Ectomycorrhizal Symbioses in Tropical and Neotropical Forests

Editors

Amadou M. Bâ
Krista L. McGuire
Abdala G. Diédhiou

CRC Press
Taylor & Francis Group
A SCIENCE PUBLISHERS BOOK

Ectomycorrhizal Symbioses
in
Tropical and Neotropical Forests

Ectomycorrhizal Symbioses in Tropical and Neotropical Forests

Editors

Amadou M. Bâ
Université Antilles-Guyane
Guadeloupe (French West Indies)
France

Krista L. McGuire
Barnard College
Columbia University
New York
USA

Abdala G. Diédhiou
Université Cheikh Anta Diop
Dakar
Sénégal

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business
A SCIENCE PUBLISHERS BOOK

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

© 2014 by Taylor & Francis Group, LLC
CRC Press is an imprint of Taylor & Francis Group, an Informa business

No claim to original U.S. Government works
Version Date: 20140117

International Standard Book Number-13: 978-1-4665-9469-2 (eBook - PDF)

This book contains information obtained from authentic and highly regarded sources. Reasonable efforts have been made to publish reliable data and information, but the author and publisher cannot assume responsibility for the validity of all materials or the consequences of their use. The authors and publishers have attempted to trace the copyright holders of all material reproduced in this publication and apologize to copyright holders if permission to publish in this form has not been obtained. If any copyright material has not been acknowledged please write and let us know so we may rectify in any future reprint.

Except as permitted under U.S. Copyright Law, no part of this book may be reprinted, reproduced, transmitted, or utilized in any form by any electronic, mechanical, or other means, now known or hereafter invented, including photocopying, microfilming, and recording, or in any information storage or retrieval system, without written permission from the publishers.

For permission to photocopy or use material electronically from this work, please access www.copyright.com (http://www.copyright.com/) or contact the Copyright Clearance Center, Inc. (CCC), 222 Rosewood Drive, Danvers, MA 01923, 978-750-8400. CCC is a not-for-profit organization that provides licenses and registration for a variety of users. For organizations that have been granted a photocopy license by the CCC, a separate system of payment has been arranged.

Trademark Notice: Product or corporate names may be trademarks or registered trademarks, and are used only for identification and explanation without intent to infringe.

Visit the Taylor & Francis Web site at
http://www.taylorandfrancis.com

and the CRC Press Web site at
http://www.crcpress.com

Foreword

***Ectomycorrhizal symbioses in tropical and neotropical forests*: A major step toward to a neglected research imperative.**

In a 2009 paper in the *New Phytologist*, Ian Alexander and myself called the mycorrhizal research in the tropics '**a neglected research imperative**'. Although Janse's '*Les endophytes radicaux de quelques plantes javanaises*' in 1896 was published shortly after the description of ectomycorrhizas by Frank in 1885, the research on tropical mycorrhizas attracted too limited attention in the next 100 years. Although papers dealing with mycorrhiza and tropic continuously accumulated in the last decade (*ca*. +10% per year, according to ISI Web of Knowledge), they represented only, each year between 2009 and 2012, a remarkably constant 0.6% fraction of the papers dealing with mycorrhiza.

Yet, one should wish more than such a constant progression. Tropical ecosystems represent more than 0.6% of land ecosystems and have pivotal role in the Earth's biogeochemical cycle and climate; moreover, threats on biodiversity in tropical forests should encourage faster study in tropical latitudes. In this framework, a book devoted to '***Ectomycorrhizal symbioses in tropical and neotropical forests***' is very timely.

Indeed, we are far from the simple view that the tropics are dominated by arbuscular mycorrhizas, as popularized by Malloch et al. in *PNAS* in 1975. However, what determines the success of ectomycorrhizas in tropical ecosystems remains unclear. One of the most striking features of tropical ectomycorrhizal symbioses is their frequent occurrence in 'monodominant' forests, where a single species dominates 60 to 100% of the canopy. The present book investigates these exciting monodominant forests by comparing their mycorrhizal diversity with that of more host-diverse ectomycorrhizal forests (Diédhiou et al., Chapter 1), and by questioning the role of ectomycorrhizas among the positive feedbacks that maintain the dominant species over generations (McGuire, Chapter 10).

Beyond such questions, basic descriptions of these insufficiently studied ectomycorrhizal symbioses are still required. This book nicely describes ectomycorrhizas of Nyctaginaceae (Haug et al., Chapter 2) and

Gnetum (a liana probably related to Pinaceae; Bechem, Chapter 8) on the plant side, and of Sebacinales (Moyersoen, Chapter 5) on the fungal side, in a timely revival of often overlooked morphological and microscopic investigations. The *Gnetum* chapter illustrates a more functional approach for studying ECM fungi based on physiology and ecophysiology. These functional aspects are also central in the chapter evaluating the role of the ectomycorrhizal symbiosis in protection of *Coccoloba uvifera* against sea salt (Bâ et al., Chapter 9), and in plant adaptation to soils highly concentrated in heavy metals (Jourand et al., Chapter 6).

More ecological questions, such as the response of the ectomycorrhizal community to disturbance (Onguene et al., Chapter 3), open the door to the use of ectomycorrhizal symbioses for reforestation, especially in physiologically constraining environments. The possibility of facilitation by 'nurse plants', which pre-cultivates a fungal community suitable for installing a target tree species, receives supports in Madagascarian sclerophyllous forests (Baohanta et al., Chapter 4) and the tree performances after inoculation of *Afzelia* by *Scleroderma* and *Thelephora* raises good hopes in Western Africa (Sanon et al., Chapter 7). In Asiatic dipterocarps forests, inoculation, using mycoflora from soil sampled under conspecific parents is promising for forest rehabilitation (Tata, Chapter 11). In an overview of the inoculation methods and results, Duponnois et al. (Chapter 12) rightly point out that ectomycorrhizal symbioses are very promising tools—yet, more long-term assessments of inoculant survival and of host growth promotion are required, especially after out planting to field sites. Encouraging results obtained from some models should not dissimulate that the method needs independent optimization for each plant-fungus model. Anarchic use of any inoculant or any method could introduce undesirable exotic fungi, and discredit ectomycorrhizal inoculations by yielding low results. In this framework, the careful case studies reported in this book are very relevant.

Finally, beyond trees and forests, edible fungal fruit bodies represent often neglected and potentially threatened resources, with high cultural and economical importance (Yorou et al., Chapter 13). '***Ectomycorrhizal symbioses in tropical and neotropical forests***' is a rich milestone in our knowledge of fungal and ectomycorrhizal functioning diversity over all the tropics, from Africa to America, including Pacific islands, with rich insights into functional and applied questions. The reader clearly feels emergent models for further studies and practices: *Scleroderma* and *Pisolithus* spp. on the fungal side, *Coccoloba*, *Afzelia*, *Gilbertiodendron*, *Uapaca*, *Dicymbe* and *Shorea* on the plant side. Researches must now go on, in order to develop robust methods ensuring environmental protection and sustainability of ecosystems services.

