

## The Exotic Legume Tree Species *Acacia holosericea* Alters Microbial Soil Functionalities and the Structure of the Arbuscular Mycorrhizal Community<sup>∇</sup>

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**The response of microbial functional diversity as well as its resistance to stress or disturbances caused by the introduction of an exotic tree species, *Acacia holosericea*, ectomycorrhized or not with *Pisolithus albus*, was examined. The results show that this ectomycorrhizal fungus promotes drastically the growth of this fast-growing tree species in field conditions after 7 years of plantation. Compared to the crop soil surrounding the *A. holosericea* plantation, this exotic tree species, associated or not with the ectomycorrhizal symbiont, induced strong modifications in soil microbial functionalities (assessed by measuring the patterns of in situ catabolic potential of microbial communities) and reduced soil resistance in response to increasing stress or disturbance (salinity, temperature, and freeze-thaw and wet-dry cycles). In addition, *A. holosericea* strongly modified the structure of arbuscular mycorrhizal fungus communities. These results show clearly that exotic plants may be responsible for important changes in soil microbiota affecting the structure and functions of microbial communities.**

Forest loss and degradation through human disturbance as well as deterioration of land productivity is a major problem in large areas of arid and semiarid environments. Degraded soils are characterized by loss or disturbance of the vegetation cover, increased soil erosion, decreased in-water infiltration, loss of available nutrients and organic matter, loss of microbial propagules, and/or diminution in microbial activity (25, 35, 44). Restoration of ecosystem health and productivity was traditionally achieved via abandonment of land and subsequent natural forest succession. In recent decades, management options for acceleration of the recovery and restoration of the productivity and biodiversity of disturbed ecosystems have been considered, since fallow periods have been shortened or eliminated due to increased anthropogenic pressure and agriculture intensification (49). Concerning the techniques used for rehabilitating degraded areas, there is increasing evidence that forest plantations can play a key role in ecosystem rehabilitation or restoration (52). Among candidate plantation species, fast-growing leguminous tree species (e.g., Australian *Acacia* species) should have preference.

*Acacia* is the largest mimosoid genus, with 1,200 species (53), and their presence in savannas and arid regions of Australia, Africa, India, and the Americas has been frequently

recorded. These multipurpose trees are useful for revegetation of degraded ecosystems that have low availability of nitrogen and phosphorus (4, 6, 13, 14, 55) because of their ability to form symbiotic associations with both rhizobial bacteria and mycorrhizal fungi. These bacterial and fungal symbionts are known to be key components of natural systems (8, 64, 65, 66), since they are involved in governing the cycles of major plant nutrients and in sustaining the vegetation cover in natural habitats (63). However, the success of revegetation programs may be limited by the low density of mycorrhizal propagules generally observed in degraded soils in semiarid and arid ecosystems (25). Hence, it may be necessary to reinforce or replace the native inoculum potential of mycorrhizal fungi by appropriate inoculation technologies (29, 30). Fast-growing leguminous trees belonging to the *Acacia* genus, brought from Australia and introduced to Western Africa, appear to be well adapted to sahelian and Sahelo-Sudanian climatic conditions (11, 57). In addition to rhizobial symbiosis, this tree species can form arbuscular mycorrhiza (AM) and/or ectomycorrhiza (19, 31). As with many N<sub>2</sub>-fixing trees and shrubs, Australian *Acacia* species are especially dependent on mycorrhizas to absorb mineral nutrients required for plant growth and efficient N<sub>2</sub> fixation. It has been previously demonstrated that controlled mycorrhizal inoculation could improve the development of these tree species in glasshouse conditions (24, 26, 27) and also after outplanting into the field (10, 29, 30). All these studies have been mainly focused on the biomass production of *Acacia* plantations, and little is known about the influence of mycor-

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rhizal inoculation on the functional diversity of native microbial communities involved in soil functioning, especially when these soils are planted with exotic tree species.

The present study addressed the following questions: (i) does the microbial functional diversity of a site planted by exotic tree species differ from that of an adjacent unplanted site, and if so, (ii) does ectomycorrhizal inoculation influence this microbial response? To answer these questions, the effect of the presence of an ectomycorrhizal fungus, *Pisolithus albus* IR100, was determined by examining the development of an Australian acacia, *A. holosericea*, in a field plantation in a dry tropical environment. In addition, modifications induced by this exotic tree species, associated or not with *P. albus* IR100 treatment, have been measured in microbial functional capabilities and in the structure of native arbuscular mycorrhizal fungus (AMF) communities, an essential component of sustainable soil-plant systems (39).

#### MATERIALS AND METHODS

**(i) Plant material and ectomycorrhizal inoculation.** Seeds of *A. holosericea*, ex G. Don, provenance Bel Air (Dakar, Senegal), were surface sterilized with concentrated 36 N sulfuric acid for 60 min. They were then rinsed for 12 h in sterile distilled water and transferred aseptically to petri dishes filled with 1% (wt/vol) agar/water medium. After 8 days of incubation at 25°C in the dark, the germinating seeds were used when rootlets were 1 to 2 cm long.

The ectomycorrhizal fungus *Pisolithus albus* IR100 was routinely maintained on modified Melin-Norkrans (MMN) agar medium at 25°C (48). Glass jars (1 liter) were filled with 600 ml of a mixture of vermiculite and peat moss (4/1 [vol/vol]) and autoclaved (120°C for 20 min). The substrate was then moistened to field capacity with 300 ml of liquid MMN medium. The jars were sealed with cotton floats and autoclaved at 120°C for 20 min. After cooling, the substrate was inoculated with fungal plugs taken from the margin of the fungal colonies and incubated for 6 weeks at 28°C in the dark (23).

