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Changes in fungal diversity and composition along a chronosequence of *Eucalyptus grandis* plantations in Ethiopia

Carles Castaño [a, 1], Tatek Dejene [b], Olaya Mediavilla [a], József Geml [c], Juan Andrés Oria-de-Rueda [a], Pablo Martín-Pinto [a, *]

[a] *Sustainable Forest Management Research Institute, University of Valladolid, Avda. Madrid 44, 34071, Palencia, Spain*
[b] *Central Ethiopia Environment and Forestry Research Center, P.O. Box 30708, Addis Ababa, Ethiopia*
[c] *Biodiversity Dynamics Research Group, Naturalis Biodiversity Center, P.O. Box 9517, 2300, RA Leiden, the Netherlands*

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ABSTRACT

Eucalyptus tree species are widely used in Ethiopian plantations, but the impact of these plantations on the soil fungal communities is still unknown. We assessed the changes in diversity, species composition and ecological guilds of the soil fungal communities across tree ages of *Eucalyptus grandis* plantations by DNA metabarcoding of ITS2 amplicons. Changes in soil fungal species composition, diversity and ecological guilds were related to stand age but also to fertility changes. The relative abundance of saprotrophs and pathogens were negatively correlated with stand age, and positively with soil fertility. In contrast, the relative abundance and diversity of ectomycorrhizal species were higher in older, less fertile stands, including well-known cosmopolitan species but also species associated with *Eucalyptus*, such as *Scleroderma albidum* and *Descomyces albellus*. We show that soil fungal community changes are linked to progressive soil colonization by tree roots but are also related to soil fertility changes.

1. Introduction

Ethiopian natural forest cover has dramatically decreased during recent decades in an estimated deforestation rate of up to 200,000 ha/y (Zewdie et al., 2010). As a result, natural forests represent today less than 3% of the total land (Taddese, 2001; Lemenih and Bekele, 2008). Plantations with fast-growing tree species have been established to obtain timber and reduce pressure on natural Ethiopian forests (Zewdie et al., 2010; Bekele, 2011). These plantations are mainly composed of *Eucalyptus*, *Cupressus*, *Pinus* and *Acacia* species (Moges et al., 2010; Bekele, 2011), which represent around 506,000 ha in Ethiopia (FAO, 2011). *Eucalyptus* species are the most widely used species, representing more than half of the total national plantation area (Bekele, 2011). *Eucalyptus* species are often chosen for their adaptation to different ecological conditions, management and fast-growing nature, serving as the main source of firewood, poles, posts and farm implements in Ethiopia (Kelemu and Tadesse, 2010).

Impacts of *Eucalyptus* on native flora have already been reported in other studies; e.g. *Eucalyptus* competes with native species for nutrients and moisture, inhibits the understorey by exudating phytotoxic chemicals (Jaleta et al., 2016) and may promote nutrient depletion (Temesgen et al., 2016). In contrast to these negative effects, other studies have shown that these plantations host some native herbaceous species and can promote natural regeneration of Ethiopian flora (Yirdaw, 2002; Lemenih, 2004), allowing the regeneration of understorey vegetation under natural successional dynamics (Onaindia et al., 2013). *Eucalyptus* plantations are a source of non-timber forest products, such as edible mushrooms (Dejene et al., 2017a), although information on the impact caused by these plantations on soil fungal communities is still unknown.

Soil microbes are essential components of forest ecosystems. Among soil fungi, mycorrhizal species are especially important because they form a beneficial symbiotic association with plants, providing them nutrients in return for photosynthetically fixed carbon (C) (Smith and Read, 2008). Ectomycorrhizal (ECM) fungi are also key players in the alleviation of drought stress for trees (Mohan et al., 2014), and the role of these organisms is especially relevant for nutrient uptake by plants under nutrient-limited

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* Corresponding author.
E-mail address: pmpinto@pvs.uva.es (P. Martín-Pinto).

[1] Present address: Department of Forest Mycology and Plant Pathology, Uppsala BioCenter, Swedish University of Agricultural Sciences, SE-750 07, Uppsala, Sweden.

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conditions (Read and Perez-Moreno, 2003). Arbuscular mycorrhizal (AM) species are also efficient in uptake and transfer nutrients, especially phosphorus (Read and Perez-Moreno, 2003).

