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The exotic legume tree species, *Acacia mearnsii*, alters microbial soil functionalities and the early development of a native tree species, *Quercus suber*, in North Africa

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ABSTRACT

Acacia mearnsii is one of the most planted Australian Acacia around the world but is known to be highly invasive, threatening native habitats by competing with indigenous vegetation. The introduction of this species in the Algerian El Kala Biosphere reserve led to the invasion of natural formations to the detriment of *Quercus suber*, a native tree species. We hypothesized that shifts in soil microbial functions and ectomycorrhizal (EcM) fungal community structure triggered by this exotic Acacia species might correlate with a decrease of the early growth of *Q. suber*. Soil samples were thus collected from 3 different sites where the exotic species was at different stages of invasion in the Algerian El Kala Biosphere reserve, (i) a *Q. suber* forest free of *A. mearnsii* (site S1), (ii) a *Q. suber*/*A. mearnsii* mixed forest where the Australian Acacia has been recently detected (site S2) and (iii) pure stands of *A. mearnsii* formed more than 20 years ago (site S3). Plant growth, EcM community structure associated with *Q. suber* roots and soil microbial functionalities were assessed for 6 month-old cultures of *Q. suber* in glasshouse conditions. The results clearly demonstrated a strong deleterious impact of *A. mearnsii* invasion level on soil chemical characteristics, microbial functions and EcM community structure and colonization, correlated to a decrease in the early growth of *Q. suber* seedlings. The current study gives new insights into both the negative impact of exotic species on soil functioning and their effect on indigenous vegetation growth. These results may be used as a basis for improving the conservation practices of native tree species in such degraded areas as a complement of ecological strategies using indigenous ectotrophic early-successional shrub species (eg. *Cistus* spp.) that our findings have shown to promote EcM multiplication and the early growth of native tree species.

1. Introduction

The resort to exotic trees has often been recommended in the past as a management option to enhance the productivity and biodiversity of disturbed ecosystems (Cossalter, 1987). Hence tree species such as *Pinus* spp., *Eucalyptus* spp. and *Acacia* spp. have been largely exported outside their natural range over the 18th and 19th centuries (Evans, 1992). Among these fast-growing tree species, the potential economic value of Australian *Acacia* species has been systematically assessed (Midgley and Turnbull, 2003). The Australian acacias, defined as the 1012 species formerly placed in *Acacia* subgenus Phyllodineae DC., are mostly native to Australia (Richardson et al., 2011; Murphy et al., 2010; Miller et al., 2011). These multipurpose trees can help to fix sand dunes, prevent wind and rain erosion, provide wood or fodder for browning livestock and produce very valuable pulp and paper (Cossalter, 1987; Le Houerou, 2000; Midgley et al., 2003). Owing to their nitrogen-fixing ability, *Acacia* species have the potential as pioneer tree legumes to grow on very infertile soils (Cossalter, 1987). It has been also suggested that these leguminous trees could promote

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biodiversity rehabilitation on degraded lands through the « catalytic effect hypothesis » (Parrotta, 1993).

Although these species are recommended to restore degraded ecosystems, it is now well established that this group of leguminous woody plants includes some of the most important plant invaders (Richardson and Rejmanek, 2011). About one-third of Australian acacias have been introduced outside Australia but surprisingly only 23 have become invasive (Richardson and Rejmanek, 2011; Richardson et al., 2011). These exotic species alter ecological interactions (Callaway and Ridenour, 2004) and plant biodiversity (Thébaud and Simberloff, 2001) in the invaded area, as well as a range of environmental parameters such as the water use, fire regime and soil nitrogen levels. Overall, they threaten the structure and composition of plant cover and the vegetation dynamics (succession and dominance) (del Moral and Muller, 1970). In addition, it has also been shown that exotic plants interact with the soil microbial community and modify mutualistic interactions within the native vegetation (Callaway and Ridenour, 2004; Kisa et al., 2007; Remigi et al., 2008). Moreover, it has been recently reported in Madagascar that *Pinus patula* and *Eucalyptus camaldulensis*, two exotic species, induced significant changes of biotic and abiotic soil properties, and more precisely of ectomycorrhizal (EcM) fungal community structure and EcM colonization of *Uapaca bojeri*, an endemic tree species, leading to its reduce early growth (Baohanta et al., 2012). Alteration of mycorrhizal community is of particular importance because is considered as a key component of the sustainable soil-plant system (Schreiner et al., 2003; Dickie and Reich, 2005) and EcM vegetation is highly dependent on EcM fungi for its growth and survival (Smith and Read, 2008). Hence their absence is a major impediment to efficient reforestation programs of areas composed of EcM vegetation (Marx, 1991).

Acacia mearnsii is one of the most planted Australian Acacia around the world (Asia, North, Central and South America, Africa (Griffin et al., 2011)), often used as a commercial source of tannin or firewood for local populations. This species is also documented as highly invasive, threatening the ecology of a broad range of habitats by competing with indigenous vegetation, replacing grass communities, reducing native biodiversity and increasing water loss from riparian zones (Richardson and Rejmanek, 2011). This extremely high invasive potential induces significant effects at the vegetation biodiversity in North Africa and more specifically in the Algerian El Kala Biosphere reserve. This area is known for its rich biodiversity harboring many endemic species which are also threatened and the introduction of exotic species, *A. mearnsii* and Eucalypts, in the 1970s mainly to produce paper pulp, led to the invasion of natural formations to the detriment of *Quercus suber*, the main endemic tree species in this region (Ouelmouhoub, 2005). The understanding of *A. mearnsii* plantation effects on soil microbial functioning is thus crucial to develop ecological strategies aiming at limiting the negative impact of such invasive species and restore native habitats, but its remains undocumented.

The aim of this study was to determine, in glasshouse conditions, the impact of *A. mearnsii* plantations on soil chemical characteristics, microbial activities and EcM community. We hypothesized that shifts in soil microbial functionalities and EcM community structure triggered by this exotic Acacia species might correlate with a decrease of the early growth of *Q. suber* seedlings.

