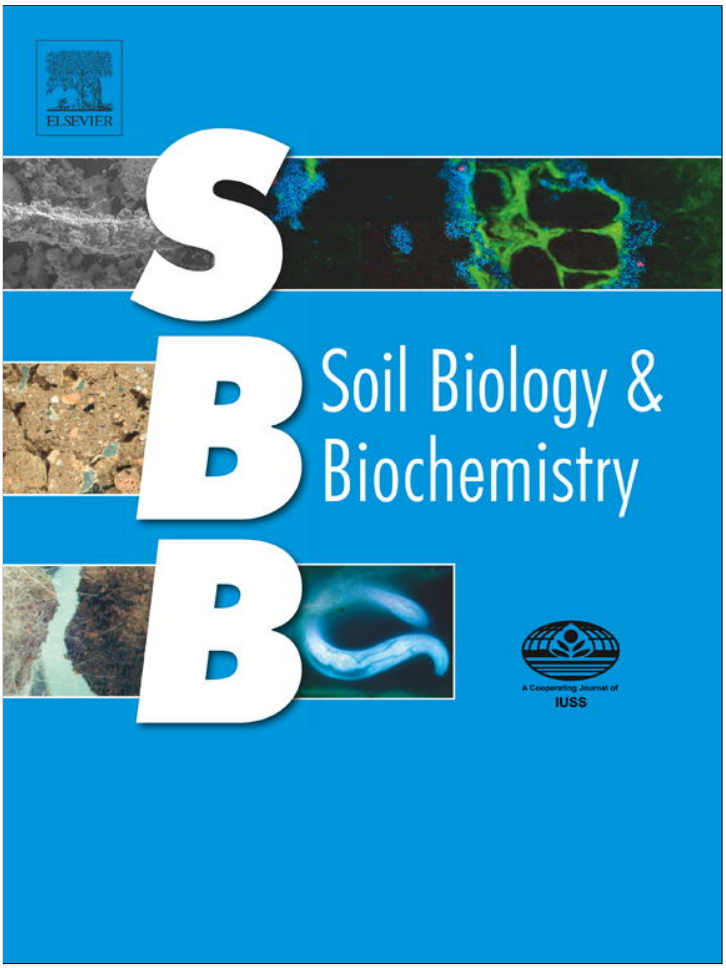
Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



(This is a sample cover image for this issue. The actual cover is not yet available at this time.)

This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/copyright

Author's personal copy

Soil Biology & Biochemistry 57 (2013) 468–476

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Ectomycorrhizal diversity enhances growth and nitrogen fixation of *Acacia mangium* seedlings

N. Diagne [a,b], J. Thioulouse [c], H. Sanguin [d], Y. Prin [d], T. Krasova-Wade [a], S. Sylla [a,b], A. Galiana [d], E. Baudoin [e], M. Neyra [e], S. Svistoonoff [f], M. Lebrun [e], R. Duponnois [e,*]

[a] IRD, Laboratoire Commun de Microbiologie IRD/ISRA/UCAD, Centre de Recherche de Bel Air, BP 1386, Dakar, Senegal
[b] Université Cheikh Anta Diop (UCAD), Faculté des Sciences et Techniques, Département de Biologie Végétale, BP 5005, Dakar, Senegal
[c] Université Lyon 1, CNRS, UMR5558, Laboratoire de Biométrie et Biologie Evolutive, F-69622 Villeurbanne, France
[d] CIRAD, Laboratoire des Symbioses Tropicales et Méditerranéennes (LSTM), UMR 113 CIRAD/INRA/IRD/SupAgro/UM2, Campus International de Baillarguet, TA A-82/J Montpellier, France
[e] IRD, Laboratoire des Symbioses Tropicales et Méditerranéennes (LSTM), UMR 113 CIRAD/INRA/IRD/SupAgro/UM2, Campus International de Baillarguet, TA A-82/J Montpellier, France
[f] IRD, UMR 188 – DIADE, INRA/IRD/SupAgro/UM2, Centre IRD France-Sud, BP 64501, Montpellier, France

ARTICLE INFO

Article history:
Received 26 March 2012
Received in revised form
25 August 2012
Accepted 29 August 2012
Available online 27 September 2012

Keywords:
Ectomycorrhizal fungal diversity
Nitrogen fixation
Tree growth

ABSTRACT

Increasing interest has been given so far to the role of mycorrhizal symbiosis on plant diversity and ecosystem productivity. However much remains unknown about the effect of ectomycorrhizal fungal diversity on plant growth and rhizobial symbiosis. The purpose of this study is to investigate the influence of ectomycorrhizal diversity on root nodulation and plant nutrient uptake during plant growth. A gradient of ectomycorrhizal diversity was created (ranged from one to six fungal isolates) on Australian Acacia (*Acacia mangium*) seedlings grown on a phosphate-deficient soil in controlled conditions. The results clearly show (i) an enhancement of *A. mangium* growth due to ectomycorrhizal fungal inoculation, (ii) as well as significant effects of increasing ectomycorhizal diversity and soil ectomycorrhizal mycelium density on whole plant biomass and plant nutrient uptake. They also reveal an effect of ectomycorrhizal diversity effect on the functioning of the nitrogen-fixing symbiosis. These results emphasize the need to manage soil ectomycorrhizal potential (abundance and diversity of ectomycorrhizal mycelium) in forest management practices in order to optimize N_2 fixation and seedling growth, leading thus to dramatic improvements in outplanting performances with fast-growing tree legumes on various reafforestation sites, more particularly in tropical and Mediterranean areas.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

The nature of relationships between biodiversity and plant productivity has aroused general interest but the majority of experimental studies have primarily focused on aboveground plant diversity (Daft, 1991; Tilman et al., 1996; Hooper and Vitousek, 1997, 1998; Wardle et al., 2000). The crucial role of belowground microbial communities in terrestrial ecosystem functioning (van der Heijden et al., 2008), such as nutrient uptake (Sprent, 2001), carbon cycling (Högberg et al., 2001), nitrogen cycling (Kowalchuk and Stephen, 2001), and soil formation (Rillig and Mummey, 2006) has only been recently investigated. Mycorrhizal fungi form an important component of the sustainable soil–plant-system (Bruno et al., 2003; Finlay, 2008) forming symbiotic relationships with about 80% of terrestrial plant species (Smith and Read, 2008) and mainly enhancing plant nutrient uptake, especially of phosphorus (Smith and Read, 2008). The benefits of mycorrhizal symbiosis to the host plant not only result from a close relationship between the fungal symbiont and plant species but also from the interactions between soil bacterial and fungal communities (Garbaye, 1991; Founoune et al., 2002a,b; Frey-Klett et al., 2005; André et al., 2005). It is well known for instance that mycorrhizal symbiosis can dramatically increase rhizobial symbiosis (nodulation and N_2 fixation) (André et al., 2005).

Deciphering the relationships between mycorrhizal symbiosis, plant diversity and ecosystem productivity is of major interest. van der Heijden et al. (1998, 2002, 2003) demonstrated that species composition and species richness of arbuscular mycorrhizal communities are essential factors in determining the plant composition and the ecosystem variability and productivity. The

* Corresponding author. Tel.: +33 4 67 59 37 86; fax: +33 4 67 59 38 02.
E-mail address: Robin.Duponnois@ird.fr (R. Duponnois).
URL: http://www.mpl.ird.fr/lstm/

0038-0717/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.soilbio.2012.08.030

Author's personal copy

other major form of mycorrhizas, the ectomycorrhizas, is also considered as an important ecological factor influencing plant community dynamics since the vegetation with which they are associated is unlikely to survive without ectomycorrhizal symbionts (Smith and Read, 2008). Ectomycorrhizal fungi affect trees and shrubs, gymnosperms (Pinaceae) and angiosperms, and usually this symbiosis results from the association of Homobasidiomycetes with about 20 families of mainly woody plants. It has been reported that a relatively high diversity of ectomycorrhizal fungi (estimated 5000–6000 species worldwide, mainly Basidiomycetes and Ascomycetes) contracted symbiotic relationships with these woody species (Molina et al., 1992; Allen et al., 1995; Valentine et al., 2004). On a root system and within a few centimeters may coexist more than 5–10 different ectomycorrhizal fungal species (Allen et al., 1995). However, few studies have been performed to test the influence of multiple ectomycorrhizal fungal inoculations on plant growth (Baxter and Dighton, 2001, 2005). For instance, it has been reported that the growth of Douglas fir seedlings inoculated with two ectomycorrhizal species was larger than that recorded with plants inoculated with a single fungal species (Parlade and Alvarez, 1993). Jonsson et al. (2001) have investigated the influence of ectomycorrhizal diversity on the growth of *Betula pendula*. They reported that the tree species growth was higher when seedlings were inoculated with eight ectomycorrhizal fungi compared to the plants inoculated with single fungal species. All these data suggest that ectomycorrhizal diversity plays a significant role in the host development. However, these experiments remain very scarce. They have been performed with only a few tree species such as *Pinus radiata* (Chu-Chou and Grace, 1985), *Pinus patula* (Reddy and Natarajan, 1997), and *B. pendula* (Jonsson et al., 2001). It is well known that many N_2-fixing trees are especially dependent on mycorrhizas to take up nutrients other than N required for plant growth and efficient N_2 fixation (Smith and Read, 2008). However the influence of ectomycorrhizal diversity on growth response and on the rhizobial symbiosis development of fast-growing leguminous trees remains largely unknown.

