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Macrofungal diversity in Yangambi Biosphere reserve and Yoko reserve rainforests of the Democratic Republic of the Congo

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Detecting and understanding patterns in the distribution of taxa is a fundamental element in implementing biodiversity conservation. Regarding fungi, understanding how functional diversity varies with forest types helps to define their niche and allows predicting the impact of forest degradation on communities of macrofungi. It also allows better understanding of the way in which they realize their ecological function and nourishment purpose. This study aimed to assess the species richness and functional diversity of macrofungi within rainforests from Yangambi Biosphere reserve and Yoko reserve in the Democratic Republic of the Congo. Results from this study show significant differences in number of macrofungi species between forest stand types (p-value<0.001). Based on all macrofungi functional groups, the most species-rich forest stand was the *Gilbertiodendron dewevrei.* Of the five reported functional groups (saprotrophic fungi, ectomycorrhizal fungi, insect parasitic fungi, plant parasitic fungi and termites' symbiotic fungi), the saprotrophic fungi were the most abundant trophic group represented 210 species of a total of 341 fungal taxa. The results revealed also that woody decaying and terrestrial saprotrophic are mainly characteristic of mixed forests while the occurrence of ectomycorrhizal taxa depends on the presence of ectomycorrhizal trees. In addition, strong relation has been demonstrated between fungal composition and the composition in vascular plants. Each forest stand type is characterized by specific floristic composition which has a corresponding and fairly predictable mycological composition. The study gave evidence that species richness and functional diversity of macrofungi are strongly influenced by vascular plants composition. The various reported functional groups differently intervene in nutrients cycling, and then play key role in forests functioning.

Key words: Species richness, functional diversity, macrofungi, rainforests, DR Congo.

INTRODUCTION

Detecting and understanding patterns in the distribution of taxa is a major topic in ecological studies and a fundamental element in implementing biodiversity conservation (Gaston, 2000; Purvis and Hector, 2000; Tilman, 2000; Schmit 2005; Caiafa et al., 2017; Dvorack et al., 2017). Regarding fungi, the most important aspect of biodiversity assessment is the measures of species richness (Schmit et al., 1999; Hawksworth, 2001). Insight into fungal species richness is vital for biodiversity management, especially when the conservation status

needs to be evaluated (Caiafa et al., 2017). Next to a number of classical qualitative and quantitative traits, functional traits are now also being used for evaluation (Caiafa et al., 2017). Understanding how functional diversity varies with forest types helps to define their niche and allows predicting the impact of forest degradation on communities of macrofungi (Caiafa et al., 2017).

Several studies (Hawksworth, 1991; Hawksworth, 1997; Hawksworth, 2001; Mueller and Schmitz, 2007; Schmitz and Mueller, 2007; Caiafa et al; 2017; Dvorack et al., 2017) have reported that species richness and composition of fungal communities are strongly related to the diversity and composition of the vegetation. As fungi developed different strategies to interact with plants and their habitats, they have been classified into three major functional groups (Read and Perez-Moreno, 2003; Lindahl and Borberg, 2008; Lonsdale et al., 2008): Saprotrophic fungi that grow on scraps on organic matter and decompose litter and dead trees or branch, mutualistic fungi that develop mutualistic relationship with host plants or other living organisms and parasitic fungi which grow in or inside other living organisms and lives totally depending on them (Read and Perez-Moreno, 2003; Lindahl and Borberg, 2008; Lonsdale et al., 2008; Tedersoo et al., 2010; Härkönen et al., 2015; Piepenbring, 2015).

However, some fungal species are selective with numerous plants trees, creating habitat filtering on their occurrence (Härkönen et al., 2015; Piepenbring, 2015). Functional trait-based approaches allow determining the relative importance of habitat filters and fungal species ability in fungal community assembly (Aguilar-Trigueros et al., 2015). Regarding this, specific groups of fungi may colonize particular groups of vascular plants or may occur on particular substratum (Munguia et al., 2005; Gómez-Hernández and Williams-Linera, 2011; Härkönen et al., 2015; Piepenbring, 2015). In addition, different fungal species develop in association with wide range of host plant or on various substratums (Lodge et al., 2004; Piepenbring, 2015). Hence, substratum reflects the strategy of macrofungi nourishment and therefore indicates the way in which fungi intervene in nutrient cycling.