2. Materials and methods

2.1. Study area, soil collection and analysis

The experimental area was located in the Algerian El Kala Biosphere reserve (36°51′–36°90′N; 08°16′–08°43′E). The National Park was established in 1983 and covers an area of 76,438 ha. Located North–East of Algeria, it is bounded on the east by the Algerian–Tunisian border, on the north by the Mediterranean sea, on the west by Cape Rosa, to the south by the foothills of Jebel El Ghorra. The mean annual precipitation and temperature are 700 mm and 17.6 °C, respectively. Soil samples were collected from 3 sites, (i) a *Q. suber* forest free of *A. mearnsii* (site S1; 8°20′E, 36°55′N), representative of the natural *Q. suber* massif of the Park, with an understorey mainly composed of *Arbutus unedo*, *Erica arborea*, *Calicotome villosa*, *Cistus monspeliensis* and *Cistus salvifolius*, *Pistacia lentiscus*, *Myrtus communis* and *Lavandula stoechas*, (ii) a *Q. suber*/*A. mearnsii* mixed forest, representing about 312 ha, recently colonized by the Australian Acacia (site S2; 8°21′E, 36°52′N) with an understorey composed of the same plant species as S1, from (iii) *A. mearnsii* stands that have spontaneously and progressively invaded an area covering 62 ha (site S3; 8°21′E, 36°52′N). The site S3 itself originally derives from a mixed *E. camaldulensis*/*A. mearnsii* stand planted in the 1970's, and the invasion of S3 by *A. mearnsii* has probably started more than 20 years ago. Currently, this site is characterized by almost pure stands of *A. mearnsii* (frequently representing 100% of the cover) rarely including, as understorey, native plant species like *Chamaerops humilis*.

In each site, nine 2 × 2 m plots were selected, to be as representative as possible of the area. In any cases, the plots were at least distant of 10 m from each other. In each plot, soil samples were randomly collected in February 2011. Each sample was composed of two 300 g sub-samples, randomly taken 1 m apart at a depth of 10–20 cm. For each soil sample, pH (soil water extract method), total organic carbon (TOC) (ANNE method [Aubert, 1978]) total nitrogen (TN) (Kjeldahl method) and available phosphorus (P) (Olsen et al., 1954) were determined. Then all soil samples collected in a given site, were pooled, crushed, sieved (2 mm) and kept at room temperature in a clean area for further use.

2.2. Bioassays of soils sampled from each targeted site and EcM assessment

Seeds of *Q. suber* collected in the El Kala Biosphere reserve were surface sterilized in 2.6% (v:v) hydrogen peroxide for 1 min, rinsed and soaked in sterile distilled water for 12 h. Then they were pregerminated for 7 days in Petri dishes on humid filter paper at 25 °C in the dark. The germinating seeds were used when rootlets were 1–2 cm long. One pre-germinated seed was planted per 1 L pot (Diameter: 9 cm, height: 20 cm) filled with soil collected from each site. The plants were arranged in a randomized, complete block design with 15 replicates per treatment. Seedlings were grown under natural light (day length approximately 10 h, mean Temperature 22 °C). They were watered daily with distilled water to avoid any contaminations, without fertilization.

The *Q. suber* seedlings were harvested after 6 months of culturing and their root systems were washed under running tap water, cut into short pieces and mixed. A soil sample (100 g) was collected from each pot and stored at 4 °C in order to determine the patterns of *in situ* catabolic potential (ISCP). The percentage of ectomycorrhizal colonization of lateral roots was determined for each treatment (number of ectomycorrhizal root tips/total number of root tips × 100) under a stereomicroscope at × 160 magnification. In each treatment, EcM root tips were classified by morphotypes according to the characteristics of their mantle and extra-matrical mycelium (branching, surface color, texture, emanating hyphae, and rhizomorphs) (Agerer, 1995). For each *Q. suber* seedlings, the oven dry weight (1 week at 65 °C) of the aerial and root part was then measured. After drying, plant tissues were ground, ashed (500 °C), digested in 2 ml HCl 6N and 10 ml HNO_3 N for nitrogen and then analyzed by colorimetry for

phosphorus (John, 1970). For nitrogen determination (Kjeldahl method), they were digested in 15 ml H_2SO_4 (36 N) containing 50 g l^{-1} of salicylic acid.

2.3. Impact of A. mearnsii on soil microbial functionalities

The soil microbial catabolic diversity (patterns of ISCP) was assessed according to Campbell et al. (2003) by a micro-respirometry method performed in 96-well microtiter plates. Soil (400 mg per well) was delivered to deep-well plates with a capacity of 1.2 mL (Thermo Scientific ABgene, Illkirch, France). In order to ensure the reactivation of the microbial activity, sterile distilled water was added (28 μL per well) to reach 30% of the water-holding capacity, and plates were incubated 3 days in the dark at 28 °C in a humid atmosphere to prevent excessive soil dehydration. The volume of dehydrated water per well was daily measured to ensure that no more than 16 μL had been dehydrated per well at the end of the third day. The fourth day, soil wells were spiked with 31 organic substrates (3 wells per substrate). Stock solutions for 10 carbohydrates (D-mannose, D-mannitol, D-trehalose, L-arabinose, D-xylose, D-sucrose, D-galactose, meso-inositol, D-sorbitol, L-rhamnose), 9 carboxylic acids (succinic acid, glutamic acid, citric acid, maleic acid, D,L-malic acid, Na-gluconate, L-ascorbic acid, α-ketoglutaric acid, oxalic acid) and 12 amino acids (L-asparagine, D,L-valine, L-methionine, L-glutamine, N-acetylglucosamine, D,L-alanine, D,L-serine, D,L-histidine, L-proline, L-leucine, L-lysine, L-arginine) were prepared with distilled water and their concentrations were calculated to reach respectively 0.03, 0.04 and 0.004 mmol g^{-1} soil by pipetting 16 μL of carbon sources per well (30% final water-holding capacity). Basal respiratory activity was determined in triplicate with distilled water (16 μL per well). The colorimetric detection plate consisted in a flat bottom-well plate (Thermo Scientific Nunc, Illkirch, France) with wells filled with 150 μL of the indicator gel containing cresol red (25 ppm, w/w), potassium chloride (300 mM) and sodium bicarbonate (5 mM) in 1% Noble-agar.

Detection plates were filled according to the MicroResp™ recommendations and stored several days after their set-up in sealed-jar containing soda lime for CO_2 absorption and water to prevent desiccation of the gel. The deep-well plates containing the soil were sealed to the rubber MicroResp™ seals and the detection plates sealed by a clamp immediately after the carbon sources were added. Absorbance of the last was measured at 572 with a Tecan infinite M200 Plate Absorbance reader before substrates spiking (t0) and after 6 h hours of incubation at 28 °C (t6). For a given well, the absolute respiratory activity was calculated by subtracting the absorbance value at t0 to the value at t6. The average basal respiration value was then subtracted to all the individual substrate respiration values. For each carbon source, this substrate-specific respiratory activity was averaged and this value was finally divided by the sum of all the mean substrate-specific respiratory activities (*pi* value). This standardization procedure minimizes the bias in respiration responses resulting from differences in microbial biomass between soil origins. The final values represent a relative measure of the contribution of a substrate to the activity of all substrates and differences in total activity do not overshadow the relative importance of each substrate. Catabolic evenness, *E* (variability of substrate used among the range of substrates tested), was calculated using the Simpson–Yule index, $E = 1/\Sigma p^2{}_i$ with $p_i =$ (respiration response to individual substrates)/(total respiration activity induced by all substrates for a soil treatment) (Magurran, 1988). Data were calculated for the individual responses to substrates but also for the average responses with carbohydrates, carboxylic acids and amino acids.

