Project Update: January 2021

Introduction

Biodiversity is a key element in science and has acquired an interest in the conservation of genetic resources. Fungi are components of biodiversity actively involved in ecological roles as parasites or symbionts (Duffy et al. 2007). The overall diversity of fungi is quite high, around 0.5-10 million species (Bass and Richards 2011; Blackwell 2011). In Benin, this diversity has been estimated at 18,000 species of higher fungi (Yorou 2010; Yorou and De Kesel 2011). With this diversity, fungi are used in food, agriculture and health. However, despite their importance in food, medical and ecology, fungi remain unrecognised by decision makers and forest managers, which means that they are neglected in forest management programmes.

Harvesting and marketing of fungi are important sources of income for local people, but today these potentialities are diminishing because of population growth, land clearing, vegetation fires which destabilise forests and cause decline or even disappearance of certain fungal species. Thus, it is therefore essential now to limit certain works in protected areas such as the galleries forest of Koussoucoingou and Kota, which constitute favourable areas of fungal production in Benin. In this way, the second part of this project aims to: (1) highlight diversity of ectomycorrhizal (EcM) fungi in the galleries forest of Koussoucoingou and Kota, (2) estimate diversity of EcM trees in Benin and (3) propose a forestry management plan by considering diversity and abundance of fungi as well as their partner trees in target forests.

Material and methods

From June to September 2020 every 3 days, a transect line of 500 m x 10 m has been traced in each forest, in the vegetation dominated by ectomycorrhizal trees such as *Berlinia grandiflora* (Vahl.) Hutch. & Dalz., *Isoberlinia doka* Craib & Stapf, *Isoberlinia tomentosa* (Harms) Craib & Stapf or *Uapaca togoensis* Pax. So, 40 transects lines in each forest have been traced starting from the east to the centre and then to the west. Therefore, in each forest an area estimated at 200,000m² was sampled during the data collection period. The frequency of transect visits have been recorded during the whole mycological season. During these surveys, all ectomycorrhizal fungi have been geo-referenced, photographed, collected, described morphologically, cleaned and dried with electronic dryers Dörrex Stöckli (A. & J. Stöckli AG) at 40°C for 24 hours (Fig 1). All samples are deposited at the mycological herbarium of the University of Parakou, UNIPAR (Fig 1). Species that are not identified in the field have been examined microscopically in laboratory.

Figure 1. Conservation and preservation of specimens

DNA Extration and PCR

DNA was extracted using minipcr (Annex, Fig 4b) by placing 10-50mg of fresh tissue in modified ROSE buffer (Steiner et al. 1995); 10mM Tris-HCl, pH 8.0; 312.5mM EDTA, pH 8.0; 1% sodium lauryl sarkosyl), breaking up tissue with some forceps, and heating at 90°C for 20 min, followed by placing on ice for 5 minutes. Raw extracts were diluted 900:1 with water. PCR was carried out in 25µL reactions including 5µL diluted DNA extract, 20µM each of primers ITS1F and ITS4 (White et al. 1990), and one PCR bead (Illustra PuReTaq Ready-To-Go PCR Beads, GE Healthcare), according to the protocol 4min at 95°C followed by 32 cycles of 30s at 90°C, 30s at 52°C, and 60s at 72°C, followed by a final 10min at 72°C.

Data analyses

To assess the diversity of fungi in the Koussoucoingou gallery forest, we first calculated the alpha diversity indices including the specific richness, the Shannon diversity index accompanied by the J equitability index from Piélou using the BiodiversityR package (Kindt 2014). Gamma diversity estimators such as chao, Jack1 and Jack2 were calculated to estimate the actual fungal diversity of the gallery forest of Koussoucoingou and Kota.

Results

Diversity of Ectomycorrhizal trees in Benin Family

Mycorrhizal symbiosis plays essential role to maintain the vitality of the forest. In Benin, eight large families Fabaceae, Cesalpiniaceae, Dipterocarpaceae, Phyllantaceae, Mimosaceae, Casuarinaceae, Lauraceae and Myrtaceae constitute the most representative families of the diversity of ectomycorrhizal trees with eighteen species (Table 1).

Table 1. Diversity of Ectomycorrhizal trees in Benin

Family	species	Habitat	References
Fabaceae	*Isoberlinia doka* Craib & Stapf	Woodland	Ducousso et al. 2003 ; Bâ et al. 2014
	Isoberlinia tomentosa (Harms) Craib & Stapf	Woodland	Ducousso et al. 2003 ; Bâ et al. 2014
	Berlinia grandiflora (Vahl) Hutch. & Dalziel	Woodland	Ducousso et al. 2003 ; Bâ et al. 2014
Cesalpiniaceae	*Afzelia africana* Sm. ex Pers.	Woodland and dense forest	Ducousso et al. 2003 ; Bâ et al. 2014
	Burkea africana Hook.	Woodland, Dense forest, Savana	Yorou et De kesel 2011
	Anthonotha crassifolia (Baill.) J. Léonard	Woodland	Ducousso et al. 2003 ; Bâ et al. 2014, Adjakpa et al. 2011
	Anthonotha fragrans (Baker f.) Exell & Hillc.	Woodland	Ducousso et al. 2003 ; Bâ et al. 2014

