



Fungal diversity and succession under *Eucalyptus grandis* plantations in Ethiopia



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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 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 m²) 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 the taxa collected were saprophytic and one third of them were classified as edible. Taxa richness, species composition, the Shannon diversity values, and sporocarp yield were positively correlated with plantation age. The PERMANOVA showed that the stands are significantly different ($P < 0.05$) in terms of their fungal species composition. An analysis of similarity percentage (SIMPER) also identified influential fungal taxa such as *Lepiota* aff. *cristata* and *Marasmius* sp. that best differentiated between paired stands. This preliminary study extends our knowledge of fungal community structure in plantation forests and provides a starting place in broadening *Eucalyptus* stands management objectives for Non Timber Forest Products (NTFPs) in the country, mainly of mushrooms that could provide complementary incomes for the rural people.

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). The change in natural forest cover is estimated between 150,000 and 200,000 ha (ha) of land per year (Zewdie et al., 2009). Today, the area covered with the natural forest is less than 3% of the country's total lands (Lemenih and Bekele, 2008; Taddese, 2001). 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 to household income (Asnake, 2002; Kebebew, 2002; Mesfin, 2002). Furthermore, *Eucalyptus* species have been used in biological soil conservation works such as erosion control and soil stabilization where it is planted in degraded and gully areas (Jaleta et al., 2016; Lemenih and Kassa, 2014).

Despite all benefits provided by plantations of *Eucalyptus* 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 (Jaleta et al., 2016; Teketay, 2000). 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,

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the knowledge and the status of fungal communities under *Eucalyptus* canopy in Ethiopia are unknown. Such information is essential to encourage 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 important 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* plantations are managed mainly for industrial wood production purposes (Hunde et al., 2003). Although the rotation periods range between 7 and 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 (Mekonnen et al., 2007; Pohjonen and Pukkala, 1990). 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 develops, 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, production and their conservation. 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 changes in fungal diversity and taxa composition with *E. grandis* plantation age classes and (2) to analyze sporocarp yields for total and edible taxa.

2. Methodology

2.1. Study area

The study was conducted at Wondo Genet 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 with an altitudinal range between 1600 and 2580 m above sea level (Belaynes, 2002; Thomas and Bekele, 2003). 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 is 1210 mm, which peaks in July. The average annual temperature is 20 °C (Belaynes, 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

Following our previous work (Dejene et al., 2017a), three different *Eucalyptus grandis* stands were selected in the study area based on their age i.e. 10-, 19- and 37-years-old stands, here after AG10, AG19 and AG37 respectively. Stand characteristics of the three plantations are given in Table 1. Fungal diversity and production were obtained by using transect methods (Smith et al., 2002; Dejene et al., 2017a). For this purpose, three 2 × 50 m (100 m²) plots were established at each stand, i.e. nine plots in total according to Hernández-Rodríguez et al. (2013) and Dejene et al. (2017a). Within each of the selected stands, plots were placed systematically about 120 m apart (Dejene et al., 2017a; Luoma et al., 1991). The plots were similar in terms of their ecological conditions such as climate and altitude.

All 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 fw ha⁻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 h. 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.

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.

2.3. Taxa identification and classification

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 re-hydrated and mounted in 5% KOH. The following keys were mainly used for the purpose: Heinemann (1956), Singer (1965), Pegler (1968), Pegler (1969, 1977), Morris (1990), Rammeloo and Walley (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 checked 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 sporocarp 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 ITS1 F (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

