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Fungal communities from forest systems in Ethiopia

Comunidades fúngicas procedentes de sistemas forestales en Etiopía

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Abstract

Afromontane forests originally dominated the highland parts of Ethiopia, delivering a wide range of social, economic and environmental benefits including key components of biodiversity. However, anthropogenic disturbances are causing a dramatic decline of these forests and gradually changed the scene. Among the factors, forest fires are increasingly contributing to the loss to these forests systems in the country. On the other hand, plantations of exotic trees are also another form of forests in the highland parts of Ethiopia. They are also serving as sources of industrial wood, firewood, farm implements, poles and posts. Owing to their rapid growth, plantations are subjected to traditional management systems mainly of a “plant, clear fell and replant” cycle method or "coppicing". For the last decades, researches have been conducted to assist the development, management and conservation of both forest systems in the country. However, fungal communities associated with these forests have been neglected and their knowledge is limited by lack of studies in Ethiopia. Previous studies indicated that forest fire and plantation forest management affected the macrofungal community in forest systems where the gestation period is long, although such impacts are yet understudied in Ethiopia. Furthermore, the information about ethnomycology and mushroom cultivation practices is scarce and the available works are even fragmented and limited in their scops to represent the country adequately. Thus, we aimed to compile and assess the existing literature on mushrooms in Ethiopia, and also, to conduct field systematic research to know fungal diversity and production according to stand development and fires in plantations and natural forests respectively. Both review of literature and field studies were carried out for the purpose. The review made included all information available in the country while the field study was conducted at Wondo Genet forest area. In the field we established plots taking into consideration the similarity of the areas in terms of ecological conditions such as climate, altitude, and soil. For the purpose, in the Dry Afromontane forests, two burned and one unburned forest areas were selected. A total of nine sample plots, i.e. three in each area, were established. Similarly, a total of 18 plots were also established in *Pinus patula* (9 plots) and *Eucalyptus grandis* (9 plots) stands. In all cases the plots covered an area of 100 m^2, with a rectangular shape (2 m x 50 m). All sporocarps found in the plots were fully harvested weekly during the rainy season in July and August in 2015. Fresh weight measurements were carried out *in situ* and the data are given in kilograms per hectare per year. Also, abundance, the number of sporocarps per species, data was taken from each plot. Sample fruit bodies from each species were taken to the laboratory and dried. Some samples were preserved as herbaria specimens, and they were used for molecular and microscopic taxa identification. Composite soil samples were also collected from each plot to determine main edaphic variables that could explain the fungal communities. Richness, Shannon index, and Fresh weight (for the edible and total taxa) estimates were subjected to one-way ANOVA analysis and a post-hoc least square means difference test (LSD, $P \leq 0.05$) in order to test for differences among treatments. Fungal species accumulation curves and the Rényi diversity profile were also generated to compare fungal richness and diversity among different age groups in the plantation forests. An ordination analysis based on fungal fresh weight data was also used in order to identify and correlate significant edaphic explanatory variables related to taxa composition. The review summarizes issues related to the diversity of wild mushrooms, main ecological niches and their associated fungal species, ethnomycology and mushroom cultivation practices in Ethiopia. Threats and the need for future conservation of wild mushrooms in the country are also reported. The results from field observations indicated that a total of 61 taxa were collected from the Dry Afromontane forests, of which 22 were edible, belonging to *Basidiomycota* division. In this study, fungal diversity and richness were affected just after fire. Fire also affected the production of edible fungal species. Fungal community composition was significantly correlated with edaphic variables such as Organic Matter, Ca and P values. Similarly, we also found a total of 53 and 29 fungal taxa belonging to *Basidiomycota* division, with the exception of *Xylaria hypoxylon* which is *Ascomycota* in *P. patula* and *E. grandis* plantations respectively. Majority of the collected taxa in plantations were saprophytic. However, about 6% of the taxa collected in *P. patula* were ectomycorrhizal fungi. Of the total taxa collected, about 36% and 31% were classified as edible in *P. patula* and *E. grandis* tree plantations respectively. Taxa richness, Shannon diversity, and sporocarp yields showed significantly increasing trends with age of both stands. The fungal community composition in *P. patula* stands also correlated significantly with N, K, and pH. Generally, the findings from review paper can serve as base line information and indicator for further mycological studies in Ethiopia. On the other hand, the results from preliminary field studies have important implication to describe the status of fungal biodiversity, sporocarps production and their succession in natural and the plantation forest systems in Ethiopia. The results also provide management implications to conserve Dry Afromontane forests in Ethiopia through adding economic value from mushrooms. The deliberate presence of mature trees in the plantations could lead to an increase in the production and fungal diversity, with their associated economic and ecological benefits.

Key words: *Ethiopia, Fungal communities, Dry Afromontane forest, Fire, Pinus, Eucalyptus, fungal succession.*

Resumen

Los bosques afromontanos dominaron originariamente las tierras altas de Etiopía, ofreciendo una amplia gama de beneficios sociales, económicos y ambientales incluyendo componentes clave de la biodiversidad. Sin embargo, las perturbaciones causadas por el hombre están causando una dramática disminución de estos bosques y poco a poco un cambio del paisaje. Entre estos factores, los incendios forestales están contribuyendo cada vez más a la pérdida de estos sistemas forestales. Por otro lado, las plantaciones de especies exóticas también son frecuentes en las tierras altas del país, sirviendo como fuente de recursos necesarios como son: la madera industrial, leña, herramientas agrícolas y postes. En estas plantaciones se emplean especies de crecimiento rápido que son sometidas a sistemas de manejo tradicionales. Durante las últimas décadas, se han realizado investigaciones para ayudar al desarrollo, manejo y conservación de los sistemas forestales en el país. Sin embargo, las comunidades de hongos asociadas a estos montes han sido desatendidas y su conocimiento está limitado por la falta de estudios. Estudios previos indican que tanto el manejo forestal de los bosques como las perturbaciones entre las que pueden destacar os incendios forestales, afectan de modo significativo a las comunidades fúngicas asociadas a esos bosques, aunque por el momento esto no se ha estudiado en Etiopía. Además, la información sobre la etnomicología y las prácticas de cultivo de hongos en el país es escasa y la disponible está muy fragmentada y limitada en sus objetivos como para representar adecuadamente el país. Por tanto, nuestro objetivo fue recopilar y evaluar la literatura existente sobre hongos en el país, así como realizar investigaciones sistemáticas sobre el terreno para conocer la diversidad y producción fúngica en función del desarrollo de las masas forestales y de los incendios forestales existentes, en plantaciones y bosques naturales respectivamente. El trabajo de recopilación bibliográfica trató de encontrar toda la información disponible en el país, mientras que los estudios de campo se llevaron a cabo en el área forestal de Wondo Genet. Las parcelas de estudio se establecieron teniendo en cuenta la similitud de las áreas en términos de condiciones ecológicas. Para ello, en los bosques afromontanos secos, se seleccionaron dos áreas forestales afectadas por el fuego y una zona control no quemada. En estos bosques naturales, se establecieron un total de nueve parcelas de muestreo, tres en cada área. Del mismo modo, se analizaron 9 parcelas en plantaciones dominadas por *Pinus patula* y otras 9 en masas de *Eucalyptus grandis*. En todos los casos las parcelas cubrieron una superficie de 100 m^2, con una forma rectangular (2 m x 50 m). Todos los carpóforos encontrados en las parcelas se recolectaron semanalmente durante la temporada principal de lluvias, en julio y agosto de 2015. Se hicieron mediciones de peso fresco *in situ* y además se anotó el número de carpóforos por especie en cada parcela que fueron conservados y llevados a laboratorio para su procesado. Algunos ejemplares se conservaron en una colección para la posterior identificación molecular y microscópica. También se recogieron muestras de suelo de cada parcela para determinar las principales variables edáficas que podrían explicar las comunidades fúngicas presentes en las parcelas. Las estimaciones de riqueza, índice de Shannon y peso fresco (para los taxones comestibles y totales) se sometieron a análisis de ANOVA y a un test post-hoc (LSD, $P \leq 0,05$) para estimar las diferencias entre tratamientos. También se generaron curvas de acumulación de especies de hongos y perfiles de diversidad de Rényi para comparar la riqueza y diversidad de hongos entre diferentes grupos de edad en las plantaciones. Por otra parte, se utilizaron Análisis de Ordenación, CCA y DCA, para correlacionar las variables edáficas significativas con la composición de los taxones. En cuanto a los resultados obtenidos, la recopilación bibliográfica resume la información disponible sobre diversidad de hongos silvestres y sus principales nichos ecológicos, así como etnomicología y prácticas de cultivo de hongos en Etiopía. También se destacan cuáles son las principales amenazas y la necesidad de conservar este recurso. Por otra parte, de los trabajos en campo se obtuvieron los siguientes resultados. Se encontraron un total de 61 taxones en los bosques afromontanos secos, de los cuales 22 eran comestibles, pertenecientes a la división de Basidiomycota. Se observó un efecto del fuego en la diversidad y riqueza fúngica, así como en la producción de especies fúngicas comestibles. La composición de la comunidad fúngica se correlacionó significativamente con las variables edáficas Materia Orgánica, Ca y P. En el trabajo llevado a cabo en las plantaciones se encontraron un total de 53 y 29 taxones asociados a *P. patula* y *E. grandis* respectivamente. La mayoría de los taxones recogidos en las plantaciones son saprófitos. Sin embargo, el 6% de los encontrados en la plantación de *P. patula* son ectomicorrícicos. Del total de taxones recogidos, aproximadamente el 36% y el 31% se clasificaron como comestibles en las plantaciones de *P. patula* y *E. grandis*, respectivamente. Tanto la riqueza, como la diversidad y la producción fúngicas mostraron tendencias significativamente mayores con la mayor edad de las plantaciones en ambos estudios. La composición de la comunidad fúngica asociada a *P. patula* se correlacionó significativamente con N, K y pH. Los hallazgos del documento de revisión pueden servir como base de información de cara a futuros estudios sobre micología forestal aplicada en Etiopía. Por otra parte, los resultados encontrados en los estudios de campo, aún siendo preliminares, pueden tener una gran relevancia de cara a describir el estado de la biodiversidad y la producción fúngica, así como su sucesión en los bosques naturales y plantaciones en Etiopía. Además se pueden obtener algunas consideraciones interesantes desde el punto de vista del manejo de estos ecosistemas. Así, una adecuada gestión puede ayudar a la conservación de estas masas tanto naturales como artificiales y de sus recursos asociados, generando al mismo tiempo rentas complementarias hasta el momento prácticamente desaprovechadas. En este sentido, el mantenimiento al final del turno, de rodales con árboles maduros y/o sobre maduros en las plantaciones podría suponer un incremento en términos de producción y diversidad fúngica con las correspondientes consecuencias positivas desde los puntos de vista económico y ecológico.

Palabras clave: *Etiopía, Comunidades fúngicas, Bosque Afromontano Seco, Fuego, Pinus, Eucalyptus, sucesión fúngica.*

List of original articles

This thesis is based on five original works, which are referred to in the text with Roman numerals (I – V). The first, the third and the fourth of them are already published and the second article is accepted in Science Citation Index (SCI) journals. The fifth article is submitted and under peer review in the SCI journal:

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

I. **Tatek Dejene, Juan Andrés Oria-de-Rueda, Pablo Martín-Pinto**, 2017. Wild mushrooms in Ethiopia: a review and synthesis for future perspective. Forest systems 26 (1), eR04. https://doi.org/10.5424/fs/2017261-10790.

II. **Tatek Dejene, Juan Andrés Oria-de-Rueda, Pablo Martín-Pinto**, 2017. Edible wild mushrooms of Ethiopia: neglected non-timber forest products. (**Accepted** in Revista Fitotecnia Mexicana)

III. **Tatek Dejene, Juan Andrés Oria-de-Rueda, Pablo Martín-Pinto**, 2017. Macrofungal species diversity and sporocarp production following wildfire in Dry Afromontane forests of Ethiopia. Forest Ecology and Management 398: 37-47. https://doi:10.1016/j.foreco.2017.05.011

IV. **Tatek Dejene, Juan Andrés Oria-de-Rueda, Pablo Martín-Pinto**, 2017. Fungal diversity and succession following stand development in *Pinus patula* Schiede ex Schltdl. & Cham. plantations in Ethiopia. Forest Ecology and Management 395: 9–18. http://dx.doi.org/10.1016/j.foreco.2017.03.032

V. **Tatek Dejene, Juan Andrés Oria-de-Rueda, Pablo Martín-Pinto**, 2017. Fungal communities under *Eucalyptus grandis* W.Hill ex Maiden plantations of different ages in Ethiopia. (Under peer review in Forest Ecology and Management)

Outline of the thesis

This thesis consisted of five studies crucial to describe the status of fungal biodiversity, ethnomycological use, sporocarps production and their succession in natural and plantation forest systems in Ethiopia. Of these, the first two studies (I & II) are reviews that discussed all-purpose information about wild mushrooms in Ethiopia which help to create awareness for mycological uses and studies in the country. Since they are complementing each other, we tried to merge them in the compendium result and discussion for straightforwardness.

In the third study (III), the fungal communities in relation to forest fire in Dry Afromontane forest systems is evaluated. The diversity, richness, and sporocarp productions following fire are discussed. The fungal taxa composition in this forest system also explained in terms of edaphic variables. Thus the findings have implications for the conservation and use of Dry Afromontane forests through the application of NTFPs production, mainly of mushrooms and also the role of sustaining biological diversity of this forest system in the study area.

In the fourth and fifth studies (IV & V), the fungal succession in relation to stand development of *Pinus patula* and *Eucalyptus grandis* plantations were discussed. The associated fungal richness, diversity and the sporocarp yield are assessed. The taxa composition also explained in terms of edaphic variables and age groups of plantations. The finding will have implication for the use of plantation forests through mycosilvicultural approach that could provide complementary income for the local communities in the study area. Conceptual map of the study including the five studies pretend below (Figure 1).

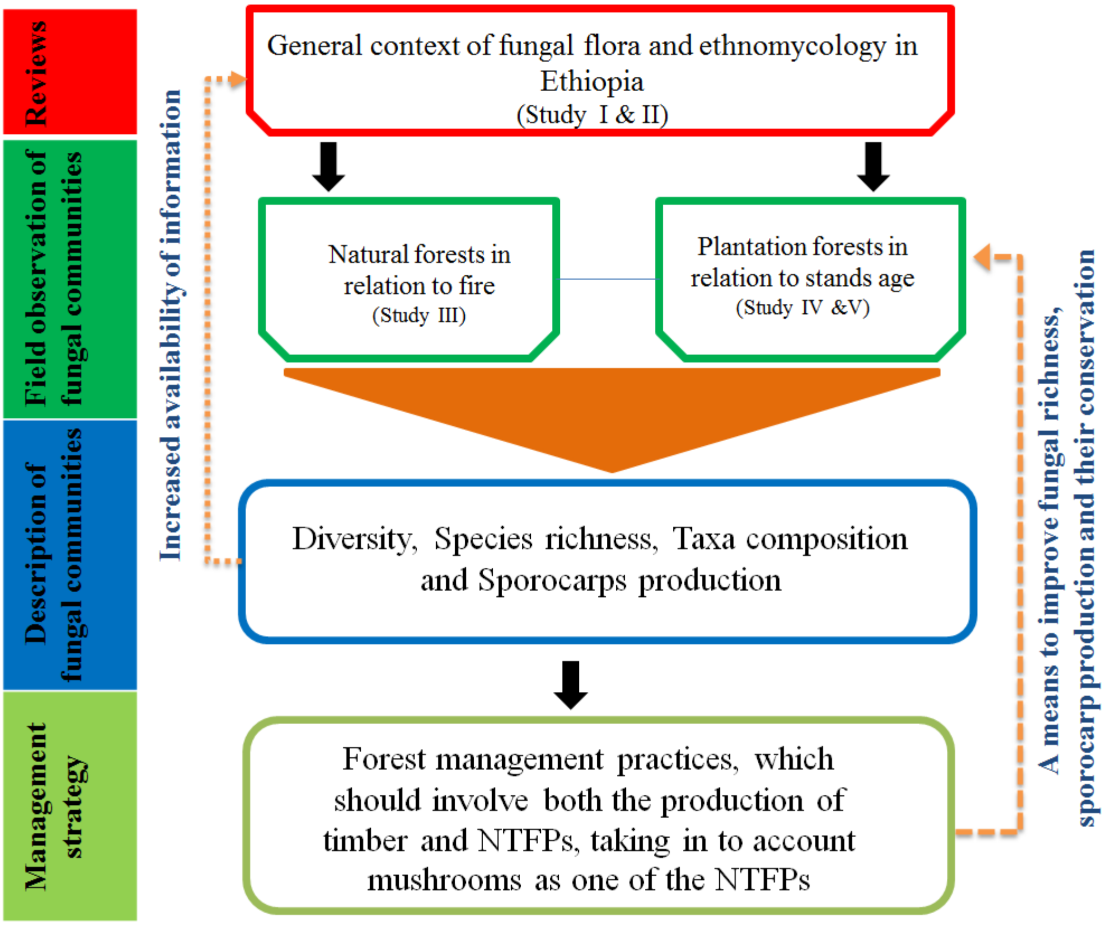

Figure 1: Conceptual map of the thesis including the five studies.

1. Introduction

1.1. General ecology and vegetation in Ethiopia

Ethiopia is ecologically very diverse country owing to the varied topographic features and altitudinal variations, range from 110 m below sea level to 4620 m above sea level (m.a.s.l) (Bongers and Tenngkeit, 2010). The country also experiences a very high variation in macro and micro-climatic conditions that have contributed to the formation of diverse ecosystems inhabited with a great diversity of life forms in flora and fauna. The mean annual rainfall of the country ranges from 500 to 2800 mm with high variation in temperature >30 ^{o}C and < 10 ^{o}C (Demissew and Nordal, 2010). Such varied ecological conditions enabled Ethiopia to inhabit unique vegetation resources (Tewolde, 1991).

According to Friis et al. (2010), the vegetation resources of Ethiopia classified into 12 types based on the altitudinal gradients in which they occurred. These include (i) Desert and semi-desert shrub land, (ii) Acacia-Commiphora woodland and bush land, (iii) Wooded grassland of the Western Gambela Region, (iv) Combretum- Terminalia woodland and wooded grasslands, (v) Dry Afromontane forest and grasslands complex, (vi) Moist Afromontane forest, (vii) Transition rainforest, (viii) Ericaceous belt, (ix) Afroalpine vegetation, (x) Riverine vegetation, (xi) Fresh water, lakes, lakes shores, marshes, swamps and flood plain vegetation and (xii) Salt-water, lakes, lakes shores, salt marshes and plain vegetations. Out of which, the natural high forests that include the Afromontane vegetations are exclusively found in the highland regions of Ethiopia.

1.2. Ethiopian highland natural forests and plantations

Ethiopia highland regions cover more than 44% of the country's land area. In most cases, these areas are found at an altitude higher than 1500 m.a.s.l (Figure 2). They form the largest continuous massive lands in Africa and they are also called "*Roof of Africa*" due to its little surfaces falling at 1500 m.a.s.l, while the summits reach heights of up to 4620 m.a.s.l. (Bongers and Tenngkeit, 2010). The highlands are more suitable for human habitation and agricultural production purposes. The annual rainfall amount and distribution in these regions are also higher as compared to others parts in the country (Tesfaye et al., 2015). Thus, the highland region accommodates about 90% of the total population; over 93% of the cropped lands, around twothirds of livestock and over 90% of the national economic activity in the country (Tesfaye et al., 2016).

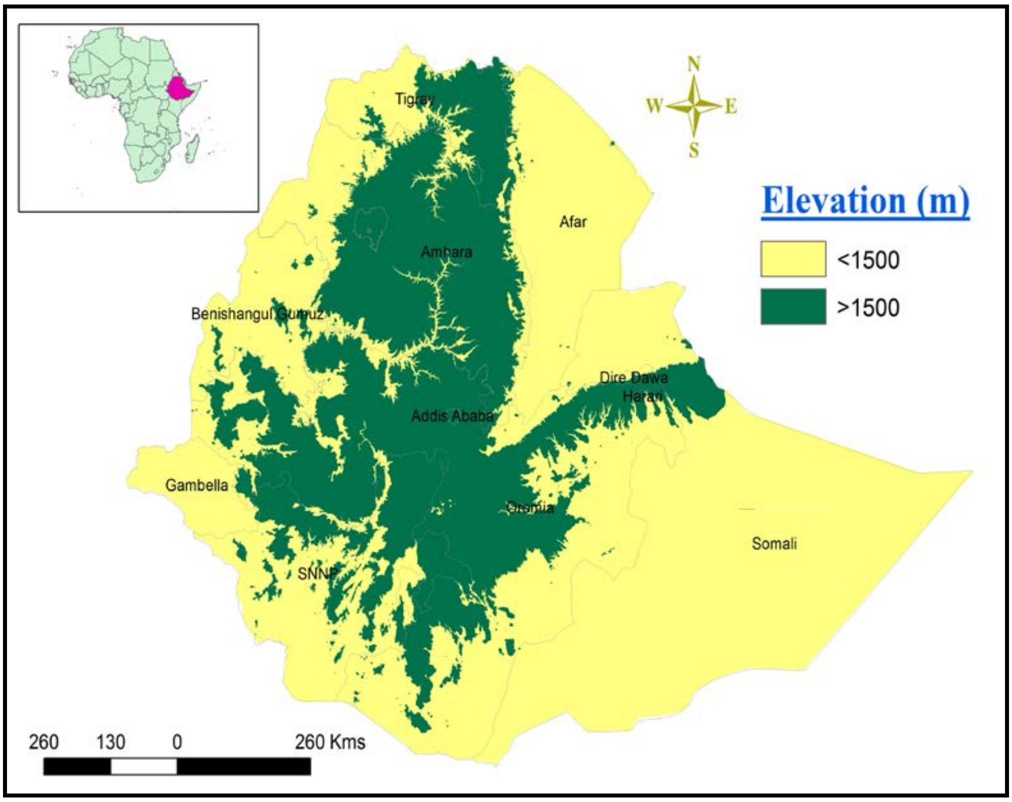

Figure 2: Map of Ethiopia showing the highland regions in which the Afromontane vegetations found in the country (Adapted from Bekele 1994).

Afromontane forests have originally dominated the highland regions of Ethiopia that can be classified as either Dry or Moist Afromontane forest types (Friis et al., 2010). Most of the highland areas are dominated by the Dry Afromontane forests which are distributed mainly in the Central, Northern and Western parts of the country (Eshete et al., 2011; Friis et al., 2010). The existence of high humidity with a variable rainfall pattern and a prolonged dry season characterize the Dry Afromontane forests and make them very complex and rich in biodiversity (Wassie et al., 2005), including macrofungal species (IBC, 2014). The main tree species characterizing the Dry Afromontane forests include *Juniperus procera*, *Hagenia abyssinica*, *Podocarpus falcatus* and *Olea africana*. These trees serve as main sources of timber to the country (Kassa et al., 2009). Also several types of NTFPs such as coffee, highland bamboo, mushrooms, different spices, etc., are found in these forests, which are vital for the socio-economic conditions of the local people as well as for the economy of the country.

In Ethiopia, highland natural forests cover was about 40% of the total land area some decades ago, delivering a wide range of social, economic and environmental benefits, including key components of biodiversity (Badege, 2001; Thomas and Bekele, 2003). However, anthropogenic disturbances are causing a dramatic decline of these forests and the scenario is gradually changing. Today, the area covered by natural high forests is less than 3% of the country's total lands (Lemenih and Bekele, 2008; Taddese, 2001). The remaining of these forests are highly fragmented and they are being modified by plantations of non-native tree species (Bekele, 2011; Moges et al., 2010; Tesfaye et al., 2016).

Figure 3: Highland natural Afromontane forests (A) and plantation of *Eucalyptus* tree forests (B) in Ethiopia

Nowadays, manmade plantations of fast growing exotic trees species are also becoming another form of vegetation found in highland regions of Ethiopia. Plantation forestry in these regions began near the turn of the 19th century (FAO, 2009; Teketay, 2000). During this period, *Eucalyptus* was the first introduced species, followed by *Cupressus*, and *Pinus* tree species (Bekele, 2011; Tesfaye et al., 2015).

The importance of tree plantation sector in Ethiopia is increasing as the demand for woody raw materials is rising and the supply from the natural forests is decreasing. Accordingly, the areas under exotic tree plantations have been increasing from an estimated 190,000 hectare (ha) in 1990 to 972,000 ha in 2011 (Bekele, 2011) and thereby reduce the pressure on the natural forests in the country. Of the estimated area under plantation forests, 190,400 ha, approximately 20%, are classified as commercial plantations (Bekele, 2011). The remaining 80% are non-industrial plantations, mainly woodlots and trees on farms. These plantations have a considerable potential for sustainable production of high value timber and NTFPs (Lemenih and Bekele, 2008).

In terms of tree species, Ethiopia holds the largest *Eucalyptus* tree plantations in East Africa region (Bekele, 2011). *Eucalyptus* plantations are being widely expanded and dominating both rural and urban landscapes in the country (Kelemu and Tadesse, 2010). They roughly cover about 56% of the total plantations by area (Bekele, 2011). Most of them are established as community forests. On the other hand, *Pinus* tree plantations are established mainly in state-owned forests, occupying the third position in terms of plantation area coverage (Bekele, 2011; Gezahgne, 2003). They contribute to the production of round wood for sawn timber, poles and posts owing to their rapid growth (Gezahgne, 2003).

In general, plantation forests serve as sources of round wood for sawn timber, farm implements, poles and posts in the country. They are also important to meet wood requirements for local use, such as for construction material and for wood fuel (Gezahgne, 2003). They are also helping the rural people improving their livelihood through the contribution to household economy (Asnake, 2002; Kebebew, 2002; Mesfin, 2002). Furthermore, plantations of some species, for example *Eucalyptus* spp*.,* have been used in biological soil conservation works where they are used in degraded and gully areas (Jaleta et al., 2016; Lemenih and Kassa, 2014). Plantation forests can also provide important recognized NTFPs in the country including forest fungi (Abate, 2008).

1.3. Fungi in forest ecosystems

Fungi are the most diverse group of organisms found in terrestrial ecosystems, playing essential roles in their development, functioning and stability. In this sense, fungi can act as either symbionts or saprotrophs in forest systems (Ferris et al., 2000; Nick et al., 2006; Nordén et al., 2004). Since fungi are unable to make their own food, they are dependent on other sources for sugars and carbohydrates. Mycorrhizal species do this by invading live trees and other plants, and forming intimate relationships with plants where both the fungus and plant benefit from the relationship (Ferris et al., 2000). Such symbiotic relationship is intimately established inside and around the plant roots (Dickie, 2007; Lindahl et al., 2007; Wardle, 2006). The fungi act as an extended root system for the plants and help the trees by improving their ability to acquire mineral nutrients and water from the soil and improve the plant resistance to pathogens (Dahlberg et al., 2001; Dalong et al., 2011). In similar way, fungi benefit from the host tree by obtaining carbohydrates (Lindahl et al., 2007). Other kinds of fungi, the saprotrophic form, also play essential ecological roles, i.e. the decomposition of dead organic matter, and therefore nutrient cycling in the forest ecosystems (Ferris et al., 2000). Thereby fungi influence plant diversity, productivity and ecosystem functions (Pietras et al., 2013; Van Der Heijden et al., 2008). Given their importance for ecosystem processes, fungi can also be used as indicators of ecological continuities or stability in forest systems (Butler et al., 2002).

In addition to their ecological functions, forest fungi are also becoming strategic components in the conservation and management of forest resources (Bonet et al., 2014). Fungi have been used as food and medicine by humankind (Boa, 2004). They are also becoming part of the livelihoods of rural people throughout the world (Cai et al., 2011; Sarma et al., 2010). Many of the wild mushrooms are being collected as valuable NTFPs (Boa, 2004; Chang and Lee, 2004). They provide economic complementary alternatives for the forest sector (Bonet et al., 2014). Mushrooms help people to reduce vulnerability to poverty and strengthen their livelihoods through a reliable source of income. This is because of their economic value, as during the last decade there has been an increasing demand for mushrooms in the world (Pettenella et al., 2007), occasionally generating even higher economic benefits than timber productions (Martín-Pinto et al., 2006).

The interaction of forest fungi with other organisms in the forest systems also cannot be ignored as the macrofungi provide food and habitat for invertebrates such as fungus gnats, molluscs, slugs and coleopterans (Jonsell and Nordlander, 2000) and to other organisms in the forest.

1.4. Context of mycology in Ethiopia

Despite their importance, fungi are among the lest studied components of the forest systems in Ethiopia (Dejene et al., 2017a; Megersa et al., 2017; Sitotaw et al., 2015b). As a result, the taxonomy and ecology are very poorly known compared with the majority of other organisms present in the forest ecosystem in Ethiopia. However, Pegler and Rayner (1969) and Pegler (1977) noted that the East Africa region, including Ethiopia, is rich in fungal species. The high diversity associated with the forest systems supposed to contribute to such diversity of fungal species (Sitotaw et al., 2015b). Many of the fungal species in Ethiopia are presumed again to be either cosmopolitan or widespread across Africa (Pegler, 1977). However a systematic estimation of fungi diversity is not yet available (IBC, 2014) and likely as a result; fungi are not included in the biodiversity database of the country. Furthermore, reports regarding fungal biodiversity rarely exist in the country (Dejene et al., 2017a). These are due to a lack of research infrastructures in mycology and less attention given to the fungal resources. This poor knowledge in fungi in Ethiopia is worrying as fungi are highly sensitive towards habitat disturbances, namely anthropogenic threats that are rife across the country (Goldmann et al., 2015; IBC, 2005).

Despite this lack of scientific knowledge on fungal resource, wild mushroom collection and utilization has been a traditional common practice among the different rural communities in Ethiopia (Semwal et al., 2014; Tuno, 2001). Fungi have been used for their nutritional, traditional and medicinal functions, and are also involved in local mythology (Abate, 2014; Tuno, 2001). Equally to other wild edibles, they have also been used as a coping food during food shortage periods (Alemu et al., 2012; Lulekal et al., 2011). Wild mushrooms are also available in some local markets (Abate, 2014), where they are sold by the local people to earn some income to supplement the household economy. Although there have been some attempts to document the ethnomycological and socio economic aspects of mushroom in Ethiopia, the available information were found to be insignificant when compared to the resources wealth and the diverse ethnicity. Given the conservation and maintenance of wild mushrooms is commonly a major goal for contemporary forest management, the presence of anthropogenic factors affecting them calls for immediate action so as to document, produce a development plan and utilization of the species in the country.

1.5. Why this study?

In Ethiopia, wild mushroom collection is a common cultural practice which is hitherto engaged in by local rural communities. However, the available information on this topic is limited while the traditional knowledge and its cultural background are being eroded through accumulation of habitat degradation and loss of biodiversity. Documentation of the knowledge that includes cultivation, mythological values, mycopharmaceuticals, mycophagy, folk taxonomy and the dynamics of inherited folk knowledge of mushrooms is very scanty and the available information is also scattered. Currently, there is a global interest in documenting ethnobotanical information on wild edible food sources (Bharucha and Pretty, 2010), including mushrooms. Hence, it worthwhile to go through the context of Ethiopian mushroom flora, their ethnomycological uses and their socioeconomic aspects to evoke consciousness on the status and wider use of edible and medicinal species in the country.

On the other hand, the forest history of Ethiopia indicates that forest land degradation and deforestation is a continuous process (Wassie et al., 2005). Anthropogenic disturbances such as illegal logging, agricultural land expansion and forest fire are main factors that have been contributing for the degradation of the forest resources in the country (Lemenih and Bekele, 2008; Tadesse, 2001). Among these factors, fire is mostly contributing to the loss of natural forests, affecting their distribution, diversity, and composition (Lemenih and Bekele, 2008; Wassie et al., 2005). For instance, the most devastating wave of forest fires, which occurred in 2000 due to an extended drought, damaged over 150,000 hectares of forested lands throughout the country (Senbeta and Teketay, 2001). This trend is more pronounced in the Dry Afromontane forest areas compared to other ecosystems and has a direct implication on the loss of biological diversity, including macrofungal species, in the these forests (Lemenih and Bekele, 2008).

Figure 4: Part of natural forest of the study area affected by fire in Wondo Genet (Ethiopia)

Although the knowledge on fungi-fire relationship is limited in Ethiopia by lack of previous studies, it has been reported that fire strongly affects fungal communities in the forest ecosystems (Bastias et al., 2006). Differences in the return interval of fire can modify the composition and diversity of fungal communities (Buscardo et al., 2010). A change in ecological conditions due to fire could also trigger fungal succession depending on the intensity and duration of fire (Hart et al., 2005). Furthermore, fire also produces direct effects on fungal communities by affecting belowground organisms (Buscardo et al., 2012). Thus, fire can impose an impact on the subsequent structure of fungal communities following succession patterns mainly driven by the dynamics of post-fire plant communities (Cairney and Bastias, 2007). On the other hand, some fungi might also be benefited from fire since they fruit as a result of fire (Hart et al., 2005). Hence, some level of fire in the ecosystem could also provide higher abundance of fire-loving fungal species. However, such impacts of fire on Ethiopian forest fungi resources are not yet studied.