As a 'primarily temperate' mycorrhizologist, I have always been fascinated by the lessons and exquisite diversity offered by tropical mycorrhizal symbioses, and I feel greatly honoured to write these introductory lines. Closing the pages of '***Ectomycorrhizal symbioses in tropical and neotropical forests***', I feel more fascinated than ever, and hope all readers will share this great excitement!

Marc-André SELOSSE
Professor at Muséum national d'Histoire naturelle and
President of the Société botanique de France
Observatoire de la Structure et de l'Evolution de la Biodiversité
(UMR7205 CNRS – Muséum), Département Systématique et Evolution,
45 rue Buffon, 75005 Paris, France

Preface

Ectomycorrhizal (EcM) symbioses are mutualistic plant-fungus associations that play a major role in function, maintenance and evolution of biodiversity and ecosystems stability and productivity. EcM associations are integral to the biology and ecology of forest trees, affecting growth, water and nutrient absorption and protection against pathogens. They are also a research imperative in tropical and neotropical forest ecosystems because they concern ecologically and economically important tree species (e.g., Ceasalpinioid subfamily in Africa, family of Dipterocarpaceae in Asia and South America). The book is an overview of the knowledge of EcM symbioses in tropical and neotropical ecosystem forests. The contents address diversity and function of ectomycorrhiza (ECM) associated with forest plants, impacts of ectomycorrhiza on plant diversity and composition, regeneration and dynamics of ecosystems, and biomass production in forestry, adaptation of EcM plants to nutrient deficient, salted and ultramafic soils.

Diédhiou et al. (Chapter 1) has provided valuable information on the diversity and community structure of EcM fungi from mixed and monodominant forests in tropical Africa. They found that monodominant forest tends to harbor more EcM-forming lineages than the mixed forest, while there was no significant difference in terms of richness of EcM fungal species between these two forest types. In the monodominant *Dicymbe corymbosa* forest in Guyana, it appears that positive plant-soil feedbacks function to maintain EcM monodominance through seedling facilitation and alterations of decomposer communities and nutrient cycling pathways. It is also possible that the EcM monodominant trees are able to support a greater diversity of EcM fungi compared to non-dominant EcM trees, which may partially explain why these trees attain such extreme dominance. With a greater diversity of symbionts, trees have access to more diverse pools of soil resources (McGuire, Chapter 10). EcM forest communities could contribute not only to the preservation of monodominant clump refuges in South Cameroon with endemic species, but also to the protection of the biodiversity hot spots of Africa's rain forests. Maintenance of such EcM forest communities with their associated edible mushrooms also helps to preserve a valuable source of alternative protein-rich food for local communities who depend on non-timber forest products (Onguene et al., Chapter 3).

Different approaches exist to characterize EcM. Morpho-anatomical features are important information, together with ultrastructure and DNA sequences of EcM are used to confirm the EcM status of Sebacinales species. These results are not only important for the taxonomy of Sebacinales EcM but also for the understanding of Sebacinales ecology (Moyersoen, Chapter 5). Haug et al. (Chapter 2) showed that although the common ancestor of Nyctaginaceae may have been involved with one thelephoroid taxon, coevolution of Nyctaginaceae and ectomycorrhizal Thelephoraceae in different habitats may have led to the evolution of several different species.

On the other hand, EcM fungi are an integral part of plant physiology. For example, there is a possibility that the fungus *Scleroderma sinnamariense* can access some organic P sources in nature when inorganic P is limiting, an ability which would be beneficial to the *Gnetum africanum*, a notable liana for its edible leaves (Bechem, Chapter 8). Bâ et al. (Chapter 9) that *Scleroderma bermudense* possess considerable resistance to salinity. Tolerance to salt stress was considerably enhanced by *S. bermudense*. Otherwise, the focus on EcM *Pisolithus albus* isolated from soils in New Caledonia highlighted the identification of an ultramafic nickel-tolerant ecotype, showing specific and adaptive molecular response to this metal. In thus, this fungus plays a key role in plant host adaptation to toxic nickel concentrations as found in these soils (Jourand et al., Chapter 6).

From ecological point of view, the EcM fungi could be from an adult tree of *Uapaca bojeri*, an endemic tree of the Malagasy sclerophyllous forest, where various putative EcM fungi were observed, or from endemic and pioneer shrub species (*Leptolenabojeriana* and *Sarcolaenaoblongifolia*) that persist on disturbed sites and facilitate the survival of EcM fungi propagules that could potentially infect roots of *U. bojeri* seedlings (Baohanta et al., Chapter 4). Sanon et al. (Chapter 7) already propose to use two effective EcM fungi, *S. dictyosporum* and *Thelephora* sp., to inoculate *Afzelia africana*, a timber tree for the reforestation programme in West Africa. Similar results were obtained in Asian forests, where diptercarp seedlings are inoculated by mycoflora from soil sampled under conspecific parents in order to rehabilitate forests (Tata, Chapter 11). Duponnois et al. (Chapter 12) present an overview of the inoculation methods and results rightly point to ectomycorrhizal symbioses as very promising tools, but more long-term assessments of inoculant survival and of host growth promotion are required, especially after outplanting to field sites. Finally, beyond trees and forests, edible fungal fruitbodies represent often neglected and potentially threatened resources, of high cultural and economic importance (Yorou et al., Chapter 13).

From this finding, it should be evident that further research is necessary in tropical and neotropical forests to (i) assess and compare the morphological and phylogenetic community composition between monodominant and mixed forests, (ii) to determine the role of EcM fungi in the recruitment and establishment of seedlings of EcM tree species, (iii) to use EcM symbioses for reforestation programmes.

