Project Update: January 2019

Introduction

Human impact on vegetation cover has increased in recent decades due to dependence of rural populations on non-timber forest products. In addition, forest exploitation isn't always compensated by natural regeneration and forest plantations. These pressures are leading to a decline in forest cover, a reduction in timber and non-timber resources, and a significant loss of biodiversity.

Benin has a great diversity of larger fungi with more than 18,000 species according to Yorou et al. (2002). So far, only a small part of this diversity has been explored, while habitat and host tree destruction actions continue to multiply. With the current rate of forest degradation, many fungi will disappear without being known if nothing is done.

That is why it is important to increase the inventory of wild fungi to know the endangered species to better protect them, and also to raise awareness and educate the local population to awaken their conscience on the risk of extinction of wild fungi. The present mid-term report aims to determine the species of fungi currently in critical danger of extinction in Benin, their host trees and fungal diversity hotspots in order to propose concrete actions to save them.

Methodology

Study area

A documentary research was carried out in the Laboratory of Ecology, Botany and Plant Biology (University of Parakou). This concerns the compilation of all the data collections of Bachelor's and Master's thesis, as well as the publications of all mycologist researchers who worked on Benin's ecosystems. Field data collection was then carried out in the Sudanian region of Benin because of its great diversity of fungi. This zone, which occupies about 2/3 of the total area of Benin, is located at the geographical position of 9 ° 10 'to 11 ° 52' of northern latitude and 1 ° 50 'to 3 ° 22' of western latitude, a forest region of excellence, it is mainly occupied by forest reserves and parks. The choice of collection sites was based on their accessibility, the presence of symbiotic tree species and the mycodiversity reported by previous work (Yorou and De Kesel, 2001, De Kesel and Yorou 2011, Yorou et al 2014, Boni and Yorou 2015, Fadéyi et al 2017. Thus, the collections were carried out in the Wari Maro forest reserves (8 ° 80 to 9 ° 10N and 1 ° 55 to 2 ° 25E), the forest reserves of Mount Kouffé (8 ° 20 'to 8 ° 50' N and 1 40 ° to 2 ° 15 'E), Bassila Forest Reserves (9 ° to 9 ° 20 'and 1 ° 30 to 1 ° 50' E) and the gallery forest of Kota (10 ° 12 to 10 ° 3N and 1 ° 20E to 1 ° 30E). Figure 1 presents a map of the forests visited.

