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Some Mediterranean plant species (*Lavandula* spp. and *Thymus satureioides*) act as potential 'plant nurses' for the early growth of *Cupressus atlantica*

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Abstract

The mycorrhizal status of several representative shrub species (*Lavandula* spp. and *Thymus satureioides*) in Moroccan semiarid ecosystems, was evaluated as well as their contribution to the mycorrhizal potential of the soil. Furthermore, the rhizosphere soils collected under these target species were tested for their influence on the growth of *Cupressus atlantica*, a tree species whose natural stands has declined in this area. Soil samples were collected from the rhizosphere of *L. stoechas*, *L. dentata* and of *C. atlantica* existing in the experimental area. Control samples were randomly collected from bare soil sites, away from plant influence.All the target species formed AM symbiosis and the extent of AM fungal colonization was not significantly different between plant species. No significant difference was detected between the total number of AM fungal spores of the bare soil and those recorded in the root zones of target species and *C. atlantica*. Three genera of AM fungi (*Scutellospora*, *Glomus* and *Acaulospora*) were present in the rhizospheres of the plant species and in the bare soil.The number of mycorrhizal propagules in soil originating from around the four target plant species was significantly higher than the one in the bare soil (Figure 1). The most probable number (MPN) of mycorrhizal propagules per 100 g of dry soil ranged from 7.82 (bare soil) to 179.7 (*L. dentata* and *C. atlantica*) and 244.5 (*L. stoechas* and *T. satureioides*). As the total number of spores was not different for the soil of different origins, the increase of the mycorrhizal soil infectivity (MSI) mainly resulted from larger AM mycelial networks that constituted the main source of AM fungal inoculum. In addition, this MSI enhancement was linked with changes in the functioning of soil microbial communities. In a glasshouse experiment, the growth of *C. atlantica* seedlings was significantly higher in the *C. atlantica* and in the shrub species soils than in the bare soil. Although the AM inoculum potential is not sufficient to ensure the development of forest trees in Mediterranean ecosystems, the use of plant nurses such as *T. satureioides* or *Lavandula* spp. could be of great interest to restore a self-sustaining vegetation cover to act against desertification.

Introduction

In semiarid Mediterranean ecosystems, desertification processes have occurred following scarce and irregular rainfall, long dry and hot summers and man-mediated degradative activities (overgrazing, non-regulated cultivation techniques, deforestation, etc). These anthropogenic activities degrade the natural plant communities in terms of population structure, successional patterns and species diversity (Barea and Jeffries 1995). In addition, these disturbances are often accompanied by degradation of physico-chemical and biological soil properties (Albaladejo et al. 1998; Requena et al. 2001) that largely determine soil quality and fertility. For instance, it has been clearly demonstrated that land degradation was associated with reductions in the belowground microbial diversity and/or activity (Kennedy and Smith 1995; Garcia et al. 1997). Mycorrhizal symbioses are known to be key components of natural systems, particularly in semiarid ecosystems (Carpenter and Allen 1988; Brundrett 1991). They are involved in governing the cycles of major plant nutrients and in sustaining the vegetation cover in natural habitats (Requena et al. 2001). These disturbances generally result in the loss or reduction of mycorrhizal propagules in the soil and, consequently, decrease the mycorrhizal potential in the degraded areas (Jasper et al. 1991; Herrera et al. 1993; Mc Lellan et al. 1995). Because of these main ecological functions, loss or diminution of mycosymbiont propagules from degraded ecosystems can limit both natural and artificial processes of revegetation (Requena et al. 2001). Therefore, an increase in the number of soil mycorrhizal propagules is needed. In revegetation schemes, two main reclamation strategies could be proposed: (i) inoculation of plants with selected mycosymbionts (Duponnois et al. 2005, in press) and/or (ii) management of the native soil mycorrhizal potential through drought-tolerant, native and highly mycotrophic plant species (Duponnois et al. 2001; Azcon-Aguilar et al. 2003).

