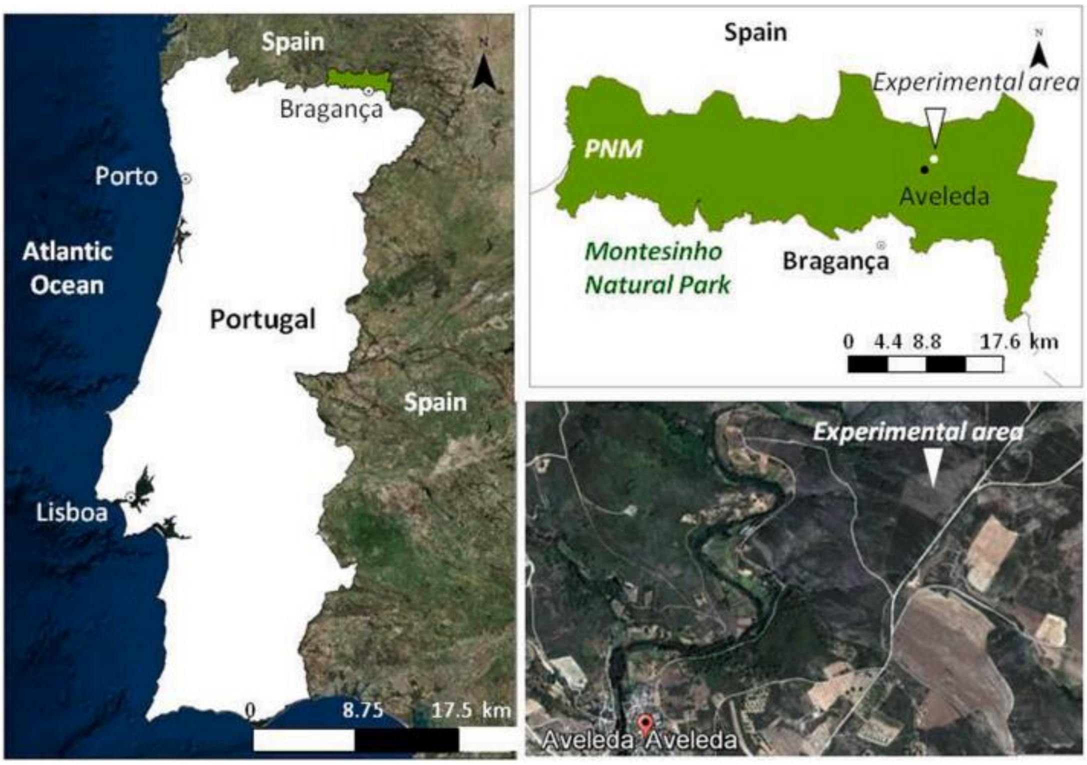

Figure 3: Location of the study area in Montesinho Natural Park (PNM), NE Portugal. Source: Fonseca *et al*. (2017).

Briefly, a low-intensity prescribed fire was implemented in the study area in March 2021, with temperatures not exceeding 200°C. Prior to the prescribed fire, the area exhibited patches with a clear dominance of species representative of the shrub communities of the PNM (IPB/ICN, 2007; Fonseca, Figueiredo & Ramos, 2012). The relative abundance of these species was assessed, and as a result, before the prescribed fire, the area was covered by *Chamaespartium tridentatum* (62% of the surface), *Erica australis* (27%), *Cystus ladanifer* (6%), *Lavandula stoechas* (3%), and *Cistus monspeliensis* (2%) (Fonseca, Figueiredo & Ramos, 2012). The soils are classified as humic leptosols of schistose origin, typically exhibiting a medium texture, acidic nature, and medium to high organic matter content (Fonseca *et al*., 2017). After fire, eight plots, each with 4 m² and labelled from 1 to 8, were marked in the burnt soil using metal barriers, arranged to follow the slope of the terrain (Figure 4). Two additional plots, each with 500 m², were established in unburnt area (plot 9) and burnt area (plot 10) soil, but they were not physically marked. On the day following the application of each controlled fire, each soil conditioners, namely polyacrylamide, olive pomace compost with almond shells, and technosol, were applied to two burnt plots (Figure 5). The amount of added soil conditioners is displayed in Table 1. In plot 9, no treatment was applied, therefore being the original soil. In plot 10, the same amount as in plots 4 and 8, of technosol was applied, however, in an area of 500 m² without physical delimitation, so, this treatment was named as superficial technosol (Table 1).

Figure 4: Distribution of the ten plots (from 1 to 10) in the study area to evaluate the effect of soil conditioners on the recovery of burnt soil. Treatments: Bur – Burnt; Poly - Polyacrylamide; Oliv - Olive pomace compost with almond shells; Tec – Technosols; STec - Superficial Technosols; NBur - Non burnt. Source: image extracted from Google Earth® software, June 2024.

Table 1: Soil conditioners added in each plot and their concentration.

Plot	Treatment	Treatment	Concentration
1 and 5	Bur	Burnt	-
2 and 6	Poly	Polyacrylamide	0.005kg/m²
3 and 7	Oliv	Olive pomace compost with almond shells	1 kg/m²
4 and 8	Tec	Technosols	25 kg/m²
9	NBur	Non burnt	-
10	STec	Superficial Technosols	25 kg/m²

27 months after the application of soil conditioners, soil samples were collected from each plot for the analysis of fungal community. Accordingly, for plots 1 to 8, two composite soil samples were collected per plot, while for plots 9 and 10, four composite samples were collected. Each composite sample was formed by combining three individual soil subsamples collected at a depth of 10 cm under the surface, using a measuring shovel (Figure 5). Overall, 24 composite soil samples were collected.

Figure 5: Collection of soil samples from the plots. A) Collecting samples. B) Measuring shovel.

The collected soil samples were transported to the laboratory and were sieved through a 2.00 mm mesh sieve to eliminate roots and soil debris. The soil samples were then stored in a refrigerated room at 4ºC, and subsequently utilized for fungal isolation and diversity assessment.

2.2. ASSESSMENT OF FUNGAL COMMUNITY

The fungal community composition and diversity of soil samples were assessed by using a culture-dependent approach. Accordingly, the dilution-to-extinction method was employed to isolate fungi, involving serial dilutions starting from 1 g of soil in 9 mL of Phosphate-Buffered Saline (PBS) solution (8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4, dissolved in 1 L H_2O, pH 7.4). Aliquots of 100 microliters from the 10^{-3} and 10^{-4} dilutions were inoculated onto 9 mm Petri dishes containing Potato Dextrose Agar (PDA) (39 g PDA per liter of water; Himedia), supplemented with chloramphenicol antibiotic (100 mg L^{-1}; OXOID - SR0078E). Each dilution was inoculated in duplicate (Figure 6).

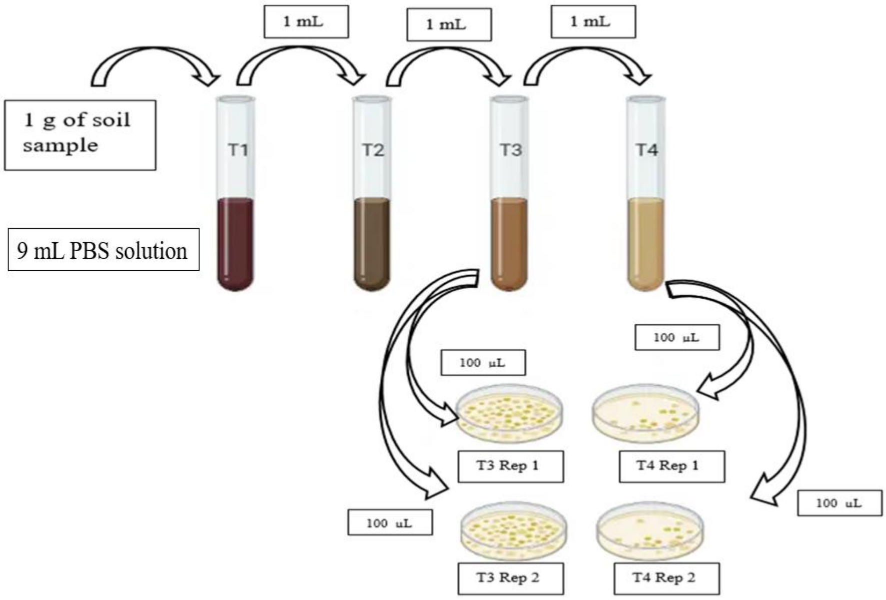

Figure 6: Dilution-to-extinction method used to isolate fungi from soil samples.

Petri dishes were incubated in the dark, at room temperature (22 ± 3°C) and checked daily for fungal growth and colony counting. Single colonies were enumerated and transferred to fresh PDA medium to establish pure cultures. Fungal colonies were then grouped according to their similarity in morphology (shape, edge/margin, surface and reverse color, and texture of the colonies as well as the production of exudates). One representative isolate of each fungal morphotype was then selected for further molecular identification.

2.3. MOLECULAR IDENTIFICATION AND PHYLOGENETIC ANALYSIS OF FUNGAL STRAINS

2.3.1. PCR AMPLIFICATION OF THE REGION ITS

The internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (rDNA) was chosen as the molecular marker for the identification of the different fungal isolates, as it is the recognized DNA barcode for fungi (Schoch *et al*., 2012). Accordingly, total genomic DNA was extracted from harvested mycelia/spores after 5-10 days of culture growth using the REDExtract-N-AmpTM Plant PCR Kit (Sigma-Aldrich, USA) following the manufacturer's instructions. Following extraction, the ITS region of rDNA comprising the ITS1-5.8S-ITS2, were amplified, employing the ITS1 as the forward primer (5' - TCCGTAGGTGAACCTGCGG - 3') and the ITS4 as the reverse primer (5' - TCCTCCGCTTATTGATATGC - 3'), as described by White *et al.* (1990). PCR amplification was conducted in a 20 µL reaction mixture, detailed in Table 2. The reactions were carried out in a BioRad T100TM Thermal Cycler (Hercules, CA, USA). The PCR program included an initial denaturation step at 95°C for 5 minutes, followed by 30 cycles consisting of denaturation at 95°C for 40 seconds, annealing at (52ºC – 56ºC) and extension at 72°C for 45 seconds, concluding with a final extension at 72°C for 7 minutes. Sterile distilled water was included as a negative control during the genomic DNA amplification process.

Table 2: Components, volumes and concentrations used for the 20 µL PCR reactions

Components	Volume (µL)	Final concentration
ddH_2O	11.9	-
PCR reaction buffer (10X)	4	1X
$MgCl_2$ (25 µM)	0.8	1.0 µM
Primer forward (10 µM)	0.4	0.2 µM
Primer reverse (10 µM)	0.4	0.2 µM
dNTPs (10 mM)[1]	0.4	0.2 mM
DNA polymerase (5U/µl)[2]	0.1	0.025 U/µl
DNA template (5-50 ng/µL)	2.0	0.4-4 ng/µL

[1] dNTPs - Deoxyribonucleotide triphosphate solution mix; equimolar solution of dATP, dCTP, dGTP and dTTP, at 2.5 mM each

[2] GoTaq® DNA Polymerase (PROMEGA, Germany)

All PCR products were analyzed through agarose gel electrophoresis (1.3%, w/v; Sigma) in Tris-Borate-EDTA (TBE) buffer (1X) (89 mM Tris base, 89 mM boric acid, and 2 mM EDTA, pH 7.6) in BioRad horizontal electrophoresis chambers. Samples consisting of PCR products (5 µL), along with the molecular marker 1Kb DNA Ladder (Bioron, Germany), were loaded onto the gel supplemented with Loading Buffer (6X) (2 µL, Bioron, Germany). Electrophoresis was conducted at 100 V for 30-45 minutes, and the separated DNA bands were visualized by staining with an aqueous solution of GelRed^{TM} Biotum (3X) for 20-30 minutes. The amplified DNA was visualized by fluorescence under ultraviolet light using a ChemiDocTM XRS+ system with Image LabTM Software from BioRad (Hercules, California). The amplified DNA fragments were subsequently sequenced by Macrogen Inc. (Madrid, Spain).

2.3.2. SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSES

The obtained sequences were analyzed using the bioinformatic software Bioedit (Biomatters, 2021). Initial identification of fungal isolates was conducted via Nucleotide Basic Local Alignment Search Tool (BLASTn) against the NCBI database (https://www.ncbi.nlm.nih.gov/) using sequences from type materials. This tool determines sequence similarity by performing alignments with entries in the database, ranking results based on the highest percentage of identity and lowest E-value. All the sequences were assigned to an operational taxonomic unit (OTU), and further classified according to the *Index Fungorum* Database (www.indexfungorum.org).