Fungi are also becoming integral part of plantation forest management since they provide significantly complementary incomes (Bonet et al., 2014). Tree retention as a forest management practice has been undertaken in different parts of the world to achieve multifunctional goals, mainly of wood and NTFPs production and biodiversity conservation (Nyland, 2002). This approach has also important implications for forest floor microhabitats improvements, such as moisture, temperature, and substratum (Smith et al., 2008), which are crucial for macrofungal species. However, such management approach is poorly represented into plantation forest management systems in Ethiopia. Furthermore, owing to the short rotation periods, plantation in Ethiopia are totally depend on traditional management systems i.e. a “plant, clear fell and replant” cycle method or "coppicing" (Gezahgne, 2003; Teshome, 2011). Retention of trees in some structure classes for ecosystem maintenance and NTFPs production is not a common practice. This indicates that the relatively short rotation period of trees and their management practices may impact the biodiversity and sporocarp production of important fungal species (Paillet et al., 2010). On the other hand, as forests develop, changes in succession of the associated fungal species occur (Gassibe et al., 2011; Luoma et al., 1991; Smith et al., 2002). Thus, the fungal species could be early-stage, which develop from the spore bank present in the soil before the development of the stand, and late-stage fungi, whose fruiting is enhanced by the new conditions (Hernández-Rodríguez et al., 2013). Suck fungal succession following stand developments has been previously studied (Fernández-Toirán et al., 2006; Gassibe et al., 2015, 2011; Mediavilla et al., 2014). They reported an increase of richness and sporocarp productions following stand development in Mediterranean ecosystems, where the rotation period is typically long. However, such impacts are also yet understudied in Ethiopian plantation forests where the rotation is short.

Finally, fire and forest development affect soil parameters which are deeply linked to the fungal communities compositions. Indeed, fungal species composition in the forest tends to be correlated with some edaphic variables (Straatsma et al., 2001), especially saprotrophic fungi which are more dependent upon their respective substrates than mycorrhizal fungi (Reverchon et al., 2010). The change in soil physical, chemical and biochemical properties (Bastias et al., 2006; Longo et al., 2011) could have also an effect on the composition of fungal communities.

Therefore, in this thesis, we aimed to compile and assess the existing literature on mushroom resources in Ethiopia, but also, to conduct field systematic studies to know fungal diversity and production according to stand development and fires effects in plantations and natural forests respectively. Then, the provided compiled information (Studies I &II) may serve as a basis for further investigations into the resources and it could also contribute to the identification of the most important and widely used species among the different rural communities in a country.

Figure 5: *Pinus patula* plantations of different age classes in the study area, Wondo Genet (Ethiopia)

Similarly, the field observation (Studies III, IV & V) could provide basic knowledge on fungal succession following fire and along the development of plantation stands, and the influence of soil variables on fungal communities. This information may also be a means to understand how to improve fungal natural richness and production in both forest systems in Ethiopia. Focusing specifically on the implications of these factors for fungal diversity will help to facilitate conservation of economically and ecologically important mushrooms in the country. Furthermore, the information generated from plantation forests might also help to compare and provide basic knowledge about the fungal succession in relation to areas with longer gestation periods, and thereby help to suggest alternative plantation management techniques in the country.

1.6. Scope of the study

To our knowledge, this work represents the first compilation of the available information about wild mushrooms, their uses and cultural practices in the country. Also, this research is the first systematic attempts focused to describe the fungal communities' structures and sporocarp production in natural and plantation forest systems in the country. The field studies were based upon observations from sporocarps collections during the main rainy season, to describe the diversity of fungi, assuming that sporocarp production reflects the relative abundance and importance of fungal species (Vogt and Bloomfield, 1992) in the studied forests. However, this approach needs to be further completed with a longer time sampling period and even including an analysis of the below-ground fungi community structures. As a preliminarily, the information generated from the reviews can serve as a basic document for further mycological studies in the country and the reached findings from field observation are also must be regarded as an indication due to sampling limitations. However, the results provide a starting place in broadening management objectives for NTFPs in the forest systems of the country.

2. Objectives of the thesis

The overall aim of this thesis was to generate baseline information about mushrooms in Ethiopia, through both the review of literature and the report of fungal communities from field systematic studies. Thus, this general goal was assessed through the following specific objectives;

- To provide all the existing information about wild mushrooms in Ethiopia (Studies I & II).
- To assess the fungal taxa richness, diversity and sporocarp production after fire in Dry Afromontane forests in Ethiopia (Study III).
- To study fungal diversity and succession following stand development in *Pinus patula* plantations in Ethiopia (Study IV).
- To study fungal communities in *Eucalyptus grandis* plantations in relation to age groups in Ethiopia (Study VI).

3. Material and methods

3.1. Data sources

This thesis is based on literature reviews (Studies I & II) and field observations (Studies III, IV & V) to compile the information necessary to describe the ethnomycology and fungal biodiversity in the country. Both review of literature and field studies were carried out for the purpose. The review made include all available information i.e. published and unpublished secondary sources such as reports, journals and books. They are used to compile the information about the context of wild mushrooms in Ethiopia (Studies I & II). All sources are duly cited in the text where appropriate. On the other hand, the primary sources included field data collection (Studies III, IV & V).

3.2. The study area

The literature review (Studies I & II) was carried out including all the information from any region in the country. The field studies (Studies III, IV & V) were conducted at Wondo Genet forest area, where the remnant Dry Afromontane natural forests and large plantations of non-native tree forests are found. Wondo Genet is located in Southern Ethiopia about 265 km from Addis Ababa, the capital city of Ethiopia (Figure 6). Geographically it is located at 7°06' – 7º07'N latitude and 38°37'– 38 º42'E longitudes with an altitudinal range between 1,600 and 2,580 m a.s.l. (Fenta, 2014). The climate of the study area is characterized by Woyna Dega agro-climatic type. The rainfall pattern is bi-modal, with minor rainfall during spring and the major rain season during summer. The average annual rainfall which peaks in July, and the temperature is 1210 mm and is 20°C respectively (Fenta, 2014; Kebede et al., 2013). The topography is slightly undulating. The soils are young and of volcanic origin, characterized by well-drained loam or sandy loam (Eriksson and Stern, 1987), and are shallow on steep convex slopes but deeper at lower altitudes with a pH average value of 5.7 (Zewdu and Högberg, 2000).

The natural forest of the study area is estimated about 797 hectares of lands (Fenta, 2014), characterized by remnant Dry Afromontane forest types and is home to important fauna and flora (Dessie, 2007). The tree species such as *Juniperus procera*, *Albizia gummifera*, *Afrocarpus falcatus*, *Bersama abyssinica*, *Prunus africana*, *Podocarpus falcatus*, *Cordia africana*, *Croton macrostachys* and *Olea africana* are mainly characterizing the natural forests of the study area (Bekele et al., 2013; Fenta, 2014; Kebede et al., 2013). The natural forest is severely threatened by heavy anthropogenic disturbance and has declined from a 16% catchment land base to 2.8% within the past three decades alone, mainly driven by the expansion of small-scale agriculture, commercial farms, and logging (Kebede et al., 2013). Current land use is predominantly small-holder agriculture with an average landholding size of less than one hectare per household (Kebede et al., 2013). Forest fires are also recurrent phenomenon occurring yearly in small patches in natural forest of the study area and thereby affect the forest biodiversity. The human induced fires are known to be the main causes for forest fire in the study area (Ango and Bewket, 2007; Bekele et al., 2013; Teshome, 2011).

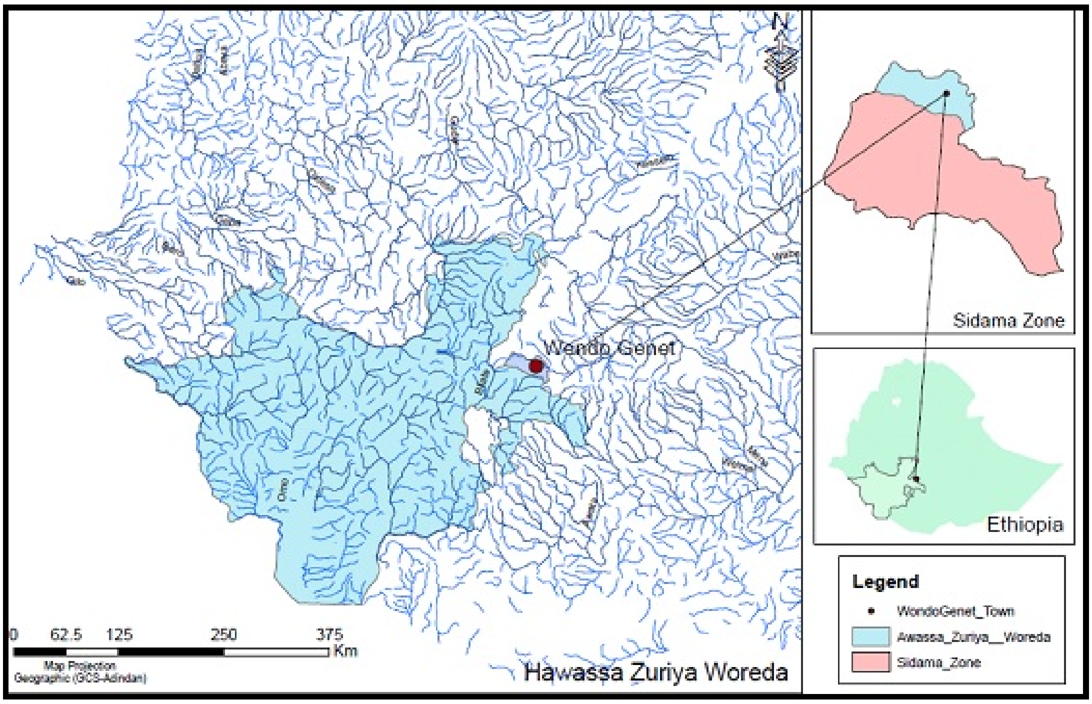

Figure 6: Location map of the study area, Wondo Genet

Plantation forests of fast growing non-native tree species are also the other forms of forests in the study area. About 100 ha of plantations of different tree species are found as commercial plantation forests (Zewdie et al., 2010). The major tree species predominantly planted include *Cupressus lustianica*, *Gravilla robusta, Pinus patula* and *Eucalyptus* spp. (Teshome, 2011). These plantation forests are subjected to common silvicultural practices such as weeding, slashing, pruning and thinning. The rotation period for most of the tree species is short and they are managed using a “clear cut and replanting” method except coppicing is the preferred management technique for *Eucalyptus* species (Teshome, 2011). Among the exotic tree species, *P. patula* and *E. grandis* were selected for our purpose as they are dominantly occupying the plantation areas of our study area.

3.3. Field plots establishments (Studies III, IV & V)

In order to achieve our objectives, we established study plots in Dry Afromontane natural forests and plantations of *P. patula* and *E. grandis* forests of the study area. The plots were established taking into consideration the similarity of the areas in terms of ecological conditions such as climate, altitude, and soil in both forest systems. Information from the Department of Forest Management in Wondo Genet College of Forestry (WGCF) was used to find areas with similar fire history in natural forests and stand ages classes in plantations in order to establish the plots. Within each of the selected areas, plots were placed systematically (Luoma et al., 1991) far enough from each other in order to provide relatively independent estimates as possible (Buscardo et al., 2014; Dias et al., 2010; Lilleskov et al., 2004). Thus, the works in this study should be considered as case studies since the plots were established in a similar stands for each forest types, and conclusions regarding other studies need to be taken with caution.

3.3.1. Plot establishment in Dry Afromontane forests to study fungal community succession and sporocarp production after fire occurrence (Study III)

For this purpose, the burned and unburned natural forest patches were selected. The unburned (control) areas were areas representative of the original natural forest not affected by fire. Burned areas were patches of forests affected similarly by high fire severity, with canopy and understory burned, and the soil organic layer consumed (Rincón and Pueyo, 2010).

Three areas could be clearly differentiated: (1) unburned natural forest area, hereafter UB area, (2) one-year-old burned forest area, hereafter B-1 area (3) ten-year-old burned forest area, hereafter B-10 area.

A total of nine sample plots i.e. three in each area, established as described in Gassibe et al. (2011) and Hernández-Rodríguez et al. (2013). Each plot covered an area of 100 m^2, with a rectangular shape (2 x 50 m). Within each of the selected areas, plots were established far enough from each other about 250 m. Differences in fungal diversity and productivity among areas prior to fire were thus unlikely.

3.3.2. Plot establishment in *Pinus patula* and *Eucalyptus grandis* stands to study fungal diversity and succession following stand development (Studies IV & V)

For this purpose, three different *P. patula* stands, i.e., 5-, 11- and 36-years-old stands, and three *E. grandis* stands, i.e. 10-, 19- and 37-years-old stands, were selected based on their ages. The main stand characteristics of both plantations are summarized (Table 1 and Table 3 in Study IV & V). Following previous studies, data were collected by using transect methods (Luoma et al., 1991; Smith et al., 2002). Within each of the selected stands, three plots with an area of each 2 x 50 m (100 m^2), were established based on the methods used by Gassibe et al. (2011) and Hernández-Rodríguez et al. (2013). Plots within stands were placed systematically far enough about 120 m from each other.

3.4. Sampling

3.4.1. Sporocarp sampling (Studies III, IV & V)

The sporocarps were collected during the major rainy season between July and August in 2015. The sporocarps sampling were conducted once in a week as described in Gassibe et al. (2011) and Hernández-Rodríguez et al. (2013). During sampling, the sporocarps were fully harvested from each plot in order to facilitate fresh weight measurements and taxa identifications. Fresh weight measurements were carried out *in situ* and the data are given in kilograms per hectare per year (kg fw/ha/year). Also, abundance, the number of sporocarps per species, data was taken from each plot as the fruit bodies were collected. Sample fruit bodies from each species were taken to the laboratory and dried. Herbaria specimens were used for molecular and microscopic taxa identification. Furthermore, in the field, specimens were photographed and their ecological characteristics were noted in order to assist and facilitate taxa identification processes.

3.4.2. Soil sampling for edaphic analysis (Studies III & IV)

To relate taxa composition to explanatory edaphic variables, soil samples were taken from each study plot established in both forest systems. Composite soil samples, from the center and the four corners of each plot, were taken by clearing plant matter and debris from the surface. The soil was extracted to a depth of 20 cm with the aid of an auger and spade. Then the samples were mixed thoroughly, and approximately 500 g of soil was finally taken in a plastic bag for laboratory analysis. After air drying of the soil in shade, important soil chemical and physical properties were determined using the test methods of DTPA extraction, KH_2PO_4 extraction, Olsen, Kjeldahl digestion Walklay Black, Ammonium Acetate and instrumental respectively. The analysis was conducted in Water Works Design and Supervision Enterprises, laboratory service sub process, soil fertility section at Addis Ababa, Ethiopia. Main edaphic variables for the natural forest (Table 1 in Study III) and *P. patula* stands (Table 2 in Study IV) are summarized.

3.5. Taxa identification and classification (Studies III, IV & V)

Both morphological and molecular feature analysis were used for taxa identification. Taxonomic classification was aided by close microscopic examination of tissues and spores with an Optika B-350PL microscope. Small samples of dried specimens were re-hydrated and mounted in 5% KOH. The following keys were mainly used for the purpose: Heinemann (1956); Singer (1965); Pegler (1968), (1969); Pegler (1977); Morris (1990); Rammeloo and Walleyn (1993); Ryvarden et al. (1994); Antonin (2007) and Hama et al. (2010). Specimens were deposited in the laboratory herbarium at the University of Valladolid. Up-to-date fungal taxa names and authors' names were obtained from Mycobank database (http://www. mycobank.org).

Molecular identification involved sequencing of the ITS region of the nuclear ribosomal genes (rDNA). For this, fungal DNA was extracted from dry sporocarps using an EZNA® Plant DNA kit (Omega Bio-Tek, USA) according to the manufacturer's instructions. Final elutions were done in a total volume of 100 µl. The internal transcribed spacer (nrITS) was amplified with primers ITS1F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990).

Taxa edibility classification was accomplished by adapting the criteria used by Bonet et al. (2004). If the taxon is described in the literature as both non-edible and edible, we classified it as a non-edible. If the taxon is described in the literature as having doubtful edibility, we classified it as a non-edible. As edible (E) are classified all species that are listed as such in the large majority of the literature consulted.

3.6. Statistical analysis

Shannon’s H’ diversity index (Shannon and Weaver, 1949) was estimated for each plot (Studies III, IV, V) using the following formula, where pi indicated the relative abundance of each macrofungal taxa.

$$H = -\sum Pi(lnpi)$$

"Richness" (number of taxa), was defined as the total number of species found per plot.

Richness, Shannon index and Fresh weight (for the edible and total taxa) estimates were subjected to one-way ANOVA analysis and a post-hoc least square means difference test (LSD, $P \leq 0.05$) in order to test for differences among areas. Data were log-transformed when needed to achieve the parametric criteria of normality and homoscedasticity that ANOVA requires. All analyses were done with SAS software (SAS Institute Inc., 2012) (Studies III, IV & V).

Fungal species accumulation curves were also constructed for *P. patula* and *E. grandis* stands to compare the rate at which new fungal species are weekly found among the different age classes and to provide an estimate of species richness (Studies IV & V). The curves were generated using sample based estimator of EstimateS Version 9 (Colwell, 2013).

The Rényi diversity profile (Tóthmérész, 1995), which depends upon a parameter alpha, was used to depict the diversity curves of the three stands in *E. grandis* plantations (Study V). For alpha=0, this function gives the total species number, alpha=1 gives an index proportional to the Shannon index. PAST software was used to plots the diversity profiles (Hammer et al., 2001).

An ordination technique based on fungal fresh weight data was used in order to identify significant edaphic explanatory variables related to taxa composition (Studies III & IV). Firstly, the fresh weight data per taxa were subjected to a Detrended Correspondence Analysis (DCA) (Ter Braak and Prentice, 1988). We used a Redundancy Analysis (RDA) (Study III) and Canonical Correspondent Analysis (CCA) (Study IV) based on the extracted gradient lengths (Ter Braak, 1986). Forward selection was used to select significant explanatory variables and only those significant at $P<0.05$ levels were included in the models. The statistical significance of the

Canonical axes was evaluated by Monte Carlo permutation tests (499 permutations). In order to test for differences in fungal species composition and abundance amongst areas differing in successional stage in *E. grandis* (Study V), we also performed a detrended correspondence analysis (DCA) (Ter Braak and Prentice, 1988). The analysis was conducted using CANOCO for Windows v.4.5 (Ter Braak and Šmilauer, 2002). The results were displayed by ordination diagrams drawn with Cano Draw 4.1 (Ter Braak and Šmilauer, 2002).

4. Results

4.1. The context of wild mushrooms in Ethiopia (Studies I & II)

Based on the review that summarizes issues related to wild mushrooms in Ethiopia, the most important scholar references in indigenous forests come from the comprehensive works of Hjortstam and Ryvarden (1996) who reported fifteen *Corticiaceae* species. Decock et al. (2005) also reported a total of four taxa from the highland forests regions. Some other taxa have also been reported by Tuno (2001); Abate (2008), (2014); Alemu (2013); Muleta et al. (2013); Sitotaw et al. (2015a) from different parts of the country. Most recently, Alemu et al., (2012) and Megersa et al. (2017) reported a number of fungal taxa from Western dryland forests and Degaga natural forests respectively (Table 1 in Study I).The taxa reported by Alemu et al. (2012) were provided only in their local names. On the other hand, Abate (1999), (2014); Alemu (2013) and Sitotaw et al. (2015a) also listed some taxa from highland grazing areas (Table 1 in study I). The genus *Agaricus* was the dominant so far reported in most of these references from grazing areas in Ethiopia. The *Termitomyces* fungi associated with termite mounds are also reported from the lowland areas of the country (Table 1 in study I), where termite mounds are more abundant (Abate, 2014; Muleta et al., 2013; Sitotaw et al., 2015b). In plantation forests, the existence of mycorrhizal mushrooms originated from Mediterranean and Temperate climates associated with non-native trees also reported (Megersa et al., 2017).

In spite of the poor ethnomycological notes, Tuno (2001) described the traditional use of wild mushroom by the "*Majangir*" ethnic groups as a subsidiary food gathered from forests in Southern region of Ethiopia. Muleta et al. (2013) and Abate (2014) also documented such traditional culture by the "*Kaffa*" ethnic group from the south region in the country. Furthermore, a noticeable interests on wild edible mushrooms also reported from Bonga area (Muleta et al., 2013) and Benihsnagul Gumz región (Alemu et al., 2012). Interestingly, the traditional medicinal uses of mushrooms are also reported by Abate (2014) from the "*Kaffa*" ethnic groups in Southern region. Among other species, the *Laetiporus sulphureus* reported commonly used for lessening childbirth by the "Kaffa" people in Southern part of Ethiopia (Abate, 2014).

The most important published reports on wild edible and medicinal mushrooms (Table 1 in study II) in Ethiopia comes from those works of Abate (1999), (2008), (2014); Tuno (2001); Alemu (2013); Muleta et al. (2013) and Sitotaw et al. (2015a). Wild edible mushrooms are also available in some local markets (Abate, 2014), where they are sold by the local people to earn some income to supplement their household economy. For example, in Bonga and Assosa, mushrooms are available in association with other vegetal products. *Agaricus* spp., *Laetiporus* spp., and *Termitomyces* spp are among the most available species in local market (Abate, 2014).

The practice of mushroom cultivation is a recent activity in Ethiopia, mostly restricted to urban areas (Abate, 2014; Yehuala, 2008). In spite of the limitations such as lack of awareness, lack training on cultivation of mushrooms, locally available substrates have been used at a small scale to produce three most commonly cultivated mushrooms: *Agaricus bisporus*, *Lentinula edodes*, and *Pleurotus ostreatus* (Yehuala, 2008).

Many threats affecting wild mushrooms are similar to those that globally affect the biodiversity in Ethiopia (IBC, 2014). The most important of all, deforestation, comes as a consequence of anthropogenic change to which global environmental and climate change also add (Lulekal et al., 2011). According to Teketay (2001), deforestation is immense and estimated between 150000 – 200000 ha of land per year. This adversely influences the macrofungi and diminishes their diversity and production (Miller and Lodge, 1997). Such impact also limits the benefits that can be obtained from fungal resources from the forests. Thus, urgent conservation strategies and actions are needed, giving special consideration to those species currently used by the local communities.

4.2. The effect of fire (Study III) and stand age (Studies IV & V) on fungal richness, diversity and production

A total of 61, 53 and 29 macrofungal taxa were collected from the Dry Afromontane natural forests (Table 2 in study III), plantation of *P. patula* (Table 3 in study IV) and *E. grandis* (Table 2 in study V) forests respectively in our study area. All of the collected taxa are belonged to *Basidiomycota,* except *Xylaria hypoxylon* (L.) Grev, which is in the division *Ascomycota*. Majority of the taxa collected are saprophytic, only 6% of the taxa collected in *P. patula* plantations are ectomycorrhizal fungi. The distribution of the taxa in families and genera are presented (Figure 2 in studies III, IV and IV).

In evaluation of the effect of fire on taxa richness, we found fire had a significant effect on fungal taxa richness in Dry Afromontane forests ($P<0.0001$). The highest richness value was recorded for the unburned area. The lowest value was recorded for the area most recently affected by fire, showing significant differences with the other burned and unburned areas (Figure 3A in study III; $P< 0.001$). There was differences between the B-10 and UB areas in their richness values (Figure 3A in study III; $P=0.003$). The Shannon's H´ diversity index also followed the same trend as in richness vale in this forest system (Figure 3B in study II).

Plantation age also showed significant differences in taxa richness in our study area ($P=0.001$). In both *P. patula* and *E. grandis* plantations, the highest richness was found in older stands (Figure 3A in study IV and V), indicating an increasing trend towards the more matured stands. The Shannon's H´ diversity index also followed the same trend as in richness vale in both plantation forests (Figure 3B in study IV and V).

Concerning production, in the Dry Afromontane forests we found no significant difference in total sporocarp yields between the burned and unburned areas ($P= 0.214$, Figure 4 in study III). However, the smallest yield was obtained from the recently burned area while the highest is from the ten years old burned area (Figure 4 in study III). In *P. patula* plantations, we found significant differences in mean total sporocarp production ($P=0.007$) among the three age class. The lowest yield was obtained from the 5-year-old stand (7.53 kg ha-1yr-1), which was significantly different from those of the 11- and 36-year-old stands (Figure 4 in study IV, $P_5 - P_{11}=0.017$, $P_5 - P_{36} = 0.003$). However, the 36- and 11-year-old stands showed no significant difference in their total sporocarps production (Figure 4 in study IV, $P = 0.051$). In *E. grandis* a significant highest fresh weight production was collected from 37-year-old stands (Figure 4 in study V; P_{AG37}-P_{AG19}=0.038, P_{AG37}-P_{AG10}= 0.017) while no differences were found between 19- and 10-year-old stands.

Considering edible species separately, we found the same trend as in total sporocarp production from the Dry Afromontane natural forests (Figure 4 in study III). In *P. patula* and *E. grandis* plantations, the average edible sporocarp yield showed an increasing significant trend ($P<0.001$) following the stand ages in both forests systems (Figure 4 in studies IV and V). In terms of number of edible species, the lowest edible specie was obtained from *E. grandis* (Table 2 in study V) while the highest is from the Dry Afromontane forests (Table 2 in study III).

4.3. Taxa composition (Studies III, IV & V)

Regarding taxa composition, the fungal community assemblies among the three areas in the Dry Afromontane forests can be analyzed from the results obtained in the Detrended Correspondence Analysis (DCA) (Table 3 in study III). The results are displayed in ordination bi-plots (Figure 5 in Study III), where a total of three edaphic variables such as Organic matter, Ca and P were found to be significantly related to the saprophytic fungal taxa composition in the ordination ($P < 0.05$, Table 4 in study III). The model was significant according to Monte Carlo permutation test for the first axis ($P = 0.002$, $F = 3.372$) and for all canonical axes ($P = 0.002$, $F = 2.158$). In this case, axis one was negatively correlated with all the three edaphic variables while axis-2 was positively correlated with them (Figure 5 in Study III). The eigenvalues also indicated that the variability in terms of taxa composition, explained by the gradients associated with the first two axes is about 50.7% of the accumulative variance of fungal taxa data, and an accumulative variance for the interaction between fungal taxa and environment of 89.8% (Table 3 in study III).

The CCA (Figure 5 in Study IV) and DCA (Figure 5 in Study V) revealed that there are clear differences between fungal communities among the three stands of age class both in the *P. patula* and *E. grandis* plantations respectively. According to the forward selection process, three edaphic variables were significantly ($P < 0.05$, Table 5 in study IV) correlated with the fungal taxa composition in *P. patula* stands. The model was significant according to Monte Carlo permutation test for the first axis ($P = 0.002$, $F = 2.683$) and for all canonical axes ($P = 0.002$, $F = 3.533$). In this case, axis one was positively correlated with Nitrogen (N), and negatively correlated with pH and Potassium (K). Axis two also correlated positively with all the three significant edaphic variables i.e. N, K and pH (Figure 5 in study IV).

5. Discussion

5.1. The context of wild mushrooms in Ethiopia (Studies I & II)

Pegler and Rayner (1969) and Pegler (1977) noted that the East Africa region that includes Ethiopia is rich in macrofungal species. Many of these species are presumed to be either cosmopolitan or to be widespread across Africa (Munyanziza, 1994; Pegler, 1977). However, most regions and habitats of fungi in Ethiopia have been seldom studied. Furthermore, reports regarding fungal diversity and list of important species are rarely exist (Megersa et al., 2017; Sitotaw et al., 2015b). This is due to a lack of research infrastructures as well as to a lack of fungal taxonomists and specialists in fungal ecology. Finally, and likely as a result, fungi are not included in the biodiversity database of the country (IBC, 2014). The poor knowledge on fungal flora in Ethiopia is also worrying as fungi are highly sensitive towards habitat disturbances, namely anthropogenic threats that are rife across the country (IBC, 2014). The data generated from the review of existing literature provided basic information about fungal flora in the country that will be helpful to create awareness for conservation, a wider use of mycological resources and also serve as baseline information for further mycological studies in the country. The diversity and availability of fungal species reported (Table 1 in studies I & II) from different habitats might be a lot richer than what has been so far reported. Thus something that further complementary study could confirm.

Despite poor information on fungal resources, ethnomycology has been a traditional common practice among the local tribes in Ethiopia (Tuno 2001; Semwal et al. 2014). Mushrooms have been used for their nutritional, traditional and medicinal properties (Abate, 2014; Tuno, 2001). However, the ethnomycological and socioeconomic experiences were not assessed for majority of the ethnic groups, indicating the information contained is inadequate and lack to convey the full picture of the country, as Ethiopia is characterized by many communities that might have long history of wild mushroom usage with wider traditional knowledge on the use. Thus, more studies and documentations are important to reveal the human mushroom relationship, and the traditional experiences in depth in the country.

Mushroom cultivation practice in Ethiopia is still very low and limited numbers of species have been cultivated commercially. Owing to their flavor and nutritional value, the consumption of cultivated mushrooms is now constantly increasing in the urban area. However, lack of mushroom cultivation skills still leave plenty of room for improvement and growth (Muleta et al., 2013; Yehuala, 2008). The conservative eating habits, on the other hand, are also hindering the transfer of cultivation technology at a local level, particularly in areas where mushroom consumption is not a common practice. Thus much has to be done to promote the cultivation and wider use of mushroom in the country, taking in to consideration those commonly collected and used edible wild mushrooms by the local communities in different part of the country. Furthermore, more efforts have to be done to widen the cultivation in large scale production system through mycorrhization of plantation trees.

5.2. The effect of fire (Study III) and stand age (Studies IV & V) on fungal richness, diversity and production

Reports on macrofungal species from Ethiopian forest systems are very limited. This study is the preliminarily attempts explaining fungal community compositions in Dry Afromontane natural forest system and plantations of *P. patula* and *E. grandis* forests in the country. The taxonomic classification in the present study was challenging, as from the collected taxa about ~45% could only be identified to the genus level despite our best efforts. This was an indication of both the uniqueness of particularly the Dry Afromontane forest ecosystems in terms of diversity of yet undescribed macrofungi species as well as the lack of scientific studies on the local fungal flora. Previous studies reported fungal assemblages in Ethiopian habitats (Alemu, 2013; Decock et al., 2005; Hjortstam and Ryvarden, 1996), however, none of these reported ECM fungi from the natural forests systems. This is not surprising as most tropical woody tree species are unable to form associations with ectomycorrhizal fungi (Brundrett, 2009). Thus, we believe this to be the first report on ECM fungi from plantation forests in Ethiopian and it presented important ramification of the non-native tree forests for the maintenance of functional fungal diversity in Ethiopia.

Forest fires have typically both short and long-term effects on fungal communities. As a short-term effect, fire causes a reduction in richness (Kutorga et al., 2012) and as a long-term effect, fire also causes a shift in the presence or relative frequencies of fungal species in the forest system (Rincón and Pueyo, 2010). In this study, we also observed an immediate negative effect of fire on fungal taxa richness since a significant decrease was observed in recently burned area in the Dry Afromontane forests. This negative effect could be associated with the reduction of substrates in the forest floor after fire (Smith et al., 2008) and also due to the negative effect of fire on organic matter deposited in the soil depending on its intensity, leading to indirect effect on fungal growth and perpetuation (Kennedy et al., 2014). The loss of topsoil by erosion after fire could also reduce the infectivity of fungal propagules (Rashid et al., 1997), and thereby impacted taxa richness immediately following fire.

The trend of fungal taxa richness in plantation forests was towards more matured stands. This trend is in agreement with other studies which demonstrated mature stands could favor more macrofungal species and thus higher richness (Fernández-Toirán et al., 2006; Gassibe et al., 2015, 2011; Kranabetter et al., 2005; Mediavilla et al., 2014; Visser, 1995). This might be explained due to the suitability of the environments created along the development of the stands. Although our plantations existed in close vicinity, they were isolated stands at different stages of forest development. Thus the difference in relative microhabitat variation created in each stand following canopy closure (Crabtree et al., 2010; Dove and Keeton, 2015; Oria-de-Rueda et al., 2010), i.e. relatively high humidity and organic matter accumulation, may enhance the high occurrence and fructification of fungi particularly towards the higher age classes. This assumption coincides with Dighton et al. (1986); Smith et al. (2002); Sysouphanthong et al. (2010) who noted higher fungal richness in well-developed stands having higher canopy closure. This difference also further reflected in the diversity value of the three stands. The Shannon diversity values also showed increasing trend as in richness values towards more matured stands. This might be due to the disproportional distribution of taxa that appeared in each successional stage in *P. patula* plantations (Hernández-Rodríguez et al., 2013).

