Highlighting the diversity of the rhizosphere mycobiome of five native West African trees

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Soil microbial communities play a vital role in ecosystem functioning by enhancing mineral nutrition and protecting forest trees against pathogens through mycorrhizal symbiosis. However, knowledge of the diversity and assemblage of belowground fungal communities associated with native host trees in tropical Africa is incomplete. Using high-throughput sequencing, this study examined soil fungal communities in the rhizosphere of five ectomycorrhizal trees (EcM) from (5) countries using ITS and LSU regions. Unconstrained ordination of fungal species was performed using principal component analysis based on their EcM tree rhizosphere affiliation. The ANOSIM test assessed the similarity between the fungal community composition associated with the EcM trees. Overall, 90 species belonging to 84 genera, 71 families, 40 orders and 4 phyla were identified. Soil fungal communities were host specific (P = 0.001). Basidiomycota were more frequently observed in the rhizosphere of Fabaceae, except for I. doka, whereas Ascomycota are more abundant in the rhizosphere of Phyllanthaceae (U. togoensis) and Dipterocarpaceae (M. kerstingii). The genus Sebacina is predominantly linked to M. kerstingii and I. tomentosa, while Russula is dominant under B. grandiflora and, Inocybe with I. tomentosa. This study provides new insights into the rhizosphere of native forest trees in West Africa and highlights areas for future research.

Key words: DNA metabarcoding, ectomycorrhizal association, molecular species, Soil microorganisms, soil fungi, timber trees.

INTRODUCTION

The rhizosphere is considered to be the narrow zone of soil immediately surrounding plant roots (Marschner et al., 2004; Olahan et al., 2016). This area is home to a wide range of interactions between plant roots and microorganisms, which affect soil physical, chemical, and biological processes that sustain biodiversity and ecosystems (Nihorimbere et al., 2011; Sathya et al., 2016; Lu et al., 2018). A major group of microorganisms found in the rhizosphere are fungi, responsible in part for colonizing the roots of a plethora of plant species.
have relied primarily on fruiting bodies surveys, mycelia isolations, and spore identification (Straatsma et al., 2001; Luo et al., 2020). Fruit bodies-based surveys do not allow a total evaluation of the fungal community (Kubartová et al., 2012; Shirouzu et al., 2016), because even if a fungus has basidiomata large enough to be spotted, they may go unnoticed because fruiting body formation is both seasonal and ephemeral (Shirouzu et al., 2016). Many taxa such as mycorrhizal and parasitic fungi may not grow or produce reproductive structures on artificial media even if they are potentially culturable (Allen et al., 2003; Senanayake et al., 2020). In addition to the aforementioned methods, spore identification is traditionally used to identify the rhizosphere arbuscular mycorrhizal fungi (Rodríguez-Morelos et al., 2014; Xavier and Rodrigues, 2020). However, even though this method is important in fungal taxonomy, it is time- and energy-consuming and susceptible to variability in spore morphology description, because host species and microbial age may be very challenging to differentiate spores of similar species (Bhat et al., 2014; Senanayake et al., 2020). Recent studies using high-throughput sequencing of environmental samples have greatly improved our understanding of the community and diversity of rhizosphere soil fungi (Tedersoo et al., 2014; Qin, 2018; Zhu et al., 2018; Nilsson et al., 2019; Tremblay et al., 2019; Meidl et al., 2021).

One of the most accepted methods for high throughput sequencing is the generation of the amplicon sequence variants (ASVs). So far, this method has been mainly used to study soil mycobiome in temperate and boreal regions (Wu et al., 2019; Lance et al., 2020; Rosas-Medina et al., 2020), while very few studies have comprehensively assessed the diversity, and community composition of soil fungi in tropical African forest ecosystems (Meidl et al., 2021). Here, PacBio sequencing was employed to assess the diversity and community composition of fungi found in the rhizosphere of five West African native trees.

MATERIALS AND METHODS

Study area

The soil samples used in this study were collected across five West African countries namely Benin, Burkina Faso, Guinea, Côte d’Ivoire, and Mali. In total, nine forests containing at least one of the targeted ECM tree species were selected. The different forests range from woodlands to gallery forests: The gallery forests and the woodland of Kota in Benin, the Kou gallery forest and the Niangoloko forest reserve in Burkina-Faso, the Farako1 forest reserve and the Farako15 forest reserve in Mali, the Bissandougou forest reserve and Moussaya forest reserves in Guinée and the Kouadianikro gallery forest in Côte d’Ivoire (Figure 1).