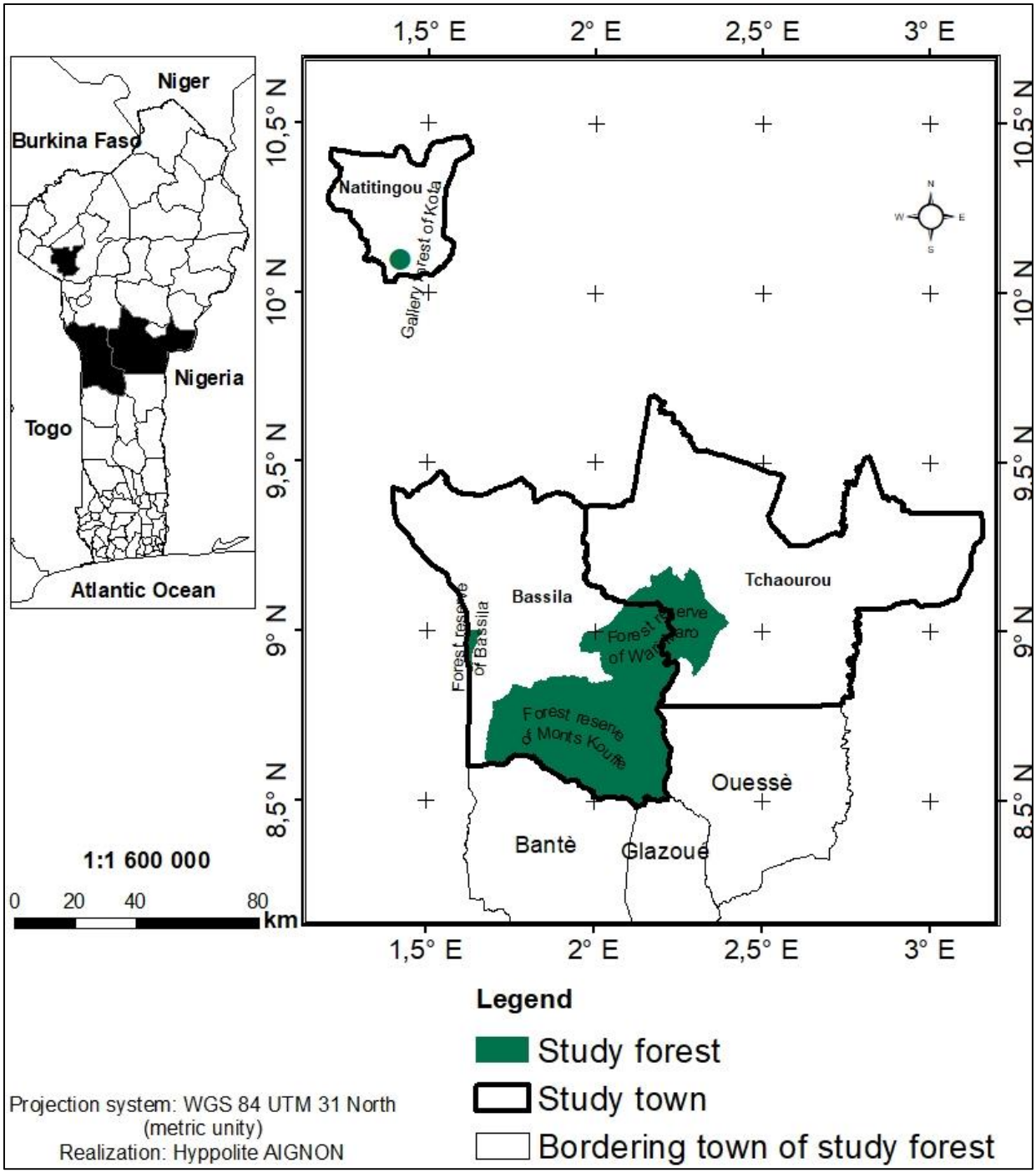

Figure 1: Map of study area

Surveys

Considering the most abundant symbiotic woody species in Benin (*Afzelia africana* Sm Huche. & Dalz. *Berlinia grandiflora* (Vahl), *Isoberlinia doka* Craib. & Stapf, *Isoberlinia tomentosa* (Harms) Craib & Stapf, Uapaca somon Aubrév. & Léandri Syn. and Uapaca togoensis Pax), we identified six groups of vegetation dominated by these species for prospecting and mushroom picking. In each vegetation dominated by those tree, three transects of 3 km long and 100 m wide have been mapped for optimal site coverage.

The inventories are based on mainly qualitative and quantitative descriptions of the mycofloristic composition of the plant communities selected according to the technique of Yorou et al. (2001). The propections were made during the rainy season (August-October). During these surveys, all the sporophores visible to the naked eye were georeferenced, photographed, collected and followed by a careful cleaning to rid the specimen of plant debris, described morphologically and a small part was taken from the lamellae and stored in the CTAB for further molecular analysis for complete identification. The specimens are then dried using Dorrex Stockli dryer. The dried specimens are deposited in the mycological herbarium of the University of Parakou.

Data compilation and analysis

After the data collection, a local database taking into account the information from the literature and the precedent field surveys was made. Method of classifying endangered species in Benin has allowed us to identify endangered species. Thus, three main factors were considered in this choice: the rarity of the species expressed by the number of collection sites of the species, the area of the habitats expressed in zones of occupation and the potential threats weighing on these habitats (degree of regression of the zone species occurrence and sensitivity to this modification).The software R allowed us to perform the statistical analyzes, among other we estimated the similarity index of Sorensen in order to highlight the similarity between the collection sites. In addition, the abundance of each species was calculated according to the forests reserves and the rarity of the species expressed by the number of collection sites and the potential threats weighing on each habitat allowed us to determine the rare species. A matrix was carried out on the rare species according to each forest reserve then the Canonical Correlation Analysis (CCA) was realized and allowed us to determine the preferable symbiotic trees of each species endangered fungi specie.