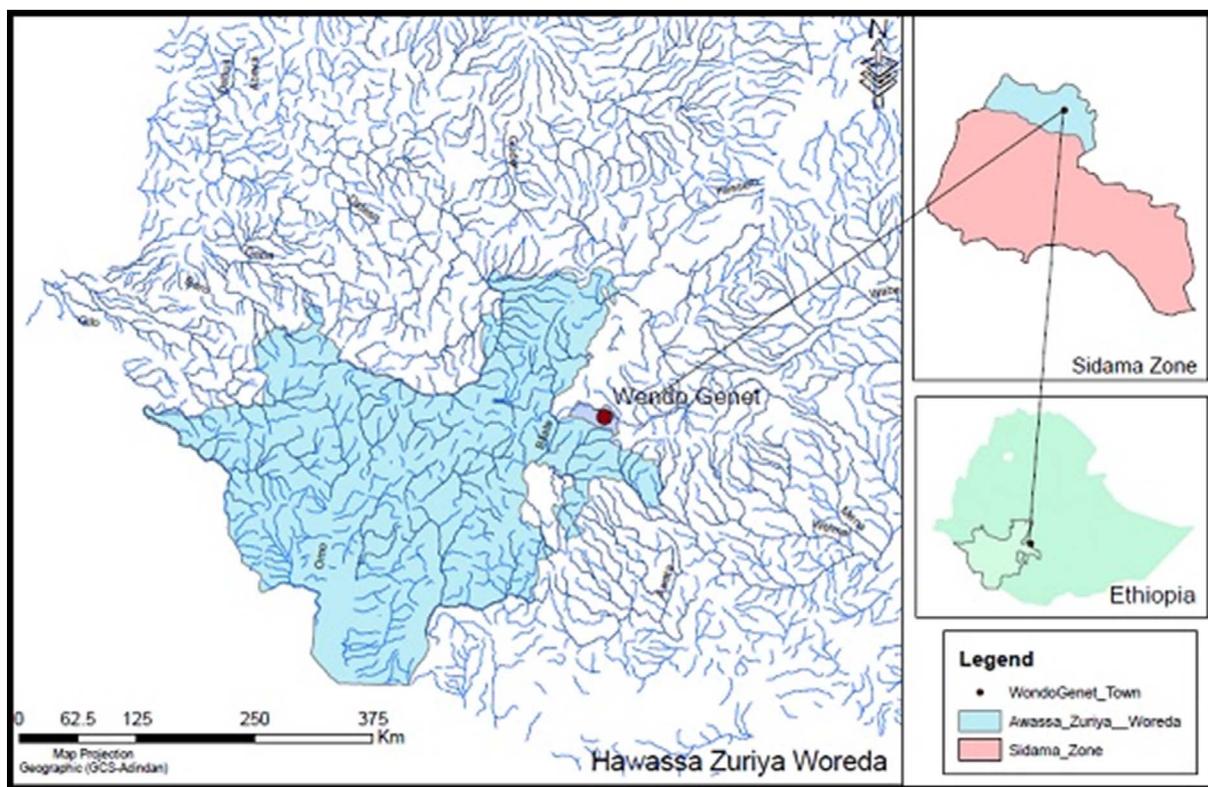


Fig. 1. Location map of the study area, Wondo Genet, Ethiopia.

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

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. Edibility (E) was classified as the species is considered as edible species in the literature consulted (Boa, 2004).

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 diversity values 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 sampling data sets.

An ordination technique was 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 C-ANOCO for Windows version 4.5 (Ter Braak and Šmilauer, 2002). Analyses were performed on the full data set (29 taxa and 9 plots). The DCA result was displayed by ordination diagrams drawn with Cano Draw 4.1 (Ter Braak and Šmilauer, 2002). Then, we tested whether fungal communities were statistically different among plantation stand ages using permutation-based nonparametric ANOVA (PERMANOVA) (Anderson, 2001). Finally, we also performed an analysis of similarity percentages (SIMPER; Clarke, 1993) to identify the fungal species that are most responsible for the observed patterns and also used to determine the percentage contribution of the fungal taxa to the significant dissimilarities among the three *E. grandis* stands (Parravicini et al., 2010).

3. Results

3.1. General data

In a single rainy season, a total of 1013 sporocarps were collected

Table 2

Total taxa list collected from *Eucalyptus grandis* stands in the study area, Wondo Genet, Ethiopia. The correct scientific name of the species and their authors were checked using mycobank.org. AG10: stand at the age of 10; AG19: stand at the age of 19; AG37: stand at the age of 37; E: edible; NE: non edible.