The ITS sequences obtained were used to performed phylogenetic trees, in an attempt to distinguish fungal OTUs from the same genus. These trees were constructed using the Maximum Likelihood (ML) method and General Time Reversible model, based on the Kimura 2-parameter (Kumar *et al*., 2018), with 1000 bootstrap repetitions. Raw sequences from reference strains showing the best % similarity (as determined by BLAST) were aligned alongside sequences obtained in this study using ClustalW. The selected replacement models for phylogenies were determined using the Corrected Akaike information criterion, with model details provided in the figures. The trees with the highest log likelihood were selected. Initial trees for heuristic searches were generated using Neighbor-Join and BioNJ algorithms, based on pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, with the topology yielding the highest log likelihood value chosen. Trees were drawn to scale, with branch lengths representing substitutions per site. Evolutionary analyses were conducted using MEGA X (Kumar *et al*., 2018).

Detailed information regarding characterized fungal sequences is summarized in Table S1 (Supporting information). All the identified pure fungal cultures were deposited at the *Centro de Investigação de Montanha* Culture Collection (CIMO-CC), of *Instituto Politécnico de Bragança*, Portugal, being preserved in 30% glycerol (v/v) at -80º C.

2.3.3. FUNGAL DIVERSITY AND COMPOSITION ANALYSIS

Each fungal taxon was classified taxonomically based on the Index Fungorum database (www.indexfungorum.org). A Krona chart illustrating the relative abundance of operational taxonomic units (OTUs) within the fungal community was generated using the Krona tool (Ondov, Bergman & Phillippy, 2011).

The diversity of fungal community in soil samples across the different treatments was evaluated at the level of their richness (number of different taxa), abundance (expressed as colony-forming units – CFU- per gram of soil), and Shannon_H and Simpson_1-D diversity indexes. These two indexes were estimated using PAST (Paleontological Statistics) software v4.03 (Hammer *et al*., 2001). The results were presented in boxplots showing the respective medians and quartiles. Differences on diversity parameters between treatments were assessed by using the same Past software version 4.03 (Hammer *et al*., 2001). The normality of the data was assessed using the Shapiro-Wilk test. For non-normally distributed data, the non-parametric Kruskal-Wallis test followed by Dunn's post hoc test was applied. Data with normal distributions were analyzed using one-way ANOVA with Tukey's pairwise comparison test for multiple comparisons ($p < 0.05$).

FUNGuild (Nguyen *et al*., 2016) was used to identify trophic modes and ecological guilds of the fungi, at the genus level. Relative abundance charts for trophic mode and ecological guilds at the level of fungal genus were created using Excel®.

Changes in fungal community composition between the different treatments were assessed using non-metric multidimensional scaling (NMDS) analysis with the Bray-Curtis distance similarity index, considering both species presence/absence and abundance. The Kruskal stress value was used to validate the NMDS model, where values below 0.2 indicate good model fit. Analysis of similarity (ANOSIM) was performed to test significant differences in fungal community dissimilarities derived from NMDS ordination, using Bray-Curtis distance matrices. ANOSIM generates a p-value (significance level < 0.05) and a correlation coefficient (R-value) ranging from 0 (completely similar) to 1 (completely different) (Clarke & Gorley, 2015). The species primarily responsible for the observed differences between treatments were identified using similarity percentages (SIMPER). NMDS, ANOSIM and SIMPER analyses were conducted using the Community Analysis Package (CAP) v. 4.0 (Henderson & Seaby, 2007).

3. RESULTS AND DISCUSSION

In this work, soil samples from burnt and non-burnt soil either treated or not with different soil conditioners (polyacrylamide, olive pomace composted with almond shells, and technosol) were analysed in terms of fungal diversity. At the time of the collection of soil samples from the different treatments, the dominant shrubs species in the survey area were *Chamaespartium tridentatum*, *Erica australis*, *Cystus ladanifer*, *Lavandula stoechas*, and *Cistus monspeliensis*, as shown in Figure 8.

These species are typical of the Montesinho Natural Park (Faria *et al*., 2023) and were widely present across all treatments at the time of sampling; however, their abundance was heterogeneous.

Figure 7: General view of the plots at the time of the soil sampling from the different treatments. (1, 5) burnt soil, (2, 6) polyacrylamide, (3, 7) olive compost, (4, 8) technosol, (9) non burnt soil and (10) superficial technosol.

The fungal community inhabiting the soil samples from the different treatments were assessed by using a culture-dependent approach. This approach is useful for obtaining living fungal cultures and detailed functional studies, but often biased towards fungi that grow well under laboratory conditions, potentially overlooking a significant portion of the fungal community that is not easily cultured (Kapinusova *et al*., 2023). The diversity detected is limited by the specific culture media (Potato Dextrose Agar - PDA) and conditions used in the present work, which may not support the growth of all fungal species inhabiting the soil. PDA is widely used due to its simple formulation and its ability to support the mycelial growth of a broad spectrum of fungi (Devi *et al*., 2018). Nonetheless, it is well-established that many microorganisms cannot be cultured in the laboratory for various reasons (Kapinusova *et al*., 2023). Culture-independent approach (metabarcoding), offers, in general, a broader and quicker overview of fungal diversity, including unculturable species, but lacks direct functional insights and can be influenced by DNA extraction and PCR biases (Reynolds *et al*., 2022). A combination of both culture- and uncultured-dependent methods has been suggested to provide a more holistic understanding of soil fungal communities (Wijayawardene *et al*., 2021).

3.1 GENERAL DESCRIPTION OF THE FUNGAL COMMUNITY

The ITS region (ITS1, 5.8S, ITS2), with approximately 600 bp in length, was utilized for the molecular identification of the isolated fungi from soils treated with the different soil conditioners. This DNA region is preferred over other molecular markers for the identification of fungi due to its high informative content and ease of amplification, and it has been designated as the official fungal barcode (Schoch *et al*., 2012). However, in this study, the ITS region alone was insufficient to identify the fungal isolates at the species level. This result was expected, as secondary barcode markers or multi-locus approaches are required to accurately identify fungi at the species level (Lucking *et al*., 2020).

Phylogenetic trees (Supporting Information) were constructed using the fungus *Colletotrichum tabaci* CPC 18945 (NR_144804.1) as an outgroup. These trees were performed for each fungal genus to determine whether isolates represent distinct species or variations within a single species. Overall, the phylogenetic trees (Figures S1-S17) allowed the classification of the 48 fungal strains (Table S1) into 31 OTUs. From the identified OTUs, *Paecilomyces* sp1, *Paecilomyces* sp2 and *Purpureocillium* sp1, showed to be closely related being clustered together on the tree (Figure S13). This result is in accordance with studies that reclassify *Paecilomyces lilacinus* into the newly proposed genus *Purpureocillium*, specifically as *Purpureocillium lilacinum* (Luangsa-ard *et al*. 2011). The reclassification of *Paecilomyces lilacinus* to *Purpureocillium lilacinum* is supported by detailed analyses of 18S rRNA genes, ITS and partial translation elongation factor 1-alpha (TEF) sequences, that differentiate *Paecilomyces lilacinus* from the genus *Paecilomyces* (Luangsa-ard *et al*. 2011). Currently, other authors, such as Lopez *et al*. (2014), Bennett & Shah (2022), and Nucci & Nouér (2023), also refer to the new name

Purpureocillium lilacinum, often indicating the former classification as a synonym in parentheses.

A similar reclassification situation occurred in the phylogenetic tree of the genus *Alternaria* (Figure S1). When performing BLASTn search, the sequence of *Ulocladium atrum* showed similarity to our isolates classified as *Alternaria* as well as with other sequences of *Alternaria* species in the database. The sequence similarities observed in the phylogenetic tree and BLASTn results between *Ulocladium atrum* and *Alternaria* species are due to the reclassification of *Ulocladium* into *Alternaria* based on phylogenetic evidence provided by Woudenberg *et al*. (2013).

The 31 OTUs identified in this work belong to 17 genera, 13 families, 9 orders, 6 classes, and 2 phyla (Figure 8). Most of the fungal isolates belong to the phylum Ascomycota (85%), with the genera *Aspergillus* (37%) and *Penicillium* (24%) accounting together for 61% of all isolates. This finding is in accordance with Egidi *et al*. (2019), who noted that Ascomycota is the most ubiquitous and diverse phylum of eukaryotes in soils worldwide. Interestingly, similar patterns were observed by Tian *et al*. (2021), who used an culture-independent method to evaluate fungal communities in soil samples and found the phylum Ascomycota to be dominant. Alem *et al*. (2020) reported comparable results, identifying Ascomycota as the prevailing phylum in both burned and unburned soils. According to Egidi *et al*. (2019), the predominance of certain ascomycetes over other taxonomic fungi can be attributed to factors such as their dispersal capabilities, modes of life, and functional traits.

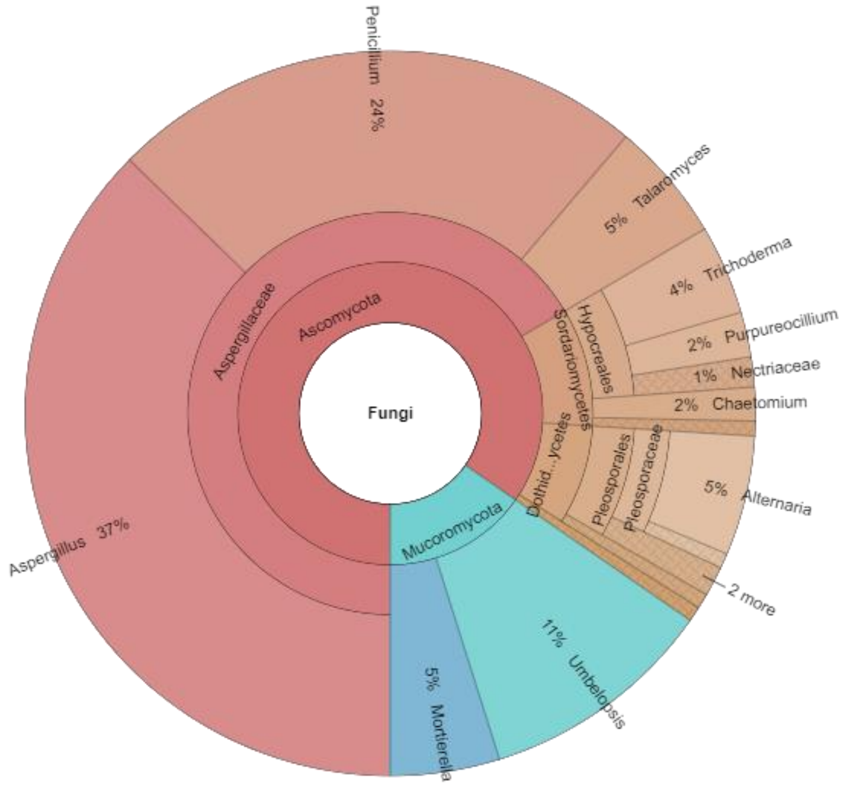

Figure 8: Krona chart illustrating the relative abundance (%) of total fungi isolated from the soil, until the genus level.

Within the Mucoromycota, which accounted for 15% of the total fungal isolates, the genus *Umbelopsis* emerged as the dominant group, comprising 11% of the isolates (Figure 9). This result is in accordance with Richardson (2009), who noted that Mucoromycota communities typically consist of only a few individuals, underscoring their ecological significance in processes like biodegradation, primary decomposition, and nutrient cycling.

