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DOCTORAL DISSERTATION / TESIS DOCTORAL

Fungal communities associated with forests in the Afromontane region of Ethiopia

Comunidades de hongos asociadas a los bosques en la región de Afromontana de Etiopía

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Dedication

Those who lost their lives due to Corona Virus (COVID-19) all over the world and conflicts in Ethiopia

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Abstract

The Afromontane region of Ethiopia has natural and plantation forest systems that provide high socioeconomic and ecological value, including the biodiversity conservation. However, the natural forests in this region are facing challenges in which their degradation is framed for decades. In response to this, exotic tree species have been introduced to decrease the pressure on the natural forests. Thus, the plantations are managed to maximize the value of the wood and reduce the gap between wood demand and supply in the country. Accordingly, some natural forests have been conserved as priority forests, but without generating tangible benefits for the local community.

In this context, some non-timber forest products, notably mushrooms, are neglected and not included in Ethiopia's forest management plans and strategies of the country. Consequently, studies on the effects of forest management on the diversity and composition of fungal communities are very limited. Therefore, our objective was to generate information on the composition and diversity of the fungal community in the forest systems of the Afromontane region, including natural and plantation forests. In the natural forests, the spatial distribution of fragmented church forests and the time after disturbance in the forest were taken into consideration for the study. In the plantation forests, the stand age was taken into account to analysis the existing fungal communities and succession in *Pinus patula* plantations. We studied the soil fungal communities in all the forests systems. Specifically, we also conducted the fruit body collection in the fragmented natural forests of the Northwest of the country, since this particular information was already available for the rest of the systems included in this study. In the fragmented Dry Afromontane forests in Northwestern Ethiopia, three forest types were selected. A total of 27 plots (2 m × 50 m), nine in each forest type, were established for the collection of the sporocarps and soil fungi. To assess the effect of fire on soil fungi, three similar plots were sampled in each of the three forests that differed in their fire history (unburned, 10-years old burned and 36-years old burned stands). Likewise, a total of nine plots were established in the plantation forests in three age categories (5-, 11- or 36- year old stands).

Abstract

For the fruit bodies study, we collected the whole sporocarps found in each of the plots established in the studied forests. The collection was conducted weekly during the rainy season in 2019. The fresh weight measurements were taken *in situ*. Also, the number of sporocarps and the abundance of each species encountered in the plots were taken. For the soil fungal community study, the molecular analysis was carried out using composite soil samples taken in each plot. Also, composite soil samples were taken for the physical-chemical analysis of the main edaphic variables that could explain the composition of the fungal community for each study. Additional data on climatic and vegetation variables were also collected from the study areas. Then, the diversity indices and species richness values were calculated and compared among forest stands. Different sorting techniques and statistical tests were carried out depending on the nature of each study. The effects of above ground vegetation, edaphic and climatic variables on the composition and diversity of the fungal community were also examined.

As main findings, the soil analysis indicated that forests in the Afromontane region of Ethiopia harbor great soil fungal diversity. A total of 2,898, 5,152 and 2,262 fungal OTUs were identified from the soil of natural Dry Afromontane forests in Southwestern Ethiopia, the fragmented church forests of Northwestern Ethiopia, and *P. patula* plantations respectively. Regarding the sporocarps study, a total of 258 taxa were collected from the fragmented church forests in the Northwest part of the country. The OTUs found in the soil fungal community were dominated by Ascomycota and most of them were saprotrophs. However, many of the taxa found could not be classified to the genus and species level, indicating the lack of information about the fungal communities from tropical and subtropical forest ecosystems. Differences in the composition and diversity of the fungal community due to the respective treatments were observed in each of our studies. A higher relative proportion of ectomycorrhizal fungi (ECM) were recorded in church forests. In the study of fruiting bodies, important ECM species in the general of *Tricholoma*, *Rhizopogon* and *Suillus* were found Also, valuable edible species belonging to the genera *Calvatia*, *Laetiporus*, *Pleurotus*, *Termitomyces* and *Macrolepiota* were collected. Also, we have found that the edaphic, spatial,

aboveground vegetation and climatic variables as important factors shaping fungal community composition in the studied forest systems.

This study contributes significantly to the knowledge about soil fungal communities in Ethiopia. Also, it provides relevant information required for the management and conservation of the studied forest systems in the Afromontane region of the country. The macrofungi existed also can play a significant role in the management of the forests and provide information for the conservation and optimization of sporocarps production, which can also provide a new source of food for the poor population during food shortage in the rural areas of the country. We recommend that the effect of forest management practices such as thinning and harvesting on soil fertility should be taken into consideration owing to the important relationship between these ecological parameters and the soil fungal composition in the dry Afromontane forests of Ethiopia. Promotion of vascular tree diversity in fragmented forest systems by enrichment plantings or assisted natural regeneration management systems would offer suitable habitats with variable microclimates that should assist fungal species. The fragmented church forests are important reservoirs of fungal diversity in Ethiopia and and this offers a complementary argument for their conservation and protection. Production of valuable wild mushrooms could be incorporated into management and conservation strategies in these fragmented forest systems.

Key words: *Aboveground vegetation, Dry Afromontane forest; climatic variables; Edaphic; Ethiopia; fire; fungal community; Pinus patula;*

Resumen

La región afromontana seca de Etiopía presenta sistemas forestales naturales y plantaciones que proporcionan un alto valor económico, social y ecológico destacando la alta biodiversidad que albergan. Desde hace décadas, los bosques naturales se enfrentan desde hace años a una enorme devastación y se han introducido y plantado especies de árboles exóticos para reducir la brecha entre la oferta y la demanda de madera. Estas plantaciones se gestionan para maximizar el valor de la madera. Por otra parte, algunos bosques naturales han sido conservados como bosques prioritarios pero sin generar beneficios tangibles para la comunidad local.

En este contexto, algunos productos no maderables, entre los que se destacan los hongos, no se incluyen en los planes y estrategias de ordenación forestal de Etiopía. Los estudios sobre los efectos de la ordenación forestal sobre la diversidad y la composición de las comunidades fúngicas son muy limitados. Por lo tanto, nuestro objetivo ha sido generar información sobre la composición y diversidad de la comunidad fúngica en la región afromontana seca incluyendo bosques naturales y plantaciones. En los bosques naturales se tuvo en cuenta en un caso la distribución espacial de los bosques de iglesia fragmentados y en otro caso el tiempo transcurrido tras la perturbación causada por los incendios forestales. En el análisis de las comunidades existentes en plantaciones de *Pinus patula,* se ha tenido en cuenta el desarrollo de la masa para entender la sucesión fúngica.

Se abordó el estudio de las comunidades de hongos de suelo en todos los ecosistemas y específicamente se llevó a cabo un estudio de los cuerpos de fructificación en los bosques naturales fragmentados del noroeste del país, ya que se disponía de esta información para el resto de los sistemas incluidos en el estudio. En los bosques secos afromontanos fragmentados en el noroeste de Etiopía, se seleccionaron tres tipos de bosque y se establecieron 27 parcelas (2 m × 50 m), 9 en cada tipo de bosque, para el estudio de cuerpos de fructificación y hongos del suelo. Para el estudio del efecto de los incendios forestales en los hongos del suelo, se establecieron tres parcelas (2 m × 50 m) de cada uno de los tres bosques históricos (sin quemar, diez años después de la

quema y 36 años después de la quema). Asimismo, en las plantaciones se establecieron un total de 9 parcelas (tres para cada categoría de edad) con una dimensión de 2 m × 50 m en tres categorías de edad (5, 11 o 36 años).

Para el estudio de los cuerpos de fructificación, en cada parcela se recolectaron semanalmente durante la temporada de lluvias en 2019 y se tomaron mediciones de peso fresco *in situ*. También se tomaron datos sobre abundancia y número de esporocarpos. Para los estudios de la comunidad de hongos en suelo, el análisis molecular se realizó en base a un pool de muestras de suelo tomadas en cada parcelas. Para todos los estudios, se tomaron muestras compuestas de suelo para el análisis físico-químico de las principales variables edáficas que podrían explicar la composición de la comunidad fúngica. También se tomaron datos sobre variables climáticas y la vegetación de las parcelas estudiadas. Se calcularon índices de diversidad y valores de riqueza de especies y se compararon entre tratamientos. Se realizaron diferentes técnicas de ordenación y pruebas estadísticas en función de la naturaleza de cada estudio. Se examinaron los efectos de la vegetación aérea, las variables edáficas y climáticas sobre la composición y diversidad de la comunidad fúngica.

Como resultados destacados, descubrimos que los bosques de la región afromontana seca de Etiopía albergan una gran diversidad fúngica de suelo. Se recolectaron un total de 2.898, 5.152 y 2.262 taxones de hongos del suelo de los bosques naturales Dry Afromontane en el suroeste de Etiopía, en los bosques de iglesia fragmentados en el noroeste de Etiopía, y en las plantaciones de *P. patula* respectivamente. También se recolectaron un total de 258 taxones en el estudio de los cuerpos de fructificación llevado a cabo en los bosques de iglesia fragmentados del noroeste del país. Los taxones encontrados en la comunidad de hongos del suelo estuvieron dominados por Ascomycota y la mayoría de los taxones eran saprótrofos. Muchos de los taxones encontrados no pudieron ser clasificados a nivel de género y especie, lo que indica la falta de datos de ecosistemas forestales tropicales y subtropicales. Se observaron diferencias en la composición y diversidad de la comunidad fúngica debidas a los respectivos tratamientos en cada uno de nuestros estudios. Se registró una mayor

proporción relativa de hongos ectomicorrícicos (ECM) en bosques de iglesia. En el estudio de cuerpos de fructificación se encontraron especies importantes de ECM como *Tricholoma*, *Rhizopogon* y *Suillus* y valiosas especies comestibles pertenecientes a los géneros *Calvatia*, *Laetiporus*, *Pleurotus*, *Termitomyces* sp. y *Macrolepiota*. Encontramos que el tipo de vegetación de suelo, la localización y las variables climáticas fueron los factores que influyeron de forma significativa en la conformación de la composición de la comunidad fúngica.

Este estudio contribuye significativamente al conocimiento sobre las comunidades de hongos en el suelo en Etiopía y brindó información relevante requerida para el manejo y conservación de los sistemas forestales estudiados en la región afromontana seca del país. Los hongos pueden jugar un rol significativo a la hora de gestionar estos bosques de cara a su conservación y a la optimización de su producción, que además puede proporcionar una nueva fuente de alimento para la población pobre en épocas de escasez de alimentos. Recomendamos que se tome en consideración el efecto de las prácticas de manejo forestal como el aclareo y el aprovechamiento sobre la fertilidad del suelo debido a la importante relación entre estos parámetros ecológicos y la composición fúngica del suelo. La promoción de la diversidad de árboles vasculares en los bosques naturales fragmentados mediante plantaciones de enriquecimiento o sistemas de gestión de regeneración natural asistida ofrecería hábitats adecuados con variables micro climáticas que promoverían una mayor diversidad y producción fúngica. Los bosques de iglesia fragmentados son importantes reservorios de diversidad fúngica en Etiopía y esto ofrece un argumento complementario para su conservación. La producción de valiosos hongos silvestres podría incorporarse a las estrategias de gestión y conservación en estos sistemas forestales.

Palabras clave: vegetación aérea, bosque seco Afromontano; variables climáticas; Edáfica; Etiopía; fuego; comunidad de hongos; *Pinus patula*;

List of original articles

This thesis is based on **four** original works, which are referred in the text with Roman numerals (I – IV). All except the forth one are already published. The fourth is a manuscript.

Authors, coauthors, and the stage of the publication are presented below:

I. **Demelash Alem**, Tatek Dejene, Juan Andrés Oria-de-Rueda, József Geml, Carles Castaño, Jane E. Smith, Pablo Martín-Pinto. 2020. Soil fungal communities and succession following wildfire in Ethiopian Dry Afromontane forests, a highly diverse underexplored ecosystem. *For. Ecol. Manage*. 474, xx–xx.(118328) https://doi.org/10.1016/j.foreco.2020.118328

II. **Demelash Alem**, Tatek Dejene, Juan Andrés Oria-de-Rueda, József Geml, Pablo Martín-Pinto. 2020. Soil Fungal Communities under *Pinus patula* Schiede ex Schltdl. & Cham. Plantation Forests of Different Ages in Ethiopia. *Forests* 11, 1109. https://doi.org/10.3390/f11101109

III. **Demelash Alem**, Tatek Dejene, Juan Andrés Oria-de-Rueda, Pablo Martín-Pinto. 2021. Survey of macrofungal diversity and analysis of edaphic factors influencing the fungal community of church forests in Dry Afromontane areas of Northern Ethiopia. For. Ecol. Manage. 496, xx–xx.(119391). https://doi.org/10.1016/j.foreco.2021.119391

IV. **Demelash Alem**, Tatek Dejene, József Geml, Juan Andrés Oria-de-Rueda and Pablo Martín-Pinto. 2021**.** Soil fungal communities in fragmented Dry Afromontane Church forests in Northern Ethiopia (Manuscript)

Outline of the thesis

This thesis consisted of **four** studies important to describe the status of fungal communities from two forest systems in the Dry Afromontane region of Ethiopia. The first study **(Study I)** focused on the community composition, diversity and richness of soil fungi under different successional stages after fire in the Dry Afromontane forest systems of Southern Ethiopia where the recurrent forest fire is common. The second **(Study II)** is on fungal succession in relation to stand development of *Pinus patula* where the effects of stand age of *P. patula* on the community composition, diversity and richness of soil fungi are discussed. The third and the fourth **(Study III and IV)** are focused on macrofungi and soil fungi composition of the Dry Afromontane church forest systems of Northwestern Ethiopia respectively. In the third paper, the effects of aboveground vegetation, climatic, spatial and edaphic variables on the community composition, diversity and richness of total macrofungi as well on functional groups are discussed. In paper IV, the impacts of the variables indicated in paper III on the community composition, diversity and richness of soil fungi and functional guild are studied. The findings have implications for the sustainable conservation and use of both the natural and plantation forests in Ethiopia through mycosilvicultural management approaches. Also, the conservation of biological diversity of the forest system through the provision of complementary income for the local communities through sporocarps production was emphasized. The taxa composition is also explained in terms of edaphic variables, stand age, vegetation types and climatic variables. Conceptual map of the study including the four studies is shown below (Fig. 1).

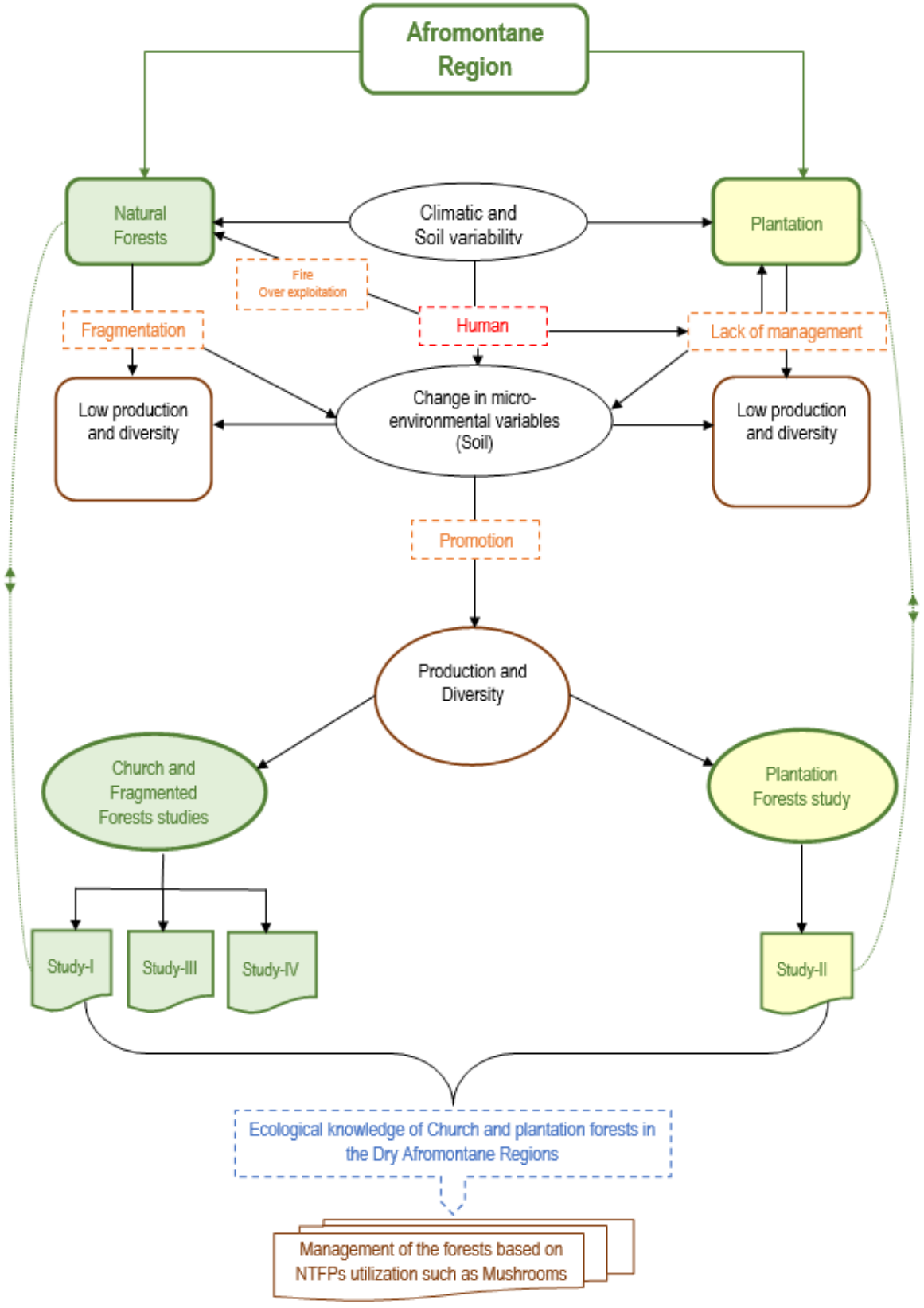

Figure 1: Conceptual map of the thesis including the 4 studies

Introduction

1. Introduction

1.1. Climate, Geology and Ecology of Ethiopia

Ethiopia is situated in central part of the horn of Africa and it is a land locked country which spans 3^0 24' to 14^0 53'N and 33^0 00' to 48° 00'E, encompassing approximately 1270 kms in North-south and 1650 kms in East-west directions. The country has varied topography ranging from 123 m below sea level to 4533 m above sea level and majority of area have > 2000 m elevation unlike other countries in Africa (Friis et al., 2010). The mean annual rainfall ranges from 500 to 2800 mm and temperature 10ºC to 30ºC (Demissew and Nordal, 2010). The highland of the country is dissected by the East African rift valley. The oldest rocks in Ethiopia are part of the crystalline basement, which is pre-Cambrian in origin. The original igneous and sedimentary rocks are interblended with schists and gneisses and subsequent igneous intrusions. The whole system is referred to as the basement complex (Friis et al., 2010). Furthermore, the country is an ecologically diverse country owing to the varied topographic features and altitudinal variations (Gebretsadik, 2016).

1.2. Natural forests in Ethiopia

According to Friis et al. (2010), the vegetation of Ethiopia is classified into 12 types based on the elevation zones in which they occurred: (1) Desert and semi-desert shrub land, (2) *Acacia-Commiphora* woodland and bush land, (3) Wooded grassland of the Western Gambela Region, (4) *Combretum-Terminalia* woodland and wooded grasslands, (5) Dry Afromontane forest and grasslands complex, (6) Moist Afromontane forest, (7) Transition rainforest, (8) Ericaceous belt, (9) Afroalpine vegetation, (10) Riverine vegetation, (11) Fresh water, lakes, lakes shores, marshes, swamps and flood plain vegetation and (12) Salt-water, lakes, lakes shores, salt marshes and plain vegetation.

The natural high-elevation forests (Fig 2), that include the Afromontane vegetation, are exclusively found in the highland regions of Ethiopia between 1500 to

3400 m above sea level (Lemenih and Bekele, 2008) that occupy more than 44% of the country's land area (Kidanu, 2004; McCann, 1995). Dry Afromontane forests is a complex ecosystem characterized by high humidity, a variable rainfall pattern, and a prolonged dry season (Friis et al., 2010). These forests provide important ecosystem services such as watershed protection and carbon sequestration (Wassie et al., 2005). The dominant tree species in these forests are *Juniperus procera*, *Podocarpus falcatus*, *Hagenia abyssinica* and *Olea africana*, which are the main source of timber in the country. These forests also harbour various types of non-timber forest products (Fig 2B), including wild edible mushrooms (Dejene et al., 2017b).

Figure 2: The natural forests (A) and the collected non timber forests products (B) in Ethiopia

High levels of historical human landscape alteration and land-use pressure have resulted in widespread deforestation and the degradation of Ethiopian forests (Aerts et al., 2016; Aynekulu et al., 2016; Darbyshire et al., 2003; Nyssen et al., 2014). A recent review of forestry in Ethiopia revealed that deforestation is a continuous process (Gebru, 2016). When all forest types were included, a deforestation rate of 0.93% per year was calculated in 2010 (FAO, 2020; Zewdie et al., 2010). The ever-increasing demand for wood products as well as crop and grazing land expansion, stimulated by rapid population and livestock growth are the factors aggravating the degradation of the Dry Afromontane forests in the country (Bekele and Lemenih, 2008). Human-induced fire is also one of the most important reasons for the depletion and degradation of natural resources in Ethiopia (Lemenih and Bekele, 2008; Wassie et al., 2005). Fire is more common in the highland areas, where the dry Afromontane forest is found, and

has a direct impact on the biodiversity in the forest ecosystem (Lemenih and Bekele, 2008). As a result, many physical and biological changes have occurred in these forest systems in Northern Ethiopia. These forest currently are the most fragmented ecosystems (Dessie, 2007; Lemenih and Bekele, 2008; Miles et al., 2006; Wassie et al., 2010). Loss of biodiversity could also occur in the forest soil, which harbours a great diversity of microbial organisms (Fierer and Jackson, 2006), including fungi. Depending on the severity and frequency, fire could directly or indirectly affect edaphic variables in the forest ecosystem (Reazin et al., 2016), which in turn could have an impact on fungal communities dwelling in the soil (Cairney and Bastias, 2007; Dahlberg et al., 2001; Rincón and Pueyo, 2010).

Figure 3: The fragmented Dry Afromontane church forests in Northern part of Ethiopia

Studies have evaluated the conservation value of fragmented forests in the Northern landscapes of Ethiopia (Aerts et al., 2016; Aynekulu et al., 2016; Wassie et al., 2010, 2005; Nyssen et al., 2014). Most of these fragment forests survive in the landscape because of the cultural or religious values held by local communities that are found as forest islands (Aynekulu et al., 2016). These forests belong to the church or are located around church forest territories (Aerts et al., 2016; Aynekulu et al., 2016; Wassie et al., 2009) (Fig 3). Forest fragmentation affects biodiversity (Lemenih and Bongers, 2011; Wassie et al., 2005) and the population viability in the long-term (Fernández et al., 2020). The condition probably reflected on the fungal communities constituting the forests systems. Additionally, the forest fragmentation impacts soil

properties and the belowground soil organisms (Martínez et al., 2009; Tilman et al., 2002). The variation in the environmental variables such as temperature (Newsham et al., 2016), precipitation (L. Tedersoo et al., 2014), altitude (Bahram et al., 2012), soil pH (Rousk et al., 2010), nutrient availability (He et al., 2017), and plant community (Tedersoo et al., 2016b) also influence fungal diversity and the community compositions.

1.3. Plantation forest in Ethiopia

In response to declining natural forest resource and increasing wood demand in Ethiopia, private, community woodlots and commercial plantations started in 1970s. The fast-growing non-native trees have been introduced and planted at wider scale over the country (Bekele, 2011; Jenbere et al., 2012) (Fig 4). FAO (2005) stated that the area of plantations in Ethiopia increased from 3.2% to 3.8% between 1990 and 2005. The more recent estimate indicated that the coverage of plantation forest in the country has been increasing and reached 1 million ha in 2010 (Bekele, 2011; Tesfaye et al., 2020).

Ethiopia plants billions of seedlings every year (Asmelash et al., 2019). For example, in 2019, the country planted over 350 million trees in just 12 hours (Kiruga, 2019). There is a plan to restore 15 million hectares of degraded land by 2025 (MEFCC, 2018). Following this, billions of seedlings, both native and indigenous have been planted every year and an estimated 300 million $USD is being invested each year for seedling production and plantation (Asmelash et al., 2019). Of the total area of plantation forests, 80 percent are non-industrial plantations, mainly woodlots and trees on farm while the remaining 20 percent is commercial plantations. These plantations produce fuel wood and construction timber, as well as NTFPs (Bekele, 2011) . The dominant tree species planted in the country is *Eucalyptus* (Aklilu et al., 2019; Bekele, 2011) and low proportion of indigenous tree seedlings are included (Negash, 2019). However, the technical and silvicultural failures combined with other related governance and management pitfalls constrained the success of plantation forest development (Le et al., 2012; Tadesse et al., 2019). This resulted in little in-country wood product supply and created huge gap between demand and supply of wood products. This gap is

compensated by importing a huge volume of wood from abroad (Alem, 2015) which created additional challenge in earning foreign currency (Lemenih and Kassa, 2014). Moreover, most of the forests resource in the country are managed for timber products while other alternative products and services such as NTFPs are neglected (Desalegn and Tadesse, 2004). Edible mushrooms exist in exotic plantation of Ethiopia (Dejene et al., 2017c, Sitotaw et al., 2020). Varied topography, climatic zones, and remnant natural forest in the country could result in rich and diverse mushroom resources (Hu et al., 2019) and would provide an opportunity to contribute more for food security and solve social and econmic problems of the country (Semwal et al., 2014), despite the resource is underutilized in the country (Gebrelibanos et al., 2016; Weldekiros et al., 2017; Zeleke et al., 2020).

Figure 4: The plantation forests of different exotic tree species in Ethiopia

P. patula Schiede ex Schltdl. &Cham. is one of the introduced tree species which is mainly grown to meet the increasing demand for woody raw materials in the country (Bekele, 2011; Gezahgne, 2003; Jenbere et al., 2012). *P. patula* has received attention largely as a suitable tree for the production of round wood (timber), poles, and posts (Belay, 2016; Tesfaye et al., 2020) because of its fast growing nature in Ethiopia (Fig 4). *Pinus* trees form obligate symbiotic and form association with mycorrhizal fungi, which are vital for host nutrition, survival and productivity in forest systems (Fernandez et al., 2016). However, the impact of stand age of Pinus plantation on fungal community composition not yet studied under Ethiopian condition.

1.4. What are fungal resources?

Despite the superficial resemblance of some fungi to plants (e.g. having rooted, stalked structures), their non-photosynthetic, absorptive method of feeding and their different cell walls, cell membrane chemistry, methods of food storage and DNA indicate that they form an independent kingdom (Kew, 2018). Fungi are the largest organisms next to insects (Hasan and Gupta, 2012). Fungi are vital in nutrient cycling and enable the existence of life on earth (Kew, 2018). Mushrooms are seasonal fungi, which occupy diverse niches in nature in forest ecosystem (Krishanu Singha Amrita Banerjee, 2017). Mushrooms mainly occur in the rainy season, especially in forests, where the shade form trees provide moisture and organic material which is important for germination and growth of mushrooms. The latest best estimate suggests that the total number of fungal species on Earth is between 2.2 and 3.8 million, a number that exceeds the estimated number of plants by more than 6 times. However, only 120,000 fungal species (8%) are described, the majority being in temperate regions (Hawksworth and Gardens, 2017). Molecular surveys of fungi in environmental samples have applied the fungus-to-plant ratio method and yielded estimates of 5-6 million species (O'Brien et al., 2005; Taylor et al., 2014).

1.5. Importance of fungi

Fungi play important roles in maintaining balance of forest ecosystems by degrading wood or forest litter enabling nutrient cycling and taking direct roles in soil formation or form symbiotic relations with trees enabling them to reach unavailable nutrients and water (Marshall and Nair, 2009; Quoreshi, 2008; Radomir et al., 2018). Some fungi form fruiting body such as mushrooms which are important for bioremediation, biodegradation, biopesticidal and pharmacological values that could be exploited (Krishanu Singha Amrita Banerjee, 2017). Wild mushrooms are also highly important for maintenance of forest ecology and indicating forest management and current status of an ecosystem (Krishanu Singha Amrita Banerjee, 2017).

Mushrooms can be used to generate income, improve food security (Abdulla, 2013; Degreef et al., 2016), treat diseases (Zeleke et al., 2020) and sustainably manage forest resources (Amma et al., 2018; Saha and Sundriyal, 2012). Previous studies in various parts of the world showed that mushrooms have good nutritional content, though varies with the species type (Tripathi et al., 2018). Because of their high content of vitamin, protein and minerals, fibers, trace elements and no/low calories, fat and cholesterol (Wani et al., 2010), mushrooms are important for good health. These nutritional properties make mushrooms a very good dietary food and recommended for patients with cardiac problem or at risk with lipid. Also, mushroom can play significant role in providing food and generate additional intermediate income from the forest for the local community (Solomon, 2016). They are also medicinally important for various types of diseases (Biswas et al., 2017) as they possess antioxidative (Woldegiorgis et al., 2014), antibacterial, antifungal, antiviral, antiparasitic, anti-inflammatory, antiproliferative, anticancer, anti-HIV, hypo-cholesterolemic, antidiabetic and anticoagulant (Krishanu Singha Amrita Banerjee, 2017; Tripathi et al., 2018) activity.

Apart from the food values, fungi are important in restoring degraded lands and enhancing tree growth. Mycorrhizal fungi can enhance plant biomass under drought conditions (Herk et al., 2002) by either decreasing water consumption or by increasing water absorption and mobilising water from deeper layers of soil (Hawksworth and Rose, 1970). For example, optimum plant growth of *Faidherbia albida* (Del.) A. Chev was reported under low water stress areas in dryland farming systems in north Ethiopia through inoculation of seedlings with indigenous AMF (Birhane et al., 2018b). Fungi are associated with the roots of almost all plants, including forest trees and act as living intermediaries between the plant and the surrounding soil. The plant benefits from the greater capacity of the fungus to absorb water and nutrients and to mobilise minerals that would otherwise be unavailable, and the fungus benefits from a steady source of carbohydrates from the plant (Clemmensen et al., 2013; Kew, 2018). The use of fungi in Ethiopian plantation forest development is important since most of the lands allocated for afforestation programs in Ethiopia are degraded and marginal lands in their soil fertility and most seedlings are planted without adding fertilizers. The majority of sites

are also moisture stress areas. Rehabilitation of such degraded sites has been a challenge for the country. Previous studies revealed that application of relevant ECM fungal species could improve the rehabilitation success (Beenhouwer et al., 2015; Birhane et al., 2017a; Quoreshi, 2008; Sewnet and Tuju, 2013; Welemariam et al., 2018). AMF biotechnology significantly improves the restoration success of degraded lands, improves soil attributes, increases above and belowground biodiversity, improves tree/shrub seedling survival, and enhances trees growth and establishment on moisture and nutrient stressed soils. AMF have also been shown to drive plant succession and may prevent invasion by alien species. Therefore, AMF spore density and colonization could be considered as an indicator of restoration in measuring success of restoration in the drylands (Birhane et al., 2017a, 2017b).

1.6. Factors governing fungal distribution

Fungal distribution is influenced by biotic and abiotic variables (Bahnmann et al., 2018) such as climate and habitat types (Djelloul and Samraoui, 2011; Khan and Chandra, 2017), host plant (Dobo et al., 2016; Khan and Chandra, 2017), soil physical and chemical properties (Drenovsky et al., 2004; Lauber et al., 2008; Lauber et al., 2009) resulted in high spatial variation of fungal diversity and relative abundance (Collins et al., 2018). However, findings on the effect of these factors on fungal community structure are not consistent since the effect depends on the type of fungi (Djelloul and Samraoui, 2011), life stage of fungi (Tomao et al., 2020) and habitat types (Danks et al., 2013), which necessitates analyzing the fungal diversity by functional groups at a site to understand the response of fungal communities to biotic and abiotic variables (Caiafa et al., 2017). It was also noted that the vegetation usually sets limits to fungal ranges (Shay et al., 2015; van der Heijden et al., 2008). As a habitat, the forests soil environment also impacts the fungal communities (Lauber et al., 2008; Rasche et al., 2011; Richter et al., 2018). These impacts differ with climate, topography, vegetation type, and the magnitude of disturbances in the forests (Day et al., 2019; Kardol et al., 2010; Monkai et al., 2017).

Human factors such as disturbance and management also affect the abundance and distribution of mushrooms. Dejene et al. (2017b) noted the impact of fire and stand age of plantations on sporocarps production from the natural and plantation forests in Ethiopia. It was also reported that older age plantations harbor more fungal diversity than younger plantation of the same species (Dejene et al., 2017a). Megersa et al. (2017) obtained similar result under plantations of *Cupressus lusitanica* in south central Ethiopia. In these regards, matured tree retention management approach could be used so as to create habitat variability for more fungal diversity and production (Dejene et al., 2017b) as management tool. Similarly, the growth stage of trees also affects the composition and diversity of fungi. As a forest develops, the composition of the associated fungal communities also changes (Gassibe et al., 2011; Luoma et al., 1991). For example, early-stage fungi emerge from spores in the spore bank that were already in the soil prior to the establishment of the plantation, whereas late-stage fungi are more prevalent as conditions change as the plantation matures (Hernández-Rodríguez et al., 2013). Both early- and late-stage fungi are involved in the stabilization of the soil and the rehabilitation of soil microflora (Claridge and Trappe, 2004). Furthermore, changes in edaphic variables due to forest growth affect the abundance and diversity of soil fungi and, thereby, dictate the fungal community composition (Reazin et al., 2016; Yang et al., 2017). Previous studies have assessed changes in soil fungal communities across stand ages by performing DNA analyses (Blaalid et al., 2012; Clemmensen et al., 2015). The fungal community composition differs at different stages of forest development in Ethiopian forests (Castaño et al., 2019; Dejene et al., 2017b). However, the effect of all the above factors such as forest fragmentation and forest fire as well as plant species diversity on fungal community composition and diversity were not studied. Moreover, the effects of age of stand in *P. patula* plantation under Ethiopian condition were not studied.

1.7. Research and conservation status of fungi

Compared to plants and animals, fungi are poorly studied from most countries, regions and habitats (Crous et al., 2006). The vast majority (over 93%) of fungal species are currently unknown to science. The conservation status of only limited number of

fungal species (56) had been evaluated for the International Union for conservation of Nature (IUCN) Red List compared to plants (25,452 species) and animals (68,054) (Kew, 2018). Despite recent advances in determining the diversity and composition of forest fungi in various biomes, fundamental questions regarding their distribution and function, and the factors that influence them remain unanswered, particularly in under-sampled biomes (Guo et al., 2013; Krashevska et al., 2015). The majority of soil fungi are unexplored and, the functional relationship between fungi, soil, and plants remains understudied (Bridge and Spooner, 2011; van der Heijden et al., 2008). Previous investigations have estimated that there are about 5.1 million fungal species worldwide (Taylor et al., 2014). Of these, 2–6% have been described (O'Brien et al., 2005) and ~1200 new species are described each year (Hibbett and Thorn, 2001), indicating that there are many more fungal species to be explored, named, and identified. Furthermore, to date, most studies of soil fungal communities have focussed on temperate and Mediterranean forest ecosystems; less consideration has been given to soil fungal communities in tropical forest ecosystems (Taudière et al., 2017). Therefore, further studies are required to increase our understanding of the dynamics of soil fungi and their community structure (Dhruba et al., 2015) and the impact of various environmental and anthropogenic factors. Also, most studies of soil fungal communities currently have focussed on temperate and Mediterranean forest ecosystems; less consideration has been given to soil fungal communities in tropical forest ecosystems (Taudière et al., 2017). Therefore, further studies are required to increase our understanding of the dynamics of soil fungi and their community structure (Dhruba et al., 2015) and the impact of various environmental and anthropogenic factors.

On the other hand, fungi are facing threats related with climate change, pollution, over-exploitation, and habitat destruction and fragmentation (Dahlberg et al., 2010; Kew, 2018). Despite these, the fungi are often neglected in conservation due to knowledge gap between mycologists and conservationists in fungal distributional and ecological data. This problem is more complicated mainly due to the invisible, indeterminate form and their tendency to switch between forms (Dahlberg and Mueller, 2011). Although the noticeable and abundant spectacles of fruiting structures of fungi

(e.g. mushrooms) produced by some fungal species, in general fungi are difficult to identify and count since, when not fruiting, most are composed of nothing more substantial than a wispy network of mycelium. Fungi therefore contain a great, and yet largely obscured, presence within soil and inside other living things (Kew, 2018). This makes them difficult subjects to characterize, survey, and monitor them. Fortunately, a general shift towards conserving whole ecosystems is now underway due to their ecological importance in nutrient cycling (Heilmann-Clausen et al., 2015). In general, long-term and large-scale data, data from tropical, experimental data from fungi associated with trees and data from multiple simultaneous drivers of change for fungal community change are the major information gaps on global fungal resources (Kew, 2018).

1.8. Overview of mycological studies in Ethiopia

Despite fragmentation and deforestation, studies have evaluated the conservation value of different forests in the Dry Afromontane regions in Ethiopia (Aerts et al., 2016; Aynekulu et al., 2016; Nyssen et al., 2014; Wassie et al., 2010). Most of these studies focused on the above story components of these forest systems and very limited numbers of studies have investigated fungal communities from the Dry Afromontane region of Ethiopia. These studies were focused on above-ground fungal communities (Dejene et al., 2017a) while the soil fungal communities associated with the dry Afromontane forests in Ethiopia are undescribed. Furthermore, the potential effect of fire, edaphic, aboveground vegetation, climate and spatial variables on soil fungal communities in these ecosystems has not yet been evaluated. Limited focuses have been given to the ecology and conservation status of the soil fungal and macrofungal diversity of the Dry Afromontane forests system despite their wider distribution in the country. Consequently, the fungal taxonomy and ecology are very poorly described and, hence, fungi are neglected when decisions need to be made regarding forest management and conservation actions in this region.

Previous studies conducted on plantation forest resources in the Dry Afromontane regions of the country focused on ways to assist the management and

development of these plantations for wood products. The study of soil fungal communities in these forest systems in the Dry Afromontane region is scarce. Recently, Castaño et al. (2019) investigated the soil fungal community and ecological guilds associated with *Eucalyptus grandis* plantations in Ethiopia. However, currently there has been an interest in surveying fungi in particular habitats (Alem et al., 2020b), to describe and predict the extent of their diversity on a larger scale (Danielsen et al., 2005; Peay, 2014). However, studies on the soil microbial community, including fungi associated with *Pinus* plantations, are very limited. Sporocarps associated with a *P. patula* plantation during a single rainy season have previously been reported (Averill et al., 2014). Although sporocarps represent a unique step in the complex life of fungi (Averill et al., 2014), they do not reflect the entire soil biota (Ortega-Martínez and Martínez-Peña, 2008).

Aboveground vegetation affects the community composition and diversity of fungi. The practice of using plant communities as surrogates to predict fungal diversity has been reported by previous studies (McMullan-Fisher et al., 2010; Rudolf et al., 2013). Despite fragmentation, the forests in the study areas are suggested to be relatively rich in plant species (Aerts et al., 2016; Wassie et al., 2010). The tree species composition of the fragmented forests also varied with their status, topography and altitude (Bongers and Tenngkeit, 2010), with a wide distribution over the landscapes (Aerts et al., 2016). However, there is no evidence that the high level of plant diversity in the church Dry Afromontane forests system anticipates correspondingly high macrofungal diversity. Moreover, there is no information on how habitat fragmentation may affect fungal communities or limit fungal processes is relatively limited (Edman et al., 2004; Grilli et al., 2012; Mangan et al., 2004). In addition, as yet, the environmental variables that govern fungal communities in these fragmented forest systems have not been identified given that these forests vary in their status (size, density, species composition etc.), topography and altitude (Bongers and Tenngkeit, 2010).

Previous studies in Ethiopia have been focused on ethnomycological knowledge and mushroom consumption habit of the local people, diversity and community composition of macrofungi and soil fungi. More attention has been given to studies on

AMF associations and edible fungi. Less emphasis was given to total fungal studies, both above ground and below ground. In terms of study locations, more studies were conducted in southern Ethiopia (Alem et al., 2020a; Beenhouwer et al., 2015; Castaño et al., 2019; Chauhan et al., 2019; Dejene et al., 2017b, 2017a; Dobo et al., 2018b, 2018a, 2016; Hailemariam et al., 2013; Megersa et al., 2017; Michelsen, 1993; Muleta et al., 2013, 2008; Sewnet and Tuju, 2013; Tuno, 2001; Wubet et al., 2009) followed by north Ethiopia (Birhane et al., 2018b, 2018a, 2017a, 2012, 2010; Delelegn et al., 2018; Weldekiros et al., 2017; Welemariam et al., 2018). Northwestern and central Ethiopia had been given less attention in mycological studies. Since Ethiopia has diversified climatic, vegetation, topographic and edaphic features (Friis et al., 2010; Gebretsadik, 2016) which in turn affect the type of fungi that exist in the area (Semwal et al., 2014) additional studies in understudied areas of the country are required.

Location specific studies carried out in Ethiopia showed the existence of huge potential of mushrooms (Abate, 1999; Weldekiros et al., 2017) and high species numebr per unit area (Dejene et al., 2017a). These studies also indicated the potential of the resources for the sustainable management of forest resources and contribution to food security of the local community living around these forests in general. It was also indicated that more number of macrofungal species and new ones can be explored if different forest systems are included (Dejene et al., 2017b, 2017d; Megersa et al., 2017). In many countries, edible wild mushrooms have been identified, cultivated and incorporated as staple foods (Boa, 2004) and extensive collections and herbarium data have also been documented (Beluhan and Ranogajec, 2011). However, in Ethiopia the mushroom cultivation practices and consumption is very low compared to other countries in Europe and Asia (Gebrelibanos et al., 2016) despite the country possesses numerous species of wild mushrooms (Abate, 1999; Weldekiros et al., 2017). The forest management in the country neglected such important resources. The overall review of the available published data indicated that, still there is a gap in quantifying the fungal diversity of the country (Dejene et al., 2017d).

1.9. Why the current study?

Sustainable development though the conservation of natural resources is a major challenge for developing countries like Ethiopia. Ethiopia is mentioned as one of the countries rich in diverse plant and animal species (Gebretsadik, 2016). However, the forest resource of the country are vanishing at an alarming rate because of various reasons such as high human and animal pressure and unsustainable use of forest resources (Gebru, 2016). To avert the problem, different forest development approaches have been tried and symbolic achievements have been recorded in some localities (Winberg, 2011). However, many remnant natural forests in Ethiopia have been closed as “reserve forests” to promote the conservation of the natural forests. Plantation forests especially fast growing tree species have been introduced in the country and planted mainly for fuelwood and timber. However, in the management of Ethiopian forests, the value of forest resources other than timber have been overlooked (Desalegn and Tadesse, 2004) and the NTFPs such as mushroom are not included in forest management plan of both natural and plantation forests resource of the country despite their potential contribution to the sustainable management of forest ecosystems (Amma et al., 2018) and ensuring food security of the nation. Because of this problem sustained deforestation and degradation has been recorded in Ethiopia (Asfaw and Etefa, 2017). Devising cheap and ethically widely accepted inocula production methods and better ways of management for effective restoration of degraded lands will also remain to be important research areas (Asmelash et al., 2016). Therefore, alterative forest management approaches that bring economic benefits to the local community and provides intermediate income from forest resources (Melesse and Abtew, 2015) are important for the sustainable management of Ethiopian forest resources (Yadav and Mekonnen, 2013). One of these alternative forest management interventions is the use of NTFPs such as gums and resin, wild honey, medicinal plants and fungi/mushrooms. However, the fungal resources and the tradition of mushroom consumption are being disappearing parallel with the forest resource of the country (Weldekiros et al., 2017). These resources are also under dynamic change due to sever habitat degradation in the country (Dejene et al., 2017d).