It has been shown in controlled glasshouse conditions that mycorrhizal symbiosis can significantly increase the growth of Australian Acacia which are frequently used in agroforestry plantations to rehabilitate degraded soils in the tropics (Duponnois et al., 2000; Duponnois and Plenchette, 2003). The *Acacia* species are able to grow on low-N soils thanks to their capacity to form root symbiosis with rhizobial bacteria. As other tree genera native from Australia (i.e. *Casuarina* and *Eucalyptus*), they can be associated with either ectomycorrhizal and/or arbuscular mycorrhizal fungi (Ducousso, 1991). The sustainability of the mycorrhizal promoting effect has also been assessed in field conditions (Duponnois et al., 2005, 2007). However, these experiments have been mainly conducted with plants mycorrhized with a single efficient fungal species.

The aim of this study was to investigate the influence of ectomycorrhizal diversity on *Acacia mangium* plant growth, root nodulation by a strain of *Bradyrhizobium* sp. as well as plant nutrient uptake by creating a gradient of ectomycorrhizal fungal diversity (ranged from one to six fungal isolates). We hypothesized that the influence of ectomycorrhizal fungal diversity on plant performance and root nodulation was not an additive positive effect of each fungal isolate but result from complex interactions between the ectomycorrhizal symbionts, the bradyrhizobial strain and the host plant.

2. Materials and methods

2.1. Plant species

Seeds of *A. mangium* Willd collected in the Bayotte Forest (Casamance, Sénégal) were surface sterilized with 95% concentrated sulfuric acid for 60 min. Then, the acid solution was removed and the seeds were rinsed for 12 h four times with sterile distilled water. Surface-sterilized seeds were then aseptically sown in Petri dishes filled with 1% (w/v) agar and germinated in the dark at 25 °C for 4 days. The seedlings were used when taproots were 1–2 cm long.

2.2. Fungal inocula and rhizobia

The origins of the ectomycorrhizal fungi are indicated in Table 1. The fungal strains were maintained in Petri dishes on Modified Melin-Norkrans (MMN) agar medium at 25 °C in the dark. Fungal inoculum was grown in vermiculite–peat moss (4:1, v/v) moistened to field capacity with 450 mL MMN per liter of vermiculite (Duponnois and Garbaye, 1991). The inoculum was incubated at 28 °C in the dark for 6–8 weeks until the substrate was fully colonized. The rhizobial strain *Bradyrhizobium* sp. Aust 13C was isolated from nodules collected in natural stands of *A. mangium* in Australia (Galiana et al., 1990, 1994). The strain was cultured in glass flasks containing liquid yeast extract-mannitol medium at 28 °C on an orbital shaker (Vincent, 1970).

2.3. Glasshouse experiments

2.3.1. Experiment 1: relationships between fungal inoculum density, plant growth and nodulation parameters

The aim of these experiments was to determine the relationships between the abundance of ectomycorrhizal propagules in the soil and the growth response of the host plant. To obtain a scale of inoculum density, the disinfected soil was mixed with 0, 1.25, 2.5, 5

Table 1
Origins of the six ectomycorrhizal isolates tested in the present study.

Species and herbarium ID	Host tree	Origin	Collectors
Pisolithus albus IR100 (Martin et al., 2002)	*Acacia mangium*	Casamance, Senegal	R. Duponnois & A.M. Bâ[a]
Scleroderma dictyosporum pat. IR109	*Afzelia africana*	Houet, Burkina Faso	A.M. Bâ[a]
S. verrucosum Bull. (Pers.) sensu Grev. IR500	*Uapaca somon*	Comoé, Burkina Faso	K. Sanon[b]
Scleroderma sp1 IR409	*Uapaca guineensis*	Houet, Burkina Faso	K. Sanon[b]
Scleroderma sp1 IR406	*Uapaca guineensis*	Houet, Burkina Faso	K. Sanon[b]
Scleroderma sp. SC2	*Uapaca bojeri*	Arivonimamo, Madagascar	H. Ramanankierana[c]

[a] Université des Antilles et de la Guyane, BP 592, 97159 Pointe à Pitre, Guyane, France. Present address: IRD, Laboratoire Commun de Microbiologie IRD/ISRA/UCAD, Centre de Recherche de Bel Air, BP 1386, Dakar, Sénégal.
[b] INERA, Laboratoire de Microbiologie forestière, BP 7047, Ouagadougou, Burkina Faso.
[c] Laboratoire de Microbiologie de l'Environnement, Centre National de Recherches sur l'Environnement, BP 1739, Antananarivo, Madagascar.

Author's personal copy

and 10% (v/v) fungal inoculum, and for each inoculum rate, the amount of peat–vermiculite mixture added to the cultural substrate was adjusted to 10% (v/v) with moistened (MMN medium) vermiculite–peat mixture (4:1; v/v) without fungal mycelium.

For each soil fungal strain and each inoculum density, *Acacia* seedlings were individually grown in 0.25 L pots filled with a sandy soil mixed with the required volume of fungal inoculum. The soil was collected from a stand of *Acacia holosericeae* located east of Dakar. After sampling it was crushed, passed through a 2 mm sieve, autoclaved for 60 min at 120 °C to eliminate the native microbiota and stored for a week in a dry room to avoid any soil toxicity. After autoclaving, soil physico-chemical characteristics were as follows: pH (H_2O) 5.3; clay (%) 3.6; fine silt (%) 0.0; coarse silt (%) 0.8; fine sand (%) 55.5; coarse sand (%) 39.4; carbon (%) 0.17; nitrogen (%) 0.02; total phosphorus 39 mg kg^{-1}; soluble phosphorus (Olsen) 4.8 mg kg^{-1}. The pots were arranged in a randomized complete block design with five replicates per treatment. The plants were placed in a glasshouse (28 °C day, 18 °C night, 10-h photoperiod) and watered regularly with distilled water. After one week of plantation, the young seedlings were inoculated with 5 mL of late log-phase *Bradyrhizobium* strain Aust 13C (10^7 CFU mL^{-1} approximately).

After 4 months of culturing, the height of the plants and the oven-dried weight (1 week at 65 °C) of shoot were recorded. Then the root systems were gently washed in tap water. Root nodules were counted. The root systems of individual seedlings were cut into 1-cm root pieces. The percentage of ectomycorrhizal root tips [colonization index = (number of ectomycorrhizal root tips/total number of root pieces) × 100] was assessed under a stereomicroscope (magnification ×40) on a random sample of at least 100 root tips per plant. Then root dry weight (1 week at 65 °C) was determined for each plant.

2.3.2. Experiment 2: ectomycorrhizal diversity effect on the growth and nodulation of A. mangium seedlings

The cultural conditions of *A. mangium* seedlings and the experimental design were the same as described above (see experiment 1). The ectomycorrhizal species combination treatments provided a diversity gradient from one to six species with all the species previously tested in monoculture (Table 1). This experimental design allowed comparing the effect of the multiple fungal species with that recorded with each of the fungal species in monoculture. It also allowed avoiding the "sampling effect" (Huston, 1997; Wardle, 1999). For the single fungal species treatments, the soil was mixed with 10% (v/v) fungal inoculum or 10% moistened (MMN medium) vermiculite–peat mixture (4:1; v/v) for the control treatment. For the other ectomycorrhizal diversity treatments, the soil was mixed using an additive design where the amount of fungal inoculum was the same across treatments as indicated in Table 2. All the pots were inoculated with *Bradyrhizobium* sp. strain Aust 13C as described above.