Eucalyptus tree species have dual mode of mycorrhizal symbiosis, with both ECM and AM taxa (Adjoud-Sadadou and Halli-Hargas, 2017), although association with AM species seems to be restricted to seedlings or younger trees (Adams et al., 2006). Changes in soil chemistry, fertility or host tree status may change the balance of such mycorrhizal associations. For example, depletion of inorganic soil N could negatively affect AM taxa due to their preference for inorganic N forms, although evidence of access of AM fungi to organic sources has been shown (Thirkell et al., 2016). ECM fungi are especially adapted to mobilize N from organic forms through oxidation chemistry (Tunlid and Lindahl, 2014). Studies on *Eucalyptus* plantations' impacts on the surrounding environment have neglected the soil microbial communities, especially saprotrophs. Saprotrophic fungi have a paramount role in litter and SOM degradation (Baldrian et al., 2011). Understanding soil fungal community structure and functioning in forest ecosystems is important, because these fungi determine many important ecosystem processes such as C storage and nutrient cycling (Kyaschenko et al., 2017). Interguild fungal interactions and fungal community shifts may be modulated by changes in the environment such as pH (Rincón et al., 2015), nitrogen (N) (Kjøller et al., 2012), climate (Geml et al., 2016; Castaño et al., 2018), but also human disturbances, such as tree harvesting (Kohout et al., 2018). Thus, such environmental changes potentially cause direct and indirect alterations to ecosystem functioning with respect to SOM decomposition, plant nutrition and C cycling, by affecting the soil-associated microbiome (Averill et al., 2014; Clemmensen et al., 2015; Kohout et al., 2018).

Replacing native vegetation or grasslands with exotic trees, such as *Eucalyptus* plantations in Ethiopia, is expected to result in changes in soil physico-chemical properties (Temesgen et al., 2016) as well as in the composition of the associated soil microbial communities and related ecosystem processes. Here, we investigated the changes in fungal diversity, community composition and ecological guilds along a chronosequence of *Eucalyptus grandis* plantations. We expected that soil fertility would decrease with the age of *E. grandis* stands, with expected lower N and P contents and higher C/N ratios in older stands. In addition, we expected that soils from older *E. grandis* stands would be more dominated by root-associated fungal species than in younger stands due to an increasing soil colonization of roots and associated symbionts. Thus, we hypothesize that (i) decreasing fertility and increasing dominance of *Eucalyptus* along the chronosequence will result in an increase in the diversity and abundance of ECM species. In contrast to old stands, young stands are expected to be more fertile and trees will be exerting less influence on the fungal microbiome, thus we hypothesise that (ii) at younger stands there will be a higher abundance and diversity of other functional groups such as saprotrophs, pathogens or AM species.

2. Material and methods

2.1. Study area

The study was carried out in the Wondo Genet plantation forest area in Southern Ethiopia (coordinates 7° 05′ 02″ N 38° 37′ 08″ E, altitude between 1600 and 2580 m above sea level), located approximately 265 km from Addis Ababa. The site has a mean annual rainfall of 1210 mm, with most of the rainfall recorded during summer. The mean annual temperature is 20 °C. Three distinct age classes of *E. grandis* plantations were selected for this study: i.e. 10, 19 and 37 y. All these had historically been natural forests, which were cut and converted to grasslands before the introduction of *E. grandis* trees. Interestingly, the sampled plots did not contain any significant regeneration of native vegetation neither estimated cover of species other than *E. grandis*. Three plots of each stand age class were selected for soil sampling, resulting in a total of nine plots. These plots were separated from each other by 120 m. Hence, the plots were similar in terms of their ecological conditions such as climate and altitude.

2.2. Soil samplings

All nine plots were sampled in July 2015. In each plot, five soil cores (20 cm deep and 4 cm in diameter) were taken using a cylindrical soil borer extracted along the centreline of each transect at 5 m distance from each other. We sampled well-decomposed organic layers and mineral soil, but we discarded the litter layer (intact and partially decomposed leaves), given that the fungal community composition in the leaves tends to diverge from that in soil (Voříšková et al., 2014). Soil cores were dried, sieved through a 1 mm mesh and grounded to a fine powder using a mortar and pestle. A subsample was stored at −20 °C for molecular analysis whereas another subsample was taken for determination of soil physico-chemical parameters (Table 1). The soil pH and electrical conductivity were measured using a soil:water (1:2.5) suspension and in the supernatant, using a pH meter and an Electrical Conductivity meter, respectively from the same suspension (Reeuwijk, 2002). Organic carbon content was determined using wet digestion (Walkley and Black, 1934). Total N content in soils was determined using the Kjeldahl procedure following Kim (1996). Available P was determined using sodium bicarbonate (0.5 M $NaHCO_3$) as extraction solution (Olsen and Sommer, 1982). The color intensity was measured spectrophotometrically at 882 nm. For soil particle size analysis, the hydrometer method (Bouyoucos, 1951) was employed, using sodium hexametaphosphate (Calgon solution) as the dispersing agent. Once the sand, silt, and clay separates were calculated as a percent, the soil was assigned a textural class name based on ASTM Software.