Review of existing literature on fungal studies of the country indicated that the fungal resource of the country is poorly studied, documented and not properly utilized. This implies the need for conducting more detailed and wider scale fungal studies to document the fungal resource of the country including fungal community compositions, mushroom production and driving factors for their distribution (Dejene et al., 2017d). Such information will help to devise strategies for the sustainable management and utilization (Hailemariam et al., 1990), formulate effective and integrated policies, provide prioritized management recommendations (Dahlberg et al., 2010) of these resources. Conservation of biodiversity depends on reliable information about the kinds of organism present, total number of species in each of these group, their genetic diversity, their habitats, distribution pattern, ecology, population size, evolutionary history, and their trends both in time and space (Bhandari and Jha, 2018). The biodiversity data of fungi are useful indicators to assess the current status of an ecosystem and important for maintaining and managing the ecosystem of a forest.

It was in the light of this background information that the present study was conducted in different agro-ecologies and different forest systems of Ethiopia so as to generate valuable information on total species richness, abundance, diversity, evenness and composition of soil and macrofungi community and factors affecting these. Due to the key ecological role that fungi play in ecosystem functioning, the information about how ecological factors affect the fungal communities in the church fragmented forests can be crucial to enable the integration of these forests into global biodiversity conservation strategies and to understand what actions must be undertaken to conserve these forests, and their biological components. Information generated from this research is believed to help design conservation and sustainable use of fungi in particular and of forest resource of Ethiopia in general. The output of the research will also add valuable information to the local, national and global fungal community diversity. It will also contribute to the achievement of national environmental and forest related goals as well as to the national GDP, food security of the nation and the country's vision to become middle income country by 2030. This research in line with the international and national development strategies of the country such as Climate

Resilient Green Economy of Ethiopia, Millennium Development Goals, and Forest Sector development of Ethiopia, Growth and transformation plan of Ethiopia and other forest related national and international conventions.

Thus, study the effect of various environmental variables soil on the fungal community composition and sporocarps production in different ecosystems is of paramount importance to have a general understanding of processes in the forests ecosystems (Hanson et al., 2012; Hazard et al., 2013) to set up their management and conservation strategies. Investigating the fungal community composition and how this community changes across sites in fragmented forests should help us to understand different aspects of fungal interaction within these systems and their function in the ecosystem (Genevieve et al., 2019). This information would also enable the integration of fragmented forests into global biodiversity conservation strategies (Hundera et al., 2013; Aerts et al., 2016; Aynekulu et al., 2016) and to understand what actions are required to conserve these forest systems their biological components, including fungi (Burgess et al., 2006). Furthermore, the macrofungal study in fragmented Dry Afromontane forests is also a means to understand how to improve natural fungal richness and sporocarp production and help us to facilitate the conservation of economically and ecologically important macrofungal species in these fragmented high priority forest systems. Such information could help to guide management and conservation strategies for these priority forests and supplement our knowledge of macrofungal species in Ethiopia. Similarly, the knowledge of fungi and their community structure in relation to different age categories of plantation forests also helps to determine proper management strategies of plantation forest in Ethiopia. Furthermore, knowledge of the edible mushrooms produced in these forests could provide an opportunity for harvesting edible mushrooms for either subsistence or commercial use.

1.10. Hypotheses and research questions

In our study regarding the effect of fire on soil fungal community composition and diversity, we hypothesized that the richness and composition of the entire and functional soil fungal communities would change substantially during a post-fire forest succession

and would differ from those in unburned forest and the community turnover would partially be explained by edaphic variables. As a consequence, we expected ruderal, generalist saprotrophic fungi be more abundant and species-rich shortly after fire than in unburned forests. By contrast, root-associated symbiotic fungi were expected to be more diverse in older burned forests and unburned forests.

In our soil fungal study under *P. patula* plantation, we hypothesized that substantial change in the composition of soil fungal communities would be detected along the chronosequence of *P. patula* plantations. Specifically, we hypothesized that there would be changes in the total and functional fungal diversity and community composition along the chronosequence of the plantations.

In our macrofungal and soil fungal studies in the three fragmented church forests of the Dry Afromontane areas in North Ethiopia, we hypothesized that the fungal diversity of the church forests would be high in terms of total fungal species and functional status given that fungal diversity is related positively to plant richness (Tedersoo et al., 2014b). We also hypothesized that the composition of macrofungal communities would differ among the studied forests, resulting in an overall higher richness value for the study sites and their community composition is driven by vegetation and site conditions such as soil fertility (Castaño et al., 2018; Vašutová et al., 2017), climatic and spatial variability (Glassman et al., 2017; Li et al., 2020; Tedersoo et al., 2014a; Tedersoo et al., 2014b).

1.11. Scope of the study

To our knowledge, this research is the first systematic attempts focused to describe the fungal communities' structures and sporocarp production in fragmented forests in Dry Afromontane region of Ethiopia in relation to multiple environmental factors such as forest fire, vascular plant diversity, edaphic characteristics, climatic and other spatial variables. This research is also the first attempt to describe the soil fungal community composition in relation to stand development stage of *P.patula* plantation under Ethiopian condition. The field studies in the Dry Afromontane forests were based

both on soil fungal sampling and sporocarp collections. Sporocarp collections were done during the pick rainy season of the year in the studied forests. However, the study was conducted during the main rainy season of a single year in the Dry Afromontane forests and *P.patula* plantation in Ethiopia. Therefore, its wider application in other forest systems of the country shall be taken with caution. Long term sampling period and wider scale studies are required to know more about the fungal resource of the country. However, the results provide relevant information for developing sustainable management strategies of Dry Afromontane forests in Ethiopia by integrating fungi/mushrooms which in turn help to conserve these forest systems and their biological components, including fungi. The study on sporocarps including edible mushrooms in fragmented church forests could also provide an opportunity for harvesting edible mushrooms for either subsistence or commercial use and help for sustainable management of these forest systems.

Objectives

2. Objectives of the thesis

General objective

The general objective of the study was to provide information about fungal diversity and production associated to different forests systems in the dry afromontane region of Ethiopia as well as identifying the main factors driving their communities in the forest systems. This general objective was assessed through the following specific objectives;

Specific objectives

- To assess the effect of fire on soil fungal communities, taxa richness and diversity in dry Afromontane natural forest system in Southern Ethiopia (study I)
- To study the soil fungal communities, taxa richness and diversity along the stand development of *Pinus patula* plantation in Southern Ethiopia (study II)
- To explore macrofungal diversity, taxa richness and production and the environmental variables structuring macrofungal community composition in fragmented church forests of Dry Afromontane natural forest systems of north Ethiopia (study III)
- To elucidate the soil fungal diversity and factors governing their community composition in fragmented church forests of Dry Afromontane natural forest systems of northwestern Ethiopia (study IV)

Methodology

3. Material and methods

3.1. Data sources

All studies in this thesis (study I-IV) are totally based on field observations to provide relevant information important to describe the fungal community composition and diversity in various agro-ecologies of Ethiopia. Therefore, field data collections were conducted for the purpose. In relation to the current study, all available information i.e. published and unpublished secondary sources such as reports, journals and books were thoroughly reviewed. All referred published data sources are properly cited in the text where appropriate.

3.2. The study areas

The studies were conducted in dry Afromontane forest systems and *P. patula* plantations of Ethiopia located in south western Ethiopia at Wondo Genet (studies I and II) and in fragmented dry Afromontane church forests of North Ethiopia in Taragedam, Alemsaga and Banja forests in Amhara National Regional State (studies III and IV) (Fig. 5).

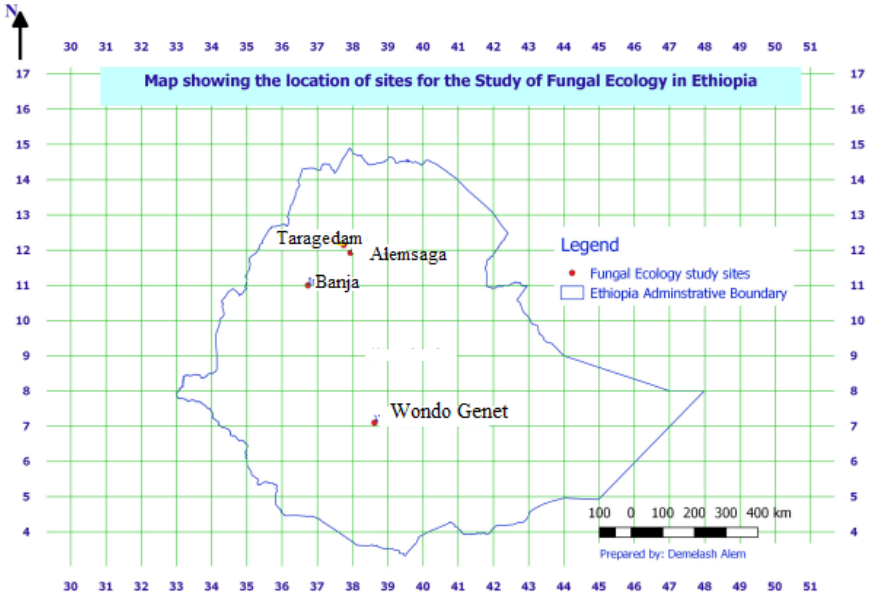

Figure.5. Location of the study sites

Wondo Genet natural forest area, where the study on soil fungal community in relation to fire conducted, covers about 797 ha of natural forests land (Ango and Bewket, 2007; Belaynesh, 2002; Fenta, 2014). The forest is characterized by remnant Dry Afromontane forest patches (Ango and Bewket, 2007; Belaynesh, 2002; Fenta, 2014) and harbours important fauna and flora (Belaynesh, 2002; Fenta, 2014). The climate is characterized by the Weyna-Dega agro-climatic zone, with a bimodal rainfall pattern: the main rainy season is in the summer and a lesser rainy season is in spring (Belay, 2016; Fenta, 2014). Part of the native vegetation in Wondo Genet study sites, where the study on effect of *P.patula* stand age on soil fungal community and diversity conducted (Fig. 6), was destroyed for cultivation (Teshome, 2011). In recent decades, a mass planting scheme of exotic tree species has been undertaken on those areas and resulted in approximately 100 ha of non-native plantations of *Cupressus lusitanica*, *Grevillea robusta* and *P. patula* (Bekele et al., 2013; Teshome, 2011).

Figure 6. Plantation of *Pinus patula* where soil fungal data was collected (photo credit, Dejene et al., 2017)

Figure 7. Dry Afromontane forests (A,Wondo Genet; B, Taragedam; C,Alemsaga; D, Banja) selected for our study (photo credit:7A, Dejene et al., 2017; 7B-7D, Demelash Alem)

The Taragedam (Fig. 7C) and Banja (Fig. 7D) forests were designated as reserves in 1979 (Zegeye et al., 2011) and 1994 (Abere et al., 2017), respectively, to prevent any kind of encroachments. The Alemsaga forest (Fig. 7B) was designated as a priority forest in 1978 to serve as a seed source, to conserve the remnant natural forest, and to rehabilitate the degraded area in the Northern part of the country (Masresha et al., 2015). Descriptions of the study forests are provided in Table 1.

Table 1. Characteristics of the study sites

Descriptions	Forests			
	Taragedam	Alemsaga	Banja	Wondo Genet
Geographical location	12°06'–12°07' N 37°46'–37°47' E	11°54'–11°56'N 37°55'–37°57'E	10°57'–11° 03'N 36°39'– 36°48'E	7°06' –7°07' N 38°37' –38°42' E
Altitude range (m asl)	2142–2484	2180–2470	1870–2570	1600-2580
Mean annual precipitation (mm)	1098	1926	1884.3	1210
Mean annual temperature (°C)	19.5	15.8	18.7	20
Forest area (ha)	875	814	897	797
Sand (%)	58.89(2.93)	51.78(2.99)	68.67(2.21)	56.55(2.14)
Silt (%)	28.44(2.38)	32.44(2.13)	20.00(1.76)	20.83(1.87)
Clay (%)	12.67(1.37)	15.78(1.93)	11.33(1.33)	23.23(1.81)
pH H2O 1:2.5	7.04(7.03)	5.85(6.59)	5.60(6.24)	6.46(0.12)
EC (dS/m)	0.43(0.05)	0.28(0.03)	0.81(0.14)	0.17(0.03)
Ex.Ca (cmol(+)/kg)	13.95(0.60)	9.19(0.52)	13.55(0.87)	22.03(2.48)
Ex.Mg (cmol(+)/kg)	6.16(0.10)	4.58(0.15)	5.54(0.20)	7.49(0.89)
Ex.Na (cmol(+)/kg)	1.95(0.05)	2.05(0.10)	1.82(0.12)	0.97(0.09)
Ex.K (cmol(+)/kg)	0.73(0.06)	0.61(0.04)	0.77(0.06)	0.60(0.07)
CEC (cmol(+)/kg)	47.21(1.36)	34.89(0.92)	44.51(1.96)	42.8(3.25)
Organic matter (%)	4.46(0.60)	3.35(1.34)	4.87(0.10)	9.14(1.03)
Nitrogen (%)	0.23(0.01)	0.17(0.02)	0.26(0.01)	0.49(0.05)
P (ppm)	17.18(5.72)	7.8(0.73)	17.64(6.05)	32.38(2.85)
Dominant species in each plots	*Maytenus obscura*, *Carissa edulis*, *Olea* sp.	*Acacia abyssinica*, *Buddleja polystachya*, *Acacia nilotica*	*Albizia gummifera*, *Prunus africana*, *Brucea antidysenterica*	*Juniperus procera, Podocarpus falcatus, Hagenia abyssinica Olea africana*
References	Gedefaw and Soromessa (2014) Zegeye et al. (2011) Zerihun et al. (2013)	Birhane et al. (2017) Masresha et al. (2015) Wubet et al. (2004)	Abere et al. (2017)	(Alem et al., 2020a; Belaynesh, 2002; Costa et al., 2014)

Note: Numbers in parentheses are standard error of the mean

3.3. Establishment of field plots

Plots in Dry Afromontane natural forests (study I) and plantations of *P. patula* (study II) of Wondo Genet study area were established by considering the similarity of the areas in climate, altitude, soil and other ecological conditions. We used the information from the Department of Forest Management in Wondo Genet College of Forestry (WGCF) to find areas with similar fire history in natural forests and stand ages classes in plantations. The plots established in Taragedam, Alemsaga and Banja forests (study III and IV) were based on their history and the composition of vascular plants. Field observations and published data sources were used to describe the sites. Within each of the selected areas in all studies, plots were placed systematically (Luoma et al., 1991) far enough from each other in order to provide relatively independent estimates as possible.

3.3.1. Plot establishment in Dry Afromontane forests to study soil fungal community composition and diversity in relation to forest fire (study I)

Sample plots were established in the forest in 2015. The control stand of unburned natural forest (UB) was representative of the original natural forest and had not been affected by fire for at least 40 years. Burned stands selected for the study were similar in terms of fire severity, *i.e*., the canopy and understory had burned and the soil organic layer had been consumed (Rincón and Pueyo, 2010). In these burned areas, two forest stands were selected based on fire history: (1) one-year-old burned forest (B1); and (2) ten-year-old burned forest (B10). Within each of these forest stands, three transects (a total of 9 plots) were established about 250 m apart from each other. Each transect covered an area of 100 m^2, with a rectangular shape (2 m $\times$ 50 m).

3.3.2. Plot establishment in *P. patula* plantation forest of different age to study the soil fungal communities and succession (study II)

In *P. patula* plantations, stands of three different age groups (5-, 11- and 36-years-old stands) were selected and three 2 m $\times$ 50 m plots were established in each age category (Gassibe et al., 2011) for soil sampling both for molecular work and for

soil physico-chemical analysis. A minimum distance of about 120 m was used between plots within a stand (Luoma et al., 1991).

3.3.3. Plot establishment to study sporocarps diversity and production (study III) and soil fungal community composition and diversity (study IV) in fragmented church forests in Dry Afromontane forest systems in Northern Ethiopia

In total, 27 sample plots were established, nine in each of the three church forests, as described in Gassibe et al. (2011) and (Hernández-Rodríguez et al., 2013). Each plot was rectangular in shape (2 m $\times$ 50 m). Within each of the selected church forests, we studied three different sites including three plots per site. The plots were established about a minimum distance of 500 m apart.

3.4. Sampling

3.4.1. Sporocarp sampling (study III)

All fungal fruit bodies found in each plot were harvested weekly. Fresh weight measurements were taken *in situ* to determine fruit body production in kilograms per hectare per year. The number of individuals of each species in each plot was also recorded. Specimens were photographed in the field and their morphological features and ecological characteristics were noted to facilitate taxonomic identification processes in the laboratory (Adeniyi et al., 2018). Specimens of each macrofungus were taken to the laboratory and dried to preserve as herbaria specimens, and then used for morphological taxa identification.

3.4.2. Soil sampling for DNA extraction (Study I, II & IV)

Five cores were extracted in each plot using a cylindrical (2 cm radius, 20 cm deep, 250 cm3) soil borer (De la Varga et al., 2012; Taylor, 2002) along the centerline of each transect and 5 m apart to collect spatial variability and minimize the probability of sampling the same genet repeatedly. Soil sample cores from each plot were pooled

to form a composite sample for DNA extraction. Soil cores were dried, sieved through a 1 mm mesh and grounded to a fine powder using a mortar and pestle. A subsample was stored at -20ºC until submitted for molecular analysis.

3.4.3. Soil sampling for physico-chemical analysis (Studies I, II, III & IV)

To relate soil fungal composition to edaphic variables, additional soil samples were collected from each transects. Soil samples, from the center and from the four corners of each plot in each study, were extracted to a depth of 20 cm with the aid of an auger and spade after clearing plant matter and debris. A composite soil sample of approximately 500 g from each plot was placed in a plastic bag and transported to the laboratory for the determination of edaphic variables.

After air drying the soil in shade, important chemical and physical properties of the soil were determined using DTPA extraction, KH_2PO4 extraction, Olsen, Kjeldahl digestion, Walkley–Black, ammonium acetate and instrumental methods respectively. The analysis was conducted by Water Works Design and Supervision Enterprises, laboratory service sub process, soil fertility section at Addis Ababa, Ethiopia (Studies I and II) and Amhara Design and Supervision Works Enterprise at Bahir Dar, Ethiopia (studies III & IV). Main edaphic variables for the natural forest in Wondo Genet (Table 1 in study I), *P. patula* stands (Table 1 in Study II) and church forests of fragmented natural forests of in Northern Ethiopia (Table 1 in study III & Table 1 in study IV) are summarized.

3.4.4. Vegetation and climate data collection (Studies III & IV)

To relate the vegetation characteristics to macrofungal (study III) and soil fungal (study IV) richness and diversity, vegetation inventories were conducted in the plots established for fungal sampling. Vascular plant identified in each plots were recorded using their vernacular names. For those species difficult to identify their scientific name in the field, specimens were collected and their taxonomic identification was conducted using published volume of the flora of Ethiopia and Eritrea (Hedberg and Sue, 1989). Large trees growing outside the plots were included in the survey if their crowns

overhung the plots because tree crown projection areas can affect fungal occurrence (Collins et al., 2018). Furthermore, large trees create their own microhabitat and develop a large root system, providing more space for fungal associations (Schön et al., 2018). Vascular plant species richness and diversity parameters were determined (Table 3 in study III). Plant parameters and their correlations were also used for further interpretation of fungal pattern from each study areas. The mycorrhizal status of the vascular tree species found in each of the studied plots were checked using freely accessible databases (Soudzilovskaia et al., 2020).

Since rainfall and temperature affect fungal community composition and diversity (Bahram et al., 2012; Djelloul and Samraoui, 2011), rainfall and temperature data of the nearby meteorological stations were procured from the Ethiopian National Meteorological Agency (NMA), Bahir Dar meteorological service center. This was done to relate climatic variables with soil fungal species composition and diversity.

3.5. Laboratory analysis and taxa identification

3.5.1 Sporocarp taxa identification and classification (Study III)

In the laboratory, the morphological features of the fruit bodies were examined using appropriate monographs, including Antonin (2007), Hama et al. (2010), Heinemann (1956), Hjortstam and Ryvarden (1996), Morris (1990), Pegler (1968, 1969, 1977), Rammeloo and Walleyn (1993), and Singer (1965), to determine the genus and species of the macrofungal specimens. Up-to-date fungal taxa names and authors' names were obtained from the Mycobank database (http://mycobank.org). Ecological functions at the genus level were identified using a FUNGuild (www.funguild.org) search and provided (Table 2, study III). In addition, the edibility of the fruiting bodies collected from the study sites was assessed following the criteria used by Bonet et al. (2004). Taxa described in the literature as both non-edible and edible in the literature were classified as non-edible. Taxa described in the literature as having doubtful edibility were classified as non-edible. Only species classified as edible by a large majority of the literature consulted were classified as edible fungi (E).

3.5.2. Molecular analysis (Studies I, II & IV)

DNA was extracted from 0.25 g of soil per sample using a PowerSoil™ DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA). PCR reactions were performed in triplicate for each sample to minimize PCR biases. PCR reactions were performed in 20 µl reaction volumes containing 11.22 µl of MQ water, 1.60 µl of DNA template, 2.00 µl of 10× buffer, 1.40 µl of $MgCl_2$ (50 mM), 1.60 µl dNTPs (10 mM), 0.50 µl BSA (2%), 0.80 µl of reverse and forward primers (10 µM) and 0.08 µl Platinum Taq polymerase (Invitrogen, Carlsbad, CA, USA). We used the following PCR conditions: an initial denaturation step at 94°C for 3 min; then 35 cycles of 94°C for 45 s, 50°C for 1 min and 72ºC for 1.5 min; and a final cycle of 72°C for 10 min. The ITS2 rDNA region was amplified using the forward primer fITS7 (Ihrmark et al., 2012) and the barcoded reverse primer ITS4 (White et al., 1990). The ITS4 primer was labelled with sample-specific Multiplex Identification DNA-tags. A negative control consisting of MQ water instead of DNA was included in each PCR run. The absence of bands on gels indicated that negative controls were amplicon free. Ion Torrent sequencing was carried out at the Naturalis Biodiversity Center. The sequencing Ion 318^{TM}Chip was used to allow for the highest possible sequencing coverage.

3.6. Data analysis

3.6.1 Bioinformatics analysis (Studies I, II & IV)

Raw sequence reads comprising demultiplexed sample reads were obtained from the Ion Torrent output. Primers and poor-quality ends were removed based on a 0.02 error probability limit in Geneious Pro 8.1.8 (BioMatters, New Zealand). Next, all sequences were truncated to 200 bp and then filtered with USEARCH v.8.0 (Edgar, 2010) to discard sequences with an expected error of >1. The remaining sequences were collapsed into unique sequence types on a per-sample basis using USEARCH v.8.0 (Edgar, 2010) while preserving read counts. First, we discarded singleton sequence types before grouping the remaining high-quality sequences into operational taxonomic units (OTUs) with USEARCH at a 97% sequence similarity level while simultaneously excluding OTUs with <70% similarity or <150 bp pairwise alignment

length to a fungal sequence. Sequences were assigned to taxonomic groups based on pairwise similarity searches against the curated UNITE+INSD fungal ITS sequence database, which contains identified fungal sequences with assignments to species hypothesis groups (Kõljalg et al., 2013). Up-to-date fungal taxa names and authors' names were obtained from Mycobank database (http://www. mycobank.org). The FUNGuild database (http://www.funguild.org) was initially used to perform functional classification of OTUs at the genus level and it was manually checked afterwards. OTUs with >90% similarities to a fungal SH with known ecological function were assigned to functional groups. For genera that are known to comprise species from multiple functional guilds, their ecological function was assigned individually based on available ecological information for the matching SH in the UNITE database.

3.6.2. Statistical analysis (Studies I-IV)

All explanatory environmental variables were subjected to appropriate data transformation when needed to achieve the parametric criteria of normality and homoscedasticity. Shannon's H' diversity indices, $H = -\Sigma pi(\ln pi)$ (Shannon and Weaver, 1949), were estimated, where p indicates the relative abundance of fungal OTUs (Kent and Coker, 1993). The Simpson's diversity indices, $D = 1 - \Sigma (pi2)$, where pi is the importance probability in element i; and the Evenness, $J = H'/H'max$, where H′ is the number derived from the Shannon diversity index and the H′ max is the maximum possible value of H′ were also calculated (Magurran, 1988). In addition, the richness values of all fungal OTUs (S) based on treatment type were estimated. All diversity measures were calculated using the BiodiversityR package (Kindt and Coe, 2005) in R (R Core Team, 2020). For studies which ANOVA assumptions were met, diversity indices and richness were compared across treatments using one-way ANOVA using R (R Core Team, 2020) and Tukey HSD was used to determine significant differences between means ($P \leq 0.05$) among treatments. Linear Mixed Effects models (LME, Pinheiro et al., 2016) was used to prevent the false positive associations due relatedness structure in the sampling (study III). The most significant variables used for interpretation of fungal diversity and community composition were selected using the *forward.sel* function of adegraphics package (Siberchicot et al., 2017) in R. Separate

analysis was conducted on functional groups of fungi to determine whether their abundance and diversity were affected by explanatory environmental variables.

Ordination techniques based on Hellinger-transformed fungal abundance data were used to discern changes in fungal community composition among treatment and to identify significant explanatory variables related to taxa composition. The abundance data matrices against edaphic variables were subjected to a canonical correspondence analysis (CCA) using PC-ORD v. 6.0 software (McCune and Mefford., 2011) (study II). Non-parametric data analysis methods were used when transformations did not provide the appropriate results or a clear interpretation (Ágreda et al., 2014). Non-metric multidimensional scaling (NMDS) was conducted using *metaMDS* function of the vegan package in R on Hellinger-transformed abundance of fungal data matrices against explanatory variables (Study III and IV). Correlation of ordinations axes scores with edaphic, vegetation and climatic variables were assessed using linear regression. A multiple-response permutation procedure (MRPP) and a permutation-based nonparametric MANOVA (PerMANOVA) (Anderson, 2001) were run using Bray–Curtis distance and *adonis* function of the vegan package (Oksanen et al., 2019) in R (R Core Team, 2020) to analyze differences in fungal communities across forests or among treatments. An analysis of similarity percentages (SIMPER; Clarke, 1993) was also performed using the *simper* function of the vegan package (Oksanen et al., 2019) in R (R Core Team, 2020) to identify fungal species that were most responsible for the observed patterns and determine the percentage contribution of fungal taxa to significant dissimilarities between the three forests (Parravicini et al., 2010). Indicator species analysis was determined using the *multipatt* function of the indicspecies package (Caceres and Legendre, 2009) in R.

Fungal species accumulation curves and the Rényi diversity profile were also generated using a sample-based estimator of EstimateS Version 9 (Colwell, 2013) to compare fungal richness and diversity among different treatments. A Rényi diversity profile (Tóthmérész, 1995) was also used to depict the diversity curves among treatment. Unless stated, all data analysis was conducted using R Software version 4.0.3 (R Core Team, 2020).


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Results

4. Results

4.1. Taxa composition of soil fungi (Study I, Study II and study IV)

Our studies indicated that the Dry Afromontane forests in Ethiopia harbor diverse fungi species. A total of 2,898, 2262 and 5152 soil fungal OTUs, respectively, were collected from Dry Afromontane natural forests in Southwestern Ethiopia (Fig 2A in study-I), plantation of *Pinus patula* (study-II) and fragmented church forests in Northwestern Ethiopia (Fig 1A in study-IV). The OTUs in all these studies were dominated by Ascomycota. We found that Agaricales was represented by the largest number of OTUs in the Dry Afromontane church forests (study-IV). *Coprinellus* and *Marasmius* were dominant fungal taxa in this study. The distribution of fungal taxa in families and genera are presented (Fig 2 & Fig 3 in study-I; Fig 1A in study-IV). In these studies, significant number of fungal taxa was not identified down to genus and species level.

We also found that the majority of the fungal taxa in these studies were saprotrophs (Fig 2B in study-I; Fig 1B in study-IV) and considerable proportions of ECM fungi were observed (3% in study-I; 2 % in study-II; 11.5 % in study-IV).

The results form Ordinations showed the difference in fungal community composition among different age categories of *P. patula* stand (Fig 2a & Fig 3a in study-II), fire history in natural Dry Afromontane forest systems at Wondo Genet forests (Fig 7 in study-I) and the different fragmented church forests of Dry Afromontane forest systems (Table 2 & Fig 4 in study-IV). The soil fungal study in fragmented Dry Afromontane church forests showed that each forest type was characterized by unique indicator species except few fungal species in common. The species making the highest contributions to the dissimilarity between stands with different fire history was Agaricus campestroides (study-IV). This species was highly abundant in one-year-old burned stands but much less abundant in ten-year-old burned and unburned stands.

We also found distinctive soil fungal communities among the three stand age groups of P. patula. Some fungal species belonging to the genera *Tomentella*, *Ramaria* and *Inocybe* were detected in the three development stages of *P. patula*. Species of *Rhizopogon* were associated more with younger stands (5- and 11-year-old stands). Higher numbers of ECM species were observed in the 36-year-old stand and the 5-year old stand of *P. patula*.

The soil fungal studies in fragmented church forests of Dry Afromontane forest systems revealed that the relative proportion of saprotrophs was significantly highest in Taragedam forest than the other forest groups ($p < 0.05$; Figure 2 in study-IV). The relative proportions ECM and Arbuscular mycorrhizal soil fungi were significantly lower in Alemsaga forest ($p<0.01$).

The ordinations (Fig 8 & Table 2 in study-I; Table 3, Table 4 & Fig 2b in study-II; Fig 4 in study-IV) and the forward selection of the environmental variables showed that edaphic variables affected the composition of fungal communities. Mg ($F = 1.71$, $p = 0.023$), K ($F = 1.63$, $p = 0.036$) and OM ($F = 1.4$, $p = 0.047$) affected the community composition of mycorrhizal fungal species in study I. K ($F = 26.83$, $p = 0.010$) and Mg ($F = 7.52$, $p = 0.008$) affected the community composition of entire soil fungi and P ($F = 2.10$, $p = 0.052$) affected the community composition of ECM fungi in study II. Edaphic variables that affected fungal community composition in study IV were pH ($F = 0.27$, $p = 0.021$), Ca ($F = 0.46$, $p = 0.002$), Mg ($F = 0.48$, $p = 0.002$), K ($F = 0.23$, $p = 0.047$) and N ($F = 0.55$, $p = 0.001$).

Spatial variables (elevation, latitude, longitude), aboveground vegetation (plant cover, basal area, tree density, shrubs coverage, tree diversity) and climatic variables (annual rainfall, average daily max temperature and cumulative rainfall 30 days before sampling) were also important in shaping fungal community composition in study-IV (Table 3 & Fig 4 in study-IV).

4.2. Macrofungal Taxa composition (Study-III)

In total, 13,736 sporocarps composed of 258 fungal species were collected from the three church forests. The Basidiomycota was the dominant phylum and Agaricaceae was the most diverse family (Table 2 in study-III). *Termitomyces*, *Psathyrella*, *Leucoagaricus*, *Marasmius*, and *Mycena* were found as the most abundant genera (Fig 2A in study-III). The majority of species were saprophytic (81%) followed by ECM (14%). About 38 species (36%) of the total macrofungi collected were edible (Table 2 in study-III). The sporocarp productions (kg ha-^{1}) were not high.

Some important ECM species such as *Tricholoma*, *Rhizopogon*, and *Suillus;* and valuable edible species belonging to the genera *Calvatia*, *Laetiporus*, *Pleurotus*, *Termitomyces* sp., and *Macrolepiota* were found (Fig 2B in study-III).

The three church forests differed significantly in their macrofungal composition ($F=2.05$, $R^2=0.14$, $p=0.001$; Fig 6 in study-III). Edaphic, climate and location parameters were correlated to the macrofungal community composition ($p<0.05$). Among these, location variables had a strongly significant effect on macrofungal community structure ($p = 0.000$) (Table 4 in study-III). The SIMPER analysis identified macrofungal species that characterized the three forests and the *Coprinellus* species are found the most important in distinguishing all forest locations along with the others (Table 5 in study-III).

The ordination (Fig 6 in study-III) and the forward selection of the environmental variables showed that edaphic variables affected the composition of fungal communities. Soil pH ($F = 0.44$, $p = 0.004$), CEC ($F = 0.32$, $p = 0.01$) and OM ($F = 0.28$, $p = 0.017$) were identified as important factors shaping the macrofungal communities. The majority of macrofungal taxa were associated to plots with low pH and CEC values. Maximum temperature ($F = 0.34$, $p = 0.004$), minimum temperature ($F = 0.62$, $p = 0.001$) and latitude ($F = 0.62$, $p = 0.001$) also affected the macrofungal community composition in fragmented church forests (Table 4 & Fig 6 in study-III).

4.3. The effect of fire (Study-I), stand age (Study-II) and aboveground plant diversity (study-III and-IV) on fungal richness, diversity and production

Fire significantly affected the fungal taxa richness in Dry Afromontane forests in Wondo Genet area (F=12.48, *p*=0.002, Fig 4 in study-I). Higher taxa richness was observed in one and ten years after fire than unburned site. However, forest fire did not show significant effect on fungal diversity indices of functional guilds in these forest systems (Fig 5 in study-I). Relatively higher diversity index of ECM fungi was recorded in the 36-year-old stand than the other age groups of *P.patula* plantations.

There was no significance difference in the richness and diversity of macrofungal taxa among the studied fragmented church forests in North Ethiopia. Numerically higher richness and diversity indices of ECM fungal taxa were recorded in Banja forest (Table 3 in study III). The soil fungal study in study-IV showed significant differences in richness and diversity indices of soil fungi in the studied fragmented church forests (Fig 3A &Fig 3B in study-IV). Taxa richness of soil fungi in three fragmented church forests in the Dry Afromontane forest systems of north Ethiopia was significantly highest in Taragedam forest (Taragedam-Alemsaga, $p < 0.001$, Taragedam-Banja, $p < 0.05$, Alemsaga-Banja, $p <= 0.001$) (Fig 3A in study-IV). Fungal diversity value was also the highest in Taragedam forest (Fig 3B in study-IV).

Taragedam forest produced the greatest quantity of sporocarps (25.4 kg ha^{-1}), although production levels were not significantly different ($p = 0.63$) to those of Alemsaga forest (21.6 kg ha^{-1}) (Fig 5 in study-III). However, both of these forests produced significantly greater quantities of sporocarps than Banja forest ($p < 0.05$). About 38 species (36%) of the total macrofungi collected were edible (Table 2 in study-III). Banja forest produced the greatest quantity of edible fungi (1.8 kg ha^{-1}) and Alemsaga forest produced the least (0.4 kg ha^{-1}); however, the production of edible taxa did not differ significantly among the three forests (Fig 5 in study-III; $p = 0.01$).

Discussion

5. Discussion

5.1. Soil Fungal Taxa Composition (Studies-I,-II, and-IV)

Fragmentation poses major threats to Dry Afromontane forest ecosystems of Ethiopia. However, these forests are considered to be major reservoirs of biodiversity (Aerts et al., 2016; Aynekulu et al., 2016; Darbyshire et al., 2003; Nyssen et al., 2014). Similarly, we found a huge soil fungal diversity in these forest systems with clear differences in community composition among the three study areas located in Northern Ethiopia (Study IV). The diverse fungal species in such forest systems could be mainly explained by higher tree species diversity (Chen et al., 2017) and due to the improved soil fertility from added nutrients in the decomposition process of wood materials that provide the required nutrients for diverse groups of fungal species (Siciliano et al., 2014).

In all studies, the fungal taxa were dominated by Ascomycota, which is congruent with other studies in different forest ecosystems (Geml et al., 2014; Reazin et al., 2016; Smith et al., 2017; Tedersoo et al., 2014). The dominancy of Ascomycota could be due to their higher genomic potential for resource utilization, competition, and stress tolerance (Egidi et al., 2019). In all our studies it was observed that significant numbers of fungal taxa were not identified down to genus and species level indicating lack of data from understudied tropical and subtropical forest ecosystems (Tedersoo et al., 2014) such as the forest systems in Ethiopia. The largest proportions of identified fungal species in our studies were saprophytic which play a role in decomposition of organic matter, as a source of human food (Kirk et al., 2008) and as biocontrol agent in agriculture (Rossman et al., 1999; Samuels, 1996).

Our study on soil fungal community under different age groups of P. patula plantation showed that the functional groups of fungi from the whole study plots were saprotrophs (41%) followed by plant pathogenic (7%) and ECM (2%) fungi. The low proportion of ECM fungi detected under *P.patula* plantations might be due to the

conversion of the native vegetation of the area to crop cultivation many years ago (Teshome, 2011).

Studies indicated that the proportion of mycorrhizal fungi in tropical regions is low and the majority of plant species in the region do not form mycorrhizal association with fungi (Brundrett, 2009). Absence of ECM fungi in the dry Afromontane forests of Ethiopia was also reported previously (Dejene et al., 2017a). In contrast, we encountered higher relative proportion of ECM fungi in such forest systems (Fig 2B in study-I; Fig 1B in study-IV). This association may be due to the diverse vegetation (Friis et al., 2010) and the presence of more trees that host mycorrhizal fungi, or may be due to the dispersion of mycorrhizal inocula from nearby plantation forests dominated by *Eucalyptus* and *Pinus* species (Castaño et al., 2019; Dejene et al., 2017a, 2017b; Urcelay et al., 2017). High number of ECM species in such forest systems could also be justified by the older and denser forests that maintain temperature and adequate moisture (Fernández-Toirán et al., 2006; Pinna et al., 2010; Toivanen et al., 2012). The root systems of the old trees also facilitate the occurrence of ECM fungi (Mölder et al., 2014).

In study-II, some fungal species under the genera *Tomentella*, *Ramaria* and *Inocybe* were found associated with *P. patula* trees at all age stages of tree development. *Tomentella* and *Inocybe* are cosmopolitan species that inhabit *Eucalyptus* plantations in Ethiopia (Castaño et al., 2019). Species of *Rhizopogon* were also associated more with younger stands (5- and 11-year-old stands) in this study, which supports previous findings, that they are early colonizer fungal species (Tedersoo et al., 2016b). The *Rhizopogon* species are known as spore bank species that facilitate the establishment of trees in formerly non-forest habitats. Amanita, which was also found in our study site of P. patula plantation are well-known for their association with conifer forests as the genera is characteristic of late-stage pine stands that are 30–40 years old (Chu-Chou and Grace, 1982; Visser, 1995).

Previous studies indicated that the composition of ECM fungi in the soil is correlated with soil fertility and the growth status of the host trees (Cozzolino et al.,

2016; Wang and Wang, 2008). Similarly, we found that the 36-year-old stand and the 5-year old stand of P. patula had distinctive soil fungal communities. The distinct composition of ECM fungi in young and old stands under P. patula plantations (study II) might be related to site quality factors, such as soil fertility and stand age factors. Given that the amount of OM, available P and the C/N ratio of 5- and 11-year-old stands were not significantly different, this may have enabled 11-year-old stands to develop an association with only a limited number of ECM fungi, but a higher relative abundance of these ECM fungi, which could indicate increased dependence of P. patula trees on a limited number of dominant symbionts species.

We observed differences in fungal community composition and diversity due to forest fire. This could be due to a change in vegetation (Hart et al., 2005) and loss of host plants after fire (Pattinson et al., 2006; Smith et al., 2005). More fungal species were detected in the burned forest areas where the soil fertility was relatively low than in unburned areas, which could be related to depositions of ash after the fire (Hul et al., 2015). Ash depositions could create empty niches for rapid colonization of the area by early stages colonizer fungi (Fritze et al., 1993). Agaricus campestroides was highly abundant in one-year-old burned stands but much less abundant in ten-year-old burned and unburned stands. The species might be partially responsible for the differences between stands, suggesting that time after fire is also probably responsible for the variation in the dominance of some species and their exclusive occurrence in certain stands. This is supported by previous findings that, for a given stand, certain fungal species tend to be abundant and characterize its composition (Zhu et al., 2010).

Distinct fungal community composition pattern was also observed in our soil fungal study in the studied Dry Afromontane forest systems in North Ethiopia (study-IV). Such differences indicate the site specific nature of fungal assembly such as ecological gradients (Egidi et al., 2019) and vegetation (Egidi et al., 2019; Tedersoo et al., 2016).Vegetation is known to affect fungi composition along with the processes that influence the nature and quantity of resources entering into the soil (Wardle et al., 2004) which further implies that fungal community is structured through the environmental

factors (Hanson et al., 2012; Hazard et al., 2013) that regulate the assemblage of vegetation.

Studies demonstrated that fungal community composition can be governed by various environmental variables and landscape heterogeneity (Bahram et al., 2015; Ferrari et al., 2016; Peay et al., 2010; L. Tedersoo et al., 2014). Thus, evaluating the fungal communities in different ecosystems is essential to filter out the relative contributions of environmental factors to fungal diversity and composition in an ecosystem (Tian et al., 2018).

Soil characteristics strongly affect fungal community structures (Straatsma et al., 2001; Zakaria and Boddy, 2002; Lauber et al., 2008; Reazin et al., 2016; Yang et al., 2017), community composition (Castaño et al., 2019; Delelegn et al., 2018; Lauber et al., 2008) and distribution (Claridge et al., 1993). Specific fungal species are likely to respond to environmental variables, mainly edaphic parameters, in different ways (Cozzolino et al., 2016; Koide et al., 2014), and, thus, in turn, the composition of the fungal community is directly correlated with edaphic variables (Cozzolino et al., 2016).

Nitrogen and Phosphorous significantly affected the community composition of entire soil fungal in our fire experiment in Dry Afromontane forest systems (study I). Previous reports also indicated that high availability of N and P could negatively affect the structure of fungi in the soil, particularly of the mycorrhizal fungi (Zhao et al., 2018) by decreasing plant dependency on fungi and reducing carbon allocation to fungi (Liu et al., 2019) which eventually could cause competition among the fungal species and lead to the formation of distinct fungal composition (Wang and Wang, 2008; Zhao et al., 2018). Available P also influenced the composition of soil ECM fungi in *P. patula* plantation (Table 4 in study-II), which was similar to the findings reported by Rosenstock et al. (2016). However, we noted positive correlation between N and entire soil fungal community composition in Dry Afromontane forests in Ethiopia (study-IV). This most probably indicates that the majority of plant species in the studied forest are independent of mycorrhizal fungi.

Soil pH is reported as important factors governing the fungal composition (Ullah et al., 2019). Our study on soil fungi in the Dry Afromontane forests in North Ethiopia (study-IV) indicated that fungal composition is maintained at a lower pH level. However, some fungal taxa are directed towards the higher pH level which could be due to the ability and adaptability of these taxa to grow in a comparatively alkaline soil condition (Nevarez et al., 2009; Tian et al., 2018).

Cations play an important role in many physicochemical processes, such as photosynthesis (He et al., 2017) and, hence, the amount of carbon that is available to soil fungi (Shi et al., 2014) in forests soils. Our study showed significant effect of Ca, Mg, and K on soil fungal community composition in the Dry Afromontane forests (study-IV).

