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Ectomycorrhizal Fungi of Comoé National Park, a Biosphere Reserve in northeast Côte d'Ivoire: Diversity, Fruiting Phenology and Production in Relation to climate variability

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Number……07….

To my Father,

For always being besides me

ABSTRACT

The key role of ectomycorrhizal (EcM) fungi in ecosystems functioning has been demonstrated worldwide. However, their diversity, spatial distribution, fruiting phenology and production as influenced by climatic parameters variability remain poorly understood in tropical African forests. Weekly surveys were conducted from April to early October 2014 at the Comoé National Park (CNP), Côte d'Ivoire (West Africa) in nine permanent plots established in *Isoberlinia doka* (IW), *Uapaca togoensis* (UW) and Mixed (MW) woodlands. Morpho-molecular technics were used to identify collected specimens and to reconstruct their phylogeny. Non metric multidimensional scaling (NMDS) of EcM fungi fruit bodies abundance was run with forest trees´ aggregation fitted within plots using the package VEGAN. Hierarchical clustering based on dissimilarity and indicator species analysis were run to characterize communities. Final, correlation between edible species production and climatic parameters recorded per plot were performed with functions PRCOMP and COR set at Spearman method. All analyses were computed with the statistical program R. A total of 123 EcM fungi species belonging to 23 genera and nine families collected at CNP. High Simpson index of diversity (1-D) but weak evenness were respectively 0.97 and 0.54 for IW, 0.97 and 0.61 for MW, 0.96 and 0.52 for UW. Yet, weekly-based species accumulation curves did not reach an asymptote. Stem density of *Uapaca togoensis* Pax and *Isoberlinia doka* Craib & Stapf were the most important tree parameters in EcM fungi distribution (respectively $r^2 = 0.92$ / p-value = 0.002 and $r^2 = 0.83$ / p-value = 0.018). Two sites groups and four species estimated good indicators were thus highlighted. 27 species were identified as edible based on literature. They started fruiting in mid-May, 02 weeks after the first important rain. Productions varied with frequency and intensity of rainfalls. The total fresh biomass production varied from 34.72 kg/ha in UW to 25.25 kg/ha in MW, whilst IW yielded 24.11 kg/ha. Productions of ten edible species were significantly correlated to all climate parameters recorded per habitat: negatively for air and soil temperature and positively for relative humidity, soil moisture and rainfall intensity. However, production of *Gyroporus castaneus* was negatively correlated with both soil moisture and rainfall intensity recorded in UW.

Key-words: EcM fungi, fruit bodies, diversity, climate variability, indicator species.

RESUME

Le rôle clé des champignons ectomycorhiziens (EcM) dans le fonctionnement des écosystèmes a été mondialement démontré. Cependant, leurs diversité, répartition spatiale, phénologie de fructification et production naturelle, telles que influencées par la variabilité des paramètres climatiques demeurent mal comprises dans les forêts tropicales africaines. Des relevés hebdomadaires ont été réalisés d'Avril à début Octobre 2014 au Parc National de la Comoé (PNC) en Côte d'Ivoire (Afrique de l'Ouest). Neuf parcelles permanentes y ont été établies dans des forêts claires dominées respectivement par les espèces végétales *Isoberlinia doka* (IW), *Uapaca togoensis* (UW), la troisième forêt claire (MW) étant un mixte de ces espèces. Le positionnement multidimensionnel non métrique a permis la visualisation des dissimilarités entre les sites prospectés en terme d'abondance des champignons EcM telle que influencée par l'agrégation des arbres forestiers à l'aide du package VEGAN. Des classifications hiérarchiques basées sur les dissemblances et l'analyse des espèces indicatrices ont été menées pour caractériser les communautés. Enfin, la corrélation de Spearman entre la production d'espèces comestibles et les composantes principales des paramètres climatiques (enregistrés par parcelle) a été effectuée avec les fonctions PRCOMP et COR. Toutes ces analyses ont été faites avec le programme statistique R. Un effectif total de 123 espèces de champignons EcM appartenant à 23 genres et neuf familles a été récolté. Les indices de diversité spécifique et d'équitabilité de Simpson ont été respectivement de 0.97 et 0.54 pour IW, 0.97 et 0.61 pour MW, 0.96 et 0.52 pour UW. Cependant, les courbes d'accumulation hebdomadaire des espèces n'ont pas atteint une asymptote. La densité des pieds de *Uapaca togoensis* Pax et *Isoberlinia doka* Craib & Stapf ont été les déterminants floristiques les plus importants de la distribution des champignons EcM (respectivement r^2 = 0.92 / p-value = 0.002 et r^2 = 0.83 / p-value = 0.018). Deux regroupements de sites et quatre espèces estimées bonnes indicatrices ont été ainsi mis en évidence. 27 espèces ont été identifiées comme comestibles selon la littérature. Leurs fructifications ont débuté à la mi-Mai, 02 semaines après la première pluie importante tandis que les productions ont varié avec la fréquence et l'intensité des précipitations. La production totale de biomasse fraîche variait de 34.72 kg / ha dans UW à 25.25 kg / ha en MW, tandis que IW produisit 24.11 kg / ha. Les productions de dix espèces comestibles étaient significativement corrélées à tous les paramètres climatiques enregistrés par habitat: négativement pour la température de l'air et du sol et positivement pour l'humidité relative, l'humidité du sol et l'intensité des précipitations. Cependant, la production de *Gyroporus castaneus* a été corrélée négativement avec l'humidité du sol et l'intensité des précipitations enregistrées en UW.

Mots-clés: Champignons EcM, fructifications, diversité, variabilité climatique, espèces indicatrices.

OUTLINE

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ACRONYMS AND ABBREVIATIONS

AFNOR : Association Française de NORmalisation
bp: base pairs
CNP: Comoé National Park
CTAB: 3D cetyltrimethylammonium bromide
DNA: desoxyribonucleic acid
EcM: ectomycorrhiza
EFFB: Ectomycorrhizal fungi fruit body
Exo I: Exonuclease I
G + I: Gamma distribution + Invariant site
G: Gamma distribution
gDNA: genomic DNA
GHG: greenhouse gas
GPS: Global Positioning System
INSDc: International Nucleotide Sequence Database consortium
IPCC: Intergovernmental Panel on Climate Change
ITS: Internal Transcribed Spacer(s) of the ribosomal DNA
K2: Kimura 2-parameter
min: minute
ML: Maximum Likelihood
mm: millimeter
mov /s: movements per second
N: nitrogen
NCBI: National Center for Biotechnology Information
P: phosphorus
PCR: polymerase chain reaction
rDNA: ribosomal desoxyribonucleic acid
rev / min: revolutions per minute
s. l.: sensu lato
s. s.: sensu stricto
s: second
SAP: Shrimp Alkaline Phosphatase
sect.: section
sp, spp.: species
T92: Tamura 3-parameter
U.N.E.S.C.O.: United Nation Educational Scientific and Cultural Organization
UNEP-WCMC: United Nations Environment Programme World Conservation Monitoring Centre
UV: Ultra violet
vol. %: volume percent
WASCAL: West African Science Service Centre on Climate Change and Adapted Land Use
WMO: World Meteorological Organization

INTRODUCTION

Global biodiversity has been under decline since the 19th century due to serious climate, environmental and ecological changes through human activities around the globe. The global climate system is actually modified by increased greenhouse gases (GHG) in the atmosphere subsequently to unrestrained deforestation, fossil fuel combustion and other anthropogenic activities **(WMO, 2007)**. Few key parameters of global change are among other trend towards warming (increasing temperature), increase of atmospheric CO_2 and disturbance in the distribution, seasonality and amount of rainfalls. It is predicted that Earth surface temperature will increase from 0.3°C to 1.7°C under scenario RCP2.6 by the end of the 21st century (2081–2100) whilst the atmospheric carbon level is continuously increasing **(IPCC, 2014)**. Though the impact of global change on ecosystems is not yet adequately addressed, it is expected that many changes in global biodiversity and ecosystem functions will occur. High temperature is expected to alter tree phenology, plant growth and distribution **(Montoya and Raffaelli, 2010)** but also to increase the length of the growing season **(Walther *et al.*, 2002; Morin *et al.*, 2007)**. Globally, rising temperature will possibly increase plants' growth rate and alter species distribution toward migration and adaptation ecozones. An increase of aboveground growth and reproductive effort of plants has even been evidenced by **Hollister *et al.* (2005)**. At the other side, elevated atmospheric CO_2 and nitrogen will likely increase the rate of net photosynthesis by 40 to 80 % **(Körner *et al.*, 2005)**, the allocation of carbon to the plant roots **(Janssens *et al.*, 2005)** and the production of leaves, wood and coarse roots **(Hyvönen *et al.*, 2007)**. It is actually difficult to predict the exact response of plant diversity to climate change as many investigations are still needed to understand their resilience, adaptation and/or migration following fluctuation of climatic parameters.

It has been demonstrated that the productivity, diversity and composition of plant communities are indirectly and directly influenced by belowground microbes from which plant symbionts play a key role **(Van Der Heijden *et al.*, 2008; Van Der Heijden and Horton, 2009)**. Indeed, over 90 % of terrestrial plants depend upon an ecological relationship with soil fungi for their regeneration and growth **(Smith and Read, 2008; Singh *et al.*, 2011; Dickie *et al.*, 2014)**. This relationship termed mycorrhiza is the most prevalent symbiosis on Earth, including cultivated plants, herbaceous species and forest trees as well. Thus, as both partners are living more or less obligatory and intimately, any possible change that affect plant partner (called host plants) is also expected to influence the symbiotic fungi. During recent years, impressive works were attempted in temperate and boreal zones to understand the response of ectomycorrhiza (EcM) fungi communities to global warming and environmental changes. First studies explicitly focused on mycorrhizae in that context of

global change **(Perry *et al.*, 1989; 1990)** and on the effects of increasing atmospheric CO_2 and temperature on all mycorrhizal classes **(Staddon *et al.*, 2002)**. At fungal level, it has been demonstrated that global warming shifts the composition and structure of EcM fungi communities **(Godbold *et al.*, 1997; Fransson *et al.*, 2001)**. As increased temperature likely affects photosynthesis rate and carbon allocation to roots, it is more likely that warming will increase fungal production **(Clemmensen *et al.*, 2006)**. Of particular interest are findings that both aboveground fungal biomass (fruit bodies productions) and respiration respond positively to increased temperature and moisture **(Vargas *et al.*, 2010)**. At the other hand, decline in fruit bodies production have been attributed to acid rains **(Arnolds, 1991)**. From those numerous investigations, local temperature and rainfall distribution (along with soil humidity) are among the most important drivers of species richness, composition and functional shift in EcM community structure as demonstrated in a recent review **(Pickles *et al.*, 2012)**. Furthermore, long term observations of fungal phenology in temperate forests reveal that fruit bodies production and temporal changes are strongly influenced by either increasing temperature **(Kauserud *et al.*, 2008; 2010)** and/or rainfalls **(Krebs *et al.*, 2008)**.

Although the response of EcM communities to global warming and environmental changes is scarcely addressed in tropical zones and especially in tropical Africa, the correlation between phenology, diversity of EcM fungi, temperature and rainfall may be also strong with contrasted seasons. In Sudanian woodlands for example, a strong variability has been noticed regarding species richness and community structure throughout the fruiting season and natural productions of EcM fungi were unevenly distributed over time sequences. More than 80 % of natural production of EcM fungi were recorded during the first two months (May-June) of the fruiting period whilst the lowest fungal biomasses coincide with the highest rainfalls **(Yorou *et al.*, 2001)**. Nevertheless, the authors failed to link species composition, community structure and productivity patterns of EcM with either the local temperature or soil humidity. To our knowledge, that study is the only one in West Africa addressing the impact of climate parameters on wild EcM fungi phenology and natural productions.

West African ecosystems are characterized by a rapid global change as a consequence of bad management strategies, drought, unrestrained deforestation and annual bushfire with release of large amount of GHG. Because of their sensitivity to temperature and strong seasonality **(Bahram *et al.*, 2012)**, EcM fungi represent, therefore good candidates to investigate for a better understanding of tropical ecosystems response to climate change. To predict ecosystem dynamic following climate change, it is actually very important to assess

the EcM fungi diversity (species richness and abundance, community structure and its variability), and the behavior of fungi (phenology, natural productions) as they are affected by current climate parameters.

1. Scientific questions addressed in this study

Through this PhD project, we attempted to answer the following questions:

- What species, genera and families of EcM fungi occur in CNP? And how abundant are they?
- Are the collected EcM fungal species endemic to CNP or also represented in other part of Africa?
- What environmental drivers (soil and plants parameters) underpin their distribution?
- What are the climatic determinants (temperature, humidity) that drive fungal fructification, phenology and primary biomass?

2. Objectives

2.1. General objective

The main goal of this study was to understand and predict the relevance/significance of climatic parameters (rainfalls intensity and frequency, air and soil temperature and humidity) on the temporal dynamic of EcM fungi and their associated forest trees.

2.2. Specific objectives

Specific objectives are enumerated as follows:

1. Assess the diversity of EcM fungi species through the diversity of fruit bodies;
2. Investigate taxonomic affinities between studied fungi and species from other regions of the world, and their phylogenetic position;
3. Determine the influence of plants and soil parameters on the spatial distribution of EcM fungi fruit bodies (EFFB);

4. Demonstrate the influence of climate parameters (air and soil humidity, rainfall distribution/intensity, temperature of air and soil) on the fruiting phenology and natural production of edible collected EcM fungi fruit bodies (EFFB).

3. Research hypotheses:

Our research hypotheses are enumerated as follow:

1. The CNP harbours diverse EcM fungal species.
2. Many EcM fungi are probably endemic to the CNP.
3. The spatial distribution of the fungi is determined by the diversity of habitat (host plants diversity and dendrometric data; soil structure and texture, minerals content).
4. Air and/or soil temperature and humidity are the main drivers of fruiting sequence of EcM fungi.

4. Structure of the thesis

This dissertation is composed of four sections including ten chapters. First, Section I presents Overview on Ectomycorrhizae. Its first chapter gives Generalities on Mycorrhiza. Specificities of the Ectomycorrhiza and its ecological importance are presented in Chapter II. Final for this section I, developed techniques of identification of symbiotic fungi are exposed in Chapter III. Second, Section II indicates the sets of Materials and Methodological approches used. Within it, the study site The Comoé National Park is presented in Chapter IV whilst target biological materials and related sampling and/or processing protocols are given respectively in chapters V and VI. Third, Results for each specific objective assessment are presented in section III. The Diversity of EcM fungi fruit bodies (Specific objective 1) are described in Chapter VII. Phylogenetic analyses of those fungi (Specific objective2) demonstrate their taxonomic affinities with African species and are presented in Chapter VIII. Their spatial distribution in relation to habitat types' floristic and edaphic characteristics (Specific objective 3) is studied in Chapter IX. Chapter X Fruiting phenology and natural production deals with the influence of climate variability on edible EcM fungi fruit bodies (Specific objective 4). Final, Discussion (Section IV) and Conclusion with research perspectives end this dissertation. References have been cited to highlight authors' contribution.

SECTION I: OVERVIEW ON ECTOMYCORRHIZAE

CHAPTER I: GENERALITIES ON MYCORRHIZAE

1. Definition

The name *mycorrhiza* means literally *fungus-root* and was invented by the German botanist Albert Bernhard Frank **(Frank, 1885; Trappe, 2005)**. It refers to a mutualistic relationship between roots of plants and soil fungi in which both partners benefit. Usually, the fungi scavenge nutrients and water from the soil with their hyphae finer than roots' hairs. In exchange, carbohydrates are supplied from plants roots.

It is hypothetised that symbiotic fungi played a key role in land colonization by first plants **(Hawksworth, 1991)**. The association had evolved some 450 to 600 million years ago as evidenced by first fossil record **(Remy *et al.*, 1994)**. Currently, symbiotic fungi represent 0.5 to 0.7 % of the global diversity of fungi estimated to 1.5 million **(Hawksworth, 2001)**. They involve members of the kingdom fungi (fig. 1) divisions Glomeromycota, Zygomycota, Ascomycota and Basidiomycota **(Piepenbring, 2015)**.

2. Mycorrhizae types

Fungus' hyphae colonise plants roots' hairs whilst its rest continues to grow through the soil, digesting and absorbing nutrients and water, and sharing them with its host plants. This colonisation follows various modes that determine two main mycorrhizae types and five derived subtypes (Fig. 2).

Mycorrhizae' first type refers to an intracellular and endotrophic colonization in which hyphae enter into the plant cells. Such mycorrhizae are termed Endomycorrhizae and encompass one basic type and two derived subtypes:

- **Arbuscular mycorrhiza is** the predominant and ancestral type **(Remy *et al.*, 1994; Wang and Qiu, 2006)**. Formerly known as vesicular-arbuscular mycorrhiza (VAM), it involves only fungi of the division Glomeromycota which hyphae form structures called arbuscules (sometimes associated with vesicles) in colonised plants cells. More than 90 % of all vascular plants including many crop plants are involved in that symbiosis **(Brundrett, 2004)**;

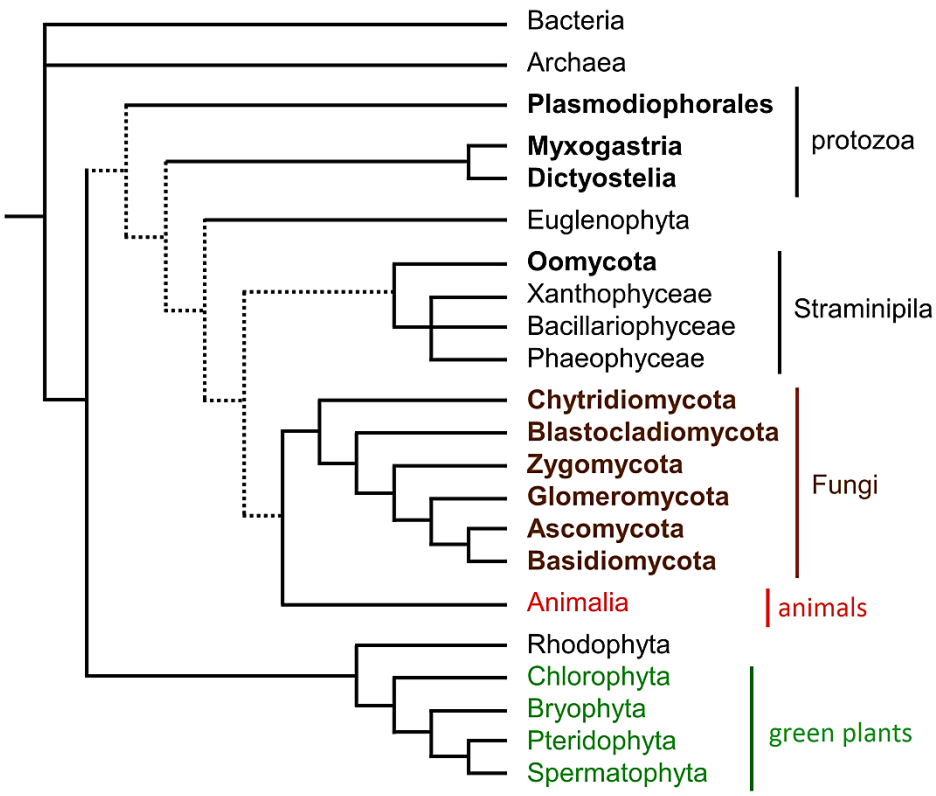

Figure 1: Simplified evolutionary tree showing genetic affinity between fungal phyla: (Ascomycota, Basidiomycota, Glomeromycota, Zygomycota Blastocladiomycota and Chytridiomycota) and with other living organisms. Source: **(Piepenbring, 2015)**

- **Ericoid mycorrhiza** is found on plants belonging to Ericaceae family. These plants are mostly found on acidic soils and harsh conditions of temperate and cold regions, and in mountainous area of the tropics. This stress-tolerance ability is conferred to these plants by their symbiotic fungi, mostly Ascomycetes that covered rootlets with a sparse network of hyphae that do not extend very far into the surrounding soil;
- **Orchid mycorrhiza** is formed on the roots of plants of the family Orchidaceae and a variety of basidiomycetes. These fungi come from a range of taxa including Ceratobasidium (Rhizoctonia), Sebacina, Tulasnella and Russula species **(Piepenbring, 2015)**. Within the cells of cortical layers of the roots and the protocorm, the fungal hyphae form a dense mass of coiled hyphae called a peloton. Each intracellular peloton lasts only a few days before it degenerates. The fungal hyphae collapse and are digested by the orchid cell. Orchid mycorrhizae are said mycoheterotrophic because unlike the other classes, symbiotic fungi provide carbohydrates to plants partners.

On the other hand, mycorrhizae second type refers to an extracellular and ectotrophic colonization called **Ectomycorrhiza** *sensus lato* (*s. l.*) in which hyphae do not enter plants cell. They encompass also one basic type and three derived subtypes:

- **Ectomycorrhiza** *sensu stricto* (*s. s.*) The hyphae penetrate between the outermost cell layers forming the so-called Hartig net. From this latter, hyphae, strands and/or rhizomorphs extend out to explore the soil. Ectomycorrhizae start to develop when hyphae infect the secondary or tertiary roots of woody species, which they seem to prefer, especially on trees **(Brundrett, 1991)**. Hyphae from the Hartig net grow inwards between epidermal cells and cortical cells, by forcing their way mechanically and by excreting pectinases; however, they never penetrate into cells or the stele (Fig. 3). Mycelium continues growing and extending surface area of exchange of substances between plant and fungus. It forms a network within the soil and leaf litter. The present dissertation deals exclusively with **Ectomycorrhizae** *s. s.* and all other mentions refer to it.

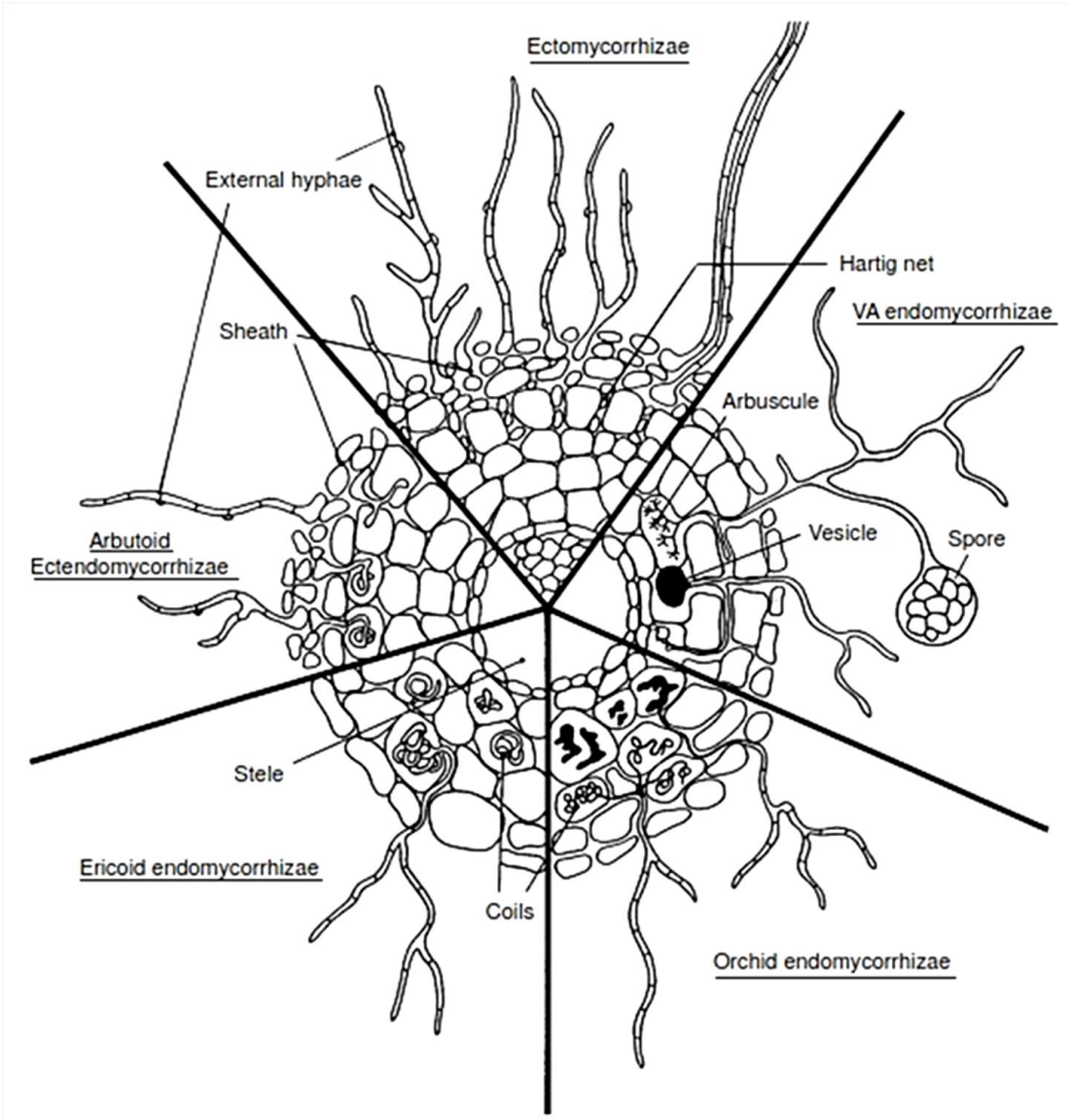

Figure 2: Mycorrhizae types modified after Selosse et Le Tacon (1998). **Source: Moore *et al.* (2011)**

- **Ectendomycorrhiza** exhibits characteristics of both ectomycorrhizae and endomycorrhizae. They are essentially restricted to the plant genera Pinus (pine), Picea (spruce) and, to a lesser extent, Larix (larch). They differ from the latter type by the penetration of the fungal hyphae into living cells of host plants' root. Symbiotic fungi are Ascomycota; most belong to the genus Wilcoxina.
- **Arbutoid mycorrhiza** derives from ectendomycorrhizae but involve plants of the subfamily Arbutoideae of Ericaceae and Basidiomycetes fungi.
- **Monotropoid mycorrhizae** are formed by the achlorophyllous plants of the subfamily Monotropoideae of the Ericaceae and are also mycoheterotrophs. Their main feature is the invagination of host plants rootlets cells walls by individual growing hypha inwards the cortical cell. These invaginations are called fungal pegs that proliferate and increase the surface area within the cell.

3. Importance of mycorrhizae in ecosystems

The ecological importance of mycorrhizae is stressed by the huge number of terrestrial plants and fungi engaged in these associations as well as the diversity of ecosystems where they occur. The long-dated co-evolution of both partners and the resulting land colonization are other proofs.

The major fact in mycorrhizae is the nutritional partnership in which both plants and fungi mutually supply to their nutritional needs. In general, it is a bidirectional movement where carbohydrates move from plant to fungus whilst nutrients (especially nitrogen, phosphorus) and water uptake is enhanced and translocated from fungus to plant. This exchange was hypothetised and confirmed by impressive studies which described the underlying physiological mechanisms **(Duddridge *et al.*, 1980; Read and Perez-Moreno, 2003; Simard and Austin, 2010)**. Thus, nutrient exchange is considered as basis of these mutualistic associations. However, the exchange direction is not always as described above. In fact in monotroip and orchid mycorrhizae, it is rather the fungi which provide carbohydrates from other surrounding plants species to their achlorophyllous plants partners. Those fungi are either parasitic like *Rhizoctonia* sp. in orchidaceous symbiosis; or ectomycorrhizal such as *Russula* sp. in monotroip symbiosis **(Moore *et al.*, 2011)**.

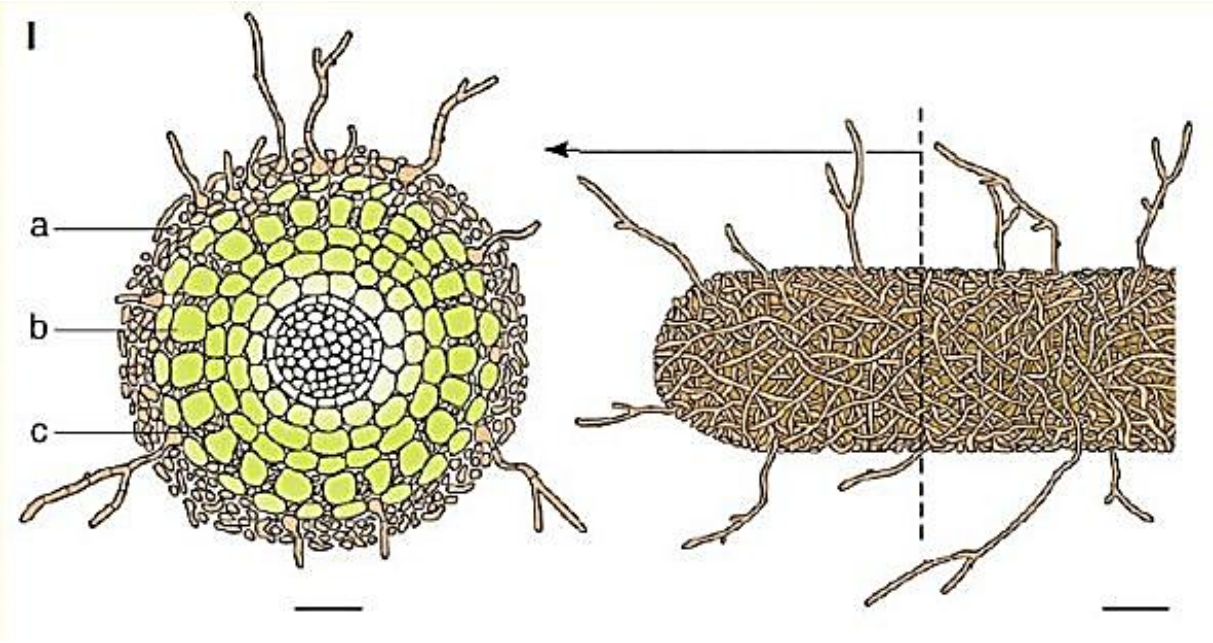

Figure 3: Ectomycorrhizae *sensu stricto* structure. a : hyphal sheath; b : cortical cells ; c : Hartig net **(Smith and Read, 2002)**

The direct consequence of nutrient uptake enhancement is the promotion of growth of colonised plants versus non infected ones of the same species. Tolerance of colonised plants to harsh environment conditions has been also reported in various ecosystems of the world (high level of soil acidity in temperate and boreal regions, drought stress in desert) where host plants are usually dominant species. Though mycorrhizal symbiosis is not an obligate symbiosis, their crucial role in development and stability of plant communities makes them ecologically obligate **(Buscot *et al.*, 2000)**.

CHAPTER II: THE ECTOMYCORRHIZAL SYMBIOSIS

1. Plant and fungi species involved in the symbiosis

Ectomycorrhizae are the most advanced symbiotic association between higher fungi and higher plants. Those plants are usually dominant in large areas of the world (Fig. 4) and have important economic value as the source of timber. About 8000 plant species **(Piepenbring, 2015)** belonging to 140 plant genera and 43 plant families form ectomycorrhizae. They include species of Pinaceae, Fagaceae, Betulaceae, Nothofagaceae, Leptospermoideae of Myrtaceae, Dipterocarpaceae, Phyllanthaceae, Gnetaceae Sapotaceae Proteaceae Sarcolaenaceae and Asteropeiaceae and Amhersteae of Caesalpiniaceae in temperate, subtropical and tropical climates **(Ducousso *et al.*, 2008; Moore *et al.*, 2011)**. Ectomycorrhizae involve 7 - 10,000 fungal species, members of the kingdom fungi divisions Zygomycota, Ascomycota and Basidiomycota **(Taylor and Alexander, 2005)**.

2. Fruit body diversity

Ectomycorrhizae fungal species belong to 82 lineages and 256 genera **(Tedersoo and Smith, 2013)**. Their teleomorph state result in epigeous (aboveground) or hypogeous (belowground) fruit bodies in a wide variety of forms, colour and size. Former classification of species was based on macroscopic characters such as the type of hymenophore as illustrated in fig. 5.

Ectomycorrhizal fungi include common aboveground woodland mushrooms with gills (Amanitaceae, Russulaceae, etc.), mushrooms with tubes (Boletaceae), sequestrate mushrooms (gasteromycetes), coral like fungi (Clavariaceae), mushrooms like fungi (Cantharellaceae) and most belowground fungi such as truffles. Such species belong to phylum Basidiomycota. EcM fungi are solitary, gregarious, caespituous or fasciculate fungi (Fig. 6).

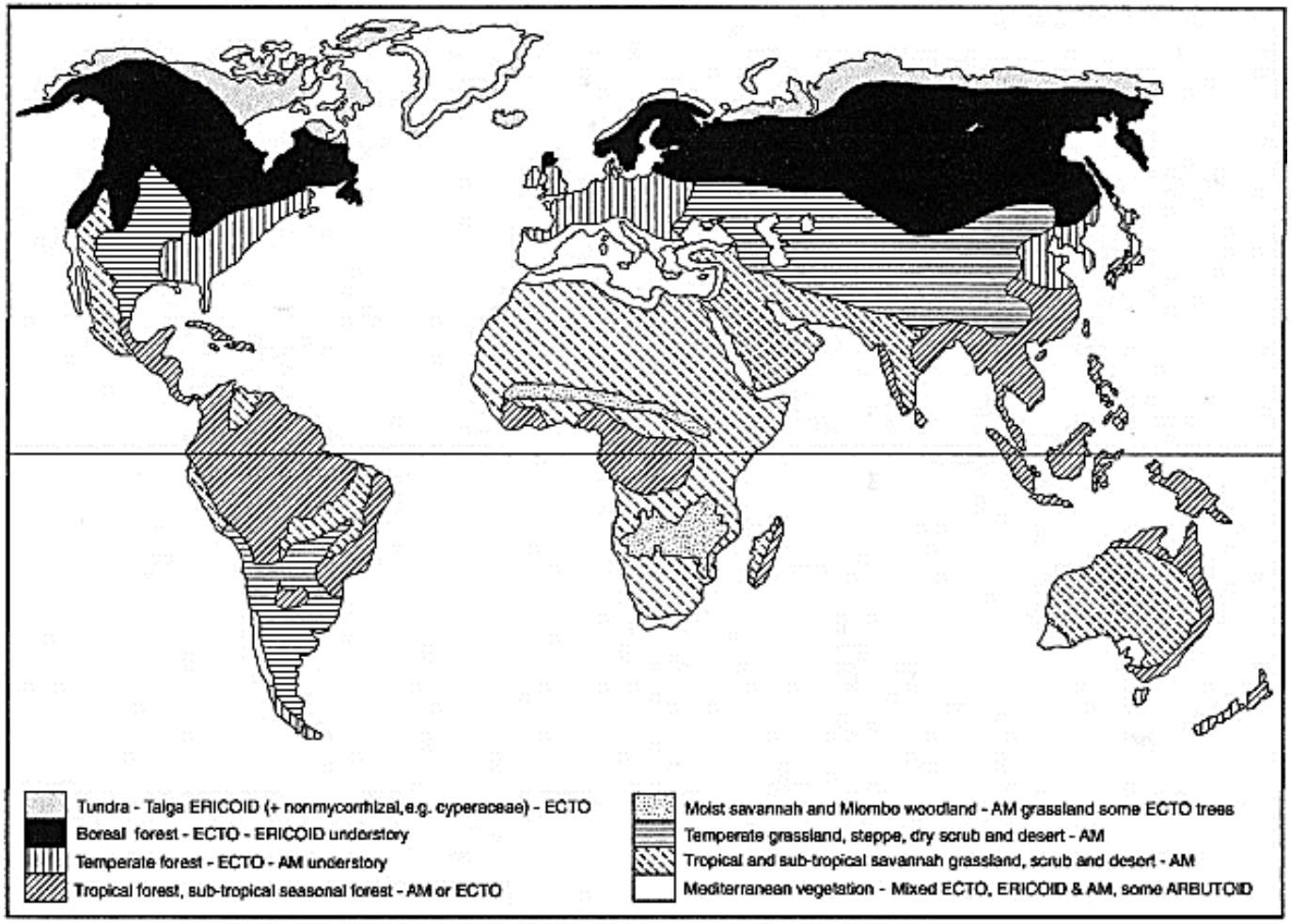

Figure 4: Map showing distribution of boreal forest (black) and heathland-type (grey stipple) communities and the patterns of dominance of ectomycorrhizal (ECTO) and ericoid mycorrhizal plants within these biomes. AM: arbuscular mycorrhiza. Source **(Read *et al.*, 2004)**

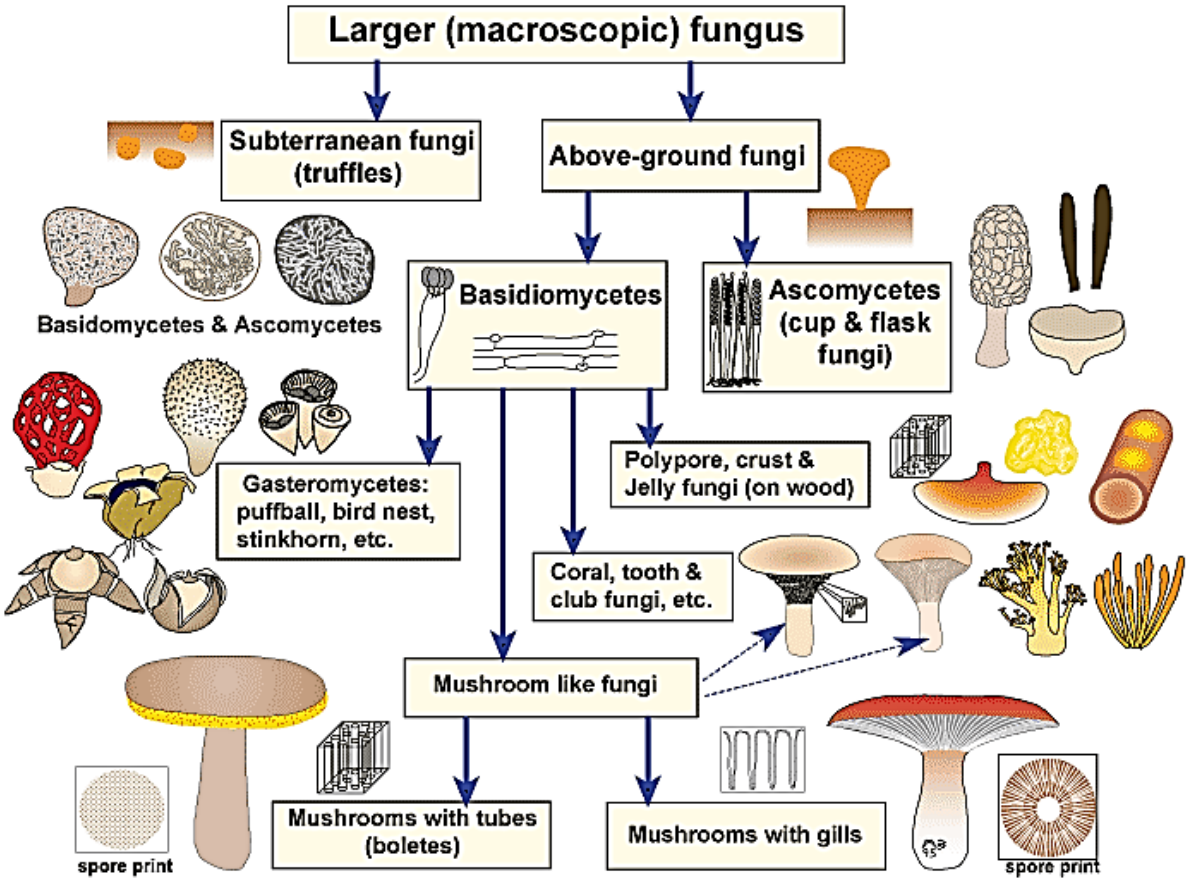

Figure 5: Main macroscopic characteristics of larger fungi according to fruit body types. Source: Brundrett (2008), http://mycorrhizas.info/ecmf.html#

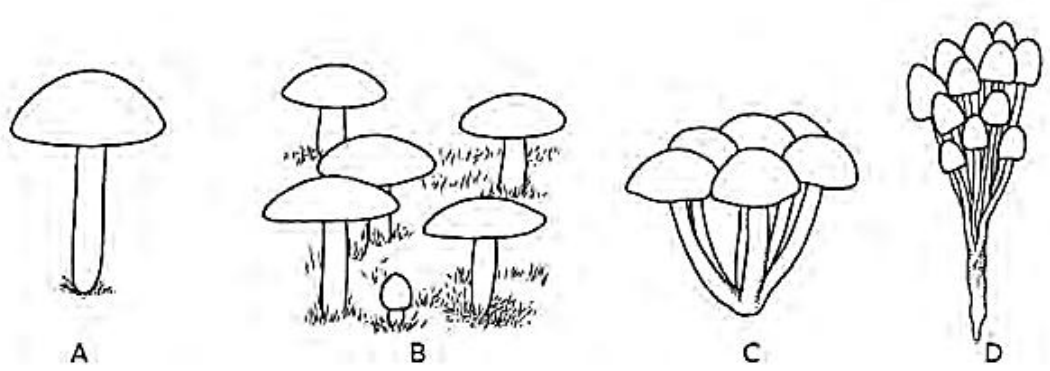

Figure 6: Mode of growth of fungi. A: solitary; B: gregarious; C: caespituous; D: fascilulate. Source: **(Eyi Ndong *et al.*, 2011)**

3. Ectomycorrhizae and exploratory types

Colonised host root tips called ectomycorrhizae are usually shorter and thicker than uncolonised ones because the fungal sheath reduces root growth. Ectomycorrhizae have usually the same colour with fruit bodies.

The extracellular Hartig net is the interface of exchanges between plant and fungi. Single hyphae or specialised aggregates called rhizomorphs radiate from the Hartig net into soil in more or less branched networks to scavenge nutrients and water **(Buscot *et al.*, 2000; Agerer, 2001)**. **Agerer (2001)** attempted the ecological classification of ectomycorrhizae into exploration types based on the development and differentiation of the extramatrical mycelium (amount of emanating hyphae or the presence and differentiation of rhizomorphs). Thus, exploration types are ***contact exploration types*** (hyphae close to sheath) or ***short to long distance types*** (more or less far-reaching rhizomorphs) with subtypes ***fringe, mat, smooth or pick-a-back exploration*** (Fig. 7)**.**

4. Interaction specificity within ectomycorrhizae

Mycological studies conducted in temperate and boreal zones but also in paleo- and neotropical zones indicated that one EcM fungus may associate with many host plants whilst one tree partner may host many symbiotic fungi **(Molina and Trappe, 1982; Molina *et al.*, 1992; Riviere *et al.*, 2007)**.

Consequently, EcM fungi have been classified into three groups according to their host specificity:

- **Low specificity** indicates that a symbiotic fungal species may associate with diverse hosts plants **(Diédhiou *et al.*, 2010)**;
- **Intermediate specificity** refers to limited host diversity**(Molina and Trappe, 1982)**;
- **Narrow or high specificity** refers to an association with a relatively high interaction between the host and the fungal species. The symbiotic fungus is usually in association with limited host species or with species within a particular genus **(Den Bakker *et al.*, 2004)** or a single plant family **(Bruns *et al.*, 2002)**.

Host specificity may limit some host plants migration suggesting a determinant influence of rates and directions of ecosystem change **(Cullings *et al.*, 2000)**.

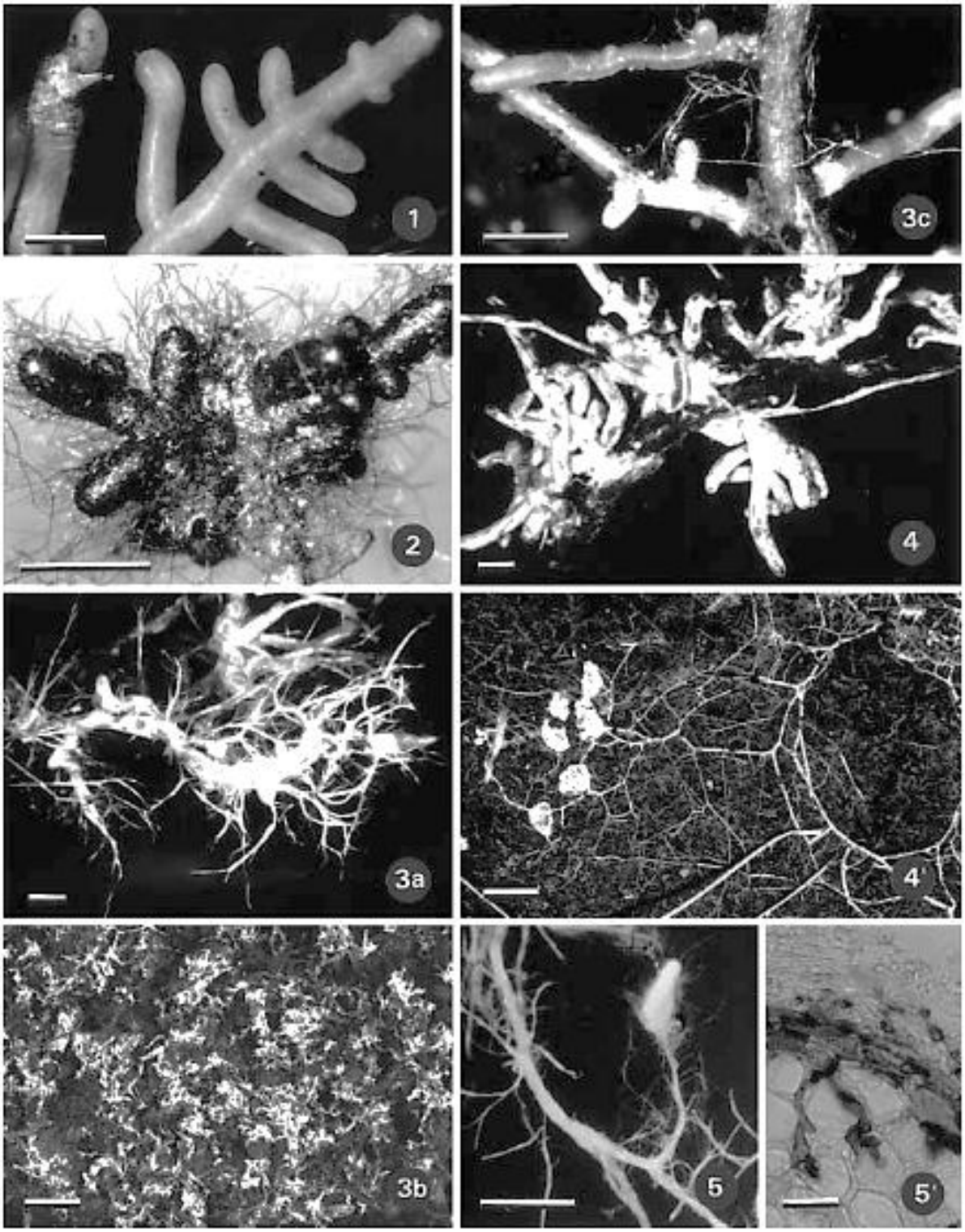

Figure 7: Examples of exploration strategies. 1: contact; 2: short-distance exploration; 3a: medium distance fringe exploration; 3b: medium-distance mat exploration; 3c: medium-distance smooth exploration; 4: long-distance exploration, monopodial ectomycorrhizal system; 4': long-distance exploration, tuberculate mycorrhizal system; 5: pick-a-back exploration; 5': hyphae stained blue with Melzer's reagent in ectomycorrhiza of Rhizopogon sp.: pick-a-back exploration. Bars: 1, 2, 3a, c = 4 1 mm; 3b = 10 mm; 4', 5 = 5 mm; 5' = 30 µm. source: **(Agerer, 2001)**

5. Importance of ectomycorrhizal symbiosis

5.1. Nutrient recycling

As many other soil inhabiting fungi, EcM fungi produce and exude in their habitats secondary compounds that help breaking down various complex molecules and soil aggregates. Similarly to saprotrophic fungi, they play a key role at the root - soil interface in various biogeochemical cycles such as in phosphorus cycle **(Plassard and Fransson, 2009)** and carbon cycle **(Simard and Austin, 2010; Orwin *et al.*, 2011)**. They are also important in the transport and storage sink of these chemical elements **(Talbot *et al.*, 2008; Talbot and Treseder, 2010)**.

5.2. Nutrient and water uptake improvement

Nutrient and water uptake improvement is the main contribution of symbiotic fungi to the mutualistic association. Though this function is shared by all mycorrhizae types (basic and derived subtypes), EcM symbiosis appear to be the most efficient one by the abundance of ectomycorrhizae and the development of exploration types **(Agerer, 2001)**. Uptake improvement is achieved by the mycelium that increases considerably host absorbing surface of minerals and water. Moreover, EcM fungi mobilise nutrients from environment by enzymatic digestion and make them available to their host plants. In addition to the high efficient translocation of nutrients back to host root, fungal sheath serves as sink for nutrients that are supplied to plant when needed. As result, host performance is enhanced leading to better growth and survival rates of host and especially of seedlings establishment. However this function effectiveness is species specific and even strain specific **(Burgess *et al.*, 1994)**.

5.3. Plant networking

Hyphae and rhizomorphs grow within soil and infect individuals of different age of the same plant species but also individuals of other species. They create a hyphal network linking EcM adults trees and seedlings **(Brownlee *et al.*, 1983; Ishida *et al.*, 2007; Bâ *et al.*, 2012)** through which nutrients and water are shared **(McKendrick *et al.*, 2000)**. Improved growth and performance result in a better establishment of seedlings, resilience to stress and disturbance, and lead to the usually observed dominant communities of EcM trees in infested

habitats **(Dickie *et al.*, 2002; Van Der Heijden and Horton, 2009; Dickie *et al.*, 2014)**. The network establishment is underpinned by the low association specificity **(Newbery *et al.*, 2000; Onguene and Kuyper, 2002)**.

5.4. Disease resistance of host plants

Ectomycorrhizal fungi increase disease resistance of host species by the physical barrier of the hyphal sheath, the production and exudation of antibiotics (antibacterial and/or antifungal molecules) as organics acids **(Duchesne *et al.*, 1988; Whipps, 2004)**. Root tips colonisation by symbiotic fungi induces biochemical changes in plant defence mechanisms resulting in resistance to further infection by pathogens.

5.5. Mycoremediation

Nutrients recycling and/or scavenging by symbiotic fungi permit the removal and storage of toxic heavy metals like zinc, cadmium and arsenic from soil. In such harsh conditions, host plants can grow without any tissue damage **(Hartnett and Wilson, 2002)**. That property is useful in highly polluted land areas restoration.

5.6. Other ecological services

All above advantages of ectomycorrhizal symbiosis are exploited in forestry (forest management, adaptation of exotic plant species to new environments, reforestation). In addition, aesthetical and cultural values of associate edible fruit bodies are always exploited worldwide **(Boa, 2004)** and in Africa **(Yorou *et al.*, 2001; De Kesel *et al.*, 2002; Härkönen *et al.*, 2003; Boa, 2006; Eyi Ndong *et al.*, 2011; Yorou *et al.*, 2014)**.