	Anthonotha macrophylla P. Beauv.	Woodland	Ducousso et al. 2003 ; Bâ et al. 2014
Dipterocarpaceae	*Monotes lutambensis* Verdc.	Woodland	Ducousso et al. 2003 ; Bâ et al. 2014
	Monotes kerstingii Gilg	Woodland	Ducousso et al. 2003 ; Bâ et al. 2014
Phyllantaceae	*Uapaca togoensis* Pax	Woodland	Ducousso et al. 2003 ; Bâ et al. 2014
	Uapaca somon Aubrév. & Leandri	Woodland and dense forest	Ducousso et al. 2003 ; Bâ et al. 2014
	Uapaca guineensis Müll. Arg.	Gallery forest and dense forest	Ducousso et al. 2003 ; Bâ et al. 2014
	Uapaca heudelotii Baill.	Dense forest	Ducousso et al. 2003 ; Bâ et al. 2014
Mimosaceae	*Acacia auriculiformis* A. Cunn. ex Benth.	-	Ducousso et al. 2003 ; Bâ et al. 2014
Casuarinaceae	*Casuarina equisetifolia* J.R. Forst. & G. Forst.	-	Ducousso et al. 2003
Lauraceae	*Cinnamomum zeylanicum* Blume	-	Ducousso et al. 2003
Myrtaceae	*Eucalyptus camaldulensis* Dehnh.	-	Ducousso et al. 2003

Diversity of macromycetes in the gallery forest of Koussoucoingou and Kota

Shannon diversity index calculated for the mycoflora of the gallery forest of Koussoucoingou and Kota is respectively H'= 3.30 Bits and H' = 2.30 Bits (table 1). These values justify the great fungal diversity of these two forests. Also, the calculated Pielou's evenness gives a value of R = 1 Bits for each forest, which reflects a strong distribution fairness of the fungal communities of the gallery forest of Koussoucoingou and Kota.

In total, 111 different taxa were collected in the two forests, 43 taxa divided into 20 genera in Koussoucoingou and 68 taxa divided into 20 genera 12 families in kota were identified during the mycological season (July-September). Let us point out that among the taxa encountered, some are news to science. Among the new taxa are *Mallocybe africana* Aïgnon, Yorou & Ryberg (Aïgnon et al. 2021a) and *Inosperma africanum* Aïgnon, Yorou & Ryberg (Aïgnon et al. 2021b) which we have just been published thanks to the contributions of this project. On the other hand, in general, the most dominant genera are *Lactarius, Cantharellus, Russula, Amanita* and *Lactafluus*. The abundance of these fungal genera confirms the strong presence of EcM trees (*Berlinia grandiflora, l. doka, l. tomentosa, M. kerstingii B. grandiflora, U. togoensis*) in these gallery forests. These forest species are considered as host plants for ectomycorrhizal fungi. as a result, the actual fungal diversity of each forest was estimated with gamma diversity indices such as Chao, Jackknif 1 and Jackknif 2 were calculated (Table 2). it shows that the fungal specific richness of

the forest of Koussoucoingou is between 64 and 76 species and the fungal specific richness of Kota is between 47 and 53 (Table 2).

Table 2: Gamma diversity estimators

Overall diversity	Chao	Jackknif 1	Jackknif 2
Koussoucoingou	67,796875	64,5625	76,520833
Kota	47.91	56.5	53.7

Management plan related to EcM fungal diversity

Kota gallery forest has a management plan. Thus, here we present a management plan for Koussoucoingou gallery forest only. The plan proposed takes into account EcM fungi and fungal partner trees such as *Berlinia grandiflora, Isoberlinia doka, Isoberlinia tomentosa* (Harms) Craib & Stapf and *Uapaca togoensis* Pax and *Uapaca guineensis* (Fig 3).

This forest management plan follows: (1) the analysis of fungal diversity as well as the diversity of the partners trees, (2) synthesis allowing to define or adjust management objectives over time with the local populations, (3) a reforestation programme to be implemented by training local communities on the production of plans that can be purchased and enriched the forest, (4) an economic and financial report which will be based on the economic contribution of the production of the plan, and (5) a mechanism for implementing the programme based on requests additional fund for implementation and high monitoring and evaluation. In addition, this plan will ensure the safety and improvement of conservation of EcM fungi and their partner trees through extension and attracting the attention of local communities.