Results

Global view on macromycetes diversity in Benin

The mycodiversity data set (2003 to 2017) was compiled to facilitate the protection and monitoring of endangered species and to predict new endangered species. More than 9803 specimens were collected, herborised and stored in the mycological herbarium of the University of Parakou. The most represented genera are:

- Amanita with more than 40 species (*Amanita citrina* sensu Stevenson, *Amanita congolensis* (Beeli) Tulloss, BE Wolfe, KW Hughes, Kudzma & D. Arora, *Amanita craseoderma* Bas, *Amanita crassiconus* Bas, *Amanita loosei* Beeli, *Amanita masasiensis* Härk. & Saarim., *Amanita pulverulenta* Beeli, *Amanita rubescens* Pers., *Amanita strobilacea* (Cooke) Sacc., *Amanita strobiliformis* (Paulet ex Vittad.) Bertill., *Amanita subviscosa* Beeli, *Amanita viridis* Pers , *Amanita xanthogala* Bottom ect ...);
- Lactifluus genus, with more than 45 different species (*Lactifluus acrissimus* Verbeken & Van Rooij, *Lactifluus gymnocarpoides* Verbeken, *Lactifluus luteopus* (Verbeken) Verbeken, *Lactifluus sesemotani* (Beeli) Buyck, *Lactifluus densifolius* Verbeken & Karhula, *Lactifluus edulis* (Verbeken & Buyck) Buyck, *Lactifluus medusae* (Verbeken) Verbeken, *Lactifluus brachystegiae* (Verbeken) Verbeken,

Lactifluus volemoides (Karhula) Verbeken, *Lactifluus xerampelinus* (Karhula & Verbeken) Verbeken ect…);

- Lactarius (*Lactarius baliophaeus* Pegler, *Lactarius alboscrobiculatus* H.T. Le & Verbeken, *Lactarius kabansus* Pegler & Piearce, *Lactarius saponaceus* Verbeken, *Lactarius scrobiculatus* (Scop.) Fr., *Lactarius subdulcis* (Pers.) Gray ect…) and
- Russula genus, with more than 48 species (*Russula acriannulata* Buyck, Karstenia; *Russula cellulata* Buyck, *Russula compressa* Buyck, *Russula congoana* Pat, *Russula cyanoxantha* (Schaeff.) Fr., *Russula grisea* Fr., *Russula luteopulverulenta* Beeli, *Russula meleagris* Buyck, *Russula ochracea* Fr., *Russula oleifera* Buyck, *Russula pellucida* (Gooss.-Font. & R. Heim) Buyck, *Russula pseudopurpurea* Buyck, *Russula rubroalba* (Singer) Romagn, *Russula termitaria* Buyck, *Russula rubroalba* (Singer) Romagn, *Russula velutina* Buyck, *Russula virescens* (Schaeff.) Fr etc.).

In addition, there is a large amount of species that are not yet identified up to the species level. The use of the molecular and anatomy studies will be the best option to identify with certainty these species to avoid errors.

Fungi species most abundant in the ecosystems of Benin

The diagram shows that five species come first for all the sites visited. These are *Cantharellus addaiensis* (4.97 %), *Scleroderma* sp (5.71 %), *Lactifluus gymnocarpoides* (6.99 %), *Amanita subviscosa* (9.12 %), and *Russula congoana* (22.46 %).