Expansion of mycelium in soil improves soil structure by aggregating its particles **(Simard and Austin, 2010)** whilst many small animals (microarthropods and rodents in particular) feed on mycelium and/or hypogeous fruit bodies **(Malajczuk *et al.*, 1987)**. Those animals are primary vectors of spore dissemination through faeces **(Maser *et al.*, 1978; Colgan and Claridge, 2002; Garkaklis *et al.*, 2003)**

CHAPTER III: IDENTIFICATION TECHNICS OF ECTOMYCORRHIZAL FUNGI SPECIES

1. Morphological identification of EcM fungi

The fisrt step of a fungus determination requires the observation and recognition of macroscopic characters visible to the naked eye or with a magnifying glass. Thougth it is a tedious task requiring sound taxonomic skills, it is essential to obtain a reference specimen of scientific value and determine many species.

EcM fungi grow directly on soil in the contrary of parasitic and saprotophic fungi. However, some saprotrophic fungi are observed growing on dead plants materials buried in soil. Nevertheless, they can be discriminated from EFFB taking into account some macroscopic features. Whilst harvesting EFFB, one should pay attention on the presence / absence of the fungus mycelium and / or ectomycorrhizae, and note their color. One should also ensure that no ephemeral feature is lost during harvest and handling of specimens. Such features should be noted in field notebook as well as changes in color. In situ and ex situ technical photographs are therefore useful to document fungi morphology in nature and any change after removal from field.

EFFB general shape (the so-called habitus) encompasses basic organs such as the cap (pileus) and the stipe. Each organ has other structures that allow fungus determination. The colors of the various structures, their possible changes and the time of occurrence of these changes are other discriminants of species, genera and families (annex 2). In mycology, the 'Methuen Handbook of color' **(Kornerup and Wanscher, 1978)** is a reference; however, it is currently almost impossible to find it **(Eyi Ndong *et al.*, 2011)**

1.1. Pileus and context

The cap or *pileus* is a very variable structure in terms of shape, dimensions, topography, color (and its possible changes), the margin and cuticule or *pileipellis* (as well as its separability, its elasticity, its viscosity). The cap flesh or context is also considered.

In Boletaceae, The cuticule may vary from glabrous to fibrillose, squarrose, squamulose, velutinous, tomentose or floccose. When aging, pilei may become rimose on the margin and areolate, and even frustose in prolonged dry weather periods. Young pileus margin is usually entire, smooth and with no ornamentations except for genera such as *Strobilomyces* **(Gelardi *et al.*, 2013)** and *Veloporphyrellus*.**(Li *et al.*, 2014)** Those genera bear velar remnants at the

margin. Boletaceae context is usually soft and spongy with shades of yellow or white even red. Pileus color may be whitish, black or different shades of brown, pink, red, yellow, orange, purple or green, alone or in combination. The pileus color is constant for a given species thought slight changes may occur with aging. Color changes of cuticule is observed in many species when bruised **(Chan, 2010; Wu *et al.*, 2014)**.

In Amanitaceae, EcM symbiosis occurs only with species belonging to genus *Amanita*. The pileus is more or less dry and the remnant of universal veil is often evident in the form of warts or patches. Species have the margin of their pileus appendiculate or never, or may be lined with prominent striations according to the genus sections. When present, appendiculate margin is decorated with more or less floccose or powdery, hanging materials that disappear when the fruit body matures **(Neville and Poumarat, 2004)**. The color of the pileus varies from white to shades of gray, yellow, red, orange and brown. In addition, pilei are usually easily separable from stipe. Their caps feel dry rather than slimy.

In Sclerodermataceae, only genus *Scleroderma* fruit bodies were observed at studied habitats. The general habitus in that genus is gasteroid that refers to a more or less reniform-shape fungus with very thick and tough outer wall called peridium **(Sanon *et al.*, 2009)**. The peridium may be smooth or scaly warted. Scleroderma context is thin and whitish to yellowish. It may stain pink to red when the fruit body is cut open according to the species. When mature, the peridium dries, cracks irregularly and leaves a large opening by which mature spores are released.

EFFB in Russulaceae are most represented in genera *Lactifluus*, *Lactarius* and *Russula*. They differ from other gilled genera by their granular and brittle context due to the presence of rounded cells called sphaerocytes **(Buyck, 1993, 1994, 1997; Verbeken and Walleyn, 2010)**. They break easily like a piece of chalk. Those genera produce fleshy fruit bodies that exude latex when crumpling or cutting in fresh material especially in genera Lactifluus and Lactarius. This is due to the presence of lactiferous hyphae intermixed with trama **(Cairney and Chambers, 2013)**. The color, taste, viscosity and abundance of this latex are important species determination features. This latex is also called milk when white but it can be watery or brightly coloured. Color changes of latex may occur with time or may be immutable (constant). All genera have species that pilei displaying dull to very brightful colors with size ranging from few mm to many cm in diameter. According to **Wang *et al.* (2015)**, pileus color

in genus *Russula* is highly variable within a species making difficult to distinguish many closely related species within this genus **(Miller and Buyck, 2002)**.

Cantharellaceae contains species with fleshy and trumpet-shaped or triangular in cross-section fruitbodies. Their pilei grow indefinitely, resulting in ruffled and wavy shape in age. Their fruit bodies are brightly colored **(Cairney and Chambers, 2013)**. The context is usually concolor to the pileus. It is not the case for Inocybaceae that have small to medium-sized fruit bodies identifiable by their silky or slightly scaly radially-fibrillose pilei **(Larsson *et al.*, 2009)**. Cortinariaceae are characterized by the sliminess of the gilled cap and / or stipe that delimit subgenera. The pileus may be hygrophanous or not, changing considerably of color in dry weather conditions. In the contrary to other EFFB, Clavulinaceae do not have a pileus **(Duhem and Buyck, 2007)**.

1.2. Hymenophore

The hymenophore is the part of the fruit body carrying the fertile hymenium on which the spores are formed. It can be smooth, wrinkled, laminated, tubular or protruding. Hymenium can be easily detachable from pilei or not. It differs from a family to another as presented below.

The gasteroid genus *Scleroderma* produces its spores through an internal spore bearing tissue calle the gleba. It is white or light brown at young and turn dark purple to brownish purple when mature. The dried and mature gleba is powdery and spores are passively discharge in the air **(Jeffries, 1999; Cairney and Chambers, 2013)**.

Boletaceae differ i by their hymenophore made of soft, moist, putrescent tubes. The tissue bearing spore, the hymenium is the inner lining of tubes and spores are released in the air thougth pores. The pores diameter, shape (roundish, regularly or not angular, elongate angular, irregular or labyrinthoid), color and its changes in time when bruised or cut, and the number of pores per cm of radius are important characters to record **(Heinemann, 1954, 1966)**.

In the contrary to the previous family, Amanitaceae have gills that are eitherentirely free from the stipe (free lamellae) or attached only by fine lines (emarginate lamellae). Most Amanitas' gills are white or pallid, though some species have gills that are either entirely

yellow or that have yellow edges. Russulaceae are also gilled fungi with attached, sub decurrent to decurrent lamellae. As in the context, gills are also granular or brittle in the most common **(Hutchison, 1999; Buyck *et al.*, 2008; Verbeken and Nuytinck, 2013)**. Cortinariaceae have gills with very variable color at young. At maturity, those gills turn to ochre or bright orange brown (rusty brown) due to spore color. A partial veil composed of fibers called cortina (from which the name of the family derives) covers their gills when young. Those tiny fibers of the cortina are ephemeral and disappear, or they frequently collapse against the stem to create a ring zone

In Cantharellaceae, the hymenophore / hymenium is either smooth or folded, and differs from true gills by gill-like ridges that run deeply down the stipe **(Cairney and Chambers, 2013)**. In the contrary, Clavulinaceae differ by the absence of pileus. However, they bear on branchs tips amphigenous hymenia **(Uehling *et al.*, 2012)**.

1.3. Stipe

A stipe is the stem-like organ supporting the cap of some fungi. It is composed of sterile hyphal tissue like the other parts of the fruit body. It is present in many macrofungi that are said stipitate but some other macrofungi do not have stipe are called gasteromycetes or sequestrate fungi. Most of the families have both kinds of fungi.

At the beginning of its development, an Amanita species is more or less enclosed in a specialised tissue called the universal veil that may remain on its pileus' cuticule and at the base of its stipe **(Neville and Poumarat, 2004)** Stipe in genus Amanita is generally central with base varying from a species to another. Indeed, stipe base can be more or less abruptly bulbous, elongate, radicant or tapering gradually. Stipe can also present at its base a universal veil in the form of a volva. Velar remnants may also stay on the stipe such as partial veil forming a ring. The ring (also called annulus) may be fragile and ephemeral or not. The volva may have different shapes, consistency, inner and outer sides colors **(Beeli, 1935)**.

Boletaceae have also central stipes that can be cylindrical, clavate, subclavate or ventricose. Their colors range from light (white, yellowish, pinkish) to darker colors (brown, red, black) and even sometimes a blending of colors. The stipe cuticule, the stipitipellis, may present innate structures (texture) and have different wetness touch (dry, viscid, moist). As for the texture, the stipe can be glabrous, floccose, reticulate, fibrillose or tomentose. The

fungus mycelium may be found at the base of the stipe. The presence of partial veil in Boletaceae is an important taxonomic feature. This veil may be in the form of pulverulent and detersile layer as in genus *Pulveroboletus* **(Degreef and De Kesel, 2009)** or annulus (membranous) covering the hymenium at early stage as in genus *Veloporphyrellus* **(Li *et al.*, 2014)** and *Strobilomyces* **(Gelardi et al., 2013)**. The context of the stipe is typically the same as in the pileus. As other agaricoid, Cortinariaceae have central stipe and are characterized by the presence of fibrous partial veil, the cortina, from which derived the family name. The ephemeral fibers of cortina disappear at maturity except for some species where limited remnants stay on the stipe **(Itoo *et al.*, 2015)**. The genus *Cortinarius* is also characterized by the shape of the stipe base, bulbous or marginately bulbous while the stipe general shape is cylindrical or clavate. Among other agaricoid fungi, Inocybaceae have stipes that may be of uniform thickness or with distinctly bulbous base **(Larsson *et al.*, 2009)**.

As for Russulaceae, they have brittle stipes that breaks like a piece of chalk, and either flow latex in *Lactifluus* and *Lactarius* or not in *Russula*. The brittle structure of Russulaceae often results in hollow or chambered stipe; however, the stipe can be solid. It is cylindrical or tapering downwards. They never have volva but a more or less fibrous or membranous partial veil is observed in some tropical species **(Heim, 1938)**. In Clavulinaceae, the generic concept encompasses coralloid and branched fruit bodies **(Uehling *et al.*, 2012)**. Generally, fruit bodies are solitary or subcaespitose and gregarious. They vary in shape from relatively simple or sparsely branching (the clavarioid forms) to much more complex, profusely branched, and well-developed (the ramarioid structures). Unbranched, infundibuliform or resupinate fruit bodies have been also described in the family **(Henkel *et al.*, 2005)**.

Genus Scleroderma, stipes are not well developped and are usually replaced by more or less thick rhizomorphs **(Sanon *et al.*, 2009)**.

1.4. Color changes

Many colors changes are observed in EFFB. They occur with fruit body aging but also in case of bruise or cut. Pileus and stipe context may change when exposed to the air or damaged. Color changes occur in numerous species of Boletaceae and are either bluing or browningblacking **(Chan, 2010; Wu *et al.*, 2014)**. As for few species of genus *Amanita*, they

blush pink or reddish when bruised, or develop reddish discolorations with age. They are said rubescent.

1.5. Smell, taste and edibility

Taste is detected by chewing chewed briefly in the front of the mouth without swallowing it. It may be neutral, mild to acrid, pungent, acidic, sweet fruity, fungal, soapy, acidulous, earthy and farinaceous. The speed of appearance of taste in the mouth and its possible change with time are also recorded. Similarly, the odors are sometimes very pronounced, from fungal to fruity, nauseating, fetid, rancid, spermatic, farinaceous, coconut, etc. **(Eyi Ndong *et al.*, 2011)**. It is worth mentioning that some fungi are poisonous and may cause severe damages up to death. It is the case for many Amanita species.

1.6. Spore print and basidiospores shape

The color of spores is one of the most important characteristics for the identification of Basidiomycetes. It is usually determined by performing a spore print or direct observation of spores deposit in mass on the top of the stipe at the maturity. The collected spore print is usually dried and any color change is recorded. Then, prints are stored in a paper envelope attached to the specimen and kept in the herbarium **(Eyi Ndong *et al.*, 2011)**.

Basidiospores display various shape and ornementations according to species. They are usually evidenced using appropriate reagents such as Melzer reagent (iodo-iodide chlorate). Indeed, non-amyloid spores stain light yellow to brownish yellow color whislt amyloid ones stain bluish-gray to blackish shade. For spores containing glycogen or dextrins (dextrinoids or pseudo-amyloids spores), they take a brown mahogany to vinous brown tint **(Cairney and Chambers, 2013)**. The spores may present or not ornamentations such as spiny, sub-reticulate and reticulate, smooth to strongly verrucose, ellipsoid, amygdaliform or nodulose / angular **(Larsson and Larsson, 2003; Cannon and Kirk, 2007)**

2. Molecular identification of EcM fungi

2.1. Limits of morphological identification methods

Morphological identification of ECM sporocarps is difficult and requires profound knowledge and experience. Mistakes are frequent due to the homoplasy of phenetic characters. Many ambiguities were observed in some genera classification where closely related species could not be discriminated based on morphology **(Itoo *et al.*, 2015)**. Moreover, identification criteria as used in temperate zones might not be applicable for tropical zones **(Wang *et al.*, 2015)**.

However, as many studies demonstrated the limits of morphological-based identification **(Gibson and Deacon, 1988; Danielson and Pruden, 1989; Dahlberg, 1991)**, molecular techniques were efficiently used to identify symbiotic fungi by amplification and sequencing of specific regions of their respective DNA **(White *et al.*, 1990; Gardes and Bruns, 1993)**. Using standardised methods, about 80 % of the estimated 100 000 described fungal species were sequenced and their ribosomal DNA (rDNA) deposited in public databases **(Hibbett *et al.*, 2007)**. However, all this impressive work was mainly done in temperate and boreal zones, and tropical zones out of Africa.

2.2. Development of molecular markers

The use of the polymerase chain reaction (PCR) in EcM communities identification led to a rapid development of that area. Primary amplification targets were ribosomal genes and spacers that combine the advantages of high copy number, highly conserved sequence tracks that can serve as sites for primer design, and variable regions between the priming sites **(Horton and Bruns, 2001)**. Various primers were designed for various taxonomic levels **(Cullings, 1992; Bruns *et al.*, 1998; Egger and Hibbett, 2004)**.

Several DNA regions were tested as molecular markers for fungi studies. Among them, many successful regions used for other organisms such as animals and plants failed to amplify in fungi because of inclusion large introns, low PCR amplification and sequencing success. Those regions included the mitochondrial Cytochrome C Oxidase subunit 1, the nuclear ribosomal RNA cistron the largest subunit of RNA polymerase II, the second largest subunit of RNA polymerase II, and mini chromosome maintenance protein **(Schoch *et al.*,**

2012). Thus, the nuclear ribosomal large subunit was widely preferred based on higher species resolution.

2.3. Internal transcribed spacer (ITS) region, the primary fungal barcode marker

The ITS region (Fig. 8) refers to the region of ribosomal DNA delimited by the 3P end of the 18S gene (the small subunit, SSU) and the 5P end of the 28S gene (the large subunit, LSU). That region encloses the ITS1 spacer, the 5.8S gene and the ITS2 spacer **(Buscot *et al.*, 2000)**. The fungi ribosomal DNA unit (rDNA unit) varies from 650 – 900 bp in size **(Gardes and Bruns, 1993)**.

ITS1 and ITS2 regions are variable non-coding regions nested within the rDNA repeat, and between the highly conserved and coding small subunit 5.8S and large subunit rRNA genes. That polymorphism by juxtaposition of conserved and variable regions, and its high copy number, is the base of the efficiency of ITS region in fungi identification **(Horton and Bruns, 2001)**. However, variation in length has been observed within as well as between species **(Feibelman *et al.*, 1994)**. The total ITS size rangs from 500 to 800 bp in various Ascomycetes and Basidiomycetes **(Toju *et al.*, 2012)** even 415-character-long in genus *Tomentella* **(Jakucs *et al.*, 2005)**.

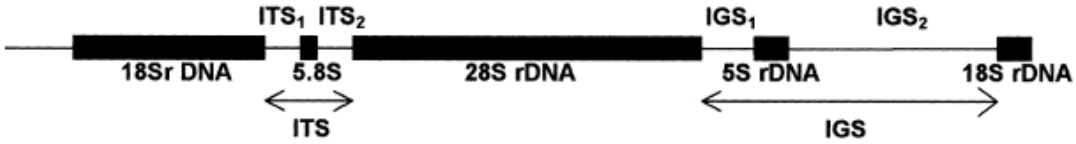

Figure 8: Schematic representation of the ribosomal DNA unit with the ITS and the IGS regions. The dark bars represent genes with highly conserved sequences, whilst the sequences of the spacers (thin lines) are more variable. Source **(Buscot *et al.*, 2000)**.

SECTION II: MATERIALS AND METHODS

CHAPTER IV: THE STUDY AREA

1. Generalities on Comoé National Park

The present study was carried out at Comoé National Park (CNP). It is located at latitude 8°32' - 9°32' North and longitude 3°01' - 4°24' West in the far North-East of Cote d'Ivoire, south to the border with Burkina Faso. It is in the Boukani region, between the towns of Bouna and Dabakala. The CNP covers about 11 500 km² **(Hennenberg, 2005)** and is presently one of the largest parks in tropical West Africa **(Sayre *et al.*, 2011)**.

The section east of the Comoé River was protected as a game park (the 'Refuge Nord de la Côte d'Ivoire') since 1926, then extended as the 'Réserve Totale de Faune de Bouna' in 1953. In 1968, the Comoé National Park was established, including forest areas west to the Comoé River **(Poilecot *et al.*, 1991)**. The CNP was internationally approved in 1983 and declared as Biosphere Reserve and World Nature Heritage by the UNESCO **(Hennenberg, 2005)**. Unfortunately, the recent unrest in Côte d'Ivoire, natural resources use pressures and mineral extraction activities had a very adverse effect on the site, with extensive poaching of wildlife and fires caused by poachers, intensive logging and over-grazing by large herds of cattle, and the absence of effective management over two thirds of the Park. All these factors cause the CNP to be listed among the 21 endangered World Heritage Sites in Africa **(Sayre *et al.*, 2011)**.

Located on the large granite stand of West Africa, Northern part of Côte d'Ivoire is characterized by a smooth and level relief with mean altitude of 300 m **(Poilecot *et al.*, 1991)**. Soils are impoverished sandy to loamy ferralsols above Precambrian granites with small areas of lateritic crusts or banks outcrop at some places **(Hennenberg *et al.*, 2005)**. The climate is a Guinea-Congolia/Sudanian transitional type, a sub-humid tropical climate **(Chidumayo *et al.*, 2010)** with mean annual rainfall of 1 011 millimeters (mm) falling mainly between March and October. The mean annual temperature is 26.5° to 27 °C **(Koulibaly, 2008)**. A single dry season lasts six months in the south and eight months in the north, with a cool and dry wind called "Harmattan" blowing in the region for three months.

The park is an area of transitional habitats ranging from forests to savannas including riparian grasslands and forests due to the presence of the Comoé River. The park is a semi-natural mosaic of forest-savanna with main anthropogenic impact being uncontrolled annual fire by poachers. Savannas in CNP cover 84.2 % of the park and include almost all types of savanna ranging from grassy to savanna woodlands. Gallery forests are found along the River

and its tributaries with estimated coverage of 2.3 %. Islands of dry forests cover 8.4% whilst a particular type of grassland growing on ferricrest covers 4.9 % of the park **(Poilecot *et al.*, 1991; Hennenberg *et al.*, 2005)**.

2. Selection of habitat types and establishment of permanent plots

Using a vegetation map of the CNP **(Poilecot *et al.*, 1991; Lauginie, 2007)**, one week-survey was undertaken in the park in order to select habitat types suitable for our study. According to numerous studies undertaken in neighboring countries (Benin, Togo and Guinea), Caesalpinioid- and Phyllantioid-dominated habitats are the most appropriate to monitor EcM fungi. Numerous similar habitats are present in northern and central part of the CNP, according to the vegetation map. However because of their inaccessibility, we could neither visit them nor selected them for this study because of their location. Indeed, habitat should be installed at a reasonable distance from the base camp in order to allow weekly surveys of fixed plots. Taking into consideration all restriction, three habitat types were selected at about 5-6 km north-east of the Ecology Research Station of Comoé (our base camp) with regard to (1) the presence and abundance of confirmed EcM tree partners, and (2) the distance to the station.

Distant of 300 m each other, the three selected habitat types (Fig. 9) are as follow:

- Habitat type 1: *Isoberlinia doka* Craib & Stapf dominated woodlands. Those woodlands harbour stems of other host plants namely *Monotes kerstingii* Gilg and *Uapaca togoensis* Pax;
- Habitat type 2: mixed woodlands dominated by *Uapaca togoensis* with few stems of *Isoberlinia doka* and *Monotes kerstingii;*
- Habitat type 3: *Uapaca togoensis* dominated woodlands. Though those woodlands harbour stems of *Monotes kerstingii*, no stems of *Isoberlinia doka* was recorded within them.

In each selected habitat type, three permanent plots of 30 m x 30 m each were established by mean of a 100-m decameter, making a total of nine plots (Fig. 10). They were labelled *FiPi* with *Fi* representing the habitat type and *Pi* the plot. All nine (09) plots were geo-referenced by recording the coordinates of each corner with a GPS Garmin GPSMAP® 62stc (Garmin International Inc., Olathe, KS, USA).

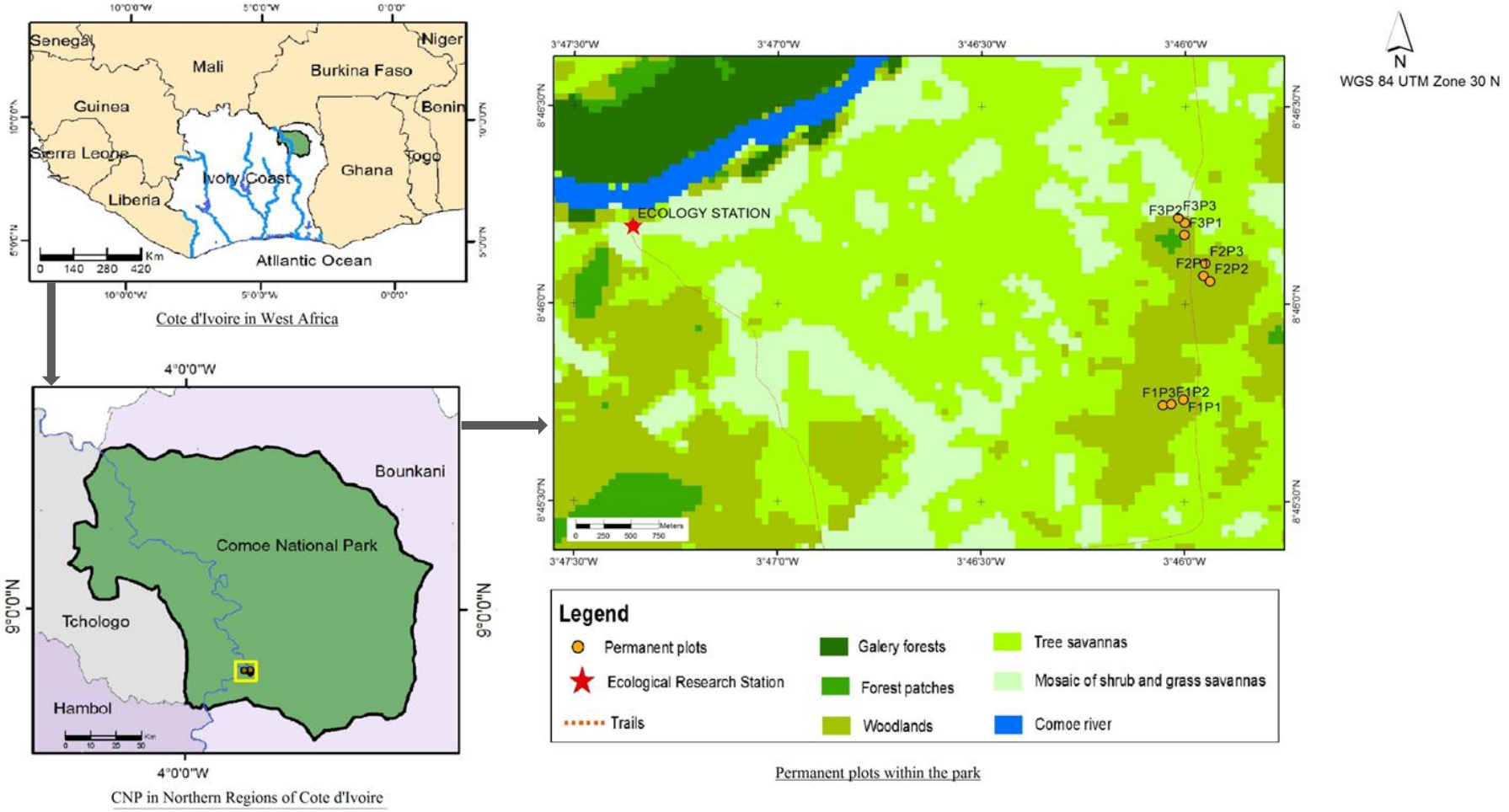

Figure 9: Location of Comoé National Park (north east of Côte d'Ivoire) and established permanent plots within it (south west of the park).

Plots were marked either by slashing the nearest tree at each corner or driving a stake that bore a colorful ribbon on its upper extremity into the ground. Plots within a habitat type were spaced at least by 10 m each other, according to tree partners' presence and density (table I).

Furthermore, understorey habitat in IW and MW were burned either totally or partially according to plot by the annual fire that passed in December 2013, four months before our arrival at the park. However, no plot in UW was burnt.

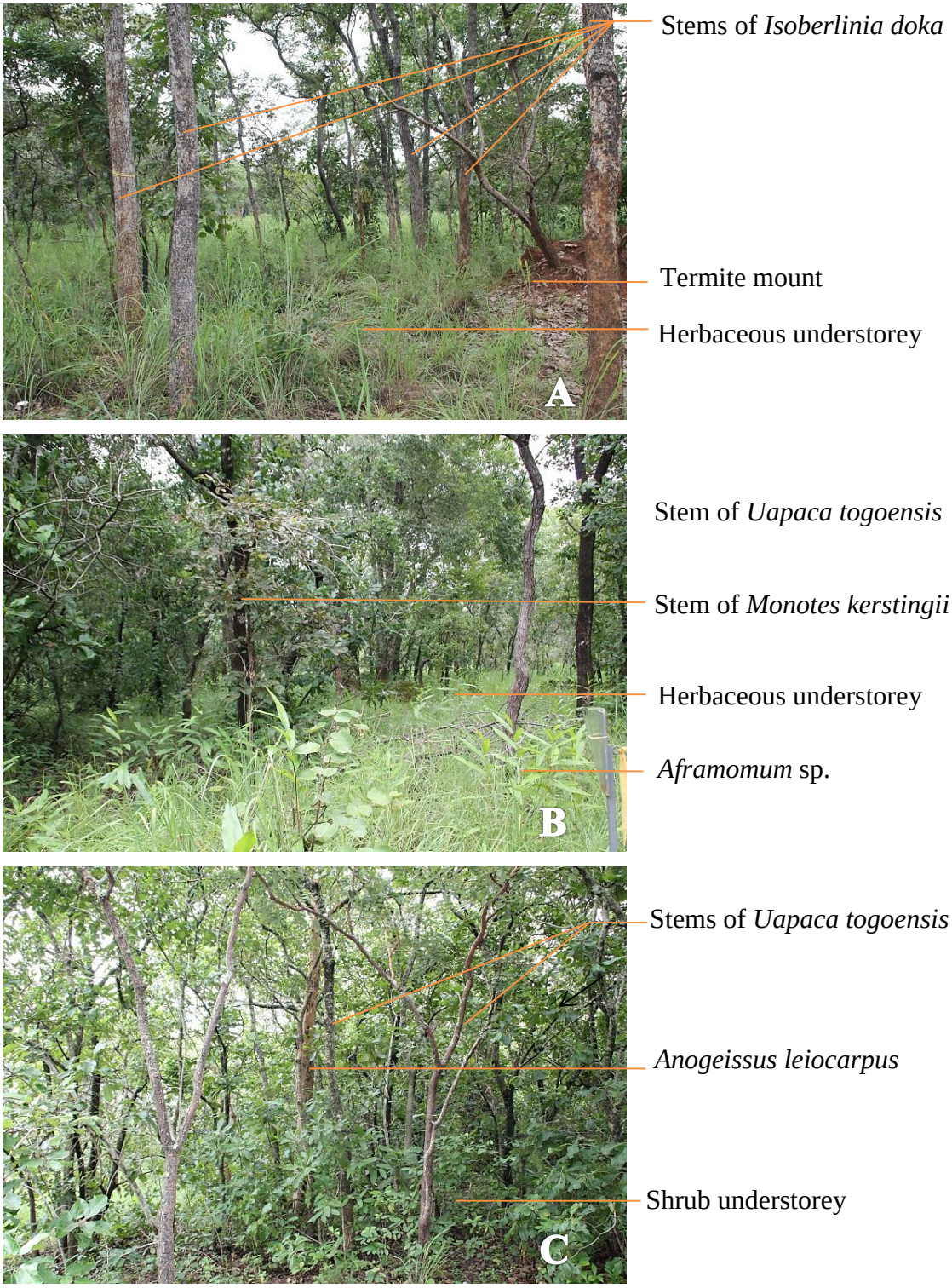

Figure 10: Structure of selected habitat types within Comoé National Park (CNP). A: *Isoberlinia* Woodland (IW); B: Mixed Woodland (MW); C: *Uapaca* Woodland (UW)

Table I: Positions of permanent plots within selected habitat types at Comoé National Park. dd: decimal degrees; m: meter

	Isoberlinia Woodland (IW)			Mixed Woodland (MW)			*Uapaca* Woodland (UW)		
Plot	F1P1	F1P2	F1P3	F2P1	F2P2	F2P3	F3P1	F3P2	F3P3
Latitude (dd)	8.76264	8.762447	8.762408	8.767876	8.7676	8.768387	8.769594	8.770105	8.7703
Longitude (dd)	- 3.7667	- 3.76719	- 3.76754	- 3.76588	- 3.766	- 3.76581	- 3.76668	- 3.76665	- 3.767
Altitude (m)	235.13	233.17	232.64	230.40	230.79	248.19	216.23	213.81	213.62

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CHAPTER V: MATERIALS

1. Biological material

Target biological materials were aboveground fruit bodies of ectomycorrhizal fungi species. However, plant specimens were also collected to make a herbarium for the accurate identification of partner trees.

2. Field equipment

To adequately record field data, following field material have been used:

- Rope rollers to establish plots;
- Strong field knives to harvest fungal fruit bodies;
- Baskets and aluminium paper to wrap and preserve fruit bodies during transportation to base camp;
- Journal papers for plants samples preservation;
- Field notebooks and writing material for recording preliminary morphological data of fresh specimens;
- A Canon EOS 1000D digital camera for making high resolution *in situ* and professional pictures of sampled specimens;
- A GPS Garmin GPSMAP® 62stc for recording geographic coordinates of sampling sites;
- Dataloggers VOLFRAFT DL-120 TH for measuring air temperature and relative humidity;
- TMS-3 Dataloggers soil moisture sensor for soil temperature and moisture recording;
- Non-recording rain gauges and their metal supports;
- A metal drill to collect soil cores.
- Field guides and illustrated monographs for preliminary identification of fungi;
- International standardized description sheets for macroscopic description of collected fungi;
- Lens (x20 and x 50 magnification) to observe tiny structures;
- Sharp cutters for longitudinal section of fruit body.

3. Laboratory equipment and reagents

At base camp, fungal and plant species handling and conservation required:

- Field guides and illustrated monographs for preliminary identification of fungi;
- International standardized description sheets for macroscopic description of collected fungi;
- Lens (x20 and x 50 magnification) to observe tiny structures;
- Sharp cutters for longitudinal section of fruit body;
- A weighting machine type Voltcraft TS-5000/1 portal scales for measuring fungal biomass;
- White paper and plastic cups for spore print making;
- An electric dryer type Stöckli Dorex to dry fungal specimens;
- Plastic bags type minigrip to conserve dried fungal specimens;
- Adapted presses for drying and pressing plants materials during sunny days;
- Incubator Binder for drying plants and avoid going mouldy during wet days.

Soil analyses required globally the following equipment and reagents (details given in Chapter VI: Methods):

- Weighting machine ;
- Grinder;
- pHmeter;
- Oven;
- Magnetic and electric agitators ;
- Autoclave Sano clav;
- Robinson-Kohn pipette;
- Spectrophotometer;
- Sieve columns;
- Beakers and capsules;
- Centrifuge type KUBOTA / 702 KR;
- Extension tubes;
- Distilled water;
- Potassium dichromate;
- Hydrogen peroxide;

- Sodium pyrophosphate;
- Acetate solution;
- Potassium hydroxide.

In addition, molecular analyses were performed to confirm fungal species identification but also to study their phylogenetic affinities and placement. Equipment used was as follows:

- FastPrep 24 Ultra-Rapid Thorough Sample Homogenization (MP Biomedicals, USA);
- Retsch Tissue Lyser Mixer Mill Grinder Homogenizer Cell Disrupter Retsch MM200;
- H122091 Velp Scientifica Wizard X Vortex Mixer F202a0175
- Water bath incubator;
- Fume hood ;
- Eppendorf Microcentrifuge 5424;
- Eppendorf 5804R refrigerated benchtop centrifuge for DNA extraction;
- Bio-RAD T100^{TN} Thermal cycler;
- ABI 3730 capillary sequencer;
- NanoDrop ND-1000 spectrophotometer for DNA quantification;
- Refrigerated benchtop centrifuge with microplate rotor to mix PCR tubes;
- An ultraviolet (UV) transilluminator;
- Freezers (+4˚ and -20˚ C);
- Flat Cap Tubes ;
- Quali-PCR-Tubes ;
- Gloves and tips ;
- Horizontal gel electrophoresis apparatus;
- D.C. power supply;
- Automatic micropipets with tips;
- Balance;
- Microwave;
- 250 ml flasks or beakers;
- Hot gloves, vinyl gloves and safety goggles;

Reagents used during molecular analyses are detailed in annexes III and IV.

CHAPTER VI: METHODS

1. Fungal survey

1.1. Sampling design

EcM fungi fruit bodies (EFFB) were sampled in each plot following parallel bands of 2 m large. To avoid missing short living species, each plot was visited once a week as implemented by **Yorou *et al.* (2001)** from April to early October 2014. They were identified based on growing substrate (soil) and known EcM habitus (amanitoid, boletoid, russuloid, etc.). They were also discriminated from other soil-growing lamellate fungi according to gills and fresh spore print colour for example.

1.2. Collection and handling of aboveground fungal fruit bodies

We recorded the nearest EcM tree partners to each sampled fruit body and geographic coordinates using GPS Garmin GPSMAP® 62stc (Garmin International Inc., Olathe, KS, USA). To facilitate future comparison and morphological identification of species, technical photographs of most representative fruit bodies per species (at different development stage, when applicable) were taken on field and at the base camp using a Canon EOS 1000D digital camera. Fresh morphological features were then recorded from specimens, following standardised description sheets (Annex 1) for tropical African fungi **(De Kesel *et al.*, 2002; Eyi Ndong *et al.*, 2011)**. Afterwards, fruit bodies per collection were enumerated, weighted, labelled and representative specimens were dried at 40 ˚C for 24 hours with a time-caliber food Dehydrator (Stockli, Switzerlands). Labelled collections were conserved with basic ecological data (habitat type, substrate, putative nearest partner tree, soil type, etc.) in plastic bags type minigrip as herbarium materiel at the West African Science Service Centre on Climate Change and Adapted Land Use (W.A.S.C.A.L.) Graduate Research Program (G.R.P.) Climate Change and Biodiversity of the University Felix Houphouet-Boigny, Côte d'Ivoire (U.F.H.B.-C.I.).

1.3. Morphological identification of fungal species

The identification of collected fungal species was performed based on morphological features as described with standardized sheet (Annex 1) at the Systematic Botany and Mycology Institute of University of Munich (LMU) in Germany and Botanic Garden Meise in Belgium by experts (**De Kesel and Yorou**, personal communication). Appropriate keys on

tropical mycology and illustrated monographs on fungi of Central and Western Africa (series of "Flore Iconographique des Champignons du Congo" and "Flore illustrée des Champignons d'Afrique Centrale") were used. These series include monographs on *Amanita* spp. **(Beeli, 1935)**, *Boletineae* and *Cantharellus* spp. **(Heinemann, 1954, 1959, 1966)**, *Scleroderma* spp.**(Dissing and Lange, 1963)** and *Russula* spp. **(Buyck, 1993, 1994, 1997)** and *Lactarius* spp. **(Heim, 1955)**. An additional monograph on *Lactarius* spp. **(Verbeken and Walleyn, 2010)** was also used. Species names and nomenclatural aspects were checked in *Index Fungorum* (http://www.indexfungorum.org/Names/Names.asp).

1.4. Molecular-based identification of fungal species

1.4.1. DNA extraction

DNA was first extracted using the ChargeSwitch® gDNA Plant Kit (Invitrogen, Darmstadt, Germany) as outlined in the user's guide. Its basic steps were as follows:

- **Sample lysing**. Small pieces of dried materiel (0.5 mm × 0.5 mm) were taken in Eppendorf tubes 0.2 ml containing different sizes of bulk beads and added at room temperature with 100 µl of pre-chilled (4 ˚C) Lysis Buffer (L18). Tissues were grinded with FastPrep machine set at 4500 g for 1 min. Then, 10 µl 10 % SDS and 40 µl pre-chilled Precipitation Buffer (N5) were added to lysate.
- **Magnetic beads binding.** After centrifugation, supernatants in new tubes were added with 10 µl 10 % Detergent (D1) plus 4 µl resuspended magnetic Beads. Gentle pipetting without bubbles forming evenly distributes the beads within supernatants;
- **Contaminants removal**. After 1 min incubation, tubes were placed in MagnaRack until the magnetic beads have formed a tight pellet. Supernatants were then removed carefully from tubes without moving them from racks. Afterwards, each pellet was immediately wash by adding 100 µl Wash Buffer (W12) to the tube that now were removed from the magnet. That step was repeated twice;
- **DNA elution from beads**. After complete discard of supernatant, tubes containing pelleted magnetic beads were removed from the rack. DNA was immediately eluted by adding 15 µl of Elution Buffer (E6). After gentle mixing and 1-min-incubation at room temperature, tubes were placed again on the magnetic rack until the beads have formed a tight pellet and supernatants were clear. Supernatants containing the DNA were carefully transferred to sterile microcentrifuge tube without disturbing the pellet and purified

genomic DNA (gDNA) were stored at – 20 °C until needed. Detailed procedure of that extraction method is given in Annex 3.

Because molecular study could not be completed during one scientific stay (short sojourn and large amount of samples) and non-availability of magnetic rack in laboratory back home, commercial kit extraction method was replaced by a modified CTAB method after **Gardes and Bruns (1993)**.

gDNA was isolated from dried sporocarps by CTAB method . For this 0.10 mg of material was taken in 2.5 mL Eppendorf tubes and to this 800 µl pre-warmed lysis buffer (2 % CTAB; 20 mM EDTA (pH 8); 100 mM Tris–HCl (pH 9); 1.4 mM NaCl and 0.3 % β-mercaptoethanol) was added. Dry samples were grinded with a 5 - mm bead in the Retsch Mill with several rounds of 45 s at 30 movements per second (mov /s). After incubation at 60 °C for 45 min, samples were extracted with an equal volume of chloroform: isoamyl alcohol and centrifuged for 5 min at 13,000 g at room temperature. This step was repeated twice, and then DNA was precipitated with a volume of 0.8 x of isopropanol at 4 ˚C and incubated for 10 min at - 20 °C. Afterwards, DNA was pelleted by centrifugation at 4 ˚C and 16,000 g for 5 min, washed with 70 % ethanol, air-dried and resuspended in 50 - 100 µl TE (pH 8) during 10 min at 60 ˚C. 2µl diluted (10 x) RNase A was added to samples, mixed and incubated 2 min at room temperature. Samples were kept cool hereafter until needed. Detailed steps are given in Annex 4.

1.4.2. DNA amplification

Polymerase Chain Reaction (PCR) was performed for amplification of the Internal Transcribed Spacers (ITS) region of the nuclear ribosomal DNA using a combination of primers, the fungi-specific primer ITS1F and the basidiomycetes-specific primer ITS4B **(Gardes and Bruns, 1993)**. Prior to amplification and to prevent inhibition, raw DNA extracts were diluted 0-, 10- and 100-fold or 25- to 1000-fold in sterile double-distilled water respective to the extraction method used.

With a final volume of 25 µl, reaction mixture was composed of 1 µl gDNA, 15.65 µl of sterile distilled water, 2.5 µl 10 x Buffer; 2.5 µl dNTPs (2 mM), 1.25 µl forward primer ITS1F (10 µM), 1.25 µl reverse primer ITS4B (10 µM), 0.75 µl $MgCl_2$ (50 mM) and 0.10 µl

Taq DNA polymerase (5 U/µl) (Invitrogen) as recommended by the manufacturer. Standard PCR amplification started by initialization step (94 °C for 2 min), followed by 5 cycles of denaturation (94 °C for 0.5 min) - primer annealing (52 °C for 2 min) - elongation (72 °C for 2 min), other 30 cycles with a decreased annealing temperature (50 °C for 1min), and a final elongation (72 °C for 5min).

However, to increase sensitivity and specificity of reactions, the Touchdown polymerase chain reaction TD-PCR **(Don *et al.*, 1991; Korbie and Mattick, 2008)** was used latter on. It is a modified PCR protocol that consists in a very high annealing temperature (2-6 degrees above melting temperature Tm of used primers) that decreases every cycle till the Tm of primers. In addition, DreamTaq DNA Polymerase (Thermo Scientific) was preferred since it provides higher PCR sensitivity and yields. Reaction mixture was made of 4 µl gDNA, 10.1 µl sterile distilled water, 2 µl 10 x DreamTaq Buffer (already supplemented with 20 mM $MgCl_2$); 1.6 µl dNTPs (2 mM), 0.3 µl forward primer ITS1F (10 µM), 0.3 µl reverse primer ITS4B (10 µM) and 0.10 µl DreamTaq DNA polymerase (5 U/µL). In the contrary of the previous reaction mixture, 1.6 µl Bovine serum albumen was added as a stabilizer (according to manufacturer). The final reaction was 20 µl volume. Therefore, TD-PCR steps were as follows: an initial denaturation (95 °C for 3 min.) was followed by a phase of 19 cycles of denaturation (95 °C for 30 s) - primers annealing (60 °C for 1 min with decreasing temperature, 0.5 ˚C lower every cycle) - elongation (72 °C for 1 min). A second phase of 30 cycles of denaturation (95 ˚C for 30 s) - primers annealing (50 °C for 30s) - elongation (72 °C for 1 min) followed. Process ended with a final elongation (72 °C for 7 min) followed by halt reaction. This thermocycler program was slightly modified (in terms of step duration and number of cycles per phase) after **Korbie and Mattick (2008).**

1.4.3. Agarose gel electrophoresis

Agarose gel electrophoresis was carried out to separate DNA molecules based on their size and charge. Separation was done directly from either raw/diluted extracts or amplified PCR products. DNA was stained with Ethidium Bromide either used as bath in which gels were soaked for 5 min. or directly added into agarose gels. Then, bands were visualized under UV by fluorescence. A DNA ladder of 100 bp was used as fragment sizes marker.

1.4.5. PCR products purification, quantification and sequencing

Successful extracts revealed by fluorescent DNA bands was purified prior to sequencing. This purification aims to remove leftover primers and any remaining dNTPs. The method differed according to the PCR method used. For standard PCR, amplicons were precipitated during 24 hours at room temperature after addition of 16 µl of isopropanol 100 % and 2.3 µl of NaCl 5M. Pure DNA products were isolated by centrifugation (at 14000g and 18 °C, 30 min.). After air drying, PCR products were resuspended with 15 µl of sterile distilled water and conserved at - 20 ˚C. As for TD-PCR, successful bands were purified by enzymatic clean-up with 0.4 µl exonuclease I Exo I at 10U and 0.8 µl Shrimp Alkaline Phosphatase SAP at 1U (Thermo Scientific) added with 0.24 µl 10 x SAP buffer and 0.96 µl sterile distilled water. DNA extracts were incubated in the PCR machine at 37 ˚C for 1 hour and then 80 ˚C for 15 min. Afterwards, pure DNA were store at -20 ˚C until needed.

DNA quantification was performed using NanoDrop ND-1000 spectrophotometer (PecLab, Thermo Fisher Scientific) prior to sequencing. Indeed, template amount to use in a cycle sequencing reaction should vary from 5 – 20 ng for PCR products of 500 - 1000 base pairs (bp) as recommended by manufacturer. Therefore, PCR products were diluted with sterile distilled water accordingly and added with 0.75 µl of primer for a final volume of 7 µl of template that were sent to sequencing. However, when NanoDrop ND-1000 spectrophotometer was not available, successful bands were identified according to the abovementioned desired length. Then, 5 µl of purified PCR products added with 5 µl of 5 µM of primer, making a final mix of 10 µl, were sent to sequencing. That last procedure was done with TD-PCR products.

DNA sequencing was based on Sanger sequencing by capillary electrophoresis. It was performed by the sequencing service of the Department of Biology I (Ludwig-Maximilians-Universitat, Munchen, Germany) using BigDye Terminator Ready Reaction Cycles Sequencing Kit v 3.1 (Applied Biosystems, Foster City, CA, USA) or by Macrogen using Applied Biosystems 3730XL DNA Analyzer.

2. Habitat characterisation

2.1. Climate variables recording

Climate parameters recorded for this study were essentially climatic parameters of air and soil. Different data loggers were used for that goal. They were set according to manufacturer and installed in each plot per habitat type to record continuously from the 8th April 2014 morning and removed the 3rd October 2014 morning. One item of each type of logger was installed as much as possible in the center of each plot and mostly under dominant EcM partners trees. The data loggers were respectively as follows:

- Air Temperature and Relative Humidity were recorded with Voltcraft DL-121TH (Conrad, Germany) set at thirty-minute interval. Each logger (fig 11-1a) was fixed at about 15 centimeters (cm) above the soil on a host plant, at the center of each plot (fig. 11-1b);
- Soil Temperature and Moisture were recorded with TMS-3 data loggers soil moisture sensor (TOMST, Czech Republic) set at basic mode corresponding to fifteen-minute interval. Each logger (fig. 11-2a) was plugged in the soil (fig. 11-2b) according to manufacturer in order to record soil moisture and temperature with specific sensor at 10 cm belowground, at soil surface and 10 cm above soil surface.

In addition to above-mentioned parameters, rainfalls intensity and frequency were also recorded over the same period, namely from April to early October 2014. Two non-recording rain gauges were installed per habitat type, one in each plot at the edges of the habitat type. Each rain gauge was fixed on a metal support at one meter (1m) above the soil in an open portion of permanent plot (fig. 11-3). Thus, rainfall intensity was recorded after each rain event during the whole rainy season.

2.2. Phytosociological studies within established plots

First, systematic inventory of plant species and total canopy cover estimation within plots were performed in April 2014, according to **Braun-Blanquet (1932)**. Each plot (30m x 30m) has been divided into nine quadrats of 10m x 10m within which each plant species encountered has been collected. Primary identification of plants specimens were done with field guide **(Arbonnier, 2004)** and completed on collected herbarium materials by experts from the Laboratoire de Botanique of the University Felix Houphouet-Boigny in Abidjan, Côte d'Ivoire. Plants materials are deposited at W.A.S.C.A.L. GRP.

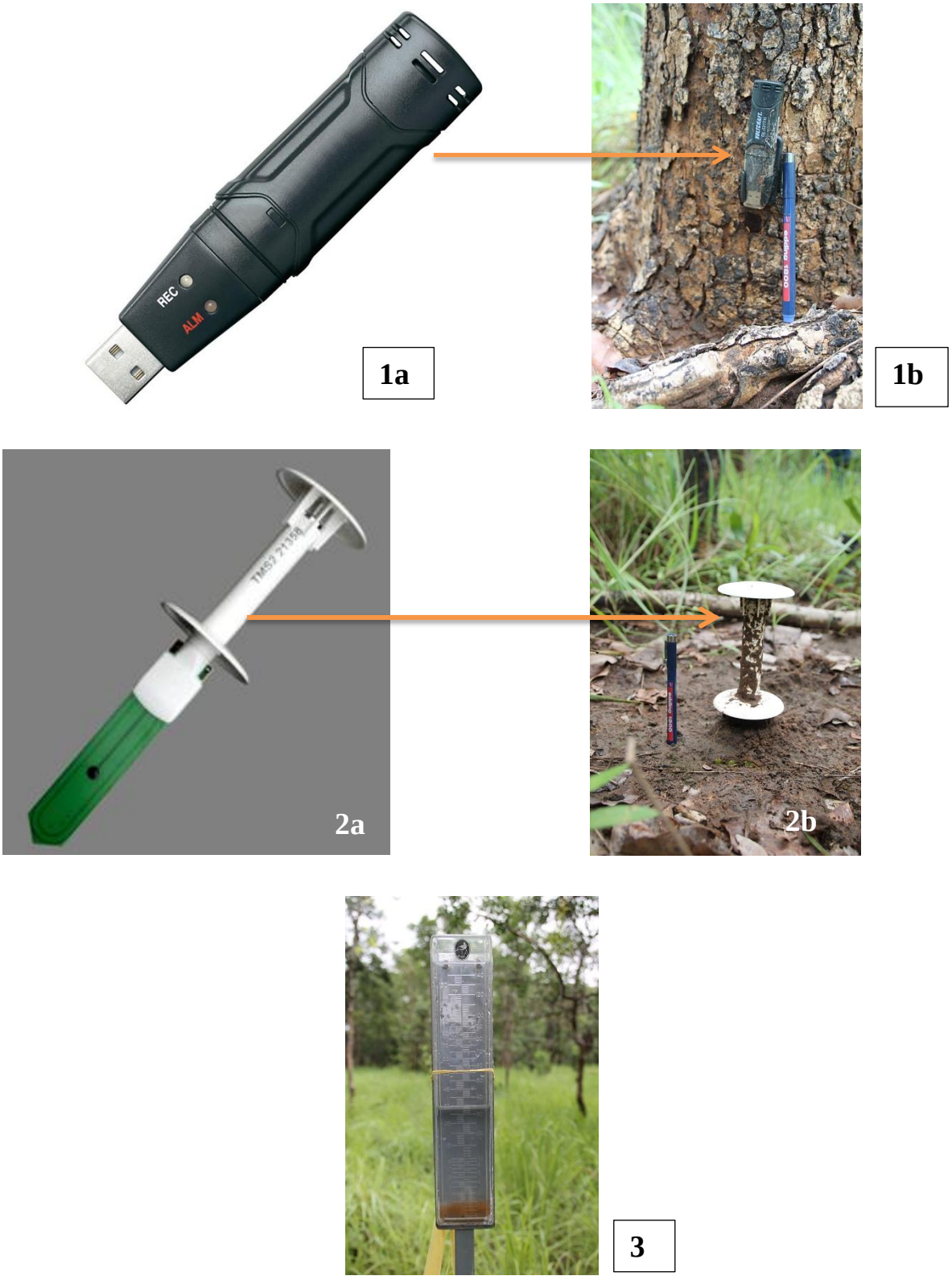


Figure 11: Climate data loggers devices (a) and their set-up within permanent plot (b). 1: Voltcraft 121TH; 2: TMS-3 Data loggers soil moisture sensor; 3: rain gauge

2.3. Soil cores sampling and analyses

2.3.1. Composite soil making

Soil cores were collected with a 10 cm x 10 cm - 10 cm depth drill at each corner and the center of each plot at mid-rainy season (late July). Those five cores per plot were mixed to make composite soil cores. After air drying in the laboratory of the base camp and sieving (2 mm size), samples of 200 g were taken from each composite soil to assess soil physical and chemical characteristics (texture, pH and mineral contents).