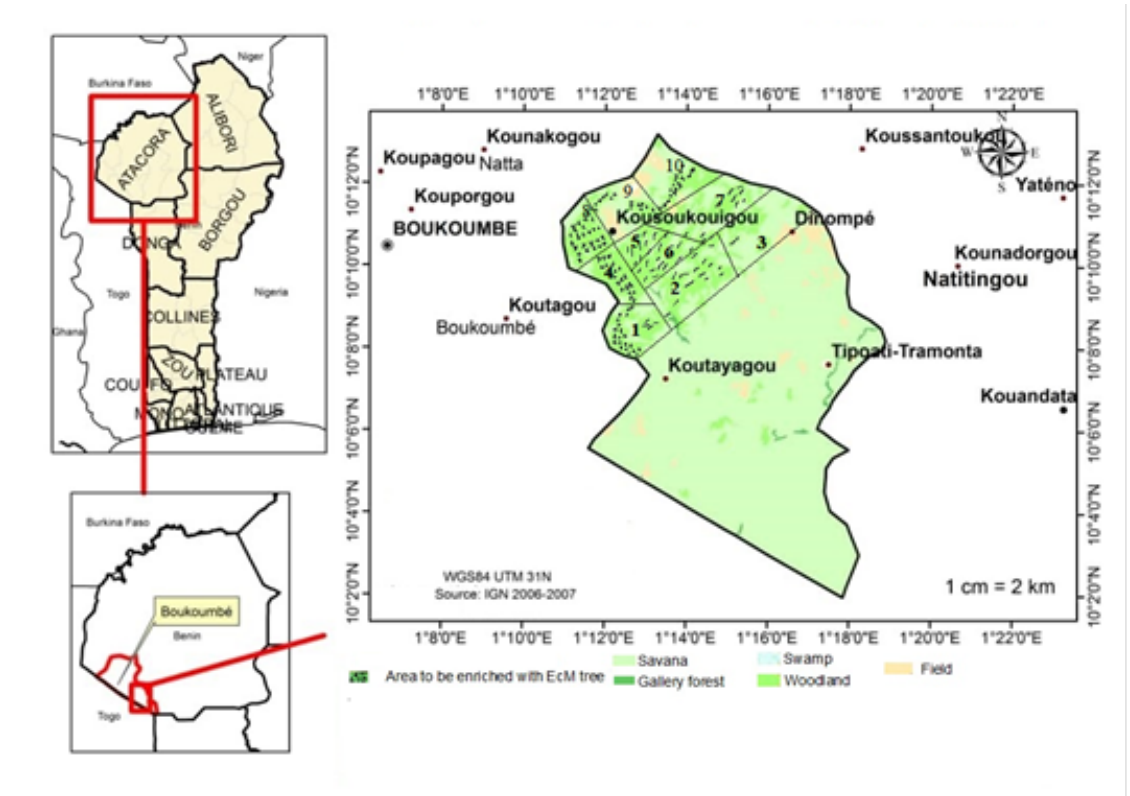

Figure 3. Management plan of Koussoucoingou gallery forest

Indeed, the plan fits with the objectives of the producers because it allows the maintenance of agricultural cultivation areas but envisages the sustainable management and maintenance of EcM trees within the gallery and also the enrichment of degraded forest areas around the gallery forest. Thus, the plan will be maintained for a period of 10 years, if possibly the forest administration does not propose a comprehensive plan for all biodiversity. In case, the administration intends to introduce a new plan, the fungi will be incorporated.

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EcM fungal diversity and indentification using molecular tools

Figure 3. A. Inosperma africanum, B. Mallocybe africana, C. Amanita subviscosa and D. Boletus sp.

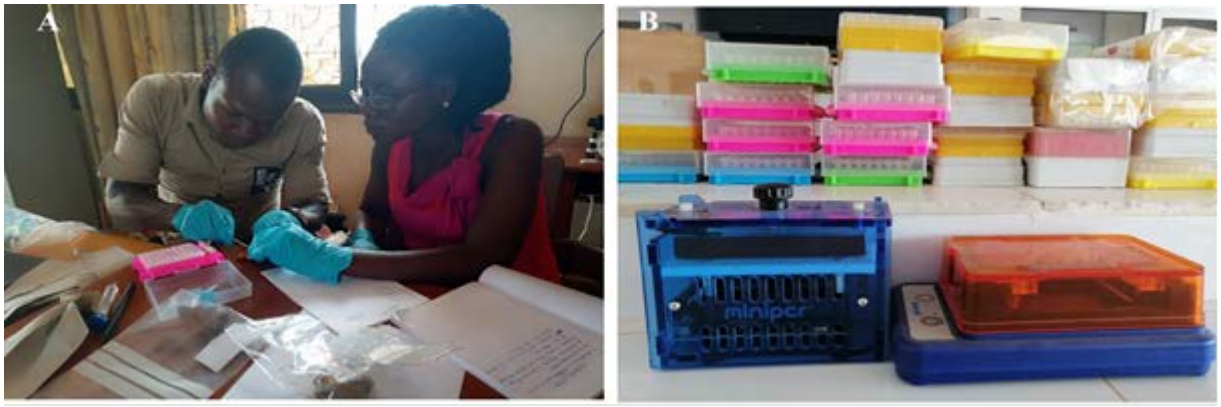

Figure 4. A – B. DNA extraction and PCR using MiniPCR