The climatic and vegetation characteristics are reported to influence the spatial variation and thus, the composition of fungal communities (Newsham et al., 2016). In our soil fungal study in the Dry Afromontane forest systems in North Ethiopia (study IV), we observed a strong association of soil fungi with daily temperature and annual rainfall. In line with our results, other studies indicated that temperature shapes the composition of soil fungal communities in forest ecosystems (Newsham et al., 2016) since air temperature together with the moisture in the soil increases the metabolic activity of fungi, extends the period for which fungi are active each year, and enables a switch from survival to growth strategies. Thus, the distinct soil fungal communities found in each of the church forests (Study-IV) may follow the variation of the rainfall of the three areas, the plant community in each of the forests, or both (Hawkes et al., 2011).

5.2. Macrofungal Taxa composition (Study-III)

Despite habitat fragmentation is reported as negatively affecting the fungi community in forest systems (Sapsford et al., 2017), we found a high number of macrofungal species in fragmented church forests in Dry Afromontane region of Ethiopia. The diverse fungal species in such forest systems could be mainly explained by higher tree species diversity (Chen et al., 2017) and due to the improved soil fertility

from added nutrients in the decomposition process of wood materials that provide the required nutrients for diverse groups of fungal species (Siciliano et al., 2014).

Significant numbers of fungal taxa were not identified down to genus and species level indicating lack of data from understudied tropical and subtropical forest ecosystems (Tedersoo et al., 2014) such as the forest systems in Ethiopia. The largest proportions of identified macrofungal species in our study were saprophytic. Such functional groups are important for decomposition of organic matter and are valuable food sources for humans (Kirk et al., 2008). Some of the species are also important in agriculture as biological control agents (Rossman et al., 1999; Samuels, 1996).

The sporocarp productions obtained in this study were not high. Although further research is needed, this could partially be explained by the single tone species. Some of the species were collected in a single time during the collection period. Moreover, majority of the species were saprophytic fungi which are characterized by low biomass productions (Gassibe et al., 2011; Mediavilla et al., 2014). However, valuable edible macrofungal species belonging to the *Calvatia*, *Laetiporus*, *Pleurotus*, *Termitomyces* sp., and *Macrolepiota* genera were also collected in this study. Among these edible species, *Termitomyces* sp. is highly regarded by local people in southwest Ethiopia because of its good taste and aroma (Abate, 2014). This also provides a starting point in terms of broadening the management and conservation of fragmented forests for the production of non-timber forests products in Ethiopia.

Although, previous studies indicated the low proportion of ECM species in the tropics (Dejene et al., 2017a; Tedersoo et al., 2014), we found more ECM taxa (14% of the total). This could be attributed to higher vascular plant diversity (Friis et al., 2010), availability of more trees that host mycorrhizal fungi (Hailemariam et al., 2013; Wubet et al., 2003) or the dispersion of mycorrhizal inocula from the nearby plantation forests. This finding indicates the important implication of the indigenous forest system for the maintenance of functional fungal diversity in Ethiopia (Dejene et al., 2017a).

Soil pH is known to be the most critical edaphic variable affecting the composition and structure of fungal communities (Docherty et al., 2015; Fierer and Jackson, 2006b; Zhang et al., 2016). Similarly, in this study, soil pH correlated with fungal species composition and the presence of greater numbers of macrofungal species was associated with lower pH values. This was in line with the findings of Puangsombat et al. (2010) and Zhang et al. (2016) who reported negative influence of higher pH levels on fungal community structure, probably because a higher pH restrains the expansion of fungi and the production of sporocarps.

Organic matter influences mycelial outgrowth and network formation (Zakaria and Boddy, 2002) and fungal community through its impact on the water-holding capacity and soil and nutrient availability (Harrington, 2003). Thus, a high level of organic matter accumulation implies a high level of macrofungal assembly, particularly of saprophytic species. Similarly, we found OM associated with the composition of macrofungi in the studied fragmented church forests of Dry Afromontane forests systems (Figure 6 in study III).

We also found that CEC associated with macrofungal composition. Crabtree et al. (2010) observed that fungal species richness was low, particularly when the CEC was high. This is probably because the CEC influence nutrient availability, soil pH, and soil reactions to other ameliorants in the soil (Ogeleka et al., 2017). CEC is vital in many physicochemical processes, such as photosynthesis (He et al., 2017) and thereby influence the amount of carbon available to fungi in the soil (Shi et al., 2014).

Maximum and minimum temperatures also affected macrofungal composition. This may be due to the fact that the mycelium of the fungal species is more readily affected by atmospheric changes (Salerni et al., 2002), being more superficial specifically for those saprotrophs species that constitute the majority of macrofungal taxa in our studied forests. Temperature can also play important role in nutrient cycling process (Geng et al., 2017) which in turn results in the formation of distinct fungal communities, particularly of the fungi that are soil dependent as a substrate (Nicolás et al., 2019).

5.3. Effect of fire (study-I), stand age (study-II), and aboveground plant diversity (study-III and study-IV) on fungal richness, diversity and production

Our study on the effect of fire on soil fungal community (study-I) revealed that higher total richness values were found in forest stands recently affected by fire than unburned stands. This could be attributed to the new ecological conditions created by differences in fire severity, which may incite or support spore germination of several fungal species in the soil (Heino, 2012) following the fire in the investigated forests. In addition, the mycelium of fungal species in the rhizosphere may persist (Cowan et al., 2016; Shen et al., 2016) or the fungal community may be resilient to the effects of fire to some extent (Cowan et al., 2016; Jennings et al., 2012).

The study also revealed absence of significant difference in fungal diversity between fire affected areas. This could be due to the less fuel consumed and heat produced during fire (Reazin et al., 2016; Semenova-Nelsen et al., 2019). Rather the change might have been driven by indirect effect of fire in soil properties or by the change in the plant communities (Oliver et al., 2015; Ponder et al., 2009; Trappe et al., 2009). Also, fungi in a recurrent forest ecosystem may be adapted to frequent fires (Semenova-Nelsen et al., 2019) (Dean et al., 2015; Hart et al., 2005) (Semenova-Nelsen et al., 2019). Furthermore, the intensity of the fire might not have been high enough to affect the below-ground fungal communities (Bárcenas-Moreno et al., 2009; Egidi et al., 2016). Thus, the responses of soil fungi to recurring low-intensity fire also appear to be minimal (Johnson et al., 2013; Oliver et al., 2015) and ephemeral (Hart et al., 2005).

Time since the fire occurrence did not affect fungal guild diversity. In agreement with our finding, Egidi et al. (2016) noted absence of a significant change in fungal diversity following fire. This might be due to low fuel loads that resulted in little heat transferred to the soil (Lunt and Morgan, 2002)..

Our study also revealed that both the richness and diversity of ECM fungi were higher in in the recently burned stands, which could indicated an immediate post-fire mycorrhizal colonization in fire-affected forest stands (Dahlberg, 2002; Rincón et al., 2015). The ECM taxa may also have established dominance immediately after burning owing to their tolerance of fire effects (Dahlberg, 2002; Kipfer et al., 2010) or they may have survived in a mycelial state during the fire event (Hewitt et al., 2013). In contrast, , other studies reported that older stands support greater diversity of ECM fungi (Fernández-Toirán et al., 2006; Pinna et al., 2010; Toivanen et al., 2012) and generally have higher abundance of ECM (Mölder et al., 2014).

The relatively higher diversity indices of ECM fungi in the 5-year-old stand than in the 11-year-old stand under P. patula may possibly be attributed to the less developed tree canopy at the earlier stage of stand development, which may have allowed diverse ECM fungi to interact extensively with the root systems of understory plants (Dang et al., 2017) and/or the previous land use of the plantation area (agricultural crop production). Deacon and Fleming, (1992) demonstrated that when afforestation takes place on land initially used for other purposes, the ECM fungal spores are the fundamental inoculum during the early stage of ECM succession. The spore banks might have also derived from other nearby mycorrhizal-associated plantations such as Eucalyptus plantations (Castaño et al., 2019; Dejene et al., 2017d).

The higher diversity indices of ECM species detected in the 36-year-old stand than in the 11-year-old stand may be due to the thinning carried out in this stand unlike the other two age groups of P. patula (Dejene et al., 2017b). Chen et al. (2015) because thinning could increase the relative abundance of mycorrhizal fungi as it opens up the forest canopy, which in turn, increases soil temperature and moisture (Wang et al., 2019). Thinning may therefore have a positive effect on microbial activity (Pang et al., 2013) because soil temperature and moisture influence the reaction of microbial enzymes and, thus, shift the microbial community composition by altering substrates and extracellular enzyme activity (Hassett and Zak, 2005). Dove and Keeton (2015) and Tomao et al. (2020) suggested that fungal diversity can be conserved or even increased using forest management practices that enhance the structural complexity of stands and

the late-successional characteristics of the forest and by carrying out low-impact logging operations. Castaño et al. (2018) observed that the species diversity of soil fungi remained stable after thinning, regardless of its intensity, when sufficient host trees and functional roots from thinned trees were retained.

The variations in macrofungal taxa among the studied fragmented church forests in Dry Afromontane forest systems in North Ethiopia (Study-III) probably reflect the heterogeneity of these habitats, resulting in variations in microclimate such as in moisture, temperature, and other factors (Suggitt et al., 2011) that influence the richness and productivity of fungi (Gómez-Hernández and Williams-Linera, 2011). In addition, the difference in the availability of suitable substrates such as plant residues on the forest floor could explain the variation in fungal taxa among the three forests. Plant residues promote moisture retention and provide organic carbon (Blumfield and Xu, 2003) which in turn influence diversity and richness of macrofungal species (Reverchon et al., 2010).

Our study on soil fungal community in fragmented church forests of Dry Afromontane forest systems in North Ethiopia (study-IV) showed variation in the overall soil fungal richness and diversity among the studied forests. The highest richness and diversity values were for the Taragedam forests, followed by the Banja and the Alemsaga forests. The observed pattern in fungi diversity may reflect the difference in ecological features (Dang et al., 2018) or plant species diversity differences of the studied forests (Gilbert et al., 2002). Plant host, depending on plant substrate type and functional groups of fungi, influences fungal diversity (Craig et al., 2016) and abundance (Djelloul and Samraoui, 2011) through its impact on the quantity and quality of carbon resources (Shi et al., 2014).

The macrofungal and soil fungal studies in the fragmented church forests of Dry Afromontane forest systems in North Ethiopia (study-III and study-IV respectively) showed that the highest fungal diversity indices were estimated for forests with highest tree species diversity, which suggests that the diversity of vascular plants can be used as a factor for fungal diversity which is in line with Gabel and Gabel (2007) and

McMullan-Fisher et al. (2010) who reported positive correlations between plant and fungal diversity. This association is particularly evident for saprotrophic fungi because higher plant diversity provides a wide variety of substrates important for such functional groups (Gessner et al., 2010; Wu et al., 2019; Zhang et al., 2018). In addition, plant diversity influences the nature, variability, and quantity of resources on which fungi depend for their survival and growth (Wardle et al., 2004). This is probably why the diversity of tree species is a useful proxy for macrofungal diversity at a global scale (Schmit et al., 2005). Highly diverse plant communities may harbor more ecological niches that can be occupied by fungi due to an increase in the diversity of organic substrates entering soils (Meier et al., 2008; Waldrop et al., 2006; Zak et al., 2003). Furthermore, the observed diversity of plants and fungi could be influenced by habitat microheterogeneity, causing a positive correlation between both plant diversity and fungal diversity (Rudolf et al., 2013). .

The soil fungal study in the fragmented church forests of the Dry Afromontane forest systems in North Ethiopia (study-IV) showed that the proportion of saprotrophs significantly differ among the studied forests. A higher proportion of saprotrophs was recorded in Taragedam forest where soil fertility is relatively higher (Table 1 in study-IV). Relatively higher carbon content was also found in the soil of the Taragedam forest, which may contribute to the higher saprotrophic fungi. The result is in line with Barnes et al. (2016) and Mundra et al.(2015), who found a higher proportion of saprotrophs in soils where the carbon content of the soil is higher.

The relative proportion of root-associated guilds is also found higher in the Taragedam forest. The species richness of ECM fungi was the highest in Taragedam forests. The result we found in this study might be associated with the relative availability of a broader host range (Roy et al., 2008; Smith and Read, 2008) in this forest. Hence, there may be more trees that can act as hosts for mycorrhizal fungi (Hailemariam et al., 2013; Wubet et al., 2003). The lower abundance, diversity indices and species richness of total and functional groups of fungi recorded in Alemsaga forest could be attributed to the land use history of the forest. Alemsaga forest was changed to agricultural land in 1990 and this could have deleteriously impacted the fungal resource

of the area (Tervonen et al., 2019). Loss of vegetation and constant removal of dead wood from forests result in loss of associated fungi (Berg et al., 2002). Landuse change can threaten fungi to the level of extinction (Dahlberg et al., 2010). Traditional agricultural practice also promotes deterioration of the soil quality and decline of fungal diversity (Delelegn et al., 2018). The lower diversity and lower species richness of fungi in Alemsaga forest could also be due to the lower soil fertility (Table in study IV) and absence of other forests in the vicinity which serve as a source of fungal propagules (Redondo et al., 2020).

In general, our study showed that the Dry Afromontane forests in Ethiopia harbor diverse fungi species and their fungal community composition is affected by edaphic, aboveground vegetation, spatial and climatic variables. The study also significantly contributes to the body of knowledge regarding soil fungal communities in Ethiopia and provided relevant information required for the management and conservation of these forest systems including provision of food resources for poor populations during times of food scarcity.

Conclusions

6. Conclusions

1. The study revealed that the fragmented church forests of the Dry Afromontane forest systems in Ethiopia harbor many and diversified fungi important for the sustainable management of these forest systems. Thus, the result presents an insight into the conservation and management of valuable functional guilds in soils of the fragmented Dry Afromontane church forest systems of Ethiopia The diversity of vascular plants was found as a factor for soil fungal diversity. Important ECM fungi were found in these forest systems Ethiopian forest systems which could provide support for the importance of fungal conservation in the dry Afromontane forest systems in Ethiopia. It is inevitable that the fragmented church forests of the dry Afromontane forest systems need connection either through the establishment of new protection forest adjacent to remnant forests or establishment of mycorrhizal associated plantation forests. This would also provide diverse niches for the growth and development of different fungal species and enable easy movement of spores among forest types.
2. Our studies highlighted that fungal composition differed across a chronosequence after fire in fragmented dry Afromontane forests, varied with stand age in *P.patula* plantations, differed due to the tree species composition of Dry Afromontane forests in Ethiopia and their composition is explained by vascular tree diversity, edaphic, spatial and climatic variables. Therefore, the effect of forest management practices such as thinning and harvesting should be taken into consideration owing to the important relationship between these ecological parameters and the soil fungal composition in Ethiopian forest systems.
3. Our survey of macrofungi revealed the presence of valuable edible macrofungal species belonging to the *Tricholoma*, *Suillus*, and *Termitomyces* genera, which could potentially be marketed and, hence, could provide supplementary incomes to forest-dependent local people and forest managers. Therefore, the production

of valuable wild mushrooms should be incorporated into management and conservation strategies in these fragmented forest systems.

4. Our soil fungal and macrofungal studies in fragmented church forests of the dry Afromontane forest systems indicated that the promotion of vascular tree diversity in these forest systems through enrichment plantings or assisted natural regeneration management systems would offer suitable habitats with variable microclimates that should assist fungal species. In addition, the effects of the aforementioned management practices on soil fertility should be taken into consideration owing to the important relationship between edaphic variables and fungal composition in these forests.
5. Data obtained in all the studies in this thesis will significantly contribute to the body of knowledge regarding soil fungal communities in Ethiopia and provided relevant information required for the management and conservation of these forest systems and the central roles played by fungi in the management and conservation of such forests systems including provision of food resources for poor populations during times of food scarcity.
6. In all our studies it was observed that significant number of fungal taxa was not identified down to genus and species level. This indicates that the fungal diversity in Ethiopian forest systems is as yet largely undescribed and likely includes many taxa unknown to science. Thus, we advise that additional long term scientific investigations are needed to consolidate the Ethiopian fungal biodiversity database.

7. Conclusiones

1. El estudio reveló que los bosques eclesiásticos fragmentados de los sistemas forestales secos afromontanos en Etiopía albergan muchos hongos diversificados importantes para el manejo sostenible de estos sistemas forestales. Por lo tanto, el resultado presenta una visión de la conservación y el manejo de gremios funcionales valiosos en los suelos de los sistemas forestales fragmentados de la iglesia Dry Afromontane de Etiopía. La diversidad de plantas vasculares se encontró como un factor para la diversidad fúngica del suelo. Se encontraron hongos ECM importantes en estos sistemas forestales. Sistemas forestales etíopes que podrían respaldar la importancia de la conservación de los hongos en los sistemas forestales afromontanos secos de Etiopía. Es inevitable que los bosques eclesiásticos fragmentados de los sistemas forestales secos afromontanos necesiten una conexión, ya sea mediante el establecimiento de un nuevo bosque de protección adyacente a los bosques remanentes o el establecimiento de plantaciones forestales asociadas con micorrizas. Esto también proporcionaría diversos nichos para el crecimiento y desarrollo de diferentes especies de hongos y permitiría un fácil movimiento de esporas entre los tipos de bosques.
2. Nuestros estudios destacaron que la composición de los hongos difería en una cronosecuencia después de un incendio en los bosques secos fragmentados de Afromontano, variaba con la edad del rodal en las plantaciones de P. patula, difería debido a la composición de especies de árboles de los bosques secos de Afromontano en Etiopía y su composición se explica por factores vasculares. diversidad arbórea, variables edáficas, espaciales y climáticas. Por lo tanto, el efecto de las prácticas de manejo forestal como el aclareo y la cosecha debe tenerse en cuenta debido a la importante relación entre estos parámetros ecológicos y la composición fúngica del suelo en los sistemas forestales de Etiopía.
3. Nuestro estudio de macrofungos reveló la presencia de valiosas especies de macrofungos comestibles pertenecientes a los géneros Tricholoma, Suillus y

Termitomyces, que potencialmente podrían comercializarse y, por lo tanto, podrían proporcionar ingresos suplementarios a la población local y los administradores forestales que dependen de los bosques. Por lo tanto, la producción de valiosos hongos silvestres debe incorporarse a las estrategias de manejo y conservación en estos sistemas forestales fragmentados.

4. Nuestros estudios sobre hongos y macrofúngicos del suelo en bosques eclesiásticos fragmentados de los sistemas forestales secos afromontanos indicaron que la promoción de la diversidad de árboles vasculares en estos sistemas forestales mediante plantaciones de enriquecimiento o sistemas de gestión de regeneración natural asistida ofrecería hábitats adecuados con microclimas variables que deberían ayudar especies. Además, deben tenerse en cuenta los efectos de las prácticas de manejo antes mencionadas sobre la fertilidad del suelo debido a la importante relación entre las variables edáficas y la composición fúngica en estos bosques.
5. Los datos obtenidos en todos los estudios de esta tesis contribuirán significativamente al cuerpo de conocimiento sobre las comunidades de hongos en el suelo en Etiopía y proporcionaron información relevante requerida para el manejo y conservación de estos sistemas forestales y los roles centrales que juegan los hongos en el manejo y conservación de dichos sistemas forestales, incluida la provisión de recursos alimentarios para las poblaciones pobres en épocas de escasez de alimentos.
6. En todos nuestros estudios se observó que no se identificó un número significativo de taxones de hongos hasta el nivel de género y especie. Esto indica que la diversidad de hongos en los sistemas forestales de Etiopía aún no se ha descrito en gran medida y probablemente incluye muchos taxones desconocidos para la ciencia. Por lo tanto, advertimos que se necesitan investigaciones científicas adicionales a largo plazo para consolidar la base de datos de biodiversidad fúngica de Etiopía.

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Original articles

Original article I

Comunidades de hongos del suelo y sucesión después de los incendios forestales en los bosques secos Afromontanos de Etiopía, un ecosistema muy diverso y poco explorado

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Resumen

Los bosques secos afromontanos de Etiopía son ecosistemas complejos que tienen importantes funciones económicas y ecológicas. Sin embargo, los incendios recurrentes han sido una fuente de perturbación para estos bosques. Evaluamos el efecto del fuego en las comunidades de hongos del suelo en un bosque afromontano seco remanente en Wondo Genet, en el sur de Etiopía, mediante el análisis de muestras de suelo recolectadas de rodales no quemados y de rodales uno y diez años después del incendio utilizando metabarcoding de ADN del ADNr ITS2. El análisis indicó que la comunidad de hongos del suelo era más diversa poco después de una perturbación por incendio y disminuyó con el tiempo. La composición de la comunidad fúngica también difirió entre los rodales. Nuestros resultados también indicaron que las diferencias en la diversidad de hongos dependían del rodal y no de la cronología de la historia de los incendios en este sistema forestal. Encontramos un mayor número de especies de micorrizas en rodales quemados, lo que sugiere que estos simbiontes de hongos podrían compensar los efectos del estrés nutricional causado por el fuego en estas áreas. La composición de la comunidad fúngica también se correlacionó significativamente con el contenido de materia orgánica, potasio y magnesio en el suelo. Este trabajo podría considerarse como un estudio de caso ya que las parcelas se establecieron en un solo rodal para cada tratamiento en los bosques secos afromontanos de Etiopía. Por lo tanto, recomendamos estudios adicionales y las conclusiones con respecto a otros rodales deben tomarse con precaución.

Palabras clave: *Variable edáfica, Etiopía, Incendio forestal, grupos funcionales de hongos, secuenciación de torrente de iones, trópicos*.

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Soil fungal communities and succession following wildfire in Ethiopian dry Afromontane forests, a highly diverse underexplored ecosystem

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ABSTRACT

Ethiopian dry Afromontane forests are complex ecosystems that have important economic and ecological roles. However, recurrent fire has been a source of disturbance for these forests. We assessed the effect of fire on soil fungal communities in a remnant dry Afromontane forest in Wondo Genet, southern Ethiopia, by analysing soil samples collected from unburned stands and from stands one and ten years after fire using DNA metabarcoding of the ITS2 rDNA. The analysis indicated that the soil fungal community was most diverse soon after a fire disturbance and declined over time. Fungal community composition also differed among stands. Our results also indicated that differences in fungal diversity were stand dependent rather than due to the chronology of the fire history in this forest system. We found higher numbers of mycorrhizal species in burned stands, suggesting that these fungal symbionts could compensate for the effects of nutrient stress caused by fire in these areas. Fungal community composition was also significantly correlated with organic matter content, potassium and magnesium in soil. This work could be considered as a case study since the plots were established in a single stand for each treatment in the dry Afromontane forests of Ethiopia. Thus, we recommend further studies and conclusions regarding other stands need to be taken with caution.

1. Introduction

Ethiopia is an ecologically diverse country owing to the varied topographic features and altitudinal variations (Bongers and Tenngkeit, 2010). The country also experiences a high variation in macro- and mesoclimatic conditions that have contributed to the formation of biologically diverse ecosystems. According to Friis et al. (2010), the vegetation of Ethiopia is classified into 12 types based on the elevation zones in which they occurred. Out of which, the natural high-elevation forests, that include the Afromontane vegetation, are exclusively found in the highland regions of Ethiopia between 1500 and 3400 m above sea level (Lemenih and Bekele, 2008).

The highlands of Ethiopia occupymore than 44% of the country's land area (Kidanu, 2004; McCann, 1995). Dry Afromontane forests dominate these highlands and are found mainly in the central, northern and western parts of the country (Friis et al., 2010). This forest is a complex ecosystem and is characterized by high humidity, a variable rainfall pattern, and a prolonged dry season (Friis et al., 2010). The dry Afromontane forest provides important ecosystem services such as watershed protection and carbon sequestration (Wassie et al., 2005). The dominant tree species in this forest are *Juniperus procera*, *Podocarpus falcatus*, *Hagenia abyssinica* and *Olea africana*, which are the main source of timber in Ethiopia. In addition, the dry Afromontane forest harbours various types of non-timber forest products, including wild edible mushrooms (Dejene et al., 2017b).

Anthropogenic factors are negatively affecting the forest resources in Ethiopia (Lemenih and Bekele, 2008). Fire is a potentially destructive disturbance, affecting the distribution, diversity and composition of the

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forest resources (Lemenih and Bekele, 2008; Wassie et al., 2005). Human-induced fire, mainly for subsistence and economic reasons, is the most important reason for the depletion and degradation of natural resources in Ethiopia. For instance, the most destructive forest fires, which occurred in 2000, destroyed over 150,000 ha of forest (Senbeta and Teketay, 2001). More recently, in 2019, a fire in the northern part of Ethiopia affected 340 ha of forest (New Business Ethiopia, 2019). This trend is more common in the highland areas, where the dry Afromontane forest is found, and has a direct impact on the biodiversity in the forest ecosystem (Lemenih and Bekele, 2008). This loss of biodiversity could also occur in the forest soil, which harbours a great diversity of microbial organisms (Fierer and Jackson, 2006), including fungi. Depending on the severity and frequency, fire could directly or indirectly affect edaphic variables in the forest ecosystem (Reazin et al., 2016), which in turn could have an impact on fungal communities dwelling in the soil (Cairney and Bastias, 2007; Dahlberg et al., 2001; Rincón and Pueyo, 2010).

Forest fungi comprise a crucial functional component and contribute to the high ecological significance of forest ecosystems (Crabtree et al., 2010). Their roles can be described in terms of nutrient addition and cycling, the carbon pool, soil formation, and the formation of symbiotic links with plants (Claridge et al., 2009; Fontaine et al., 2007; Van Der Heijden et al., 2008). Saprotrophic fungi play a key role in the decay of organic matter (Hobbie et al., 1999), whereas mycorrhizal fungi form symbiotic associations with plants, and can form up to 80% of the fungal biomass in forest soils in northern temperate regions (Nehls, 2008). The fungal mycelium also plays an essential role in soil stabilization and helps to increase the water-holding capacity of the soil (Kennedy and Gewin, 1997). Furthermore, some species of fungi are pathogenic, causing disease in both above- and belowground components of the forest system (Narayanasamy, 2011).

Despite recent advances in determining the diversity and composition of forest fungi in various biomes, fundamental questions regarding their distribution and function, and the factors that influence them remain unanswered, particularly in under-sampled biomes (Guo et al., 2013; Krashevska et al., 2015). The majority of soil fungi are unexplored and, the functional relationship between fungi, soil, and plants remains understudied (Bridge and Spooner, 2011; Van Der Heijden et al., 2008). Previous investigations have estimated that there are about 5.1 million fungal species worldwide (Taylor et al., 2014). Of these, 2–6% have been described (O'Brien et al., 2005) and ~1200 new species are described each year (Hibbett and Thorn, 2001), indicating that there are many more fungal species to be explored, named, and identified. In addition, further studies are required to increase our understanding of the dynamics of soil fungi and their community structure (Dhruba et al., 2015) and the impact of various environmental and anthropogenic factors. Furthermore, to date, most studies of soil fungal communities have focussed on temperate and Mediterranean forest ecosystems; less consideration has been given to soil fungal communities in tropical forest ecosystems (Taudière et al., 2017).

The effects of fire on soil fungi are determined by the fire severity, changes in soil properties and post-fire environmental conditions (Bastias et al., 2006; Buscardo et al., 2012, 2010; Neary et al., 1999; Reazin et al., 2016). A change in vegetation following a fire may also impact on fungi living in a symbiotic or saprophytic relationship with trees. Thus, the subsequent structure of fungal communities might be influenced by the dynamics of post-fire plant communities (Cairney and Bastias, 2007). In addition, factors such as the fuel load (Dahlberg et al., 2001), soil moisture and temperature (Bonet et al., 2010) and other environmental and weather conditions during the fire event can also influence the effects on soil biota. Furthermore, differences in the sensitivity of fungal propagules to fire determine the degree to which the composition of fungal communities changes after the fire (Hernández-Rodríguez et al., 2013). However, the inconsistency of results from individual studies makes it difficult to provide a general conclusion regarding the possible dynamics of fire, fungi and ecosystem function. Consequently, location-based studies are necessary to obtain a better understanding of the effect of fire on the soil fungal community at a specific location (Taudière et al., 2017).

A limited number of studies have investigated fungal communities in the forest systems of Ethiopia; however, these were mainly focused on above-ground fungal communities (Dejene et al., 2017b, 2017a). Recently, Castaño et al. (2019) also investigated the soil fungal community and ecological guilds associated with *Eucalyptus grandis* plantations in Ethiopia. However, the soil fungal communities associated with the dry Afromontane forests in Ethiopia are undescribed and the potential effect of fire on soil fungal communities in these ecosystems where forest fire is a recurrent phenomenon has not yet been analysed. Forest fires are expected to change the edaphic variables on which the fungi depend for their trophic as well as their community composition. In addition, it is important to understand how fungal communities respond to the post-fire environment and to identify which are the most important environmental factors driving fungal community structure and function to supplement our knowledge of Ethiopian fungal resources as well as to promote their conservation and development. Thus, the aim of this study was to provide baseline information on soil fungal communities in the dry Afromontane forests along a post-fire, secondary succession gradient in Ethiopia.

We hypothesized that the richness and composition of the entire soil fungal communities would change substantially during a post-fire forest succession and would differ from those in unburned forest (Hypothesis 1). Specifically, we expected that soils would be more fertile immediately after fire with a gradual decrease in soil fertility over time and that the community turnover would partially be explained by these changes in edaphic conditions (Hypothesis 2). As a consequence, we expected ruderal, generalist saprotrophic fungi, mainly utilizing simple and easily absorbable forms of nutrients, to be more abundant and species-rich shortly after fire than in unburned forests (Hypothesis 3). By contrast, root-associated symbiotic fungi were expected to be more diverse in older burned forests and unburned forests (Hypothesis 4).

2. Materials and methods

2.1. The study area description

The study was conducted in Wondo Genet natural forest area, which is located in southern Ethiopia, approximately 265 km from Addis Ababa (Fig. 1) (between 7°06′ N–7°07′ N and 38°37′ E–38°42′ E) at 1,600 to 2,580 m above sea level (Belaynesh, 2002; Fenta, 2014). Wondo Genet is characterized by remnant dry Afromontane forest patches (Ango and Bewket, 2007; Belaynesh, 2002; Fenta, 2014). The climate is characterized by the Weyna-Dega agro-climatic zone, with a bimodal rainfall pattern: the main rainy season is in the summer and a lesser rainy season is in spring. The mean annual rainfall and mean annual temperature of the study area are 1210 mm and 20 °C, respectively (Belay, 2016; Fenta, 2014). The soil is an Andisol with a sandy loam texture (Eriksson and Stern, 1987) and an average pH value of 5.7 (Eshetu and Högberg, 2000). The study area covers about 797 ha of natural forests lands (Ango and Bewket, 2007; Belaynesh, 2002; Fenta, 2014) that are characterized by remnant Dry Afromontane forest patches, home to important fauna and flora. The dominant tree species in the study forest are *Juniperus procera*, *Albizia gummifera*, *Afrocarpus falcatus*, *Bersama abyssinica*, *Prunus africana*, *Podocarpus falcatus*, *Cordia africana*, *Croton macrostachys* and *Olea africana* (Ango and Bewket, 2007; Zerga and Berta, 2016). These trees are reported to be associated with arbuscular mycorrhizal fungi (Wubet et al., 2003). Human-induced fire is a recurrent phenomenon, occurring yearly in small patches of this natural forest (Bekele et al., 2013; Dejene et al., 2017b).

Sample plots were established in the forest in 2015 in stands with similar environmental conditions such as climate, altitude and soil. Information about the forest fire history of these stands was obtained from the Department of Forest Management at the Wondo Genet

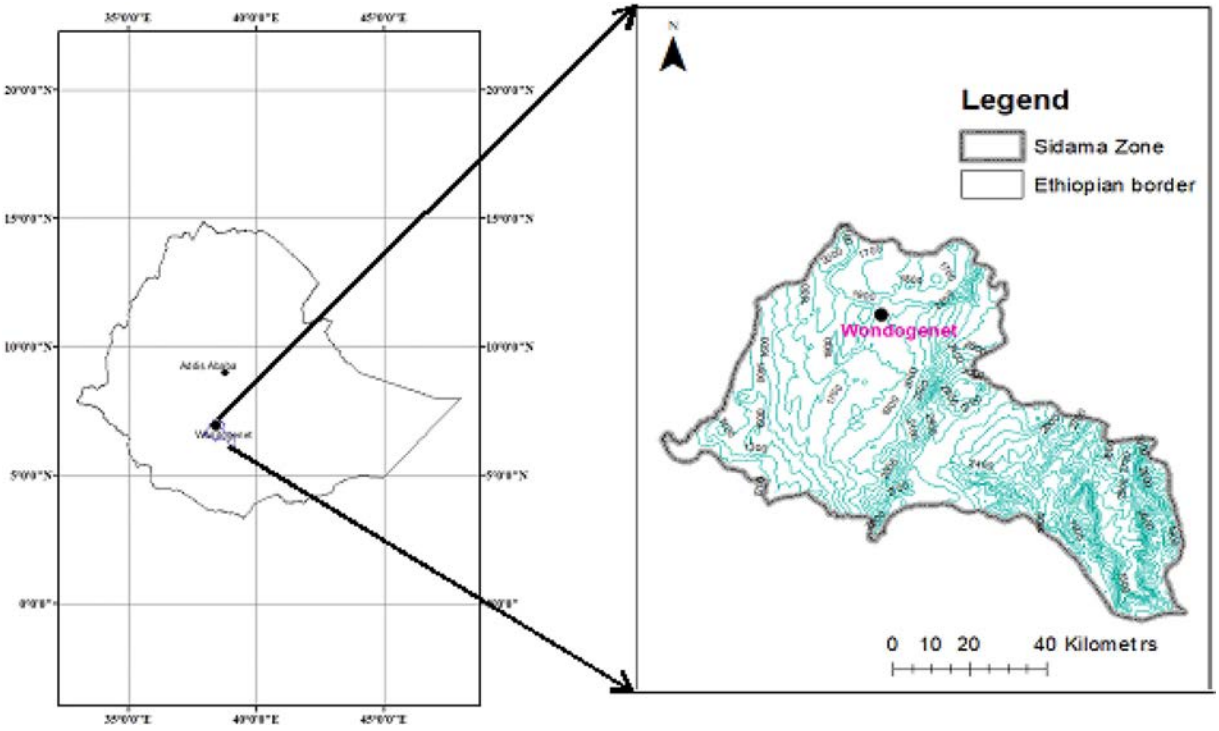

Fig. 1. Location of the study area, Wondo Genet, Ethiopia.

College of Forestry. The control stand of unburned natural forest (UB) was representative of the original natural forest and had not been affected by fire for at least 40 years. Burned stands selected for the study were similar in terms of fire severity, i.e., the canopy and understory had burned and the soil organic layer had been consumed (Rincón and Pueyo, 2010). In these burned areas, two forest stands were selected based on their fire history: (1) one-year-old burned forest (B1); and (2) ten-year-old burned forest (B10). Within each of these forest stands, three transects were established about 250 m apart from each other. Each transect covered an area of 100 m^2, with a rectangular shape (2 m × 50 m). Because the plots were established in a single stand for each treatment, this work could be considered as a case study and conclusions regarding other stands need to be taken with caution.

2.2. Soil sampling for molecular work

A total of nine (2 m × 50 m) transects, three per each studied stands (UB, B1 and B10), were established perpendicular to the slope following Luoma et al. (1991) and Smith et al. (2002). Five soil cores were extracted 5 m apart along the centreline of each transect using a cylindrical (2 cm radius, 20 cm long, 250 cm^3) soil borer (De la Varga et al., 2012; Taylor, 2002) to sample spatial variability and to minimize the probability of sampling the same genet repeatedly. From these cores, well-decomposed organic layers and mineral soils were sampled. The litter layer (intact and partially decomposed leaves) was discarded because the fungal community composition in litter tends to diverge from that in soil (Voříšková et al., 2014). The five cores from each transect were pooled to obtain a composite soil sample for each transect for the final DNA extraction. Soil cores were dried, sieved through a 1 mm^2 mesh and ground to a fine powder using a mortar and pestle. A subsample of each pooled sample was stored at −20 °C for molecular analysis and the rest of the sample was used for determining selected physical and chemical properties of the soil (Table 1).

2.3. Molecular analysis

DNA was extracted from 0.25 g of soil per sample using a PowerSoil™ DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA). PCR reactions were performed in triplicate for each sample to minimize PCR biases. PCR reactions were performed in 20 µl reaction volumes containing 11.22 µl of MQ water, 1.60 µl of DNA template, 2.00 µl of 10 × buffer, 1.40 µl of $MgCl_2$ (50 mM), 1.60 µl of dNTPs (10 mM), 0.50 µl of BSA (2%), 0.80 µl of reverse and forward primers (10 µM) and 0.08 µl of Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA). We used the following PCR conditions: an initial denaturation step at 94 °C for 3 min; then 35 cycles of 94 °C for 45 s, 50 °C for 1 min and 72 °C for 1.5 min; and a final cycle of 72 °C for 10 min. The ITS2 rDNA region was amplified using the forward primer fITS7 (Ihrmark et al., 2012) and the barcoded reverse primer ITS4 (White et al., 1990). The ITS4 primer was labelled with sample-specific Multiplex Identification DNA-tags. A negative control consisting of MQ water instead of DNA was included in each PCR run. The absence of bands on gels indicated that negative controls were amplicon free. Ion

Table 1
Selected soil physic chemical properties of study plots in the dry Afromontane forest of the Wondo Genet area (Ethiopia).

Soil properties	Stand age after burn[a]		
	Unburned	One year	Ten years
Clay	1.60 (0.01)	1.72 (0.02)	1.27 (0.03)
Silt	1.30 (0.1)	1.40 (0.10)	1.38 (0.12)
Sand	1.76 (0.08)	1.38 (0.12)	1.79 (0.04)
pH-H_2O (1:2.5)	6.51 (0.30)	6.46 (0.1)	6.81 (0.30)
Na (meq 100 g^{-1} of soil)	0.83 (0.07)	1.00 (0.4)	0.99 (0.10)
K (meq 100 g^{-1} of soil)	0.55 (0.12)	0.62 (0.35)	0.80 (0.08)
Ca (meq 100 g^{-1} of soil)	28.43 (13.67)	20.85 (5.18)	24.15 (4.98)
Mg (meq 100 g^{-1} of soil)	9.77 (5.18)	7.42 (1.42)	8.05 (1.50)
CEC (meq 100 $g^{-1}$of soil)	52.44 (14.91)	43.97 (10.9)	42.66 (5.10)
OM	6.05 (1.77)	2.93 (0.36)	5.08 (1.88)
Nitrogen (%)	0.67 (0.17)	0.40 (0.06)	0.54 (0.11)
C/N	5.25 (0.20)	4.25 (0.03)	5.46 (3.89)
P (mg P_2O_5/kg soil)	43.33 (12.72)	28.89 (4.36)	32.59 (5.18)

Abbreviations: CEC, cation exchange capacity; OM, organic matter.
[a] Numbers in parentheses are the standard deviation of the mean.

Torrent sequencing was carried out at the Naturalis Biodiversity Center. We used the Ion 318™Chip to allow for the highest possible sequencing coverage.

2.4. Soil sampling and edaphic variables analysis

To relate soil fungal composition to explanatory edaphic variables, additional soil samples were collected from each transects. Composite soil samples, from the centre and from the four corners of each plot, were extracted after clearing plant matter and debris from the soil surface. Soil was extracted to a depth of 20 cm with the aid of an auger and spade. After mixing the samples thoroughly, approximately 500 g of soil was placed in a plastic bag for transport back to the laboratory for analysis. After air drying the soil in shade, the chemical and physical properties of the soil were determined using DTPA extraction, KH_2PO_4 extraction, Olsen, Kjeldahl digestion, Walkley–Black, ammonium acetate and instrumental methods. The analysis was conducted by Water Works Design and Supervision Enterprise, a laboratory test service for soil fertility at Addis Ababa, Ethiopia. A soil: water (1:2.5) suspension and the supernatant of the same suspension were measured using a pH meter and an electrical conductivity meter, respectively, to determine the soil pH (Reeuwijk, 2002). Organic carbon (C) content was determined using wet digestion (Walkley and Black, 1934). Total nitrogen (N) content was determined using the Kjeldahl digestion procedure (Kim, 1996). Available phosphorus (P) was determined using sodium bicarbonate (0.5 M $NaHCO_3$) as an extraction solution (Olsen and Sommer, 1982). Available sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) were extracted using ammonium acetate. Soil particle size was analysed with a hydrometer (Bouyoucos, 1951), using sodium hexametaphosphate (Calgon solution) as the dispersing agent. Once the proportion of sand, silt and clay separates were calculated, the soil was assigned textural class name using ASTM software. We also used the following formula to convert organic carbon to organic matter. Organic matter (%) = Total organic carbon (%) × 1.72. The selected soil properties of the studied plots are provided in Table 1.

2.5. Bioinformatic analysis

Raw sequence reads were obtained from the Ion Torrent output that comprised demultiplexed sample reads. Primers and poor-quality ends were trimmed off based on a 0.02 error probability limit in Geneious Pro 8.1.8 (BioMatters, Auckland, New Zealand). Subsequently, sequences were filtered using USEARCH based on the following settings: all sequences were truncated to 200 bp and sequences with an expected error of > 1 were discarded. The remaining sequences were collapsed with USEARCH v.8.0 (Edgar, 2010) into unique sequence types on a per-sample basis while preserving read counts. Singleton sequence types were discarded; the resulting 305,520 high-quality sequences were grouped into 3,286 operational taxonomic units (OTUs) with USEARCH at 97% sequence similarity while simultaneously excluding 181 chimeric sequences. We assigned sequences to taxonomic groups based on pairwise similarity searches against the curated UNITE + INSD fungal ITS sequence database containing identified fungal sequences with assignments to species hypothesis groups (Kõljalg et al., 2013). After excluding OTUs with < 70% similarity or with < 150 bp pairwise alignment length to a fungal sequence, the dataset contained 2,898 fungal OTUs, representing a total of 296,384 high-quality sequences. Functional classification of OTUs at the genus level was performed using the FUNGuild database.

2.6. Statistical analysis

We normalized the OTU table for subsequent statistical analyses by rarefying the number of high-quality fungal sequences to the smallest library size (8,361 reads). Shannon's H' diversity index, $H = -\Sigma pi\ (lnpi)$ (Shannon and Weaver, 1949), was estimated for each stand, where p_i indicates the relative abundance of fungal OTUs (Kent and Coker, 1993). The Simpson's diversity, $D = 1 - \Sigma\ (p_i^2)$, where p_i is the importance probability in element i; and the Evenness, $J = H'/H'max$, where H' is the number derived from the Shannon diversity index and the H' max is the maximum possible value of H' were also calculated (Magurran, 1988). In addition, the richness values of all fungal OTUs (S) based on stand type were estimated. All diversity measures were calculated using the BiodiversityR GUI package in R version 3.5.3 (R Core Team, 2019). Diversity indices and richness were compared across stands using one-way ANOVA using R (R Core Team, 2019). Data were scaled using R when needed to normalize for ANOVA. Tukey HSD was used to determine significant differences between means ($P \leq 0.05$) among stands.

