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ORIGINAL PAPER

Arbuscular mycorrhizas and ectomycorrhizas of *Uapaca bojeri* L. (Euphorbiaceae): sporophore diversity, patterns of root colonization, and effects on seedling growth and soil microbial catabolic diversity

Naina Ramanankierana · Marc Ducousso · Nirina Rakotoarimanga · Yves Prin · Jean Thioulouse · Emile Randrianjohany · Luciano Ramaroson · Marija Kisa · Antoine Galiana · Robin Duponnois

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Abstract The main objectives of this study were (1) to describe the diversity of mycorrhizal fungal communities associated with *Uapaca bojeri*, an endemic Euphorbiaceae of Madagascar, and (2) to determine the potential benefits of inoculation with mycorrhizal fungi [ectomycorrhizal and/or arbuscular mycorrhizal (AM) fungi] on the growth of this tree species and on the functional diversity of soil microflora. Ninety-four sporophores were collected from three survey sites. They were identified as belonging to the ectomycorrhizal genera *Afroboletus*, *Amanita*, *Boletus*, *Cantharellus*, *Lactarius*, *Leccinum*, *Rubinoboletus*, *Scleroderma*, *Tricholoma*, and *Xerocomus*. *Russula* was the most frequent ectomycorrhizal genus recorded under *U. bojeri*. AM structures (vesicles and hyphae) were detected from the roots in all surveyed sites. In addition, this study showed that this tree species is highly dependent on both types of mycorrhiza, and controlled ectomycorrhization of this *Uapaca* species strongly influences soil microbial catabolic diversity. These results showed that the complex symbiotic status of *U. bojeri* could be managed to optimize its development in degraded areas. The use of selected mycorrhizal fungi such the *Scleroderma* Sc1 isolate in nursery conditions could be of great interest as (1) this fungal strain is very competitive against native symbiotic microflora, and (2) the fungal inoculation improves the catabolic potentialities of the soil microflora.

Keywords Ectomycorrhizas · Arbuscular mycorrhizas · Fungal diversity · Microbial functionalities · *Uapaca bojeri* · Madagascar

N. Ramanankierana · N. Rakotoarimanga · E. Randrianjohany · L. Ramaroson
Laboratoire de Microbiologie de l'Environnement, Centre National de Recherches sur l'Environnement, P.O. Box 1739, Antananarivo, Madagascar

M. Ducousso · Y. Prin · A. Galiana
CIRAD, UMR 113 CIRAD/INRA/IRD/AGRO-M/UM2, Laboratoire des Symbioses Tropicales et Méditerranéennes (LSTM), TA10/J, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France

J. Thioulouse
CNRS, Laboratoire de Biométrie et Biologie Evolutive, UMR 5558, Université Lyon 1, 69622 Villeurbanne Cedex, France

M. Kisa · R. Duponnois
IRD, UMR 113 CIRAD/INRA/IRD/AGRO-M/UM2, Laboratoire des Symbioses Tropicales et Méditerranéennes (LSTM), TA10/J, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France

R. Duponnois (✉)
IRD, Laboratoire Commun de Microbiologie IRD/ISRA/UCAD, Centre de Recherche de Bel Air, P.O. Box 1386, Dakar, Senegal
e-mail: robin.duponnois@ird.sn

Introduction

A high botanical diversity and a high degree of endemism characterize Madagascarian forests (Lowry et al. 1997), but they are often deforested for their conversion to agriculture. Deforestation rates were estimated to be 0.11 Mha year^{-1} between 1950 (7.6 Mha) and 1985 (3.8 Mha; Green and

Sussman 1990). Disturbances of the vegetation cover are often accompanied by rapid erosion of surface soil that induces a loss or reduction of major physicochemical and biological soil properties (Vagen et al. 2006a,b). In particular, it has been shown that mycorrhizal soil potential was drastically reduced (Marx 1991; Jasper et al. 1991; Herrera et al. 1993; Dickie and Reich 2005). Hence, an increase of this fungal inoculum potential is needed in both natural and artificial revegetation processes (McGee 1989). However, the mycorrhizal status of the Madagascarian flora is poorly known. Typical ectomycorrhizal fungi were reported more than 60 years ago (Heim 1970). More recently, mycological surveys show the large diversity of the associated ectomycorrhizal fungi (Buyck et al. 1996; Ducousso et al. 2004). The mycorrhizal inoculation of plants is very efficient in establishing plants on disturbed soils (Estaun et al. 1997; Duponnois et al. 2001, 2005). The management of mycorrhizal symbiosis needs to investigate the presence, abundance, and community composition of mycorrhizal fungi associated with plants. Furthermore, efficient fungal strains have to be selected to help tree establishment and also to improve soil quality (Franson and Bethlenfalvay 1989; Duponnois and Plenchette 2003; Diédhiou et al. 2005; Duponnois et al. 2005).

The benefits of mycorrhizal symbiosis to the host plant have usually been considered a result from the close relationship between fungal symbionts and plant species. However, it has been demonstrated that mycorrhizal symbiosis has a great influence on the soil bacterial and fungal communities in natural conditions (Frey et al. 1997; Founoune et al. 2002a,b; Mansfeld-Giese et al. 2002; Frey-Klett et al. 2005). This microbial compartment is commonly named "mycorrhizosphere" (Linderman 1988) and is usually divided in two different zones: one is subjected to the dual influence of the root and the mycorrhizal symbionts (the mycorrhizosphere) and, the other, under the influence of mycorrhizal hyphae (the hyphosphere). The microbial activities that occur in the hyphosphere are different from those recorded in the mycorrhizosphere (Andrade et al. 1998). Hyphosphere microorganisms may influence mycorrhizal functions such as nutrient and water uptake carried out by the external hyphae of the mycorrhizal fungi (Duponnois, unpublished data). Hence, the association between the fungus and the host plant has been enlarged to the soil microflora to form a multitrophic mycorrhizal complex (Frey-Klett et al. 2005). The microbial functional diversity of each soil compartment includes a vast range of activities (nutrient transformations, decomposition, etc.) and can be characterized by the measurement of catabolic response profiles (CRPs; Degens and Harris 1997; Degens et al. 2001). The measurement of CRPs directly assesses the catabolic diversity of microbial communities involved in decomposition activities by adding a range of simple organic substrates directly to the soil and measuring the short-term catabolic responses (Degens and Harris 1997). Catabolic evenness, a component of microbial functional diversity is defined as the uniformity of substrate use and can be calculated from the CRPs (Degens and Harris 1997).