2.3.2. Physico-chemical analyses

The analyses were locally performed at the Laboratoire Enval in Abidjan, Côte d'Ivoire. Soil chemical include pH (H_2O), Carbon, Nitrogen, soil organic carbon (SOC), ratio C/N, Total Phosphorus (TotalP), Available Phosphorus (AvailP), Calcium (Ca), Potassium (K)) whilst physical features refer to clay, fine silt, coarse silt, fine sand, coarse sand. They were determined after AFNOR methods.

2.3.3. pH_20 measurement

It was performed with a soil solution at a ratio 2/5 **(Duchaufour and Blum, 1997)**. A volume of 50 mL of distilled water was added to twenty grams (20 g) of the soil sample in a beaker. The whole was stirred using a magnetic agitator for ten minutes (10 min) at 300 revolutions per minute (rev / min). The mixture was then allowed to stand for 30 minutes. The pH was determined by immersing the probe of the pHmeter in the supernatant. Finally, the reading was performed after stabilization of the digital display of the pHmeter.

2.3.4. Determination of extractable cations

Exchangeable cations (Ca^{2+}; K^{+}) were determined according to standard NFX 31-130 **(AFNOR, 1999)**. 5 g of soil sample were put in a centrifuge tube added with 25 ml of acetate solution. That solution is obtained by dissolving 77.08 g of acetate in 1000 ml of distilled water, shaking back and forth for 30 min. and then centrifuging for 5 min. using a centrifuge type KUBOTA / 702 KR. The supernatant was discarded and the process was repeated four (4) times. Final supernatant was collected for the measurement by reading on the digital display of spectrophotometer of cations.

2.3.4.1. Determination of organic and total carbon

The total carbon content in soil is determined after dry combustion. The soil's organic carbon content is calculated according to the method NF ISO 10694 **(AFNOR, 1995)**. This method is based on the oxidation of organic carbon by potassium dichromate ($K_2Cr_2O_7$) in acidic medium. The titrating of non-reduced dichromate in excess determines the amount of organic carbon neutralized.

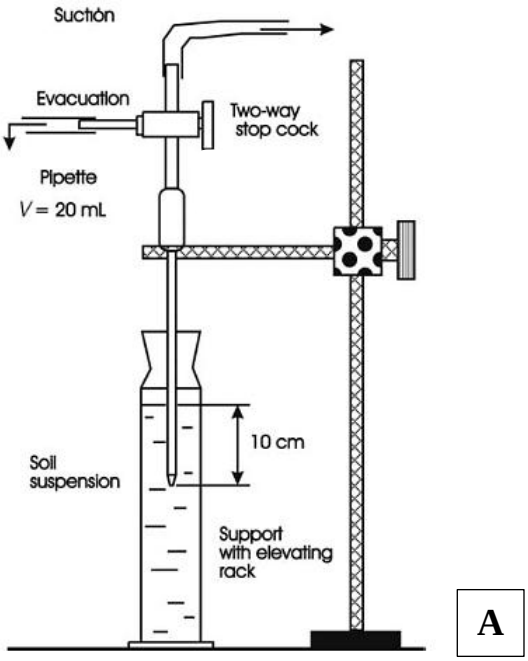
2.3.4.2. Particle size determination by sedimentation – the pipette method

The particle size analysis is based on the classification of mineral soil particles by size categories to determine soil texture. Soil particles refer to clays (diameter d < 0.002 mm), fine silt (0.002 < d <0.02 mm), coarse silt (0.02 < d <0.05 mm), fine sand (0.05 < d <0.2 mm) and coarse sand (0.2 <d <2 mm). The different fractions were separated by destruction of organic matter using hydrogen peroxide. That destruction stabilizes clays and silts. Soil sample was suspended in a solution of water and sodium pyrophosphate according to the NF X 31-107 **(AFNOR, 2003)**. Sodium pyrophosphate neutralizes the flocculating action of colloids. The determination follows different steps:

(1) The pipette was immersed 10 centimeters deep in an extension tube containing prepared soil solution (Fig. 12). 20 mL of the solution were taken in a capsule and heated in an oven at 105 °C until complete evaporation of the water. The fraction obtained (clay and silt) was weighed.

(2) After sedimentation during 6 hours at 26 °C, the pipette was again immersed 10 centimeters deep in the extension tube and a second aliquot of 20 mL was taken. The aliquot is poured in a capsule that was then heated in an oven at 105 °C until complete evaporation of water. That second fraction obtained (Clay) was weighed.

(3) The sand was obtained after washing the remaining content of the each extension tube. Indeed, the bottom has been washed until clear liquid and heated in a capsule in an oven at 105 °C until complete evaporation of the water. The fraction obtained was then passed through a sieve column composed of two levels (d_1 = 0.2 mm and d_2 = 0.05 mm). The sand retained on the 0.2 mm sieve is coarse sand and that retained on the sieve of 0.05 mm is fine sand. The fraction passed through the two sieves is the coarse silt.



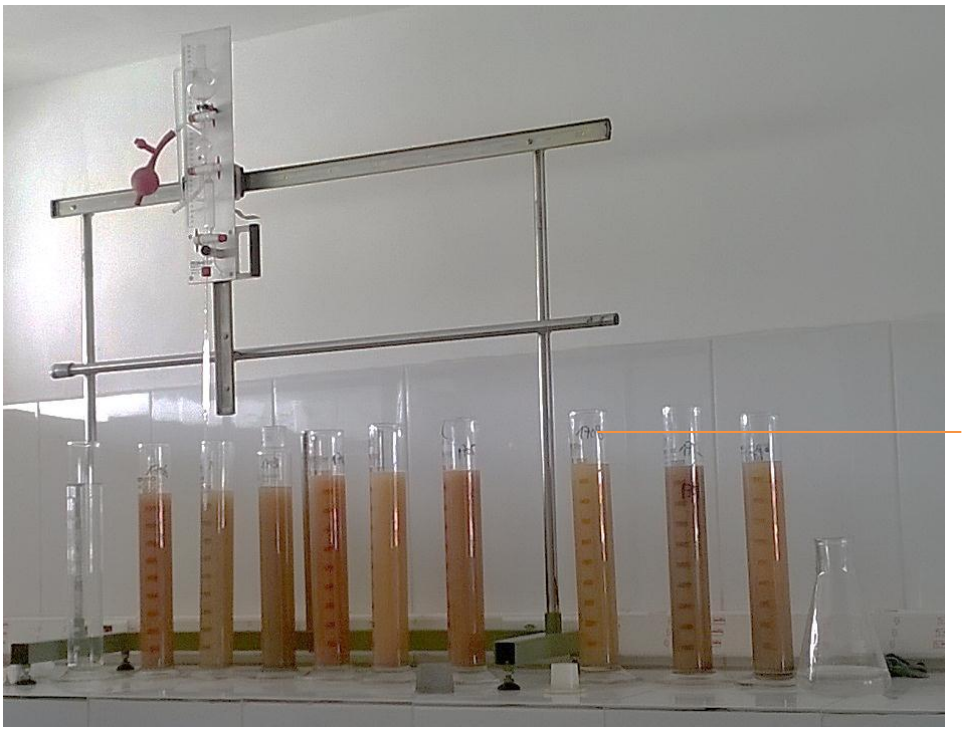


Figure 12: Particles size determination by pipette method. A: Modified sketch of Robinson-Köhn pipette (Pansu and Gautheyrou, 2007), B: determination of collected composite soils

3. Data analysis

Analyses were performed according to specific objectives and encompassed all analyses useful to attain these objectives.

When applicable, Analysis of variance (ANOVA) test at $\alpha < 0.05$ was performed at habitat type level using package lawstat of R software **(Hui *et al.*, 2008).** When requirement of distribution and homogeneity of variance were not met, Kruskal-Wallis test **(Kruskal and Wallis, 1952)** was also performed in R software.

3.1. EcM fungi fruit bodies (EFFB) diversity

3.1.1. Molecular-based identification of EFFB

Generated DNA sequences were analyzed, assembled and edited using the software FINCH TV version 1.4.0 (http://www.geospiza.com) that gives a graphic view of chromatogram files (ABI files) of sequences. It allows assessing the fine-scale sequence quality based on Phred quality score assigned to each nucleotide base call during sequencing **(Ewing *et al.*, 1998)**. In our study, a threshold of Phred quality score of 20 corresponding to a base call accuracy of 99 % was selected.

BlastNucleotide (BlastN) investigation **(Altschul *et al.*, 1997)** was performed against sequences of the UNITE database http://unite.ut.ee **(Kõljalg *et al.*, 2005)** including sequences from the *International Nucleotide Sequence Database consortium* (INSDc). That consortium encompasses the *National Center for Biotechnology Information GenBank* (NCBI), the *European Molecular Biology Laboratory* (EMBL) and the *DNA Data Bank of Japan* (DDBJ). To support the taxonomic affinity of collected specimens, homologous sequences of each generated sequence were retrieved with focus on African species including sequences of uncultured/environmental samples. Taking into account percentage of identity, best matches of sequences of taxa identified up to species level and/or genus level were considered and downloaded.

Generated sequences were labelled ***Taxon_LPxxx(CNP)*** whilst downloaded homologous sequences were labelled ***Taxon_accession_number(country)***. Generated sequences were named according to homologous sequences having the best percent of identity (PI). Generated and homologous sequences were automatically aligned in the computer program *Molecular Evolutionary Genetics Analysis version 6.0* MEGA6 **(Tamura *et al.*, 2013)** using

MUltiple Sequence Comparison by Log-Expectation (MUSCLE) method with Align DNA option **(Edgar, 2004)**. The clustering method used was UPGMB, a variant of *Unweighted Pair-Group Method with Arithmetic Mean* (UPGMA). Alignment was done with sequences dataset grouped per family, examined and manually adjusted. Similarities of generated sequences were then estimated by pairwise distance in the same software to remove duplicates and check accuracy of multiple alignments for reliable phylogenetic analyses.

3.1.2. Fungi species richness estimation

3.1.2.1. Observed richness and diversity indices

Presence/absence data of EFFB was used to determine (1) the observed species richness (SR: number of species) and composition (SC: list of species) per habitat; (2) the total observed species richness and composition as cumulative data of all habitat types. Thereby, the frequency of occurrence (percentage of total weeks during which a species was recruited) of fungal species was used to highlight the contribution of each species in the community **(Horton and Bruns, 2001)**. The relative frequency of each species was calculated as the percentage of total frequency.

Assessment of fungi diversity and evenness of frequency of species within habitat types was achieved respectively by computing Simpson's Index of Diversity (1 – D) and Simpson' Evenness with the program Ecological Methodology **(Krebs and Kenney, 2002)**. Simpson's Index of Diversity (1 – D) refers to the probability that two individuals randomly selected from a sample will belong to different species. Its value ranges between 0 and 1, greater value corresponding to high diversity. As for Simpson evenness, it refers to the variability in species abundance and highlight equitability of species abundance **(Magurran, 2004)**. It expresses the dominance of a species when it tends towards 0, or the co-dominance of several species when it tends towards 1 **(Grall and Coïc, 2006)**.

3.1.2.2. Sampling representativeness: Species accumulation curves and Similarity assessment

Sample-based species accumulation curves were constructed in EstimateS ver. 9.1.0 **(Colwell, 2013)** using presence / absence (incidence) data. The sample order was randomized 500 times without replacement for the statistical representation of the EcM fungi community.

In this study, "*sample"* referred to frequency of survey, a week-interval, against which Observed and Estimated Chao 2 species accumulation curves were plotted.

The similarity of sampling to the community was estimated by measuring the autosimilarity **(Cao *et al.*, 2002)** among plots of each habitat type. This was calculated as mean Jaccard coefficient computed with EstimateS ver. 9.1.0 software. Autosimilarity index varies from 0 (low autosimilarity) to 1 (high autosimilarity). The more taxa are common to all plots, the more plots are similar and thus the sampling has captured the total taxon richness.

Constructed week-based species accumulation curves, Simpson's Index of Diversity (1 – D) and Simpson's evenness along with autosimilarity index served to assess the sampling representativeness of fungal communities of study sites.

3.2. Phylogeny of EFFB

Phylogenetic inference was performed to highlight the evolutionary relationship (1) between EcM fungal species collected at CNP and (2) with homologous from various parts of the world. Final dataset of sequences per family were used to draw most likely phylogenetic trees by the Maximum Likelihood (ML) statistical method using MEGA6. The Best substitution model and rates among sites were sought for each case *via* the Bayesian Information Criterion (BIC). Models with the lowest scores are considered to describe the substitution pattern the best. Model selection was performed prior to reconstruction with partial deletion of gaps and site coverage cutoff of 95 %. Tree inference options were the nearest-neighbor-interchange (NNI) as ML heuristic method and Neighbor-Joining NJ / BioNJ as automatical initial tree. Robustness of individual branches was tested by bootstrap method **(Felsenstein, 1985; Stamatakis, 2006)** with 2000 replications.

3.3. Spatial distribution of EFFB

3.3.1. Habitats characterisation

3.3.1.1. Floristic richness and dendometric parameters assessment

Plants species were inventoried per plot and habitat type for floristic richness assessment. Number of stems and diameter at breast height (dbh) per species underwent basic statistical analyses as follows:

- Plant species density (D_i), the number of stems per species per plot surface in square meters (m^2), converted later in hectares (ha);
- Individual stem basal area (BA_i). $BA_i = \pi \times 10^{-4} \times (dbh_i/2)^2$, where tree dbh in cm and BA_i in m^2. This formula is simplified as: $BA_i = 0.00007854 \times (dbh)^2$;
- Species basal area (BA_{sp}) that equals to the sum of all BA_i of stems of the same plant species within a plot, converted later in hectares (ha);
- Total basal area (TBA), summing up the all calculated BA_{sp} within a plot;
- Species relative dominance (SRD): $SRD\% = \left(BA_{sp}/TBA\right) \times 100$.

3.3.1.2. Soil chemical and texture analyses

3.3.1.2.1. Determination of organic and total carbon

The content of organic matter was determined according to equation:

$$M.org = 1.724 \times C.org$$

With M.org = Organic matter (mg / kg); C.org = Organic Carbon (mg / kg).

3.3.1.2.2. Particle size determination by sedimentation – the pipette method

From the different aliquots, the percentages of different mineral fractions were determined by the following equations: (a), (b), (c), (d), (e), (f) and (g)

$$C + St\% = [(Pc + s) - (p1) - (Pb)] \times 5000 \times k/Pe \times Fh \quad \text{(a)}$$

$$C\% = [(Pa) - (P1) - (Pb)] \times 5000 \times k/Pe \times Fh \quad \text{(b)}$$

$$FSt\% = (C + St)\% - C\% \quad \text{(c)}$$

$$TSd\% = (Tt - P1) \times 100/Pe \times Fh \quad \text{(d)}$$

$$CSd\% = (Tc - P1) \times 100/Pe \times Fh \quad \text{(e)}$$

$$FSd\% = (Tf - P1) \times (100/Pe) \times Fh \quad \text{(f)}$$

$$CSt\% = TSd \times (CSd + FSd) \times Fh \quad \text{(g)}$$

With

C= Clay P_{C+St}= Tare weight + clay + silt

St= Silt

FSt= Fine Silt

TSd= Total Sand

CSd= Coarse sand

FSd= fine Sand

CSt= coarse silt

Tt = cap weight + the Total Sand

Tc= cap weight + coarse sand

Tf = cap weight + fine sand

P1 = Weight of empty tare (capsule)

P2 = Weight of empty tare + white

$Pb = P2 - P1$

$k = 20N/V$

V = Volume of the pipette

Pe = aliquot intake

Fh = humidity factor

Pc= cap weight + clay

The texture of each soil was determined using TRIANGLE, A Program For Soil Textural Classification **(Gerakis and Baer, 1999)**. It followed percentage of particles within studied soils.

3.3.2. Influence of environmental parameters on fungi distribution

To visualize the spatial distribution of EFFB, non-metric multidimensional scaling NMDS ordination was performed on matrix of fungi species relative frequency per plot using function *metaMDS* of package Vegan **(Oksanen *et al.*, 2015)** of R software version 3.3.0 (2016-05-03). Fungi relative frequencies were first transformed by Wisconsin double standardization using function *Wisconsin* to improve ordination. A distance matrix generated by Bray–Curtis dissimilarity index with function *vegdist* was used as input for the NMDS whilst function *metaMDS* used Jaccard index. Then, main environment variables (host communities and soil parameters) influencing the fungi communities structure were evidenced by fitting them to the ordination plot using function *envfit* of the Vegan package. Statistical significance was based on 999 random permutations and plotting was limited to most significant variables with argument *p.max* set at 0.1.

To better depict the similarity of habitat types, a hierarchical clustering based on Bray–Curtis dissimilarity index was conducted in R software version 3.3.0 (2016-05-03) using function *hclust* and average-linkage. Subsequently, each fungi community was characterized by conducting indicator species analysis using the *Multipatt* function in the R package Indicspecies **(De Cáceres and Legendre, 2009; De Cáceres and Jansen, 2015)**. Indicator

Value (IndVal) index **(Dufrêne and Legendre, 1997)** was computed to measure the association between a species and a site group. Statistical significance of association was tested by running 999 random permutations. In addition, the specificity (the so-called IndVal Component A) and the fidelity (second component B of IndVal) of a species as indicator of a target site group were inspected. Component A or specificity commonly referred to "the probability that the surveyed site belongs to the target site group given the fact that the species has been found" whilst component B referred to "the probability of finding the species in sites belonging to the site group" according to **Dufrêne and Legendre (1997)** and **De Cáceres and Legendre (2009).** Final, ecological distance between generated site groups was calculated by Jaccard index using the R package Fossil **(Vavrek, 2011)**.

3.4. Fruiting phenology and production of edible EFFB in relation with microclimate variability

Influence of climatic parameters variability was addressed especially on collected EcM fungi identified as edible according to literature **(De Kesel *et al.*, 2002; Eyi Ndong *et al.*, 2011)**. Indeed, numerous studied pointed out the importance of wild EcM fungi as renewable non-timber forest product (NTFP). Wild mushrooms are widely consumed by local population **(Härkönen and Vainio-Mattila, 1998; Yorou *et al.*, 2001; Härkönen, 2002; Härkönen *et al.*, 2003; Eyi Ndong *et al.*, 2011)** as substitute to meat and fish. Moreover, picking wild edible mushrooms is a lucrative business that involves hundreds of rural women worldwide **(Boa, 2006; Koné *et al.*, 2011; Yorou *et al.*, 2014)**. Knowing temporal change in the phenology and production distribution as affected by fluctuation of climatic parameters is essential to the valorisation of wild EcM fungi.

3.4.1. Climate parameters variability

After removal from field, minutes-interval rough data were downloaded from Voltcraft DL-121TH and TMS-3 loggers and re-arranged according to day and week with software Excel 10. Then, average values of each parameter were extracted at week-interval with package plyr of software R. These weekly-based average dataset per plot were used to assess significance of variance between habitat types using function repeated measures ANOVA in Statistica 7.1 **(StatSoft, 2006)**. Assumption of sphericity of Mauchly was tested with the same software prior to these ANOVA tests. These latter were performed for the effects

habitat type, *week* and *interaction of both factors*. Similarly, rainfalls intensity was calculated at week interval with software Excel 10; then submitted to sphericity test of Mauchly and ANOVA with repeated measures in Statistica 7.1 (**StatSoft, 2006**).

3.4.2. Fruiting phenology of edible EFFB

Number of occurrence weeks and number of habitat where the species occurs per week were used to evaluate commonness/rarity and fruiting phenology of species. Time laps between rain events and fruiting was also used to appreciate hydric requirement for species fruiting.

3.4.3. Production of edible EFFB

Cumulative fresh biomass per species, per week and per habitat type served to evaluate natural production of edible EcM fungi species. Values obtained at plot size were converted to hectare.

3.4.4. Influence of climate variability on edible EFFB production:

Collinearity between recorded climate parameters was tested in Statistica 7.1 **(StatSoft, 2006)** and, when significant, principal component analysis (PCA) was run to extract independent variables **(Dormann *et al.*, 2013)**. Those latter, called principal components, were correlated to weekly fungi species production. Correlation method to be used was determined after test of normality of fungi production. When normality was not met, Spearman correlation was run. PCA was computed with function *prcomp* and correlation of extracted principal components (PC) to initial variables was tested with function *corr.test* of package psych of R software version 3.3.0 (2016-05-03). Afterwards, correlation between PC and Fresh Biomass of edible fungi was tested using the same function as above-mentioned. That function allows testing correlation significance at $\alpha > 0.05$.

SECTION III: RESULTS

CHAPTER VII: ECTOMYCORRHIZAL FUNGI FRUIT BODIES DIVERSITY

1. **Molecular-based identification of EFFB**

A cumulative number of 134 pure sequences were generated from the 239 best representative specimens, making a success rate of 62.76 %. The final length of generated sequences after analysis and editing varied from 509 to 914 base pairs (bp).

BlastN search confirmed the EcM habitus of 126 of studied specimens whilst the other 8 specimens were assigned to non EcM lineages. Alignments of all generated EcM fungi sequences indicated 72 different sequences after removal of duplicates ones. Thus, those different sequences evidenced a diversity of 72 EcM fungal species.

Identification at species level was possible with 11 DNA sequences representing 15.27 % of all generated sequences. That included species such as *Amanita subviscosa, A. masasiensis, Lactarius tenellus, Lactifluus flammans, Lactifluus gymnocarpoides* and *Lactifluus volemoides* with percentage of identity varying from 98 to 100. Formerly identified as *Amanita* aff. *rubescens*, that species has been described and the distinct species name of *A. congolensis* has been proposed. The 61 other generated sequences were identified only at genus level with percentage of identity varying from 71 to 100, inclusive of suspected novel species (Annex 5).

However discrepancies of morphological- and molecular-based identifications were noticed in some cases. In fact, some generated DNA sequences did not match with holotypes' DNA sequences available in public databases. These discrepancies enabled also to identify and discriminate cryptic species mostly in genera *Amanita, Lactifluus* and *Cortinarius*. In those cases, species were named after holotypes with the mention *cf.* or *aff.*.

2. Fungi species richness estimation

2.1. Observed species richness and Diversity indices

EcM fungal fruiting started in mid-May and was still continuing in early October making a cumulative total of 21 weeks of occurrence. 2814 fruit bodies have been collected and were sorted into 123 species belonging to 23 genera and 09 families (table II). The most frequently recorded family was Russulaceae with 53 species composed of 36 *Russula* species, 11 *Lactifluus* species and 6 *Lactarius* species. The second frequently observed family was Boletaceae represented by 13 genera with a total of 32 species.

Table II: Richness of ectomycorrhizal fungi per habitat

Cumulative parameters	Isoberlinia Woodland (IW)	Mixed Woodland (MW)	Uapaca Woodland (UW)	Total
Numbers of fruit bodies	1565	513	736	2814
Numbers of species	75	65	56	123
Numbers of genus	21	15	16	23
Numbers of family	8	6	5	8

The Amanitaceae ranked third most important recorded family with a total of 26 species. The less recorded other families included Cantharellaceae, Cortinariaceae, Gyroporaceae, Inocybaceae, Sclerodermataceae and Clavulinaceae. These families were represented each by only one genus with respectively 1, 3, 1, 1, 5 and 1 species (fig 13). From the total species richness, 57 taxa (46.34 % of the total) were identified up to species level with 19 of them being related to known species from temperate and tropical zones. The remaining 66 species (53.66 % of the total) were identified only at the genus level with some of them suspected new to science.

The most frequent species per habitat type included *Russula congoana* Pat. (13 weeks corresponding to the relative frequency of 2.53 %), *Amanita* aff. *craseoderma* (11 weeks, relative frequency = 2.14 %) and *Lactarius tenellus* Verbeken & Walleyn (10 weeks, relative frequency = 1.95 %) in IW; *Amanita annulatovaginata sensu lato* Beeli and *Lactarius tenellus* (both with 8 weeks, relative frequency = 1.56 %) in MW; *Cantharellus addaiensis* Henn. and *Amanita* aff. *subviscosa* Beeli (both with 11 weeks, relative frequency = 2.14 %), *Amanita* aff *virosa* and *Amanita strobilaceovolvata sensu lato* Beeli (both in 10 weeks, relative frequency = 1.95 %) in UW (Annex 6).

22 species were found common to the three habitat types and represented 17.89 % of total observed species richness (Annex 6). On the other hand, 72 species accounting for 58.53 % of the species richness were specific to one habitat type. Many of these specific species were observed and collected only once from May to early October 2014 (Annex 6) and are uniques species. Specific species such *Inocybe* sp 1 and *Cortinarius* subgenus *telamonia* sp 1 have been picked under *Isoberlinia doka* trees in IW. Meanwhile, *Russula annulata* R. Heim, *R. discopus* R. Heim (a rare species) and *Veloporphyrellus africanus* Watling were collected beneath *Uapaca togoensis.* Final, 29 species (23.58 %) were shared by two habitat types. In addition with species common to all habitat types, 38 species were shared by IW and MW (e.g. *Amanita afrospinosa* Pegler & Shah-Smith, *Lactarius saponaceus* Verbeken); 28 species shared by IW and UW (e.g. *Gyroporus castaneus* (Bull.) Quél., *Amanita strobilaceovolvata* sensu lato) and 29 species shared by MW and UW (e.g. *Amanita. congolensis* Pers., *Boletus loosii* Heinem.).

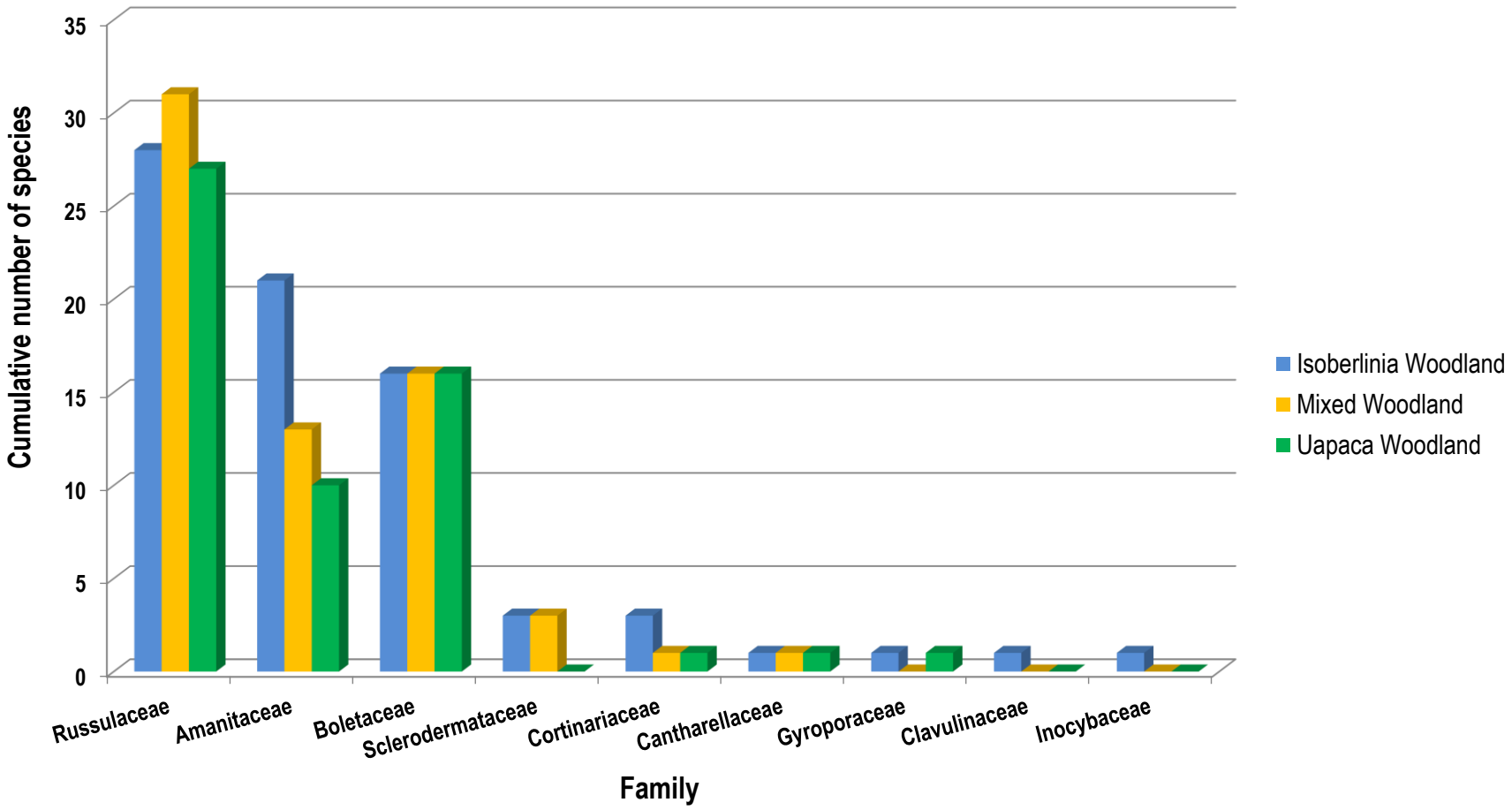


Figure 13 : Representativeness of ectomycorrhizal fungal families per habitat type

2.2. Similarity and sampling representativeness

Computed Simpson's Index of Diversity 1 – D of IW was 0.97 with an autosimilarity index calculated to 0.40. Therefore, plots in IW were found non similar likewise for, plots within habitat types MW and UW with respectively 0.33 and 0.29. In those latter habitats, higher diversity indices were respectively 0.97 and 0.96.

Weekly-based species accumulation curves of the different habitat types have almost the same shape in observed and estimated species richness (Fig. 14). Accumulation curves of IW were generally above those of the other habitat types through weeks except for the estimated species richness where curve of MW outdid the other curves from the fourteenth week till the end of the survey. Globally, all curves were ascendant and did not reach an asymptote of total richness.

Sample coverage highlighted the percentage of species detected by our study on the overall estimated species richness. Thus, 75.25 % of species was detected in IW against 81.88 % in MW and 58.78 % in UW (table III). Furthermore, 38, 32 and 36 unique species have been collected in the different habitat types respectively.

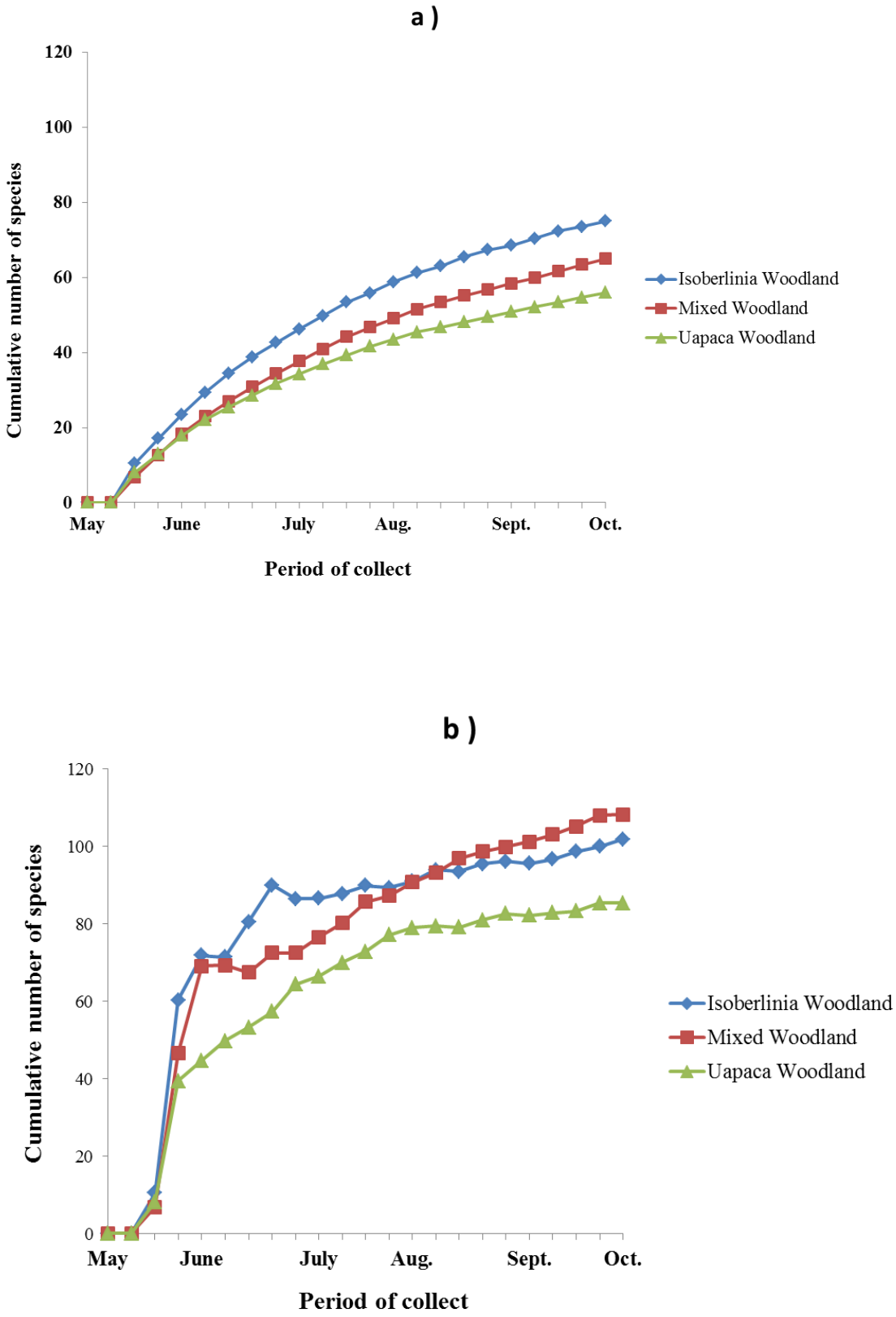


Figure 14 : Week-based accumulation curves of observed (a) and estimated (b) fungal species richness of EcM fungi during fruiting season 2014 (mid-May to early-October). Aug.= August, Sept.= September, Oct.= October.

Table III: Sampling representativeness estimators. Sample coverage: proportion of observed species richness (S_{obs}) as per cent of estimated species richness (S_{est}); Auto-similarity: mean similarity between plots of the same habitat type; Uniques: number of species collected only once during the whole period.

Habitat type	Number of fruit bodies	Observed species richness S_{obs}	Estimated species richness (Chao 2) S_{est}	Sample coverage	Auto-similarity	Simpson's Index of Diversity 1- D	Simpson's Evenness	Uniques
Isoberlinia Woodland	1542	75	99.67	75.25	0.40	0.97	0.06	38
Mixed Woodland	502	65	79.38	81.88	0.33	0.97	0.25	32
Uapaca Woodland	775	56	95.27	58.78	0.29	0.96	0.19	36

Sample coverage = $\frac{\text{Sobs}}{\text{Sest}} * 100\%$

CHAPTER VIII: PHYLOGENETIC PLACEMENT OF COLLECTED ECM FUNGI

1. Russulaceae

For the most recorded family Russulaceae, the final dataset included 81 sequences with 27 generated (labelled LP) sequences, 52 homologous sequences from which 2 sequences belonging to genus *Amanita* as outgroup for tree rooting. The best substitution model selection indicated Kimura 2-parameter model plus Gamma-distributed rates + invariant sites (K2+G+I) for that latter family with Bayesian Information Criterion (BIC) scores being 3676.335.

The constructed plylogenetic tree was successfully rooted at 99 % of boostrap indicating that Russulaceae originated from a signle ancestor and constitued a monophyletic group (fig. 15).

Most of the sequences felt within the identified genera *Lactifluus*, *Lactarius* and *Russula*. However, two ambiguous positions were observed. The collected specimen LP 137 which was assigned to genus *Russula* according to its best match with the unidentified *Russula* sequence KM594829 (P.I = 88; e-value = 0.0), felt within genus *Lactifluus*. That specimen has a yellowish stipe and its pileus ranged from yellow at the margin to orange at the center. Though that colour is usually found in genus *Lactifluus*, the specimen did not exudate any latex. Similarly, the whitish specimen LP 190 did not exudate latex; however, it has 99 PI and e-value = 0.0 with an unidentified *Lactarius* from Zambia (accession number UDB016890). Nevertheless, its sequence felt within sequences of genus *Russula*. In addition for that specimen LP 190 and though the best match was that unidenfied *Lactarius* sequence, all other homologous sequences suggested by the Blast output belonged to genus *Russula* with P.I. decreasing from 86.

Similarly, the specimen LP728 was previously identified as *Lactifluus heimii* based on morphology; however, its generated sequence did not nested with available homologous sequence in database. In the contrary, it nested with *Lactifluus sesemontani*.

Taking into account percent identity, the distributions rang from west-african countries (Benin, Guinea, etc.) like with LP441_*Lactifluus luteopus* but also wide up to Madagascar like for LP457, *Lactifluus pelliculatus* (Fig. 15).

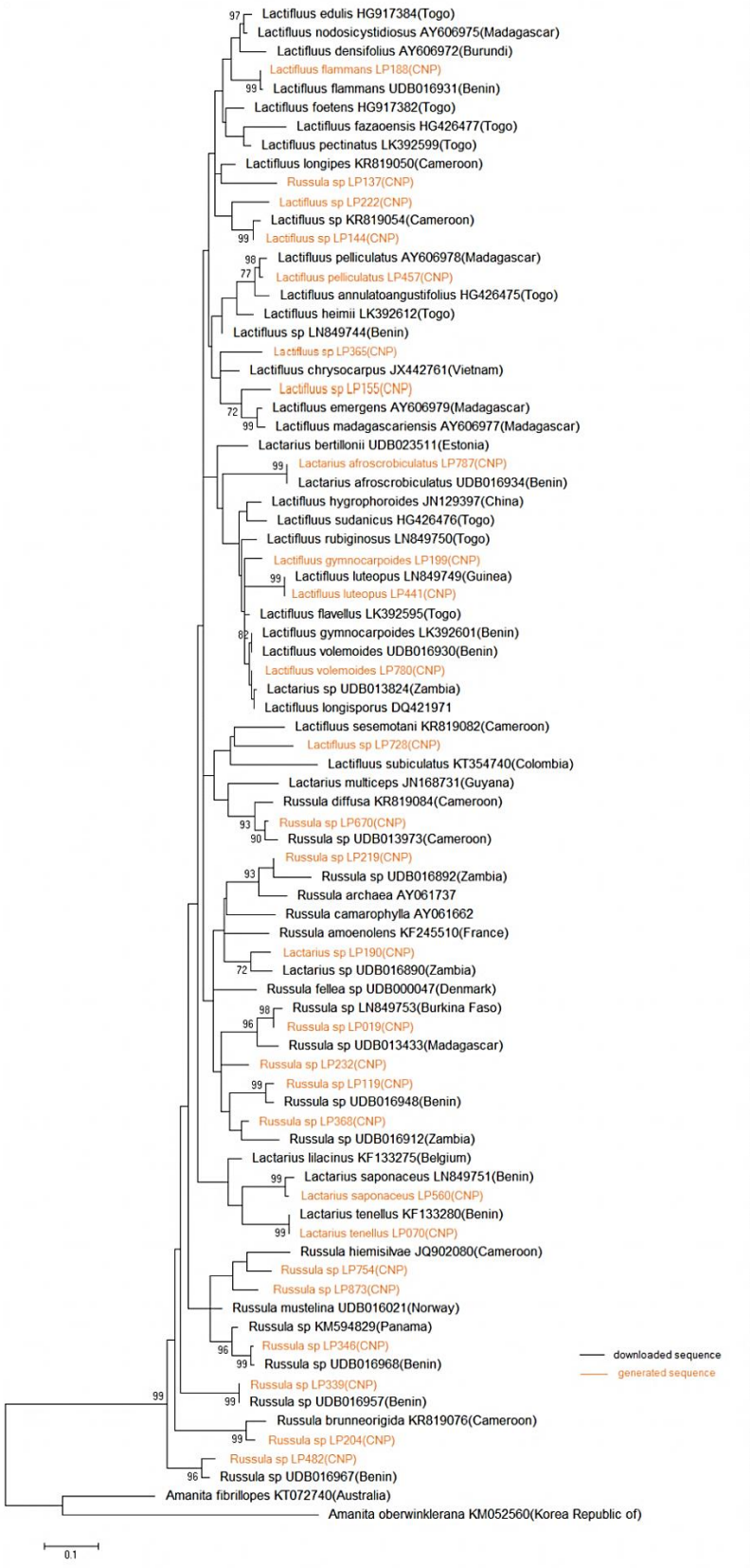


Figure 15: Phylogeny tree of Russulaceae constructed by Maximum Likelihood with Kimura -2 parameter model with Gamma-distributed rates + invariant sites (K2+G+I). Generated sequences are in red. CNP: Comoe National Park

2. Boletaceae

Their final dataset of 62 sequences encompassed 22 LP sequences, 38 homologous sequences from which 02 sequences belonging to genus *Craterellus* as outgroup. The best substitution model was Kimura 2-parameter model with Gamma-distributed rates (K2+G+I) with BIC at 2915.857.

The studied Boletaceae family is a well supported monophyletic group with 100 %. However, branchs of the tree were not supported with boostrap values generally below threshold of 70 % (fig. 16).

In the contrary of the Russulaceae, no generated sequences was identified at species level. They were named at genus level according to the best match; however, sometimes the low percentage of identity with that best match raised questions about those molecular-based identifications. Indeed, position of generated sequences within reconstructed tree is ambigous in most of the case. For example, sequences named Boletus sp did not assemble in the same clade as they should.

While considering percentage of identity and species distribution, 08 LP species were likely also collected in other african countries. Species LP276 and LP297 have a west-african distribution and occur also in Benin (PI = 99 and 96 respectively) whilst some other like LP322 and LP134 have a wide distribution with respective PI of 99 and 98 with *Boletus* spp. fruiting in Zambia.

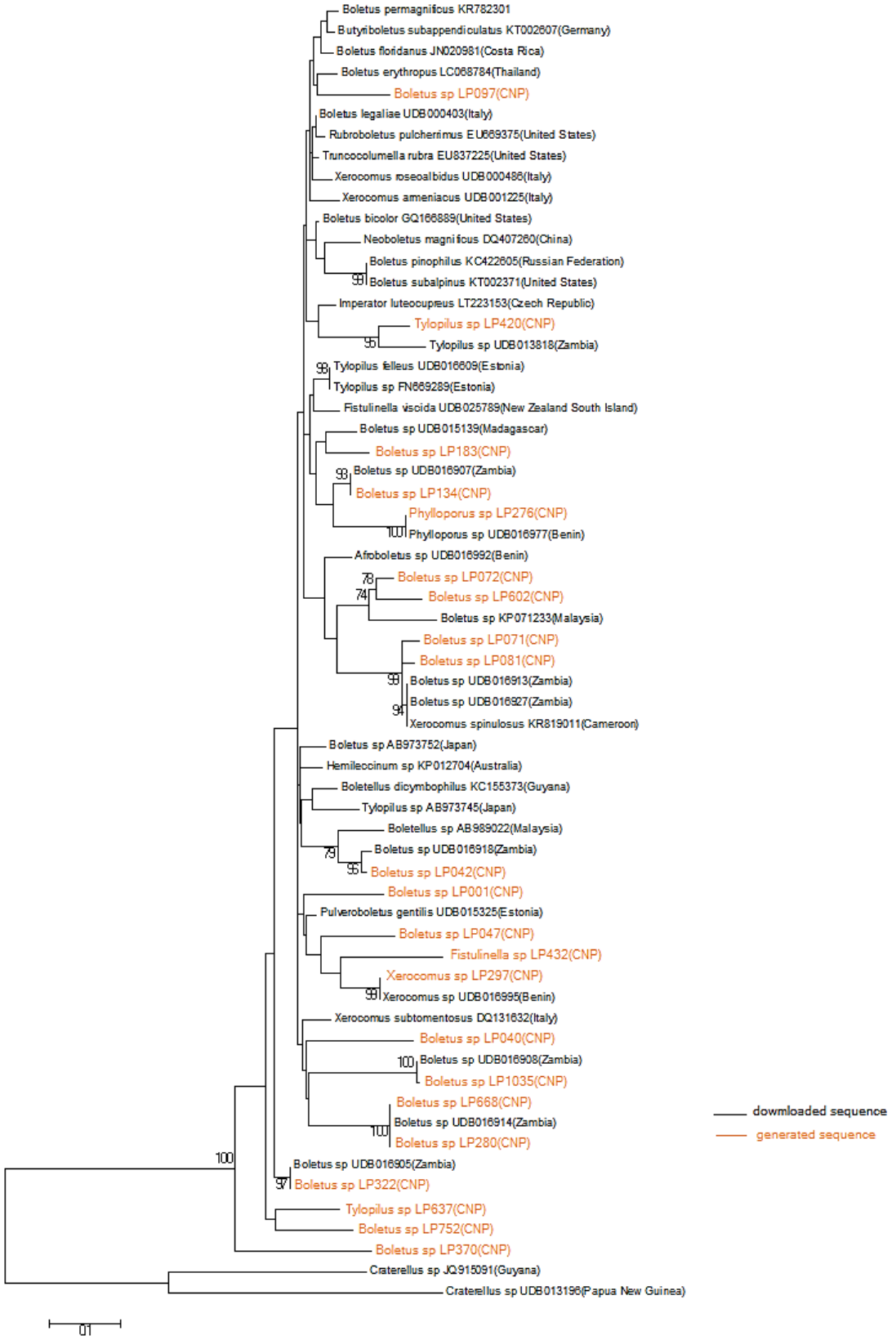


Figure 16: Phylogeny tree of Boletaceae constructed by Maximum Likelihood with Kimura 2-parameter model with Gamma-distributed rates (K2+G). Generated sequences are in red. CNP: Comoe National Park

3. Amanitaceae

In Amanitaceae, 13 generated sequences plus downloaded 26 homologous sequences made a final dataset of 41 sequences with 02 sequences belonging each to *Cantharellus* sp and *Craterellus* sp as outgroup. In that family, the best substitution model was Tamura 3-parameter model with Gamma-distributed rates (T92+G) with BIC score at 2958.144.

The maximum likelihood analysis resulted in a totally unresolved tree with multifurcation of the phylogenetic tree in Amanitaceae although a good support of tree rooting (fig. 17). This suggested a rapid radiation of Amanitaceae species in the park. Some generated species nested within supported clades suggesting those species belong to their specific morphological section. Thus, species such as *A. masasiensis* LP620 (CNP) nested with species of the *Amanita* sect. *Caesareae.*

LP110 and LP156 were formelly identified as *A. subviscosa* according to their morphology. However, molecular analysis revealed that they are distinct species, LP110 identified as *A. subviscosa* with 99 percentage of identity. Therefore, LP156 were labelled *A.* aff *subviscosa*. This species has 81 percentage of identity with best match, *A. volvata*.

Like in previous studies, high percentage of identity highligthed the large distribution of some Amanitaceae. Some of them fruited also in different countries of west (Benin) and austral Africa (Zambia).

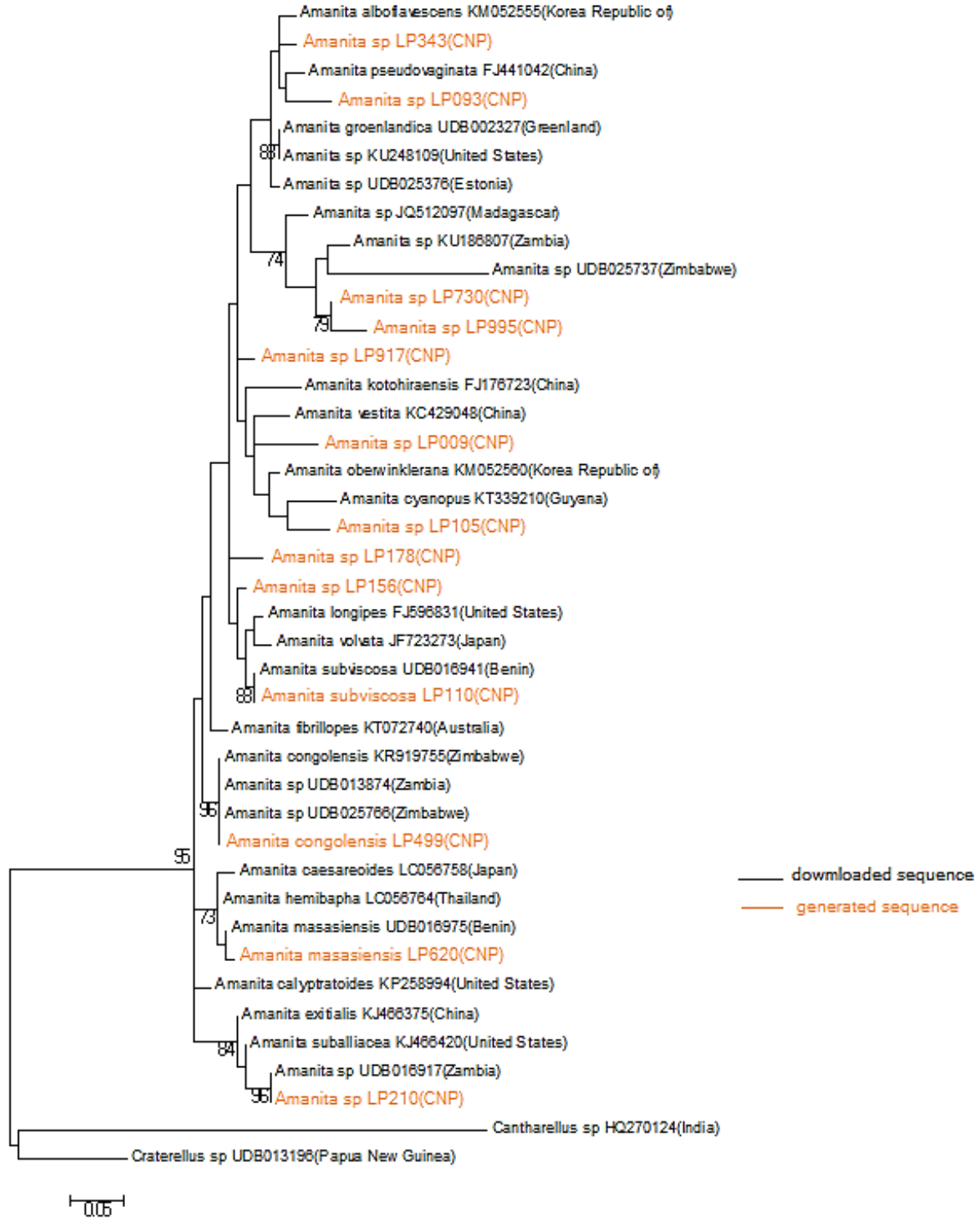


Figure 17: Phylogeny tree of Amanitaceae constructed by Maximum Likelihood with Tamura 3-parameter model with Gamma-distributed rates (T92+G). Generated sequences are in red. CNP: Comoe National Park

4. Sclerodermataceae

Final matrix of Sclerodermataceae has 16 sequences including 3 LP ones and 13 homologous with 01 outgroup sequence belonging to genus *Rhizopogon.* The best substitution model was Kimura 2-parameter model plus Gamma-distributed rates (K2+G) with BIC score at 3247.592.

