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Controlled ectomycorrhization of an exotic legume tree species *Acacia holosericea* affects the structure of root nodule bacteria community and their symbiotic effectiveness on *Faidherbia albida*, a native Sahelian Acacia

A. Faye [a,b], T. Krasova-Wade [b], M. Thiao [b,c], J. Thioulouse [d], M. Neyra [e], Y. Prin [f], A. Galiana [f], I. Ndoye [b,c], B. Dreyfus [e], R. Duponnois [b,e,*]

[a] *ISRA, Institut Sénégalais de Recherche Agricole, BP 3120 Dakar, Senegal*
[b] *IRD, Laboratoire Commun de Microbiologie IRD/ISRA/UCAD, Centre de Recherche de Bel Air, BP 1386 Dakar, Senegal*
[c] *UCAD, Faculté des Sciences et Techniques, Département de biologie végétale, BP 5005 Dakar, Senegal*
[d] *Université de Lyon, F-69000 Lyon, Université Lyon 1, CNRS, UMR5558, Laboratoire de Biométrie et Biologie Evolutive, F-69622 Villeurbanne, France*
[e] *IRD, UMR 113 CIRAD/INRA/IRD/SUP-AGRO/UM2, Laboratoire des Symbioses Tropicales et Méditerranéennes (LSTM), TA10/J, Campus International de Baillarguet, Montpellier, France*
[f] *CIRAD, UMR 113 CIRAD/INRA/IRD/SUP-AGRO/UM2, Laboratoire des Symbioses Tropicales et Méditerranéennes (LSTM), TA10/J, Campus International de Baillarguet, Montpellier, France*

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ABSTRACT

Many fast growing tree species have been introduced to promote biodiversity rehabilitation on degraded tropical lands. Although it has been shown that plant productivity and stability are dependent on the composition and functionalities of soil microbial communities, more particularly on the abundance and diversity of soil symbiotic micro-organisms (mycorrhizal fungi and rhizobia), the impact of tree introduction on soil microbiota has been scarcely studied. This research has been carried in a field plantation of *Acacia holosericea* (Australian *Acacia* species) inoculated or not with an ectomycorrhizal fungus isolate, *Pisolithus albus* IR100. After 7 year's plantation, the diversity and the symbiotic properties of Bradyrhizobia isolated from the plantation soil or from the surrounding area (*Faidherbia albida* (Del.) a. Chev. parkland) and able to nodulate *F. albida*, a native Sahelian *Acacia* species, have been studied. Results clearly showed that *A. holosericea* modified the structure of Bradyrhizobia populations and their effectiveness on *F. albida* growth. This negative effect was counterbalanced by the introduction of an ectomycorrhizal fungus, *P. albus*, on *A. holosericea* root systems.

In conclusion, this study shows that exotic plant species can drastically affect genotypic and symbiotic effectiveness of native Bradyrhizobia populations that could limit the natural regeneration of endemic plant species such as *F. albida*. This effect could be counterbalanced by controlled ectomycorrhization with *P. albus*. These results have to be considered when exotic tree species are used in afforestation programs that target preservation of native plants and soil ecosystem rehabilitation.

1. Introduction

Soil degradation and desertification processes of arid and semi-arid environments result from particular climatic conditions (irregular rainfall and long dry period) in addition to anthropogenic impacts (clearing, tillage, cultivation and harvesting) (Matson et al., 1997). The first symptom of ecosystem degradation is the lack or scarcity of plant cover associated with increased soil erosion, decreases in water infiltration, plant nutrient availability, organic matter content and loss of microbial activity (Garcia et al., 1997; Lal, 1996; Remigi et al., 2008). Since there is increasing evidence that forest plantations can play a key role in ecosystem rehabilitation or restoration especially in arid and semi-arid environment, trials of many Australian *Acacia* species were conducted by a number of research organizations in the semi-arid Sahelian region of West Africa in order to assess their potential to ease fuelwood shortages and serve as effective windbreaks (Cossalter, 1986). *Acacia* is the largest mimosoid genus with 1200 species (Pedley, 1986) and they are frequently recorded in savannas and arid regions of Australia, Africa, India and the Americas. For instance, in the 400–700 mm

* Corresponding author. Present address: IRD, Laboratoire Commun de Microbiologie IRD/ISRA/UCAD, Centre de Recherche de Bel Air, BP 1386 Dakar, Senegal. Tel.: +221 849 33 22; fax: +221 849 33 02.
E-mail address: robin.duponnois@ird.sn (R. Duponnois).