Since fungal diversity is affected by host plant diversity (Härkönen et al., 2015; Piepenbring, 2015), rainforests from the Democratic Republic of the Congo are expected to host high numbers of fungal species. Although fungi are omnipresent and highly diverse within rainforests from the Democratic Republic of the Congo, little attention has been given to the assessment of fungal species richness. This paper is the first attempt to assess the species richness and functional diversity of macrofungi within various types of rainforests found in the Yangambi biosphere reserve and the Yoko forest reserve. The aim of this study was to investigate patterns of distribution of macrofungi species and their functional diversity associated with different forest stand types.

MATERIAL AND METHODS

Study area

The two study sites (The Yangambi Biosphere reserve and the Yoko reserve) are located in the Tshopo province of the Democratic Republic of the Congo. Extended on the two sides of equator, the province of Tshopo is located in the central Congo basin, between -2° of south latitude and +2° of north latitude, and from 22° up to 28° Eastern longitude (Lejoly et al., 2010). As reported by Lejoly et al. (2010), the Yangambi Biosphere reserve is located within the Congo River Basin west, laying around 90 km west of Kisangani (Isangi territory) while the Yoko site is located in the Ubundu territory 32 km south-East of Kisangani in the Democratic Republic of the Congo (Figure 1). The biosphere reserve is home to a widespread range of virgin tropical rainforests hosting about 32,000 tree species and with about 2200 km² of surface (https://www.protectedplanet.net/yangambi-biosphere-reserve).

As part of the equatorial region, the Yangambi Biosphere reserve is characterized by a rainy and hot climate (Lejoly et al., 2010). The climate is characterized by monthly average temperature between 22.4 and 29.3°C, and annual average surrounding 25°C. The annual rainfall ranges from 1600 to 2200 mm with an average surrounding of 1828 mm (Mohymont and Demarée, 2006). Rainfall is irregular throughout the year. The average year has a long rainy season interrupted by two small drier seasons from December till January and from Jun till August (Mohymont and Demarée, 2006).

Fungal sampling and identification

The mycological inventories were carried out in November of the year 2012 and 2013, and during the main rainy season (between March and May) of the years 2015 and 2016. Fungal sampling was performed every two weeks within different types of forests (forests dominated by *Gilbertiodendron dewevrei, Brachystegia laurentii, Julbernardia serretii, Uapaca heudelotii* and *Uapaca guineensis*, and mixed forests) and along the main forestry roads trails and transects (Hueck, 1951; Lodge et al. 2004). Plots (100 x100 m) divided in 20 x 20 grid were demarcated in each forest type, except the *Uapaca heudelotii* in which plots were less than 100 x 100 m, that is 25 m x 50 m. The locations of all sampling plots were recorded with a Global Position System (GPS) device using the WGS-84 geographical coordinate system.

In each plot, the above ground encountered fungi fruiting bodies of Basidiomycetes and Ascomycetes have been surveyed and harvested (Arnolds, 1981). The fresh collected fruiting bodies were dried and voucher specimens were deposited at the Botanic Garden Meise (Belgium). Species identification was based on macro and micromorphological features referring to the description found in several documents (Heinemann, 1954; Heim, 1955;