3.2. FUNGAL DIVERSITY

The species richness (number taxa) and the abundance (CFU/g soil) were evaluated, also Shannon_H and Simpson_1-D diversity indices were calculated to compare the different soil treatments (Figure 10). Regarding the richness, which represents the number of fungal taxa per treatment, the overall Kruskal-Wallis test revealed no statistically significant differences between treatments. Statistically differences between treatments were only noticed at the level of fungal abundance and diversity indices. Indeed, fungal abundance was significantly higher in burnt soils not treated or treated with olive pomace compost when compared to burnt soils treated with superficial technosol. This result suggests that the amount of nutrients added by technosol appears to be insufficient to increase the abundance of fungi in burned soils. This is contradictory, given that technosols can be rich in nutrients, which can improve the growth and abundance of fungi (Soria *et al.*, 2022). The results also showed that fungal communities have the capacity to recover after fire. This capacity might be linked to the recovery of vegetation. At the time of soil sampling, the burnt soil plot was covered with a number of typical shrubs species. These plants can provide essential organic matter, improve soil structure, and create favorable conditions for fungal growth. This hypothesis is supported by the similar fungal abundance observed between the burnt soil (without any soil conditioners) and non-burnt soil. These results align with the conclusions from Dooley & Treseder's (2012) meta-analysis on the effects of fire on microbial biomass in pasture ecosystems, which noted no significant changes in fungal abundance following prescribed burns. Another interestingly observed result was the inability of olive pomace compost to promote fungal growth in burnt soils. Comparing the non burnt soil with the treatment olive pomace compost, both Simpson and Shannon indices showed statistical differences. This finding contradicts Yang *et al*. (2017), who reported that the addition of compost did not alter diversity indices compared to the control. This discrepancy could be attributed to the type of compost used: this study utilized composted olive pomace with almond husk, while Yang *et al*. (2017) used composted cow manure with vegetable residues.

The results observed for the diversity indices were slightly different compared to those of fungal abundance. Indeed, non-burnt soils were shown to harbour a significantly higher fungal diversity (Shannon_H and Simpson_1-D) than burnt soils not treated or treated with polyacrylamide or olive pomace compost (Figure 10). This finding suggests that fire may have a detrimental impact on fungal diversity, and the use of both polyacrylamide and olive pomace compost in burned soils does not appear effective in promoting fungal diversity. This contradicts partly with Cao *et al*. (2023), who found that a soil conditioner containing attapulgite and polyacrylamide influenced the fungal diversity compared to the control. Cao *et al*. (2023) reported a significantly higher Shannon index in the soil conditioner treatment, while the Simpson index showed no significant differences.

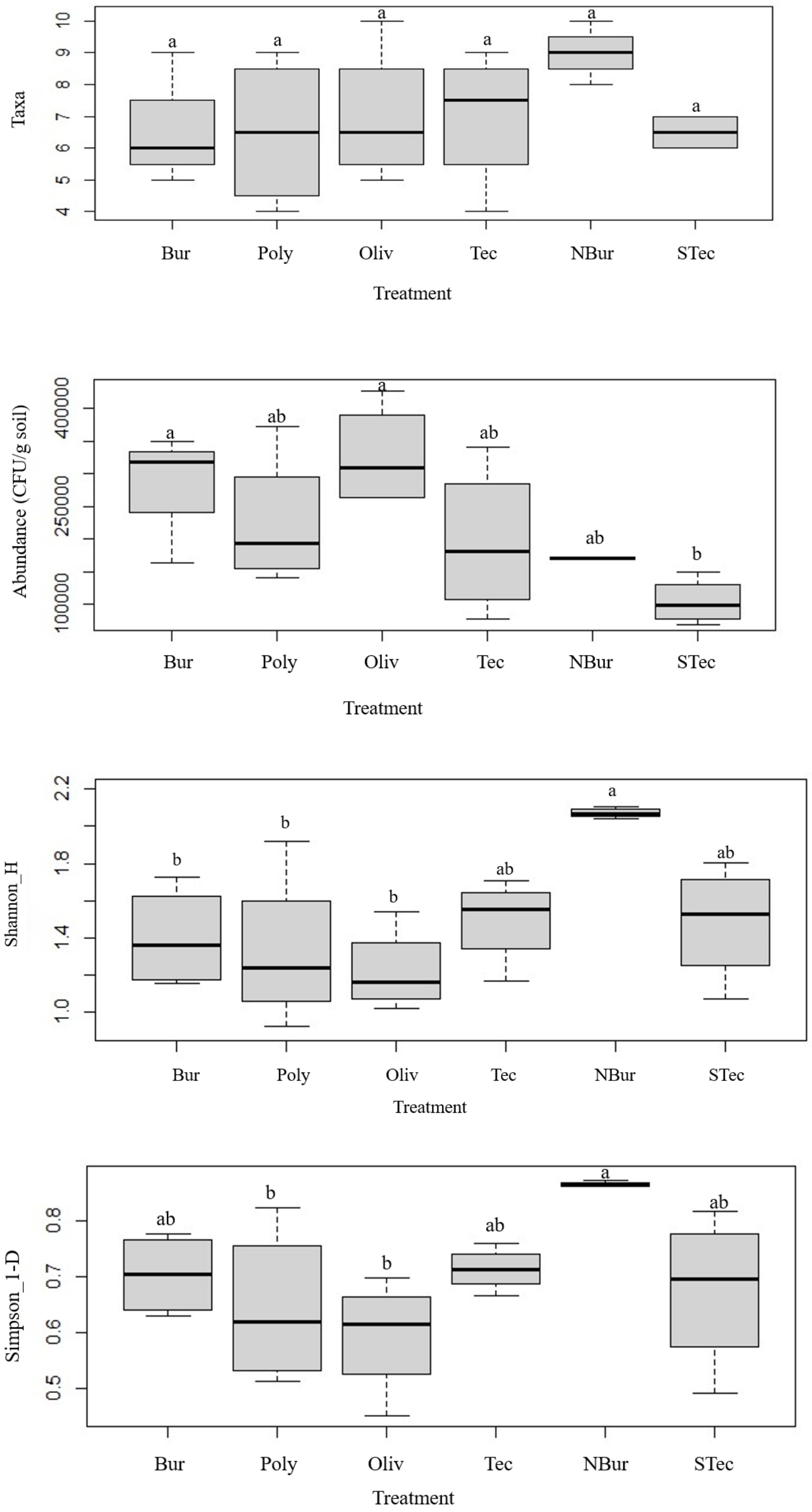

Figure 9: Comparison of fungal diversity of soils between the different treatments. Diversity was evaluated by determining fungal richness, abundance, and Shannon_H and Simpson_1-D diversity indices. Treatments: Bur – Burnt; Poly - Polyacrylamide; Oliv -

Olive pomace compost with almond shells; Tec – Technosols; STec - Superficial Technosols; NBur - Non burnt. Box plots depict medians (central horizontal lines), the inter-quartile ranges (boxes), and 95% confidence intervals (whiskers). Different letters above the bars indicated significant differences among soil treatments ($p<0.05$).

It is important to note that Cao *et al*. (2023) used 0.00204 kg/m² of polyacrylamide, whereas this study used 0.005 kg/m². The lack of significant differences in fungal diversity between non-burnt soil and burnt soil treated with technosol or superficial technosol, suggests that this soil conditioner might be important in the recovery of fungal diversity after fire. This is consistent with the findings of Epelde *et al*. (2019), who examined various technosols composed of construction and demolition waste, bentonite, and sewage sludge. When compared with a control, they found no differences in Shannon and Simpson indices, suggesting that the technosols developed a microbial community similar to that of the control.

Overall, the results indicate that prescribed fire negatively impacts fungal diversity but does not affect fungal abundance. However, with the restoration of soil cover vegetation and the application of suitable soil conditioners, fungal communities are likely to recover in burnt soils. The net effect of soil conditioners on the fungal community of burnt soils showed to be highly variable, depending on the type of soil conditioners and the method of their application. Among the three soil conditioners tested, technosols seem to be the most effective in recovering both fungal abundance and diversity in burnt soils, especially when applied mixed thoroughly into the soil. The application of olive pomace compost into burnt soils showed to favoured the abundance of fungi but did not succeed in restoring fungal diversity. Polyacrylamide was less effective in recovering fungal communities in burnt soils, in particular fungal diversity. Nonetheless, further studies are still required to determine accurately the effect of these three soil conditions in the recovery of burnt soils, using fungal communities as bio-indicators.

3.3. FUNGAL COMPOSITION AT TAXONOMIC LEVEL

For the fungal compositional analysis, a non-metric multidimensional scaling (NMDS) plot was performed (Figure 10) to visualize species distribution patterns across the various soil treatments. The results indicate that the superficial technosol treatment exhibits the most distinct fungal community composition compared to the other treatments, showing a complete separation. This result was corroborated by the ANOSIM analysis, revealing that the application of superficial technosol on burnt soils affected the composition of total fungal communities, being significantly different from the other treatments ($R \geq 0.885$, $p=0.014$; Table 3), including from the technosol treatment ($R=1$, $p=0.014$). This finding is intriguing, given that the only difference between "superficial technosol" and "technosol" treatments is the surface area they cover. While the technosol treatment was applied over a plot of 4 m^2, the superficial technosol treatment spans an area of 500 m^2. One possible explanation for this phenomenon is that the extensive coverage of the 500 m^2 superficial technosol treatment may have induced changes in the fungal community, establishing a distinct microbiome within that plot. Another hypothesis to explain this finding is that samples from the superficial technosol treatment may more accurately reflect the fungal community due to the larger sampling area. This perspective is supported by Zheng *et al*. (2021), who observed a significant increase in fungal community beta diversity with larger sampling areas.

Although to a lesser extent, the prescribed fire also showed to influenced significantly the fungal community composition when compared to unburnt soil not treated ($R=0.479$, $p=0.014$; Table 3). Interestingly, the fungal community composition of burnt soils treated with polyacrylamide ($R=0.302$, $p=0.014$), olive pomace compost ($R=0.334$, $p=0.04$) and technosol ($R=0.312$, $p=0.014$) was very similar to those found on unburnt soil (Figure 11). This suggests that using these soil conditioners could enhance the recovery of fungal communities following a fire, potentially restoring the fungal composition of burned soils to a state more similar to that of unburned soils.

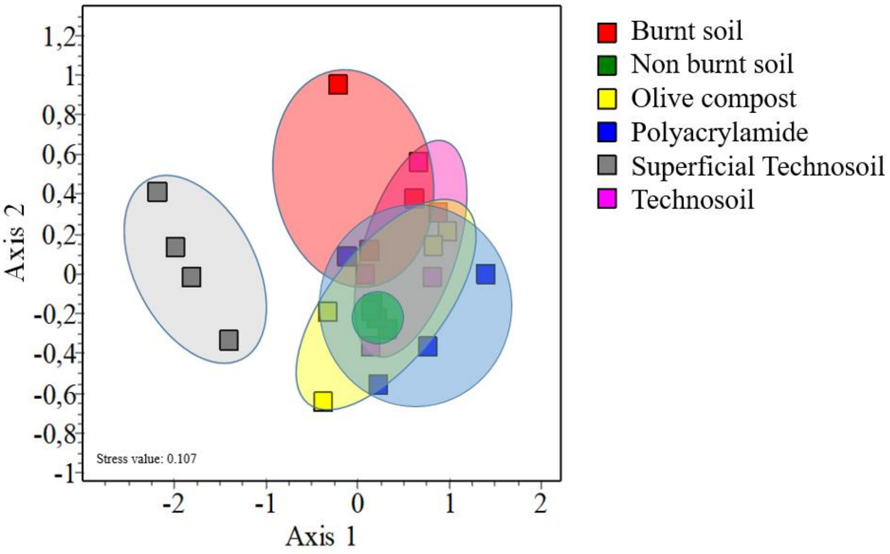

Figure 10: Non-metric multidimensional scaling (NMDS) plot corresponding to the clustering of fungal communities across the different soil treatments. The clustering analysis was performed using the Bray-Curtis coefficient.

Table 3: Analysis of similarity (ANOSIM), based on Bray-Curtis distance, showing the differences of the fungal community composition between the different soil treatments.