*Acacia* seedlings were grown in 1-liter pots filled with an autoclaved sandy soil (120°C for 40 min) collected from a stand of *A. holosericea* trees located east of Dakar (Senegal). After sampling, the soil was crushed, passed through a 2-mm-pore-size sieve, and autoclaved for 40 min at 120°C to eliminate the native microbiota. After autoclaving, its physicochemical characteristics were as follows: H<sub>2</sub>O pH, 5.3; clay, 3.6%; fine silt, 0.0%; coarse silt, 0.8%; fine sand, 55.5%; coarse sand, 39.4%; carbon, 0.17%; total nitrogen, 0.02%; soluble phosphorus, 4.8 mg kg<sup>-1</sup>; total phosphorus, 39 mg kg<sup>-1</sup>. The ectomycorrhizal inoculation was performed by mixing the fungal inoculum with the soil (10/1 [vol/vol]). The control treatment (without fungus) included an autoclaved mixture of vermiculite and peat moss moistened in MMN medium at the same rate. The pots were arranged in a randomized complete block design. The plants were grown in a glasshouse under natural light conditions (approximately 12 h of daylight, mean daily temperature 30°C) and watered daily with tap water.

After 4 months of culturing, 10 plants were randomly chosen from each treatment. They were uprooted and their root systems gently washed. The oven dry weight (1 week at 65°C) of the shoot was measured. Then, the root systems were cut into 1-cm-long root pieces and mixed. The percentage of ectomycorrhizal short roots (number of ectomycorrhizal short roots/total number of short roots) was determined using a random sample of at least 100 short roots per treatment under a stereomicroscope (magnification ×40). Some nodules were observed along the root systems despite disinfection of the soil and the seed surface. Root nodules were counted and their dry weight (after 65°C for 1 week) determined. The dry weight of roots (after 1 week at 65°C) was then measured.

**(ii) Experimental design, tree growth measures, and soil sampling.** The study site was located in Senegal at Ngane village (17°50'W to 14°10'N) (15 km at the west of Kaolack) on a ferruginous soil (29, 30). The climate is Sahelo-Sudanian and tropically dry, with an average annual rainfall of 700 mm and a mean annual temperature of 25°C. Two seasons can be distinguished: (a) the long dry season from October to June and (b) the rainy season from July to September. The physicochemical characteristics of the soil were as follows: H<sub>2</sub>O pH, 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<sup>-1</sup>; and total phosphorus, 54.7 mg kg<sup>-1</sup>.

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 a nonplanted area 6 m wide. In July 2000, *A. holosericea* seedlings were planted in individual holes (20 cm diameter and 30 cm depth) at 3 m apart in an area of 900 m<sup>2</sup>. They were at least 27 seedlings per treatment and replication (nine plants and two treatments in each block). The dead plants were replaced in each treatment block during the first months of plantation. After 7 years of plantation, the stem diameter of each tree was measured at 0.1 m above the soil. Then, phyllode, small branch, wood, and litter biomasses were evaluated according to allometric relations previously established (30).

A transect was marked out along the diagonal of each plot with two sets of soil samples (four samples per set that were pooled together). In addition, six soil samples were taken from the surrounding cultivated area (millet plantation) of the *A. holosericea* plantation. These soil samples (250 cm<sup>3</sup> each) were collected during the wet season from the 0 to 10 cm layer and stored in sealed plastic bags with field moisture content at 4°C for further measurements. All soil samples were characterized by measuring total C, total N, total P, and soluble P levels (50) in the Laboratoire des Moyens Analytiques laboratory (Certifié International Standard for Organization 9001, version 2000, Dakar, Senegal; US Imago [Unité de Service Instrumentations, Moyens Analytiques, Observatoires en Géophysique à Océanographie], Institut de Recherche pour le Développement [www.lama.ird.sn]).

**(iii) Soil characteristics.** (a) *Measurement of microbial functional diversity.* Microbial functional diversity of soil treatments was assessed by measuring the patterns of in situ catabolic potential of microbial communities (15). This method is based on the measurement of short-term respiration responses of soils amended with a range of simple organic compounds (15). One gram of equivalent dry soil was moistened with each of the 33 (15) substrates suspended in 2-ml sterile distilled water in 10 ml bottles (68). CO<sub>2</sub> production from basal respiratory activity in the soil samples was measured by adding 2 ml sterile distilled water to 1 g equivalent dry weight of soil. The bottles were sealed with a Vacutainer stopper and incubated at 28°C for 4 h in the dark. The amount of respired CO<sub>2</sub> in the headspace of each bottle was determined by taking a 1-ml syringe sample. The CO<sub>2</sub> concentration was analyzed for each sample by using an infrared gas analyzer (Polytron IR CO<sub>2</sub>; Dräger) in combination with a thermal flow meter (38). Carbon dioxide measurements were subtracted from the CO<sub>2</sub> basal production measurements and were expressed as micrograms of CO<sub>2</sub> per gram of soil per hour. Among the 33 substrates, there were nine amino acids (L-arginine, L-serine, L-glutamic acid, L-phenylalanine, L-asparagine, L-lysine, L-cysteine, L-tyrosine, and L-histidine), two amines (D-glucosamine and L-glutamine), two amides (N-methyl-D-glucamine and L-succinamide), three carbohydrates (D-mannose, D-sucrose, and D-glucose), and 17 carboxylic acids (α-ketobutyric acid, α-ketoglutaric acid, fumaric acid, oxalic acid, tartaric acid, gluconic acid, ascorbic acid, D,L-malic acid, malonic acid, quinic acid, 3-OH-butyric acid, formic acid, gallic acid, succinic acid, trisodium citric acid, uric acid, and citric acid). The amines and amino acids were added at 10 mM, whereas the carbohydrates were added at 75 mM and the carboxylic acids at 100 mM (16). Catabolic evenness (a measure of relative variability in the catabolic functions) was calculated using the Simpson-Yule index  $E = 1/p^2$ , with  $p_i$  = (respiration response to individual substrates)/(total respiration activity induced by all substrates for a soil treatment) (47). Microbial biomass C levels calculated using the substrate-induced respiration method as described by Sparling (60). One gram of oven-dry weight of soil was suspended in 2 ml of a 75 mM glucose solution in 10 ml bottles sealed with a Vacutainer stopper and incubated at 25°C for 4 h. After correction for CO<sub>2</sub> produced in bottles with only deionized water added, the microbial biomass C levels were calculated as follows: (micrograms of C per gram of soil) = 50.4 × respiration rate (micrograms of C per gram of soil per hour).