2.3. Molecular analysis

DNA extraction was performed from 0.25 g of soil per sample with the PowerSoil™ DNA Isolation Kit (MoBio laboratories Inc., Carlsbad, CA, USA). PCR reactions were performed in triplicate for each sample to minimize PCR biases. PCR reactions were performed in 40 μl reaction volumes containing 24 μl of sterile water, 1.00 μl of DNA template, 4.00 μl of 10x buffer, 5.60 μl of $MgCl_2$ (25 mM), 1.50 μl dNTPs (10 mM), 0.50 μl BSA (2%), 1.50 μl of reverse and forward primers (10 μM) and 0.4 μl Taq polymerase (Invitrogen, Carlsbad, CA). We used the following PCR conditions: an initial denaturation step at 95 °C for 5 min; then 35 cycles of 95 °C for 20 s, 54 °C for 30 s and 72 °C for 1.5 min; and ending with one cycle of 72 °C for 10 min. The ITS2 rDNA region was amplified using the forward primer fITS7 (Ihrmark et al., 2012) and reverse primer ITS4 (White et al., 1990). These primers were designed to detect a wide-range of fungal species, but they amplify fewer AMF families in comparison with SSU primers (Lekberg et al. 2018). Similarly, copy gene number per biomass unit in AM species is much lower than in ECM species. Therefore, a reliable description of AM species is not possible, nor a comparison between AM and ECM species, although an evaluation of the changes in relative abundance of AM species across samples is still possible. To be able to identify each sample, the ITS4 primer was labelled with sample-specific Multiplex Identification DNA-tags. A negative control consisting of sterile water was included in each PCR replicate and underwent the PCR under the same experimental conditions and was shown on a gel to

Table 1
Soil physico-chemical characteristics of the *E. grandis* plots from Wondo Genet (Ethiopia). OM= Organic matter, N=Nitrogen, P= Phosphorus, K=Potassium, Ca=Calcium, Mg = Magnesium, Na= Sodium. Values indicate means and standard deviations.

Age (years)	Sand (%)	Silt (%)	Clay (%)	pH	OM (%)	N (%)	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Na (mg kg^{-1})
10	59.5 ± 5.8	21.8 ± 11.1	18.8 ± 6.8	6.2 ± 0.1	7.7 ± 1.9	0.54 ± 0.08	60.9 ± 22.3	0.70 ± 0.06	27.2 ± 4.6	9.7 ± 1.0	0.9 ± 0.06
19	61.5 ± 12.3	22 ± 3.3	13.2 ± 7.3	5.2 ± 0.2	5.5 ± 0.1	0.26 ± 0.00	30.7 ± 8.7	0.42 ± 0.04	14.3 ± 1.8	4.9 ± 0.86	1.6 ± 0.11
37	50.1 ± 1.8	24.3 ± 3	25.7 ± 1.5	6.2 ± 0.3	4.6 ± 0.7	0.27 ± 0.06	26.7 ± 5.7	0.48 ± 0.10	16.4 ± 1.2	5 ± 0.87	1.7 ± 0.11

be amplicon free. Ion Torrent sequencing was carried out at the Naturalis Biodiversity Center. We used the sequencing Ion 318TMChip to allow for highest possible sequencing coverage.

2.4. Quality control and bioinformatics

Raw sequence reads were obtained from the Ion Torrent output that comprise demultiplexed sample reads. Primers and poor-quality ends were trimmed based on 0.02 error probability limit in Geneious Pro 8.1.8 (BioMatters, New Zealand). Subsequently, sequences were filtered using USEARCH v.8.0 (Edgar, 2010) based on the following settings: all sequences were truncated to 200 bp and sequences with expected error >1 were discarded. The remaining sequences were collapsed with USEARCH v.8.0 (Edgar, 2010) into unique sequence types on a per-sample basis while preserving read counts. Singleton sequence types were discarded and the resulting 305,520 high-quality sequences were grouped into 3286 operational taxonomic units (OTUs) with USEARCH at 97% sequence similarity while simultaneously excluding 181 chimeric sequences. We assigned sequences to taxonomic groups based on pairwise similarity searches against the curated UNITE + INSD fungal ITS sequence database containing identified fungal sequences with assignments to species hypothesis groups (Kõljalg et al., 2013). After excluding OTUs with <70% similarity or <150 bp pairwise alignment length to a fungal sequence, the dataset contained 2886 fungal OTUs, representing total of 296,384 high quality sequences. Ecological guilds of identified taxons at species or genera level was performed using FUNGuild (Nguyen et al., 2016).