Taxa	Family	Code	AG10	AG19	AG37	Edible
<i>Agaricus campestroides</i>	Agaricaceae	Acamps		x	x	E
<i>Agaricus</i> sp ₁	Agaricaceae	Agarsp			x	NE
<i>Agaricus</i> sp ₂	Agaricaceae	Agaricu			x	NE
<i>Agaricus subedulis</i>	Agaricaceae	Asubed			x	E
<i>Agrocybe</i> sp	Strophariaceae	Agrocyb	x	x	x	E
<i>Calvatia subtomentosa</i>	Agaricaceae	Csubto	x	x	x	E
<i>Collybia piperata</i>	Tricholomataceae	Cpiper	x	x	x	NE
<i>Conocybe</i> sp	Bolbitiaceae	Conocy	x	x	x	NE
<i>Coprinellus domesticus</i>	Psathyrellaceae	Cdomes	x	x	x	E
<i>Coprinellus</i> sp	Psathyrellaceae	Coprin	x		x	E
<i>Coprinopsis</i> sp	Psathyrellaceae	Copsis	x	x	x	E
<i>Crepidotus</i> sp	Crepidotaceae	Crepdo	x	x		NE
<i>Gymnopilus pampeanus</i>	Strophariaceae	Gpampe			x	NE
<i>Hymenoagaricus</i> sp	Agaricaceae	Hymcus	x	x		E
<i>Infundibulicybe</i> aff. <i>mediterranea</i>	Tricholomataceae	Imedit			x	NE
<i>Lepiota</i> aff. <i>cristata</i>	Agaricaceae	Lepiot		x	x	NE
<i>Leucoagaricus holosericeus</i>	Agaricaceae	Lholos	x	x	x	E
<i>Marasmius buzungolo</i>	Marasmiaceae	Mbuzun			x	NE
<i>Marasmius katangensis</i>	Marasmiaceae	Mkatan	x	x	x	NE
<i>Marasmius</i> sp	Marasmiaceae	Marasm	x	x	x	NE
<i>Parasola</i> sp	Psathyrellaceae	Parasol	x	x		NE
<i>Polyporus</i> aff. <i>badius</i>	Polyporaceae	Pbadiu			x	NE
<i>Polyporus</i> aff. <i>tuberaster</i>	Polyporaceae	Ptuber		x	x	NE
<i>Psilocybe merdaria</i>	Hymenogastraceae	Pmerda	x		x	NE
<i>Psilocybe</i> sp	Hymenogastraceae	Ppsybe		x	x	NE
<i>Rhodocollybia</i> aff. <i>maculata</i>	Marasmiaceae	Rmacul			x	NE
<i>Stropharia</i> sp	Strophariaceae	Stroph			x	NE
<i>Tremella mesenterica</i>	Tremellaceae	Tmesen			x	NE
<i>Xylaria hypoxylon</i>	Sphaeriaceae	Xhypox			x	NE
Total			14	16	27	9