In degraded semiarid ecosystems, shrub and tallgrass species grow following a patchy distribution. The vegetation patches usually constitute 'fertility islands' (Garner and Steinberger 1989) or 'resource islands' (Schlesinger et al. 1996) which could promote the native plant species development

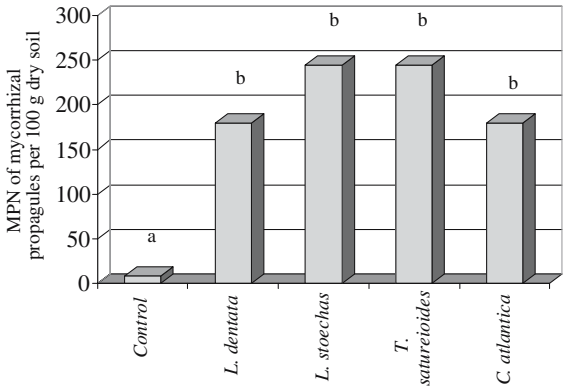

Figure 1. Most probable number (MPN) of mycorrhizal propagules in rhizosphere soils from target species and in bare soil (Control). Columns indexed by the same letter are not significantly different ($p < 0.05$).

(Callaway 1995, 1997). It has been previously assessed that some native plant species improve their own environment (self-promoting changes in water infiltration, organic matter, etc.) (Bochet et al. 1999; Valladares and Pugnaire 1999) and could act as 'nurse plants', through their positive impacts in the survival of other native plant species Carrillo-Garcia et al. (2000). Some representative plant species have a high capacity to enhance the development of mycorrhizal propagules in their rhizosphere (Azcon-Aguilar et al. 2003), suggesting that mycorrhizal propagules are involved in the functioning of the resource islands developing around plant roots.

In Morocco, overgrazing of pastoral resources, deforestation caused by demographic pressure, irregularity of rainfall distribution and changes in cultural practices limited the natural diversity of several species. In particular, the area of natural cypress stands (*Cupressus atlantica* Gaussen and *C. sempervirens* L.) has declined and observations have indicated a complete absence of natural regeneration (Ouahmane, personal communication). Attempts were made to replant these species, but the rate of success was very low.

The general objective of the current study was to determine the potential impacts of several representative shrub species (*Lavandula* spp. and *Thymus satureoides*) in Moroccan ecosystems on the early growth of *C. atlantica* seedlings and the potential contribution of arbuscular mycorrhizal (AM) symbiosis and the soil microflora to this 'plant nurse' effect.

Materials and methods

Experimental ecosystem area

The experimental area was located in the N'Fis valley (Haut Atlas, Morocco). Soil sampling was carried out at the Idni station (8°17′02″ W, 31°54′34″, 1700 m above sea level). The climate is semiarid Mediterranean with hot and dry summers and with rainfall occurring mostly in autumn. The mean annual precipitation is 634 mm. The plant cover is sparse and degraded due to overgrazing. The vegetation in this area is composed of grasses (i.e. *Stipa nitens* Ball.) and various shrub species, such as *Cistus salviifolus* L., *Lavandula dentata* L ., *L. stoechas* L., *Thymus pallidus* Coss., *Polygala balansae* Coss., *Globularia alypum* L. and *T. satureioides* Coss. Three shrub species (*L. dentata*, *L. stoechas* and *T. satureioides*) were selected as target species, as they were more abundant than the others (>50% of plant population) in the sampling area and always recorded in the vicinity of *C. atlantica* adult.

Field sampling

Soil samples were collected from the rhizosphere of the 3 target species and of *C. atlantica* existing in the experimental area. For each target species, 10 individual plants were randomly chosen and rhizosphere soil samples were taken. For *C. atlantica*, soil samples were taken at 2 m from the trunk, under the canopy. Each sample consisted of five sub-samples (100 cm^3) collected at the depth of 10–20 cm. Control samples were randomly collected from bare soil sites, away from plant influence.

Chemical assessments of the rhizosphere of the target species and of the bare soil

For each soil sample, pH of a soil suspension in water (10 g dry soil/15 ml deionized water) was determined. The total organic carbon (TOC) was measured according to the ANNE method (Aubert 1978) and the total nitrogen (TN) by the Kjeldhal method. The available phosphorus was determined calorimetrically (Olsen et al. 1954).

Determination of mycorrhiza in the rhizosphere of the target species and in the bare soil

Roots of target species were randomly collected within the experimental area (about 100 g fresh root per species) at 10 cm depth. They were washed with tap water, cleared and stained according to the method of Phillips and Hayman (1970). They were then placed on a slide for microscopic observation at 250× magnification (Brundrett et al. 1985). About one hundred 1-cm root pieces were observed per plant. The extent of mycorrhizal colonization was expressed as (the number of mycorrhizal root pieces)/(total number of root pieces) × 100. In addition, the frequency of arbuscules and vesicles per cm of root was also recorded.