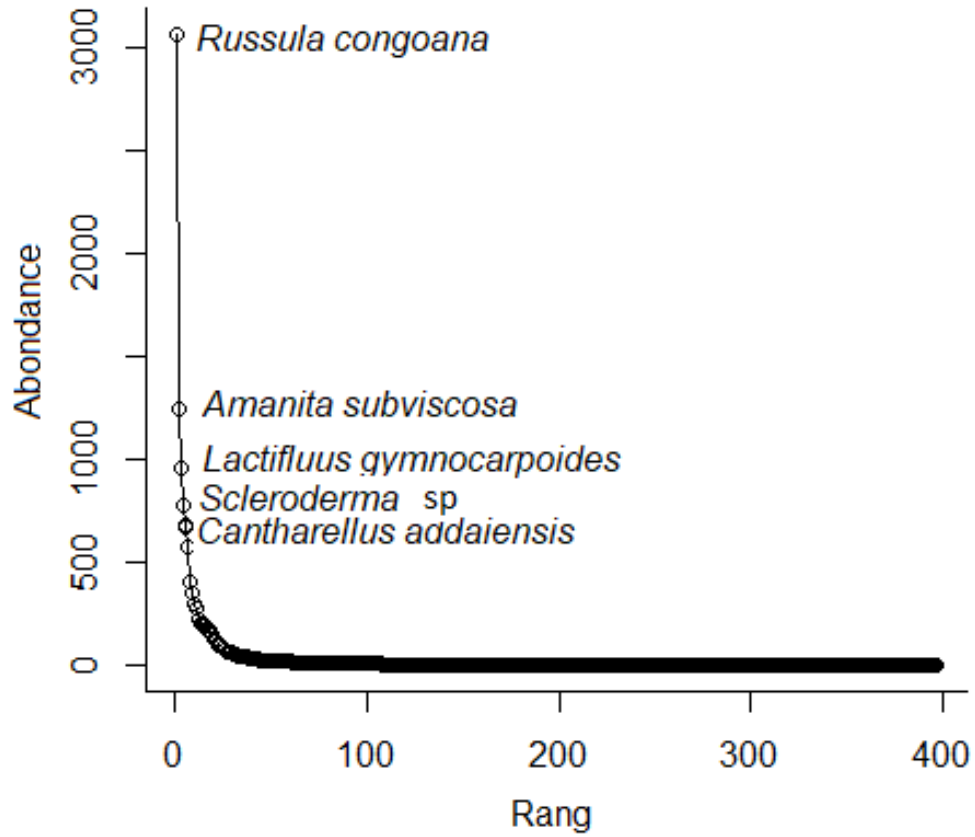

Figure 2: Rang-frequency curve for all species

Comparison of fungi diversity between different forests in Benin

The profile of Renyi shows that the individuals are equitably distributed among species for the site of Mont Kouffé noticed by the horizontal form of its curve. The distribution is irregular for the other five sites but appears to be less irregular for the Bassila and Wari Maro sites, which have a similar profile. The value of alpha = 1 corresponding to the diversity of Shannon and suggests that for this value the site of forest reserve of "Ouémé superieur" is more diversified than the other five sites. For alpha equal to unity, the following rankings may be established in ascending order of diversity: MK <OF <BF <WM <KW <OS