Comparison	R	*p-value*
Non burnt vs. Burnt	0.479	0.014
Non burnt vs. Superficial Technosol	1	0.014
Superficial technosol vs. Burnt	0.989	0.014
Superficial technosol vs. Olive compost	0.885	0.014
Superficial technosol vs. Polyacrylamide	0.958	0.014
Superficial technosol vs. Technosol	1	0.014
Non burnt vs. Olive compost	0.334	0.04
Non burnt vs. Polyacrylamide	0.302	0.014
Non burnt vs. Technosol	0.312	0.014

The analysis of the relative abundance of fungal genera in the soils of the different treatments (Figure 12) reveals that the superficial technosol treatment is particularly enriched on both *Alternaria* and *Chaetomium* species. Moreover, the genera *Chaetomium*, *Fusarium*, *Preussia*, and *Pseudopithomyces* were exclusive to this treatment, indicating a distinct community composition compared to other treatments. These differences can be attributed to the specific characteristics of technosols. Indeed, technosols are human-made soils, often created by combining various organic and inorganic materials, and thus potentially supporting distinct microbial communities. This hypothesis is supported by a study on fungal communities in various technosols applied to green roofs by Joimel *et al*. (2022), which also identified distinct fungal communities with some underrepresented genera. Similarly, Šimonovičová *et al*. (2017) examined technosols formed from mining activities and identified only 13 fungal genera, many of which were alkaliphilic and/or alkali-tolerant. The most dominant genera found in this treatment, specifically *Fusarium*, *Alternaria,* and *Chaetomium*, are known not only for their saprophytic lifestyles but also for comprising several opportunistic plant pathogen species that impact agriculture and horticulture globally (Summerell *et al*., 2010; Mamgain, Roychowdhury & Tah, 2013). In the remaining soil treatments (*i.e*., polyacrylamide, olive pomace compost, technosol), including burnt soil non treated and unburnt soil, was found an enrichment of fungal species belonging to the genera *Aspergillus* and *Penicillium* (Figure 12). This observation is consistent with several studies on soil fungal diversity, which highlight the dominance of *Aspergillus* and *Penicillium* species (Gomez *et al*., 2007; Coutinho *et al*., 2010; Demirel *et al*., 2013; Haas *et al*., 2016). Several members of these two genera have been reported to be essential for soil health and ecosystem functioning through their roles in nutrient cycling (Wang *et al*., 2022) and disease suppression (Win *et al*., 2021). The genus *Talaromyces* was found in all treatments except in the superficial technosol. This genus is significant due to its ubiquity, with some species being heat-resistant and others known for their enzymes used in saccharide synthesis, the preparation of chiral building blocks, and their application in pest biocontrol (Zhai *et al*., 2016).

Interestingly, the genera *Curvularia* and *Cladosporium* were isolated exclusively in the burnt soil treated with olive pomace compost (Figure 12). Both genera are cosmopolitan; with *Curvularia* comprising species that can be detrimental to plants, by acting as plant pathogens (Marin-Felix *et al*., 2020) but others provide plant benefits, by establishing mutualistic interaction as endophytes (Mehta, Meena & Nagda. 2022). *Cladosporium* includes some species that are pathogens of various crops, with others being of only endemic importance (Ogórek *et al*., 2012), other species have also been reported as growth promoters (Yang *et al*., 2023). Similarly, the genus *Coniochaeta* was uniquely isolated from the burnt soils treated with polyacrylamide (Figure 12). This genus comprises filamentous fungi involved in the degradation of decomposing wood in soil and likely participates in hemicellulose degradation (Lopez *et al*., 2007).

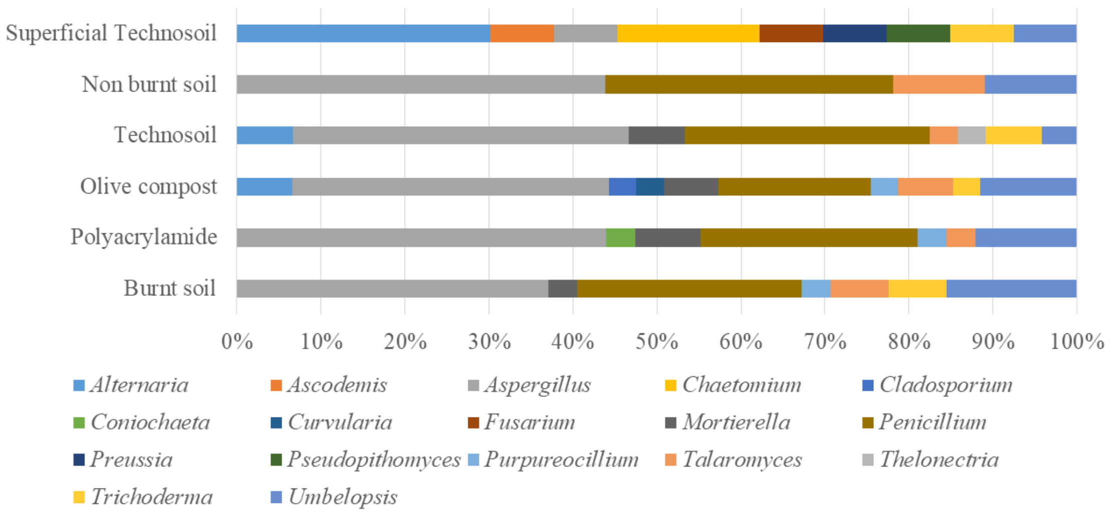

Figure 11: Relative abundance of fungal genera inhabiting the soils of the different treatments.

The comparison of fungal community composition between the different soil treatments at lower taxonomic levels (i.e., OTUs) also reveals interesting results (Table S2 – Table S10). According to the SIMPER analysis, burnt soil is distinct from the non-burnt soil, due to the higher abundance of the OTUs *Umbelopsis* sp1, *Aspergillus* sp2, and *Penicillium* sp1 in the unburned soil (Table S2).

Also, according to the SIMPER analysis, both *Chaetomium* sp1 and *Alternaria* sp5 were the OTUs that mainly contributed to the dissimilarity between fungal community's composition of superficial technosols and the other soil treatments (Table S3, S4, S5, S6 and S7). These two OTUs were exclusively detected in burnt soils treated with superficial technosols. *Chaetomium*, known for its cosmopolitan distribution, exhibits broad substrate colonization capabilities (Abdel-Azeem *et al*., 2021). Similarly, *Alternaria* is associated with diverse substrates such as seeds, plants, agricultural produce, animals, soil, and the atmosphere, with certain species recognized as significant plant pathogens (Woudenberg, 2013). The treatment of the superficial technosol differs also from the other treatments, due to the absence of the genera *Aspergillus* and *Penicillium,* that were present in varying proportions in the remaining treatments.

The comparison between unburnt soil and burnt soil treated with olive compost, polyacrylamide, or technosol, indicated differences mostly at the level of *Aspergillus* sp2 and *Penicillium* sp1 (Tables S8, S9 and S10). These two OTUs were more abundant in the unburnt soil.

Overall, these findings suggest that across the technosol, polyacrylamide, olive compost, burnt soil, and non-burnt soil treatments, the fungal community has reached a stable state. This observation is consistent with the conclusions drawn by Epelde *et al*. (2019), who studied the microbiota of three technosol formulations and observed that after three years, the microbial community and soil properties resembled those of undisturbed forest soil nearby. This indicates that the evaluated technosols were developing structural and functional characteristics similar to healthy soil, thereby contributing positively to ecosystem services.

3.4. FUNGAL COMPOSITION AT FUNCTIONAL LEVEL

Fungi play a crucial role in the soil, performing essential ecological functions such as decomposition, transformation, soil structure improvement, symbiotic relationships, disease suppression, and efficient utilization of organic substrates, which facilitate nutrient cycling processes (Mehta, Meena & Nagda. 2022). Because of the vital functions played by this group of microorganisms in the soil, in this work was used the FUNGuild annotation tool to analyse the functional guilds of fungal communities inhabiting the soils of the different treatments. This tool, proposed by Nguyen *et al*. (2016), classified the fungal OTUs into various trophic groups. These groups were further divided into specific ecological guilds, comprising fungi with similar lifestyle modes.

The classification of fungi into the different trophic groups, reveal that saprotrophic-pathotrophic-symbiotrophic fungi were the most dominant in all the treatments, accounting for more than 60% of the total fungal abundance (Figure 12). Saprotrophic fungi contribute approximately to 20% of the total abundance. These results contrast with those reported by Langa-Lomba *et al*. (2023), who examined fungal communities in two commercial vineyards. In their study, saprotrophic fungi predominated, constituting an average of 42% of the community, whereas fungi exhibiting all three trophic modes represented an average of 10.5% of the total community.

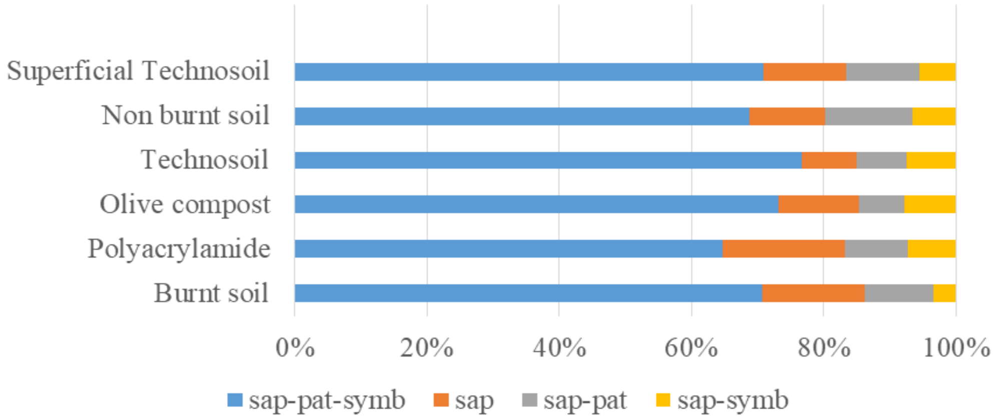

Figure 12: FUNGuild analysis of fungal functional groups. Relative abundance of the different trophic groups in each soil treatment. Abbreviations: sap-pat-symb - saprotrophic-pathotrophic-symbiotrophic; sap –saprotrophic; sap-pat - saprotrophic-pathotrophic; and sap-symb - saprotrophic-symbiotrophic.

The trophic groups identified were then further divided into specific ecological guilds (Figure 14). Members of an ecological guild share common strategies for acquiring food, habitat use, or other ecological functions, regardless of their taxonomic classification. Following the ANOVA analysis, no statistically significant differences were found between soil treatments across the assessed guilds (Figure 13). Within the trophic group pathotrophic, guilds of animal pathogens followed by plant pathogens were the most dominant across all soil treatments. The present study was performed in the Montesinho Natural Park. This park hosts a diverse fauna, including the Iberian wolf, deer, water mole, wildcat, greater horseshoe bat, water vole, and approximately 160 bird species (ICNF, 2023). The high abundance of animal pathogen fungi in the experimental plots may be attributed to the presence of these animals.

At the symbiotrophic trophic group, the endophytes were the most abundant fungal guild across all the soil treatments (Figure 14). Endophytes are microorganisms, including fungi, that live within a plant for all or part of their life cycle without causing any apparent harm to the host (Hardoim *et al.*, 2015). Fungal endophytes inhabiting the plants not only promote the growth of the host but also enhance its resistance to biotic and abiotic factors (Hardoim *et al.*, 2015). Their interactions with plants and other soil organisms contribute significantly to the overall health and productivity of the soil ecosystem.

At the saprotrophic trophic group, undefined saprotroph were the most abundant fungal guild across the treatments (Figure 13). This result agrees with the results found by Nguyen *et al.*, (2016) who also found undefined saprotrophs as the most abundant ecological guild for forest, grassland and wood environments.

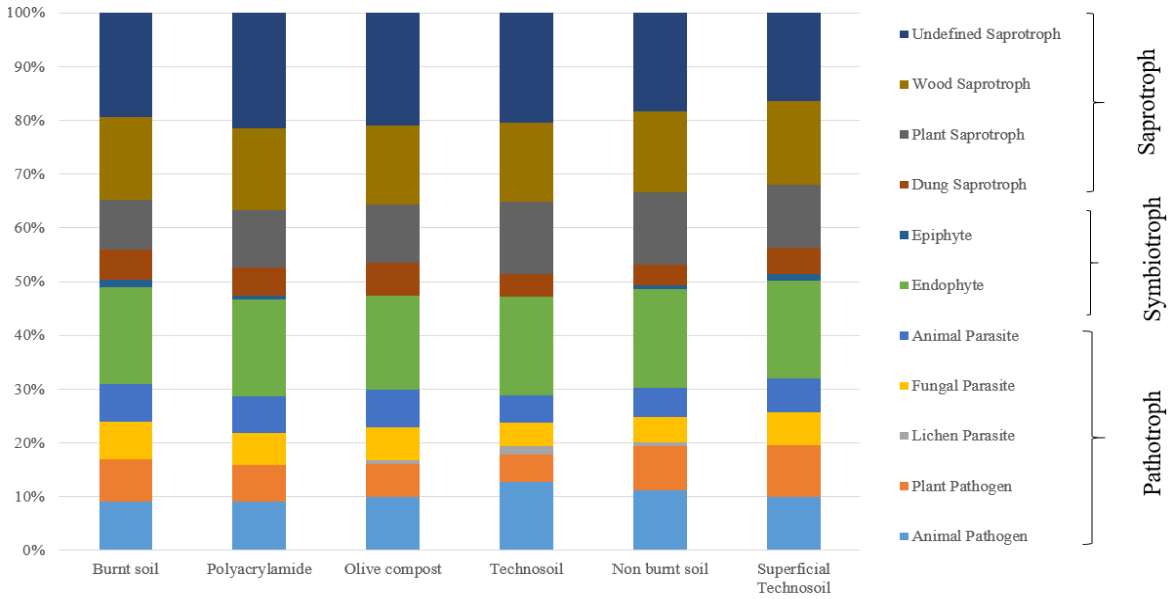

Figure 13: Relative abundance of each ecological guilds of the fungi inhabiting the soils of the different treatments.