The negative effect of fire on fungal sporocarp production has been investigated in previous studies by Hart et al. (2005); Bastias et al. (2006); Cairney and Bastias (2007); Hernández-Rodríguez et al. (2013); and Mediavilla et al. (2014) from different geographical areas. Post fire fruiting and the relative effects of fire on fungi fruiting, with a special emphasis on the saprophytic species also deeply reviewed (Taudière et al., 2017). In our study, lower fungal production was also collected from recently burned areas in the Dry Afromontane forest of our study area. However, such difference was not significantly different between the burned and unburned areas. The absence of differences was previously reported by Mediavilla et al. (2014) who studied the effect of fire on saprophytic species associated to *Pinus nigra* stands in the

Mediterranean. This could be explained by the existence of fungal species whose ephemeral fruit bodies may cover forest soil in recently burned areas (Hart et al., 2005; Taudière et al., 2017), and produces more biomass taking advantage of the condition created (Bean et al., 2009). For example, *Armillaria* sp. was fruited most abundantly in a recently burned area as compared to other fungal species. This species might survive in the early stage of after fire and accumulate biomass either by persisting on remnant plant bodies or uses other organic matter after fire (Bonello et al., 1998). Furthermore, the quick recovery rates of some plant species like *Cordia africana*, an early colonizer in forest rehabilitation in the burned areas might also contribute to the quick accumulation of organic matter in the soil, which in turn benefits saprophytic fungi (Bonello et al., 1998).

Similarly, in plantation forests the highest total mushroom fresh weight was obtained from older stands while the lowest was from younger stands (Figure 4 in studies VI & V), indicating that sporocarp yield also increases towards the more matured *P. patula* and *E. grandis* stands. According to Oria-de-Rueda et al. (2010) this kind of increasing trend can be related to the different availability of substrata along the stand's development. As a forest stand matures, the humus layer develops (Dove and Keeton, 2015; Pinna et al., 2010; Toivanen et al., 2012) and the forest soil increases its capacity to buffer temperature and moisture. Such conditions could enhance more fungal growth and fruiting, especially saprotrophic fungi (Fernández-Toirán et al., 2006). Apart from the suitability of the environment created along the stand ages, such increasing association of a relative higher yield in older stands might be explained in terms of the abundance of some taxa like *Agaricus* sp., *Agrocybe* sp., *Coprinopsis* sp., *Hygrophoropsis aurantiaca*, *Leucoagaricus rubrotinctus*, and *Tylopilus niger*.

5.3. Taxa composition (Studies III, IV & V)

Distinctive fungal communities were observed between the burned and unburned areas in the study forests. The effect of fire on taxa composition was observed and they formed a distinctive fungal community in the ordination (Figure 5 in Study III). The non-litter decomposer fungal species are more favored and exclusively found in the recently burned area. This is likely the case for the species of *Favolaschia calocera* and *Hygrophoropsis aurantiaca*. These species are reported commonly fruit in areas disturbed by human activities such as in burned forests (Smith and Read, 1977; Vizzini et al., 2009). This highlighted the perturbative effects that fire has on both the type and number of species, only those able to resist or adapt to the new conditions after fire in the forest (Greeshma et al., 2016). Distinctive fungal communities were also observed among the three age groups in *P. patula* and *E. grandis* plantations (Figure 5 in studies IV & V). More taxa were found towards the more matured stands in both plantations. This probably was due to the positive effect of stand development that can be expressed in terms of age, which could harmonize high soil humification and a thicker litter layer along stand development (Mediavilla et al., 2014; Pinna et al., 2010; Toivanen et al., 2012) which is more suitable for the fructification and development of fungal species.

It is well established that fungal communities as a whole are significantly influenced by edaphic variables (Straatsma et al., 2001; Zakaria and Boddy, 2002), although information about particular species is scarce. This is because soil nutrients have been shown to affect mycelial development and hence sporocarp occurrence (Zakaria and Boddy, 2002). In the present study, there seems to be cumulative effect of edaphic variables on the composition and distribution of macrofungi in the Dry Afromontane natural forests. The soil Organic matter (OM), Phosphorous (P) and Calcium (Ca) were found to correlate significantly with the fungal taxa composition. Among these edaphic variables, OM appeared to be the most important factor related to saprophytic fungal composition (Figure 5 in study III). This is because fungi typically extend their mycelia at the soil-litter interface (Boddy et al., 2009) and thereby the organic matter influences mycelia outgrowth and network formation (Zakaria and Boddy, 2002). Organic matter also influences the fungal community through its impact on water holding capacity and nutrient availability in the soil (Harrington, 2003). Thus, organic matter could favor more saprophytic fungal assembly in an area. On the other hand, edaphic variables such as nitrogen (N), Potassium (K), and pH were found to be correlated significantly with the fungal taxa composition in *P. patula* stands (Figure 5 in study IV). The result was in agreement with those of Gassibe et al. (2015) who noted the correlation of edaphic variables with fungal taxa composition in *Pinus* plantations forest systems. Among these elements, nitrogen can influence the formation of extra radical mycelium in the soil, and play a vital role in sporocarp formations (Trudell and Edmonds, 2004). Furthermore, K is also important for the growth and sporulation of fungi (Nonzom and Sumbali, 2014), thus potentially can shape the fungal community composition.

Soil pH is the other edaphic variable we found correlated with fungal taxa composition in *P. patula* plantations. A higher occurrence of taxa was observed around lower pH values and it seemed that pH had an effect on fungal community composition in our study area. This coincides with the findings of Puangsombat et al. (2010) who noted that the number of fungal species tends to be higher in areas where pH value is low. However, some taxa like *Infundibulicybe mediterranea*, *Cyptotrama asprata*, *Gerronema hungo*, *Lepiota cristata* and *Marasmius* sp showed exceptional ordination towards the higher end point of the pH gradient. This might be associated with their adaptability of the species to higher pH values in the soil.

6. Conclusions

1. The results from the review papers highlighted the existence of valuable wild mushrooms and their ethnomycological uses in Ethiopia. This analysis indicates a lack of information to have an adequate description of mycological resources in the country. Most of the studies conducted so far were focused on just a very limited number of species and on the ethnomycology of few ethnic groups in the country. Some of the taxa reported in some of the literatures are even not properly identified as they are only registered with their local names. This indicates the need to accomplish adequate scientific studies through examination of different habitats in order to glimpse the existing diversity of fungal species and the indigenous knowledge associated with them across the country.
2. According to our review, there are many options to widen the cultivation of mushroom at small and large scales in the country. On one hand, homestead cultivation is possible using locally available substrates. On the other, mycorrhized plantations inoculating demanded taxa such as *Boletus pinophilus*, *B. edulis* and *Lactarius deliciosus* can also provide opportunities to introduce appreciated mushroom species and they can be used for large scale mushroom cultivation purposes. Also, wood waste could be used to cultivate important medicinal species as *Pleurotus ostreatus*, *Ganoderma lucidum* and *Coriolus versicolor*. Thus, adopting and scaling up of such technologies may offer incentives for widening mushroom cultivation practices and thus this could also be a major future research in plantation forest areas in the country.
3. In our study, we also provided data on the occurrence of macrofungi species related to successional stages after fire in a Dry Afromontane forest of Ethiopia. The forests are characterized by a notable diversity of saprophytic macrofungal taxa, likely including many taxa still not known to science as well as several edible species. We could also observe that fire strongly affected to fungal richness and diversity. However, fungal community was recovered in a short period after the fire when we could also find the highest edible species production. Although the result obtained is preliminary, it represents the first systematic investigation of fungal communities and sporocarp productions in natural forest systems in Ethiopia. The result provided a starting place in broadening forests management objectives, which should involve both the production of timber and NTFPs, taking in to account edible wild mushrooms production as one of the NTFPs.

4. We also attempted to describe the fungal communities and their succession in plantation forests systems in relation to their age in Ethiopia. So far, only saprophytic species have been reported from forest systems in the country. We found EM fungal species in matured *Pinus patula* stands that could increase the fungal functional group diversity in plantation forest systems in Ethiopia. The result also showed matured *P. patula* and *E. grandis* stands provided the highest fungal diversity as compared to the middle and young age groups. The oldest stands were also characterized by higher sporocarp productions, particularly in *P. patula* stands, almost half of it corresponding to edible taxa. Thus, leaving patches of matured trees through tree retention management approach in plantation forests, could increase habitat availability, such as substratum for saprophytic fungi and live standing trees for mycorrhizal species after final rotation cut, and thereby provide higher fungal diversity and edible sporocarps production. This could serve as a means to deliver complementary incomes to rural population from mushroom as potential NTFPs while the mature trees could serve as a bridge for providing fungal inocula to the new plantations.

5. Conclusiones

1. Los resultados de los trabajos de revisión revelaron la existencia de valiosos hongos silvestres y su uso etnomicológico en Etiopía. Este análisis indicó la ausencia de información que permita tener una adecuada descripción de los recursos micológicos en el país. La mayoría de los estudios realizados hasta ahora se centran en un número muy limitado de especies y en la etnomicología de un número de grupos étnicos también muy reducido. Algunos de los taxones que aparecen reflejados en la literatura ni siquiera son apropiadamente identificados ya que sólo están registrados con sus nombres locales. Este contexto indica la necesidad de realizar estudios científicos adecuados mediante el examen de diferentes hábitats con el fin de describir adecuadamente la diversidad fúngica existente y poder asociar el conocimiento popular.
2. Según nuestra revisión, hay muchas opciones para ampliar el cultivo de hongos en el país a pequeña y gran escala. Por un lado, es posible el cultivo familiar utilizando substratos localmente disponibles. Por otra parte, las plantaciones micorrizadas con taxones tan demandados como *Boletus pinophilus*, *B. edulis* y *Lactarius deliciosus* pueden ofrecer también oportunidades para introducir especies apreciadas de hongos y pueden utilizarse para su producción a gran escala. Además, los restos de madera podrían utilizarse para cultivar especies medicinales importantes como *Pleurotus ostreatus*, *Ganoderma lucidum* y *Coriolus versicolor*. Por lo tanto, la adopción y ampliación de estas tecnologías puede ofrecer incentivos para ampliar las prácticas de cultivo de hongos y, en consecuencia, esto podría suponer también una importante línea de investigación futura en el país.
3. En este estudio, también se proporciona información sobre las especies de hongos relacionadas con etapas sucesionales después de incendios forestales en un bosque afromontano seco de Etiopía. Se ha observado una notable diversidad fúngica, fundamentalmente dominada por especies saprófitas, probablemente incluyendo muchos taxones aún no conocidos por la ciencia, así como varias especies comestibles. También se ha observado que el fuego afecta fuertemente a la riqueza y diversidad de los hongos existentes. Sin embargo, la comunidad de hongos se recuperó en un período corto de tiempo tras el incendio, momento en el que se pudo encontrar también la mayor producción de especies comestibles. Aunque el resultado obtenido es preliminar, este trabajo representa la primera investigación sistemática sobre comunidades fúngicas y sus producciones en sistemas forestales naturales en Etiopía. Este resultado proporciona un buen punto de

partida en la ampliación de los objetivos de manejo de los bosques, que debería incluir tanto la producción de madera como los PFNM, teniendo en cuenta la producción de hongos silvestres comestibles como un valioso recurso.

4. También se han descrito las comunidades de hongos y su sucesión en plantaciones de *Pinus* y *Eucalyptus* muy importantes en Etiopía. Hasta el momento, no se han encontrado referencias de especies micorrícicas en los sistemas forestales etíopes. Sin embargo, sí que pudimos observar hongos EM en rodales maduros de *Pinus patula* que podrían aumentar la diversidad funcional de hongos en estos sistemas forestales. Nuestros resultados también mostraron que las masas maduras de *P. patula* y *E. grandis* proporcionaron la mayor diversidad de hongos en comparación con las masas de edad media y joven. Los rodales más maduros también se caracterizaron por proporcionar mayores producciones fúngicas, particularmente en los rodales de *P. patula*, donde casi la mitad de los taxones eran comestibles. Por lo tanto, dejando rodales de árboles maduros siguiendo el sistema denominado “green tree retention” se podría aumentar la disponibilidad de microhábitats, proporcionando más substrato para hongos saprófitos y árboles en pie vivos para especies micorrizas después del corte de rotación final. Esto podría servir como un medio para proporcionar ingresos complementarios a la población rural a partir de los hongos comestibles. Además, los árboles maduros podrían servir como puente para proveer inóculo fúngico a las nuevas plantaciones.

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Original article I

Setas salvajes en Etiopía: Revisión y síntesis para perspectivas futuras

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Resumen

Objetivo del estudio: Revisar y proveer todo tipo de información con respecto a las setas silvestres de Etiopía y crear conciencia con respecto a la conservación y el uso de recursos micológicos

Area de estudio: El estudio se ha realizado principalmente el Etiopía, lugar en donde la información disponible sobre las zetas salvajes is escasa y su estatus es desconocido dada la degradación de hábitats naturales.

Resultados principales. Se revisaron todas las referencias bibliográficas relevantes con respecto a las zetas salvajes, nichos ecológicos, practicas de cultivoy especies cultivadas, así como los factores antrópicos que afectan la conservación de la diversidad fúngica.

Aspectos destacados de la investigación: Esta revisión resume temas relevantes para la diversidad de hongos silvestres, los principales nichos ecológicos, las especies de zetas, y las prácticas de cultivo de hongos en Etiopía. Por otra parte, se reportan amenazas y necesidades para la futura conservación de las setas silvestres en el país. Esta revisión puede tomarse como referencia para otros estudios micológicos en Etiopía, así como en otros países en vías de desarrollo con recursos micológicos similares.

Palabras clave: Diversidad; Nichos ecológicos; Etiopía; Cultivo de hongos; Hongos silvestres

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REVIEW ARTICLE

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Wild mushrooms in Ethiopia: A review and synthesis for future perspective

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Abstract

Aim of study: To review and provide all-purpose information about wild mushrooms in Ethiopia and to create awareness for conservation and use of mycological resources.

Area of study: We focused mainly on Ethiopia, where information about wild mushrooms is scanty and their status is unknown under the rampant degradation of the habitats.

Main results: We reviewed all relevant references related to wild mushrooms and their ecological niches, cultural practices and species used for cultivation as well as the anthropogenic factors affecting the conservation of fungal diversity.

Research highlights: This review summarizes issues related to the diversity of wild mushrooms, the main ecological niches and their associated fungal species, and mushroom cultivation practices in Ethiopia. Moreover, threats and the need for future conservation of wild mushrooms in the country are also reported. This review paper can serve as base line information and indicator for further mycological studies in Ethiopia as well as in other developing countries with similar scenarios.

Keywords: Diversity; ecological niches; Ethiopia; mushroom cultivation; wild mushrooms.

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Introduction

Wild mushrooms are either epigeous or hypogeous heterotrophic organisms that belong to the *Basidiomycota* and *Ascomycota* divisions (Chang & Lee, 2004; Crous *et al*., 2006). Many of the wild mushroom species are becoming important parts of the livelihoods of rural people in different parts of the world (Sarma *et al*., 2010; Cai *et al*., 2011), being collected as valuable Non Timber Forest Products (NTFPs) (Chang & Lee, 2004; Boa, 2004). They help people to reduce vulnerability to poverty and strengthen the livelihoods through a reliable source of income. Globally, about 140,000 important mushroom species have been reported (Boa, 2004). These can serve as sources of food (Boa, 2004), medicine (Ferreira *et al*., 2010), enzymes and various industrial compounds (Gryzenhout *et al*., 2010).

Pegler & Rayner (1969) and Pegler (1977) noted that the East Africa region is rich in macrofungal species. Many of these species are presumed to be either cosmopolitan or to be widespread across Africa (Pegler, 1977; Munyanziza, 1994). Like other East African countries, Ethiopian fungal flora remains unexplored (Sitotaw *et al*., 2015b), as most regions and habitats in the country have been seldom studied (Sitotaw *et al*., 2015a). Reports regarding wild mushroom diversity rarely exist (Sitotaw *et al*., 2015b; Megersa *et al*., 2017). This is due to a lack of research infrastructures as well as to a lack of fungal taxonomists and specialists in fungal ecology (Osarenkhoe *et al*., 2014). Finally, and likely as a result, fungi are not included in the biodiversity database of the country (IBC, 2005). This poor knowledge on Ethiopian fungal species is worrying as fungi are highly sensitive towards habitat disturbances, namely anthropogenic threats that are rife across the country (IBC, 2005; Goldmann *et al*., 2015).

Despite poor scientific knowledge on fungal diversity, wild mushroom hunting and utilization is a traditional common practice among the different tribes in the country (Tuno, 2001; Semwal *et al*., 2014). Mushrooms have been used for their nutritional, traditional and medicinal properties, and are also involved in local mythology (Tuno, 2001; Abate, 2014). Equally to other wild edibles, they have also been used as a coping food during food shortage periods (Lulekal *et al*., 2011, Alemu *et al*., 2012). In some local markets mushrooms are also available (Abate, 2014), where they are sold by the local people to earn some income to supplement the household economy.

In this review, we aimed to provide basic information about wild mushrooms in Ethiopia by assessing the available documents and to create awareness for conservation and a wider use of mycological resources in the country. This paper aims to serve as a basic document for further mycological studies in the country, and elsewhere in the region with similar scenarios.

Diversity of wild mushrooms

Functionally wild mushrooms are categorized as saprophytes, that obtain nutrients from dead organic materials; parasitic which depend on living plants and mycorrhizal, that form associations with host plants from which each partner gets benefits from each other (Ferris *et al*., 2000).

Mushrooms also tend to be linked to the vegetation of an area. Hence, understanding the ecology of host or keystone species helps to find the possible associated taxa in any habitat (Härkönen *et al*., 2003). In Ethiopia there are diverse habitats (Friis *et al*., 2010) characterized by a high richness of species, including the fungi (Sitotaw *et al*., 2015b). However, the published literature to which we had access lack to portrait the country's mycoflora profile but only focuses on a handful of species. Here we summarize and present a short overview of wild mushrooms and their related habitats in Ethiopia (Table 1). The discussion analyses the following categories: (1) mushrooms in indigenous forests, (2) mushrooms in grazing lands, (3) mushrooms in termite mounds and (4) mushrooms in exotic tree plantations.

Mushrooms in indigenous forests

Indigenous forests are a typical part of Ethiopian landscape (Friis *et al*., 2010), covering a range of environments. The occurrence of mushrooms in these forests is widespread during the rainy season (Abate, 2014). Some taxa like *Lentinus* spp. are also unique as they fruit during the dry season (Tuno, 2001).

The most important scholar references on fungal diversity come from the comprehensive works of Hjortstam & Ryvarden (1996) who reported fifteen *Corticiaceae* species (List of species not given), of which *Mycoacia brunneofusca* and *Vuilleminia obducens* were new to science. Decock *et al*. (2005) also reported a total of four taxa from the highland forests region, and of which *Fomitiporia tenuis* and *F. aethiopica* were newly reported to the world. Some other taxa have also been reported by Tuno (2001), Abate (2008), Alemu (2013), Muleta *et al*. (2013), Abate (2014) and Sitotaw *et al*. (2015a) from indigenous forests in different parts of the country.

Most recently, Megersa *et al*. (2017) reported 49 fungal taxa from Degaga natural forest in three years of collections (Complete list of the taxa not given). Also, our research team collected 64 macrofungal species in a single rainy season, suggesting the presence of moderate diversity of fungi in the dry Afromontane forests in the Southern region (Pers.obs). Interestingly, some of the taxa like *Agaricus* spp., *Agrocybe* spp., and *Calvatia* spp. in our collections could only be identified at the genus level, while some others couldn't be identified at all, indicating the likely presence of species new to science. Based on a survey report of NTFPs from the Benishnagul Gumz Region, Alemu *et al*. (2012) also reflected a wider diversity of macrofungal species in Western dryland forests. The species reported there were described using local names only and lack their precise Latin names.

All of the taxa reported in indigenous forests were saprophytic (Table 1). Unfortunately, most valuable ectomycorrhizal (ECM) species common in African forests like *Lactarius* spp. and *Amanita* spp. (Okhuoya *et al*., 2010), were not reported in any of the references assessed. This was not surprising as most of the tropical woody tree species are unable to form associations with ECM fungi (Brundrett, 2009), particularly those indigenous tree species of Ethiopia.

Mushrooms in grazing areas

Upland grazing areas are found on the highland plateaus about 2000-3000 m above sea level. The farming systems in these areas are characterized by livestock rearing in addition to crop production. Abate (1999), Alemu (2013), Abate (2014) and Sitotaw *et al*. (2015a) cited some taxa in these areas. The saprophytic species belong to the genus *Agaricus* spp. was the dominant so far reported in the upland grazing areas. Despite valuable, the diversity of fungal species in Ethiopian grazing lands might be a lot richer than what has been so far reported; something that further complementary studies could confirm.

Mushrooms associated with termites

The symbiotic association of *Termitomyces* fungal species with termites is a remarkable example of the

Table 1. Resum of taxa of wild mushrooms reported so far from Ethiopia and with reference to their associated habitats.

List of taxa	Habitat	Sources
Agaricus arvensis Schaeff.	NF, GA	Abate (2014), Alemu (2013)
Agaricus campestris L.	NF, GA	Abate (1999), Abate (2008), Alemu (2013), Sitotaw *et al*. (2015a)
Agaricus xanthodermulus Callac & Guinb.	GA	Sitotaw *et al*. (2015a)
Agaricus xanthodermus Genev.	GA	Sitotaw *et al*. (2015a)
Amanita spp. Pers.	No avail	Megersa *et al*. (2017)
Armillaria spp. (Fr.) Staude	NF	Abate (2008), Abate (2014)
Auricularia spp. Bull. exJuss.	NF	Abate (2008), Abate (2014)
Bjerkandera adusta (Willd.) P. Karst.	No avail	Megersa *et al*. (2017)
Catathelasma ventricosum (Peck) Singer	No avail	Megersa *et al*. (2017)
Chlorophyllum molybdites (G. Mey.) Massee	NF, PT	Abate (2008), Abate (2014), Megersa *et al*. (2017)
Climacodon septentrionalis (Fr.) P. Karst.	No avail	Megersa *et al*. (2017)
Clitocybe nuda (Bull.) H.E. Bigelow & A.H. Sm.	NF	Alemu (2013)
Coprinus spp. Pers.	NF	Abate (2014)
Corticiaceae spp. Herter	NF	Hjortstam & Ryvarden (1996)
Craterellus spp. Pers.	No avail	Megersa *et al*. (2017)
Dictyophora indusiata (Vent.) Desv.	NF	Tuno (2001)
Diplomitoporus rimosus (Murrill) Gilb. & Ryvarden	NF	Hjortstam & Ryvarden (1996)
Fomitiporia aethiopica Decock, Bitew & G. Castillo	NF	Decock *et al*.(2005)
Fomitiporia pseudopunctata (A. David, Dequatre & Fiasson) Fiasson	NF	Decock *et al*.(2005)
Fomitiporia robusta (P. Karst.) Fiasson & Niemelä	NF	Decock *et al*.(2005)
Fomitiporia tenuis Decock, Bitew & Castillo	NF	Decock *et al*.(2005)
Ganoderma applanatum (Pers.) Pat.	No avail	Megersa *et al*. (2017)
Geastrum triplex Jungh.	No avail	Megersa *et al*. (2017)
Gymnopilus spp. P.Karst.	NF	Abate (2008)
Gymnopus eucalyptorum (Pers.) Roussel	No avail	Megersa *et al*. (2017)
Gyromitra spp. Fr.	NF	Alemu (2013)
Hypholoma spp. (Fr.) P.Kumm.	NF	Abate (2008)
Laetiporus sulphureus (Bull.) Murrill	NF	Abate (2008), Muleta *et al*. (2013), Abate (2014)
Lentinellus cochleatus (Pers.) P. Karst.	No avail	Megersa *et al*. (2017)
Lentinus spp. Fr.	NF	Tuno (2001), Abate (2008)
Lenzites betulina (L.) Fr.	No avail	Megersa *et al*. (2017)
Lepista spp. (Fr.) W.G. Sm.	No avail	Megersa *et al*. (2017)
Macrolepiota procera (Scop.) Singer	No avail	Megersa *et al*. (2017)
Macrolepiota spp. Singer	NF, GA	Abate (2008), Abate (2014)
Morchella esculenta (L.) Pers.	No avail	Megersa *et al*. (2017)
Mycoacia brunneofusca Hjortstam & Ryvarden	NF	Hjortstam & Ryvarden (1996)
Omphalotus olearius (DC.) Singer	NF	Abate (2008)
Onnia tomentosa (Fries) P. Karsten	No avail	Megersa *et al*. (2017)
Phallales spp. E. Fisch	NF	Tuno (2001)
Phellinus populicola Niemelä	No avail	Megersa *et al*. (2017)
Pholiota adipose (Fr.) P. Kumm.	No avail	Megersa *et al*. (2017)
Pholiota spp. (Fr.) P. Kumm.	NF	Abate (2014)

Table 1. Continued

List of taxa	Habitat	Sources
Physisporinus rivulosus (Berk. & M.A. Curtis) Ryvarden	NF	Hjortstam & Ryvarden (1996)
Polyporus cinnabarinus (Jacq.) Fr.	No avail	Megersa *et al*. (2017)
Polyporus spp. P. Micheli ex Adans.	NF	Alemu (2013)
Polyporus squamosus (Huds.) Fr.	No avail	Megersa *et al*. (2017)
Pycnoporus spp. P. Karst.	NF	Alemu (2013)
Ramaria stricta (Pers.) Quél.	No avail	Megersa *et al*. (2017)
Russula spp. Pers.	No avail	Megersa *et al*. (2017)
Schizophyllum commune Fr.	NF	Tuno (2001), Abate (2008), Alemu (2013)
Stereum rugosum Pers.	No avail	Megersa *et al*. (2017)
Suillus luteus (L.) Roussel	PT	Abate (2008)
Termitomyces aurantiacus (R. Heim) R. Heim	TM	Sitotaw *et al*. (2015b)
Termitomyces clypeatus R. Heim	TM	Muleta *et al*. (2013), Sitotaw *et al*. (2015b)
Termitomyces eurrhizus (Berk.) R. Heim	No avail	Megersa *et al*. (2017)
Termitomyces eurrhizus (Berk.) R. Heim	TM	Sitotaw *et al*. (2015b)
Termitomyces letestui (Pat.) R. Heim	TM	Sitotaw *et al*. (2015b)
Termitomyces microcarpus (Berk. & Broome) R. Heim	TM	Muleta *et al*. (2013), Abate (2014), Sitotaw *et al*. (2015b)
Termitomyces robustus (Beeli) R. Heim	TM	Sitotaw *et al*. (2015b)
Termitomyces schimperi (Pat.) R. Heim	TM	Sitotaw *et al*. (2015b)
Thelephora terrestris Ehrh.	No avail	Megersa *et al*. (2017)
Trametes gibbosa (Pers.) Fr.	No avail	Megersa *et al*. (2017)
Trametes versicolor (L.) Lloyd	NF, PT	Alemu (2013), Megersa *et al*. (2017)
Trichaptum biforme (Fr.) Ryvarden	No avail	Megersa *et al*. (2017)
Vascellum spp. F. Marda	GA	Abate (2008)
Vuilleminia obducens Hjortstam & Ryvarden	NF	Hjortstam &Ryvarden (1996)

GA: grazing area, NF: natural forest, TM: termite mounds and PT: plantation forest, *No avail*: habitat not available in the document. Fungal taxa names and authors' names were obtained from Mycobank database (http://www. mycobank.org)

coexistence of fungi with insects (Frøslev *et al*., 2003; Yamada *et al*., 2005; Damian, 2012). The fungus produces small nodules, which are consumed by termites along with the degraded substrate piles, named combs. During rainy periods, the mycelium that grows degrading the termite combs produces mushrooms, which penetrate the termite nests and soil to reach the surface and thus spread their spores (Frøslev *et al*., 2003). In Ethiopia, most of the *Termitomyces* fungal species (Table 1) are reported from the lowland areas of the country, where termite mounds are more abundant (Muleta *et al*., 2013; Abate, 2014; Sitotaw *et al*., 2015b; Megersa *et al*., 2017).

Mushroom in exotic tree plantations

Plantation forests are dominated by exotic tree species, mainly of the *Eucalyptus, Cupressus, Pinus* and *Acacia* genera (Bekele, 2011). The mass introduction and expansion of these trees in the country implies as a consequence, the indirect introduction of associated exotic fungal species too. This is the case of several ECM mushrooms (Table 1) originated from Mediterranean and temperate climates associated with these non-native trees (Megersa *et al*., 2017). For example, *Suillus luteus* is common in *Pinus* tree species plantations (Abate, 2008). Such mushroom species have a potential to diversify the value of plantation forests through mycosilvicultural management approaches (Boa, 2004; Peredo *et al.,* 1983) and can be produced in high quantities.

Mushroom cultivation

Mushroom cultivation can contribute towards the goal of habitat conservation and food security. Around the world about 60 mushroom species have been cultivated commercially (Chang & Miles, 2004). The most common ones include *Agaricus bisporus, Lentinula edodes, Pleurotus ostreatus, Flammulina velutipes, Volvariella volvacea, Grifola frondosa,* and *Pholiota nameko* (Gizaw, 2010). In Ethiopia, the practice of mushroom cultivation is a recent activity, mostly restricted to urban areas (Yehuala, 2008; Abate, 2014). Agricultural and agro-industrial wastes have been used at a small scale to produce four most commonly cultivated mushrooms: *A. bisporus, L. edodes, P. ostreatus* (Yehuala, 2008; Gebrelibanos *et al*., 2016) and *P. florida* (Gebrelibanos *et al*., 2016). The levels of essential and non-essential metals in cultivated mushrooms such as *P. ostreatus* and *P. florida* were also studied in Haramaya, Oromia Region (Gebrelibanos *et al*., 2016). However, lack of awareness and cultivation skills still leave plenty of room for improvement and growth regarding mushroom cultivation (Yehuala, 2008; Muleta *et al.,* 2013).

Owing to their flavor and nutritional value, the consumption of cultivated mushrooms is now constantly increasing, particularly in the main cities. On the other hand, conservative eating habits are also hindering the transfer of cultivation technology at a local level, particularly in areas where mushroom consumption is not a common practice.

Threats and the need for conservation

Many threats affecting wild mushrooms are similar to those that globally affect the biodiversity in Ethiopia (IBC, 2005). The most important of all, deforestation, comes as a consequence of anthropogenic change to which global environmental and climate change also add (Lulekal *et al*., 2011). According to Teketay (2001), deforestation is immense and estimated between 150000 – 200000 ha of land per year. Factors contributing to habitat degradation, such as fires, are also affecting the fungal communities in forest systems (Vásquez-Gassibe *et al*., 2016), which is also a recurrent phenomenon in the natural forest systems in the country. This adversely influences the macrofungi and diminishes their diversity and production (Miller & Lodge, 1997). Such impact also limits the benefits that can be obtained from fungal resources. Thus, urgent conservation strategies and actions are needed, giving special considerations to those species currently used by the local people.

Conclusions and prospects

The reviewed references in this document highlight the existence of a valuable mycoflora in Ethiopia. Also, reveal some ecological niches in which important wild mushrooms exist. However, they miss to portrait the fungal diversity profiles in the country overall. Some of the taxa reported in some references are also not properly identified as they are only registered with their local names (Alemu *et al*., 2012). This also applies to other countries in the Region that supposed to have high potential linked to this natural resource as well, and reveals the need to accomplish thorough scientific studies in order to get a glimpse of the vast number of fungal species across the Region. Furthermore, the status of many wild mushroom species is also unclear, as habitat degradation is immense. We believe that further studies involving close examinations of different habitats are needed, since there might be yet unknown species with valuable potential but equally unknown uses.

According to our review, many are the possible options to widen the cultivation of mushrooms at small and large scales. At a small-scale level, homestead cultivation is possible using locally available substrates. Plantation forests in Ethiopia and also in others countries in the region could provide opportunities to introduce important mushroom species and can be used for large scale cultivation purposes. Globally highly appreciated taxa such as *Boletus pinophilus, B. edulis* and *Lactarius deliciosus* could be produced in plantation forests by means of seedling mycorrhization (Högberg & Piearce, 1986; Perry *et al*., 1987; Águeda *et al*., 2008; Mediavilla *et al*., 2016). Thus, adopting and scaling up of mycorrhization technologies may offer incentives for widening mushroom cultivation practices. This could also be a major future research area. Also, wood waste could be used (Sefidi & Etemad, 2015) to cultivate important medicinal species such as *Pleurotus ostreatus, Ganaoderma lucidum* and *Coriolus versicolor* in natural and plantation forest systems.