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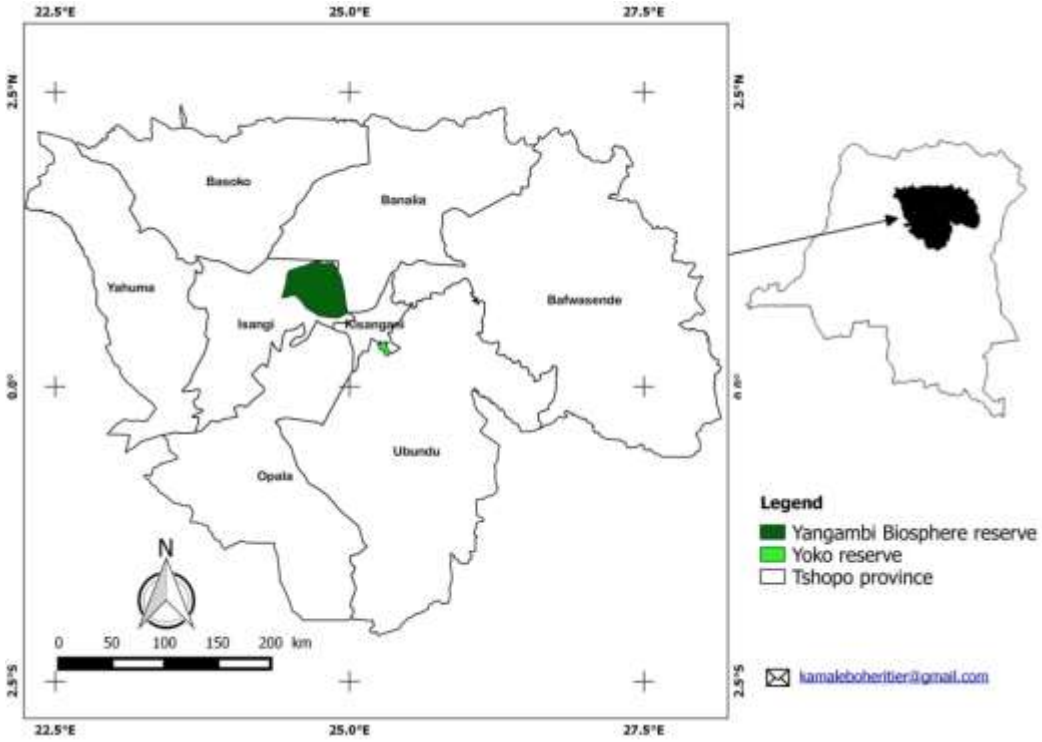

Figure 1. Study site location.

Pegler, 1977; Heinemann and Rammeloo, 1983; Heinemann and Rammeloo, 1987, 1989; Buyck, 1993; Buyck, 1994a, 1997; De Kesel et al., 2002; Verbeken and Walleyn, 2010, Eyi et al., 2011). Species names and author's abbreviations were annotated using the Index Fungorum site (http://www.indexfungorum.org/Names/Names.aspp). All unidentified species but identified to the genus level were noted 'sp.' Before confirming identifications, doubtful records were indicated by the abbreviation 'cf.' or 'aff.' before the epithet. Unidentified taxa (no genus, no species name) were excluded from the analysis.

Analysis of fungal diversity

Species richness was calculated as the number of fungal species collected from each type of forest (Hueck, 1951; Baptista et al., 2010; Caiafa et al., 2017). Based on their mode of nutrition and referring to the studies of Caiafa et al. (2017), all recorded species were classified into trophic groups. Three main trophic groups were defined: saprotrophic fungi (including woody-decaying), parasitic fungi and mutualistic fungi (Read and Perez-Moreno, 2003; Lindahl and Borberg, 2008; Lonsdale et al., 2008; Tedersoo et al., 2010). Saprotrophs are fungi which grow on scraps on organic matter, on dead organisms or on non-living part of a living organism such animal dung, dead straw, decomposed litter and dead trees or branch. The parasitic fungi constitute a group of fungi which grow either on living plants, animals or on other living fungi and lives totally depending on its host organisms. Furthermore, the mutualistic fungi include the mycorrhizal fungi associated with vascular plants and fungi living in mutualism with termites (Read and Perez-Moreno, 2003; Lindahl and Borberg, 2008; Lonsdale et al., 2008; Tedersoo et al., 2010).

The diversity of macrofungi was determined using the Shannon (H) index (Fisher et al., 1943). Hierarchical clustering and Correspondence Analysis (CA) among forest types were performed with the packages FactoMineR found in R software (Cornillon et al., 2012). The Chi-squared test (α=5%) permitted to assess the difference between the number of fungi species between different forest types and fungi families.

RESULTS

A total of 341 taxa of macromycetes were recorded in the seven different forest stand types from rainforest of Tshopo. Of all 341 recorded fungi, 193 were determined to species level and 148 to the genus level. The most species-rich group of macromycetes was the class of *Basidiomycetes* (with 310 species), while the class of *Ascomycetes* was only represented by 31 species. Of all *Basidiomycetes*, the *Marasmiaceae* family was the most diverse (with 65 species) followed by the *Russulaceae* (35 species), *Agaricaceae* (23 species), *Cantharellaceae* (18 species), *Polyporaceae* (18 species), *Amanitaceae* (14 species), *Boletaceae* (14 species), *Tricholomataceae* (14 species) and *Pluteaceae* (8 species) (Figure 2). Likewise, the *Xylariaceae* was the most diverse family of *Ascomycetes* (12 species).