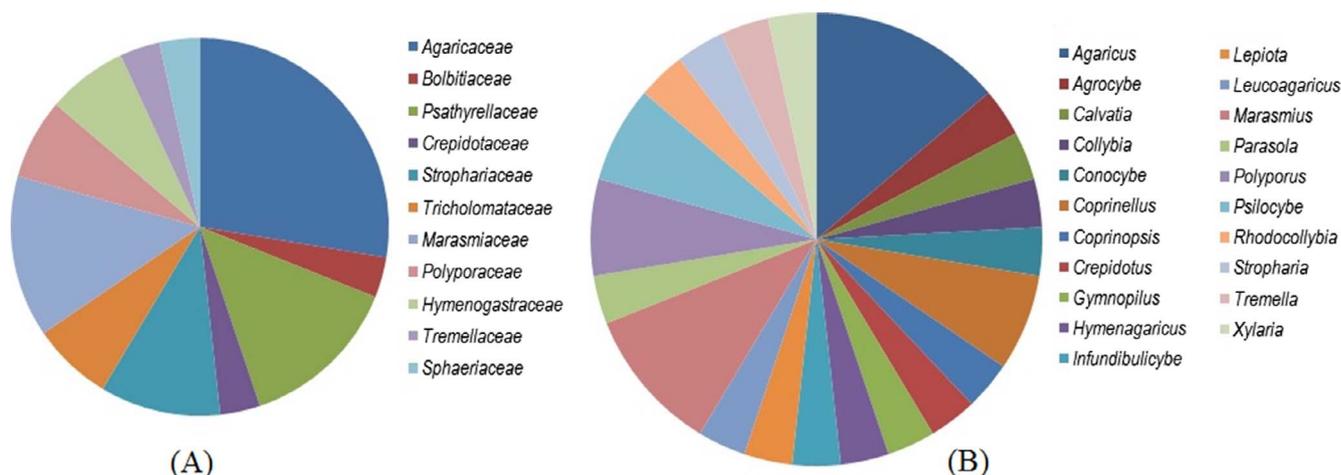


Fig. 2. Distribution of the total macrofungal taxa by family (A) and by genus (B), collected from *Eucalyptus grandis* stands of successive ages in Wondo Genet, Ethiopia.

and classified into a total of 29 fungal taxa (Table 2). All of the taxa collected were 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 (Fig. 2). The largest contribution of taxa came from Agaricaceae family, which it was represented by eight species, followed by Psathyrellaceae and Marasmiaceae with four species per each (Fig. 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 (Fig. 2B). All the collected taxa were saprophytic and could be identified at least at the genus level. Of which, 58% were identified to the species level.

The species accumulation curves (Fig. 3A) portrayed how the fungal taxa were accumulating progressively for each additional weekly

sampling in all the stands. The curve in the 37-year-old stand showed a relatively steep rising initial slope and reached higher values than the others. The curves in the 10- and 19-year-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 stand than in the others ($P = 0.001$). No significant differences were observed when comparing the taxa richness between 10- and 19-year-old stand ($P = 0.448$).

The highest Shannon diversity value (2.94) was recorded at the 37-year-old stand followed by the 19- (2.37) and 10-year-old stands (2.28), indicating a positive correlation of fungal diversity with *E. grandis* stand age in the study area. The values were further elucidated by the Rényi diversity profiles indicated that the 37-year-old stand was significantly

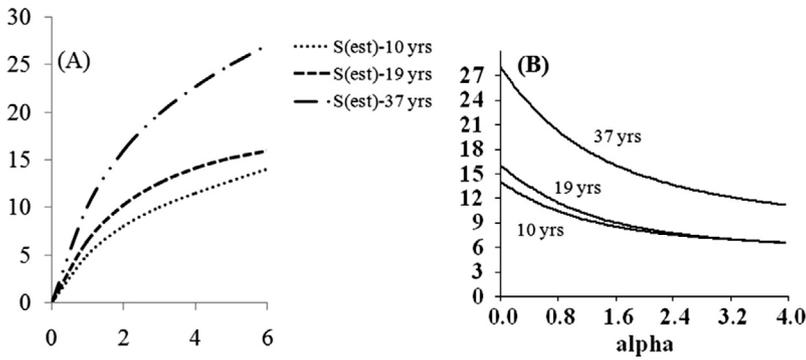


Fig. 3. Species accumulation curves generated using the rarefaction sample-based estimator of EstimateS (A) and Rényi diversity profiles (B) of fungal community found in the entire *Eucalyptus grandis* stands of three age classes in Wondo Genet, Ethiopia. The species richness curves (S(est)) are based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

different from the 10-year-old stands ($P = 0.025$) and the 19-year-old stand ($P < 0.039$) (Fig. 3B). The diversity profiles of the 10- and 17-year-old stands crossed each other, indicating that they are not different in their diversity values ($P = 0.657$).

3.2. Sporocarp productions

The highest fresh weight production was collected in the 37-year-old stand (7.94 kg ha^{-1}). This value 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}) (Fig. 4; $P = 0.425$).