To compare the entire fungal OTU community composition across the studied stands, we used PC-ORD v. 6.0 (McCune and Grace, 2002) to run detrended correspondence analyses (DCAs) on the presence-absence data matrix. Data were also analysed using PERMANOVA (Adonis) in R to determine the effect of forest types and edaphic variables on the fungal community (Anderson, 2001). We performed an analysis of Similarity Percentages (SIMPER) to identify fungal species that are most responsible for the observed patterns (Clarke, 1993) and to determine the percentage contribution of fungal taxa to significant dissimilarities among the three stands (Parravicini et al., 2010). Canonical correspondence analysis (CCA) was also used to relate the selected edaphic variables (Table 1) with the mycorrhizal fungal community composition using CANOCO version 5.0 (Smilauer and Lepš, 2014). The statistical significance of the environmental variables was tested using a Monte Carlo permutation test (999 permutations).

3. Results

3.1. Sequencing outputs and fungal diversity

A total of 2,898 fungal OTUs of a total of 296,384 sequences were found across all samples before rarefaction. The taxonomic classification revealed that Ascomycota was the most diverse fungal phylum, with 1708 OTUs; 52% of the total (Fig. 2A). The ranking of taxonomic orders in Ascomycota, based on the number of representative OTUs, was as follows Chaetothyriales (128), Pleosporales (106) and Hypocreales (77), followed by many other orders with less than 50 fungal OTUs (Fig. 3A). In Basidiomycota, Agaricales was the most species-rich order followed by other orders with less than 50 OTUs each. Unidentified fungi were classified down to kingdom level and represented about 645 OTUs; 20% of the total (Fig. 2A). The number and proportional distribution of fungal OTUs describing all known taxonomic phyla and orders are shown in Fig. 3.

About 42% (1217 OTUs) of the fungal OTUs were classified to the genus level. These genera were assigned to ecological functional groups, i.e. symbionts (ectomycorrhizal and arbuscular mycorrhizal fungi) and non-mutualistic fungi, including saprotrophs, parasites and pathogens. The proportion of fungal OTUs at the genus level sharing the same ecological function is presented in Fig. 2B.

3.2. Fungal richness and diversity changes after fire

The observed total fungal OTU richness was significantly affected by fire ($P = 0.002$; $F = 12.48$; Fig. 4) and was higher in one and ten years after fire, compared with the unburned site. The highest richness value was observed in one-year-old burned stands, whereas the lowest was observed in unburned stands, which had an average richness value that was 50% and 18% lower than that of one- and ten-year old burned stands, respectively.

Diversity indices for each ecological guild did not differ significantly among treatments ($P > 0.05$; Fig. 5). The observed evenness values also did not differ significantly among treatments ($F = 0.18$, $P = 0.72$; Fig. 5). However, the trend observed for evenness values indicated that

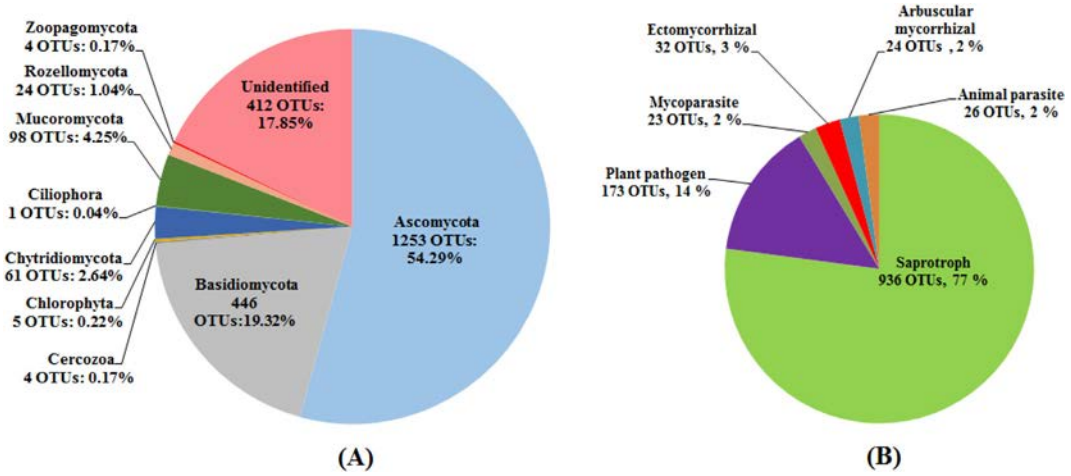

Fig. 2. Relative proportions of fungal operational taxonomic units (OTUs): (A) taxonomical classification at the phylum level (name of phylum; the number of OTUs; percentage); (B) classification of fungal ecological function at the genus level (ecological function; the number of OTU; percentage) based on a FUNGuild (www.funguild.org) search.

ecological guilds are distributed more uniformly in B10 and UB stands than in B1 stands (Fig. 5).

When the relative proportions of each of the ecological guilds in each of the different types of forest stand were considered separately, only the proportions of plant pathogens differed significantly in the different types of forest stand. The relative proportion of plant pathogens in B10 soils was significantly greater than in UB or B1 soils ($P = 0.001$; $F = 5.04$; $n = 3$ transects). The relative proportions of the other ecological guilds were not significantly different ($P > 0.05$) in burned and unburned forest stands (Fig. 6).

3.3. Soil fungal communities and environmental variables

The DCA showed that the variation in fungal community composition can be partially explained by the successional stage following fire (Fig. 7), indicating distinct fungal compositions in each treatment. The PERMANOVA analyses confirmed that the composition of the fungal OTUs in the three stand types were significantly different ($F = 1.54$, $P = 0.02$), indicating that the fungal communities are differently associated with the three forest stands due primarily to soil fertility. With respect to the edaphic variables, Nitrogen (N) ($R^2 = 0.5685$), C/N ration ($R^2 = 0.6355$), and Phosphorus (P) ($R^2 = 0.6387$) correlated most strongly with fungal community composition ($P < 0.05$). The SIMPER analysis identified fungal species that typified and distinguished between the three treatments (Table S1). The overall between-group dissimilarity was 79.59% for UB and B1 treatments, 74.47% for UB and B10 treatments and 72.91% for B1 and B10 treatments. *Agaricus campestroides* and *Gymnopilus ochraceus* were the most influential species and, along with other species such as *Hypocreales* sp. and *Onygenales* sp., made the greatest cumulative contribution towards differences between the three stands, often accounting for more than 25% of the observed value of dissimilarity (Table S1). Furthermore, the one-year-old burned treatment (B1) dataset contained a relatively higher number of OTUs as compared to the ten-year old burned (B10) and unburned (UB) treatments.

CCA of ectomycorrhizal fungal OTUs based on simple term effects revealed that edaphic variables such as the Mg, K and OM were significantly correlated with ectomycorrhizal fungal community composition in our forest study area (Table 2; Fig. 8). The cumulative contribution of the explained variation data for the interaction between ectomycorrhizal soil fungal composition and soil variables are shown in Fig. 8.

The response of the ectomycorrhizal species was unique to the different types of stands. In contrast to our expectation, number ectomycorrhizal taxa were associated with recently burned stands, which had lower soil fertility levels compared with unburned and 10-year-old burned stands (Table 1). Species such as *Tricholoma* sp. and *Inocybe* sp. were among the observed taxa associated to these 1-year-old burned stands. In older stands i.e. the 10-year-old burned and unburned stands, where the soil fertility was high, species such as *Amanita* sp. and *Laccaria* sp. were found together with other species. Other species such as *Entoloma* sp. and *Cortinarius* sp. were associated with all stand age groups in the forest study area.

4. Discussion

4.1. Fungal OTU diversity

Afromontane forests in Ethiopia are considered to be a major reservoir of biodiversity (Lemenih and Bekele, 2008), and may support a high level of microorganism diversity, including fungi. Our study revealed that the dry Afromontane forest in our study area harbours many more fungal species than previously reported in studies based on sporocarps sampling at a given period of time (Dejene et al., 2017b). However, the taxonomic classification was challenging owing to the lack of matches in the database. Thus, only about 48% and 24.13% of the OTUs detected could be identified to the genus and species level, respectively, indicating that the majority of fungal in this region had not been sequenced before this study and that many possibly are undescribed species. This may also be due to the uniqueness of the dry Afromontane forests in terms of the diversity of soil fungi as well as the lack of scientific studies that have investigated the local mycota (Dejene et al., 2017a). Furthermore, about 20% of sequences were not identified even at the phylum level, highlighting the current lack of data from understudied tropical and subtropical forest ecosystems (Tedersoo et al., 2014), such as the forest systems in Ethiopia. In light of this, the present study provides important information that contributes to the Ethiopian fungal biodiversity knowledge base.

The largest group of fungi found in this study belonged to the Ascomycota; previous studies of different forest ecosystems have also reported a dominance of Ascomycota taxa in the soil (Dhruba et al., 2015; Geml et al., 2014; Reazin et al., 2016; Smith et al., 2017). The Ascomycota is the most species-rich phylum of fungi (Araujo and Hughes, 2016), which may explain its dominance. The Hypocreales, Pleosporales, and Chaetothyriales were the three largest orders of Ascomycota found in this study. The order Hypocreales is a group whose taxonomy has been relatively well studied and identification aids are widely available (Chaverri and Samuels, 2003; Schroers, 2001). The

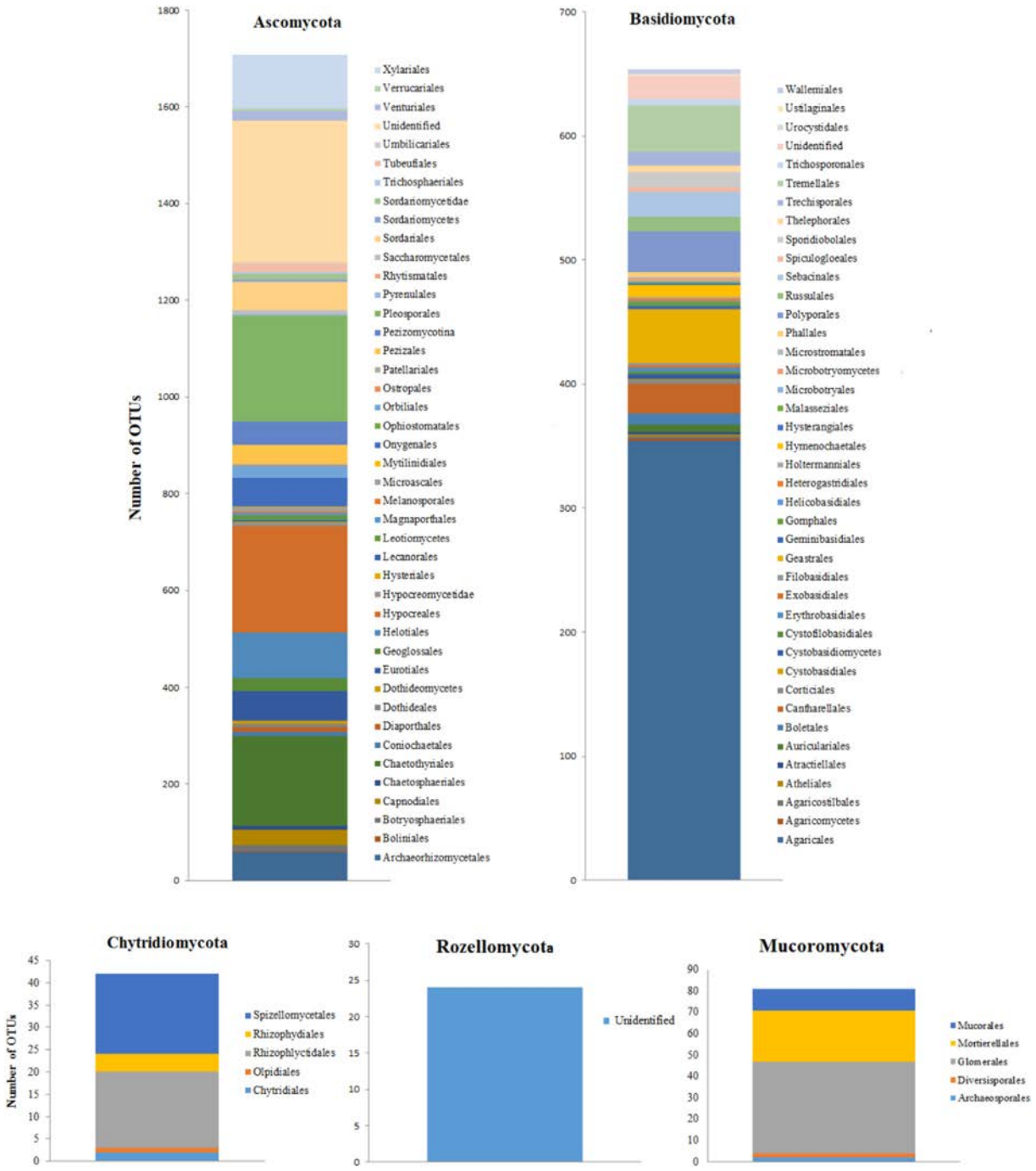

Fig. 3. Numbers and proportional distribution of fungal operational taxonomic units (OTUs) representing all taxonomic phyla and orders found in soil samples collected from the dry Afromontane forest of Wondo Genet, Ethiopia.

fungi in this order can also be saprotrophic, entomopathogenic, and mycoparasitic (Rossman et al., 1999). In addition to their ecological and economic importance, the Hypocreales are also considered to be the most important regulators of insect and fungal populations and, therefore, are used in agriculture as biocontrol agents (Carruthers and Hural, 1990; Esser and El-Gholl, 1993; Rossman et al., 1999; Samuels, 1996). The second largest order of Ascomycota detected in this study was the Pleosporales. This order comprises saprotrophs or fungi that are parasites of vascular plants (Kruys et al., 2006). Some species from this order are also found on animal dung (Kruys et al., 2006), a small number occur as lichens (Semenova-Nelsen et al., 2019) and as rock-inhabiting fungi (Ruibal et al., 2009). The epiphytic or endophytic fungi of the Pleosporales are mainly saprotrophic but also play a key role in causing plant diseases such as stem canker (Zhang et al., 2009). A considerable number of fungi belonging to the order Chaetothyriales were also detected in this study. This order includes fungi that are known to be epiphytes, colonizing the leaves and the bark of trees in tropical forest ecosystems (Arnold et al., 2000; Batista and Ciferri, 1962).

The order Agaricales was the largest order of Basidiomycota detected in this study: members of this order produce the familiar gilled mushroom (Binder et al., 2005; Hibbett and Thorn, 2001; Stajich, 2015). Agaricales are widespread in diverse ecosystems (Kirk et al., 2008) and many form ectomycorrhizae by engaging in mutualistic symbioses with vascular plants (Alexopoulos et al., 1996). Some Agaricales are known to be termite symbionts, some are valuable as a

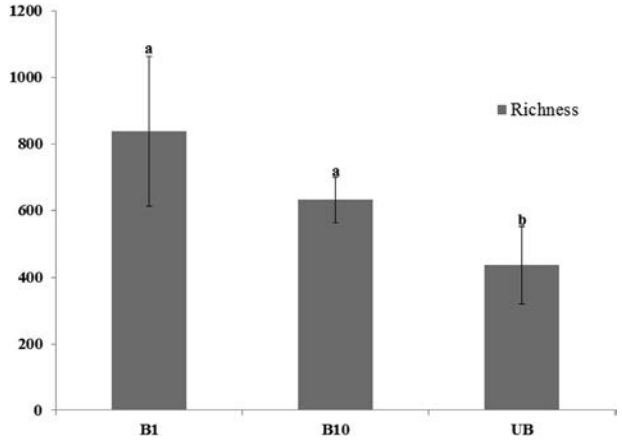

Fig. 4. Mean total fungal community richness values in a dry Afromontane forest of Wondo Genet, Ethiopia, following fire. Abbreviations: UB, unburned stand; B1, one-year-old burned stand; B10, ten-year-old burned stand. Bars denote standard deviation. Different letters above the bars indicate a significant difference in richness between stand types ($P < 0.01$, $n = 3$ transects per stand).

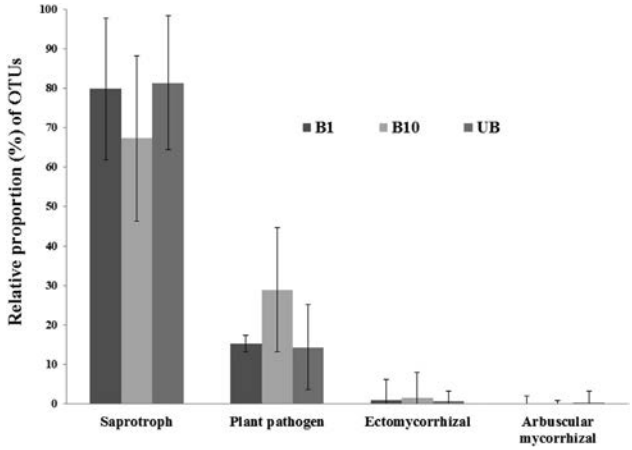

Fig. 6. Relative proportions of fungal operational taxonomic units (OTUs) in different ecological guilds in unburned stands (UB), one-year-old burned stands (B1) and ten-year-old burned stands (B10). Bars denote standard deviation.

source of food for animals, including humans (Kirk et al., 2008), whereas others have hallucinogenic properties or produce toxins lethal to humans (Nichols, 2003). Most of the species of Agaricales detected in this study are well known soil saprotrophs, such as those belonging to the genera *Agaricus*, *Calvatia*, *Coprinellus*, *Gymnopilus*, *Leucoagaricus*, *Lycoperdon*, *Marasmius*, *Psathyrella* and *Psilocybe*, have been reported previously as fruit bodies from our study area (Dejene et al., 2017b) providing validation of our molecular techniques.

4.2. Fungal richness and diversity changes after fire

Our results from the post-fire successional chronosequence showed that soil fungal richness was related to fire. In this study, we found higher total richness and diversity values in the forest stands recently affected by fire than unburned stands. This may be attributed to the new ecological conditions created owing to differences in fire severity, which may incite or support spore germination of several fungal species in the soil (Heino, 2012) following the fire in the investigated forests. In addition, the mycelium of fungal species in the rhizosphere may persist (Cowan et al., 2016; Shen et al., 2016) or the fungal community may be resilient to the effects of fire to some extent (Cowan et al., 2016; Jennings et al., 2012). Furthermore, the intensity of the fire might not have been high enough to affect the below-ground fungal communities given that low-intensity fires may have little effect on below-ground fungal communities (Bárcenas-Moreno et al., 2009; Egidi et al., 2016). Thus, the responses of soil fungi to reoccurring low-intensity fire also appear to be minimal (Johnson et al., 2013; Oliver et al., 2015) and ephemeral (Hart et al., 2005). Contrary to our expectations, we found that the amount of time since the fire did not seem to affect fungal guild

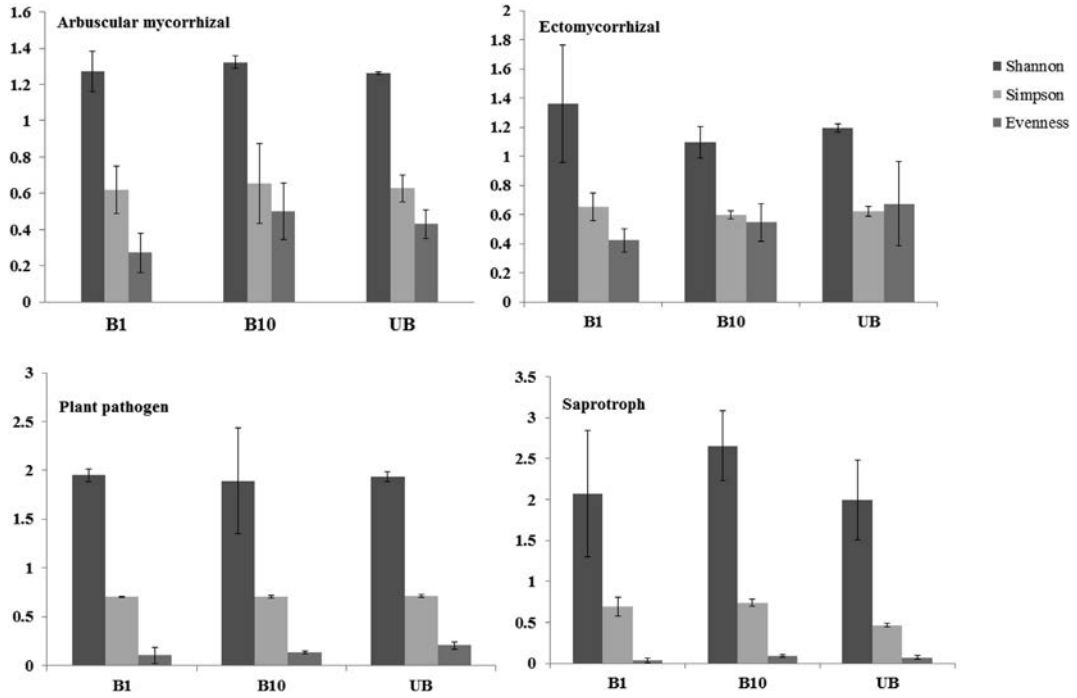

Fig. 5. Mean soil fungal community diversity and evenness estimated for functional guilds detected in three types of forest stand with different fire histories. Abbreviations: UB, unburned stand; B1, one-year-old burned stand; B10, ten-year-old burned stand; Key: Shannon = Shannon diversity values; Simpson = Simpson diversity values; Evenness = evenness values. Bars denote standard deviation, n = 3 transects per stand.

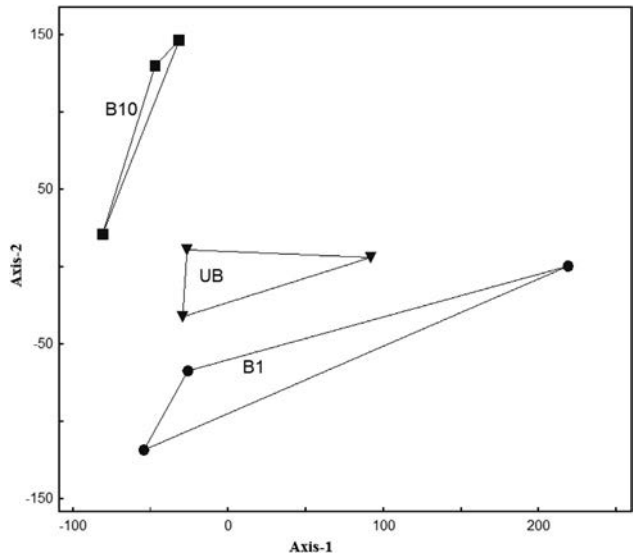

Fig. 7. . Detrended Correspondence Analysis ordination plot for soil fungal communities detected in the three treatment groups: B1, plots in one-year-old burned stands; B10, ten-year-old burned stands; UB, unburned stands. Plots with the same symbol are in the same treatment group.

Table 2
Canonical correspondence analysis showing the significance ($P < 0.05$) of edaphic variables based on simple term effects on the mycorrhizal fungal species.

Variable	Simple term effects		
	Explains %	pseudo-F	P
Mg	22.6	1.71	0.032
K	19.7	1.63	0.036
OM	17.0	1.40	0.047

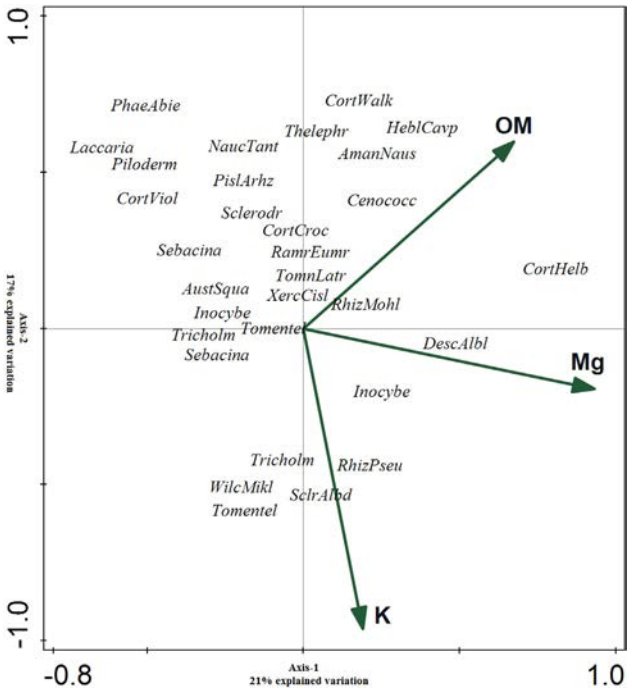

Fig. 8. . Canonical Correspondence Analysis (CCA) of the species level community composition of ectomycorrhizal fungi in a dry Afromontane forest in Ethiopia. Abbreviations: OM, organic matter; Mg, magnesium; K, potassium. Species names are abbreviated (the full names of the ectomycorrhizal species used in the ordination are provided in supplementary Table S2).

diversity. These results agree with the findings of a meta-analysis of fire effects on soil fungi (Egidi et al., 2016), which highlighted the absence of a significant change in fungal diversity following fire. This might be because little heat is transferred to the soil because fuel loads are low (Lunt and Morgan, 2002) or might indicate that the fungal guild communities in burned and unburned forest stands shared similar gene profiles, which may promote functional similarities among fungal communities with differing compositions (Mundra, 2015). On the other hand, the absence of significant difference in fungal richness and diversity in fire affected areas might be due to the fact that recurrent fires consume less fuel and produce less heat, which does not penetrate into soil as deeply as during high-intensity fires (Reazin et al., 2016; Semenova-Nelsen et al., 2019). Accordingly, fungal community shifts in such recurrent fire ecosystems, like that of the dry Afromontane forest, may be relatively modest (Choromanska and DeLuca, 2001; Korb et al., 2004) and the change may be driven by indirect fire-induced changes in soil properties or by the change in the plant communities (Hart et al., 2005; Oliver et al., 2015; Ponder et al., 2009; Trappe et al., 2009). Also, fungi in a recurrent forest ecosystem may be adapted to frequent fires. Some fungi produce heat- and smoke-activated spores (Semenova-Nelsen et al., 2019) and some may benefit from post fire ash deposits (Dean et al., 2015; Hart et al., 2005) or reduced competition from other species (Semenova-Nelsen et al., 2019). However, factors other than fire might have a greater effect on the richness and diversity of soil fungal communities. Therefore, further research is needed to better understand the dynamics and characteristics of soil fungal communities.

A previous study reported the absence of ectomycorrhizal fungi in the dry Afromontane forests of Ethiopia (Dejene et al., 2017a). This finding was not exceptional as the majority of tropical woody tree species are unable to form associations with ectomycorrhizal fungi (Brundrett, 2009). However, in this study, we observed different groups of mycorrhizal fungi and they were identified and classified as ectomycorrhizal and arbuscular mycorrhizal (Fig. 2). This association may be due to the diverse vegetation (Friis et al., 2010) and, hence, there may be more trees present that can act as hosts for mycorrhizal fungi, or may be due to the dispersion of mycorrhizal inocula from nearby plantation forests, which are dominated by *Eucalyptus* and *Pinus* species (Castaño et al., 2019; Dejene et al., 2017a; Urcelay et al., 2017). Thus, the findings presented here may have important implifications for the indigenous forest system for the maintenance of functional guild diversity in Ethiopia given that mycorrhizal fungi have previously only been reported from exotic tree plantations (Dejene et al., 2017a). However, the importance of ectomycorrhizae and arbuscular mycorrhizae in indigenous forest systems in Ethiopia needs empirical data to confirm. In addition, the coexistence of these fungi has many practical advantages, such as the exchange of water and nutrients through mycorrhizal hyphal networks (Brundrett, 2002, 2004). Thus, our analysis of the fungal communities in these forest soils presents an insight into the conservation of functional guilds in the forest system in the study area.

The vegetation changes after a fire may affect the soil microbial community (Hart et al., 2005). Previous studies have reported that the loss of host plants after fire decreases mycorrhizal fungal diversity (Pattinson et al., 2006; Smith et al., 2005). In our study, both the richness and diversity of ectomycorrhizal fungi increased in the recently burned stands, which could indicated an immediate post-fire mycorrhizal colonization in fire-affected forest stands (Dahlberg, 2002; Rincón et al., 2015), while the saprotrophic fungi mineralize nutrients and stabilize the soil moisture after the fire (Dighton et al., 1986). The ectomycorrhizal taxa may also have established dominance immediately after burning owing to their tolerance of fire effects (Dahlberg, 2002; Kipfer et al., 2010) or they may have survived in a mycelial state during the fire event (Hewitt et al., 2013). However, the

colonization of mycorrhizal fungi could also be governed by burn severity and by the depth of burning in the soil profile (Hewitt et al., 2013). Thus, the effect of fire on mycorrhizae could be reduced when the fire only occurs at the soil surface, and the effect of the fire reduces with soil depth (Danielson, 1984; Pattinson et al., 2006; Visser, 1995). Thus, the fire that occurred in our forest study area might not have been strong enough to affect the mycorrhizal fungi or may have only affected fungi on the soil surface. It may also be influenced by the host plant's response to fire. However, in Ethiopia the mycorrhizal-associations for most plants are not yet well known. Thus, this should be investigated in future studies, including ectomycorrhizal root-tip samples, to learn more about the diversity of ectomycorrhizal host tree species and their associated fungi in dry Afromontane forests.

4.3. Soil fungal communities and environmental variables

The DCA indicated that the fungal communities detected in the three stand types were different. The SIMPER analysis also distinguished the total dissimilarity between stands and the relative contribution of each fungal species to the observed dissimilarity. The species making the highest contributions to the dissimilarity between the one- and ten-year-old burned stands (10.95%) and the one-year-old burned stands and the unburned stands (10.92%) was *Agaricus campestroides*. This species was highly abundant (N ~ 14916) in one-year-old burned stands but much less abundant in ten-year-old burned and unburned stands (N = 2 and N = 386, respectively). The contribution of the species might be partially responsible for the differences between stands, suggesting that time after fire is also probably responsible for the variation in the dominance of some species and their exclusive occurrence in certain stands. This is supported by previous findings that, for a given stand, certain fungal species tend to be abundant and characterize its composition (Zhu et al., 2010). Thus, a species with a consistently high contribution to the dissimilarity is a good discriminating species (Clarke, 1993).

Soil microorganisms, including fungi, are influenced by edaphic parameters (Drenovsky et al., 2004; Lauber et al., 2009, 2008). Our edaphic data from the dry Afromontane forest showed that more fungal species were detected in the burned forest areas (B1 and B10) where the soil fertility was relatively low than in unburned areas, which could be related to depositions of ash after the fire (Hul et al., 2015). Ash depositions could create empty niches that provide opportunities for the area to be rapidly colonized by fungal species at the early stages of succession (Fritze et al., 1993). However, the dry Afromontane forest area has suffered erosion caused by heavy rainfall soon after the fire events. As a result, there is a potential for sediment transportation from fire-affected areas and, thus, changes in soil fertility levels among stands. For instance, pH was assumed to be increased in newly burned areas, owing to the production of oxides and hydroxides (Hul et al., 2015). However, in our study forests, we recorded slightly high soil pH values in unburned forest stands. We found also a significant influence of the N, C/N ration and P on the entire fungal community in this study. For example, N and P reported could affect the structure of fungi in the soil, particularly of the mycorrhizal fungi (Zhao et al., 2018). The higher availability of these elements could decrease plant dependency on mycorrhizal fungi. This condition could also reduce the carbon allocation to fungi (Liu et al., 2019), which could increase competition and affect community composition (Wang and Wang, 2008; Zhao et al., 2018). Our result also confirmed that the fungal richness is low in stands where the soil C/N ratio is higher.

Previous studies have reported that after fire, the abundance of ectomycorrhizal fungi is reduced owing to the loss of host plants (Hart et al., 2005). However, in our study, the total fungal OTU richness in fire-affected stands, which had poor soil fertility, was high compared with that of unburned stands (Fig. 4), although such conditions remain to be interpreted. However, Castaño et al. (2019), reported high levels of ectomycorrhizal fungi in stands with poor soil quality. The occurrence of mycorrhizal species in poor quality soils suggests that the nutrient stress created in the fire-affected area could be compensated for by the increased dependency of trees on fungal symbionts (Read and Perez-Moreno, 2003). In this regard, the mycorrhizal ruderal guild in the spore bank would play an important role by quickly colonizing roots of plants, and will likely aid the survival of trees after the fire (Glassman et al., 2016). Species of *Wilcoxina*, *Tomentella*, *Tricholoma* and *Laccaria* were among the ectomycorrhizal species represented in the fire-affected stands, where soil fertility was low. Some of these genera such as *Laccaria* are considered ruderal species (Ishida et al., 2007) and are known to form an ectomycorrhizal association with several host tree species (Glassman et al., 2016; Hul et al., 2015).

4.4. Conclusions

This pioneer study is the first attempt to describe the soil fungal community in a dry Afromontane forest system of Ethiopia using next-generation sequencing and to investigate the effect of fire disturbance on these fungal communities. Data obtained in this study will significantly contribute to the body of knowledge regarding soil fungal communities in Ethiopia; however, the taxonomy of these fungi remains challenging and about 20% of the fungal species detected have not been described even at the phylum level. We conclude that, in general, the fungal diversity in Ethiopian forest systems is as yet largely undescribed and likely includes many taxa unknown to science. Thus, we advise that additional scientific investigations of this highly diverse but unexplored forest ecosystem are needed to consolidate the Ethiopian fungal biodiversity database. Also in this study, the soil fungal communities expected to be changed substantially along a post-fire forest succession and in comparison to those in unburned forest. However, we found that fire did not have a significant negative effect on fungal richness and diversity in the burned stands. Our study also highlighted that soil fungal composition differed across a chronosequence after fire and was correlated with soil fertility conditions and the changes would be explained partially by the edaphic conditions. Contrary to our expectation, root-associated symbiotic fungi like that of the mycorrhizal fungi were not lacking in fire-affected stands. We assume that mycorrhizal fungi present in the spore bank were able to colonize the roots of plants that survived the fire. In the fire-affected forests, we also found fungi that are known to form ectomycorrhizal associations with several host tree species. This key ecological role could provide support for the importance of fungal conservation in the dry Afromontane forest systems in Ethiopia. Similarly, vital edaphic variables such as OM and K also appear to be important in shaping the composition of mycorrhizal soil fungi in different ways. Thus, the effect of forest management practices such as thinning and harvesting on soil fertility should be taken into consideration owing to the important relationship between these ecological parameters and the soil fungal composition in the dry Afromontane forests of Ethiopia.

CRediT authorship contribution statement

Demelash Alem: Formal analysis, Writing - review & editing. **Tatek Dejene:** Methodology, Writing - review & editing. **Juan Andrés Oria-de-Rueda:** Supervision. **József Geml:** Formal analysis, Writing - review & editing. **Carles Castaño:** Writing - review & editing. **Jane E. Smith:** Writing - review & editing. **Pablo Martín-Pinto:** Conceptualization, Methodology, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2020.118328.

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Original article II

Comunidades de hongos del suelo bajo *Pinus patula* Schiede ex Schltdl. &amperio; Cham. Bosques de plantaciones de diferentes edades en Etiopía

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Resumen

Es probable que el cultivo de plantaciones forestales cambie la diversidad y composición de las comunidades de hongos del suelo. En la actualidad, hay poca información sobre estas comunidades en los sistemas forestales de plantaciones de Etiopía. Evaluamos las comunidades de hongos del suelo en *Pinus patula* Schiede ex Schltdl. & Cham. Stands de 5, 11 o 36 años de edad utilizando metabarcoding de ADN de amplicones ITS2. Las condiciones ecológicas de cada parcela, como clima, altitud y suelo, fueron similares. La edad del rodal y la fertilidad del suelo influyeron en la diversidad de especies de hongos del suelo y los gremios ecológicos. En total, se identificaron 2262 unidades taxonómicas operativas de hongos, de las cuales el 2% eran ectomicorrízicas (ECM). La diversidad de hongos ECM fue mayor en los rodales de 5 y 36 años que en los rodales de *P. patula* de 11 años. Contrariamente a nuestras expectativas, se observó un alto nivel de diversidad de especies de ECM en rodales jóvenes, lo que sugiere que estas especies de ECM podrían compensar los efectos del estrés nutricional en estos rodales. Nuestros resultados también sugirieron que la abundancia de patógenos vegetales y saprótrofos no se vio afectada por la edad del rodal. Este estudio proporciona información de referencia sobre los cambios en las comunidades de hongos en los rodales de árboles de diferentes edades en las plantaciones de *P. patula* en Etiopía que probablemente estén relacionados con los hongos ECM en rodales jóvenes donde prevalece una fertilidad del suelo relativamente baja. Sin embargo, dado que las parcelas se establecieron en un solo rodal para cada clase de edad para cada tratamiento, este estudio debe considerarse como un caso de estudio y, por tanto, se debe tener precaución al aplicar las conclusiones a otros rodales.

***Palabras clave**: hongos ectomicorrízicos; Secuenciación ion torrent; metabarcoding; Pinus patula; diversidad de hongos en el suelo; soportar la edad*

forests

MDPI

Article

Soil Fungal Communities under *Pinus patula* Schiede ex Schltdl. & Cham. Plantation Forests of Different Ages in Ethiopia

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Abstract: The cultivation of plantation forests is likely to change the diversity and composition of soil fungal communities. At present, there is scant information about these communities in Ethiopian plantation forest systems. We assessed the soil fungal communities in *Pinus patula* Schiede ex Schltdl. & Cham. stands aged 5, 11, or 36-years-old using DNA metabarcoding of ITS2 amplicons. The ecological conditions of each plot, such as climate, altitude, and soil, were similar. Stand age and soil fertility influenced soil fungal species diversity and ecological guilds. In total, 2262 fungal operational taxonomic units were identified, of which 2% were ectomycorrhizal (ECM). The diversity of ECM fungi was higher in the 5 and 36-year-old stands than in the 11-year-old *P. patula* stands. Contrary to our expectations, a high level of ECM species diversity was observed in young stands, suggesting that these ECM species could compensate for the effects of nutrient stress in these stands. Our results also suggested that the abundance of plant pathogens and saprotrophs was not affected by stand age. This study provides baseline information about fungal community changes across tree stands of different ages in *P. patula* plantations in Ethiopia that are likely related to ECM fungi in young stands where relatively low soil fertility prevails. However, given that the plots were established in a single stand for each age class for each treatment, this study should be considered as a case study and, therefore, caution should be exercised when applying the conclusions to other stands.

Keywords: ectomycorrhizal fungi; Ion torrent sequencing; metabarcoding; *Pinus patula*; soil fungal diversity; stand age

1. Introduction

A recent review of forestry in Ethiopia revealed that deforestation is a continuous process [1]. When all forest use was included, a deforestation rate of 0.93% per year was calculated in 2010 [2,3]. Despite this, establishing plantations of fast-growing exotic tree species is becoming a major part of forestry practice in Ethiopia [4,5]. Exotic tree species plantations are now estimated to cover 1,000,000 ha of land [5,6]. One of these introduced tree species is *Pinus*, which is mainly being grown to meet the increasing demand for woody raw materials [6–8]. As a consequence, *Pinus patula* Schiede ex Schltdl.

& Cham. has received attention largely as a suitable tree for the production of round wood (timber), poles, and posts [5,9], although it also used as a source of resin and turpentine oil constituents [10].

Soil fungi drive many ecosystem processes and, hence, are a vital part of the soil microbial community [11,12]. *Pinus* trees form obligate symbiotic relations with mycorrhizal fungi, which are vital for host nutrition, survival and productivity in forest systems [13]. Root associations with mycorrhizal fungi are beneficial for most plants because they not only increase their access to nutrients and water in the soil but also enhance their stress tolerance [14]. Similarly, mutualistic fungi use carbohydrates from the host tree for their survival [15]. Mycorrhizal associations also increase host tree resistance to pathogens [16,17]. Previous studies have also reported that there tends to be a greater accumulation of organic matter belowground in forests with ectomycorrhizal (ECM) associations than in other forests [18,19]. Furthermore, decomposition in forests is often limited by competition for nitrogen (N) between ECM and free-living saprotrophic fungi [18,20,21]. Mycorrhizal fungi mediate interactions between plants and other soil microorganisms, such as pathogens and mycorrhizosphere mutualists that produce vitamins, and provide protection against antagonists [14]. Saprotrophic fungi also play a crucial role in the decomposition of organic materials [22]. Thus, knowledge of fungi and their community structure in the soil of plantation forests are important because the trees and fungal synergies could determine many vital ecosystem services such as nutrient cycling [23].

The composition and structure of fungal communities are affected by diverse interacting biotic and abiotic factors [11,23], including host plant succession [24–26]. As a forest develops, the composition of the associated fungal communities also changes [27,28]. For example, early-stage fungi emerge from spores in the spore bank that were already in the soil prior to the establishment of the plantation, whereas late-stage fungi are more prevalent as conditions change as the plantation matures [29]. Both early- and late-stage fungi are involved in the stabilization of the soil and the rehabilitation of soil microflora [30]. Furthermore, changes in edaphic variables due to forest growth affect the abundance and diversity of soil fungi and, thereby, dictate the fungal community composition [31,32]. Previous studies have assessed changes in soil fungal communities across stand ages by performing DNA analyses [33,34] and fruit body surveys [35]. We have also previously shown that the fungal community composition differs at different stages of forest development in Ethiopian forests [8,24,36].

Ethiopian fungal flora remains unexplored, as most regions and habitats in the country have been seldom studied and reports regarding fungi diversity rarely exist. This is due to a lack of research infrastructures as well as to a lack of fungal taxonomists and specialists in fungal ecology. Likely as a result, fungi are not included in the biodiversity database of the country. Previous studies conducted on *Pinus* tree species in Ethiopia have focused on ways to assist the management and development of *Pinus* plantations. However, studies on the soil microbial community, including fungi associated with *Pinus* plantations, are very limited. Sporocarps associated with a *P. patula* plantation during a single rainy season have previously been reported [8]. Although sporocarps represent a unique step in the complex life of fungi [8], they do not reflect the entire soil biota [37,38]. However, the maintenance of a vital soil biota is essential for many woodland ecosystems because energy reaches the forest floor mainly through the degradation of organic matter and the recycling of vital elements found in animal and plant remains [24,39]. Furthermore, a rich soil biodiversity guarantees the presence of vital habitats, the integrity of the soil structure and water-holding capacity, soil fertility, and plant growth [40]. In the present study, we sampled soil from different age groups of *P. patula* plantations. We hypothesized that, as stand age increased, soil fertility would decrease. In addition, we expected that soil would become increasingly colonized by roots and associated symbionts and, hence, that root-associated fungal species would be more dominant in soils in older *P. patula* stands than in younger stands. Thus, in older stands, there is more organic matter and lower N than other edaphic elements. Given the potential changes in edaphic variables with increasing stand age [41], we expected that changes in the soil fungal community would also occur. Thus, the main aim of this study was to provide baseline information about the soil fungal communities that occur along a chronosequence of *P. patula* plantations in Ethiopia. We hypothesized that substantial change in the composition of soil fungal communities would be

detected along the chronosequence of *P. patula* plantations. Specifically, we hypothesized that: (1) there would be changes in the total fungal diversity and community composition, and (2) that the ecological guilds would change along the chronosequence of *P. patula* plantations in the study area.