Significant difference was observed in number of macromycetes species between forest stand types (p-value<0.001). In all investigated forest stands, the forest dominated by *Gilbertiodendron dewevrei* was reported the most species-rich (total species number = 166,

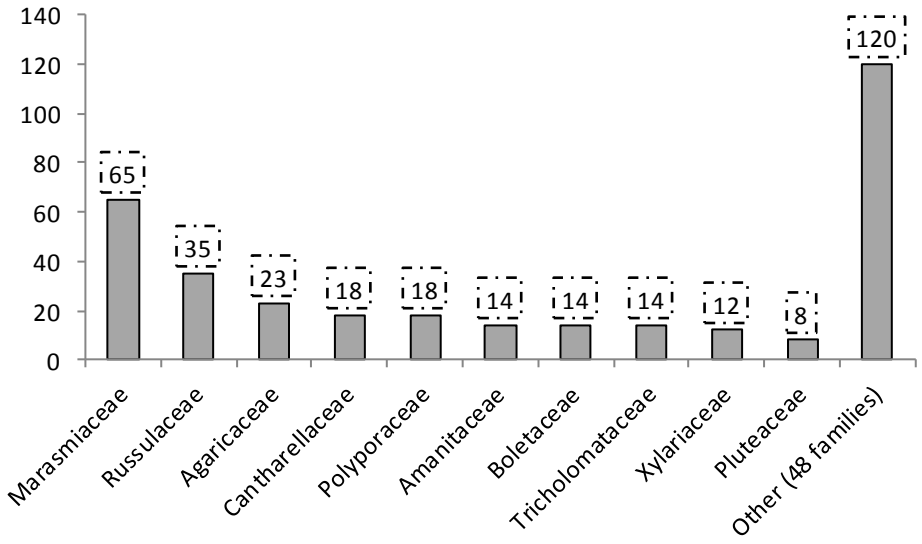

Figure 2. Numbers of macrofungi species in fungal families.

Shannon index value=5,1), followed by the mature mixed forests (total species number=157, Shannon index value=5,1), the disturbed mixed forests (total species number= 101, Shannon index value= 4,6), *Brachystegia laurentii* dominated forest (total species number= 92, Shannon index value= 4,5) and *Julbernardia seretti* dominated forests (total species number= 36, Shannon index value= 3,6). The lowest species richness was observed in *Uapaca heudelotii* dominated forests (with 15 species and 2,7 as value of the Shannon index).

Whereas the numbers of macromycetes significantly differ between forests, several types of forest stands have shown strong variation in the composition of macrofungi (Figure 3). Of all the 166 species of macrofungi recorded in the *Gilbertiodendron dewevrei* dominated forests for example, only an average of 79±3 species was reached per plot. A similar pattern has been observed in the other types of forests. In the mature mixed forests; an average of 54±17 species were reported per plot for a total of 157 species, 60±24 species in *Brachystegia laurentii* dominated forest (with a total of 92 species), 38±19 species in disturbed forests (total species number=101) and 19±3 species in *Julbernardia seretti* dominated forests (total species number=36). The lowest mean number of macromycetes species was reached by the *Uapaca guineensis* dominated forest (8±2 species per plot for a total number of 16 species).

Likewise, significant difference on fungal species numbers was observed in fungal functional groups of each forest stand type (Figure 4). In all forest stand types, the saprotrophic fungi (wood decaying and terrestrial saprotrophic fungi) were the most abundant trophic group represented by 210 species. The following large trophic group was the ectomycorrhizal fungi (93 species), followed by the plant parasitic fungi (7 species) and the insect parasitic fungi (5 species). The lowest species-rich trophic group was the termites' symbiotic fungi represented by only 4 species. While the highest numbers of wood decaying and terrestrial saprotrophic fungi were found in both mature mixed and disturbed forests, the ectomycorrhizal species were most diverse and abundant in the EcM dominated forests.