2.5. Data analysis

Statistical analyses were implemented in the R software environment (version 2.15.3; R Development Core Team 2013) using the *vegan* package for multivariate analysis (Oksanen et al., 2015), *nlme* package for linear mixed models (LME: Pinheiro et al., 2016) and *indicspecies* for indicator species analysis (De Cáceres and Legendre, 2009). *iNEXT* package (Hsieh et al., 2016) was used for diversity analysis and interpolation of fungal diversity data. Ordination of community data (Detrended Correspondence Analysis and Canonical Correspondence Analysis: DCA and CCA, respectively) was carried out using CANOCO version 5.0 (Biometris Plant Research International, Wageningen, The Netherlands).

First, we investigated whether there was a strong correlation between some of the measured environmental variables, especially soil variables (OM, P, Mg, N, K, Ca), with variance inflation factor (VIF) values > 20, except for pH (Fig. S1). Accordingly, the first axis (PCA1) of a PCA considering these variables was used as a soil chemistry index. Soil chemistry index also represented a fertility gradient (Fig. S1). Similarly, the first axis (PCA1) of a PCA considering soil texture values (sand, silt, clay) was used as a soil texture index.

DCA considering the fungal species composition was used to obtain graphical representations of fungal community similarity across stand ages and the parameters related to soil chemistry and textures. CCA was used to relate fungal community composition with the selected environmental variables (soil chemistry, texture and tree age), and significance was studied by both simple and conditional effects. Simple term effects are the effects of an individually tested factor, whereas conditional effects are the effects of each factor before removing the effects of the other factor of factors included in the model. Significance of the environmental variables was tested by a Monte Carlo permutations test (999 permutations). Similarly, significance of the environmental variables was also confirmed using Permutational multivariate analysis of variance based on distance matrices (function *adonis* and Bray-Curtis distance). Responses of specific fungal taxa to either stand age or soil fertility were tested by running species response curves using Generalized Linear Models (GLM), and indicator species analysis was used to identify taxa associated to a certain stand age. The same analyses (DCA, CCA) were carried out using the relative proportions of each guild as a response variable, and the environmental variables (stand age, fertility index) as an explanatory variable. Significant effects of these environmental variables to each guild were specifically tested by ANOVA and confirmed by Linear Mixed Effects Models (LME), in which the effect of stand age was specifically tested by defining this variable as a fixed factor and the soil fertility as a random variable. Similarly, the effect of soil fertility over each specific guild was tested by defining this variable as a fixed factor and tree age as a random variable. Effects of these LME were tested by ANOVA ($P < 0.05$ was considered a significant effect).

Hill's series of diversity indices (Hill, 1973) was used to compare differences in diversity values across fertility indices and tree ages. Hill's diversity consists of three numbers: N0 is species richness; N1 is the antilogarithm of Shannon's diversity index; and N2 is the inverse of Simpson's diversity index. These tests were performed over the whole fungal diversity values, but also separate diversity analyses were carried out considering only species belonging to specific guilds (e.g. ECM, saprotrophs). N0, N1 and N2 Hill's diversity indices were calculated from the asymptotic estimates implemented in *iNEXT.* Diversity comparison between tree ages and soil fertility indices were performed using ANOVA.

3. Results

3.1. Sequencing output and fungal community composition

Clustering resulted in 2886 OTUs, of which 350 were singletons. We obtained an average of 24,711 ± 6325 reads in each site. Overall, saprotrophs (dung saprotrophs, other saprotrophs) were the most abundant across the whole dataset, representing 47% of the community. The dominance of these groups was followed by ECM species (15%), plant pathogens (12%) and endophytes (12%). Less dominant groups were represented by animal pathogens, AM, wood saprotrophs and soil saprotrophs.

3.2. Environmental drivers affecting the fungal community composition

Simple effects of explanatory variables revealed that soil fertility, pH and tree age, but not texture, significantly affected the fungal community composition (Fig. 1A and B; Table 2). Conditional