Spores of AM fungi were extracted from rhizosphere and from bare soils by wet sieving and decanting which was followed by sucrose centrifugation (Sieverding 1991). Then the supernatant was poured through a 50 μm 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 to genus. Spore identification was assessed mainly using spore size and colour, wall structure and hyphal attachment (Walker 1983; INVAM 1997). Mycorrhizal fungal spore diversity was calculated using Simpson–Yule's diversity index (Krebs 1989).

The mycorrhizal potential of the rhizosphere soil samples was measured using the dilution technique (Sieverding 1991). Six dilutions were made of each soil treatment by thoroughly mixing the original soil in 1:4 proportions with an autoclaved sandy soil (140 °C, 40 min). After autoclaving, its physical and chemical characteristics were as follows: pH (H_2O) 5.6; clay (%) 4.6, fine silt (%) 0.0; coarse silt (%) 0.8; fine sand (%) 25.5; coarse sand (%) 69.1; carbon (%) 2.04; total nitrogen (%) 0.04; Olsen phosphorus 4.3 mg kg^{-1}; total phosphorus 116 mg kg^{-1}. Five replicates were prepared for each dilution. Seeds of *Sorghum vulgare* Pers, previously surface sterilized with 10% sodium hypochlorite, were pre-germinated for 2-days in Petri-dishes on humid filter paper. A germinated seed was then transplanted into each of small plastic pots (5.5 cm diameter; 6 cm high)

filled with 100 g of different soil dilutions. The pots were placed in a glasshouse under natural light (daylight approximately 12 h, mean temperature 30 °C) and watered daily with deionized water. After 1 month of growth, the entire root system of each seedling was collected, washed under tap water, cleared and stained as described above. Each entire root system was mounted on a microscope slide and observed at a 250× magnification under a compound microscope to observe presence of AM structures. Data were expressed as the number of AM propagules in 100 g of dry soil and the confidence limits were assigned according to Fisher and Yates (1970).

Measurement of the catabolic diversity of microbial communities in the rhizosphere of the target species and in the bare soil

Microbial functional diversity in rhizosphere and in bare soil was assessed by measuring of the patterns of *in situ* catabolic potential (ISCP) of microbial communities (Degens and Harris 1997). Three soil samples were randomly chosen among each original soil. Thirty-three substrates, comprising a range of amino-acids, carbohydrates, organic acids and amides, were screened for differences in SIR (substrate induced respiration) responsiveness of the soil samples. The substrate concentrations providing optimum SIR responses are indicated in Table 1 (Degens and Harris 1997). One gram equivalent of dry weight soil was mixed to each substrate suspended in 2 ml sterile distilled water (West and Sparling 1986) in 10 ml bottles. CO_2 production from basal respiratory activity in the soil samples was also determined, by adding 2 ml of distilled water to 1 g equivalent dry weight of soil. After the addition of substrate solutions to soil samples, bottles were immediately closed and kept at 28 °C for 4 h. CO_2 fluxes from the soils were measured using an infrared gas analyser (IRGA) (Polytron IR CO_2, Dräger^{TM}) in combination with a thermal flow meter (Heinemeyer et al. 1989). Results were expressed as μg CO_2 g^{-1} soil h^{-1}. Catabolic diversity was measured by catabolic richness and catabolic evenness. Catabolic richness, R, is the number of substrates used by microorganisms in each soil treatment. 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 = (respiration response to individual substrates)/(total respiration activity induced by all substrates for a soil treatment) (Magurran 1988).

Glasshouse experiment

Soil samples were packed in 1 dm^3 pots. Seeds of *C. atlantica* (Provenance Idni, Morocco) were immersed in distilled water at 4 °C for 24 h. Then they were transferred into Petri-dishes on humid filter paper. The plates were incubated for 1 week at 20 °C. When rootlets of germinating seeds were 1–2 cm long, they were individually transplanted to the pots.

The plants were arranged in a randomized, complete block design with 5 replicates per treatment. They were screened from the rain and grown under natural light (day length approximately 10 h, mean temperatures 25 °C) and watered daily with tap water during 5 months of growth.