Referring to Figure 4, it appears clearly that wood decaying and terrestrial saprotrophic fungi are present in all forest types. Nevertheless, the correspondence analysis (Figure 5) revealed that woody decaying and terrestrial saprotrophic fungi mainly characterize the mature mixed forests and the disturbed mixed forests dominated by many dead woody and fallen trunks to be decomposed. Plant parasitic fungi also occur mostly in mature forests characterized by several fragile old trees. Furthermore, the ordination analysis confirmed the main evidence that ectomycorrhizal fungi are characteristics of ectomycorrhizal trees dominated forests. Figure 5 revealed also that the *Brachystegia laurentii* tree had a prominent influence on the occurrence of insect parasitic fungi. Definitely, strong relationship was observed between fungal trophic groups and vascular plants distribution.

The composition of macromycetes' species is highly correlated with the composition of vascular plants. The hierarchical clustering (Figure 6) revealed that each forest stand type, characterized by a specific floristic composition, has a corresponding and fairly predictable mycological composition. Based on the composition of vascular plants species, all plots of *Gilbertiodendron dewevrei* dominated forests clustered together, while plots of other type of forest stand formed separated groups. Mature mixed stands formed a separate cluster as all plots of disturbed forests highly clustered together

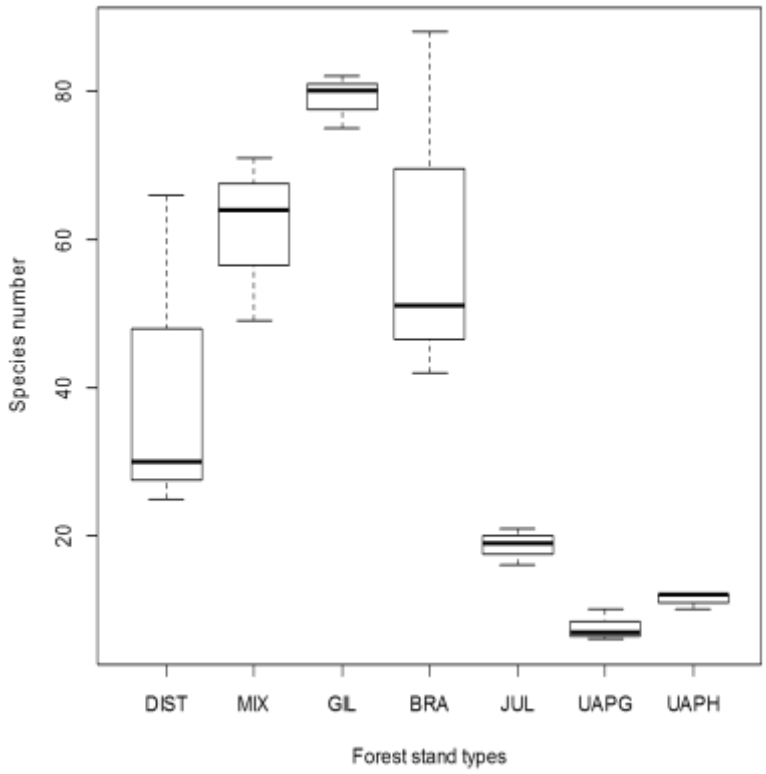

Figure 3. Number of species of macrofungi within studied plots. DIST=Disturbed or degraded mixed forest, MIX= Mature mixed forest; GIL= *Gilbertiodendron dewevrei* dominated forest; BRA= *Brachystegia laurentii* dominated forest; JUL= *Julbernardia seretii* dominated forest; UAPG= *Uapaca guineensis* dominated forest; UAPH= *Uapaca heudelotii* dominated forest.

as a whole. A similar pattern was also observed in separate ordinations of plots based on the composition in macrofungi. All plots with same pattern in the composition of vascular plants, present almost same predictable fungal composition. *G. dewevrei* dominated forests plots formed a distinct group which corresponded to the same cluster based on vascular plants composition. Similar pattern was observed in clustering plots of mature mixed forests and disturbed forests based on both floristic and mycological composition.

DISCUSSION

Results from this study showed significant difference of species richness and functional diversity of macrofungi within forest stand types. Species richness and functional diversity are strongly influenced by the occurrence of some particular vascular plants. As reported by several other studies (Hawksworth, 1991; Hawksworth, 1997; Hawksworth, 2001; Mueller and Schmitz, 2007; Schmitz and Mueller, 2007; Caiafa et al., 2017; Dvorack et al., 2017), the species richness and mycocoenose are affected by the diversity and composition in host plants trees. Munguia et al. (2005) and Gómez-Hernández and Williams-Linera (2011) have also reported that some species of fungi occur on particular substratum or grow in particular type of forest as several other fungal species develop in association with wide range of host plant or colonize diverse types of substrates (Lodge et al., 2004).

