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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 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 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 (Belaynesh, 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 (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

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 m^2) 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 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 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