2.4. Statistical analysis

All the data were treated with one-way analysis of variance (ANOVA). Means were compared using the Newman–Keul's test ($P < 0.05$). The percentages of mycorrhizal colonization were transformed by arcsin ($\sqrt{x}$) prior statistical analysis. The patterns of ISCP of microbial communities in the soil treatments after 6 months of cultivation with *Q. suber* were analyzed using the between-group analysis (BGA, Dolédec and Chessel, 1989; Culhane et al., 2002). BGA is an ordination method considered as a robust alternative to the discriminant analysis (Huberty, 1994). A permutation test (Monte-Carlo method) was used to check the statistical significance of the between-group differences. BGA computations were performed with the free ADE 4 software (Dray and Dufour, 2007) for the R software for statistical computing (R Development Core Team, 2010). The distributions of EcM morphotypes were compared between each soil with 2 × 2 contingency tables and chi-square test (χ^2 test) and Yates correction for small numbers.

3. Results

3.1. Impact of soil origins on growth of Q. suber seedlings

The soil properties used for *Q. suber* growth experiments showed significant differences between the 3 sites, for most of the parameters measured. All soils were acid (pH < 6) but significantly different between the site S3 (pH = 5.59) and S1 (pH = 5.91), and intermediary value was obtained for the site S2 (Table 1). As described in the materials and methods, the site S3 is a long impacted habitat constituted by pure stands of *A. mearnsii* whereas the site S1 is a *Q. suber* forest, still free of *A. mearnsii*. For the total carbon, organic matter, total nitrogen and total phosphorus, the highest values ranged as follow: S2 > S1 > S3 (Table 1). The soluble P was significantly higher for soils from the sites S1 and S2 as compared to the site S3, whereas the lowest value for C/N ratio was recorded for the site S2 (Table 1).

The analysis of *Q. suber* growth parameters from glasshouse experiments using the soils characterized above (S1, S2 and S3) revealed *Q. suber* seedling mortality rates significantly higher with soils from sites S2 and S3 (presence of *A. mearnsii* on natural sites) compared to S1 (absence of *A. mearnsii* on natural site) (Table 2). All the growth parameters (excepted N and P leaf contents and shoot biomass) were significantly higher with soil from site S1 compared to the two other soils (Table 2).

Table 1
Chemical characteristics of soils collected from the three sampling areas (site S1: *Q. suber* forest free of *A. mearnsii*; site S2: *Q. suber*/*A. mearnsii* mixed forest recently impacted by the Australian *Acacia*; site S3: long impacted area where *A. mearnsii* has been detected for more than 20 years).

	Sampling areas		
	S1	S2	S3
pH	5.93 (0.01)[(1)]b[(2)]	5.61 (0.02)ab	5.59 (0.01)a
Total carbon (%)	2.92 (0.05)b	3.98 (0.02)c	2.09 (0.03)a
Organic matter (%)	5.04 (0.12)b	6.86 (0.15)c	3.60 (0.14)a
Total nitrogen (%)	1.67 (0.11)b	2.91 (0.12)c	1.28 (0.08)a
Total phosphorus (mg kg^{-1})	155.0 (1.23)b	192.5 (1.32)c	65.0 (1.53)a
Soluble phosphorus (mg kg^{-1})	2.0 (0.21)b	2.4 (0.12)b	1.20 (0.11)a
C/N	17.51 (1.21)b	13.67 (0.43)a	16.26 (1.15)b

(1) Standard error.
(2) Data in the same line followed by the same letter are not significantly different according to the Newman–Keul's test ($P < 0.05$).

Table 2
Effect of soil origins on growth and EcM colonization of 6 month-old *Q. suber* seedlings in glasshouse conditions.

	Soil origins		
	S1[1]	S2	S3
Mortality (%)	6.6	46.7	40.0
Height (cm)	63.7 (4.9)[2]b[3]	45.3 (3.07)a	44.3 (5.4)a
Shoot biomass (g dry weight)	2.72 (0.38)a	2.71 (0.34)a	2.16 (0.37)a
Root biomass (g dry weight)	5.57 (0.37)b	3.01 (0.30)a	3.45 (0.39)a
Total biomass (g dry weight)	8.29 (0.86)b	5.71 (0.64)a	5.61a
Root/shoot ratio	2.19 (0.16)b	1.14 (0.05)a	1.75 (0.17)b
N leaf content (mg g^{-1} dry weight)	5.14 (0.59)b	5.88 (0.74)b	3.49 (0.43)a
P leaf content (mg g^{-1} dry weight)	2.35 (0.27)b	2.27 (0.29)b	1.54 (0.26)a
EcM colonization (%)	62.5 (4.33)b	37.5 (9.46)a	27.8 (7.51)a

[1] For the legend, see Table 1.
[2] Standard error.
[3] Data in the same line followed by the same letter are not significantly different according to the Newman–Keul's test ($P < 0.05$).

3.2. Impact of soil origins on ectomycorrhizal colonization and community composition

After 6 month trapping, the extent of ectomycorrhizal (EcM) colonization was significantly lower in soils where *A. mearnsii* was originally present, sites S2 and S3, compared to S1 (absence of *A. mearnsii*) (Table 2). Fifteen EcM morphotypes were distinguished from all treatments according to EcM morphology/anatomy data (Table 3). Five EcM morphotypes were related to *Coenococcum geophilum*, *Scleroderma*-like sp. and *Tomentella*-like sp. (Table 3). The structure of EcM communities associated with *Q. suber* were significantly different ($P < 0.05$; Fig. 1) between the 3 conditions (soils S1, S2 and S3).