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Original article II

Setas silvestres comestibles de Etiopía: productos forestales no maderables infravalorados

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Resumen

Este artículo resume los trabajos previos existentes sobre Etnomicología en Etiopía. Aunque los documentos a los que se ha tenido acceso son escasos y limitados en cuanto a sus objetivos, se presenta una valiosa y extraordinaria información sobre la recolección y consumo de hongos silvestres comestibles en el país. Además, se presenta una listado de los hongos utilizados por su valor comestible y medicinal y se hace referencia a los ecosistemas en los que se recogen. Se ha observado que los hongos silvestres representan un reducido valor en comparación con otros recursos forestales no madereros RFNM. Este hecho se debe en parte al desconocimiento taxonómico. De hecho todos los hongos reciben el mismo nombre local “Enguday”. Este es un recurso muy poco estudiado si se compara con otros RFNMs, lo que hace que esté especialmente amenazado debido a los pocos estudios etnomicológicos llevados a cabo hasta el momento en el país. Por todo ello, pensamos que este estudio puede suponer un punto de partida fundamental para posteriores trabajos que contribuyan a un mayor conocimiento de este recurso en Etiopía, lo que conllevará una mayor promoción y conservación de los hongos silvestres comestibles y medicinales en el país.

Palabras clave: *Hongos silvestres, “Enguday”, etnomicología, producto forestal no maderero, Etiopía*

Edible wild mushrooms of Ethiopia: neglected non-timber forest products

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Abstract

This review summarizes existing ethnomycological work in Ethiopia. Although the literature to which we had access were scanty and limited in their scope, comprehensive issues such as the culture of collection and use of edible wild mushrooms in the country are summarized. In this review, moreover, a check list of available wild edible and medicinal mushrooms and their niches are also documented. The review reveals that wild mushrooms are the most neglected non-timber forest products (NTFPs). They are poorly studied and undocumented in Ethiopia as compared to other NTFPs and their importance for the livelihood of the local communities is also overlooked. Recognition of this resource is also hampered by the lack of taxonomic studies; most of the species are simply known locally by the shared common name "Enguday". Thus, this document can serve as baseline information and indicator for further studies to facilitate the wider use, promotion and conservation of wild mushrooms in Ethiopia.

Keywords: *Wild mushrooms, Enguday, ethnomycology, Non-timber forest products, Ethiopia*

1. Introduction

Ethiopia encompasses a broad range of ecosystems, with great ranges of altitude, rainfall patterns and soil variability which contribute to the occurrence of different life forms both in flora and fauna (Bongers and Tenngkeit, 2010; Friis *et al*., 2010). The existence of high variation in macro- and micro-climatic conditions has also contributed to the formation of diverse vegetation types in the country. Friis *et al*. (2010) classified the vegetation resources of Ethiopia into 12 types that range from Afro-alpine formations through dense high canopy montane forests to savanna, scrubland and deserts. An important features of these vegetation types is that their richness in valuable types of Non-Timber Forest Products (NTFPs) (Adilo, 2007; Seyoum, 2007; Sultan, 2009). The most important NTFPs include honey and bees-wax, bamboo, gum arabic, resin, coffee and spices, incense, edible plant products like fruits, seeds, fodder, medicinal plants, mushrooms, various extractives and flavorings (Asfaw and Tadesse, 2001; Lulekal *et al*., 2011).

Wild mushrooms are parts of the livelihood of people in different parts of the world (Boa, 2004; Manoharachary *et al*., 2005; Sarma *et al*., 2010). They have long been collected as a valuable NTFPs and play significant role in terms of generating cash income gained by selling in the market, and of subsistence local use such as food and traditional medicine (Boa, 2004; Chang and Lee, 2004; Mau *et al*., 2004). This practices help rural people to reduce vulnerability to poverty and strengthen their livelihoods through a reliable foundation of income, and have brought ethnomycology as a discipline in different part of the world (Boa, 2004). In Ethiopia, however, wild edible mushrooms are the most neglected NTFPs resources. Despite their importance, wild mushrooms have been given little attention and they are less studied and rarely documented (Alemu, 2013; Muleta *et al*., 2013). Then again, the justification behind forest resource management in the country has been primarily based on the production of wood products (Yemiru *et al*., 2010). The values and roles of NTFPs like mushrooms have been neglected and all activities related to forest management are focused on maximizing the wood products (Asfaw and Tadesse, 2001; Lulekal *et al*., 2011; Melaku *et al*., 2014). However, if managed and conserved properly, mushrooms have a potential to support the livelihoods of the rural people as major sources of food, medicine, and means of cash income for rural communities. Interestingly, other studies in different part of the world indicated that wild mushrooms could play roles in local economic developments. In some cases, they could generate even higher economic benefits than timber productions (Oria-de-Rueda *et al*., 2008). Thus, this could help to reconcile the social, economic and ecological values of the forests and really could encourage the rural people to rationally manage and conserve the forest resources in their locality.

Ethnobotanical and various studies undertaken in Ethiopia already have revealed long lists of NTFPs, indicating their importance, contribution and how they are traditionally utilized by the rural communities (Lulekal *et al*., 2011; Melaku *et al*., 2014). However, reports on mushroom rarely exist, while those that do exist contain only scanty and basic information to demonstrate their existence and use at some community levels (Abate, 2014; Muleta et al., 2013; Tuno, 2001). Efforts are needed to integrate wild mushrooms into the mainstream of NTFPs in the country to ensure their conservation and thereby enhance their value in human welfare. One of the strategies could be documentation and dissemination of existing information relating to important edible and medicinal mushrooms and their ethnomycological uses to help transform the uses from subsistence to development through promotion in the country. Therefore, in this paper, an attempt has been made to review and compile the diversity of wild edible and medicinal mushrooms in Ethiopia. Comprehensive information on the traditional practices regarding the use by certain ethnic tribes in the country was also documented through reviewing the available literatures. Thus, this paper could serve as a baseline document for further studies and investigations in Ethiopia.

2. Mushroom collection and use

Wild mushrooms have been utilized as important sources of food and medicine by rural communities, who mostly depend on forests for their livelihoods. Such ethnomycological usage has been also traditional among the forest dweller communities in Ethiopia. The term “*Enguday*” or "*Yejib tila*" is commonly used by people to call wild edible mushrooms in the country. Of these, the literal meaning of "*Yejib tila*" is "shadow of the hyena" (“*yejib*”: hyena and “*tila*”: shadow) implying that wild mushrooms appear in places where the hyena shadow is cast, indicating the growth of wild mushroom is somewhat mysterious (Abate, 2014). Although wild mushrooms have been used as sources of food and traditional medicine (Asfaw and Tadesse, 2001; Semwal *et al*., 2014), their indigenous knowledge among the different tribes has not been given significant attention, and documented information on the collection, use and list of valuable mushrooms is limited. In this section, we summarize the available ethnomycological notes to serve as a compressive reference and for further investigations.

2.1. Mushroom use as food

The available ethnomycological literature provided a good illustration of how certain ethnic groups use wild mushrooms as a source of food. For example, Tuno (2001) described the traditional use of wild mushrooms by the *Majangir* ethnic groups as a subsidiary food gathered from the natural forests in southern Ethiopia. Muleta *et al*. (2013) and Abate (2014) also documented the culture of hunting and traditional use of mushrooms by the *Kaffa* ethnic groups in the southwest part of the country. In both cases mushroom has a long history as part of their livelihood because of the nutritional value and good taste they have (Muleta *et al*., 2013; Tuno, 2001). Those mushrooms collected from the forest are eaten fresh and efforts to preserve are not a common practice in either of the two ethnic groups.

Other important ethnomycological resources also come from Muleta *et al*. (2013) who indicated the existence of mushroom hunting cultural practices in Bonga area in southern region. In his documentation, Muleta *et al*. (2013) mentioned wild edible mushrooms are important sources of food supplement for rural communities, and the rural people preferred mushrooms primarily because of their unique flavor and texture. Mushrooms used to make soup, salad and other traditional meals. Rare practice of mushroom drying and preservation existed among the local communities in the Bonga area (Muleta *et al*., 2013).

A noticeable interest on wild mushrooms was also reported from the Benihsnagul Gumz region, western Ethiopia (Alemu *et al*., 2012). In this region, the main tribal groups i.e. *Gumz* and *Berta* are practicing mushroom collection for subsistence use and they appreciate mushrooms as valuable food sources. They also use mushrooms as seasonal coping food, during food shortage period, mainly in rainy season when the grain scarcity occurred (Alemu *et al*., 2012).

Wild mushroom fruiting and collection are restricted to the rainy season, mainly from June to September in Ethiopia. For some species like *Lentinus* sp., collection could be made during the dry season (Tuno, 2001). Summary of the most commonly used species presented in the table (Table 1) include *Agaricus arvensis*, *A. campestroides*, *Laetiporus sulphureus*, *Termitomyces microcarpus*, *T. clypeatus*, *Lentinus* sp., *Schizophyllum commune* and *Dictyophora* *indusiata* (Abate, 2014, 2008; Muleta *et al*., 2013; Tuno, 2001). Among these species, *L. sulphureus* and *S. commune* are well exploited and documented for their ethnomycological use in Ethiopia (Tuno, 2001). Habitat information for commonly used species was also noted (Table 1); they grow mainly in natural forests, grazing areas and termite mounds (Abate, 2014). However, taxonomic description and voucher collections of most of the species are lacking, indicating further work is needed in fungi taxonomy and classification. The information from the literature is very limited and does not reflect the wider cultural usage in the country. Thus, as there are a number of benefits that can be derived from traditional uses, further ethnomycological work is clearly needed to maximize the benefits from traditional knowledge and use of wild mushroom resources in Ethiopia.

2.2. Mushrooms as traditional medicine

Wild mushrooms are known to be rich sources of various bioactive substances (Lindequist *et al*., 2005) and many of them have been reported to be used in folk medicine worldwide (Hobbs, 1995). Interestingly, traditional medicinal uses of mushrooms are reported by Abate (2014) from the *Kaffa* ethnic groups in southern Ethiopia. Among other species, *Laetiporus sulphureus* is reported to be commonly used for treating the pain during childbirth and its powder is usually preserved for long periods of time to use as drug during child delivery in Kaffa area (Abate, 2014). The medicinal use of mushrooms, whose traditional knowledge for the practice is handed down generational lines via oral communications, has also been reported by Alemu et al. (2012) from the Benihsnagul Gumz region, western Ethiopia. In all cases, most of the knowledge on medicinal uses was found to be confined to elderly people of the village or handled secretly by traditional healers of the locality. They both are also key informants for the identification of medicinal species since they use different mushrooms for their traditional medicinal practices (Abate, 2008). Consequently it is difficult to get list of species that have been used traditionally for medicinal purpose from an ordinary person (Abate, 2014). Thus, further documentation and ethnomedicinal uses study will be important for enhancing the understanding of indigenous knowledge systems and the list of species used in folk medicine in Ethiopia. List of species used for traditional medicinal purpose is provided (Table 1).

2.3. Mushrooms for market sale

Wild mushrooms are generally not among NTFPs for sale in Ethiopia. They are collected mostly for subsistence use (Yehuala, 2010). The major reason is that the season for wild mushroom growth is short and during this season everyone can collect from the wild for their own consumption. However, in some places wild edible mushrooms can provide additional income to households when sold in the markets. For example, in local markets of Bonga and Assosa, mushrooms are available occasionally in association with other vegetal products (Abate, 2014) which the local people sell to earn some income to supplement their household economy. The common species found fresh in local markets includes *Agaricus* sp., *Laetiporus* sp*., and Termitomyces* sp., (Abate, 2014). However, their market value is very low as the buyers are restricted among the local tribes and many people were found to be quite ignorance about their edibility (Abate, 2014). Moreover, awareness to use mushrooms as a potential source of income in most rural part of the country is very low and complete lists of species for the local markets is also lacking in all the literature. Traditional taboos such as considering wild edibles collections as a sign of poverty also hinder wider mushrooms utilization in majority of part of the country, indicating that increasing awareness through collaborative efforts are essential to assist the nationwide efforts to combat food insecurity and ensure nutritional diversity in Ethiopia. List of species used for sale in some localities is presented in Table 1.

3. Gender and mushrooms

In Ethiopia women are usually involved in collection of mushrooms and they recognize more fungi species than men. Children are also involved in the collection of some specific taxa like *Termitomyces* sp. (Abate, 2014; Alemu, 2013). Women have also basic knowledge of mushrooms in terms of habitats, niche, and associated substrates. They have also good expertise to distinguish between edible and poisonous species. This might be due to the fact that they are more often involved in preparing and cooking mushrooms than are men. Although it differs place to place, the most recognized ways used to classify mushrooms as edible and non-edible are the colors, shapes and the presence or absence of strong bad smell (Tuno, 2001). These all help for the successful collection of wild edible mushrooms in the locality. The traditional processing knowledge, including handling and cooking are also well known and handled by women (Abate, 2014; Muleta *et al*., 2013).

4. Checklist of wild edible mushrooms

The majority of the rural population in Ethiopia is dependent on forest resources, either in the form of subsistence or as a cash income derived from NTFPs (Lulekal *et al*., 2011). Although mushroom is part of the NTFPs, information on the diversity is hardly ever documented and the country remains mycologically unexplored (Sitotaw *et al.*, 2015b). So far limited number of species with saprobic or ectomycorrhizal habits have been reported from different regions.

The most important published reports on list of mushrooms in Ethiopia comes from the work of Abate (2014), (2008); Alemu (2013) and Muleta *et al*. (2013) from natural forests in the highland parts of the country. Some specific *Agaricus* species were reported by Abate (1999) and Sitotaw *et al*. (2015a) from the Afro-montane forest regions in central Ethiopia. Moreover, Tuno (2001) also reported few edible mushrooms from the moist Afro-montane forest systems in the southwest part of the country. Most recently, Sitotaw *et al*. (2015b) described seven edible *Termitomyces* species in the western lowland part, where the *Combretum* – *Terminalia* woodland vegetations are dominant. A compilation of the wild edible or medicinal mushrooms and their associated habitats reported from Ethiopia are presented (Table 1). Up-to-date fungal taxa names and authors' names were obtained from Mycobank database (http://www. mycobank.org).

Table 1: Summary of wild edible and medicinal mushrooms and their associated habitats in Ethiopia

List of taxa	Use	Referred sources
Agaricus arvensis Schaeff.	F, S	Abate (2008); Alemu (2013); Muleta *et al*., (2013); Sitotaw et al. (2015a)
Agaricus campestroides Heinem & Gooss.-Font.	F, S	Abate (2014); Alemu (2013); Dejene *et al*. (2017), Sitotaw *et al*. (2015a); Woldegiorgis *et al*. (2014)
Agaricus subedulis Heinem.	F	Dejene *et al*. (2017), (2016)
Agrocybe pediades (Fr.) Fayod	F	Dejene *et al*., 2016
Armillaria heimii Pegler.	FM	Abate (2014), Dejene *et al*. (2016); Osarenkhoe *et al*. (2014)
Auricularia sp. Bull. ex Juss.	FM	Abate (2008); Osarenkhoe *et al*. (2014); Woldegiorgis *et al*. (2014)
Calvatia rubroflava (Cragin) Lloyd	FM	Dejene *et al*. (2016)
Coprinellus domesticus (Bolton) Vilgalys, Hopple & Jacq. Johnson.	F	Dejene *et al.* (2017), (2016)
Coprinopsis nivea (Pers.) Redhead, Vilgalys & Moncalvo	F	Dejene *et al.* (2016)
Coprinus pseudoplicatilis Voglino	F	Abate (2014), (2008)
Hygrophoropsis aurantiaca (Wulfen) Maire.	F	Dejene *et al.* (2017), (2016)
Hymenagaricus sp. Heinem.	F	Dejene et al. (2017), (2016)
Laetiporus sulphureus (Bull.) Murrill	FM, S	Alemu (2013); Dejene *et al*. (2016); Muleta *et al*. (2013); Osarenkhoe *et al.* (2014); Tuno (2001);

List of taxa	Use	Referred sources
		Woldegiorgis *et al*. (2014)
Lentinus sp. Fr.	FM, S	(Osarenkhoe et al., 2014; Tuno, 2001)
Leucoagaricus holosericeus (J.J. Planer) M.M. Moser.	F	Dejene *et al.* (2017), (2016)
Leucoagaricus leucothites (Vittad.) Wasser.	F	Dejene *et al*. (2017), (2016)
Leucoagaricus rubrotinctus (Peck) Singer.	F	Dejene *et al*. (2017), (2016)
Leucocoprinus birnbaumii (Corda) Singer.	F	Dejene *et al*. (2016)
Leucocoprinus cepistipes (Sowerby) Pat.	F	Dejene *et al*. (2017), (2016)
Lycoperdon sp. Pers.	F	Dejene *et al*. (2016)
Macrolepiota sp. Singer	F	Abate (2008); Alemu (2013); Woldegiorgis et al. (2014)
Pholiota sp. (Fr.) P. Kumm.	F	Abate (2014), (2008)
Schizophyllum commune Fr.	FM	Alemu (2013); Osarenkhoe *et al*. (2014); Tuno (2001)
Suillus luteus (L.) Roussel	F	Abate (2008); Semwal *et al*. (2014); Woldegiorgis *et al*. (2014)
Termitomyces aurantiacus (R. Heim) R. Heim	F	Sitotaw *et al*. (2015b)
Termitomyces clypeatus R. Heim	F, S	Alemu (2013); Muleta *et al*. (2013); Sitotaw *et al*. (2015b)
Termitomyces eurrhizus (Berk.) R. Heim	F	Sitotaw *et al*. (2015b)
Termitomyces le-testui (Pat.) R. Heim	F	Sitotaw *et al*. (2015b)
Termitomyces microcarpus (Berk. & Broome) R. Heim	FM, S	Abate (2014); Alemu (2013); Muleta *et al.* (2013); Osarenkhoe *et al*. (2014)
Termitomyces robustus (Beeli) R. Heim	FM	Osarenkhoe *et al*. (2014); Sitotaw *et al.* (2015b)
Termitomyces schimperi (Pat.) R. Heim	F	Sitotaw *et al*. (2015b)
Tylopilus niger (Heinem. & Gooss.-Font.) Wolfe	F	Dejene et al. (2017)
Vascellum sp. F. Smarda	F	Abate (2014); Alemu (2013)

Note: GA: grazing area, NF: natural forest, TM: termite mounds and PT: plantation forest, F: food and FM: Food and Medicine, and S: For sale.

Some other documents have also reported the potential availability of wild edible mushrooms in plantation forest systems from Ethiopia (Megersa *et al*., 2017; Semwal *et al*., 2014). These documents highlight the existence of valuable genera such as *Suillus*, *Lactarius*, *Lepista*, and *Cantharellus*. In a similar way, a survey report of NTFPs from the *Combretum*–*Terminalia* woodland vegetations in Western Ethiopia, reflected wider availability of edible mushrooms (Alemu *et al*., 2012). However, this report lacks field observations to justify the claims and the species mentioned here are not properly identified. Almost all of the species lack scientific names, underlining the fact that the majority of wild mushrooms in Ethiopia remains unnamed or awaits scientific description.

More recently, our research team reported a total of 26 wild edible mushrooms from natural and exotic forest plantations in southern Ethiopia (Dejene *et al*., 2017, 2016). Most these species are not known by the local communities and their edibility was assessed from other countries experiences. Interestingly, we found some edible mycorrhizal mushroom in non-native tree plantation forests. List of species reported are presented (Table 1) and photographs of some the edible mushrooms collected are illustrated (Figure 1).

Figure 1: Pictures of some wild Edible mushrooms in Ethiopian forests: (A) *Agaricus campestroides*, (B) *Agaricus subedulis*, (C) *Agrocybe pediades,* (D) *Armillaria heimii*, (E) *Calvatia rubroflava* (F) *Coprinellus domesticus*, (G) *Coprinopsis nivea*, (H) *Coprinus pseudoplicatilis* (I) *Hygrophoropsis aurantiaca*, (J) *Hymenagaricus fuscobrunneus* (K) *Leucoagaricus holosericeus*, (L) *Leucoagaricus leucothites*, (M) *Leucoagaricus rubrotinctus*, (N) *Leucocoprinus birnbaumii*, (O) *Leucocoprinus cepistipes,* (P) *Lycoperdon perlatum* and (Q) *Tylopilus niger* (Photos by Tatek, 2015).

5. Conclusions

By and large, the reviewed literature showed that wild mushroom collection as accessible food and in traditional medicine is a common practice in Ethiopia and that wild mushrooms have economic importance to local people in some localities. However, all the available ethnomycological notes focused on specific areas such as the West and Southwest parts of the country and the majorities are still remaining unexplored. This indicates that the information contained is inadequate and does not convey the full ethnomycological picture of the country, as Ethiopia is characterized by many communities that might have long histories of wild mushroom usage with wider traditional knowledge in the culture. Thus, we encourage more research and documentation to reveal the human-mushroom relationships in depth in the country.

On the other hand, the reviewed literature highlighted the potential existence of wild edible and medicinal mushrooms in Ethiopia. More interestingly, some lists of common species used in the country have been indicated even though lots of species and their diverse uses remain unidentified, undescribed, and undocumented. This indicates that wild mushrooms have been suffered many years of neglect and some of the species might face extinction or extirpation as their habitats are being altered in the country. Consequently, mushroom in Ethiopia under-represented in the literature. Thus further studies are needed for effective and potential utilization of wild mushrooms as well as focusing on wild mushroom conservation in the country.

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Original article III

Sucesión de comunidades fúngicas y producción de esporocarpos ante la ocurrencia de fuegos en bosques secos Afromontanos de Etiopía

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Resumen

El fuego es una de las principales amenazas para los ecosistemas forestales de Etiopía y está afectando la biodiversidad de los bosques, incluyendo las comunidades de fúngicas. Este estudio tuvo como objetivo examinar los efectos del fuego sobre la riqueza de los taxones macrofúngicos, la diversidad y la producción de esporocarpos en bosques secos Afromontanos de Etiopía. Se recolectaron esporocarpos de nueve parcelas permanentes (100 m2) establecidas en rodales quemados con edades entre uno y diez años de edad, así como en un área no quemada. Los datos utilizados para cuantificar la riqueza fúngica fueron los pesos secos de los esporocarpos. Se utilizaron análisis morfológicos y moleculares para identificar los hongos. También se recogieron muestras de suelo compuestas para determinar las principales variables edáficas que explican la composición de los taxones. Como resultado se reportaron 61 taxones de hongos, pertenecientes a la división de Basidiomycota, de los cuales 22 son comestibles. La diversidad de hongos, su riqueza y producción de esporocarpos fueron afectadas por el fuego. La composición de la comunidad fúngica estuvo correlacionada de manera significativa con la materia orgánica, así como por las concentraciones de P y Ca. Estos resultados son alentadores desde el punto de vista de la conservación fúngica pues proporcionas nueva información sobre las comunidades macrofúngicas de los bosques secos Afromontanos de Etiopía, incluyendo probablemente diversos taxones aún desconocidos para la ciencia, así como varias especies comestibles que podrían favorecer a las poblaciones rurales en el área de estudio.

Palabras clave:*Bosque Afromontanoseco, Diversidad, Fuego, Comunidad fúngica, Hongos, Riqueza*

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Fungal community succession and sporocarp production following fire occurrence in Dry Afromontane forests of Ethiopia

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ABSTRACT

Fire is among the main threats to forest ecosystems in Ethiopia and is affecting the forest biodiversity, including fungal communities. This study was aimed to examine the effects of fire on macrofungal taxa richness, diversity and sporocarp production in the Dry Afromontane forests in Ethiopia. Sporocarps were collected from nine plots (100 m^2) established in one- and ten-year-old burned stands, and in an unburned stands. The data were used to quantify fungal richness and sporocarp fresh weights. Morphological and molecular analyses were used to identify the fungi. Composite soil samples were also collected from each stand and used to determine main edaphic explanatory variables for taxa composition. A total of 61 fungal taxa, belonging to *Basidiomycota* division were reported, of which 22 were edible. Fungal diversity, richness and sporocarp production were affected just after the fire. Fungal community composition was significantly correlated with Organic matter, P and Ca. Generally, the result is encouraging from the point of view of fungal conservation. It provides novelty information about the macrofungal communities in Ethiopian dry Afromontane forests, likely including many taxa are still unknown to science as well as several edible species which could supply complementary incomes for the rural populations in the study area.

1. Introduction

The Ethiopian highlands cover more than 44% of the country's land area. Afromontane vegetation originally dominated these highlands, characterized by high-altitude natural forests classified as either Dry or Moist Afromontane forests (Friis et al., 2010). Of these two, the Dry Afromontane forests form the largest part and are distributed mainly in the Central, Northern and Western parts of Ethiopia (Eshete et al., 2011; Friis et al., 2010). The existence of high humidity with a variable rainfall pattern and a prolonged dry season characterize the Dry Afromontane forests making them complex and rich in biodiversity (Wassie et al., 2005). The main tree species found in these forests include *Juniperus procera*, *Podocarpus falcatus*, *Hagenia abyssinica* and *Olea africana*. These tree species serve as the main sources of timber to the country (Kassa et al., 2009). The Dry Afromontane forests also harbor various types of Non-Timber Forest Products (NTFPs) (Shumi, 2009), including edible fungi (Dejene et al., 2017).

Fungi are key components of the forest ecosystems (Lindahl et al., 2007), since they are responsible for the decomposition of organic materials and recycling of nutrients (Ferris et al., 2000). Fungi play a key role in the mobilization, uptake and translocation of nutrients in forest soils. They can also improve plant water uptake and resistance to abiotic stresses; thereby influencing plant diversity, productivity and ecosystem functions (Pietras et al., 2013; Van Der Heijden et al., 2008). In addition to ecological functions, fungi have become a strategic component in the conservation and management of forest systems. This is because of their economic value, as during the last decade, there has been an increasing demand for wild edible fungi (Pettenella et al., 2007), which are becoming an important source of rural income (Boa, 2004). In fact, in some cases forest fungi may generate even higher economic benefits than timber production (Martín-Pinto et al., 2006).

Although reliable data on cover change is scarce, the forest history of Ethiopia indicates that forest land degradation and fragmentation is a continuous process, notably in the Dry Afromontane forests (Wassie et al., 2005). The ever-increasing demand for wood products as well as crop and grazing land expansion, stimulated by rapid population and livestock growth are factors aggravating the degradation of these forests in Ethiopia

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(Lemenih and Bekele, 2008). In addition, fire is also responsible for the loss of forest in the country, affecting the distribution, diversity and composition of forests resources (Lemenih and Bekele, 2008; Wassie et al., 2005). For instance, the most devastating wave of forest fires, which occurred in 2000 due to an extended drought, damaged over 150,000 ha (ha) of forested lands throughout the country (Teketay, 2001). This trend is more pronounced in the Dry Afromontane forests compared to other ecosystems, and has a direct implication on the loss of biological diversity of these forest ecosystems (Lemenih and Bekele, 2008).

As an ecological factor, fire is affecting forest fungal communities (Bastias et al., 2006). Differences in its return interval can modify the composition and diversity of fungi (Buscardo et al., 2010). A change in ecological conditions effects fungal growth and perpetuation depending on the intensity and duration of fire in the forests (Hart et al., 2005). Furthermore, fire produces direct effects on fungal communities by affecting belowground organisms (Buscardo et al., 2012). Thus, the subsequent structure of fungal communities following succession patterns will be affected, mainly driven by the dynamics of post-fire plant communities (Cairney and Bastias, 2007). On the other hand, some fungi might also benefit from fire since they fruit as a result of fire (Hart et al., 2005). Hence, some level of fire in the ecosystem could provide higher abundance of fire-loving fungal species. Also, fungal communities are closely influenced by other biotic and abiotic factors such as soil characteristics. Indeed, fungal species composition in the forest tends to be correlated with edaphic variables (Straatsma et al., 2001), especially saprotrophic fungi which are more dependent upon their respective substrates than mycorrhizal fungi (Reverchon et al., 2010).

A recent review work on the effects of forest fire on fungal association reveal that fungal-fire relations studies were mostly located in temperate and Mediterranean forest ecosystems (Taudière et al., 2017). The tropical forests are yet understudied, and comprehensive studies are recommended to improve understanding of the fungi–fire relationships with current global scale changes. Despite their ecological and economic importance, fungal communities are the most neglected components of Ethiopian forest systems, mainly of the Dry Afromontane forests (Dejene et al., 2017). This poor knowledge is worrying as fungi are highly sensitive towards anthropogenic threats like human induced fire, which are common in the Dry Afromontane forest ecosystems (IBC, 2014). Off course, the influence of fire on the diversity and sporocarp production of fungi remains understudied in the country overall. Thus, a close examination of fungal ecology in relation to fire factor may facilitate the diversity conservation and production of economically important fungal species in our study area.

This pioneering work was designed to provide baseline information about macrofungi assemblage, diversity and sporocarp production in the Dry Afromontane forests which helps to derive benefits through management strategies, and also to supplement the current knowledge about the fungal community in Ethiopia. The specific objectives included; (i) to analyze fungal richness after fire, (ii) to analyze the total and edible sporocarp yields after fire and (iii) to relate taxa composition to explanatory edaphic variables.

2. Material and method

2.1. Study area

The study was conducted at Wondo Genet natural forest area, one of the remnant Dry Afromontane forests, located in Southern Ethiopia. Wondo Genet is found approximately 265 km from Addis Ababa, the capital city of Ethiopia (Fig. 1). It is located at 7°06′–7°07′N latitude and 38°37′–38°42′E longitudes with an altitudinal range between 1600 m and 2580 m above sea level (m.a.s.l.) (Belaynesh, 2002; Fenta, 2014). The climate of the study area is characterized by the Woyna Dega agro-climatic type, and the rainfall pattern is bi-modal, with minor rainfall during spring and the major rain season during summer. The average annual rainfall of the study area is 1210 mm, which peaks in July. The average annual temperature of the study area is 20 °C (Belay, 2016; Fenta, 2014).

The topography is slightly undulating and the soils are young and of volcanic origin, characterized by sandy loam (Eriksson and Stern, 1987) with a pH average value of 5.7 (Eshetu and Högberg, 2000). The soil physical and chemical properties of the study plots are presented in Table 1.

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. This forest also provides a variety of important ecosystem services that can be expressed in terms of watershed protection and carbon sequestration. *Juniperus procera*, *Albizia gummifera*, *Afrocarpus falcatus*, *Bersama abyssinica*, *Prunus africana*, *Podocarpus falcatus*, *Cordia africana*, *Croton macrostachys* and *Olea africana* tree species mainly characterize the natural forests of our study area (Ango and Bewket, 2007; Belay, 2016). Forest fire is a recurrent phenomenon, occurring yearly in small patches in the natural forest of the study area (Bekele et al., 2013).

We established our study plots in the natural forests in 2015, taking into consideration the similarity of the stands in terms of ecological conditions such as climate, altitude and soil. Information from the Department of Forest Management in Wondo Genet College of Forestry was used to find patches of forest stands with similar fire history. The control stand was patches of forest representative of the original natural forest not affected by fire at least in the last 40 years. Burned stands were patches of forests affected similarly by high fire severity, with canopy and understory burned, and the soil organic layer consumed (Rincón and Pueyo, 2010). Three stands could be clearly differentiated: (1) unburned natural forest stand, hereafter UB stand: no fire occurred previously since the inception of the nearby college of forestry (1976) where it is located, (2) one-year-old burned forest stand, hereafter B-1 stand: mainly characterized by different kind of shrubs species and burned standing trees and logs, (3) ten-year-old burned forest stand, hereafter B-10 stand which resembles the unburned stands in terms of vegetation composition but without reaching the maturity and complexity of the unburned stand. Within each of the selected stands, plots were established systematically about 250 m apart. Differences in fungal diversity and productivity among stands prior to fire were thus unlikely.