After 4 months of plantation, the seedlings were harvested and the growth parameters (height, shoot biomass, root biomass) were measured. The ectomycorrhizal colonization index was determined as described above. Root nodules were counted and surface sterilized by immersion in 0.1% (w/v) $HgCl_2$ for 30 s, washed in sterile water and then in 96% ethanol for 2–3 min to finally be rinsed in sterile water and stored at −80 °C in 20% glycerol (v/v) for further analyses. Dried ground plant tissues were ashed at 500 °C, digested in 2 mL of HCL (6 N) and 10 mL of HNO_3 (N), then analyzed by colorimetry for phosphorus (John, 1970). For nitrogen (Kjeldhal) determination, dried ground plant tissues were digested in 15 mL H_2SO_4 (36 N) containing 50 g L^{-1} of salicylic acid.

Table 2
Fungal species combinations and fungal inoculum rates used in the ectomycorrhizal diversity experiment.

Treatment	Species richness	Inoculation rates of each ectomycorrhizal strains (%)					
		IR100[a]	IR109[b]	IR500[c]	IR409[d]	IR406[e]	SC2[f]
A	0	0	0	0	0	0	0
B	1	10	0	0	0	0	0
C	2	5	5	0	0	0	0
D	3	3.3	3.3	3.3	0	0	0
E	4	2.5	2.5	2.5	2.5	0	0
F	5	2	2	2	2	2	0
G	6	1.66	1.66	1.66	1.66	1.66	1.66

[a] *Pisolithus albus* IR100.
[b] *Scleroderma dictyosporum* pat. IR109.
[c] *S. verrucosum* Bull. (Pers.) sensu Grev. IR500.
[d] *Scleroderma* sp1 IR409.
[e] *Scleroderma* sp1 IR406.
[f] *Scleroderma* sp. SC2.

2.4. Assessment of Bradyrhizobium sp. strain Aust 13C root colonization

PCR and Southern hybridization methods were used for studying the distribution of *Bradyrhizobium* sp. Aust 13C in each treatment.

2.4.1. DNA extraction

Twenty nodules were randomly collected in each treatment and crushed in a drop of sterile distilled water. For the DNA extraction, 150 μL of 2 × CTAB/PVPP buffer (0.2 M Tris–HCl, pH 8; 0.04 M EDTA; pH 8; 2.8 M NaCl; 4% (w/v) CTAB; 2% (w/v) PVPP) were added to 300 μL of crushed nodule. The homogenate was incubated at 65 °C for 60 min and centrifuged for 10 min at 11,000 g to remove cell debris. Supernatant was taken and an equal volume (300 μL) of phenol–chloroform–isoamyl alcohol (25:24:1; v/v/v) was added. The samples were homogenized and centrifuged for 15 min at 13,000 g. The aqueous phase containing nucleic acids was transferred in a new tube and 300 μL of chloroform–isoamyl alcohol (24:1; v/v) was added, then centrifuged for 15 min at 13,000 g. DNA was finally precipitated overnight at −20 °C in a solution containing 0.1 volume of sodium acetate and 2.5 volumes of absolute ethanol. The samples were centrifuged for 30 min at 13,000 g at 4 °C. The resulting DNA pellet was washed with 70% (v/v) ethanol by centrifugation for 15 min at 13,000 g at 4 °C, vacuum dried, and solubilized in 20 μL of ultrapure water. The DNA purity and quantity were assessed by spectrophotometry.

2.4.2. Probe design

A strain-specific DNA oligonucleotide probe targeting the ITS 16S–23S rDNA of the strain Aust 13C was designed and its theoretical specificity was evaluated by alignment against the GenBank database by using the algorithm BLAST (Altschul et al., 1997). The probe 5′-CGCTTGTTCATCGCGGCTCATCG-3′ has been synthesized and labeled by with 5′, 3′ and internal Dig-labeling (Eurogentec, Seraing, Belgium). The hybridization conditions have been determined empirically by probe hybridization on strain DNA with a hybridization temperature of 52 °C.

2.4.3. Slide preparation by "dot blotting"

The DNA extracted from nodules randomly collected in each treatment was denatured by incubation at 100 °C for 10 min and cooled in ice for 10 min before dotting to 0.45 μm Biodyne Plus nylon membrane (PALL, Saint-Germain-en-Laye, France) at a rate of 1 μg per dot with Dot blotting Fisher Bioblock Scientific system. The DNA was fixed on filter for 30 min at 80 °C.

Author's personal copy

2.4.4. Probe hybridization

Filters were pre-incubated in a hybridization buffer (750 mM NaCl, 75 mM Sodium Citrate, 1% w/v blocking reagent, 0.1% w/v N-laurylsarkosine sodium salt, 0.02% w/v SDS) for 2 h at 68 °C. The hybridization step was performed overnight at 52 °C in the hybridization buffer containing 2.5 pmole L^{-1} of probe.

2.4.5. Anti-DIG recognition and signal detection

Filters were washed at hybridization temperature twice for 5 min in a first washing solution (2 × SSC, 300 mM NaCl, 30 mM citrate de sodium, 0.1% w/v SDS) and twice for 5 min in a second washing solution (0.1 × SSC, 15 mM NaCl, 1.5 mM Sodium Citrate, 0.1% w/v SDS). Filters were then incubated in a washing buffer (150 mM NaCl, 100 mM Tris–HCl, pH 7.5) for 1 min at room temperature (RT) and transferred in blocking solution (150 mM NaCl, 100 mM Tris–HCl, pH 7.5, 0.5% blocking powder [Roche Diagnostics, Meylan, France]) for 30 min at RT. Stock anti-DIG-labeled alkaline phosphatase (Roche Diagnostics) was diluted 5000 times in washing buffer (150 mM NaCl, 100 mM Tris–HCl, pH 7.5) and each filter was then incubated with 10 mL of diluted anti-DiG-labeled alkaline phosphatase solution for 30 min, and washed twice in washing buffer for 15 min. The signal was detected by using CDP-Star Chemiluminescence substrate (Roche Diagnostics) in 0.13 mM detection buffer (100 mM NaCl, 100 mM Tris–HCl, pH 9.5) per 100 cm^2 filter and incubated in plastic bags for 5 min in the dark. Positive signal were detected by imprint on Lumi-film chemiluminescent detection film (Roche Diagnostics) after a 10 min exposition.

2.5. Statistical analysis

Plant growth measurements were treated with one-way analysis of variance and means were compared with the Newman–Keuls multiple range test ($P < 0.05$). The fungal colonization indexes were transformed by arcsin (sqrtx) before statistical analysis. The numbers of *Bradyrhizobium* sp. strain Aust 13C nodules along the root systems in each treatment were compared with 2 × 2 contingency tables and chi-square test (χ^2-test) and Yates correction for small numbers.

Data from experiment 1 (fungal monoculture treatment) were analyzed with Principal Component Analysis (PCA). The data table used in this PCA had 174 rows and seven columns. The 174 rows corresponded to the five replicates of the four different inoculum densities, plus nine controls, for each of the six fungal species. The seven columns were the plant growth and nodulation parameters.

Data from experiment 2 (multiple fungal isolate treatment) were projected as additional elements in the PCA of the first experiment. The projection of supplementary data as additional elements in a PCA is useful to assess the position of these supplementary data with respect to the original data set. The projection of additional elements does not modify the results of the PCA. It simply gives row scores that can be used to display these new elements on the factor map. Here, this technique allowed us to compare the effects of single and multiple fungal inoculations on plant growth parameters. PCA and additional elements computations were done with the ade4 package for the R software (Thioulouse and Dray, 2007; R Development Core Team, 2010).

3. Results

3.1. Experiment 1: relationships between fungal inoculum density, plant growth and nodulation parameters

A. mangium seedlings were colonized by all the fungal isolates. For all the tested ectomycorrhizal strains, inoculation with increased ectomycorrhizal rates significantly increased shoot, root and total biomasses, ectomycorrhizal colonization and the total number of nodules per plant as well as phosphorus and nitrogen foliar contents (Table 3).