(b) *Assessment of soil microbial resistance to stress and disturbance.* Each soil sample, maintained at field moisture content (50% of maximum water holding capacity), was subjected to one stress and three disturbance treatments according to the methods described by Degens et al. (18). The stress treatment consisted of increasing soil salinity. The disturbance treatments consisted of increasing soil temperature and consecutive wet-dry and freeze-thaw cycles. Control treatments were performed by a continuous incubation at 25°C in the dark to verify whether the incubation period imposed to soil samples in the wet-dry and freeze-thaw treatments (24 days) had any effects on microbial activity.

Three levels of the salinity stress treatment were obtained using a solution of NaCl to modify the soil salinity, which was increased from an electrical conductivity level of 0.04 mS cm<sup>-1</sup> (control without NaCl addition) to 0.3, 0.7, and 1.1 mS cm<sup>-1</sup>.

TABLE 1. Effect of *P. albus* inoculation on the growth of *A. holosericea* seedlings and on nitrogen fixative symbiosis after 4 months of culture growth under glasshouse conditions<sup>a</sup>

Treatment	Shoot biomass (mg dry wt)	Root biomass (mg dry wt)	No. of nodules per plant	Total nodule biomass per plant (mg dry wt)	Ectomycorrhizal colonization index (%)
Control (no inoculation)	650a	351a	8.6a	2.8a	
<i>P. albus</i> IR100 inoculation	1,545b	795b	12.3b	10.5b	30.5

<sup>a</sup> Data followed by the same letter in each column are not significantly different according to results of the Newman-Keuls test ( $P < 0.05$ ).

For temperature disturbance treatment, soil samples were incubated at 25°C (control), 37°C, 45°C, and 50°C in the dark for 1 week.

Each wet-dry cycle consisted of air drying at 25°C for 24 h followed by a rapid rewetting to initial field moisture content and incubation for 48 h in the dark at 25°C. One, two, four, six, and eight successive wet-dry cycles were imposed on soil samples. Freeze-thaw cycles consisted of freezing at -20°C for 24 h followed by thawing and incubation at 25°C for 48 h. Soil samples were subjected to one, two, four, six, and eight successive freeze-thaw cycles.

There were three replicates per stress level. After each stress or disturbance treatment, soil samples were kept at 25°C in sealed plastic bags for 2 weeks to permit equilibration of the microbial communities and decomposition of organic C from microorganisms destroyed by the treatments (18).

(c) **Total soil microbial activity (FDA).** After each stress or disturbance treatment, total microbial activity in soil samples was estimated using a fluorescein diacetate (3',6'-diacetylfluorescein [FDA]) hydrolysis assay (58). FDA (Sigma-Aldrich Chemie, France) was dissolved in acetone and stored as a stock solution (5 mg ml<sup>-1</sup>) at -20°C. Soil samples (1 g equivalent dry weight) were suspended in 200 µl FDA and 15 ml of sterile 60 mM sodium phosphate buffer (pH 7.6). The mixture was incubated at 25°C for 1 h on a rotary shaker. Then, the FDA hydrolysis reaction was stopped by adding 750 µl acetone. Soil suspensions were centrifuged (2,400 × g for 10 min), and the supernatant was sampled and passed through a 45-µm-pore-size filter. Then, the absorbance readings were taken at 490 nm. Three replicates were prepared for each treatment, and a fourth received 15 ml of buffer without substrate; this served as a control to correct for background. A standard fluorescein concentration curve, ranging from 0 to 0.5 mg liter<sup>-1</sup>, was prepared fresh using the stock solution of fluorescein diacetate in sodium phosphate buffer. The rate of fluorescein diacetate hydrolysis was calculated (in micrograms of product corrected for background fluorescence per hour per gram of soil) to determine total microbial activity for each soil origin.

(d) **Description of the structure of AMF communities.** Spores of AM fungi were extracted from soil samples by wet sieving and decanting followed by sucrose centrifugation (59). Then, the supernatant was poured through a 50-µm-pore-size sieve and rinsed with tap water. Spores were counted using a stereomicroscope and grouped according to morphological characteristics. The uniformity of morphological groups was confirmed under the optical microscope, and the different morphotypes were identified with respect to genus. Spore identification was assessed, mainly using spore size and color, wall structure, and hyphal attachment (67) (<http://www.invam.caf.wvu.edu/>). Mycorrhizal fungal spore diversity was calculated using the Simpson-Yule diversity index (43).

(iv) **Statistical analysis.** Data were analyzed using one-way analysis of variance. Means were compared using the Newman-Keuls test ( $P < 0.05$ ). The distribution of AMF species was compared between sets of soil origins using two-by-two contingency tables and  $\chi^2$  tests and Yates correction for small numbers.