Mycorrhizal fungi are ubiquitous components of most ecosystems throughout the world and are considered key ecological factors in governing the cycles of major plant nutrients and in sustaining the vegetation cover (van der Hejden et al. 1998; Requena et al. 2001; Schreiner et al. 2003). Two major forms of mycorrhizas are usually recognised: the arbuscular mycorrhizas (AM) and the ectomycorrhizas (ECMs). AM symbiosis is the most widespread mycorrhizal association type with plants that have true roots, i.e. pteridophytes, gymnosperms and angiosperms (Read et al. 2000). They affect about 80–90% land plants in natural, agricultural, and forest ecosystems (Brundrett 2002). ECMs affect trees and shrubs, gymnosperms (Pinaceae) and angiosperms, and are usually the result of the association of Homobasidiomycetes with about 20 families of mainly woody plants (Smith and Read 1997). These woody species are associated with a larger (compared to the AM symbiosis) diversity of fungi, comprising 4,000 to 6,000 species, mainly Basidiomycetes and Ascomycetes (Allen et al. 1995; Valentine et al. 2004).

The main objectives of this study were (1) to describe the diversity of mycorrhizal fungal communities associated with *Uapaca bojeri*, an endemic Euphorbiaceae of Madagascar and (2) to determine the potential benefits of inoculation with mycorrhizal fungi (ectomycorrhizal and/or AM fungi) on the growth of this tree species and on the functional diversity of soil microflora.

Materials and methods

Site description and sporophore sampling

Three forests in Madagascar were visited at 2- to 3-week intervals during the sampling seasons, mid-November to early February 1993, July–August 1994, and July to mid-September 1995, to collect ectomycorrhizal fungi fruiting under *U. bojeri*. The forests were located 50 km to the west of Antananarivo (Arivonimamo site as site A), 20 km to the south of Antsirabe (Ambositra site as site B), and 100 km to the east of Toliara (Isalo site as site C). The mean annual rainfall varied from 912.4 mm (site C), 1,428.8 mm (site A), to 1,554.4 mm (site B). The vegetation sampled included savannas (sites A and B) and deciduous forests (site C). The main chemical characteristics of the upper soil layer (0–20 cm) of these sites are shown in Table 1.

Table 1 Main-chemical characteristics of the upper soil layer (0–20 cm)

Site	Site A	Site B	Site C
pH (H_2O)	4.96	5.37	4.54
pH (KCl)	4.75	5.23	4.45
Total C (%)	1.12	3.09	1.33
Total N (%)	0.07	0.15	0.91
Total organic matter (%)	1.92	5.31	2.28
C/N	16.0	21.0	14.6
Total P (mg kg^{-1})	15.2	15.2	17.3
Available P (mg g^{-1}, Olsen et al. 1954)	3.42	7.01	5.24

Sporophores of putative epigeous ectomycorrhizal fungi were collected under *U. bojeri*, photographed, described as fresh material, preserved by oven-drying, and deposited at the herbarium at Laboratoire de Microbiologie de l'Environnement (LME, Madagascar). In addition, roots of *U. bojeri* were collected in each site, and fine roots were stained for AM according to the procedure of Phillips and Hayman (1970) and examined with light microscopy.

Time sequence of mycorrhizal colonization on *U. bojeri* in glasshouse conditions

Surface forest soil (0- to 20-cm depth) was collected from the native stand of *U. bojeri* in site A, crushed, passed through a 2-mm sieve, carefully mixed, and distributed in 1-l pots. The seeds of *U. bojeri* collected in site A were surface sterilized in hydrogen peroxide for 10 min, rinsed and soaked in sterile distilled water for 12 h, and germinated on 1% agar. After 1 week of incubation at 30°C in the dark, one pre-germinated seed was planted per pot. The seedlings were screened from the rain and grown under natural light (daylight of approximately 12 h, average daily temperature of 25°C). They were watered regularly with tap water without fertilizer.

During 5 months, four plants per month were randomly sampled, uprooted, and their root systems gently washed with tap water. About 30 lateral roots were randomly chosen along the tap root of each plant, cut into short pieces, and observed under a stereomicroscope (magnification ×40). All ECMs were counted on each root fragment. Other root samples were collected from each plant to detect AM structures using the same procedure as before (Phillips and Hayman 1970).

Assessment of *U. bojeri* mycorrhizal dependency

A strain of *Scleroderma* sp. (strain Sc1) was isolated from a sporocarp collected in site A. This fungal isolate was previously tested for its compatibility with *U. bojeri* in axenic conditions (data not shown). The fungal strain was maintained in Petri dishes on modified Melin–Norkrans (MMN) agar medium at 25°C (Marx 1991). The fungal inoculum was prepared according to Duponnois and Garbaye (1991).

The AM fungus *Glomus intraradices* (Schenk and Smith, DAOM 181602, Ottawa Agricultural Herbarium) was multiplied on leek (*Allium porrum* L.) on Terragreen (Oil Dri UK) in glasshouse conditions. The culture substrate was an attapulgite (calcined clay; average particle size, 5 mm) from Georgia used for the propagation of AM fungi (Plenchette et al. 1996). After 12 weeks of culturing, the leek plants were uprooted and gently washed, and the roots were cut into 0.5-cm pieces bearing around 250 vesicles per centimeter. Non-mycorrhizal leek roots prepared as above were used for the control treatment without AM inoculation.

The seeds of the *U. bojeri* were surface sterilized as described above. The germinated seeds were individually grown in 1-l polythene bags filled with sterilized sandy soil (140°C, 40 min) in which *G. intraradices* and/or *Scleroderma* Sc1 were already inoculated. A control treatment without fungi was included. After autoclaving, the soil chemical characteristics were as follows: pH 5.01 (H_2O); total carbon, 9.3%; total nitrogen, 0.06%; total phosphorus, 120.6 mg kg^{-1}. For ectomycorrhizal inoculation, the soil was mixed with fungal inoculum (10/1; v/v). The treatments without fungus received an autoclaved mixture of moistened (MMN medium) vermiculite/peat moss at the same rate. For endomycorrhizal inoculation, one hole (1×5 cm) was made in each pot and filled with 1-g fresh leek root (mycorrhizal for the experimental treatment or non-mycorrhizal for the control treatment without fungus). The holes were then covered with the same autoclaved soil. They were watered regularly with tap water without fertilizer. The pots were arranged in a randomized complete block design with 25 replicates per treatment. The seedlings were screened from the rain and grown under natural light (daylight of approximately 12 h, average daily temperature of 25°C).

After 5 months of culture, the *Uapaca* plants were uprooted, and the oven dry weight (1 week at 65°C) of the shoot was measured. The root systems were gently washed, cut into 1-cm root pieces, mixed, and the percentage of ectomycorrhizal short roots (number of ectomycorrhizal short roots/total number of short roots) was determined on a random sample of at least 100 short roots under a stereomicroscope (magnification ×40). Then these root pieces were cleared and stained according to the method of Phillips and Hayman (1970). The root pieces were placed on a slide for microscopic observation at 250× magnification (Brundrett et al. 1985). About 100 1-cm root pieces were observed per plant. The extent of mycorrhizal colonization was expressed in terms of the fraction of root length with the internal fungal structures (vesicles and arbuscules). The relative mycorrhizal dependency was determined by expressing the difference between the shoot dry weight of the mycorrhizal plant and the shoot dry weight of the non-

mycorrhizal plant as a percentage of the shoot dry weight of the mycorrhizal plant (Plenchette et al. 1983).