2. Materials and Methods

2.1. Study Area

The study was conducted in *P. patula* stands in Wondo Genet, Ethiopia. Wondo Genet is approximately 265 km from the Ethiopian capital, Addis Ababa (Figure 1). The study site ranged from 7°06′ North to 7°07′ North and from 38°37′ East to 38°42′ East, and was 1600 to 2580 meter (m) above sea level [42,43]. The climate of the study area is characterized by the Woyna Dega agro-climatic zone, which has a bimodal rainfall pattern: a rainy season in spring followed by the main rainy season in summer, with a mean annual rainfall of 1210 millimeter (mm) [43,44]. The mean annual temperature is 20 °C [43,44]. The study area has a slightly undulating topography and an Andisol soil with a sandy loam texture [45] that has an average pH value of 5.7 [46].

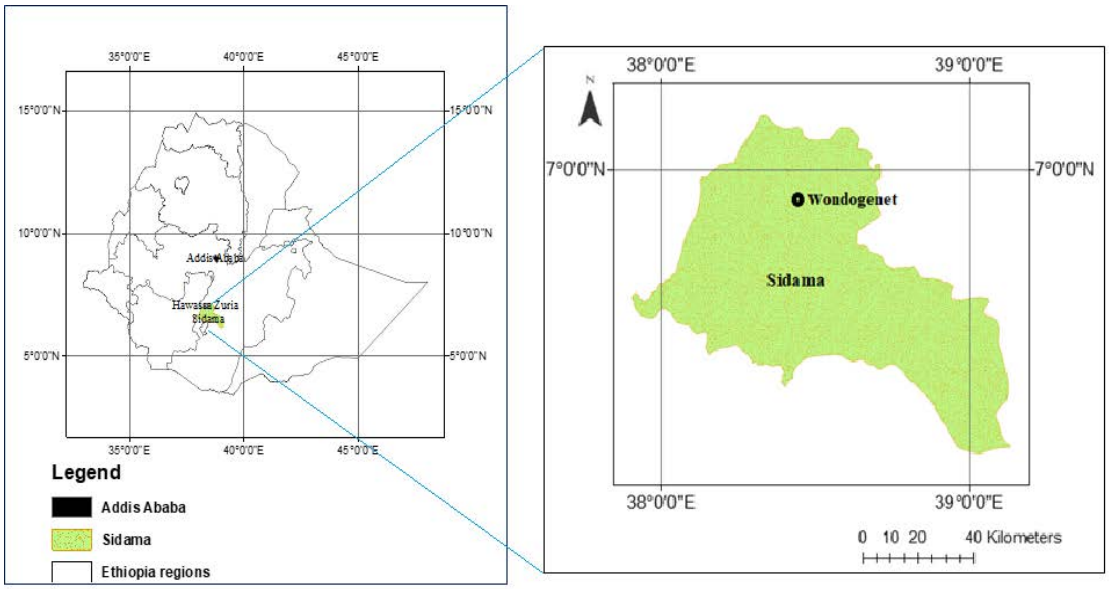

Figure 1. Maps showing the location of the study area, Wondo Genet, in the Sidama region of Ethiopia.

The native vegetation that originally grew in the study area was destroyed before the inception of the nearby college of forestry (1976) by logging and clearance for cultivation [10]. However, in recent decades, a mass planting scheme of exotic tree species has been undertaken, resulting in approximately 100 ha of non-native plantations of *Cupressus lusitanica* Mill., *Grevillea robusta* A. Cunn. ex R.Br., and *P. patula* in the study area [10,47]. In *P. patula* plantations, stands of three different ages (i.e., 5, 11, and 36-years-old) were selected and three 2 × 50 m plots were established in each stand age category [27,29], with a minimum distance of approximately 120 m between plots within a stand [28]. All plots were similar in terms of their ecological conditions such as climate, altitude, and soil.

The three stands were far enough apart (minimum 4 km) that they are not interacting and they could be clearly differentiated with the silvicultural treatments applied to them. For example, weeding and slashing were conducted for the 5 and 11-year-old stands while thinning was applied for the 36-year-old stands in the study area [8].

2.2. Collection of Soil Samples for Molecular Analysis

In total, nine transects, one in each of the 2 × 50 m plots, running perpendicular to the slope, were established [28,48]. Five cores 5 m apart were extracted along the centerline of each transect using a cylindrical soil borer (2 cm radius, 20 cm deep, and 250 cm^3) [49] to collect samples with spatial variability while minimizing the likelihood of repeatedly sampling the same genet. Before these cores were sampled, the litter layer, which comprised intact and partially decomposed leaves, was removed because the fungal community in leaves tends to differ from that in soil [50]. The five cores from each transect, comprising well-decomposed organic layers and mineral soils, were pooled to produce a composite soil sample of each transect. Next, the cores were dried, sieved through a 1-mm mesh and then ground to a fine powder using a mortar and pestle. A subsample of each composite sample was stored at −20 °C while awaiting molecular analysis.

2.3. Molecular Analysis

A PowerSoil™ DNA Isolation Kit (MoBio laboratories Inc., Carlsbad, CA, USA) was used to extract DNA from 0.25 g of soil per sample. PCR reactions of each sample were carried out in triplicate to minimize PCR biases. PCR reactions were performed in 20 µL reaction volumes containing 11.22 µL of Modified Quantization (MQ) water, 1.60 µL of DNA template, 2.00 µL of 10× buffer, 1.40 µL of $MgCl_2$ (50 mM), 1.60 µL of dNTPs (10 mM), 0.50 µL of Bovine Serum Albumin (BSA) (2%), 0.80 µL of reverse and forward primers (10 µM), and 0.08 µL of Platinum Taq polymerase (Invitrogen, Carlsbad, CA, USA). The following PCR conditions were used: an initial denaturation step at 94 °C for 3 min; then 35 cycles of 94 °C for 45 s, 50 °C for 1 min, and 72 °C for 1.5 min; ending with one cycle of 72 °C for 10 min. To amplify the ITS2 rDNA region, we used the forward primer fITS7 [51] and the barcoded reverse primer ITS4 [52]. Sample-specific Multiplex Identification DNA-tags were used to label the ITS4 primer. Each set of PCR replicates also included a negative control comprising MQ water instead of DNA that underwent PCR under the same experimental conditions and was shown to be amplicon-free on a gel. Ion torrent sequencing was performed at the Naturalis Biodiversity Center using the sequencing Ion 318^{TM} Chip to obtain the greatest possible sequencing coverage.

2.4. Collection of Soil Samples for Chemical Analysis

To examine the relationship between soil fungal composition and edaphic variables, soil samples were extracted from the center and the four corners of every plot after removing plant material and debris from the surface. Soil down to a depth of 20 cm was extracted using an auger and spade. The samples were mixed thoroughly to create a composite sample before placing approximately 500 g of soil in a plastic bag for laboratory analysis. After air-drying the soil in the shade, standard extraction methods (i.e., diethylene triamine pentaacetic acid extraction, KH_2PO_4 extraction, Olsen, Kjeldahl digestion Walkley Black, and ammonium acetate) and instrumental analysis were used to determine the pH, organic matter, cation exchange capacity, sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), N, available phosphorus (P), and the physical properties (% of sand, silt, and clay) of the soil.

Soil analysis was conducted by Water Works Design and Supervision Enterprises, Laboratory Service Subprocess, Soil Fertility Section at Addis Ababa, Ethiopia. Soil pH and electrical conductivity were determined by analyzing a soil:water (1:2.5) suspension and the supernatant from the same suspension with the aid of a pH meter and an electrical conductivity meter, respectively [53]. The organic carbon content of the soil was determined using wet digestion [54]. The Kjeldahl procedure was used to determine the total N content in soils [55]. Sodium bicarbonate (0.5 M $NaHCO_3$) was used as an extraction solution to determine the available P [56]. Available Na, available K, Ca, and Mg were also determined. To assess soil particle size we used a hydrometer [57] and sodium hexametaphosphate (Calgon solution) was used as a dispersing agent. After calculating the proportions of sand, silt, and clay, the soil was assigned a textural class name using ASTM free software, Version 4, Available: http://www.astm.org. The results of the soil analysis are presented in Table 1.

Table 1. Selected chemical properties of soil samples from the study plots in Pinus patula plantations in Wondo Genet area, Ethiopia.

Soil Chemical Properties	Stand Age		
	5 Years	11 Years	36 Years
pH-KCl (1:2.5)	6.04 ± 0.19	5.42 ± 0.20	5.44 ± 0.44
Exch. Na^+ (meq/100 g of soil)	0.85 ± 0.03	0.78 ± 0.05	1.10 ± 0.49
Exch. K^+ (meq/100 g of soil)	1.32 ± 0.25	0.42 ± 0.08	0.77 ± 0.05
Exch. Ca^{2+} (meq/100 g of soil)	22.62 ± 3.82	13.66 ± 2.99	16.01 ± 7.23
Exch. Mg^{2+} (meq/100 g of soil)	7.96 ± 1.16	4.36 ± 0.42	6.66 ± 1.15
Organic matter	5.96 ± 0.68	7.27 ± 0.84	13.33 ± 0.98
Nitrogen (%)	0.83 ± 0.07	0.71 ± 0.06	0.34 ± 0.02
Available P (mg P_2O_5/kg soil)	35.70 ± 5.02	36.99 ± 8.10	38.56 ± 7.46
C/N	3.61 ± 0.28	5.17 ± 0.41	19.56 ± 2.11

Note: Values shown are means ± the standard deviation.

2.5. Bioinformatic Analysis

Raw sequence reads comprising demultiplexed sample reads were obtained from the Ion Torrent output. Primers and poor-quality ends were removed based on a 0.02 error probability limit in Geneious Pro 8.1.8 (BioMatters, Auckland, New Zealand). Next, all sequences were truncated to 200 bp and then filtered with USEARCH v.8.0 [58] to discard sequences with an expected error of >1. The remaining sequences were collapsed into unique sequence types on a per-sample basis using USEARCH v.8.0 [58] while preserving read counts. First, we discarded singleton sequence types before grouping the remaining 187,332 high-quality sequences into 2262 operational taxonomic units (OTUs) with USEARCH at a 97% sequence similarity level while simultaneously excluding 3161 sequences representing 67 OTUs with <70% similarity or <150 bp pairwise alignment length to a fungal sequence. Sequences were assigned to taxonomic groups based on pairwise similarity searches against the curated UNITE+INSD fungal ITS sequence database, which contains identified fungal sequences with assignments to species hypothesis groups [59]. The FUNGuild database (http://www.funguild.org) was initially used to perform functional classification of OTUs at the genus level and it was manually checked afterwards. OTUs with >90% similarities to a fungal SH with known ecological function were assigned either to plant pathogens, mycoparasites, animal parasite, ECM fungi, arbuscular mycorrhizal, and saprotrophs functional groups. For genera that are known to comprise species from multiple functional guilds, their ecological function was assigned individually based on available ecological information for the matching SH in the UNITE database.

2.6. Statistical Analysis

To normalize the OTU table for subsequent statistical analyses, we rarefied the number of high-quality fungal sequences to the smallest library size (1951 reads). The resulting matrix contained 1216 fungal OTUs representing 17,559 high-quality sequences. Thus, the analyses used sequence read abundance data, which is not necessarily equivalent to true biological abundance. Shannon's diversity index, $H = -\Sigma pi(lnpi)$ [60], was estimated for each stand, where pi indicates the relative abundance of fungal OTUs [61]. We also calculated Simpson's diversity, $D = 1 - \Sigma$ (pi2), where pi is the importance probability in element i, and the Evenness, $J = H'/H'max$, where H' is the number derived from the Shannon diversity index and the H'max is the maximum possible value of H' [62]. We also estimated the richness values of all fungal OTUs (S) based on stand type. The Biodiversity RGUI package [63] in R was used to calculate all the diversity measures [64], such as diversity indices and richness.

Ordination of community data was carried out by performing detrended correspondence analysis (DCA) and canonical correspondence analysis (CCA) using CANOCO version 5.0 [65] based on a Hellinger-transformed abundance data matrix. The relative sequence read abundance contribution of each ecological group to community composition was assessed using the relative abundance of the fungal OTUs. A Monte Carlo Permutation test was used to test the statistical significance of the

environmental variables (999 permutations). Correlation between the CCA axes scores and edaphic variables were assessed using linear regression. We also tested whether fungal compositions were statistically different across forest stands of different ages using the multiple-response permutation procedure (MRPP) and permutation-based nonparametric MANOVA (PerMANOVA) [66]. Data were subjected to 4999 iterations per run using the Sørensen similarity (Bray–Curtis index) and a random number to start. To identify which fungal species were most responsible for the patterns observed and to calculate the percentage contribution of fungal taxa to significant dissimilarities among the three stands, we undertook an analysis of similarity percentages (SIMPER) [67,68].

3. Results

3.1. Diversity of Fungal OTUs

A total of 187,332 high-quality sequences were grouped into 2262 OTUs. We obtained between 1951 and 37,245 high-quality reads from each sample. The OTUs were assigned to six fungal phyla. The majority of the OTUs belonged to the Ascomycota (58%), followed by the Basidiomycota (26%). The Chytridiomycota, Glomeromycota, Rozellomycota, and Zygomycota made up to 4%, 3%, 1% and 2% of the total OTUs, respectively, and approximately 7% of OTUs were not assigned to a fungal phylum.

Saprotrophs were the most abundant species found in *P. patula* plantations, comprising 41% of the fungal community, followed by plant pathogens (7%) and ECM species (2%). Less abundant groups were categorized as animal parasites, arbuscular mycorrhizal fungi, mycoparasites, and endophytes. These groups were excluded from further analysis. About 48% of the species were not categorized into any of these functional groups.

The highest mean values for Shannon and Simpson diversity indices for the ECM and saprotrophic fungi were found in 5-year-old stands. Contrary trend was observed for plant pathogens which showed higher diversity in 36-year-old stands. On the other hand, the estimated evenness values of the ECM (range 0.24 to 0.30), plant pathogens (range 0.21 to 0.24), and saprotrophic fungi (range 0.12 to 0.25), showed that each functional groups distributed uniformly in each of the three stands.

The relative abundance of ECM fungi was greater in the 11-year-old *P. patula* stand, whereas the plant pathogens obtained higher mean values in the 5-year-old stands. By contrast, the relative abundances of saprotrophic fungi were homogeneous along the chronosequence of *P. patula* stands.

3.2. Fungal Community Composition and Edaphic Variables

PerMANOVA analyses indicated that the composition of fungal OTUs in stands of different ages were significantly different ($F = 2.76$, $p = 0.013$). MRPP tests also confirmed that the plantation age effect was significant, with a distance effect size of $A = 0.017$ and $p = 0.006$. More specifically, the composition of 5 and 36-year-old stands differed from the 11-year-old stand, as indicated by the Sørensen average distance measures (Table 2). In addition, SIMPER analysis indicated that the overall between-group dissimilarity based on Bray-Curtis measures was 76.09% for the 5 and 11-year-old stands, 77.67% for the 5 and 36-year-old stands and 83.58% for the 11 and 36-year-old stands (Table S1).

Table 2. Average forest group distances calculated using Sørensen distance matrices to determine whether soil fungal compositions were statistically different across *Pinus patula* stands of three different ages in Wondo Genet, Ethiopia. The average value of all three stand ages ± the standard deviation is also shown.

Group	Forest Stand Type	Sørensen Average Group Distance
1	5-year-old stand	0.60
2	11-year-old stand	0.39
3	36-year-old stand	0.63
Average		0.54 ± 0.13

From the canonical correspondence analysis (CCA) (Figure 2), the eigenvalues indicated that the variability in terms of fungal guild composition, explained by the gradients associated with the first two axes, is high. Together they explained 29.47% of the accumulative variance of fungal guild data. With respect to the edaphic variables, potassium (K) and magnesium (Mg) correlated the most strongly with fungal community composition (Figure 2). The correlation of these edaphic variables with CCAs was evident based on fungal guild Hellinger-transformed abundance data (Table 3).

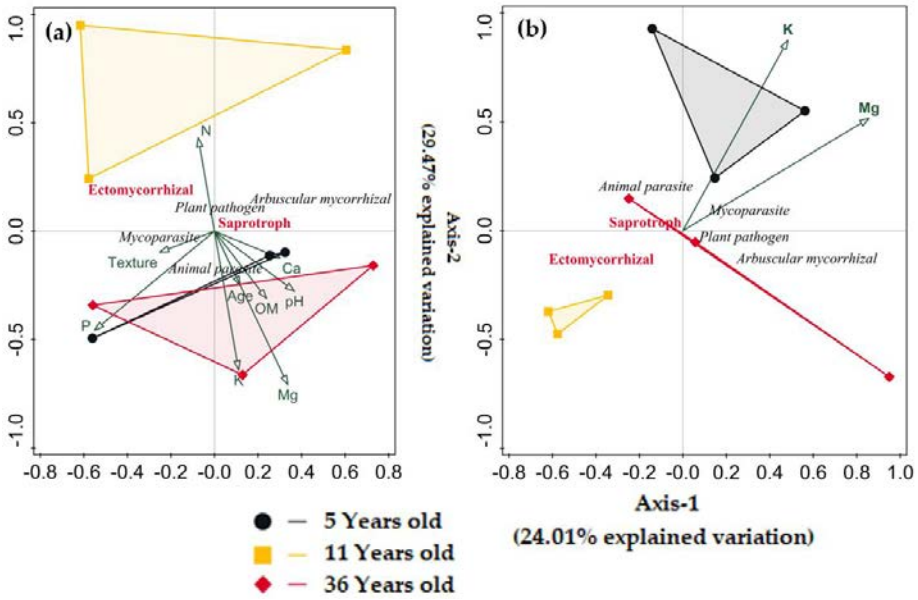

Figure 2. (**a**) Detrended correspondence analyses and (**b**) canonical correspondence analysis (CCA) ordination plots based on Hellinger-transformed abundance data of fungal guilds detected in the soil of three *Pinus patula* stands of different ages. Plots shown in the same color are in the same stand (black, plots in the 5-year-old stand; yellow, plots in the 11-year-old stand; red, plots in the 36-year-old stand). Edaphic variables are shown in green. The percentages of explained variation by each axis are shown in (**b**).

Table 3. Canonical correspondence analysis based on simple term effects showing the significance ($p < 0.05$) of edaphic variables when considering the Hellinger transformed data of the entire fungal guilds at the level of fungal community in the study area.

Edaphic Variables	Df	Sum of Squares	R^2	F	p
K	1	0.018	0.365	26.825	0.010
Mg	1	0.021	0.425	7.518	0.008

Compared with the 5 and 36-year-old stands, we found that a relatively greater proportion of the ECM guild was associated with the 11-year-old stand. By contrast, the saprophytes, mycoparasites, and animal parasites were mainly found associated with the 5- and 36-year-old stands. Based on the cumulative contribution to the dissimilarity of stands, the SIMPER analysis also identified fungal species that typified and distinguished between the soil fungal communities associated with the three *P. patula* plantation age groups (Table S1). *Wilcoxina* sp., *Schizothecium* sp., and *Pustularia* sp. were among the most influential species detected in the soil analyses and cumulatively made the greatest contribution to the differences observed between the three stands, accounting for more than 38.63% of the observed dissimilarity value (Table S1).

When ECM fungi were analyzed separately, the simple term effects in CCA (Figure 3b) revealed that plantation age and the soil fertility variable P significantly influenced the composition of the ECM fungal community in the forest study area (Table 4). The CCA revealed that both axes explained about 45.82% of the cumulative variation in interactions between ECM fungal composition, soil fertility

variables and plantation age. The PerMANOVA analysis also indicated that there were species compositional differences between stands in terms of ECM fungi (F = 3.21, p = 0.003).

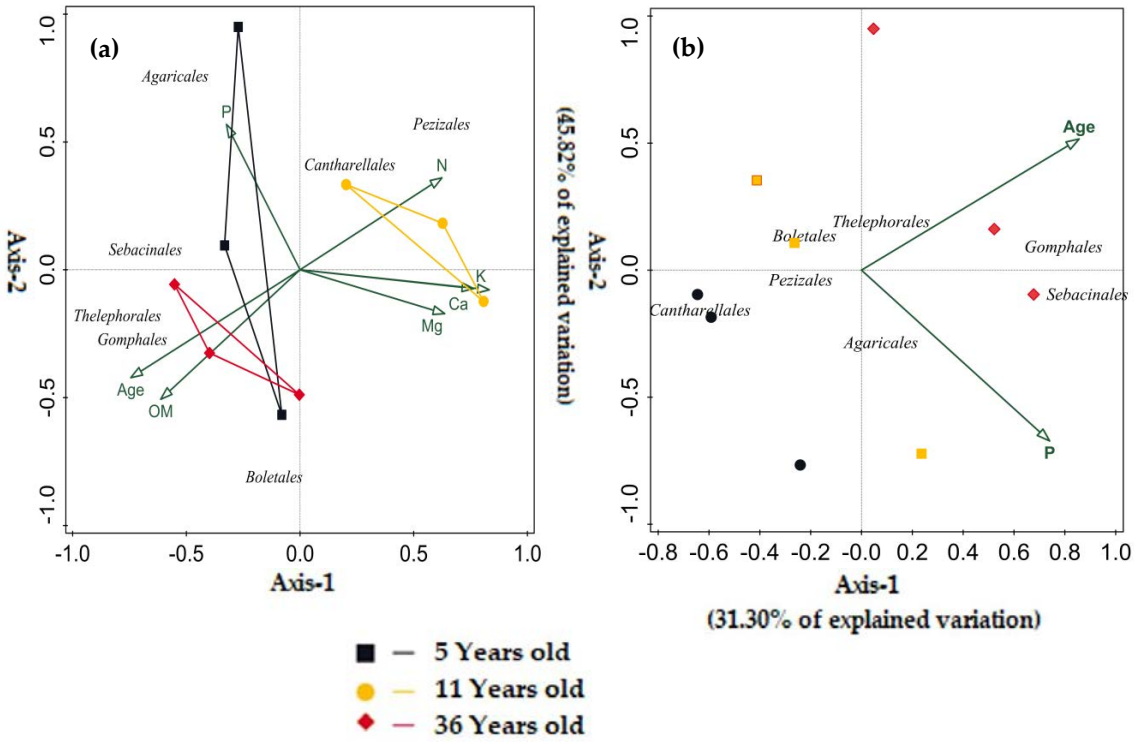

Figure 3. (**a**) Detrended correspondence analyses and (**b**) canonical correspondence analysis (CCA) ordination plots based on Hellinger-transformed abundance data of mycorrhizal fungal species at the order level of fungal communities associated with three age groups of *Pinus patula* stands in the study area in Ethiopia. Plots shown in the same color are in the same stand (yellow, plots in the 5-year-old stand; black, plots in the 11-year-old stand; red, plots in the 36-year-old stand). Edaphic variables are shown in green. The percentages of cumulative explained variation by each axis are shown in (**b**).

Table 4. Canonical correspondence analysis based on simple term effects showing the significance ($p < 0.05$) of edaphic variables when considering the Hellinger transformed data of ectomycorrhizal species at the order level of fungal community in the study area.

Variable	Simple Term Effects		
	Explains %	Pseudo-F	p
Age	26.8	2.60	0.010
Phosphorus	19.0	2.10	0.052

Several species belonging to the orders Gomphales, Sebacinales, and Thelephorales were associated with plots in the oldest stand, where the soil is characterized by relatively high fertility (based on the organic matter content and the C/N ratio). In 5 and 11-year-old stands, which are characterized by low soil fertility (based on organic matter content and the C/N ratio), the species belonging to the order Cantharellales formed an association with other fungal species (Figure 3). Species in the orders Boletales, Pezizales and Agaricales were associated with all age groups of *P. patula* plantations (Figure 3).

4. Discussion

4.1. Diversity of Fungal OTUs

Fungi have been described as the most interesting, enigmatic and species-rich organisms on Earth [69]. The use of molecular methods in recent years has dramatically increased the number of fungal OTUs detected worldwide [70]. In this study, we detected a total of 2262 high-quality OTUs, of which 1303 OTUs (58%) belonged to the Ascomycota, the largest phylum of fungi [71], indicating the dominance of Ascomycota in the *P. patula* forests investigated in this study. Fungi often interact with other organisms, forming beneficial or mutualistic associations. Conifers in the Pinaceae usually form symbiotic relationships with ECM fungi. Thus, ECM fungi were likely to play an important role in the *P. patula* plantations in our study area. However, of the total OTUs identified at genus level and classified by ecological function, 41% were saprotrophs, 7% were plant pathogens, and only 2% were ECM fungi. An explanation for the small proportion of ECM fungi detected might be that the plantation area was located in a non-ECM biome area. The native vegetation that originally grew in the study area was destroyed many years ago by logging and clearance for crop cultivation [10]. Therefore, fungal symbionts compatible with *P. patula* may be absent in the native fungal community in the soil, enabling ECM fungi introduced to the plantation area along with *P. patula* to co-invade the soil habitat [72]. However, the ecological function of 48% of the fungi identified in this study is unknown, indicating that we have hardly scratched the surface in terms of understanding the role played by fungi in these plantation forest systems. It might also an indication for the lack of scientific studies on the local fungal flora in the country. Thus, they are highlighting the need for further studies in the study area.

4.2. Diversity of Functional OTUs along the Chronosequence of Stands

The Shannon and Simpson diversity indices were determined to explain variations in the soil fungal functional groups at different stages of *P. patula* stand development. Only ECM fungi showed differences in their diversity among the stand age groups. Initially, we expected that as stands developed, soil fertility would decrease over time and that these conditions would lead to higher ECM fungal diversity along the chronosequence. By contrast, the soil fertility of young *P. patula* stands was expected to be higher than that of old stands and, therefore, the trees were expected to exert less influence on the fungal microbiome. However, our investigations revealed that the 5 and 36-year-old stands had more diverse ECM fungi in their soils than the 11-year-old stand and were more fertile. Despite this, the relative proportion of ECM abundance was higher in the 11-year-old stand than in the 5 and 36-year-old stands due to the dominance of some ECM species in the 11-year-old stand.

Soil microorganisms, including fungi, are influenced by stand development in several ways [73,74]. As the stand develops, the amount of tree cover directly modifies the amount of light available, which affects the composition of the understory, which regulates carbon allocation, nutrient cycling and soil water content [75]. The relatively higher diversity indices of ECM fungi in the 5-year-old stand than in the 11-year-old stand may possibly be explained by the less developed tree canopy at the earlier stage of stand development, which may have allowed diverse ECM fungi to interact extensively with the root systems of understory plants [75]. Another explanation for the relatively higher diversity indices of ECM fungi in the 5-year-old stand may be the previous land use of the plantation area. Deacon and Fleming [76] demonstrated that when afforestation takes place on land initially used for other purposes, the ECM fungal spores are the fundamental inoculum during the early stage of ECM succession. In this case, prior to the establishment of *P. patula* plantations, the study area was previously used for agricultural purposes, which might have contributed to the diversity of ECM fungi in young stands of *P. patula* trees due to the primary succession of ECM fungi through inocula in the spore bank derived from other nearby mycorrhizal-associated plantations such as *Eucalyptus* sp. L'Hér. plantations [24,36].

The relatively higher diversity indices of ECM species in the 36-year-old stand than in the 11-year-old stand may reflect a difference in the management of the 36-year-old stand. Chen et al. [77] indicated that thinning could increase the relative abundance of mycorrhizal fungi because thinning opens up the forest canopy, which increases soil temperature and moisture [78]. Thinning may therefore have a positive effect on microbial activity [79] because soil temperature and moisture influence the reaction of microbial enzymes and, thus, shift the microbial community composition by altering substrates and extracellular enzyme activity [80]. Dove and Keeton [81] and Tomao et al. [82] have suggested that fungal diversity can be conserved or even increased using forest management practices that enhance the structural complexity of stands and the late-successional characteristics of the forest and by carrying out low-impact logging operations. In this study, the 36-year-old stand had undergone thinning as part of a management operation in the study area [8], which could have enhanced the root growth of the remaining trees [83,84], providing new environments for soil microbes, which could lead to an improvement in fungal diversity through root attachments. Castaño et al. [12] observed that the species diversity of soil fungi remained stable after thinning, regardless of its intensity, when sufficient host trees and functional roots from thinned trees were retained. This finding has been supported by a number of different reports. For example, according to Mölder et al. [85], thinning could maintain a high level of ECM fungal diversity in mature stands. Chen et al. [86] and Dang et al. [75] also hypothesized that when thinning operations are performed, light availability, water, and nutrients increase, which improves the forest microclimatic conditions and, hence, the diversity of ECM fungi could be improved by this type of management practice. Furthermore, Goldmann et al. [87] reported that ECM fungi were less diverse in unmanaged forests than in highly managed stands. Thus, we suggest that forest management practices such as thinning could be one of the factors that impact ECM fungal diversity along the chronosequence of *P. patula* stands, although this should be further studied.

4.3. Fungal Composition and Edaphic Variables

Forest soils contain a diverse range of fungal species. The composition of soil fungal communities is influenced by different factors, such as dispersal, plant diversity, soil properties, land use, and climate, which are key components of forest systems [88,89]. Specifically, different fungal species are likely to respond to environmental drivers in different ways, depending on their characteristic traits [90,91], and, thus, in turn, the composition of soil fungal communities is directly correlated with soil fertility and plant growth status [92]. Mycorrhizal species are a particularly important part of the soil fungal community because they form a beneficial symbiotic association with plants, providing them with nutrients in return for photosynthetically fixed carbon [93], which is especially relevant under nutrient-limited conditions [94]. ECM fungi also play a key role in alleviating the drought stress of the host tree [95]. The composition of ECM fungi in the soil is also correlated with soil fertility and the growth status of the host trees [92,96]. In this study, we found that both stand age and soil fertility were factors that affected the fungal community composition in our study area. Our ordination analysis indicated that the 36-year-old stand and the 5-year-old stand had distinctive soil fungal communities, characterized by a relatively high number of ECM species. Previous studies have related similar findings regarding fungal community composition to several factors, such as changes in soil fertility [24], changes in root density [97], specific life-history events that have occurred since the stand was established or changes in microclimate conditions [12]. For example, less litter accumulation in young *P. patula* stands resulted in less organic matter and a lower C/N ratio in the 5 and 11-year-old stands in this study compared with the 36-year-old stand. This situation leads to trees having a greater dependence on mycorrhizal fungal associations for enhanced nutrient and water uptake and availability [24]. Similarly, the greater diversity of ECM species in young stands may indicate that suitable symbionts are present. However, older stands have a greater capacity to reduce fluctuations in temperature and to maintain adequate moisture levels [98–100], which is particularly important for the occurrence of ECM fungi. The abundance of ECM fungi in older stands is generally greater than in younger stands, which could be facilitated by the root systems of old trees, which could increase the

chances of ECM associations forming [85], thereby facilitating the easy uptake of nutrients by trees [94]. Thus, the distinct composition of ECM fungi in young and old stands might not only be related to site quality factors, such as soil fertility, but also to stand age factors (e.g., the increasing area of tree root exploration in the soil with stand age); however, this needs to be investigated further. Given that the amount of organic matter, available P, and the C/N ratio of 5 and 11-year-old stands showed no greater difference in their values, this may have enabled 11-year-old stands to develop an association with only a limited number of ECM fungi, but a higher relative abundance of these ECM fungi, which could indicate increased dependence of *P. patula* trees on a limited number of dominant symbiont species. In any case, the survival of *P. patula* in soils in which ECM species comprise only a small proportion of the microbial community, together with other factors, supports the view that *P. patula* is well adapted to the conditions in this study area; however, this also needs further study.

Fertile soil contains nutrients that enable the growth and development of a soil fungal community [101]. Thus, in turn, the fungi are directly influenced by edaphic parameters [102–104]. In this study, edaphic cation elements, such as Mg and K, were also correlated with the overall fungal community from the whole data set, which indicates that soil cation concentrations could influence the composition of the fungal community [105]. Cations in general play an important part in a number of physicochemical processes, such as photosynthesis [106] and, thus, can affect plant photosynthesis and, hence, the amount of carbon that is available to soil fungi and bacteria [107]. Of the various cations, Ca is one of the main edaphic factors that influence the structure of soil fungal communities worldwide [108]. Other edaphic elements have also been reported to influence the composition of fungal communities in forest systems. For example, in this study, available P and tree age influenced the composition of mycorrhizal fungi (Table 4), which was similar to the findings reported by Rosenstock et al. [109]. The composition of fungi in the soil particularly that of mycorrhizal fungi, can also be influenced by N availability [110]. High levels of available N could decrease the dependency of the host plant on mycorrhizal fungi, which could reduce the amount of carbon allocated to fungi [111], which eventually could cause competition among the fungal species and could lead to changes in their composition [96,110]. In this study, the C/N ratio of soil in 5 and 11-year-old stands was relatively low compared with that of 36-year-old *P. patula* stands (Table 1). This result is inconsistent with Wang and Wang [96] who reported that a high C/N ratio negatively influenced fungal community structure, probably because a high concentration of N restrains the expansion of fungi. Our results also confirmed this, in the sense that the fungal diversity was low in stands where the soil C/N ratio was high, indicating that a high C/N ratio might not favor the fungal community in the forests in the study area. Soil organic matter could also impact the composition of soil fungal communities because fungi generally extend their mycelia at the soil–litter interface [112]. The amount of organic matter affects the water holding capacity of soil and nutrient availability, which could affect mycelial outgrowth and network formation [113,114]. However, soil acidity can also influence the composition of soil fungal communities [115,116] through its influence on spore germination and mycelial development [117].

Fungi have different life-history strategies and, in plantations, early colonizer fungal species that relish disturbance colonize first, followed by superior competitors that can outcompete the early colonizer species in older stands where resources are getting scarcer. In this study, we found that some fungal species associated with *P. patula* trees were detected at all age stages of tree development, such as those belonging to the genera *Tomentella*, *Ramaria*, and *Inocybe*. These genera have several hundreds of species, many still undescribed, and their strategies do not seem to be conserved at the genus level. Of these, *Tomentella* and *Inocybe* are cosmopolitan species that inhabit *Eucalyptus* plantations in Ethiopia [24]. Species of *Rhizopogon* were also associated more with younger stands (5- and 11-year-old stands) in this study, which supports previous findings that they are early colonizer fungal species [118]. The *Rhizopogon* species are known as spore bank species that facilitate the establishment of trees in formerly non-forest habitats. Other taxa belonging to the genera *Amanita* have also been reported in our *P. patula* plantation forests. These fungi species are well-known for their association with conifer forests as the genera is characteristic of late-stage pine stands that are 30–40 years old [119,120].

5. Conclusions

Our study explained the soil fungal community composition associated with a *P. patula* plantation in Ethiopia. The diversity value of vital fungi such as ECM was relatively higher in the youngest stand than in the two older stands. Stand age and soil fertility were also found to affect fungal community composition. Some ECM fungi were found as early colonizer species in the young stand and were replaced by superior competitor species as the *P. patula* stands developed.

The overall low level of ECM species richness detected in the *P. patula* stands is probably because this is a plantation of an ECM tree in a non-ECM biome. Due to the importance of fungi in plantation forest system, the results of this study could be relevant for the promotion and conservation of forests in Ethiopia through the promotion of non-wood forest products such as fungi, which could also provide food resources for poor populations during times of food scarcity. Thus, it is imperative to investigate how soil fungal communities respond to management regimes such as thinning and clear-cutting. The high diversity and relative abundance of plant pathogenic fungi detected in this study also highlights the need to protect Ethiopian plantations from plant diseases and pests.

Although the ecological conditions of all studied plots were similar in terms of climate, altitude, and soil, the results of this study should be considered as a case study, given that the plots were established in a single stand for each age class for each treatment and, therefore, the applicability of any conclusions to other stands should be treated with caution. Furthermore, additional scientific investigations of the plantation forest ecosystem are needed in order to consolidate the Ethiopian fungal biodiversity database.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/11/10/1109/s1, Table S1: Summary of the Similarity Percentage (SIMPER) analysis showing contrasts between the cumulative total contribution (50% cut-off) and the contribution (%) of the most influential fungal operational taxonomic units to the dissimilarity of the soil fungi detected in three *Pinus patula* stands of different age groups in a plantation in Wondo Genet, Ethiopia.

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Original article III

Estudio de la diversidad macrofúngica y análisis de los factores edáficos que influyen en la comunidad fúngica de los bosques eclesiásticos en las áreas secas afromontanas del norte de Etiopía

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Resumen

Los bosques secos afromontanos del norte de Etiopía se han talado para la agricultura y se han reducido a fragmentos pequeños y aislados. La mayoría de estos bosques se encuentran alrededor de los territorios de las iglesias y se denominan bosques de iglesia. Se sabe que los bosques de iglesia son islas de biodiversidad y brindan servicios ecosistémicos clave a las comunidades locales. Sin embargo, hasta la fecha, los recursos fúngicos de estos bosques no han sido evaluados y, por lo tanto, se desconoce la contribución de los hongos a su valor de conservación. En 2019, investigamos la diversidad fúngica de tres bosques de iglesia en la región afromontana seca. En cada bosque, establecimos nueve parcelas permanentes (2 m × 50 m), que fueron muestreados semanalmente durante la época de lluvias para cuantificar la diversidad fúngica y los niveles de producción de esporocarpos. También se analizaron variables explicativas para determinar su relación con la composición de las especies encontradas. Recolectamos 13,736 esporocarpos correspondientes a 188 especies. De éstas, el 81% eran saprotróficas y el 14% ectomicorrícicas. Sesenta y ocho especies eran comestibles, incluidas especies económicamente valiosas como *Tricholoma* y *Termitomyces*. Esto sugiere que estos sistemas forestales fragmentados podrían manejarse para proporcionar valiosos productos forestales no maderables como hongos y beneficios socioeconómicos para las comunidades locales. Aunque muchas especies estaban presentes en los tres bosques, algunas solo se encontraron en un bosque, lo que destaca la importancia de conservar los bosques individuales. La correlación de los índices de diversidad de Shannon y Simpson de la comunidad fúngica y vegetal mostró una tendencia positiva a pesar de la correlación negativa entre su riqueza. Las comunidades de hongos en su conjunto se vieron influidas por variables edáficas, espaciales y climáticas. Este estudio indica que los bosques de iglesia sustentan una amplia diversidad de hongos, incluidas especies de hongos potencialmente nuevas, y destaca la necesidad de que los gestores forestales consideren la importancia de los hongos en el manejo de estos ecosistemas, proporcionando hábitats que mantengan la diversidad de hongos y la producción de esporocarpos al planificar estrategias de conservación.

Palabras clave: *Conservación, variables edáficas, bosques fragmentados, macrofungi, bosques de iglesia, esporocarpos*.

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Survey of macrofungal diversity and analysis of edaphic factors influencing the fungal community of church forests in Dry Afromontane areas of Northern Ethiopia

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ABSTRACT

The Dry Afromontane forests in Northern Ethiopia have been cleared for agriculture and reduced to small and isolated fragments. Most of these forests are located around church territories and are they called church forests. The church forests are known to be biodiversity islands and provide key ecosystem services to local communities. However, to date, the fungal resources of these forests have not been assessed and, therefore, the contribution of fungi to their conservation value is unknown. In 2019, we investigated the fungal diversity of three Dry Afromontane church forests. In each forest, we established nine permanent plots (2 m × 50 m), which were surveyed weekly during the rainy season to quantify the fungal diversity and sporocarp production levels. Explanatory variables were also analyzed to determine their relationship with macrofungal species composition. We collected 13,736 sporocarps corresponding to 188 taxa. Of these, 81% were saprotrophic and 14% were ectomycorrhizal. Sixty-eight species were edible, including economically valuable species such as *Tricholoma* and *Termitomyces*. This suggests that these fragmented forest systems could be managed to provide valuable non-timber forest products such as mushrooms and socioeconomic benefits for local communities. Although many species were present in all three forests, some were only found in one forest, highlighting the importance of conserving individual forests. The correlation of the Shannon diversity indices of the two communities showed a positive trend in spite of the lack of correlation between their richness. Macrofungal communities as a whole were influenced by edaphic, spatial and climate variables. This study indicates that church forests support a wide diversity of fungi, including potentially novel fungal species, and highlights the need for forest managers to consider the importance of fungi in forest ecosystem management and to provide habitats that will maintain fungal diversity and sporocarp production when planning conservation strategies.

1. Introduction

The Ethiopian highlands constitute large parts of the Afromontane regions in Africa (Aynekulu et al., 2016; Nyssen et al., 2014). These highlands are dominated by Dry Afromontane forests and are mainly found in the Northern part of Ethiopia (Eshete et al., 2011; Friis et al., 2010a,b). Dry Afromontane forests are rich in biodiversity and are dominated by pioneers, shrubs, and high-quality trees (Abiyu et al., 2018; Lemenih et al., 2011) that are able to grow at high altitudes (Friis et al., 2010a,b). The main tree species that constitute the Dry Afromontane forests include *Juniperus procera*, *Podocarpus falcatus*, *Hagenia abyssinica* and *Olea africana* (Kassa et al., 2009). These trees serve as a vital source of timber to the country (Kassa et al., 2009) and thus an indication of a need for the sustainable management of these forests. The Dry Afromontane forests also produce several Non-Timber Forest Products (NTFPs) such as wild edible fruits and medicinal plants that are vital for the socioeconomics of the local communities (Shumi, 2009). Furthermore, edible mushrooms from the forest systems have been utilized as important sources of food and medicine by rural communities for their livelihoods in few specific regions in Ethiopia (Abate, 2008). However, high levels of historical human landscape alteration and land-use pressure have resulted in widespread deforestation and the degradation of forest land (Aerts et al., 2016; Aynekulu et al., 2016; Darbyshire et al., 2003; Nyssen et al., 2014). The ever-increasing demand for

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wood products as well as crop and grazing land expansion, stimulated by rapid population and livestock growth are also factors aggravating the degradation of the Dry Afromontane forests in the country (Bekele and Lemenih, 2008). As a result of the many physical and biological changes to the Dry Afromontane forests in Northern Ethiopia, natural forests have been reduced to small and isolated fragments, most of which belong to the church or are located around church forest territories (Aerts et al., 2016; Aynekulu et al., 2016; Wassie et al., 2009). These forest fragments have survived because of the cultural or religious values held by local communities, which have contributed to the conservation of their biodiversity. Owing to the small size of these forest fragments, there are variations in their biodiversity between forest fragments (Lemenih et al., 2011; Wassie et al., 2005) and probably of their fungal communities.

Fungal communities are an important component of forest ecosystems and have a broad range of functions (Song et al., 2019; Tedersoo et al., 2014a; Wagg et al., 2014). As decomposers, they are important for the degradation of organic matter and play a vital role in nutrient cycling (Chen et al., 2019; Ferris et al., 2000). Mycorrhizal fungi form symbiotic associations with higher plants, facilitating plant uptake of water and nutrients (Egli, 2011; Hall et al., 2003; Tedersoo et al., 2020). Fungi can also be used as bioindicators to assess the quality of forests (Egli, 2011; Van Bruggen and Semenov, 2000). In addition to their ecological roles, fungi have been used by humans for thousands of years in different ways (Boa, 2004; Oria-De-Rueda et al., 2008) and are sold in markets worldwide, providing an important source of rural income (Boa, 2004; Pettenella et al., 2007). Indeed, in some cases, forest fungi provide significant complimentary benefits to forest managers (Bonet et al., 2014; Martín-Pinto et al., 2006). Fungi also provide food and habitats for other organisms and, therefore, interactions between fungi and other organisms in forest systems cannot be overlooked (Jonsell and Nordlander, 2000). Pathogenic fungi also impact ecosystems, mainly by acting as natural population regulators, thereby influencing productivity, species diversity, and composition (Ruiz-Almenara et al., 2019). For all these reasons, fungi are considered a strategic component in the conservation and management of forest systems (Bonet et al., 2014).