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rainfall zone, *Acacia holosericea* was found to be the best-adapted Australian species, showing excellent survival rate and rapid early growth (Harwood, 1994; Rinaudo et al., 1995). In addition, leaves from the pruning can be used as mulch on the soil surface, and pruned twigs and branches make excellent fuelwood. This multipurpose *Acacia* species has the additional benefit of being able to fix nitrogen, thus constituting an N input into the ecosystem (Cossalter, 1986) and it has been suggested that this Australian *Acacia* species could be used to improve soil characteristics through maintenance of soil organic matter or soil aggregation (Cossalter, 1986, 1987). Hence it has been proposed that fast growing leguminous trees could be good candidates to promote biodiversity rehabilitation on degraded tropical lands through the "catalytic effect hypothesis" (Parrotta, 1993). Tree plantations can facilitate, or 'catalyze', forest succession by inducing changes in under-story microclimatic conditions, increased plant community and soil microbiota structural complexity, and development of litter and humus layers that occur during the early years of plantation growth. In addition to rhizobial symbiosis, this tree species can form arbuscular mycorrhiza (AM) and/or ectomycorrhiza (De La Cruz and Garcia, 1991; Founoune et al., 2002). As for many N_2 fixing trees and shrubs, Australian *Acacia* species are especially dependent on mycorrhizas to absorb mineral nutrients required for plant growth and efficient N_2 fixation. It has been previously demonstrated that controlled mycorrhizal inoculation could improve the performance of these tree species in glasshouse conditions (Duponnois et al., 2000a, 2001; Duponnois and Plenchette, 2003) and also after outplanting into the field (Duponnois et al., 2005, 2007).

Increasing concern has been given on the devastating ecological impacts resulting from the widespread anthropogenic dispersal of exotic plants (Rejmanek, 2000; Kourtev et al., 2002, 2003). Plant species composition aboveground is related to the structure and functional diversity of microbial communities (Grayston et al., 2001; Priha et al., 1999; van der Heijden et al., 1998a,b). Numerous studies have shown that microbial communities were structurally and functionally different under different plant species (Bossio et al., 1998; Degens and Harris, 1997; Marilley and Aragno, 1999) but the impact of the establishment of exotic plant species on the structure and function of native microbial communities is not well studied. In view of this, and because it is being recognized that mutualistic interactions between plants and microbes play a key role in ecosystems (Bruno et al., 2003; van der Heijden et al., 1998a,b), these investigations have to be performed in order to evaluate the real potential of exotic fast growing leguminous trees to rehabilitate degraded soil and facilitate revegetation with native plant species.

A field experiment has been carried out in Senegal with *A. holosericea* inoculated or not with an ectomycorrhizal strain, *Pisolithus albus* IR100 (Duponnois et al., 2005, 2007). This plantation was located in a degraded parkland of *Faidherbia albida* (syn *Acacia albida*). One of the objectives of this tree plantation was to rehabilitate soil characteristics to help the native plant species become established. Among the targeted native species, the leguminous tree *F. albida* has been selected since it plays a major role in the agro-sylvo-pastoral balance of the Sahelian regions of Africa (Dupuy and Dreyfus, 1992).

After 7 years plantation, the performance of *A. holosericea* was significantly enhanced by the ectomycorrhizal fungus. It was also demonstrated that, compared to the *F. albida* parkland soil surrounding the *A. holosericea* plantation, this exotic tree species associated or not with the ectomycorrhizal symbiont, induced disturbances in soil microbial functionalities and strongly altered the structure of arbuscular mycorrhizal fungus communities (Remigi et al., 2008). The influence of exotic plant species on rhizobia diversity has not been investigated although rhizobia have been proposed as keystone species (Wardle, 2002) and ecosystem engineers (Crooks, 2002). In fact symbiotic soil bacteria act as drivers of plant community structure and plant productivity and could be involved in natural regeneration process of native legume species (van der Heijden et al., 2006).

This study focussed on the effects of rhizobia selected or not by the exotic tree species, on the early growth of *F. albida*, a native *Acacia* species encountered in Sahelian regions of Africa. Since young *F. albida* seedlings only nodulate with slow-growing *Bradyrhizobium* species (Dreyfus and Dommergues, 1981), we hypothesized that the native rhizobial communities in the initial soil will differentiate under *A. holosericea* inoculated or not with the ectomycorrhizal *P. albus*, and that will in turn lead to changes in the impact of the Bradyrhizobia strains on biomass allocation of *F. albida* young seedlings.

2. Materials and methods

2.1. Sampling sites

The study site was located in Senegal at Ngane village (17° 50′ W–14° 10′ N) (15 km at the west of Kaolack) on a ferrugineous soil (Remigi et al., 2008). The climate is Sahelo-Sudanian, tropical dry with an average annual rainfall of 700 mm and a mean annual temperature of 25 °C. The physicochemical characteristics of the soil were as follow: pH (H_2O) 5.2; clay (%) 8.7; fine silt (%) 6.5; coarse silt (%) 17.6; fine sand (%) 40.8; coarse sand (%) 25.6; carbon (%) 0.4; total nitrogen (%) 0.033; soluble phosphorus 4.6 mg kg^{-1}, total phosphorus 54.7 mg kg^{-1}. More detailed information on the experimental area and design can be found in Remigi et al. (2008). Briefly, the field trial had a randomized block design with one factor and three replication blocks. The factor was the direct ectomycorrhizal inoculation of *A. holosericea* seedlings with *P. albus* IR100 or not (control) in the glasshouse. The plot size was 6 m by 6 m. Each plot was separated from the other plots by an unplanted area 6 m wide. *A. holosericea* seedlings were planted at 3 m apart. A transect was marked out along the diagonal of each plot with eight soil samples taken 1 m apart that were pooled together. There were three replicates for each soil origin (soil collected under ectomycorrhized or uninoculated *A. holosericea* trees). In addition, 6 soil samples were taken under *F. albida* trees from the surrounding area of *A. holosericea* plantation (within 100 m of plots). These soil samples (250 cm^3 each) were collected during the wet season from the 0–10 cm layer and stored in sealed plastic bags at field moisture content at 4 °C for further measurements.