Influence of ectomycorrhizal inoculation on soil microbial catabolic diversity

The *Uapaca* seedlings were grown in 1-l pots filled with natural soil collected in site A. One part of the soil was autoclaved (140°C, 40 min) and the other part was not disinfected before use. After autoclaving, its chemical characteristics were as follows: pH 5.2 (H_2O); total C, 1.01%; total N, 0.08%; organic matter, 1.55%; C/N, 13.2; total P, 11.9 mg kg^{-1}. The native chemical characteristics of this soil are indicated in Table 1. The ectomycorrhizal inoculation with the *Scleroderma* isolate Sc1 was performed as described above, and the same treatment was performed for the control treatment. They were watered regularly with tap water without fertilizer. The pots were arranged in a randomized complete block design with ten replicates per treatment. The seedlings were screened from the rain and grown under natural light (daylight of approximately 12 h, average daily temperature of 25°C).

After 5 months of culture, *Uapaca* plants were uprooted, the shoot biomass and the ectomycorrhizal colonization were measured as described before. Most of the soil from 3 randomly chosen pots in each treatment was carefully mixed and kept at 4°C for further analysis.

The microbial catabolic diversity was measured by adding a range of simple organic compounds to the soil and determining the short-term respiration responses (Degens and Harris 1997; Degens et al. 2001). Each of the 31 substrates suspended in 2-ml sterile distilled water was added to 1 g of moist soil in 10-ml bottles (West and Sparling 1986). The CO_2 production from the basal respiratory activity in the soil samples was measured by adding 2-ml sterile distilled water to 1 g of the equivalent dry weight of soil. After the addition of the substrate solutions to the soil samples, the bottles were immediately sealed with a vacutainer stopper and incubated at 28°C for 4 h in darkness. After 4 h, respired CO_2 in the headspace of each bottle was determined by taking a 1-ml syringe sample and analysing the CO_2 concentration using an infrared gas analyser (Polytron IR CO_2, Dräger™) in combination with a thermal flow meter (Heinemeyer et al. 1989). The results were expressed as μg CO_2 g^{-1} soil h^{-1}. There were 10 amino acids (L-glutamine, L-serine, L-arginine, L-asparagine, L-cystein, L-histidine, L-lysine, L-glutamic acid, L-phenylalanine, L-tyrosine), 3 carbohydrates (D-glucose, D-mannose, sucrose), 2 amides (D-glucosamine and succinamide), and 16 carboxylic acids (ascorbic acid, citric acid, fumaric acid, gluconic acid, quinic acid, malonic acid, α-ketoglutaric acid, α-ketobutyric acid, succinic acid, tartaric acid, uric acid, oxalic acid, malic acid, hydroxybutyric acid). The amines and amino acids were added at 10 mM, whereas the carbohydrates were added at 75 mM and the carboxylic acids at 100 mM (Degens and Vojvodic-Vukovic 1999). The catabolic richness and catabolic evenness were calculated to evaluate the catabolic diversity of both soil treatments. The catabolic richness, R, expressed the number of substrates used by the microorganisms in each soil treatment. The catabolic evenness, E, representing the variability of used substrates amongst the range of the substrates tested was calculated using the Simpson–Yule index $E = 1/p_i^2$ with p_i=respiration as the response to individual substrates/total respiration activity induced by all substrates for a soil treatment (Magurran 1988).

Statistical analysis

The data were treated with one-way analysis of variance. The means were compared using the Newman and Keuls test ($p < 0.05$). The percentages of the mycorrhizal colonization were transformed by arcsin(sqrt) before the statistical analysis.

The between-group analysis (BGA, Dolédec and Chessel 1987; Culhane et al. 2002) was used to analyse the surface insulation resistance (SIR) responses in soil samples inoculated with *Scleroderma* Sc1 and samples without inoculation. BGA is a multivariate analysis technique derived from principal components analysis (PCA). The aim of PCA is to summarize a data table by searching orthogonal axes on which the projection of the sampling points (rows of the table) has the highest possible variance.

From a theoretical point of view, BGA is the particular case of PCA with respect to instrumental variables (principal component analysis with instrumental variables, Rao 1964; Lebreton et al. 1991) where the instrumental variables table is reduced to just one qualitative variable. This variable defines groups of rows in the data table, and BGA consists of the PCA of the table of the means by groups. This table has a number of rows equal to the number of groups, and the same number of columns as the original table. The aim of this analysis is to separate the groups. This is also the aim of discriminant analysis (also called canonical variates analysis), but whilst discriminant analysis is limited to tables that have a high number of samples compared to the number of variables, BGA can be used even when the number of rows is less than the number of variables. BGA can, thus, be considered as a robust alternative to discriminant analysis when the number of samples is low.

A Monte Carlo test (permutation test) can be used to check the significance of the differences between groups. This method consists, in performing many times, a random permutation of the rows of the table (but not of the qualitative variable defining the groups) followed by the recomputation of the between-class inertia. By comparing the between-class inertia obtained in the normal analysis with the between-class

inertia obtained after randomization, we get an estimation of the probability of meeting a situation similar to the observed situation without differences between groups (i.e. a significance test of the differences between groups).

The computations and graphical displays were made with the free ADE-4 software (Thioulouse et al. 1997) available in the Internet at http://www.pbil.univ-lyon1.fr/ADE-4/.

Results

Sporophore survey

We collected 94 sporophores in three survey sites (S 1). They were identified as belonging to the ectomycorrhizal genera *Afroboletus*, *Amanita*, *Boletus*, *Cantharellus*, *Leccinum*, *Gyroporus*, *Rubinoboletus*, *Russula*, *Scleroderma*, *Suillus*, *Tricholoma*, and *Xerocomus* (S 1). The highest fungal diversity of the above-ground sporophores was recorded in site A (40 species), whereas only 27 and 29 fungal species were detected in sites B and C, respectively (S 1). *Russula* was the most frequent ectomycorrhizal genus recorded under *U. bojeri* (32.9% of the above-ground sporophore diversity) followed by the genera *Amanita* (17.1%) and *Cantharellus* (Fig. 1a). Twenty-one different species were recorded for *Russula* followed by *Amanita* (14 species) and the genera *Cantharellus* and *Boletus* (10 species; Fig. 1b). AM structures (vesicles and hyphae) were detected from the roots in all surveyed sites.