The rooted tree is not supported though four well-supported clades were obtained (fig. 18). However, closer homologous were not identified at species level and we could not therefore identify our collected species. LP381 is likely a sister species of *S. dictyosporum.*

High PI indicated that collected species have also west african distribution (Burkina Faso) and central african distribution (Cameroon and Zambia).

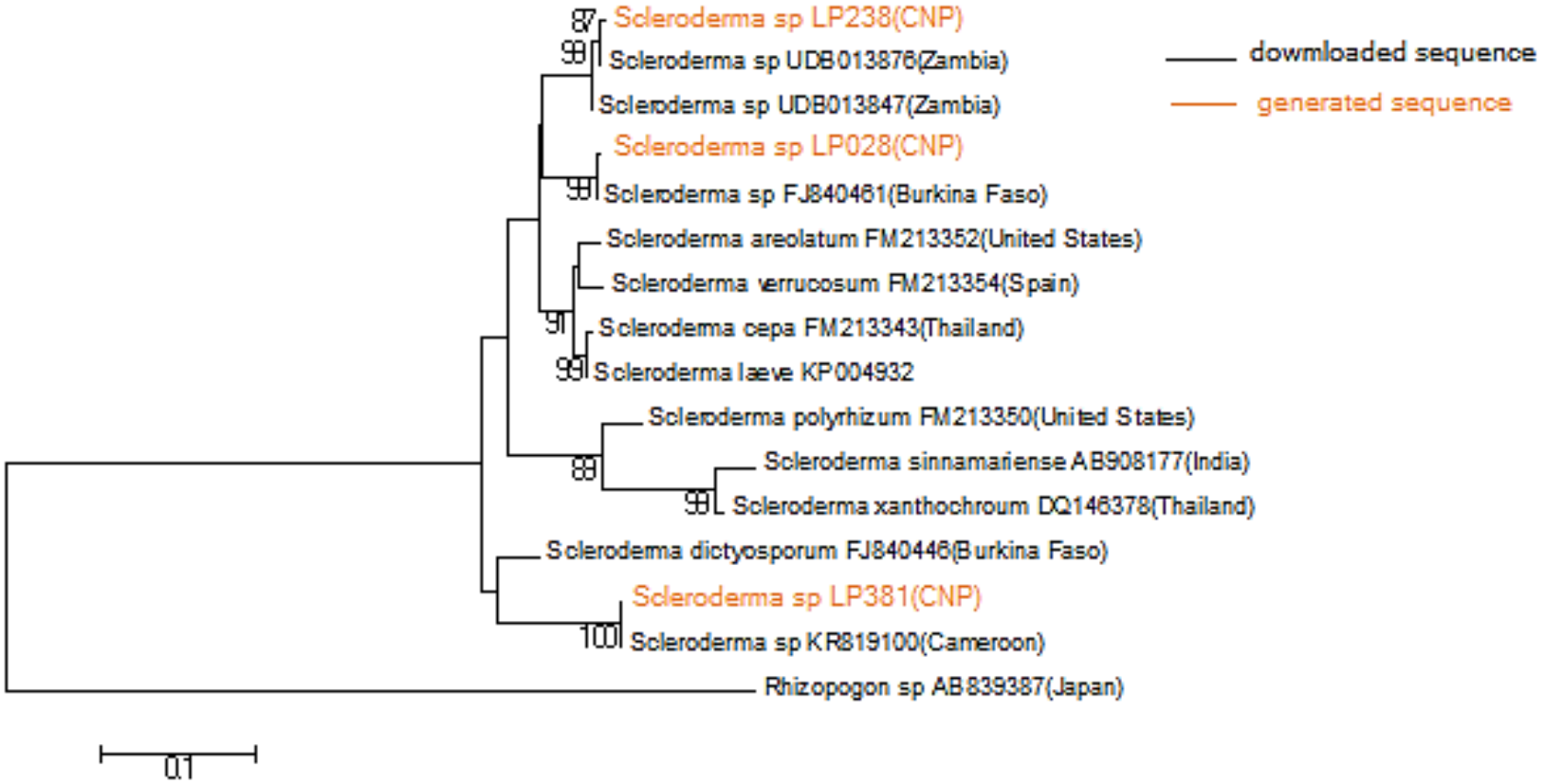


Figure 18: Phylogeny tree of Sclerodermataceae constructed by Maximum Likelihood with Kimura -2 parameter model with Gamma-distributed rates (K2+G). Generated sequences are in red. CNP: Comoe National Park

5. Cortinariaceae

Final matrix of Cortinariaceae has 13 sequences including 2 LP ones and 11 homologous with 02 outgroup sequences belonging to genus *Pisolithus.* The best substitution model was Kimura 2-parameter model plus Gamma-distributed rates (K2+G) with BIC score at 2683.352

Constructed tree is well supported with two main clades supported also. However, the lack of precise identification did not allow to name the clade within which generated sequences are nested. The high PI indicated that collected species occurred also in Benin and may have west african distribution (fig. 19).

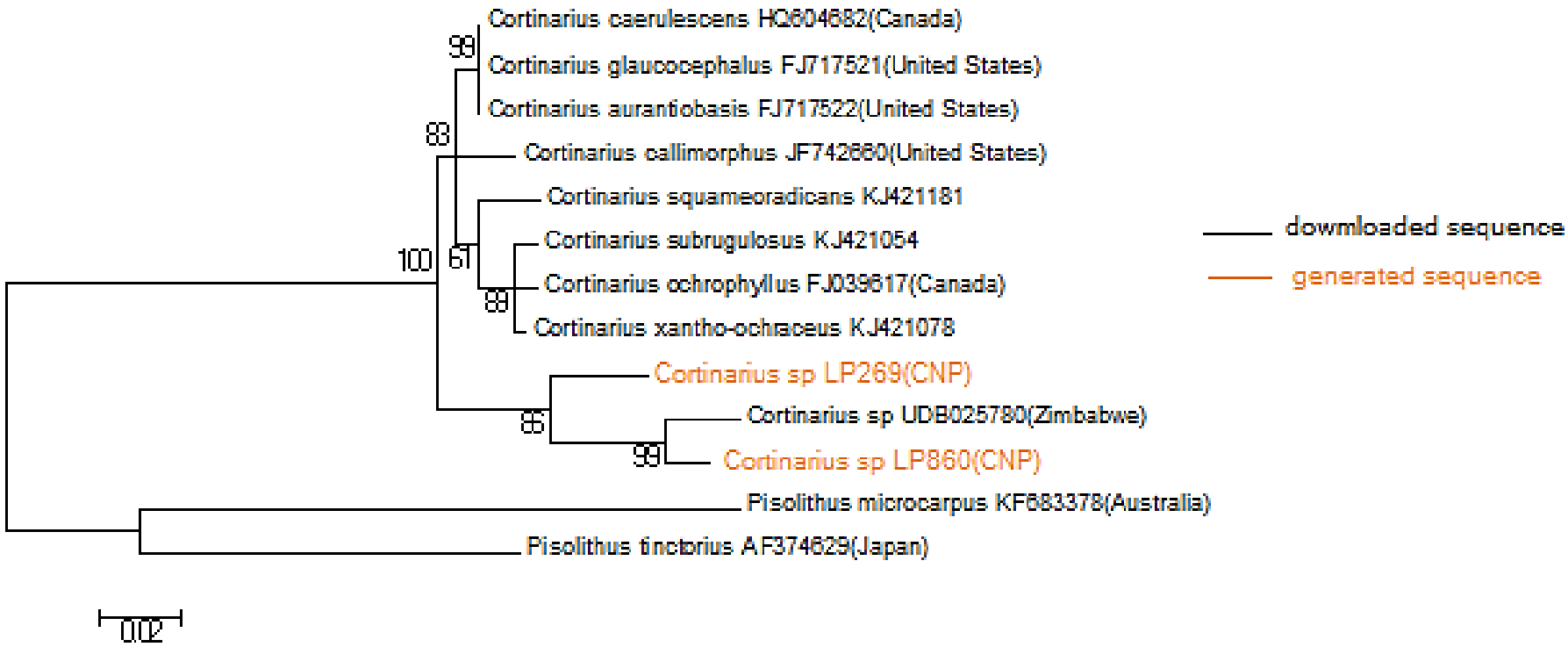


Figure 19 : Phylogeny tree of Cortinariaceae constructed by Maximum Likelihood with Kimura 2-parameter model plus Gamma-distributed rates (K2+G). Generated sequences are in red. CNP: Comoe National Park

6. Inocybaceae

Final matrix of Inocybaceae has 19 sequences including 1 LP ones and 18 homologous with 02 outgroup sequences from *Cortinarius callimorphus* and *C. caerulescens.* The best substitution model was Tamura 3-parameter model plus Gamma-distributed rates (T92+G) with BIC score at 3981.975.

The reconstructed tree was rooted suggesting a monophyletic family (fig.20); however, the tree was not supported. It appears that species from African continent diverged earlier from those from european and american zones.

Our generated sequence nested with African specimen with 96 PI and illustrated the distribution of those species in Africa namely in Benin (west Africa).

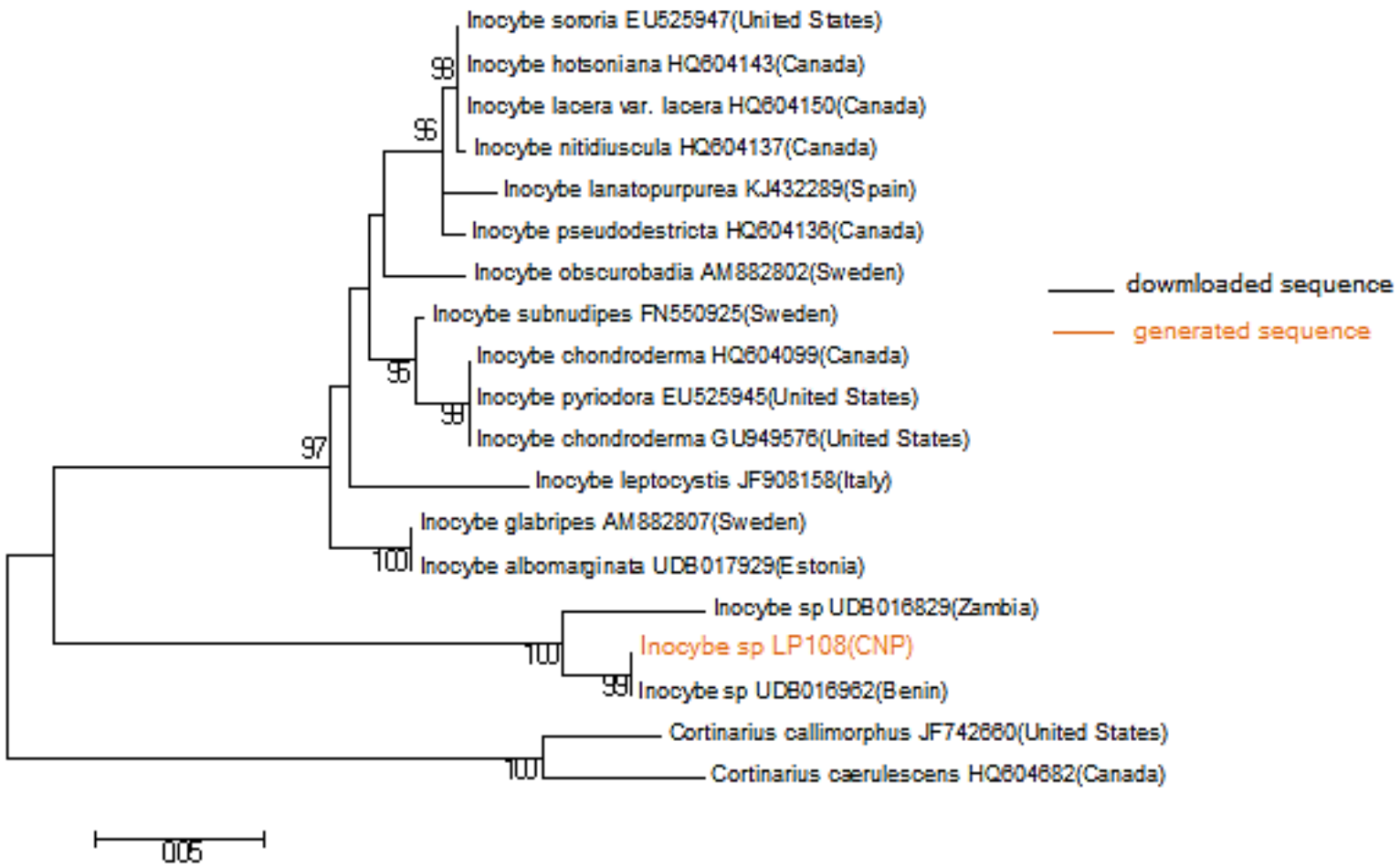


Figure 20: Phylogeny tree of Inocybaceae constructed by Maximum Likelihood with Tamura 3-parameter model plus Gamma-distributed rates (T92+G). Generated sequences are in red. CNP: Comoe National Park

7. Clavulinaceae

Final matrix of Clavulinaceae has 15 sequences including 1 LP ones and 14 homologous with 02 outgroup sequences belonging to genera *Cantharellus* and *Craterellus*. The best substitution model was Tamura 3-parameter model plus Gamma-distributed rates (T92+G) with BIC score at 4456.707

The tree was well-supported and displayed three well-supported clades. In the contrary to the previous family, african, european and south american species nested in the same clade (fig. 21). PI indicated that this species occurs also in austral Africa, namely in Zambia.

Here also, the phylogeny reconstruction of this family suggests a monophyletic family well supported (Fig. 25). 3 supported clades suggest also a hemispheric distribution: north and south hemispheres. However, the African continent is separated from the "south hemisphere" clade.

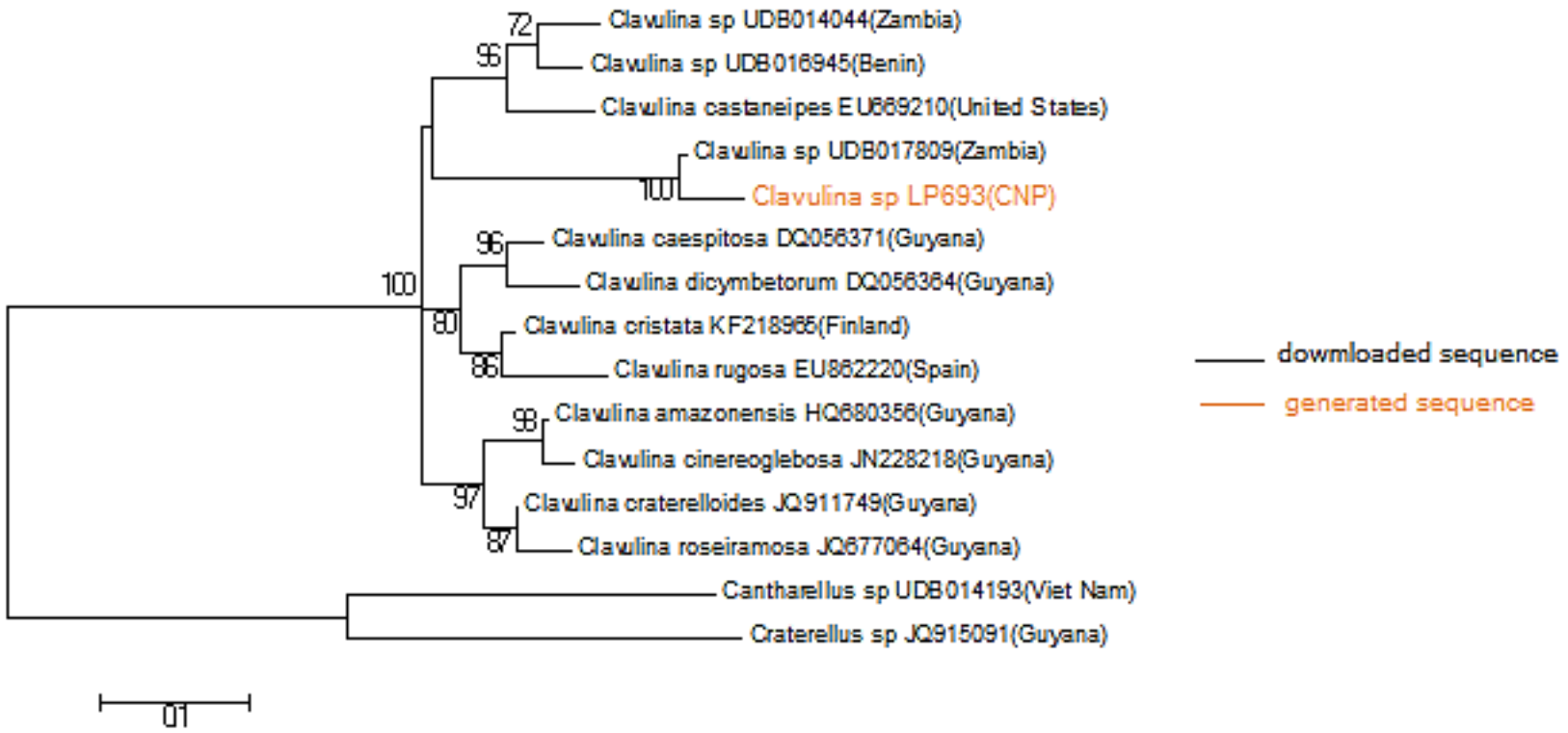


Figure 21: Phylogeny tree of Clavulinaceae constructed by Maximum Likelihood with Tamura -3 parameter model with Gamma-distributed rates (T92+G). Generated sequence is in red. CNP: Comoe National Park

8. Other families

Cantharellaceae and Gyroporaceae were represented each by one collected species. However, DNA sequencing of those species was unsuccessful so phylogenetic reconstruction could not be performed.

CHAPTER IX: SPATIAL DISTRIBUTION OF EFFB

1. Habitat characterisation

1.1. Floristic and dendrometric parameters

A cumulative number of 822 stems with dbh ≥ 10 cm belonging to 50 woody species and 49 families were detected for all habitat types (Annex 7 and 8). 18, 19 and 31 species were inventoried respectively in IW, MW and UW. The total density and basal area of all tree species, and the dendrometric parameters (density and SRD) of cores EcM forest trees in each plots is provided in table IV.

Kruskal-Wallis test demonstrated that floristic richness and total basal area did not differed significantly from one habitat type to another (chi-squared= 1.55 / p-value= 0.46 and chi-squared= 0.62 / p-value= 0.73 respectively). Considering EcM tree partners, density and SRD of *Isoberlinia doka* differed significantly between the habitat types (chi-squared = 6.72; p-value = 0.03), IW harboring the highest values. Density and SRD of *Uapaca togoensis* were also significant (respectively F = 20.73 / p-value = 0.002 and chi-squared =5.95 / p-value = 0.05), decreasing from MW to UW and finally IW. At the opposite, the densisty and SRD of *Monotes kerstingii* does not significantly differed from one habitat type to another (chi-squared = 0.62 / p-value= 0.73 and chi-squared = 2.51 / p-value= 0.28 respectively).

1.2. Soil chemical and physical parameters

pH(H_2O) measurement indicated that soils in plots were generally neutral, ranging from 6.52 to 6.78. As for texture analysis, soils in plots were generally silt loamy with regard to soil particles size (table V). However, differences among both chemical and physical parameters of the different habitat types were not significant at 0.05, pointing out an absence of soil gradient.

Table IV: Mean values of density of woody species, Species relative dominance (SRD) of identified EcM trees and total basal area per habitat

Parameters		*Isoberlinia* Woodland	Mixed Woodland	*Uapaca* Woodland
Cumulative number of stems (three plots)		276	246	300
Forest tree species richness SR		18	19	31
Total tree density TD (stem/ha)		3066.66	2733.33	3333.33
Total basal area TBA (m^2/ha)		179.75	158.43	186.89
Mean canopy cover (%)		66.67	73.33	80
EcM tree partners density(stem/ha)	*Isoberlinia doka*	171.11	5.56	0.00
	Monotes kerstingii	35.56	18.89	23.33
	Uapaca togoensis	10.00	167.78	153.33
EcM tree partners SRD (%)	*Isoberlinia doka*	62.29	3.68	0.00
	Monotes kerstingii	10.28	4.13	6.57
	Uapaca togoensis	0.99	53.48	40.50

Table V: Soil chemical and physical parameters variations per habitat type

Parameters	Habitat type			F	Chi-square	Df	p-value
	IW	MW	UW				
pH(H_2O)	6.7 ±0.14	6.52±0.4	6.78±0.2		2.039	2	0.361
Carbon (%)	1.96±0.089	1.85±0.15	1.71±0.13		4.392	2	0.111
Nitrogen (%)	0.09±0.05	0.09±0.01	0.12 ± 0.02	0.495		2	0.632
Available Phosphorus (ppm)	1.34±0.32	1.63±0.12	1.20±0.12		3.586	2	0.166
Calcium (cmol/kg)	1.71±0.42	1.45±0.31	1.07±0.17	3.078		2	0.12
Potassium (cmol/kg)	0.06±0.04	0.09±0.03	0.07±0.03	0.936		2	0.443
Clay (%)	8.67±2.08	10±2.64	9.33±0.58		0.858	2	0.651
FineSilt (%)	9.33±3.51	5±0.00	8.66±3.05		5.727	2	0.057
CoarSilt (%)	44.33±12.1	42.66±3.05	45.67±5.86		0.291	2	0.864
FineSand (%)	34.333±8.14	37±2.64	33.67±3.79		1.195	2	0.550

IW: *Isoberlinia* Woodland; MW: Mixed Woodland; UW: *Uapaca* Woodland

2. Correlation between EcM fungal species fruting and habitat characteristics

In absence of soil gradient between habitat types, soil variables were excluded from initial environmental matrix that was finally reduced to five plant variables after multicollinearity test. Those variables were Plant species richness (PlantSp), Total basal area (TBA), *Isoberlinia doka* Density (IDDen), *Monotes kerstingii* Density (MKDen) and *Uapaca togoensis* Density (UTDen).

Environment variables fitting into NMDS result indicated that *Isoberlinia doka* Density (IDDen) and *Uapaca togoensis* Density (UTDen) are the main statistically significant variables driving the EFFB spatial distribution (fig. 22). UTDen was positively correlated with both axes ($r^2 = 0.92$; p-value = 0.002) whilst IDDen was negatively correlated to the first axis only ($r^2 = 0.83$; p-value = 0.018).

Hierarchical analysis of study sites evidenced two sites groups (fig. 23). The first group (G1) encompassed all plots of habitat 1 (*Isoberlinia* woodland IW) and the two first plots of the second habitat, Mixed woodland (MW). The secont group is composed of the last plot of habitat 2 (MW) and all plots of the third habitat *Uapaca* Woodland (UW). The indicator species analysis showed that four species were significantly associated to just one group on a total of 123 species. 03 species were associated to G1 and 01 species to G2 (table VI).

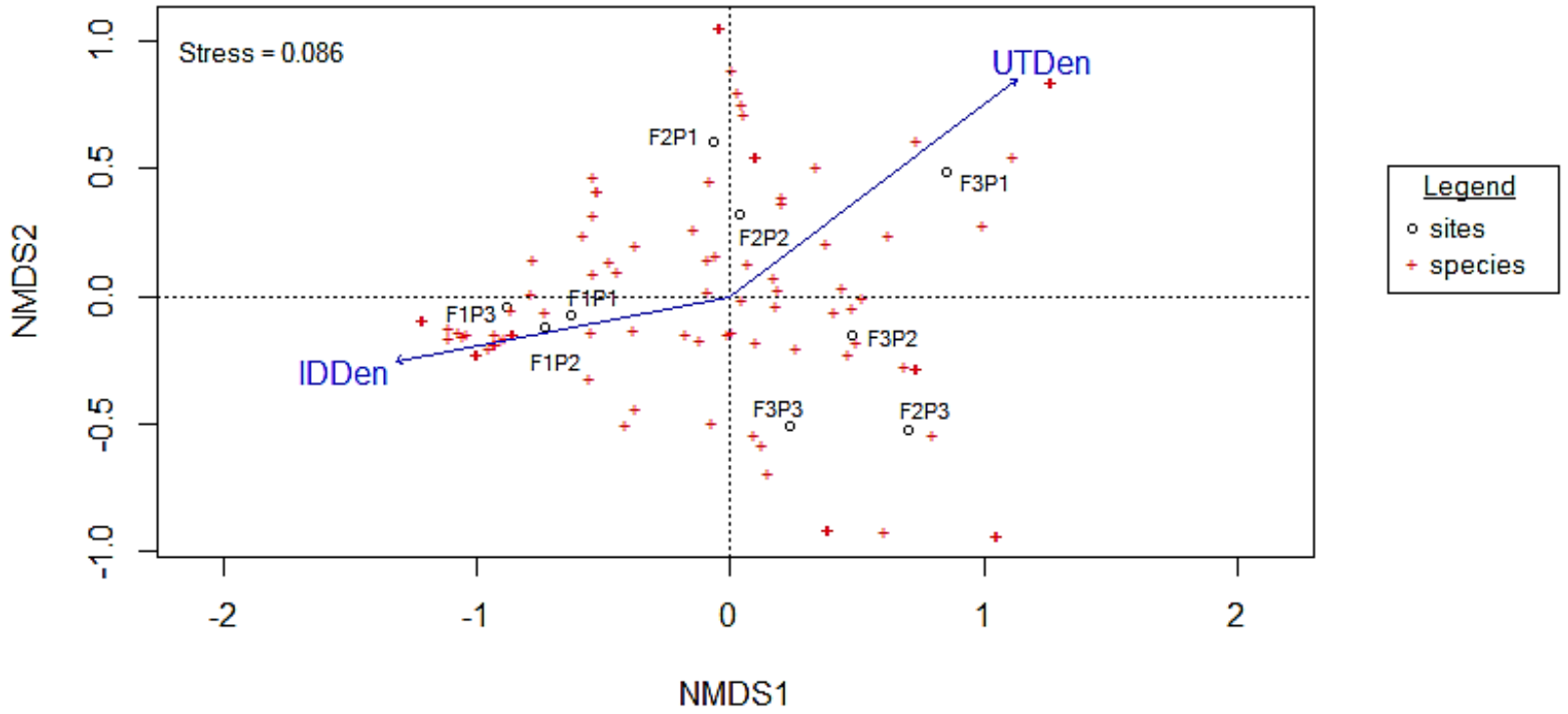


Figure 22: EcM fungi distribution at Comoé National Park driven by main EcM tree partners density. IDDen: *Isoberlinia doka* density; UTDen: *Uapaca togoensis* density. FiPi: plot *i* within habitat type *i*

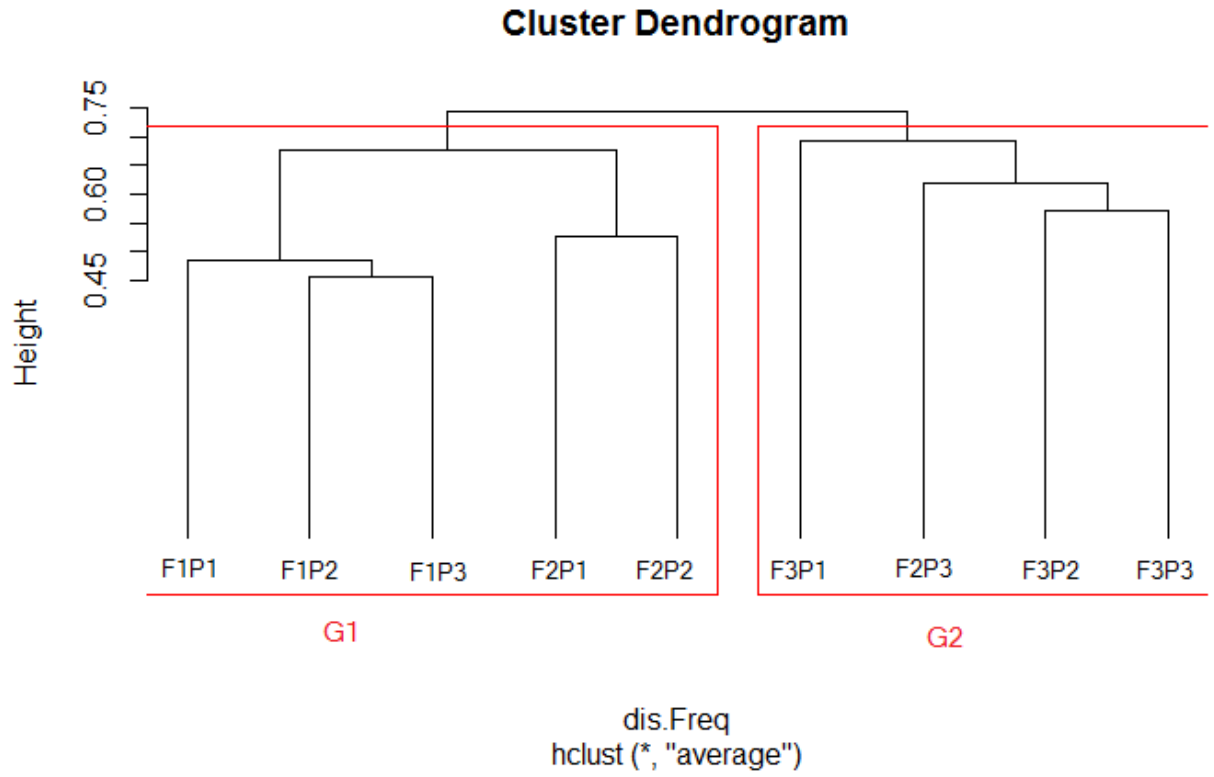


Figure 23: Hierarchical clustering of permanent plots based of dissimilarity. FiPi: plot *i* within habitat type *i;* Gi: group *i*

Table VI : List of species associated to each group

Site group	Component A	Component B	Stat	p.value
Group 1 # sps. 3				
RusCon	0.9573	1.0000	0.978	0.013 *
Pulve1	0.9057	1.0000	0.952	0.028 *
AmaXa	0.8276	1.0000	0.910	0.040 *
Group 2 # sps. 1				
AcfVir	1	1	1	0.013 *

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

RusCon: *Russula congoana*; Pulve1: *Pulveroboletus* sp 1; AmaXa: *Amanita xanthogala;* AcfVir: *A.* cf *virosa*

CHAPTER X: FRUITING PHENOLOGY AND PRODUCTION OF EFFB IN RELATION TO CLIMATE VARIABILITY

1. Climate parameters variability

Climatic data were collected over 26 weeks from 8th April to early October 2014. However, only 23 weeks were considered to assess influence of climate variability on EcM fungi fruiting phenology and production, namely from May to early October 2014. That required checking the significance of climate variability.

1.1. Air temperature

Minimum and maximum values recorded were respectively 27.43 and 34.60 ˚C for IW, with a mean value of 30.56 ˚C. In MW, minimum and maximum were 25.37 and 31.42 ˚C for a mean temperature of 27.62 ˚C 66. As for UW, those values were respectively 25.10 ˚C and 30.50 ˚C for a mean of 26.85 ˚C.

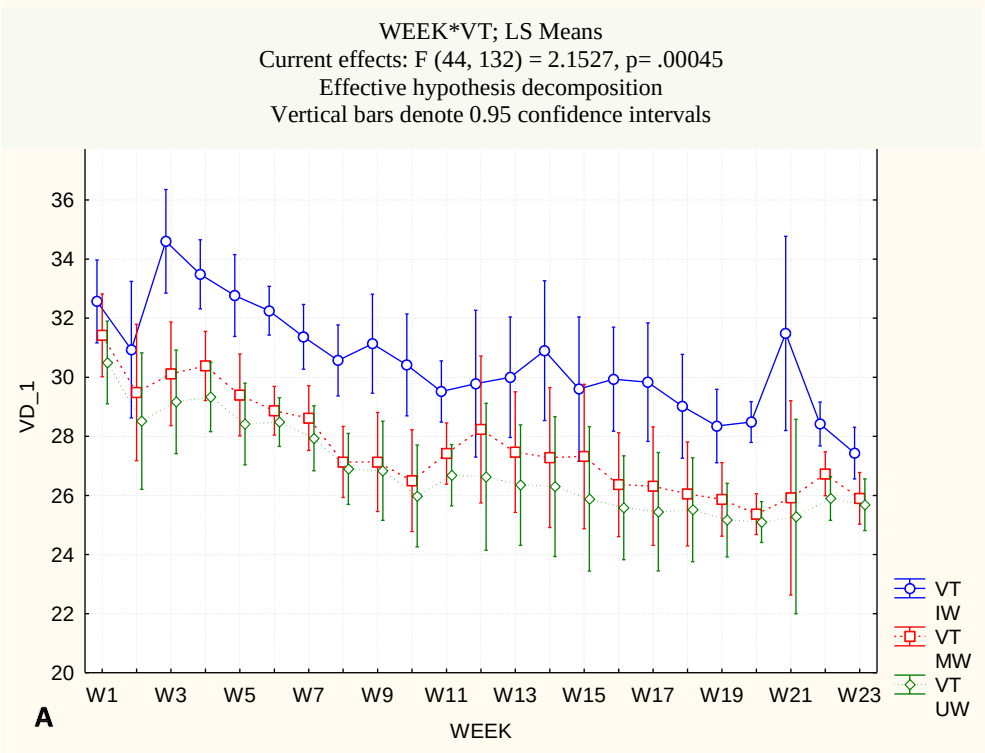
Generally, air Temperature (AirTemp.) decreased in all habitat types (fig. 24A). However, slight increases were observed in IW respectively at the third week of May (34.6 ˚C) and September (31.5 ˚C). Maxima of that factor were recorded at IW where they were above 30 ˚C from May till early August whilst they were below 30˚C from last June at MW. For UW, the decrease below 30 ˚C was recorded earlier, from second week of May (fig. 24B).

Probability of Mauchly's Test indicated that assumption of sphericity was not violated for air temperature (table VI). Moreover, repeated measures ANOVA revealed that variability of Air Temperature was statistically significant respectively for the three effects *habitat type* (VT) , *week* and *interaction week*VT*. Thus for effect *habitat type* (VT), IW was the hottest habitat followed by MW and then UW ($F(2, 6) = 11.461$, p- value = 0.00893**). Similarly, the variability during period of collect as reflected by the effect *week* was significant (table VI). Final, significance of *interaction week*VT* ($F(44, 132) = 2.1527$, p= 0.00045***) allowed the assessment of influence of air temperature on edible EcM fungi production.

Table VII : Repeated measures ANOVA of climate parameters recorded in permanent plots of CNP

Parameter	Habitat type		Week		Habitat type*Week	
	F(2,6)	p-value	F(22,132)	p-value	F(44,132)	p-value
Air Temperature	11.461	0.00893**	40.582	0.0000****	2.1527	0.00045***
Rainfall	F(2,3) = 2.0632	0.27314	F(20,60) = 916.88	0.0000****	F(40,60) = 6.4554	0.0000****
Relative humidity	9.3881	0.01420*	46.062	0.0000****	1.5687	0.02690*
Soil temperature	21.754	0.00178**	728.86	0.0000****	9.9614	0.0000****
Soil moisture	0.41158	0.67998	70.503	0.0000****	0.87916	0.68248

$\alpha > 0.001$ (***) ; $\alpha > 0.01$ (**); $\alpha > 0.05$ (*)



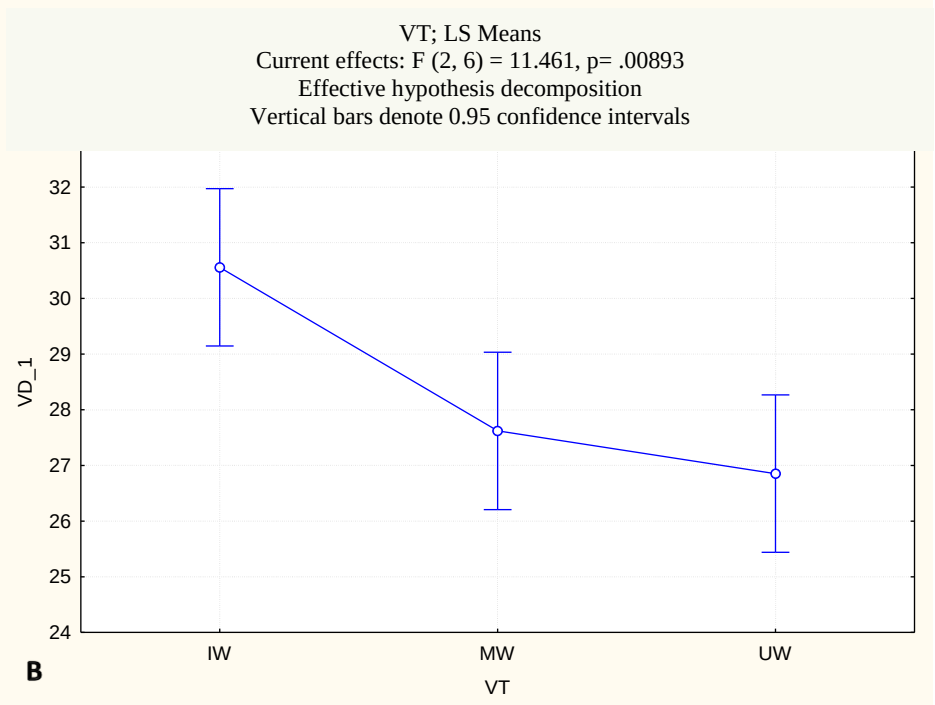


Figure 24 : Air Temperature variability for effect *interaction week*VT* (A) and between habitat types (B)

1.2. Relative humidity

Similarly to air temperature, recorded minimum and maximum relative humidity were respectively 63.88 and 76.13 % for IW, with a mean value of 71.34 %. In MW, minimum and maximum were 70.03 and 85.98 % for a mean value of 79.33 %. As for UW, those values were respectively 71.73 and 89.32 % for a mean of 81.75 %.

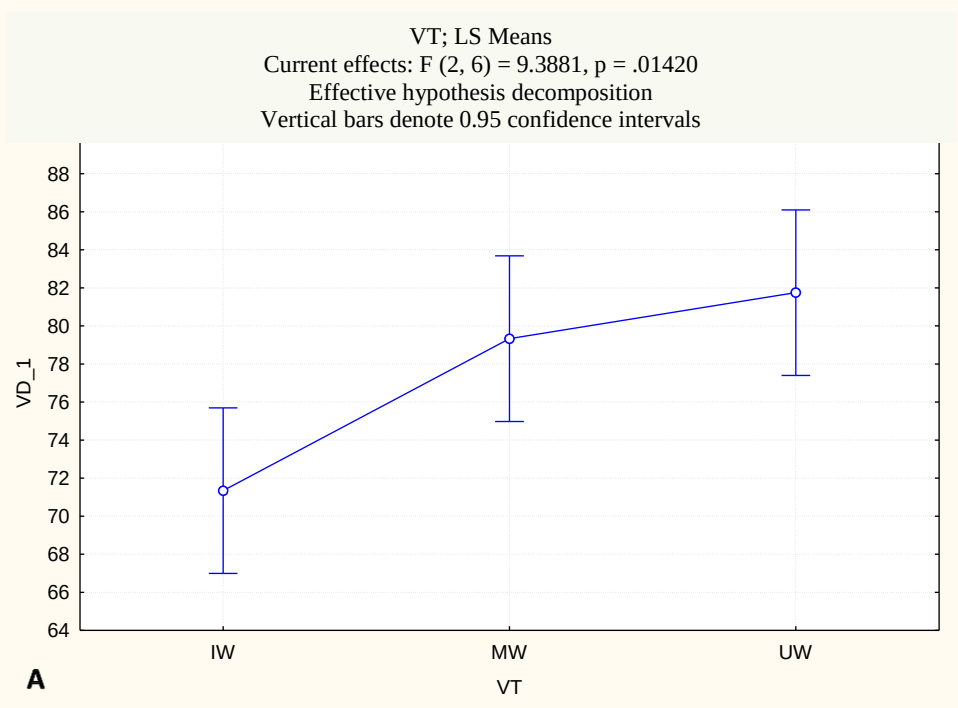
During the collect period, relative humidity (RelHum) generally increased continuously at all habitat types reaching almost 90 % at UW and MW whilst it was below 80 % in IW. Nevertheless, a slight decrease of RelHum was observed at all habitat type that corresponded to the last week of May and first week of June due to minima and absence of rainfalls (fig. 25A).

Since the assumption of sphericity was not violated also for that parameter (table VI), repeated measures ANOVA revealed that variability was also significant for all tested effects. As expected, relative humidity was increasing significantly from IW to UW but also from the beginning till the end of season (fig. 25B) as expressed by effect *interaction VT*Week* (F (44, 132) = 1.5687, p = 0.02690*).

1.3. Rainfall intensity

Rainfall varied globally in eigth cycles of increased and decreased intensities, falling at all habitat types during almost all weeks except the first week of June and second week of August. Higher values were observed in early October with 102.9 mm in IW against 98.8 mm in MW and 101.1 mm in UW.

Rainfall intensity ranged from 0.25 to 102.90 mm for IW, with a mean value of 32.03 mm. In MW, amplitude was 0.10 to 98.80 mm for a mean value of 31.10 whilst in UW it ranged from 0.10 to 101.10 mm for a mean of 31.45. Thus, higher mean value was obtained in IW followed by a decrease of intensity in MW and an increase recorded in UW. Nevertheless, rainfall intensity did not differed statistically (fig. 26A) from one habitat another (effect *VT*: F (2, 3) = 2.0632, p = 0.27314). However, its variability was significant for effect *week* (table VI). Fortunately, *interaction VT*Week* (fig. 26B) was also significant (F (40, 60) = 6.4554, p = 0 .00000***), allowing assessment of influence on fungi production.



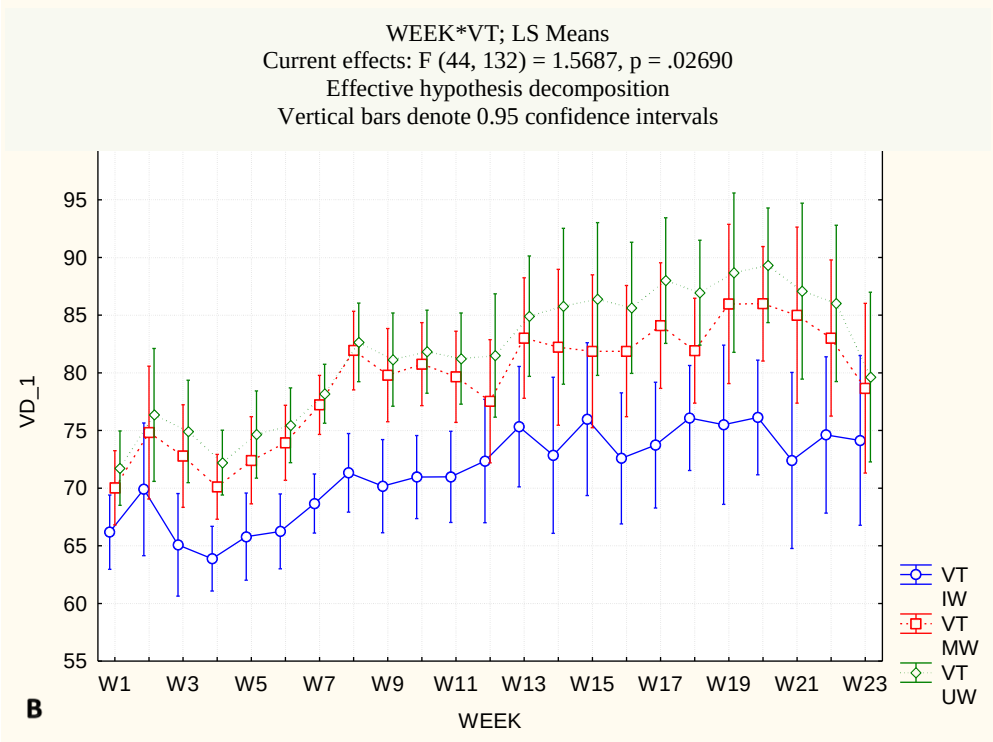


Figure 25: Relative humidity variability between habitat types (A) and for effect *interaction week*VT* (B)

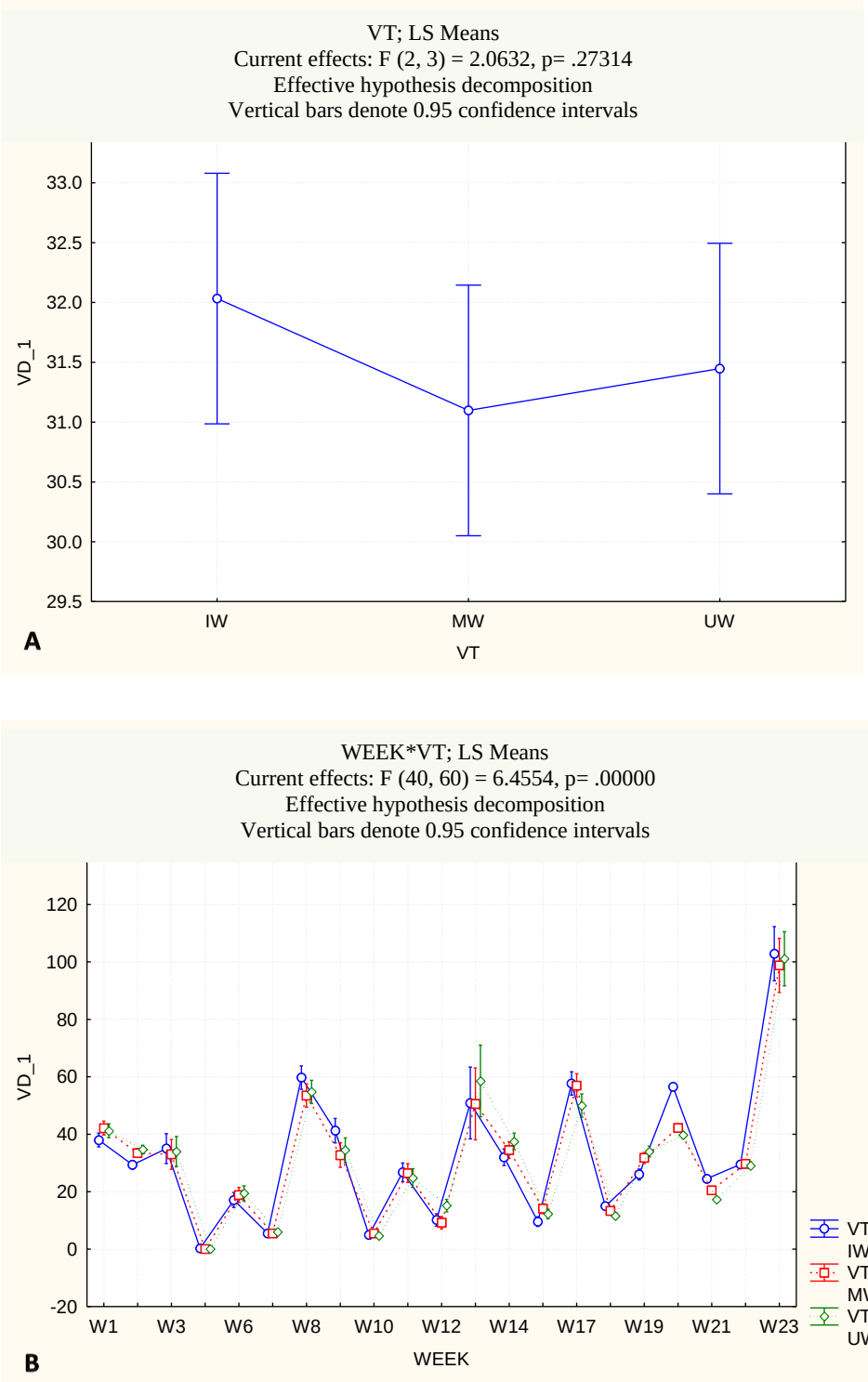


Figure 26: Rainfalls variability between habitat types (A) and for effect *interaction week*VT* (B)

1.4. Soil temperature

Minimum and maximum values recorded were respectively 24.24 and 30.24 ˚C for IW, with a mean value of 26.12 ˚C. In MW, minimum and maximum were 23.82 and 28.16 ˚C for a mean temperature of 25.43 ˚C. As for UW, those values were respectively 23.64 and 27.74 ˚C for a mean of 25.13 ˚C. Mean temperature was high at IW and was above 30 ˚C. Indeed, higher values of soil temperature were recording in IW and decreased to UW. That variability at habitat type level was significant (fig. 27A) according to repeated measures ANOVA ($F(2, 6) = 21.754$, $p = 0.00178$**).