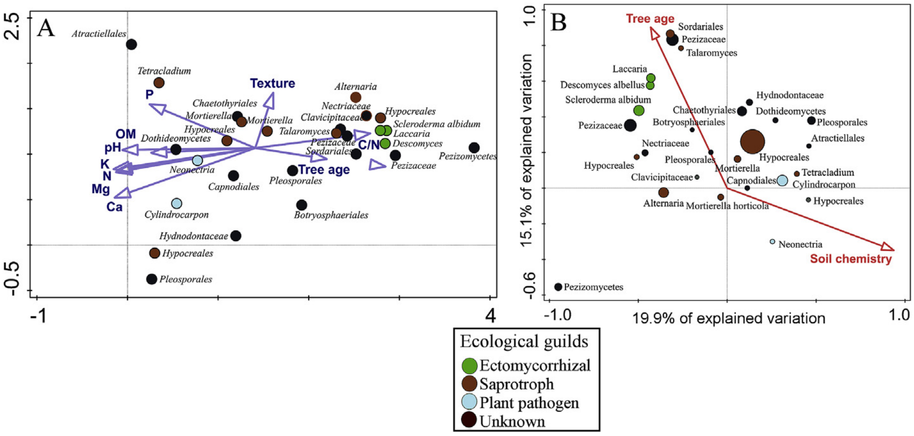

Fig. 1. (A) Detrended correspondence analyses (DCA) and (B) Canonical Correspondence Analysis (CCA) of the species level community composition of soil fungi in Ethiopian forests, as analysed by sequencing internal transcribed spacer 2 amplicons. Environmental parameters are shown as supplementary variables in (A), whereas soil chemistry and age are constrained parameters in (B). In (B), species symbol sizes are proportional to the average relative abundance. The figure shows the 26 most abundant species hypotheses coloured according to their ecological guild. Here, only ectomycorrhizal, saprotrophs and plant pathogens are shown because other fungal guilds were less abundant.

Table 2
Significance of the CCA analysis based on simple term effects and conditional effects, considering the Hellinger transformed fungal community data at species level. Numbers in bold indicate significant effects ($P < 0.05$).

Variable	Simple Term Effects			Conditional effects		
	Explains %	pseudo-F	P	Explains %	pseudo-F	P
Soil chemistry	**19.3**	**1.7**	**0.004**	**19.3**	**1.7**	**0.002**
Age	**16**	**1.3**	**0.037**	**15.7**	**1.4**	**0.017**
Texture	13.2	1.1	0.273	11.9	1.1	0.411

effects also identified soil chemistry and tree age as significantly affecting the soil fungal community composition (Table 2). Significance of soil chemistry and stand age were also confirmed by PERMANOVA analyses, with both stand age ($F = 2.93$, $P = 0.007$, $R^2 = 0.23$) and soil chemistry ($F = 3.52$, $P = 0.002$, $R^2 = 0.28$) affecting the fungal community composition. Indicator species analyses and GLMs showed that several ECM taxa (e.g. *Laccaria* sp., Thelephoraceae, *Descomyces*, *Scleroderma*; Fig. 1) were associated with older, less fertile stands. In contrast, specific AM species were more associated with younger, more fertile stands, together with many saprotrophic species such as *Mortierella, Tetracladium*, or fungi belonging to Pleosporales (Fig. 1), but also plant pathogens (*Neonectria*, Fig. 1). When considering only the ECM community, there were significant compositional changes across stands of different ages ($P = 0.05$), with specific taxa such as *Laccaria* sp. and *Thelephora* sp. being more prevalent in older stands.

3.3. Fungal functional changes across tree ages and soil fertility

When considering the composition of ecological guilds, simple effects of explanatory variables revealed again that soil chemistry (~fertility) was responsible for up to 41% of the total fungal functional variation ($F = 4.9$, $P = 0.005$). Tree age was responsible for up to 33.4% of the total variation ($F = 2.7$, $P = 0.01$), sharing 15.3% of the variance with soil chemistry. However, there were specific guild responses to either tree age and soil fertility, with some of these functional groups responding differently to each these parameters (Fig. 2). For example, the relative abundance of undefined saprotrophs was only significantly increased according to a fertility index, whereas the relative abundance of plant pathogens decreased across tree ages and increased with more fertility (Table 3; Figs. 2 and 3). Finally, ECM species decreased with soil fertility but

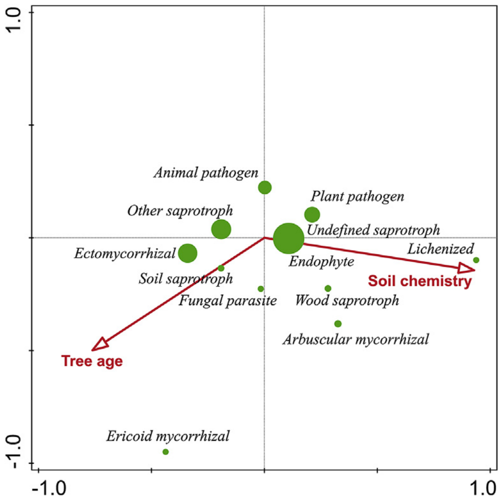

Fig. 2. DCA plots considering the relative proportions of fungal guilds in relation to tree age and soil chemistry in Ethiopian forests, as analysed by sequencing internal transcribed spacer 2 amplicons. Symbol sizes are proportional to average relative abundances of each guild.

Table 3
Significance of the effects between the relative proportions of specific fungal guilds and diversity indices as measured with Hill's numbers, and the tree age and soil chemistry. Hill's diversity consists of three numbers: N0 is species richness; N1 is the antilogarithm of Shannon's diversity index; and N2 is the inverse of Simpson's diversity index. Here, Numbers in bold indicate significant effects ($P < 0.05$).