2.2. Enumeration of Bradyrhizobia

The number of Bradyrhizobia able to nodulate *F. albida* seedlings in the collected soils was estimated by the plant infection most-probable-number technique (Brockwell, 1980) using a 10-fold dilution series and *F. albida* as the trap host. Seeds of *F. albida* (collected from the *F. albida* trees located near the *A. holosericea* plantation) were surface sterilized with 95% concentrated sulphuric acid for 60 min. The acid solution was then decanted off and the seeds were rinsed and soaked for 12 h in sterile distilled water. Seeds were then transferred aseptically to Petri dishes filled with 1% (w/v) agar water medium. These plates were incubated at 30 °C in the dark. The germinating seeds (1–2 cm long rootlets) were transferred to tubes containing agar slants of Jensen medium (Vincent, 1970). Four replicate plant infection tubes were inoculated with 1 ml aliquots of each dilution step (up to 10^{-6}). The tubes were placed in a controlled environment growth chamber (12 day-length at 28 °C, 40,000 lx, 75% relative humidity and 20 °C at nights). After 4 week's culture, all tubes were examined for

nodulation. No nodules were recorded with uninoculated plants indicating an absence of contamination from exogenous rhizobia.

2.3. Isolation, cultivation and genotypic characterization of Bradyrhizobia

Nodules formed in tubes inoculated with the highest dilution of each soil sample were collected and re-hydrated in sterile water. Then they were surface sterilized by immersion in 0.1% (w/v) $HgCl_2$ for 30 s, rinsing in sterile water and then in 96% ethanol for 2–3 min following by rinsing in sterile water and crushed in a drop of sterile distilled water. One part of crushed nodule suspensions was plated onto yeast Mannitol agar (YMA) plates (Vincent, 1970). Culture purity was verified by repeated streaking of single colony isolates. Bradyrhizobia strains were checked for nodulation on *F. albida*, and stored at −80 °C in YM adjusted to 20% (v/v) glycerol (Dupuy and Dreyfus, 1992). The other part of crushed nodule suspensions was used for genotypic characterization of nodulated Bradyrhizobia.

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) (Sigma) 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 then extracted with an equal volume (300 μl) of phenol–chloroform–isoamyl alcohol (25/24/1; v/v/v) and centrifuged for 15 min at 13,000 g. DNA from the aqueous phase was purified from phenol with 300 μl of chloroform–isoamyl alcohol (24/1; v/v) and centrifuged for 15 min at 13,000 g. Supernatant was centrifuged one more time for 5 min. DNA from the aqueous phase was precipitated overnight at −20 °C with the addition of 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 solubilised in 20 μl of ultrapure water. The purity and the quantity of extracted DNA were estimated by spectrophotometry (Pharmacia Biotech) in the range of 200 nm–340 nm.

Primers FGPS1490-72 (5′-TGCGGCTGGATCCCCTCCTT-3′), corresponding to positions 1521–1541 of *Escherichia coli* and FGPL132-38 (5′-CCGGGTTTCCCCATTCGG-3′), corresponding to position 114–132 of *E. coli* (Normand et al., 1996) were used for PCR amplification. The oligonucleotides were purchased from Pharmacia. PCR was carried out in 25 μl reaction volume containing 50 ng of pure total DNA extract, one lyophilised bead (Ready-to-Go PCR beads, Pharmacia Biotech) containing 1.5 U of *Taq* polymerase, 10 mM Tris–HCl, (pH 9 at room temperature), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM of each dNTP and 2.5 μl of each primer (1 mM). Amplification was carried out in a GeneAmp PCR System 2400 (Perkin Elmer) using the following program: initial denaturation at 95 °C for 5 min; 35 amplification cycles of denaturation (30 s at 95 °C), annealing (1 min at 55 °C) and extension (1 min at 72 °C) and a final extension (3 min at 72 °C). PCR-amplified DNAs were visualized by electrophoresis of 3 μl of the amplified mixture on 1% (w/v) horizontal agarose gel (type II, Sigma) in TBE 1X buffer (1/1; w/v Tris–HCl; 0.1% w/v $Na_2EDTA\ 2H_2O$; 0.55% w/v boric acid) at 4 V cm^{-1} for 1 h. The gels were stained for 30 min in an aqueous solution of ethidium bromide (1 μg ml^{-1}) and integrated with Image Analysis software BIOCAPT (Vilber Lourmat) under a 260 nm UV source.