The high fungal species richness in *Gilbertiodendron dewevrei* dominated forests suggests that fungal host plant trees in this forest stand have developed mutualistic relationship with wide range of fungal species. In addition, habitats and local environment in the *G. dewevrei* dominated forests are expected to be favorable for the development of numerous species of macrofungi. Several studies (White, 1983; Eyi et al., 2011) have reported that the *G. dewevrei* is the most important ectomycorrhizal tree which develops wide mycorrhizal symbiosis with numerous species of EcM fungi from rainforests of the central Africa. The abundant aboveground litter found in forests dominated by *G. dewevrei* (Bartholomew et al., 1953; White, 1983) should

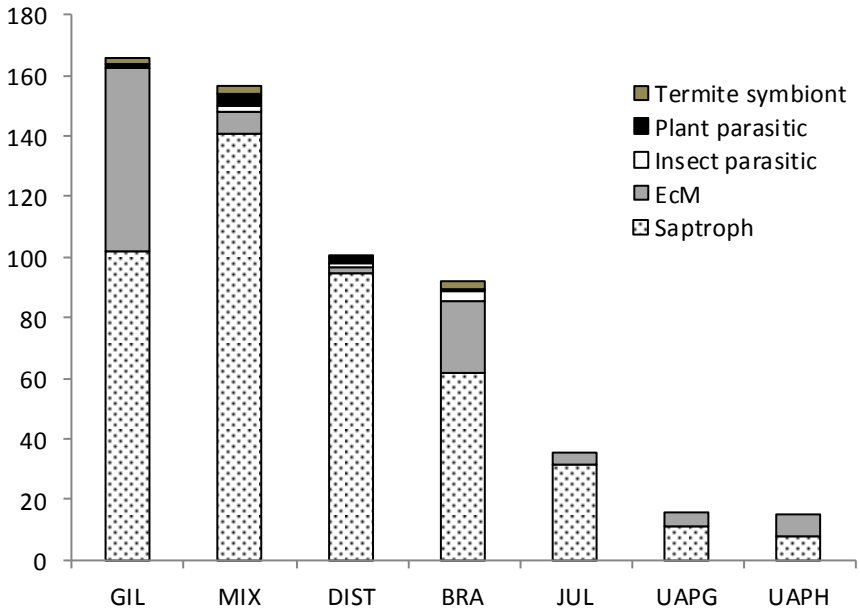

Figure 4. Frequency of fungal trophic groups within forest stands (EcM= Ectomycorrhizal).