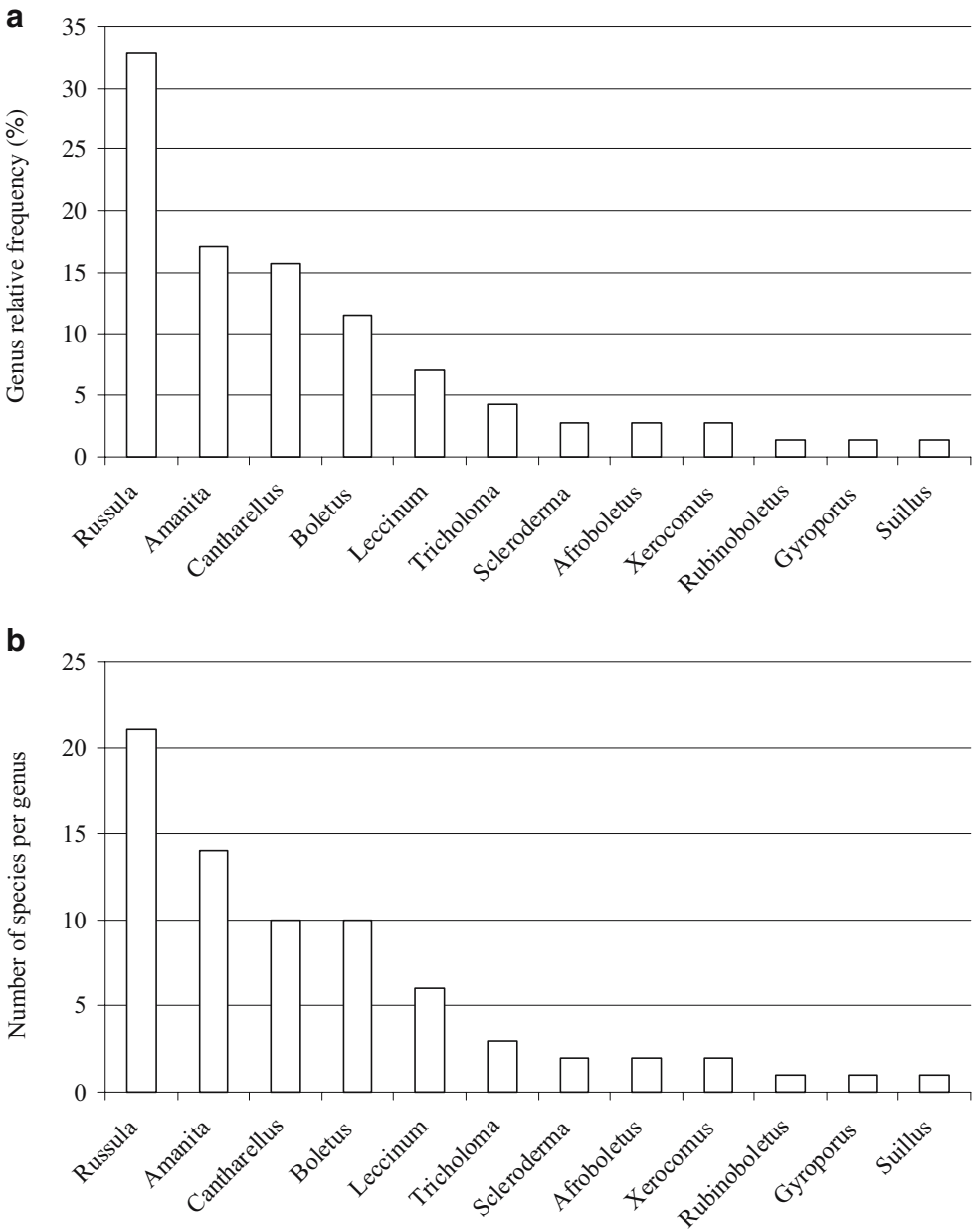

Fig. 1 **a** Structure of the ectomycorrhizal community (above-ground diversity) expressed as genus relative frequency (**b**). Number of species per genus

Time sequence of mycorrhizal colonization on *U. bojeri*

First, ECMs were recorded after 2 months (Fig. 2). Native ectomycorrhizal fungi colonized approximately 50% of the lateral roots sampled after 5 months of culture (Fig. 2). AM structures were also observed after 2 months of culturing (Fig. 2).

Mycorrhizal dependency of *U. bojeri* seedlings

The shoot dry weight of the plants inoculated with *G. intraradices* or *Scleroderma* sp. Sc1 was significantly higher than in the control treatment (Tables 2 and 3). Compared to the control treatment, the shoot growth of ectomycorrhized plants was stimulated 1.9 times, whereas it was 1.7 times for plants inoculated with *G. intraradices* (Table 2). When both fungal symbionts were co-inoculated, the shoot dry weight significantly increased over the single inoculation treatments (Table 2). The shoot dry weight increased 2.1 times compared to the mean shoot dry weight of the single fungus treatments (*G. intraradices* alone or *Scleroderma* sp. Sc1 alone). The dual fungal inoculation did not significantly modify the establishment of ectomycorrhizal and AM symbioses compared to the ectomycorrhizal or AM colonization rates measured in the single inoculation treatments (Table 2).

Influence of ectomycorrhizal inoculation on soil microbial catabolic diversity

The growth of *U. bojeri* seedlings was significantly higher in the native soil than in the autoclaved soil (Table 3). Ectomycorrhizal fungal inoculation significantly increased shoot biomass of *U. bojeri* seedlings. There were no significant interactions between the autoclaving and the inoculum treatments (Table 3).

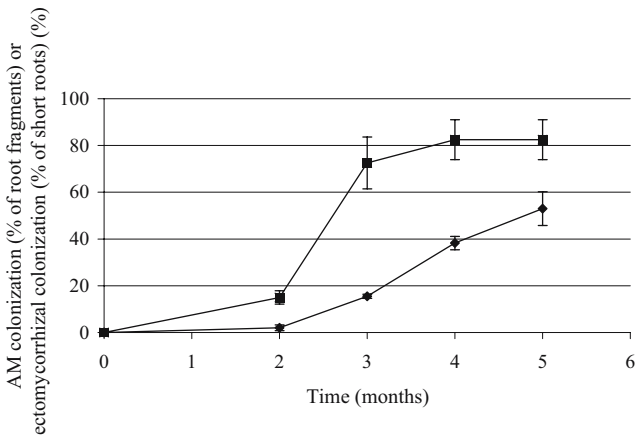

Fig. 2 Sequence of mycorrhizal colonization on *U. bojeri* seedlings in experiment 1 (*square*, AM colonization; *diamond*, total ectomycorrhizal colonization)

Table 2 Shoot growth, mycorrhizal development, and relative mycorrhizal dependency of *U. bojeri* seedlings 5 months after *G. intraradices* and/or *Scleroderma* sp. Sc1 inoculation in pot culture

Treatments	Shoot biomass (mg per plant)	Ectomycorrhizal colonization (%)	AM colonization (%)	RMD (%)[a]
Control	91.1a[b]	0a	0a	–
Scleroderma sp. Sc1	181.2b	8.7b	0a	47.6a
G. intraradices	160.1b	0a	77.5b	42.7a
Scleroderma sp. Sc1 + *G. intraradices*	360.3c	11.5b	82.5b	70.7b

[a] *RMD* Relative mycorrhizal dependency

[b] Data in the same column followed by the same letter are not significantly different according to the one-way analysis of variance ($p<0.05$).

Catabolic richness did not differ between the treatments (Table 3). However, catabolic evenness was significantly influenced by the soil treatments (autoclaved or not) and by the fungal inoculation (Table 4).