Today, biodiverse remnants of the Dry Afromontane forests survive in the agricultural landscape and church areas as forest islands (Aynekulu et al., 2016). Several studies have evaluated the conservation value of these fragmented forests in the Northern landscapes of Ethiopia (Aerts et al., 2016; Aynekulu et al., 2016; Wassie et al., 2010, 2005; Nyssen et al., 2014). However, the ecology and conservation status of the macrofungi in these isolated and fragmented forest systems is unknown. Consequently, the fungal taxonomy and ecology are very poorly described and, hence, fungi are neglected when decisions need to be made regarding forest management and conservation actions. Recently, there has been an interest in surveying fungi in particular habitats (Alem et al., 2020), to describe and predict the extent of their diversity on a larger scale (Danielsen et al., 2005; Peay, 2014). This information is important to enable the integration of fragmented forests into global biodiversity conservation strategies (Hundera et al., 2013; Aerts et al., 2016; Aynekulu et al., 2016) and to understand what actions are required to conserve fragmented forests and their biological components, including fungi, which are known for their exceptionally high diversity levels (Burgess et al., 2006).

The practice of using plant communities as surrogates to predict fungal diversity has been reported by previous studies (McMullan-Fisher et al., 2010; Rudolf et al., 2013). Fungal communities have also been associated with essential ecosystem parameters such as edaphic variables (Chen et al., 2018), as well as other relevant drivers of fungal richness at a global level such as climate (Tedersoo et al., 2014b). Furthermore, fungal diversity is considered to reflect niche diversity given that reducing niche similarity drives species assemblage (Silvertown, 2004). The Dry Afromontane church forests in Ethiopia are reported to have high levels of plant species diversity (Mokria et al., 2015; Tsegaye et al., 2010). However, there is no evidence that the high level of plant diversity in the church Dry Afromontane forests system anticipates correspondingly high macrofungal diversity. In addition, as yet, the environmental variables that govern fungal communities in these fragmented forest systems have not been identified given that these forests vary in their status (size, density, species composition etc.), topography and altitude (Bongers and Tenngkeit, 2010). Thus, investigating the fungal community composition and how this community changes across sites in fragmented forests should help us to understand different aspects of fungal interaction within these systems and their function in the ecosystem (Genevieve et al., 2019). This information may also be a means to understand how to improve natural fungal richness and sporocarp production and also help us to facilitate the conservation of economically and ecologically important macrofungal species in these fragmented forest systems.

This study is the first attempt to provide baseline information about macrofungi assemblage, diversity, and sporocarp production in Dry Afromontane church forests with priority status in Northern Ethiopia. Priority forests are those that have been designated as reserves to give them additional protection. The information generated should help to guide management and conservation strategies for these priority forests and supplement our knowledge of macrofungal species in Ethiopia. Furthermore, determining whether edible mushrooms are produced in these forests could provide an opportunity for harvesting edible mushrooms for either subsistence or commercial use. Despite fragmentation, the forests in the study areas are suggested to be relatively rich in plant species (Aerts et al., 2016; Wassie et al., 2010). On average, there are 25 vascular tree species per forest patch (Aerts et al., 2016). The tree species composition of these forests also varied with their status, topography and altitude (Bongers and Tenngkeit, 2010), with a wide distribution over the landscapes (Aerts et al., 2016). Given that fungal diversity is related positively to plant richness (Tedersoo et al., 2014b), we hypothesized that the fungal diversity of the study plots in the church forests would be high in terms of total fungal species. In addition, the edaphic conditions, vascular plant richness and diversity would follow given the differences in climate and topography among the different fragmented forests, we also hypothesized that the composition of macrofungal communities would differ among the studied forests, resulting in an overall higher richness value for the study sites because fungi will be driven mainly by vegetation condition and site conditions such as soil fertility conditions (Castaño et al., 2018; Vašutová et al., 2017). Thus, our specific aims were to study three church forests in Dry Afromontane areas of Northern Ethiopia: (1) to describe fungal species richness, diversity, and sporocarp production; (2) to correlate the macrofungal and plant diversity of the three church forests; and (3) to determine whether the macrofungal community composition was governed by the soil fertility status of the three forest sites.

2. Materials and methods

2.1. Description of the study areas

The study was conducted in three church forests located in three different districts of the Amhara region, namely the Taragedam forest located in *Libokemkem* Woreda, the Alemsaga forest located in *Farta* Woreda, and the Banja forest located in *Banja* Woreda (Fig. 1). These forests are fragments of the remnant Dry Afromontane forests in Northern Ethiopia (Gebeyehu et al., 2019; Masresha et al., 2015; Zegeye et al., 2011). The Taragedam and Banja forests were designated as reserves in 1979 (Zegeye et al., 2011) and 1994 (Abere et al., 2017), respectively, to prevent any kind of encroachments. The Alemsaga forest was designated as a priority forest in 1978 to serve as a seed source, to conserve the remnant natural forest, and to rehabilitate the degraded area in Northern part of the country (Masresha et al., 2015). Comprehensive descriptions of the forests are provided in Table 1. Within each of the forests, plots were established systematically about 500 m apart in 2019. The plots were laid out randomly in the forests to avoid

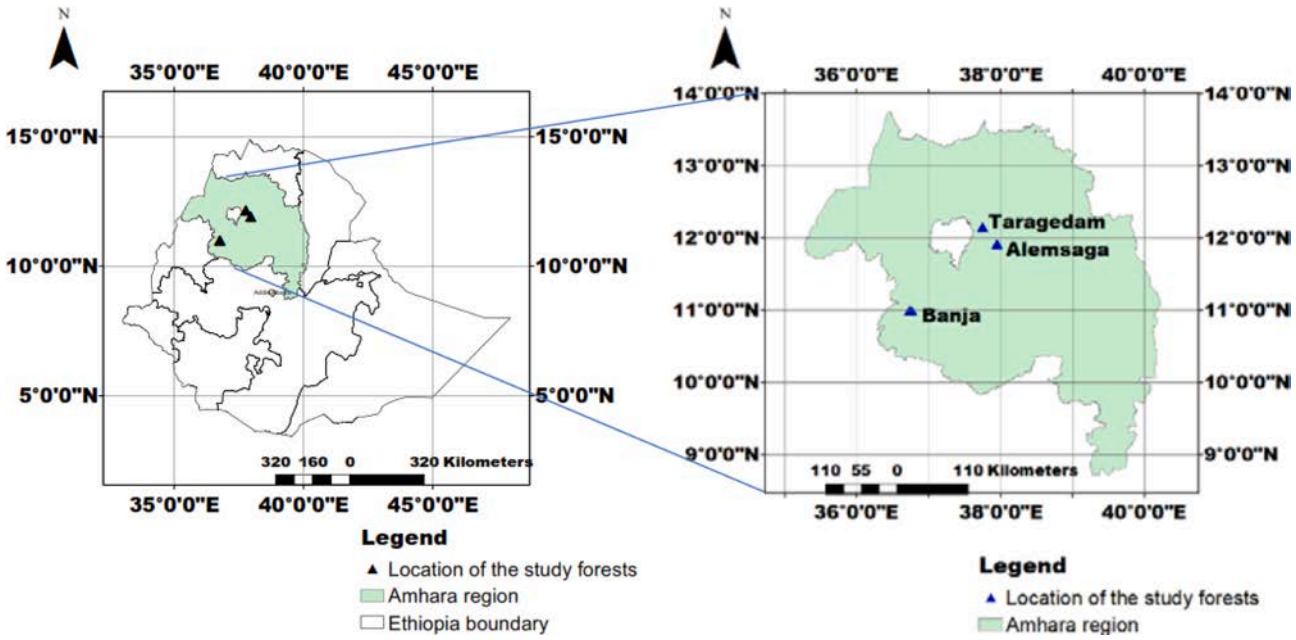

Fig. 1. Map of the Amhara region in Northern Ethiopia showing the location of the three church forests in which the study plots were located.

confounding spatial effects inherent to such a plot-based design (Hiiesalu et al., 2017; Rudolph et al., 2018). The plots were analyzed as independent samples as suggested by Ruiz-Almenara et al.(2019). However, the present study provides a starting place in broadening management objectives for NTFPs in the Dry Afromontane church forests. Thus, the result should be considered as a case study and as a preliminary indication and conclusions regarding other similar studies need to be taken with caution.

2.2. Sporocarp sampling

In total, 27 sample plots were established, nine in each of the three church forests, as described in Gassibe et al. (2011) and Hernández-Rodríguez et al. (2013). Each plot was rectangular in shape (2 m × 50 m) and covered an area of 100 m^2. Within each of the selected church forests, we studied three different blocks including three plots per block. The plots were established about a minimum distance of 500 m apart. All fungal fruit bodies found were harvested weekly during the major rainy season in July and August of 2019. Fresh weight measurements were taken *in situ* using a digital sensitive balance (SF-400) to determine fruit body production in kilograms per hectare per year. The number of sporocarps of each species in each plot was also recorded. Specimens were photographed in the field and their morphological features and ecological characteristics were noted to facilitate taxonomic identification processes in the laboratory (Adeniyi et al., 2018). Specimens of each macrofungus were taken to the laboratory and dried to preserve as herbaria specimens, and then used for morphological species identification.

2.3. Species identification

In the laboratory, the morphological features of the fruit bodies were examined using appropriate monographs, including Antonin (2007), Hama et al. (2010), Heinemann (1956), Hjortstam and Ryvarden (1996), Morris (1990), Pegler (1968, 1969, 1977), Rammeloo and Walleyn (1993), and Singer (1965), to determine the genus and species of the macrofungal specimens. Up-to-date fungal species names and authors' names were obtained from the Mycobank database (http://mycobank.org). Ecological functions at the genus level were identified using a FUNGuild (www.funguild.org) search and provided in Table 2. In addition, the edibility of the fruiting bodies collected from the study sites was assessed following the criteria used by Bonet et al. (2004). Species described in the literature as both non-edible and edible in the literature were classified as non-edible. Species described in the literature as having doubtful edibility were classified as non-edible. Only species classified as edible by a large majority of the literature consulted were classified as edible fungi (E).

2.4. Soil sampling and analysis

To relate macrofungal composition to edaphic variables, soil samples were collected from each of the sample plots established in the three forests. Composite samples were collected by grouping each plot into relatively homogeneous subsamples. After clearing and removing plant matter and debris from the soil surface, five soil cores were extracted from the center and the four corners of each plot using an auger (2 cm radius, 20 cm deep and 250 cm^3). Subsamples collected from each plot were mixed thoroughly and a composite sample of approximately 500 g was placed in a plastic bag for analysis. Soil samples were dried under a shed until a constant weight was obtained and ground to < 2 mm sieved soil is used in the analysis. The soil pH and electrical conductivity were determined by analyzing a soil:water (1:2.5) suspension and the supernatant from the same suspension with the aid of a pH meter and an electrical conductivity meter, respectively (Reeuwijk, 2002). The organic carbon content of the soil was determined using wet digestion (Walkley and Black, 1934). The Kjeldahl procedure was used to determine the total N content in soils (Kim et al., 2005). Sodium bicarbonate (0.5 M NaHCO3) was used as an extraction solution to determine the available phosphorus (P) (Kim, 1996). Available sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) were also determined. To assess soil particle size we used a hydrometer (Bouyoucos, 1951) and sodium hexametaphosphate (Calgon solution) was used as a dispersing agent. After calculating the proportions of sand, silt, and clay, the soil was assigned a textural class name using ASTM free software, Version 4, Available: http://www.astm.org. The results of the soil analysis are provided in Table 1. The soil analysis was conducted by the Amhara Design and Supervision Works Enterprise at Bahir Dar, Ethiopia.

Table 1
Characteristics of the study sites and selected edaphic properties.

Descriptions	Forests		
	Taragedam	Alemsaga	Banja
Geographical location	12°06′–12°07′ N 37°46′– 37°47′ E	11°54′–11°56′N 37°55′–37°57′E	10°57′–11° 03′N 36°39′– 36°48′E
Altitude range (m asl)	2142–2484	2180–2470	1870–2570
Mean annual precipitation (mm)	1098	1926	1884.3
Mean annual temperature (°C)	19.5	15.8	18.7
Forest area (ha)	875	814	897
Density of trees ha^{-1}	48.11	17.19	43.13
Sand (%)	58.89 ± 2.93b	51.78 ± 2.99b	68.67 ± 2.21a
Silt (%)	28.44 ± 2.38a	32.44 ± 2.13a	20.00 ± 1.76b
Clay (%)	12.67 ± 1.37a	15.78 ± 1.93a	11.33 ± 1.33a
pH H2O 1:2.5	7.04 ± 7.03a	5.85 ± 6.59b	5.60 ± 6.24c
EC (dS/m)	0.43 ± 0.05b	0.28 ± 0.03b	0.81 ± 0.14a
Ex.Ca (cmol (+)/kg)	13.95 ± 0.60a	9.19 ± 0.52b	13.55 ± 0.87a
Ex.Mg (cmol (+)/kg)	6.16 ± 0.10a	4.58 ± 0.15c	5.54 ± 0.20b
Ex.Na (cmol (+)/kg)	1.95 ± 0.05a	2.05 ± 0.10a	1.82 ± 0.12a
Ex.K (cmol (+)/kg)	0.73 ± 0.06a	0.61 ± 0.04a	0.77 ± 0.06a
CEC (cmol (+)/kg)	47.21 ± 1.36a	34.89 ± 0.92b	44.51 ± 1.96a
Organic matter (%)	4.46(0.60)a	3.35(1.34)b	4.87(0.10)a
Nitrogen (%)	0.23 ± 0.01a	0.17 ± 0.02b	0.26 ± 0.01a
P (ppm)	17.18 ± 5.72a	7.8 ± 0.73b	17.64 ± 6.05a
Dominant species in each plots	*Maytenus obscura*, *Carissa edulis*, *Olea* sp.	*Acacia abyssinica*, *Buddleja polystachya*, *Acacia nilotica*	*Albizia gummifera*, *Prunus africana*, *Brucea antidysenterica*
References	Gedefaw and Soromessa (2014), Zegeye et al. (2011), Zerihun et al. (2013)	Birhane et al. (2017), Masresha et al. (2015), Wubet et al. (2004)	Abere et al. (2017)

Note: Values shown are means; standard errors of the means are indicated in parentheses. Values with different lowercase letters are significantly different ($p < 0.05$). The mean annual precipitation and mean annual temperature are given based on nearby stations data of each study area by the year 2019. Abbreviations: EC, electrical conductivity; CEC, cation exchange capacity; m, meter; mm, millimeter; asl, above sea level. The references listed are related to the climatic and geographical descriptions of the study areas.

2.5. Vegetation sampling

To relate the vegetation characteristics to the macrofungal richness and diversity, vegetation inventories were conducted in the plots established for macrofungal species sampling as described above. Vascular plants identified in each plots were recorded using their vernacular names. For those species difficult to identify their scientific name in the field, specimens were collected and their taxonomic identification was conducted using published volume of the flora of Ethiopia and Eritrea (Hedberg and Sue, 1989). Large trees growing outside the plots were included in the survey if their crowns overhung the plots because tree crown projection areas can affect macrofungal occurrence (Collins et al., 2018). Furthermore, large trees create their own microhabitat and develop a large root system, providing more space for fungal associations (Schön et al., 2018). Then, the vascular plant species richness and diversity parameters were determined (Table 3). Plant parameters and their correlations were also used for further interpretation of macrofungal pattern from each study areas. The mycorrhizal status of the vascular tree species found in each of the studied plots were checked using freely accessible databases (Soudzilovskaia et al., 2020) and the data is provided (Table S1).

2.6. Statistical analysis

Data were transformed when needed to achieve the parametric criteria of normality and homoscedasticity. The macrofungi data were normalized by rarefying the abundance data to the smallest number of macrofungi per plot. Also, the data from soil variables were scaled using base R and used for subsequent statistical analyses. Shannon's H′ diversity index, $H' = -\Sigma pi\ (lnpi)$ (Shannon and Weaver, 1949), was estimated for each forest, where pi indicates the relative abundance of the species (Kent and Coker, 1993). Simpson's diversity, $D = 1 - \Sigma(pi2)$, where pi is the importance probability in element i; and the evenness, $J = H'/H'max$, where H′ is the number derived from the Shannon diversity index and the H′ max is the maximum possible value of H′ were also calculated (Magurran, 1988). In addition to species richness values, macrofungi biomass production levels in each forest were estimated and converted in to Kg bases. All diversity measures for macrofungi and vascular plants were analyzed using the Biodiversity R package (Kindt and Coe, 2005) in R version 4.0.3 (R Core Team, 2020). The difference in the soil, vegetation and sporocarps variables across forests were assessed by Linear Mixed Effects models (LME, Pinheiro et al., 2016), where block (a set of plots in a same site in each forest) was defined as random and forest was defined as fixed factor. The LME used to prevent the false positive associations due relatedness structure in the sampling. Tukey Test was later used to check significant differences ($p \leq 0.05$) between forests when needed.

Species accumulation curves were constructed to compare the rate at which new fungal species were found in the three forests and to provide an estimate of macrofungal species richness. Curves were generated using a sample-based estimator of EstimateS Version 9 (Colwell, 2013). The number of fungal species collected during each weekly visit to a plot within a forest constituted the sample. Curves were generated based on the total of the weekly sampling datasets. A Rényi diversity profile (Tóthmérész, 1995) was also used to depict the diversity curves of the three church forests. When parameter alpha = 0, this function gives the total species number and when alpha = 1, this gives an index proportional to the Shannon index.

The relationship of macrofungal composition with the edaphic, climate and location parameters was visualized using non-metric multidimensional scaling (NMDS), based on absence and presence species data matrix and environmental scaled data. A permutation-based nonparametric MANOVA (PerMANOVA) (Anderson, 2001) using Bray–Curtis distance was conducted to analyze differences in macrofungal communities across forests. The isolines of the elevation also plotted on the NMDS ordinations using the ordisurf function. The correlation of NMDS axes scores with explanatory variables was assessed using envfit function in R. To test the influence of categories of the edaphic, climate and location variables on the fungal community, we used Mantel Test (Bray-Curtis distance) on total species matrix and scaled environmental parameters. Also, an analysis of similarity percentages (SIMPER; Clarke, 1993) was performed to identify macrofungal species that were most responsible for the observed patterns and was also used to determine the percentage contribution of macrofungal species to significant dissimilarities between the three forests (Parravicini et al., 2010). The SIMPER analysis was performed using the sim function of the Vegan package in R (R Core Team, 2020).

3. Results

3.1. Macrofungal richness and diversity

In total, 13,736 sporocarps were collected from the three church

Table 2
Fungal sporocarps collected in July and August in three church forests in Northern Ethiopia.

Taxa	Order	Family	T	A	B	E	LM
Agaricus augustus Fr.	Agaricales	Agaricaceae		x		E	SS
Agaricus bitorquis (Quél.) Sacc.	Agaricales	Agaricaceae	x			E	SS
Agaricus campestris L.	Agaricales	Agaricaceae	x	x	x	E	SS
Agaricus cupreobrunneus (Jul.Schäffer & Steer ex F.H.Møller) Pilát	Agaricales	Agaricaceae		x	x	E	SS
Agaricus megalosporus J. Chen, R.L. Zhao, Karun. & K.D. Hyde	Agaricales	Agaricaceae	x	x	x	E	SS
Agaricus moelleri Wasser	Agaricales	Agaricaceae	x		x	E	SS
Agaricus murinaceus Bull.	Agaricales	Agaricaceae			x	E	SS
Amanita vaginata (Bull.) Lam.	Agaricales	Amanitaceae	x		x		EM
Amanita sp. Pers.	Agaricales	Amanitaceae	x		x	E	EM
Amanita verna (Bull.) Lam.	Agaricales	Amanitaceae	x	x	x	E	EM
Ampulloclitocybe clavipes (Pers.) Redhead, Lutzoni, Moncalvo & Vilgalys	Agaricales	Tricholomataceae		x	x	E	LS
Artomyces pyxidatus (Pers.) Jülich	Russulales	Amylostereaceae	x	x	x		WS
Auricularia auricula-judae (Bull.) Quél.	Auriculariales	Auriculariaceae	x			E	WS
Bisporella citrina (Batsch) Korf & S.E.Carp.	Helotiales	Helotiaceae			x		WS
Bjerkandera adusta (Willd.) P.Karst.	Polyporales	Meruliaceae	x		x		WS
Bolbitius sp. Fr.	Agaricales	Bolbitiaceae		x	x		DS
Bovista aestivalis (Bonord.) Demoulin	Agaricales	Agaricaceae	x		x		SS
Bovista plumbea Pers.	Agaricales	Agaricaceae		x			SS
Calvatia cyathiformis (Bosc) Morgan.	Agaricales	Agaricaceae		x	x	E	SS
Calvatia gigantea (Batsch) Lloyd	Agaricales	Agaricaceae	x			E	SS
Calvatia sp. Fr.	Agaricales	Agaricaceae			x	E	SS
Cantharellula umbonata (J.F.Gmel.) Singer	Agaricales	Tricholomataceae		x	x		LS
Cantharellus cinnabarinus (Schwein.) Schwein.	Cantharellales	Hydnaceae	x			E	EM
Chlorophyllum molybdites (G. Mey.) Massee	Agaricales	Agaricaceae	x	x	x	E	LS
Chlorophyllum rhacodes (Vittad.) Vellinga	Agaricales	Agaricaceae	x	x	x	E	LS
Clavaria falcata Pers.	Agaricales	Clavariaceae		x			SS
Climacodon septentrionalis (Fr.) P. Karst.	Polyporales	Phanerochaetaceae	x				WS
Clitocybe carolinensis H.E. Bigelow & Hesler	Agaricales	Tricholomataceae	x	x		E	LS
Clitocybe cistophila Bon & Contu	Agaricales	Tricholomataceae	x			E	LS
Clitocybe foetens Melot.	Agaricales	Tricholomataceae	x	x	x	E	LS
Clitocybe fragrans (With.) P.Kumm.	Agaricales	Tricholomataceae	x	x	x	E	LS
Clitocybe geotropa (Bull.ex DC.) Quél	Agaricales	Tricholomataceae		x		E	LS
Clitopilus hobsonii (Berk. & Broome) P.D. Orton	Agaricales	Entolomataceae	x	x	x		LS
Conocybe apala (Fr.) Arnolds	Agaricales	Bolbitiaceae			x		SS
Conocybe aurea (Jul.Schäff.) Hongo	Agaricales	Bolbitiaceae	x	x			SS
Conocybe dumetorum (Velen.) Svrcek	Agaricales	Bolbitiaceae		x	x		SS
Conocybe tenera (Schaeff.) Fayod	Agaricales	Bolbitiaceae	x	x	x		SS
Conocybe velutipes (Velen.) Hauskn. & Svrcek	Agaricales	Bolbitiaceae	x	x	x		SS
Coprinellus disseminatus (Pers.) J.E.Lange	Agaricales	Psathyrellaceae		x	x		SS
Coprinellus micaceus (Bull.) Vilgalys, Hopple & Jacq. Johnson	Agaricales	Psathyrellaceae	x	x	x		SS
Coprinopsis sp. P. Karst.	Agaricales	Coprinaceae	x		x		SS
Coprinus comatus (O.F.Müll.) Pers.	Agaricales	Coprinaceae	x	x	x	E	DS
Coprinus lagopus (Fr.) Fr.	Agaricales	Coprinaceae		x	x		DS
Coprinus micaceus (Bull.) Fr.	Agaricales	Coprinaceae	x			E	DS
Coprinus niveus (Pers.) Fr.	Agaricales	Coprinaceae	x	x	x	E	DS
Cortinarius rubellus Cooke	Agaricales	Cortinariaceae		x	x		EM
Craterellus ignicolor (R.H. Petersen) Dahlman, Danell & Spatafora	Cantharellales	Hydnaceae	x			E	EM
Crepidotus applanatus (Pers.) P. Kumm.	Agaricales	Inocybaceae	x	x	x	E	WS
Crepidotus mollis (Schaeff.) Staude	Agaricales	Inocybaceae	x	x	x	E	WS
Crucibulum laeve (Huds.) Kambly	Agaricales	Agaricaceae			x		LS
Cystodermella granulosa (Batsch) Harmaja	Agaricales	Agaricaceae	x		x		LS
Dacrymyces palmatus (Schwein.) Burt	Dacrymycetales	Dacrymycetaceae			x		WS
Daedaleopsis confragosa (Bolton) J.Schröt.	Polyporales	Polyporaceae	x		x		WS
Daldinia concentrica (Bolton) Ces. & De Not.	Xylariales	Hypoxylaceae			x		WS
Deconica montana (Pers.) P.D. Orton	Agaricales	Strophariaceae	x	x	x		LS
Entoloma asprellum (Fr.) Fayod.	Agaricales	Entolomataceae	x	x	x		SS
Entoloma olivaceohebes Noordel. & Hauskn.	Agaricales	Entolomataceae		x	x		SS
Entoloma poliopus (Romagn.) Noordel.	Agaricales	Entolomataceae		x	x		SS
Entoloma sp. Fr. ex P. Kumm.	Agaricales	Entolomataceae	x		x		SS
Entoloma undatum (Gillet) M.M. Moser	Agaricales	Entolomataceae		x	x		SS
Favolaschia calocera R. Heim	Agaricales	Marasmiaceae			x		WS
Galerina badipes (Pers.) Kühner.	Agaricales	Strophariaceae	x	x	x		WS
Geastrum triplex Jungh.	Geastrales	Geastraceae	x	x	x		LS
Geoglossum sp. Pers.	Geoglossales	Geoglossaceae	x	x			SS
Gymnopilus sp1. P.Karst.	Agaricales	Omphalotaceae		x	x		WS
Gymnopilus sp2. P.Karst.	Agaricales	Omphalotaceae	x		x		WS
Gymnopilus sp3. P.Karst.	Agaricales	Omphalotaceae	x				WS
Gymnopus dryophilus (Bull.) Murrill	Agaricales	Omphalotaceae	x	x	x		LS
Gymnopus luxurians (Peck) Murrill	Agaricales	Omphalotaceae	x				LS
Gymnopus putillus (Fr.) Antonín, Halling & Noordel.	Agaricales	Omphalotaceae		x	x		LS
Hebeloma eburneum Malençon	Agaricales	Strophariaceae			x		EM
Hemimycena delectabilis (Peck) Singer.	Agaricales	Tricholomataceae	x	x	x		LS
Hexagonia tenuis (Hook.) Fr.	Polyporales	Polyporaceae	x	x	x		WS
Hohenbuehelia petalodes (Bull.) Schulzer.	Agaricales	Pleurotaceae	x				WS

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Table 2 (*continued*)

Taxa	Order	Family	T	A	B	E	LM
Hygrocybe chlorophana (Fr.) Wünsche	Agaricales	Hygrophoraceae	x	x	x	E	SS
Hygrocybe chlorophana var. *aurantiaca* Bon.	Agaricales	Hygrophoraceae			x	E	SS
Hygrophoropsis aurantiaca (Wulfen) Maire	Boletales	Hygrophoropsidaceae	x	x	x		LS
Hygrophorus hypothejus Fr. (Fr.)	Agaricales	Hygrophoraceae	x	x	x	E	EM
Hymenagaricus sp1. Heinem.	Agaricales	Agaricaceae			x	E	SS
Hymenagaricus sp2. Heinem.	Agaricales	Agaricaceae	x				SS
Inocybe viridiumbonata Pegler	Agaricales	Inocybaceae			x		EM
Laccaria glabripes McNabb.	Agaricales	Hydnangiaceae	x				EM
Laccaria laccata (Scop.) Cooke	Agaricales	Hydnangiaceae		x			EM
Laetiporus sulphureus (Bull.) Murrill	Polyporales	Fomitopsidaceae	x	x	x	E	PP
Lentinellus cochleatus (Pers.) P. Karst.	Russulales	Auriscalpiaceae		x	x	E	WS
Lepiota cristata (Bolton) P.Kumm.	Agaricales	Agaricaceae			x		LS
Lepiota ermine (Fr.) P.Kumm.	Agaricales	Agaricaceae		x	x		LS
Lepiota himalayensis Khalid & Razaq	Agaricales	Agaricaceae	x	x			LS
Lepiota sp1. (Pers.) Gray	Agaricales	Agaricaceae			x		LS
Lepiota sp2. (Pers.) Gray	Agaricales	Agaricaceae	x				LS
Lepiota sp3. (Pers.) Gray	Agaricales	Agaricaceae		x	x		LS
Leptonia lampropus (Fr.) Quél.	Agaricales	Entolomataceae	x	x	x		SS
Leucoagaricus americanus (Peck) Vellinga.	Agaricales	Agaricaceae	x	x	x	E	SS
Leucoagaricus purpureolilacinus Huijsman	Agaricales	Agaricaceae	x	x	x	E	SS
Leucoagaricus sp1. Locq. ex Singer	Agaricales	Agaricaceae	x		x	E	SS
Leucoagaricus sp2. Locq. ex Singer	Agaricales	Agaricaceae			x		SS
Leucocoprinus cepaestipes (Sowerby) Pat.	Agaricales	Agaricaceae	x		x		SS
Leucocoprinus fragilissimus (Berk. & M.A.Curtis) Pat.	Agaricales	Agaricaceae			x		SS
Lyophyllum infumatum (Bres.) Kühner	Agaricales	Lyophyllaceae			x		EM
Macrolepiota procera (Scop.) Singer	Agaricales	Agaricaceae			x	E	LS
Macrolepiota sp. Singer	Agaricales	Agaricaceae	x			E	LS
Marasimus sp1. Fr.	Agaricales	Marasmiaceae	x			E	LS
Marasmiellus chamaecyparidis (Hongo) Hongo	Agaricales	Omphalotaceae			x		LS
Marasmius arborescens (Henn.) Beeli	Agaricales	Marasmiaceae	x	x			LS
Marasmius candidus Fr.	Agaricales	Marasmiaceae	x			E	LS
Marasmius guyanensis Mont.	Agaricales	Marasmiaceae	x	x	x	E	LS
Marasmius oreades (Bolton) Fr.	Agaricales	Marasmiaceae	x	x		E	LS
Marasmius purpureostriatus Hongo	Agaricales	Marasmiaceae	x	x	x	E	LS
Marasmius scorodonius (Fr.) Fr.	Agaricales	Marasmiaceae			x	E	LS
Marasmius siccus Schwein. ex Fr.	Agaricales	Marasmiaceae	x	x		E	LS
Marasmius sp2. Fr.	Agaricales	Marasmiaceae	x	x	x	E	LS
Marasmius sp3. Fr.	Agaricales	Marasmiaceae	x		x	E	LS
Marasmius undatus (Berk.) Fr.	Agaricales	Marasmiaceae	x	x	x	E	LS
Micropsalliota sp. Höhn.	Agaricales	Agaricaceae		x			SS
Mycena griseoviridis A.H. Sm.	Agaricales	Mycenaceae		x	x		LS
Mycena interrupta (Berk.) Sacc.	Agaricales	Mycenaceae			x		LS
Mycena rhenana Maas Geest. & Winterh.	Agaricales	Mycenaceae	x	x	x		LS
Mycena rosea Gramberg	Agaricales	Mycenaceae			x		LS
Mycena sp1. (Pers.) Roussel	Agaricales	Mycenaceae			x		LS
Mycena sp2. (Pers.) Roussel	Agaricales	Mycenaceae		x			LS
Mycena stipata Maas Geest. & Schwöbel	Agaricales	Mycenaceae	x	x	x		LS
Mycena tenerrima (Berk.) Quél.	Agaricales	Mycenaceae		x			LS
Neopaxillus plumbeus Singer & Lodge.	Boletales	Serpulaceae		x	x		SS
Onnia tomentosa (Fr.) P.Karst.	Hymenochaetales	Hymenochaetaceae	x	x			WS
Panaeolina foenisecii (Pers.) Maire	Agaricales	Psathyrellaceae	x	x	x		SS
Panaeolus fimicola (Fr.) Quél.	Agaricales	Psathyrellaceae		x			DS
Panaeolus papilionaceus (Bull.) Quél	Agaricales	Psathyrellaceae		x	x		DS
Panellus mitis (Pers.) Singer	Agaricales	Mycenaceae	x	x			WS
Phaeolus schweinitzii (Fr.) Pat.	Polyporales	Fomitopsidaceae		x	x		WS
Phellinus noxius (Corner) G. Cunn.	Hymenochaetales	Hymenochaetaceae	x	x			PP
Phellinus populicola Niemelä	Hymenochaetales	Hymenochaetaceae			x		PP
Pholiota aurivella (Batsch) P. Kumm.	Agaricales	Strophariaceae		x	x	E	WS
Pleurotus luteoalbus Beeli	Agaricales	Pleurotaceae	x	x	x	E	WS
Pleurotus populinus O.Hilber & O.K.Mill.	Agaricales	Pleurotaceae		x	x	E	WS
Pleurotus pulmonarius (Fr.) Quél.	Agaricales	Pleurotaceae	x	x		E	WS
Pluteus longistriatus (Peck) Peck	Agaricales	Pluteaceae			x		LS
Pluteus mammillatus (Longyear) Minnis, Sundb. & Methven.	Agaricales	Pluteaceae	x				LS
Pluteus umbrosus (Pers.) P. Kumm.	Agaricales	Pluteaceae	x	x	x		LS
Polyporus brumalis (Pers) Fr.	Polyporales	Polyporaceae	x	x	x		WS
Polyporus tenuiculus (P. Beauv.) Fr.	Polyporales	Polyporaceae	x		x		WS
Polyporus varius (Pers.) Fr.	Polyporales	Polyporaceae	x	x	x		WS
Psathyrella candolleana (Fr.) Maire	Agaricales	Psathyrellaceae	x	x	x		WS
Psathyrella corrugis (Pers.) Konrad & Maubl.	Agaricales	Psathyrellaceae	x	x			WS
Psathyrella multipedata (Peck) A.H. Sm.	Agaricales	Psathyrellaceae	x	x	x		WS
Psathyrella gracilis (Fr.) Quél.	Agaricales	Psathyrellaceae	x	x	x		WS
Psathyrella ammophila (Durieu & Lév.) P.D. Orton	Agaricales	Psathyrellaceae	x	x	x		WS
Psathyrella piluliformis (Bull.) P.D.Orton	Agaricales	Psathyrellaceae	x	x	x		WS
Psathyrella sp1. Fr. ex Quél.	Agaricales	Psathyrellaceae		x			WS
Psathyrella sp2. Fr. ex Quél.	Agaricales	Psathyrellaceae	x				WS
Psathyrella sp3. Fr. ex Quél.	Agaricales	Psathyrellaceae	x	x			WS

(*continued on next page*)

Table 2 (*continued*)

Taxa	Order	Family	T	A	B	E	LM
Psathyrella sp4. Fr. ex Quél.	Agaricales	Psathyrellaceae	x				WS
Psathyrella sp5. Fr. ex Quél.	Agaricales	Psathyrellaceae	x	x	x		WS
Psathyrella sp6. Fr. ex Quél.	Agaricales	Psathyrellaceae			x		WS
Pseudoclitocybe cyathiformis (Bull.) Singer	Agaricales	Tricholomataceae	x				LS
Pseudohydnum gelatinosum (Scop.) P.Karst.	Auriculariales	Exidiaceae	x	x	x		WS
Pseudoomphalina pachyphylla (Fr.) Knudsen.	Agaricales	Tricholomataceae	x				LS
Psilocybe ovoideocystidiata Guzmán & Gaines	Agaricales	Strophariaceae	x		x		LS
Psilocybe samuiensis Guzmán, Bandala & J.W.Allen	Agaricales	Strophariaceae		x			LS
Ramaria stricta (Pers.) Quél.	Gomphales	Gomphaceae	x	x	x	E	EM
Rhizopogon luteolus Krombh.	Boletales	Rhizopogonaceae	x	x	x	E	EM
Rhizopogon pseudoroseolus A.H. Sm.	Boletales	Rhizopogonaceae	x			E	EM
Russula gracillima Jul. Schäff.	Russulales	Russulaceae			x		EM
Russula ochroleuca Pers.	Russulales	Russulaceae	x	x	x		EM
Sarcoscypha occidentalis (Schwein.) Sacc.	Pezizales	Sarcoscyphaceae	x	x	x		WS
Scleroderma areolatum Ehrenb.	Boletales	Sclerodermataceae			x		EM
Scleroderma aurantium (L.) Pers.	Boletales	Sclerodermataceae			x		EM
Sebacina concrescens (Schwein.) P. Roberts	Auriculariales	Exidiaceae			x		EM
Skeletocutis carneogrisea A.David	Polyporales	Polyporaceae		x	x		WS
Suillus luteus (L.) Roussel	Boletales	Suillaceae			x	E	EM
Suillus sp. Gray	Boletales	Suillaceae			x		EM
Terfezia leonis (Tul. & C.Tul.) Tul.	Pezizales	Terfeziaceae	x		x	E	EM
Termitomyces clypeatus R.Heim	Agaricales	Lyophyllaceae	x	x	x	E	LS
Termitomyces microcarpus (Berk. & Broome) R. Heim	Agaricales	Lyophyllaceae	x		x	E	LS
Termitomyces robustus (Beeli) R. Heim	Agaricales	Lyophyllaceae	x	x		E	LS
Termitomyces sp. R. Heim	Agaricales	Lyophyllaceae	x	x	x	E	LS
Termitomyces schimperi (Pat.) R.Heim	Agaricales	Lyophyllaceae	x	x	x	E	LS
Trichaptum biforme (Fr.) Ryvarden	Polyporales	Polyporaceae		x			WS
Tricholoma portentosum (Fr.) Quél.	Agaricales	Tricholomataceae			x	E	EM
Tricholoma saponaceum (Fr.) P.Kumm.	Agaricales	Tricholomataceae	x			E	EM
Tricholoma sp. (Fr.) Staude	Agaricales	Tricholomataceae	x			E	EM
Tricholomopsis rutilans (Schaeff.: Fr.) Sing.	Agaricales	Tricholomataceae	x	x	x		WS
Volvariella speciosa (Fr.) P.Kumm.	Agaricales	Pluteaceae	x		x		LS
Wilcoxina mikolae (Chin S. Yang & H.E. Wilcox) Chin S. Yang & Korf	Pezizales	Pyronemataceae	x		x	E	EM
Xeromphalina caulicinalis (Bull.) Kühner & Maire	Agaricales	Mycenaceae	x	x	x		WS
Xeromphalina tenuipes (Schwein.) A.H.Sm.	Agaricales	Mycenaceae	x	x	x		WS
Xerula radicata (Relhan) Dörfelt	Agaricales	Physalacriaceae	x	x			PP
Xylaria hypoxylon (L.) Grev.	Xylariales	Xylariaceae	x				WS
Xylaria scruposa (Fr.) Fr.	Xylariales	Xylariaceae	x		x		WS

Note: Abbreviations: T = the Taragedam forest group; A = the Alemsaga forest group; B = the Banja forest group; x = sporocarp production; E = edible; LM = mode of life; PP = Plant pathogen; EM = ectomycorrhizal, SS = Soil saprotroph, WS = Wood saprotroph, LS = Litter saprotroph, DS = Dung saprotroph.

Table 3
Macrofungal and vascular plant richness and diversity indices in three church forests in Northern Ethiopia.

Forest status	Banja forest	Taragedam forest	Alemsaga forest
All macrofungi			
Richness	22.56 ± 3.02a	18.44 ± 2.34a	22.67 ± 1.84a
Shannon	2.03 ± 0.23a	2.57 ± 0.13a	2.06 ± 0.20a
Simpson	0.73 ± 0.05b	0.88 ± 0.02a	0.77 ± 0.05ab
Evenness	0.38 ± 0.03c	0.60 ± 0.03a	0.47 ± 0.02b
Vascular plants			
Richness	5.78 ± 0.55c	16.89 ± 1.25a	12.67 ± 1.04b
Shannon	1.38 ± 0.12b	2.18 ± 0.08a	2.04 ± 0.07a
Simpson	0.67 ± 0.05b	0.83 ± 0.02a	0.82 ± 0.02a
Evenness	0.73 ± 0.04a	0.55 ± 0.03b	0.63 ± 0.03ab
Ectomycorrhizal fungi			
Richness	3.88 ± 0.64a	2.57 ± 0.3a	2.67 ± 0.21a
Shannon	1.09 ± 0.1a	0.80 ± 0.06a	0.90 ± 0.07a
Simpson	0.61 ± 0.03a	0.53 ± 0.02a	0.57 ± 0.02a
Evenness	0.85 ± 0.05a	0.91 ± 0.05a	0.94 ± 0.03a

Note: Values shown are means ± the SE of the mean. Different lowercase letters indicate a significant difference ($p < 0.05$) in richness or diversity between forests.

forests and classified into 258 fungal taxa (Table 2). Although identification of sporocarps down to species level was not possible, out of the total taxa collected, 155 (60%) were identified to species level, 33 (13%) to genus level and further 69 (27%) were completely unidentified. The unidentified sporocarps were excluded from further analysis. The Basidiomycota was the dominant phylum and was represented by 10 orders, 62 families, 90 genera, and 180 species. Ascomycota was represented by three orders, seven families, seven genera, and eight species (Table 2).

Among the taxa identified, the Agaricaceae was the most diverse family with 58 different taxa, followed by Psathyrellaceae (26), Tricholomataceae (22), and Mycenaceae (20), which together accounted for 33.6% of the total collected taxa (Table 2). The most abundant genera were *Termitomyces*, *Psathyrella*, *Leucoagaricus*, *Marasmius*, and *Mycena*. The proportions of macrofungal taxa at the genus level are provided (Fig. 2A). The Agaricales was the most prevalent order in the three forests (77.66%). Since many Agaricales are conspicuous macrofungi, it is not surprising to find a higher abundance during sampling. The family to genus and genus to species ratios were 0.70 and 0.50, respectively. Total numbers of fungal taxa per family encountered in the three studied forests are provided (Fig. 2B). In terms of the trophic groups, the majority of species were saprophytic (81%) followed by ectomycorrhizal (14%) and parasitic taxa (4%).