Among all the EcM morphotypes characterized, fourteen different morphotypes were recorded on the *Q. suber* seedlings grown in soil S1, whereas only 7 and 5 in the soils S2 and S3, respectively (Fig. 1). The morphotypes MT1 (*Scleroderma*-like sp.1), MT3 and MT4 were recorded in all conditions. No morphotype specific of S2 or S3 conditions was retrieved from *Q. suber*. By contrast, the morphotypes MT6, MT9 (*Tomentella*-like sp.2) as well as MT11–MT13 were specifically identified when soil S1 (absence of *A. mearnsii* in the study site) was used. Several morphotypes were also shared between two soil conditions MT5, MT7 (*Tomentella*-like sp.1) and MT8 (*C. geophilum*) for soils S1 and S2, MT14 for soils S1 and S3, and MT15 for soils S2 and S3 (Fig. 1). Because of the typical morphology/anatomy of EcM with *C. geophilum*, its colonization rate was analysed for the 3 conditions (soils S1, S2 and S3). *C. geophilum* colonization rate was significantly higher on *Q. suber* seedlings grown in soil S1 (10.8%) than S2 (1.7%) (Fig. 2). No *C. geophilum* ectomycorrhiza has been retrieved with soil S3.

3.3. Impact of soil origins on soil microbial functionalities

Between-group analysis (BGA) of soil microbial catabolic diversity data revealed a significant effect ($P < 0.001$; permutation test) of soil origin (S1, S2 and S3) (Fig. 3A). This difference was mainly explained by the preferential used of leucine and methionine as substrates in soil S1, α-ketoglutaric acid in S2, and lysine and malic acid in S3 (Fig. 3B). Regarding the most different soils in terms of soil origin, i.e. S1 (*Q. suber* forest with absence of Acacia) and S3 (pure stand of Acacia), major differences were also observed, with a preferential used in S1 of carbohydrates (D-trehalose, D-xylose, D-sucrose, meso-inositol, L-rhamnose) and amino acids (L-methionine, N-acetylglucosamine, D,L-alanine, D,L-serine and L-leucine) and on the contrary mainly of carboxylic acids (Citric acid, D,L-malic acid, Na-gluconate, L-ascorbic acid) in S3 (Fig. S1). The catabolic evenness (variability of substrate used among the range of substrates tested) was also investigated for each soil origin after 6 month-old cultures of *Q. suber* revealing a significantly higher value in S1 than in the two other conditions, S2 and S3 (Fig. 4).

4. Discussion

The study, conducted in glasshouse conditions, clearly shows that the presence of *A. mearnsii* highly impact soil properties, microbial functions and ectomycorrhizal (EcM) community (structure and colonization rate) of natural habitats. In the current case, it leads to a decrease of the early growth of *Q. suber* seedlings.

Table 3
Description of the main phenotypical characteristics of the morphotypes.

Sample reference	EcM anatomotype[1]	Macroscopic description	Outer mantle	Emanating hyphae
MT 1	*Scleroderma*-like sp. 1	Light brown	Plectenchymatous	Scarce; hyaline
MT 2	nd	Yellowish white	Plectenchymatous	Scarce; hyaline
MT 3	nd	White to light brown	Plectenchymatous and pseudoparenchymatous	Irregularly hyphae
MT 4	nd	Light to dark brown	Plectenchymatous	Irregularly white hyphae
MT 5	nd	Light to dark brown	Pseudoparenchymatous	Numerous white hyphae forming an abundant matrical net
MT 6	*Scleroderma*-like sp.2	Yellowish white to light brown	Pseudoparenchymatous	Scarce; hyaline
MT 7	*Tomentella*-like sp. 1	Light to dark gray	Plectenchymatous	Numerous white hyphae
MT 8	*Coenococcum geophilum*	Black	Plectenchymatous	Numerous thick black hyphae
MT 9	*Tomentella*-like sp. 2	Black	Pseudoparenchymatous	Scarce; hyaline
MT 10	nd	White to light brown	Pseudoparenchymatous	White. Presence of Rhizomorphs
MT 11	nd	Light to dark brown	Pseudoparenchymatous	Regularly shaped hyphae forming a coarse net
MT 12	nd	Dark brown. Dichotomous ectomycorrhizas	Plectenchymatous	None
MT 13	nd	White to dark brown	Plectenchymatous	Scarce; hyaline
MT 14	nd	Light to dark brown	Pseudoparenchymatous	Irregularly shaped hyphae forming a coarse net
MT 15	nd	White to light brown	Plectenchymatous	Irregularly shaped hyphae forming a coarse net

[1] nd : not determined.

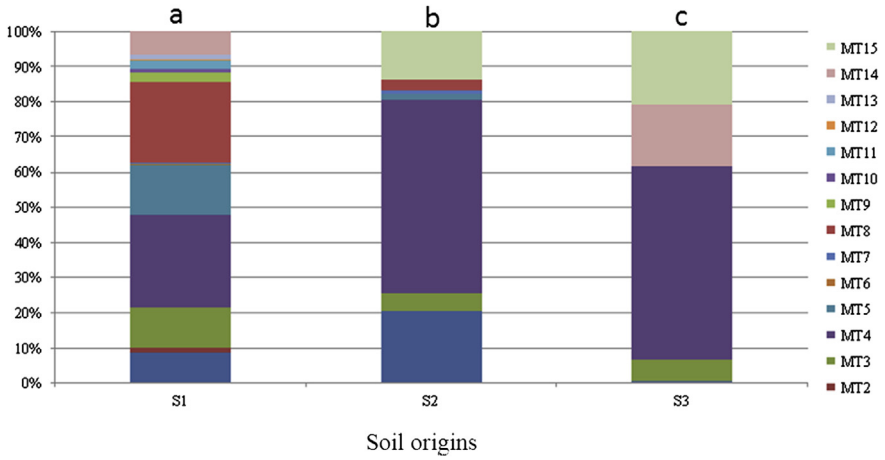

Fig. 1. Relative abundance of the most abundant EcM morphotypes associated with *Q. suber* after 6 month-old cultures in each soil origin. S1 indicates a *Q. suber* forest free of *A. mearnsii*, S2 a *Q. suber*/*A. mearnsii* mixed forest recently impacted by the Australian *Acacia* and S3 a long impacted area where *A. mearnsii* has been detected for more than 20 years. The distributions of EcM morphotypes in columns indexed by different letters are significantly different according to the χ^2 test ($P < 0.05$).

It has been frequently reported that the introduction of an exotic tree species induced strong modifications on soil characteristics (i.e. ph, soil nutrient contents, water dynamics, etc.) but with contrasting results. For instance, the invasion of Acacia in native oak habitats was shown to be related to soil acidification (Gonzales-Munoz et al., 2012) and the introduction of *Acacia holosericea* and eucalypts were shown to decrease in particular C and N content and soil microbial activity (Bargali et al., 1993; Sicardi et al., 2004; Bilgo et al., 2012). In addition, Bargali et al., 1993 noted that these effects were plantation age-related. All these observations are in accordance with the current results since the long-impacted soil (20-year old pure stands of Acacia) displayed the lowest pH and nutrient contents compared to the recently impacted soil (Cork oak/Acacia stands) and the native habitat (Cork oak forest).