3.2. Experiment 2: effect of ectomycorrhizal diversity on plant growth and root nodulation

All *A. mangium* seedlings inoculated with a single strain of ectomycorrhizal fungus were mycorrhized after 4 month culturing (Table 4). Ectomycorrhizal colonization ranged from 35% (*Scleroderma dictyosporum* IR109) to 79.4% (*Scleroderma* sp. IR406) (Table 4). All single fungal isolate inoculations significantly enhanced plant growth (height, shoot and root biomasses) as well as foliar mineral (N and P) contents (Table 4). For total biomass, the highest promoting effect was recorded with *Scleroderma* sp. SC2 (+78.6%). The strongest fungal effect on plant growth parameters was obtained with *Scleroderma* sp. SC2 and the weakest with *Scleroderma* sp. IR406 (Table 4). A similar pattern was observed for root infection by *Bradyrhizobium* Aust 13C (Table 4). All the fungal strains have

Table 3
Regression analysis between the rate of ectomycorrhizal inoculation and plant growth, ectomycorrhizal colonization and rhizobial nodulation of *A. mangium* seedlings for each fungal strain after a 4-month plantation (Experiment 1).

Fungal strains	Parameters	Equation	r	P
IR100	Shoot biomass	$y = 53.2 + 41.4x$	0.90	<0.0001
	Root biomass	$y = 19.1 + 11.8x$	0.80	<0.0001
	Total biomass	$y = 72.2 + 53.2x$	0.89	<0.0001
	Ectomycorrhizal colonization	$y = 12.1 + 4.3x$	0.67	<0.0001
	Number of nodules per plant	$y = -1.04 + 1.97x$	0.95	<0.0001
	Shoot N content	$y = 0.03 + 0.001x$	0.92	<0.0001
	Shoot P content	$y = 35.8 + 16.7x$	0.97	<0.0001
IR109	Shoot biomass	$y = 57.9 + 38.4x$	0.84	<0.0001
	Root biomass	$y = 22.8 + 8.6x$	0.86	<0.0001
	Total biomass	$y = 80.7 + 47.1x$	0.86	<0.0001
	Ectomycorrhizal colonization	$y = 12.2 + 4.3x$	0.74	<0.0001
	Number of nodules per plant	$y = 0.19 + 2.3x$	0.94	<0.0001
	Shoot N content	$y = 0.004 + 0.001x$	0.87	<0.0001
	Shoot P content	$y = 40.2 + 19.6x$	0.95	<0.0001
IR500	Shoot biomass	$y = 44.1 + 48.1x$	0.86	<0.0001
	Root biomass	$y = 14.4 + 17.5x$	0.85	<0.0001
	Total biomass	$y = 58.4 + 65.5x$	0.86	<0.0001
	Ectomycorrhizal colonization	$y = 5.5 + 3.6x$	0.81	<0.0001
	Number of nodules per plant	$y = -0.51 + 2.2x$	0.95	<0.0001
	Shoot N content	$y = 0.004 + 0.001x$	0.92	<0.0001
	Shoot P content	$y = 32.5 + 33.5x$	0.97	<0.0001
IR409	Shoot biomass	$y = 22.6 + 75.3x$	0.79	<0.0001
	Root biomass	$y = 15.6 + 20.1x$	0.76	<0.0001
	Total biomass	$y = 38.2 + 95.5x$	0.80	<0.0001
	Ectomycorrhizal colonization	$y = 7.1 + 3.4x$	0.59	0.0006
	Number of nodules per plant	$y = -0.88 + 1.1x$	0.80	<0.0001
	Shoot N content	$y = 0.03 + 0.002x$	0.46	0.0126
	Shoot P content	$y = 45.1 + 25.3x$	0.96	<0.0001
IR406	Shoot biomass	$y = 57.1 + 37.7x$	0.92	<0.0001
	Root biomass	$y = 25.5 + 6.8x$	0.82	<0.0001
	Total biomass	$y = 82.6 + 44.5x$	0.92	<0.0001
	Ectomycorrhizal colonization	$y = 0.59 + 5.1x$	0.93	<0.0001
	Number of nodules per plant	$y = -0.67 + 1.4x$	0.85	<0.0001
	Shoot N content	$y = 0.004 + 4.10^4x$	0.77	<0.0001
	Shoot P content	$y = 47.4 + 14.2x$	0.94	<0.0001
SC2	Shoot biomass	$y = 48.5 + 44.6x$	0.88	<0.0001
	Root biomass	$y = 26.3 + 9.6x$	0.59	0.0008
	Total biomass	$y = 74.8 + 54.2x$	0.86	<0.0001
	Ectomycorrhizal colonization	$y = 7.2 + 5.7x$	0.83	<0.0001
	Number of nodules per plant	$y = 2.6 + 5.5x$	0.91	<0.0001
	Shoot N content	$y = 0.003 + 0.001x$	0.93	<0.0001
	Shoot P content	$y = 56.9 + 46.3x$	0.96	<0.0001

Author's personal copy

Table 4
Effect of ectomycorrhizal isolate inoculation on mycorrhiza formation, rhizobial development, shoot mineral contents and growth of *A. mangium* after 4 months of plantation.

	Ectomycorrhizal strains						
	Control	IR100	IR109	IR500	IR409	IR406	SC2
Height (cm)	8.6 (0.6)[a] a [b]	11.3 (0.55) bc	11.3 (0.84) bc	12.9 (1.11) c	13.1 (0.93) cd	10.3 (0.46) b	15.6 (0.90) d
Shoot biomass (mg dry weight)	160 (46) a	487 (27) bc	385 (47) b	717 (107) cd	508 (54) c	318 (36) b	808 (83) d
Root biomass (mg dry weight)	60 (12) a	150 (23) b	123 (15) b	197 (17) bc	163 (17) b	117 (9) b	220 (33) c
Total biomass (mg dry weight)	220 (45) a	637 (44) c	508 (62) bc	913 (122) cd	670 (70) c	435 (45) b	1028 (114) d
Shoot P content (μg per plant)	44.2 (12.6) a	209.2 (11.5) b	236.8 (28.9) b	375.3 (56.1) bc	295.4 (31.3) bc	183.5 (20.7) b	509.7 (52.1) c
Shoot N content (mg per plant)	3 (0.001) a	10 (0.001) c	9 (0.001) bc	15 (0.002) d	11 (0.001) cd	7 (0.001) b	14 (0.001) d
Ectomycorrhizal colonization (%)	0 a	71.1 (6.1) c	35.0 (4.2) b	68.3 (4.4) c	42.8 (13.7) b	79.4 (4.4) c	46.6 (5.6) b
Number of nodules per plant	7.3 (0.3) a	18.8 (2.5) b	21.2 (3.5) b	21.3 (4.9) bc	11.1 (4.8) ab	13.8 (4.5) a	56.8 (12.7) c

[a] Standard error of the mean.
[b] Data in the same line followed by the same letter are not significantly different according to the Newman–Keuls test ($P < 0.05$).

significantly stimulated the root nodulation of *A. mangium* by *Bradyrhizobium* sp. Aust 13C (Table 4). The highest number of nodules per plant was recorded with *Scleroderma* sp. SC2 whereas the lowest was measured with *Scleroderma* sp. IR409 (Table 4).

Significant effects of increasing ectomycorrhizal diversity were obtained on whole plant biomass, plant nutrient acquisition and root nodulation (Table 5, Fig. 1). However no significant relationship was observed between ectomycorrhizal colonization and the increase of ectomycorrhizal diversity, whole plant biomass, nutrient acquisition and root nodulation (Table 5).

Although ectomycorrhizal inoculation (single and multiple fungal isolate inoculations) had significantly enhanced root nodulation with *Bradyrhizobium* sp. Aust 13C, the frequencies of Aust 13C nodules decreased from one to six fungal isolates (Fig. 2).

The PCA of the single fungal strain experiment data (black dots and ellipses) and the multiple fungal strain experiment data (color dots and ellipses) is shown in Fig. 3. In the single fungal strain experiment, black ellipses represented the dispersion of the samples inoculated with one of the six fungal species. Each ellipse summarized the five replicates (black dots) for the four inoculums' rates and the nine controls (not inoculated). The PCA correlation circle (top left) showed that the first PCA axis (horizontal) was highly correlated with the plant biomass parameters, and that the second axis was correlated with the nodulation parameters.