2.2. Sporocarps sampling

A total of nine sample plots, three per stand (UB, B-1 and B-10), were established as described in Gassibe et al. (2011) and Hernández-Rodríguez et al. (2013). Each plot covered an area of 100 m^2, with a rectangular shape ($2 m \times 50 m$). All sporocarps found in the plots were fully harvested weekly during the major rainy season in July and August in 2015. Fresh weight measurements were carried out *in situ* and the data are given in kilograms per hectare per year (kg fw/ha/year). Also abundance data of each species was taken from each plot. Sample fruit bodies from each species were taken to the laboratory and dried. Herbaria specimens were used for molecular and microscopic taxa identification. Furthermore, in the field, specimens were photographed and their ecological characteristics were noted in order to assist and facilitate taxa identification processes. This work could be considered as a case

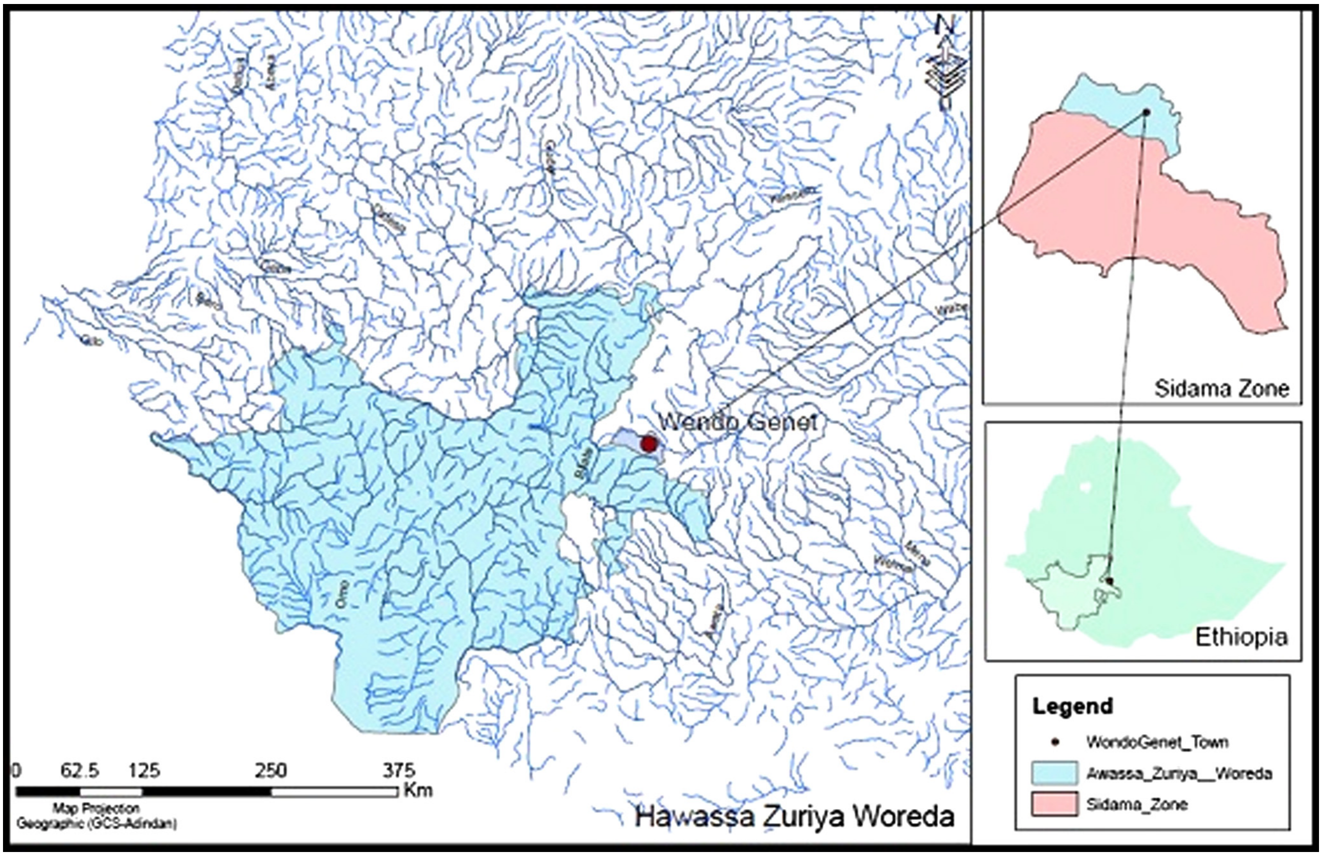

Fig. 1. Location map of the study area, Wondo Genet, Ethiopia.

Table 1
Selected soil properties of the study plots in Dry Afromontane forest of Wondo Genet (Ethiopia).

Soil parameters	Plots		
	B-1	B-10	UB
Na (meg/100 gm soil)	1.00 (0.4)	0.99 (0.1)	0.83 (0.07)
K (meg/100 gm soil)	0.62 (0.35)	0.8 (0.08)	0.55 (0.12)
Ca (meg/100 gm soil)	20.85 (5.18)	24.15 (4.98)	28.43 (13.67)
Mg (meg/100 gm soil)	7.42 (1.42)	8.05 (1.5)	9.77 (5.18)
CEC (meg/100 gm soil)	43.97 (10.9)	42.66 (5.1)	52.44 (14.91)
Om (%)	2.93 (0.36)	5.08 (1.88)	6.05 (1.77)
N (%)	0.40 (0.06)	0.54 (0.11)	0.67 (0.17)
P (mg P_2O_5/kg soil)	28.89 (4.36)	32.59 (5.17)	43.33 (12.72)

Note: Numbers in parenthesis are standard deviation of the mean, B-1: one year old burned stand, B-10: ten year old burned stand, UB: unburned stand, CEC: Cations Exchange Capacity; and Om: organic matter.

study since the plots were established in a single stand for each treatment, and conclusions regarding other stands need to be taken with caution.

2.3. Soil sampling

To relate taxa composition to explanatory edaphic variables, soil samples were taken from each study plot. Composite soil samples, from the center and the four corners of each plot, were taken by clearing plant matter and debris from the surface. The soil was extracted to a depth of 20 cm with the aid of an auger and spade. Then the samples were mixed thoroughly, and approximately 500 g of soil was finally taken in a plastic bag for laboratory analysis. After air drying of the soil in shade, soil chemical properties such as Organic matter (OM), Cation Exchange Capacity, Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), Nitrogen (N) and Phosphors (P) were determined using the test methods of Diethylen Triamine Penta Acetic acid (DTPA) extraction, KH_2PO_4 extraction, Olsen, Kjeldahl digestion Walklay Black, Ammonium Acetate and instrumental respectively. The analysis was conducted in Ethiopian Water Works Design and Supervision Enterprises, laboratory service subprocess, soil fertility section at Addis Ababa, Ethiopia.

2.4. Taxa identification and classification

Both morphological and molecular analyses were used for taxa identification. Morphological classification was aided by close microscopic examination of tissues and spores with an Optika B-350PL microscope. Small samples of dried specimens were rehydrated and mounted in 5% KOH. The following keys were mainly used for the purpose: Heinemann (1956), Singer (1965), Pegler (1968, 1969, 1977), Morris (1990), Rammeloo and Walleyn (1993), Ryvarden et al. (1994), Antonin (2007) and Hama et al. (2010). Specimens were deposited in the laboratory herbarium at the University of Valladolid. Up-to-date fungal taxa names and authors' names were obtained from Mycobank database (http://mycobank.org).

Molecular identification involved sequencing of the ITS region of the nuclear ribosomal genes (rDNA). For this, fungal DNA was extracted from dry sporocarps using an EZNA® Plant DNA kit (Omega Bio-Tek, USA) according to the manufacturer's instructions. Final elutions were done in a total volume of 100 µl. The internal transcribed spacer (nrITS) was amplified with primers ITS1F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990). For PCR reactions, HotBegan™ Hot Start Taq DNA Polymerase (Canvax

Biotech, Cordoba, Spain) was used following manufacturer's instructions, adding 1 μl of genomic DNA to a final reaction volume of 25 μl. PCR conditions were: 5 min initial denaturation at 94 °C followed by 40 cycles of: 45 s denaturation at 94 °C, primer annealing at 56 °C for 30 s, and extension at 72 °C for 40 s, followed by a final extension step of 10 min at 72 °C. The PCR products were checked on a 2% agarose gel. Sequences were obtained in the laboratories of Macrogen (Amsterdam, Netherlands) using the primer ITS4 as a template.

Taxa edibility classification was accomplished by adapting the criteria used by Bonet et al. (2004). If the taxon is described in the literature as both non-edible and edible, we classified it as a non-edible. If the taxon is described in the literature as having doubtful edibility, we classified it as a non-edible. As edible (E) are classified all species that are listed as such in the large majority of the literature consulted.

2.5. Statistical analysis

Shannon's H′ diversity index (Shannon and Weaver, 1949) was estimated for each plot using the following formula, where p_i indicated the relative abundance of each macrofungal taxa. This index increases with both the number of species and the evenness of their distribution. It usually ranges between 1.5 and 3.5 and rarely exceeds 4.5 (Kent and Coker, 1992).

$$H = -\sum Pi(lnpi)$$

"Richness" (number of taxa), was defined as the total number of species found per plot.

Richness, Shannon index, and Fresh weight (for the edible and total taxa) estimates were subjected to one-way ANOVA analysis and a post hoc least square means difference test (LSD, $P \le 0.05$) in order to test for differences among stands. Data were log-transformed when needed to achieve the parametric criteria of normality and homoscedasticity that ANOVA requires. All analyses were done with SAS software (SAS Institute Inc., 2012).

An ordination technique based on fungal fresh weight data was used in order to identify significant edaphic explanatory variables related to taxa composition. Firstly, the fresh weight data per taxa were subjected to a detrended correspondence analysis (DCA) (Ter Braak and Prentice, 1988). Since the length of the extracted gradient was less than 3 SD units, a redundancy analysis (RDA) (Ter Braak, 1986) was used to assess the correlation of edaphic variables and fungal taxa composition. Forward selection was used to select significant explanatory variables and only those significant at $P < 0.05$ levels were included in the models. The statistical significance of the Canonical axes was evaluated by Monte Carlo permutation tests (499 permutations). The analysis was conducted using CANOCO for Windows v.4.5 (Ter Braak and Šmilauer, 2002). The RDA result was displayed by ordination diagrams drawn with Cano Draw 4.1 (Ter Braak and Šmilauer, 2002).

3. Results

3.1. Fungal taxa richness and diversity

A total of 61 taxa were collected from the entire study forest area. All of the taxa were saprophytic, belonging to the *Basidiomycota* division (Table 2). No mycorrhizal taxa were found. Out of the total collected taxa, 28 (46%) were identified to species level, 29 (48%) to genus level and 4 (6%) were completely unidentified. The identified taxa were distributed in 13 families and 31 genera (Fig. 2). The families that contained the highest number of species were *Agaricaceae* (20) and *Psathyrellaceae* (12), which all together accounted for 52.5% of the total surveyed taxa (Fig. 2).

Fire had a significant effect on macrofungal taxa richness in the studied stand ($P < 0.0001$). The highest richness value was recorded for the unburned stand. The lowest value was recorded for the forest stand most recently affected by fire (B-1), showing significant differences with the other burned and unburned stands (Fig. 3A; $P < 0.001$). The B-10 and UB stands also showed significant difference in their richness values (Fig. 3A; $P = 0.003$). We also observed some taxa to be exclusive from some specific stands. For example, four taxa were exclusive for B-1, four species were solely found in B-10, and two *Psathyrella* spp. were found exclusively in the UB stand (Table 2).

Shannon's H′ diversity index also showed significant differences between fire affected stands ($P < 0.009$). The lowest Shannon's value was obtained from the stand most recently affected by fire (Fig. 3B). This value was significantly different from that of the ten-year-old burned stand ($P_{B\text{-}1} - P_{B\text{-}10} = 0.008$) and unburned stand ($P_{B\text{-}1} - P_{UB} = 0.005$). However, the B-10 and UB stands showed no significant difference in their Shannon's values (Fig. 3B; $P = 0.059$).

3.2. Sporocarp production

There was no significant difference in total sporocarp production among the three studied stands ($P = 0.214$, Fig. 4). We found an average sporocarp production of 26.16 kg ha^{-1} from the entire area. The lowest average fresh weight production (22.03 kg ha^{-1}) was obtained from the one-year-old burned stand while the highest (35.22 kg ha^{-1}) was from the B-10 stand.

A total of 22 edible taxa were identified (Table 2). The average fresh weight production of edible taxa was significant among the three studied stands (Fig. 4; $P = 0.028$). The highest average production, 7.42 kg ha^{-1}, was from B-10 stand. This value was significantly higher than that of the recently burned stand (B-1) ($P_{B\text{-}10} - P_{B\text{-}1} = 0.011$) but not that of unburned stand ($P_{B\text{-}10} - P_{UB} = 0.061$). The lowest production was collected from B-1 stand, with the average production amount of 3.46 kg ha^{-1} but the value was not significantly different from that of the unburned stand ($P_{B\text{-}1} - P_{UB} = 0.136$).

3.3. Taxa composition

Fungal community assemblies among the three studied stands can be analyzed from the results obtained in the Detrended Correspondence Analysis (DCA) (Table 4). The results are displayed in ordination bi-plots (Fig. 5). Axis-1 separated recently burned stands (B-1) from B-10 and unburned (UB) stands. Axis-2 also showed further differences between B-10 and UB stands where there was a relative higher species overlap. Majority of the taxa in B-10 and UB stands tended to concentrate towards the middle, except some taxa which are dispersed towards the axis two. The taxa in B-1 stand were dispersed towards axis-1 (Fig. 5).

The eigenvalues indicated that the variability in terms of taxa composition, explained by the gradients associated with the first two axes is higher. They together explained about 50.7% of the accumulative variance of fungal taxa data, and an accumulative variance for the interaction between fungal taxa and environment of 89.8% (Table 3). The third and fourth ordination axes with eigenvalues less than 0.1 were less important in ecological terms and not considered further.

A total of three edaphic variables such as Organic matter, Ca and P were found to be significantly related to the saprophytic fungal taxa composition in the ordination ($P < 0.05$, Table 4). The model was significant according to Monte Carlo permutation test for the first axis ($P = 0.002$, $F = 3.372$) and for all canonical axes ($P = 0.002$, $F = 2.158$). In this case, axis one was negatively corre-

Table 2
Total taxa collected from the Dry Afromontane forest of the study area, Wondo Genet (Ethiopia).

Taxa name	Code	B-1	B-10	UB	Edibility
Agaricus aff. *Ampestroides* Heinem & Gooss.-Font.	*Agcam*		x	x	E
Agaricus sp$_1$. L.	*Agar$_1$*		x	x	
Agaricus sp$_2$. L.	*Agar$_2$*		x	x	
Agaricus sp$_3$. L.	*Agar$_4$*		x	x	
Agaricus sp$_4$. L.	*Agar$_5$*		x	x	
Agaricus sp$_5$. L.	*Agar$_6$*		x		
Agaricus subedulis Heinem.	*Agsub*		x		E
Agaricus aff. *trisulphuratus* Berk.	*Agtris*		x	x	
Agrocybe sp. Fayod.	*Agroc*		x	x	E
Amauroderma sp. Murrill.	*Amaur*		x		
Armillaria heimii Pegler.	*Armihe*	x	x	x	E
Armillaria sp. (Fr) Staude.	*Armisp*	x	x	x	E
Calvatia sp. Fr.	*Calva*		x	x	E
Collybia piperata (Beeli) Singer.	*Colpip*		x	x	
Conocybe sp. Fayod.	*Conoy*		x	x	
Coprinellus domesticus (Bolton) Vilgalys, Hopple & Jacq. Johnson.	*Copdm*		x	x	E
Coprinellus sp. P. Karst.	*Coprll*	x	x	x	E
Coprinopsis nivea (Pers.) Redhead, Vilgalys & Moncalvo	*Coprni*		x	x	E
Coprinopsis sp$_1$. P. Karst.	*Copr$_1$*		x	x	E
Coprinopsis sp$_2$. P. Karst.	*Copr$_2$*	x	x	x	E
Coprinus sp. Pers.	*Coprsp*	x	x	x	E
Crepidotus sp. (Fr.) Staude.	*Crepid*	x	x	x	
Cyptotrama asprata (Berk.) Redhead & Ginns.	*Cypas*		x	x	
Favolaschia calocera R. Heim.	*Favcal*	x			
Gerronema hungo (Henn.) Degreef & Eyi.	*Gerhug*	x	x	x	
Gymnopilus junonius (Fr.) P.D. Orton.	*Gymjun*	x	x	x	
Gymnopilus pampeanus (Speg.) Singer.	*Gympa*	x	x	x	
Hygrophoropsis aurantiaca (Wulfen) Maire.	*Hygau*	x			E
Hymenagaricus sp. Heinem.	*Hymsp*		x	x	E
Hypholoma fasciculare (Huds.) P. Kumm.	*Hyphfas*	x	x	x	
Lepiota aff. *cristata* (Bolton) P.Kumm.	*Lepcri*	x	x	x	
Leucoagaricus holosericeus (J.J. Planer) M.M. Moser.	*Leuhol*		x	x	E
Leucoagaricus leucothites (Vittad.) Wasser.	*Leuleu*		x		E
Leucoagaricus aff. *rubrotinctus* (Peck) Singer.	*Leurub*		x	x	E
Leucoagaricus sp$_1$. Locq. ex Singer.	*Leucoa$_1$*		x	x	E
Leucoagaricus sp$_2$. Locq. ex Singer	*Leucoa$_2$*	x	x	x	E
Leucocoprinus birnbaumii (Corda) Singer.	*Leucbir*		x	x	E
Leucocoprinus cepistipes (Sowerby) Pat.	*Leucep*		x	x	E
Lycoperdon sp. Pers.	*Lycoper*	x	x	x	E
Marasmius buzungolo Singer.	*Marbuz*	x	x		
Marasmius katangensis Singer.	*Markat*	x	x	x	
Marasmius aff. *Rotalis* Berk & Broome.	*Marrot*	x	x		
Marasmius sp$_{1.}$ Fr.	*Marsp*	x	x	x	
Microporus sp .P. Beauv.	*Microsp*		x	x	
Parasola sp$_1$. Redhead, Vilgalys & Hopple.	*Paras$_1$*	x	x	x	
Parasola sp$_2$. Redhead, Vilgalys & Hopple.	*Paras$_2$*	x		x	
Polyporus aff. *badius* (Pers.) Schwein.	*Polbad*	x	x	x	
Psathyrella sp$_1$. Fr.ex Quél.	*Psath$_1$*			x	
Psathyrella sp$_2$. Fr.ex Quél.	*Psath$_2$*			x	
Psathyrella sp$_3$. Fr.ex Quél.	*Psath$_3$*		x	x	
Psathyrella sp$_4$. Fr.ex Quél.	*Psath$_4$*		x	x	
Psilocybe cyanescens Wakef.	*Psicya*		x	x	
Psilocybe merdaria (Fr.) Ricken.	*Psilmed*	x	x	x	
Psilocybe sp. (Fr.) P. Kumm.	*Psilsp*	x	x	x	
Trametes versicolor (L.) Lloyd.	*Traves*		x		
Tremella mesenterica (Schaeff.) Retz.	*Tremes*	x			
Undescribed sp$_1$.	*Unkn$_1$*	x	x	x	
Undescribed sp$_2$.	*Unkn$_2$*	x			
Undescribed sp$_3$.	*Unkn$_3$*	x	x	x	
Undescribed sp$_4$.	*Unkn$_4$*		x	x	
Xerula sp. Maire.	*Xerusp*		x		

Note: B-1 and B-10 = One and ten years old burned plots, UB = unburned plots, E = Edible.

lated with all the three edaphic variables while axis-2 was positively correlated with all of them (Fig. 5).

4. Discussion

4.1. Taxa richness and diversity

Reports on macrofungal species from Ethiopian forests are limited. This study is the first to explain fungal communities in the Dry Afromontane forest system in the country. The taxonomic classification in this study was particularly challenging, as from the collected taxa, about 48% could be identified only to the genus level. This was an indication of both the uniqueness of the Dry Afromontane forest ecosystems in terms of diversity of yet undescribed macrofungi species as well as the lack of scientific studies on the local fungal flora in the country. All of the taxa collected were saprophytic, i.e. no ectomycorrhizal species were found. This was not surprising as the majority of tropical woody tree species are

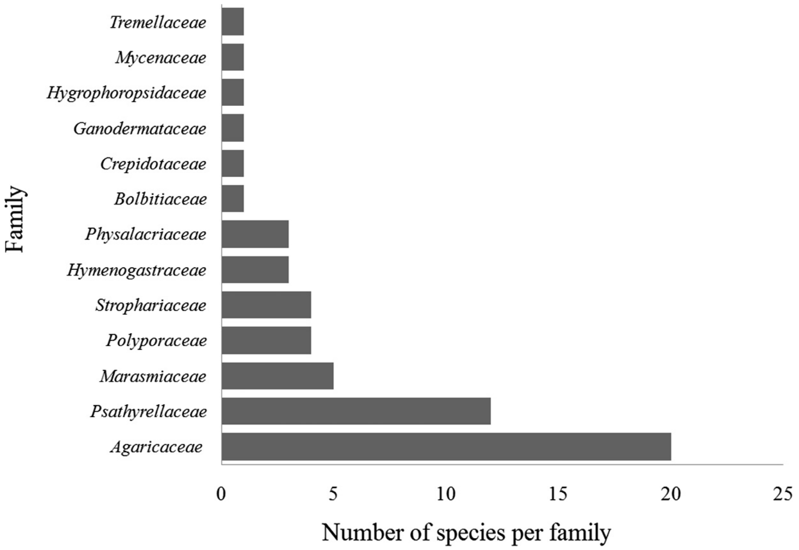

Fig. 2. Total number of fungal species per family encountered in the Dry Afromontane forest area of Wondo Genet (Ethiopia).

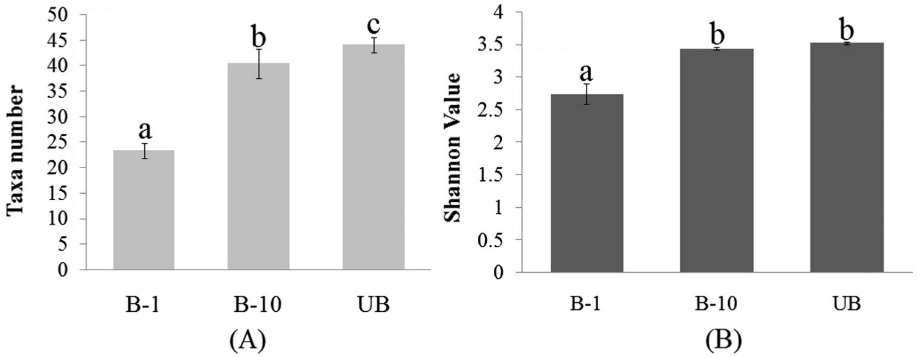

Fig. 3. Number of taxa (A) and the Shannon diversity index (B) in the Dry Afromontane forest area of Wondo Genet (Ethiopia). The data are mean results ± standard error of the mean. Values with the same letter are not significantly different.

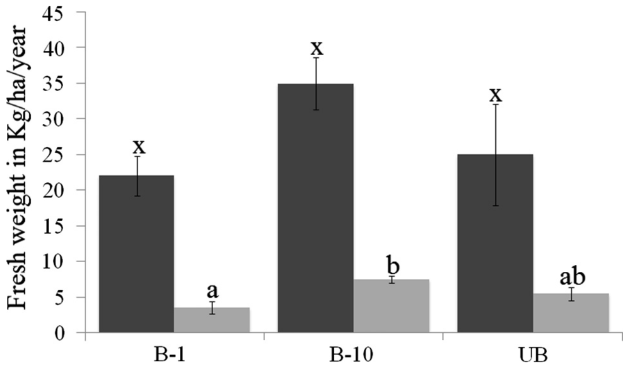

Fig. 4. Production of sporocarps according to total taxa (Dark colour) and edibility (Gray colour), in kg ha^{-1}collected from the dry Afromontane forest area of Wondo Genet (Ethiopia). The data are mean results ± standard error of the mean. Values with the same letter are not significantly different.

unable to form associations with ectomycorrhizal fungi (Brundrett, 2009), particularly those tree species of our study area.

The present study revealed occurrence of 61 macrofungal species considering fire-affected and unburned stands together. The number of fungal taxa reported here is the highest among the literature in Ethiopian natural forest areas. For example, Hjortstam and Ryvarden (1996) reported 15 taxa, Alemu (2013) reported seven taxa, and Decock et al. (2005) reported four taxa from the highland forest areas in the country. There are no previous works studying fire-fungal relation in Ethiopia. However, research in the Mediterranean region where forest fire is a dominant factor, have characterized the fungal communities. Unlike our result, lower numbers of saprophytic taxa were reported by Martín-Pinto et al. (2006), Oria-de-Rueda et al. (2010) and Mediavilla et al. (2014). This difference might be due to the variation in ecological factors such as climate and soils, which are among the most important factors that could affect saprophytic fungal taxa richness (Oria-de-Rueda et al., 2010). In the Mediterranean region, for example, rainfall is much lower (≤600 mm) than that of the Afromontane region where we carried out this study in Ethiopia (≥1200 mm). This difference in rainfall likely had an impact on richness as saprophytic fungi are dependent on available moisture in the soil for their fructification (Høiland, 2012). Furthermore, the reason could also be explained by the type of litter coverage on the forest soil, and thus on the species composition of the stands. A reduction in plant species richness (substrate richness) has been found to influence the diversity and richness of saprophytic species (Reverchon et al., 2010) as they depend on the available substrates. This probably indicates that the richness and complexity of the Dry Afromontane forests supply diversified substrates for saprophytic fungi to occur, as compared to the monotypic *Quercus* forest and/

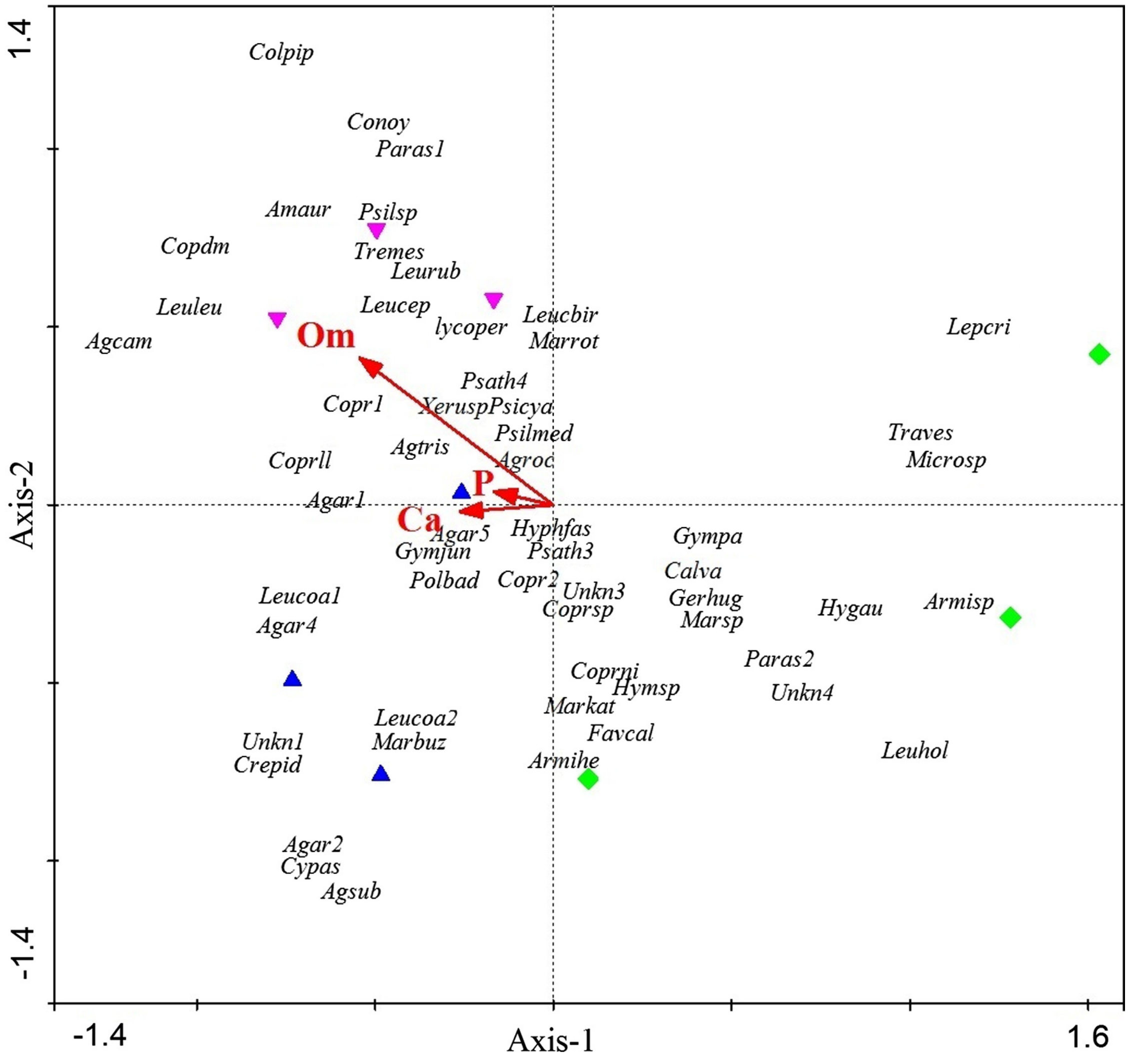

Fig. 5. RDA ordination bi-plots showing: fungal taxa abbreviated shown in Table 2, Plots in similar colour are in a group (Green triangle (B-1), Blue upward triangle (B-10), and Purple downward triangle (UB)) and environmental factors (arrows).

Table 3
Summary of constrained principal component analysis of fungal taxa presence and environmental factors for the study area, Wondo Genet (Ethiopia).

Axes	1	2
Eigenvalues	0.403	0.104
Taxa-environment correlations	0.925	0.892
Cumulative percentage variance of		
taxa data	40.3	50.7
taxa-environment relation	71.4	89.8

Table 4
Significant edaphic variables resulted from the forward selection process in Redundancy analysis (RDA).

Variables	*F*-ratio	*p*-value
OM	0.69	0.038
Ca	2.14	0.040
P	0.43	0.042

or *Pinus* plantation stands assessed by Martín-Pinto et al. (2006), Oria-de-Rueda et al. (2010) and Mediavilla et al. (2014) in the Mediterranean region.

High numbers of saprophytic taxa were also reported from studies in other eco-regions. For example, Tibuhwa et al. (2011) reported 91 taxa in Serengeti-Masai Mara ecosystem in Tanzania and Kenya, in Africa. Similarly, 72 saprophytic taxa were reported by Reverchon et al. (2010) from Mexico. O'Hanlon and Harrington (2012) also reported high numbers of saprophytic species from Atlantic region where rainfall is high. In all these cases, the sampling was done for extensive periods of time. Such systematic studies likely have a positive effect on macrofungal taxa numbers as fungi occurrence show considerable seasonal and yearly variation and in some cases individual taxa may not appear every year (Tibuhwa et al., 2011).

Forest fires typically have both short- and long-term effects on fungal communities. As a short-term effect, fire causes a reduction in richness (Kutorga et al., 2012; Oliver et al., 2015; Reazin et al., 2016) and as a long-term effect, fire also causes a shift in the presence or relative frequencies of fungal species in the forest system (Rincón and Pueyo, 2010; Smith et al., 2017). In this study, we also observed the least fungal taxa richness in the recently burned stand. This negative effect could be associated with the reduction of substrates in the forest floor after fire (Smith et al., 2008) and also due to the negative effect of fire on organic matter deposited in the soil depending on fire intensity, leading to indirect effect on fungal growth and perpetuation (Kennedy et al., 2014). The loss of topsoil by erosion after fire could also reduce the infectivity of fungal propagules (Rashid et al., 1997), and thereby reduce taxa richness immediately following fire.

Fungal taxa richness was highest in UB stand (Fig. 3A). This is in line with Ratkowsky and Gates (2009) and Mediavilla et al. (2014) who noted succession of macrofungi related to time since fire in the *Eucalypt* and *Pinus* forests of Southern Tasmania and Spain respectively. Such increasing trend might be explained by the high soil humification and litter layer in relatively developed and canopy closed forest systems, which are particularly relevant for more saprophytic fungi occurrence. This assumption coincides with Dighton et al. (1986), Sysouphanthong et al. (2010) and Toivanen et al. (2012) who noted high fungal richness in matured stands with canopy closure.

When comparing diversity values among stands, the results were consistent with those from our richness analysis i.e. the lowest value was recorded at the one-year-old burned stands (Fig. 3B). The diversity value in early stage of fire might be due to the typical effects of fire to limit the type and number of fungal species appearing in an area. Thus, only a few fungi establish early in post-fire conditions, appearing adapted to the environment created after fire (Hansen et al., 2013; Reazin et al., 2016; Smith et al., 2017). In the following successional stage, however, the diversity values showed non-significant differences with the unburned stand (Fig. 3B). This might suggest that the environmental conditions in both stands are less likely to be hostile for a variety of fungal species. Thus, a large number of fungi are uniformly fruiting and distributed within any of these stands (Hernández-Rodríguez et al., 2013).