Amadou M. Bâ
Krista L. McGuire
Abdala G. Diédhiou

Contents

CHAPTER 1

Diversity and Community Structure of Ectomycorrhizal Fungi in Mixed and Monodominant African Tropical Rainforests

Abdala Gamby Diedhiou,[1,2,*] *Helvyne Christelle Michaella Ebenye,*[2,3,4] *Marc-André Selosse,*[3,4] *Nérée Onguene Awana*[5] and *Amadou Mustapha Bâ*[2,6]

1. Introduction

Mycorrhizal symbioses play a prominent role in the biology and ecology of forest trees. They involve soil fungi and roots of trees, which together as a symbiosis provide the fungi with carbohydrates and enhance the uptake of water and nutrients for the trees, and also have a major protective role for the roots (Smith and Read 2008). Forest trees are primarily associated with two types of mycorrhizas: arbuscular mycorrhizas which include fungi from the phylum of Glomeromycota and ectomycorrhizas (EcMs) mainly formed by members of Ascomycota and Basidiomycota. It is currently estimated that 6,000–10,000 plant species (Smith and Read 2008, Brundrett 2009) and 20,000–25,000 fungal species (Rinaldi et al. 2008) are involved in

---

Authors' affiliations given at the end of the chapter.

ectomycorrhizal symbioses. EcMs are the most widespread mycorrhizal type in the forests of cool-temperate and boreal latitudes (Molina et al. 1992). Conversely, tropical forests are dominated by arbuscular mycorrhizas (Malloch et al. 1980), a situation that once constrained the introduction of EcM *Pinus* species in the tropics (see Pringle et al. 2009 for review). EcMs being found only in a minority of ecologically and economically important tree species that can form stands where they dominate (Hart et al. 1989, Alexander 1989, 2006, Alexander and Lee 2005, McGuire 2007). Moreover, the species richness of EcM fungi appears to have a unimodal relationship with latitudinal gradient (Tedersoo and Nara 2010, Tedersoo et al. 2012).

In tropical Africa, EcMs are mainly distributed in open forests, gallery forests, and rainforests of the Guineo-Congolian basin, Zambezian Miombo woodlands of East and South-Central Africa, and Sudanian savannah woodlands of the sub-Sahara (Fig. 1, Bâ et al. 2012).

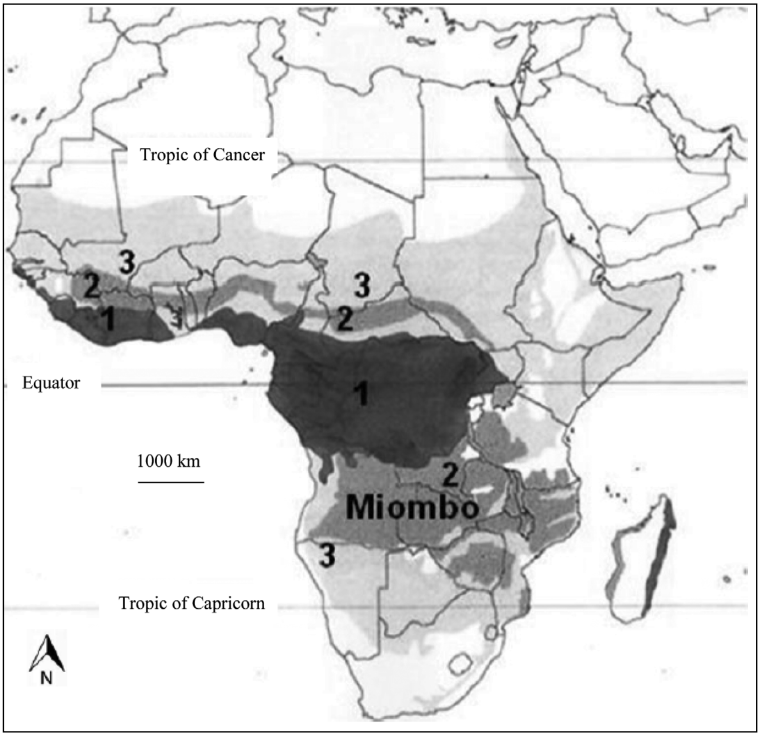

Fig. 1. Distribution of EcM trees in tropical Africa: (1) rainforests in the Guinea-Congo region; (2) open forests in the Sudanian and Zambezian regions; (3) savanna woodlands in the Sudanian and Zambezian regions (Bâ et al. 2012).

Color image of this figure appears in the color plate section at the end of the book.

EcM associations are found mainly on Caesalpinioideae, Dipterocarpaceae, Phyllantaceae, Sarcolaenaceae, Asteropeiaceae, Sapotaceae, Papilionoideae, Gnetaceae (now considered to be close to Pinaceae; Burleigh and Mathews 2004), and Proteaceae (Bâ et al. 2012). In their natural habitats, some African EcM tree species tend to aggregate in patches forming monodominant or mixed stands where they significantly contribute to the forest basal area (Newbery et al. 2004). For instance, *Microberlinia bisulcata*, *Tetraberlinia bifoliolata*, and *T. moreliana* form up to 70% of local patches in the Korup National Park in Cameroon (Newbery et al. 1997). Similarly, in the Southern Guinea rainforests (West Africa), Caesalpinioideae and Phyllanthaceae trees are the most abundant native EcM species, growing in mixed patches with high regeneration of seedlings (Diédhiou et al. 2010). In the Congo basin, *Gilbertiodendron dewevrei* forms monodominant forests where it represents more than 90% of trees in some stands, with abundant seedlings and saplings (Hart et al. 1989, Hart 1995).

It has been documented that EcM fungi contribute to the establishment and structure of these African EcM plant communities (Högberg and Piearce 1986, Newbery et al. 2000, Onguene and Kuyper 2001). However, the diversity and specificity of African EcM fungi have remained inadequately understood. Until recently, available African EcM fungi data was mainly obtained from sporocarp collections, *ex situ* fungal baiting, inoculation of seedlings, and pure culture synthesis trials (Thoen and Bâ 1989, Thoen and Ducousso 1989, Bâ and Thoen 1990, Bâ et al. 1991, Buyck et al. 1996, Sanon et al. 1997, Diédhiou et al. 2004, 2005). The use of molecular tools, such as barcoding with ITS (Nilsson et al. 2008) for *in situ* identification and phylogenetic analysis substantially improved our knowledge of the diversity and specificity of African EcM fungi (Rivière et al. 2007, Tedersoo et al. 2007, 2010a, 2011, Diédhiou et al. 2010, Jairus et al. 2011).

Indeed, a high diversity of EcM fungal species belonging to more than 25 phylogenetic lineages has been revealed from Continental Africa, Madagascar, and the Seychelles. As in temperate forests, the /russula–lactarius and /tomentella–thelephora lineages dominated the EcM fungal flora in these African forests. Furthermore, a low level of host preference and dominance of multi-host fungal species have been revealed from mixed EcM forests (Diédhiou et al. 2010, Tedersoo et al. 2011), suggesting that some EcM tree species may facilitate the recruitment and establishment of conspecific and non-conspecific seedlings. However, little is known about EcM fungal diversity in African tropical monodominant forests. Based on these different observations we addressed the following question: Do mixed forests and monodominant forests in tropical Africa have similar diversity and community structure patterns of EcM fungi? In a monodominant forest of South-Eastern Cameroon we described the EcM and sporocarp diversity, and compared these results to those found from a mixed forest of Southern Guinea (Rivière et al. 2007, Diédhiou et al. 2010).