Sampling design and methods

Within each study site, we established a plot of 50 m × 50 m (2500
m²) in woodlands and a rectangular plot of 30 m × 80 m (2,400 m²) within gallery forests due to their shape. Within each plot, five EcM trees were targeted, namely *I. doka*, *I. tomentosa*, *U. togoensis*, *M. kerstingii*, and *B. grandiflora*. Ten trees were chosen in proportion to their abundance, while ensuring that each of the EcM trees in the plot is represented at least once and that all sampled trees were at least eight meters apart. Under each targeted tree, two soil samples of about 200 g around 1 m was taken from each side of the trunk using a small shovel to collect the first 15 cm of soil. The two soil samples were pooled in a plastic bag. A total of 90 (5 EcM trees × 2 samples × 9 sites) soil samples were collected at a rate of 10 samples per site. Later on, the collected soil samples were processed following the protocol described by Tedersoo et al. (2014).

**DNA extraction, sequencing and bioinformatics analyses**

For the DNA extraction and sequencing, soil samples were sent to the Department of Ecology and Genetics, Evolutionary Biology, Uppsala University. A subsample of approximately 250 mg was placed in separate 2.0 ml tube containing 750 µl of field lysis and preservation buffer (Xpedition Soil/Fecal DNA miniprep, Zymo Research Corporation, Irvine, California, USA) and lysed in the field using a portable bead beater (TeraLyser, Zymo Research Corporation).

Extraction, amplification, sequencing, and clustering of sequences into amplicon sequence variants (ASVs) were performed as described by Meidl et al. (2021). For more details, see the methodology of Meidl et al. (2021). The taxonomic attribution of the different ASVs was carried out on the PlutoF platform (Kõljalg et al., 2019) using the PROTAX software (Somervuo et al., 2016) (publication date 2020-10-21), configured by the Index Fungorum taxonomic database and the UNITE reference sequence database (Nilsson et al., 2016). We recorded for each query ASV the most likely taxonomic identity at the phylum, class, order, family, genus, and species levels, as well as the uncertainty of these assignments, measured by probabilistic placement. The authors note that the PROTAX uncertainty estimates explain the possibility that the species is unknown to science (that is, not included in the taxonomic database), or known to science but lacking sequences reference (Somervuo et al., 2016; Abarenkov et al., 2018).

**Data processing and analysis**

To illustrate the fungal taxonomic composition associated with the rhizosphere of the target EcM trees, we constructed a Krona wheel for each tree using Protax-fungi in PlutoF platform from ASV diversity. Alpha diversity was determined for each EcM tree by calculating species richness and the Shannon diversity index. The similarity analysis (ANOSIM) was used to assess the similarity between the fungal communities associated with EcM trees. Through principal component analysis, we highlight fungal species affiliation with each EcM tree, and to identify the potential fungal species which better characterize each EcM tree. Finally, the Jaccard similarity index was calculated to compare the proportion of species shared by different EcM trees. All these analyses were carried out using the vegan package (Oksanen et al. 2022) with the statistical software R version 3.6.2 (R Core Team, 2019) and the ggplot2 package (Wickham, 2016) was used to create the nMDS graph.
Figure 2. Krona-Wheels illustrating the taxonomic distribution of fungi in soil samples. Results of samples associated with the rhizosphere of *Berlinia grandiflora* (A) and *Isoberlina doka* (B).
Source: Authors

RESULTS

Taxonomic composition of fungal communities associated with the rhizosphere of targeted EcM trees