Overall, the results suggest that the different soil conditioners and the prescribed fire did not affect the relative abundances of fungal guilds. Thus, although the differences found on fungal diversity and composition among the different treatments, no significant differences were observed in ecological guilds. This observation supports the hypothesis proposed by Allison & Martiny, (2008), suggesting that despite shifts in microbial composition, functional similarities to the original community can persist.

4. CONCLUSIONS AND FUTURE PERSPECTIVES

The study addressed the taxonomic and functional diversity of the fungal community under different soil conditions, including burned, unburned, and treated with various conditioners, in Montesinho Natural Park. The fungal community was assessed using a culture-dependent approach, which, despite its limitations, provided valuable insights into the fungal diversity. The ITS region was employed for the molecular identification of the fungi, revealing that a single genetic marker was insufficient for species-level identification.

Phylogenetic analysis categorized 48 fungal strains into 31 OTUs, belonging to 17 genera, 13 families, 9 orders, 6 classes, and 2 phyla. Overall, the genera *Aspergillus* and *Penicillium*, were the most predominant.

The diversity analysis indicated that fire had a detrimental impact on fungal diversity but not on fungal abundance. Fungal composition analysis highlighted that superficial technosol treatment led to a distinct fungal community composition, enriched with genera like *Alternaria* and *Chaetomium*. This uniqueness was attributed to the specific properties of technosols, which are man-made soils with varied organic and inorganic materials. The results indicate that the fungal community in soils treated with technosol, polyacrylamide, and olive compost achieved a stable state, similar to undisturbed soil. Nevertheless, the efficacy of these three soil conditioners on the recovery of burnt soil varies. Technosols are likely to be the most effective for restoring both fungal abundance and diversity. Olive pomace compost enhances fungal abundance but does not restore diversity, whereas polyacrylamide is the least effective, particularly in recovering fungal diversity.

At the functional level, the FUNGuild annotation revealed that the dominant trophic mode across all treatments was saprotrophic-pathotrophic-symbiotrophic fungi. Within this group, animal and plant pathogens were prevalent, likely due to the diverse fauna in the Montesinho Natural Park. The study found no significant differences in the relative abundances of fungal guilds across treatments, suggesting functional resilience in the fungal community despite changes in diversity and composition.

Overall, this study demonstrates that while prescribed fire negatively affects fungal diversity, the application of suitable soil conditioners, can aid in the recovery of fungal communities in burnt soils. Further research is required to optimize the use of these conditioners and better understand their long-term impacts on soil health and ecosystem function, and to understand the physicochemical variables that may influence microbial communities at different application scales.

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SUPPORTING INFORMATION

Table S1: Blast results showing the final taxonomic classification of the fungal isolates based on ribosomal marker ITS (ITS1, 5.8S and ITS2).

Final classification (accession number)	**Query cover (%)**	**E value**	**Percentage of identity (%)**	**BLAST classification (accession number)**
Aspergillus sp1	100	0.0	100	*Aspergillus felis* CBS 130245 (NR_138341.1)
Aspergillus sp2	100	0.0	100	*Aspergillus felis* CBS 130245 (NR_138341.1)
Penicillium sp1	100	0.0	99.58	*Penicillium glaucoroseum* NRRL 908 (NR_138338.1) *Penicillium ludwigii* CBS 417.68 (NR_138339.1)
Aspergillus sp3	100	0.0	100	*Aspergillus felis* CBS 130245 (NR_138341.1)
Umbelopsis sp1	100	0.0	100	*Umbelopsis dimorpha* CBS 110039 (NR_111664.1)
Umbelopsis sp2	100	0.0	100	*Umbelopsis dimorpha* CBS 110039 (NR_111664.1)
Aspergillus sp4	100	0.0	100	*Aspergillus felis* CBS 130245 (NR_138341.1)
Mortierella sp1	98	0.0	98.49	*Mortierella rishikesha* CBS 652.68 (NR_111564.1)
Penicillium sp3	99	0.0	98.72	*Penicillium camponotum* CBS 140982 (NR_158823.1) *Penicillium reticulisporum* NRRL 3447 (NR_121231.1)
Aspergillus sp5	100	0.0	100	*Aspergillus felis* CBS 130245 (NR_138341.1)
Aspergillus sp6	100	0.0	98.80	*Aspergillus felis* CBS 130245 (NR_138341.1)
Aspergillus sp7	100	0.0	100	*Aspergillus felis* CBS 130245 (NR_138341.1)
Paecilomyces sp1	88	0.0	98.13	*Paecilomyces lilacinus* ATCC 10114 (AY213665.1)
Umbelopsis sp3	100	0.0	95.80	*Umbelopsis angularis* CBS 603.68 (NR_137072.1)
Penicillium sp4	100	0.0	100	*Penicillium soli* KUMCC 18-0202 (MT152337.1) *Penicillium tengii* CS27-03 (OQ870735.1) *Penicillium cremeogriseum* CBS 223.66 (NR_111505.1)
Penicillium sp5	100	0.0	100	*Penicillium radiatolobatum* CBS 340.79 (NR_163679.1) *Penicillium murcianum* CBS 161.81 (NR_138358.1) *Penicillium echinatum* DTO 189-B8 (KP016840.1)

				Penicillium dunedinense DTO_244G1 (KJ775678.1) *Penicillium canescens* NRRL910 (NR_121256.1)
Aspergillus sp8	99	0.0	100	*Aspergillus felis* CBS 130245 (NR_138341.1)
Trichoderma sp1	100	0.0	99.47	*Trichoderma neokoningii* CBS 120070 (NR_138446.1) *Trichoderma dorotheae* CBS 119089 (NR_166014.1)
Purpureocillium sp1	100	0.0	99.82	*Paecilomyces lilacinus* ATCC 10114 (AY213665.1) *Purpureocillium lilacinum* NRRL 895 (NR_165946.1)
Trichoderma sp2	100	3e-173	100	*Trichoderma nordicum* ACCC 39713 (NR_184883.1) *Trichoderma scalesiae* CBS 120069 (PP732322.1) *Trichoderma atroviride* CBS 142.95 (AF456917.1) *Trichoderma uncinatum* YMF 1.04622 (MK795994.1) *Trichoderma obovatum* YMF 1.06211 (MN977803.1)
Aspergillus sp9	100	0.0	100	*Aspergillus felis* CBS 130245 (NR_138341.1)
Alternaria sp1	99	0.0	100	*Alternaria multiformis* CBS 102060 (MH862776.1) *Alternaria consortialis* CBS 104.31 (MH855147.1)
Aspergillus sp10	100	0.0	100	*Aspergillus felis* CBS 130245 (NR_138341.1)
Aspergillus sp11	100	0.0	100	*Aspergillus sojae* CBS 100928 (MH862715.1) *Aspergillus toxicarius* CBS822.72 (MH860619.1) *Aspergillus parasiticus* NRRL 502 (NR_121219.1) *Aspergillus transmontanensis* CBS 130015 (NR_137520.1)
Thelonectria sp1	100	0.0	100	*Thelonectria nodosa* CBS 132327 (NR_160260.1)
Trichoderma sp3	99	2e-169	100	*Trichoderma saturnisporum* (NR_103704.1) *Trichoderma pseudokoningii* (NR_120296.1)
Penicillium sp10	100	0.0	100	*Penicillium radiatolobatum* CBS 340.79 (NR_163679.1) *Penicillium murcianum* CBS 161.81 (NR_138358.1) *Penicillium echinatum* DTO 189-B8 (KP016840.1) *Penicillium dunedinense* DTO_244G1 (KJ775678.1)

				Penicillium canescens NRRL910 (NR_121256.1) *Penicillium jensenii* NRRL 909 (NR_121297.1)
Talaromyces sp1	100	0.0	99.81	*Talaromyces yunnanensis* (MT152339.1) *Talaromyces verruculosus* NRRL1050 (KF741994.1)
Aspergillus sp12	100	0.0	100	*Aspergillus subramanianii* CBS 138230 (OL711825.1)
Talaromyces sp2	100	0.0	99.82	*Talaromyces coalescens* CBS 103.83 (NR_120008.1)
Coniochaeta sp1	99	0.0	99.78	*Coniochaeta marina* (MK458764.1) *Coniochaeta cipronana* CBS 144016 (NR_157478.1)
Aspergillus sp13	100	0.0	100	*Aspergillus papuensis* CBS 841.96 (OL711748.1)
Chaetomium sp1	100	0.0	100	*Chaetomium cochliodes* CBS 155.52 (NR_151835.1) *Chaetomium spiculipilium* CBS 373.66 (NR_144836.1) *Chaetomium spirochaete* CBS 730.84 (NR_144823.1)
Preussia sp1	100	0.0	100	*Preussia flanaganii* CBS 112.73 (NR_077168.1)
Trichoderma sp4	100	0.0	99.47	*Trichoderma zeloharzianum* YMF 1.00268 (NR_165872.1) *Trichoderma afarasin* CBS 130755 (NR_137301.1) *Trichoderma simmonsii* CBS 130431 (NR_137297.1)
Chaetomium sp2	100	0.0	99.81	*Chaetomium cochliodes* CBS 155.52 (NR_151835.1) *Chaetomium spiculipilium* CBS 373.66 (NR_144836.1) *Chaetomium spirochaete* CBS 730.84 (NR_144823.1)
Trichoderma sp5	100	0.0	99.47	*Trichoderma zeloharzianum* YMF 1.00268 (NR_165872.1) *Trichoderma afarasin* CBS 130755 (NR_137301.1) *Trichoderma simmonsii* CBS 130431 (NR_137297.1)
Alternaria sp2	100	0.0	100	*Alternaria multiformis* CBS 102060 (MH862776.1) *Alternaria consortialis* CBS 104.31 (MH855147.1) *Ulocladium atrum* ATCC 18040 (AF229486.1)
Alternaria sp3	100	0.0	100	*Alternaria tellustris* CBS 538.83 (NR_135961.1)
Ascodesmis sp1	99	3e-139	90.23	*Ascodesmis rosicola* GACP GUCC 190035.1 (NR_184935.1)
Cladosporium sp1	92	4e-148	89.36	*Cladosporium grevilleae* (NR_119960.1)

				Cladosporium kenpenggii CPC 19248 (KY646222.1) *Cladosporium brigadeirensis* COAD 2257 (MZ318435.1) *Cladosporium chusqueae* COAD 2258(MZ318430.1) *Cladosporium aulonemiae* COAD 2269 (MZ318427.1) *Cladosporium hillianum* ICMP 17529 (OL639132.1) *Cladosporium cucumerinum* CBS 171.52 (MW810260.1)
Paecilomyces sp2	100	0.0	99.42	*Paecilomyces lilacinus* ATCC 10114 (AY213665.1)
Curvularia sp1	99	0.0	98.92	*Curvularia coatesiae* BRIP 24261 (NR_158444.1)
Alternaria sp4	100	0.0	100	*Alternaria angustiovoidea* CBS 195.86 (MH861939.1) *Alternaria astragalicola* JZB3180064 (MW793891.1)
Alternaria sp5	100	0.0	99.06	*Alternaria eureka* ATCC 66974 (NR_136016.1)
Pseudopithomyces sp1	99	0.0	98.57	*Pseudopithomyces angolensis* (NR_161121.1)
Aspergillus sp14	99	0.0	100	*Aspergillus sojae* CBS 100928 (MH862715.1) *Aspergillus toxicarius* CBS822.72 (MH860619.1) *Aspergillus parasiticus* NRRL 502 (NR_121219.1) *Aspergillus transmontanensis* CBS 130015 (NR_137520.1)
Fusarium sp1	99	0.0	99.60	*Fusarium foetens* CBS 110286 (NR_159865.1)