Aliquots (6–10 μl) of PCR products were digested with the restriction endonuclease MspI as specified by the manufacturer (Amersham Pharmacia Biotech) with an excess of enzyme (10 U per 20 μl reaction volume) for 2 h. Restricted DNA was analyzed by horizontal electrophoresis in 2.5% w/v agarose gel (Metaphor®; FMC BioProducts, Rockland, Marine USA). Electrophoresis was carried out at 80 V for 3 h in 14 cm gels. Gels were stained and integrated as described above.

2.4. Greenhouse experiment

Seeds of *F. albida* were treated as described before. The germinated seeds were individually grown in 0.5 L pots filled with a sandy soil collected from a 20-year-old plantation of *A. holosericea* in an experimental station at Sangalkam (50 km east of Dakar). Before use, the soil was crushed, passed through a 2-mm sieve, autoclaved for 40 min at 120 °C and stored for 1 week in a dry room to avoid any soil toxicity. One week after autoclaving, its 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; C/N 8.5; total P (mg kg^{-1}) 39 and soluble P (mg kg^{-1}) 2.1. The plants were placed in a glass-house (28 °C day, 18 °C night, 10-h photoperiod) and watered regularly with sterile distilled water. The pots were arranged in a randomized complete block design with eight replicates per treatment. For bacterial inoculation, fourteen Bradyrhizobia isolates, randomly chosen from the bacterial strain collection among soil origins, were cultured in glass flasks containing liquid yeast extract–mannitol extract medium at 37 °C on an orbital shaker. The bacterial suspensions were 10^7 CFU ml^{-1} approximately. After 1 week's culture, the young seedlings were inoculated with 5 ml of late log-phase Bradyrhizobia culture or 5 ml of the culture medium without bacteria for the control treatment. After 3 month's culture, the plants were uprooted and the shoots and roots separated. Shoot and root dry matters were determined after drying at 65 °C for 1 week. Root nodules were counted on each plant and weighed (65 °C for 1 week).

2.5. Statistical analysis

Data were subjected to a one-way analysis of variance and means were compared with the Newman–Keul's test ($p < 0.05$). The distribution of PCR-RFLP groups among soil treatments was compared with 2 × 2 contingency tables and chi-square test (χ^2 test) and Yates correction for small numbers. Between-Group Analysis (BGA, Dolédec and Chessel, 1987; Culhane et al., 2002) was used to analyse the relationships between Bradyrhizobia isolate effectiveness on *F. albida* (shoot and root biomass, number and dry weight of nodules per plant) and their soil origins: PS (soil samples collected from the soil of the *F. albida* parkland surrounding the *A. holosericea* plantation), NIS (soil samples collected under not inoculated *A. holosericea* trees), and IR100S (soil samples collected under *A. holosericea* trees inoculated with *P. albus* IR100).

BGA is a very simple multivariate analysis method, 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 sampling points (rows of the table) has the highest possible variance. This characteristic ensures that the associated graphs (principal component maps) to represent the initial data. In PCA, the principal components (PCs) are linear combinations of the original variables (columns of the data table), and they have the property of having the highest possible correlation with all these variables.

Unlike PCA, BGA is a classification method: groups of samples are defined before the analysis is run, and, like discriminant analysis (DA), BGA looks for functions that best separate the groups. DA suffers from several drawbacks, particularly the fact that the number of individuals must be high compared to the number of variables and that high correlations between the variables can lead to spurious results. BGA is a very robust method, that can be used even when the number of individuals is

Table 1
Number and percentages of Bradyrhizobia nodulating *F. albida* per PCR-RFLP group isolated inside the *A. holosericea* plantation from each treatment (not inoculated plots and *P. albus* inoculated plots) and outside the *A. holosericea* plantation (soil of *F. albida* parkland).

PCR-RFLP groups	Total number and percentages of isolates collected from		
	F. albida Parkland soil (30 isolates)[a]	Uninoculated *A. holosericea* soil (38 isolates)	*P. albus*/*A. holosericea* soil (32 isolates)
A	14 (46.6)[b]	4 (10.5)	19 (59.4)
B	0	15 (39.5)	3 (9.4)
C	8 (26.7)	18 (47.4)	0 (0)
D	8 (26.7)	1 (2.6)	10 (31.2)

[a] Total number of Bradyrhizobia analyzed by PCR-RFLP from each treatment.
[b] Total number and percentage of isolates of each RFLP-PCR group from each soil treatment.

less than the number of variables, or when variables are highly correlated. From a technical point of view, BGA is very simple: it is just the PCA of the table of group means. The mean of each variable in each group is computed to build the table of group means, and a PCA is done on this table. Row scores of the original table are computed by projection on the subspaces generated by principal components.

Finally, a Monte-Carlo test (multivariate permutation test) is used after BGA to check the significance of the differences between groups. This is also an advantage compared to DA, as the significance tests of DA need several hypotheses that are often difficult to confirm. BGA permutation tests can be used even when the number of individuals is low (even lower than the number of variables), when the variables are correlated, or when they have irregular distributions. Computations and graphical displays were realised with the free ade4 package for R (http://pbil.univ-lyon1.fr/ade4/) (R Development Core Team, 2007).