Between-group analysis (BGA) (12, 22) was used to analyze the relationships between substrate-induced responses (SIRs) and the three soil origins: crop soil (i.e., soil samples collected from the crop soil surrounding the *A. holosericea* plantation), soil samples collected under uninoculated *A. holosericea* trees, and soil samples collected under *A. holosericea* trees inoculated with *P. albus* IR100. BGA is a multivariate analysis technique derived from principal components analysis (PCA). The aim of PCA is to summarize a data table by searching orthogonal axes to determine the projection of the sampling points (rows of the table) that has the highest possible variance. This characteristic ensures that the associated graphs (principal component maps) will best represent the initial data. These principal components have the property of having the highest possible correlation with the original variables (as shown in the columns of the data table).

From a theoretical point of view, BGA is a particular case of PCA with respect to instrumental variables (45, 54) in which the instrumental variable table is reduced to just one qualitative variable. This variable defines groups of rows in the data table, and BGA consists of the PCA of the table of the means by groups. This table has a number of rows equal to the number of groups and the same number of columns as the original table. The objective of this analysis is to separate the groups. This is also the aim of discriminant analysis (also called canonical variate analysis), but while discriminant analysis is limited to tables that have a high number of samples compared to the number of variables, BGA can be used even when the number of rows is less than the number of variables, as in the present study (33 SIRs versus nine soil samples). BGA can thus be considered a robust alternative to discriminant analysis in experiments with a low number of samples. A Monte-Carlo test (permutation test) was used to check the significance of the differences between groups. Computations and graphical displays were realized with the free ADE-4 software (62) available on the Internet at <http://pbil.univ-lyon1.fr/ADE-4/>.

## RESULTS

**Plant growth.** After 4 months of culture growth under glasshouse conditions, ectomycorrhizal inoculation significantly enhanced shoot and root growth (Table 1). Compared with the control results, the levels of shoot and root growth of *P. albus* IR100 plants were stimulated to 2.4-fold and 2.3-fold increases, respectively (Table 1). Although soil was autoclaved and seeds of *A. holosericea* were surface sterilized, some rhizobial nodules have been observed on the root systems. It was assumed that the tap water used during this experiment contained contaminant rhizobia. The number of nodules per plant and the total nodule biomass per plant were significantly increased in the *P. albus* IR100 treatment compared to the control results (Table 1).

Seven years after tree transplantation, the height, diameter, and phyllode, small branch, and wood biomasses as well as litter biomass in the ectomycorrhizal treatment samples were significantly higher than in the control samples (Table 2). For instance, litter biomass and wood biomass were increased 3.1-fold and 5.6-fold in the *P. albus* IR100 treatment samples, respectively (Table 2).

**Chemical and microbial characteristics of soils.** Soil nitrogen, carbon, and soluble phosphorus contents were significantly higher in the *P. albus* IR100 treatment samples, whereas

TABLE 2. Effect of *P. albus* IR100 inoculation on the growth of *A. holosericea* after 7 years of plantation in the field<sup>a</sup>

Treatment	Height (m)	Stem diam (cm)	Leaf biomass (kg per tree)	Small branch biomass (kg per tree)	Wood biomass (kg per tree)	Total aboveground biomass (kg per tree)	Litter biomass (kg m <sup>-2</sup> )
Control (no inoculation)	4.84a	11.8a	19.1a	16.9a	27.1a	63.1a	1.98a
<i>P. albus</i> IR100 inoculation	6.43b	23.2b	82.6b	75.1b	153.2b	310.9b	6.19b

<sup>a</sup> Data followed by the same letter in each column are not significantly different according to results of the Newman-Keuls test ( $P < 0.05$ ).

TABLE 3. Microbial biomass and chemical characteristics of the soils sampled inside the *A. holosericea* plantation from each treatment (uninoculated plots and *P. albus*-inoculated plots) and outside the *A. holosericea* plantation (crop soil)<sup>a</sup>

Soil origin	Total nitrogen (%)	Total carbon (%)	Total phosphorus (mg kg <sup>-1</sup> )	Soluble phosphorus (mg kg <sup>-1</sup> )	Microbial biomass (μg C g <sup>-1</sup> soil)
Crop soil	0.032a	0.398a	67.0a	4.6a	278.7b
Plantation with uninoculated trees	0.024a	0.338a	73.2ab	4.9a	227.5a
Plantation with <i>P. albus</i> IR100-inoculated trees	0.046a	0.600b	84.2b	7.1b	231.2a

<sup>a</sup> Data followed by the same letter in each column are not significantly different according to results of the Newman-Keuls test ( $P < 0.05$ ).

under inoculated *A. holosericea* trees, the microbial biomass was significantly lower than the level seen with the other treatments (soil collected from the uninoculated *A. holosericea* treatment and outside the tree plantation from the cultivated area) (Table 3). Soil total phosphorus contents were significantly different for soil sampled from the cultivated field compared to the results seen with the *P. albus* IR100 treatment (Table 3).

The catabolic evenness of the crop soil was significantly higher than those recorded in the other treatments (Table 4). The highest average respiration SIRs to amides and carbohydrates were recorded with the crop soil (Table 4), whereas the lowest SIR to amino acids was recorded with the uninoculated *A. holosericea* treatment (Table 4). Significant differences were found in the average SIRs to amines and carboxylic acids between the crop soil treatment and uninoculated *A. holosericea* treatment results, but for amino acids the highest SIR was found in the crop soil and for carboxylic acids in the uninoculated *A. holosericea* treatment (Table 4).