4.2. Sporocarp production

The negative effect of fire on sporocarp production has been investigated in previous studies by Hart et al. (2005), Bastias et al. (2006), Cairney and Bastias (2007), Hernández-Rodríguez et al. (2013); and Mediavilla et al. (2014) from multiple geographical areas. Post fire fruiting and the relative effects of fire on fungi fruiting, with a special emphasis on the saprophytic species are also deeply reviewed (Taudière et al., 2017). In the present study lower fungal production was also collected from recently burned stand. However, no significant differences were found. The absence of difference in sporocarp production between a recently burned and an unburned stands was previously reported by Mediavilla et al. (2014) who studied the effect of fire on saprophytic species associated to *Pinus nigra* stands in the Mediterranean. This could be explained by the existence of fungal species whose ephemeral fruit bodies may cover forest soil in recently burned areas (Hart et al., 2005; Taudière et al., 2017), taking advantage of the condition created (Bean et al., 2009). For example, *Armillaria* sp. fruited most abundantly in a recently burned stand as compared to other fungal species. This species might survive in the early stage of after fire and accumulate biomass either by persisting on remnant plant bodies or uses other organic matter after fire (Bonello et al., 1998). Furthermore, the quick recovery rates of some plant species like *Cordia africana*, an early colonizer in forest rehabilitation in the burned stand might also contribute to the quick accumulation of organic matter in the soil, which in turn benefits saprophytic fungi (Bonello et al., 1998).

Edible sporocarp production was also lower in B-1 stand (Fig. 4). However, only differences between B-10 and B-1 stands were observed. This result seems to contrast with those obtained for the total sporocarp production comparing B-10 and B-1 stands, where the total biomass production didn't significantly differ. An explanation for this apparent contradiction could be due to the existence of some exclusive taxa in B-10 that we characterized as higher biomass producer (e.g. *Agaricus subedulis*). This species accounted for up to 25% of the total edible sporocarp fresh biomass in B-10 stand of the study area. On the other hand, the p-value observed between B-10 and UB ($P = 0.06$) suggests a slight difference in sporocarp production. This should be ensured through further sampling efforts enough to detect practically existing differences between the two stands.

4.3. Taxa composition

We found the recently burned stand to have distinctive fungal communities (Fig. 5). The non-litter decomposer fungal species are more favored and exclusively found in this stand. This is likely the case for the species of *Favolaschia calocera* and *Hygrophoropsis aurantiaca*. These species are reported to commonly fruit in areas disturbed by human activities such as in burned forests (Smith and Read, 1997; Vizzini et al., 2009). Fungal species richness is lowest in the recently fire-impacted stand. This highlighted the perturbative effects that fire has on both the type and number of species, only those able to resist or adapt to the new conditions (Greeshma et al., 2016).

Similarities were found between B-10 and UB stands. Both are found on the right side of the ordination, characterized by high taxa richness. *Agaricus* spp. and most *Leucoagaricus* spp. were exclusively found in these stands. The possible explanation for such exclusive occurrence and high number of taxa in both stands could be due to the increased complexity of the forest system, featuring high soil humification and thickness of the litter layer (Toivanen et al., 2012; Mediavilla et al., 2014), particularly relevant for higher saprophytic fungi occurrence. Furthermore, the presence of a high number of taxa shared between B-10 and UB stands might be a reason for such occurrence. Some species occurring in the extreme points of the ordination axis are probably responding to specific ecological requirements of the species.

When analyzing saprotrophic taxa by family, we found *Agaricaceae*, *Psathyrellaceae*, and *Marasmiaceae* appearing in all three studied stands. The largest number of fungal taxa we found from the entire forest area was also represented by these families. The main reason for such wider representation might be the species in these families are able to fruit in different ecological conditions and have wide substrate requirements. Species such as *Armillaria heimii*, *Coprinellus* sp., *Coprinus* sp., *Crepidotus* sp., *Gerronema hungo*, *Gymnopilus junonius*, *Gymnopilus pampeanus*, *Hypholoma fasciculare*, *Lepiota cristata*, *Leucoagaricus* sp., *Lycoperdon* sp., *Marasmius buzungolo*, *Parasola* sp., *Psilocybe merdaria* were common to all study stands evaluated in this study. Interestingly, *Marasmius* sp. and *Lepiota* sp. were reported as common species in both fire affected and unburned stands by Greeshma et al. (2016).

It is well established that fungal communities as a whole are significantly influenced by edaphic variables (Straatsma et al., 2001; Zakaria and Boddy, 2002), although information about particular species is scarce. This is because soil nutrients have been shown to affect mycelial development and hence sporocarp occurrence (Zakaria and Boddy, 2002). In the present study, there seems to be a cumulative effect of edaphic variables on the composition and distribution of macrofungi in the studied forest stands. The soil OM, P and Ca were found to correlate significantly with the fungal taxa composition. Among these edaphic variables, OM appeared to be the most important factor related to saprophytic fungal composition (Fig. 5). This is likely because fungi typically extend their mycelia at the soil-litter interface (Boddy et al., 2009) and thereby the organic matter influences mycelia outgrowth and network formation (Zakaria and Boddy, 2002). Organic matter also influences the fungal community through its impact on water holding capacity and nutrient availability in the soil (Harrington, 2003). Thus, OM may favor more saprophytic fungal assembly in an area.

Different fungal species seemed to have divergent responses to different soil factors. In this study we found that *Amauroderma* spp., *Agaricus campestroides*, *Coprinopsis* spp., *Psilocybe* spp., *Leucocoprinus birnbaumii*, *Leucoagaricus leucothites*, and *Parasola* spp., appeared to be in the higher end points of OM gradient in the ordination suggesting that the composition of these species could be highly associated with OM in the soil. Some of the species listed above (e.g. *L. birnbaumii*) are reported to be common in areas with abundant decayed plant matter (Dutta et al., 2011).

In this study we also found that the edaphic variables of P and Ca were correlated with saprophytic taxa composition. The result coincides with Gassibe et al. (2015) who noted the correlation of P with saprophytic taxa under *Pinus* stands in the Mediterranean. Cairney (2011) also noted the significant influence of P on soil fungal taxa assemblages. Such correlation might be due to the fact that P, among other elements, is essential for the growth and sporulation of fungal species (Nonzom and Sumbali, 2014), and thereby could influence sporocarp production. Likewise, Ca has been also been implicated as one of the major factors associated with fungal composition in the present study, as Ca is also crucial for fungal metabolism (Bindschedler et al., 2009) and growth (Griffin, 1994). Thus, some saprophytic species that are related to OM were also associated with the P and Ca in the ordination. In this sense, species such as *Agaricus campestroides*, *A. trisulphuratus, Psathyrella* sp., *Parasola* sp., *Psilocybe* sp., *Coprinellus* sp., and *Leucoagaricus* sp., were also closely related with P and Ca content of the soils in our study, indicating that their fructification is probably influenced by these elements. The fructification of these species was higher in UB and B-10 stands where the nutrients availability was higher. Some *Agaricus* and *Collybia* species were previously reported in unburned stands, where there was a higher availability of a pool of nutrients, when comparing to fire affected stands (Mediavilla et al., 2014).

5. Conclusion

This study represents the first systematic research providing noticeable contribution to the knowledge of fungal communities in the Dry Afromontane forests in Ethiopia and their relation with fire perturbation. The work provides a starting place in broadening management objectives for NTFPs in the Dry Afromontane forests. However, the results should be regarded as a preliminary indication due to sampling limitations.

The result indicated that an unburned mature natural stand provided high fungal richness as compared to the burned stand. The low fungal diversity was reported in the recently burned stands. An indication of differences in edible fungal yields was also observed among the studied stands. Finally, we also observed the noticeable presence of the edible species like *Agaricus subedulis* which could be potentially marketed in rural areas providing supplementary incomes. Taxa compositions in unburned and 10 years old stands were significantly explained by higher amounts of soil OM, Ca and P.

The results of this preliminarily case study are also encouraging from a conservation point of view. It provides novelty information about the diversity of macrofungal species in Ethiopian Dry Afromontane forests, likely including many taxa which are still unknown to science as well as several edible species which could supply complementary incomes for the rural populations in the study area.

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Original article IV

Diversidad fúngica y sucesión ante el desarrollo de rodales de *Pinus patula* Schiede ex Schltdl. & Cham en Etiopia

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Resumen

Se conoce poco sobre las comunidades fúngicas asociadas con coníferas de bosques tropicales en contraste con la información disponible sobre poblaciones fúngicas en coníferas de bosques templados. El presente estudio examinó la sucesión macrofúngica, así como su correspondiente producción de carpóforos, en rodales de *Pinus patula* en Etiopía. Se establecieron nueve parcelas permanentes (100 m^2) en rodales de *P. patula* con edades de cinco, once y treinta y seis años de edad. Se establecieron la riqueza de taxones, la diversidad y la producción de carpóforos. También se colectaron muestras de suelo compuestas en cada parcela para determinar las variables edáficas explicativas de las composiciones de los taxones de hongos. Se encontraron 53 taxa de hongos (Basidiomycota), con excepción de *Xylaria hypoxylon* (Ascomycota). La mayoría de los taxones encontrados fueron saprofíticos y cerca de 6% fueron ectomicorricicos. Alrededor del 36% de los taxa fueron comestibles. La riqueza de taxones, el índice de diversidad de Shannon y los rendimientos de carpóforos mostraron tendencias significativamente crecientes hacia los rodales de *P. patula* con mayor edad. La composición de la comunidad fúngica también estuvo correlacionada con los niveles de N, K y de pH. Esta investigación arrojó nuevos conocimientos sobre la diversidad fúngica y la estructura de la comunidad en plantaciones manejadas de *P. patula*. Se encontró unas mayores diversidades y producción de carpóforos en los rodales más antiguos. Se concluyó que la presencia deliberada de árboles maduros en Pinares podría proporcionar incentivos para la producción y conservación de especies fúngicas de importancias tanto ecológicas como comerciales en el área de estudio.

Keywords: *Factor edáfico, comunidad fúngica, Pinus, carpóforos, edad de plantación, sucesión.*

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Fungal diversity and succession following stand development in *Pinus patula* Schiede ex Schltdl. & Cham. plantations in Ethiopia

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ABSTRACT

Fungal communities associated to conifers have been typically studied in temperate conifer forests, but little is known about tropical habitats. The present study examined the macrofungal succession and corresponding sporocarp production in *Pinus patula* stands in Ethiopia. For this purpose, we surveyed nine permanent plots (100 m^2) established in five-, eleven- and thirty-six years old *P. patula* plantations and estimated fungal taxa richness, diversity and sporocarp yields. Composite soil samples were also collected from each plot to determine explanatory edaphic variables for fungal taxa compositions. We found a total of 53 fungal taxa belonging to *Basidiomycota*, with the exception of *Xylaria hypoxylon* which is *Ascomycota*. The majority of the collected taxa were saprophytic and about 6% were ectomycorrhizal fungi. About 36% of the taxa were classified as edible. Taxa richness, the Shannon diversity index and sporocarp yields showed significant increasing trends towards the more mature *P. patula* stands. Fungal community composition was also correlated significantly with N, K, and pH. This study extends our knowledge on fungal diversity and community structure in managed *P. patula* plantations. Higher diversity and sporocarp production was found in older stands. Thus, the deliberate presence of mature trees in *Pinus* stands could provide incentives for the production and conservation of ecologically and commercially important fungal species in the study area.

1. Introduction

In Ethiopia, natural forests cover was about 40% of the total land area, delivering a wide range of social, economic and environmental benefits including key components of biodiversity (Badege, 2001; Thomas and Bekele, 2003). Anthropogenic disturbances, however, are causing a dramatic decline of the natural forests and gradually changed the scene. Today the area covered with natural forests is less than 3% of the country's total lands (Lemenih and Bekele, 2008; Taddese, 2001). Illegal logging, agricultural land expansion and forest fires are the main factors that have been affecting forests in the country. The remaining natural forests are highly fragmented and are being modified by plantations of non-native tree species (Bekele, 2011; Moges et al., 2010; Tesfaye et al., 2016).

Plantation forestry in Ethiopia began near the turn of the 19th century (FAO, 2009; Teketay, 2000). During this period, *Eucalyptus* was the first introduced species, followed by *Cupressus*, and *Pinus* tree species (Bekele, 2011; Tesfaye et al., 2015). The importance of the plantation sector in the country is increasing as the demand for woody raw materials is rising and the supply from the natural forests is decreasing. Accordingly, the areas under plantation have been increasing from an estimated 190,000 ha (ha) in 1990 to 972,000 ha today (Bekele, 2011). Most of the plantations were established as community forests and they have a considerable potential for sustainable production of high value timber and Non Timber Forest Products (NTFPs) (Lemenih and Bekele, 2008). *Pinus* tree plantations, however, are established mainly in state-owned forests, occupying the third position in terms of plantation area coverage (Bekele, 2011; Gezahgne, 2003). They contribute to the production of round wood for sawn timber, poles and posts owing to their rapid growth (Gezahgne, 2003).

Pinus trees are well known to form symbiotic associations with mycorrhizal fungi crucial for their growth and survival (De Mendonça Bellei et al., 1992). Colonization by fungi improves the ability of trees to acquire mineral nutrients and water from the soil and plant pathogen resistance (Dahlberg et al., 2001; Dalong et al., 2011). In return, fungi benefit from the host tree by obtaining carbohydrates (Lindahl et al., 2007). Saprotrophic fungi also play essential ecological roles in the decomposition of dead organic matter and nutrient cycling in the forest ecosystems (Ferris et al., 2000). Some mycorrhizal and saprotrophic fungi are edible, and

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represent economically important NTFPs in plantation forest systems (Boa, 2004). Edible fungi provide supplementary economical incomes as a complement to those obtained from timber, and in some cases, they could generate higher benefits than timber productions (Oria-de-Rueda et al., 2008).

As forests develop, changes in succession of the associated fungal species occur (Gassibe et al., 2011; Luoma et al., 1991; Smith et al., 2002). Thus, the fungal species could be early-stage, which develop from the spore bank present in the soil before the development of the stand, and late-stage fungi, whose fruiting is enhanced by the new conditions (Hernández-Rodríguez et al., 2013). Both early and late stage fungi play a role in soil stabilization and restoration of soil microflora (Claridge and Trappe, 2004). Fungal succession in *Pinus* plantations have been studied previously (Fernández-Toirán et al., 2006; Gassibe et al., 2011; Mediavilla et al., 2014). These studies reported an increase of fungal richness and their sporocarps production following stand development in Mediterranean ecosystems, where the rotation period is typically long.

Green-tree retention as a forest management practice has been undertaken in different parts of the world to achieve multifunctional goals, mainly of wood production and biodiversity conservation (Nyland, 2002). This approach has also important implications for forest floor microhabitats, such as moisture, temperature, and substratum (Smith et al., 2008). However, such management approach is poorly represented into forest management systems in Ethiopia as the plantation forests mainly depend on the traditional silvicultural systems. For instance, the rotation time for *Pinus* trees is usually about 26–30 years (Bekele, 2011). In some cases, the trees are deliberately left for more years than mentioned in order to provide large-sized logs. *Pinus* plantations are managed using a ‘‘plant, clear fell and replant” cycle method (Teshome, 2011). Retention of trees in some structure classes for ecosystem maintenance is not a common practice. This indicated that the relatively short rotation period of the tree and the management practices may impact the succession and sporocarp production of ecological and commercially important macrofungal species (Mediavilla et al., 2014; Paillet et al., 2010), though such impacts are yet understudied in Ethiopia. Thus, understanding of the fungal succession along stand ages may be a means to improve biodiversity, sporocarp production, and fungal conservation in plantation forest systems in Ethiopia. This might also help to compare the fungal succession in *Pinus* plantations relative to other areas with longer gestation periods.

This pioneering work was designed to generate information helpful for integrated forest management aimed at combining both timber and NTFPs production in Ethiopia, especially mushrooms for both economic and ecological benefits. Thus, the general objective was to characterize the fungal communities among *P. patula* stands of three age classes and explain the fungal compositions based on edaphic variables in Southern Ethiopia. The specific objectives include: (1) to evaluate fungal taxa richness and diversity variation among stands of three age classes, (2) to examine sporocarps biomass yield among stands based on edibility and total production, and (3) to correlate explanatory edaphic variables with fungal taxa compositions.

2. Methodology

2.1. Study area

The study was conducted at Wondo Genet College of forestry plantation forest area in Southern Ethiopia. The study area is found approximately 265 km from Addis Ababa, the capital city of Ethiopia (Fig. 1). It is located at 7°06′–7°07′ N latitude and 38°37′–38°42′ E longitude with an altitudinal range between 1600 and 2580 m above sea level (m.a.s.l.) (Belaynesh, 2002). The climate of the study area is characterized by Woyna Dega agro-climatic type. The rainfall pattern is bimodal, with minor rainfall during spring and the major rain season is during summer. The average annual rainfall and temperature of the study area is 1210 mm and 20 °C respectively (Belaynesh, 2002; Fenta, 2014). The topography is slightly undulating and the soils are young and of volcanic origin, characterized by sandy loam. The soil is shallow at steep convex slopes but deeper at lower altitudes (Eriksson and Stern, 1987) where most of the plantations are located.

The original vegetation of the study area was destroyed long ago as a result of clearance for cultivation and logging (Teshome, 2011). Consequently, mass plantation of exotic tree species was established and currently about 100 ha (hectares) of non-native forests of different tree species are found in the study area. The three predominantly planted species are *Cupressus lustianica*, *Gravilla robusta* and *P. patula* (Bekele et al., 2013; Teshome, 2011).

2.2. Sporocarps sampling

Three different *P. patula* stands were selected based on their ages: 5-, 11- and 36-years-old stands (Table 1). Following previous studies, fungal diversity and production was obtained by using transect methods (Smith et al., 2002). In our case, three 2×50 m (100 m^2) plots were established at each stand, i.e. nine plots in total according to Gassibe et al. (2011) and Hernández-Rodríguez et al. (2013). Within each of the selected stands, plots were placed systematically about 120 m apart from each other (Luoma et al., 1991). All plots were similar in terms of their ecological conditions such as climate, altitude, and soil. This work could be considered as a case study since the plots were established in a single stand for each age class, and conclusions regarding other stands need to be taken with caution.

All sporocarps found in the plots were fully harvested weekly during the rainy season in July and August in 2015. Fresh weight measurements were carried out *in situ* and the data are given in kilograms per hectare per year (kg fwt/ha/year). Also, abundance, the number of sporocarps per species, data was taken from each plot. Sample fruit bodies from each species were taken to the laboratory and dried. Herbaria specimens were used for molecular and microscopic taxa identification. Furthermore, in the field, specimens were photographed and their ecological characteristics were noted in order to assist and facilitate taxa identification processes.

2.3. Soil sampling

To relate taxa composition to explanatory edaphic variables, soil samples were taken from each study plot. Composite soil samples, from the center and the four corners of each plot, were taken by clearing plant matter and debris from the surface. The soil was extracted to a depth of 20 cm with the aid of an auger and spade. Then the samples were mixed thoroughly, and approximately 500 g of soil was finally taken in a plastic bag for laboratory analysis. After air drying in shade, soil chemical properties such as pH, organic matter, Cation Exchange Capacity, Na, K, Ca, Mg, N, P and soil physical properties such as sand, silt and clay were determined using the test methods of DTPA extraction, KH_2PO_4 extraction, Olsen, Kjeldahl digestion Walklay Black, Ammonium Acetate and instrumental respectively (Table 2). The analysis was conducted in Water Works Design and Supervision Enterprises, laboratory service sub-process, soil fertility section at Addis Ababa, Ethiopia.

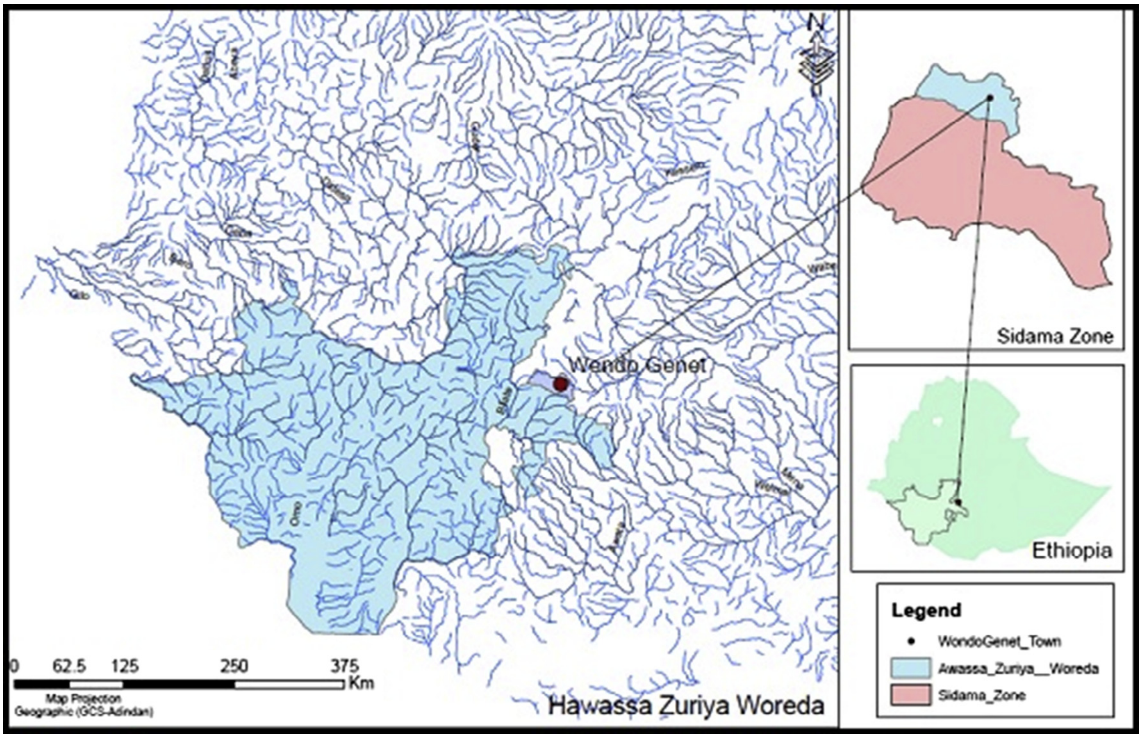

Fig. 1. Location map of the study area, Wondo Genet, Ethiopia.

Table 1
Characteristics of *Pinus patula* study stands in Wondo Genet (Ethiopia), DBH, diameter at breast height, Ht, height, ha, hectares. *Source:* Zewdie et al. (2010).

Plantation time	Stand age (year)	Area (ha)	Density per ha	Mean DBH (cm)	Mean Ht (cm)	Silvicultural treatments
2010	5	5	1958	–	–	Weeding and slashing
2004	11	4.8	1880	7.0	7.3	Weeding and slashing
1979	36	1.7	239	34.8	24.9	Thinning and pruning

Table 2
Selected edaphic variables of the three *Pinus patula* stands in Wondo Genet area (Ethiopia).

Soil parameter	Stand age groups		
	5-Year	11-Year	31-Year
Sand (%)	66.63^a	50.10^b	52.39^b
Silt (%)	19.93^a	23.33^a	24.06^a
Clay (%)	14.44^a	19.89^{ab}	21.88^b
pH	6.04^a	5.42^a	5.44^a
Na (meg/100 g soil)	0.85^a	0.78^a	1.10^a
K (meg/100 g soil)	1.32^a	0.42^b	0.77^c
Ca (meg/100 g soil)	22.62^a	13.66^a	16.01^a
Mg (meg/100 g soil)	7.96^a	4.36^b	6.67^a
CEC (meg/100 g soil)	49.54^a	28.07^b	42.04^c
% OM	5.96^a	7.27^a	13.33^b
N (%)	0.83^a	0.71^b	0.34^c
P (mg P_2O_5/kg soil)	35.70^a	36.99^a	38.56^a

Note: Lower case letters indicate differences among stand ages. Values with the same letter are not significantly different.

2.4. Taxa identification and characterization

Both morphological and molecular analyses were used for taxa identification. The taxonomic classification was aided by close microscopic examination of tissues and spores with an Optika B-350PL microscope. Small samples of dried specimens were rehydrated and mounted in 5% KOH. The following keys were mainly used for the purpose: Antonin (2007), Hama et al. (2010), Heinemann (1956), Morris (1990), Pegler (1969, 1968, 1977), Rammeloo and Walley (1993), Ryvarden et al. (1994), Singer (1965). Specimens were deposited in the laboratory herbarium at the University of Valladolid. Up-to-date fungal taxa names and authors' names were obtained from Mycobank database (http://mycobank.org).

Molecular identification involved sequencing of the ITS region of the nuclear ribosomal genes (rDNA). For this, fungal DNA was extracted from dry sporocarps using an EZNA® Plant DNA kit (Omega Bio-Tek, USA) according to the manufacturer's instructions. Final elutions were done in a total volume of 100 μl. The internal transcribed spacer (nrITS) was amplified with primers ITS1F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990). For PCR reactions, HotBegan™ Hot Start Taq DNA Polymerase (Canvax Biotech, Cordoba, Spain) was used following manufacturer's instructions, adding 1 μl of genomic DNA to a final reaction volume of 25 μl. PCR conditions were: 5 min initial denaturation at 94 °C followed by 40 cycles of: 45 s denaturation at 94 °C, primer annealing at 56 °C for 30 s, and extension at 72 °C for 40 s, followed by a final extension step of 10 min at 72 °C. The PCR products were checked on a 2% agarose gel. Sequences were obtained in the laboratories of Macrogen (Amsterdam, Netherlands) using the primer ITS4 as a template.

Taxa edibility classification was accomplished by adapting the criteria used by Bonet et al. (2004). If the taxon is described in the literature as either non-edible or edible, we classified it as a non-edible. If the taxon is described in the literature as having doubtful edibility, we classified it as a non-edible. As edible (E) were classified all species that are listed as such in the large majority of the literature consulted.

2.5. Statistical analysis

Shannon's H′ diversity index (Shannon and Weaver, 1949) was estimated for each plot using the following formula, where p_i indicated the relative abundance of each macrofungal taxa. This index increases with both the number of species and the evenness of their distribution. It usually ranges between 1.5 and 3.5 and rarely exceeds 4.5 (Kent and Coker, 1993).

$$H = -\sum Pi(\ln pi)$$

"Richness" (number of taxa), was defined as the total number of species found per plot.

Richness, Shannon index, and Fresh weight (for the edible and total taxa) estimates were subjected to one-way ANOVA analysis and a post hoc least square means difference test (LSD, $P \leq 0.05$) in order to test for differences among stand ages. Data were log-transformed when needed to achieve the parametric criteria of normality and homoscedasticity that ANOVA requires. All analyses were done with SAS software (SAS Institute Inc., 2012).

Species accumulation curves were also constructed to compare the rate at which new species are weekly found among the three studied stands and to provide an estimate of species richness. The curves were generated using sample based estimator of EstimateS Version 9 (Colwell, 2013). The cumulative number of species encountered during each weekly visit to plots within a stand constituted the sample represented in the output axis. These curves were generated based on a total of 6 weekly samplings.

An ordination technique based on fungal fresh weight data was used in order to identify significant edaphic explanatory variables related to taxa composition. Firstly, the fresh weight data per taxa were subjected to a Detrended Correspondence Analysis (DCA) (Ter Braak and Prentice, 1988). Since the length of the extracted gradient was greater than 3 SD units (3.03), the Canonical Correspondent Analysis (CCA) (Ter Braak, 1986) was used to assess the correlation of edaphic variables and fungal taxa composition. Forward selection was used to select significant explanatory variables and only those significant at $P < 0.05$ levels were included in the models. The statistical significance of the Canonical axes was evaluated by Monte Carlo permutation tests (499 permutations). The analysis was conducted using CANOCO for Windows v.4.5 (Ter Braak and Šmilauer, 2002). The CCA result was displayed by ordination diagrams drawn with Cano Draw 4.1 (Ter Braak and Šmilauer, 2002).

3. Results

3.1. Taxa richness and diversity

In a single rainy season, a total of 1129 sporocarps were collected and classified into a total of 53 fungal taxa (Table 3). All of the taxa belonged to *Basidiomycota* except *Xylaria hypoxylon* (L.) Grev, which is in the division *Ascomycota*. Complete identification of the taxa to species level was not always possible; 28 (53%) were identified to species level, 24 (45%) to genus level and 1 (2%) remained completely unidentified. The identified taxa were represented by 15 families (Fig. 2A) and 31 genera (Fig. 2B). Family to genus and genus to species ratios were 0.48 and 0.58 respectively.

The families that contained the highest number of species were *Agaricaceae* (18), *Psathyrellaceae* (9), *Marasmiaceae* (5), and *Strophariaceae* (5), which together accounted for about 70% of the total surveyed taxa (Fig. 2A). The distribution of the identified taxa by trophic groups revealed a dominance of saprophytic species (94%) while the ectomycorrhizal (ECM) taxa were lower in numbers (6%). According to edibility status, 19 (36%) of the total taxa collected were edible (Table 2).

The fungal species accumulation curve for 36-years-old stand showed a relatively steep rising initial slope and tends to leveled off for each additional taxa added in this stand as most of the taxa found in this stand were collected just in the earlier weekly samplings. The curves for the 5- and 11-years-old stands portrayed a relatively low inflection point and accumulate fungal taxa progressively as fungal species were added with each additional sampling. A significance difference in taxa richness was also observed among the studied stands (P_5–P_{11} = 0.002, P_5–P_{36} = 0.001, P_{11}–P_{36} = 0.002). The highest richness is for 36-years old stand, followed by the 11- and 5-years-old stands (Fig. 3A), indicating an increasing trend towards the more matured *P. patula* stands (Table 3). Since the number of ECM taxa was very small, separate analyses of ECM and saprophytic taxa was not considered. The ECM taxa were collected exclusively from the 11- and 36-years-old stands (Table 3).

The Shannon's H′ diversity value also showed the same trend as in richness. The lowest Shannon's value was obtained from the 5-years-old *P. patula* stand (Fig. 3B) and this value was significantly different from those of the 11- and 36-years-old stands (Fig. 3B, P5–P_{11} = 0.003, P_5–P_{36} = 0.001). The difference in Shannon's value was also significant between the 11- and 36-years-old stands (Fig. 3B, P = 0.002).

3.2. Sporocarp productions

We found significant differences in mean total sporocarp production (P = 0.007) among the three age class stands. The lowest yield was obtained from the 5-year-old stand (7.53 kg ha^{-1} yr^{-1}). This value was significantly different from those of the 11- and 36-year-old stands (Fig. 4, P_5–P_{11} = 0.017, P_5–P_{36} = 0.003). The highest yield was obtained from the 36-years-old stand, with an average production of 35.86 kg ha^{-1} yr^{-1}, but the value was not significantly different when compared with that of the 11-year-old stand (Fig. 4, P = 0.051).

The average edible sporocarp yield also showed an increasing significant trend following the stand ages (P = 0.004). The highest yield was obtained from the 36-years-old stand, with a production of 16.30 kg ha^{-1} yr^{-1}. This value was significantly higher than that of the yield from the 5- and 11-years-old stands (Fig. 4, P_{36}–P_5 = 0.002, P_{36}–P_{11} = 0.043). The lowest production was from the 5-year-old stand, with a production of 1.5 kg ha^{-1} yr^{-1}. This value was also significantly different from the 11-year-old stand (Fig. 4, P = 0.008).

3.3. Taxa composition

Based on similarity and productivity of the taxa, the three *P. patula* stands tended to form distinct groups of taxa composition (Fig. 5). The 36- and 11-years-old stands showed distinct groups characterized by relatively high taxa richness (Fig. 5). The 5-years-old plots also clustered together, but characterized by a lower number of species. Furthermore, in the ordination diagram (Fig. 5), axis one separated the 11-years-old stands taxa from 36-years-old stands. Axis two also further differentiated the 5-years-old stands taxa from the remaining stands.

The eigenvalues indicated that the variability in terms of taxa composition, explained by the gradients associated with axis one is higher as compared to axis two. It explained 34.9% of the variation in the taxa data and an accumulative variance for the interaction between taxa and environment of 54.1% (Table 4).

According to the forward selection process, three edaphic variables were significantly (P < 0.05, Table 5) correlated with the fungal taxa composition. The model was significant according to

Table 3
Total taxa list collected from *Pinus patula* stands in the study area, Wondo Genet, Ethiopia.