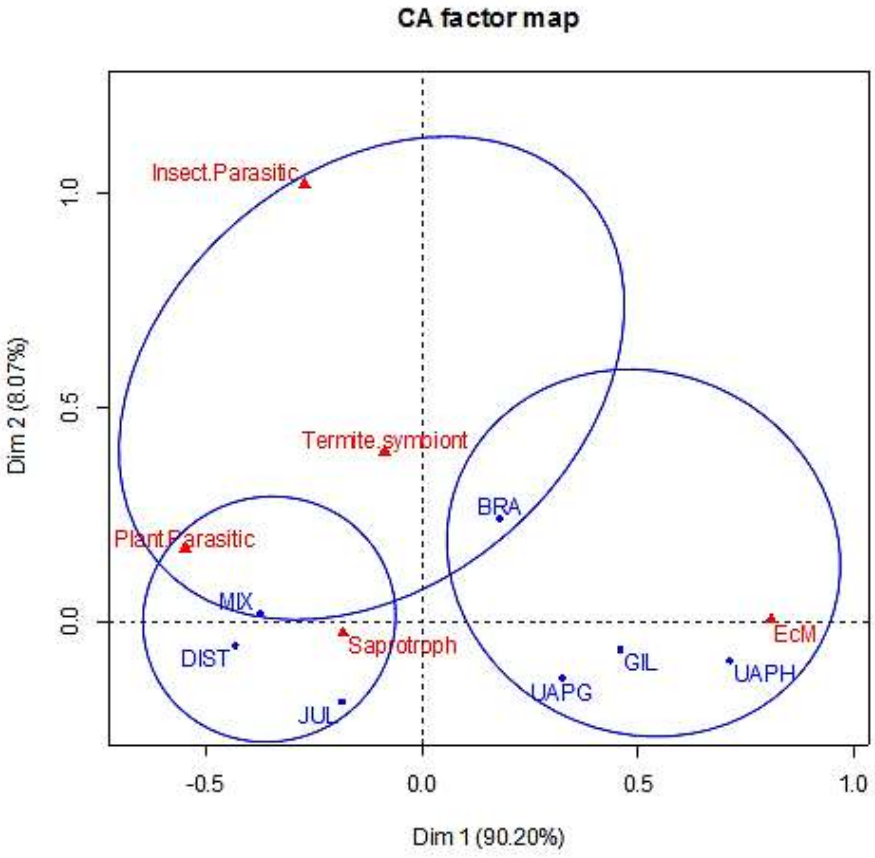

Figure 5. First two axes of the correspondence analysis (CA) showing the ordination of fungal trophic groups (red abbreviated names) within forest stand types (blue abbreviated names). The two axes explain 98.27% of ordination (90.20 % for the first axis and 8.07% for the second axis).

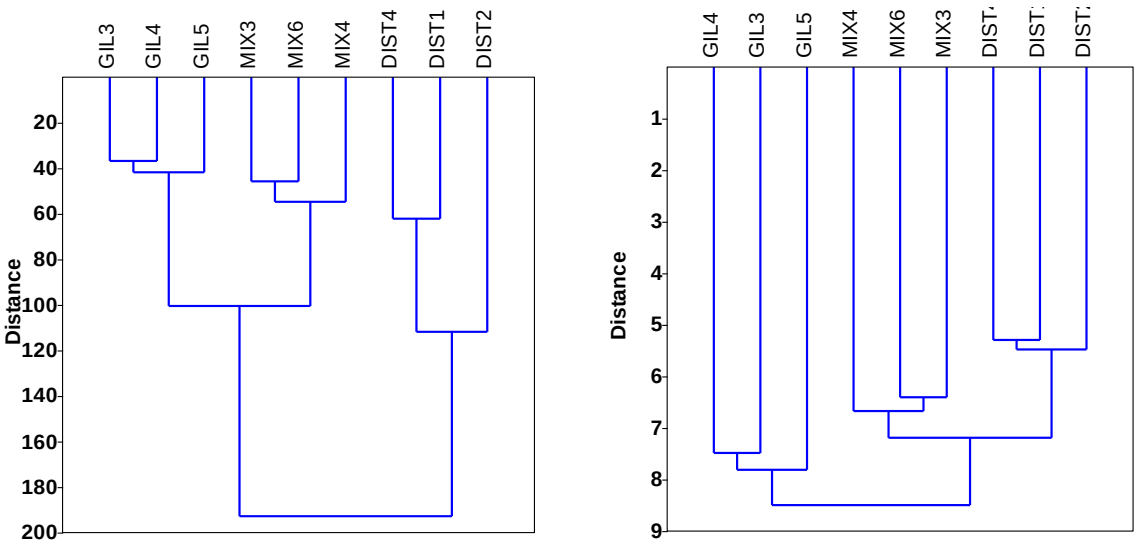

Figure 6. Hierarchical clustering of plots based on fungal composition (on the right side) and the composition in vascular plants (on the left side) of three selected forest stand. GIL= *Gilbertiodendron dewevrei* dominated forest, MIX= Mature mixed forest, DIST= Disturbed young mixed forest.

be normally a favorable habitat for numerous species of saprotrophic fungi (Mayor and Henkel, 2005). However, despite the presence of abundant dried aboveground biomass, high numbers of EcM fungi have been reported than saprotrophic fungi.

