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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Article info

Article history:
Received 4 February 2013
Received in revised form 7 May 2013
Accepted 8 May 2013
Available online 10 June 2013

Keywords:
Exotic invasive plant
*Acacia mearnsii*
Soil microbial community
Ectomycorrhiza

A B S T R A C T

*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 browsing 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
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 (Scheiener 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 Eucalyps, 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 (Ouelmouhouh, 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 salviifolius, 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 x 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 x 100) under a stereomicroscope at x 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 HNO3 N for nitrogen and then analyzed by colorimetry for
phosphorus (John, 1970). For nitrogen determination (Kjeldahl method), they were digested in 15 ml H₂SO₄ (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-xyllose, D-sucrose, D-galactose, meso-inositol, D-sorbitol, L-rhamnose), 9 carboxylic acids (succinic acid, glutamic acid, citric acid, maleic acid, cis-malic acid, Na-glutamate, L-ascorbic acid, N-ketoglutaric acid, fumaric 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 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 Nune, Illkirch, France) with wells filled with 150 μL of the indicator gel containing cresol red (25 ppm, w/v), 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 CO₂ 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 nm 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 (πi 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, \( E = 1 / \sum p_{i}^{2} \), with \( p_{i} = ( \text{respiration response to individual substrates} ) / ( \text{total respiration activity induced by all substrates for a soil treatment} ) \) (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–Keuls test (\( P < 0.05 \)). The percentages of mycorrhizal colonization were transformed by arcsin(√% ) 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 (χ² 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 acidic (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 forest 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>
<thead>
<tr>
<th>Sampling areas</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.91 (0.01)±1</td>
<td>5.61 (0.02)ab</td>
<td>5.59 (0.01)a</td>
</tr>
<tr>
<td>Total carbon</td>
<td>2.92 (0.05)b</td>
<td>3.98 (0.02)c</td>
<td>2.09 (0.03)a</td>
</tr>
<tr>
<td>Organic matter</td>
<td>5.04 (0.12)b</td>
<td>6.86 (0.15)c</td>
<td>3.60 (0.14)a</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>1.67 (0.11)b</td>
<td>2.91 (0.12)c</td>
<td>1.28 (0.08)a</td>
</tr>
<tr>
<td>Total phosphorus (mg kg⁻¹)</td>
<td>155.0 (1.23)b</td>
<td>192.5 (1.32)c</td>
<td>65.0 (1.53)a</td>
</tr>
<tr>
<td>Soluble phosphorus (mg kg⁻¹)</td>
<td>2.0 (0.21)b</td>
<td>2.4 (0.12)c</td>
<td>1.20 (0.11)a</td>
</tr>
<tr>
<td>C/N</td>
<td>17.51 (1.21)b</td>
<td>13.67 (0.43)a</td>
<td>16.26 (1.15)b</td>
</tr>
</tbody>
</table>

(1) Standard error.
(2) Data in the same line followed by the same letter are not significantly different according to the Newman–Keuls 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.

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

(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-Keuls 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 Coenococcus 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 use 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 (β-trehalose, β-xylene, β-sucrose, meso-inositol, l-rhamnose) and amino acids (l-methionine, N-acetylglucosamine, ωωωω-alanine, ωωωω-serine and l-leucine) and on the contrary mainly of carboxylic acids (Citric acid, D,L-malic acid, Na-gluconate, l-aspartic 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.

<table>
<thead>
<tr>
<th>Sample reference</th>
<th>EcM anamototype(1)</th>
<th>Macroscopic description</th>
<th>Outer mantle</th>
<th>Emanating hyphae</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT 1</td>
<td>Scleroderma-like sp. 1</td>
<td>Light brown</td>
<td>Plectenchymatous</td>
<td>Scare; hyaline</td>
</tr>
<tr>
<td>MT 2</td>
<td>nd</td>
<td>Yellowish white</td>
<td>Plectenchymatous</td>
<td>Scare; hyaline</td>
</tr>
<tr>
<td>MT 3</td>
<td>nd</td>
<td>White to light brown</td>
<td>Plectenchymatous and pseudoparenchymatous</td>
<td>Irregularly hyphae</td>
</tr>
<tr>
<td>MT 4</td>
<td>nd</td>
<td>Light to dark brown</td>
<td>Plectenchymatous</td>
<td>Irregularly white hyphae</td>
</tr>
<tr>
<td>MT 5</td>
<td>nd</td>
<td>Light to dark brown</td>
<td>Pseudoparenchymatous</td>
<td>Numerous white hyphae forming an abundant matrical net</td>
</tr>
<tr>
<td>MT 6</td>
<td>Scleroderma-like sp.2</td>
<td>Yellowish white to light brown</td>
<td>Pseudoparenchymatous</td>
<td>Scare; hyaline</td>
</tr>
<tr>
<td>MT 7</td>
<td>Tomentella-like sp. 1</td>
<td>Light to dark gray</td>
<td>Plectenchymatous</td>
<td>Numerous white hyphae</td>
</tr>
<tr>
<td>MT 8</td>
<td>Coenococcus geophilum</td>
<td>Black</td>
<td>Plectenchymatous</td>
<td>Numerous thick black hyphae</td>
</tr>
<tr>
<td>MT 9</td>
<td>Tomentella-like sp. 2</td>
<td>Black</td>
<td>Pseudoparenchymatous</td>
<td>Scare; hyaline</td>
</tr>
<tr>
<td>MT 10</td>
<td>nd</td>
<td>White to light brown</td>
<td>Pseudoparenchymatous</td>
<td>White. Presence of Rhizomorphs</td>
</tr>
<tr>
<td>MT 11</td>
<td>nd</td>
<td>Light to dark brown</td>
<td>Pseudoparenchymatous</td>
<td>Regularly shaped hyphae forming a coarse net</td>
</tr>
<tr>
<td>MT 12</td>
<td>nd</td>
<td>Dark brown. Dichotomous ectomycorrhizas</td>
<td>Plectenchymatous</td>
<td>None</td>
</tr>
<tr>
<td>MT 13</td>
<td>nd</td>
<td>White to dark brown</td>
<td>Plectenchymatous</td>
<td>Scare; hyaline</td>
</tr>
<tr>
<td>MT 14</td>
<td>nd</td>
<td>Light to dark brown</td>
<td>Pseudoparenchymatous</td>
<td>Irregularly shaped hyphae forming a coarse net</td>
</tr>
<tr>
<td>MT 15</td>
<td>nd</td>
<td>White to light brown</td>
<td>Plectenchymatous</td>
<td>Irregularly shaped hyphae forming a coarse net</td>
</tr>
</tbody>
</table>

(1) nd : not determined.
soil microbial activity (Bargali et al., 1993; Sicardi et al., 2004; Bilgo eucalypts were shown to decrease in particular C and N content and nutrient contents compared to the recently impacted soil (Cork year old pure stands of Acacia) displayed the lowest pH and dance with the current results since the long-impacted soil (20-...all these observations are in accor-...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; Velenik 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.

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 soils 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...
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 successions (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* 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.

Acknowledgments

This study was financially supported by the Centre de coopération internationale en recherche agronomique pour le développement (CIRAD).

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


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