The three treatments gave very different SIR profiles (Fig. 1 and Fig. 2). The highest SIRs have been obtained with  $\alpha$ -ketobutyric and  $\alpha$ -ketoglutaric acids (Fig. 1). The permutation test of BGA showed that microbial functionalities were very different according to the soil treatment ( $P < 0.001$ ) (Fig. 2). The three treatments were very well separated on the BGA second axes (Fig. 2). The substrates preferentially used in samples collected from the field were L-asparagine, succinamide, cysteine, tyrosine, and trisodium citrate (Fig. 2). In contrast, the substrate preferentially used in the soil collected under uninoculated *A. holosericea* trees was malonic acid whereas it was L-arginine in the soil sampled under *A. holosericea* trees inoculated with *P. albus* IR100 (Fig. 2).

**Total microbial activities in stressed and disturbed soils.** Before any stresses or disturbances were imposed to the soil treatments, their total microbial activities were not significantly different (mean, 0.389 μg of hydrolyzed fluorescein diacetate

h<sup>-1</sup> g<sup>-1</sup> of soil). In addition, the longer incubation period required in imposing wet-dry and freeze-thaw treatments did not significantly affect the total microbial activities of soil samples. The effects of each stress and disturbance regimen on total microbial activities caused significant differences between soils with the highest catabolic evenness (crop soil) and those with the lowest catabolic evenness (soil sampled from *A. holosericea* plantation).

Increasing the salt stress caused a higher increase in the total microbial activities of crop soil up to 0.7 mS cm<sup>-1</sup>; after that, microbial activities decreased (Fig. 3A). At 0.7 mS cm<sup>-1</sup>, microbial activities ranged among the treatments as follows: crop field → plantation soil with *P. albus* IR100-inoculated trees → plantation with uninoculated trees and, at 1.0 mS cm<sup>-1</sup>, crop field → *A. holosericea* plantation (with or without *P. albus* inoculation).

Increasing soil temperature induced similar changes in each soil (Fig. 3B) but with a higher response seen with the crop soil (Fig. 3B). There was an increase of soil microbial activities for each treatment with all the temperatures imposed on the soil samples and a slight decrease for the crop soil after 45°C treatment (Fig. 3B).

Responses to wet-dry cycles were significantly higher with the crop soil than with the other soil treatments (Fig. 3C). Microbial activities increased up to two cycles and then decreased (Fig. 3C). At six cycles, the microbial activity was higher in the soil collected under inoculated *A. holosericea* trees than in that sampled under uninoculated *A. holosericea* trees.

Freeze-thaw cycles had effects opposite those recorded when the soils were imposed to wet-dry cycles (Fig. 3D). Microbial activities decreased up to two cycles and afterward decreased slightly (Fig. 3C). At eight cycles, the microbial activity was higher in the soil collected under inoculated *A. holosericea* trees than in that sampled under uninoculated *A. holosericea* trees (Fig. 3D).

TABLE 4. Catabolic evenness and average SIR with each substrate group (carboxylic acids, amino acids, amides and carbohydrates) in the soil treatments<sup>a</sup>

Soil origin	Catabolic evenness	Avg SIR (μg CO <sub>2</sub> g <sup>-1</sup> soil h <sup>-1</sup> )				Carboxylic acids
		Amino acids	Amides	Amines	Carbohydrates	
Crop soil	16.5b	8.7b	9.3b	9.8b	18.5b	14.3a
Plantation with uninoculated trees	9.8a	6.3a	4.3a	7.9a	10.9a	16.6b
Plantation with <i>P. albus</i> IR100-inoculated trees	9.5a	7.9b	3.6a	8.3ab	12.9a	14.8ab

<sup>a</sup> Data followed by the same letter in each column are not significantly different according to results of the Newman-Keuls test ( $P < 0.05$ ).

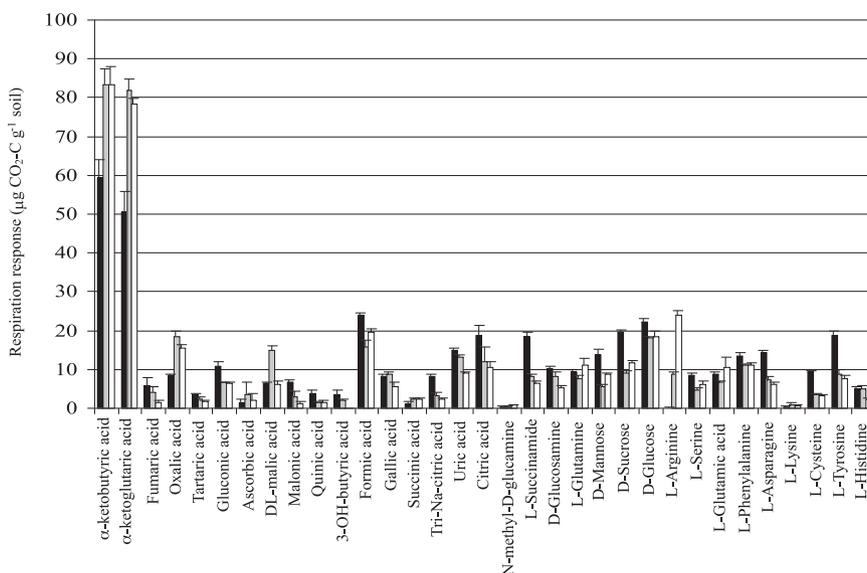


FIG. 1. Catabolic response profiles for each soil origin. Error bars represent standard errors ( $n = 6$ ). Black bars, crop soil; gray bars, plantation soil with uninoculated trees; white bars, plantation soil with *P. albus* IR100-inoculated trees.