Root/shoot ratio data are presented as a measure of plant biomass allocation. The non-parametric Wilcoxon two-sample test was used to compare the location of the median of the distribution of Bradyrhizobia groups according to their effects on plant biomass allocation and their soil treatment origins (Hollander and Wolfe, 1999).

3. Results

Population densities of Bradyrhizobia nodulating *F. albida* ranged among the treatments as follows: soil collected under ectomycorrhized *A. holosericea* trees (12.4 Bradyrhizobia per g of soil) > soil collected under uninoculated *A. holosericea* trees (7.9 Bradyrhizobia per g of soil) > soil collected from the *F. albida* parkland soil (4.9 Bradyrhizobia per g of soil).

All the isolated Bradyrhizobia were slow-growing strains. Total DNA was extracted and amplified from 100 crushed nodules (Krasova-Wade et al., 2003). Single IGS PCR products were usually observed, ranged from 600 bp to 1170 bp. According to the RFLP profile obtained for each nodule, the 100 nodules were divided into four groups, corresponding to four distinct restriction patterns named PCR-RFLP group from A to D (Table 1). Three different groups were detected from the parkland soil and from the *P. albus*/*A. holosericea* soil whereas 4 groups were recorded from the uninoculated *A. holosericea* soil treatment (Table 1). The distribution of Bradyrhizobia PCR-RFLP groups was highly dependent on the soil treatment origin according to the χ^2 test (Parkland soil *vs* uninoculated *A. holosericea* soil, $\chi^2 = 29.3$, $p < 0.001$; Parkland soil *vs P. albus*/*A. holosericea* soil: $\chi^2 = 11.9$, $p < 0.007$) and uninoculated *A. holosericea* soil *vs P. albus*/*A. holosericea* soil, $\chi^2 = 42.9$, $p < 0.0001$).

After 3 month's culture in glasshouse conditions, only one Bradyrhizobia isolated from the *F. albida* parkland soil (PS4) stimulated the shoot growth of *F. albida* seedlings (Table 2). Compared to the control (without Bradyrhizobia inoculation), the root biomass of the *F. albida* seedlings was significantly enhanced by five bacterial strains isolated from the *F. albida* parkland soil (PS7, PS8, PS10, PS11 and PS14), five from the uninoculated *A. holosericea* soil (NIS1, NIS5, NIS8, NIS11 and NIS14) and three from the *P. albus*/*A. holosericea* soil (IR100S7, IR100S8 and IR100S9) (Table 1). For the symbiotic nodulation development, the average total number of nodules per *F. albida* seedlings was significantly higher with the Bradyrhizobia strains isolated from the *A. holosericea* soil plantation (with or without *P. albus*) (Table 2) whereas the mean total nodule biomass per *F. albida* seedlings was significantly higher with Bradyrhizobia isolated from *P. albus*/*A. holosericea* soil plantation

Table 2
Symbiotic performance (shoot, root and total biomass) of Bradyrhizobia strains isolated from soils sampled inside the *A. holosericea* plantation (not inoculated plots and *P. albus* inoculated plots) and outside the *A. holosericea* plantation (soil of *F. albida* parkland), on *F. albida* growth after 3 month's culture in controlled conditions.

Strains	SB (mg)[a]	RB (mg)[b]	Strains	SB (mg)	RB (mg)	Strains	SB (mg)	RB (mg)
Control[c]	289 (21)[d]	524 (25)	Control	289 (21)	524 (25)	Control	289 (21)	524 (25)
PS (1)[e]	288 (15)	605 (64)	NIS (1)[f]	332 (41)	**663*[h]**(39)	IR100S (1)[g]	345 (39)	602 (37)
PS (2)	290 (35)	608 (36)	NIS (2)	365 (30)	**702*** (**52**)	IR100S (2)	270 (13)	570 (46)
PS (3)	260 (22)	587 (51)	NIS (3)	260 (24)	582 (33)	IR100S (3)	240 (32)	562 (47)
PS (4)	**388*** (**27**)	573 (58)	NIS (4)	318 (49)	568 (12)	IR100S (4)	392 (64)	587 (67)
PS (5)	283 (27)	607 (46)	NIS (5)	280 (23)	**658*** (**53**)	IR100S (5)	257 (34)	633 (76)
PS (6)	342 (47)	560 (56)	NIS (6)	330 (37)	558 (44)	IR100S (6)	293 (17)	573 (72)
PS (7)	355 (39)	**737*** (**57**)	NIS (7)	323 (37)	622 (74)	IR100S (7)	303 (36)	**673*** (**50**)
PS (8)	262 (13)	**707*** (**27**)	NIS (8)	363 (51)	**625*** (**23**)	IR100S (8)	335 (44)	**653*** (**24**)
PS (9)	373 (89)	523 (56)	NIS (9)	350 (34)	608 (51)	IR100S (9)	282 (24)	**655*** (**43**)
PS (10)	322 (35)	**660*** (**46**)	NIS (10)	313 (37)	613 (69)	IR100S (10)	212 (31)	622 (89)
PS (11)	283 (15)	**652*** (**45**)	NIS (11)	367 (28)	**667*** (**61**)	IR100S (11)	362 (44)	540 (51)
PS (12)	262 (33)	572 (69)	NIS (12)	322 (24)	593 (36)	IR100S (12)	215 (14)	603 (11)
PS (13)	287 (25)	648 (89)	NIS (13)	255 (18)	462 (23)	IR100S (13)	347 (48)	607 (28)
PS (14)	353 (34)	**667*** (**53**)	NIS (14)	345 (34)	**632*** (**44**)	IR100S (14)	252 (23)	603 (66)