The BGA of the SIR responses for the four soil treatments are presented in Fig. 3. The map of the soil samples (Fig. 3b) shows that the four treatments (NDNI, NDI, DNI, and DI) were clearly separated. This result indicates that the microbial communities were different (in composition or at least in activity), according to the soil treatment. The map of the substrates (Fig. 3a) shows that, on the first axis, the use of four organic acids was highest in non-autoclaved soil samples and in inoculated samples (left part of the figure: ketobutyric, ketoglutaric, oxalic, and citric acids). The Monte Carlo test is significant ($p=0.025$). The soil autoclaving involved a lower rate of use of these four organic acids, whereas fungal inoculation led to a higher rate. Moreover, the effect of inoculation seemed stronger in non-disinfected soil samples.

Discussion

The main results of this study show that (1) a large diversity of sporophores was recorded under *U. bojeri*, (2) *U. bojeri* formed AMs and ECMs in natural soils, (3) this tree species is highly dependent on both types of mycorrhiza, and (4) controlled ectomycorrhization of *U. bojeri* strongly influences soil microbial catabolic diversity.

Our investigations show that forests dominated by *U. bojeri* contain a wide range of sporophores belonging to at least four different fungal families: Russulaceae, Cantharellaceae, Boletaceae, and Amanitaceae. In tropical forests, these families of putative ectomycorrhizal fungi have been

Table 3 Shoot growth, mycorrhizal development, and relative mycorrhizal dependency of *U. bojeri* seedlings 5 months after *Scleroderma* sp. Sc1 inoculation in disinfected or nondisinfected soil

Treatments	Shoot biomass (mg per plant)	Ectomycorrhizal colonization (%)	RMD (%)[a]	R[b]	E[c]
Disinfected soil					
Control	135a[d]	0a	–	28.7a	4.7a
Scleroderma sp. Sc1	192c	62.1c	29.1a	30.3a	6.9c
Nondisinfected soil					
Control	165b	18.2b	–	29.7a	6.1b
Scleroderma sp. Sc1	240d	58.6c	30.4a	30.7a	7.7d
Soil Treatment (ST)	S[e]	NS		NS	S
Fungal inoculation (FI)	S	NS		S	S
FI × ST	NS[f]	NS		NS	NS

[a] *RMD* Relative mycorrhizal dependency
[b] Catabolic richness
[c] Catabolic evenness
[d] Data in the same column followed by the same letter are not significantly different according to the one-way analysis of variance ($p<0.05$).
[e] Significant ($p<0.05$)
[f] Nonsignificant ($p<0.05$)

described under *Afzelia africana*, *Monotes kerstingii*, *Uapaca guineensis*, and *U. somon* in Africa (Thoen and Bâ 1989; Sanon et al. 1997) and in Asia under dipterocarps (Lee 1998). It is also well known that Russulaceae are often dominant in tropical rainforests of Africa, Asia, and Madagascar (Buyck et al. 1996; Lee et al. 1997; Watling and Lee 1998; Riviere et al. 2006). The identification of this group in the tropics remains problematic as many species are new and undescribed. A high diversity of ectomycorrhizal fungi was associated with *U. bojeri*. With other tropical ectomycorrhizal tree species, Lee et al. (1997) recorded only 28 fungal species under *Shorea leprosula*, and Sanon et al. (1997) had identified 14 fungal species under *U. guineensis* and 11 species under *U. somon* in Burkina Faso. However, numerous studies in temperate areas indicate little correlation between above-ground (sporophores) and below-ground (ECMs) fungal diversity (Buscot et al. 2000; Horton and Bruns 2001). Further molecular-based studies are needed to determine the fungal diversity of ECMs associated with *U. bojeri* in natural conditions.

Most mycorrhizal species are generally associated with only one type of mycorrhiza, usually either AMs or ECMs (Moyersoen and Fitter 1999). It has also been reported that some plant species formed both AM and ECM (Molina et al. 1992). The dual symbiotic association is well documented for *Populus* (Lodge and Wentworth 1990), *Salix* (Dhillion 1994), *Eucalyptus* (Lapeyrie and Chilvers 1985), *Alnus* (Molina et al. 1994), *Acacia* (Founoune et al. 2002a,b), Pinaceae (Cazares and Trappe 1993), *Quercus* (Egerton-Warburton and Allen 2001), and Casuarinaceae (Duponnois et al. 2003), but it was unknown for *U. bojeri*, although it was usually stated that this tree species was only colonized by ectomycorrhizal fungi (Moyersoen and Fitter 1999). But it has also been reported that roots of *U. guineensis* seedlings growing in a forest soil were only colonized by AM fungi (Moyersoen and Fitter 1999). The results of the present study confirmed the high occupancy of AM fungi recorded on young seedlings (3-month-old root systems) and that AM structures appeared for the first time on the plant culture followed by ECM colonization (Chilvers et al. 1987).

A synergistic effect of dual AM/ECM inoculation was described for *Acacia holosericea* inoculated with *G. fasciculatum* and *Pisolithus albus* (Founoune et al. 2002a, b), but the involved mechanisms remained unknown. In contrast, in 1-year-old field seedlings of *Quercus agrifolia* with a high glomalean and ectomycorrhizal fungal load, coexistent mycorrhizal types constituted a cost during the establishment of young oaks and potentially limited their development (Egerton-Warburton and Allen 2001). These authors suggested that the progressive shift to predominantly ectomycorrhizal colonization with increasing plant age become beneficial over time as it has been recorded with *U. bojeri* after AM/ECM inoculation in the present study.

Pirozynski and Malloch (1975) hypothesised that the AM habitat was a prerequisite for the early development of land flora. Soil nutrient distribution in natural environments is typically heterogenous (Farley and Fitter 1999), and mycorrhizas may allow plants growing in low nutrient patches to access resources in adjacent rich nutrient patches (Casper and Cahill 1998). In addition, ectomycorrhizal fungi are not uniformly distributed in terms of their presence, abundance, or community composition (Dickie and Reich 2005), and a lack of ectomycorrhizal fungi may slow the invasion of disturbed sites by ectomycorrhizal plants. Young seedlings of *U. bojeri* that form AM could survive in sites with low availability of ectomycorrhizal

Table 4 Description of putative ectomycorrhizal fungi collected from the three studied sites beneath *U. bojeri*