The accumulation curves (Fig. 3A) generated for the taxa identified in the three forests show that the saturation of macrofungal richness was not reached during the survey given that the curves showed a steady increase with additional samplings. Although there was no significant difference in species richness between the three forests ($p > 0.05$), the taxa accumulation curve for Banja forest showed a relatively steeper rising slope and yielded higher macrofungal richness values than the other forests. The highest macrofungal diversity values were obtained for Taragedam forest; however, diversity was not significantly different to that of the other two forests (Fig. 3B). The occurrence of macrofungi was more uneven in Banja forest than in the other forests (Table 3), with no fungal species found at all sampling events and certain macrofungal

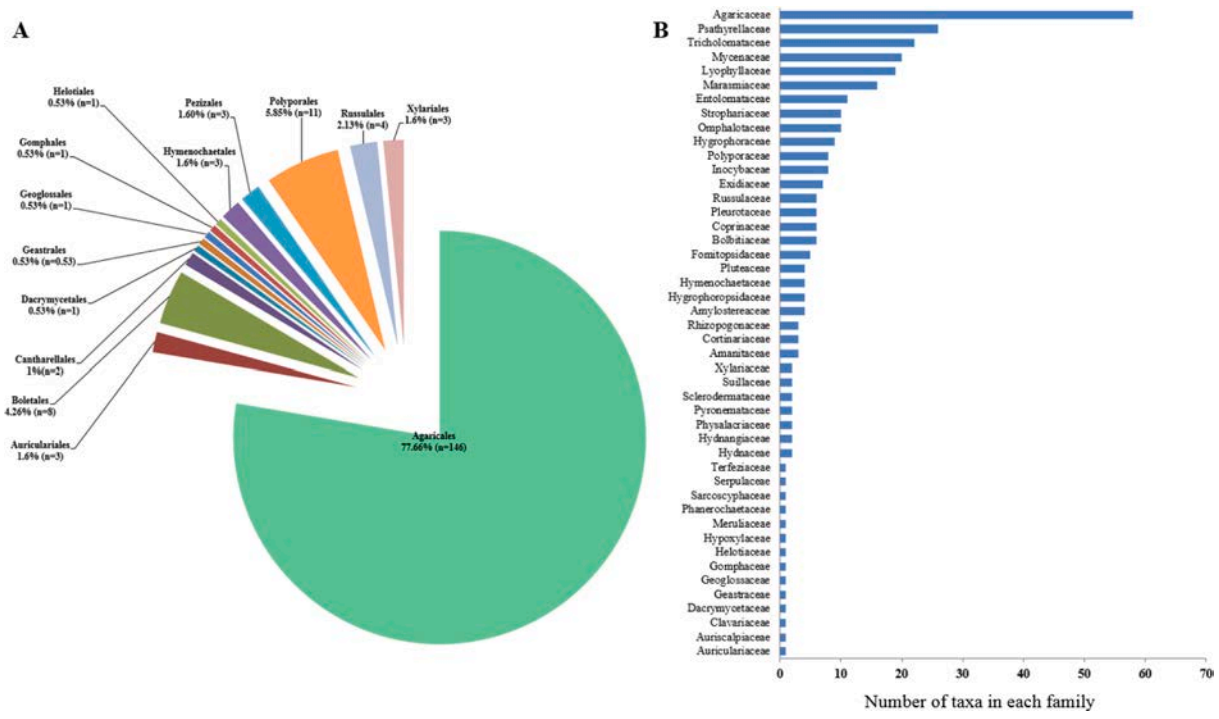

Fig. 2. (A) The proportions of macrofungal taxa at the genus level (name of genus; the number of species; percentage); and (B) total numbers of fungal taxa per family encountered in the three studied forests.

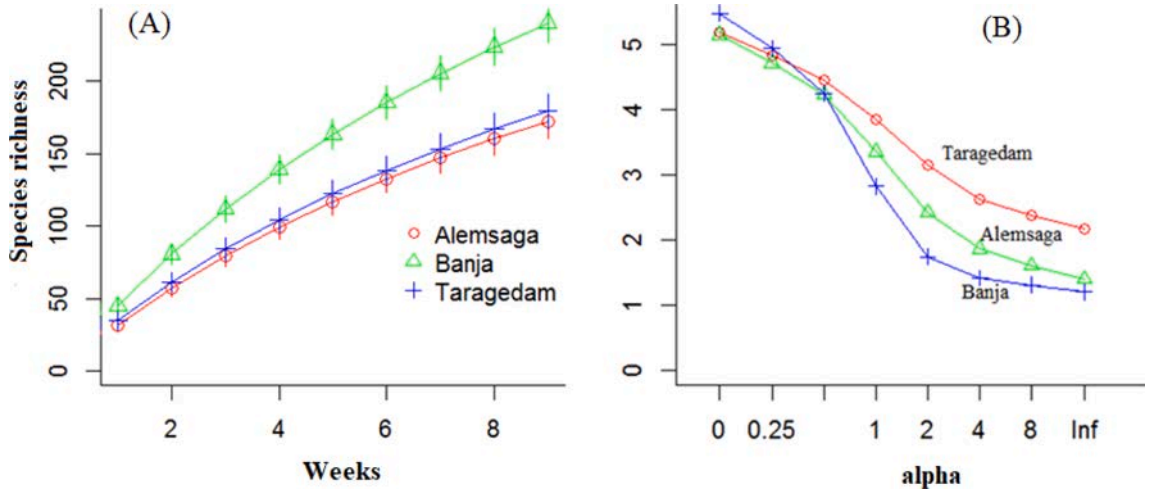

Fig. 3. Taxa accumulation curves generated for the fungal community found in the three studied forests using a rarefaction sample-based estimator (A) and Rényi diversity profiles (B).

species were more dominant in Banja forest than in the other two forests.

The Shannon index and richness for vascular plants were significantly correlated with Shannon and Simpson diversity indices for the fungal communities (Fig. 4). Interestingly, for all these variables, the highest values were found in Taragedam forests and the lowest values were observed in Banja forests (Table 3).

Although the three forests were not significantly different ($p > 0.05$; Table 3) in terms of measure of diversity of their ectomycorrhizal fungal species and richness, more ectomycorrhizal species were collected from Banja forest (20) than from Taragedam (15) or Alemsaga (7) forests (Table 2).

3.2. Sporocarp production

Taragedam forest produced the greatest quantity of sporocarps (25.4 kg ha^{-1}), although production levels were not significantly different ($p = 0.63$) to those of Alemsaga forest (21.6 kg ha^{-1}; Fig. 5). However, both of these forests produced significantly greater quantities of sporocarps than Banja forest ($p < 0.05$).

Sixty eight (36%) of the total macrofungi collected were deemed to be edible (Table 2). Banja forest produced the greatest quantity of edible fungi (mean fresh weight, 1.8 kg ha^{-1}) and Alemsaga forest produced the least (0.4 kg ha^{-1}); however, the production of edible species did not differ significantly among the three forests (Fig. 5; $p = 0.01$).

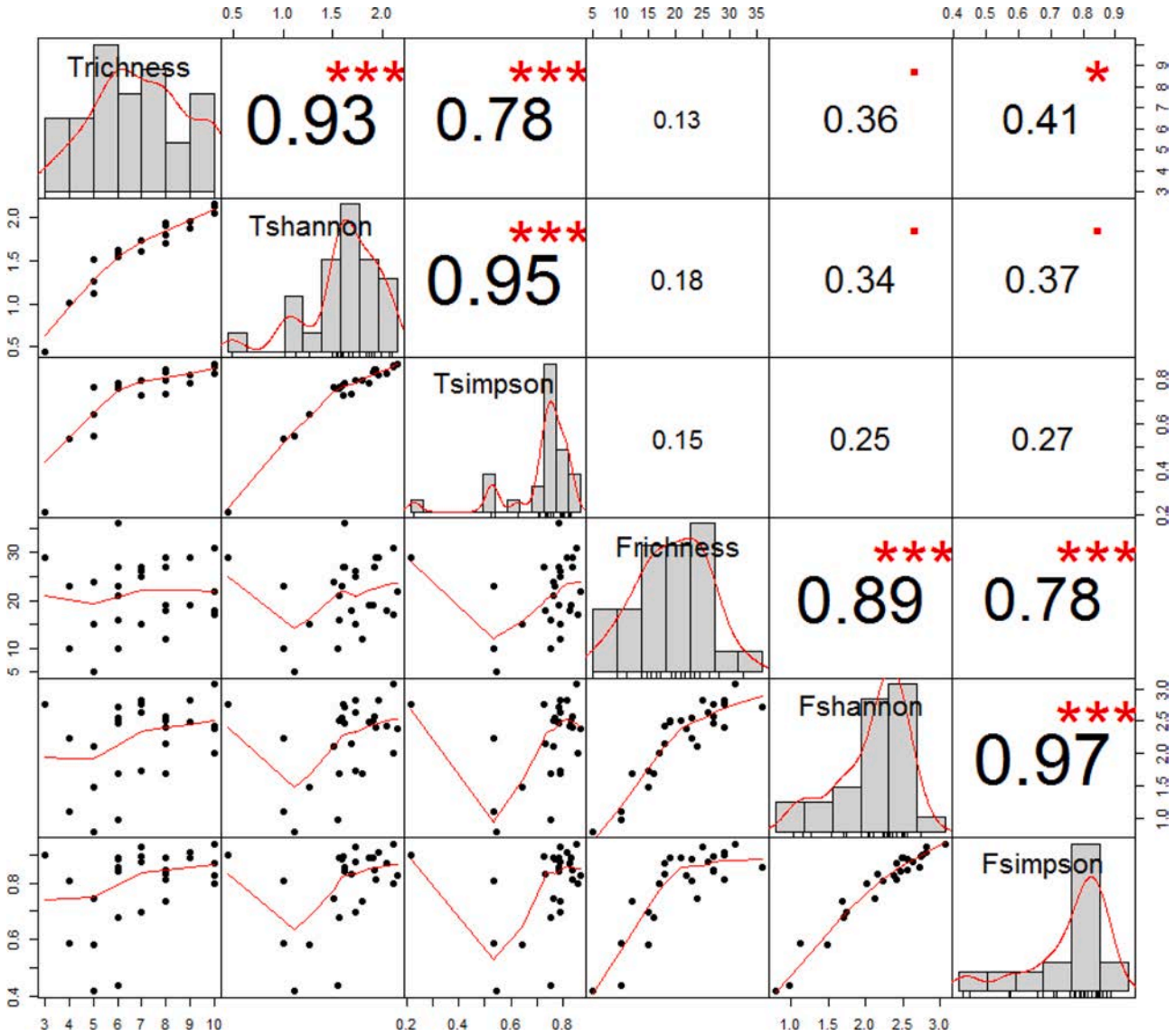

Fig. 4. Scatter plot matrices showing correlation coefficients between the entire tree and macrofungal variables and their significance levels. Abbreviations: T = tree, F = fungi. On the bottom of the diagonal, bi-variate scatter plots with a fitted line are displayed. On the top of the diagonal, the value of the correlation is shown, plus the significance level of the *p*-values, which are indicated by red asterisks. *p-values*: ***, <0.001; **, <0.01; and *, <0.05; *<0.1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Macrofungal communities and edaphic variables

The perMANOVA analyses indicated the three church forests differed significantly in their macrofungal composition ($F = 2.05$, $R^2 = 0.14$, $p = 0.001$; Fig. 6). With respect to the explanatory variables, categorized edaphic, climate and location parameters were correlated to the macrofungal community composition ($p < 0.05$; Table 4). Of these, Mantel test confirmed that location variables aggregately had a strongly significant effect on macrofungal community structure ($p = 0.000$) than that of the climate ($p = 0.009$) and the edaphic variables ($p = 0.112$). The significance of each explanatory variable and their aggregated contribution to the difference of the macrofungal community compositions is provided (Table 4).

The SIMPER analysis also identified macrofungal species that distinguished between the three forests (Table 5). The overall between-group dissimilarity (Sørensen) was 88.73% for Taragedam and Alemsaga forests, 94.44% for Alemsaga and Banja forests, and 93.76% for Taragedam and Banja forests. In this regard, the *Coprinellus* species are found the most important in distinguishing all forest locations along with the others (Table 5). The cumulative contribution of the most influential macrofungal species for the dissimilarity between these forests is shown in Table 5.

4. Discussion

Although fragmentation poses major threats to forest ecosystems, the Dry Afromontane forests in the highland region of Ethiopia, including forest fragments owned by the church or located around church forest territories, are considered to be major reservoirs of biodiversity (Aerts et al., 2016; Aynekulu et al., 2016; Darbyshire et al., 2003; Nyssen et al., 2014). This study provides a comprehensive analysis of macrofungal communities and showed the differences in fungal community compositions of the fragmented forest systems in Northern Ethiopia. The difference in macrofungal species among the three forests might be due to the difference in vegetation composition or the variation in ecological factors such as soils, which are among the most important factors that could affect macrofungal species (Oria-de-Rueda et al., 2010). The availability of suitable substrates due to the difference in plant inputs on

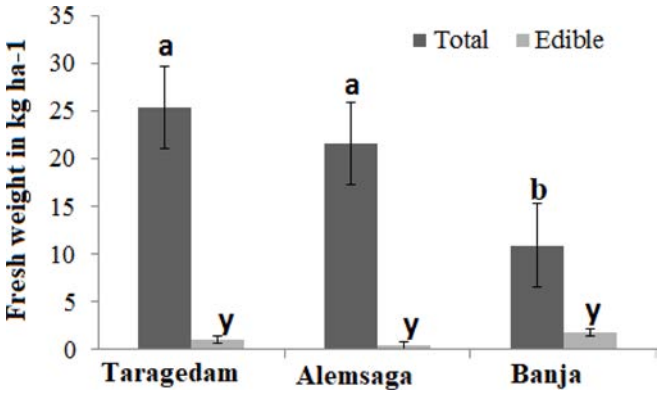

Fig. 5. Fresh weight of sporocarps collected from three forests in Northern Ethiopia. Dark-gray bars indicate the total fungal species collected; light-gray bars indicate edible fungal species. The data shown are means ± the SE of the mean. Values with different lowercase letters are significantly different ($p < 0.05$).

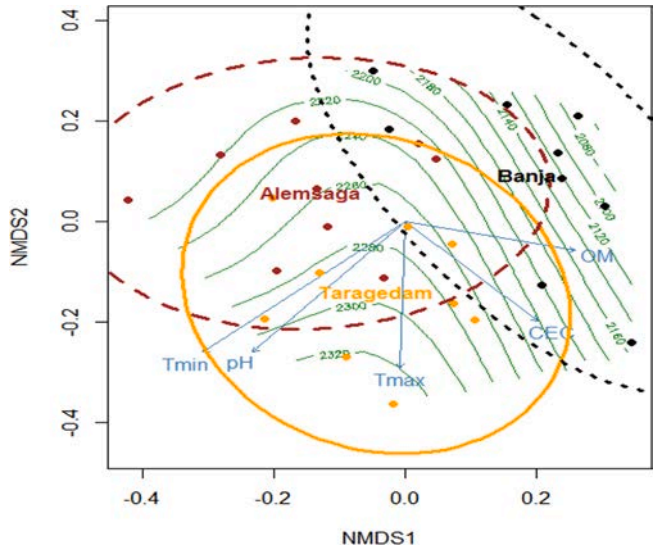

Fig. 6. Non-metric Multidimensional Scaling (NMDS) ordination graph with fitted explanatory variables based on dissimilarities calculated using the Bray–Curtis index of macrofungal communities compositions from plots in the three forests in Northern Ethiopia with altitude displayed as isolines. Arrows represent environmental variables that were most significantly ($p < 0.005$) related to ordination. Ellipses indicate forest groups with the names indicated. The explanatory variables are shown in blue color: CEC, cation exchange capacity; OM, organic matter; Tmax, maximum daily temperature; and Tmin, minimum daily temperature. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 4
Significance of the explanatory variables for macrofungal community compositions. Numbers in bold indicate a highly significant effects ($p < 0.001$).

Sources	Contribution%	Variables	pseudo-F	*p*
Edaphic variables	7.13%	pH	0.4358	**0.004**
		CEC	0.3191	0.010
		OM	0.2767	**0.017**
Climate	14.11%	Tmax	0.3441	0.004
		Tmin	0.6150	**0.001**
Spatial factors	33.92%	Latitude	0.6162	**0.001**

Note: the variables are: CEC, cation exchange capacity; OM, organic matter; Tmax, maximum daily temperature and Tmin, minimum daily temperature.

Table 5
Summary of similarity percentage (SIMPER) results showing the cumulative total contribution (50% cut-off) and the contribution (%) of the most influential species to the dissimilarity between stands in the three forests in Northern Ethiopia.

Species	Individual contribution to the dissimilarity	Cumulative contribution to the dissimilarity	Edibility status
Alemsaga and Banja forests			
Coprinellus disseminatus	13.07	13.07	
Coprinellus micaceus	10.37	23.44	
Coprinellus micaceus	6.49	29.93	
Geastrum triplex	5.35	35.27	
Marasmius guyanensis	4.19	39.46	edible
Psathyrella sp.	3.33	46.31	
Agaricus megalosporus	3.06	49.37	edible
Taragedam and Alemsaga forests			
Coprinellus micaceus	7.42	7.42	
Sarcoscypha occidentalis	6.02	13.44	
Geastrum triplex	5.36	18.80	
Psathyrella sp3.	3.83	30.81	
Psathyrella candollena	3.60	34.41	
Gymnopus dryophilus	3.14	37.55	
Marasmius guyanensis	2.92	40.47	edible
Phellinus noxius	2.74	43.20	
Termitomyces robustus	2.24	45.44	edible
Psathyrella sp.	2.04	47.49	
Marasmius guyanensis	1.71	49.19	edible
Polyporus varius	1.69	50.88	
Taragedam and Banja forests			
Coprinellus disseminatus	13.21	13.21	
Coprinellus micaceus	10.45	23.67	
Sarcoscypha occidentalis	5.68	29.34	
Marasmius guyanensis	3.90	33.24	edible
Geastrum triplex	3.50	40.63	
Agaricus megalosporus	3.08	43.71	edible
Crepidotus mollis	2.37	46.08	edible
Xylaria scruposa	1.96	48.04	
Crepidotus applanatus	1.85	49.89	edible
Psathyrella corrugis	0.93	67.45	
Psathyrella candollena	0.89	68.35	
Psathyrella candolleana	0.87	69.22	
Psathyrella sp.	0.84	70.05	

the forest floor could be also a factor explaining the variation in fungal species composition among the three forests. The retention of plant residues is thought to enhance fungal activity by promoting moisture retention and providing a source of organic carbon, which is important for fungal survival and growth (Blumfield and Xu, 2003). Thus, the differences in substrate richness among these three forests can influence the diversity and richness of macrofungal species (Reverchon et al., 2010). Besides, the variation in macrofungal species among the three forests probably reflect the heterogeneity of these habitats, resulting in

variations in microclimate and, hence, variations in moisture, temperature, and other factors among these different forest systems (Suggitt et al., 2011) that influence the richness and productivity of fungi (Gómez-Hernández and Williams-Linera, 2011). However, the characteristics of the macrofungi themselves could also explain the variation in fungal species among the three forests. Many macrofungal species are believed to fruit spontaneously, with no consistent pattern of occurrence at any time given favorable environmental conditions and suitable substrates (Piepenbring et al., 2012; Tibuhwa et al., 2011). Furthermore, fungal sporocarps are short-lived and may last only a few days before decomposing or being eaten and, therefore, may not have been observed during our weekly surveys (Maurice et al., 2021).

Habitat fragmentation can influence the fungal communities in forests (Sapsford et al., 2017). Lack of symbiotic fungal colonization in these systems may be a limiting factor for seedling establishment, which is the main regeneration ecological process in the studied forests. Thus, trees species more dependent on mycorrhizal fungi could potentially have a substantial decrease in recruitment, particularly in the rehabilitation or conservation scheme of the forests (Tonn and Ibáñez, 2017). Although, recently studies reported the availability of Ectomycorrhizal (ECM) hosts plant from the tropic regions (Tedersoo et al., 2010), the ECM associations has long been considered rare or absent from tropical forest ecosystems (Corrales et al., 2018), particularly of the African forests like that of Ethiopian. A previous study also reported the absence of ECM fungi in the Dry Afromontane forests of Ethiopia (Dejene et al., 2017a). Though the majority of the species collected in this study were saprophytic, about 14% were characterized as ectomycorrhizal. Species from the general of *Amanita, Entoloma, Geastrum, Laccaria, Russula* and *Rhizopogon* were reported from these studied forests. Although the mycorrhizal status of each tree species in the study area are unknown (Table S1), the existence of ECM species may be due to the diverse vegetation (Friis et al., 2010a,b) and, hence, there may be more trees present that can act as hosts for mycorrhizal fungi (Hailemariam et al., 2013; Wubet et al., 2003). Also, the existence of mycorrhizal species in the studied forests can be explained by the dispersion of mycorrhizal inocula from nearby plantation forests that are supposed to host trees. The plantations are constituted by *Eucalyptus camaldulensses*, *Eucalyptus globulus*, *Pinus patula* and other highland *Aacacia* species. Thus, the findings presented here may have important implication for the indigenous forest system for the maintenance of functional fungal diversity in Ethiopia (Dejene et al., 2017a). Besides, the coexistence of mycorrhizal fungi with natural forests has many practical advantages, such as the exchange of water and nutrients through hyphal networks (Brundrett, 2002; Brundrett, 2004). They are also commonly the key determinants of plant population and community dynamics in the forests systems (Tedersoo et al., 2020). This result presents an insight into the conservation of fungal functional groups in the forest system in the study areas as these functional groups are important for the rehabilitation and conservation of these fragmented forests as the fungi, particularly of the ECM, species could potentially have a substantial role in recruitments seedlings (Tonn and Ibáñez, 2017). Thus, further studies on tropical ectomycorrhizae are deeply needed, particularly in Africa where the vegetation resource is immense with significant livelihood and environmental benefits.

In Ethiopia, wild mushrooms have been used for their nutritional and medicinal properties (Abate, 2014; Dejene et al., 2017b; Tuno, 2001). Equally to other wild edibles, they have also been used as a coping food during food shortage periods (Alemu et al., 2012; Sitotaw et al., 2020). In some local markets mushrooms are also available where they are sold by the local people to earn some income to supplement the household economy (Abate, 2014). The sporocarp productions obtained in this study were not high. Although further research is needed to verify the claim, the lower biomass yield reported here could be explained by the single tone species and the species composition. Some of the species were collected in a single time during the collection period. Majority of the species were saprophytic fungi and are characterized by low biomass productions (Gassibe et al., 2011; Mediavilla et al., 2014). However, valuable edible macrofungal species belonging to the *Calvatia*, *Laetiporus*, *Pleurotus*, *Termitomyces* sp., and *Macrolepiota* genera were also collected in this study. Among these edible species, *Termitomyces* sp. is highly regarded by local people in southwest Ethiopia because of its good taste and aroma (Abate, 2014). Although the overall quantity of sporocarp biomass produced in the studied forests was low, the most productive species had biomass values of approximately 0.46 kg $ha^{-1}yr^{-1}$, which provides an insight into the potential production levels of valuable sporocarp species. This also provides a starting point in terms of broadening the management and conservation of fragmented forests for the production of non-timber forests products in Ethiopia. In addition, important ectomycorrhizal species such as *Tricholoma*, *Rhizopogon*, and *Suillus* were also found in this study. The presence of these species in the study areas may be due to the high level of plant diversity in church forests, which may provide ectomycorrhizal fungi with a very broad host range (Roy et al., 2008; Smith and Read, 2008) and, hence, there may be a number of trees that can act as hosts for mycorrhizal fungi (Hailemariam et al., 2013; Wubet et al., 2003). Interestingly, some of the fungi in the genera of *Trichoderma* could also function as biocontrol activity (Vinale et al., 2008) in the forests. In addition, the overall landscape connectivity of exotic tree plantations to nearby fragmented natural forests could also contribute to the presence of ectomycorrhizal fungi in the fungal community assembly (Boeraeve et al., 2018; Peay and Bruns, 2014; Vannette et al., 2016). In these kind of plantations, the local communities in Ethiopia are collecting edible mushrooms, particularly in the Southwest part of the country for their subsistence use or to generate income in some cases (Dejene et al., 2017c). However, this finding may have important implications for indigenous forest systems in terms of the maintenance of valuable macrofungal species for commercial production in Ethiopia (Dejene et al., 2017a). Thus, our survey of macrofungi provides an insight into the valuable fungal functional groups present in the fragmented Dry Afromontane forest system of Ethiopia, which may aid their conservation and management through increasing their economic outputs through NTFPs production in addition to other forests products.

Vascular plants are often used as a surrogate for total biodiversity (Schmit et al., 2005; Sætersdal et al., 2004). Thus, the vascular plants have also been considered a useful indicator of fungal diversity in management programs based on the fact that a species-rich plant community assumed to have more ecological niches or microhabitats available for fungi than a species-poor community (Chiarucci et al., 2005). However, in this study reported a lack of congruence between the species richness of vascular plants and macrofungi in line with Rudolf et al. (2013) who indicted the negative correlation between the two communities regarding species richness. Such correlation might be due to the fact that higher species richness of vascular plants could cause variation of light availability for the ground species, including macrofungi, due to canopy (Härdtle et al., 2003). Thus, the fungal community and their species richness could be influenced by the amount and variation of light availability on the forest floor (Rudolph et al., 2018). The low correlation of species richness of vascular plant and fungi might be due to the fact that the pooled plant species richness not always maximize species richness of other organisms, including all macrofungi (Chiarucci et al., 2005). This is probably because of the special ecological requirements of the fungal that constitute the composition of the community, which are linked to substrate or other factors related to habitats such as edaphic variables (Liang et al., 2015; Rillig et al., 2015). In contrary to this, however, we found an indication of the positive correlation in the Shannon diversity index values of the two communities. Such association could suggests that the tree species identity can be used as a factor for macrofungal diversity (Otsing et al., 2021). Gabel and Gabel (2007) and McMullan-Fisher et al. (2010) also reported positive correlations between plant identities and fungal diversity based on abundance as a measure of diversity. This association is particularly evident for saprotrophic fungi because saprotrophic fungi increase their

community diversity through the provision of wider variety of substrates from the diverse vegetation to establish facilitative interactions in the systems (Gessner et al., 2010; Wu et al., 2019; Zhang et al., 2018), which in turn promote their higher levels of diversity (Ye et al., 2019). The observed correlation of plants and macrofungi diversity indices may suggest the influence of habitat microheterogeneity, causing a positive correlation between both plant diversity and macrofungal diversity (Rudolf et al., 2013). Thus, the promotion of vascular tree plantations in these fragmented forest systems, such as enrichment plantings or assisted natural regeneration systems, should offer suitable habitats with variable microclimates that would influence and/or assist the diversity and productivity of fungal species in the fragmented Dry Afromontane forests of Ethiopia.

Studies demonstrated that fungal community composition can be governed by various environmental variables and landscape heterogeneity (Bahram et al., 2015; Ferrari et al., 2016; Peay et al., 2010; Tedersoo et al., 2014b). Thus, evaluating the fungal communities in different ecosystems is essential to filter out the relative contributions of environmental factors to fungal diversity and composition in an ecosystem (Tian et al., 2018). In this study, the NMDS ordination against the environmental variables is shown distinct macrofungal pattern of the three studied forests. Of the categorized variables, the spatial factors contributed highly for driving the macrofungi assembly together with climate and edaphic variables. This may an indication that the climate, and soil characteristics together are vital in setting spatial variation (Chen et al., 2015), reflecting the combined effects of these variables on the vegetation and thus on macrofungal community (Li et al., 2020). Although the relative degree to which organisms can move is determined by multiple factors, Golan and Anne (2017) indicated that distances as a spatial factor could affect the dispersal of fungal propagules. This could affect the large scale connectively of the different fungal species to form similarity in community structure or morphology (Calhim et al., 2018). However, this needs further investigation to provide an ecological meaningful explanation from our study areas. Conversely, specific fungal species are likely to respond to environmental variables, mainly edaphic parameters, in different ways (Cozzolino et al., 2016; Koide et al., 2014), and, thus, in turn, the composition of the fungal community is directly correlated with edaphic variables (Cozzolino et al., 2016). In particular, pH is known to be the most critical soil characteristic affecting the composition and structure of fungal communities across different continents (Docherty et al., 2015; Fierer and Jackson, 2006; Zhang et al., 2016). Similarly in this study also, soil pH appeared to be correlated with fungal species composition. We found that the presence of greater numbers of macrofungal species was associated with lower pH values. A relatively lower pH values were found in the Alemsaga and Banja forests. This supports the findings of Puangsombat et al. (2010) and Zhang et al. (2016) who reported that higher pH levels negatively influenced fungal community structure, probably because a higher pH restrains the expansion of fungi and the production of sporocarps. However, the species from the Taragedam forests showed exceptional ordination towards a relatively higher end point of the pH gradient. This might be associated with their adaptability of the species to higher pH values in the soil. We also found that CEC and EC are explanatory factors for macrofungal composition. Although the exact role that the CEC and EC play in macrofungal composition and sporocarp production is not fully understood, Crabtree et al. (2010) observed that fungal species richness was low, particularly when the CEC was high. This is probably because the CEC and EC influence nutrient availability, soil pH, and soil reactions to other ameliorants in the soil (Ogeleka et al., 2017). The majority of species in our ordination were directed towards plots with low CEC and EC values. This is probably also because soils with a high CEC are less susceptible to the discharge of base saturation as base saturation is an important factor in the distribution of macrofungal species. Base saturation indicates the proportion of sites occupied by basic cations such as Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} (Zheng et al., 2019). These elements are vital in many physicochemical processes, such as photosynthesis (He et al., 2017) and, thus, can affect plant photosynthesis and, hence, the amount of carbon that is available to fungi in the soil (Shi et al., 2014).

Organic matter also appeared to be an important factor associated with the composition of macrofungi in the studied forests. This is likely because fungi typically extend their mycelia at the soil–litter interface (Boddy et al., 2009) and, thereby, organic matter influences mycelial outgrowth and network formation (Zakaria and Boddy, 2002). Organic matter also influences the fungal community through its impact on the water-holding capacity of soil and nutrient availability (Harrington, 2003). Thus, a high level of organic matter accumulation implies a high level of macrofungal assembly, particularly of saprophytic species. However, the accumulation of organic matter in some cases may also attract the ectomycorrhizal fungi as some of the ECM species can be benefit from organic matter decomposition in a similar manner to free-living saprotrophs; that is, as a source of reduced C compounds to support metabolism (Lindahl and Tunlid, 2015). Nitrogen was also correlated with the composition of fungal species. This finding is in line with those of Kranabetter et al. (2009) and Reverchon et al. (2010), who reported that fungi assembly increased along soil N gradients. This is because nitrogen can influence the formation of mycelium in the soil and play a role in sporocarp formation (Trudell and Edmonds, 2004). Furthermore, many fungal species can adapt to more nitrogen-rich sites (Kranabetter et al., 2009; Toljander et al., 2006). In addition to the edaphic variables, the analysis also showed a significant role of max and minimum temperature on the composition of macrofungal composition. This may be due to the fact that the mycelium of the fungal species is more readily affected by atmospheric changes (Salerni et al., 2002), being more superficial specifically for those saprotrophs species that constitute mainly the community composition of our studied forests. Furthermore, the temperature can play role in nutrient cycling process (Geng et al., 2017). An increase in temperature generally facilitates the decomposition organic matter in the soil and accelerates the availability of nutrients. Thus, the fungal species likely are responding to this condition and form distinct communities, particularly of the fungi that are soil dependet as a substrate (Nicolás et al., 2019).

5. Conclusions

We investigated the diversity and composition pattern of macrofungi in three church forests in Northern Ethiopia to help us to understand the strategies required for the management and conservation of these remnant Dry Afromontane forests and the crucial roles played by fungi in the management and protection of these forest systems. The diversity indexes and community composition of macrofungi in the study areas were influenced by site conditions, including vascular plant diversity and soil fertility gradients. From the analysis on fungi and plant diversity indices, we can see that the species richness of macrofungi is independent of the diversity and richness of vascular plant communities. In our analysis, no correlations were observed for richness, suggesting that richness of vascular plants cannot be used as a proxy for macrofungi richness. However, a positive correlation was found between the two communities for their diversity Shannon index, indicating the tree identity might be used as a factor for macrofungal diversity as there was a highest fungal diversity value in forests with the highest level of tree diversity values. Unsurprisingly, macrofungal communities as a whole were influenced by edaphic variables given that edaphic variables are the main factors affecting mycelial development and, hence, the production of sporocarps by different macrofungal species. Thus, the promotion of vascular tree diversity in fragmented forest systems by enrichment plantings or assisted natural regeneration management systems would offer suitable habitats with variable microclimates that should assist macrofungal species diversity and productivity in the fragmented Dry Afromontane forests of Ethiopia. In addition, the effects of the aforementioned management practices on soil fertility should be taken into consideration owing to the important relationship between

edaphic variables and macrofungal composition in these forests. Forests and sites showed a significant influence in the composition of the fungal communities associated. Therefore, conservation of a higher number of these fragmented forests, can lead to the conservation of a higher fugal richness in an overall landscape scale. Our survey also revealed the presence of valuable edible macrofungal species belonging to the *Tricholoma*, *Suillus*, and *Termitomyces* genera, which could potentially be marketed and, hence, could provide supplementary incomes to forest-dependent local people and forest managers. Thus, we suggest that the production of valuable non-timber forest products such as wild mushrooms should be incorporated into management and conservation strategies for these fragmented forest systems. Moreover, the application of the baseline information provided in this study could assist other countries that are facing similar forest conservation issues due to deforestation and forest fragmentation.

CRediT authorship contribution statement

Demelash Alem: Investigation, Data curation, Writing - original draft. **Tatek Dejene:** Supervision, Investigation, Writing - review & editing. **Juan Andrés Oria-de-Rueda:** Conceptualization, Methodology. **Pablo Martín-Pinto:** Supervision, Conceptualization, Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2021.119391.

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Original article IV

Comunidades fúngicas asociadas a bosques de iglesia remanentes y fragmentados en la región afromontana del norte de Etiopía

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Resumen

Evaluamos las comunidades de hongos del suelo en tres bosques de iglesia utilizando metabarcoding de ADNr ITS2. En total, se identificaron 5152 OTU fúngicas, que representan 16 filos fúngicos. Los saprótrofos seguidos de ectomicorrícicos y patógenos animales dominaron las comunidades de hongos. Se observaron diferencias de diversidad y riqueza entre bosques. Además, el análisis NMDS confirmó que las comunidades de hongos se asocian de manera diferente con cada bosque. Por otra parte, la composición estuvo influenciada por las variables espaciales, climáticas, edáficas y de vegetación. Se identificaron especies indicadoras asociadas a cada bosque. En general, los bosques de iglesias albergan diferentes comunidades fúngicas y los factores ambientales influyen significativamente en la composición de estas comunidades. Así, las estrategias de manejo forestal que consideren factores variaciones micro ambientales ofrecerían hábitats adecuados para conservar y promover la diversidad y la producción fúngica en los sistemas forestales estudiados. La aplicación de la información de referencia proporcionada en este estudio podría ayudar a otros países que enfrentan problemas similares de conservación forestal debido a la deforestación y la fragmentación de sus bosques.

***Palabras clave**: Variables ambientales; Bosques afromontanos; Hongos; Diversidad; Secuencias; ITS2*

Soil fungal communities in fragmented Dry Afromontane Church forests in Northern Ethiopia

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Abstract

We assessed soil fungal communities in three church forests using ITS2 rDNA metabarcoding. In total, 5152 fungal OTUs, representing 16 fungal phyla were identified. Saprotrophs followed by ectomycorrhizal and animal pathogens dominated fungal communities. The diversity and richness difference was observed between forests. Also, NMDS confirmed that fungal communities are associated differently with each forest. Besides, the composition was influenced by the climatic, edaphic, vegetation and spatial variables of the studied church forests. Furthermore, indicator species associated with each forest were identified. Valuable ectomycorrhizal fungi indicator species found more in forests were enrichment plantation conducted. Linear relations were found between basal area and abundance of total fungi and functional guilds. Generally, church forests support diverse fungi and important environmental factors are maintaining the fungi composition. Thus, forest management strategies that consider the cover, tree density, enrichment plantations of host tree species including the environmental factors would offer suitable habitats with variable microclimates for fungi diversity, production, and function in the studied forest systems. The application of the baseline information provided in this study could assist other countries with similar forest conservation issues due to deforestation and forest fragmentation.

Keywords: *Environmental variables, Fragmented forests, Fungi, Diversity, ITS2 sequences*

Introduction

Fungi occupy a wide range of ecological niches (Balami et al., 2020). Forest soil likely represents the utmost reservoir of fungi (Deacon, 2009). According to their functions, fungi play a vital role in many forest ecosystem functions (Balami et al., 2020). The organic matter and plant litter decomposition are conducted mainly by saprotrophic fungi and thereby influencing nutrient cycling (Deacon, 2009; Ruiz-Almenara et al., 2019). Fungi provide soil carbon resources to sustain plant growth in the forest ecosystems (Moore et al., 2004). The fungal symbiotic connections with plants are also helping plant uptake of water and nutrients (Egli, 2011; Hall et al., 2003). As pathogens, soil fungi influence the species diversity, composition, dynamics, and productivity of plant communities (Deacon, 2006; Westover and Bever, 2001).

Furthermore, fungi serve as symbols of conservation and healthful functioning status of most terrestrial ecosystems (Fernandez et al., 2017; Heilmann-Clausen et al., 2015), including the forest ecosystems (Deacon, 2009). Despite these, the vegetation usually sets limits to fungal ranges (Shay et al., 2015; Van Der Heijden et al., 2008). As a habitat, the forests soil environment also impacts the fungal communities (Lauber et al., 2008; Rasche et al., 2011; Richter et al., 2018). These impacts differ with climate, topography, vegetation type, and the magnitude of disturbances in the forests (Day et al., 2019; Kardol et al., 2010; Monkai et al., 2017). Thus, given their role in the ecosystem, it is imperative to study fungi in different ecosystems to gain a rigorous understanding of how fungi response to the ecosystem properties to set up their management and conservation strategies.

The Ethiopian highlands comprise the largest part of the Afromontane regions in Africa (White, 1983), where many biodiversity hotspots are existed (Aynekulu et al., 2016). However, the Afromontane forests are the most fragmented ecosystems, which need to be harmonized by high conservation priority in Ethiopia (Dessie, 2007; Lemenih and Bekele, 2008; Miles et al., 2006; Wassie et al., 2010). These forest fragments have survived because of the cultural or religious values held by local communities, which have contributed to the conservation of the biodiversity of the fragmented forests. Owing to the small size of these forest fragments, there are variations in their biodiversity (Lemenih and Bongers, 2011), as the fragmentation could result in variation in species richness and their composition (Wassie et al., 2010). These modifications might also be shown by the patterns of associated biological resources in several ways, including microhabitats change and habitat isolation (Fernández et al., 2020). Besides, fragmentation results in limitations which affect the population viability in the long-term (Fernández et al., 2020). Several studies have evaluated the conservation value of the fragmented Dry Afromontane church forests in the Northern landscapes of Ethiopia (Aerts et al., 2016; Aynekulu et al., 2016; Nyssen et al., 2014; Wassie et al., 2010). However, no accounts have been given to the ecology and conservation status of the fungi diversity in these fragmented forest systems, which is helpful to facilitate the conservation of economically and ecologically important species in these fragmented forest systems.

Recently, there has been an interest in surveying fungi in particular habitats (Fernández et al., 2020), to describe and predict the extent of their diversity on a larger scale (Danielsen et al., 2005; Peay and Bruns, 2014). This information is important to enable the integration of fragmented Dry Afromontane church forests into global

biodiversity conservation strategies (Aerts et al., 2016; Aynekulu et al., 2016; Hundera et al., 2013) and to understand what actions are required to conserve fragmented forests and their biological components, including fungi, which are known for their exceptionally high diversity levels (Burgess et al., 2006). On the other hand, there is a consensus that fragmentation has an impact on soil properties and the belowground soil organisms (Martínez et al., 2009; Tilman et al., 2002). The variation in the environmental variables such as temperature (Newsham et al., 2016), precipitation (Tedersoo et al., 2014), altitude (Bahram et al., 2012), soil pH (Rousk et al., 2010), nutrient availability (He et al., 2017a), and plant community (Tedersoo et al., 2016b) also influence fungal diversity and the community compositions. However, these impacts on fungal composition in fragmented forest systems in tropical forest areas, like that of the Ethiopian Dry Afromontane forests, are not well understood (Alem et al., 2020; Dejene et al., 2017). Thus, exploring how the environmental variables affect fungal communities may be meaningful for managing fragmented church forests and their ecosystem components (Li et al., 2020), yet information on how habitat fragmentation may affect fungal communities or limit fungal processes is relatively limited (Edman et al., 2004; Grilli et al., 2012; Mangan et al., 2004). Thus, emphasizing the need for study on soil fungal communities which perform a variety of essential functions (Fierer and Jackson, 2006; Jinhong et al., 2017), through identifying the spatial, vegetation, and environmental factors including climate and soil that regulate the distribution and assemblage of fungi is of paramount importance to have a general understanding of processes in ecosystems (Hanson et al., 2012; Hazard et al., 2013). The general objective of this study would be to provide scientific insight into the fungal communities which could promote the conservation of these valuable Dry Afromontane fragmented church forests in Northern Ethiopia. Thus, in this research we evaluated the soil fungal diversity and community composition associated to these relictic ecosystems by sampling of soils from church forests in Northern Ethiopia. Despite fragmentation, church forests are suggested to be rich in plant species diversity (Aerts et al., 2016; Wassie et al., 2010). Fungal diversity is related to vegetation characteristics (Tedersoo et al., 2014a), and so, a previous research based on the sporocarps collected in these forests, reported that the macrofungal sporocarps composition differs among church forests (Alem et al., 2021). In order to have a full picture of the fungal diversity and composition in this kind of forests, this partial knowledge from the taxa that are able to fruit has to be supported and complemented with a deeper analysis of the total fungal community present in the soil. Accordingly, we expect that the soil fungal community differs among church forests resulting in an overall higher richness and diversity value for the entire study sites, as fungi will be

driven also by site conditions such as climate and soil fertility (Glassman et al., 2017; Li et al., 2020; Tedersoo et al., 2014a; Tedersoo et al., 2014b). Thus, our specific aims were to study three church forests in Dry Afromontane areas of Northern Ethiopia: (1) to describe fungal OTUs richness and diversity; and (2) to determine whether and how the soil fungal community composition was governed by the spatial characteristics including vegetation, climate, and soil variability of the studied fragmented church forests.

Methodology

Description of the study areas

This study was conducted on three church forests (Gebeyehu et al., 2019; Masresha et al., 2015; Zegeye et al., 2011) that were selected to evaluate the fungal communities in a set of plots that were randomly distributed in each of the church forest areas, namely the Taragedam forest located in Libokemkem district, the Alemsaga forest located in Farta district, and the Banja forest located in Banja district of the Amhara region, Northern Ethiopia. These forests are fragments of the remnant Dry Afromontane forests in Northern Ethiopia (Gebeyehu et al., 2019; Masresha et al., 2015; Zegeye et al., 2011). The Taragedam and the Banja forests were designated as reserve forests in 1979 (Zegeye et al., 2011) and 1994 (Abere et al., 2017), respectively, to prevent any kind of encroachments. The Alemsaga forest was designated as a priority forest in 1978 to serve as a seed source, to conserve the remnant natural forest, and to rehabilitate the degraded area in the Northern part of the country (Masresha et al., 2015). Comprehensive descriptions of the forests are provided in Table 1.

Soil sampling for molecular and edaphic analysis

Within each of the church forests, nine 2 × 50 m plots were established systematically about 500 m apart in 2019. The plots were laid out randomly in the forests to avoid confounding spatial effects inherent to such a plot-based design (Hiiesalu et al., 2017; Rudolph et al., 2018). Then, the plots were analyzed as independent samples (Ruiz-Almenara et al., 2019). In each plots, soil sample of five cores of 5 m apart were extracted along the centerline of each plot using a cylindrical soil borer (2 cm radius, 20 cm deep, and 250 cm^3) (De la Varga et al., 2012) to collect samples with spatial variability while minimizing the likelihood of repeatedly sampling the same genet. Litters and twigs were removed from the surface before soil cores were taken (Voříšková and Baldrian, 2013). The entire cores from each transect were pooled to produce a

composite soil sample of each plot established in each forest. The samples were transported to the laboratory in sterile plastic bags and stored at 4 °C. Next, the samples were dried, sieved through a 1-mm mesh, and then ground to a fine powder using a mortar and pestle. Each sample was subjected to genomic DNA analysis, which was performed using 100 g of soil, and chemical analysis, which was performed using two 20 g samples of each soil sample,

Standard extraction methods (i.e., diethylene triamine pentaacetic acid extraction, KH2PO4 extraction, Olsen, Kjeldahl digestion Walkley Black, and ammonium acetate) and instrumental analysis were used to determine the pH, organic matter, cation exchange capacity, sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), N, available phosphorus (P), and the physical properties (% of sand, silt, and clay) of the soil. The soil analysis was conducted by Amhara Design and Supervision Works Enterprises, Laboratory at Bahir Dar, Ethiopia. Results of some of the soil variable analyses from each of the church forests are provided in Table 1. In addition to soil, to obtain vegetation descriptive variables from each 20 x 50 m plots, we inventory the number of vascular plants and shrubs were conducted in the entire plots. Big trees outside the plots but their canopy within the sample plots were also included in the survey as the big trees could create their microhabitat and provide a large root system to provide more space for fungal association (Schön et al., 2018). Vascular plant species richness and diversity parameters were determined and used for further interpretation of fungal community composition. Also, Basal Area (BA) was calculated from the vegetation inventory data for each plot.