2. Site Description

The mixed forest was located in Southern Guinea, one of the last regions of West Africa retaining a primary tropical rainforest. Above- and below-ground EcM fungal diversity surveys were conducted in typical evergreen rainforests covering hills and mountains ranging in altitude from 500 m in the Ziama forest (8°51′N, 9°31′W) to 1,752 m on the Mount Nimba forest (7°60′N, 8°49′W). The evergreen rainforests are characterized by a mean annual rainfall of 2,500–3,000 mm and a dry season (mean rainfall <15 mm) from January to March. Temperatures are generally above 24°C with relative humidity up to 80%. The soils are generally poor, lateritic and prone to heavy leaching (McGinley 2008). Canopy trees are at least 30 m tall, with some emergent individuals reaching 50–60 m in height. The canopy is dominated by *Cryptosepalum tetraphyllum* (EcM Caesalpinioideae, Fabales), as well as the AM trees *Erythrophleum ivorense* (Fabaceae) and *Heritiera utilis* (Malvaceae, Malvales). The other EcM trees are represented by Caesalpinioideae (*Afzelia bella, Anthonotha fragans, A. macrophylla, Gilbertiodendron limba, Paramacrolobium coeruleum* and *Pelligriniodendron diphyllum*), and Phyllanthaceae (a family formerly classified within Euphorbiaceae: *Uapaca esculenta, U. chevalieri, U. guineensis* and *Uapaca heudelotii*). Some of these Caesalpinioideae and Phyllanthaceae tree species grow in mixed patches where they dominate (Rivière et al. 2007, Diédhiou et al. 2010). Sporocarps (fruit-bodies) and ECMs of mature trees and seedlings were collected within 3 plots of 0.5–1 ha each in the mixed forest. For the mature trees, root tips were sampled around trees by tracing roots from the base of trunk whenever possible.

The monodominant forest of *G. dewevrei* is located in the Dja Faunal Reserve (2°49′ to 3°23′N; 12°25′ to 13°35′E) in the Southeastern region of Cameroon, which extends from Mouloundou to south of Bertoua. The climate is characterized by two wet seasons from March to June and from August to November with a mean annual rainfall varying from 1600 to 1700 mm. The two dry seasons are December–February (18–59 mm) and July (54 mm). Mean annual temperature varies from 23.7° to 24.3°C. Soils are clayey ferralitic, acidic, and poor in nutrients (Peh et al. 2011). The vegetation has a main canopy of 30–40 m with highest trees rising to 60 m (Letouzey 1985). The monodominant forests are surrounded by mixed forests including many tree and shrub species, which form mainly arbuscular mycorrhiza (e.g., *Afrostyrax lepidophyllus, Afzelia bipendensis, Anthonotha ferruginea, Baphia pubescens, Beilschmiedia louisii, Cryptosepalum congolum, Drypetes paxii,* *Entangrophragma congoensi, Erismadelphus exsul* var. *platyphyllus, Fernandoa ferdinandii* and *Hesteria trillesiana*). Sporocarps and EcMs of mature trees, saplings and seedlings of *G. dewevrei* were collected within 3 plots of 0.5 ha each in a monodominant stand. EcMs from mature trees and saplings were sampled by extracting cylindrical soil cores from around trees.

3. General Traits of the EcM Fungal Communities from the Two Forest Types

A total of 856 samples, 463 from the monodominant forest and 393 from the mixed forest, were collected and analyzed by morphological and molecular approaches. Of the 856 samples 343 (145 and 198 for the monodominant forest and mixed forest, respectively) were represented by sporocarps and 513 (354 and 159 for the monodominant forest and mixed forest, respectively) were represented by EcM tips. Most of the morphologically identified sporocarps sampled from the mixed forest were vouchered and stored in the Museum National d'Histoire Naturelle, France (Rivière et al. 2007). The voucher specimens of sporocarps from the monodominant forest are kept at the herbarium of the Institute of Agricultural Research for Development (IRAD), Yaounde, Cameroon. EcMs were classified into morphotypes (MTs) for molecular analyses based on distinctive macroscopic and microscopic features: branching, color and texture of the mantle, presence or absence of emanating hyphae, rhizomorphs, and sclerotia linked to EcMs (Diédhiou et al. 2004, 2009). The molecular identification of EcM fungi from sporocarps and MTs was conducted by DNA sequencing of the internal transcribed spacer (ITS) region (Diédhiou et al. 2010, H.C.M. Michaella Ebenye unpublished data) and a portion of mitochondrial rDNA large subunit (mtLSU) (Rivière et al. 2007). Hence, from the 856 samples collected, 613 were successfully sequenced and the sequences were aligned with the Clustal X program (Thompson et al. 1997). Alignments were manually optimized with the Genedoc program (Nicholas et al. 1997). BlastN searches of obtained sequences against the INSD were conducted to determine their taxonomical affiliation (Altschul et al. 1990). ITS sequences (OTUs, operational taxonomic units) displaying >90% and >97% full-length similarity to identified fungal taxa were considered congeneric and conspecific, respectively. These sequence similarities are arbitrary, but were deemed as adequate screening for DNA barcoding DNA barcoding thresholds for fungal taxonomy at genus and species levels, respectively (Smith et al. 2007, Nilsson et al. 2008, Hughes et al. 2009, Tedersoo et al. 2010b).

Hence, 625 samples were successfully identified at the family, genus or species level by combining DNA barcoding and morphological analyses of sporocarps and EcMs collected from the two forest types. The identified fungi fell into three phyla: Ascomycota, Basidiomycota and Zygomycota (Table 1). The Basidiomycota was the most abundant phylum and the Zygomycota the least abundant. The identified fungi were then assigned to the phylogenetic lineages of EcM fungi predefined by Tedersoo et al. (2010b); however, we excluded from the analysis the genera *Chalciporus*, *Geastrum* and *Leptodontidium* as their EcM status is still debatable (see Rinaldi et al. 2008, Tedersoo et al. 2010b, Comandini et al. 2012). The names of the identified EcM-forming lineages are written in lower case, non-italicized font with a slash (e.g., /russula-lactarius; Moncalvo et al. 2002). Thus, 18 EcM-forming lineages (Table 1) accounting for 88% of the total abundance of identified samples were recorded from both African tropical forests. This result is consistent with those obtained from other tropical forests where a considerable number of fungi of unknown trophic status have been revealed from EcMs (Tedersoo et al. 2010a, Peay et al. 2010, Smith et al. 2011, 2013). The fungi of unknown trophic status included members of Agaricomycetes, Sordariomycetes, Zygomycetes, Leotiomycetes, Dothideomycetes and Tremellomycetes (Table 1). Although these latter fungi were molecularly identified from EcMs, they are traditionally considered to be parasitic, saprophytic, endophytic, or of unknown trophic status. However, some of these fungi, particularly Mortierellaceae (Zygomycetes), Hypocreaceae (Sordariomycetes) and Polyporaceae (Agaricomycetes) found on many healthy EcMs (data not shown) may represent new EcM-forming lineages in tropical ecosystems (Peay et al. 2010, Smith et al. 2011, 2013) or even on larger scales. Further work is thus necessary to clearly determine the EcM lifestyle of not only these tropical fungal taxa but also of many unexplored fungi which were often revealed in EcMs by DNA barcoding. Indeed, it was only ten years ago that important EcM taxa such as Sebacinales were confirmed to associate with tree roots (Selosse et al. 2002). The EcM-forming lineage inventory is a continuous challenge and requires extensive careful work from field to laboratory, particularly in the fungal taxa where EcM associations are uncommon (de Roman et al. 2005, Rinaldi et al. 2008, Tedersoo et al. 2010b, Comandini et al. 2012).