The height and the stem diameter were measured. Then, the *C. atlantica* plants were uprooted and the root systems gently washed with tap water. The extent of AM fungal colonization was assessed as describe above. The dry weight (60 °C, 1 week) of shoots and roots was measured. After drying, sub-samples of ground shoot tissues were ashed (500 °C), digested in 2 ml HCl 6 M and 10 ml HNO_3 1 M and analysed by colorimetry for P (John 1970) and by flame emission for K. Plant tissues were digested in 15 ml H_2SO_4 18 N containing 50 g l^{-1} salicylic acid for N (Kjeldhal) determination.

Statistical analysis

Data were treated with one-way analysis of variance. Means were compared using PLSD Fisher test ($p < 0.05$). The percentages of mycorrhization were transformed by arcsin (sqrt) before statistical analysis. Between-Group Analysis (BGA, Dolédec and Chessel 1987; Culhane et al. 2002) was used to analyse the relationships between SIR responses and the five soil origins: *L. stoechas (Last)*, *L. dentata (Lade)*, *T. satureioides (Tysa)*, *C. atlantica (Cuat)*, and bare-soil (BS). BGA is a multivariate analysis technique derived from principal components analysis (PCA). The aim of PCA

Table 1. Organic compounds and their appropriate concentrations used to assess patterns of *in situ* catabolic potential (ISCP) of soil treatments.

Organic substrates	Conc. (mM)
Amino-acids	
L-Phenylalanine	15
L-Glutamine	15
L-Serine	15
L-Arginine	15
L-Asparagine	15
L-Histidine	15
L-Lysine	15
L-Glutamic acid	15
L-Tyrosine	15
L-Cysteine	15
Carbohydrates	
D-Glucose	75
D-Mannose	75
Sucrose	75
Amides	
D-Glucosamine	15
N-methyl D-glucamine	15
Succinamide	15
Carboxylic acids	
Ascorbic acid	100
Citric acid	100
Fumaric acid	100
Gluconic acid	100
Quinic acid	100
Malonic acid	100
Formic acid	100
α-ketoglutaric acid	100
Succinic acid	100
Tartric acid	100
Uric acid	100
Oxalic acid	100
Gallic acid	100
Malic acid	100
Tri-citrate	100
DL-α-Hydroxybutyric acid	100
Polymers	
Cyclohexane	100

is to summarize a data table by searching orthogonal axes on which the projection of the sampling points (rows of the table) has the highest possible variance. This characteristic ensures that the associated graphs (principal component maps) will best represent the initial data. These principal components (PCs) have the property of having the highest possible correlation with the original variables (columns of the data table).

From a theoretical point of view, BGA is the particular case of PCA with respect to instrumental variables (PCAIV, Rao 1964; Lebreton et al. 1991) where 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 aim of this analysis is to separate the groups. This is also the aim of discriminant analysis (also called canonical variates 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. This is the case in this paper (33 SIR responses vs. 15 soil samples), and this is why we used BGA. BGA can thus be considered as a robust alternative to discriminant analysis when the number of samples is low.

A Monte-Carlo test (permutation test) can be used to check the significance of the differences between groups.

Computations and graphical displays were made with the free ADE-4 software (Thioulouse et al. 1997), available on Internet at http://www.pbil.univ-lyon1.fr/ADE-4/.

Results

Chemical and mycorrhizal characterization of the soils from around target plant species

The soil samples had a neutral to neutral-alkaline reaction (Table 2). The total carbon content of the *C. atlantica* soil was significantly higher than in the other soils, and the highest soluble P content was measured in the bare soil (Table 2). The total nitrogen content was significantly higher in the *C. atlantica* soil than in the bare and *T. satureioides* soils, whereas medium values were recorded for *Lavandula* soils. No significant differences were found between different soils for the C/N ratios.

All the target species formed AM symbiosis (Table 3). The extent of AM fungal colonization was not significantly different between plant species (Table 3). On the contrary, *L. dentata* showed the lowest frequency of vesicles and the highest frequency of arbuscules (Table 3).

The total number of spores of AM fungi found in the root zone of *L. dentata* was significantly

Table 2. Chemical characteristics of the rhizosphere soils collected from *L. dentata*, *L. stoechas*, *T. satureioides*, *C. atlantica* and the bare soil (Control).