For the multiple fungal strain experiment, color ellipses represent the dispersion of the samples inoculated with increasing ectomycorrhizal diversity. Each ellipse summarized the four replicates (color dots) for the different number of inoculated fungal strains (from zero species to six species). The plant biomass and notably the nodulation parameters were much higher in samples inoculated with multiple fungal strains and this increase was related to the number of inoculated ectomycorrhizal fungi. Treatments with two and three fungal isolates seemed to have a similar effect, and the effect of four to six isolates treatments was also comparable. It is worth noticing that the dispersion of plant growth and nodulation parameters for the four replicates also increased with the number of fungal species inoculated (Fig. 3).

4. Discussion

Several experimental studies have demonstrated that plant diversity, plant productivity and ecosystem functioning can be greatly enhanced by plant species richness (Tilman et al., 1996; Hector et al., 1999), symbiotic bacteria (van der Heijden et al., 2006) and mycorrhizal fungi (Grime, 1997; van der Heijden et al., 1998; Clay and Holah, 1999; Rudgers et al., 2004).

However, the data resulting from these studies are subjected to debates (Huston, 1997; van der Heijden et al., 1999; Wardle, 1999; Hector et al., 2000; Huston et al., 2000) since the "diversity effect" could result from a "sampling effect" or from a resource use complementary. In the present study, this "sampling effect" could very likely be the result of a fungal species that induced a large growth effect in all or most of the plant species included in the experiment (van der Heijden et al., 1999). However, the relationships between the ectomycorrhizal diversity and the enhancement of plant biomass and nodulation parameters after a 4 month cultivation of *A. mangium* cannot be explained by a "sampling effect" because (i) only one plant species has been used and (ii) the presence of the most effective fungal species (*Scleroderma* sp. SC2) identified in experiment 1 cannot explain the enhancement of *A. mangium* growth in samples inoculated with a gradient of ectomycorrhizal diversity. These experimental designs followed the recommendations addressed by Garnier et al. (1997) and Huston et al. (2000) to unambiguously differentiate the effects of diversity from those related to the sampling effect.

The present study clearly shows (i) a strong enhancement of *A. mangium* growth due to ectomycorrhizal fungal inoculation, (ii) significant effects of ectomycorrhizal density and diversity on

Table 5
Effect of ectomycorrhizal diversity on growth variables, shoot mineral contents, rhizobial development and ectomycorrhizal colonization in *A. mangium* plants after 4 months of plantation.

	Treatments						
	Control[a]	IR100	C	D	E	F	G
Height (cm)	8.6 (0.6)[b] a[c]	11.3 (0.55) b	16.2 (0.57) b	15.3 (0.53) b	19.6 (0.43) c	21.2 (0.42) d	17.4 (0.74) b
Shoot biomass (mg dry weight)	160 (45) a	487 (27) b	902 (163) cd	805 (85) c	1175 (176) cd	1227 (45) d	1137 (105) cd
Root biomass (mg dry weight)	60 (12) a	150 (23) b	282 (41) c	220 (25) bc	280 (50) c	330 (44) c	297 (38) c
Total biomass (mg dry weight)	220 (55) a	637 (44) b	1185 (204) cd	1025 (110) c	1455 (215) c	1557 (85) d	1435 (132) d
Shoot P content (μg per plant)	44.2 (12.6) a	209.1 (11.5) b	565.9 (102.1) c	648.8 (68.6) c	1108.9 (166) d	1283.9 (47.1) d	1074.9 (99.5) d
Shoot N content (mg per plant)	3.3 (0.96) a	10.5 (0.58) b	18.5 (3.34) c	18.3 (1.93) c	25.1 (3.74) cd	29.2 (1.07) d	25.5 (2.35) d
Ectomycorrhizal colonization (%)	0	71.0 (6) ab	74.4 (3.4) b	75.1 (0.5) b	68.8 (1.8) a	76.9 (2.6) b	78.4 (1.4) b
Number of nodules per plant	7.3 (0.3) a	18.8 (2.5) b	39.2 (1.1) cd	33.2 (4.1) c	89.0 (23.1) de	132.5 (20.9) e	91.5 (13.8) e

[a] See Table 2.
[b] Standard error of the mean.
[c] Data in the same line followed by the same letter are not significantly different according to the Newman–Keuls test ($P < 0.05$).

Author's personal copy

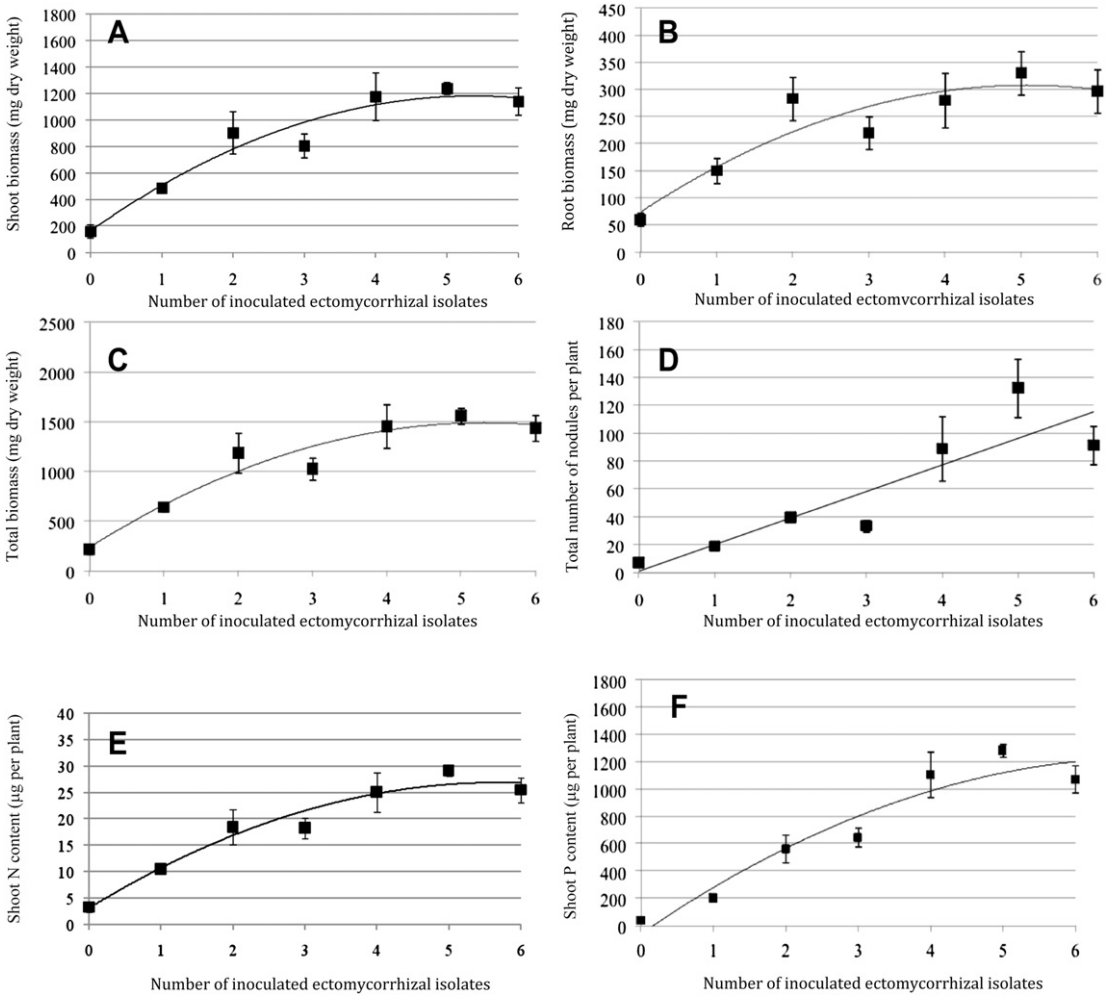


Fig. 1. Relationships between plant growth variables, rhizobial development, mycorrhizal formation and ectomycorrhizal fungal diversity. A: Shoot biomass ($y = -35.23x^2 + 378.6x + 164.6$; $r = 0.85$; $P < 0.0001$). B: Root biomass ($y = -9.09x^2 + 92.4x + 72.8$; $r = 0.74$; $P < 0.0001$). C: Total biomass ($y = -44.27x^2 + 470.5x + 238$; $r = 0.84$; $P < 0.0001$). D: Number of nodules per plant ($y = 19.1x + 1.34$; $r = 0.77$; $P < 0.0001$). E: Shoot N content (mg per plant) ($y = -0.73x^2 + 8.32x + 3.18$; $r = 0.88$; $P < 0.0001$). F: Shoot P content (µg per plant) ($y = -25.6x^2 + 362.5x - 53.8$; $r = 0.96$). Squares represent means (±standard error of the mean).