Among the 18 phylogenetic lineages of EcM fungi revealed from the mixed and monodominant forests, only one (/tuber-helvella) has not yet been reported from other African tropical forests (Tedersoo et al. 2007, 2010a, 2011, Jairus et al. 2011, Bâ et al. 2012). This corroborates the hypothesis of a lower diversity of phylogenetic lineages of EcM fungi in tropical forests relative to temperate forests where individual sites usually support more than 20 lineages (Tedersoo and Nara 2010). Besides the genus *Tuber*, known only from the Holarctic realm (Bonito et al. 2010), the /tuber-helvella lineage includes many separate taxa which are widely distributed in the Austral regions (Tedersoo et al. 2010b). However, the members of this EcM-forming lineage would be poorly represented in the African tropical forests

Table 1. Fungal species identified from EcMs and sporocarps collected from the two forest types (EcM-forming lineages are written in lower case, non-italicized font preceded with a slash (e.g.,/russula-lactarius)).

Type of forest:			**Mixed**		**Monodominant**	
Fungi:						
Phylum	**Class/EcM lineage**	**Family/Genus**	**sporocarps**	**EcMs**	**sporocarps**	**EcMs**
Basidiomycota	/russula-lactarius	*Russula*	+	+	+	+
		Lactarius	+	+	+	+
	/tomentella-thelephora	*Tomentella*	+	+	+	+
		Thelephora		+		+
	/boletus	*Boletus*	+	+	+	+
		Leccinum	+	+		
		Strobilomyces	+	+		
		Xerocomus	+		+	
		Tubosaeta	+			
	/sebacina	*Sebacina*				+
	/pisolithus-scleroderma	*Scleroderma*	+	+	+	
	/amanita	*Amanita*	+	+	+	+
	/tricholoma	*Tricholoma*	+	+		
		Pseudobaeospora			+	
	/clavulina	*Clavulina*		+	+	+
	/coltricia	*Coltricia*	+			
	/cantharellus	*Cantharellus*	+		+	
		Sistotrema				+
	/cortinarius	*Cortinarius*			+	
	/inocybe	*Inocybe*	+		+	

Table 1. contd....

Table 1. contd.

Type of forest:			**Mixed**		**Monodominant**	
Fungi:						
Phylum	**Class/EcM lineage**	**Family/Genus**	**sporocarps**	**EcMs**	**sporocarps**	**EcMs**
	/ramaria-gautieria	*Ramaria*			+	
	/suillus-rhizopogon	*Truncocolumella*			+	
	Agaricomycetes	*Chalciporus*	+		+	
		Geastrum			+	
		Polyporaceae				+
	Tremellomycetes	*Trichosporon*				+
		Tremellaceae				+
Ascomycota	/marcelleina-peziza gerardii	*Marcelleina*				+
	/tuber-helvella	*Helvella*			+	
	/helotiales	Helotiales				+
	/elaphomyces	*Elaphomyces*				+
	Sordariomycetes	Nectriaceae				+
		Chaetosphaeria				+
		Cercophora				+
		Sordariomycete				+
		Hypocreaceae				+
	Leotiomycetes	*Leptodontidium*				+
		Leohumicola				+
		Hyaloscyphaceae				+
	Dothideomycetes	Botryosphaericeae				+
		Leptosphaeria				+
Zygomycota	Zygomycetes	Mortierellaceae				+

as observed in the suillus-rhizopogon lineage assumed to be specific to Pinaceae (Molina et al. 1992, Bruns et al. 2002). On the other hand, there is a noticeable absence of some Holarctic and Austral EcM-forming lineages such as /cenococcum and /laccaria, and some panglobal EcM-forming lineages (e.g., /entoloma, /hebeloma-alnicola and /hysterangium) as well.

The/russula-lactarius, and /tomentella-thelephora, were the most abundant EcM-forming lineages, accounting for 43.17%, and 17.49% of the total abundance of the identified EcM fungi, respectively (Fig. 2). The relative abundances of the /boletus, /amanita, /sebacina and /pisolithus-scleroderma lineages were 10.38%, 6.58%, 5.65% and 5.28% respectively. The remaining EcM-forming lineages accounted for 11.47% of the total abundance, each contributing less than 4% (Fig. 2).

In addition, the below-ground fungal diversity and abundance differed from those observed above-ground. For instance, the /russula-lactarius, /boletus and /amanita lineages dominated the sporocarps, while the /russula-lactarius, /tomentella-thelephora, and /sebacina lineages dominated the EcMs (data not shown). Other EcM-forming lineages such as the /elaphomyces, /helotiales, and /marcelleina-peziza gerardii were

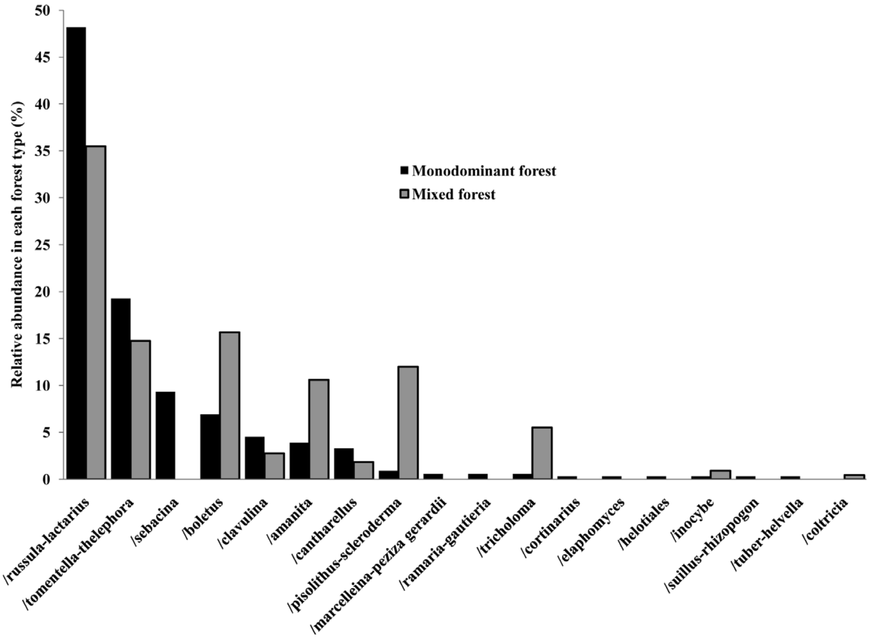

Fig. 2. Relative abundance of the EcM-forming lineages revealed from EcMs and sporocarps collected from each forest type.