Species	Prominent features	Habitat	Sites		
			Site A	Site B	Site C
Amanitaceae					
Amanita rubescens Gray	White pinkish cap (8-cm diameter) covered with white powdered and flat scales, remnant veil visible at the margin, white stem reddening by wound, often eaten by insect larvae	Solitary, scarce		x	
Amanita virosa (Fr.) Bertillon	White yellowish fruiting body (7- to 12-cm diameter), white and chinated stem (1.2-cm diameter) with ring and cup at the base	Patch of 5 to 6 individuals	x	x	x
Amanita phalloides var. *verna* Bull	White fruiting body (5.5- to 11-cm diameter), stem (0.6 diameter by 9.5 cm high) with a large pendant ring and a bulbous cup at the base	Patch of 5 to 7 individuals	x	x	X
Amanita strobiliformis Bertillon	White and big fruiting body (10 to 12 cm diameter), fleecy remnant veil on the cap, club-shaped stem (2.2-cm diameter) with a ring	Solitary, scarce	x		
Amanita cf. *Baccata* (Fr.) Gillet	Big white fruiting body similar features than previous species but with no ring, stem (2-cm diameter by 7 cm high)	Solitary, scarce		x	
Amanita sp1	White finely scaled fruiting body (4- to 6- cm diameter) turning yellowish when ageing or by wound, concoloured gills and flesh	Solitary, scarce	x		
Amanita cf. *Strobiloceovolvata* Beeli	White fruiting body (8.5- to 11-cm diameter), stem (1.2-cm diameter by 10.5 cm high) without ring, well-developed bulbous cup at the base	Patch of 3 to 4 individuals	x	x	x
Amanita sp2	White and big species with a convex scaly cap (10- to 13-cm diameter by 9 to 10 cm high), strong bulbous stem (3- to 4-cm diameter) with a pendant ring	Solitary, scarce		x	
Amanita sp3	Pale grey cap (4.5-cm diameter) with few veil remanences on surface, bulbous stem (0.7 to 6 cm) with grey chinates	Solitary, scarce	x		
Amanita sp4	Yellow conical and mucronated cap (2.5- to 3-cm diameter), paler to whitish gills and stem (0.5-cm diameter by 12 cm high), white scaly basal cup	Solitary, scarce	x		
Amanita cf. *cecilia* (Berk. et Broome) Bas	Yellow grey cap (4- to 5-cm diameter) with rised scales, white gills and concoloured stem (0.7-cm diameter to 6 cm high), bulbous base covered by grey chinates and veil remanences	Solitary, scarce	x		
Amanita sp5	Convex and grey purplish-blue cap (4 to 4.5 cm diameter) with grey flat scales at the centre and hairy ones at the margin, white flesh and gills, white bulbous stem (0.9-cm diameter by 6 cm high) turning to grey by touch with a pendant ring	Solitary, scarce	x		
Amanita sp6	Small white species (2- to 3-cm diameter) with yellowish scales, bulbous based stem with pendant ring	Solitary, scarce		x	
Amanita sp7	Big white flat cap species (9- to 13-cm diameter) with veil remanences at the margin, strong bulbous stem (3- to 4-cm diameter) with a ring	Patch of 2 to 3 individuals	x	x	x
Boletaceae					
Rubinoboletus griseus	Big red-pink and grey-brownish dry and smooth cap (10- to 12-cm diameter by 8 to 9 cm high), white flesh (1.8 cm thick) partially burnishing after sectioning, pale reticulated hairy scaled stem, burnishing like pores by touch	Patch of 5 to 6 individuals	x	x	x
Gyroporus cf. *cyanescens* (Bulliard Fr.) Quélet	Big white yellowish smooth cap (10- to 12-diameter by 8–9 cm high), concoloured tubes and stem turning to blue by wound	Patch of 3 to 4 individuals	x	x	x
Boletus sp1	Brownish to brown cap, with large darker flat scales, cylindrical and dark stem, red reticulated, becoming yellow at the base like rhizomorph, flesh and pores turning blue by wound	Patch of 3 to 4 individuals	x		

Leccinum sp1	Small grey boletus (1.8- to 3-cm diameter by 3 to 4 cm high), yellow pores, red hairy scales on the stem, base of the stem yellow like the rhizomophs	Patch of 3 to 4 individuals	x	x	x
Boletus sp2	Big brownish-brown wet cap (7- to 8-cm diameter to 12 to 15 cm high), white and smooth flesh	Patch of 5 to 6 individuals	x		
Xerocomus sp1	Brown scaly cap (8.5-cm diameter) showing white flesh between scales, white stem (1.4-cm diameter by 5 to 6 cm high) with some red zone	Solitary, scarce	x		
Leccinum sp2	Yellow grey scaled boletus (4.5- to 6-cm diameter by 6 to 7 cm high), stem yellow at the base and red in its upper part, yellow blueishing pores	Solitary, scarce	x		
Boletus sp3	Brown cap (7.5-cm diameter) with red-pink pigments, yellow and red pores, greenishing and blueishing tubes, yellowish stem with some red pigments	Solitary, scarce	x		
Leccinum sp3	Red purplish-blue wet cap (7-cm diameter), yellow burnishing stem (0.8-cm diameter by 6 cm high), concoloured yellow flesh and pores, blueishing after air exposure	Solitary, scarce	x		
Boletus sp4	Big smooth and shiny red boletus (8- to 12-cm diameter by 7 to 8 cm high), yellowish stem with some pink pigments, concoloured flesh (1.6 cm thick)	Patch of 2 to 3 individuals	x	x	x
Xerocomus sp2	Pale to dark brown scaly dry cap (5-cm diameter), white dirty stem (0.8-cm diameter by 4 cm high) with a white-yellowish flesh, yellow greenish and pink pores	Solitary, scarce	x		
Boletus sp5	Yellowish brown cap (8-cm diameter) with flat partially pink scales, yellow pores and stem (1.2-cm diameter by 6 cm high), white flesh (1.6 cm thick)	Solitary, scarce		x	
Boletus sp6	Dark brown scaly cap showing yellow flesh, pale concoloured pores and stem	Solitary, scarce			x
Boletus sp7	Brown boletus with dry and silky cap (4.5-cm diameter), concoloured dark stem (2.2-cm diameter by 5.2 cm high), white flesh (1.6 cm thick) rapidly turning to red, then black after air exposure	Solitary, scarce			x
Boletus sp8	Pale brown boletus with silky cap (5-cm diameter), white stem (1.5-cm diameter by 5.2 cm high) and flesh (1.3 cm thick) turning purplish-blue after air exposure	Solitary, scarce			x
Leccinum sp4	Yellow and wet cap (3.5 cm) with hairy grey scales, yellow pores, yellow and red stem (0.5-cm diameter) with dark scales and a narrow base	Solitary, scarce			x
Leccinum sp5	Yellowish-brown dry cap (3.5-cm diameter), red pores and lighter stem (0.6-cm diameter by 4 cm high) turning to dark-brownish in section, white flesh turning burnish after air exposure	Solitary, scarce			x
Suillus sp2	Yellow and grey scaly cap (5-cm diameter), yellow pores covered by a yellow partial veil when young, yellow stem (1.4-cm diameter by 4.5 cm high) with greenish grey scales, becoming very slimy	Patch of 2 to 3 individuals		x	
Boletus sp9	Yellow brownish boletus (7- to 8-cm diameter) with a sticky surface, yellow pores and stem, yellowish flesh (1.7 cm thick)	Solitary, scarce		x	
Leccinum sp6	Pale brown cap (5- to 4-cm diameter) with red brownish scales at the centre, white pores and white flesh turning rapidly to red, then black by wound	Solitary, scarce		x	
Boletus sp10	Yellow brown boletus (4.5- to 5.7-cm diameter) with wet and smooth surface, yellow pores, yellow stem (1.2 diameter by 3 cm high), white flesh (1 cm thick)	Solitary, scarce		x	
Cantharellaceae					
Cantharellus sp1	Tall thick and lobed fasciculate bright yellow caps (4- to 6-cm diameter) forming patches of 4 to 5 individuals (12 cm), grained gills, pale yellow stem (1.8 cm), white flesh	Patch of 8 to 10 individuals	x	x	x
Cantharellus sp2	Small orange-brownish cap (2- to 2.2-cm diameter), white pinkish gills, pink stem and white flesh	Solitary, scarce	x		
Cantharellus sp3	Yellowish to pale brown cap (3.5- to 3.2-cm diameter), yellow grained gills, pale yellow stem (0.6 to 2.5 cm)	Solitary, scarce	x	x	x