PHYLOGENETIC TREES

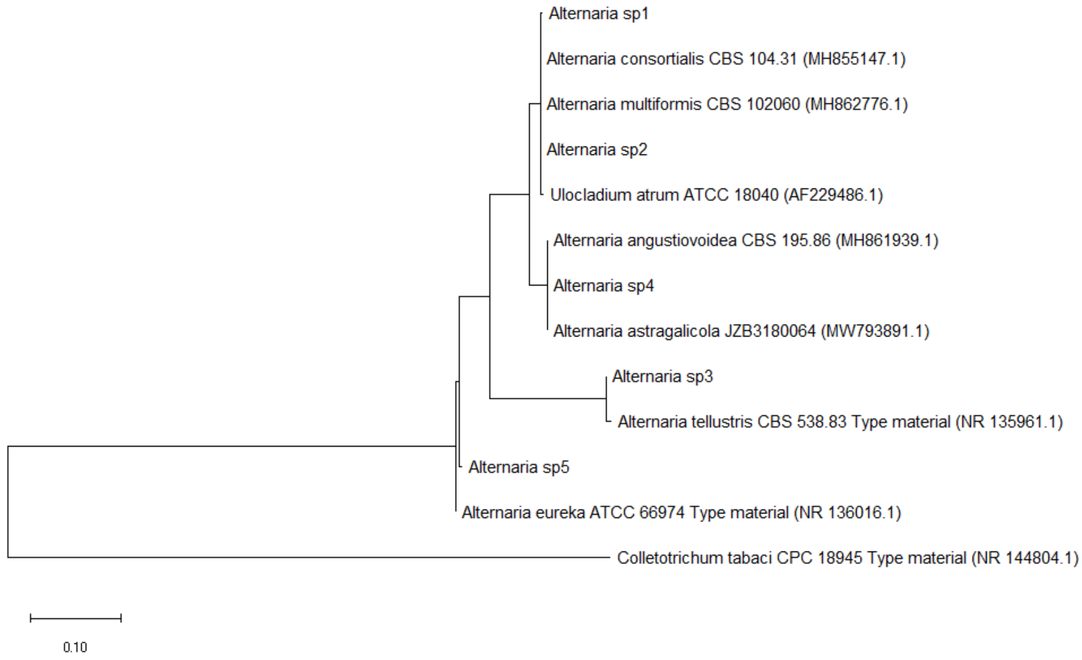

Figure S1: Maximum likelihood tree of ITS sequences of fungal strain of the genus *Alternaria*. The tree used K2+G model. *Colletotrichum tabaci* was used as the outgroup.

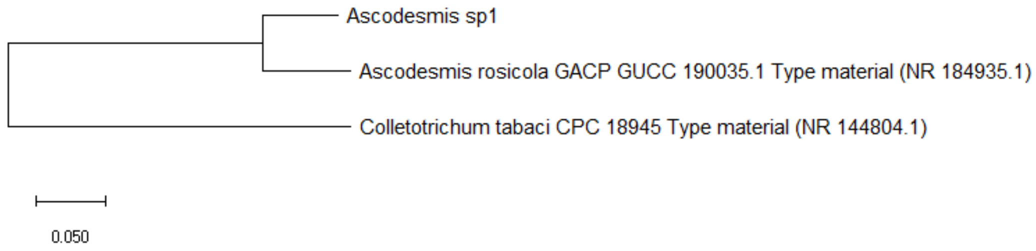

Figure S2: Maximum likelihood tree of ITS sequences of fungal strain of the genus *Ascodesmis*. The tree used K2 model. *Colletotrichum tabaci* was used as the outgroup.

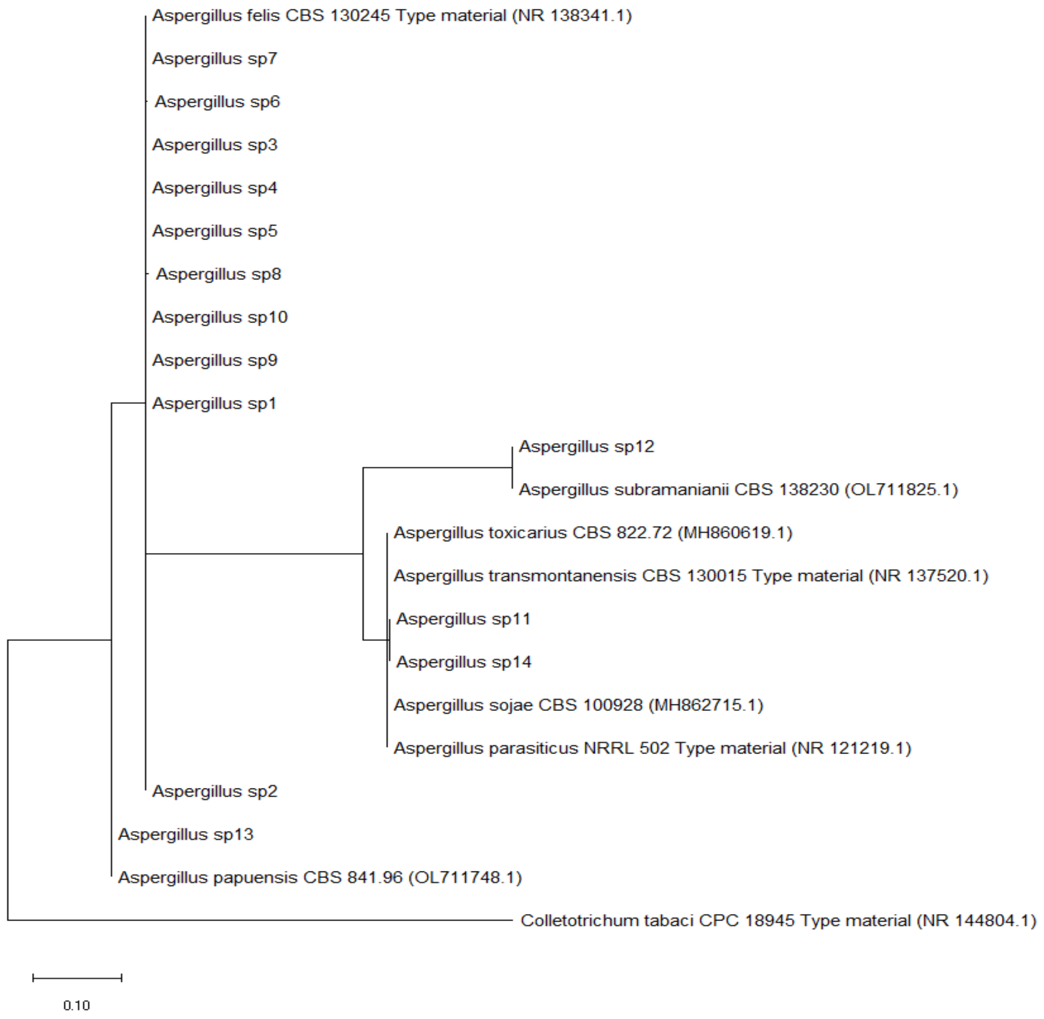

Figure S3: Maximum likelihood tree of ITS sequences of fungal strain of the genus *Aspergillus*. The tree used T92 + G model. *Colletotrichum tabaci* was used as the outgroup.

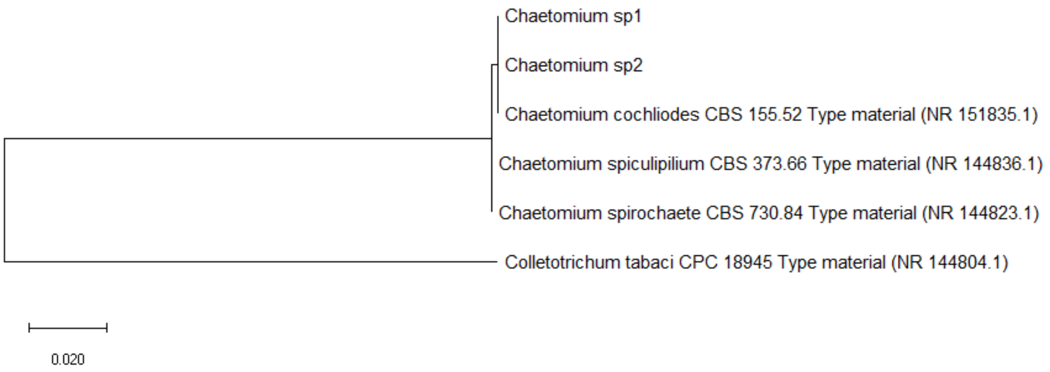

Figure S4: Maximum likelihood tree of ITS sequences of fungal strain of the genus *Chaetomium*. The tree used JC model. *Colletotrichum tabaci* was used asthe outgroup.

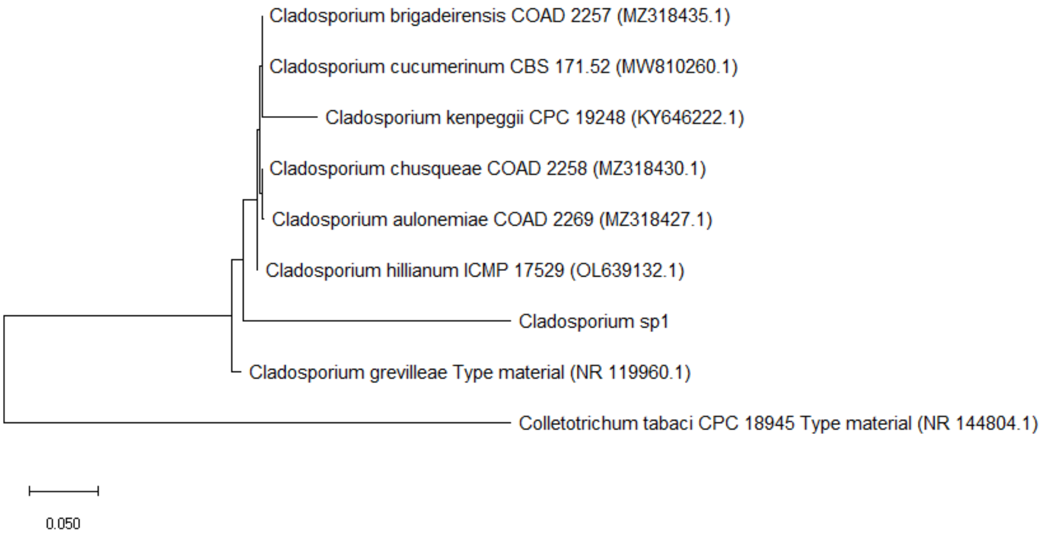

Figure S5: Maximum likelihood tree of ITS sequences of fungal strain of the genus *Cladosporium*. The tree used K2+G model. *Colletotrichum tabaci* was used as the outgroup.

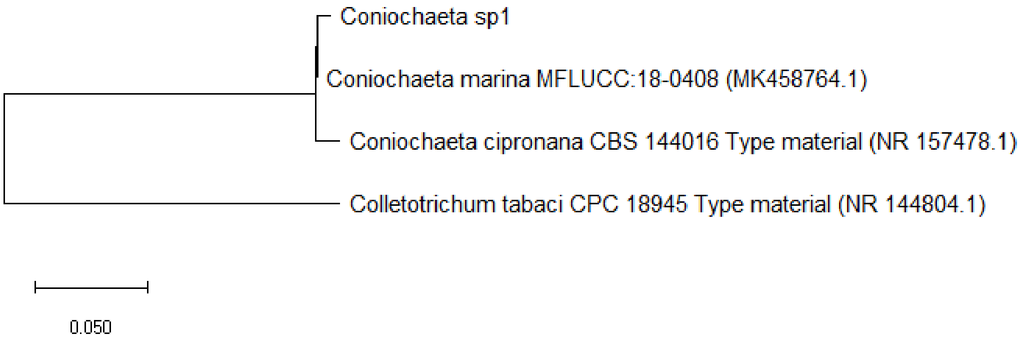

Figure S6: Maximum likelihood tree of ITS sequences of fungal strain of the genus *Coniochaeta*. The tree used K2 model. *Colletotrichum tabaci* was used as the outgroup.