Species	Code	5 yr	11 yr	36 yr	Mode	Edible
Agaricus aff. *campestroides* Heinem & Gooss.-Font.	*Agc*			x	S	E
Agaricus sp.$_1$.L.	*Ags*			x	S	
Agaricus sp.$_2$.L.	*Agpf*			x	S	
Agaricus sp.$_3$.L.	*Agpv*			x	S	
Agaricus sp.$_4$.L.	*Agps*			x	S	
Agaricus subedulis Heinem.	*Agub*			x	S	E
Agrocybe sp. Fayod.	*Agy*			x	S	E
Bovista dermoxantha De Toni.	*Bova*			x	S	
Calvatia sp. Fr.	*Calvs*		x		S	E
Calvatia subtomentosa Dissing & M. Lange.	*Calsu*			x	S	E
Collybia piperata (Beeli) Singer.	*Coia*			x	S	
Conocybe sp.Fayod.	*Cosp*			x	S	
Coprinellus domesticus (Bolton) Vilgalys, Hopple & Jacq. Johnson.	*Codo*	x	x	x	S	E
Coprinellus sp. P.Karst.	*Cops*	x		x	S	E
Coprinopsis sp.$_1$. P.Karst.	*Copo*			x	S	E
Coprinopsis sp.$_2$. P.Karst.	*Copst*	x	x	x	S	E
Cyptotrama asprata (Berk.) Redhead & Ginns.	*Cypas*	x	x	x	S	
Geastrum aff. *saccatum* Fr.	*Gesa*		x	x	EM	
Gerronema hungo (Henn.) Degreef & Eyi.	*Geru*	x	x	x	S	
Gymnopilus pampeanus (Speg.) Singer.	*Gypa*			x	S	
Hygrophoropsis aurantiaca (Wulfen) Maire.	*Hygu*			x	S	E
Hymenagaricus sp. Heinem.	*Hyag*			x	S	E
Hypholoma fasciculare (Huds.) P. Kumm.	*Hyol*	x	x	x	S	
Infundibulicybe aff. *mediterranea* Vizzini, Contu & Musumeci.	*Indib*	x	x	x	S	
Lepiota aff. *cristata* (Bolton) P.Kumm.	*Lepri*	x	x	x	S	
Lepiota sp. (Pers.) Gray	*Lepio*	x	x	x	S	
Leucoagaricus holosericeus (J.J. Planer) M.M. Moser.	*Lehol*			x	S	E
Leucoagaricus leucothites (Vittad.) Wasser.	*Lleu*			x	S	E
Leucoagaricus aff. *rubrotinctus* (Peck) Singer.	*Leub*			x	S	E
Leucoagaricus sp.$_1$. Locq.ex Singer.	*Lepo*			x	S	E
Leucoagaricus sp.$_2$. Locq.ex Singer.	*Lespt*		x	x	S	E
Leucocoprinus cepistipes (Sowerby) Pat.	*Leep*			x	S	E
Lycoperdon sp. Pers.	*Lycer*		x		S	E
Marasmius buzungolo Singer.	*Mabu*	x	x	x	S	
Marasmius katangensis Singer.	*Mark*	x		x	S	
Marasmius sp. Fr.	*Mars*	x	x	x	S	
Parasola sp.$_1$. Redhead, Vilgalys & Hopple.	*Parpo*	x	x	x	S	
Parasola sp.$_2$. Redhead, Vilgalys & Hopple.	*Parpt*	x	x	x	S	
Polyporus aff. *badius* (Pers.) Schwein.	*Polba*	x	x	x	S	
Polyporus aff. *tuberaster* (Jacq. ex Pers.) Fr.	*Polyt*		x		S	
Psathyrella sp.$_1$. Fr.ex Quél.	*Psptr*		x	x	S	
Psathyrella sp.$_2$. Fr.ex Quél.	*Psspf*		x	x	S	
Psathyrella sp.$_3$. Fr.ex Quél.	*Pspfi*		x	x	S	
Psilocybe cyanescens Wakef.	*Psilcy*		x		S	
Psilocybe merdaria (Fr.) Ricken.	*Psile*	x	x	x	S	
Psilocybe sp. (Fr.) P. Kumm.	*Psisp*	x	x	x	S	
Rhodocollybia aff. *maculata* (Alb. & Schwein.) Singer.	*Rhma*			x	S	
Scleroderma bovista Fr.	*Sclbo*			x	EM	
Stropharia sp.$_1$. (Fr.) Quél.	*Stpo*	x		x	S	
Stropharia sp.$_2$. (Fr.) Quél.	*Stpt*	x		x	S	
Tylopilus niger (Heinem. & Gooss.-Font.) Wolfe.	*Tylop*			x	EM	E
Undescribed sp.	*Unkn*			x	S	
Xylaria hypoxyloni (L.) Grev.	*Xyhy*			x	S	

Note: 5 yr: stands at the age of 5; 11 yr: stands at the age of 11; 36 yr: stands at the age of 36; EM: ectomycorrhizae; S: saprotrophic; E: edible.

Monte Carlo permutation test for the first axis (P = 0.002, F = 2.683) and for all canonical axes (P = 0.002, F = 3.533). In this case, axis one was positively correlated with Nitrogen (N), and negatively correlated with pH and Potassium (K). Axis two also correlated positively with all the three significant edaphic variables i.e. N, K and pH (Fig. 5).

4. Discussion

4.1. Taxa richness and diversity

In this study, a total of 53 different fungal taxa were collected associated with three different *Pinus patula* stands. The taxonomic classification of the collected taxa was challenging and we could only identify about 48% of the taxa to genus level. This might be an indication that the majority of the fungal biodiversity in Ethiopian forests is yet undescribed and more scientific studies on the local fungal flora are needed. The number of fungal taxa reported here is the highest among the literature in Ethiopia. Although with a different study aim, Hjortstam and Ryvarden (1996) reported 15 taxa, Alemu (2013) reported seven taxa, and Decock et al. (2005) reported four taxa. Most recently, Dejene et al. (2017) also reported an equivalent number of fungal taxa from the dry Afromontane forests in the country. This could indicate the important ecological role in terms of diversity which plantations can play in Ethiopian forests, as fungal richness is as high as that observed in natural forests.

Similarly, in studies carried out in *Pinus* stands other countries, a lower number of fungal taxa were also reported. For example 49 taxa were reported by Oria-de-Rueda et al. (2010) and 39 taxa

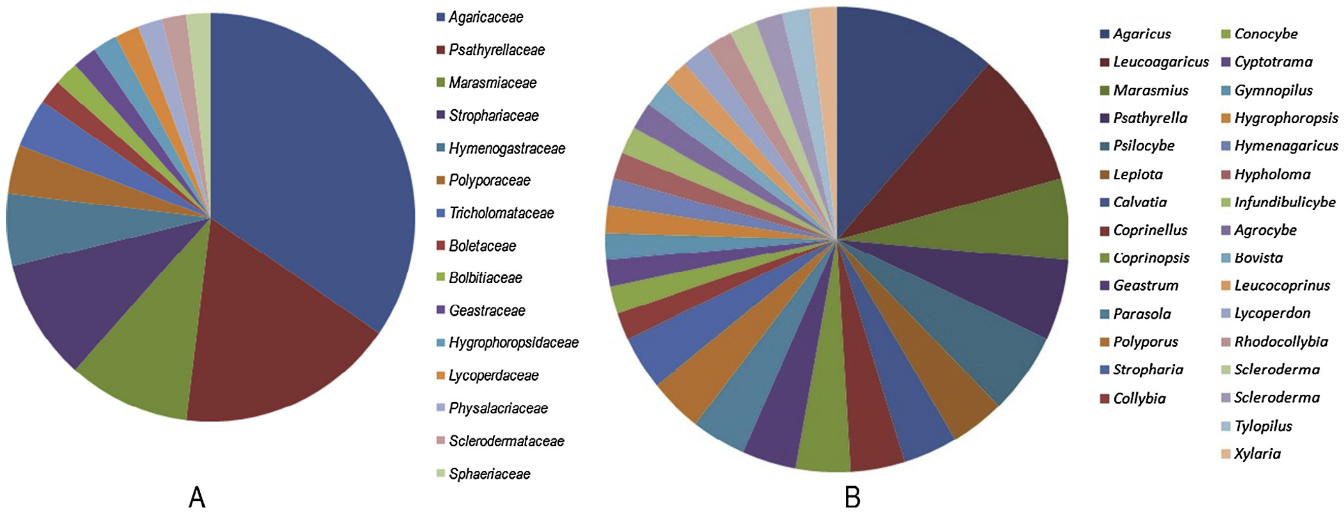

Fig. 2. Distribution of the total macrofungal taxa by family (A) and by genus (B) collected from *Pinus patula* stands of successive ages in Wondo Genet, Ethiopia.

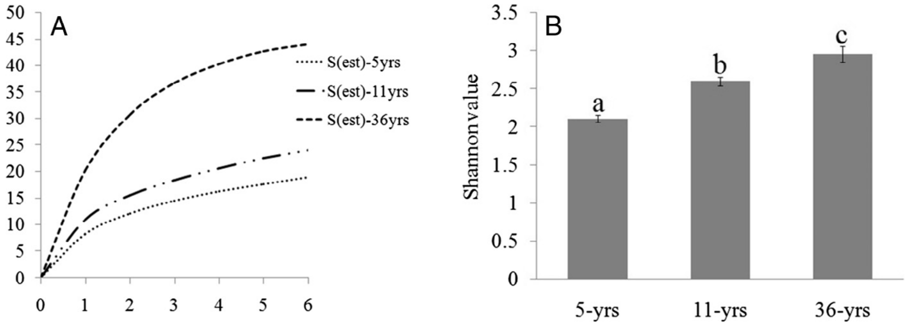

Fig. 3. Species accumulation curves generated using the rarefaction sample-based estimator of EstimateS (A) and Shannon diversity indices (B) of fungal community found in the entire *Pinus patula* stand of three age classes in Wondo Genet, Ethiopia. The species richness curves (S(est) are based on a total of 6 weekly samples. The data in Shannon diversity (B) are in mean ± SE. S(est)-5 yrs (A) & 5-yrs (B): stands at the age of 5; S(est)-11 yrs (A) & 11-yrs (B): stands at the age of 11; S(est)-36 yrs (A) & 36-yrs (B): stands at the age of 36.

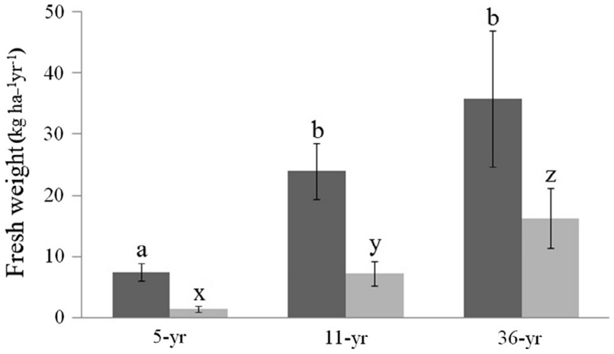

Fig. 4. Production of sporocarps according to total production (black) and edible taxa (gray) collected from *Pinus patula* stands in Wondo Genet, Ethiopia. The data are in mean results ± SE. Values with the same letter are not significantly different. 5 yr: stands at the age of 5; 11 yr: stands at the age of 11; 36 yr: stands at the age of 36.

were also reported by Martín-Pinto et al. (2006) from *P. pinaster* stands in the Mediterranean regions. Such difference in taxa number might be due to differences in site ecological factors that can be expressed in terms of soil, climate and host species, which are decisive factors affecting the fungal richness in an area (Oria-de-Rueda et al., 2010). On the other hand, higher number of fungal taxa associated with *Pinus* stands was also reported by Bonet et al. (2004), Gassibe et al. (2015, 2011), and Kutorga et al. (2012). In all these cases, the collection was done for longer periods of time. Such prolonged studies likely have a positive effect on taxa numbers as fungi occurrence show considerable seasonal and yearly variation (Tibuhwa et al., 2011). Although our results are based on a single rainy season sampling, we could provide preliminarily general information about the fungal association and their succession along stand development of *P. patula* plantation during the study period. However, a follow up study including observations of the same stands along different years would be highly desirable as this would avoid any uncontrolled confounding effect unevenly affecting our stands.

The majority of the taxa collected in this study were saprophytic. Only about 6% were ectomycorrhizal (ECM) (Table 3). Different studies reported fungal assemblages in Ethiopian habitats (Alemu, 2013; Decock et al., 2005; Hjortstam and Ryvarden, 1996), however, none of them reported ECM fungi. This result is not surprising as most tropical woody tree species are unable to form associations with ectomycorrhizal fungi (Brundrett, 2009). Thus, we believe this to be the first report on ECM fungi and it presented important ramification of the non-native tree forests for the maintenance of functional fungal diversity in Ethiopia. The lower proportion of ECM fungi and their exclusive occurrence in older stands could be explained in terms the nature of ECM fungi dynamics (Ashkannejhad and Horton, 2006; Widden, 1981). Typically, several ECM fungi inhabit the root system of an individual

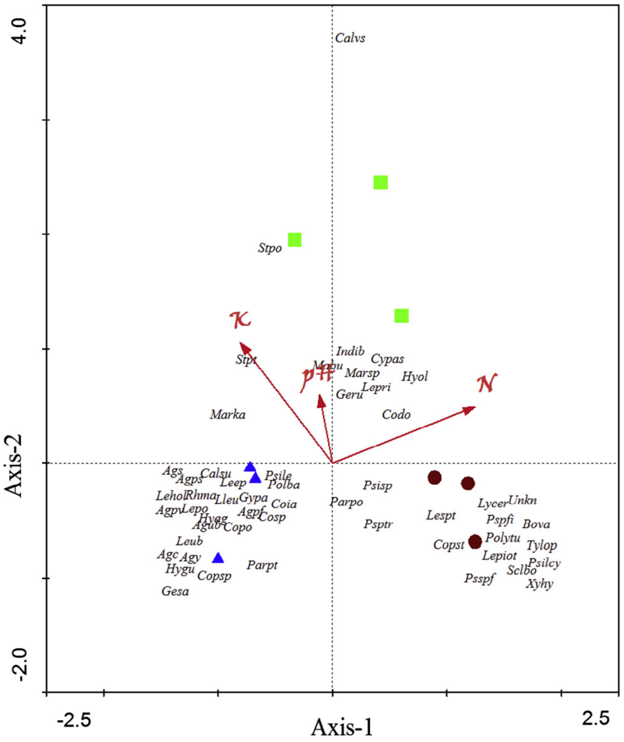

Fig. 5. CCA ordination bi-plot showing: fungal taxa abbreviated shown in Table 1. Plots in similar color are in a group (Green square: 5-years-old stands, Brown Circle: 11-years-old stands and Blue up triangle: 36-years-old stands), and environmental factors (arrows)). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 4
Summary of CCA of fungal taxa presence and environmental factors for the study area, Wondo Genet, Ethiopia.

Axes	1	2
Eigenvalues	0.537	0.303
Taxa-environment correlations	0.987	0.959
Cumulative percentage variance of		
taxa data	34.9	54.6
taxa-environment relation	51.4	80.4

Table 5
Significant edaphic variables associated with fungal taxa composition in *Pinus patula* plantations in Wondo Genet, Ethiopia.

Variables	*F*-ratio	*p*-value
N	3.01	0.002
K	2.89	0.002
pH	2.37	0.028

tree (Hui et al., 2011) and practically all fine roots of coniferous trees are colonized by ECM fungi (Taylor et al., 2000). Along with the development of the forest stands, ideal microhabitats could be created at the soil level (Dighton et al., 1986) and continuous root-hyphal contacts could be initiated (Kranabetter et al., 2005). Thus these conditions are important for their occurrence and thereby govern their spatial and temporal abundance in the forest systems. Also, the use of our study plots as agricultural land before plantation could potentially have had a negative impact on the occurrence and diversity of ECM fungi. Furthermore, *Pinus* is an exotic tree species to the area, and there might be no inocula of compatible ECM fungi in the surroundings, irrespective of the previous land use.

The species accumulation curves indicated a rapid increase in initial number of fungal species caught in all the three stands (Fig. 3A). Latter, however, the 36-years-old curve goes higher and demonstrated a tendency of stabilization than the other curves as more sampling are considered. Thus, this suggests that the sampling period is more representative for total fungal taxa richness in the 36- than in 5- and 11-years-old stands. In general terms, our study underestimates fungal richness since the progressive propensity in the curves for 5- and 11-years-old stands indicated that more samplings are required to capture sufficient number of fungal taxa to accurately predict the richness in these stands (Thompson and Withers, 2003).

The trend of fungal taxa richness is towards more matured stands. The maximum number of fungal taxa was counted from the 36-years old stands, while the lower is from the 5-years-old stands. This trend is in agreement with other studies which demonstrated mature stands could favor more macrofungal species and thus higher richness (Fernández-Toirán et al., 2006; Gassibe et al., 2015, 2011; Kranabetter et al., 2005; Mediavilla et al., 2014; Visser, 1995). This might be explained due to the suitability of the environments created along the development of the stands. Although our *P. patula* plantations existed in close vicinity, they were isolated stands at different stages of forest development. Thus the difference in relative microhabitat variation created in each stand following canopy closure (Crabtree et al., 2010; Dove and Keeton, 2015; Oria-de-Rueda et al., 2010), i.e. relatively high humidity and organic matter accumulation, may enhance the high occurrence and fructification of fungi particularly towards the higher age classes. This assumption coincides with Dighton et al. (1986), Smith et al. (2002), Sysouphanthong et al. (2010) who noted higher fungal richness in well-developed stands having higher canopy closure. This difference also further reflected in the diversity value of the three stands. The Shannon diversity values also showed increasing trend as in richness values towards more matured stands. This might be due to the disproportional distribution of taxa that appeared in each successional stage in *P. patula* plantations (Hernández-Rodríguez et al., 2013).

4.2. Sporocarp production

Mushroomharvesting is an important NTFP resource and their demand has been increasing for the last decade (Boa, 2004). This indicates that the commercial value of a forests can be increased through managed timber harvesting that may improve the habitat for valuable mushroom productions (Bonet et al., 2004). In this study, the highest total mushroom fresh weight was obtained from older stands while the lowest was from younger stands (Fig. 4), indicating that sporocarp yield increases towards the more matured *P. patula* stands. According to Oria-de-Rueda et al. (2010) this kind of increasing trend can be related to the different availability of substrata along the stand's development. As a forest stand matures, the humus layer develops (Dove and Keeton, 2015; Pinna et al., 2010; Toivanen et al., 2012) and the forest soil increases its capacity to buffer temperature and moisture. Such conditions could enhance more fungal growth and fruiting, especially for saprotrophic fungi (Fernández-Toirán et al., 2006).

Wild mushroom utilization is a traditional common practice among the different tribes in Ethiopia (Dejene et al., 2017; Semwal et al., 2014). Mushrooms have been used for their nutritional, traditional and medicinal properties, and are also involved in local mythology (Tuno, 2001). In some local markets mushrooms are also available (Abate, 2008), where they are sold by the local people to earn some income to supplement the household economy. From our study area we collected a total of 19 edible fungal taxa (Table 1), representing 20%, 31% and 46% of the total yield from 5-, 11- and 36-years-old stands respectively, indicating that a higher yield of edible mushrooms is also found in the older *P. patula* stands. Apart from the suitability of the environment created along the stand ages, such increasing association of a relative higher yield in older stands might be explained in terms of the abundance of taxa like *Agaricus* sp., *Agrocybe* sp., *Coprinopsis* sp., *Hygrophoropsis aurantiaca*, *Leucoagaricus rubrotinctus*, and *Tylopilus niger*. Among the collected edible taxa, *Agaricus* species are commonly reported in the literatures used by the local people in the rural area of Ethiopia (Abate, 2008).

4.3. Taxa composition

Distinctive fungal communities were observed among the stands (Fig. 5). The 11- and 36-years-old stands showed to have distinctive fungal communities characterized by high taxa numbers (Table 3). They also shared about 20 taxa in common. This result probably was due to the positive effect of stand development that can be expressed in terms of age, which could harmonize high soil humification and a thicker litter layer along stand development (Mediavilla et al., 2014; Pinna et al., 2010; Toivanen et al., 2012). Most of the taxa found in these stands are characterized as being generalists and associated with litter decomposition. However, some exclusive taxa were found in either of the specific age class. For instance, *Calvatia* sp., *Lycoperdon* sp., *Polyporus tuberaster* and *Psilocybe cyanescens* were exclusive to 11-years-old stands, while the *Agaricus* sp., *Agrocybe* sp., *Bovista dermoxantha*, *Collybia piperata*, *Gymnopilus pampeanus*, *Leucoagaricus holosericeus*, *Leucoagaricus leucothites*, *Leucocoprinus cepistipes*, *Rhodocollybia maculata*, *Scleroderma bovista* and *Xylaria hypoxylon* were exclusive to the 36-year-old stands (Table 3). This indicated the effects of stand age to limit the type of taxa, appearing only those able to adapt to the new conditions due to the development of the stands (Greeshma et al., 2016). Some of the taxa collected from these age groups like *Agaricus* sp., *Psilocybe* sp., *Leucoagaricus* sp., *Lycoperdon* sp., and *Collybia* sp., were also reported as common in relatively well-developed and older forest stands (Gassibe et al., 2011; Mediavilla et al., 2014), indicating that these genera might also be characteristic of late stage stands.

The 5-years-old stand plots showed variability, and were more or less scattered in the ordination (Fig. 5). The possible explanation for such scattered occurrence might be the sampling variability and/or the existence of a high number of shared taxa with other plots, characterized by diverse ecological requirements. In this stand age, we could not find exclusive taxa. All of the taxa from this stand were broad-based and common to other age groups. Out of the collected taxa in this group, *Marasmius* sp. and *Hyholoma* sp. were reported as common both in young and old forest stands (Gassibe et al., 2011; Hernández-Rodríguez et al., 2013), indicating that these genera are also multi-stage species that might be able to fruit in different ecological conditions and have wide substrate requirements.

Fungal taxa compositions are sensitive to soil conditions (Setälä and McLean, 2004) as soil nutrients have been shown to be important factors in mycelial development and hence sporocarp occurrence (Harold et al., 2005; Zakaria and Boddy, 2002). In the present study, edaphic variables such as nitrogen (N), Potassium (K), and pH were found to be correlated significantly with the fungal taxa composition. The result was in agreement with those of Gassibe et al. (2015) and Dejene et al. (2016) who noted the correlation of edaphic variables with fungal taxa composition in the forest systems.

Finding nitrogen (N) as an important factor correlated with the fungal taxa compositions is in accordance with Gassibe et al. (2015), Kranabetter et al. (2009), Reverchon et al. (2010), who noted the influence of N on fungal richness distribution patterns. In these reports, fungi showed community specialization towards more soil N gradients. This might be due to the fact that nitrogen can influence the formation of extra radical mycelium in the soil, and play a vital role in sporocarp formations (Trudell and Edmonds, 2004). Furthermore, other authors also noted that fungal communities can be adapted to more nitrogen-rich sites (Kranabetter et al., 2009; Toljander et al., 2006). Although different fungal species have diverse responses towards a certain soil parameter, we found that mostly *Coprinellus domesticus*, *Hypholoma fascicular*, *Lycoperdon* sp., *Psathyrella* sp., *Bovista dermoxantha*, *Lepiota* sp., and *Coprinopsis* sp., occurred towards the higher end points of N gradient in our ordination (Fig. 5).

Our results also showed Potassium (K) to be correlated with fungal composition. The result coincides with Gassibe et al. (2015) and Dejene et al. (2016) who noted fungal assembly towards the K gradients. This might be due to the fact that K is, along with other elements, important for the growth and sporulation of fungi (Nonzom and Sumbali, 2014), thus potentially can shape the fungal community composition. Other authors also demonstrated the fungal distribution and community compositions to be influenced by available K in the soil (Bisset and Parkinson, 1979). Taxa such as *Marasmius buzungolo*, *Stropharia* sp., *Psilocybe merdaria*, *Polyporus badius*, *Leucocoprinus cepistipes*, and *Agaricus subedulis* were closely related to soil K content in our study. Out of those, the species belonging to the genera *Agaricus* and *Leucocoprinus* have been cited as correlated with K (Dejene et al., 2016).

Soil pH is the other edaphic variable we found correlated with fungal taxa composition (Ferris et al., 2000; Talbot et al., 2013). We found a higher occurrence of taxa around lower pH values and it seemed that pH had an effect on fungal community composition in our study area. This coincides with the findings of Puangsombat et al. (2010) and (Dejene et al., 2016) who noted that the number of fungal species tends to be higher in areas where pH value is low. However, some taxa like *Infundibulicybe mediterranea*, *Cyptotrama asprata*, *Gerronema hungo*, *Lepiota cristata* and *Marasmius* sp. showed exceptional ordination towards the higher end point of the pH gradient. This might be associated with their adaptability of the species to higher pH values in the soil.

This work represents the first systematic investigation of mushroom diversity and production in Ethiopia providing a starting place in broadening management objectives for NTFPs in these pine plantations. However reached findings must be regarded as a preliminary indication due to sampling limitations.

5. Conclusion

This case study is the first preliminary attempt to describe the fungal community and their succession in plantation forest systems in Ethiopia. So far, only saprophytic species have been reported from forest systems in Ethiopia. In this study we also found ECM fungal species in matured stands that could increase the functional group diversity in plantation forest systems in the country. The result also showed matured *P. patula* stands provided the highest fungal diversity as compared to the middle and young age groups. They were also characterized by higher sporocarps productions, almost half of it corresponding to edible taxa. Thus, leaving patches of matured trees through green-tree retention management approach, could increase habitat availability, such as substratum for saprophytic fungi and live standing trees for mycorrhizal species after final rotation cut, and thereby provide higher fungal diversity and edible sporocarps production. This could serve as a means to deliver complementary incomes to rural population from mushroom as potential NTFPs while the mature trees could serve as a bridge for providing fungal inocula to the new plantations.

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Original article V

Comunidades fúngicas bajo la influencia de plantaciones de *Eucalyptus grandis* W.Hill ex Maiden del sur de Etiopía.

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Forest Ecology and Management Submitted

Resumen

Las plantaciones forestales de Etiopía se encuentran representadas principalmente por árboles de Eucalipto. Sin embargo, se conoce poco sobre la flora asociada con este tipo de bosques. Se carece especialmente de estudios enfocados en la flora fúngica. En esta investigación, se estudió la riqueza de especies de hongos, su diversidad y composición, así como la producción de carpóforos, en relación con la edad de plantación de *Eucalyptus grandis* en el sur de Etiopía. Para tal fín, se establecieron nueve parcelas (100 m^2) en rodales de *E. grandis* con edades de diez, nueve y treinta y siete años. Se encontraron 29 taxones de hongos (Basidiomycota), con la excepción de *Xylaria hypoxylon* (Ascomycota). Todos los taxones encontrados fueron saprofitos y un tercio de los taxones fueron comestibles. La riqueza de taxones, los índices de Shannon y el rendimiento de los carpóforos se correlacionaron positivamente con la edad de plantación. Además, la composición fúngica se asoció con las etapas sucesivas de desarrollo de los rodales. Este estudio preliminar ofreció nuevos conocimientos sobre la estructura de la comunidad de hongos en bosques plantados y proporcionó un punto de partida para mejorar los objetivos de manejo de bosques de Eucalipto para los PFNMs en el país, principalmente de hongos que podrían proporcionar ingresos complementarios para la población rural.

Palabras clave: *Factores edáficos, comunidades fúngicas, Pinus, carpóforos, edad, sucesión*

Fungal communities under *Eucalyptus grandis* W.Hill ex Maiden plantations of different ages in Southern Ethiopia

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Forest Ecology and Management Submitted

Abstract

In Ethiopia, plantation forestry is dominated by *Eucalyptus* tree species. However, there is a very limited knowledge on the associated flora. Specifically, any study focused on fungal flora is lacking. In this study, we investigated the fungal species richness, diversity, composition and sporocarp production in relation to plantation age of *Eucalyptus grandis* stands in Southern Ethiopia. For this purpose, we surveyed nine plots (100 m^2) established in ten-, nineteen-, and thirty-seven years old *E. grandis* stands. We found a total of 29 fungal taxa belonging to *Basidiomycota*, with the exception of *Xylaria hypoxylon* which is *Ascomycota*. All taxa collected were saprophytic and one third of the taxa were classified as edible. Taxa richness, the Shannon diversity values, and sporocarps yield were positively correlated with plantation age. Also, fungal composition was correlated with successive stages of stand development. This preliminary study extends our knowledge of fungal community structure in plantation forests and provides a starting place in broadening *Eucalyptus* management objectives for NTFPs in the country, mainly of mushrooms that could provide complementary incomes for the rural people.

Keywords: *Ethiopia, Eucalyptus grandis, fungal community, sporocarp, stand age, succession.*

1. Introduction

Ethiopia has been facing rapid deforestation (Badege, 2001). The natural forest cover of the country has declined considerably in the last decades (Kuru, 1990) and the change in natural forest cover is estimated between 150,000 and 200,000 hectares (ha) of land per year (Zewdie et al., 2009). Increasing demands for fuelwood and construction materials are among the major causes for such changes in Ethiopia (Jaleta et al., 2016). As a result, plantation of fast-growing trees has become a major forestry practice, thereby reducing pressure on the natural forest resources (Bekele, 2011; Zewdie et al., 2009). Hence, it has led to a rapid expansion of exotic tree species and more than 506,000 ha of land have been planted in the last decades (FAO, 2011).

Plantation forests in Ethiopia are mainly dominated by *Eucalyptus, Cupressus, Pinus* and *Acacia* genera (Bekele, 2011; Moges et al., 2010). Among these, *Eucalyptus* species hold the largest share and they roughly cover about 56% of the total plantation by area (Bekele, 2011). *Eucalyptus* is preferred owing to its fast-growth nature, coppicing ability and wider adaptation to different ecological conditions (FAO, 2009). It also serves as main source of firewood, farm implements, poles and posts in Ethiopia (Kelemu and Tadesse, 2010). Economically, *Eucalyptus* helps the rural people in improving their livelihood through its contribution in household income (Asnake, 2002; Kebebew, 2002; Mesfin, 2002). Furthermore, *Eucalyptus* species have been used also in biological soil conservation works where it is planted in degraded and gully areas (Jaleta et al., 2016; Lemenih and Kassa, 2014).

Despite all benefits provided by plantations of *Eucalypts* species, the alleged negative environmental impact is still a narrative in Ethiopia (Davidson 1995; FAO 2011). Among the criticisms, the impediment of the establishment of other plants by out-competing for moisture and nutrients, as well as by direct inhibition of understory flora through phytotoxic exudates from leaves and litter are most cited (Teketay 2000; Jaleta et al. 2016). In contrast to this view, many plantations of *Eucalyptus* have been also found to host a high richness of herbaceous species and foster natural regeneration of native flora in Ethiopia (Lemenih, 2004; Yirdaw, 2002) and, thus, contributing to biodiversity rehabilitation (Moges, 2010). In all these views, however, the knowledge and the status of fungal communities under *Eucalyptus* canopy in Ethiopia are unknown. Such information is vital to promote an alternative plantation forest management including the conservation and production of mushrooms through a mycosilvicultural approach (Castellano and Molina, 1989; Trappe, 1977).

Fungi are considered key factors in plantation forest ecosystems (Butler et al., 2002; Heilmann-Clausen, 2003; Lindahl et al., 2007). Mycorrhizal fungi are required for the survival and growth of forest trees (Smith and Read 1997). They play roles in mobilization, uptake and translocation of nutrients in forest soils. Furthermore, they can also improve plant water uptake and resistance to abiotic stresses; thereby influencing plant productivity (Pietras et al., 2013; Van Der Heijden et al., 2008). Other fungal species behave as saprophytes and are responsible for the decomposition of organic materials and thus recycling of nutrients (Ferris et al., 2000). In addition to important ecological functions, edible fungi have also become a strategic component in the management of plantation forests. This is because of their economic value, as during the last decade, there has been an increasing demand for edible fungi from the forests (Pettenella et al., 2007). In fact, in some cases mushrooms may generate even higher economic benefits than timber production (Martín-Pinto et al., 2006) and they are also becoming an important source of rural incomes (Abate, 2008; Boa, 2004).

In Ethiopia, *Eucalyptus grandis* W.Hill ex Maiden plantations are managed mainly for industrial wood production purposes (Hunde et al. 2003). Although rotation periods range between 7 – 25 years, depending on the purpose, the maximum wood production from the species can be attained at ~18 years (Pohjonen and Pukkala, 1990). According to FAO (2009), *Eucalyptus* plantation management in Ethiopia depends on traditional silvicultural systems, and coppicing is the preferred management technique (Pohjonen and Pukkala 1990; Mekonnen et al. 2007). Tree retention as a management approach aiming at perpetuating ecosystem integrity while providing wood and non-wood values (Lindenmayer et al., 2012; Nyland, 2002) is poorly represented in the country. This approach has also important implications for forest floor microhabitat improvements, such as moisture, temperature and substratum (Smith et al., 2008), important for the fructification and growth of macrofungal species.

Although the impacts are yet understudied, the relatively short rotation period of *Eucalyptus* plantations and their management practices might have impacts on the associated fungal communities. As the stand growth, changes in the fungi communities occur (Luoma et al., 1991; Smith et al., 2002). Thus, understanding fungal ecology and fruiting patterns along the development of *E. grandis* stands may be a means to improve fungal richness and production.

This might also help to provide basic information about the management of *Eucalyptus* plantations in order to conserve fungal communities and to promote the production of demanded edible taxa. With these concerns in mind, the broad scope of this pioneer systematic case study was characterizing fungal communities in *E.grandis* stands and explaining the sporocarp production linked to stand development in Southern Ethiopia. The specific objectives include: (1) to evaluate the fungal taxa richness and diversity variation among three age plantations, (2) to analyze sporocarp yields for total and edible taxa, (3) to examine the fungal composition based on stand age classes.