[a] SB: Shoot biomass (mg dry weight).
[b] RB: Root biomass (mg dry weight).
[c] Control: uninoculated treatment.
[d] Standard error.
[e] PS: Bradyrhizobia strains isolated from the soil of *F. albida* parkland outside the *A. holosericea* plantation.
[f] NIS: Bradyrhizobia strains isolated from the soil sampled under uninoculated *A. holosericea* trees.
[g] IR100S: Bradyrhizobia strains isolated from the soil sampled under *A. holosericea* trees inoculated with *P. albus* strain IR100.
[h] For each soil origin of Bradyrhizobia strains, data followed by the asterisk and in bold are significantly different from the control according to the Newman–Keul's test ($p < 0.05$).

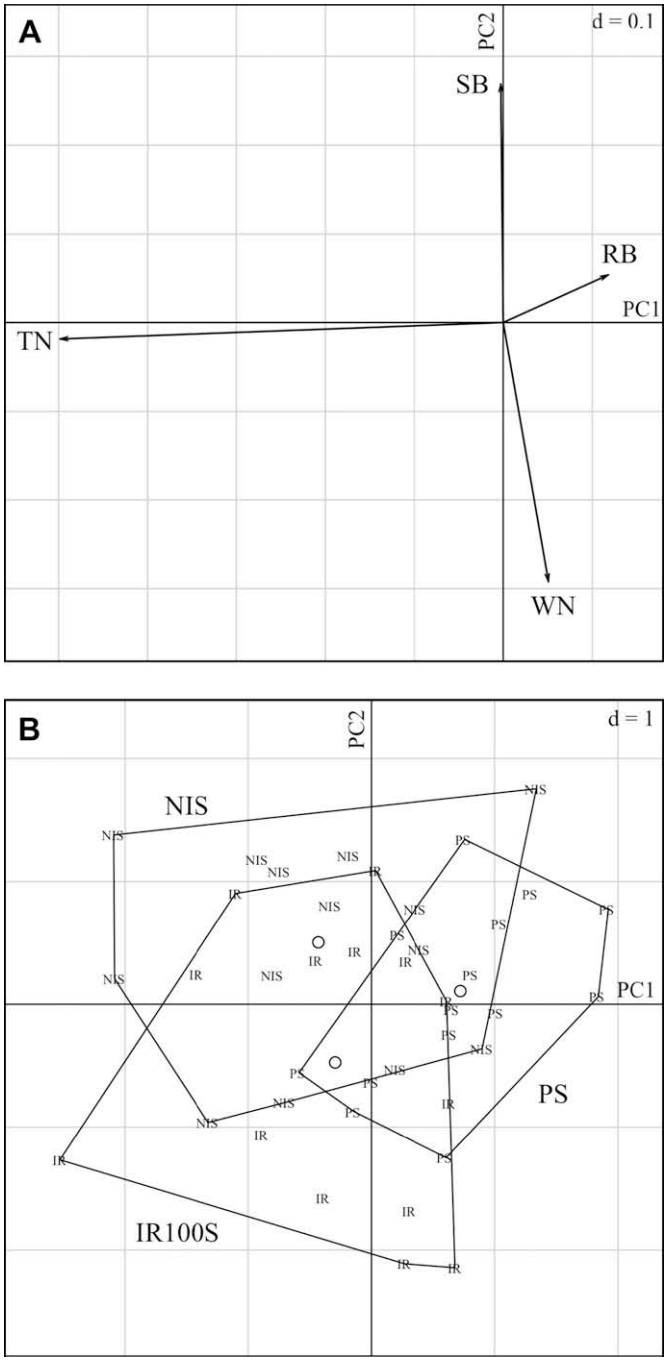

Fig. 1. Between-group analysis (BGA) of *Faidherbia albida* growth (shoot and root biomass: SB and RB, respectively) and nodule formation (total number and dry weight of nodules per plant: TN and WN, respectively). A: plot of variable loadings. B: plot of sample scores. The scale is given by the value in the upper right corner: this value represents the length of the side of background grid squares. The second principal component opposes the shoot biomass (up) to the nodule dry weight (down). The plot of sample scores (B) is split in three groups, according to the origin of the Bradyrhizobia isolates: PS, soil of *F. albida* parkland collected outside the *A. holosericea* plantation, NIS, soil of plantation with not inoculated trees, and IR100S, soil of plantation with IR100-inoculated trees. The circle inside each convex hull gives the position of the gravity center of each group.

compared to that from uninoculated *A. holosericea* soil plantation (Table 2).