Guilds	Tree age		Soil chemistry	
	F	P-value	F	P-value
Ectomycorrhizal	**11.16**	**0.012**	**7.88**	**0.038**
Other saprotrophs	2.13	0.187	6.05	0.057
Plant pathogen	**50.24**	**<0.001**	**7.83**	**0.038**
Undef. saprotrophs	0.35	0.575	**7.83**	**0.037**
Endophytes	0.73	0.42	1.34	0.3
Animal pathogen	0.69	0.432	0.41	0.549
Arbuscular	0.05	0.833	2.41	0.181
Hill's				
N0	**11.57**	**0.011**	**7.02**	**0.045**
N1	2.40	0.165	**9.96**	**0.025**
N2	0.30	0.599	4.34	0.091

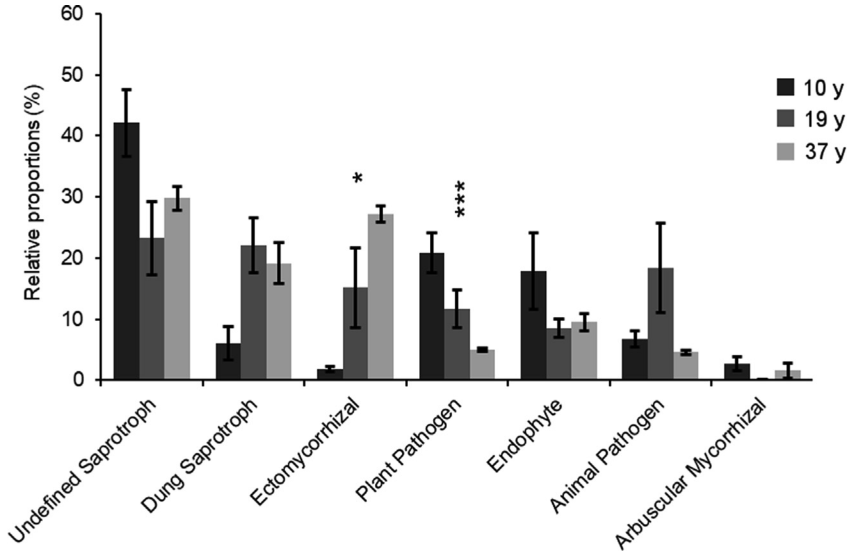

Fig. 3. Fungal functional changes showing the most abundant guilds in 10, 19 and 37 y stands. Here, the seven most abundant guilds are shown, as classified by Nguyen et al. (2016). Significance levels: ***P < 0.001, **P < 0.01, *P < 0.05.

increased with tree age, with a higher ECM abundance in older stands (Table 3; Figs. 2 and 3).

3.4. Fungal diversity changes across tree ages and soil fertility

Stand age significantly affected the total fungal alpha diversity N0, which represents the fungal richness (Table 3, Fig. S2). N0 decreased with tree age, with a 35% decrease in fungal richness from 10 y to 39 y (Fig. 4). Despite the decreasing trend of N1 and N2 values with increasing stand age, these differences were not significant (Table 3). Both N0, N1 and marginally N2 values increased with increasing soil fertility (Table 3), especially in the very fertile sites. However, diversity changes across tree ages and soil fertility were distinct across functional groups. Thus, for ECM species, there was an increase in alpha diversity with increasing stand age (Fig. 4), concurrent with an increase of their relative abundance (Fig. 3). Although the relative abundance of AM fungi was neither affected by stand age nor soil fertility (Fig. 3), this guild was much more