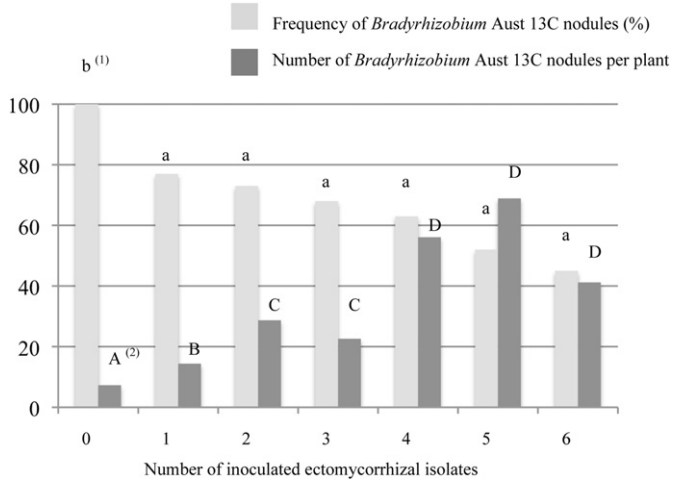


Fig. 2. Frequencies and enumeration of *Bradyrhizobium* Aust 13C nodules in each treatment assessed by PCR-amplified 16S–23S rDNA spacer region and Southern hybridization methods. (1) Data indexed by the same lowercase letter are not significantly different according to the χ^2 test ($P < 0.05$). (2) Data indexed by the same uppercase letter are not significantly different according to the Newman–Keuls test ($P < 0.05$).

whole plant biomass and plant nutrient acquisition and (iii) an ectomycorrhizal diversity effect on the functioning of the nitrogen-fixing symbiosis.

It is well known that many N_2-fixing trees and shrubs are especially dependent on mycorrhizas to uptake mineral nutrients and to ensure an efficient N_2 fixation (Cornet and Diem, 1982; Duponnois et al., 2001; Duponnois and Plenchette, 2003). It has been already reported that *A. mangium* was effectively colonized by ectomycorrhizal fungi (Duponnois and Bâ, 1999; Duponnois et al., 2002). Our results also showed that different ectomycorrhizal strains differ in their effect on plant growth and more particularly on plant nutrient acquisition (Chapin, 1980; Smith and Read, 2008). These data are in accordance with other studies performed on *B. pendula* inoculated with eight ectomycorrhizal fungi and from which it has been concluded that ectomycorrhizal fungi were important determinants of seedling root morphology and nutrient foraging ability (Jonsson et al., 2001).

It has been clearly demonstrated that the mycorrhizal effect on plant growth was dependent on the abundance of infective ectomycorrhizal propagules in soil (Garbaye et al., 1988). Our results are in accordance with these authors since the intensity of root colonization by the ectomycorrhizal fungi, the plant response, the rhizobial nodulation and the nutrient acquisition are positively correlated to the ectomycorrhizal inoculum densities in soil.

Author's personal copy

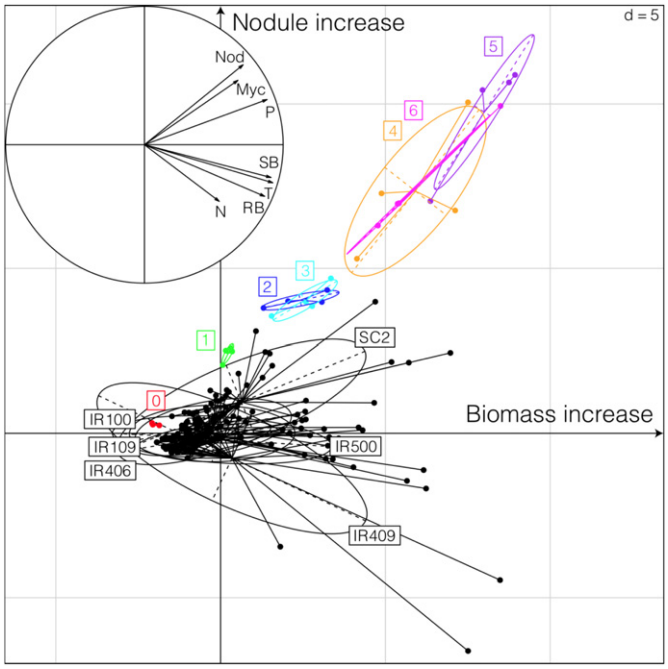


Fig. 3. Comparative effects of single fungal isolate treatments (black dots and ellipses) with multiple fungal isolate treatments (color dots and ellipses) on plant growth parameters and nodulation process analyzed with Principal Component Analysis (PCA). The multiple fungal isolate treatments were projected as additional elements in the PCA computed on the single fungal isolate treatments. The correlation circle of the seven variables is given in the upper-left corner of the figure. The code for variables is as follows. SB: shoot biomass, RB: root biomass, T: total biomass, N: nitrogen rate in plant tissues, Nod: number of nodules per plant, Myc: mycorrhization rate, P: phosphorus rate in plant tissues. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Numerous studies have also reported that mycorrhizas could dramatically increase root nodulation (Founoune et al., 2002a,b,c; Duponnois et al., 2002; Duponnois and Plenchette, 2003). It has been suggested that the mycorrhizal symbiosis enhanced nodulation and N_2 fixation by enabling plant P uptake (Cornet and Diem, 1982; André et al., 2005) or by improving root growth that facilitated rhizobial colonization and infection. It is also well known that mycorrhizas modify root functions (in particular, root exudation) and, therefore, could influence the microbial community structure (commonly named the "mycorhizosphere effect") (Katznelson et al., 1962; Linderman, 1988; Frey-Klett et al., 2005). Specific relationships occur between mycorrhizal fungi and mycorhizosphere microflora (Garbaye, 1991; Garbaye and Bowen, 1989; Marshner et al., 2001; Frey-Klett et al., 2005). Moreover, the extraradical mycelium of ectomycorrhizal fungi could also influence the chemical composition and pH of the surrounding soil by excreting chemical substances (Frey et al., 1997; Caravaca et al., 2002). Frey-Klett et al. (2005) have proposed that the ectomycorrhizal symbiosis could be considered as a microbial complex where the fungal symbiosis has a direct effect on plant growth (nutritional and hormonal mechanisms). They further argued that the fungal symbiosis has an indirect positive effect *via* a selective pressure on bacterial communities resulting, for instance, in a higher abundance of Plant Growth Promoting Rhizobacteria (PGPR). In the current study, all these physical and chemical modifications could increase the development of the inoculated bradyrhizobial strain and, consequently, improve the nodulation process. However, these hypotheses are not limited to the environment of the host plant/ectomycorrhizal fungus symbiosis. More specific relationships could be involved during the development of the tripartite symbiosis, at the physiological and molecular level (van Rhijn et al., 1997; Blilou et al., 1999; Parniske, 2000). All these experimental studies have been undertaken with single fungal strain inoculations and our study showed that these interactive effects between rhizobia and ectomycorrhizal fungi depend on the amount and diversity of ectomycorrhizal mycelium.

The *Bradyrhizobium* sp. strain Aust 13C has been previously tested in field trials to determine the effect of selected *Bradyrhizobium* strains on the growth of *A. mangium* (Galiana et al., 1990, 1994). Nineteen months after tree transplantation, Aust 13C provided a stimulating effect on tree growth and was predominant in nodules collected from *A. mangium* root systems (Galiana et al., 1994). Using PCR and Southern hybridization methods, our data confirmed the Aust 13C high competitive ability against indigenous N_2-fixing bacteria and its persistence in soil. However, Aust 13C frequencies decreased with an increasing mycorrhizal fungal diversity. About seven Aust 13C nodules have been recorded in the control treatment whereas 66 Aust 13C nodules have been numbered in the mycorrhizal treatment performed with five ectomycorrhizal isolates. This result suggests that the persistence in soil of this bradyrhizobial strain and its nodulation process are promoted with an increasing ectomycorrhizal fungal diversity.