Results from the BGA of the *F. albida* growth and nodule formation with respect to the three Bradyrhizobia origins are presented in Fig. 1. The Monte-Carlo test demonstrated that the differences between Bradyrhizobia origins were statistically significant ($p \sim 0.01$). The BGA clearly indicated that the increase of root biomass of *F. albida* was positively linked to the inoculation of Bradyrhizobia isolated from the *F. albida* parkland soil. The total number of nodules per *F. albida* seedlings was positively linked to the inoculation of Bradyrhizobia isolated from the *A. holosericea* soil plantation (with or without *P. albus*). The classification of Bradyrhizobia with respect to their effect on root/shoot ratios of *F. albida* seedlings revealed that the highest numbers resulted from the inoculation of Bradyrhizobia isolated from the *P. albus*/*A. holosericea* soil plantation whereas the lowest were recorded from the uninoculated *A. holosericea* soil plantation (Fig. 2). The distributions of Bradyrhizobia isolated from the Parkland soil and from the *P. albus*/*A. holosericea* soil plantation were significantly different to that recorded for the Bradyrhizobia isolated from the uninoculated *A. holosericea* soil plantation according to their influence on *F. albida* root/shoot ratio (Wilcoxon test, $p < 0.05$).

4. Discussion

From this research work, two main points deserve discussion: (i) whether an exotic fast growing tree species, *A. holosericea*, can modify the structure of Bradyrhizobia populations and their effectiveness on the native *Acacia* species, *F. albida*, in a Sahelian soil and (ii) whether these effects can be counterbalanced by the introduction of an ectomycorrhizal fungus, *P. albus*, on *A. holosericea* root systems (Table 3).

4.1. Responses of Bradyrhizobia populations to the exotic tree plantation

In Sahelian areas, it has been previously found that surface nodulation of *F. albida* is lacking and surface Bradyrhizobia populations are low. For instance, in the Sahelian ecoclimatic zone of Senegal (100–500 mm of annual rainfall), Dupuy and Dreyfus (1992) found that population densities of Bradyrhizobia were low or negligible at the soil surface that corroborate the data of the present study. It has been also demonstrated that *A. holosericea* was preferentially nodulated with slow-growing strains (Dreyfus and Dommergues, 1981; Duponnois et al., 2000a,b). Hence since this exotic tree species develops an extensive root system and more particularly near the soil surface (Duponnois, pers. com.), it facilitates the Bradyrhizobia multiplication and increases Bradyrhizobia population densities. In addition to the positive effect on Bradyrhizobia multiplication, the introduction of the uninoculated *A. holosericea* trees induced severe disturbances in Bradyrhizobia genotypic diversity by promoting the predominance of PCR-RFLP profiles such as IGS groups B and C. Numerous studies have clearly showed that the structure and functional diversity of microbial communities in the soil were mainly dependent on aboveground plant composition (Grayston et al., 2001). Previous sequencing analysis of 16S–23S rDNA intergenic spacer (ITS) indicated that representatives of the groups B and C belong to genospecies IV of Willems et al. (2001). These authors showed that genospecies IV comprised Bradyrhizobia mostly isolated from the tree *F. albida* in West Africa. In this last study, DNA–DNA hybridizations were performed between *Bradyrhizobium* strains, isolated mainly from *F. albida* and *Aeschynomene* species. The results indicated the genus *Bradyrhizobium* consisted of at least 11 genospecies. The genospecies IV comprised Bradyrhizobia strains ranged from highly effective strains (ORS188), effective strains (ORS101), partially effective strains (ORS187) and ineffective strains (ORS146) (Dupuy and Dreyfus, 1992). However Bradyrhizobia strains positively selected by *A. holosericea* trees were less effective on the *F. albida* growth compared to the other bacterial isolate origins since root/shoot

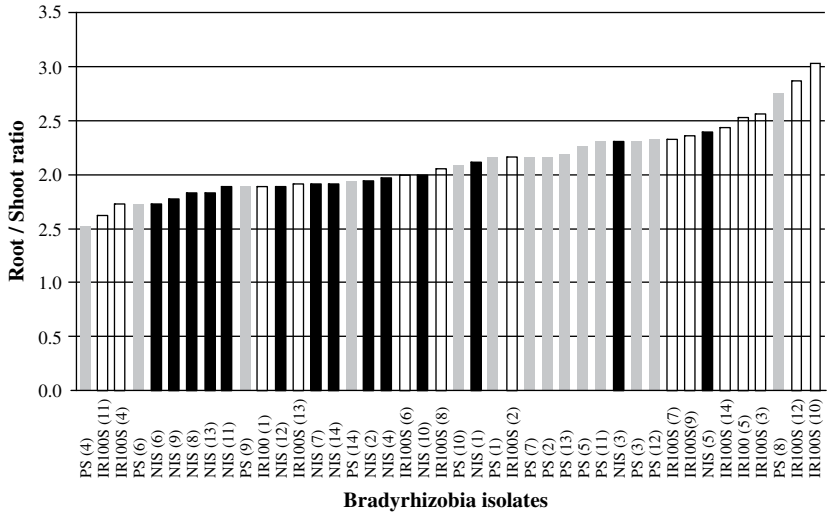

Fig. 2. Effect of Bradyrhizobia strains isolated from soils sampled inside the *A. holosericea* plantation (not inoculated plots and *P. albus* inoculated plots) and outside the *A. holosericea* plantation (soil of *F. albida* parkland collected outside the *A. holosericea* plantation) on the biomass allocation of *F. albida* seedlings (measured by root/shoot ratio) after 3 month's culture in controlled conditions.