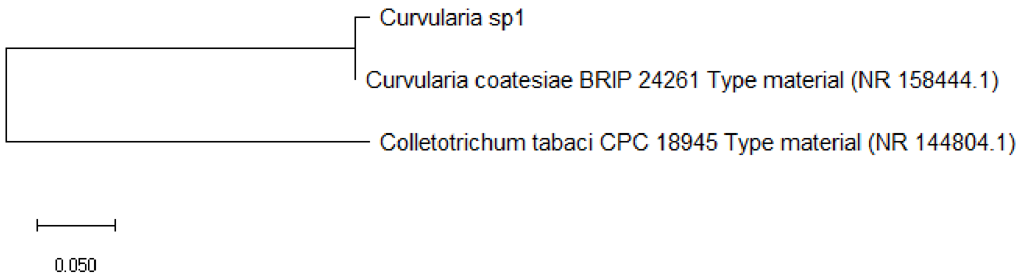

Figure S7: Maximum likelihood tree of ITS sequences of fungal strain of the genus *Curvularia*. The tree used K2 model. *Colletotrichum tabaci* was used as the outgroup.

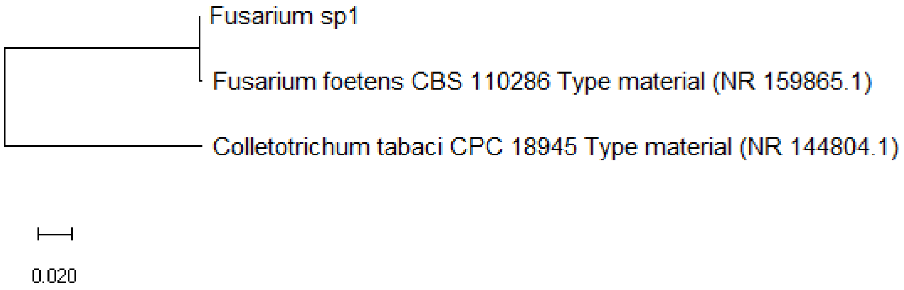

Figure S8: Maximum likelihood tree of ITS sequences of fungal strain of the genus *Fusarium.* The tree used K2 model. *Colletotrichum tabaci* was used as the outgroup.

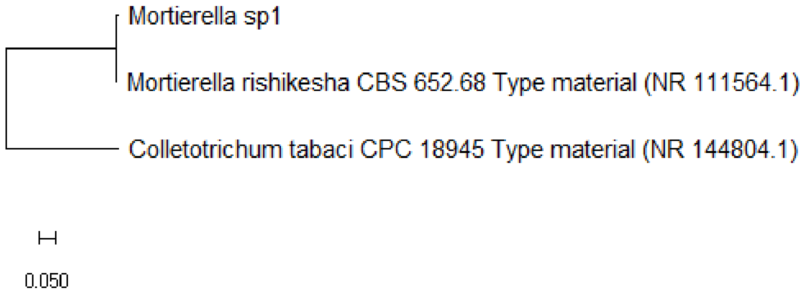

Figure S9: Maximum likelihood tree of ITS sequences of fungal strain of the genus *Mortierella*. The tree used K2 model. *Colletotrichum tabaci* was used as the outgroup.

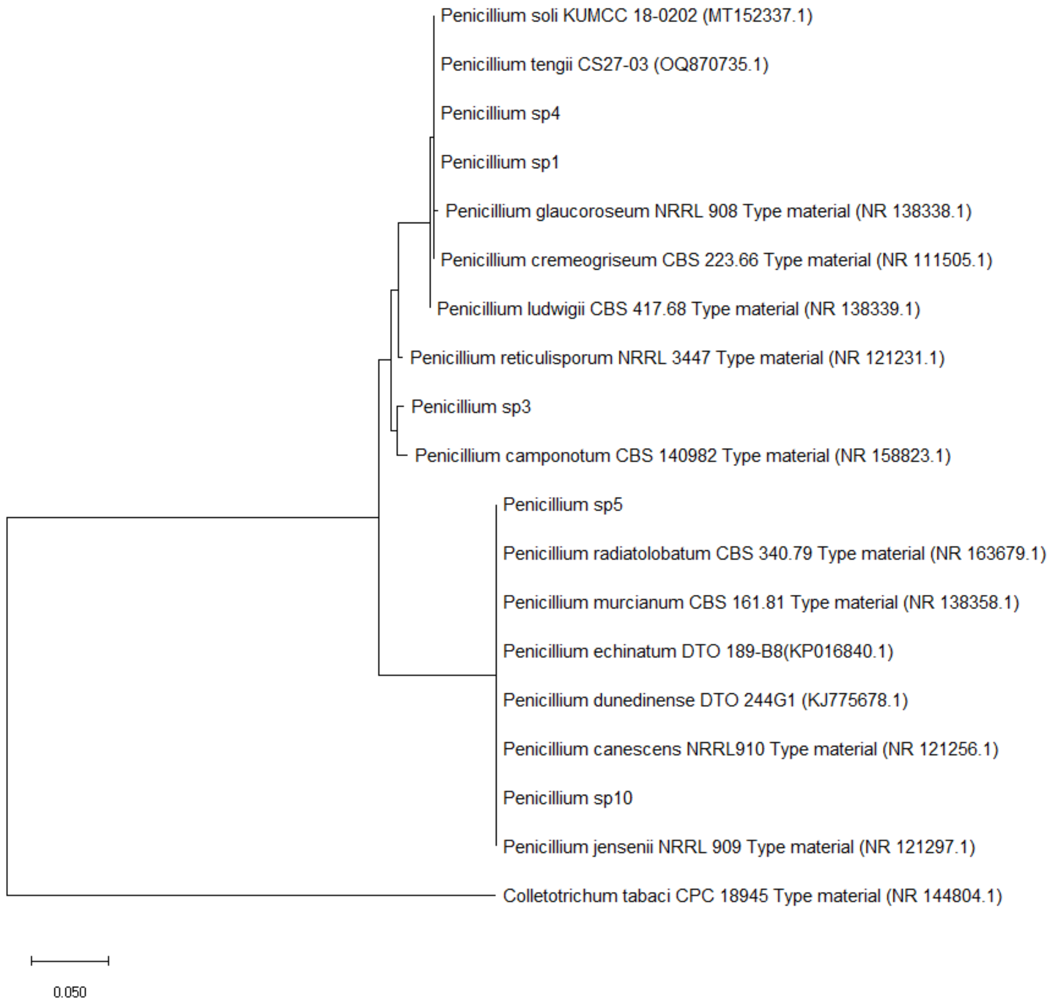

Figure S10: Maximum likelihood tree of ITS sequences of fungal strain of the genus *Penicillium*. The tree used K2+I model. *Colletotrichum tabaci* was used as the outgroup.

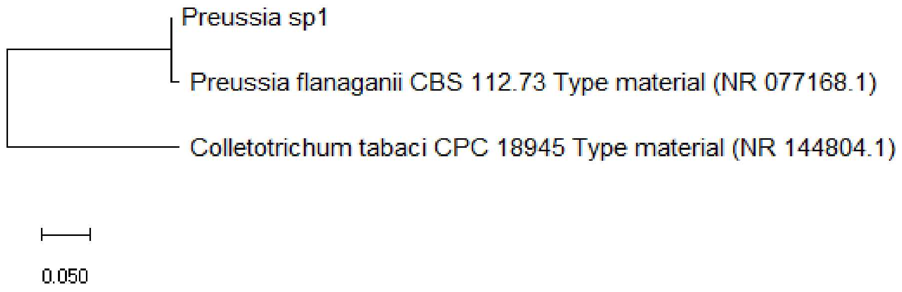

Figure S11: Maximum likelihood tree of ITS sequences of fungal strain of the genus *Preussia*. The tree used JC model. *Colletotrichum tabaci* was used as the outgroup.

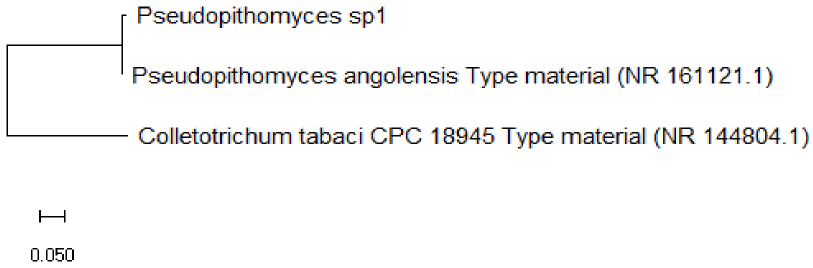

Figure S12: Maximum likelihood tree of ITS sequences of fungal strain of the genus *Pseudopithomyces*. The tree used K2 model. *Colletotrichum tabaci* was used as the outgroup.

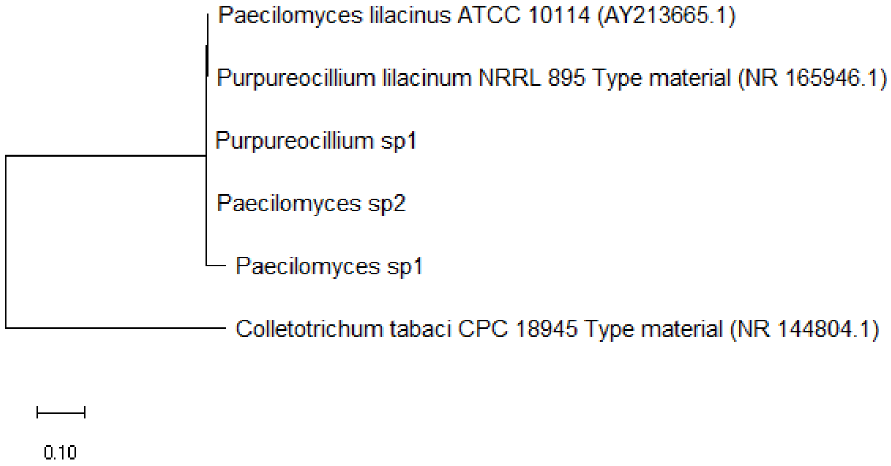

Figure S13: Maximum likelihood tree of ITS sequences of fungal strain of the genus *Purpureocillium*. The tree used JC+G model. *Colletotrichum tabaci* was used as the outgroup.

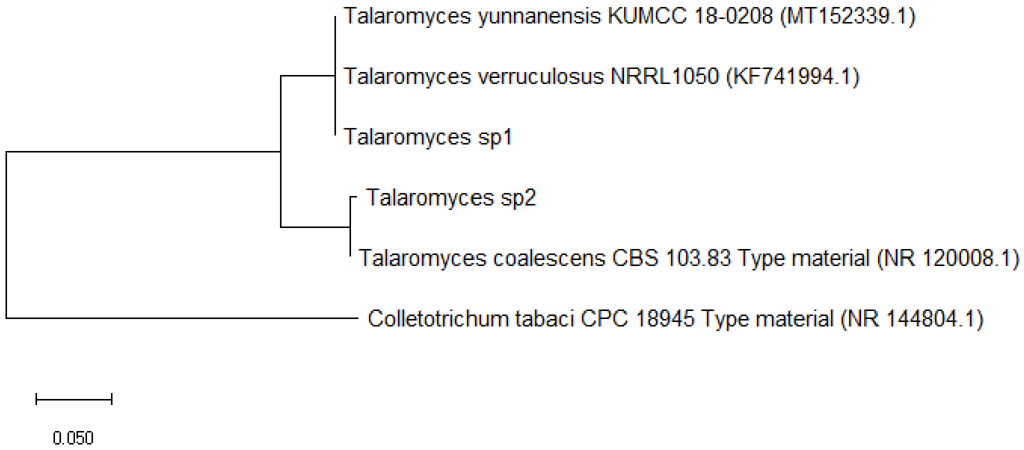

Figure S14: Maximum likelihood tree of ITS sequences of fungal strain of the genus *Talaromyces*. The tree used K2 model. *Colletotrichum tabaci* was used as the outgroup.