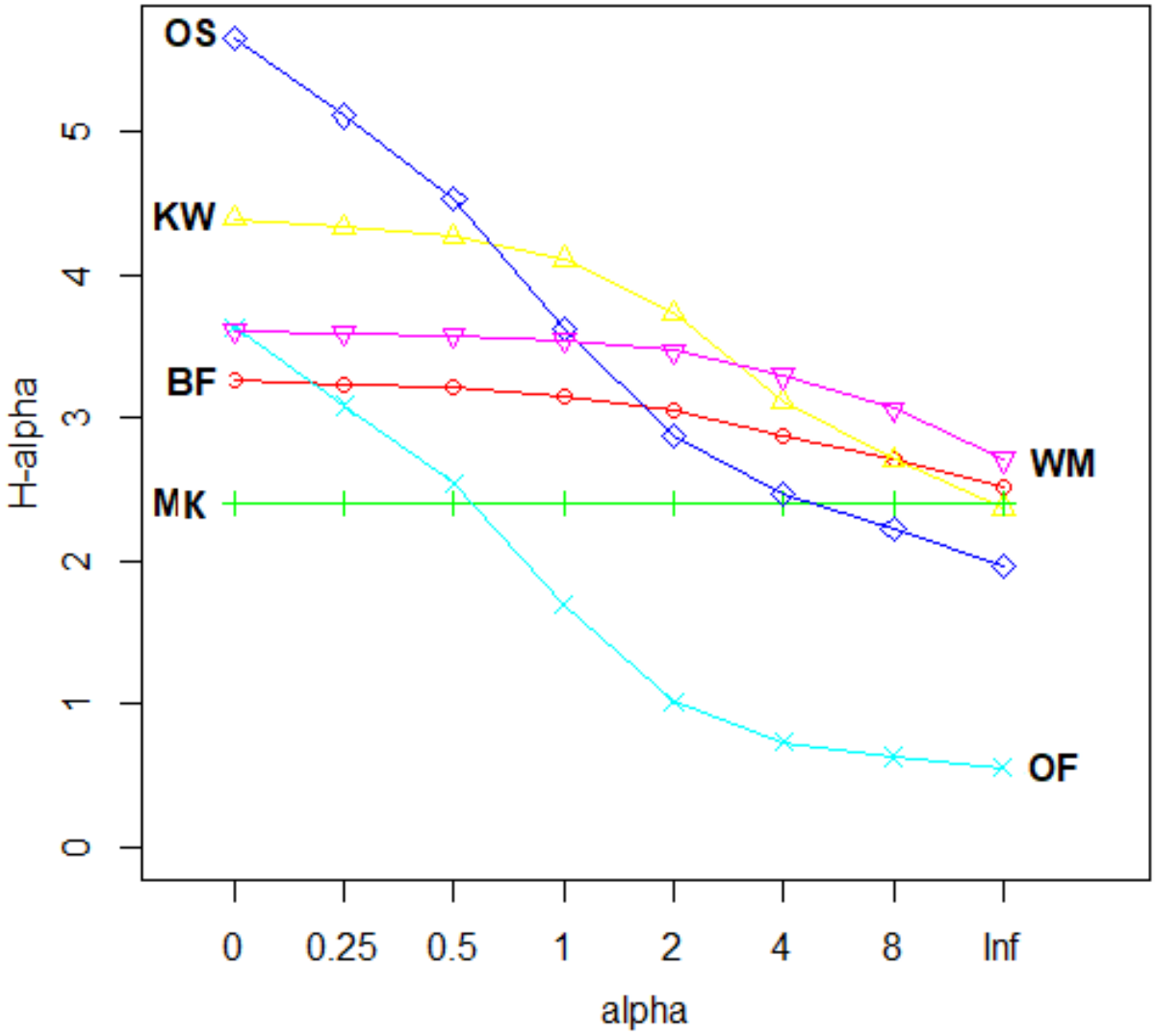

Figure 3: Profil de diversité de Renyi pour chaque site

Diversity and distribution of the fungi in Kota and Bassila Galleries forests

A total of 244 macofungi were harvested during the collection period, distributed among thirteen different families. The russulaceae family comes first with more than 26 species identified, Xylariaceae, Polyporaceae, Gyroporaceae and Clavulinaceae are the least families represented. Moreover, we group in the others families all the families represented by a single species, we can thus mention: Bolbitiaceae, Dacrymycetaceae,

Ganodermataceae, Gomphaceae, Hymenochaetaceae, Hymenogastraceae, Leotiaceae, Omphalotaceae, Sclerodermataceae, Thelephoraceae, Tricholomataceae and also the species identified with little certainty of which only later molecular studies can situate us if it is a new species or not. Figure 2 shows the distribution of species by family. These species are *Berlinia grandiflora*, *Isoberlinia doka*, and Uapaca togoensis