	Control	Plant species			
		L. dentata	*L. stoechas*	*T. satureioides*	*C. atlantica*
pH	7.5 a[A]	7.0 a	7.5 a	7.4 a	7.7 a
Total carbon (%)	1.58 a	1.60 a	1.73 a	1.80 a	3.15 b
Total nitrogen (%)	0.09 a	0.10 ab	0.12 ab	0.10 a	0.14 b
C/N	17.2 a	15.7 a	14.7 a	18.8 a	22.9 a
Soluble P (mg kg^{-1})	19.7 d	7.9 a	9.8 ab	11.8 bc	13.1 c

[A]Data in the same line followed by the same letter are not significantly different according to the one-way analysis of variance ($p < 0.05$).

Table 3. Main plant species in the experimental area and their extent of Arbuscular Mycorrhizal Fungal (AMF) colonization.

Plant species	AMF colonization (%)	Arbuscules (%)	Vesicules (%)
L. dentata	61.6 a[A]	31.3 b	68.8 a
L. stoechas	66.9 a	0 a	100 b
T. satureioides	56.2 a	0 a	100 b
C. atlantica	57.3 a	0 a	100 b

[A]Data in the same column followed by the same letter are not significantly different according to the one-way analysis of variance ($p < 0.05$).

higher than found around *T. satureioides* (Table 4). Nevertheless, no significant difference was detected between the total number of AM fungal spores of the bare soil (control) and those recorded in the root zones of target species and *C. atlantica* (Table 4). Three genera of AM fungi (*Scutellospora*, *Glomus* and *Acaulospora*) were present in the rhizospheres of the plant species and in the bare soil (Table 4). No significant difference was found in the abundance of *Glomus* and *Acaulospora* spores in different soils. In contrast, the number of *Scutellospora* spores was significantly higher in the rhizosphere of *L. dentata* and *C. atlantica* than in the soil originating from around *T. satureioides* (Table 4). The Simpson–Yule's diversity indexes, calculated for each of the soils, were not significantly different between plant species (mean value: 2.085).

The number of mycorrhizal propagules in soil originating from around the four target plant species was significantly higher than the one in the bare soil (Figure 1). The MPN of mycorrhizal propagules per 100 g of dry soil ranged from 7.82 (bare soil) to 179.7 (*L. dentata* and *C. atlantica*) and 244.5 (*L. stoechas* and *T. satureioides*).

Catabolic diversity of microbial communities in the soils originating from around target plant species

The average substrate-induced respiration (SIR) responses with amino-acids, amides and carbohydrates were significantly higher in the bare soil (control) than in the other soils (Table 5). With carboxylic acids, the highest values were recorded for *T. satureioides* and *C. atlantica* soils and the lowest for *L. dentata* soil (Table 5). The catabolic richness of the tested soils was in the following order: control > *C. atlantica* > *L. dentata* = *L. stoechas* > *T. staureioides* and, for the catabolic eveness, control > *L. dentata* > *L. stoechas* > *T. satureioides* > *C. atlantica* (Table 5).

BGA of the SIR responses with respect to the five soil types appears in Figure 2. The map of the five soil types (Last, Lade, Tysa, Cuat, and BS) is superimposed to the map of substrates. Only the 12 most important substrates have been represented, to avoid cluttering of the graphic. The Monte-Carlo test shows that the differences between soil types are statistically significant ($p < 0.01$).

Figure 2 clearly shows that the microbial communities are very different according to the soil

Table 4. Abundance of spores of three AMF in the rhizosphere of the target plant species and in the bare soil (control).

Origins	*Glomus* spp. (100 g^{-1} dry soil)	*Scutellospora* sp. (100 g^{-1} dry soil)	*Acaulospora* sp. (100 g^{-1} dry soil)	Total number of AMF spores (100 g^{-1} dry soil)
Control (bare soil)	315.1 a[A]	174.3 ab	48.4 a	537.8 ab
L. dentata	342.5 a	255.5 b	57.0 a	655.0 b
L. stoechas	288.2 a	228.1 ab	39.9 a	556.1 ab
T. satureioides	304.4 a	123.1 a	33.9 a	461.4 a
C. atlantica	294.2 a	276.8 b	33.3 a	604.3 ab

[A]Data in the same column followed by the same letter are not significantly different according to the one-way analysis of variance ($p < 0.05$).

Table 5. Substrate-induced respiration (SIR) responses (μg CO_2 g^{-1} soil h^{-1}) with each substrate group (Carboxylic acids, amino-acids, amides and carbohydrates) and catabolic richness and evenness in the rhizosphere of the target plant species and in the bare soil (control).