**AMF communities.** The number of spores was significantly higher in the soil collected under inoculated *A. holosericea* trees (253 spores per 100 g of soil) than in both of the other soil categories (104 spores and 112 spores per 100 g of soil in the crop soil and in the soil collected under uninoculated *A. holosericea* trees, respectively). Nine AM species were detected in

the soils: *Scutellospora gregaria*; *S. verrucosa*; *Scutellospora* sp. strain 1; *Gigaspora* sp. strain 1; *Gigaspora* sp. strain 2; *Glomus rubiforme*; *G. aggregatum*; *Glomus* sp. strain 1; and *Glomus* sp. strain 2 (Fig. 4). Simpson indices (SI) ranged as follows: crop soil ( $7.66 \pm 1.1$ )→soils from *A. holosericea* plantation (inoculated,  $SI = 4.7 \pm 0.2$ ; uninoculated,  $SI = 5.2 \pm 0.4$ ). Spores of *Glomus* sp. strain 1 were mainly recorded in the soils collected from the *A. holosericea* plantation (Fig. 4). The distributions of AM species within soil origins were significantly different between the crop soil and the soil collected under uninoculated *A. holosericea* trees ( $\chi^2 = 63.9 [P < 0.0001]$ ) and between the crop soil and the soil collected under inoculated *A. holosericea* trees ( $\chi^2 = 106.6 [P < 0.0001]$ ). No significant differences were recorded between the AM species distributions of AM communities under inoculated and uninoculated trees.

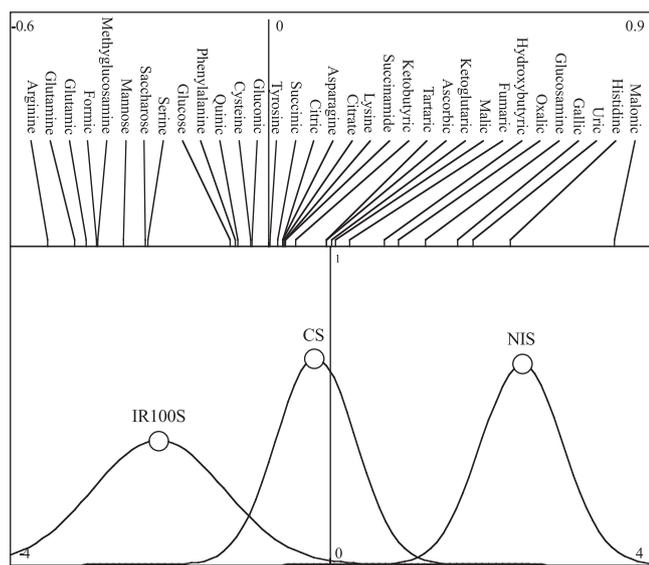


FIG. 2. Graphical display of the second BGA axis showing the SIRs with respect to the soil treatments. Only the second axis is used here, as the first axis merely separated the crop soil samples. The upper part of the figure shows the scores of the 33 substrates on the second BGA axis. In the lower part, the three Gauss curves represent the mean and variance of the scores of the nine soil samples (three repetitions for three treatments) on the second BGA axis. CS, crop soil; NIS, soil of plantation with uninoculated trees; IR100S, soil of plantation with *P. albus* IR100-inoculated trees. Substrates represented by lines curved in the same direction as corresponding Gauss curves tended to be used more in the corresponding soil samples.

**DISCUSSION**

This study clearly shows that (i) *A. holosericea* growth is greatly enhanced by the presence of the ectomycorrhizal fungus *P. albus* IR100 after 7 years of plantation; (ii) ectomycorrhizal inoculation induces significant changes in the functions of soil microbial communities; and (iii) the introduction of an exotic tree species significantly alters soil microbial functionalities (i.e., resistance to stress and disturbance) and the structure of AMF communities.

Inoculation with *P. albus* IR100 was highly beneficial to the growth of *A. holosericea* in the disinfected sandy soil, and this stimulating effect was retained during 7 years of culture growth in field conditions. This result is in accordance with other studies in which it was demonstrated that this fungal isolate was very efficient with respect to enhancement of the growth of Australian *Acacia* species (1, 27, 29, 30) and *Casuarina* species (28). Under field conditions, this positive effect of fungal inoculation on *A. holosericea* growth was previously recorded in the same region but after shorter periods (18 or 30 months of