However, as observed by Ehrenfeld (2003), the impact of invasive species on soil nutrient cycling is plant- and site-dependant. Indeed, the effect of long-impacted Mediterranean areas by *Acacia longifolia* showed adverse effects (Marchante et al., 2008) on nutrient contents, but a similar trend for C/N ratio, i.e. the lowest C/N ratio for the recently invaded soil compared to the two other conditions (Marchante et al., 2008). This last result suggests an easier decomposition of the organic matter in the recently impacted area and this difference in C/N ratio may be due to the type of forest cover. As observed in Marchante et al. (2008), the highest litter accumulation on the soil surface was obtained in long-invaded areas. This observation is not surprising because both of the N-fixing status of *Acacia* species that thus produce an abundant litter, but slowly degradable (Li et al., 2001; Yelenik et al., 2007; Castro-Diez et al., 2012), and the fact that in the long-impacted area, the main source of litter is from *A. mearnsii*. Furthermore, an extensive study conducted in Mediterranean ecosystems tended to show a slower decomposition of litter from invasive species (Godoy et al., 2010), as for example *Acacia saligna*, compared to native species.

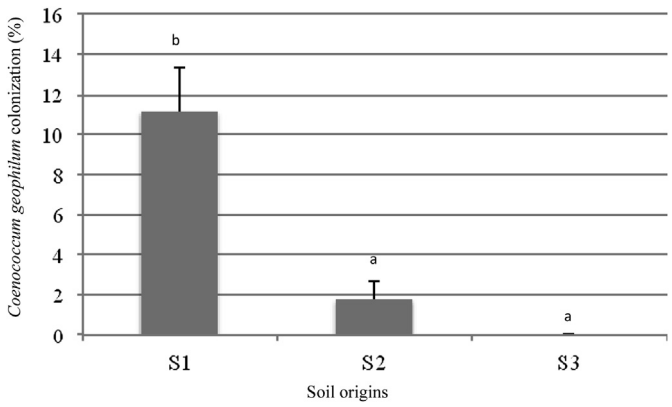

Fig. 2. Ectomycorrhizal colonization of *Q. suber* seedlings by *Coenococcum geophilum* after 6 month-old cultures of *Q. suber* in each soil origin. The percentages of mycorrhizal colonization were transformed by arcsin ($\sqrt{x}$) prior statistical analysis. Bars with the same letters are not significantly different according to the Newman–Keul's test ($P < 0.05$). See Fig. 1 for the origins of S1, S2 and S3.

On the other hand, the higher nutrient contents observed in soils from natural (Cork oak forest) and recently impacted habitat (mixed Cork oak/Acacia stands) could explained the progressive invasion by *A. mearnsii*. Indeed it has been reported that plant invasion was correlated with elevated or fluctuating resource levels (Daehler, 2003; Ehrenfeld, 2003). It has also been shown that exotic species can generate their own-rich sites thus possibly promoting their own growth (Vitousek et al., 1987; Ehrenfeld et al., 2001; Sanon et al., 2009b). In our study, the most favorable condition for *A. mearnsii* establishment is thus recorded in the recently impacted soil that displays the highest nutrient contents.

In the El Kala Biosphere reserve, the strong litter accumulation of *A. mearnsii* is probably one of the main factors altering the vegetation development, with the further action of allelochemicals (Richardson and Rejmanek, 2011). The drastic decrease of early growth of *Q. suber* seedlings in the long-impacted soil was probably related to this allelopathic effect. Indeed, one of the allelopathic effects is the inhibition of root system development (Hierro and Callaway, 2003) leading to a lower nutrient acquisition, as observed in our study. In addition, the lowest root/shoot ratio has been measured in the two impacted soils. The control of this ratio is recognized as playing a major role in the plant uptake of nutrients and water under limited conditions, as well as in the regeneration process of native tree species (Reader et al., 1992). Hence in the first steps of *A. mearnsii* invasion, the exotic tree species could limit the development of *Q. suber* young regeneration. In addition, the release of allelopathic compounds by Acacia and Eucalypts was shown to strongly modify soil microbial functions (Sanon et al., 2009a; Lorenzo et al., 2013).

We hypothesized that the allelochemicals released by *A. mearnsii* might thus be one of the factors leading to the strong