In conclusion, our results provide evidence that mycorrhizal diversity has a great impact on plant growth and biological nitrogen-fixing symbiosis. They also underline the necessity to manage ectomycorrhizal symbiosis and/or to associate tree seedlings with multiple mycorrhizal symbionts in order to conserve an efficient abundance and diversity of ectomycorrhizal propagules in plantation soils. These management practices would optimize forest plantation performance by promoting the efficiency of nitrogen-fixing symbiosis for legume tree species and depleting the transplant shock, a critical step in successful plantation programs. It is now unquestionable that seedlings inoculated with multiple fungal isolates show better growth parameters as well as better nutrient acquisition than those associated with a single isolate.

Acknowledgments

We would like to thank M. Cheikh Ndiaye for his technical assistance.

References

Allen, E.B., Allen, M.F., Helm, D.J., Trappe, J.M., Molina, R., Rincon, E., 1995. Patterns and regulation of mycorrhizal plant and fungal diversity. Plant and Soil 170, 47–62.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25, 3389–3402.

André, S., Galiana, A., Le Roux, C., Prin, Y., Neyra, M., Duponnois, R., 2005. Ectomycorrhizal symbiosis enhanced the efficiency of two *Bradyrhizobium* inoculated on *Acacia holosericea* plant growth. Mycorrhiza 15, 357–364.

Baxter, J.W., Dighton, J., 2001. Ectomycorrhizal diversity alters growth and nutrient acquisition of grey birch (*Betula populifolia*) seedlings in host–symbiont culture conditions. New Phytologist 152, 139–149.

Baxter, J.W., Dighton, J., 2005. Phosphorus source alters host plant response to ectomycorrhizal diversity. Mycorrhiza 15, 513–523.

Blilou, I., Ocampo, J.A., Gracia-Garrido, J.M., 1999. Resistance of pea roots to endomycorrhizal fungus or *Rhizobium* correlates with enhanced levels of endogenous salicylic acid. Journal of Experimental Botany 50, 1663–1668.

Bruno, J.F., Stachowicz, J.J., Bertness, M.D., 2003. Inclusion of facilitation into ecological theory. Trends in Ecology and Evolution 18, 119–125.

Caravaca, F., Barea, J.M., Figueroa, D., Roldan, A., 2002. Assessing the effectiveness of mycorrhizal inoculation and soil compost addition for reafforestation with *Olea europaea* subsp. *sylvestris* through changes in soil biological and physical parameters. Applied Soil Ecology 20, 107–118.

Chapin III, F.S., 1980. The mineral nutrition of wild plants. Annual Review of Ecology and Systematics 11, 223–260.

Chu-Chou, M., Grace, L.J., 1985. Comparative efficiency of the mycorrhizal fungi *Laccaria laccata*, *Hebeloma crustuliniforme* and *Rhizopogon* species on growth of radiate pine seedlings. New Zealand Journal of Botany 23, 417–424.