Table 4 (continued)

Species	Prominent features	Habitat	Sites		
			Site A	Site B	Site C
Cantharellus sp4	Red orange cap (3.2- to 3.5-cm diameter), largely spaced yellowish grained gills, pale yellow to reddish stem (0.9 cm)	Patch of 8 to 10 individuals	x	x	x
Cantharellus sp5	Pale brown cap (3.2- to 3.5-cm diameter), pale pink grained gills, white stem and flesh, turning to yellow by touch or sectioning	Solitary, scarce	x	x	x
Cantharellus sp6	Red pinkish fasciculate caps (2.5-cm diameter) forming small patch (3.5 to 4 cm), yellowish grained gills, pink orange stem and white fibrous flesh	Patchy			x
Cantharellus sp7	Small and fragile bright yellow cap (2- to 3.2-cm diameter), pale yellow gills, concoloured short stem (0.3 cm)	Solitary, scarce			x
Cantharellus cf *decolorans* Eyss. et Buyck	Small pink orange cap (0.7- to 1.5-cm diameter, 2.5 to 3.5 cm high), concoloured gills and short stem (0.2 cm)	Patch of 5 to 6 individuals		x	
Cantharellus cf. *Cyanoxanthus* R. Heim	Yellow and purple cap (4-cm diameter), pale pink grained gills, pale yellow stem (1.8 cm), fibrous flesh	Patch of 2 to 3 individuals			x
Cantharellus rubber R. Heim	Pale pink cap (3.5- to 4-cm diameter), concoloured stem and gills	Patch of 2 to 3 individuals			x
Russulaceae					
Russula subfistulosa Buyck	White-greyish (darker at the centre) umbilicated cap (3- to 12-cm diameter)	Solitary to patch of 3 individuals	x	x	x
Russula ochraceorivulosa	Greyish to purplish-blue grey cap (7- to 8-cm diameter), convex cap with an undulating margin	Solitary	x	x	x
Russula patouiillardi	Pale yellow and purple (darker at the centre) dry scaly cap, white and purple stem	Solitary to patch of 5 individuals	x	x	x
Russula liberiensis Buyck	White-greyish fibrillose cap (3- to 12-cm diameter) turning brown when ageing, closely spaced decurrent gills	Solitary to patch of 3 individuals	x	x	x
Russula cf. *Cyanoxantha*	Pink to purple-red cap (5- to 15-cm diameter), white stem	Patch of 2 to 3 individuals	x		
Russula cellulata Buyck	Brown scaly cap (3- to 9-cm diameter), closely spaced decurrent gills	Patch of 2 to 3 individuals	x		
Russula cf. *archae R. Heim*	White smooth and flat cap (4.5- to 6-cm diameter)	Solitary	x		
Russula cf. *nigricans*	White-greyish cap turning to brown when ageing, white flesh turn rapidly pink to red by air exposure	Solitary	x		
Russula cf. *subfistulosa*	White-greyish convex cap (3- to 8-cm diameter)	Solitary to patch of 4 individuals	x		
Russula sp3	White to pale yellow gluey and convex cap (3- to 13-cm diameter)	Solitary to patch of 3 individuals	x		
Russula sp5	Yellow smooth umbilicated cap (6- to 12-cm diameter), with a very regular margin	Patch of 2 to 3 individuals	x	x	x
Russula sp6	White-yellowish flat or slightly umbilicated cap (4- to 10-cm diameter), white flesh turning reddish after air exposure	Patch of 3 to 5 individuals	x		
Russula sp7	Dark grey to brown convex cap (3.5- to 8-cm diameter), involucrated margin, wet surface covered by orange to yellow layers, white-yellowish flesh	Solitary, rarely patchy	x		
Russula sp8	White convex to slightly umbilicate cap (4- to 13-cm diameter) turning brown when ageing, smooth surface with involucrated margin, white flesh turning reddish after air exposure	Solitary, scarce	x		

Russula sp10	Dark grey to brown when fully mature convex to flat cap (4- to 9-cm diameter), white flesh	Patch of 2 to 4 individuals	x		
Russula sp11	Small purple to purple-reddish umbilicate when young to flat when ageing cap (2- to 7-cm diameter), sticky surface, regular margin, adnate white to yellowish closely spaced gills, white flesh	Solitary to patch of 3 individuals	x		
Russula sp13	Brown-reddish convex and smooth glutinous cap (6- to 15-cm diameter), decurrent gills, white flesh turning greyish by air exposure	Solitary		x	
Russula sp14	Dark yellow to brown convex to flat sticky cap (4- to 10-cm diameter), adnate closely spaced gills	Patch of 3 to 5 individuals			x
Russula sp15	Yellow to orange-yellow flat slightly umbilicated with an involucrated yellow margin cap (2- to 8-cm diameter) with a smooth surface with small strias	Patch of 2 to 5 individuals			x
Russula sp16	Pink to reddish (darker at the centre) fragile convex glutinous cap, (2- to 6-cm diameter) with a smooth or dusty surface, white flesh	Patch of 2 to 4 individuals			x
Russula sp17	Slightly umbilicated convex and glutinous cap (4- to 10-cm diameter), dark yellow tending to brown, yellow to pale orange closely spaced gills	Solitary to patch of 4 individuals			x
Strobilomycetacea					
Afroboletus sp1	Brown-purple scaly cap (3- to12-cm diameter), fibrous stem, pale yellow flesh turning purplish by air exposure	Patch of 3 to 5 individuals	x		
Afroboletus sp2	Flat-convex dusty cap (3 to 10 cm diameter) with dark-brown to black scales, fibrous stem inflated at the base, greyish-yellow flesh	Patch of 2 to 3 individuals			x
Sclerodermataceae					
Scleroderma sp1	Whitish to yellowish small pyriformic fruit bodies, size below 3 cm in diameter, dark grey gleba	Solitary to patch of 5 individuals			x
Scleroderma sp2	Whitish to yellowish 3- to 7-cm diameter fruit bodies with grey spots at the top, dark grey gleba	Solitary, rarely patchy	x		
Tricholoma sp2	Yellow cap (3- to 9-cm diameter), dry surface, involucrated margin, thick widely spaced gills, yellow flesh keeping yellow even after exposure to air	Solitary to patch of 4 individuals	x		
Tricholoma sp3	Yellow-greyish cap (3- to 12-cm diameter), dry surface, white yellowish stalk, white flesh	Solitary to patch of 4 individuals		x	
Tricholoma sp4	Dark-grey cap (3- to 15-cm diameter), smooth dry surface, thick gills, white flesh	Solitary		x	