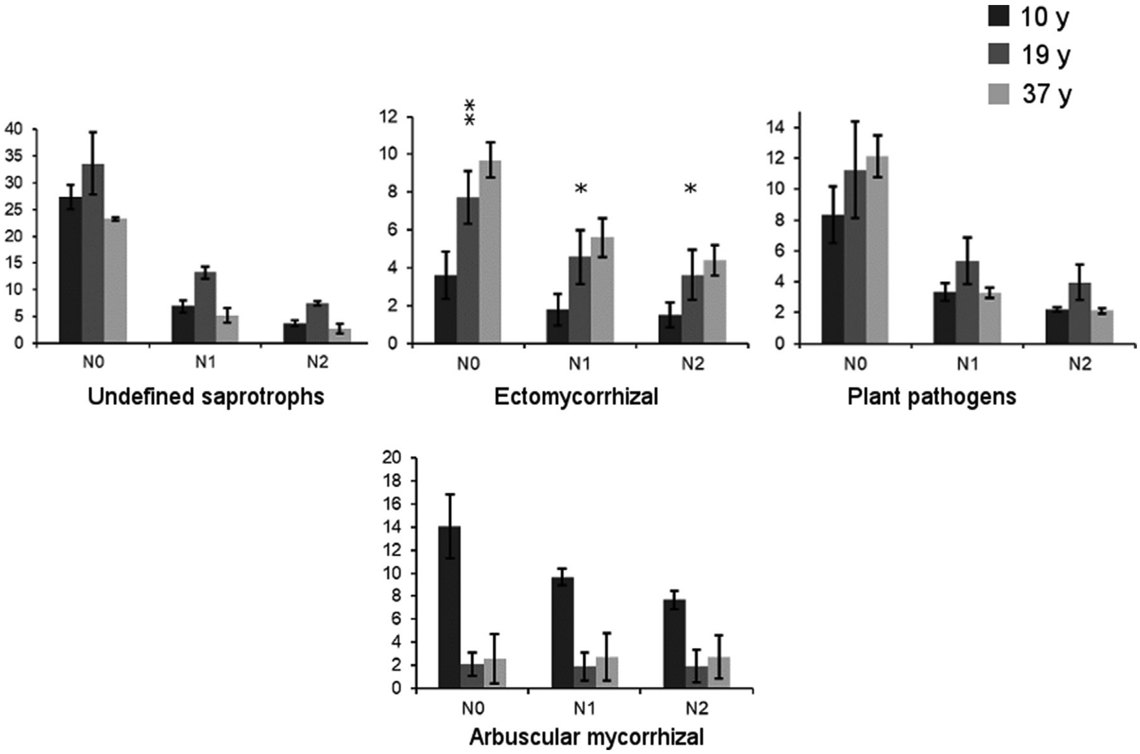

Fig. 4. Changes in interpolated diversity values for each guild, as measured with Hill's numbers, across the three age classes (10, 19 and 37 years old stands) and considering root-associated species and saprotrophs. Hill's diversity consists of three numbers: N0 is species richness; N1 is the antilogarithm of Shannon's diversity index; and N2 is the inverse of Simpson's diversity index. Significance levels: ***P < 0.001, **P < 0.01, *P < 0.05.

diverse at the 10-y old sites (Fig. 4). For undefined saprotrophs, there were no significant changes in alpha diversity nor in abundance across years, although they significantly increased in the most fertile plots (Fig. 4; Table 3). Finally, despite plant pathogens having a high relative abundance in younger stands (Fig. 3; Table 3), the diversity of this group was not affected by tree age nor soil fertility (Fig. 4).

4. Discussion

Our study showed considerable changes in the composition of the soil fungal community and ecological fungal guilds along a chronosequence of *E. grandis* stands. Development over time of *E. grandis* plantations was concurrent with a decrease in soil fertility, especially N and P. Thus, fungal compositional and functional changes across tree ages parallel the changes in soil fertility, suggesting that both drivers (tree aging and soil fertility) may potentially be interrelated. It seems that such changes had distinct effects on the functional community depending on the guild, with both diversity and abundance of ECM species increasing over time, and either abundance and/or diversity of pathogens, saprotrophs or AM fungi decreasing with stand age but increasing with greater soil fertility. A reliable description of the AM taxa found in our study is not possible, and so a comparison between diversity of AM and ECM species cannot be made. However, we believe that between sample comparison of the total relative abundance of AM OTUs is possible.

We observed considerable changes in soil fungal communities across the *E. grandis* chronosequence. Changes in fungal communities across stand ages has been reported previously for soil fungal communities based on DNA analysis (Blaalid et al., 2012; Clemmensen et al., 2015) and on fruit body surveys (Bonet et al., 2004; Dejene et al., 2017a, 2017b). Fungal community shifts along chronosequences have been related to several factors, such as changes in soil chemistry or fertility (Blaalid et al., 2012; Clemmensen et al., 2015), changes in root density (Peay et al., 2010), specific life-history events since tree establishment (Blaalid et al., 2012), or changes in microclimate conditions (Castaño et al., 2018). Our results suggest that observed changes and shifts in community composition of soil fungi may be both related to changes in soil chemistry or fertility and stand age (i.e. increasing tree root cover in soil). The decreasing soil fertility and increasing plant nutrient stress over time could be compensated by an increasing tree dependency on their fungal symbionts (Read and Perez-Moreno, 2003).