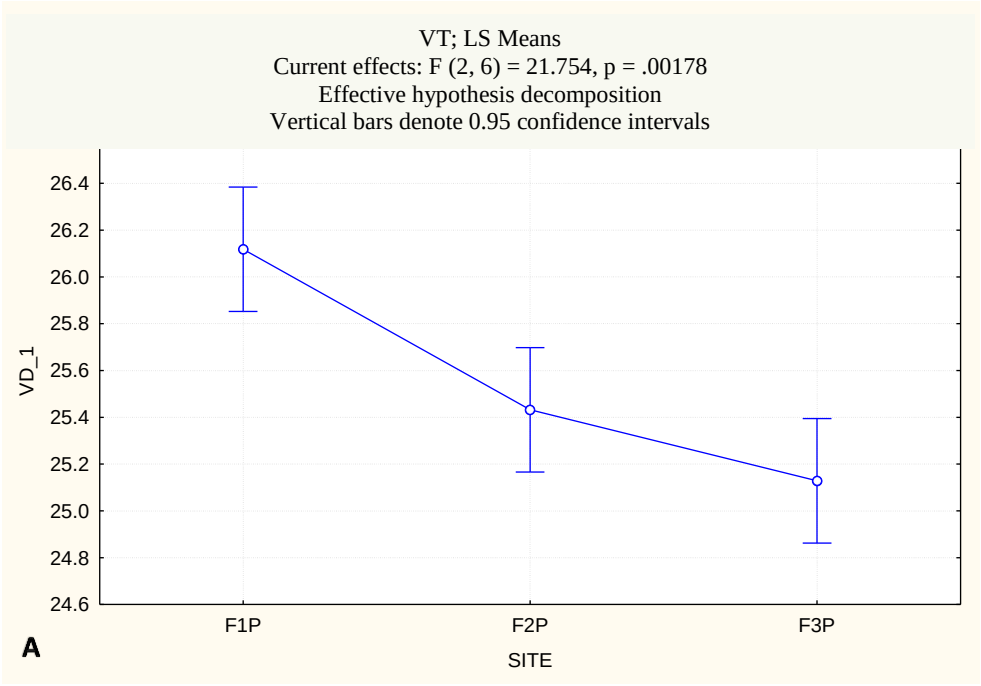
Soil temperature was generally below 30 ˚C at all habitat types but a slight increase was recorded in late May and early June (fig. 27B). That corresponded to the weak intensity and absence of rainfall at that period of season 2014. Similarly to air temperature, soil cooled significant during the season, likely due to rainfalls. Moreover, interaction was also statistically significant ($F(44, 132) = 9.9614$, $p = 0 .0000$***).

1.5. Soil moisture

Recorded minimum and maximum of soil moisture were respectively 1.51 and 20.31 volume percent (vol. %) for IW, with a mean value of 12.90 vol. %. In MW, they were 2.52 and 22.40 vol. % for a mean value of 14.51 vol. %. As for UW, those values were respectively 1.47 and 16.76 volume % for a mean of 10.26 vol. %.

Globally, soil moisture increased during season 2014. However, a decrease was also recorded from late May to early June as consequence of the weak and / or absence of rainfall at that period of rainy season 2014. Minima of soil moisture for the season were recorded during those weeks. Though general minimum was observed in UW (1.47 vol. %) the first week of June, it was recorded one week earlier in IW (1.5 vol. %). In addition, maxima were above 20 vol. % at MW whilst equal or below that value respectively at IW and UW.

Soil moisture was statistically significant from a week to another (table VI) but not for effect *habitat type VT* and *interaction VT*week* (fig. 28).



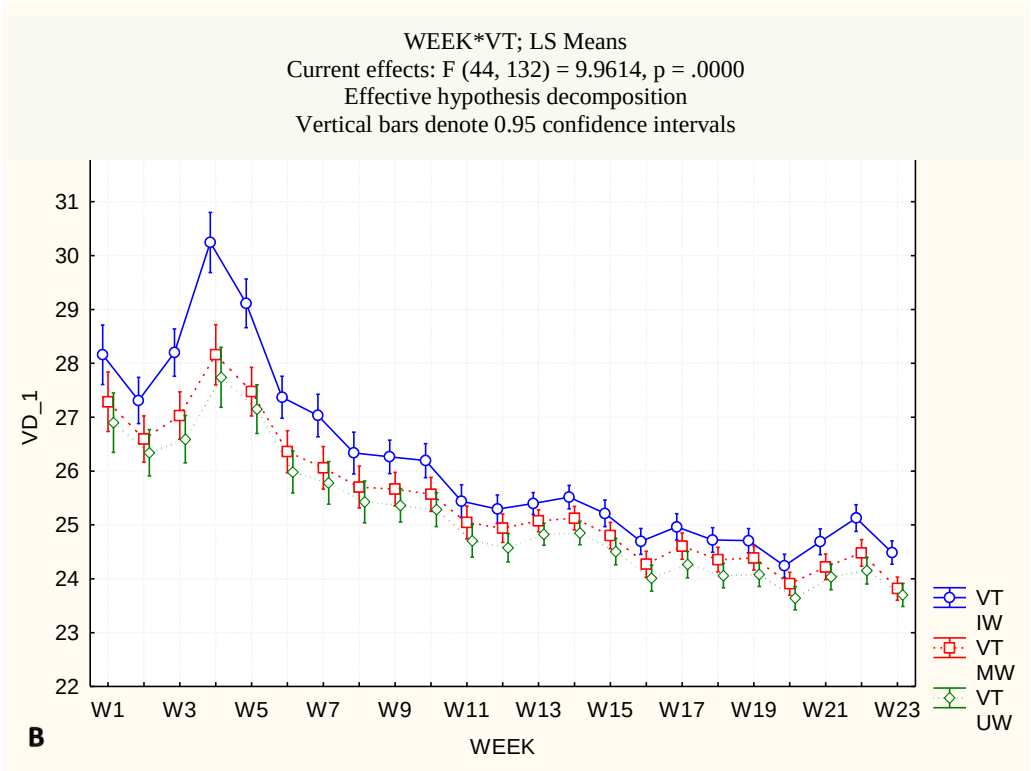
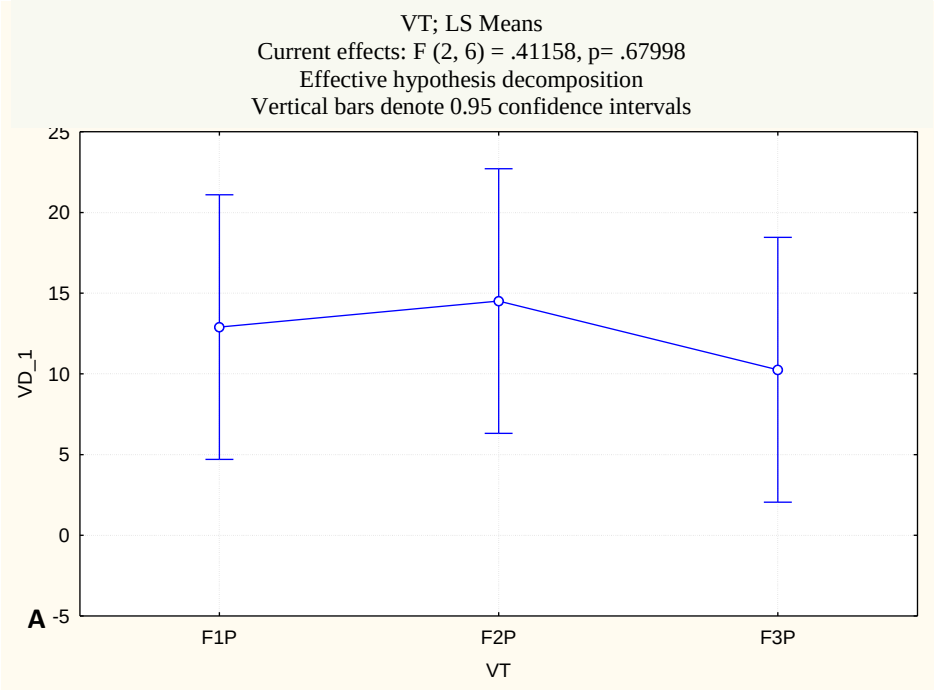


Figure 27: Soil Temperature variability between habitat types (A) and for effect *interaction week*VT* (B). F1P = IW; F2P = MW; F3P = UW.



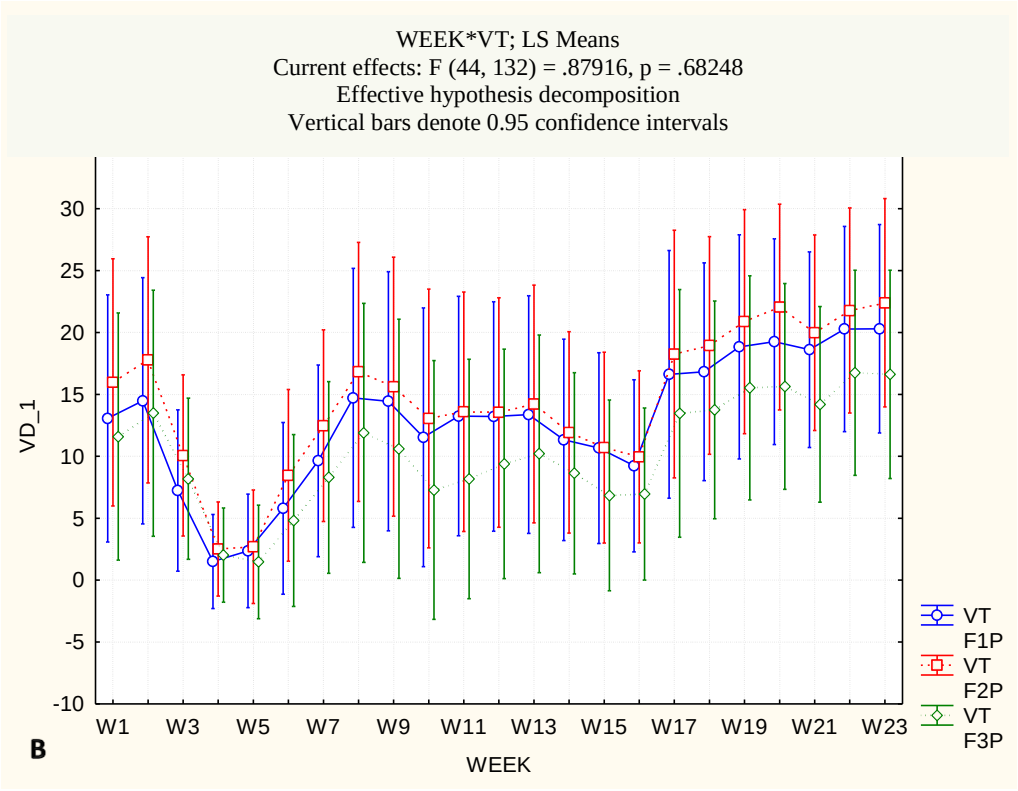


Figure 28: Soil moisture variability between habitat types (A) and for effect *interaction week*VT* (B).

2. Edible EcM fungi and their fruiting phenology

According to literature **(Yorou *et al.*, 2001; De Kesel *et al.*, 2002; Eyi Ndong *et al.*, 2011)**, 27 EcM fungal species from our collection are edible (table VIII). They belong to 9 genera and 5 families with a cumulative number of 1810 fruit bodies. The first collected edible EcM fungi belonged to genus *Amanita* and started fruiting in mid-May with *A. cf. crassiconus* during two weeks in MW whilst *A. strobilaceovolvata s.l.* and *A. xanthogala* fruited in UW, each of them during one week at the end of June 2014. The other fungi fruited from the last week of June until early October, more or less repeatedly and with different durations depending on the species. According to their occurrence in the different habitat types, edible species were distributed in different groups as in chapter III. 9 species were common to all habitat types, 10 were shared by two habitat types and 8 species were specific to one habitat type. 9 shared species and 5 specific species were recruited in MW.

In general, fruiting was observed during 15 weeks in IW (Fig. 29) and MW (Fig. 30), and 16 weeks in UW (Fig. 31). The first edible EcM fungi fruited during weeks 3 and 4, namely during the last weeks of May. That occurred during the third week of effective rainfalls at CNP in 2014. Meanwhile, no fruit bodies was recruited in IW but also during week 5 to week 8 likewise MW and UW. Fruiting started again in all habitat types at week 9 and lasted till week 23, namely from late June to early October 2014. However, no recruitment was done in MW at week 22. Week 21 was deleted from analysis as mentioned previously because technical issue did not allow survey in all habitat types.

3. Production of edible EFFB

A cumulative fresh biomass of 84.09 kg/ha was yielded during rainy season 2014. The highest production was obtained in UW with 34.72 kg/ha, followed by 25.25 kg/ha in MW whilst 24.11 kg/ha was collected in IW.

Peaks of production followed peaks of fruiting in UW and MW but not in IW. Indeed, in those previous habitat types, both peaks were attained the same week whilst in the latter peak of production was obtained the twelfth week of season that was the third week of July. So globally, peaks of production were attained during July 2014.

Table VIII: Edible EcM fungi records in Comoé National Park. IW: *Isoberlinia* Woodland; MW: Mixed Woodland; UW: *Uapaca* Woodland

Parameter	IW	MW	UW	Total
Number of fruit bodies	1096	298	416	1810
Number of species	19	22	14	27
Number of genera	7	7	6	9
Number of families	5	4	5	5
Cumulative fresh biomass (kg/ha)	24.11	25.25	34.72	84.09

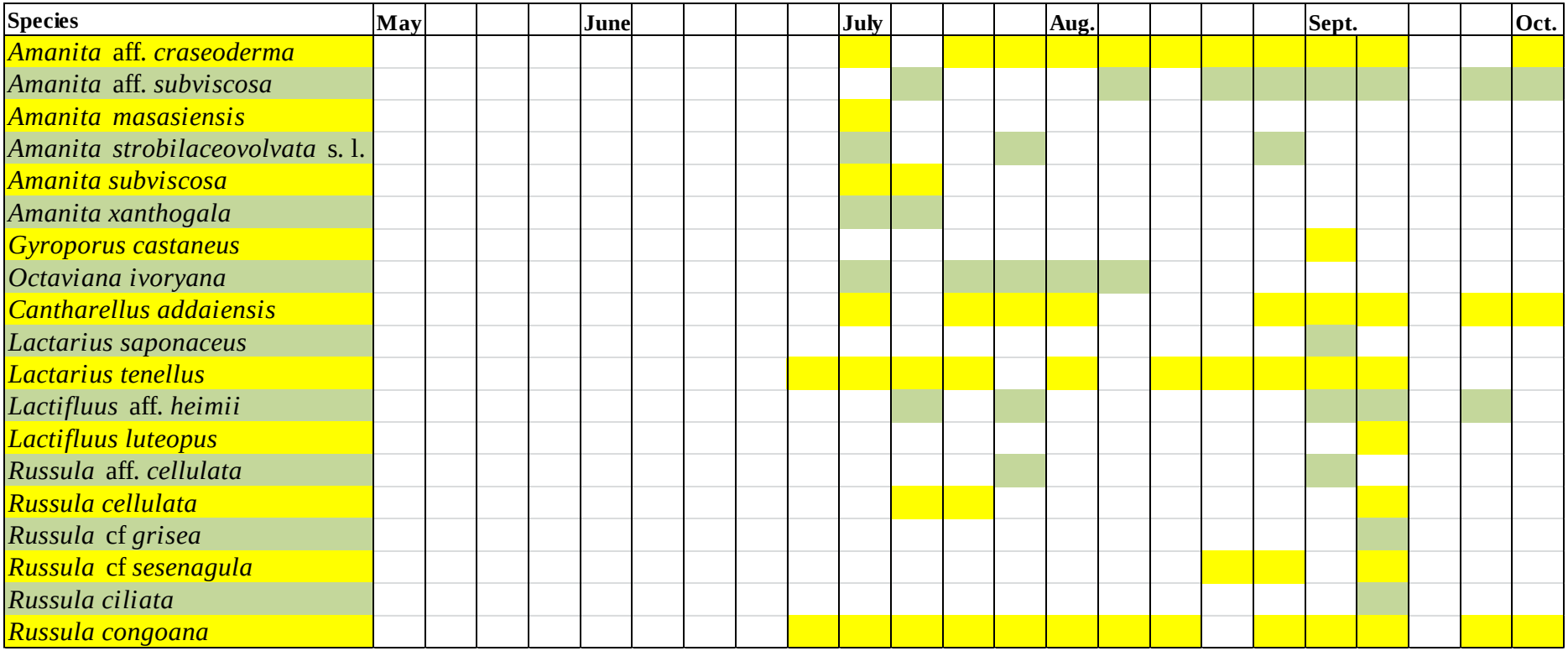


Figure 29: Fruiting phenology of edible EcM fungi in *Isoberlinia* Woodland (IW). Aug.: August, Sept.: September, Oct.: October. Colours are only indicative.

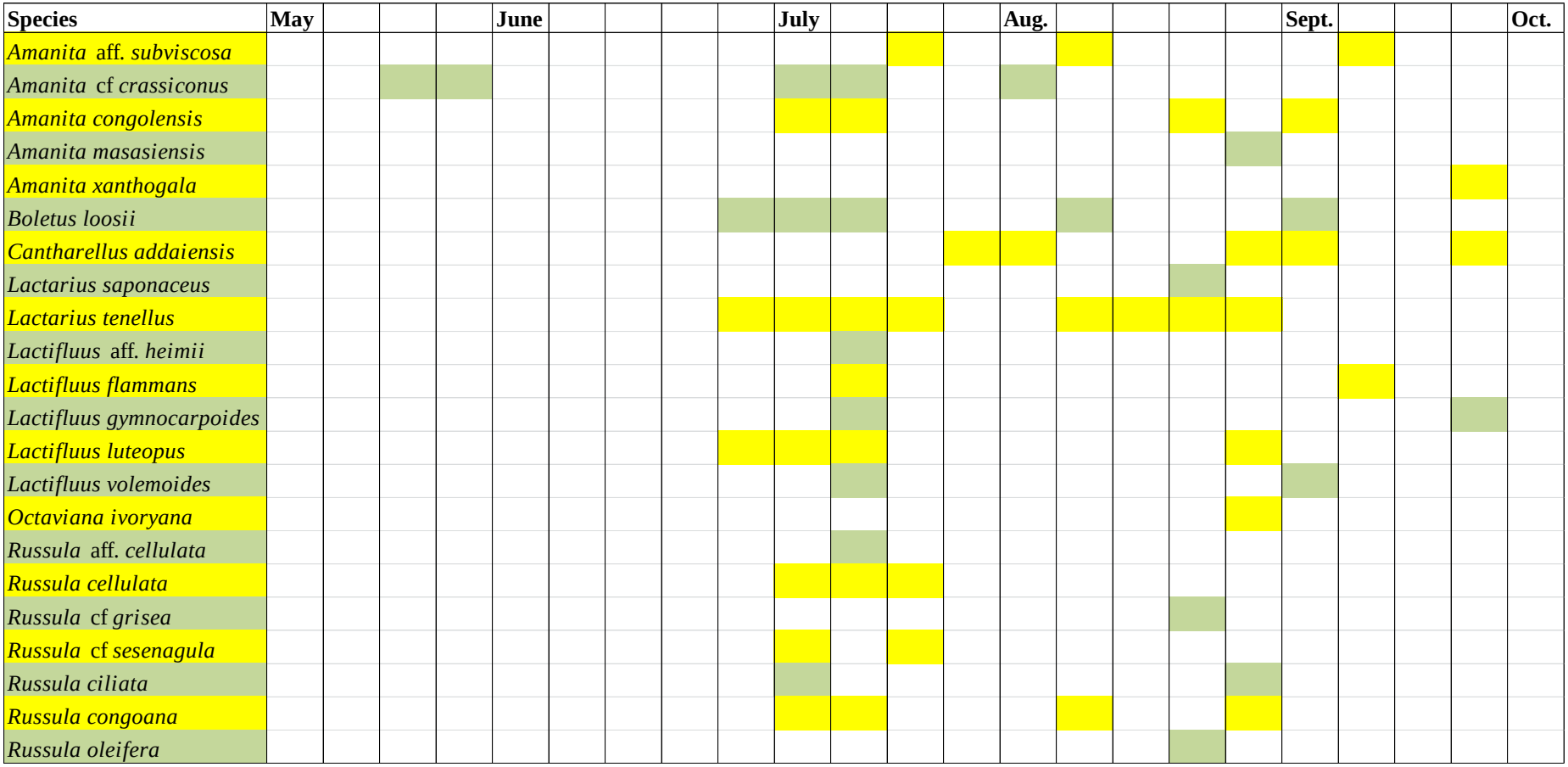


Figure 30: Fruiting phenology of edible EcM fungi in Mixed woodland (MW). Aug.: August, Sept.: September, Oct.: October. Colours are only indicative.

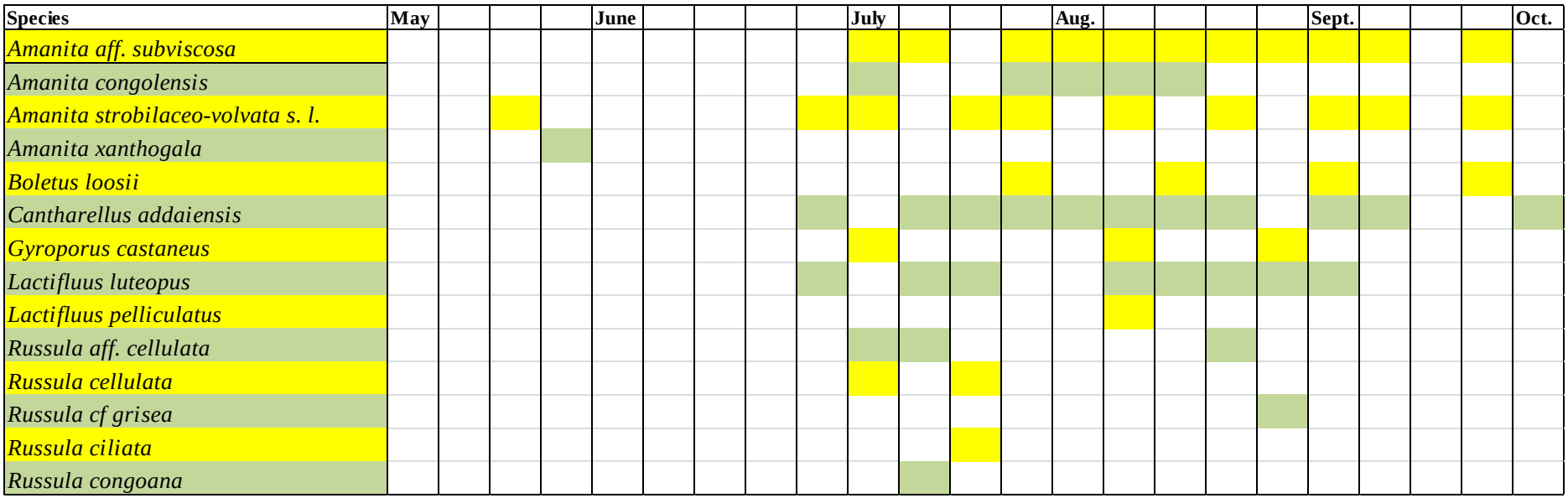
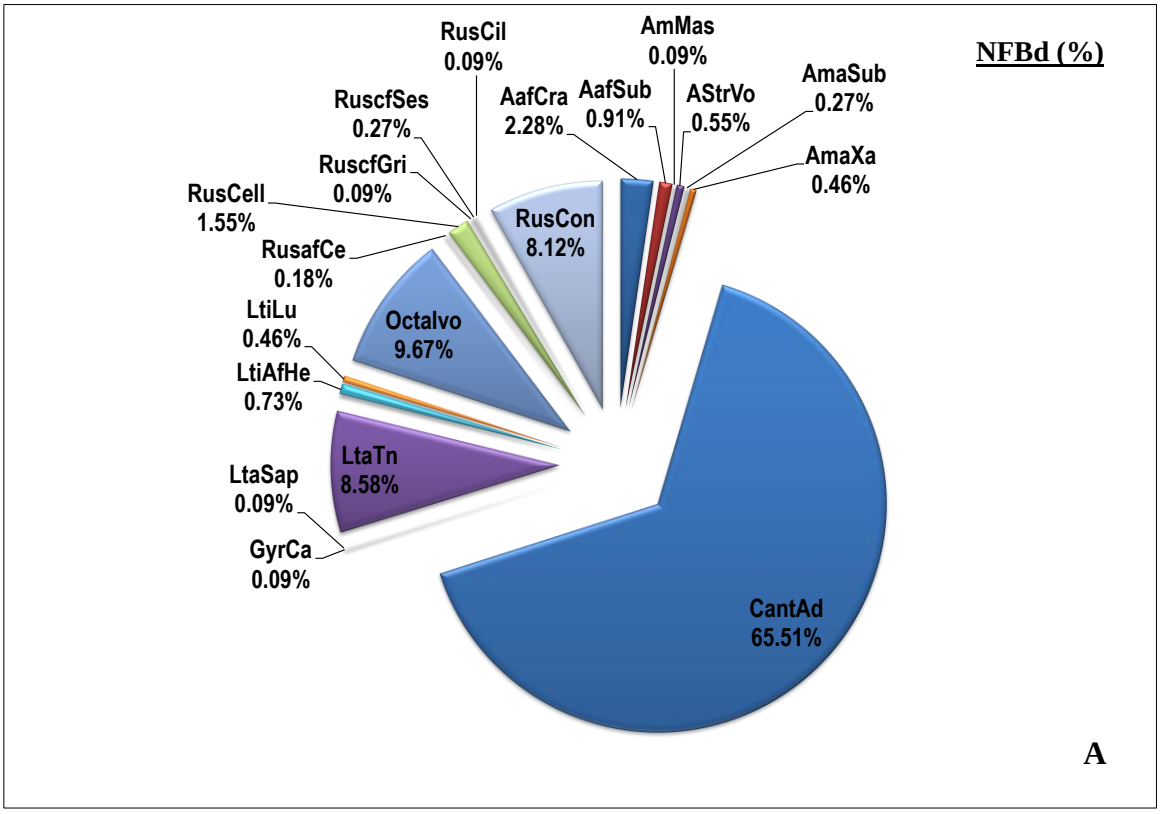


Figure 31: Fruiting phenology of edible EcM fungi in *Uapaca* woodland (UW). Aug.: August, Sept.: September, Oct.: October. Colours are only indicative.

Peak of fruiting differed from a habitat type to another. In IW, it was reached later in the season at the twentieth week (second week of September) with 22.99 % of total fruiting. Eleven edible species were recorded but maximum was acquired with *Cantharellus addaiensis* that with its 206 fruit bodies represented 18.8 % of cumulative fruiting at IW (Fig. 32-A). In the contrary, the peak in MW was reached second week of July. 24.83 % of total fruiting was obtained there the second week of July (corresponding to the eleventh week of the season) with 12 fruiting species. However, maximum was attained with *Lactifluus flammans* with 15 fruit bodies representing 5.03 % of cumulative fruiting at MW (Fig. 33A). It was reached earlier in UW with 34.13 % of total fruit bodies, the first week of July that makes the tenth week of the season. Only six species fruited that week and maximum fruiting was obtained with *Russula cellulata* (62 fruit bodies representing 14.90 % of cumulative fruiting at UW, Fig. 34A).

For production, peak in IW was obtained earlier as abovementioned with *Octaviania ivoryania* that yielded 1.77 kg / ha for 11.84 % of cumulative production. Moreover, *R. cellulata* ranked second with 1.51 kg / ha (11.10 % of cumulative production, Fig.32B). Similarly to fruiting, maximum production was also obtained the eleventh week of the season in MW but with *Russula cellulata* instead of *Lactifluus flammans* like in fruiting. In MW, *R. cellulata* yielded 1.91 kg / ha corresponding to 8.05 % of cumulative production of the season in MW (Fig. 33B). The main difference was observed with a change in both week and species. In UW, maximum production was still obtained the tenth week of season with *Russula cellulata* that yielded 4.94 kg / ha representing 40.2 % of cumulative production (Fig. 34B).

The most abundant (number of fruit bodies) edible EcM fungal species collected in all habitat types was *Cantharellus addaiensis* with 65.51, 28.52 and 44.47 % of total number of fruit bodies respectively for IW, MW and UW. However, *C. addaiensis* is one of the less represented EcM in term of natural production with 4.39, 0.94 and 0.63 % of total fresh biomass for the same habitat types.



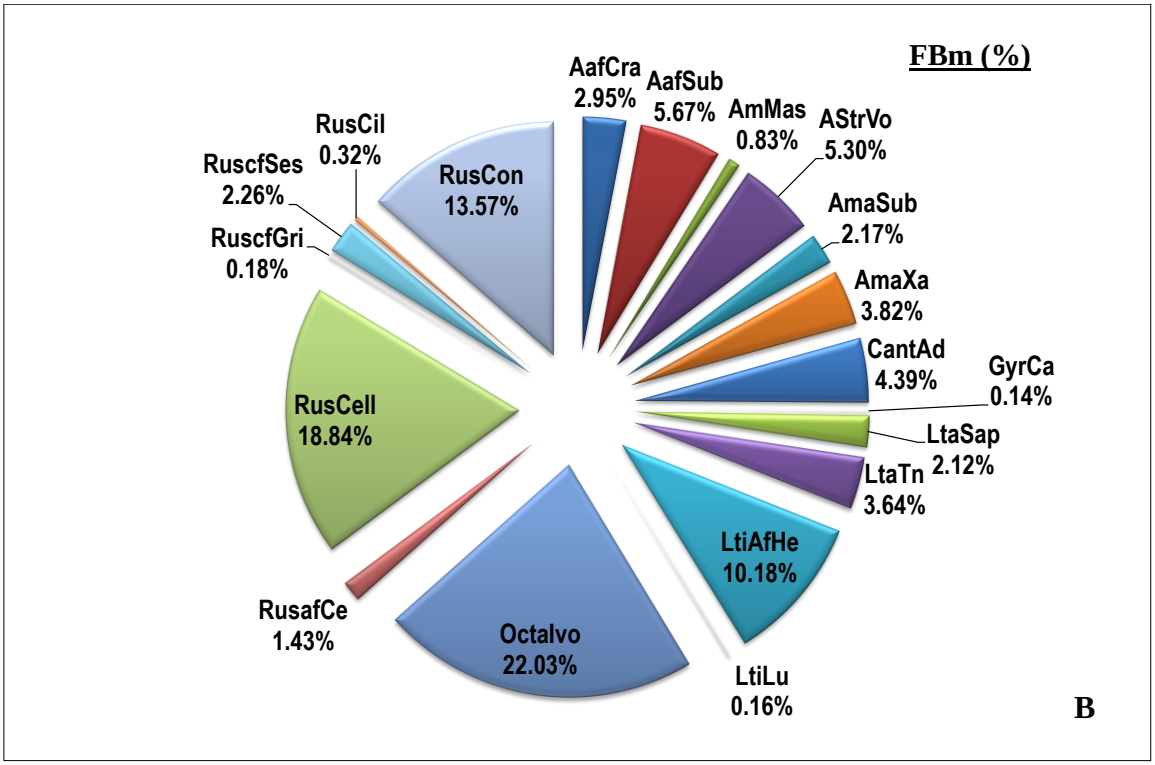


Figure 32: Proportions of edible ectomycorrhizal fungal species collected in *Isoberlinia* Woodland (IW). A: Number of Fruit Bodies NFBd; B: fresh biomass FBm (kg / ha). AafCra: *Amanita aff. craseoderma*; AafSub: *Amanita aff. subviscosa*; AmMas: *Amanita masasiensis*; AStrVo: *Amanita strobilaceovolvata sensu lato*; AmaSub: *Amanita subviscosa*; AmaXa: *Amanita xanthogala*; CantAd: *Cantharellus addaiensis*; GyrCa: *Gyroporus castaneus*; LtaSap: *Lactarius saponaceus*;LtaTn: *Lactarius tenellus*; LtiAfHe: *Lactifluus aff. heimii*; LtiLu: *Lactifluus luteopus*; OctaIvo: *Octaviana ivoryana*; RusafCe: *Russula aff. cellulata*; RusCell: *Russula cellulata*; RuscfGri: *Russula cf grisea*; RuscfSes: *Russula cf sesenagula*; RusCil: *Russula ciliata*; RusCon: *Russula congoana*

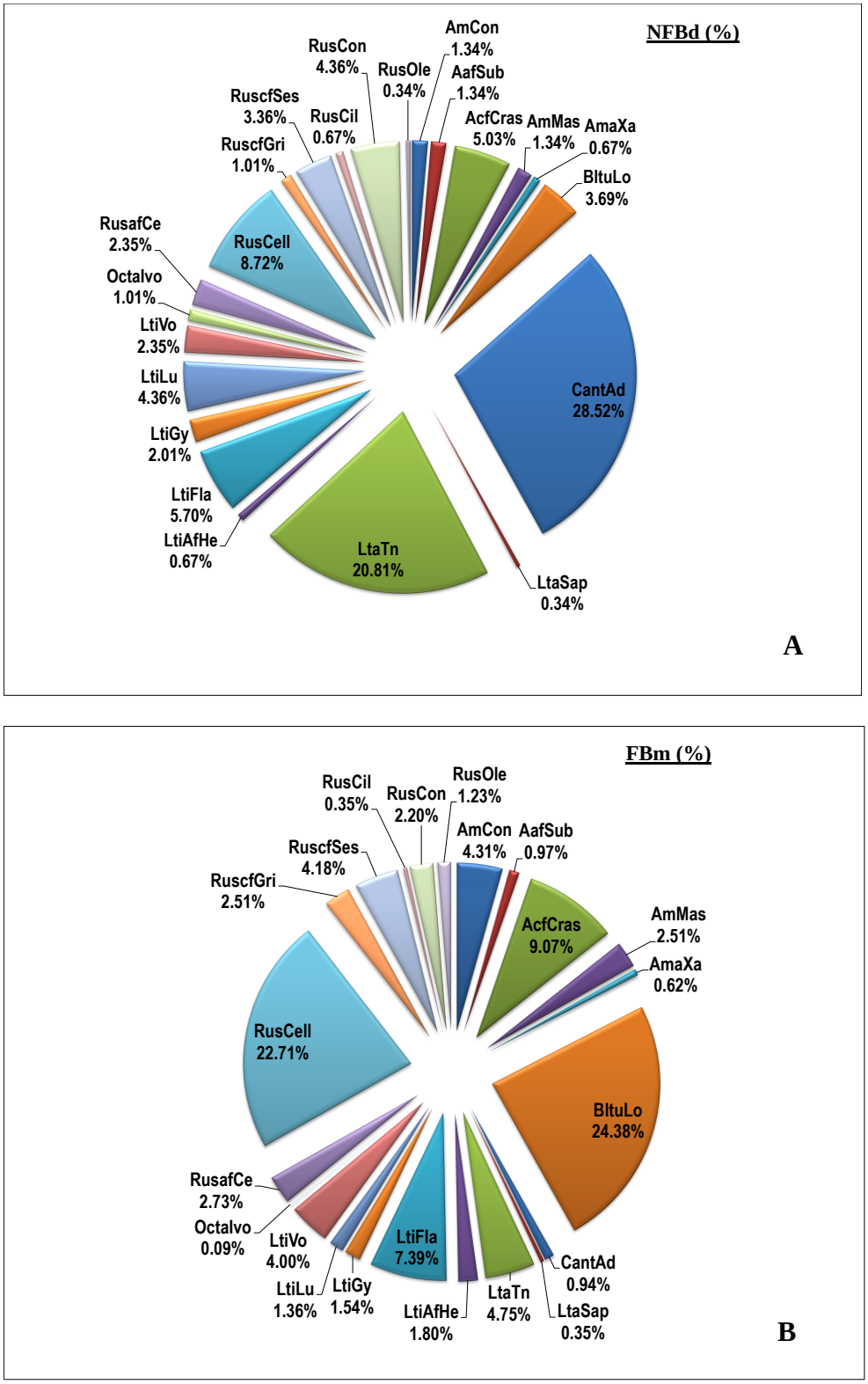


Figure 33: Proportions of edible ectomycorrhizal fungal species collected in Mixed Woodland (MW). A: Number of Fruit Bodies NFBd; B: fresh biomass FBm (kg / ha). AafSub: *Amanita aff. subviscosa*; AcfCras: *Amanita cf crassiconus;* AmCon: *Amanita. Congolensis*, AmMas: *Amanita masasiensis*; AmaXa: *Amanita xanthogala*; BltuLo: *Boletus loosii;* CantAd: *Cantharellus addaiensis*; LtaSap: *Lactarius saponaceus*; LtaTn: *Lactarius tenellus*; LtiAfHe: *Lactifluus aff. heimii*; LtiFla: *Lactifluus flammans;* LtiGy: *Lactifluus gymnocarpoides;* LtiLu: *Lactifluus luteopus*; LtiVo: *Lactifluus volemoides;* OctaIvo: *Octaviana ivoryana*; RusafCe: *Russula aff. cellulata*; RusCell: *Russula cellulata*; RuscfGri: *Russula cf grisea*; RuscfSes: *Russula cf sesenagula*; RusCil: *Russula ciliata*; RusCon: *Russula congoana;* RusOle: *Russula oleifera*

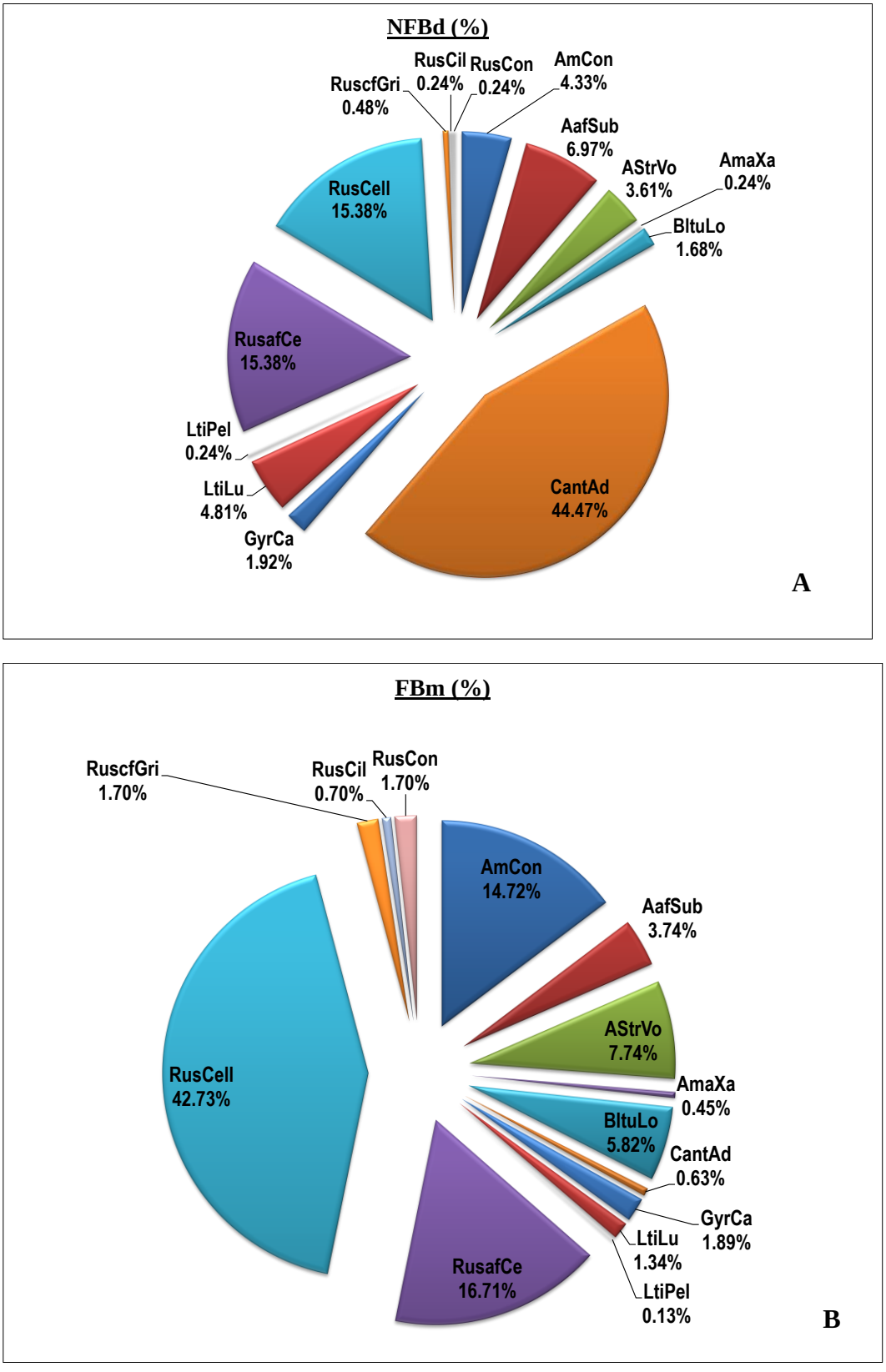


Figure 34: Proportions of edible ectomycorrhizal fungal species collected in *Uapaca* Woodland (UW). A: Number of Fruit Bodies NFBd; B: fresh biomass FBm (kg / ha). AafSub: *Amanita aff. subviscosa*; AmCon: *Amanita. congolensis;*. AStrVo: *Amanita strobilaceovolvata sensu lato*; AmaXa: *Amanita xanthogala*; BltuLo: *Boletus loosii;* CantAd: *Cantharellus addaiensis*; GyrCa: *Gyroporus castaneus*; LtiLu: *Lactifluus luteopus*; LtiPel: *Lactifluus pelliculatus*; RusafCe: *Russula aff. cellulata*; RusCell: *Russula cellulata*; RuscfGri: *Russula cf grisea*; RusCil: *Russula ciliata*; RusCon: *Russula congoana*

4. Influence of microclimate on edible EFFB production

4.1. Habitat type 1: *Isoberlinia doka* woodland (IW)

There is a significant relationship between extracted principal components (PCs) and initial climate variables recorded in IW. The first two PCs of the PCA express 91.88 % of the variance with 74.12 and 17.76 % respectively for PC1 and PC2. All climate variables were correlated with axis PC1. Soil moisture, rainfall and relative humidity were positively correlated whilst air and soil temperature were negatively. However, correlation of rainfall was higher with axis PC2 than PC1 (table IX).

Normality test indicated that fresh biomass was not normally distributed so correlation with generated PCs was performed using Spearman method. Thus, productions of six edible species were positively correlated with PC1 but no significant correlation was found with PC2 (table X). *A.* aff. *subviscosa, C. addaiensis* and *R. congoana* were highly correlated with p-value > 0.001 (respective correlation coefficient 0.75, 0.64 and 0.59). Correlation coefficient with *L.* aff. *heimii* equaled 0.55 with significance above $\alpha > 0.01$. Correlation coefficients with *A. aff. craseoderma* and *R.* cf *sesenagula* equaled both to 0.45; however they were found significant at $\alpha > 0.05$. Knowing that air and soil temperature decreased over period, it showed that productions increased for those species and were maintained at some level by increases in soil moisture and rainfall.

The absence of correlation of species production with PC2 which is mostly correlated with rainfall indicated that that variable has weak impact on production compared to the other variables. Production was not linearly correlated with Soil Temperature and Moisture, Air Temperature and Relative Humidity. Those results suggested that edible species specific to that habitat type are likely “drought-tolerant”.

4.2. Habitat type 2: Mixed woodland

Like in IW, a significant relationship was found between extracted principal components (PCs) and initial climate variables recorded in MW. Similarly, the first two PCs of the PCA express 91.83 % of the variance with 70.8 and 21.03 % respectively for PC1 and PC2. All climate variables were correlated with axis PC1. Soil moisture, rainfall and relative humidity were positively correlated whilst air and soil temperature were negatively.

Table IX: Spearman correlation of extracted principal components (PC) with initial variable in IW

Parameter	AirTemp	RelHum	Rainfall	TempSoil	Moist.
PC1	-0.90***	0.91***	0.44*	-0.87***	0.87***
PC2	0.26	-0.29	0.81***	0.35	0.22

$\alpha > 0.001$ (***) ; $\alpha > 0.01$ (**); $\alpha > 0.05$ (*)

AirTemp: Air temperature; RelHum: Relative humidity; SoilTemp: Soil temperature; SoilMoist: Soil moisture

Table X: Spearman correlation between principal components (PC) and fresh biomass of edible EcM fungi in IW

Species	PC1	PC2
AafCra	0.45*	-0.42
AafSub	0.75***	-0.06
CantAd	0.64***	-0.08
LtiAfHe	0.55**	-0.08
RuscfSes	0.45*	0.04
RusCon	0.59***	-0.39

$\alpha > 0.001$ (***) ; $\alpha > 0.01$ (**); $\alpha > 0.05$ (*)

AafCra: *Amanita* aff. *craseoderma*; AafSub: *Amanita* aff. *subviscosa*; CantAd: *Cantharellus addaiensis*; LtiAfHe: *Lactifluus* aff. *heimii*; RuscfSes: *Russula* cf *sesenagula*; RusCon: *Russula congola*.

Furthermore, correlation of rainfall was higher with axis PC2 than PC1whilst correlation was weaker between PC2 and soil moisture (table XI).

Normality test indicated also that fresh biomass was not normally distributed so correlation with generated PCs was performed using Spearman method (table XII). Unlike in IW, only *R. congoana* production was negatively correlated with PC2 ($\alpha > 0.05$). That means its production decreased when rainfall and soil moisture increased.

4.3. Habitat type 3: *Uapaca togoensis* woodland

Likewise in previous habitat, a significant relationship existed between extracted principal components (PCs) and initial climate variables recorded in UW. The first two PCs of the PCA express 92.17 % of the variance with 67.87 % and 24.30 % respectively for PC1 and PC2. In that habitat, PC1 was correlated positively correlated with Soil moisture and relative humidity. Correlation was negative with air and soil temperature. PC2 was positively correlated with rainfall but also with soil moisture (table XIII).

Spearman method indicated that six edible species were also correlated with the different axis. *A.* aff. *subviscosa, A. strobilaceovolvata s.l., B. loosii* and *C. addaiensis* were significantly and positively correlated with PC1 (table XIV). Productions of those species increased with soil moisture and relative humidity but inversely with air and soil temperature. In other words, decrease in temperature resulted in increase of production. *A. congolensis* and *Gyroporus castaneus* were negatively correlatively to PC2 showing that increases in soil moisture and rainfall are detrimental to their productions.

Table XI: Spearman correlation of extracted principal components (PC) with initial variable in MW

Parameter	AirTemp	RelHum	Rainfall	TempSoil	Moist.Soil
PC1	-0.93***	0.89***	0.51**	-0.87***	0.80***
PC2	0.17	-0.11	0.85***	0.28	0.41*

α > 0.001 (***) ; α > 0.01 (**); α > 0.05 (*).

AirTemp: Air temperature; RelHum: Relative humidity; SoilTemp: Soil temperature; SoilMoist: Soil moisture

Table XII: Spearman correlation between principal components (PC) and fresh biomass of edible EcM fungi in MW

Species	PC1	PC2
RusCon	-0.02	-0.48*

α > 0.001 (***) ; α > 0.01 (**); α > 0.05 (*).
RusCon: *Russula congoana*

Table XIII: Spearman correlation of extracted principal components (PC) with initial variable in UW

Parameter	AirTemp	RelHum	Rainfall	TempSoil	Moist.Soil
PC1	-0.93***	0.91***	0.39	-0.92***	0.72***
PC2	0.26	-0.22	0.88***	0.25	0.53**

$\alpha > 0.001$ (***) ; $\alpha > 0.01$ (**); $\alpha > 0.05$ (*)

Table XIV: Spearman correlation between principal components (PC) and fresh biomass of edible EcM fungi in UW

Species	PC1	PC2
AafSub	0.53**	-0.26
AmCon	0.07	-0.41*
AStrVo	0.41*	0.01
BltuLo	0.41*	0.02
CantAd	0.59***	-0.06
GyrCa	0.05	-0.53*

$\alpha > 0.001$ (***) ; $\alpha > 0.01$ (**); $\alpha > 0.05$ (*).

AafSub: *A.* aff. *subviscosa*; AmCon: *A. congolensis*; AStrVo: *A. strobilaceovolvata*; BltuLo: *Boletus loosii*; CantAd: *Cantharellus addaiensis*; GyrCa: *Gyroporus castaneus*

SECTION IV: DISCUSSION

EcM Fungi Diversity

The present study constitutes the first systematic and continuous monitoring of EFFB in Côte d'Ivoire and aimed to give a baseline for further studies. It shed light on very specious habitats within Comoé National Park (CNP) where almost all known EcM fungi fruit bodies (EFFB) families were represented. As already mentioned in various paleo- and neotropical regions **(Riviere *et al.*, 2007; Bâ *et al.*, 2012; Henkel *et al.*, 2012; Onguene and Kuyper, 2012)**, dominance of Russulaceae and specifically of genus *Russula* was also observed. Among the other frequently recruited families in tropical regions, Cantharellaceae was represented, in prospected habitats, by only one species member of genus *Cantharellus, C. addaiensis.* In the contrary, four *Cantharellus* species (*C. floridulus* Heinem., *C. platyphyllus* Heinem., *C.* cf. *platyphyllus* Heinem. and *Cantharellus* sp.) were reported in traditional systems of fallows dominated by many confirmed EcM tree partners near the city of Korhogo in North western part of Côte d'Ivoire **(Ducousso *et al.*, 1999)**. This difference may be due to higher number of tree partners in that area, namely *Afzelia Africana* Sm. ex Pers., *Anthonotha crassifolia* (Baill.) J. Léonard, *Berlinia grandiflora* (Vahl) Hutch. & Dalziel, *Isoberlinia doka* and *Uapaca togoensis*. Likewise in genus *Clavulina,* only one species was detected in *Isoberlinia* woodland (IW) suggesting that other species may have been overlooked or mistook for saprotrophic species. Few species belonging to genera *Inocybe* and *Cortinarius* were also found in CNP. This supports the trend observed in other tropical regions **(Onguene and Kuyper, 2002; Riviere *et al.*, 2007; Onguene and Kuyper, 2012)**, and strengthens the idea that those species might be adapted to temperate and boreal zones . However, the paucity of studies in tropical woodlands and forests comparative to temperate and boreal ones should be considered. Yet, the abundance of EcM fungi species was highlighted at continental level. In West Africa**, Sanon *et al* (1997**) found 37 EcM fungi during rainy season 1994 and 1995 in savanna and open riparian forests in southwestern Burkina Faso. 126 EcM species were censured after various surveys in different areas of Benin, ranging from protected areas to farms **(Yorou, 2010)**. In Southern Guinea rainforests, **Diédhiou *et al.* (2010)** identified 39 EcM fungal taxa. In central Africa, **Onguene *et al* (2012)** reported the collect of 100 EcM fungi in forest habitats of South Cameroon during a three-year survey. Numerous species have been also collected in Congo and are documented in two series, "Flore Iconographique des Champignons du Congo" and "Flore illustrée des Champignons d'Afrique Centrale".