	Origins of the soil samples				
	Control (Bare soil)	*L. dentata*	*L. stoechas*	*T. satureioides*	*C. atlantica*
Carboxylic acids	991.9 b[A]	282.7 a	739.8 ab	3129.9 c	2726.2 c
Amino-acids	375.9 b	137.8 a	139.6 a	144.5 a	200.1 a
Amides	169.9 b	67.1 a	43.2 a	46.7 a	69.4 a
Carbohydrates	163.4 b	40.1 a	35.4 a	35.1 a	60.7 a
Catabolic richness	33.1 c	32.0 b	32.0 b	31.3 a	32.7 c
Catabolic evenness	25.2 c	24.8 c	17.7 b	12.7 ab	10.3 a

[A]Data in the same line followed by the same letter are not significantly different according to the one-way analysis of variance ($p < 0.05$).

origin. Three types of samples could be separated: samples coming from *Thymus* rhizosphere soil (upper right), from *Cupressus* rhizosphere soil (lower right), and from bare soils (control) and those with *Lavandula* rhizosphere soils (left). Organic acids used in *Cupressus* rhizosphere soil were fumaric, tartric and malonic acids, while in *Thymus* rhizosphere soil, the most important acids were gallic, formic, and oxalic. In bare and *Lavandula* rhizosphere soils, these six substrates are less used, and all the other substrates are used indistinctly.

Glasshouse experiment

After 6 months of culturing in glasshouse conditions, the height, stem diameter, nitrogen, phosphorus and potassium foliar contents, and AM colonization of the *C. atlantica* seedlings were significantly higher in the soils originating from around *T. satureioides*, *Lavandula* spp. and *C. atlantica* than in the bare soil (Table 6). For the shoot and root biomass, a similar positive effect of these soil origins has been recorded except for the soil originating from around *C. atlantica* (Table 6).

Discussion

All the target plants examined in this research were highly infected by arbuscular mycorrhizal fungi. These results are in accordance with other survey studies carried out in Mediterranean areas, that have shown that most of the plant species involved were heavily mycorrhizal (Requena et al. 1996, 1997). For example, it has been previously described that lavender plants form arbuscular mycorrhizas and are mycorrhizal-dependent species (Azcon and Barea 1997). These lavender plants have been classified as 'obligatory mycorrhizal' (Brundrett 1991) or as 'highly dependent on mycorrhiza' (Habte and Manjunath 1991). To our

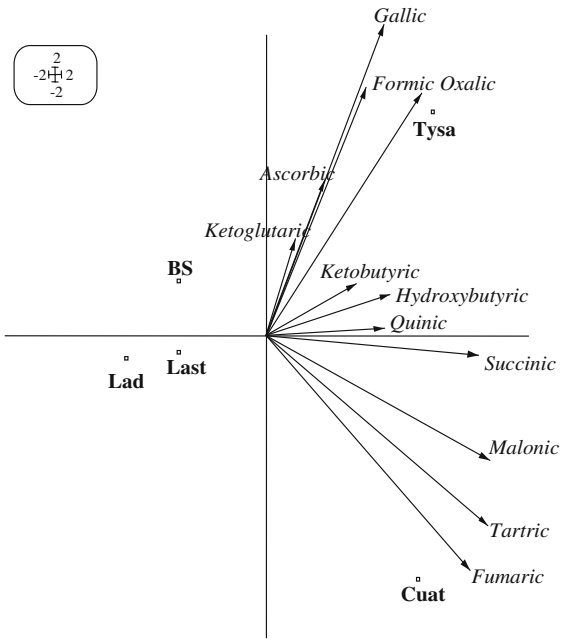

Figure 2. Between-group analysis (BGA) of the substrate induced respiration (SIR) responses with respect to the rhizosphere and the bare soils. *L. stoechas (Last)*, *L. dentata (Lade)*, *T. satureioides (Tysa)*, *C. atlantica (Cuat)*, and bare soil (BS).

knowledge, there are no available references for the mycorrhizal status of *C. arizonica* and *T. satureioides*, but their high mycorrhizal colonization permit to think that they may be also highly mycorrhizal dependent.