Table 1: Comprehensive descriptions and selected edaphic variables of the three church forests, Northern Ethiopia

Descriptions	Forests		
	Taragedam	Alemsaga	Banja
Geographical location	12°06'–12°07' N 37°46'– 37°47' E	11°54'–11°56'N 37°55'–37°57'E	10°57'–11° 03'N 36°39'–36°48'E
Altitude range (m asl)	2142–2484	2180–2470	1870–2570
Mean annual precipitation (mm)	1098	1926	1884.3
Mean annual temperature (°C)	19.5	15.8	18.7
CRF30D (mm)	1488	1926	1884
Forest area (ha)	875	814	897
Density of trees ha^{-1}	48.11	17.19	43.13
Canopy cover (%)	80a	82a	67b
Basal area m2	4.95a	1.72b	4.36a
Shrubs density ha^{-1}	146a	747b	2881b
Tree Shannon values	2.18a	2.04a	1.38b

Sand (%)	58.89±(2.93)b	51.78±(2.99)b	68.67±(2.21)a
Silt (%)	28.44±(2.38)a	32.44±(2.13)a	20.00±(1.76)b
Clay (%)	12.67±(1.37)a	15.78±(1.93)a	11.33±(1.33)a
pH H2O 1:2.5	7.04±(7.03)a	5.85±(6.59)b	5.60±(6.24)c
EC (dS/m)	0.43±(0.05)b	0.28±(0.03)b	0.81±(0.14)a
Ex.Ca (cmol(+)/kg)	13.95±(0.60)a	9.19±(0.52)b	13.55±(0.87)a
Ex.Mg (cmol(+)/kg)	6.16±(0.10)a	4.58±(0.15)c	5.54±(0.20)b
Ex.Na (cmol(+)/kg)	1.95±(0.05)a	2.05±(0.10)a	1.82±(0.12)a
Ex.K (cmol(+)/kg)	0.73±(0.06)a	0.61±(0.04)a	0.77±(0.06)a
CEC (cmol(+)/kg)	47.21±(1.36)a	34.89±(0.92)b	44.51±(1.96)a
Organic matter (%)	4.46(0.60)a	3.35(1.34)b	4.87(0.10)a
Nitrogen (%)	0.23±(0.01)a	0.17±(0.02)b	0.26±(0.01)a
P (ppm)	17.18±(5.72)a	7.8±(0.73)b	17.64±(6.05)a
Dominant species in each plots	*Maytenus obscura* *Carissa edulis* *Olea* sp.	*Acacia abyssinic* *Buddleja polystachya* *Acacia nilotica*	*Albizia gummifera* *Prunus africana* *Brucea antidysenterica*
References	Gedefaw and Soromessa (2014) Zegeye et al. (2011) Zerihun et al. (2013)	Birhane et al. (2017) Masresha et al. (2015) Wubet et al. (2004)	Abere et al. (2017)

Note: Values shown are means; standard errors of the means are indicated in parentheses. Values with different lowercase letters are significantly different ($p < 0.05$). The mean annual precipitation and mean annual temperature are given based on nearby stations data of each study area by the year 2019. Abbreviations: EC, electrical conductivity; CEC, cation exchange capacity; m, meter; mm, millimeter; asl, above sea level; CRF30D: cumulative rainfall 30 days before sampling. The references listed are related to the climatic and geographical descriptions of the study areas. On the other hand, in order to relate the vegetation characteristics to the fungal diversity, vegetation inventories were conducted. Then, the vegetation parameters were determined (Table 1). Plant parameters and their correlations with fungi community were also used for further interpretation of soil fungal pattern from each study areas.

Molecular analysis

A PowerSoil™ DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) was used to extract DNA from 0.25 g of soil per sample. PCR reactions of each sample were carried out in triplicate to minimize PCR biases. PCR reactions were performed in 20 µL reaction volumes containing 11.22 µL of Modified Quantization (MQ) water, 1.60 µL of DNA template, 2.00 µL of 10× buffer, 1.40 µL of MgCl2 (50 mM), 1.60 µL of dNTPs (10 mM), 0.50 µL of Bovine Serum Albumin (BSA) (2%), 0.80 µL of reverse and forward primers (10 µM), and 0.08 µL of Platinum Taq polymerase (Invitrogen, Carlsbad, CA, USA). The following PCR conditions were used: an initial denaturation step at 94 °C for 3 min; then 35 cycles of 94 °C for 45 s, 50 °C for 1 min, and 72 °C for

1.5 min; ending with one cycle of 72 °C for 10 min. To amplify the ITS2 rDNA region, we used the forward primer fITS7 (Ihrmark et al., 2012) and the barcoded reverse primer ITS4 (White et al., 1990). Sample-specific Multiplex Identification DNA-tags were used to label the ITS4 primer. Each set of PCR replicates also included a negative control comprising MQ water instead of DNA that underwent PCR under the same experimental conditions and was shown to be amplicon-free on a gel. The amplicon library was sequenced at BaseClear B.V. (Leiden, The Netherlands) with paired-end Illumina MiSeq platform.

Bioinformatic analysis

Primers and poor-quality ends in both directions (3´and 5´) were removed based on a 0.02 error probability limit in Cutadapt (Martin, 2011). Next, all sequences were truncated to 200 bp and then filtered with USEARCH v.8.0 (Edgar, 2010) to discard sequences with an expected error of >1. The remaining sequences were collapsed into unique sequence types on a per-sample basis using USEARCH v.8.0 (Edgar, 2010) while preserving read counts. First, we discarded singleton sequence types before grouping the remaining 105,840 high-quality sequences into 5152 operational taxonomic units (OTUs) with USEARCH at a 97% sequence similarity level while simultaneously excluding sequences representing OTUs with < 70% similarity or < 150 bp pairwise alignment length to a fungal sequence. Sequences were assigned to taxonomic groups based on pairwise similarity searches against the curated UNITE+INSD fungal ITS sequence database of version v.8.0 released on November 18th, 2018, which contains identified fungal sequences with assignments to species hypothesis groups (Kõljalg et al., 2013). Ecological functions at the genus level were assigned using the recent classification published by Põlme et al. (2020). OTUs with > 90% similarities to a fungal species hypothesis with known ecological function were assigned either to plant pathogens, animal parasites, ECM fungi, arbuscular mycorrhizal, saprotrophs functional, and other groups. For genera that are known to comprise species from multiple functional guilds, their ecological function was assigned individually based on available ecological information for the matching SH in the UNITE database. List of OTUs is provided as supplementary data to this article (Table S1).

Statistical analysis

Shannon's H' diversity index, H' = –Σpi (lnpi) (Shannon and Weaver, 1949), was estimated for each forest, where pi indicates the relative abundance of fungal species (Kent and Coker, 1993). The Simpson's diversity, D = 1–Σ(pi2), where pi is the importance probability in element i; and the Evenness, J = H´/H´max, where H´ is the number derived from the Shannon diversity index and the H´ max is the maximum possible value of H´ were also calculated (Magurran, 1988). Also, the richness values for each forest were estimated. All diversity measures were analyzed using the Biodiversity R package (Kindt and Coe, 2005) in R version 4.0.3 (R Core Team, 2020).

The difference in the soil, vegetation and fungal variables across forests were assessed by Linear Mixed Effects models (LME, Pinheiro et al., 2016), where block (plots in each forests area) was defined as random and forest was defined as fixed factor. The LME used to prevent the false positive associations due relatedness structure in the sampling. Tukey Test was later used to check significant differences ($p \leq 0.05$) between forests when needed. The relation between fungi and vegetation attributes were determined through correlation analysis whenever needed. The variation of fungi functional groups were analyzed separately. All data were transformed when needed to achieve the parametric criteria of normality and homoscedasticity.

The species accumulation curves were constructed to provide an estimate of soil fungal species richness in each forest. The curves were generated using EstimateS Version 9 (Colwell, 2013), based on total fungal OTUs data sets with 1000 permutations. The Rényi diversity profile (Tóthmérész, 1995), was also used to depict the diversity curves of the three church forests. It depends upon a parameter alpha, such that for alpha= 0, this function gives the total species number and alpha= 1 gives an index proportional to the Shannon index. To analyze correlations between Tree and Fungal parameters, we performed correlation matrix based on Pearson Test using Hmisc package. Cormat and pmat functions were also used to obtain and organize the results.

The relationship of soil fungal composition with the edaphic and vegetation parameters was visualized using non-metric multidimensional scaling (NMDS), based on Hellinger transformed OTUs matrix and environmental scaled data. The effects of forest were analyzed using permutational multivariate ANOVA (PerMANOVA) based on 999 permutations with the adonis function in package vegan. A one-way crossed analysis of similarities (ANOSIMs) was also performed to assess the significance of the differences among the groups observed in the NMDS plots. The isolines of the vascular

plants richness were also plotted on the NMDS ordinations using the ordisurf function. The strength of the difference between forests was measured by the R values generated by ANOSIM which may take a value from 0 to 1, with 1 being the strongest possible difference (Clarke et al., 2014). Permutation tests using 999 replicates established the statistical significance (P) of the R values. The nonparametric test analysis was used because of the large number of zeros in the data set. The analysis was conducted using PAST software (Hammer et al., 2001). Effects of edaphic variables on soil fungi community composition were determined based on Bray-Curtis dissimilarity after excluding single tone OTUs. The correlation of NMDS axes scores with explanatory variables was assessed using envfit function in R. To test the influence of grouping categories from the edaphic, climate, location and vegetation variables on the fungal community, we used Mantel Test using Bray-Curtis distance on total species matrix and Euclidean distance on environmental parameters. We determined any preferences of individual OTUs for specific forests using indicator species analyses (Dufrên M, 1997). The indicator species analysis was carried out using the interspecies package (Cáceres and Legendre, 2009) to determine the indicator fungal species for the three forest types.

Results

Fungal OTUs taxonomic composition

A total of 105,840 passed quality filtering, representing 5152 fungal OTUs and 16 fungal phyla. Overall, Ascomycota dominated the fungal community followed by the Basidiomycota, both accounting for 71.48% of the sequences. We found significant differences in the relative proportion of Ascomycota between forests ($p < 0.05$). The highest proportion was found in the Taragedam church forest (42.30%). This value was significantly higher than that of the Alemsaga ($p = 0.0002$) and the Banja forests ($p = 0.006$). The lowest proportion was found in the Alemsaga forest (23.51%), which was significantly different when compared with that of the Banja forest (34.19%; $p = 0.001$). The Taragedam and the Banja forests were not significantly different in their proportion of Basidiomycota ($p = 0.144$). However, the Alemsaga forest showed a significantly lower proportion (21.41%) when compared with the Taragedam (42.75%; $p = 0.0002$) and the Banja forest (35.84%; $p = 0.002$).

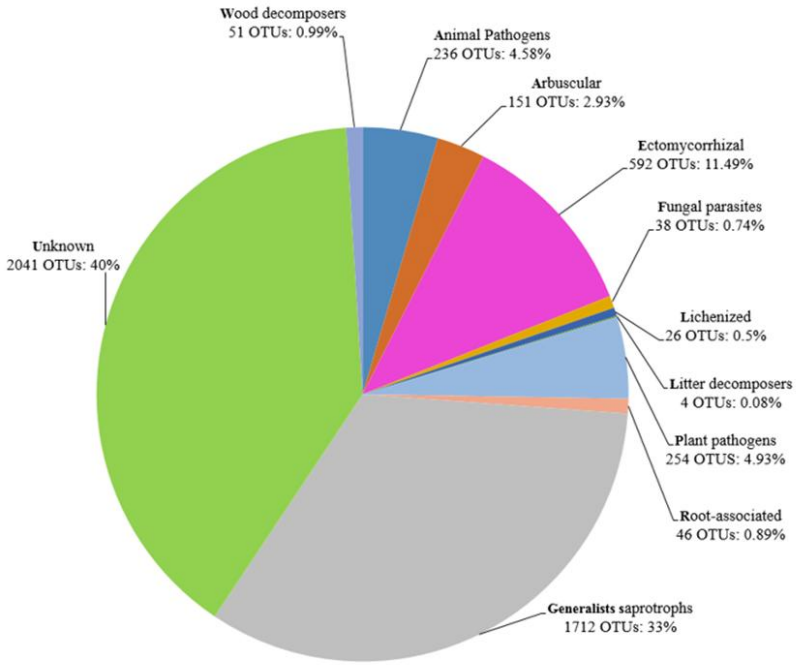

Figure 1: Relative proportions of fungal operational taxonomic units (OTUs) according to their guild assignment at the genus level (ecological function; the number of OTU; percentage).

The ranking of taxonomic orders in Ascomycota, based on the number of representative OTUs, was as follows Hypocreales (375), Pleosporales (241), Chaetothyriales (230), Eurotiales (179), Helotiales (123), Sordariales (109) followed by many other orders with less than 100 fungal OTUs (Table S1). In Basidiomycota, Agaricales (524) and Thelephorales (353) were the most species-rich order followed by other orders with less than 70 OTUs each. Unidentified fungi were classified down to kingdom level and represented about 780 OTUs; 15.14 % of the total. Also, there was a significant difference in number of OTUs identified at a genus level between forests ($p < 0.000$). The highest number was found in the Taragedam forest (42.05%), which was significantly higher than that of the Banja forests (30.77%) and the Alemsaga (27.17%) forests. Several species in the genera of *Agaricus*, *Boletus*, *Geastrum*, *Lepiota*, *Psathyrella*, *Russula*, *Termitomyces*, *Tomentella* and *Trichoderma* were also identified in this study at least 98% similarity with the reference sequences. Surprisingly some of them are ECM, despite the studied forest are considered non ECM ecosystems. The fungal OTUs describing all known taxonomic phyla, orders and genus are provided in Table S1.

The fungal OTUs were further assigned to ecological guilds (Figure 1). Overall, saprotrophs were the most abundant across the whole dataset, representing 33.24% of the community. The dominance of these groups was followed by ectomycorrhizal fungi (11.49%), animal pathogens (4.58%), plant pathogens (4.93%), and arbuscular mycorrhizal (2.93%). Less dominant groups were represented by wood and litter decomposers, root-associated fungi, lichenized fungi, and fungal parasites. About 40% (2041) of the fungal OTUs were not assigned to an ecological guild (Figure 1B). When considered the relative proportions of dominant ecological guilds separately, saprotrophs (generalists) and animal pathogens showed a significant difference between the forests ($p < 0.05$; Figure 3). Whereas, the relative proportion of ectomycorrhizal, arbuscular mycorrhizal, and plant pathogens were significantly lower in the Alemsaga forest ($p < 0.01$), while these ecological guilds showed no significant difference between the Taragedam and the Banja forests ($p > 0.05$; Figure 2).

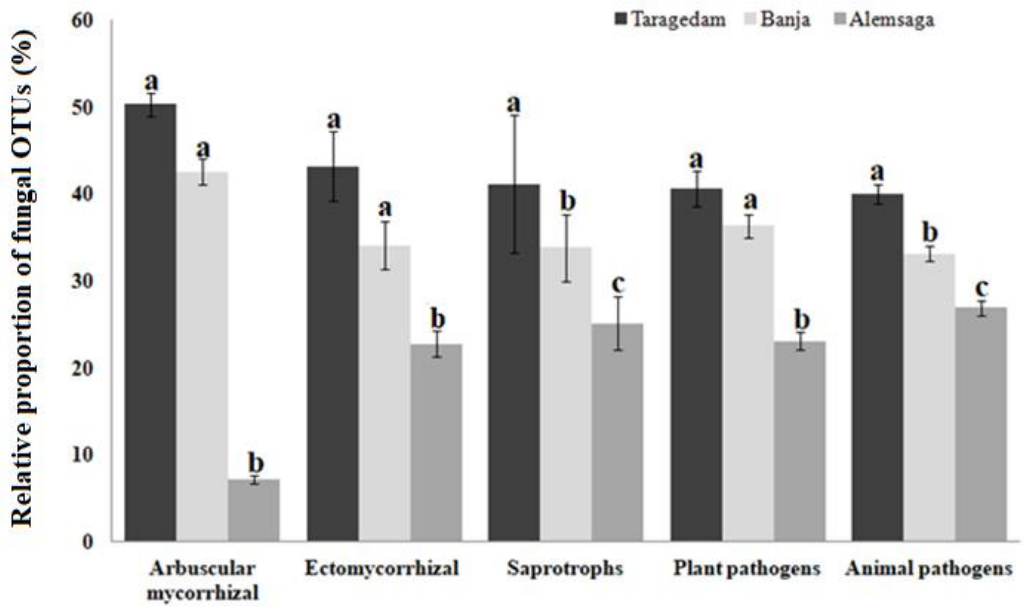

Figure 2: The relative distribution of fungal operational taxonomic units (OTUs) among the three studied Dry Afromontane church forests for each main functional group. Values with the same letter in a group are not significantly different. Bars denote standard deviation.

Soil fungal richness and diversities

The overall accumulation curves of the three forests showed saturation of fungal richness was not reached. The curves showing a steady increase even after data

extrapolation (Figure 3A). However, a significant difference in overall OTUs richness was observed among the three Dry Afromontane church forests. The highest richness is for the Taragedam forests, and this value was significantly different from those of the Banja (p = 0.010) and the Alemsaga forests (p = 0.000). The fungal OTUs richness was also significantly different between the Banja and the Alemsaga forests (p = 0.000). As in richness, fungal Shannon's H' diversity also showed a distinct trend among forests. The highest value (5.31) was recorded at the Taragedam forest followed by the Banja (2.37) and Alemsaga forests (4.73), indicating that the Alemsaga forest was significantly different from the Taragedam (p = 0.001) and the Banja forests (p = 0.010). The diversity profiles of the Taragedam and Banja forests are more asymptotes and are not different in their diversity values (Figure 3B; p = 0.182).

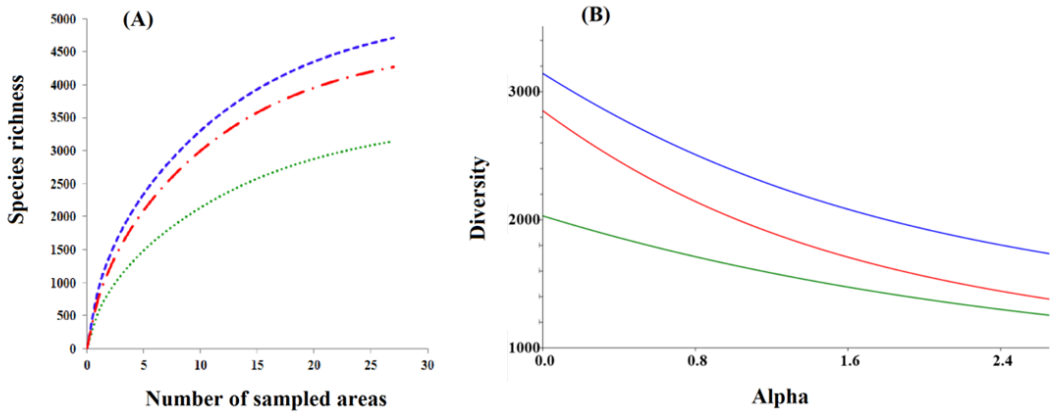

Figure 3: Patterns of fungal species recorded in the three Dry Afromontane forests in Northern Ethiopia. (A) Observed species accumulation curves across the fragmented forests using the rarefaction sample-based estimator of EstimateS. Data were extrapolated following procedures proposed in the EstimateS manual (Colwell 2013) and (B) Rényi diversity profiles with log-transformed fungal OTUs abundant data. Plots in similar colors are in a group: Blue lines: the Taragedam forest, Red lines: the Banja forest, and Green lines: Alemsaga forest.

Significant differences were found between the three forests when comparing their tree parameters such as basal area, tree canopy cover values etc. (Table 1). In addition to these differences, when considering the functional guilds, linear relations were found between basal area and abundance of total fungi (r = 0.42, p< 0.05), Arbuscular mycorrhizal (r = 0.50, p<0.01), ectomycorrhizal (r = 0.04, p< 0.05). Similarly, the Shannon diversity value of the total fugal species, animal pathogen species,

ectomycorrhizal species, and fungal parasites were also significantly correlated with tree canopy cover ($p< 0.05$). Also, the tree canopy cover correlated with the richness of the total fungi, richness of animal pathogen, and richness of ectomycorrhizal species ($p< 0.05$). The significant and the correlation values of the fungi and vegetation parameters are provided in the Table 2.

Table 2: Correlation of tree and fungi variables plus the corresponding significance *p*-values.

Tree parameters	**Fungi parameters**	**r**	***p*-values**
Tree basal area	Abundance of total fungi species	0.42	<0.05
	Abundance of Arbuscular mycorrhizal species	0.50	<0.01
	Abundance of ectomycorrhizal species	0.40	<0.05
Tree canopy cover	Shannon diversity value of total fungi species	0.46	<0.05
	Shannon diversity value of animal pathogen	0.42	<0.05
	Shannon diversity value of ectomycorrhizal	0.40	<0.05
	Shannon diversity value of fungal parasite species	0.38	<0.05
	Richness of total fungi species	0.40	<0.05
	Richness of animal pathogen species	0.45	<0.05
	Richness of ectomycorrhizal species	0.39	<0.05

Soil fungal composition and environmental variables

The NMDS based on Bray Curtis distance followed by the perMANOVA analyses confirmed that soil fungal communities differed among the three fragmented church forests ($F = 2.64$, $R^2 = 0.19$, $p = 0.010$). However, the pattern showed that the Alemsaga forest is more distinct than the other two forests than they are to each other (Figure 4). The ANOSIM pair-wise comparisons measuring the strength of the differences in fungal composition between the three church forests are provided (Table 2).

Table 2: ANOSIM pair-wise comparisons of soil fungal composition between the three fragmented forests based on Bray-Curtis distance measures (Global R-value = 0.75; $p = 0.001$).

Pair-wise comparison between	R values	*P*

forests		
Alemsaga and Banja forests	0.86	0.0002
Alemsaga and Taragedam forests	0.79	0.0001
Banja and Taragedam forests	0.58	0.0001

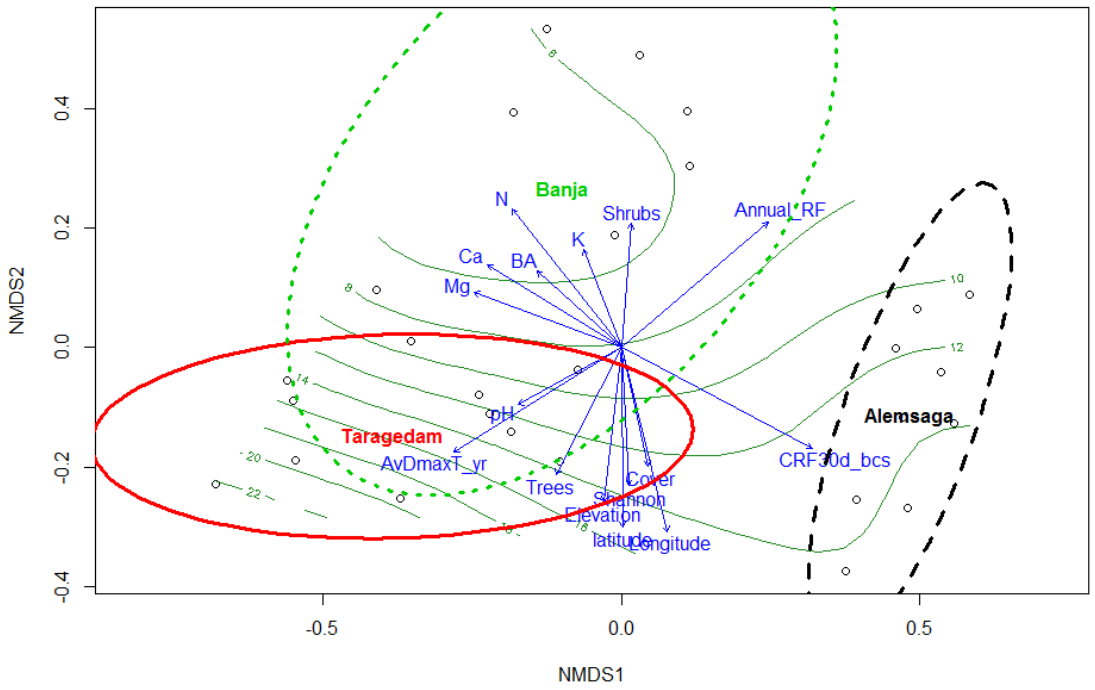

Figure 4: Non-metric Multidimensional Scaling (NMDS) ordination graph with fitted environmental variables based on dissimilarities calculated using the Bray–Curtis index of fungal communities compositions of the three Dry Afromontane church forests in Northern Ethiopia with vascular trees richness displayed as isolines. Arrows represent environmental variables that were most significantly ($p \leq 0.005$) related to ordination. Ellipses indicate forest groups with the names indicated. The explanatory variables are shown in blue color. CRF30b_bcs: cumulative rainfall 30 days before sampling.

Considering the influence of environmental variation, explanatory environmental variables grouped under the spatial, climate, vegetation, and edaphic parameters were also significantly correlated to the soil fungal community composition ($p < 0.050$; Figure 4; Table 3). Specifically, the individual spatial and climatic parameters showed a highly significant influence on soil fungi composition of the church forests (Table 3). Similarly, among the vegetation and soil parameters, the tree density and nitrogen parameters influenced highly the fungal community structures respectively. Isolines on the NMDS

ordination (Figure 4) represent vascular plant richness values across the three studied forests.

Table 3: Significance of the explanatory variables for soil fungal community compositions based on the Hellinger transformed matrix. Numbers in bold indicate a highly significant effects ($p < 0.001$). Grouped contribution is showed according Mantel Test.

Sources	Contribution	Variables	pseudo-F	*p*
Soil fertility	30.16%	pH	0.2736	0.021
		Ca	0.4626	0.002
		Mg	0.4787	0.002
		K	0.2332	0.047
		N	0.5459	**0.001**
Climate	62.87%	Annual rainfall	0.7019	**0.001**
		CRF30d_bcs	0.8736	**0.001**
		Average daily max temperature	0.7448	**0.001**
Spatial factors	6.17%	Elevation	0.4110	0.002
		Latitude	0.5126	**0.001**
		Longitude	0.5805	**0.001**
Vegetation	16.91%	Tree density	0.3887	**0.001**
		Shrubs density	0.3027	0.004
		Cover	0.2899	0.011
		Basal area	0.2319	0.046
		Tree diversity	0.3149	0.007

Indicator species generally define a trait or characteristics of an environment. Our analysis of the indicator species identified fungal OTUs that are associated with each of the forests. There were 566 significant ($p < 0.05$) indicators of fungal OTUs across the three forests (Table S2). Of these, 58 OTUs were indicators for the Banja forest, 184 OTUs were indicators for the Alemsaga forest, and 324 OTUs were indicators for the Taragedam forests. In all the three forests, the saprophytic fungi are the dominant indicator species. However, different ectomycorrhizal species in the genera of *Byssocorticium*, *Piloderma* and *Pseudotomentella* were also identified charactering the Alemsaga forests while *Russula* and *Sebacina* were indicators for the Banja forest. Finally, *Boletus* and *Tylospora* were specific for the Taragedam forests in this study at least 98% similarity with the reference sequences. Several other ectomycorrhizal species in the genera of *Cortinarius* and *Tomentella* were commonly characterizing the three forests (Table S2).

Discussion

In highland regions of Northern Ethiopia, fragmentation created forest patches of Dry Afromontane forests around churches and inaccessible mountain areas (Wassie et al., 2005). These fragmented forests are a reservoir of biodiversity (Aerts et al., 2016; Aynekulu et al., 2016; Darbyshire et al., 2003; Nyssen et al., 2014). However, the management of such kind of forests requires a quantitative and subjective assessment and description of their biodiversity components (Purvis and Hector, 2000). In particular, the knowledge of fungi is vital as fungi are continuously interacting with the plants as pathogen, mutualism, or are involved in the recycling of organic matter (Tedersoo et al., 2014; Wagg et al., 2014). In this study, the Ascomycota was the most taxa-rich (2332 OTUs) phylum, indicating its dominance in the studied forests with observed differences between forests. The higher number of the Ascomycota was not a surprising, as different studies have also reported the dominance of Ascomycota taxa from different forest ecosystems (Geml et al., 2014; Reazin et al., 2016; Smith et al., 2017; Tedersoo et al., 2014). This maybe because majorities of the fungi in the Ascomycota have a higher genomic potential for resource utilization, competition, and stress tolerance (Egidi et al., 2019). The other possible explanation for the higher Ascomycota fungi in the studied frosts is the larger amounts of organic materials material. This is because the saprotrophs from the Ascomycota phylum are decomposers, thereby promoting nutrient availability to plants (Egidi et al., 2019; Niklaus et al., 2001) and sources of organic carbon in the forests system (Ma et al., 2013; Voříšková and Baldrian, 2013). This may also confirm particularly the importance of vegetation in determining the fungi composition differences (Egidi et al., 2019; Tedersoo et al., 2016) as vegetation are known to affect fungi composition along with the processes that influencing the nature and quantity of resources entering into the soil (Wardle et al., 2004). Thus, in turn, the fungal community is structured through the environmental factors (Hanson et al., 2012; Hazard et al., 2013) that regulate the assemblage of vegetation. Furthermore, the complexity of forest seems likely stochastic factors that play a role in determining fungal species composition (Chaverri and Vilchez, 2006).

A considerable number of important fungi were observed in this study. Many of the species are saprotrophs which are ecologically important for decomposition of organic matter and are also valuable as a source of food for humans (Kirk et al., 2008). Some of the species can also be used in agriculture as biocontrol agents (Rossman et al., 1999; Samuels, 1996). Most of the species of in the general of *Agaricus* and *Termitomyces*, detected in this study, have been reported previously as fruit bodies from southern part of Ethiopia (Dejene et al., 2017a). Some species such as *Agaricus*

arvensis, *A. campestroides*, *Termitomyces microcarpus*, and *T. clypeatus* have been also reported edible and used by the local community in Ethiopia (Abate, 2014, 2008; Dejene et al., 2017b; Muleta et al., 2013). The species in the genera of *Termitomyces* are also usually collected by women and children from the fields and can be used as copping season food in some localities in Ethiopia (Dejene et al., 2017b). Similarly, several other species in the general of *Boletus*, *Russula* and *Trichoderma* were also reported. Their roles can be described by the formation of symbiotic links with vascular plants (Claridge et al., 2009; Fontaine et al., 2007; Van Der Heijden et al., 2008). In fact, some of the species form the genera of *Boletus* and *Russula* are known to produce edible sporocarps, in some cases, highly appreciated and prized all over the world.

We have observed variation in the overall soil fungal richness and diversity among the studied forests. The highest richness and diversity values were for the Taragedam forests, followed by the Banja and the Alemsaga forests. The observed pattern in fungi diversity may reflect the difference in ecological features (Dang et al., 2018) or plant species diversity differences of the studied forests (Gilbert et al., 2002). When considered dominant ecological guilds separately, the saprotrophs showed a significant difference between the forests. A higher proportion of saprotrophs were in the forests where soil fertility is relatively higher. Interestingly, the relatively higher carbon content was also found in the soil of the Taragedam forest, which may contribute to the higher saprotrophic fungi in this forest. The result is in line with Barnes et al. (2016) and Mundra et al. (2015), who found a higher proportion of saprotrophs in soils where the carbon content of the soil is higher. The relative proportion of root-associated guilds is also found higher in the Taragedam forest. Root-associated fungi are vital contributors to ecosystem functioning. However, the factors which determine their assemblies are still poorly understood (Barnes et al., 2016). The result we found in this study might be associated with the relative availability of a broader host range (Roy et al., 2008; Smith and Read, 2008) in the studied forests. Hence, there may be more trees that can act as hosts for mycorrhizal fungi (Hailemariam et al., 2013; Wubet et al., 2003). Thus, the result presents an insight into the conservation and management of valuable functional guilds in soils of the fragmented Dry Afromontane church forest systems of Ethiopia.

Evaluating the fungal communities in different ecosystems is essential to filter out the relative contributions of environmental factors to fungal composition in an ecosystem (Tian et al., 2018). In this study, the NMDS ordination plot showed clear differences in composition of soil fungi among the three studied areas located in Northern Ethiopia.

This may be due to the fact that such kind of fragmented forests have a characteristic of its own site status, thus creating differences in determining factors that could influence the fungal structures in the forest systems (Rosales-Castillo et al., 2017), indicating fungal community composition can be governed by site factors. In addition to this, we have also identified different indicator fungal species associated with each forests type and whose occurrence depicts the conservational value of that particular habitat or church forests in the study areas. For example, we found the Taragedam forests to have distinctive fungal community, and this community is characterized by the highest number of indicator species (N= 324) as compared to the other two church forests. Majority of these indicator species in the composition are non-litter decomposers, and they constitute about 57% of the total composition of the indicator species in the Taragedam church forest. In the case, climatic variables such as mean annual precipitation and temperature could be important factor driving such composition in addition to the other variables such as shrub cover, tree density, and soil variables (Tedersoo et al., 2014; Tedersoo et al., 2016). This is because the Taragedam forests is a relatively warm with the mean annual temperature 19.5 °C, and less humid site with the mean annual precipitation amount of 1098 mm as compared to the other two church forests. The result also coinciding with the relative diversity of tree species and less shrub cover in the Taragedam forests, thus there might be more fungal species associated to this specific condition and could form distinct community. The analysis also indicated that the non-litter decomposer fungal species are more favored in the Taragedam forests. This is likely the case for the species of *Ramicandelaber, Polyschema, Mortierella, Calocybe, Auxarthron, Trichoglossum* and *Geoglossum.* Among these, the higher abundance occurrence of *Polyschema* species were reported from rain but warmer site forests from Cuba (Ruiz et al., 2000). Similarly, the species of the *Trichoglossum* were also reported commonly fruit in abundant in an areas where there is a relatively warmer temperature nut mostly on humified soils (Kaygusuz, 2020). This result may be further explained by the microclimate condition of the forests ecosystems (Mayer, 2008), particularly of the temperature (Krishna and Mohan, 2017). The forests temperature condition in the Taragedam forest may have been hospitable to many of the non-litter decomposers fungi as the soil microbial activity rises exponentially with soil temperature (Kirschbaum, 1995), thus influencing the fungi community structure based on their feeding patterns (Mayer et al., 2005) in this forests systems. Although it needs further study, the plant species composition may also influencing the fungi community structure due to the less availability of decomposable material in the forest floors of the study area so that the non-litter decomposer would be promoted in the systems, as

non-hospitable conditions could shift the abundance or structure of microbial communities in the forest soil systems (Epstein et al., 2002; Sharon et al., 2001).

Although the numbers of species were very small, the fungal composition of the Banja forest was also dominated by the non-litter decomposers indicator species (35%), as we have observed in the Taragedam forests. The species in the general of *Entoloma*, *Calvaria*, *Rosasphaeria*, *Boubovia*, and *Omphalotus* were some of the species found in the Banja forest. The possible explanation for such similarity between the Taragedam and Banja forests in terms of dominant species in the composition might be due to the similarity of the available humification in the forest soil. Both forests have similar type of climatic condition and the organic matter availability in the soil which could favor similar species composition, as the diversity and composition of non-litter fungi usually amended by organic matter and moisture availability in the soil (Clocchiatti et al., 2020). Furthermore, the similarity of tree species richness and diversity might also be a reason for such occurrence. We found equivalent higher diversity and richness value of both forests. Higher tree diversity in forests should lead to a unique microbial diversity and activity due to greater niche variability such as carbon sources and root exudates in the soil, variable microclimate, spatial and age variability of trees etc (Setälä, 2002). Such conditions are suitable to change trophic level of the fungi species so that fungi could form distinct composition in a similar ecosystems (Kubartová et al., 2009; Setälä, 2002) conditions of different forests.

The Alemsaga church forest also has distinctive fungal communities dominated by litter-decomposers and mycorrhizal species respectively. Of the various species, the genera of *Tomentella*, *Piloderma*, *Byssocorticium* and *Cortinarius* are indicator ectomycorrhizal species dominating the composition of fungi in the Alemsaga forest. Of these, the genus *Tomentella* reported usually found in abundant in coniferous and deciduous forests worldwide (Kuhar et al., 2016). However, the existence of the ectomycorrhizal indicator species in the Alemsaga church forests may be due to the dispersion of mycorrhizal inocula from nearby plantation forests, which are dominated by *Eucalyptus* and *Pinus* species (Alem et al., 2020; Castaño et al., 2019; Urcelay et al., 2017). These trees are ectomycorrhizae associated species and the result may be an indicator of the ecological restoration status of the studied forests as plantation could change the trophic status of the soil (Dang et al., 2018). This is because the enrichment plantation can improve the ecological environment, plant diversity, and soil nutrient levels (Lozano et al., 2014), which could potentially alter the microclimate environment and ecosystem process, thereby influencing belowground fungal

community structures (Cao et al., 2007). Thus, the findings here may indicate that the ectomycorrhizal indicator species more preferred enriched forests with host trees. This may have important implication that enrichment plantations would offer suitable habitats with variable microclimates that should assist the ectomycorrhizal species richness in the Dry Afromontane church forests system for the maintenance of functional fungal diversity in Ethiopia (Dejene et al., 2017a). Furthermore, the findings presented here may have important implications for the indigenous forest system for the maintenance of functional guild diversity given that mycorrhizal fungi have previously only been reported from exotic tree plantations (Alem et al., 2020). However, the association of mycorrhizal species in indigenous forest systems in Ethiopia needs empirical data to confirm. The relationships among indicator fungal species, soil properties, and vegetation may also be important for further understanding the conservation and rehabilitation process of these important forests through enrichment or artificial plantations in the study area.

It is well established that fungal communities as a whole are significantly influenced by edaphic variables (Straatsma et al., 2001; Zakaria and Boddy, 2002). This is because the soil contains nutrients that enable fungi to grow and develop (Drenovsky et al., 2004) and thus, in turn, the composition of fungi is directly influenced by the soil fertility condition of a site (Drenovsky et al., 2004; Lauber et al., 2009). In this study we have found that pH and Nitrogen are correlated with the soil fungal community composition together with other cation elements such as Ca, Mg, and K. Among these, the pH and Nitrogen are considered the most influential parameters to govern the fungal composition (Ullah et al., 2019). Our forest soil samples were relatively acidic, where the pH values range between 4 and 6, indicating the fungal composition of the studied forests are maintained at a lower pH level. The lower soil pH conditions may affect the composition through its influences on spore germination and mycelial development (Rousk et al., 2010). Also, it is evident that the soil pH influences carbon (Andersson et al., 2000) and nutrient availability (Pietri and Brookes, 2008; Kemmitt et al., 2006) in the soil on which the fungal growth and biomass composition are dependent (Bååth and Anderson, 2003). In our study also found some fungal taxa are directed towards the higher pH level (example at the Taragedam forests). One of the reasons for this might be these taxa characteristically are adapted and grow in a comparatively alkaline soil condition, resulting in an increase of the community richness without inhibition of their growth (Nevarez et al., 2009; Tian et al., 2018), as some fungi can also grow well in neutral to slightly alkaline conditions (Zhang et al., 2016). Studies recognized that Nitrogen could affect microbial community structures depend on the forest types

(Tedersoo et al., 2020; Tian et al., 2017). Because the Nitrogen availability in the soil negatively influences the fungal growth, particularly of the mycorrhizal fungi, and has a significant effect on community composition (Zhao et al., 2018). In this study, we observed an obvious correlation of Nitrogen with the entire fungal community composition of the studied forests. A relatively higher value of Nitrogen was estimated from the Banja forest, where fungi composition was relatively higher. This might be an indication that the majority of plant species in the studied forest are independent of mycorrhizal fungi. Higher availability of Nitrogen in the soil could reduce the dependency of the host plant on fungi (Liu et al., 2019), which eventually could cause competition among the fungal species and could lead to their distinct composition (Wang and Wang, 2008; Zhao et al., 2018). Also, cations play an important part in many physicochemical processes, such as photosynthesis (He et al., 2017b) and, thus, can affect plant photosynthesis and, hence, the amount of carbon that is available to soil fungi (Shi et al., 2014) in the forests soils.

Conclusion

Due to the key ecological role that fungi play in ecosystem functioning, the information about how ecological factors affect the fungal communities in the church fragmented forests can be crucial to enable the integration of these forests into global biodiversity conservation strategies and to understand what actions must be undertaken to conserve these forests, and their biological components. Thus, we investigated the diversity and community composition of soil fungi to suggest management and conservation strategies of church forests through identifying crucial roles played by fungi in the management and protection of these forest systems. In this study we could find the environmental variables significantly driving fungal communities and analyzing grouped variables such as climate, soil and vegetation played role in driving fungal community assembly. This is because the climate, vegetation, and soil characteristics together are vital in setting spatial variation, reflecting the combined effects of these variables on fungal community assembly. Unsurprisingly, the soil fungi communities as a whole were also influenced by climatic and edaphic variables given that both variables could influence mycelial development in the soil. The separate analysis of the vegetation characterizes also showed fungal community can be affected by the difference in the plant community variables. Thus, in this study the soil fungal communities may follow the spatial variation, climate variables, the plant community composition of forests or all of the factors together are the mechanisms regulating soil fungal communities and they are essential for maintaining fungal biodiversity in the

studied forests. Furthermore, we have found indicator species of ectomycorrhizal species in the forest where previously conducted enrichment plantations of exotic tree species. Some of the vegetation parameters such as tree cover and basal areas were also found positively associated with the functional guilds of the studied forests. The conservation and management of fungi in the studied forests systems can be maintained through forest management since they can modify vegetation parameters like tree density, canopy cover, basal area, understory plant communities and thus could play a crucial part in shaping fungal communities of the studied forests etc. Furthermore, promotion of host diversity through taking into consideration the suitability of the climate and spatial characteristic of an area through enrichment plantation systems would offer suitable habitats with variable microclimates for fungi diversity and composition in the fragmented forest systems. We do these managements because the conservation of fungi could assist the successful rehabilitation of these fragmented forests as fungi have direct consequences on plant growth through mutualism, pathogenicity, and their effect on nutrient availability and cycling. Also, fungi have vital roles in soil organic matter stabilization and decomposition of residues so that they contributed more to the rehabilitation and conservation of the fragmented forest systems in the country. The application of the baseline information provided in this study could assist other countries that are facing similar forest conservation issues due to deforestation and forest fragmentation.

Authorship contribution statement

Demelash Alem: Data curation, Formal analysis, Writing-original draft. **Tatek Dejene**: Methodology, Formal analysis, Writing-review and editing, Supervision. **Jozsef Geml**: Supervision, Writing-review. **Juan Andrés Oria-de-Rueda**: Supervision, Writing-review, and editing. **Pablo Martín-Pinto**: Conceptualization, Methodology, Supervision, Formal analysis, Writing-review, and editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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