Grouping the sequences into amplicon sequence variants (ASVs) gave a total of 1147 ASVs. In sum, 1051 ASV (91.63%) were identified as fungi. On the Krona wheels (Figures 2 to 4, Supplementary materials A, B, C, D and E for more detail), the color scales show the type and confidence level of each taxonomic placement. Color scales 1 to 3 correspond to the identified taxonomic units for which the proportion of reliable identifications ranges from 50...100% (1), 0...50% (2) or 0 % Color 3. Scales 4 to 6 correspond to unknown taxonomic units. In total, four taxonomic groups of fungi such as Basidiomycota, Ascomycota, Glomeromycota, Zygomyctota were identified from the rhizosphere of the targeted...
EcM trees. These latter are unevenly distributed for each EcM tree. For example, Basidiomycota are most dominant under B. grandiflora (42%) and I. tomentosa (49%); while Ascomycota are the most dominant under I. doka (50%), M. kerstingii (56%), and U. togoensis (50%). Glomeromyxota and Zygomycota are weakly represented under the target EcM trees. Sixteen percent of the sequences associated with B. grandiflora and I. doka are unidentified or unknown. Fourteen percent of the fungi sampled under M. kerstingii and U. togoensis are unidentified, whilst unknown taxa make up to 13% of total fungal community under I. tomentosa.

In general, Russulales is the most dominant fungal group under B. grandiflora, I. doka, U. togoensis, and M. kerstingii, while Agaricales is more abundant in the rhizosphere of I. tomentosa (Figure 5). Sebacinales are more represented under M. kerstingii than the other trees investigated in contrast to Boletales, which are

Figure 3. Krona-Wheels illustrating the taxonomic distribution of fungi in soil samples. Results of samples associated with the rhizosphere of Isoberlina tomentosa (C) and Monotes kerstingii (D).
Source: Authors
more represented under *B. grandifolia*, *I. doka* and *I. tomentosa* have the highest proportion of Pezizales. Cantharellales, an important group of edible fungi in tropical Africa, is best represented under *I. tomentosa*.

**Genera representativeness under the different forest species**

A total of 1051 ASV, including 810 (77.07%) belonging to 90 species from 84 genera, 71 families, 40 orders, 19 classes, and 04 phyla have been recorded. Moreover, 66.67% of this specific richness is observed under *B. grandiflora* (60 species), against 62.22% for *I. doka* (56 species), 53.33% for *I. tomentosa* (48 species), 48.89% for *U. togoensis* (44 species), and 47.78% for *M. kerstingii* (43 species). The real diversity is probably much higher because about 60% of the genera (50 genera for all EcM trees combined) could not be identified up to species level. About 22.93% (241) of the ASV remained unidentified and were not included in this analysis. *Russula* is better represented under *B. grandiflora*, *I. doka*, *U. togoensis*, and *M. kerstingii* unlike *Inocybe* that is much more observed under *I. tomentosa* (Figure 6).

**Diversity of belowground fungal communities of five EcM trees**

Table 1 presents the intraspecific diversity of the belowground fungal communities of the different tree species in the EcM. At the genus level, the belowground fungal communities were found to be the most diverse for *Isoberlinia doka* ($G = 63$, $H' = 2.81$, $J = 0.679$) and the least diverse for *Monotes kerstingii* ($G = 54$, $H' = 1.78$, $J = 0.447$). On the other hand, fungal generic diversity affiliated with *Uapaca togoensis* ($G = 53$, $H' = 2.39$, $J = \ldots$)
Figure 5. Representativeness of fungal taxa under target forest species.
Source: Authors
Figure 6. Distribution of the best-represented genera within the different EcM tree species. Source: Authors

Table 1. Genus level intraspecific diversity of belowground fungal community of ectomycorrhizal host trees.

<table>
<thead>
<tr>
<th>Forest trees</th>
<th>Richness</th>
<th>Shannon</th>
<th>Evenness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoberlinia doka</td>
<td>63</td>
<td>2.81</td>
<td>0.679</td>
</tr>
<tr>
<td>Isoberlinia tomentosa</td>
<td>62</td>
<td>2.48</td>
<td>0.6</td>
</tr>
<tr>
<td>Uapaca togoensis</td>
<td>53</td>
<td>2.39</td>
<td>0.603</td>
</tr>
<tr>
<td>Berlinia grandiflora</td>
<td>67</td>
<td>2.24</td>
<td>0.533</td>
</tr>
<tr>
<td>Monotes kerstingii</td>
<td>54</td>
<td>1.78</td>
<td>0.447</td>
</tr>
</tbody>
</table>

Source: Authors

Table 2. Similarity index of Jaccard among the forest trees.