Highest species richness and number of EFFB were found in IW. According to **Nara *et al.*, 2003,** such values reflected host development stage. Indeed, highest cumulative values of tree partners' stems density and basal area were found in plots of IW. Some of those tree species were estimated aging more than 200 years with regard to their dbh (Tedersoo, personal communication). In disturbed areas of tropical zones, EcM Fabaceae and Dipterocarpaceae stands (*I. doka* and *M. kerstingii* respectively in our case) are considered climax stands which establishment is facilitated by *Uapaca* spp. **(Onguene, 2000; McGuire, 2007; Tedersoo *et al.*, 2011; Onguene and Kuyper, 2012)**. According to **Poilecot *et al.* (1991)**, CNP is included of 93.3 % of fire climax vegetation from which 6.7 % is made of woodlands. Indeed, understorey vegetation in IW and MW were burned either totally or partially according to plot by the annual fire that passed in December 2013, four months before our arrival at the park. However, no plot in UW was burnt. Moreover, EcM fungi species belonging to genus *Scleroderma* previously described as characteristic of disturbed and elevated soil temperature areas **(Ingleby *et al.*, 1985; Nara *et al.*, 2003)** were collected within burnt plots of IW and MW. Three of the five *Scleroderma* species were recruited in IW and the latter two in MW. Consequently, IW is likely older than the others whilst UW is the youngest and MW at an intermediate stage. This assumption is strengthened by the different proportions of *U. togoensis* and presence/absence of *I. doka* in the different habitats. First, IW harboured many stems of the EcM tree partners *Monotes kerstingii* Gilg and *Uapaca togoensis* but it is dominated by *Isoberlinia doka.* Second, few stems of *I. doka* were censured in MW whilst the tree species is completely absent from UW plots. Another support of that assumption is the presence of *Inocybe* sp. and the number of species of genus *Cortinarius* in IW are other supports of that assumption since those EcM fungi were depicted late successional symbionts **(Nara *et al.*, 2003)**.

Sampling representativeness

Sampling representativness assessment demonstrated that a large number of symbiotic fungi were not detected in the different habitats monitored. This result is corroborated by the important values of uniques species that reflected rare species. That number of observed rare species give an estimate of the number of unseen species **(Chiarucci *et al.*, 2011)** as captured by the estimated species richness in each habitat. That result is a support of the limitation of fruit body based study of EcM fungi species **(Horton and Bruns, 2001; Taylor, 2002).**

Nevertheless, climate impact is more appreciable on fruit bodies than on below-ground tips **(Andrew and Lilleskov, 2009; Pickles *et al.*, 2012)**.

Molecular based identification and Phylogenetic reconstruction

Only 13.88 % of collected specmiens was identified at species level leaving the rest of identity at genus level. Identified species belonged mainly to genus *Lactarius* and *Lactifluus*. In fact, one of the most studied families and genera in the world and especially in Africa is Russulaceae and its genera *Russula*, *Lactarius* and *Lactifluus* **(Van Rooij *et al.*, 2003; Buyck *et al.*, 2008; Verbeken and Walleyn, 2010; Maba *et al.*, 2013; 2014; Verbeken *et al.*, 2014; Maba *et al.*, 2015)**. These studies included anato-morphological and/or molecular characterisations of russuloid species collected in various habitat of Africa.

Molecular alignment allowed the identification and the grouping of various specimens into the same species. Indeed, solitary specimens (one specimen at a time) collected at distant periods of the season displayed different morphological features according to their development stage. Thus, morphological variability in species such as *R. cellulata* and *Lactarius afroscrobiculatus* was evidenced. As result, fruiting phenology and natural production of such species were adequately addressed.

Moreover, closely related specimens displaying similar morphological features were revealed after sequences alignment. Such species are potentially cryptic species and have been observed in genera Amanita and Lactifluus. Indeed, specimen LP110 identified as *A. subviscosa* was discriminated from a sister species that was named after it. according to **Wang *et al.* (2015)** and **Itoo *et al.* (2015)**, identification criteria as used in temperate zones fail sometimes in discriminating related species of tropical zones based on morphology. Discrimination of cryptic species in mycology has been achieved by implementing molecular technics **(Sato *et al.*, 2007; Leavitt *et al.*, 2013; Balasundaram *et al.*, 2015)**. The use of marker such the ITS regions has been documented **(Kõljalg *et al.*, 2005; 2013)**. According to **Sato *et al.* (2007)**, the wide distribution of a single species is an indicateur of possible existence of cryptic species. Potential revealed cryptic species are likely species new to science and futher studies should be undertaken to characterise them.

In addition, phylogenetic reconstruction evidenced several ambiguous positions in Russulacaea and Boletaceae. Such discrepancies may be due to misidentification of homologous sequences in database as pointed out by several authors **(Nilsson *et al.*, 2006; Heinrichs *et al.*, 2012; Piepenbring, 2015)**. Indeed, specimens such as LP137 identified as a *Russula* sp based on morphology and molecular analysis (percentage of identity PI = 88), nested within clade *Lactifluus*. This questionable position may also suggest another classification for the species. According to **Buyck *et al.* (2008)**, some rare species belonging to the Russulaceae display features belonging to both genera *Russula* and *Lactarius*, the latter being splited in *Lactarius* s.s and *Lactifluus*. Such species were classified into a new genus called *Multifurca*. Up to now, no species from that genus has been reported from Africa and the ambiguous position of specimen LP137 raised many hypotheses. All things considered, the specimen needs to be deeply studied and characterise since it is likely new to science like all other unidentified specimens.

As for Boletaceae, the need of complete revision of the family has raised after several discrepancies have been observed between morphological and molecular data. According to **Wu *et al.* (2014)**, The morphology-based taxonomy for the family is controversial. Taxonomic levels of the Boletaceae were developed based on the basidiospore ornamentation, unfortunately not supported by molecular phylogenetic analysis **(Hibbett *et al.*, 2007; Nuhn *et al.*, 2013)**. The authors pointed out the fact that some genera shared characters whilst others are polyphyletic **(Nuhn *et al.*, 2013)**. Major genera Boletus, Tylopilus and Xerocomus have been suggested polyphyletic **(Drehmel *et al.*, 2008)**. Thus, phylogenetic relationships among genera of Boletaceae are not resolved yet and several molecular markers have been used at the same time to address this issue. Our reconstructed tree of Boletaceae is in accordance with above-mentioned authors. It can even be worse in tropical Africa where mycological studies and implementation of molecular technics are still new and of limited access.

In family Amanitaceae, the need of revision of African species is also needed although the species do not nested according to morphological classification. Though the split of genus Amanita has been approuved **(Redhead *et al.*, 2016)**, debate is still ongoing among authorities **(Tulloss *et al.*, 2016)**.

Many generated sequences nested with several homologous sequences from neighboring and also distant countries of Africa. In general, high percentages of identity were only

obtained with African homologous sequences. That is a direct confirmation that species differed greatly in their identity according to tree partners but mainly climatic zone **(Verbeken and Buyck, 2002)**.

Spatial distribution of EFFB

Phytosociological study of permanent plots evidenced important floristic richness and especially numerous stems with dbh above 10 cm. EcM tree partners thrive in dominant and sometimes almost mono-dominant stands. Such habitats have been demonstrated as niche for abundant EcM fungi. *Isoberlinia doka* and *Uapaca togoensis* were the main dominant species in prospected habitats. Sites grouping were correlated with their density more than stands age. Indeed, though only stems with dbh above 10 cm were considered in data analysis, numerous juveniles and sprouts were present within plots. This was favorable to the establishment of both early- and late-successional EcM fungi. In addition, the grouping also reflected fire impact within study sites evidencing the "drought-tolerant" capacity of some collected fungi species. There is therefore an urgent need to monitor such disturbed stands to adequately address that assumed capacity.

Indicator species analysis evidenced four species associated to site groups (three species associated with G1 and one species with G2). Those species, *Russula congoana*, *Pulveroboletus* sp 1 and *A. xanthogala* were good indicators of G1 and *A. cf virosa* was for G2 taking into account specificity and fidelity. Indeed, those species were collected either exclusively in plots assigned to each group or predominantly in them. Association of *R. congoana* and *A. xanthogala* to *I. doka* was also documented in Benin by **De Kesel *et al*., (2002).** Those species are mentioned in literature as edible fungi in various part of Africa **(Boa, 2004)**. As for the two remaining indicators species, they need to be characterised and compared to available monographs and / or keys to ascertain their identity at species level. However, they are likely associated to *U. togoensis*

Influence of the variability of climatic parameters on Fruiting phenology and natural production

Many wild fungi are always been consumed by local population worldwide. In Africa, 300 wild fungi are listed as edible and encompass both saprotrophic and symbiotic fungi **(Boa, 2004)**. In Democratic Republic of Congo, 39 edible fungal species were recorded including 26 EcM ones **(Degreef *et al.*, 1997)**. In Benin 26 species on 30 edible ones were also identified as EcM fungi **(Yorou *et al.*, 2001)**. 27 EcM fungi were also listed among edible wild fungi collected in rainforests in central Africa **(Eyi Ndong *et al.*, 2011)**. Based on these studies, 27 edible EcM fungi were identified among 123 collected species in CNP during rainy season 2014. As in abovementioned studies, edible EcM fungi of CNP belong to Amanitaceae, Russulaceae, Cantharellaceae, few genera of Boletaceae and one genus of Gyroporaceae.

Edible EcM fungi fruiting in CNP started in mid-May 2014, two weeks after the effective beginning of the rainy season with rainfall intensity varying from 30 to 40 mm in all habitat types. Consequently, air and soil temperature decreased whilst air relative humidity and soil moisture increased. Those trends were maintained by the regular and increasing intensity of rainfalls until early October 2014 except for the last week of May and first week of June where the lowest values of rainfalls were recorded. *Amanita* spp. fruited earlier in mid-May and continuously during the season in all habitat types. Bolets species, *Cantharellus addaiensis*, *Russula* spp. *Lactarius* spp. and *Lactifluus* spp. fruited later, particularly from late June and early July. Peaks of fruiting and production (fresh biomass) were generally in July as also observed in Benin **(Yorou *et al.*, 2001)**.

Natural production (fresh biomass) was higher in UW than in IW and MW though UW yielded the lowest number of edible species. In fact UW yielded larger species than in other habitat types. In addition, fruiting lasted more in UW. These observations suggested a differential life strategy of EcM fungi in CNP **(Dighton, 2003)**. A cumulative production of 84.09 kg / ha was yielded during season 2014. This number is far below production recorded in other countries in Africa. **Yorou *et al.*, (2001)** mentioned a mean production of 140.7 kg/ha of fresh biomass collected in 18 plots established in six habitat types (including dry forest, gallery forest, tree savanna, savanna woodland and woodlands) dominated by various trees and host plants like *Isoberlinia doka*, *I. tomentosa* and *Uapaca togoensis*. It is worth mentioning that those high productions reflected both saprotrophic and symbiotic fungi.

Moreover, sampling sites of that study were located in the center of Benin, more humide than CNP and harboured more ecM tree species. Species such *Amanita subviscosa, A. masasiensis, Lactifluus gymnocarpoides* and *Russula congoana* were reported among the most common in all prospected habitat types.

As previously mentioned, fruiting started two weeks after first rain event. This demonstrated the key role of water budget in fruiting patterns of EcM fungi In fact, soil moisture, air relative humidity and rainfall were all significant in the production of that species that fruited 11 weeks during the season in UW. Air and soil temperature were generally negatively correlated with production of edible species as evidenced in chapter. The simultaneous decrease of both soil and air temperature and increase of water balance created cooler microclimate in the different habitat types, UW being the coolest mainly due to its higher canopy closure. In temperate and boreal zone, rainfall and moisture availability have been demonstrated as critical to EcM fruiting and natural production **(O'Dell *et al.*, 2000; Gange *et al.*, 2007; Kauserud *et al.*, 2010)**. Those climate factors are also beneficial to host plants and consequently to symbiotic fungi through photosynthetic products allowance that likely influence phenology and natural production **(Straatsma *et al.*, 2001; Pinna *et al.*, 2010)**.

CONCLUSION AND PERSPECTIVES

The impacts of climate change on human beings are the center the interest of many research projects worldwide those last decades. In order to address and mititgate those impacts, various solutions have been suggested among which is the maintenance and monitoring of natural ecosystems. Although the crucial role of ectomycorrhizal fungi as been demonstrated in ecosystem functioning and resilience, very little is known about their dicervity and their determinants especially in tropical zones. Therefore, we aimed throught that PhD. project to understand and predict the relevance/significance of climatic parameters (rainfalls intensity and frequency, air and soil temperature and humidity) on the temporal dynamic of EcM fungi and their associated forest trees. The study has been conducted in the Comoe National Park, a Biosphere Reserve located in northeast of Cote d'Ivoire, West Africa.

First, the diversity of EcM fungi species has been assessed through the diversity of fruit bodies occurring in different woodlands selected according to the presence and abundance of known host plants. Thus, a six-month monitoring of EcM fungi fruit bodies (EFFB) ascertained their occurrence at Comoé National Park in the Sudanian climatic zone of Côte d'Ivoire. Woodlands of the park harboured rich plant species diversity from which known EcM tree partners were frequently dominant. In those habitat types estimated of more than 200 years old, 123 EcM fungi species fruited and were collected. Those species belong to 09 families and 23 genera. Almost all families known from tropical regions were represented with the same trend observed elsewhere. Dominant families were as usual and in order Russulaceae, Boletaceae and Amanitaceae. In the contrary of other reports in Africa, Cantharellaceae and Clavulinaceae were weakly represented whilst no species of Thelephoraceae was collected. They were likely overlooked or mistook for saprotrophic species. Molecular based identification confirmed the ectomycorrhizal status of collected specimens but did not provide accurate identification of specimen to species level in most of the case. High Simpson's Index of Diversity 1 – D and weak evenness calculated per habitat evidenced the patchy distribution of EFFB within selected woodlands. Autosimilarity based on index of Jaccard was weak within and between habitats indicating that those habitats were dissimilar, although plots and habitats were not so greatly spaced. In addition, the sampling representativeness assessment demonstrated that a large number of symbiotic fungi were not detected, although the high species richness recorded. the estimators used were Observed species richness Sobs, Estimated species richness (Chao 2) Sest, Sample coverage,

Autosimilarity, Simpson's Index of Diversity 1- D, Simpson's Evenness, number of Uniques and the accumulation curves.

Second, the taxonomic affinities were investigated between collected EFFB and species from other regions of Africa and world. Phylogenetic reconstruction was undertaken per famiy except for Cantharellaceae and Gyroporaceae for which DNA amlification was not successful. For molecular identifications, generated sequences clustered according to their respective genera; however, various ambiguous positions have been observed. Numerous previous studies highlighted the lact of accuracy of sequences identities in public databases. Those identifications were usually based on morphological classifications rather than phylogenetic classifications. Consequently, revisions of fungi in general and EcM ones in particular are needed especially for African species which were usually named in comparison of morphological affinities with temperate and boreal species. Multiple alignments indicated that many collected fungi occur in neighboring countries as well as in other regions of the continent where they are associated with other tree partners. This low specificity of symbiotic fungi has been demonstrated as driving the dominance of host plants in large areas of the world. Moreover, the greater part of collected species could not be identified at species level. This suggests that they may be new to science; however, further studies are required and results will be consequently published.

Third, the determination of the influence of plants and soil parameters on the spatial distribution of EcM fungi fruit bodies (EFFB) have been performed. Soils within selected habitats were generally neutral and silt loamy. That was favorable to the establishment of a rich plant diversity. However, homogeneity of soil has been evidenced by analysis of variance of chemical and textural parameters of the soil. As for plants parameters, the dominance of EcM host plants was demonstrated by their relative density and basal area. Indeed, three confirmed host plants have been identified within habitats and were *Isoberlina doka, Monotes kerstingii* and *Uapaca togoensis.* Among those EcM host plants, *Isoberlina doka* and *Uapaca togoensis* were significantly distributed from an habitat to other. Non metric multidimensional scaling (NMDS) revealed that only the density of stems of *Isoberlina doka* and *Uapaca togoensis* was the determinant of EFFB abundance as reflected by their relative frequency. Thus, in our study site, edaphic conditions did not drive fungi

distribution since they were homogenous along habitats. In addition, hierarchical classification evidenced two sites groups to which three and one species were significantly associated with good indicator value. *Russula congoana, Amanita xanthogala* and *Pulveroboletus* sp 1 were associated with gourp 1 defined by presence / dominance of *Isoberlinia doka* (habitat IW and two plots of habitat MW) but also fire occurrence. The two first were specific to that group whilst the last one was most frequently censure in it. The second group was characterised by the dominance of U. togoensis and the occurrence of the last indicator species, A. cf virosa, within its sites (last plot of MW and all ones of habitat UW).

Final, the influence of climate parameters variability (air and soil humidity, rainfall distribution/intensity, temperature of air and soil) on the fruiting phenology and natural production of edible collected EFFB was demonstrate. 27 species collected at Comoé National Park were identified as edible according to literature. They started fruiting two weeks after the first important rain that felt in mid-May 2014. Analyses demonstrated that climate variability was significant for most of its parameters from one habitat to another, one week to another and for the interaction habitat*week. The production of 10 edible species was significatively correlated to climate variability. In general, correlations were negative with air and soil temperature indicatind that production of those edible fungi increased whilst both air and soil temperature were cooling during the rainy season 2014. In the contrary, the increase of rainfall, soil moisture and relative humidity leaded to the increase of production; however to a certain level. Indeed, for species such as *A. congolensis* and *Gyroporus castaneus,* the increase of rainfall and soil moisture became detrimental at certain point. The cumulative productions were weaker in comparison with studies in other countries of the continent.

Our study is the first one addressing EcM diversity, their spatial distribution and influence of climate parameters in Cote d'Ivoire and in West Africa. This baseline study will serve to monitor long term spatio-temporal change in diversity, fruiting phenology and production of EcM fungi within Comoé National Park but also in other regions of Côte d'Ivoire and West

Africa. Other mycorrhizal classes, fire impact and host plants carbohydrates allocation should be included in further researches to address carbon storage capacity in tropical Africa. Knowing that fruit bodies are part of the diet of many small mammals and other invertebrates, the use of other technics are required to prevent systematic removal of fruit bodies from field. Discrepancies in morpho-molecular identification of species impose additional studies to solve them. However, discrepancies can demonstrated that many specimens are likely new to science because their DNA sequences did not match with available sequences of holotypes. Consequently, there is an urgent need to complete characterisation of such species as well as ethnomycological studies with surrounding populations to ascertain effective consumption of edible species and avoid confusion and poisoning.

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Annex 1: Standardized description sheet used during field work

Lamellae (L) – lamellulae (l) # L+l/cm (margin):
spacing (arbitrary) : remote – distant – subdistant – close – crowded
thickness (frontal view): very thin – thin – normal – thick # l per L:
arrangement: simple – furcate – intervenose – anastomosing – subporoid – collarium
attachment: free – adnexed (subadnate) – sinuate – emarginate – emarginate with decurrent tooth – seceding – adnate – adnate with decurrent tooth – subdecurrent – decurrent – deeply decurrent.
shape: linear – segmentiform – subventricose – ventricose – broadly ventricose – arcuate – triangular
surface topography: even – rugose (wrinkled) – rugulose – transvenose (veins-ribs)
surface texture: smooth (glabrous) – laccate (waxed) – pulverulent (pruinose).
surface touch (wetness) : dry – moist – waxy
consistency: normal – flexible – waxy – fragile (brittle) – deliquescent – hard
Lamella colour fresh: white (ivory) **dry :**
colour distribution : even – irregular-patchy – spotted-marmorate
edge (margin): even (entire) – undate – serrate (dentate) – crenate (indented) – incised (notched) – fimbriate – laciniate – eroded – powdery (pruinose) – lannate (wooly) – split – with setae – with droplets
concolorous: yes / no, **colour :**

Tubes & pores
tube inter-separability: tubes firmly connected – tubes (easily) separable from one another
tube attachment to stipe: free – adnexed (subadnate) – sinuate – emarginate – emarginate with decurrent tooth – seceding – adnate – adnate with decurrent tooth – subdecurrent – decurrent – deeply decurrent.
tube layer shape: linear – segmentiform – subventricose – ventricose – broadly ventricose – arcuate
tube consistency: fleshy/soft (spongy) – thin – waxy – corky – hard (woody, tough).
tube colour fresh: **colour changes when cut or bruised:**
pore diameter: **number of pores/cm at mid-radius:**
pore shape: regularly roundish – regular angular (hexa- or pentagonal) – elongated angular – irregular – labyrinthoid
near the stipe, at first: **with age:**
in the middle, at first: **with age:**
near the cap margin, at first: **with age:**
pore arrangement: regular (homogenous) – radial – concentric – irregular
pore layer topography: regular (even) – subregular – irregular (composite pores)
pore layer touch (wetness): dry – moist – waxy
pore texture: even – powdery (pruinose) – lannate (wooly) – with setae – with droplets.
pore colour fresh: **dry:**
colour distribution : even – only paler at margin – gradually/abruptly paler to margin – gradually/abruptly darker to margin
near the stipe, at first: **with age:**
in the middle, at first: **with age:**
near the cap margin, at first: **with age:**
pore specific colour changes when cut or bruised: **changing: quickly – slowly**

Spines
number of spines/cm at mid-radius: **spine length:**
spine shape: pointed – blunt – terete – compressed
near the stipe, at first: **with age:**
in the middle, at first: **with age:**
near the cap margin, at first: **with age:**
spine arrangement: regular (homogenous) – irregular
spine layer surface touch (wetness): dry – moist – waxy
spine consistency: normal – flexible – waxy – fragile (brittle) – hard
spine colour fresh: **dry :**
at first: **with age:**

Macrochemical features (colour reactions)

	Lactic acid	Phenol	FeSo4	HCl	NaOH (KOH)	NH4OH
pileus						
pileus context						
stipe						
stipe context						
hymenophore						

Nr. LP 285 **Taxon:**

specimens exsicc : # specimens in liq. :
slides : # pictures: # drawings:

Collector: LLP-J **Determinavit & date:**

Country: CI **Province:** Boukané
Locality: CNP
Collecting date: 28 Juil 2014 **Altitude:**
Geographical coordinates: F1P3

Habitat – Vegetation – Substrate :

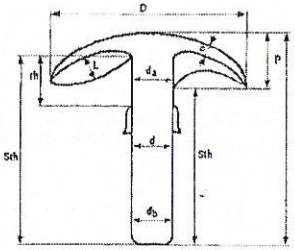
Vernacular name: **Local language:**
Significance:
Edible: yes / no Use: consumption – medicinal – religious – others:

Growth habit : solitary – caespitose – fairy ring – gregarious – fasciculate

Connection to substrate: stipitate – substipitate – sessile – imbricate – resupinate – effuso-reflexed – effused

Fruitbody general aspect: amanitoid

SPORE PRINT COLOUR fresh : **dry:**



Dimensions (in mm): Dev. development, im: immature, ma:mature, ol:old

n°	Dev.	H	shape	D	P	Sth	da	d	db	rh	c	L
1	ma	127		70		121	9	12	12	45	2	5
2												
3												
4												
5												

Pileus
shape: convex – plano-convex – plane – subdepressed – subumbonate – umbonate – conical – mammillate – papillate – pulvinate – depressed – plano-depressed – concave – campanulate – broadly convex – narrowly conical – obtusely conical – infundibuliform - umbilicate
at first: **with age:** plane
surface topography: even – rugose (wrinkled) – rugulose – pustulate (glandulose) – scabrous (rough) – verrucose – costate – venose – scrobiculate – strobiliform – rimose (cracked & creviced) – rimose areolate – rivulose – fissurate-incised – striate – sulcate – plicate – wavy.
moderator for density: moderately – minutely – densely – coarsely – slightly
moderator for organisation: regularly – irregularly – radially – concentrically – reticularly
in the centre, at first: **with age:** even
margin shape: straight – deflexed – inflexed – involute – reflexed – revolute
at first: **with age:** straight
margin topography: even – wavy - rugose (wrinkled) – rugulose – verrucose – venose – rimose – rivulose - fissurate-incised – striate – sulcate – plicate – crenate – crenulate
at first: **with age:** crenulate
perforatorium: yes / no

Pileipellis (cuticle)

fit: too short – exact – slightly exceeding – exceeding – deeply exceeding
at first: **with age:** exact
separable : not – margin only – half radius – up to centre – entirely
consistency: elastic – fibrous – tough – papery – thick
shine : shiny (polished) – dull – silky
touch (wetness) **:** dry – moist – waxy (slippery) – subviscid – viscid (sticky, tacky, gluey, resinous) – glutinous (slimy, viscous) – unctuous (oily).
velar remnants: none – pulverulent (pruinose) – micaceous – granulose – fibrils – echinate or pyramidal cones – echinulate – warts – patch(es)
at the disc: echinate **at the margin:** none **appendiculate:** yes : no
colour: light brown **persistence:** evanescent (fugacious) – fragile – persistent
surface texture (innate structures): smooth (glabrous) – laccate (waxed) – sericeous – fibrillose – squamose – squamulose – squarrose – squarrulose – velutinous (velvety) – tomentose – matted tomentose (felted) – pubescent (downy) – villose (pilose) – hispid (hirsute) – hispidulous – strigose – lannate – floccose – flocculose – arachnoid – echinate – echinulate.
moderator for density: moderately – minutely – densely – coarsely – slightly
moderator for organisation: regularly – irregularly – radially – concentrically – reticularly
in the centre, at first: **with age:** echinate
at the margin, at first: **with age:** smooth
colour fresh : white **dry :**
colour distribution : even – irregular-patchy – marmorate – guttate – virgate – zonate – gradually/abruptly paler to margin – gradually/abruptly darker to margin
hygrophanous: yes / no

Pileus context

consistency: spongy – fleshy – membranous – deliquescent – brittle (chalky) – turgid – fibrous – cartilaginous – leathery – corky - hard
context: thin (membraneous) – carnulose (slightly fleshy) – carnose (fleshy)
colour fresh: white **dry :**
colour above hymenium fresh: "
colour distribution : even – irregular-patchy – marmorate – guttate – virgate
colour changes when cut or bruised: **changing: quickly – slowly**

taste: absent – mild – bitter – acrid – very acrid – peppery – sweet fruity – farinaceous – soapy – acidulous
smell: absent – alliaceous – bitter almonds – bugs – cocos – farinaceous – fetid – fruity-sweet – fungoid – mild fungoid – orange blossom – pungent (rotting cabbage) – rancid – spermatic – spicy – rubber – earth

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Stipe

insertion (pileus): central – excentric – lateral
attachments to substrate: inserted (insititious, no visible basal hyphae) – basal tomentum (hyphae) – strigose basal mycelium – rhizoids – rhizomorphs – pseudorhiza **colour attachments :**
stipe separability from context of pileus: continuous (not easily separated) – discontinuous (easily separated)
stipe shape (longitudinal section): clavate – cylindrical – filiform – subclavate – tapering downwards – tapering upwards – ventricose – bulbous
at first: **with age:** cylindrical
stipe cross section: terete – compressed
stipe curvature: straight – curved
stipe base: normal – subbulbous – bulbous – abruptly bulbous – marginately bulbous – rooting – pseudorhiza
sclerotia : present – absent: shape, size, section, surface
annular zone: present – absent
surface topography: even – rugose (wrinkled) – rugulose – pustulate (glandulose) – scabrous (rough with short projections) – verrucose – costate (ridged, fluted) – venose (veins-ribs) – scrobiculate – rimose (cracked, creviced) – rivulose – fissurate-incised (clefted, splitted) – striate – sulcate.
upper half – above annular zone, at first: **with age:** even
lower half – below annular zone, at first: **with age:** "

Stipitipellis

shine : dull – silky – shiny (polished)
touch (wetness) **:** dry – moist – waxy (slippery) – subviscid – viscid (sticky, tacky, gluey, resinous) – glutinous (slimy, viscous) – unctuous (oily).
surface texture (innate structures): smooth (glabrous) – laccate – sericeous – fibrillose – squamose – squamulose – squarrose – squarrulose – velutinous (velvety) – tomentose – matted tomentose (felted) – pubescent (downy) – villose (pilose) – hispid (hirsute) – hispidulous – strigose – lannate – floccose – flocculose – arachnoid – echinate – echinulate – reticulate.
abundance/density moderator: moderately – minutely – densely – coarsely – slightly
organisation moderator: regularly – irregularly – longitudinally – reticularly
upper half – above annular zone, at first: **with age:** smooth
lower half – below annular zone, at first: **with age:** "
colour fresh : white **dry :**
colour distribution: even – irregular-patchy – marmorate – guttate – virgate – paler to base – darker to base
upper half – above annular zone, at first: **with age:** even
lower half – below annular zone, at first: **with age:** "
specific colour of scabers, reticulum, squamules, when fresh: **dry:**

Context of stipe (i.e. its flesh or trama)

stipe body: solid – stuffed – fistulose – hollow – chambered
at first: **with age:** fistulose
consistency: spongy – fleshy – membranous – brittle (chalky) – turgid – fibrous – cartilaginous – leathery – corky - hard
at first: **with age:** fibrous
colour fresh: white **dry :**
colour distribution : even – irregular-patchy – marmorate – guttate – virgate – paler to base – darker to base
upper half – above annular zone, at first: **with age:** even
lower half – below annular zone, at first: **with age:** "
specific colour changes when cut or bruised: : **changing: quickly – slowly**
taste: absent – mild – bitter – acrid – very acrid – peppery – sweet fruity – farinaceous – soapy – acidulous
smell: absent – alliaceous – bitter almonds – bugs – cocos – farinaceous – fetid – fruity-sweet – fungoid – mild fungoid – orange blossom – pungent (rotting cabbage) – rancid – spermatic – spicy – rubber – earth.

Partial veil – Annulus – Cortina **present / absent**
position (relative height from base of stipe): <¼ (basal) – ¼ (inferior) – ½ (central) – ¾ (superior) – >¾ (apical)
partial veil shape: cortina – thickened area – single annulus – double
annulus origin: ascending – descending – complex
annulus attachment : fixed – moveable
annulus consistency: fibrous – membraneous – thick
annulus inner side: smooth (glabrous) – striate – dotted
annulus outer side: smooth – striate – matted tomentose (felted) – lannate – echinate
colour fresh: white **dry :**

Universal veil – Volva **present / absent**
volva shape: farinose - circumsessile – zoned – scaly – flaring – saccate membranous – saccate fragile
height : 57 **width :** 21
volva consistency: powdery – fibrous – membranous – thick
volva inner side: smooth – striate
limbus internis: yes – no
volva outer side: smooth (glabrous) – velutinous-velvety – tomentose – matted tomentose-felted – pubescent-downy –echinate – echinulate – pulverulent-pruinose – granulose – strobiliform (pine cone)
volva upper edge: even – lobed – incised – eroded (irregular)
colour inside fresh: white **dry:**
colour outside fresh: white + light brow **dry:**

Latex (milk or other exudates)

presence: absent / present **flux:** weak – normal – abundant
colour at first: watery translucent – white – yellow – bright orange – reddish grey – red – green – brown
colour becoming: watery translucent – white – greyish – greyish yellow – orange – reddish – green – bluish green – brown – black
taste: absent – mild – bitter – acrid – very acrid – peppery – sweet fruity – farinaceous – soapy – acidulous

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Hymenophore : smooth – ridged/veined – lamellate – subporoid (anastomosing) – tubulose (pores) – spines – amorphous mass

Separability from pileus context: yes – no

Annex 2: Morphological diversity of EcM fungi families at Comoe National Park

Amanitaceae

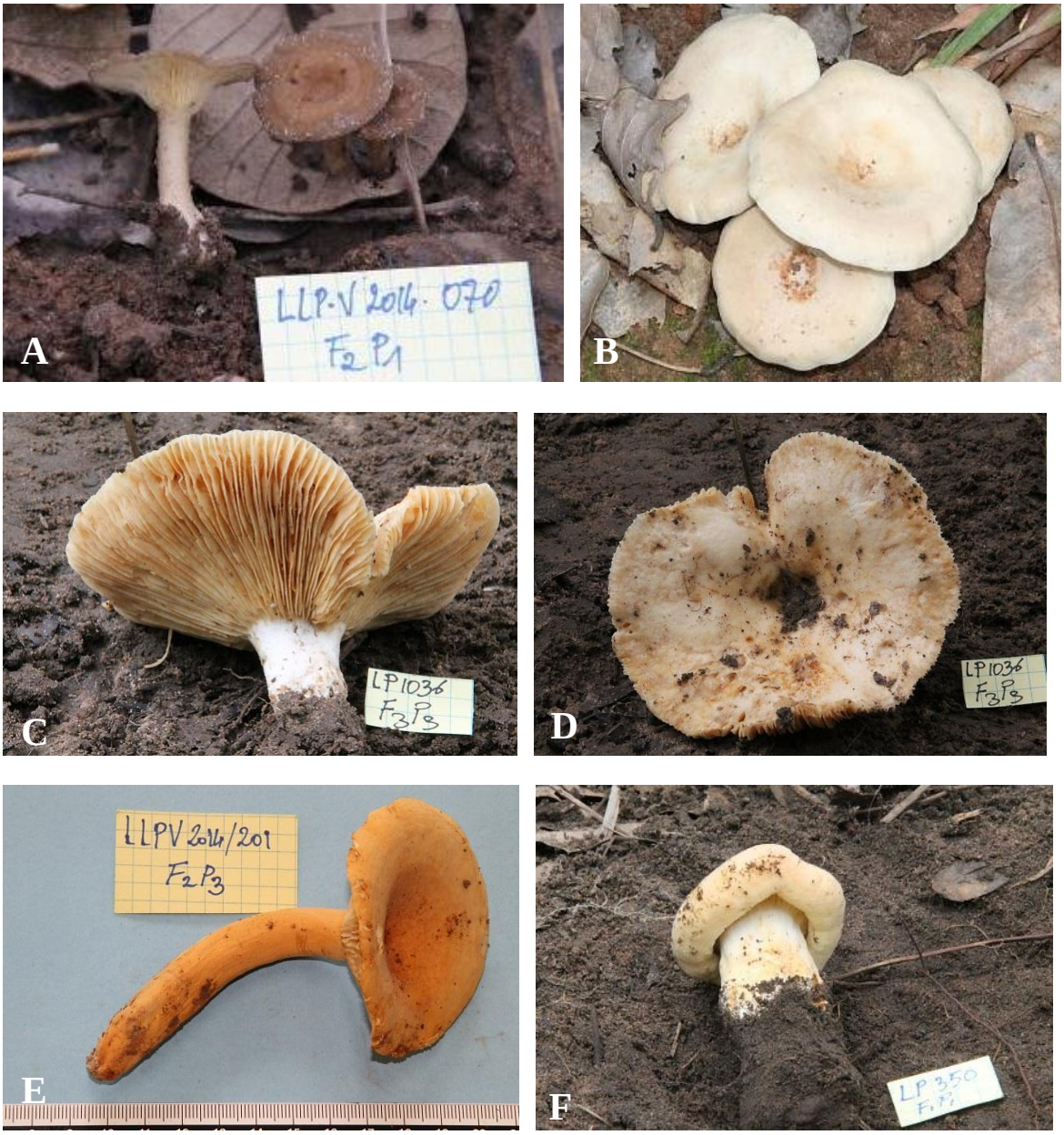
Some collected Amanita spp.: A = *A cf crassiconus; B* = *A.* sp 12*; C* = *A. sect. lepidella* sp 1; D = *A. annulatovaginata s.l.;* E = *A. subviscosa;* F = *A. aff. craseoderma;* G = *A. afrospinosa;* H = *A.* sp 7; I = *A. strobilaceovolvata s.l.;* J = *A. cf virosa;* K = *A. sect. lepidella strips xanthogala;* L = *A.* sp 13 .

Boletaceae

Some collected Boletaceae: A= *Boletellus linderi;* B = *Xerocomus* sp 3*;* C = *Pulveroboletus* sp 1*; D* = *Rubinoboletus cf griseus; E* = *Pulveroboletus* sp 2*;* F = *Tylopilus* sp 1*;* G = *Sutorius* sp 1*;* H = *Tubosaeta heterosetosa;* I = *Gyroporus castaneus;* J = *Rubinoboletus cf balloui;* K = *Rubinoboletus cf griseus;* L = *Veloporphyrellus africanus,* M = *Pulveroboletus* sp 3*;* N = *Boletus loosii;* O =*Xerocomus* sp 6

Russulaceae

Lactarius spp.



Some collected *Lactarius* spp.: A= *Lactarius tenellus;* B = *Lactarius saponaceus;* C and D = *Lactarius afroscrobiculatus;* E = *Lactarius* sp 2*;* F = *Lactarius* sp 3*;*

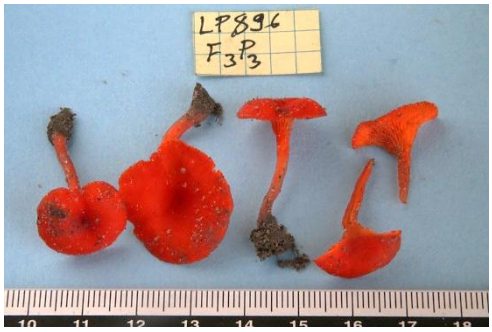
Lactifluus spp.

Some collected *Lactifluus* spp.: A= *L. luteopus;* B = *L.* sp 1*;* C = *L. aff. emergens;* D and E = *L.* sp 2 *;* F = *L. flammans;* G = *L. volemoides;* H and I = *Lactifluus* sp 4*;* J = *L. gymnocarpoides;* K = *L.* sp 3*; L = L. aff heimii*

Russula spp.

Some collected *Russula* spp.: A= *R. congoana;* B = *R.* sp 6*;* C = *R.* sp 11*;* D = *R cf grisea;* E = *R. aff. flavobrunnea ;* F = *R. sp 16; G and H = R. cf flavobrunnea.*

Cantharellaceae



Cantharellus addaiensis

Inocybaceae

Inocybe sp 1

Cortinariaceae

A = *C.* sp 1*;* B = *C. aff. violaceus;* C = *C. subgenus telamonia*

Clavulinaceae

Clavulina sp 1

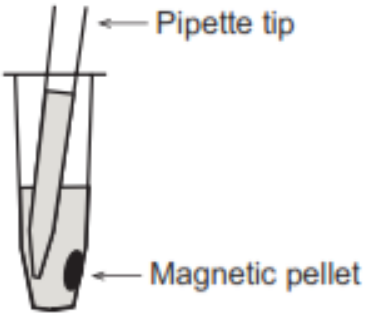
Sclerodermataceae

A = *Scleroderma* sp 1; B = *Scleroderma cf cepa*

Annex 3: Summary of DNA extraction using ChargeSwitch ® gDNA Plant Kit (Invitrogen, Darmstadt, Germany)

1. Chill the Precipitation Buffer (N5) on ice.
2. Put small pieces of EcM fungi (from hymenium and/or pileus or stipe context of 0.5 mm × 0.5 mm) in Eppendorf tubes 0.2 ml containing different sizes of bulk beads
3. At room temperature, add 100 µl of pre-chilled (4˚C) Lysis Buffer (L18) to tissues;
4. Grind tissues with FastPrep machine set at 4500g for 1 min. repeat step after 5 min pause;
5. Add 10 µl 10% SDS and leave at room temperature for 5 min. Mix by inversion ;
6. Add 40 µl pre-chilled Precipitation Buffer (N5) to the lysate. Mix by inversion;
7. Centrifuge for 5 min at and room temperature and Transfer supernatant in new and sterile 0.2 ml microcentrifuge tubes
8. Add 10 µl 10% Detergent (D1) to supernatant;
9. Add 4 µl resuspended (after thoroughly vortexing) Magnetic Beads;
10. Mix by pipetting up and down gently 15 times using a micropipette without forming any bubbles (ensure that the micropipette tip is submerged during mixing) to evenly distribute the Magnetic Beads within the solution;
11. Incubate at room temperature for 1 min, then place tubes on the MagnaRack until the magnetic beads have formed a tight pellet;

12. Without removing the tubes from the magnet, carefully aspirate and discard the supernatant without disturbing the pellet of beads by angling the micropipette such that the tip is pointed away from the pellet



13. Proceed immediately to Washing DNA by adding 100 µl Wash Buffer (W12) to the tube. Remove tubes from the magnet;
14. Gently pipet up and down 15 times without forming bubbles using micropipette to mix and resuspend the beads. Repeat once again steps 11 and 12;
15. Wash once again DNA as described above;
16. Remove the tube containing the pelleted magnetic beads from the magnet (there should be no supernatant);
17. Proceed immediately to Eluting DNA by adding 15 µl of Elution Buffer (E6);
18. Pipet up and down gently 15-30 times using an adapted micropipette until Magnetic Beads are completely dispersed and no clumps are visible;
19. Incubate at room temperature for 1 min;
20. Place again tubes on the magnet until the beads have formed a tight pellet and the supernatant is clear;
21. Without removing the tubes from the magnet, carefully transfer the supernatant containing the DNA to a sterile microcentrifuge tube without disturbing the pellet;
22. Discard the used Magnetic Beads. Do not reuse them;
23. Store the purified gDNA at -20°C or use immediately for the desired downstream application. Avoid repeated freezing and thawing of gDNA.

Annex 4: DNA extraction by modified CTAB method

1. Put small pieces of EcM fungi (from hymenium and/or pileus or stipe context of 0.5 mm × 0.5 mm) in low binding Eppendorf (LoBind eppies) tubes 2.5 ml;
2. Add 0.3% (V/V) β-mercapto-ethanol (3µl/ml) to 2x CTAB buffer and keep at 60˚C in water bath incubator;
3. Powder the dry samples with a 5 mm bead in the Retsch Mill for 45s at 30 mov./s. several rounds of grinding may be necessary (speed can be increased also).
4. Add 1600 µl pre-heated (60˚C) extraction buffer;
5. Incubates samples at 60˚C for 45 min with periodic gentle mixing;
6. Remove samples from water bath and let them cool a bite;
7. In the fume hood, add equal volume (ca. 800 µl) chloroform: isoamyl alcohol (24:1). Mix gently but thoroughly;
8. Centrifuge at 13 000g for 5 min at room temperature. Label new 1.5ml LoBind eppies;
9. Transfer the aqueous top phase (ca. 700 µl) to the new labelled eppies. Avoid interphase;
10. Repeat steps 7 to 9. Adjust volumes accordingly (700 µl chloroform:isoamyl alcohol, ca. 600 µl supernatant);
11. Add 0.8 x volume of supernatant, ca. 480 µl isopropanolal 4˚C. mix gently (~50 x) and incubate at -20˚C for 10 min;
12. Centrifuge at 4˚C and 16 000g for 5 min;
13. Cautiously discard supernatant (take care of the pellet!). drain briefly on paper towel;
14. Add 600 µl washing 70 % ethanol (EtOH). Swirl gently a few times. At this stage, samples can be stored overnight at -20˚C;
15. Centrifuge at 4˚C and 16 000g for 5 min and pour off supernatant (take care of the pellet!);
16. Invert tubes on a paper towel and allow the pellet to dry until the last traces of ethanol have evaporated (~1 hour);
17. Resuspend pellet in 50-100 µl TE Buffer (10 mM Tris, pH 8, 1 mM EDTA) pH 8 for 10 min at 60˚C (or until pellet has dissolved);
18. Add 2 µl diluted (10x) RNase A, mix and incubate 2 min at room temperature. Keep the samples cool hereafter.

Annex 5: best matches between homologous downloaded and generated sequences

Russulaceae

Species voucher	Morphological-based identification	Best match of retrieved homologous sequence	Percentage of identity	Molecular-based identification
LP019	*Russula aff. ochrocephala*	*Russula* sp_LN849753(Burkina Faso)	98	*Russula* sp LP019(CNP)
LP070	*Lactarius tenellus*	*Lactarius tenellus*_KF133280(Benin)	98	*Lactarius tenellus* LP070(CNP)
LP119	*Russula sp 16*	*Russula* sp_UDB016948(Benin)	99	*Russula* sp LP119(CNP)
LP137	*Russula cf flavobrunnea*	*Russula* sp_KM594829(Panama)	88	*Russula* sp LP137(CNP)
LP144	*Lactifluus sp 1*	*Lactifluus* sp_KR819054(Cameroon)	95	*Lactifluus* sp LP144(CNP)
LP155	*Lactifluus aff. emergens*	*Lactifluus chrysocarpus*_JX442761(Viet Nam)	83	*Lactifluus* sp LP155(CNP)
LP188	*Lactifluus flammans*	*Lactifluus flammans*_UDB016931(Benin)	99	*Lactifluus flammans* LP188(CNP)
LP190	*Lactarius sp*	*Lactarius* sp_UDB016890(Zambia)	99	*Lactarius* sp LP190(CNP)
LP199	*Lactifluus gymnocarpoides*	*Lactifluus gymnocarpoides*_LK392601(Benin)	99	*Lactifluus gymnocarpoides* LP199(CNP)
LP204	*Russula aff. cellulata*	*Russula brunneorigida*_KR819076(Cameroon)	95	*Russula* sp LP204(CNP)
LP219	*Russula sect. archaeina*	*Russula* sp_UDB016892(Zambia)	97	*Russula* sp LP219(CNP)
LP222	*Lactifluus sp 3*	*Lactifluus* sp_KR819054(Cameroon)	91	*Lactifluus* sp LP222(CNP)
LP232	*Russula cf ochrocephala*	*Russula* sp_UDB016896(Zambia)	87	*Russula* sp LP232(CNP)
LP339	*Russula sp 23*	*Russula* sp_UDB016957(Benin)	99	*Russula* sp LP339CNP)
LP346	*Russula cf flavobrunnea*	*Russula* sp_UDB016968(Benin)	100	*Russula* sp LP346CNP)
LP365	*Lactifluus aff. emergens*	*Lactifluus sesemontani*_KR819082(Cameroon)	84	*Lactifluus* sp LP365(CNP)
LP368	*Russula sp 17*	*Russula* sp_UDB016912(Zambia)	89	*Russula* sp LP368CNP)
LP441	*Lactifluus luteopus*	*Lactifluus luteopus*_LN849749(Guinea)	99	*Lactifluus luteopus* LP441(CNP)
LP457	*Lactarius pelliculatus*	*Lactarius pelliculatus*_AY606978(Madagascar)	98	*Lactarius pelliculatus* LP457(CNP)
LP482	*Russula sp 1*	*Russula* sp_UDB016967(Benin)	100	*Russula* sp LP482CNP)
LP560	*Lactarius saponaceus*	*Lactarius saponaceus*_LN849751(Benin)	98	*Lactarius saponaceus* LP560(CNP)
LP670	*Russula sp 8*	*Russula diffusa*_KR819084(Cameroon)	93	*Russula* sp LP670CNP)
LP728	*Lactifluus heimii*	*Lactifluus annulatoangustifolius*_HG426475	84	*Lactifluus* sp LP728(CNP)
LP754	*Russula cf. annulata*	*Russula hiemisilvae*_JQ902080(Cameroon)	94	*Russula* sp LP754(CNP)
LP780	*Lactifluus volemoides*	*Lactifluus volemoides*_UDB016930(Benin)	100	*Lactifluus volemoides* LP780(CNP)
LP787	*Lactarius afroscrobiculatus*	*Lactarius afroscrobiculatus*_UDB016934(Benin)	100	*Lactarius afroscrobiculatus* LP787(CNP)
LP873	*Russula sp 10*	*Russula* sp_UDB016935(Benin)	99	*Russula* sp LP873(CNP)

Boletaceae

Species voucher	Morphological-based identification	Best match of retrieved homologous sequence	Percentage of identity	Molecular-based identification
LP001	*Boletellus linderi*	*Boletus subtomentosus* _DQ131632(Italy)	86	*Boletus* sp LP001(CNP)
LP040	*Boletoid sp 1*	*Boletus permagnificus* _KR782301	83	*Boletus* sp LP040(CNP)
LP042	*Veloporphyrellus africanus*	*Boletus* sp _UDB016918(Zambia)	85	*Boletus* sp LP042(CNP)
LP047	*Boletellus longipes*	*Tylopilus* sp_FN669689(Estonia)	82	*Tylopilus* sp LP047(CNP)
LP071	*Xerocomussubspinulosus*	*Boletus* sp _UDB016927(Zambia)	97	*Boletus* sp LP071(CNP)
LP072	*Phylloporus ampliporus*	*Boletus legaliae* _UDB000403(Italy)	85	*Boletus* sp LP072(CNP)
LP081	*Xerocomus sp 3*	*Boletus* sp _UDB016913(Zambia)	94	*Boletus* sp LP081(CNP)
LP097	*Sutorius sp 1*	*Afroboletus* sp _UDB016992(Benin)	81	*Afroboletus* sp LP097(CNP)
LP134	*Rubinoboletus cf griseus*	*Boletus* sp _UDB016907(Zambia)	98	*Boletus* sp LP134(CNP)
LP183	*Rubinoboletus cf balloui*	*Boletus bicolor* _GQ166889(USA)	82	*Boletus* sp LP183(CNP)
LP276	*Phylloporus cf rhodophaeus*	*Phylloporus* sp _UDB016977(Benin)	99	*Phylloporus* sp LP276(CNP)
LP280	*Xerocomus sp 4*	*Boletus* sp _UDB016914(Zambia)	99	*Boletus* sp LP280(CNP)
LP297	*Boletus sp 2*	*Xerocomus* sp _UDB016995(Benin)	98	*Xerocomus* sp LP297(CNP)
LP322	*Boletus pallidisimus*	*Boletus* sp _UDB016905(Zambia)	99	*Boletus* sp LP322(CNP)
LP370	*Tuboeseta heterosera*	*Boletus subalpinus* _KT002371(USA)	80	*Boletus* sp LP370(CNP)
LP420	*Tylopilus sp 2*	*Tylopilus* sp _UDB013818(Zambia)	85	*Tylopilus* sp LP420(CNP)
LP432	*Pulveroboletus sp 3*	*Fistulinella viscida* _UDB025789(USA)	80	*Fistulinella* sp LP432(CNP)
LP602	*Xerocomus sp6*	*Boletus* sp _KP071233(Malaysia)	82	*Boletus* sp LP602(CNP)
LP637	*Octaviania ivoryana*	*Tylopilus* sp _AB973745(Japan)	84	*Tylopilus* sp LP637(CNP)
LP668	*Xerocomus sp 4*	*Boletus* sp _UDB016914(Zambia)	99	*Boletus* sp LP668(CNP)
LP752	*Porphyrellus sp 1*	*Hemileccinun* sp _KP012704(Australia)	78	*Boletus* sp LP752(CNP)
LP1035	*Tylopilus sect. chromapes*	*Boletus* sp _UDB016908(Zambia)	98	*Boletus* sp LP1035(CNP)