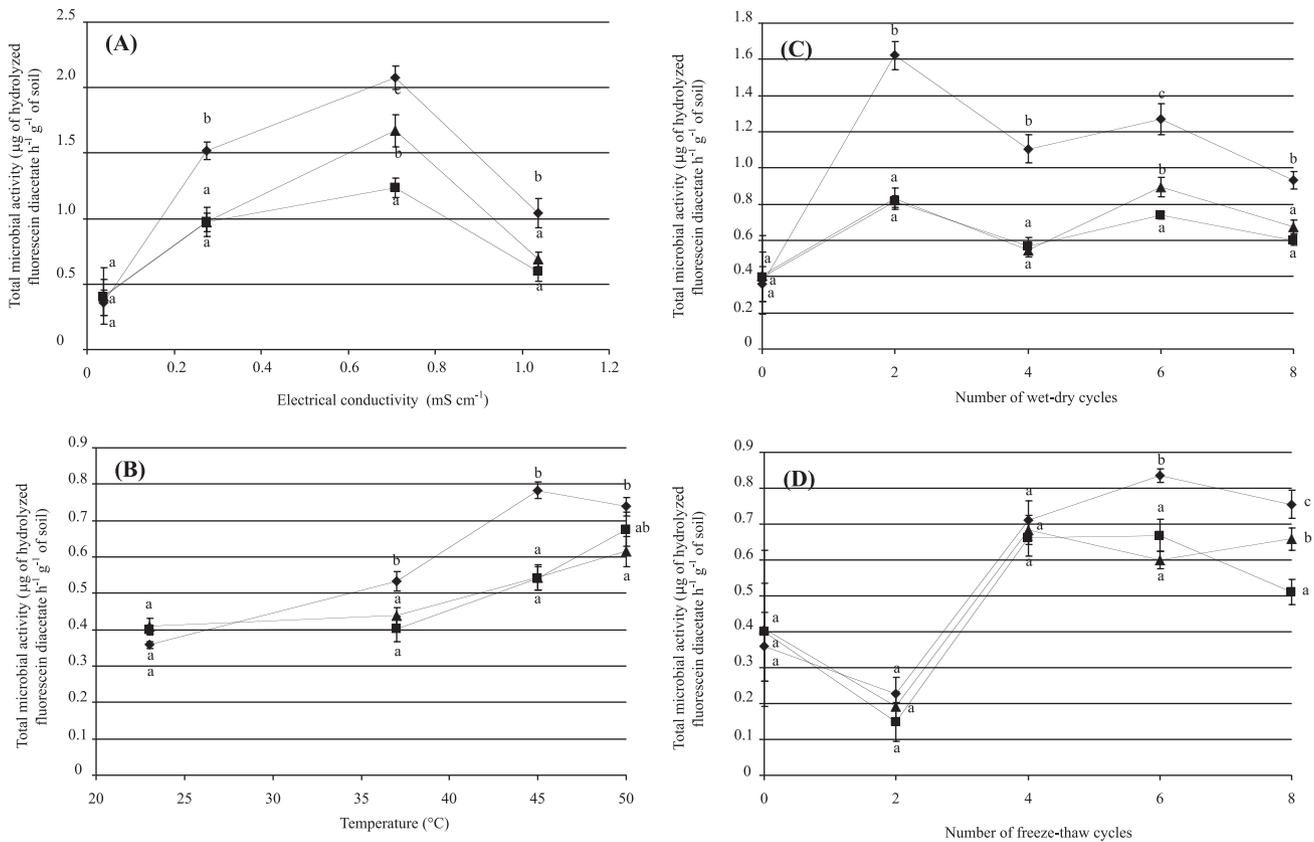


FIG. 3. Responses of total microbial activity to increased levels of stress induced by increases of NaCl (A) or disturbance by increases in temperature (B) or by wet-dry cycles (C) or freeze-thaw cycles (D). ●, crop soil; ■, soil of plantation with uninoculated trees; □, plantation soil with *P. albus* IR100-inoculated trees. Error bars represent standard errors ( $n = 6$ ). For each level of stress or disturbance, data indexed by the same letter are not significantly different according to results of the Newman-Keuls test ( $P < 0.05$ ).

plantation) (30). The measurements found in the present study show that the fungal effect on the plant growth can be kept for a longer duration. Some *Pisolithus* fruiting bodies have been detected under inoculated *A. holosericea* trees (R. Duponnois, unpublished data) after 3 to 5 years of plantation, confirming the development of the fungal inoculant in the soil. After 5 years of plantation, *Pisolithus* fruiting bodies disappeared (Duponnois, unpublished data). Quantitative and qualitative differences in the occurrences of carpophores of ectomycorrhizal fungi between stands of different ages are well known (21). According to Dighton and Mason (21), the number of fruiting bodies in the stand declined at a later stage.

Total nitrogen, carbon, and soluble phosphorus contents were significantly higher in soil collected under ectomycorrhized *A. holosericea* trees, whereas no significant differences were recorded for the C/N ratios among soil treatments. The effect of ectomycorrhizal inoculation on soil carbon content could be easily explained, as a greater biomass of litter was measured under ectomycorrhized *A. holosericea* trees. Decomposition results in the transformation of organic forms of nitrogen, phosphorus, and sulfur, which are found in litter, into mineral forms that are readily absorbed by plants (2). Numerous studies of the effects of roots on decomposition in the soil have produced contradictory results. It has been found that in forest soils, roots and ectomycorrhizal fungi significantly retard

decomposition rates of litter decomposition through the "Gadgil effect" (33, 34). In contrast, roots stimulate greater activity of the soil biota in soils with low total C and N contents and, in turn, contribute to faster litter decomposition and nutrient release (69). In the present study, although roots of *A. holosericea* (inoculated or not with *P. albus* IR100) decreased microbial biomass, soil N and P levels were enhanced in the *P. albus* treatment. Olsson et al. (51) have found that the presence of ectomycorrhizal mycelia reduced bacterial activity in a sandy soil, whereas it has been previously demonstrated that ectomycorrhizal symbiosis had a selective pressure on bacterial communities by promoting the development of bacterial strains potentially beneficial to the symbiosis and to the plant (i.e., organic phosphate-solubilizing bacteria) (32). It is generally admitted that ectomycorrhizal fungi have a reduced ability to decompose complex organic compounds of carbon (9), but the impact of ectomycorrhizal symbiosis on soil microbiota could promote the process of decomposition of organic N and P compounds, leading to higher soil P and N contents. Changes in the catabolic response profiles also revealed this mycorrhizosphere effect (46). A higher SIR with L-arginine was recorded from the soil collected under inoculated *A. holosericea* trees. Duponnois et al. (29) found that ectomycorrhizal inoculation significantly enhanced root growth of this Australian acacia in field conditions. Since L-arginine has been detected in