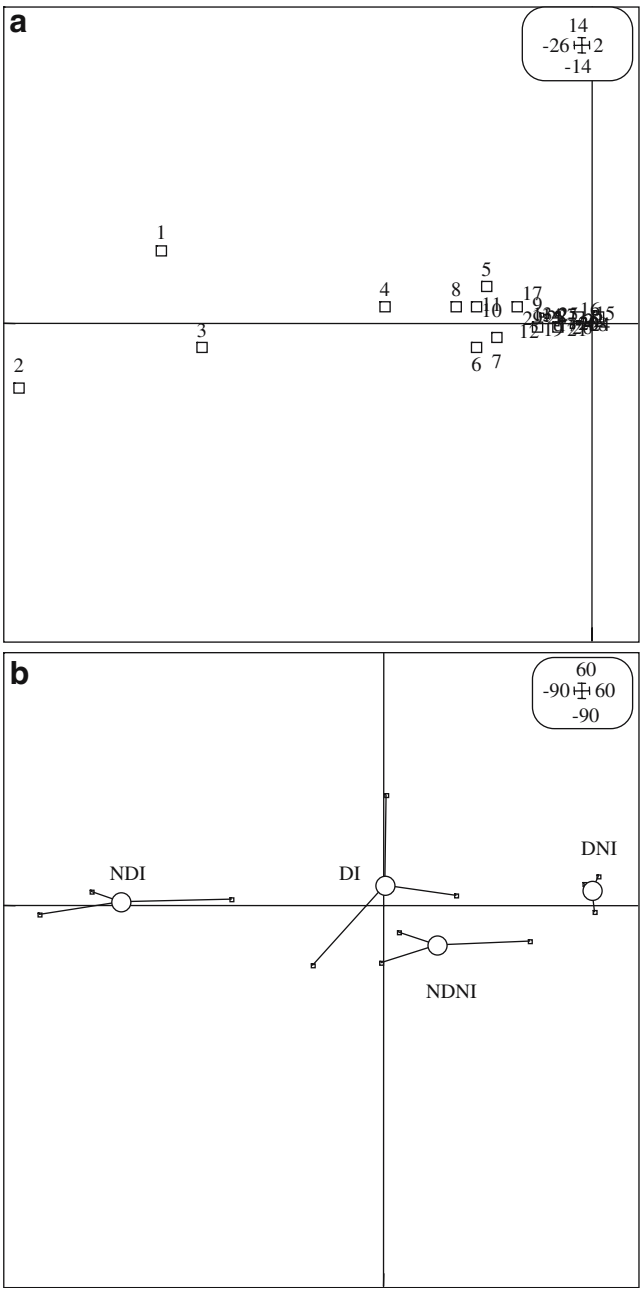

Fig. 3 BGA of the SIR responses with respect to the fungal treatments and soil treatments (*DNI* disinfected soil without fungal inoculation, *DI* disinfected soil with fungal inoculation, *NDNI* nondisinfected soil without fungal inoculation, *NDI* nondisinfected soil with fungal inoculation, *NIND*: *1* Ketobutyric acid, *2* ketoglutaric acid, *3* oxalic acid, *4* citric acid, *5* phenylalanine, *6* gluconic acid, *7* glucose, *8* uric acid, *9* malic acid, *10* asparagine, *11* tartaric acid, *12* malonic acid, *13* gallic acid, *14* formic acid, *15* cystein, *16* histidine, *17* sucrose, *18* tyrosine, *19* glutamic acid, *20* succinic acid, *21* glucosamine, *22* succinamide, *23* mannose, *24* glutamine, *25* quinic acid, *26* lysine, *27* ascorbic acid, *28* serine, *29* arginine, *30* fumaric acid, *31* hyroxybutyric acid

fungi and develop ectomycorrhizas later as roots contact residual ECM communities. This mycorrhiza successional process would promote the development of subsequent ectomycorrhizal fungus communities and facilitate the establishment or re-establishment of the seedlings of ectomycorrhizal tree species after the disturbance (Perry et al. 1989), thus, influencing plant succession from prairie or old field to savanna or woodland.

Scleroderma species are considered "early-stage" symbionts (Deacon et al. 1983; Bâ et al. 1991) and can form mycorrhizas with a wide range of tropical tree species such as *Afzelia africana* (Bâ and Thoen 1990), *A. quanzensis*, *Isoberlinia doka*, *I. dalziellii*, and *Brachystegia speciformis* (Sanon et al. 1997). In the present study, *Scleroderma* isolate Sc1 increased *Uapaca* growth in disinfected and in non-disinfected soil, suggesting that this fungal strain was highly competitive against the native ectomycorrhizal mycota at least under the conditions of this pot-based experiment. In addition, ectomycorrhizal inoculation induced strong modification of the soil microflora functionalities and increased its catabolic microbial diversity. Elliott and Lynch (1994) hypothesised that microbial communities with reduced catabolic evenness are less resistant to stress and disturbance. Microbial functional diversity is involved in a large range of activities such as nutrient transformation, decomposition, etc. (Wardle et al. 1999). In particular, ectomycorrhizal fungi mobilize P and other essential plant nutrients directly from minerals through the excretion of organic acids (Landeweert et al. 2001). Amongst the total organic acids in the soil solution, low molecular weight organic acids are considered to be the most important biological weathering agents (Ochs 1996). Oxalate, citrate, and malate produced by plant roots and soil microorganisms are the strongest chelators of trivalent metals (Landeweert et al. 2001). Oxalic acid, commonly produced by many different fungal species, has the highest acid strength (Dutton and Evans 1996). In the present study, SIR responses with all oxalic and citric acids increased in the fungal inoculated soil, suggesting that *Scleroderma* Sc1 and its associated microflora excreted higher amounts of such organic acids and induced a multiplication of microorganisms that utilize these available organic resources than noninoculated soil.

In conclusion, this study showed that *U. bojeri* has a complex symbiotic status that can be managed to optimize its development in degraded areas. In addition, the use of selected mycorrhizal fungi such the *Scleroderma* Sc1 isolate in nursery conditions could be of great interest, as (1) this fungal strain appears competitive against native symbiotic microflora and (2) the fungal inoculation improves the catabolic potentialities of the soil microflora. However, further studies are needed to describe the belowground diversity of ectomycorrhizal fungi and to demonstrate the potential interest of controlled mycorrhization in natural conditions in afforestation programs with *U. bojeri* in Madagascar.

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