Amanitaceae

Species voucher	Morphological-based identification	Best match of retrieved homologous sequence	Percentage of identity	Molecular-based identification
LP009	*Amanita cf crassiconus*	*Amanita vestita*_KC429048(China)	85	*Amanita* sp LP009(CNP)
LP093	*Amanitaannulatovaginata s.l.*	*Amanita alboflavescens*_ KM052555(Korea, Republic of)	85	*Amanita* sp LP093(CNP)
LP105	*Amanita xanthogala*	*Amanita cyanopus*_ KT339210(Guyana)	77	*Amanita* sp LP105(CNP)
LP110	*Amanita subviscosa*	*Amanita subviscosa*_ UDB016941(Benin)	99	*Amanita subviscosa* LP110(CNP)
LP156	*Amanita aff. subviscosa*	*Amanita volvata*_JF723273(Korea, Republic of)	81	*Amanita* sp LP156(CNP)
LP178	*Amanita sp 9*	*Amanita regalis*_LT594941(USA)	79	*Amanita* sp LP178(CNP)
LP210	*Amanita aff. virosa*	*Amanita* sp_UDB016917(Zambia)	99	*Amanita* sp LP210(CNP)
LP343	*Amanita sp*	*Amanita* sp_UDB025376(Estonia)	88	*Amanita* sp LP343(CNP)
LP499	*Amanita congolensis*	*Amanita congolensis*_ KR919755(Zimbabwe)	99	*Amanita congolensis* sp LP499(CNP)
LP620	*Amanita masasiensis*	*Amanita masasiensis*_ UDB016975(Benin)	100	*Amanita masasiensis* LP620(CNP)
LP730	*Amanita sp*	*Amanita* sp_KN186807(Zambia)	95	*Amanita* sp LP730(CNP)
LP917	*Amanita sp*	*Amanita oberwinklerana*_ KM052560(Korea, Republic of)	79	*Amanita* sp LP917(CNP)
LP995	*Amanita strobilaceovolvata s.l.*	*Amanita* sp_UDB025737(Zimbabwe)	88	*Amanita* sp LP995(CNP)

Sclerodermataceae

Species voucher	Morphological-based identification	Best match of retrieved homologous sequence	Percentage of identity	Molecular-based identification
LP028	*Scleroderma* aff *verrucosum*	*Scleroderma* sp_FJ840461 (Burkina Faso)	97	*Scleroderma* sp LP028(CNP)
LP238	*Scleroderma* sp 1	*Scleroderma* sp_UDB013876 (Zambia)	99	*Scleroderma* sp LP238(CNP)
LP381	*Scleroderma* sp 2	*Scleroderma* sp_KR819100 (Cameroon)	99	*Scleroderma* sp LP381(CNP)

Inocybaceae

Species voucher	Morphological-based identification	Best match of retrieved homologous sequence	Percentage of identity	Molecular-based identification
LP108	*Inocybe* sp 1	*Inocybe* sp_UDB016962(Benin)	99	*Inocybe* sp LP108 (CNP)

Clavulinaceae

Species voucher	Morphological-based identification	Best match of retrieved homologous sequence	Percentage of identity	Molecular-based identification
LP693	*Clavulina* sp 1	*Clavulina* sp_UDB017809(Zambia)	96	*Clavulina* sp LP693(CNP)

Cortinariaceae

Species voucher	Morphological-based identification	Best match of retrieved homologous sequence	Percentage of identity	Molecular-based identification
LP269	*Cortinarius* sp 1	*Cortinarius callimorphus*_JF742660(USA)	84	*Cortinarius* sp LP269(CNP)
LP860	*Cortinarius* aff. *violaceus*	*Cortinarius* sp_UDB025780(Zimbabwe)	95	*Cortinarius* sp LP860(CNP)

Annex 6: Relative frequency of occurrence of EcM fungi species within woodlands of Comoé National Park

Distribution class	Taxon	Family	IW	MW	UW
Common to all habitat types	*Amanita* aff. *subviscosa*	Amanitaceae	1.56	0.58	2.14
	Amanita annulatovaginata sensu lato	Amanitaceae	0.58	1.56	0.39
	Amanita sp 13	Amanitaceae	1.17	0.78	0.78
	Amanita xanthogala	Amanitaceae	0.39	0.19	0.19
	Cantharellus addaiensis	Cantharellaceae	1.75	0.97	2.14
	Cortinarius sp 1	Cortinariaceae	0.19	0.19	0.39
	Lactifluus aff. *emergens*	Russulaceae	0.78	0.19	0.39
	Lactifluus luteopus	Russulaceae	0.19	0.78	1.56
	Phylloporus ampliporus	Boletaceae	0.39	0.58	0.19
	Pulveroboletus sp 1	Boletaceae	1.17	0.39	0.19
	Pulveroboletus sp 2	Boletaceae	0.58	0.78	1.36
	Russula aff. *cellulata*	Russulaceae	0.39	0.19	0.58
	Russula aff. *ochrocephala*	Russulaceae	0.58	0.78	0.97
	Russula cellulata	Russulaceae	0.78	0.58	0.39
	Russula cf *amoenolens*	Russulaceae	0.78	0.39	0.58
	Russula cf *flavobrunnea*	Russulaceae	0.97	0.39	0.58
	Russula cf *grisea*	Russulaceae	0.19	0.19	0.19
	Russula ciliata	Russulaceae	0.19	0.39	0.19
	Russula congoana	Russulaceae	2.53	0.78	0.19
	Russula sp 10	Russulaceae	0.58	0.19	0.78
	Russula sp 11	Russulaceae	0.19	0.19	0.39
	Xerocomus sp 4	Boletaceae	0.19	0.19	0.19
Shared by two habitat types	*Amanita congolensis*	Amanitaceae	0.00	0.78	0.97
	Amanita sp 12	Amanitaceae	0.19	0.39	0.00
	Amanita aff. *virosa*	Amanitaceae	0.00	1.36	1.95
	Amanita masasiensis	Amanitaceae	0.19	0.19	0.00
	Amanita sect *lepidella* sp 1	Amanitaceae	0.39	0.19	0.00

	Amanita sect. *lepidella strips xanthogala* sp 1	Amanitaceae	0.19	0.00	0.19
	Amanita sp 5	Amanitaceae	0.19	0.19	0.00
	Amanita sp 8	Amanitaceae	0.19	0.00	0.39
	Amanita strobilaceo-volvata sensu lato	Amanitaceae	0.58	0.00	1.95
	Boletus loosii	Boletaceae	0.00	0.97	0.78
	Boletus sp 2	Boletaceae	0.39	0.00	0.58
	Gyroporus castaneus	Gyroporaceae	0.19	0.00	0.58
	Lactarius afroscrobiculatus	Russulaceae	0.00	0.19	0.39
	Lactarius saponaceus	Russulaceae	0.19	0.19	0.00
	Lactarius tenellus	Russulaceae	1.95	1.56	0.00
	Lactifluus aff. *heimii*	Russulaceae	0.97	0.19	0.00
	Lactifluus sp 4	Russulaceae	0.19	0.19	0.00
	Octaviana ivoryana	Boletaceae	1.17	0.19	0.00
	Rubinoboletus cf *balloui*	Boletaceae	0.00	1.17	0.19
	Rubinoboletus cf *griseus*	Boletaceae	0.39	0.39	0.00
	Russula cf *sesenagula*	Russulaceae	0.58	0.39	0.00
	Russula sect *griseineae*	Russulaceae	0.00	0.19	0.19
	Russula sect. *archaeina*	Russulaceae	0.39	0.19	0.00
	Russula sp 7	Russulaceae	0.19	0.19	0.00
	Scleroderma sp 2	Sclerodermataceae	0.58	0.19	0.00
	Sutorius sp 1	Boletaceae	0.39	0.00	0.39
	Tylopilus sp 1	Boletaceae	0.00	0.19	0.39
	Xerocomus subspinulosus	Boletaceae	0.39	0.39	0.00
Specific to one habitat type	*Amanita* aff. *craseoderma*	Amanitaceae	2.14	0.00	0.00
	Amanita cf *crassiconus*	Amanitaceae	0.00	0.97	0.00
	Amanita sp 1	Amanitaceae	0.19	0.00	0.00
	Amanita sp 2	Amanitaceae	0.00	0.19	0.00
	Amanita sp 3	Amanitaceae	0.19	0.00	0.00
	Amanita sp 4	Amanitaceae	0.00	0.00	0.19
	Amanita sp 6	Amanitaceae	0.19	0.00	0.00
	Amanita sp 7	Amanitaceae	1.75	0.00	0.00
	Amanita sp 9	Amanitaceae	0.19	0.00	0.00

Amanita sp 10	Amanitaceae	0.19	0.00	0.00
Amanita sp 11	Amanitaceae	0.19	0.00	0.00
Amanita subviscosa	Amanitaceae	0.39	0.00	0.00
Boletellus linderi	Boletaceae	0.97	0.00	0.00
Boletellus longipes	Boletaceae	0.00	0.58	0.00
Boletus pallidisimus	Boletaceae	0.00	0.19	0.00
Boletus sp 1	Boletaceae	0.00	0.00	0.19
Clavunila sp 1	Clavulinaceae	0.19	0.00	0.00
Cortinarius aff violaceus	Cortinariaceae	0.39	0.00	0.00
Cortinarius subgenus *telamonia* sp 1	Cortinariaceae	0.58	0.00	0.00
Inocybe sp 1	Inocybaceae	0.58	0.00	0.00
Lactarius sp 1	Russulaceae	0.00	0.00	0.19
Lactarius sp 2	Russulaceae	0.00	0.00	0.19
Lactarius sp 3	Russulaceae	0.00	0.39	0.00
Lactifluus flammans	Russulaceae	0.00	0.39	0.00
Lactifluus gymnocarpoides	Russulaceae	0.00	0.00	0.19
Lactifluus pelliculatus	Russulaceae	0.00	0.00	0.97
Lactifluus sp 1	Russulaceae	0.00	0.19	0.00
Lactifluus sp 2	Russulaceae	0.00	0.00	0.19
Lactifluus sp 3	Russulaceae	0.19	0.00	0.00
Lactifluus volemoides	Russulaceae	0.00	0.39	0.00
Phylloporus cf *rhodophaeus*	Boletaceae	0.58	0.00	0.00
Porphyrellus sp 1	Boletaceae	0.19	0.00	0.00
Pulveroboletus sp 3	Boletaceae	0.00	0.00	0.58
Russula aff. *flavobrunnea*	Russulaceae	0.19	0.00	0.00
russula cf *annulata*	Russulaceae	0.00	0.00	0.39
Russula cf *mairei*	Russulaceae	0.00	0.39	0.00
Russula cf *ochrocephala*	Russulaceae	0.39	0.00	0.00
Russula cf *subfistulosa*	Russulaceae	0.00	0.00	0.39
Russula discopus	Russulaceae	0.00	0.00	0.19
Russula oleifera	Russulaceae	0.00	0.19	0.00
Russula sp 1	Russulaceae	0.00	0.19	0.00

Russula sp 2	Russulaceae	0.00	0.19	0.00
Russula sp 3	Russulaceae	0.19	0.00	0.00
Russula sp 4	Russulaceae	0.39	0.00	0.00
Russula sp 5	Russulaceae	0.00	0.19	0.00
Russula sp 6	Russulaceae	0.00	0.00	0.19
Russula sp 8	Russulaceae	0.00	0.00	0.19
Russula sp 9	Russulaceae	0.19	0.00	0.00
Russula sp 12	Russulaceae	0.19	0.00	0.00
Russula sp 13	Russulaceae	0.00	0.19	0.00
Russula sp 14	Russulaceae	0.00	0.00	0.19
Russula sp 15	Russulaceae	0.00	0.00	0.19
Russula sp 16	Russulaceae	0.58	0.00	0.00
Russula sp 17	Russulaceae	0.19	0.00	0.00
Russula sp 18	Russulaceae	0.00	0.00	0.19
Scleroderma cf *cepa*	Sclerodermataceae	0.58	0.00	0.00
Scleroderma cf *citrinum*	Sclerodermataceae	0.00	0.19	0.00
Scleroderma sp 1	Sclerodermataceae	0.00	0.58	0.00
Scleroderma aff. verrucosum	Sclerodermataceae	0.19	0.00	0.00
Tubosaeta heterosetosa	Boletaceae	0.39	0.00	0.00
Tylopilus griseus	Boletaceae	0.00	0.00	0.19
Tylopilus niger	Boletaceae	0.39	0.00	0.00
Boletoid sp 1	Boletaceae	0.00	0.00	0.19
Tylopilus sp 2	Boletaceae	0.00	0.58	0.00
Tylopilus sect. chromapes sp 1	Boletaceae	0.00	0.00	0.19
Veloporphyrellus africanus	Boletaceae	0.00	0.00	0.97
Xerocomus sp 1	Boletaceae	0.00	0.19	0.00
Xerocomus sp 2	Boletaceae	0.00	0.00	0.19
Xerocomus sp 3	Boletaceae	0.19	0.00	0.00
Xerocomus sp 5	Boletaceae	0.00	0.19	0.00
Xerocomus sp 6	Boletaceae	0.78	0.00	0.00
Xerocomus sp 7	Boletaceae	0.00	0.19	0.00

IW: Isoberlinia Woodlands; MW: mixed wwoodlands: UW: Uapaca Woodlands

Annex 7: Species relative dominance (SRD) of plant species with diameter at breast height (dbh) ≥ 10 cm per plot and habitat type. Bold taxa and values are known host plants. IW: *Isoberlina* Woodland; MW: Mixed Woodland; UW: *Uapaca* Woodland. *FiPi* are plots within each habitat type

TAXON	FAMILY	IW			MW			UW		
		F1P1	F1P2	F1P3	F2P1	F2P2	F2P3	F3P1	F3P2	F3P3
Annona senegalensis	Annonaceae	0	0	0	0	0	0	0.052079	0	0
Anogeissus leiocarpa	Combretaceae	0	7.07472	0	2.11E-05	0.902768	0.362384	0	15.78371	0
Bridelia ferrugina	Euphorbiaceae	0.202674	0	0.743972	1.65E-06	1.180989	1.74627	0	0.116948	0.866907
Burkea africana	Caesalpinioideae	9.59379	0.95881	2.208639	8.92E-05	9.779555	5.936332	0.413232	0.315776	4.532828
Cassia sieberriana	Caesalpinaceae	0	20.99042	0	0	0	0	0	0	0
Combretum molle	Combretaceae	0	0	0.180639	0	0	1.775204	0	0.986948	0
Combretum nigricans	Combretaceae	0	0	0	9.84E-06	0	0	3.006922	0.330634	0
Crossopteryx febrifuga	Rubiaceae	0	0	0	0	1.668538	0	0	0.785605	0
Detarium microcarpum	Caesalpinioideae	0	0	0	0	0	0	13.05877	0.254106	1.851009
Diospyros mespiliformis	Ebenaceae	0	10.56528	0	0	5.422796	2.922625	1.924418	2.136937	0
Ficus sur	Moraceae	0	0	0.422518	0	0	1.566635	0.317664	0.246757	0
Hexalobus sp	Annonaceae	0.073653	1.035776	0.508826	0	0	0	0	0	0
Hymenocardia acida	Euphorbiaceae	0	0.062951	0	0	0	0	0	0.072294	0
Isoberlinia doka	**Caesalpinioideae**	**62.59906**	**40.22337**	**84.0345**	**7.42E-05**	**0**	**0**	**0**	**0**	**0**
Keetia cornelia	Rubiaceae	0	0	0	0	0.262753	0	0	0	0
khaya senegalensis	Meliaceae	0	0	0	0	28.87822	0	10.48244	12.31928	17.05903
Kigelia africana	Bignoniaceae	0	0	0	0	0	0	0	0.259778	0
Lannea acida	Anacardiaceae	0.594603	1.866866	0	0	1.071291	0	0	0	0.250441
Lannea barteri	Anacardiaceae	0	0	0	0	0	11.46376	0	2.559955	0
Lannea microcarpum	Anacardiaceae	0.346293	0.657312	0	2.08E-06	0	0	0.182068	5.603758	0
Lophira lanceolata	Ochnaceae	0	0	0	0	0	2.992169	0	0	0
Manilkara multinervis	Sapotaceae	0	14.68388	0	0	0	0.612862	0	9.021531	0

Maytenus senegalensis	Celastraceae	0	0	0	0	0	0	0	0	0.29474
Monotes kerstingii	**Dipterocarpaceae**	**22.08517**	**0.907412**	**7.857085**	**4.96E-05**	**1.668194**	**3.330356**	**5.646427**	**2.425193**	**11.64935**
Parinari polyandra	Chrysobalanaceae	0	0	0	2.02E-06	0.518926	0	0	0.227545	0.57455
Parkia biglobosa	Mimosoideae	0.103807	0	0	0	0	0.130785	0	6.073188	0
Pericopsis laxifolia	Papilionoideae	1.104863	0.334262	0.525658	9.88E-06	0.220131	0.907648	2.099864	0.490159	0
Piliostigma thoningii	Caesalpinioideae	0	0	0	0	0	0.117421	1.327627	0.834932	0.739899
Pseudocedrela kotschyi	Meliaceae	0	0	0	0	0	1.891327	0	1.578718	0
Psychotria rufipilis	Rubiaceae	0	0	0	0	0.434713	1.027405	0	2.411573	0
Pterocarpus erinaceus	Fabaceae	0.723993	0.125675	1.393041	0	0	0.074864	0	0.239248	0
Strycchnos innocua	Loganiaceae	0	0.106244	0.087025	0	0	0	0	0	0
Syziguium guineense var. macrocarpum	Myrtaceae	0	0	0	6.74E-06	1.313666	0	2.12301	0.518797	12.0153
Tamarindus indica	Caesalpinioideae	0	0.082533	0	0	1.916412	0	0	0	0
Terminalia vicennioides	Combretaceae	0.305743	0.03371	0	1.06E-06	0.67537	0	1.708466	0.516121	0
Terminalia glaucescens	Combretaceae	0	0	0	0	0	0.189561	0	0	0
Terminalia ternifolia	Combretaceae	0	0	0	0	0	0	0	2.360848	0
Trichilia emetica	Meliaceae	0	0	0	1.05E-06	0	0	0	0	0.71067
Uapaca togoensis	**Euphorbiaceae**	**1.966356**	**0.071928**	**0.92454**	**0.000395**	**42.59799**	**59.10492**	**46.95737**	**27.9052**	**46.63201**
undetermined sp1	undetermined	0	0	0.100848	0	0	0	0	0	0
undetermined sp2	undetermined	0	0	0.255058	0	0	0	0	0	0
undetermined sp3	undetermined	0	0	0	0	0.902768	0	0	0	0
undetermined sp4	undetermined	0	0	0	0	0.118094	0	0	0	0
undetermined sp5	undetermined	0	0	0	0	0	0	0	0.97908	0
undetermined sp6	undetermined	0	0	0	0	0	0	0	0	2.823266
Vitellaria paradoxa	Sapotaceae	0.299993	0	0	7.17E-06	0.466827	3.847464	9.394684	2.204518	0
Vitex doniana	Verbenaceae	0	0	0	0	0	0	1.304958	0.440872	0
Ximenia americana	Olacaceae	0	0.218851	0.757653	1.8E-06	0	0	0	0	0

Annex 8: Number of stems per plant species within plots per habitat type. Bold taxa and values are known host plants. IW: *Isoberlina* Woodland; MW: Mixed Woodland; UW: *Uapaca* Woodland. *FiPi* are plots within each habitat type

TAXON	FAMILY	IW			MW			UW		
		F1P1	F1P2	F1P3	F2P1	F2P2	F2P3	F3P1	F3P2	F3P3
Annona senegalensis	Annonaceae	0	0	0	0	0	0	1	0	0
Anogeissus leiocarpa	Combretaceae	0	1	0	1	0	1	1	5	0
Bridelia ferrugina	Euphorbiaceae	1	0	1	1	2	4	0	2	2
Burkea africana	Caesalpinioideae	9	4	9	3	2	1	2	1	3
Cassia sieberiana	Caesalpinaceae	0	1	0	0	0	0	0	0	0
Combretum molle	Combretaceae	0	0	0	0	0	2	0	2	0
Combretum nigricans	Combretaceae	0	0	0	1	0	0	1	1	0
Crossopteryx febrifuga	Rubiaceae	0	0	0	0	1	0	0	1	0
Detarium microcarpum	Caesalpinioideae	0	0	0	0	0	0	8	1	6
Diospyros mespiliformis	Ebenaceae	0	2	0	0	4	1	1	1	0
Ficus sur	Moraceae	0	0	1	0	0	2	2	4	0
Hexalobus monopetalus	Annonaceae	1	11	2	0	0	0	0	0	0
Hymenocardia acida	Euphorbiaceae	0	1	0	0	0	0	0	1	0
Isoberlinia doka	**Caesalpinioideae**	**48**	**52**	**54**	**5**	**0**	**0**	**0**	**0**	**0**
Keetia cornelia	Rubiaceae	0	0	0	0	2	0	0	0	0
khaya senegalensis	Meliaceae	0	0	0	0	1	0	1	1	1
Kigelia africana	Bignoniaceae	0	0	0	0	0	0	0	1	0
Lannea acida	Anacardiaceae	1	3	0	0	1	0	0	0	1
Lannea barteri	Anacardiaceae	0	0	0	0	0	1	0	1	0
Lannea microcarpa	Anacardiaceae	1	2	0	1	0	0	1	2	0
Lophira lanceolata	Ochnaceae	0	0	0	0	0	1	0	0	0
Manilkara multinervis	Sapotaceae	0	2	0	0	0	2	0	3	0
Maytenus senegalensis	Celastraceae	0	0	0	0	0	0	0	0	1

Monotes kerstingii	**Dipterocarpaceae**	**16**	**5**	**11**	**10**	**3**	**4**	**5**	**8**	**8**
Parinari polyandra	Chrysobalanaceae	0	0	0	2	1	0	0	5	3
Parkia biglobosa	Mimosoideae	1	0	0	0	0	1	0	1	0
Pericopsis laxifolia	Papilionoideae	2	1	1	1	2	2	2	1	0
Piliostigma thoningii	Caesalpinioideae	0	0	0	0	0	1	1	4	2
Pseudocedrela kotschyi	Meliaceae	0	0	0	0	0	1	0	1	0
Psychotria rufilipis	Rubiaceae	0	0	0	0	1	1	0	13	0
Pterocarpus erinaceus	Fabaceae	2	1	4	0	0	1	0	1	0
Strycchnos innocua	Loganiaceae	0	2	1	0	0	0	0	0	0
Syziguium guineense var. macrocarpum	Myrtaceae	0	0	0	3	2	0	5	2	12
Tamarindus indica	Caesalpinioideae	0	1	0	0	1	0	0	0	0
Terminalia avicennioides	Combretaceae	1	1	0	1	1	0	5	5	0
Terminalia glaucescens	Combretaceae	0	0	0	0	0	1	0	0	0
Terminalia ternifolia	Combretaceae	0	0	0	0	0	0	0	1	0
Trichilia emetica	Meliaceae	0	0	0	1	0	0	0	0	2
Uapaca togoensis	**Euphorbiaceae**	**3**	**1**	**5**	**50**	**55**	**46**	**63**	**45**	**30**
undetermined species sp1	undetermined	0	0	1	0	0	0	0	0	0
undetermined species sp2	undetermined	0	0	1	0	0	0	0	0	0
undetermined species sp3	undetermined	0	0	0	0	1	0	0	0	0
undetermined species sp4	undetermined	0	0	0	0	1	0	0	0	0
undetermined species sp5	undetermined	0	0	0	0	0	0	0	1	0
undetermined species sp6	undetermined	0	0	0	0	0	0	0	0	2
Vitellaria paradoxa	Sapotaceae	2	0	0	2	4	5	2	5	0
Vitex doniana	Verbenaceae	0	0	0	0	0	0	4	2	0
Ximenia americana	Olacaceae	0	3	3	1	0	0	0	0	0

ANNEX 9: RESUME DE LA THESE

INTRODUCTION

L'association mycorhizienne est la symbiose la plus répandue dans le monde (Smith & Read 2008). Parmi les types mycorhiziens, la symbiose ectomycorhizienne (EcM) est la plus évidente en raison de la production d'organes visibles à l'extrémité des racines des plantes partenaires et la fructification épigée de sporophores formés par les champignons symbiotiques. La symbiose EcM concerne principalement des arbres forestiers, et se produit dans divers biomes dans le monde entier, y compris les forêts claires et denses en zones tempérées, boréales et tropicales. En Afrique tropicale, il est connu que les champignons EcM sont prépondérants dans les forêts soudano-zambéziennes dominées par les Caesalpinioideae et Phyllantaceaea (**De Kesel *et al.*, 2002; Ducousso *et al.*, 2002; Diedhiou *et al.*, 2010; Bâ *et al.*, 2012**). Cependant, les forêts mono-spécifiques humides tropicales abritent également une grande diversité de ces champignons. Malgré leurs grandes valeurs économiques et écologiques, on sait très peu sur l'influence des facteurs climatiques sur l'apparition, la production naturelle et la structure des communautés de champignons EcM. L'apparition saisonnière des leurs sporophores suscite l'hypothèse que les symbiotes fongiques sont très sensibles à certains paramètres climatiques tels que les précipitations, l'humidité et la température du sol et de l'atmosphère. Pour évaluer et prédire les changements induits par les variations climatiques dans l'apparition, la production naturelle et la diversité des champignons EcM, nous avons entrepris des inventaires mycologiques dans le Parc National de la Comoé (PNC) située au Nord-Est de la Côte-d'Ivoire. Ce parc se trouve dans le Centre Soudanien d'Endémisme défini par **White (1983)**, centre dominé par de nombreux arbres forestiers EcM reconnus (*Afzelia africana*, *Isoberlinia* spp, *Uapaca* spp, *Monotes kerstingi, etc.*).

Objectifs

Objectif général

L'objectif principal de cette étude est de comprendre la significativité des paramètres climatiques (intensité et la fréquence de la pluie, température et humidité de l'air et du sol) sur la dynamique temporelle des champignons EcM et leurs arbres forestiers associés.

Objectifs spécifiques

Les objectifs spécifiques sont énumérés comme suit :

1. Evaluer la diversité des EcM champignons;

2. Examiner les affinités taxonomiques entre (1) les champignons récoltés et (2) avec des espèces provenant d'autres régions du monde, et leur position phylogénétique;

3. Déterminer les variables environnementales gouvernant leur répartition spatiale

4. Démontrer l'influence de l'humidité de l'air et du sol, la distribution des précipitations / intensité, la température de l'air et du sol sur la phénologie de fructification et de la production de champignons EcM comestibles.

MATÉRIEL ET MÉTHODES

Site d'étude

La présente étude a été réalisée dans le Parc National de la Comoé (PNC) situé à 8 ° 32 '- 9 ° 32' Nord et 3 ° 01' - 4 ° 24' Ouest dans le nord-est de la Côte d'Ivoire, au sud de la frontière avec le Burkina Faso. Le parc est situé dans la région du Boukani et entre les villes de Bouna et Dabakala.

Une semaine d'enquête exploratoire a été réalisée dans les parties accessibles du parc en Novembre 2013 pour identifier les sites d'étude appropriés. Sur la base des cartes de végétation disponibles (**Poilecot *et al.*, 1991; Lauginie, 2007**), trois types d'habitat ont été sélectionnés eu égard à (1) la présence et l'abondance des partenaires arbres reconnus, membres de Caesalpiniaceae et Phyllantaceae, et (2) la distance à la Station de Recherche en Écologie de la Comoé, notre camp de base (pour un traitement rapide des échantillons et éviter leur pourrissement). Les différents types d'habitat étaient espacé de 300 mètres les uns des autres et comprenaient:

• Type 1: Forêt claire dominée par *Isoberlinia doka* Craib & Stapf (IW);

• Type 2: Forêt mixte (MW);

• Type 3: Forêt claire dominée par *Uapaca togoensis* Pax (UW).

Trois parcelles permanentes de 30 m x 30 m chacune ont été établies dans chaque type de végétation sélectionné, faisant un total de neuf parcelles. Elles ont été labellisées FiPi, Fi représentant le type de formation végétal et Pi la parcelle. Tous les neuf (09) parcelles ont été géo-référencées à l'aide d'un GPS Garmin GPSMAP® 62stc (Garmin International Inc., Olathe, KS, USA). Les parcelles dans un type d'habitat ont été espacées l'une de l'autre d'au moins 10 m, selon la présence et la densité des arbres partenaires.

Méthodes

Échantillonnage des champignons EcM

L'échantillonage a consisté à explorer chaque parcelle suivant des bandes parallèles de 2 m de large et à récolter tous les sporophores observés. Pour éviter de manquer les espèces de cycle court, chaque parcelle a été visitée une fois par semaine d'Avril au début Octobre 2014. Ainsi, après chaque récolte, les sporophores ont été comptés, pésés et séchés. Puis, des spécimens représentatifs par espèce ont été conservés dans des sacs en plastique à fermeture zippée avec une étiquette portant le numéro de collection et des données écologiques de base sur la collection. Ce matériel d'herbier est conservé à l'École doctorale Wascal Changement Climatique et Biodiversité de l'Université Félix Houphouët-Boigny, Côte d'Ivoire. L'identification des champignons EcM collectés a été effectuée par des experts du Jardin Botanique Meise, Belgique.

Analyses moléculaire et phylogénétique

Des extractions d'ADN ont été entreprises à partir des collections en utilisant soit un kit commercial ou la méthode CTAB selon un protocole modifié d'après **Gardes et Bruns (1993)**. Le séquençage a été réalisé par la méthode de Sanger, le BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Après le traitement des séquences générées, des recherches BlastN ont été effectuées pour identifier et sélectionner les séquences les plus proches des bases de données publiques UNITE et INSD pour des alignements multiples. Une inférence phylogénétique a été réalisée avec la méthode statistique Maximum de vraisemblance (ML) du

programme informatique MEGA6 pour mettre en évidence la structure communautaire d'espèces fongiques récoltées et mieux la mieux comprendre.

Collecte de données des facteurs environnementaux

Les facteurs environnementaux biotiques et abiotiques ont été enregistrés pour évaluer leur possible influence sur l'occurrence et la production des champignons EcM. Premièrement, des pluviomètres et des unités d'enregistrement ont été installés dans chaque parcelle du 8 Avril 2014 au 3 Octobre 2014 pour recueillir respectivement l'intensité des précipitations, les paramètres climatiques de l'air et du sol (humidité et température). Deuxièmement, l'inventaire floristique complet de chaque parcelle a été effectué en Avril 2014. Toutefois, seules les espèces ligneuses dont le diamètre à hauteur de poitrine (DHP) ≥ à 10 cm ont été prises en compte pour l'analyse statistique. Le nombre de tiges par espèce et leur DHP ont été enregistrés pour évaluer la densité des espèces ligneuses, les surfaces terrières des arbres EcM identifiés et la surface terrière totale de chaque parcelle. Finalement, des sols composites de 200 g ont été faits à partir d'échantillons de sol prélevés à chaque angle et au centre de chaque parcelle à la mi-saison des pluies (fin Juillet) pour évaluer la texture et structure du sol, le pH et les teneurs des principaux minéraux.

Analyse des données

Diversité des champignons EcM

Les données de l'occurrence (présence / absence par espèce, parcelle et type d'habitat) et de l'abondance (fréquence relative par espèce, parcelle, type d'habitat et semaine) ont été enregistrées dans le logiciel de traitement de données Excel 2010. La richesse spécifique (par parcelle et par type d'habitat, et la richesse spécifique totale) et les indices de diversité (alpha et bêta-diversité) ont été calculés. Les données d'incidence ont été utilisées pour construire des courbes d'accumulation hebdomadaire avec un intervalle de confiance de 95% et 500 randomisations en utilisant le programme informatique EstimateS 9.0 (Colwell, 2006). Les courbes d'accumulation et d'autres statistiques obtenues ont été utilisées pour évaluer la représentativité de notre protocole d'échantillonnage.

Influence des facteurs environnementaux

Les moyennes hebdomadaires par parcelle ont été utilisées pour évaluer la significativité de la variabilité climatique à l'échelle du type d'habitat à l'aide de la fonction ANOVA des mesures répétées avec le programme informatique Statistica 7.1 (StatSoft, 2006). L'hypothèse de sphéricité de Mauchly a été testée au préalable avec le même logiciel. L'ANOVA des mesures répétées a été réalisée pour les effets *Type d'habitat*, *Semaine* et de l'interaction des deux facteurs.

La biomasse fraîche cumulée par espèce, par semaine et par type d'habitat a servi à évaluer la production naturelle des espèces EcM de champignons comestibles. Les valeurs obtenues à l'échelle des parcelles (m^2) ont été rapportées à l'hectare.

L'Influence de la variabilité du climat du sol et de l'air sur la production naturelle de champignons EcM comestibles a été étudiée par analyse de corrélation. Du fait de la multicollinéarité des variables climatiques, une analyse des composantes principales (ACP) a été entreprise afin d'extraire les composants indépendants et tester la significativité de leur corrélation avec les variables initiales. Des tests de corrélation selon Spearman ont permis d'etudier la relation entre ces composantes extraites et la biomasse fraiche des espèces comestibles.

RÉSULTATS ET DISCUSSION

Diversité et Distribution spatiale

2814 sporophores ont été recueillies de mi-Mai à début Octobre 2014. Ils ont été classés en 123 espèces appartenant à 23 genres et 09 familles. A l'échelle du type de végétation, 1565 sporophores appartenant à 75 espèces, 21 genres et 9 familles sont recensés dans IW, 513 sporophores pour 65 espèces appartenant à 15 genres et 6 familles ont été détectées dans MW tandis que UW a produit 736 sporophores appartenant à 56 espèces issues de 16 genres et 6 familles. Les indices de diversité de Simpson (1-D) et d'autosimilarité (moyenne des valeurs de Jaccard) calculés des parcelles étaient respectivement 0.97 et 0.40 pour IW, 0.97 et 0.33 pour MW tandis qu'UW avait des valeurs de 0.96 et 0.29. La similarité entre les types d'habitat a été

estimée à 0.34. Toutefois, les courbes d'accumulation ascendantes hebdomadaires n'ont pas atteint l'asymptote indiquant que de nombreuses espèces restent encore à recenser.

Phylogénie des champignons EcM

Bien que l'extraction d'ADN n'ait pas réussie pour l'ensemble des spécimens représentatifs sélectionnés, 150 séquences d'ADN pures ont été générées avec des longueurs variant de 500 à 914 paires de bases. Les alignements multiples indiquent 72 séquences d'ADN différentes correspondant à 72 espèces différentes. Toutefois, seulement 10 individus ont été identifiés au niveau de l'espèce à l'aide des séquences d'ADN, laissant un total de 62 espèces non identifiés (y compris les espèces soupçonnées nouveau à la science). En fait, de nombreuses séquences d'ADN générées ne correspondent pas à l'ADN des séquences de holotypes disponibles dans les bases de données publiques en dépit de l'identification morphologique. Plusieurs espèces récoltes au PNC fructifient également dans divers pays ouest-africains tandis que d'autres ont une répartition plus large s'étendant jusqu'en Afrique australe et même à Madagascar.

Distribution spatiale des champignons ectomycorhiziens

La densité des tiges d'Uapaca *togoensis* Pax (UTDen) et *Isoberlinia doka* Craib & Stapf ont été les variables environnementales influençant significativement la distribution spatiale des espèces fongiques ectomycorhiziennes récoltées. UTDen etait positivement correle ($r^2 = 0.92$; p-value = 0.002) tandis que IDDen l'etait negativement correlé au premier axe ($r^2 = 0.83$; p-value = 0.018) tel que visualisé par le positionnement multidimensionnel non-métrique (NMDS).

De même, une classification hiérarchique a mis en évidence deux groupes de sites auxquels sont associes respectivement trois et une espèce indicatrices.

Influence de la variabilité climatique sur la production des champignons comsetibles

Parmi les 123 espèces collectées, 27 ont été identifiés comme étant comestible selon la littérature. Les fructifications débutèrent à la mi-Mai excepté dans IW où elles commencèrent à la dernière semaine du mois de Juin. Les pics de production furent en général observés au mois de Juillet.

L'analyse des composantes principales montra que la production des espèces était en général négativement corrélée avec les températures de l'air et du sol tandis l'intensité de la pluie et l'humidité relative avaient une influence positive en IW et MW. Toutefois, ces dernières variables citées ont un impact négatif sur la production de certaines espèces en UW. Ce sont notamment *A. congolensis et Gyroporus castan*eus. Ainsi, les productions de six espèces en IW, une en MW et six espèces pour un nombre cumulé de 10 espèces furent significativement influencées par les variables climatiques.

CONCLUSION ET PERSPECTIVES

Les forêts claires du PNC abritent une grande diversité de champignons et arbres EcM. Bien que de nombreuses espèces fussent partagées par ces différents types d'habitat, des valeurs faibles de similarité ont été calculées. 10.57 % de la richesse spécifique totale a été identifiée comme comestible selon la littérature. Ainsi la production de dix espèces comestibles était significativement influencée par la variabilité des paramètres de microclimat. De plus, les analyses moléculaires ont montré que certaines de ces espèces comestibles sont susceptibles d'être des espèces nouvelles à la science parce que leurs séquences d'ADN ne correspondaient pas aux séquences disponibles des holotypes. Par conséquent, des études supplémentaires sont nécessaires pour résoudre cette divergence dans l'identification morphologique et moléculaire ainsi que des études ethnomycologique avec les populations environnantes. D'autres enquêtes mycologiques sont également nécessaires pour déterminer les variations de diversité, phénologies de fructification et productions naturelles au fil des années.

MANUSCRIPT

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Full Length Research Paper

Diversity of ectomycorrhizal fungal fruit bodies in Comoé National Park, a Biosphere Reserve and World Heritage in Côte d'Ivoire (West Africa)

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The key role of ectomycorrhizal (EcM) fungi in ecosystems functioning has been demonstrated worldwide. However, their diversity, spatial distribution, fruiting phenology and production as influenced by climatic parameters variability remain poorly understood in tropical African forests. Weekly surveys were conducted from April to early October 2014 at the Comoé National Park (CNP), Côte d'Ivoire (West Africa) in 09 permanent plots established in *Isoberlinia doka* (IW), *Uapaca togoensis* (UW) and Mixed (MW) woodlands. Non metric multidimensional scaling (NMDS) of EcM fungi abundance was run to assess the influence of environment tal parameters on fungi distribution using the package VEGAN. Hierarchical clustering based on dissimilarity and indicator species analysis were run to characterize fungi communities. Analyses were computed with the statistical program R. A total of 123 EcM fungi species belonging to 23 genera and 09 families were collected at CNP. Simpson diversity (1-D) and evenness were 0.97 and 0.54, 0.97 and 0.61, 0.96 and 0.52 for IW, MW and UW respectively. Yet, weekly-based species accumulation curves did not reach an asymptote. Stem density of *U. togoensis* Pax (UTDen) and *I. doka* Craib & Stapf were the most important tree parameters influencing EcM fungi distribution (respectively r^2 = 0.92 / p-value = 0.002 and r^2 = 0.83 / p-value = 0.018). Two sites groups were distinguished and four indicators species were identified.

Key words: EcM fungi, fruit bodies, diversity, indicator species.

INTRODUCTION

Productivity, diversity and composition of plant communities have been demonstrated indirectly and directly influenced by belowground micro-organisms from which plant symbionts play a key role (Van Der Heijden

et al., 2008; Van Der Heijden and Horton, 2009). Globally, over 90% of terrestrial plants depend upon an ecological relationship with soil fungi for their growth and regeneration (Smith and Read, 2008; Singh et al., 2011; Dickie et al., 2014). This relationship termed mycorrhiza is the most prevalent symbiosis on Earth, including cultivated plants, herbaceous species and forest trees. Generally, autotrophic plants provide carbohydrates to their fungi partners, which in turn improve host performance by enhancing mineral nutrient uptake from soil, especially nitrogen (N) and phosphorus (P). Symbiotic fungi enhance plant tolerance to environmental stress caused by low soil water potential, toxic heavy metals, salinity, herbivores and root pathogens (Smith and Read, 2008; Singh et al., 2011; Dickie et al., 2014). Among mycorrhizas types, ectomycorrhiza (EcM) is the most advanced one (Moore et al., 2011) involving mostly higher plants and fungi (Piepenbring, 2015). Thus, EcM fungi have an important position in the plant-soil interface (Ceulemans et al., 1999) worldwide, playing a key role in the growth and regeneration of forest trees, and in ecosystems functioning.

However, the global biodiversity is under decline since the 19th century due to serious climate, environmental and ecological changes through human activities around the globe. The global climate system is actually modified by increased greenhouse gases (GHG) in the atmosphere subsequently to unrestrained deforestation, fossil fuel combustion and other anthropogenic activities (WMO, 2007). Few key parameters of global change are among other trend towards warming (increasing temperature), increase of atmospheric CO_2 and disturbance in the distribution, seasonality and amount of rainfalls. It is predicted that Earth surface temperature will increase from 0.3°C to 1.7°C under scenario RCP2.6 by the end of the 21st century (2081–2100) whilst the atmospheric carbon level is continuously increasing (IPCC, 2014). Though the impact of global change on ecosystems is not yet adequately addressed, it is expected that many changes in global biodiversity and ecosystem functions will occur. High temperature is expected to alter tree phenology, plant growth and distribution toward migration and adaptation ecozones (Montoya and Raffaelli, 2010) but also to increase the length of the growing season (Walther et al., 2002; Morin et al., 2007), and the aboveground growth and reproductive effort of plants (Hollister et al., 2005). At the other side, elevated atmospheric CO_2 and nitrogen will likely increase the rate of net photosynthesis by 40 to 80% (Körner et al., 2005), the allocation of carbon to the plant roots (Janssens et al., 2005) and the production of leaves, wood and coarse roots (Hyvönen et al., 2007). It is actually difficult to predict the exact response of plant diversity to climate change as many investigations are still needed to understand the resilience, adaptation and/or migration following fluctuation of climatic parameters.

As both partners are living more or less obligatory and intimately, any possible change that affect host plants is also expected to influence the symbiotic fungi. In temperate and boreal zone, rainfall and moisture availability have been demonstrated as critical to EcM fruiting and natural production (O'Dell et al., 2000; Gange et al., 2007; Kauserud et al., 2010). Furthermore, long term observations of fungal phenology in temperate forests reveal that fruit bodies production and temporal changes are strongly influenced by either increasing temperature (Kauserud et al., 2008; Kauserud et al., 2010) and/or rainfalls (Krebs et al., 2008). Due to their vital role in forest ecosystems and the sensitivity of their respiration to high temperature and strong seasonality (Vargas et al., 2010; Bahram et al., 2012), EcM fungi represent best candidates to investigate for a better understanding of ecosystems response to global warming and especially in carbon sequestration capability (Simard and Austin, 2010; Orwin et al., 2011; Büntgen et al., 2012; Büntgen et al., 2013; Boddy et al., 2014). Unfortunately, the response of EcM communities to global warming and environmental changes is scarcely addressed in tropical zones and especially in tropical. In Sudanian woodlands of Africa, a strong variability has been noticed regarding species richness and community structure throughout the fruiting season (Yorou et al., 2001). Nevertheless, the authors failed to link species composition, community structure and productivity patterns of EcM with either the local temperature or soil humidity. To our knowledge, that study is the only one in tropical Africa addressing the impact of climate parameters on wild EcM fungi phenology and productions. Now, knowing temporal change in the phenology and production distribution, and their determinants is essential in the valorisation of natural productions of wild edible EcM fungi that amounts to thousand tons annually and involves many rural women (Yorou et al., 2001, 2014; Boa, 2004). However, a prerequisite to climate impact assessment is the analysis of EcM fungi diversity and the evaluation of possible other natural underlying mechanisms of richness pattern (Tedersoo and Nara, 2010). It has been demonstrated that the impacts of atmospheric carbon dioxide enrichment is more clear on fruit bodies than on below-ground tips (Andrew and Lilleskov, 2009; Pickles et al., 2012). Therefore, this study aims to (1) assess the diversity (species richness and community structure) of

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EcM fungi species through fruit bodies diversity and (2) assess the spatial variability of the community composition following habitat characteristics (plant and soil parameters) at local scale. We hypothesised that (1) African protected areas harbour a great diversity of EcM fungi with many species likely new to sciences, and (2) host plants and soil structural parameters drive the communities of EcM fungi.

MATERIALS AND METHODS

Study site

The Comoé National Park (CNP) is located in the North-East of Côte d'Ivoire (8°32' - 9°32'N, 3°01' - 4°24'W) between the towns of Bouna and Dabakala, and south of the border with Burkina Faso. The CNP covers about 11 500 km² (Hennenberg, 2004) and is presently one of the largest national park in West Africa (Poilecot et al., 1991). Initially erected as a game park since 1926 ('Refuge Nord de la Côte d'Ivoire') and then established as national park in 1968, Comoé was approved in 1983 and declared as Biosphere Reserve and World Nature Heritage by the UNESCO (Hennenberg, 2004).

The park is located on the large granite stand of West Africa and is characterized by a smooth and level relief. Soils are impoverished sandy to loamy ferralsols above Precambrian granites with small areas of lateritic crusts or banks outcrop at some places (Hennenberg et al., 2005). The climate is a Guineo-Congolian/Sudanian transitional type, a sub-humid tropical climate (Chidumayo et al., 2010) with mean annual rainfall of 1 011 mm falling mainly between March and October. The mean annual temperature is 26.5 to 27°C (Koulibaly, 2008). CNP vegetation is transitional ranging from forests to savannas including riparian grasslands (Poilecot et al., 1991; Hennenberg et al., 2005).

Selection of habitat types and establishment of permanent plots

One-week exploratory survey was undertaken within the accessible parts of the park in November 2013 to identify appropriate study sites. Based on available vegetation maps (Poilecot et al., 1991; Lauginie, 2007), three habitat types were selected with regard to; (1) The presence and abundance of known EcM partners trees, members of Caesalpiniaceae and Phyllantaceae (to ensure collection of symbiotic fungi and assess partners influence on fungal species distribution) and (2) the distance to the Ecological Research Station of Comoé, our base camp (for rapid handling of fragile specimens during hot and wet season).

The different habitat types were at least 300 m away from one another and included:

Habitat type 1: *Isoberlinia doka* Craib & Stapf Woodland (IW);
Habitat type 2: Mixed Woodland (MW);
Habitat type 3: *Uapaca togoensis* Pax Woodland (UW).

In each selected habitat type, three permanent plots of 30 m × 30 m each have been established by mean of a hectometer, making a total of nine plots (Figure 1). They have been labelled *FiPi* with *Fi* representing the habitat type and *Pi* the plot. All nine (09) plots have been geo-referenced by recording the coordinates of each corner with a GPS Garmin GPSMAP® 62stc (Garmin International Inc., Olathe, KS, USA). Plots within a habitat type were spaced at least by 10 m one another, according to tree partners' presence and density (Table 1).

EcM fungal fruit bodies collect and handling

EcM fungal fruit bodies (EFFB) were collected in each plot following parallel bands of 2 m large. To avoid missing short living species, each plot was visited once a week from April to early October 2014 as implemented by Yorou et al. (2001). We recorded the nearest EcM partner trees to each sampled fruit body and geographic coordinates using GPS Garmin GPSMAP® 62stc (Garmin International Inc., Olathe, KS, USA). To facilitate future comparison and morphological identification of species, technical photographs of most representative fruit bodies per species (at different development stage, when applicable) were taken on field and at the base camp using a Canon EOS 1000D digital cameras. Fresh macroscopic features were then recorded from specimens, using standardized descriptions sheets (size, shape; colour and any change with time; presence/absence of ephemeral structures; type of hymenophore, its colour and organization; etc.) developed for tropical African fungi (De Kesel et al., 2002; Eyi Ndong et al., 2011). Afterwards, Fruit bodies per collection were counted, weighted, labelled and representative specimens were dried at 40°C for 24 h. Labelled collections were conserved with basic ecological data (habitat type, substrate, putative nearest partner tree, exposition to sun, etc.) as herbarium materiel at the WASCAL GSP Climate Change and Biodiversity, University Felix Houphouet-Boigny (Côte d'Ivoire).

The identification of collected fungal species was performed based on morphological features at Botanic Garden of Munich in Germany and Botanic Garden Meise in Belgium by experts (De Kesel and Yorou, personal communications). Appropriate keys and numerous illustrated monographs on fungi of Central and Western Africa (series of "Flore Iconographique des Champignons du Congo" and "Flore illustrée des Champignons d'Afrique Centrale") were used. These series include monographs on *Amanita* spp. (Beeli, 1935), *Boletineae* and *Cantharellus* spp. (Heinemann, 1954, 1959, 1966), *Scleroderma* spp.(Dissing and Lange, 1963) and *Russula* spp. (Buyck, 1993, 1994, 1997) and Lactarius spp. (Heim, 1955). An additional monograph on *Lactarius* spp. (Verbeken and Walleyn, 2010) was also used. Species names and nomenclatural aspects were checked in index fungorium (http://www.indexfungorum.org/Names/Names.asp). Moreover, molecular-based identification of representative specimens per species was performed (Gardes and Bruns, 1993; Maba et al., 2013) at both abovementioned research institutes. Results of molecular analysis along with metabarcoding analyses of composite soil samples (for belowground fungi diversity assessment) will be presented in a manuscript in preparation.

Habitat types characterisation

Biotic and abiotic variables were collected to assess their possible influence on EFFB occurrence and spatial distribution.

First, systematic inventory of plant species and total canopy cover estimation within plots were performed in April 2014 according to the phytosociological method (Braun-Blanquet, 1932). Primary identification of plants specimens were done with field guide (Arbonnier, 2004) and completed with collected herbarium materials by experts from the Laboratoire de Botanique of the University Felix Houphouet-Boigny in Abidjan, Côte d'Ivoire. However, for statistical analyses, only woody species with diameter at breast height (dbh) equal or above (≥) 10 cm were considered. Therefore, in addition to plant species richness, structural parameters (number of stems and dbh per species and per plot) were recorded.