Author's personal copy

Clay, K., Holah, J., 1999. Fungal endophyte symbiosis and plant diversity in successional fields. Science 285, 1742–1744.
Cornet, F., Diem, H.G., 1982. Etude comparative de l'efficacité des souches de Rhizobium d'Acacia isolées de sols du Sénégal et effet de la double symbiose Rhizobium – *Glomus mosseae* sur la croissance de *Acacia holosericea* et *A. raddiana*. Bois et Forêts des Tropiques 198, 3–15.
Daft, M.J., 1991. Influences of génotypes, rock phosphate and plant densities on mycorrhizal development and the growth-responses of 5 different crops. Agriculture Ecosystems & Environment 35, 151–169.
Ducousso, M., 1991. Importance des symbioses racinaires pour l'utilisation des acacias d'Afrique de l'Ouest. Thèse de l'Université de Lyon 1, pp. 205.
Duponnois, R., Bâ, A.M., 1999. Growth stimulation of *Acacia mangium* Willd by *Pisolithus* sp. in some Senegalese soils. Forest Ecology and Management 119, 209–215.
Duponnois, R., Garbaye, J., 1991. Techniques for controlled synthesis of the Douglas fir-*Laccaria laccata* ectomycorrhizal symbiosis. Annales des Sciences Forestières 48, 239–251.
Duponnois, R., Plenchette, C., 2003. A mycorrhiza helper bacterium enhances ectomycorrhizal and endomycorrhizal symbiosis of Australian *Acacia* species. Mycorrhiza 13, 85–91.
Duponnois, R., Founoune, H., Lesueur, D., 2002. Influence of controlled dual ectomycorrhizal and rhizobial symbiosis on the growth of *Acacia mangium* provenances, the indigenous symbiotic microflora and the structure of plant parasitic nematode communities. Geoderma 109, 85–102.
Duponnois, R., Founoune, H., Lesueur, D., Thioulouse, J., Neyra, M., 2000. Ectomycorrhization of six *Acacia auriculiformis* provenances from Australia, Papua New Guinea and Senegal in glasshouse conditions: effect on the plant growth and on the multiplication of plant parasitic nematodes. Australian Journal of Experimental Agriculture 40, 443–450.
Duponnois, R., Founoune, H., Masse, D., Pontanier, R., 2005. Inoculation of *Acacia holosericea* with ectomycorrhizal fungi in a semi-arid site in Senegal: growth response and influences on the mycorrhizal soil infectivity. Forest Ecology and Management 207, 351–362.
Duponnois, R., Plenchette, C., Ba, A.M., 2001. Growth stimulation of seventeen fallow leguminous plants inoculated with *G. aggregatum* in Senegal. European Journal of Soil Biology 37, 181–186.
Duponnois, R., Plenchette, C., Prin, Y., Ducousso, M., Kisa, M., Bâ, A.M., Galiana, A., 2007. Use of mycorrhizal inoculation to improve reafforestation process with Australian Acacia in Sahelian ecozones. Ecological Engineering 29, 105–112.
Finlay, R.D., 2008. Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium. Journal of Experimental Botany 59, 1115–1126.
Founoune, H., Duponnois, R., Bâ, A.M., 2002a. Influence of the dual arbuscular endomycorrhizal/ectomycorrhizal symbiosis on the growth of *Acacia holosericea* (A. Cunn. ex G. Don) in glasshouse conditions. Annals of Forest Sciences 59, 93–98.
Founoune, H., Duponnois, R., Bâ, A.M., Sall, S., Branger, I., Lorquin, J., Neyra, M., Chotte, J.L., 2002b. Mycorrhiza helper bacteria stimulate ectomycorrhizal symbiosis of *Acacia holosericea* with *Pisolithus albus*. New Phytologist 153, 81–89.
Founoune, H., Duponnois, R., Meyer, J.M., Thioulouse, J., Masse, D., Chotte, J.L., Neyra, M., 2002c. Interactions between ectomycorrhizal symbiosis and fluorescent pseudomonads on *Acacia holosericea*: isolation of mycorrhiza helper bacteria (MHB) from a soudano-Sahelian soil. FEMS Microbiology Ecology 41, 37–46.
Frey, P., Frey-Klett, P., Garbaye, J., Berge, O., Heulin, T., 1997. Metabolic and genotypic fingerprinting of fluorescent pseudomonads associated with the Douglas fir *Laccaria bicolor* mycorrhizosphere. Applied and Environmental Microbiology 63, 1852–1860.
Frey-Klett, P., Chavatte, M., Clausse, M.L., Courrier, S., Le Roux, C., Raaijmakers, J., Martinotti, M.G., Pierrat, J.P., Garbaye, J., 2005. Ectomycorrhizal symbiosis affects functional diversity of rhizosphere fluorescent pseudomonads. New Phytologist 165, 317–328.
Galiana, A., Chaumont, J., Diem, H.G., Dommergues, Y.R., 1990. Nitrogen-fixing potential of *Acacia mangium* and *Acacia auriculiformis* seedlings inoculated with *Bradyrhizobium* and *Rhizobium* spp. Biology and Fertility of Soils 9, 261–267.
Galiana, A., Prin, Y., Mallet, B., Gnahoua, G.M., Poitel, M., Diem, H.G., 1994. Inoculation of *A. mangium* with alginate beads containing selected *Bradyrhizobium* strains under field conditions: long-term effect on plant growth and persistence of the introduced strains in soil. Applied and Environmental Microbiology 60, 3974–3980.
Garbaye, J., 1991. Biological interactions in the mycorrhizosphere. Experientia 47, 370–375.
Garbaye, J., Bowen, G.D., 1989. Ectomycorrhizal infection of *Pinus radiata* by *Rhizopogon luteolus* is stimulated by microorganisms naturally present in the mantle of ectomycorrhizas. New Phytologist 112, 383–388.
Garbaye, J., Delwaulle, J., Diangana, D., 1988. Growth response of eucalyptus to ectomycorrhizal inoculation in the Congo. Forest Ecology and Management 24, 151–157.
Garnier, E., Navas, M.L., Austin, M.P., Lilley, J.M., Gifford, R.M., 1997. A problem for biodiversity–productivity studies: how to compare the productivity of multispecific plant mixtures to that of monocultures. Acta Oecologica 18, 657–670.
Grime, J.P., 1997. Biodiversity and ecosystem function: the debate deepens. Science 277, 1260–1261.
Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M.C., Diemer, M., Dimitrakopoulos, P.G., Finn, J.A., Freitas, H., Giller, P.S., Good, J., Harris, R., Högberg, P., Huss-Danell, K., Joshi, J., Jumpponen, A., Körner, C., Leadley, P.W., Loreau, M., Minns, A., Mulder, C.P.H., O'Donovan, G., Otway, S.J., Pereira, J.S., Prinz, A., Read, D.J., Scherer-Lorenzen, M., Schulze, E.D., Siamantziouras, A.S.D., Spehn, E.M., Terry, A.C., Troumbis, A.Y., Woodward, F.I., Yachi, S., Lawton, J.H., 1999. Plant diversity and productivity in European grasslands. Science 286, 1123–1127.
Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M.C., Diemer, M., Dimitrakopoulos, P.G., Finn, J.A., Freitas, H., Giller, P.S., Good, J., Harris, R., Högberg, P., Huss-Danell, K., Joshi, J., Jumpponen, A., Körner, C., Leadley, P.W., Loreau, M., Minns, A., Mulder, C.P.H., O'Donovan, G., Otway, S.J., Pereira, J.S., Prinz, A., Read, D.J., Scherer-Lorenzen, M., Schulze, E.D., Siamantziouras, A.S.D., Spehn, E.M., Terry, A.C., Troumbis, A.Y., Woodward, F.I., Yachi, S., Lawton, J.H., 2000. No consistent effects of plant diversity on productivity (response). Science 289, 1255.
Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Löfvenius, M., Read, D.J., 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411, 789–792.
Hooper, D.U., Vitousek, P.M., 1997. The effects of plant composition and diversity on ecosystem processes. Science 277, 1302–1305.
Hooper, D.U., Vitousek, P.M., 1998. Effects of plant composition and diversity on nutrient cycling. Ecological Monographs 68, 121–149.
Huston, M.A., 1997. Hidden treatments in ecological experiments: re-evaluation the ecosystem function of biodiversity. Oecologia 110, 449–460.
Huston, M.A., Aarssen, L.W., Austin, M.P., Cade, B.S., Fridley, J.D., Garnier, E., Grime, J.P., Hodgson, J., Lauenroth, W.K., Thompson, K., Vandermeer, J.H., Wardle, D.A., 2000. No consistent effect of plant diversity on productivity. Science 289, 1255.
John, M.K., 1970. Colorimetric determination of phosphorus in soil and plant material with ascorbic acid. Soil Science 68, 171–177.
Jonsson, L.M., Nilsson, M.C., Wardle, D.A., Zackrisson, O., 2001. Context dependent of ectomycorrhizal species richness on tree seedling productivity. Oikos 93, 353–364.
Katznelson, H., Rouatt, J.W., Peterson, E.A., 1962. The rhizosphere effect of mycorrhizal and non-mycorrhizal roots of yellow birch seedlings. Canadian Journal of Botany 40, 377–382.
Kowalchuk, G.A., Stephen, J.R., 2001. Ammonia-oxidizing bacteria: a model for molecular microbial ecology. Annual Review of Microbiology 55, 485–529.
Linderman, R.G., 1988. Mycorrhizal interactions with the rhizosphere microflora: the mycorrhizosphere effect. Phytopathology 78, 366–371.
Marshner, P., Crowley, D.E., Lieberei, R., 2001. Arbuscular mycorrhizal infection changes the bacterial 16 S rDNA community composition in the rhizosphere of maize. Mycorrhiza 11, 297–302.
Martin, F., Diez, J., Dell, B., Delaruelle, C., 2002. Phylogeography of the ectomycorrhizal *Pisolithus* species as inferred from the ribosomal DNA ITS sequences. New Phytologist 153, 345–358.
Molina, R., Massicotte, H., Trappe, J.M., 1992. Specificity phenomena in mycorrhizal symbiosis: community-ecological consequences and practical implications. In: Allen, M.F. (Ed.), Mycorrhizal Functioning: an Integrative Plant-fungal Process. Chapman & Hall, New York, USA, pp. 357–423.
Parlade, J., Alvarez, I.F., 1993. Coinoculation of aseptically grown Douglas-fir with pairs or ectomycorrhizal fungi. Mycorrhiza 3, 93–96.
Parniske, M., 2000. Intracellular accommodation of microbes by plant: a common developmental program for symbiosis and disease? Current Opinion in Plant Biology 3, 320–3208.
R Development Core Team, 2010. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0. URL: http://www.R-project.org/.
Reddy, M.S., Natarajan, K., 1997. Coinoculation efficacy of ectomycorrhizal fungi on *Pinus patula* seedlings in a nursery. Mycorrhiza 7, 133–138.
Rillig, M.C., Mummey, D.L., 2006. Mycorrhiza and soil structure. New Phytologist 171, 41–43.
Rudgers, J.A., Koslow, J.M., Clay, K., 2004. Endophytic fungi alter relationships between diversity and ecosystem properties. Ecology Letters 7, 42–51.
Smith, S.E., Read, D.J., 2008. Mycorrhizal Symbiosis, third ed. Academic Press, London, UK.
Sprent, J.I., 2001. Nodulation in Legumes. Royal Bot. Gardens, Kew, UK.
Thioulouse, J., Dray, S., 2007. Interactive multivariate data analysis in R with the ade4 and ade4TkGUI packages. Journal of Statistical Software 22, 1–14.
Tilman, D., Wedin, D., Knops, J., 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature 379, 718–720.
Valentine, L.L., Fieldler, T.L., Hart, A.A., Petersen, C.A., Berninghausen, H.K., Southworth, D., 2004. Diversity of ectomycorrhizas associated with *Quercus garryana* in southern Oregon. Canadian Journal of Botany 82, 123–135.
van der Heijden, M.G.A., Cornelissen, J.H.C., 2002. The critical role of plant–microbe interactions on biodiversity and ecosystem functioning: arbuscular mycorrhizal associations as an example. In: Loreau, M., Naeem, S., Inchausti, P. (Eds.), Biodiversity and Ecosystem Functioning: Synthesis and Perspectives. Oxford University Press, London, pp. 181–194.

Author's personal copy

van der Heijden, M.G.A., Bakker, R., Verwaal, J., Scheublin, T.R., Rutten, M., van Logtestijn, R., Staehelin, C., 2006. Symbiotic bacteria as a determinant of plant community structure and plant productivity in dune grassland. FEMS Microbiology Ecology 56, 178–187.

van der Heijden, M.G.A., Bardgett, R.D., van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11, 296–310.

van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., Sanders, I.R., 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396, 72–75.

van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., Sanders, I.R., 1999. "Sampling effect", a problem in biodiversity manipulation? A reply to David A. Wardle. Oikos 87, 408–410.

van der Heijden, M.G.A., Wiemken, A., Sanders, I.R., 2003. Different arbuscular mycorrhizal fungi alter coexistence and resource distribution between co-occurring plant. New Phytologist 157, 569–578.

van Rhijn, P., Fang, Y., Galili, S., Shaul, O., Atzon, N., Wininger, S., Eshed, Y., Lum, M., Li, Y., To, V., Fujishige, N., Kapulik, Y., Hirsch, A.M., 1997. Expression of early nodulin genes in alfalfa mycorrhizae indicates that signal transduction pathways used in forming arbuscular mycorrhizae and Rhizobium-induced nodules may be conserved. Plant Biology 94, 5467–5472.

Vincent, J.M., 1970. A Manual for the Practical Study of the Root Nodule Bacteria. In: International Biological Program. Blackwell, Oxford. Handbook no. 15.

Wardle, D.A., 1999. Is "sampling effect" a problem for experiments investigating biodiversity–ecosystem function relationships? Oikos 87, 403–407.

Wardle, D.A., Bonner, K.I., Barker, G.M., 2000. Stability of ecosystem properties in response to above ground functional group richness and composition. Oikos 89, 11–23.