<table>
<thead>
<tr>
<th>Species</th>
<th>I. doka</th>
<th>I. tomentosa</th>
<th>M. kerstingii</th>
<th>B. grandiflora</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. tomentosa</td>
<td>0.831</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. kerstingii</td>
<td>0.692</td>
<td>0.692</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. grandiflora</td>
<td>0.658</td>
<td>0.725</td>
<td>0.725</td>
<td></td>
</tr>
<tr>
<td>U. togoensis</td>
<td>0.635</td>
<td>0.676</td>
<td>0.700</td>
<td>0.800</td>
</tr>
</tbody>
</table>

Source: Authors

0.603) was approximately equal to that of *Isoberlinia tomentosa* (G = 62, $H' = 2.48$, J = 0.6).

Considering pairwise EcM trees, Jaccard's similarity index (Table 2) indicated generally large proportions of shared fungal genera. Indeed, similarity (0.635) was obtained between *I. doka* and *U. togoensis*; but *I. doka* and *I. tomentosa* shared the largest number of taxa (Jaccard index = 0.831). Although the proportion of genera shared was greater than 0.6 in all pairwise cases, the similarity analysis (ANOSIM) supported the evidence that at the genus level, the belowground fungal community associated with the rhizosphere of at least one of the five EcM trees differed significantly from the others ($P < 0.05$, Figure 7).

**Categorization of below-ground fungal species according to EcM hosts**

Figure 8 presents the projection of fungal genera
generated for each EcM tree according to the principal components 1 and 2. Figure 8 suggests that EcM trees hardly cluster separately and share a large number of fungal genera as the similarity index of Jaccard indicated it. This makes it difficult to clearly identify the genera that characterized the fungal community of each tree. Nevertheless, through the projection of the circles, the genus Russula seems to cluster more with B. grandiflora; while Sebacina seems more associated with M. kerstingii and I. tomentosa; and the genus Inocybe clusters more with I. tomentosa.

**DISCUSSION**

To assess the diversity and community composition of fungi found in the rhizosphere of five West African native trees, high throughput sequencing was employed. Out of 1051 ASVs generated, a significant percentage of 22.93% remained unidentified. This could potentially be explained by the incompleteness of the reference databases or taxonomic placement (Somervuo et al., 2016; Abarenkov et al., 2018). Secondly, the high percentage of unknown taxa suggests that a large proportion of taxa remain to be described. In a global study on soil fungi, Tedersoo et al. (2014) estimate that about 80% of all soil fungal taxa cannot be identified to the species level, and 20% reliably assigned to known orders. The data, therefore, opens new perspectives for future work on the analysis of undescribed or at least not yet sequenced fungal species, the estimation of below-ground fungal diversity and therefore calls for a greater sampling effort in West African soils (Crous et al., 2006). Basidiomycota are better represented under I. tomentosa (49%) and B. grandiflora (42%); unlike the Ascomycota that are more recorded under M. kerstingii (56%), I. doka (50%), and U. togoensis (50%). Also, the genus Russula is most abundant under B. grandiflora, I. doka, U. togoensis, and M. kerstingii; unlike Inocybe that is more frequently observed under I. tomentosa. These results largely corroborate previous observations that EcM fungal communities in West Africa are dominated by fungi in Russulaceae families (Bâ et al., 2012; Tedersoo and Smith, 2013, 2017; Ebenye et al., 2017). Meild et al. (2021) also reported the dominance of the genera Russula and Inocybe in the same geographical areas. The high proportion of Ascomycota (Peziza) in the soil fungal community highlights the presence of trophic groups other than EcM and their potential role as important decomposers of a wide variety of dead organic matter in forest ecosystems through the production of a wide range of hydrolytic enzymes, including cellulase and...
Figure 8. Prioritization of fungal species according to the EcM tree species.

Source: Authors

Figure 8 shows the prioritization of fungal species according to the EcM tree species. The absence or low representativeness of certain groups of fungi with large fruit bodies such as Polyporales and Hymenochaetales, has also been evidenced regarding soil fungi in temperate ecosystems (Tedersoo et al., 2020). This suggests a general pattern indicative of soil fungal communities and a limitation of exchange between the fungal communities of the phyllosphere and dead wood within the soil. Moreover, the effective presence of Glomeromycota highlights the probable duality of EcM and AMF of these trees. It has been demonstrated that some local forest trees form both EcM and AMF symbioses (Hougnandan et al., 2009; Djotan et al., 2021).