Fungal community changes over time in exotic plantations such as the ones considered in the present study may also be related to the distinct colonization strategies of fungi (i.e. first colonization by pioneer species and then replaced by other new species). Pioneer species may be taxa that were already present in the soil as spores or resistant structures (Bruns et al., 2009) or taxa whose spores are efficiently dispersed over long distances by wind (Peay and Bruns, 2014). In this sense, we found that some of the fungal symbionts associated with *E. grandis* trees were well-known cosmopolitan fungal species. For example, well-known ECM genera such as *Tomentella*, *Inocybe* and *Laccaria* were highly represented even in the oldest stands, and they are among the first species found after disturbances. Some of these genera such as *Laccaria* are considered pioneer, opportunistic species (Ishida et al., 2008; Collier and Bidartondo, 2009) and are known to form ECM with several hosts (Roy et al., 2008). Other taxa, such as *Scleroderma albidum* and *Descomyces albellus*, are well-known fungal species associated with *Eucalyptus*, and probably these species were dispersed from nearby plantations or introduced via seedlings. Since studies describing the local fungal community inhabiting Ethiopian forests are scarce, we cannot discard the possibility that novel and not yet described ECM taxa were not detected in our study.

As already reported in other systems, ECM richness and diversity increased with stand age (Wallander et al., 2010). However, in our study the abundance and diversity of ECM species was surprisingly low in 10-y old stands, and dominance of mycorrhizal species was not obvious until trees were already 19-y old. Thus, despite the number of symbionts significantly increasing over time in our study, the overall low diversity of ECM species both in young and older stands suggests the low presence of suitable symbionts at the study site. Similarly, Dejene et al. (2017a) observed that fruit body diversity under *E. grandis* stands in Ethiopia was lower compared with other countries. Surprisingly, lack of symbionts seemed not to limit the development of *E. grandis*, supporting the hypothesis that a low number of available symbionts is sufficient for the growth of *Eucalyptus* plantations (Urcelay et al., 2017). It is also possible that the relatively high N and other soil chemistry values measured at the 10-y old stands allowed trees to develop with reduced symbiont dependence. In any case, the survival of *E. grandis* species under very low abundance and diversity of ECM species together with a lack of low competition support the good adaptation of this species to the environment.

As expected, stand age effects on the soil fungal community contrasted with soil fertility effects. N addition in soils has been observed to negatively affect both mycorrhizal diversity and biomass (Lilleskov et al., 2002; Kjøller et al., 2012; Ekblad et al., 2013). N addition also changed the composition of mycorrhizal species (Lilleskov et al., 2002; Kjøller et al., 2012). In our study, both the diversity and the relative abundance of ECM species increased with tree age, concomitant with a decrease in soil fertility, decrease in soil N and P values and increase in soil C/N ratios. In contrast, some saprotrophs and pathogens increased with increasing fertility. These results support recent findings from similar forest systems, in which increasing abundances of saprotrophic and/or pathogenic fungi were observed under fertilization treatments in *Eucalyptus saligna* plantations (Zheng et al., 2017). Thus, in our study saprotrophs correlated mostly with soil fertility rather than with tree age. A positive response of saprotrophs to higher soil N was also reported in a fruit body study in *Pinus patula* (Dejene et al., 2017b). These results also support the recent findings from Kyaschenko et al. (2017) and Zheng et al. (2017), that soil fertility was positively related to the abundance of fungal saprotrophs, which may grow at the expense of ECM fungi. These interguild relationships are very relevant, because they potentially affect important ecosystem processes such as C storage and nutrient cycling (Averill and Hawkes, 2016; Kyaschenko et al., 2017). Increases in C/N ratios also correlated with increasing ECM abundance, which may also be indicative of ECM species oxidizing organic matter to mine for N (Clemmensen et al., 2013). Phosphorus levels also dramatically decreased with stand age, which could be attributed to the increase of specific ECM taxa. Thus, despite AM species being especially efficient in P uptake, seedlings associated with ECM taxa such as *Scleroderma* or *Laccaria* retained more phosphorus in their roots than plants inoculated with other fungal species (Burgess et al., 1993). However, whether these ECM species were responsible for the dramatic decrease of P and the mechanisms behind this should be further studied.

5. Conclusions

This study is among the first to characterize the soil fungal communities of introduced *E. grandis* plantations in Ethiopia. Our results suggest that aging of *E. grandis* stands cause profound soil fungal community changes, concurrent with changes in soil chemistry. Such changes may potentially slow soil C cycle, promoting soil C storage, but also may potentially deplete soil nutrients. We also show fungal guild-dependent changes that will potentially affect several related ecosystem processes such as nutrient cycling. These findings are important because *E. grandis* plantations represent around half a million hectares only in Ethiopia, but to date no similar studies on the soil functional fungal community has been carried out. New studies should relate potential changes in fungal traits and guilds with changes in soil C budgets, which should be quantified in order to predict how much C is lost or gained under these plantations.

Data accessibility

Sequence data are archived at NCBI's Sequence Read Archive under accession number **PRJNA503133** (www.ncbi.nlm.nih.gov/sra). Fungal community data, environmental data and ecological guilds of fungi can be consulted in Mendeley data, link: https://doi.org/10.17632/tz32h5brhd.1.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funeco.2019.02.003.

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