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

3.3. Taxa composition

The Detrended Correspondence Analysis (DCA) revealed clustering of fungal taxa around *E. grandis* stands of the three age classes (AG10, AG19, and AG37) (Fig. 5), indicating distinct fungal compositions in each group. The PERMANOVA analyses confirmed that the three *E. grandis* stands are significantly different in their fungal composition ($F = 3.112$, $P < 0.01$). The SIMPER analysis identified the fungal species that typified and distinguished between the three stands (Table 3). The overall between-group dissimilarity was 77% between the 10- and 19-year-old stands, 82% for the 10- and 37-year-old stands and 69% for 19- and 37-year-old stands. The cumulative contribution of

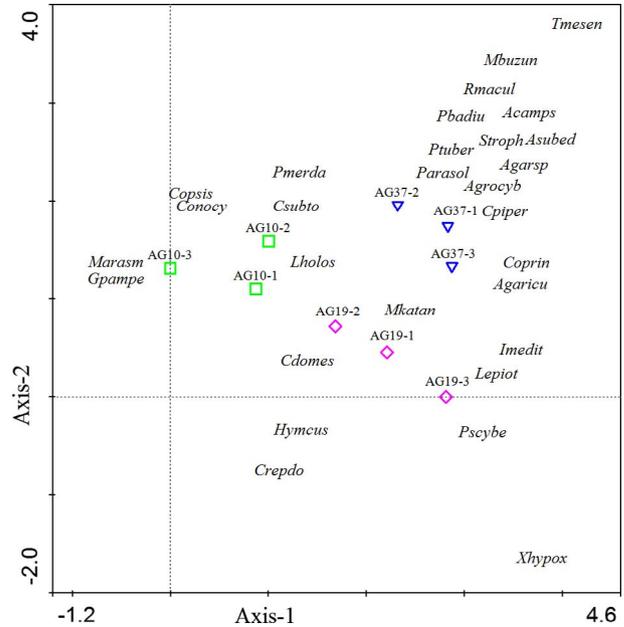


Fig. 5. DCA ordination bi-plot showing: fungal taxa identified by the code shown in Table 1. Plots in similar color are in a group (Green square (AG10): 10-year-old stand, Purple diamond (AG19): 19-year-old stand and Blue down triangle (AG37): 37-year-old stand). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

most influential specie is given (Table 3). In terms of grouping of taxa, the 37-year-old stand tended to form an assembly 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.

4. Discussion

4.1. General

This study represents the first effort to provide a systematic characterization of fungal communities under *Eucalyptus* tree plantations in Ethiopia. This pioneering work was designed to generate information helpful for integrated plantation forest management aimed at combining both timber and NTFPs production in Ethiopia, especially mushrooms for both economic and ecological benefits. In this study, 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) and Newbound et al. (2011) reported a number of fungal taxa in natural *Eucalyptus* forests from Australian. Lu et al.

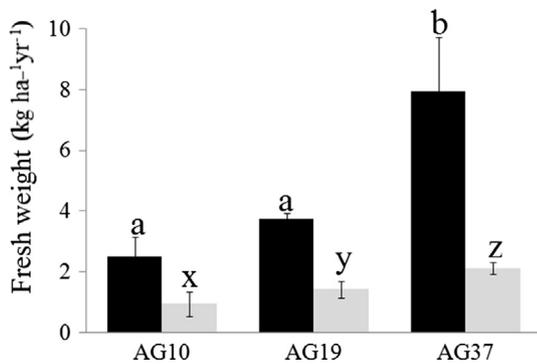


Fig. 4. Production of sporocarps according to total taxa (black) and edible taxa (gray) collected from *Eucalyptus grandis* stands in Wondo Genet, Ethiopia. The data are in mean results \pm SE. Values with the same letter are not significantly different. AG10: stand at the age of 10; AG19: stand at the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

Table 3

Summary of Similarity percentage (SIMPER) results showing contrasts of cumulative total contribution (70% cut-off) and contribution (%) of most influential species to the dissimilarity between stands of the three *E. grandis* stand ages in the study area, Wondo Genet, Ethiopia.