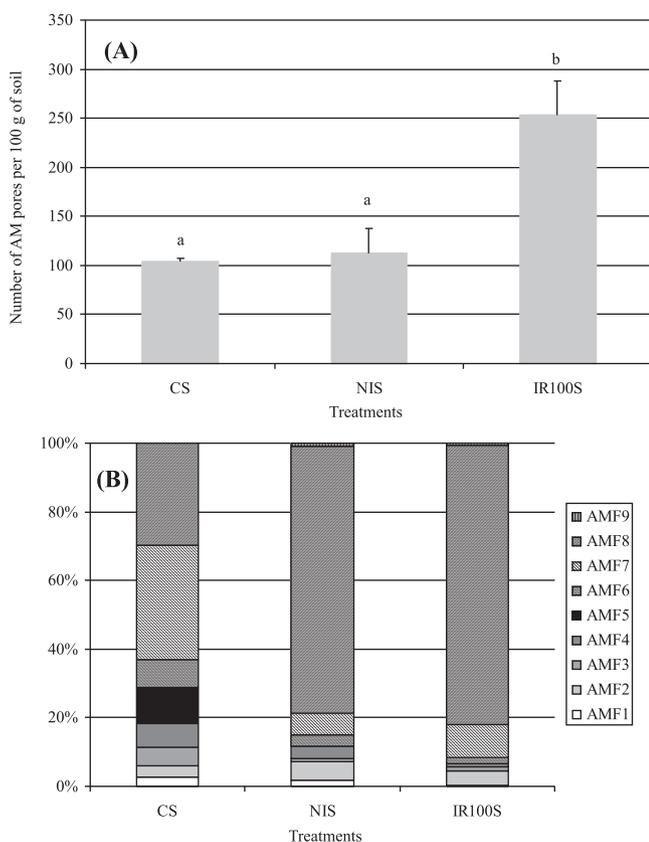


FIG. 4. Distribution in each soil origins of arbuscular mycorrhizal fungus species. CS, crop soil; NIS, soil of plantation with uninoculated trees; IR100S, soil of plantation with *P. albus* IR100-inoculated trees. AMF1, *Scutellospora gregaria*; AMF2, *S. verrucosa*; AMF3, *Scutellospora* sp. strain 1; AMF4, *Gigaspora* sp. strain 1; AMF5, *Gigaspora* sp. strain 2; AMF6, *Glomus rubiforme*; AMF7, *G. aggregatum*; AMF8, *Glomus* sp. strain 1; AMF9, *Glomus* sp. strain 2.

root exudates (5), a higher amount of this amino acid could exert a selective influence on soil microbial communities through a multiplication of arginine-catabolizing microorganisms while inducing a higher SIR.

Compared to the crop soil, the *A. holosericea* plantation induced strong modifications in soil microbial functionalities that reduced soil resistance to increasing stress or disturbance. In the present study, the catabolic evenness of the crop soil was 16.5, which was in accordance with previous studies in which values of catabolic evenness for soils under cropping conditions ranged from 16.4 to 19.6 (17). After 7 years of plantation, *A. holosericea* trees (inoculated or not with *P. albus* IR100) had significantly decreased levels of soil catabolic evenness to 9.8 (soil of plantation with uninoculated trees) and 9.5 (soil of plantation with *P. albus* IR100-inoculated trees), values that are rather low compared to the values reported for the literature (17) but that have already been reported for a glasshouse experiment with *Eucalyptus camaldulensis* (40). Stevenson et al. (61) compared the catabolic respiration responses of microbial communities from pastures and forest soils. They found that pasture soil communities had significant higher responses to carbohydrate and amino acid substrates and significant lower relative responses to carboxylic acid substrates than mi-

crobial communities from forest soils. In the present study, taking into account the measurements from the crop soil and from the soil of plantation with uninoculated trees, our results are in accordance with those of Stevenson et al. (61) for the SIRs seen with amino acids, carbohydrates (higher levels in the crop soil), and carboxylic acids (higher levels in the tree plantation soil). In addition, our results confirm the differential responses of glucose and  $\alpha$ -ketobutyric acid between crop and tree plantation (61). Ectomycorrhizal inoculation slightly modified the SIR differences between the two soil origins with respect to amino acids, amines, and carboxylic acids. It has been previously demonstrated that mycorrhizal symbiosis can counterbalance the influence of exotic tree species (*E. camaldulensis*) on the functioning of soil microbial communities in a sahelian soil (40).

Degens et al. (18) showed that soils with reduced catabolic evenness are less resistance to stress and disturbance. These results are consistent with those recorded in the present study. Soil collected outside the *A. holosericea* plantation (highest catabolic evenness) was more resistant to stress and disturbance than the soils collected inside the *A. holosericea* plantation (lowest catabolic evenness). Microbial activity showed a classical “hump-back” pattern in response to increasing stress or disturbance. This effect (predicted by the intermediate disturbance hypothesis) has been frequently reported for plant communities (3) but more rarely for soil microbial communities (18, 20). According to this hypothesis, it is assumed that under conditions of minimal stress or disturbance microbial diversity is poor and that under conditions of greater stress and disturbance this microbial diversity increases until stress or disturbance reaches levels that permit the growth of only a few species.

Numerous studies have clearly shown that structurally and functionally distinct microbial communities develop under different plant species (36, 37, 42). Several well-documented studies have shown that exotic plant species can significantly alter soil biological and chemical characteristics (40, 41, 42). In particular, it has been found that exotic plants could alter the structure of AM fungus communities (40) and disrupt mutualistic associations between existing ecological associations within native communities (7, 56). It has been suggested that introduced plant species had a selective positive influence on some AM species within the AM fungus native communities (41). In the present study, a similar process was found, as *A. holosericea* drastically promoted the multiplication of one fungal species (*Glomus* sp. strain 1) and consequently altered the species evenness of AM communities.

While the effects of microbial communities (structure and functions) on plant community diversity and soil biofunctioning have attracted considerable attention, our results clearly show that exotic plants may be directly responsible for important changes in soil microbiota affecting the structure and functions of microbial communities.

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