The diversity indices indicate a higher species diversity for *Isoberlinia doka* (*G* = 63, *H*′ = 2.81, *J* = 0.679). For the other forest species (*I. tomentosa; U. togoensis; M. kerstingii and B. grandiflora*), the diversity is low with an average distribution between genera. Fonton et al. (2012) argued that *I. doka* is a good early colonizer because it can reproduce from suckers and grows quickly. As such, *I. Doka* can connect to a larger number of below-ground fungal networks (Diédhiou et al., 2010; Gorzelak et al., 2015; Mcguire, 2017). Also, the density or uneven distribution of stands dominated by target EcM trees could explain this observation, but also other factors including different soil characteristics, altitude, and host specificity (Corrales et al., 2018). Indeed, the increasing proportion of phosphorus, clay, nitrogen, and soil pH, is negatively correlated with fungal community diversity, abundance, and composition (LeDuc et al., 2013; Zhang et al., 2016). This difference in belowground fungal community diversity among EcM trees is strongly correlated with canopy composition, stand age, EcM tree density, and canopy cover rate (Johnson et al., 2004; Gebhardt et al., 2007; Burke et al., 2009; Henry et al., 2021; Meidl et al., 2021). However, the Jaccard similarity index shows that a large proportion of genera are shared. *I. doka and I. tomentosa* share the greatest number of common genera (*J* = 0.831); unlike *I. doka and U. togoensis*, which display the lowest number (*J* = 0.635). *I. doka and I. tomentosa* are two EcM sister species within the same family (Fabaceae). Such phylogenetic proximity could explain why both tree species obtained the highest value of the similarity index. However, the similarity analysis (AnoSim) indicates that the generic fungal composition differs significantly between the five EcM trees (*P* = 0.001) at the 5% level.

Based on nMDS results, only three of the fungal genera are more specific to certain woody species. This could be explained by the preference or specificity of certain fungal partners in symbiotic relationships with EcM trees. Previous studies highlighted the close preference between certain bellowground fungal communities and their host plants (Kretzer et al., 1996; Taylor and Bruns, 1997; Taylor et al., 2002). For example, *Lactarius deliciosus* (L.) Gray, *L. deterrimus* Gröger and *L. salmonicolor* R. Heim and Leclair are specific to *Pinus*...
African trees. Mycorrhiza 22:1994; Molina and Trappe, 1994). These results corroborate those of Toju et al. (2013), who pointed out that some fungi of the Russulaceae family have been detected exclusively on specific oak species (Quercus spp.). Other research confirms the specificity of some genera of soil fungi with respect to their host plants (Ishida et al., 2007; Tedersoo et al., 2008). This is the case for fungal species such as Rhizopogon spp. and Suillus spp., which are almost exclusively associated with Pinaceae and sometimes Monotropaceae (Massicotte et al., 1994; Molina and Trappe, 1994; Kretzer et al., 1996; Taylor and Bruns, 1997; Taylor et al., 2002).

While the recent work of Meidl et al. (2021) aimed to document the effect of vegetation types on the mycobiome of soils associated with EcM trees, the present study targets the relation between selected EcM trees and the mycobiome immediately within their rhizosphere (all vegetation combined). The findings corroborate previous work by Massicotte et al. (1994), Molina and Trappe (1994), Kretzer et al. (1996), Taylor and Bruns (1997), Taylor et al. (2002), Ishida et al. (2007), Tedersoo et al. (2008), which highlighted different mechanisms of microbiome specification by host plants. The results, therefore, supplement those of Meidl et al. (2021) not only by confirming host preference but more importantly by highlighting the specialist genera partnered with valuable native tree species of West Africa.

Conclusion

Until recently, estimates of total fungal diversity did not include results from large-scale environmental sequencing methods, especially in West African regions. This study constitutes the first major exploration of the edaphic fungal communities of West African ecosystems, revealing insufficient sampling effort in currently neglected ecosystems and regions. The authors’ data provide a baseline for phylogenetic placement and taxonomic resolution of environmental sequences of five EcM trees of socio-economic importance in West Africa.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Supplementary materials C
Supplementary materials D
Supplementary materials E