found only in EcMs, whereas the /cantharellus, /coltricia, and /inocybe were identified only from sporocarps (Table 1). This result confirms the benefit of combining sporocarp and EcM surveys to predict the EcM association patterns at the root level (Richard et al. 2005, Bâ et al. 2012).

4. Comparisons of the EcM Community between the Mixed and Monodominant Forests

Of the 549 samples representing the EcM-forming lineages, 332 were collected from the monodominant forest of Cameroon and 217 from the mixed forest of Guinea. The samples from the mixed forest were dominated by sporocarps, which represented 63.30%, whereas the samples from the monodominant forest were dominated by EcMs (74.69%). Of the 18 EcM-forming lineages, nine were found in both forest types, eight were found exclusively in the monodominant forest, and one in the mixed forest (Table 1, Fig. 2). Rarefaction analysis performed using the software EstimateS ver. 8.0.0 (Colwell 2006) suggested that, for the minimal samples size $n = 217$, the richness of EcM-forming lineages was lower in the mixed forest (Chao2, Jackknife 2 and ICE were 22.09, 24.57 and 26.27, respectively for the monodominant forest *vs* 10, 11 and 10.49, respectively for the mixed forest). Three non-exclusive reasons can account for the lower richness in terms of EcM-forming lineages in the mixed forest. First, given the seasonal shifts and spatial heterogeneity that exist in EcM fungal communities, the difference in the sampling strategy (e.g., tracing roots vs. extracting soil cores) may provide two different pictures of the EcM fungal communities. Second, the age and history of hosts may influence the EcM communities through competition and selection of more adapted EcM fungal taxa (Selosse et al. 2006). Third, the lower root density of EcM hosts in the mixed forest (our personal observations) may reduce the population size of EcM fungi and subsequently result in lower richness of EcM-forming lineages (Tedersoo and Nara 2010).

Furthermore, with the exception of the /sebacina lineage which accounted for 9.34% of the total abundance of EcM fungi in the monodominant forest, the other EcM-forming lineages found exclusively in a single forest type were represented by either one or two species and displayed relative abundances ≤ 1% (Fig. 2). In this context, we cannot exclude that the members of these latter EcM-forming lineages might have been simply overlooked in one type of forest due to their apparent scarcity. The members of the /sebacina lineage are among the most common EcM fungal species in temperate and Mediterranean forests (Selosse et al. 2002, 2007, Avis et al. 2003, Weiß et al. 2004, Richard et al. 2005) and have been reported from the Neotropics (Moyersoen 2006, Morris et al. 2009, Selosse et al. 2009, Henkel et al. 2012) and the Paleotropics as well (Peay et al. 2010, Tedersoo et al. 2010a, 2011, Jairus et al. 2011). In regard to this observation and because the members of this lineage produce inconspicuous sporocarps, one could argue that they might have been missed in the mixed forest where relatively few samples were collected. However, some EcM-forming lineages such as /pisolithus-scleroderma and /tricholoma were more abundant in the mixed forest (accounting for 11.98% and 5.53%, respectively) than in the monodominant forest where they accounted for < 1% each (Fig. 2). Thus, although possibly underestimating the number and abundance of EcM-forming lineages in the mixed forest, our results show that the monodominant forest tends to harbor more EcM-forming lineages.

In terms of species richness, the lineages of /russula-lactarius (86 spp.), /tomentella-thelephora (30 spp.), /sebacina (19 spp.), and /boletus (13 spp.) dominated in the monodominant forest, while the /russula-lactarius (42 spp.), /tomentella-thelephora (21 spp.), /boletus (20 spp.), and /amanita (15 spp.) dominated in the mixed forest. Other species-rich lineages included the /pisolithus-scleroderma (10 spp.) and /tricholoma (10 spp.) in the mixed forest and the /clavulina (11 spp.) and /amanita (10 spp.) in the monodominant forest (Fig. 3).

The EcM-forming lineages included 189 and 126 putative EcM species for the monodominant forest and mixed forest, respectively (Table 2). The high EcM species richness observed from these African forests is a

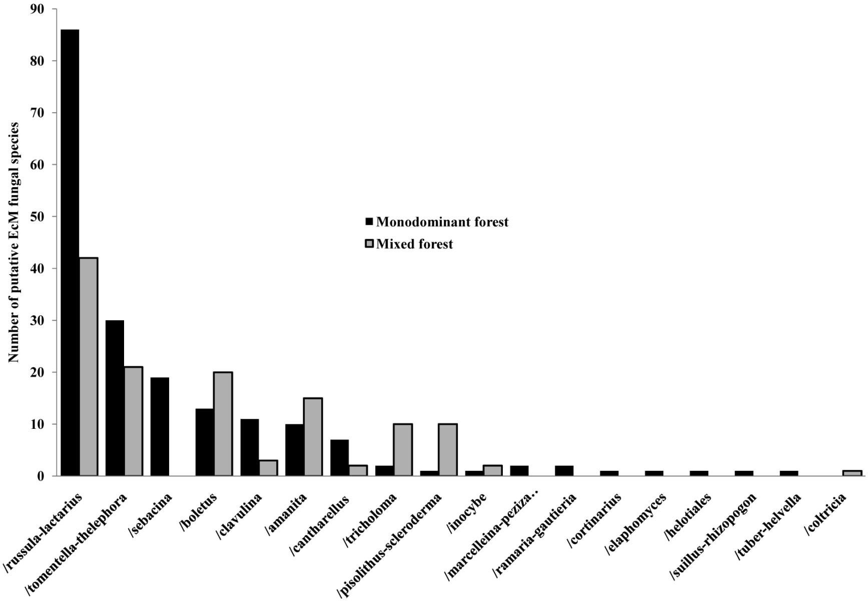

Fig. 3. Number of putative EcM fungal species identified from EcMs and sporocarps collected from each forest type.