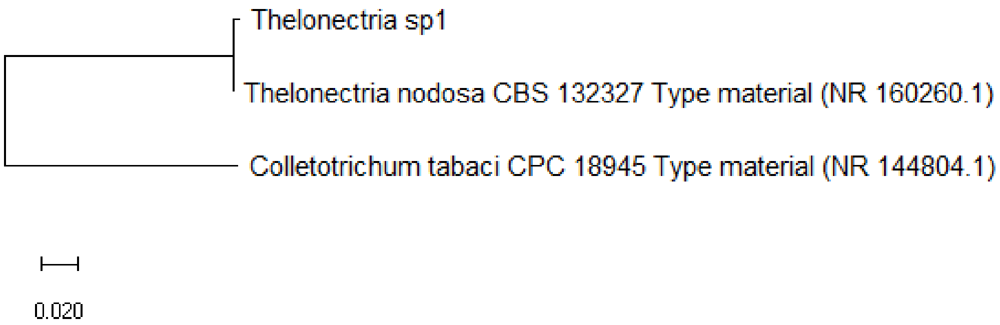

Figure S15: Maximum likelihood tree of ITS sequences of fungal strain of the genus *Thelonectria*. The tree used K2 model. *Colletotrichum tabaci* was used as the outgroup.

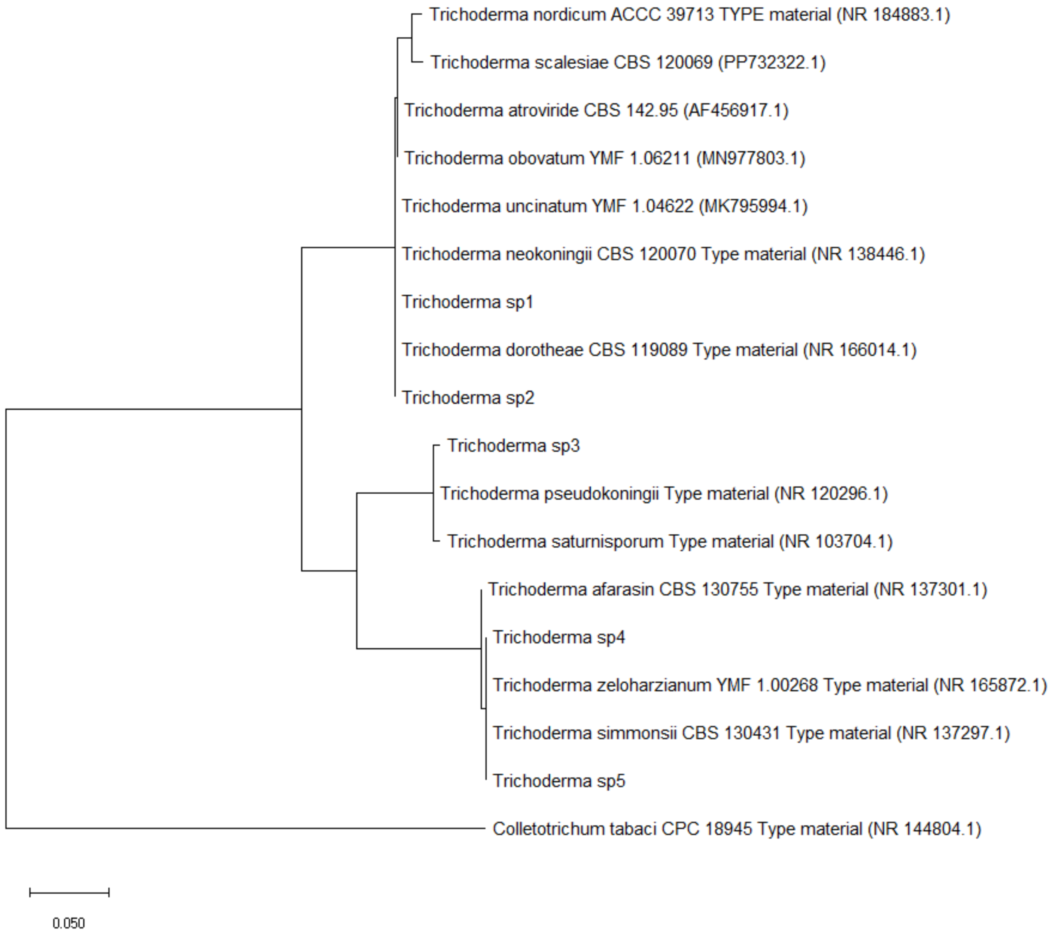

Figure S16: Maximum likelihood tree of ITS sequences of fungal strain of the genus *Trichoderma*. The tree used K2+G model. *Colletotrichum tabaci* was used as the outgroup.

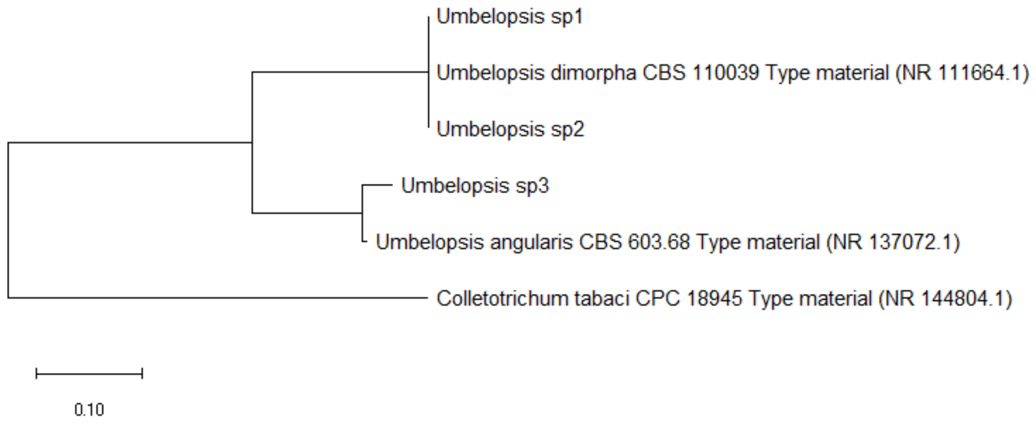

Figure S17: Maximum likelihood tree of ITS sequences of fungal strain of the genus *Umbelopsis*. The tree used T92+I model. *Colletotrichum tabaci* was used as the outgroup.

Table S2: SIMPER analysis identifying the % contribution (up to 50%) of each fungal OTU to the Bray Curtis dissimilarity metric between burnt soil and non burnt soil.

OTU	Burnt soil Ave Abund (Log_{10} CFU/g)	Non burnt soil Ave Abund (Log_{10} CFU/g)	% Contribution	% Cumulative
Umbelopsis sp1	1.25	4	9.27	9.27
Aspergillus sp2	1.25	4	9.27	18.54
Penicillium sp1	1	4	8.67	27.21
Trichoderma sp3	2.25	0	6.60	33.81
Aspergillus sp13	0	2.25	6.24	40.05
Aspergillus sp11	0	2.25	6.24	46.29
Penicillium sp5	2.25	4	5.91	52.2

Table S3: SIMPER between non burnt soil and superficial technosol. Average dissimilarity – 88.77

OTU	Non burnt soil Ave Abund (Log$_{10}$ UFC/mL)	Superficial Technosol Ave Abund (Log$_{10}$ UFC/mL)	% Contribution	% Cumulative
Chaetomium sp1	0	4.5	8.03	8.03
Penicillium sp2	4.5	0	8.01	16.04
Penicillium sp1	4	0	7.13	23.17
Penicillium sp5	4	0	7.13	30.3
Aspergillus sp1	4	0	7.13	37.43
Alternaria sp5	0	4	7.13	44.56
Aspergillus sp6	2.25	0	4.03	48.59
Aspergillus sp13	2.25	0	4.03	52.62

Table S4: SIMPER between burnt soil and superficial technosol. Average dissimilarity – 95.07.

OTU	Burnt soil Ave Abund (Log$_{10}$ UFC/mL)	Superficial Technosol Ave Abund (Log$_{10}$ UFC/mL)	% Contribution	% Cumulative
Aspergillus sp1	5	0	9.54	9.54
Chaetomium sp1	0	4.5	8.60	18.14
Alternaria sp5	0	4	7.63	25.77
Penicillium sp2	3.5	0	6.49	32.26
Aspergillus sp6	3.25	0	6.00	38.26
Trichoderma sp3	2.25	0	4.60	42.86
Penicillium sp 5	2.25	0	4.56	47.42
Ascodesmis sp1	1.25	2.25	4.53	51.95

Table S5: SIMPER between olive compost and superficial technosol. Average dissimilarity – 92.48.

OTU	Olive compost Ave Abund (Log$_{10}$ UFC/mL)	Superficial Technosol Ave Abund (Log$_{10}$ UFC/mL)	% Contribution	% Cumulative
Aspergillus sp6	4.5	0	8.71	8.71
Chaetomium sp1	0	4.5	8.64	17.35
Alternaria sp5	0	4	7.66	25.01
Penicillium sp2	3.5	0	6.67	31.68
Umbelopsis sp1	2.5	1.75	4.70	36.38
Aspergillus sp2	2.5	1.75	4.70	41.08
Aspergillus sp1	2.25	0	4.55	45.63
Pseudopithomyces	0	2.25	4.25	49.88
Alternaria sp3	0	2.25	4.26	54.14

Table S6: SIMPER between polyacrylamide and superficial technosol. Average dissimilarity – 95.06.

OTU	Polyacrylamide Ave Abund (Log_{10} UFC/mL)	Superficial Technosol Ave Abund (Log_{10} UFC/mL)	% Contribution	% Cumulative
Aspergillus sp6	5	0	9.68	9.68
Chaetomium sp1	0	4.5	8.73	18.41
Alternaria sp5	0	4	7.74	26.15
Penicillium sp5	3	0	5.39	31.54
Penicillium sp2	2.25	0	4.61	36.15
Umbelopsis sp1	2.25	1.75	4.38	40.53
Alternaria sp3	0	2.25	4.30	44.83
Alternaria sp2	0	2.25	4.30	49.13
Pseudopithomyces	0	2.25	4.30	53.43

Table S7: SIMPER between superficial technosol and technosol. Average dissimilarity – 95.48.

OTU	Superficial Technosol Ave Abund (Log_{10} UFC/mL)	Technosol Ave Abund (Log_{10} UFC/mL)	% Contribution	% Cumulative
Aspergillus sp6	0	4.75	8.92	8.92
Penicillium sp2	0	4.5	8.49	17.41
Chaetomium sp1	4.5	0	8.46	25.87
Alternaria sp5	4	0	7.51	33.38
Aspergillus sp1	0	3.25	6.20	39.58
Penicillium sp5	0	3	5.22	44.8
Alternaria sp3	2.25	0	4.17	48.97
Pseudopithomyces	2.25	0	4.17	53.14

Table S8: SIMPER between non burnt soil and olive compost. Average dissimilarity - 55.60.

OTU	Non burnt soil Ave Abund (Log$_{10}$ UFC/mL)	Olive compost Ave Abund (Log$_{10}$ UFC/mL)	% Contribution	% Cumulative
Penicillium sp5	4	1	8.56	8.56
Penicillium sp1	4	1	8.14	16.70
Umbelopsis sp1	4	2.5	7.01	23.71
Aspergillus sp2	4	2.5	7.01	30.72
Aspergillus sp6	2.25	4.5	6.18	36.90
Aspergillus sp11	2.25	0	6.13	43.03
Aspergillus sp1	4	2.25	5.79	48.82
Aspergillus sp13	2.25	1	5.76	54.58

Table S9: SIMPER between non burnt soil and polyacrylamide. Average dissimilarity - 52.33.

OTU	Non burnt soil Ave Abund (Log_{10} UFC/mL)	Polyacrylamide Ave Abund (Log_{10} UFC/mL)	% Contribution	% Cumulative
Aspergillus sp2	4	1.25	9.47	9.47
Aspergillus sp6	2.25	5	8.14	17.61
Penicillium sp1	4	2.25	7.37	24.98
Penicillium sp2	4.5	2.25	7.22	32.20
Aspergillus sp13	2.25	1.25	7.02	39.22
Umbelopsis sp1	4	2.25	6.94	46.16
Aspergillus sp1	4	2.25	6.77	52.92

Table S10: SIMPER between non burnt soil and technosol. Average dissimilarity – 46.66.

OTU	Non burnt soil Ave Abund (Log_{10} UFC/mL)	Technosol Ave Abund (Log_{10} UFC/mL)	% Contribution	% Cumulative
Umbelopsis sp1	4	1.25	10.77	10.77
Penicillium sp1	4	1.25	10.59	21.36
Aspergillus sp6	2.25	4.75	8.12	29.48
Aspergillus sp13	2.25	0	7.37	36.85
Aspergillus sp11	2.25	1	7.00	43.85
Aspergillus sp2	4	2	6.73	50.58
Mortierella sp1	0	2	6.31	56.89