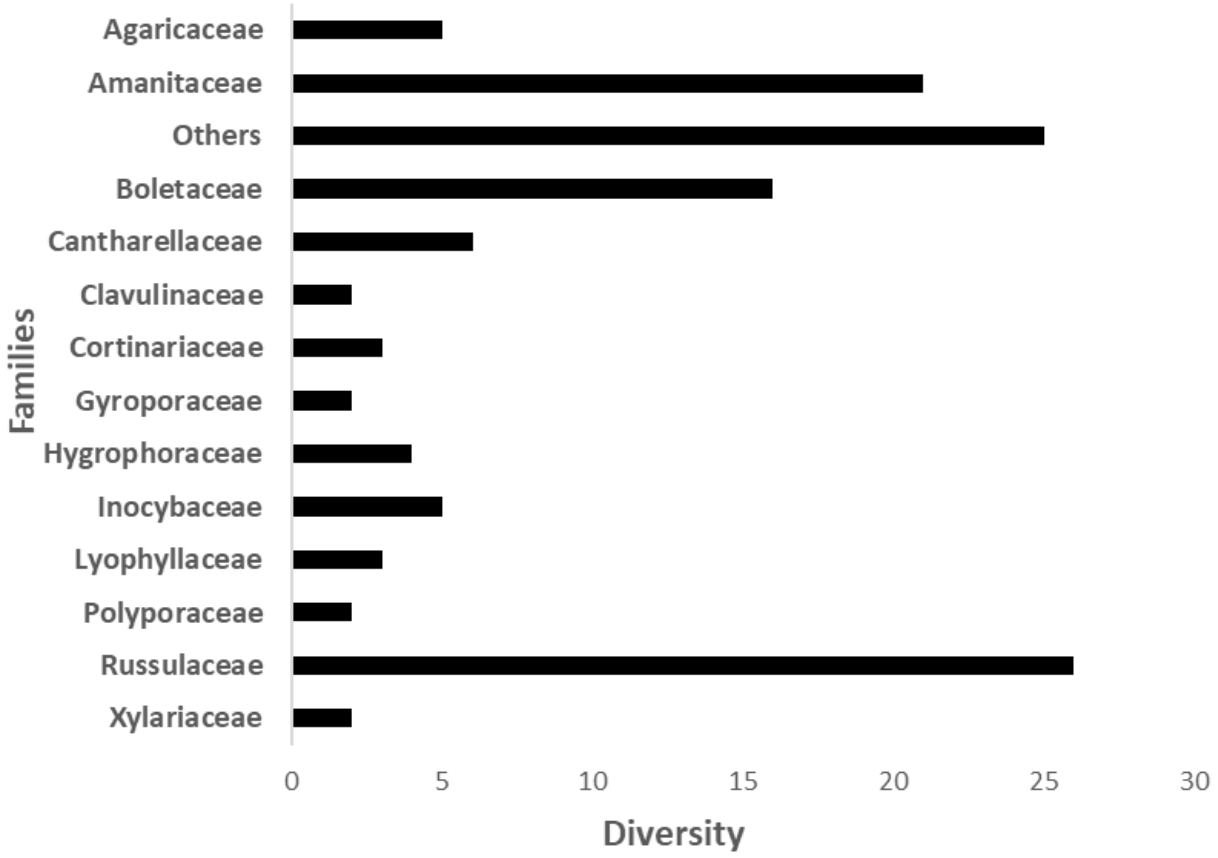

Figure 4: Numbers of macrofungi species by fungal families

Evaluating of similarity between the different forests

The similarity index of Sorensen shows that there is a strong similarity between the sites and suggests that the sites share practically the same species.

Table 1: similarity between the forests

Forests reserves	**Bassila**	**Kota waterfall**	**Mont Kouffé**	**Wari-maro**
Bassila	1			
Kota waterfall	0,957	1		
Mont Kouffé	0,998	0,998	1	
Wari-maro	0,921	0,911	0,902	1