Table 2. Number (No.) of EcM samples observed and estimated EcM species richness in each forest type. *The numbers appearing in bold were obtained after rarefaction to 217.

	Monodominant forest	**Mixed forest**
No. of EcM samples	332	217
No. of putative EcM species	189	126
	137.37*	**126**
Chao2 (95% CI)	575.42 (419.98–835.47)	249.08 (193.87–349.18)
	478.06 (321.39–768.92)	**249.08 (193.87–349.18)**
Jackknife 2	440.97	259.25
	329.82	**259.25**
ICE	675.07	282.2
	523.9	**282.2**
Fisher's alpha ± SD	182.1 ± 17.87	125.51 ± 15.38
	161.82 ± 20.93	**125.51 ± 15.38**
Shannon ± SD	4.86	4.62
	4.64 ± 0.05	**4.62**

common trend in Neotropical (Morris et al. 2009, Smith et al. 2011, 2013, Henkel et al. 2012) and Paleotropical forests (Peay et al. 2010, Tedersoo et al. 2010b, 2011). The calculation of sample-based rarefaction curves and minimal species richness estimates showed that the sampling efforts were insufficient in both types of forests. Indeed, the rarefied accumulation curves of species and minimal species richness estimates (Chao2, Jack2 and ICE) did not reach a clear asymptote with increasing sample size (Fig. 4). The Chao2, Jack2 and ICE richness estimators predicted 249.08, 259.25 and 282.2 putative EcM species, respectively for the mixed forest and 575.42, 440.97 and 675.07 putative EcM species, respectively for the monodominant forest (Table 2).

Surprisingly, when rarefying the samples from the monodominant forest to $n = 217$ to match the sample size of the mix from the mixed forest, we found no significant difference in terms of richness of putative EcM fungal species between the two forests types (Table 2, Fig. 4). Indeed, the 95% CI (confidence intervals) for Chao2 are overlapping and the indices of species diversity, Fisher's alpha and Shannon are not significantly different between the two types of forests (Table 2). This lack of difference in richness of EcM fungal species between the mixed forest and monodominant forest may be in part related to the prevalence of multi-host EcM fungi (Onguene and Kuyper 2002, Diédhiou et al. 2010) which could mask the influence of plant host species on EcM fungal community structure. Interestingly, this rejects the generalization of the idea that host diversity is the sole driver of diversity in EcM community (Dickie 2007, Ishida et al. 2007, Tedersoo

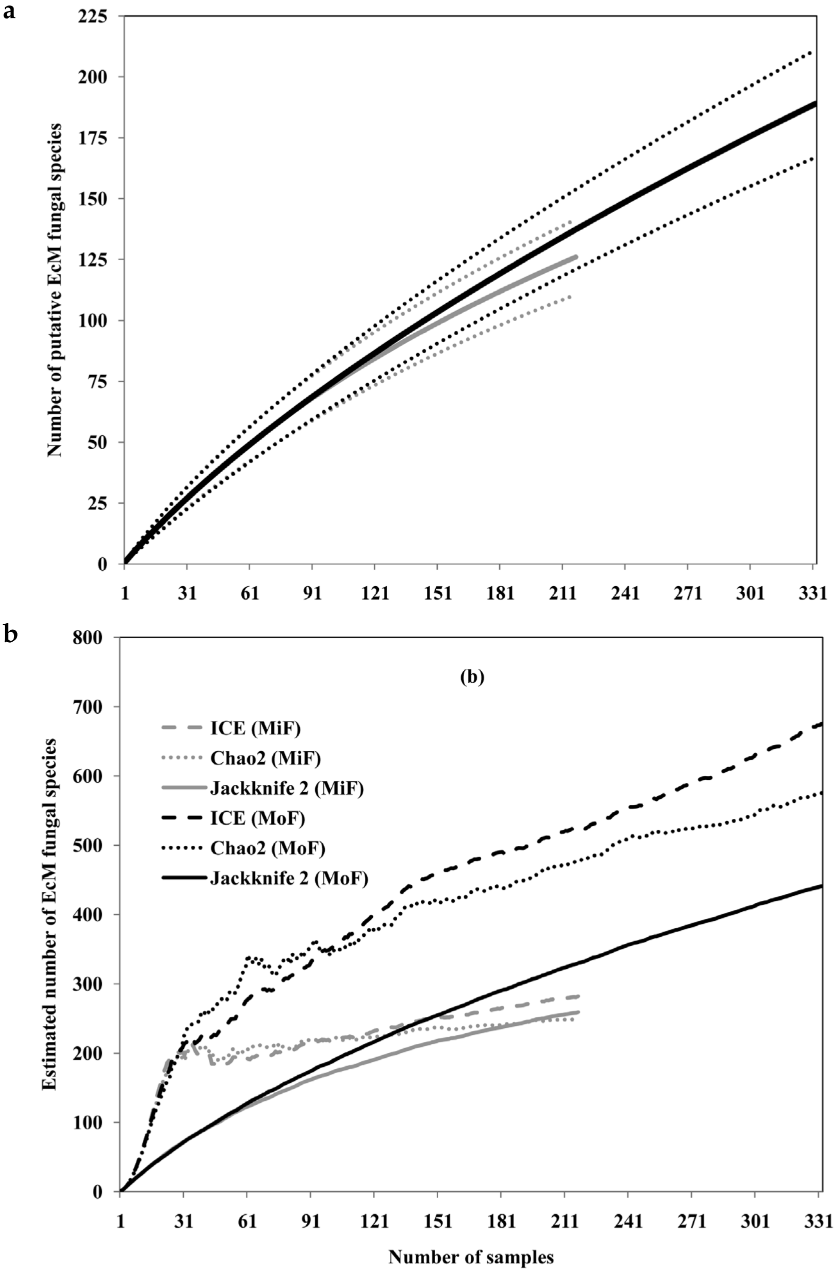

Fig. 4. Rarefied accumulation curves of (a) EcM fungal species and (b) minimal species richness estimates for the monodominant forest (MoF, black) and mixed forest (MiF, grey). Dotted lines in (a) indicate 95% CI.

et al. 2008). This idea may be supported by the finding that African EcM fungal communities are not strongly structured by soil horizon and host at the plant species and family levels (Tedersoo et al. 2011).

5. Conclusion

This study has provided valuable information on the diversity and community structure of EcM fungi from mixed and monodominant forests in tropical Africa. The monodominant forest tends to harbor more EcM-forming lineages than the mixed forest, while there was no significant difference in terms of richness of EcM fungal species between these two forest types. The dominant EcM-forming lineages are similar in the two forest types. On the other hand, a large number of fungi of unknown trophic status were recovered from healthy EcMs; some of them may represent new, overlooked tropical EcM-forming lineages. From this finding, it should be evident that further research is necessary to (i) determine the EcM lifestyle of the latter fungal taxa, (ii) rigorously assess and compare the phylogenetic community composition between the two forest types, and (ii) highlight the role of EcM fungi in the recruitment and establishment of seedlings of EcM tree species in monodominant and mixed forests.