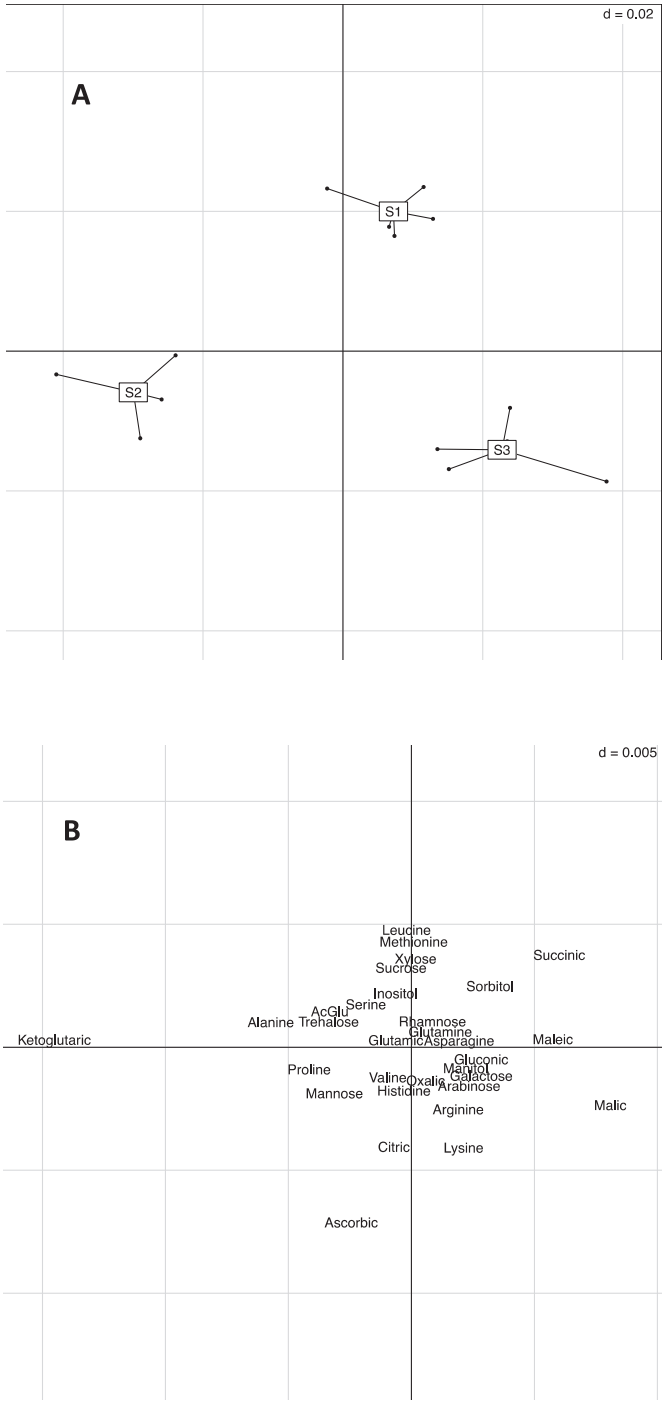

Fig. 3. Between-group analysis (BGA) of the SIR responses with respect to the soil origin. See Fig. 1 for the origins of S1, S2 and S3. A: Factor map of the soil origins. B: Factor map of SIR responses.

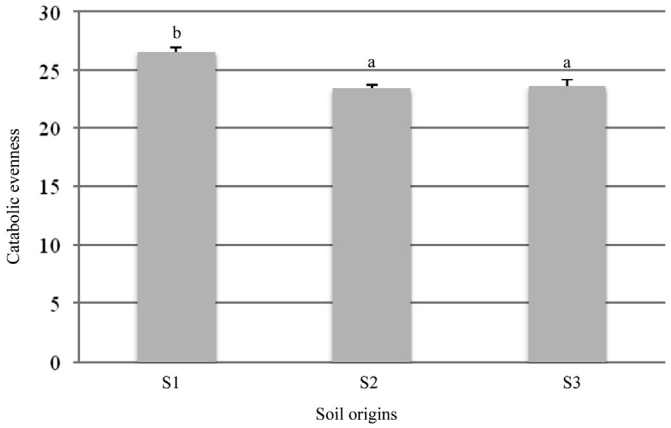

Fig. 4. Catabolic evenness after 6 month-old cultures of *Q. suber* in each soil origin. Error bars represent standard error of the mean ($n = 5$). Bars indexed by different letters indicate a significant difference according to the Newman–Keul's test ($P < 0.05$). See Fig. 1 for the origins of S1, S2 and S3.

decrease of EcM colonization of *Q. suber* seedlings in recently- and long-impacted soils. Similar effects have been recorded with two exotic species, *P. patula* and *E. camaldulensis*, which exerted an inhibiting effect on EcM colonization of *U. bojeri*, an endemic tree species of Madagascar, as well as a strong impact on its EcM community structure (Baohanta et al., 2012). Importantly, this decrease in ectomycorrhizal colonization could besides influence nutrient uptake and growth of *Q. suber* seedlings (Lilleskov et al., 2002; Dickie and Reich, 2005). A strong effect was particularly observed in impacted soils for *C. geophilum*, a drought-tolerant EcM fungus usually associated with adult oak trees (Dickie et al., 2004; Richard et al., 2009; Azul et al., 2010). It is also considered as an ultra-generalist (Kranabetter and Wylie, 1998), present in both early and late succession (Visser, 1995) and associated with a large range of host plant species (LoBuglio, 1999). Hence its role in natural regeneration of *Q. suber* seedlings is of great importance and its absence could explain the lowest growth and high mortality of *Q. suber* seedlings in the long-impacted soil.

Our results also showed that disturbances of EcM soil infectivity were accompanied with a decrease of the soil catabolic evenness in the two impacted soils compared to the native one. This decrease appears to disturb soil functioning by reducing the resistance of soils to stress and disturbances (Giller et al., 1997; Degens, 1998; Degens et al., 2001). Furthermore, major changes in soil microbial catabolic diversity were observed. The highest substrate induced respiration (SIR) responses were obtained for carboxylic acids in all soils, as observed in a similar study by Marchante et al. (2008), strengthening the importance of carboxylic acids as C source in soils. More precisely, higher SIR responses were obtained for many carboxylic acids in long-impacted areas and mostly carbohydrates and amino acids in native and recently impacted areas. Opposite trends were however observed for carboxylic acids in Marchante et al. (2008), but the comparison has to be made with caution because of the difference between the ecosystems studied (soil type, plant species, etc…). Among carbohydrates and amino acids, two substrates, trehalose and N-acetylglucosamine, are known as major components of the fungal wall and the mycelium network (Smith and Read, 2008), which might suggest larger ectomycorrhizal hyphal network in native and recently-impacted soils compared to the long-impacted one. Importantly, no ectomycorrhizal structure has been observed on *A. mearnsii* roots in the field (data not shown), which is in line with the previous hypothesis, i.e. a poor ectomycorrhizal networks in long-impacted areas, and the low ectomycorrhizal root colonization measured on the *Q. suber* seedlings in impacted soils.

To avoid the disturbances resulting from the introduction of the exotic species, one-way of intervention could be thus to reinforce the ectomycorrhizal infection potential. Recently, it has been demonstrated in Madagascar that the use of ectotrophic early-successional shrub species enhanced soil chemical characteristics and enzymatic activities, ectomycorrhizal infection and growth of young seedlings of a native tree species (*U. bojeri*) after disturbances resulting from the introduction of two exotic species (*P. patula* and *E. camaldulensis*) (Baohanta et al., 2012).

Our results clearly demonstrated a strong deleterious impact of *A. mearnsii* invasion level on soil chemical characteristics, microbial functions and EcM community structure and colonization, correlated to a decrease in the early growth of *Q. suber* seedlings. To mitigate the deleterious effects of exotic species introduction on the native flora, further studies have to be undertaken to analyze the impact of some ectotrophic shrub species (i.e. *Cistus* spp.) on ectomycorrhizal community abundance and, diversity and on the early growth of *Q. suber*, in order to improve the performances of reforestation programs with native tree species in such degraded areas.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2013.05.003.