Relationship between endangered fungi and EcM trees

The Mantel test shows that there is a significant correlation between symbiotic trees and EcM fungi (r = 1, p = 0.001). The factorial map shows that the first two canonical axes account for 56.63 % of the relationship between symbiotic trees and EcM fungi. The first axis explains the largest proportion of this total variance is 39.96 % and the second axis the smallest proportion is 16.67 %. Fungi species with a strong contribution to axis 2 are *Lactifluus luteopus* (L.l) and Amanita xanthogala (A.x) (Table 5). On axis 2, the species Cantharellus solidus (C.s) is more associated with vegetation dominated by *Berlinia grandiflora* and vegetation dominated by association of *Isoberlinia doka* and *Monotes kerstingii* in its positive part and opposes *Amanita xanthogala*, which is relatively associated with the vegetation dominated by *Uapaca guineensis* in its negative part (Figure 1). Axis 1 connects *Lactifluus luteopus* (L.l) in its positive part to the vegetation dominated by *Berlinia grandiflora* and *Uapaca guineensis* and also *Isoberlinia doka* and *Uapaca togoensis*. The same axis connects in its negative part, *Russula pellucida* (R.p) to the vegetation dominated by *Berlinia grandiflora*.

Table 2: Contributions of variables to axis formation

	CCA1		CCA2	CCA3
B.gran+U.gui	0,401		0,032	-0,320
B.gran	0,747		0,771	0,350
I.dok	-0,125		0,191	-0,089
I.dok+M.kes	0,279		1,145	0,929
I.tom	-2,633		-0,127	-0,185
U.gui	0,448		-1,527	0,432
U.tog	-0,398		-0,127	0,216
I.dok+U.tog	0,401		0,032	-0,320
A.x	-0,449		-0,821	0,175
C.s	0,179		0,735	0,596
L.l	0,161		0,000	-0,159
R.p	-0,995		-0,048	-0,070