2. Methodology

2.1. Study area

The study was conducted at Wondo Genet *Eucalyptus* plantation forest area in Southern Ethiopia. It is found approximately 265 km from Addis Ababa, the capital city of Ethiopia (Figure 1). Geographically the study area is located at 7°06'–7º07'N latitude and 38°37'–38º42'E longitude with an altitudinal range between 1,600 and 2,580 m above sea level (Belaynesh, 2002; Thomas and Bekele, 2003). The climate is characterized by Woyna Dega agro-climatic type. The rainfall pattern is bimodal, with minor rainfall during spring and the major rain season is during summer. The average annual rainfall is 1,210 mm, which peaks in July. The average annual temperature is 20°C (Belaynesh, 2002; Fenta, 2014). The topography is slightly undulating and the soils are young and of volcanic origin, characterized by sandy loam. The soil is shallow at steep convex slopes but deeper at lower altitudes (Eriksson and Stern, 1987), where most of the plantations are located.

2.2. Sampling and sporocarps collection

Three different *Eucalyptus grandis* stands were selected in the study area based on their age i.e. 10-, 19- and 37-years-old stands, hereafter AG10, AG19 and AG37 respectively (Table 1). Following previous studies, fungal diversity and production were obtained by using transect methods (Smith et al., 2002).In our case, three 2 x 50 m (100m^2) plots were established at each stand, i.e. nine plots in total according to Gassibe et al. (2011) and Hernández-Rodríguez et al. (2013). Within each of the selected stands, plots were placed systematically far enough, about 120m, from each other (Luoma et al., 1991). The plots were similar in terms of their ecological conditions such as climate, altitude, and soil. This work should be considered as a case study since the plots were established in a single stand for each age class, and despite our efforts, conclusions regarding other stands need to be taken with caution.

All the sporocarps found in each plot were fully harvested weekly during the main rainy season in July and August in 2015. Fresh weight measurements were carried out *in situ* and the data are given in kilograms per hectare per year (kg fwha^{-1}year^{-1}). Also, the number of sporocarps per taxa was collected from each plot. Sample fruit bodies from each taxon were taken to the laboratory and dried in oven at 35°C for 48 hours. Then, herbaria specimens were used for molecular and microscopic taxa identification. Furthermore, in the field, specimens were photographed and their ecological characteristics were noted to assist and facilitate taxa identification processes.

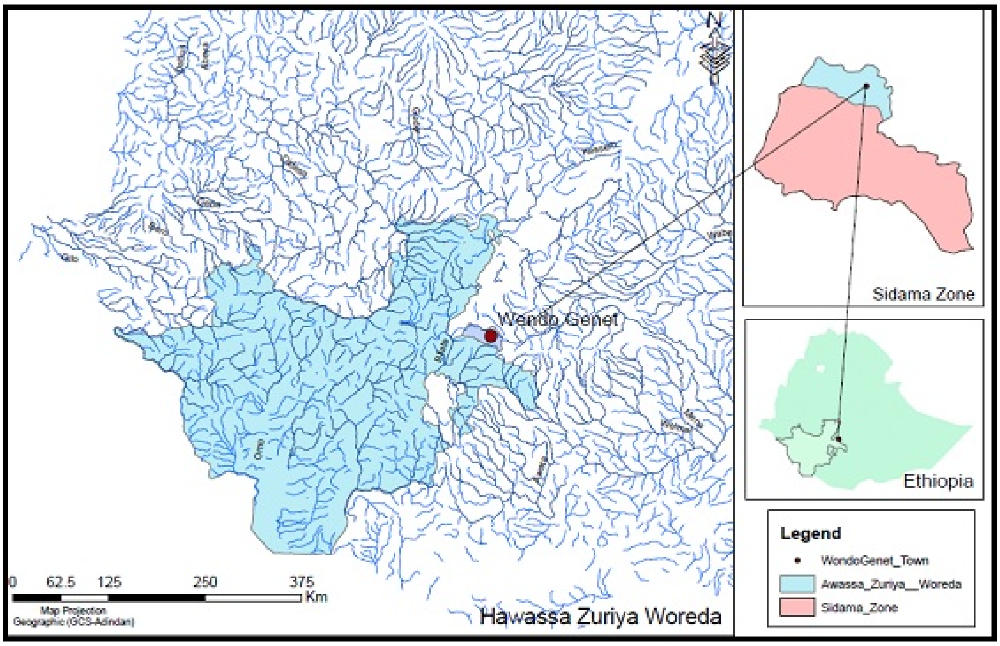

Figure 1: Location map of the study area, Wondo Genet, Ethiopia.

2.3. Taxa identification and classification

Morphological and molecular analyses were used for taxa identification. Taxonomic classification was aided by close microscopic examination of tissues and spores with an Optika B-350PL microscope. Small samples of dried specimens were re-hydrated and mounted in 5% KOH. The following keys were mainly used for the purpose: Heinemann (1956); Singer (1965); Pegler (1968), (1969), (1977); Morris (1990); Rammeloo and Walleyn (1993); Ryvarden et al. (1994); Antonin (2007) and Hama et al. (2010). Specimens were deposited in the laboratory herbarium at the University of Valladolid. Up-to-date fungal taxa names and authors' names were obtained from Myco bank database (http://mycobank.org).

Molecular identification involved sequencing of the ITS region of the nuclear ribosomal genes (rDNA). For this, fungal DNA was extracted from dry sporocarps using an EZNA® Plant DNA kit (Omega Bio-Tek, USA) according to the manufacturer's instructions. Final elutions were done in a total volume of 100 µl. The internal transcribed spacer (nrITS) was amplified with primers ITS1F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990). For PCR reactions, HotBegan™ Hot Start Taq DNA Polymerase (Canvax Biotech, Cordoba, Spain) was used following manufacturer's instructions, adding 1 µl of genomic DNA to a final reaction volume of 25 µl. PCR conditions were: 5 min initial denaturation at 94°C followed by 40 cycles of: 45 sec denaturation at 94°C, primer annealing at 56°C for 30 sec, and extension at 72°C for 40 sec, followed by a final extension step of 10 min at 72°C. The PCR products were checked on a 2% agarose gel. Sequences were obtained in the laboratories of Macrogen (Amsterdam, Netherlands) using the primer ITS4 as a template.

Table 1: Characteristics of studied *Eucalyptus grandis* stands in Wondo Genet (Ethiopia), DBH, diameter at breast height, Ht, height, ha, hectares. Source: Zewdie et al. (2010)

Plantation time	Stand age (year)	Area (ha)	Density per (ha)	Mean DBH (cm)	Mean Ht (cm)
2005	10	2.1	1075	9.2	9
1996	19	1.3	1050	7.0	6.5
1978	37	1.5	3280	42.5	55.1

Taxa edibility classification was accomplished by adapting the criteria used by Bonet et al. (2004). If the taxon is described in the literature as either non-edible or edible, we classified it as a non-edible. If the taxon is described in the literature as having doubtful edibility, we classified it as a non-edible. As edible (E) were classified all species that are listed as such in the large majority of the literature consulted.

2.4. Data analysis

Shannon's H' diversity index (Shannon and Weaver, 1949) was estimated for each plot using the following formula, where p_i indicated the relative abundance of each macrofungal taxa. This index increases with both the number of species and the evenness of their distribution. It usually ranges between 1.5 and 3.5 and rarely exceeds 4.5 (Kent and Coker, 1993).

$$H = -\sum Pi(lnpi)$$

"Richness" (number of taxa), was defined as the total number of species found per plot.

Richness, Shannon index, and fresh weight (for the edible and total taxa) estimates were subjected to one-way ANOVA analysis and a post-hoc least square means difference test (LSD, $P \leq 0.05$) to test for differences among *E. grandis* stand age classes. Data were log-transformed when needed to achieve the parametric criteria of normality and homoscedasticity that ANOVA requires. All analyses were done with SAS software (SAS Institute Inc., 2012).

The Rényi diversity profile (Tóthmérész, 1995),was used to depict the diversity curves of the three stands. 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. PAST software was used to plot the diversity profiles (Hammer et al., 2001).

Species accumulation curves were also constructed to compare the rate at which new fungal species were found between the three studied stands and to provide an estimate of species richness. The curves were generated using sample based estimator of EstimateS Version 9 (Colwell, 2013). The number of fungal species collected during each weekly visit to a plot within a stand constituted the sample. The curves were generated based on a total of six weekly samplings data set.

An ordination technique was also used to test for differences in fungal taxa composition among the three *E. grandis* stands of age classes. Fresh weight data per taxa were subjected to a Detrended Correspondence Analysis (DCA) (Ter Braak and Prentice, 1988), using the software CANOCO for Windows version 4.5 (Ter Braak and Šmilauer, 2002). Analyses were performed on the full data set (29 taxa and 9 plots). The CCA result was displayed by ordination diagrams drawn with Cano Draw 4.1 (Ter Braak and Šmilauer, 2002).

3. Results

3.1. General data

A total of 29 macrofungal taxa were collected from the entire *Eucalyptus grandis* stands (Table 2). All of the taxa collected belonged to *Basidiomycota* division except *Xylaria hypoxylon* (L.) Grev, which is in the division of *Ascomycota*. The identified taxa were classified into 21 genera and 11 families (Figure 2). The largest contribution of taxa came from *Agaricaceae* family, which it was represented by eight species, followed by the *Psathyrellaceae* and *Marasmiaceae* with four species per each (Figure 2A). The richest genus was *Agaricus* that comprised four species followed by *Marasmius* represented by three species, and *Psilocybe*, *Polyporus* and *Coprinellus* with two species at par (Figure 2B).

All the collected taxa were saprophytic and could be identified at least at the genus level. Of them, 58% were identified to the species level.

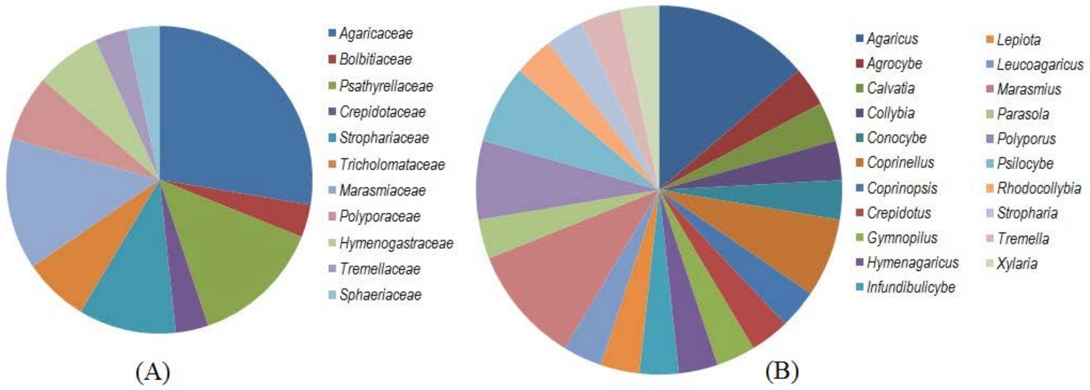

Figure 2: Distribution of the total macrofungal taxa by family (A) and by genus (B), collected from *Eucalyptus grandis* stands of successive ages in Wondo Genet, Ethiopia.

The species accumulation curves (Figure 3A) portrayed how the fungal taxa were accumulating progressively for each additional weekly sampling in all the stands. The curve in the 37-years-old stand showed a relatively steep rising initial slope and reached higher values than the others. The curves in the 10- and 19-years-old stands portrayed relatively low inflection points and they tended to level off for each additional sampling.

Fungal taxa richness was significantly higher in 37-year-old stands than in the others (P = 0.001). No significant differences were observed when comparing the taxa richness between 10- and 19-year-old stands (P = 0.448).

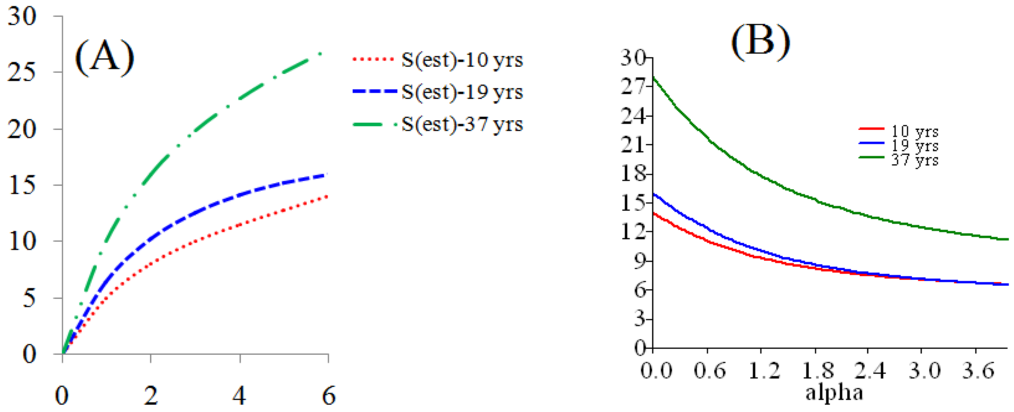

Figure 3: Species accumulation curves generated using the rarefaction sample-based estimator of EstimateS (A) and Rényi diversity profiles ((B) of fungal community found in the entire *Eucalyptus grandis* stands of three age classes in Wondo Genet, Ethiopia. The species richness curves (S(est) are based on full data set of six weekly samples. The data in Shannon diversity (B) are in mean ± SE. S(est) -10 yrs (A) &10-yrs (B): stands at the age of 10; S(est) -19yrs (A) & 19-yrs (B): stands at the age of 19; S(est)-37yrs (A) & 37-yrs (B): stands at the age of 37.

The highest diversity value (2.94) was recorded at the 37-year-old stand followed by the 11- (2.37) and 10-year-old stands (2.28), indicating a positive correlation with *E. grandis* stand age. The values were further explained by the Rényi diversity profiles, which indicated that 37-year-old stand was significantly different from the 10-year-old stands (P = 0.025) and the 19-year-old stands (P < 0.039) (Figure 3B). The fungal diversity profiles for the 10- and 17-year-old stands crossed each other, indicating that they are not different in their Shannon Diversity values (P=0.657).

Table 2: Total taxa list collected from *Eucalyptus grandis* stands in the study area, Wondo Genet, Ethiopia

Taxa	Code	AG10	AG19	AG37	Edible
Agaricus aff. *campestroides* Heinem & Gooss.-Font.	Acamps		x	x	E
Agaricus sp $_1$. L.	Agarsp			x	
Agaricus sp $_2$. L.	Agaricu			x	
Agaricus subedulis Heinem.	Asubed			x	E
Agrocybe sp. Fayod.	Agrcyb	x	x	x	E
Calvatia subtomentosa Dissing & M. Lange.	Csubto	x	x	x	E
Collybia piperata (Beeli) Singer.	Cpiper	x	x	x	

Taxa	Code	AG10	AG19	AG37	Edible
Conocybe sp. Fayod.	Conocy	x	x	x	
Coprinellus domesticus (Bolton) Vilgalys, Hopple & Jacq. Johnson.	Cdomes	x	x	x	E
Coprinellus sp. P.Karst.	Coprin	x		x	E
Coprinopsis sp. P.Karst.	Copsis	x	x	x	E
Crepidotus sp. (Fr.) Staude	Crepdo	x	x		
Gymnopilus pampeanus (Speg.) Singer.	Gpmpe			x	
Hymenagaricus sp. Heinem.	Hymus	x	x		E
Infundibulicybe aff. *mediterranea* Vizzini, Contu & Musumeci.	Imedit			x	
Lepiota aff. *Cristata* (Bolton) P.Kumm.	Lepiot		x	x	
Leucoagaricus holosericeus (J.J. Planer) M.M. Moser.	Lholos	x	x	x	E
Marasmius buzungolo Singer.	Mbzun			x	
Marasmius katangensis Singer.	Mkatan	x	x	x	
Marasmius sp. Fr.	Marsm	x	x	x	
Parasola sp. Redhead, Vilgalys & Hopple.	Parasol	x	x	x	
Polyporus aff. *badius* (Pers.) Schwein.	Pbadiu			x	
Polyporus aff. *tuberaster* (Jacq. ex Pers.) Fr.	Ptuber		x	x	
Psilocybe merdaria (Fr.) Ricken.	Pmerda	x		x	
Psilocybe sp. (Fr.) P. Kumm.	Pscybe		x	x	
Rhodocollybia aff. *maculata* (Alb. & Schwein.) Singer.	Rmacul			x	
Stropharia sp. (Fr.) Quél.	Stroph			x	
Tremella mesenterica (Schaeff.) Retz.	Tmesen			x	
Xylaria hypoxylon (L.) Grev.	Xhypox			x	

Note: AG10: stands at the age of 10; AG19: stands at the age of 19; AG37: stands at the age of 37; E: edible.

3.2. Sporocarp productions

The highest fresh weight production was collected in 37-year-old stands (7.94 kg ha^{-1}). This was significantly higher than those from the other studied stands (P_{AG37}-P_{AG19}=0.038, P_{AG37}-P_{AG10}= 0.017). No differences were found between 19- and 10-year-old stands where the lowest value was obtained (2.51 kg ha^{-1}) (Figure 4; P = 0.425).

Considering edible species separately, sporocarp productions were also related positively with stand age (Figure4). A production of 0.94 kg ha^{-1} was obtained in 10-year-old stands, showing significant differences with 19-year-old stands (1.41 kg ha^{-1}, P = 0.049) and 37-year-old stands (2.11 kg ha-^{1}, P = 0.002). Production in 19-years-old stands also differed significantly from that obtained in the 36-years-old stands (P = 0.015) (Figure 4).

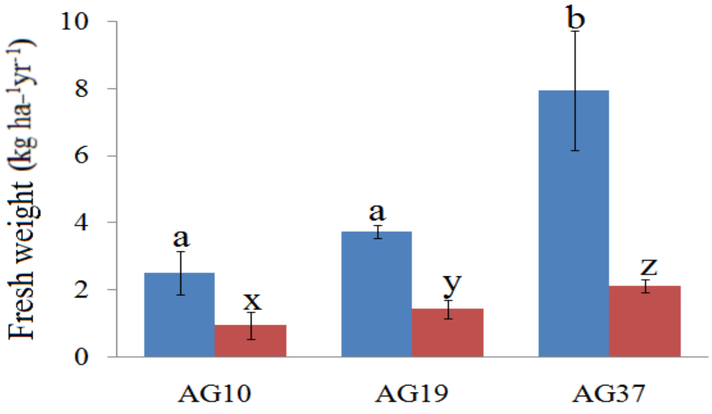

Figure 4: Production of sporocarps according to total taxa (black) and edible taxa (gray) collected from *Eucalyptus grandis* stands in Wondo Genet, Ethiopia. The data are mean results ± SE. Values with the same letter are not significantly different. AG10: stands at the age of 10; AG19: stands at the age of 19; AG37: stands at the age of 37.

3.3. Taxa composition

From the Detrended Correspondence Analysis (DCA), it is obtained that the first two axes explained more of the taxa variability than the others (Table 3). The third and fourth ordination axes, with eigenvalues less than 0.1 were less important in ecological terms and not considered further. Greater cumulative variability in terms of taxa composition, among the three stand age groups, is explained by the gradient associated with axis-1 (Table 3).

Table 3: Summary of DCA of fungal taxa presence for the study area, Wondo Genet, Ethiopia

Axes	1	2
Eigenvalues	0.549	0.201
Length of gradient	3.01	1.96
Cumulative percentage variance of taxa data	32.8	44.8

A clustering of fungal taxa around *E. grandis* stands of the three age classes (AG10, AG19, and AG37) was also observed (Figure 5), indicating distinct fungal compositions in each stand age. The 37-year-old stands tended to form a group of taxa, characterized by relatively high richness, while the 10-and 19-year-old stands were also clustered together, but characterized by a lower number of fungal taxa. However, the results should be regarded with some caution given the low number of fungal taxa in our study plantations.

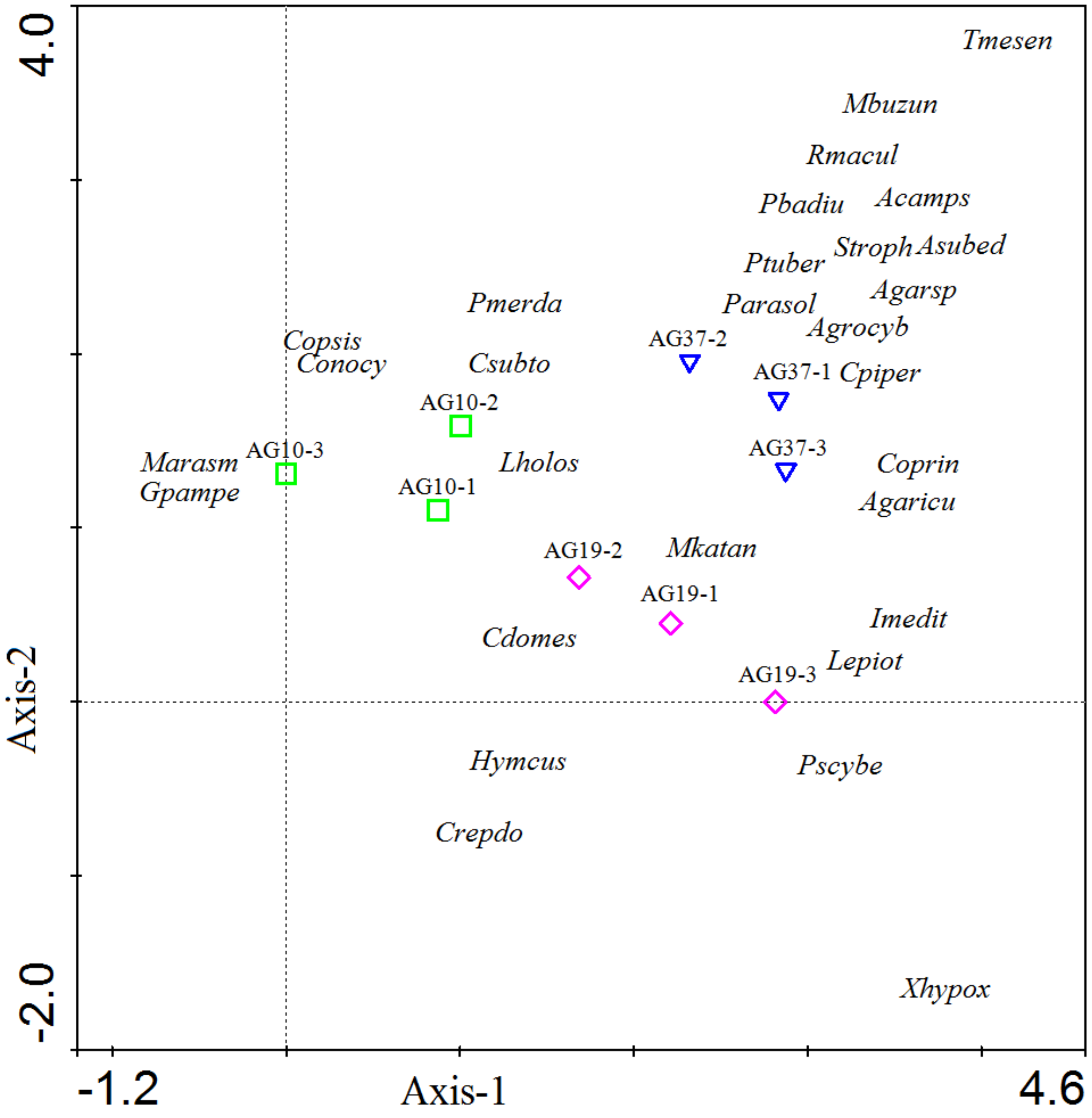

Figure 5: DCA ordination bi-plot showing: fungal taxa identified by the code shown in Table1, Plots in similar color are in a group (Green square (AG10): 10-year-old stands, Purple diamond (AG19): 19-year-old stands and Blue down triangle (AG37): 37-year-old stands).

4. Discussion

4.1. General

This study represents a primary effort to provide a systematic characterization of fungal communities under *Eucalyptus* tree plantations in Ethiopia. A total of 29 macrofungal taxa were collected in a single rainy season from the entire set of stands. According to Chilvers (2000); Parkin (1942) and Pascoe (1990), *Eucalyptus* species can support a high number of fungal taxa in the field around the world. Among them, May and Simpson (1997) reported more than 180fungaltaxain natural *Eucalyptus* forests from Australian. Lu et al. (1999) and Giachini et al. (2000) are also reported a number of fungal species from plantation of *Eucalyptus* species in and outside of Australia respectively. As compared to these, the number we found is certainly limited.

The lower number of taxa collected in our study could be linked with the lower substrate availability, explained by debris and litter coverage on the forest floor of our studied stands. This assumption coincides with Toivanen et al. (2012) and Osemwegie et al. (2010) who noted high fungal richness in forests with lots of debris and litter fall. In Ethiopia, mainly in our study area, fallen logs and other debris collection under *Eucalyptus* plantations for fuelwood is a common practice by the local people (Figure 6), which could adversely influence the fungal community by creating an artificial substrates deficit (Toivanen et al., 2012). Such effect is more prominent on saprophytic fungi that mainly constituted the taxa composition in this study, which are dependent on substrates availability in the forest floor for their occurrence (Tibuhwa et al., 2011).

Figure 6: Collected litterfall and failed logs from *Eucalyptus* plantations for fuelwood in Ethiopia

On the other hand, Megersa et al. (2017) obtained an equivalent result in a study under plantations of *Cupressus lusitanica* in Degaga area, South Central part of Ethiopia. This study was conducted during three years and reported 38 fungal taxa. A higher number of taxa would be expected if we extended the study for more years in our *E. grandis* stands. The similar number of fungal taxa found in both studies, could also further indicate that the ecological role that *Eucalyptus* species could play in terms of habitat for fungal species equally with other non-native tree species like *C. lusitanica* in Ethiopia (Megersa et al., 2017).

Moreover, *Eucalypts* species predominantly form ectomycorrhizal (EM) associations in its native geographical areas (Chilvers, 1973; Malajczuk and Hingston, 1981 and Lu et al., 1999). Also in exotic areas, Giachini et al. (2000) and Giachini et al. (2004)from Brazil, Chen et al. (2007) from China and Kluthe et al. (2016) and Ducousso et al. (2012) from Africa reported a number of EM fungi in plantations of different *Eucalyptus* species. However, in our study all of the collected taxa were classified as saprophytic, since we couldn't find EM fungi. The occurrence of EM fungi in an area is likely affected by previous land uses. Lu et al. (1999) and Postma-Blaauw et al. (2010) noted that in farm sites where agricultural crops or pasture have been grown for many years, it is unlikely to find EM propagules. In this case, the agricultural use of our studied plots before plantation could also potentially have had a negative impact on the occurrence of EM fungi in *Eucalyptus* plantations of our study area. The cryptic nature of the EM fungi, on the other hand, could also explain their absence from our study plots, as EM fungi are variable in their spatial and temporal occurrence (Ashkannejhad and Horton, 2006; Widden, 1981). However, more research is required to determine and discuss the situation and which factors are actually limiting the occurrence of EM fungi in our *E. grandis* plantations.

The species accumulation curves (Figure 3A) showed that more samplings are required to capture sufficient number of fungal taxa to accurately predict the richness in the entire stands (Thompson and Withers, 2003). However, the result indicated that fungal taxa richness is higher in the matured stands. This is in agreement with other studies which demonstrated that matured stands could favor more macrofungal taxa and thus higher richness (Dejene et al., 2017a; Lu et al., 1999). The reason might be related to the suitability of the environments created along the stand development i.e. with a relative microhabitat variation created following canopy closure (Crabtree et al., 2010). Thus, the relative difference in micro-environmental conditions, generated as a result of canopy closure in the stands after different stage developments, such as soil temperature, humidity and organic matter accumulation (Crabtree et al., 2010; Dove and Keeton, 2015) may provide congenial conditions for fungal occurrence, mainly towards the more matured stands (Dighton et al., 1986; Sysouphanthong et al., 2010). However, further studies including long-term dynamics rather than single-year observations would be highly desirable. This would provide more information for describing the fungal successional along plantation development and year-to-year meteorological variation that affects fungal taxa richness.

In line with the richness value, the Rényi diversity profiles (Figure 3B) also explained that the 37-years-old stands appear relatively with higher fungal diversity. Thus, this might lead to the management of *Eucalyptus* stands through matured green-tree retention approach that could increase habitat availability for diverse fungal species, giving more emphasis for those edible species which mostly were collected in the older stands.

4.2. Sporocarp productions

The sporocarp productions obtained in this study were not high (Figure 4). This low yield could be explained by the taxa composition, since saprophytic fungi are characterized by low biomass productions (Gassibe et al., 2011; Mediavilla et al., 2014). In line with this, other study also reported lower sporocarp production from natural *Eucalyptus* forests in South-eastern Australia (Claridge et al., 1993). Although further research is needed to verify the claim, the lower yield reported also suggested that *Eucalyptus* plantations may offer low mushroom productions.

Wild edible mushroom utilization has been a common tradition among the local communities in Ethiopia (Dejene et al., 2017b; Tuno, 2001). Also, in some local markets, mushrooms are sold by local people to earn some income supplementing the household economy (Abate, 2008). In this study, we collected a total of nine edible fungal taxa (Table 1). Although some of them are not very suitable since they are too small or fragile to be marketed or consumed, other species such as *Agaricus campestroides*, and *Calvatia subtomentosa* could be potentially used for these purposes. These two species were abundantly associated with the older stands. They are also commonly reported in the literature used by the local people in the rural area of Ethiopia (Abate, 2008).

4.3. Taxa composition

Fungal community compositions have been reported to be closely linked to forest stand ages (Wallander et al., 2010; Zhu et al., 2010). The present study also portrayed distinct fungal communities under the three *Eucalyptus* stand age classes. This is consistent with our previous study, showing the correlation of stand age with fungal taxa composition in *P. patula* stands (Dejene et al., 2017a). Based on the aggregation of the fungal taxa, the 37-year-old stand showed a distinctive macrofungal community (Figure 5), characterized by a relatively high number of taxa (Table 3). In this composition, about eleven species (~38%) were exclusively recorded (Table 1). This merely arises from site condition differences that can be explained in terms of soil humification and litter layer due to stand development (Pinna et al., 2010; Toivanen et al., 2012). As a forest stand matures, the humus layer develops and the forest soil increases its capacity to maintain the temperature and adequate moisture (Fernández-Toirán et al., 2006; Pinna et al., 2010; Toivanen et al., 2012), particularly important for saprophytic fungi occurrence.

Analysis of the fungal communities at genus level in the 37-year-old stand also revealed that some genera such as *Agaricus*, *Infundibulicybe*, *Marasmius*, *Rhodocollybia*, *Stropharia*, *Tremella* and *Xylaria* were most abundant. Of which, the taxa belonging to the genera *Agaricus*, *Stropharia* and *Tremella* were collected in relatively matured stands accordingly with Mediavilla et al. (2014), indicating that these taxa could be dominant in the fungal community associated with mature stand development conditions. Some of the taxa were also characterized as being broad-based species. This is exemplified by *Agrocybe* sp., *Calvatia subtomentosa*, *Collybia piperata*, *Conocybe* sp., *Coprinellus domesticus*, *Coprinopsis* sp., *Leucoagaricus holosericeus*, *Marasmius* sp., and *Parasola* sp. The non-specificity association of these species with a given stand age is a result of their divergence potential to grow in multiple stages of forest development. Thus, those taxa can be found in different stand development conditions and then they were collected from all the studied stands, arriving in early stages of forest growth and persisting also in mature stands.

The fungal taxa in 10- and 19-year-old stands showed more similarity and were characterized by low taxa richness. The existence of more shared species between them, made their pattern of fungal composition more or less scattered in the ordination (Figure 5). Also some taxa like *Crepidotus* sp. and *Hymenagaricus* sp. were found exclusively at both stands. Furthermore, only few macrofungal species occurred in greater abundance and dominating the composition in both stands. *Conocybe* sp., *Marasmius* sp., *Psilocybe* sp., and *Coprinopsis* sp., were found abundantly and dominating the composition in the 10-yerar-old stands. While *Psilocybe* sp., *Lepiota* sp., *Hymenagaricus* sp., and *Marasmius* sp., dominated the taxa composition in 19-year-old stands. Of these species *Marasmius* sp., and *Lepiota* sp. were reported as commonly found in young forest stands (Dejene et al., 2017a).

5. Conclusion

Although this work represents the first systematic investigation of fungal diversity and production in Ethiopia under *E. grandis* canopy, the result provides a starting place in broadening management objectives for NTFPs in plantation forests in the country.

In this study, the matured stands hosted the highest fungal richness and diversity. The result indicates an influence of stand age on fungal community composition, mainly of the saprophytic species and suggests succession of taxa during forest growth in the studied area. Managing *Eucalyptus* stands through tree retention approach could provide relatively higher fungal richness and help to facilitate conservation of fungal species. The matured trees in this regard could also serve as a bridge for providing fungal inocula to the new coppice stands while providing complementary incomes from edible fungi production as potential NTFPs.

6. Acknowledgement

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