Species	Individual contribution to the dissimilarity	Cumulative contribution to the dissimilarity
	AG10 and AG19	
<i>Lepiota</i> aff. <i>cristata</i>	0.29	0.29
<i>Marasmius</i> sp	0.16	0.45
<i>Hymenoagaricus</i> sp	0.09	0.54
<i>Psilocybe merdaria</i>	0.08	0.62
<i>Calvatia subtomentosa</i>	0.03	0.65
<i>Psilocybe</i> sp	0.08	0.73
	AG10 and AG37	
<i>Lepiota</i> aff. <i>cristata</i>	0.17	0.17
<i>Marasmius</i> sp	0.10	0.27
<i>Agaricus campestris</i>	0.09	0.36
<i>Agaricus</i> sp ₁	0.07	0.43
<i>Agrocybe</i> sp	0.07	0.50
<i>Stropharia</i> sp	0.07	0.57
<i>Polyporus</i> aff. <i>badius</i>	0.06	0.63
<i>Calvatia subtomentosa</i>	0.05	0.68
<i>Parasola</i> sp	0.03	0.71
	AG19 and AG37	
<i>Lepiota</i> aff. <i>cristata</i>	0.13	0.13
<i>Agaricus campestris</i>	0.09	0.22
<i>Agaricus</i> sp ₁	0.09	0.31
<i>Agrocybe</i> sp	0.08	0.39
<i>Stropharia</i> sp	0.07	0.46
<i>Polyporus</i> aff. <i>badius</i>	0.07	0.53
<i>Hymenoagaricus</i> sp	0.06	0.59
<i>Psilocybe merdaria</i>	0.06	0.65
<i>Calvatia subtomentosa</i>	0.04	0.69
<i>Collybia piperata</i>	0.05	0.74

(1999) and Giachini et al. (2000) are also reported numerous species from plantation of *Eucalyptus* species in and outside of Australia. As compared to these, the number we found is certainly limited.

As compared to the above mentioned studies, the lower number of taxa collected in our study could be linked with the lower substrate availability that can be explained by lower debris and litter coverage on the forest floor of our studied *Eucalyptus* stands. In Ethiopia, mainly in our study area, fallen logs and other debris collection for fuel wood under *Eucalyptus* plantations is a common practice by the local people, which could adversely influence the fungal communities 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). This difference also further reflected on the chance of occurrence or lack of detection of fungal species in our study area during the collection period as fungi occurrence show considerable seasonal and yearly variation in their occurrences (Tibuhwa et al., 2011).

An equivalent number of fungal taxa, on the other hand, was also reported by Megersa et al. (2017) under *Cupressus lusitanica* plantations 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 (Newbound et al., 2010). 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).

Eucalyptus species predominantly form ectomycorrhizal (EM) associations in its native geographical areas (Chilvers, 1973; Malajczuk and Hingston, 1981; Lu et al., 1999). Also in exotic areas, Giachini et al. (2000, 2004) from Brazil, Chen et al. (2007) from China and Kluthe et al. (2016) and Ducouso et al. (2012) from Africa reported EM fungal

association in plantations of different *Eucalyptus* species. However, in our study 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 of the plantation area. 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 (Fig. 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 high in mature stands. This is in agreement with other studies which demonstrated that mature 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 mature stands (Dighton et al., 1986; Sysouphanthong et al., 2010). However, further studies including long-term dynamics rather than single-year observation 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 (Fig. 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 (Fig. 4). The value obtained also by far smaller than that of the *Pinus* stands of our previous study of the same area (Dejene et al., 2017a). 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 *Eucalyptus* forests in South-eastern Australia (Claridge et al., 1993). Although further research is needed to verify the claim, the lower yield reported here suggested that *Eucalyptus* plantations may offer low mushroom productions.