The high species richness of ectomycorrhizal fungi compared to saprotrophic fungi reported in the *G. dewevrei* dominated forests can be explain by the fact that the mycelia of some ectomycorrhizal fungi have capacity to inhibit the development of numerous saprotrophic fungi (Gadgil and Gadgil, 1971, 1975; Leake et al., 2002). This presumption is also supported by Torti et al. (2001) who reported that *G. dewevrei* dominated forest is characterized by deep leaf litter with slower decomposition. The slower decomposition of litter is however caused by the alteration of the understory environment by the plant tree physiology (Torti et al., 2001). In addition, the competition for nutrients that occurs between ectomycorrhizal fungi and saprotrophic fungi may retard the decomposition of litter by limiting the activities of the saprotrophs (Gadgil and Gadgil, 1971, 1975; Mayor and Henkel, 2005; Koide and Wu, 2003). However, by inhibiting the development of saprotrophic fungi and because of their capacity to decompose some forms of litter, apart from their role in nutrients uptake, some ectomycorrhizal fungi may supplement the decomposition capacities of saprotrophic fungi (Koide and Wu, 2003). Yet, the *Marasmiaceae* remains the most important and diverse family of litter saprotrophic fungi involved in the decomposition of the aboveground twigs and litter inside this forests.

The analysis of functional diversity allowed also to understand the potential contribution of macromycetes to ecosystem functioning. The insect parasitic fungi for example are involved in the decomposition and cycling of mineral nutrients from insects and further invertebrates. However, the ecological relationship developed between *Brachystegia laurentii* tree and numerous species insects (Okangola, 2007; Payne et al., 2016) may explain the high occurrence of insect parasitic fungi in this forest stand type. According to these authors, the *B. laurenti* tree constitutes unique favorable habitat and nest for numerous species of insects, especially for numerous larva of *Lepidoptera*. Furthermore, wood-decaying and saprotrophic fungal taxa were abundant in secondary and degraded forests as well as in old dense forests. This observation is in line with several other reports (Eyi et al., 2011; Balezi, 2013) who found that the wood-decaying fungi mostly develop on old and fragile plant trees in mature forests, and on dead wood in degraded forests (Balezi, 2013) while litter saprotrophs mainly occur on the aboveground leaf litter and twigs (Eyi et al., 2011).

The abundance of ectomycorrhizal fungi was also observed in *B. laurentii* dominated forest as in several other ectomycorrhizal trees dominated forests. This observation confirms the main presumption that ectomycorrhizal fungi are characteristics of forests dominated by ectomycorrhizal trees. In addition, the forests dominated by *Uapaca* spp. were reported the lowest species-rich. The lowest species richness in *Uapaca* spp. forests may have been caused by the fact these forests develop on flooded or swamp soil (Beernaert, 1999) which has to inhibit the development of several fungal species. The lowest species richness in *Uapaca heudelotii* can also be explained by the lower sampling intensity in less than 1 ha plots due to the limited distribution of this type of forest. Several studies (White, 1983; Beernaert, 1999; Lejoly et al., 2010) have

reported that species of *Uapaca* develop some limited groves of monodominant dense forest often on soft and spongy soil.

At the plot scale, the *G. dewevrei* dominated forests showed higher pool of species variation. This observation suggests that even small, variation of biotic and abiotic parameters at fine spatial scale has influence on the occurrence of macromycetes. Observed differences in species richness and fungal functional diversity between forest stand indicate that the communities of macromycetes present various ecological niches and a wider variation of their functional traits (Aguilar-Trigueros et al., 2015).

Conclusion

The findings of this study give evidence that species richness and functional diversity of macrofungi are strongly influenced by vascular plants composition. Woody-decaying fungi mostly develop in mature mixed forests to decompose organic matter from fragile old trees and dead wood. Likewise, ectomycorrhizal dominated forests are in particular home for wide range of ectomycorrhizal fungi that inhibit the development of numerous saprotrophic fungi. The results of this study have clearly shown that the different functional groups of fungi play key role in the functioning of natural forests, especially in nutrient cycling. However, their effectiveness depends on the particular functional trait and specific abilities developed by each group. Strategies of fungi nourishment can be also related to some abilities developed in response to various abiotic and biotic factors. Since species of macrofungi play key role in organic matter cycling, describing deeply traits related to their specific performance and abilities is of great importance to understanding the way in which they realize their ecological function and nourishment purpose. Therefore, the establishment of links between species of macrofungi and their functional diversity and traits is of great importance. Hence, wider range of functional traits should be incorporated in future studies to identify the whole range of strategies used by species of macrofungi to maintain ecosystem functioning.

CONFLICT OF INTEREST

The authors declare that they have no competing interests

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