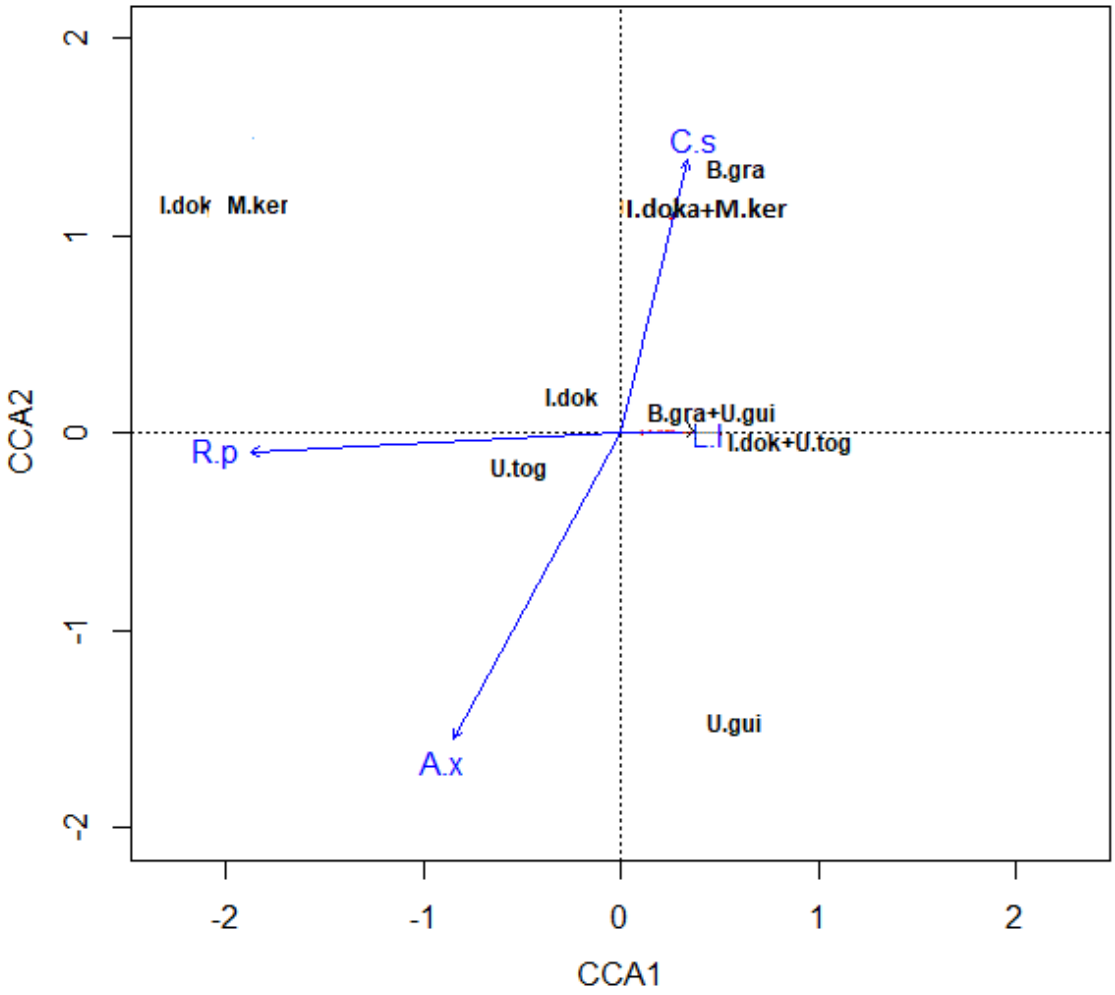

Figure 5: Factorial map showing the relationship between ECM species and fungi
Legend: (L.l) *Lactifluus luteopus*, (A.x) *Amanita xanthogala*, (C.s) *Cantharellus solidus,* (R.p) *Russula pellucida, (U.gui) Uapaca guineensis, (I.dok) Isoberlinia doka*, (B.gra) *Berlinia grandiflora, (U.tog) Uapaca togoensis*

Critically endangered species and factors causing species extinctions in Benin
This project reveals that four species of fungi are currently critically endangered. These are*: L. luteopus, A. xanthogala, C. solidus and R. pellucida*. these species are more associated with the vegetation dominated by *U. guineensis, I. doka, B. grandiflora*, and *U. togoensis* trees, well exploited by the forestry operators and also farmers. The forestry operators cut the symbiotic trees for the carpenter production and charcoal wood. As the impacts of agriculture, it is rare to note a selection of tree but a vast area is deforested for the yam and cotton fields' installation. Indeed, it destabilizes the forest reserves, all of these process cause the loss of fungi diversity and species richness such as *L. luteopus, A. xanthogala, C. solidus* and *R. pellucida*. In addition, under the effect of forest management, some hectares of natural vegetation are also destroyed in order to be replaced by plantations. This cause real harm to symbiotic fungal species because certain fungi are closely related to partner tree, thus the threats to trees cause the disappearance these fungi.

Fallen back and perspectives

Four different fungi species (*Cantharellus solidus, Lactifluus luteopus, Amanita xanthogala* and *Russula pellucida*) are in critical danger of extinction. In addition, fungi species have a diversity of tree partners. Potential partner trees of these fungi include *Berlinia grandiflora, Isoberlinia doka, Monotes kerstingii* and *Uapaca guineensis.* It is Noteworthy that the fungi species threatened here are often associated with *Isoberlinia doka* tree, but some fungal species *Cantharellus solidus* prefer the vegetation dominated by Berlinia grandiflora. Thus for the second phase, in order to better protect fungi species, we plan to plant two EcM species, the most common, currently under human pressure. These are mainly *Berlinia grandiflora* and *Isoberlinia doka* to restore degraded habitat in the project area. We must also educate the local people about the importance of these fungi in the wild even if some are not edible so that they had better save them as well as their partner trees.

Acknowledgment

We gratefully acknowledge The Rufford Foundation for financing the project N ° 25978-1

Annex

1. **Some critically endangered species**

***Amanita xanthogala* Bas**

***Cantharellus solidus* De Kesel, Yorou & Buyck**

Others species collected

Cantharellus congolensis Beeli

Lactifluus gymnocarpoides (Verbeken) Verbeken

***Amanita strobilaceovolvata* Beeli**

***Amanita strobiliformis* Gonn & Rabenh**

Professional photography on the field