Wild edible mushroom utilization has been a common tradition among the local communities in Ethiopia, mainly in South and South Western part of the country (Dejene et al., 2017b; Tuno, 2001). Also, in some local markets of these regions, 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 2). Although some of the edible species are not very suitable and also have limited priority for market since they are too small or fragile to be marketed or consumed, other species such as *Agaricus campestris*, and *Calvatia subtomentosa* could be potentially used for

these purposes. These two species were abundantly associated with the older stands. They are also reported in literature used by the local people in the rural area of Ethiopia (Abate, 2008). We believe that further studies involving close examinations of the *Eucalyptus* habitats are needed, since there might be yet unknown species with valuable marketable and social potential but equally unknown uses.

4.3. Taxa composition

Our SIMPER analysis distinguished the total dissimilarity between pairs of stands and the relative contribution of each fungal species to the observed dissimilarity (Table 3). An important component of this result we could see those fungi species that were responsible for the differences observed among the three stands (Lupatini et al., 2013). It also indicated that the overall differences between stands were due to some fungal taxa that each contributing with a relatively small percentage of the dissimilarity (Table 3). For example, the species with the highest contributions (29%) for the dissimilarity between the 10- and 19-years old stands was *Lepiota aff. cristata* (Table 3). This species occurred in high abundances in the 19-year-old stand. Thus, the more abundant a species with a consistently high contribution to the dissimilarity between groups is a good discriminating species (Clarke, 1993; Clarke and Warwick, 2001). *L. cristata* also reported exclusive abundance occurrence in older plantations (Dejene et al., 2017a), suggesting that *L. cristata* a discriminating species explaining an intermediate-to-matured stands. Thus, our findings support the notion that, for a given stand age, certain fungal species tend to be abundant and characterize its stage (Zhu et al., 2010). Similarly, the analysis also displays most important influential species for other pairs of stands (Table 3). For example, *L. cristata* and *Marasmius* sp., contributed to 27% of the average cumulative dissimilarity between AG10 and AG37 and 22% of the average cumulative dissimilarity between AG19 and AG37 (Table 3). These contributions of the species might be responsible for the difference between *E. grandis* stands and suggesting that change in stand age is probably responsible for the variation in dominance of some species and their exclusive occurrences (Dejene et al., 2017a).

Fungal composition and their succession have been reported to be closely linked to plantation forest developments (Wallander et al., 2010; Zhu et al., 2010). Based on the aggregation of the fungal taxa, our ordination analysis also indicated that the 37-year-old stand showed a distinctive macrofungal community (Fig. 5), characterized by a relatively high number of taxa (Table 2). In this composition, about eleven species (~38%) were exclusively recorded. This merely arises from site condition differences that can be explained in terms of soil humification and litter layer due to stand development and stand density (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*, *Marasmius*, *Rhodocollybia*, *Stropharia*, and *Xylaria* were most abundant. Of which, the taxa belonging to the genera *Agaricus*, and *Stropharia* were collected in relatively matured stands (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 were characterized by low taxa richness. There are also more shared taxa between these two stands (Table 2 and Fig. 5). 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-year-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

Mushroom hunting and utilization is a traditional common practice among the different tribes in Ethiopia. They have been used for their medicinal properties, and are also involved in local mythology. In some local markets mushrooms are also available, where they are sold by the local people to earn some income to supplement the household economy. However, reports on macrofungal species as mushrooms from Ethiopian plantation forests are limited. This case of study is the first to explain fungal communities in general and production under *Eucalyptus grandis* canopy in the country. The results provide a starting place in broadening management objectives for NTFPs in plantation forest systems, mainly of the edible wild mushrooms in Ethiopia.

The results showed that mature *E. grandis* stand hosted high fungal taxa richness and diversity. Also an influence of stand age was observed on fungal community composition and suggesting succession of taxa during forest developments in the studied stands. Also, our analysis identified main species that mainly characterized the development of the stands at different stages. Some of these species are may be locally relevant as food or as for their ecological functions. Thus managing *Eucalyptus* stands through matured tree retention approach could provide relatively high 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 Non Timber Forest Products (NTFPs).

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