Acknowledegments

We thank the anonymous referees for their valuable comments on this study, and Krista L. McGuire and Caitlyn Gillikin for improving the language.

References

Alexander, I.J. 1989. Mycorrhizas in tropical forests. *In*: J. Proctor (ed.). Mineral Nutrients in Tropical Forest and Savanna Ecosystems. Blackwell Scientific Publications, Oxford, pp. 169–188.

Alexander, I.J. 2006. Ectomycorrhizas-out of Africa? New Phytol. 172: 589–591.

Alexander, I.J. and S.S. Lee. 2005. Mycorrhizas and ecosystem processes in tropical rain forest: implications for diversity. *In*: D. Burslem, M. Pinard and S. Hartley (eds.). Biotic Interactions in the Tropics. Cambridge University Press, Cambridge, pp. 165–203.

Altschul, S.F., W. Gish, W. Miller, E.W. Myers and D.J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215: 403–410.

Avis, P.G., D.J. McLaughlin, B.C. Dentinger and P.B. Reich. 2003. Long-term increase in nitrogen supply alters above- and below-ground ectomycorrhizal communities and increases the dominance of *Russula* spp. in a temperate oak savanna. New Phytol. 160: 239–253.

Bâ, A.M., J. Garbaye and J. Dexheimer. 1991. Influence of fungal propagules during the early stade of the time sequence of ectomycorrhizal colonization on *Afzelia africana* Sm. Seedlings. Can. J. Bot. 66: 2442–2447.

Bâ, A.M. and D. Thoen. 1990. First syntheses of ectomycorrhizas between *Afzelia africana* Sm. (Caesalpinioideae) and native fungi from West Africa. New Phytol. 103: 441–448.

Bâ, A.M., R. Duponnois, B. Moyersoen and A.G. Diedhiou. 2012. Ectomycorrhizal symbiosis of tropical African trees. Mycorrhiza 22: 1–29.

Bonito, G.M, A.P. Gryganskyi, J.M. Trappe and R. Vilgalys. 2010. A global meta-analysis of Tuber ITS rDNA sequences: species diversity, host associations and long-distance dispersal. Mol. Ecol. 19: 4994–5008.

Brundrett, M.C. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320: 37–77.

Bruns, T.D., M.I. Bidartondo and D.L. Taylor. 2002. Host specificity in ectomycorrhizal communities: what do the exceptions tell us? Integr. Comp. Biol. 42: 352–359.

Burleigh, J.G. and S. Matthews. 2004. Phylogenetic signal in nucleotide data from seed plants: implications for resolving the seed plant tree of life. Am. J. Bot. 91: 1599–1613.

Buyck, B., D. Thoen and R. Walting. 1996. Ectomycorrhizal fungi of the Guinea–Congo region. Proc. R. Soc. Edinb. 104: 313–333.

Colwell, R.K. 2006. Estimates: Statistical Estimation of Species Richness and Shared Species from Samples, Version 8 [WWW document]. URL http://purl.oclc.org/estimates.

Comandini, O., A.C. Rinaldi and T.W. Kuyper. 2012. Measuring and estimating ectomycorrhizal fungal diversity: a continuous challenge. *In*: M.C. Pagano (ed.). Mycorrhiza Occurrence in Natural and Restored Environments. Nova Science Publishers, Hauppauge, NY, pp. 165–200.

de Roman, M., V. Claveria and A.M. de Miguel. 2005. A revision of the descriptions of ectomycorrhizas published since 1961. Mycol. Res. 109: 1063–1104.

Dickie, I.A. 2007. Host preference, niches and fungal diversity. New Phytol. 174: 230–233.

Diédhiou, A.G., A.M. Bâ, S. Sylla, B. Dreyfus, M. Neyra and I. Ndoye. 2004. The early-stage ectomycorrhizal Thelephoroid fungal sp. is competitive and effective on Afzelia africana Sm. in nursery conditions in Senegal. Mycorrhiza 14: 313–322.

Diédhiou, A.G., O. Guèye, M. Diabaté, Y. Prin, R. Duponnois, B. Dreyfus and A.M. Bâ. 2005. Contrasting responses to ectomycorrhizal inoculation in seedlings of six tropical African tree species. Mycorrhiza 16: 11–17.

Diédhiou, A.G., J.-L. Dupouey, M. Buée, E. Dambrine, L. Laüt and J Garbaye. 2009. Response of ectomycorrhizal communities to past Roman occupation in an oak forest. Soil Biol. Bioch. 41: 2206–2213.

Diédhiou, A.G., M.-A. Selosse, A. Galiana, M. Diabaté, B. Dreyfus, A.M. Bâ, S.M. de Faria and G. Béna. 2010. Multi-host ectomycorrhizal fungi are predominant in a Guinean tropical rain forest and shared between canopy and tree seedlings. Environ. Microbiol. 2: 2219–2232.

Hart, T.B. 1995. Seed, seedling and sub-canopy survival in monodominant and mixed forests of the Ituru forest, Africa. J. Trop. Ecol. 11: 443–459.

Hart, T.B., J.A. Hart and P.G. Murphy. 1989. Monodominant and species-rich forests of the humid tropics: causes for their occurrence. Am. Nat. 133: 613–633.

Henkel, T.W., M.C. Aime, M.M.L. Chin, S.L. Miller, R. Vilgalys and M.E. Smith. 2012. Ectomycorrhizal fungal sporocarp diversity and discovery of new taxa in *Dicymbe* monodominant forests of the Guiana Shield. Biodivers. Conserv. 21: 2195–2220.

Högberg, P. and G.D. Piearce. 1986. Mycorrhizas in Zambian trees in relation to host taxonomy, vegetation type and successional patterns. J. Ecol. 74: 775–785.

Hughes, K.W., R.H. Peterson and E.B. Lickey. 2009. Using heterozygosity to estimate a percentage DNA sequence similarity for environmental species' delimitation across basidiomycete fungi. New Phytol. 182: 795–798.

Ishida, T.A., K. Nara and T. Hogetsu. 2007. Host effects on ectomycorrhizal fungal communities: insight from eight host species in mixed conifer-broadleaf forests. New Phytol. 174: 430–440.

Jairus, T., R. Mpumba, S. Chinoya and L. Tedersoo. 2011. Invasion potential and host shifts of Australian and African ectomycorrhizal fungi in mixed eucalypt plantations. New Phytol. 192: 179–187.