ratios of *F. albida* seedlings inoculated with Bradyrhizobia isolated from *A. holosericea* soil plantation were lower than those recorded with Bradyrhizobia from the other soil origins. High root/shoot ratio has been identified as an important factor allowing plants to exploit reduced resource availability due to patchiness in distribution, both for water and nutrients (Reader et al., 1992). These high ratios would be of great importance in the regeneration process of native tree species especially during periods of drought or where nutrient resources are heterogeneously distributed. Hence the exotic tree species could indirectly limit the growth of young regeneration of *F. albida* by limiting the multiplication of Bradyrhizobia, highly efficient on the *F. albida* growth.

4.2. Effect of ectomycorrhizal inoculation on soil Bradyrhizobia populations

In contrast, the controlled ectomycorrhization significantly counterbalanced the effect of the exotic tree species on Bradyrhizobia populations. Many studies have shown that ectomycorrhizal symbiosis helped nodule formation and rhizobia multiplication in glasshouse conditions (Duponnois and Plenchette, 2003; Duponnois et al., 2000b, 2002) but this ectomycorrhizal effect remained unknown in field conditions. In the present study, Bradyrhizobia soil potential was higher in the soil under *P. albus*/*A. holosericea* trees. This mycorrhizal-promoting effect probably results from a better root growth that favored rhizobia colonization and infection. But, it is also well known that mycorrhizas modify root functions (in particular, root exudation) and, therefore, could modify microbial communities (commonly termed the "mycorrhizosphere effect") (Katznelson et al., 1962; Linderman, 1988). The development of a number of different organisms is influenced by the mycorrhizosphere effect, for example, protozoa (Jentschke et al., 1995; Wamberg et al., 2003), microarthropods (Cromack et al., 1988), microfungi (Neal et al., 1964) and bacteria (Andrade et al., 1997; Frey-Klett et al., 2005). Surprisingly, the influence of ectomycorrhizal symbiosis on the structure of Bradyrhizobia populations has not been studied in field conditions. In the present study, the ectomycorrhizal inoculation of *A. holosericea* drastically modified the Bradyrhizobia soil diversity by promoting the multiplication of bacteria belonging to the IGS groups A and D. The analysis of 16S–23S rDNA intergenic spacer (ITS) indicated that representatives of the group A belong to *Bradyrhizobium yuanmingense* (Yao et al., 2002) while the group D was not related to the currently known genospecies (data not published). In addition highest root/shoot ratios of *F. albida* seedlings were recorded with the Bradyrhizobia isolated from the soil collected under inoculated *A. holosericea* trees. Since the level of this ratio is linked to a higher capacity of plant species to grow under stress conditions, this "mycorrhizosphere" effect would be useful to help the natural regeneration of native plant species such as *F. albida*.

Table 3
Average symbiotic development (total number of nodules per plant, total dry weight of nodules per plant) of Bradyrhizobia strains on the *F. albida* seedlings after 3 month's culture in controlled conditions. Bradyrhizobia were isolated from soils sampled inside the *A. holosericea* plantation (not inoculated plots and *P. albus* inoculated plots) and outside the *A. holosericea* plantation (soil of *F. albida* parkland).

	Soil origin		
	Parkland soil	Plantation with uninoculated trees	Plantation with IR100-inoculated trees
Total number of nodules per plant	5.9 a[a]	8.7 b	8.4 b
Total nodule dry weight per plant (mg)	4.61 ab	4.01 a	5.31 b

[a] Data in the same line followed by the same letters are not significantly different according to the Newman–Keul's test ($p < 0.05$).

4.3. Conclusion

This study shows that exotic plant species can drastically affect structure and symbiotic effectiveness of native Bradyrhizobia populations that could limit the natural regeneration of endemic plant species such as *F. albida*. van der Heijden et al. (2006) showed that some legume species (*Lotus corniculatus*, *Trifolium repens*, etc.) require rhizobia to successfully coexist with other plants in natural communities. Disturbances of native rhizobia communities could alter plant competitive interactions and, more particularly, decrease the capacity of legumes to out-compete other plants. The present work shows that the introduction of exotic legume tree species such as *A. holosericea* strongly modifies this important group of mutualists and could minimize the potential effects of this

cultural practice on ecosystem rehabilitation process. This field-based experimental research outlights the role of mycorrhizal symbiosis in afforestation programmes with exotic tree species that target preservation of native plants. As it has been also demonstrated in controlled conditions (Kisa et al., 2007), mycorrhizal symbiosis can counterbalance the negative influence of exotic tree species (i.e. *Eucalyptus camaldulensis*) through different ways that remain unknown.

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