References

Agerer, R., 1995. Anatomical characteristics of identified ectomycorrhizas: an attempt towards a natural classification. In: Varma, A., Hock, B. (Eds.), Mycorrhiza: Structure, Function, Molecular Biology and Biotechnology. Springer, Berlin, pp. 687–734.
Aubert, G., 1978. Méthodes d'Analyse des sols. Edition CRDP, Marseille, p. 360.
Azul, A.M., Sousa, J.P., Agerer, R., Martin, M.P., Freitas, H., 2010. Land use practices and ectomycorrhizal fungal communities from oak woodlands dominated by *Quercus suber* L. considering drought scenarios. Mycorrhiza 20, 73–88.
Baohanta, R., Thioulouse, J., Ramanankierana, H., Prin, Y., Rasolomampianina, R., Baudoin, E., Rakotoarimanga, N., Galiana, A., Randriambanona, H., Lebrun, M., Duponnois, R., 2012. Restoring native forest ecosystems after exotic tree plantation in Madagascar: combination of the local ectotrophic species *Leptolena bojeriana* and *Uapaca bojeri* mitigates the negative influence of the exotic species *Eucalyptus camaldulensis* and *Pinus patula*. Biological Invasions 14, 2407–2421.
Bargali, S.S., Singh, R.P., Joshi, M., 1993. Changes in soil characteristics in eucalypt plantations replacing natural broadleaved forests. Journal of Vegetation Science 4, 25–28.
Bilgo, A., Sangare, S.K., Thioulouse, J., Prin, Y., Hien, V., Galiana, A., Baudoin, E., Hafidi, M., Bâ, A.M., Duponnois, R., 2012. Response of native soil microbial functions to the controlled mycorrhization of an exotic tree legume, *Acacia holosericea* in a Sahelian ecosystem. Mycorrhiza 22, 175–187.
Callaway, R.M., Ridenour, W.M., 2004. Novel weapons: invasive success and the evolution of increased competitive ability. Frontiers in Ecology and Environment 2, 436–443.
Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S., Potts, J.M., 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Applied and Environmental Microbiology 69, 3593–3599.
Castro-Diez, P., Fierro-Brunnenmeister, N., Gonzalez-Munoz, N., Gallardo, A., 2012. Effects of exotic and native tree litter on soil properties of two contrasting sites in the Iberian Peninsula. Plant and Soil 350, 179–191.
Cossalter, C., 1987. Introduction of Australian acacias into dry tropical West Africa. Forest Ecology and Management 16, 367–389.
Culhane, A.C., Perriere, G., Considine, E.C., Cotter, T.G., Higgins, D.G., 2002. Between-group analysis of microarray data. Bioinformatics 18, 1600–1608.
Daehler, C., 2003. Performance comparisons of co-occuring native and alien invasive plants: implications for conservation and restoration. Annual Review of Ecology, Evolution and Systematics 34, 183–211.
Degens, B.P., 1998. Decreases in microbial functional diversity do not result in corresponding changes in decomposition under different moisture conditions. Soil Biology & Biochemistry 30, 1989–2000.
Degens, B.P., Schipper, L.A., Sparling, G.P., Duncan, L.C., 2001. Is the microbial community in a soil with reduced catabolic diversity less resistant to stress or disturbance? Soil Biology & Biochemistry 33, 1143–1153.
del Moral, R., Muller, C.H., 1970. The allelopathic effects of *Eucalyptus camaldulensis*. American Midland Naturalist 83, 254–282.
Dickie, I.A., Reich, P.B., 2005. Ectomycorrhizal fungal communities at forest edges. Journal of Ecology 93, 244–255.
Dickie, I.A., Guza, R.C., Krazewski, S.E., Reich, P.B., 2004. Shared ectomycorrhizal fungi between a herbaceous perennial (*Helianthemum bicknellii*) and oak (*Quercus*) seedlings. New Phytologist 164, 375–382.
Dolédec, S., Chessel, D., 1989. Rythmes saisonniers et composantes stationnelles en milieu aquatique II – Prise en compte et élimination d'effets dans un tableau faunistique. Acta Oecologica 10, 207–232.
Dray, S., Dufour, A.B., 2007. The ade4 package: implementing the duality diagram for ecologists. Journal of Statistical Software 22, 1–20.
Ehrenfeld, J.G., Kourtev, P., Huang, W., 2001. Changes in soil functions following invasions of exotic understorey plants in deciduous forests. Ecological Applications 11, 1287–1300.
Ehrenfeld, J.G., 2003. Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6, 503–523.
Evans, J., 1992. Plantation Forestry in the Tropics, second ed. Clarendon Press, Oxford, p. 403.
Giller, K.E., Beare, M.H., Lavelle, P., Izac, A.-M.N., Swift, M.J., 1997. Agricultural intensification, soil biodiversity and agrosystem function. Applied Soil Ecology 6, 3–16.
Godoy, O., Castro-Diez, P., Van Logtestijn, R.S.P., Cornelissen, J.H.C., Valladeres, F., 2010. Leaf litter traits of invasive species slow down decomposition compared to Spanish natives: a broad phylogenetic comparison. Oecologia 162, 781–790.
Gonzales-Munoz, N., Costa-Tenorio, Espigares, T., 2012. Invasion of alien *Acacia dealbata* on Spanish *Quercus robur* forests: impact on soils and vegetation. Forest Ecology and Management 269, 214–221.
Griffin, A.R., Midgley, S.J., Bush, D., Cunningham, P.J., Rinaudo, A.T., 2011. Global uses of Australian acacias – recent trends and future prospects. Diversity and Distributions 17, 837–847.
Hierro, J.L., Callaway, R.M., 2003. Allelopathy and exotic plant invasion. Plant and Soil 256, 29–39.
Huberty, C.J., 1994. Applied Discriminant Analysis. Wiley Interscience, New York, p. 466.
John, M.K., 1970. Colorimetric determination in soil and plant material with ascorbic acid. Soil Science 68, 171–177.
Kisa, M., Sanon, A., Thioulouse, J., Assigbetse, K., Sylla, S., Spichiger, R., Dieng, L., Berthelin, J., Prin, Y., Galiana, A., Lepage, M., Duponnois, R., 2007. Arbuscular mycorrhizal symbiosis can counterbalance the negative influence of the exotic tree species *Eucalyptus camaldulensis* on the structure and functioning of soil microbial communities in a Sahelian soil. FEMS Microbiology Ecology 62, 32–44.
Kranabetter, J.M., Wylie, T., 1998. Ectomycorrhizal community structure across forest openings on naturally regenerated western hemlock seedlings. Canadian Journal of Botany 76, 189–196.
Le Houerou, H.N., 2000. Utilization of fodder trees and shrubs in the arid and semi-arid zones of West Africa and North Africa. Arid Soil Research and Rehabilitation 14, 101–135.
Li, Z-a., Peng, S.-l., Rae, D.J., Zhou, G-y., 2001. Litter decomposition and nitrogen mineralization of soils in subtropical plantation forests of southern China, with special attention to comparisons between legumes and non-legumes. Plant and Soil 229, 105–116.
Lilleskov, E.A., Hobbie, E.A., Fahey, T.J., 2002. Ectomycorrhizal fungal taxa differing in response to nitrogen deposition also differ in pure culture organic nitrogen use and natural abundance of nitrogen isotopes. New Phytologist 154, 219–231.
LoBuglio, K.F., 1999. Cenococcum. In: Cairney, J.W.G., Chambers, S.M. (Eds.), Ectomycorrhizal Fungi Key Genera in Profile. Springer, Berlin, pp. 287–309.
Lorenzo, P., Pereira, C.S., Rodriguez-Echeverria, S., 2013. Differential impact on soil microbes of allelopathic compounds released by the invasive *Acacia dealbata* Link. Soil Biology & Biochemistry 57, 156–163.
Magurran, A.E., 1988. Ecological Diversity and Its Measurement. Croom Helm, London.
Marchante, E., Kjoller, A., Struwe, S., Freitas, H., 2008. Invasive *Acacia longifolia* induces changes in the microbial catabolic diversity of sand dunes. Soil Biology and Biochemistry 40, 2563–2568.
Marx, D.H., 1991. The practical significance of ectomycorrhizae in forest establishment. In: Ecophysiology of Ectomycorrhizae of Forest Trees. Marcus Wallenberg Foundation Symposia Proceedings, vol. 7, pp. 54–90.
Midgley, S.J., Turnbull, J.W., 2003. Domestication and use of Australian acacias: case studies of five important species. Australian Systematic Botany 16, 89–102.
Midgley, S.J., Turnbull, J.W., Pinyopusarerk, K., 2003. Industrial acacias in Asia: small brother or big competitor? In: Wei, R.-P., Xu, D. (Eds.), Eucalyptus Plantations - Research, Management and Development. Proceedings of the International Symposium on *Eucalyptus* Plantations, Guangzhou/Zhao, China. World Scientific, Singapore, pp. 19–36.
Miller, J., Murphy, D.J., Brown, G.K., Richardson, D.M., González-Orozco, C.E., 2011. The evolution and phylogenetic placement of invasive Australian Acacia species. Diversity and Distributions 17, 848–860.
Murphy, D.J., Brown, G.K., Miller, J.T., Ladiges, P.Y., 2010. Molecular phylogeny of *Acacia* Mill. (Mimosoideae: Leguminosae): evidence for major clades and informal classification. Taxon 59, 7–19.
Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate. In: Circular, vol. 939. U.S. Department of Agriculture, Washington, DC, p. 19.
Ouelmouhoub, S., 2005. Gestion multi-usage et conservation du patrimoine forestier: cas des subéraies du Parc National d'El Kala (Algérie). In: CIHEAM-IAMM (Ed.), 2005. Série Master of Science, vol. 78, p. 127. Montpellier.