Second, soil cores were collected with a 10 cm × 10 cm - 10 cm depth auger at each corner and the center of each plot at mid-rainy season (late July). All five cores were mixed to make a composite soil which was air-dried and passed through a 2-mm sieve. Three

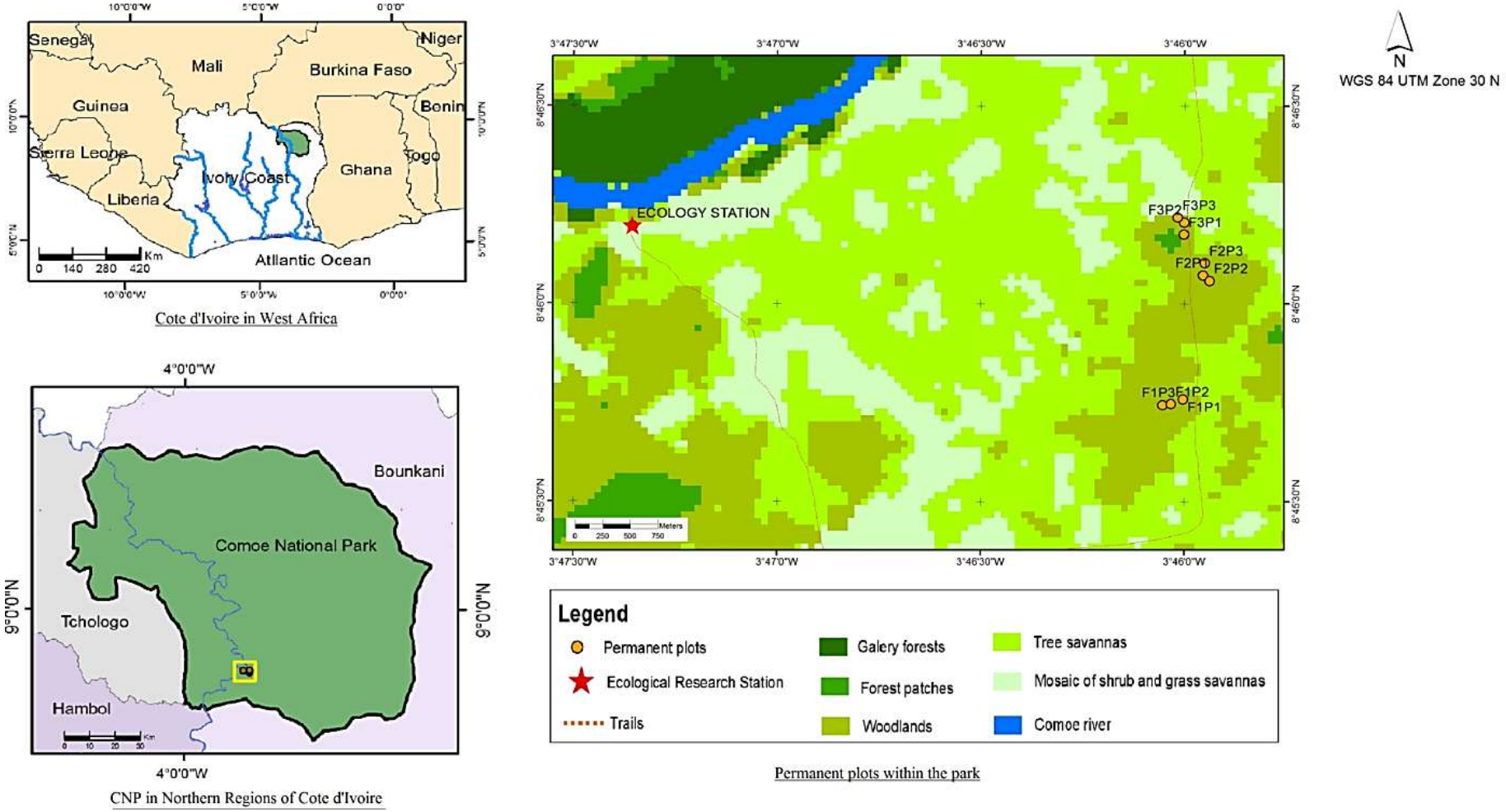

Figure 1. Location of Comoé National Park (north east of Côte d'Ivoire) and established permanent plots within it (south west of the reserve).

composite soils were thus made per habitat and 200 g per sample were used to assess soil granulometry, pH and minerals contents. Chemical parameters assessed were pH (H_2O), Carbon (C), Nitrogen (N), soil organic carbon (SOC), ratio C/N, Total Phosphorus (TotalP), Available Phosphorus (AvailP), Calcium (Ca) and Potassium (K). Physical parameters referred to soil texture: Clay, fine and coarse Silt, fine and coarse Sand. They were determined as follows:

1. pH (H_2O) measurement was performed with a soil solution at a ratio 2/5 (Duchaufour and Blum, 1997).

2. Determination of extractable cations' content was achieved according to standard NFX 31-130 (AFNOR, 1999).

3. Determination of organic and total carbon: The total carbon content in soil is determined after dry combustion. The soil's organic carbon content is calculated

Table 1. Positions of permanent plots within habitat types in Comoé National Park (CNP), Cote d'Ivoire

Habitat type	*Isoberlinia* Woodland			Mixed Woodland			*Uapaca* Woodland		
Plot	F1P1	F1P2	F1P3	F2P1	F2P2	F2P3	F3P1	F3P2	F3P3
Latitude (dd)	8.76264	8.762447	8.762408	8.767876	8.7676	8.768387	8.769594	8.770105	8.7703
Longitude (dd)	-3.7667	-3.76719	-3.76754	-3.76588	-3.766	-3.76581	-3.76668	-3.76665	-3.767
Altitude (m)	235.13	233.17	232.64	230.40	230.79	248.19	216.23	213.81	213.62

dd: decimal degrees; m: meters

according to the method NF ISO 10694 (AFNOR, 1995).
4. Particle size determination by sedimentation - the pipette method following the standard method NF X 31-107 (AFNOR, 2003).

Data analysis

EcM fungal fruiting bodies diversity assessment

Basic estimators and indices were calculated to assess the diversity of fungi species as reflected by EFFB at plot and habitat type level. They included also similarity between plots and habitat types as well as the number of shared species to compare communities.

Observed species richness and diversity assessment

Presence/absence data of EFFB was used to determine (1) the observed species richness (SR: number of species) and composition (SC: list of species) per habitat type; (2) the total observed species richness and composition as cumulative data of all habitat types. Thereby, the frequency of occurrence (percentage of total weeks during which a species was recruited) of fungal species was used to highlight the contribution of each species in the community (Horton and Bruns, 2001). The relative frequency of each species was calculated as the percentage of total frequency.

Assessment of fungi diversity and evenness of frequency of species within habitat types was achieved respectively by computing Simpson's Index of Diversity (1 – D) and Simpson' Evenness with the program Ecological Methodology (Krebs and Kenney, 2002). Simpson's Index of Diversity (1 – D) refers to the probability that two individuals randomly selected from a sample will belong to different species. Its value ranges between 0 and 1, greater value corresponding to high diversity).

Sampling representativeness: Species accumulation curves and similarity assessment

Sample-based species accumulation curves were constructed in EstimateS ver. 9.1.0 (Colwell, 2013) using presence/absence (incidence) data. The sample order was randomized 500 times without replacement for the statistical representation of the EcM fungi community. In this study, "***sample***" referred to frequency of survey, a week-interval, against which Observed and Estimated Chao 2 species accumulation curves were plotted.

The similarity of our sampling to the fungi community was estimated by measuring the autosimilarity (Cao et al., 2002) between plots of each habitat type. This was calculated as mean Jaccard coefficient computed with EstimateS ver. 9.1.0 software. Autosimilarity index varies from 0 (no species common to plots) to 1 (same species composition in plots). Constructed week-based species accumulation curves, Simpson's Index of Diversity (1 – D) and Simpson's evenness along with autosimilarity index served to assess the sampling representativeness of fungal communities of study sites.

Habitat characterisation

Floristic richness and dendometric parameters assessment: Number of stems and dbh per species underwent basic statistical analyses as follows:

1. Plant species density (D_i), the number of stems per species per plot surface in square meters (m^2), converted later in hectares (ha);
2. Individual stem basal area (BA_i). $BA_i = \pi \times 10^{-4} \times (dbh_i/2)^2$, where tree dbh in cm and BA_i in m^2. This formula is simplified as: $BA_i = 0.00007854 \times (dbh)^2$;
3. Species basal area (BA_{sp}) that equals to the sum of all BA_i of stems of the same plant species within a plot, converted later in hectares (ha);
4. Total basal area (TBA), summing up the all calculated BA_{sp} within a plot;
5. Species relative dominance (SRD): $SRD\% = \left(BA_{sp}/TBA\right) \times 100$.

Soil chemical and physical analysis

Soil parameters evaluation was performed according to standard method as follows:

1. Determination of pH (H_20) and content of extractable cations (Ca^{2+}, K^+ , NH_4^+) was performed by reading directly the digital display of the pHmeter or spectrophotometer;
2. Determination of organic and total carbon: $M.org = 1.724 \times C.org$. with M.org = organic matter (mg / kg); C.org = organic carbon (mg/kg)
3. Particle size determination by sedimentation using the pipette method. Content of different fractions was determined as follows:

$$C + St\% = [(Pc + s) - (p1) - (Pb)] \times 5000 \times k/Pe \times Fh \quad (1)$$

$$C\% = [(Pa) - (P1) - (Pb)] \times 5000 \times k/Pe \times Fh \quad (2)$$

$$FSt\% = (C + St)\% - C\% \quad (3)$$

$$TSd\% = (Tt - P1) \times 100/Pe \times Fh \quad (4)$$

$$CSd\% = (Tc - P1) \times 100/Pe \times Fh \quad (5)$$

$$FSd\% = (Tf - P1) \times (100/Pe) \times Fh \quad (6)$$

$$CSt\% = TSd \times (CSd + FSd) \times Fh \quad (7)$$

With C = clay; P_{C+St} = T are weight + clay + silt; St = silt; P1 = weight of empty tare (capsule); FSt = fine silt;P2 = Weight of empty tare + white; TSd = total sand; $Pb = P2 - P1$; CSd = coarse sand; $k = 20N/V$; FSd = fine sand; V = volume of the pipette; CSt = coarse silt; Pe = aliquot intake; Tt = cap weight + the total sand;

Table 2. Richness of EcM fungi within selected habitat types

Fungi parameters	*Isoberlinia* Woodland (IW)	Mixed Woodland (MW)	*Uapaca* Woodland (UW)	Total
Numbers of fruit bodies	1565	513	736	2814
Numbers of species	75	65	56	123
Numbers of genus	21	15	16	23
Numbers of family	9	6	6	9

Fh = humidity factor; Tc = cap weight + coarse sand; Pc = cap weight + clay; Tf = cap weight + fine sand.

The texture of each soil was determined using TRIANGLE, A Program For Soil Textural Classification (Gerakis and Baer, 1999). That texture determination followed percentage of particles within studied soils.

Gradients effectiveness

Analysis of variance (Anova) test at $\alpha < 0.05$ was performed to assess the effectiveness of gradient among soil and plant data. It was performed at habitat type level for both variables using package lawstat of R software (Hui et al., 2008). When requirement of distribution and homogeneity of variance were not met, Kruskal-Wallis test (Kruskal and Wallis, 1952) was performed in R software. Afterward, significant gradient (s) underwent a preliminary analysis to check collinearity between them and clarify the ordination. One variable among all highly collinear ones was conserved in the subset of the ordination. That preliminary analysis has been performed with software Statistica 7.1 (StatSoft France, 2006).

Ectomycorrhizal fungi fruit bodies spatial distribution

To visualize the spatial distribution of EFFB, non-metric multidimensional scaling NMDS ordination was performed based on a matrix of fungi species relative frequency per plot using function *metaMDS* of package Vegan (Oksanen et al., 2015) of R software version 3.3.0 (2016-05-03). Fungi relative frequencies were first transformed by Wisconsin double standardization using function *Wisconsin* to improve ordination. A distance matrix generated by Bray-Curtis dissimilarity index with function *vegdist* was used as input for the NMDS whilst function *metaMDS* used Jaccard index.

Then, main environment variables (host communities and soil parameters) influencing the fungi communities structure were evidenced by fitting them the ordination plot using function *envfit* of the Vegan package. Statistical significance was based on 999 random permutations and plotting was limited to most significant variables with argument *p.max* set at 0.1.

To better visualize the similarity of habitat types, a hierarchical clustering based on Bray-Curtis dissimilarity index was conducted in R software version 3.3.0 (2016-05-03) using function *hclust* and average-linkage. Subsequently, each fungi community was characterized by conducting indicator species analysis using the MULTIPATT function in the R package Indicspecies (De Cáceres and Legendre, 2009; De Cáceres and Jansen, 2015). Indicator Value (IndVal) index (Dufrêne and Legendre, 1997) was computed to measure the association between a species and a site group. Statistical significance of association was tested by running 999 random permutations. In addition, the specificity (the so-called IndVal Component A) and the fidelity (second component B of IndVal) of a species as indicator of a target site group were inspected. Component A or specificity refers to "the probability that the surveyed site belongs to the target site group given the fact that the species has been found" whilst component B refers to "the probability of finding the species in sites belonging to the site group" according to Dufrêne and Legendre (1997) and De Cáceres and Legendre (2009). Final, ecological distance between generated site groups was calculated by Jaccard index using the R package Fossil (Vavrek, 2011).

RESULTS

EcM fungi diversity

Observed species richness and diversity indices

EcM fungal fruiting started in mid-May and was still continuing in early October making a cumulative total of 21 weeks of occurrence. In total 2814 fruit bodies have been collected and were sorted into 123 species belonging to 23 genera and 09 families (Table 2). The most frequently recorded family was Russulaceae with 53 species composed of 36 *Russula* species, 11 *Lactifluus* species and 6 *Lactarius* species. The second frequently observed family was Boletaceae represented by 13 genera with a total of 32 species. The Amanitaceae ranked third most important recorded family with a total of 26 species. The less recorded other families included Cantharellaceae, Cortinariaceae, Gyroporaceae, Inocybaceae, Sclerodermataceae and Clavulinaceae. These families were represented each by only one genus with respectively 1, 3, 1, 1, 5 and 1 species (Figure 2). From the total species richness, 57 taxa (46.34% of the total) were identified up to species level with 19 of them being related to known species from temperate and tropical zones. The remaining 66 species (53.66% of the total) were identified only at the genus level with some of them suspected new to science (Supplementary Table 1).

The most frequent species per habitat type included *Russula congoana* Pat. (13 weeks corresponding to the relative frequency of 2.53%), *Amanita aff. craseoderma* (11 weeks, relative frequency = 2.14%) and *Lactarius tenellus* Verbeken & Walleyn (10 weeks, relative frequency = 1.95%) in IW; *Amanita annulatovaginata sensu lato* Beeli and *Lactarius tenellus* (both with 8 weeks, relative frequency = 1.56%) in MW; *Cantharellus addaiensis* Henn. and *Amanita aff. subviscosa* Beeli (both with 11 weeks, relative frequency = 2.14%), *Amanita aff virosa* and *Amanita strobilaceovolvata sensu lato* Beeli (both in 10 weeks, relative frequency = 1.95%) in UW.

22 species were found common to the three habitat

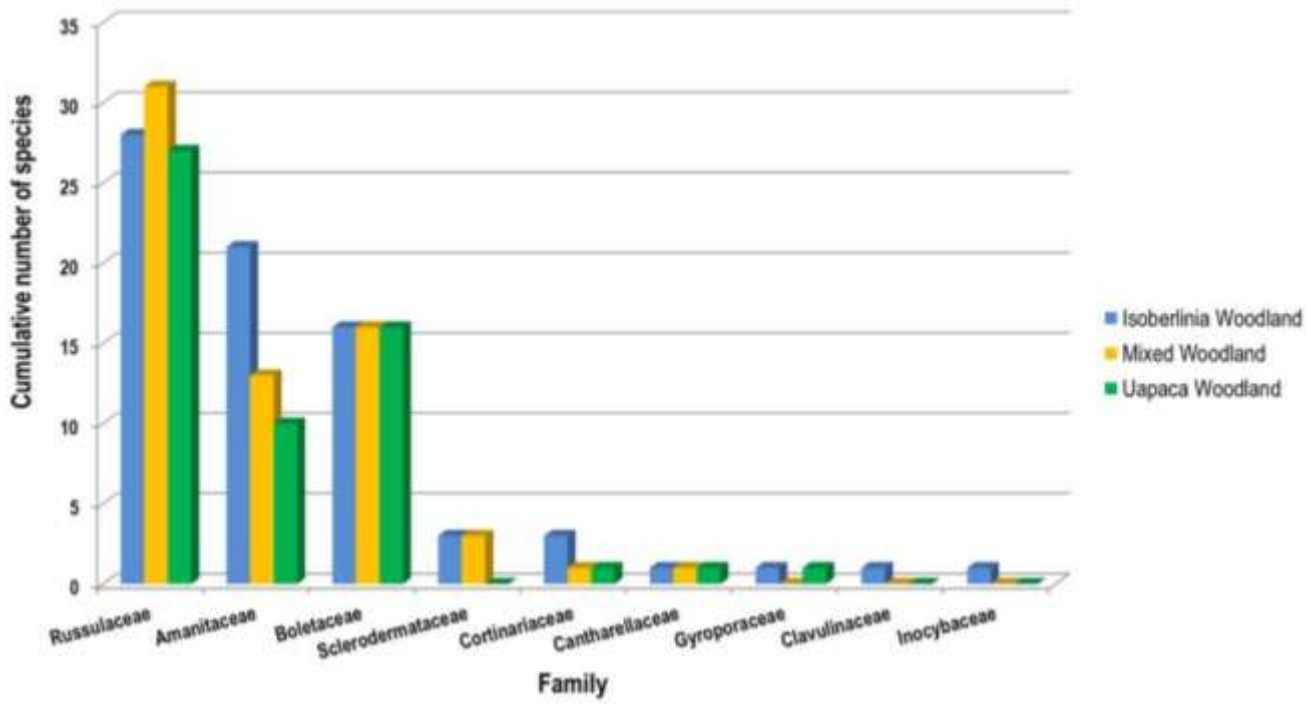

Figure 2. Families representativeness per habitat type.

types and represented 17.89% of total observed species richness (Supplementary Table 1). On the other hand, 72 species accounting for 58.53% of the species richness were specific to one habitat type. Many of these specific species were observed and collected only once from May to early October 2014 (Supplementary Table 1) and are uniques species. Specific species such *Inocybe* sp 1 and *Cortinarius* subgenus *telamonia* sp 1 have been picked under *Isoberlinia doka* trees in IW. Meanwhile, *Russula annulata* R. Heim, *R. discopus* R. Heim (a rare species) and *Veloporphyrellus africanus* Watling were collected beneath *Uapaca togoensis.* Final, 29 species (23.58%) were shared by two habitat types. In addition with species common to all habitat types, 38 species were shared by IW and MW (e.g. *Amanita afrospinosa* Pegler & Shah-Smith*, Lactarius saponaceus* Verbeken); 28 species shared by IW and UW (e.g. *Gyroporus castaneus* (Bull.) Quél*., Amanita strobilaceovolvata* sensu lato) and 29 species shared by MW and UW (e.g. *Amanita aff. rubescens* Pers*., Boletus loosii* Heinem).

Similarity and sampling representativeness

Computed Simpson's Index of Diversity 1 – D of IW was 0.97 with an autosimilarity index calculated to 0.40. Therefore, plots in IW were found non-similar likewise for, plots within habitat types MW and UW with respectively0.33 and 0.29. In those latter habitat types, higher diversity indices were respectively 0.97 and 0.96.

Weekly-based species accumulation curves of the different habitat types have almost the same shape in observed and estimated species richness (Figure 3). Accumulation curves of IW were generally above those of the other habitat types through weeks except for the estimated species richness where curve of MW outdid the other curves from the fourteenth week till the end of the survey. Globally, all curves were ascendant and did not reach an asymptote of total richness.

Sample coverage highlighted the percentage of species detected by our study on the overall estimated species richness. Thus, 75.25% of species was detected in IW against 81.88% in MW and 58.78% in UW (Table 3). Furthermore, 38, 32 and 36 unique species have been collected in the different habitat types respectively.

Habitats characterisation

Floristic and dendrometric parameters

A cumulative number of 822 stems belonging to 49 woody species with dbh ≥ 10 cm were detected for all habitat types. Those species belonged at least to 20 families, knowing that 6 species identity was undetermined. 18, 19 and 31 species were inventoried respectively in IW, MW and UW. The total density and basal area of all tree species, and the dendrometric parameters (density and SRD) of cores EcM forest trees in each plots is provided in Table 4.

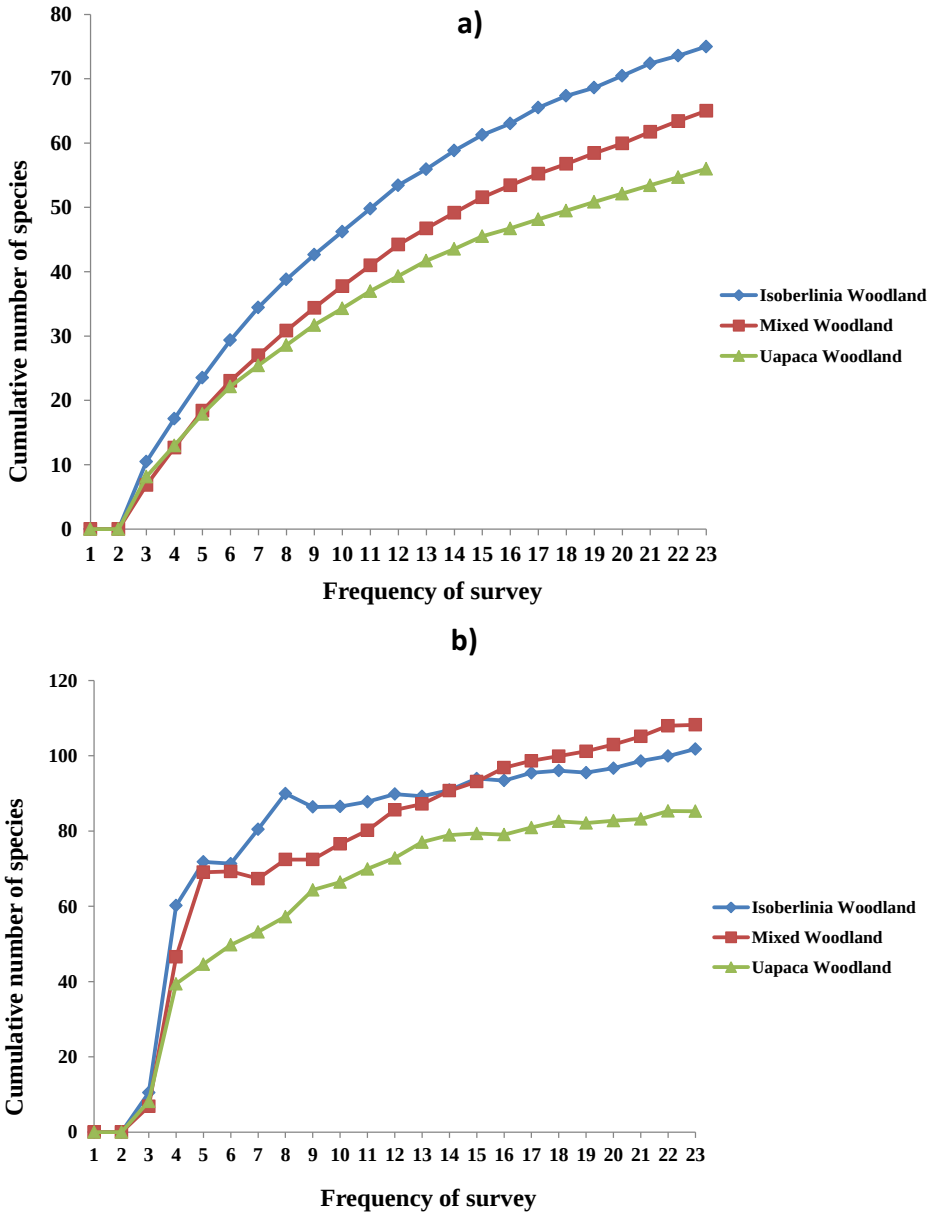

Figure 3. Week-based accumulation curves of observed (a) and estimated (b) species richness of EcM fungi during fruiting season 2014 (mid-May to early-October). Aug. = August, Sept.= September, Oct.= October.

Table 3. Sampling representativeness estimators. Sample coverage: proportion of observed species richness (S_{obs}) as per cent of estimated species richness (S_{est}); Auto-similarity: mean similarity between plots of the same habitat type; Uniques: number of species collected only once during the whole period

Habitat type	Number of fruit bodies	Observed species richness S_{obs}	Estimated species richness Chao 2 (S_{est})	Sample coverage	Autosimilarity	Simpson's Index of Diversity 1- D	Simpson's Evenness	Uniques
Isoberlinia Woodland	1542	75	99.67	75.25	0.49	0.77	0.06	38
Mixed Woodland	502	65	79.38	81.88	0.41	0.94	0.25	32
Uapaca Woodland	775	56	95.27	58.78	0.36	0.91	0.19	36

Table 4. Mean values of density of woody species, Species relative dominance (SRD) of identified EcM trees and total basal area per habitat.

Plant parameters		*Isoberlinia* Woodland	Mixed Woodland	*Uapaca* Woodland
Cumulative number of stems (three plots)		276	246	300
Forest tree species richness SR		18	19	31
Total tree density TD (stem/ha)		3066.66	2733.33	3333.33
Total basal area TBA (m^2/ha)		179.75	158.43	186.89
Mean canopy cover		66.67	73.33	80
EcM tree partners density(stem/ha)	*Isoberlinia doka*	171.11	5.56	0.00
	Monotes kerstingii	35.56	18.89	23.33
	Uapaca togoensis	10.00	167.78	153.33
EcM tree partners SRD (%)	*Isoberlinia doka*	62.29	3.68	0.00
	Monotes kerstingii	10.28	4.13	6.57
	Uapaca togoensis	0.99	53.48	40.50

Table 5. Soil chemical and physical parameters variations per habitat type.

Soil parameters	Habitat type			F	Chi-square	Df	p-value
	IW	MW	UW				
pH	6.7 ±0.14	6.52±0.4	6.78±0.2		2.0392	2	0.36
Carbon (%)	1.96±0.09	1.85±0.15	1.71±0.13		4.3922	2	0.11
Nitrogen (%)	0.09±0.05	0.09±0.01	0.12±0.02	0.495		2	0.63
Available Phosphorus (ppm)	1.34±0.32	1.63±0.12	1.20±0.12		3.5862	2	0.17
Calcium (cmol/kg)	1.71±0.42	1.45±0.31	1.07±0.17	3.078		2	0.12
Potassium (cmol/kg)	0.06±0.04	0.09±0.03	0.07±0.03	0.936		2	0.44
Clay (%)	8.67±2.08	10±2.64	9.33±0.58		0.85797	2	0.65
FineSilt (%)	9.33±3.51	5±0.00	8.66±3.05		5.7275	2	0.06
CoarSilt (%)	44.33±12.1	42.66±3.05	45.67±5.86		0.29132	2	0.86
FineSand (%)	34.33±8.14	37±2.64	33.67±3.79		1.1954	2	0.55
Type of soil	Silt loam	Silt loam	Silt loam				

Kruskal-Wallis test demonstrated that plant richness and total basal area did not differed significantly from one another habitat type (chi-squared = 1.55, p-value = 0.46 and chi-squared = 0.62, p-value = 0.73, respectively). Considering EcM tree partners, density and SRD of *I. doka* differed significantly between habitat types (chi-squared = 6.72; p-value = 0.03), IW harboring the highest values. Density and SRD of *U. togoensis* were also significant (F = 20.73, p-value = 0.002 and chi-squared = 5.95, p-value = 0.05 respectively), decreasing from MW to UW and finally IW. At the opposite, the density and SRD of *Monotes kerstingii* does not significantly differed from one another habitat type (chi-squared = 0.62, p-value= 0.73 and chi-squared = 2.51; p-value= 0.28 respectively).

Soil chemical and physical parameters

pH (H_2O) measurement indicated that soils in all plots were generally neutral, ranging from 6.52 to 6.78. As for texture analysis, soils in plots were generally silt loamy with regard to soil particles size (Table 5). However, differences among both chemical and physical parameters of the different habitat types were not significant at 0.05, pointing out an absence of soil gradient.

Ectomycorrhizal fungi fruit bodies spatial distribution

In absence of soil gradient between habitat types, soil variables were excluded from initial environmental matrix that was finally reduced to 05 plant variables after multicollinearity test. Those variables were plant species richness (PlantSp), total basal area (TBA), *I. doka* density (IDDen), *M. kerstingii* Density (MKDen) and *U. togoensis* Density (UTDen).

Environment variables fitting into NMDS result indicated that *I. doka* Density (IDDen) and *U. togoensis*

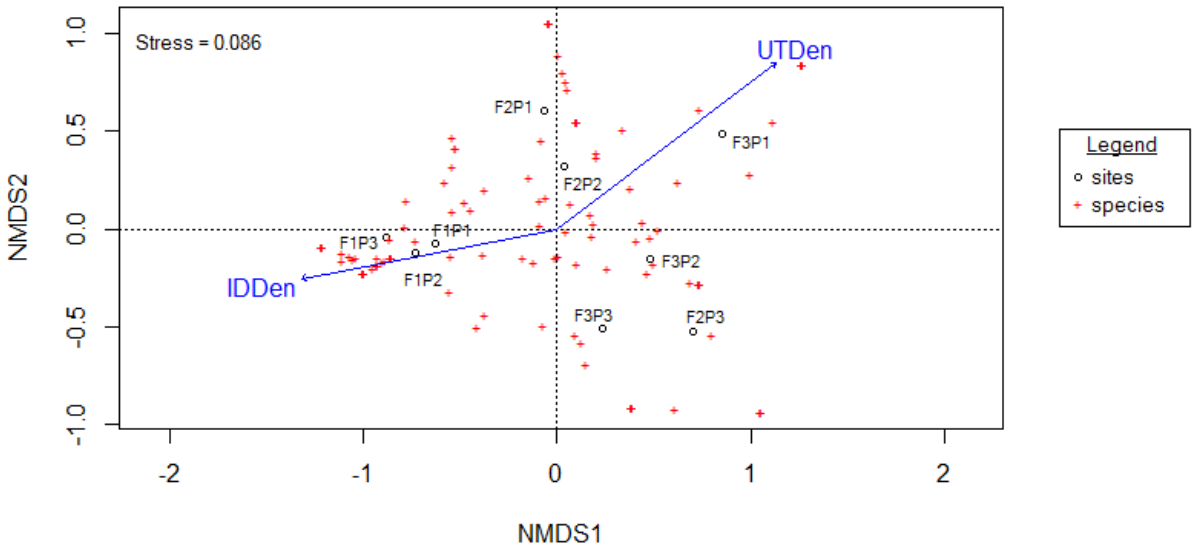

Figure 4. EcM fungi distribution at Comoé National Park according to stem density of *Uapaca togoensis* and *Isoberlinia doka.*

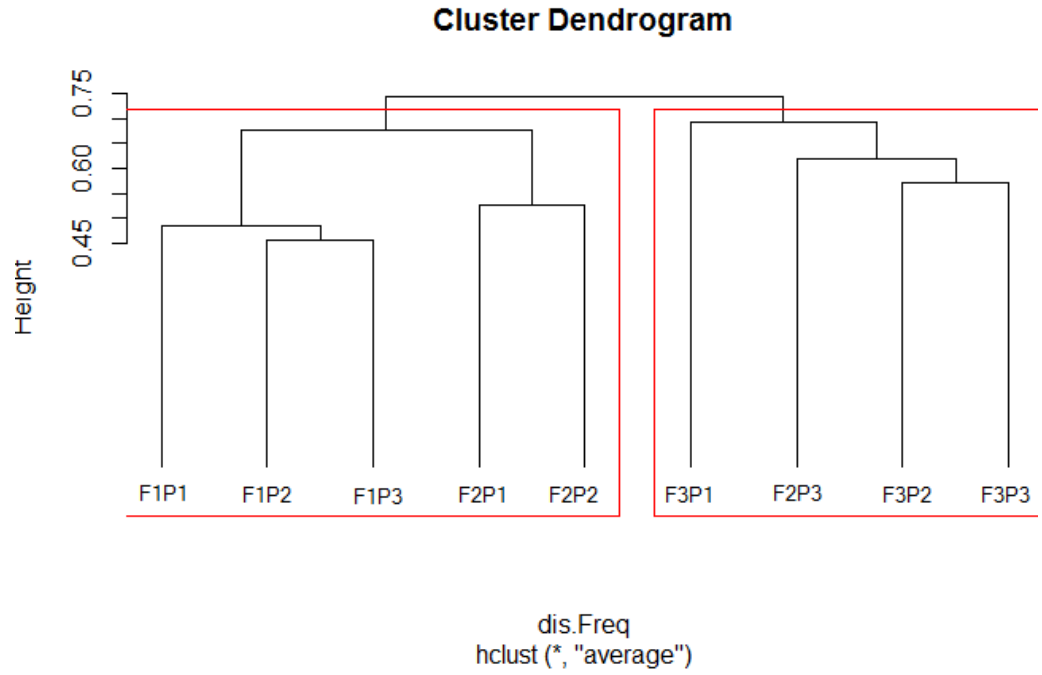

Figure 5. Hierarchical clustering of permanent plots based of dissimilarity.

density (UTDen) are the main statistically significant variables driving the EFFB spatial distribution (Figure 4). UTDen was positively correlated with both axes (r^2 = 0.92; p-value = 0.002) whilst IDDen was negatively correlated to the first axis only (r^2 = 0.83; p-value = 0.018).

Hierarchical analysis of study sites evidenced two sites groups (Figure 5). The first group (G1) encompassed all plots of habitat 1 (Isoberlinia woodland IW) and the two first plots of the second habitat, Mixed woodland (MW). The second group is composed of the remaining plot of habitat 2 (MW) and all plots of the third habitat Uapaca Woodland (UW). The indicator species analysis showed that 04 species were significantly associated to just one group on a total of 123 species. 03 species were associated to G1 and 01 species to G2 (Table 6).

Table 6. List of indicator species associated to each site group.

Site group	Component A	Component B	Stat	p.value
Group 1 #sps. 3				
RusCon	0.9573	1.0000	0.978	0.013 *
Pulve1	0.9057	1.0000	0.952	0.028 *
AmaXa	0.8276	1.0000	0.910	0.040 *
Group 2 #sps. 1				
AcfVir	1	1	1	0.013 *

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 RusCon: *Russula congoana*; Pulve1: *Pulveroboletus* sp 1; AmaXa: *Amanita xanthogala;* AcfVir*: A.* cf *virosa*

DISCUSSION

EFFB richness

Mycological monitoring within Comoé National Park (CNP) shed light on very specious habitats where almost all known EFFB families were represented. As already mentioned in various paleo and neotropical regions (Sanon et al., 1997; Riviere et al., 2007; Bâ et al., 2012; Henkel et al., 2012; Onguene and Kuyper, 2012), dominance of Russulaceae and specifically of genus *Russula* was also observed. Among the other frequently recruited families in tropical regions, Cantharellaceae was represented, in prospected habitats, by only one species member of genus *Cantharellus, C. addaiensis.* In the contrary, four *Cantharellus* species (*C. floridulus* Heinem., *C. platyphyllus* Heinem., *C.* cf*. platyphyllus* Heinem. and *Cantharellus* sp.) were reported in traditional systems of fallows dominated by many confirmed EcM tree partners near the city of Korhogo North western part of Côte d'Ivoire (Ducousso et al., 1999). This difference may be due to higher number of tree partners in that area, namely *Afzelia Africana* Sm. ex Pers*., Anthonotha crassifolia* (Baill.) J. Léonard, *Berlinia grandiflora* (Vahl) Hutch. & Dalziel, *I. doka* and *U. togoensis*. Likewise in genus *Clavulina,* only one species was detected in *Isoberlinia* woodland (IW) suggesting that other species may have been overlooked or mistook for saprotrophic species. Few species belonging to genera *Inocybe* and *Cortinarius* were also found in CNP. This supports the trend observed in other tropical regions (Onguene and Kuyper, 2002; Riviere et al., 2007; Onguene and Kuyper, 2012), and strengthens the idea that those species might be adapted to temperate and boreal zones (Buyck et al., 1996). However, the paucity of studies in tropical woodlands and forests comparative to temperate and boreal ones should be considered. Yet, the abundance of EcM fungi species was highlighted at continental level. In West Africa, Sanon et al (1997) found 37 EcM fungi during rainy season 1994 and 1995 in savanna and open riparian forests in southwestern Burkina Faso. 126 EcM species were censured after various surveys in different areas of Benin, ranging from protected areas to farms (Yorou, 2010). In Southern Guinea rainforests, Diédhiou et al. (2010) identified 39 EcM fungal taxa. In central Africa, Onguene et al (2012) reported the collect of 100 EcM fungi in forest habitats of South Cameroon during a three-year survey. Numerous species have been also collected in Congo and are documented in two series, "Flore Iconographique des Champignons du Congo" and "Flore illustrée des Champignons d'Afrique Centrale". Highest species richness and number of EFFB were found in IW. According to Nara et al., 2003, such values reflected host development stage. Indeed, highest cumulative values of tree partners' stems density and basal area were found in plots of IW. Some of those tree species were estimated aging more than 200 years with regard to their dbh (Tedersoo, personal communication). In disturbed areas of tropical zones, EcM Fabaceae and Dipterocarpaceae stands (I. doka and M. kerstingii respectively in our case) are considered climax stands which establishment is facilitated by Uapaca spp. (Lawton, 1978; Högberg and Piearce, 1986; Onguene, 2000; McGuire, 2007; Tedersoo et al., 2011; Onguene and Kuyper, 2012). According to Poilecot et al. (1991), CNP is included of 93.3 % of fire climax vegetation from which 6.7 % is made of woodlands. Indeed, understorey vegetation in IW and MW were burned either totally or partially according to plot by the annual fire that passed in December 2013, four months before our arrival at the park. However, no plot in UW was burnt. Moreover, EcM fungi species belonging to genus Scleroderma previously described as characteristic of disturbed and elevated soil temperature areas (Ingleby et al., 1985; Nara et al., 2003) were collected within burnt plots of IW and MW. Three of the five Scleroderma species were recruited in IW and the latter two in MW. Consequently, IW is likely older than the others whilst UW is the youngest and MW at an intermediate stage. This assumption is strengthened by the different proportions of U. togoensis and presence/absence of I. doka in the different habitats. First, IW harboured many stems of the EcM tree partners Monotes kerstingii Gilg and Uapaca togoensis but it is dominated by Isoberlinia doka. Second, few stems of I. doka were censured in MW whilst the tree species is completely absent from UW plots. Another support of that assumption is the presence of Inocybe sp. and the number of species of genus Cortinarius in IW are other supports of that assumption since those EcM fungi were depicted late successional symbionts (Nara et al., 2003).

Sampling representativeness

Sampling representativeness assessment demonstrated that a large number of symbiotic fungi were not detected in the different habitats monitored. This result is

corroborated by the important values of uniques species that reflected rare species. That number of observed rare species give an estimate of the number of unseen species (Chiarucci et al., 2011) as captured by the estimated species richness in each habitat. That result is a support of the limitation of fruit body based study of EcM fungi species (Horton and Bruns, 2001; Taylor, 2002). Nevertheless, climate impact is more appreciable on fruit bodies than on below-ground tips (Andrew and Lilleskov, 2009; Pickles et al., 2012).

Spatial distribution of symbiotic fungi

Phytosociological study of permanent plots evidenced important floristic richness and especially numerous stems with dbh above 10 cm. EcM tree partners thrive in dominant and sometimes almost mono-dominant stands. Such habitats have been demonstrated as niche for abundant EcM fungi. *I. doka* and *U. togoensis* were the main dominant species in prospected habitats. Sites grouping were correlated with their density more than stands age. Indeed, though only stems with dbh above 10 cm were considered in data analysis, numerous juveniles and sprouts were present within plots. This was favorable to the establishment of both early- and late-successional EcM fungi. In addition, the grouping also reflected fire impact within study sites evidencing the "drought-tolerant" capacity of some collected fungi species. There is therefore an urgent need to monitor such disturbed stands to adequately address that assumed capacity.

Indicator species analysis evidenced four species associated to site groups (three species associated with G1 and one species with G2). Those species, *Russula congoana*, *Pulveroboletus* sp 1 and *A. xanthogala* were good indicators of G1 and *A. cf virosa* was for G2 taking into account specificity and fidelity. Indeed, those species were collected either exclusively in plots assigned to each group or predominantly in them. Association of *R. congoana* and *A. xanthogala* to *I. doka* was also documented in Benin by De Kesel et al. (2002). Those species are mentioned in literature as edible fungi in various part of Africa (Boa, 2004). As for the two remaining indicators species, they need to be characterised and compared to available monographs and / or keys to ascertain their identity at species level. However, they are likely associated to *U. togoensis*.

Conclusion

A six-month monitoring of EFFB ascertained their occurrence at Comoé National Reserve in the Sudanian climatic zone of Côte d'Ivoire. Woodlands of the reserve harboured high plant species diversity from which known EcM tree partners were frequently dominant. In these habitat types estimated of more than 200 years old, 123 EFFB species fruited and were collected. Their abundance and spatial distribution were significantly correlated to the stem density of *U. togoensis* and *I. doka* that were respectively the dominant species in each site group. *R. congoana*, *Pulveroboletus* sp 1 and *A. xanthogala* were good indicators of site group G1 and *A. cf virosa* for G2. However, further studies on contrasting soil types, fungal and forest succession, site microclimate as well as fire impact are needed to improve the understanding of fungal community dynamics in West African woodlands.

Conflict of Interests

The authors have not declared any conflict of interests.

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SUPPLEMENTARY DATA

Table 1. Relative frequency of occurrence of EcM fungi species within woodlands of Comoé National Park.

Distribution class	Taxon	Family	IW	MW	UW
Common to all habitat types	*Amanita* aff. *subviscosa*	Amanitaceae	1.56	0.58	2.14
	Amanita annulatovaginata sensu lato	Amanitaceae	0.58	1.56	0.39
	Amanita sp 13	Amanitaceae	1.17	0.78	0.78
	Amanita xanthogala	Amanitaceae	0.39	0.19	0.19
	Cantharellus addaiensis	Cantharellaceae	1.75	0.97	2.14
	Cortinarius sp 1	Cortinariaceae	0.19	0.19	0.39
	Lactifluus aff. *emergens*	Russulaceae	0.78	0.19	0.39
	Lactifluus luteopus	Russulaceae	0.19	0.78	1.56
	Phylloporus ampliporus	Boletaceae	0.39	0.58	0.19
	Pulveroboletus sp 1	Boletaceae	1.17	0.39	0.19
	Pulveroboletus sp 2	Boletaceae	0.58	0.78	1.36
	Russula aff. *cellulata*	Russulaceae	0.39	0.19	0.58
	Russula aff. *ochrocephala*	Russulaceae	0.58	0.78	0.97
	Russula cellulata	Russulaceae	0.78	0.58	0.39
	Russula cf *amoenolens*	Russulaceae	0.78	0.39	0.58
	Russula cf *flavobrunnea*	Russulaceae	0.97	0.39	0.58
	Russula cf *grisea*	Russulaceae	0.19	0.19	0.19
	Russula ciliata	Russulaceae	0.19	0.39	0.19
	Russula congoana	Russulaceae	2.53	0.78	0.19
	Russula sp 10	Russulaceae	0.58	0.19	0.78
	Russula sp 11	Russulaceae	0.19	0.19	0.39
	Xerocomus sp 4	Boletaceae	0.19	0.19	0.19
Shared by two habitat types	*Amanita congolensis*	Amanitaceae	0.00	0.78	0.97
	Amanita sp 12	Amanitaceae	0.19	0.39	0.00
	Amanita aff. *virosa*	Amanitaceae	0.00	1.36	1.95
	Amanita masasiensis	Amanitaceae	0.19	0.19	0.00
	Amanita sect *lepidella* sp 1	Amanitaceae	0.39	0.19	0.00
	Amanita sect. *lepidella strips xanthogala* sp 1	Amanitaceae	0.19	0.00	0.19
	Amanita sp 5	Amanitaceae	0.19	0.19	0.00
	Amanita sp 8	Amanitaceae	0.19	0.00	0.39
	Amanita strobilaceo-volvata sensu lato	Amanitaceae	0.58	0.00	1.95
	Boletus loosii	Boletaceae	0.00	0.97	0.78
	Boletus sp 2	Boletaceae	0.39	0.00	0.58
	Gyroporus castaneus	Gyroporaceae	0.19	0.00	0.58
	Lactarius afroscrobiculatus	Russulaceae	0.00	0.19	0.39
	Lactarius saponaceus	Russulaceae	0.19	0.19	0.00
	Lactarius tenellus	Russulaceae	1.95	1.56	0.00
	Lactifluus aff. *heimii*	Russulaceae	0.97	0.19	0.00
	Lactifluus sp 4	Russulaceae	0.19	0.19	0.00
	Octaviana ivoryana	Boletaceae	1.17	0.19	0.00
	Rubinoboletus cf *balloui*	Boletaceae	0.00	1.17	0.19
	Rubinoboletus cf *griseus*	Boletaceae	0.39	0.39	0.00
	Russula cf *sesenagula*	Russulaceae	0.58	0.39	0.00
	Russula sect *griseineae*	Russulaceae	0.00	0.19	0.19
	Russula sect. *archaeina*	Russulaceae	0.39	0.19	0.00
	Russula sp 7	Russulaceae	0.19	0.19	0.00
	Scleroderma sp 2	Sclerodermataceae	0.58	0.19	0.00
	Sutorius sp 1	Boletaceae	0.39	0.00	0.39

Table 1. Contd.

	Tylopilus sp 1	Boletaceae	0.00	0.19	0.39
	Xerocomus subspinulosus	Boletaceae	0.39	0.39	0.00
	Amanita aff. *craseoderma*	Amanitaceae	2.14	0.00	0.00
	Amanita cf *crassiconus*	Amanitaceae	0.00	0.97	0.00
	Amanita sp 1	Amanitaceae	0.19	0.00	0.00
	Amanita sp 2	Amanitaceae	0.00	0.19	0.00
	Amanita sp 3	Amanitaceae	0.19	0.00	0.00
	Amanita sp 4	Amanitaceae	0.00	0.00	0.19
	Amanita sp 6	Amanitaceae	0.19	0.00	0.00
	Amanita sp 7	Amanitaceae	1.75	0.00	0.00
	Amanita sp 9	Amanitaceae	0.19	0.00	0.00
	Amanita sp 10	Amanitaceae	0.19	0.00	0.00
	Amanita sp 11	Amanitaceae	0.19	0.00	0.00
	Amanita subviscosa	Amanitaceae	0.39	0.00	0.00
	Boletellus linderi	Boletaceae	0.97	0.00	0.00
	Boletellus longipes	Boletaceae	0.00	0.58	0.00
	Boletus pallidisimus	Boletaceae	0.00	0.19	0.00
	Boletus sp 1	Boletaceae	0.00	0.00	0.19
	Clavunila sp 1	Clavulinaceae	0.19	0.00	0.00
	Cortinarius aff violaceus	Cortinariaceae	0.39	0.00	0.00
	Cortinarius subgenus *telamonia* sp 1	Cortinariaceae	0.58	0.00	0.00
	Inocybe sp 1	Inocybaceae	0.58	0.00	0.00
	Lactarius sp 1	Russulaceae	0.00	0.00	0.19
	Lactarius sp 2	Russulaceae	0.00	0.00	0.19
Specific to one habitat type	*Lactarius* sp 3	Russulaceae	0.00	0.39	0.00
	Lactifluus flammans	Russulaceae	0.00	0.39	0.00
	Lactifluus gymnocarpoides	Russulaceae	0.00	0.00	0.19
	Lactifluus pelliculatus	Russulaceae	0.00	0.00	0.97
	Lactifluus sp 1	Russulaceae	0.00	0.19	0.00
	Lactifluus sp 2	Russulaceae	0.00	0.00	0.19
	Lactifluus sp 3	Russulaceae	0.19	0.00	0.00
	Lactifluus volemoides	Russulaceae	0.00	0.39	0.00
	Phylloporus cf *rhodophaeus*	Boletaceae	0.58	0.00	0.00
	Porphyrellus sp 1	Boletaceae	0.19	0.00	0.00
	Pulveroboletus sp 3	Boletaceae	0.00	0.00	0.58
	Russula aff. *flavobrunnea*	Russulaceae	0.19	0.00	0.00
	russula cf *annulata*	Russulaceae	0.00	0.00	0.39
	Russula cf *mairei*	Russulaceae	0.00	0.39	0.00
	Russula cf *ochrocephala*	Russulaceae	0.39	0.00	0.00
	Russula cf *subfistulosa*	Russulaceae	0.00	0.00	0.39
	Russula discopus	Russulaceae	0.00	0.00	0.19
	Russula oleifera	Russulaceae	0.00	0.19	0.00
	Russula sp 1	Russulaceae	0.00	0.19	0.00
	Russula sp 2	Russulaceae	0.00	0.19	0.00
	Russula sp 3	Russulaceae	0.19	0.00	0.00
	Russula sp 4	Russulaceae	0.39	0.00	0.00
	Russula sp 5	Russulaceae	0.00	0.19	0.00
	Russula sp 6	Russulaceae	0.00	0.00	0.19
	Russula sp 8	Russulaceae	0.00	0.00	0.19
	Russula sp 9	Russulaceae	0.19	0.00	0.00
	Russula sp 12	Russulaceae	0.19	0.00	0.00

Table 1. Contd.

	Russula sp 13	Russulaceae	0.00	0.19	0.00
	Russula sp 14	Russulaceae	0.00	0.00	0.19
	Russula sp 15	Russulaceae	0.00	0.00	0.19
	Russula sp 16	Russulaceae	0.58	0.00	0.00
	Russula sp 17	Russulaceae	0.19	0.00	0.00
	Russula sp 18	Russulaceae	0.00	0.00	0.19
	Scleroderma cf *cepa*	Sclerodermataceae	0.58	0.00	0.00
	Scleroderma cf *citrinum*	Sclerodermataceae	0.00	0.19	0.00
	Scleroderma sp 1	Sclerodermataceae	0.00	0.58	0.00
	Scleroderma aff. verrucosum	Sclerodermataceae	0.19	0.00	0.00
	Tubosaeta heterosetosa	Boletaceae	0.39	0.00	0.00
	Tylopilus griseus	Boletaceae	0.00	0.00	0.19
	Tylopilus niger	Boletaceae	0.39	0.00	0.00
	Boletoid sp 1	Boletaceae	0.00	0.00	0.19
	Tylopilus sp 2	Boletaceae	0.00	0.58	0.00
	Tylopilus sect. chromapes sp 1	Boletaceae	0.00	0.00	0.19
	Veloporphyrellus africanus	Boletaceae	0.00	0.00	0.97
	Xerocomus sp 1	Boletaceae	0.00	0.19	0.00
	Xerocomus sp 2	Boletaceae	0.00	0.00	0.19
	Xerocomus sp 3	Boletaceae	0.19	0.00	0.00
	Xerocomus sp 5	Boletaceae	0.00	0.19	0.00
	Xerocomus sp 6	Boletaceae	0.78	0.00	0.00
	Xerocomus sp 7	Boletaceae	0.00	0.19	0.00

IW: Isoberlinia Woodlands; MW: mixed wwoodlands: UW: Uapaca Woodlands.

Figure 1a, b. Russula congoana.

Figure 2a, b. Amanita xanthogala.

Figure 3a, b. Pulveroboletus sp 1.

Figure 4a, b. Amanita cf virosa.

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Abidjan, le 10 Avril 2017

PERMIS D'IMPRIMER DE LA VERSION CORRIGEE DE LA THESE

Doctorat de l'Université Félix HOUPHOUËT-BOIGNY

Présentée par VANIE Louyounan Linda Patricia épouse LEABO

Thème : **Champignons ectomycorhiziens du Parc National de la Comoé, une Réserve de Biosphère au nord-est de la Côte d'Ivoire : Diversité, Phénologie de Fructification et Production en relation avec la variabilité climatique**

Vu et approuvé
Abidjan le 10/04/2017

Directeur GRP WASCAL
Coordonnateur CEA-CCBAD

Professeur KONE Daouda

Coordonnateur
National du Projet
CEA-CCBAD

Vu et permis d'imprimer
Abidjan le 10/04/2017

Le Vice-Président de l'Université
Félix HOUPHOUËT-BOIGNY

Professeur AFFIAN Kouadio