Parrotta, J.A., 1993. Secondary forest regeneration on degraded tropical lands. The role of plantations as "foster ecosystems". In: Leith, H., Lohmann, M. (Eds.), Restoration of Tropical Forest Ecosystems. Kluwer Academic Publishers, pp. 63–73.

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. http://www.R-project.org.

Reader, R.J., Jalili, A., Grime, J.P., Spencer, R.E., Matthews, N., 1992. A comparative study of plasticity in seedling rooting depth in drying soil. Journal of Ecology 81, 543–550.

Remigi, P., Faye, A., Kane, A., Deruaz, M., Thioulouse, J., Cissoko, M., Prin, Y., Galiana, A., Dreyfus, B., Duponnois, R., 2008. The exotic legume tree species *Acacia holosericea* alters microbial soil functionalities and the structure of the arbuscular mycorrhizal community. Applied and Environmental Microbiology 74, 1485–1493.

Richard, F., Selosse, M.-A., Gardes, M., 2009. Facilitated establishment of *Quercus ilex* in shrub-dominated communities within a mediterranean ecosystem: do mycorrhizal partners matter? FEMS Microbiology Ecology 68, 14–24.

Richardson, D.M., Rejmanek, M., 2011. Trees and shrubs as invasive alien species – a global review. Diversity and Distributions 17, 788–809.

Richardson, D.M., Carruthers, J., Hui, C., Impson, F.A.C., Miller, J., Robertson, M.P., Rouget, M., le Roux, J.J., Wilson, J.R.U., 2011. Human-mediated introductions of Australian acacias – a global experiment in biogeography. Diversity and Distributions 17, 771–787.

Sanon, A., Andrianjaka, Z.N., Prin, Y., Bally, R., Thioulouse, J., Comte, G., Duponnois, R., 2009a. Rhizosphere microbiota interfers with plant–plant interactions. Plant and Soil 321, 259–278.

Sanon, A., Béguiristain, T., Cébron, A., Berthelin, J., NDoye, I., Leyval, C., Sylla, S., Duponnois, R., 2009b. Changes in soil diversity and global activities following invasions of the exotic invasive plant, *Amaranthus viridis* L., decrease the growth of native sahelian *Acacia* species. FEMS Microbiology Ecology 70, 118–131.

Schreiner, R.P., Mihara, K.L., Mc Daniel, H., Benthlenfalvay, G.J., 2003. Mycorrhizal fungi influence plant and soil functions and interactions. Plant and Soil 188, 199–209.

Sicardi, M., Garcia-Prechac, F., Frioni, L., 2004. Soil microbial indicators sensitive to land use conversion from pastures to commercial *Eucalyptus grandis* (Hill ex Maiden) plantations in Uruguay. Applied Soil Ecology 27, 125–133.

Smith, S., Read, J., 2008. Mycorrhizal Symbiosis, third ed. Academic Press, San Diego, CA, USA, p. 800.

Thébaud, C., Simberloff, D., 2001. Are plants really larger in their introduced ranges? American Naturalist 157, 231–236.

Visser, S., 1995. Ectomycorrhizal fungal succession in jack pine stands following wildfire. New Phytologist 129, 389–401.

Vitousek, P.M., Walker, L.R., Whiteaker, L.D., Mueller-Dombois, D., Matson, P.A., 1987. Biological invasion by *Mirtica faya* alters ecosystem development in Hawaï. Science 238, 802–804.

Yelenik, S.G., Stock, W.D., Richardson, D.M., 2007. Functional group identity does not predict invader impacts: differential effects of nitrogen-fixing exotic plants on ecosystem function. Biological Invasions 9, 117–125.