The arid and semiarid ecosystems are generally characterized by a patchy distribution of individual plant species (Halvorson et al. 1994). Under *C. arizonica*, the soil carbon and nitrogen contents were, not surprisingly, higher than in the other sampling areas because of the leaf litter formation and the fact that in forest ecosystem, most of the soil nitrogen is in organic form (Kaye and Hart 1997). For other rhizosphere soil samples, collected around *Lavandula* spp. and *T. satureioides*, chemical characteristics were not significantly different from those of the bare soil, except for the soluble phosphorus. The principal explanation of this P depletion is that plants mainly absorb this nutrient from the soil solution, but it does not mean that there was deficiency of P in these soils. Indeed, it has been demonstrated that lavender plants accumulate high P concentrations in their tissues (Azcon and Barea 1997). For Koide (1991) momentary luxury consumption of P may act as storage function to be subsequently used when the P demand exceeds P supply.

On the contrary, the microbial characteristics of the rhizosphere soil samples were significantly different than those of the bare soil. *Cedrus atlantica* and the three target shrub species enriched the soil with mycorrhizal propagules. Although the total number of spores per 100 g of dry soil was not different for the soil of different origins, the mycorrhizal soil infectivity (MSI) was significantly higher in the rhizosphere soil than in the bare soil. In soil, AM fungi are found as spores, hyphae or infected root pieces. All these propagules can be inoculum sources (Duponnois et al. 2001). From the present work, it appears that the soil borne spores were not involved in this MSI enhancement. This result agrees with previous studies in which it has been attested that the AM mycelia network is the main source of inoculum in semiarid and arid ecosystems (Brundrett and Kendrick 1991; Bashan et al. 2000).

Table 6. Growth and AM colonization of *C. atlantica* seedlings on the rhizosphere soils collected from *L. dentata*, *L. stoechas*, *T. satureioides*, *C. atlantica* and the bare soil (Control) after 6 months of culturing in greenhouse conditions.

	Control	Plant species			
		L. dentata	*L. stoechas*	*T. satureioides*	*C. atlantica*
Height (cm)	14.2 a[A]	18.6 b	21.0 cd	23.0 d	19.4 bc
Stem diameter (mm)	2.02 a	2.72 bc	2.72 bc	2.94 c	2.54 b
Shoot biomass (mg dry weight)	330 a	634 bc	738 c	666 bc	486 ab
Root biomass (mg dry weight)	76 a	176 c	157 bc	115 abc	104 ab
N (mg per plant)	0.785 a	1.559 b	1.823 c	2.029 d	1.480 b
P (mg per plant)	0.033 a	0.107 c	0.115 c	0.147 d	0.090 b
K (mg per plant)	3.71 a	9.54 b	26.53 c	25.16 c	8.58 b
AM colonization (%)	35 a	48 b	50 b	75 c	54 b

[A]Data in the same line followed by the same letter are not significantly different according to the one-way analysis of variance ($p < 0.05$).

This MSI enhancement was linked with the changes in the functioning of soil microbial communities. It has been previously demonstrated that the composition of microbial communities is mainly determined by plant factors, such as species composition and formation age (Grayston et al. 2001), as well as various environmental factors such as soil type, nutrient status, pH and moisture (Stotzky 1997). Differentiation of microbial communities with respect to the vegetation has been investigated using methods reflecting membrane chemistry structure such as PLFA (Phospho-Lipid Fatty acid Analysis) (Kourtev et al. 2002), DNA profiles (Marilley and Aragno 1999) and assessment of the catabolic diversity of soil microbial communities (substrate-specific respiration) (Degens and Harris 1997). It is generally assumed that the changes in the microbial community structures are the consequence of differences in root inputs among plant species (rhizosphere exudates and root turnover) (Grayston et al. 1998; Coleman et al. 2000) as well as in the quantity and chemical quality of aboveground litter inputs. In the present study, SIR responses to carbohydrates, amides and amino-acids were significantly lower in the rhizosphere soils than in the bare soil. This difference could be due to the assimilation of these organic substrates by the AM symbionts, as it is known that AM symbiosis alters the amount of root exudates (Marschner et al. 1997). The trophic transfer (catabolism and anabolism) through the carbon flow between free-living microorganisms, involves a large CO_2 production, whereas mycorrhizal fungi can directly use plant compounds for anabolism without large catabolism and CO_2 production (Wamberg et al. 2003). On the contrary, SIR responses to carboxylic acids were significantly higher in the *C. atlantica* and *T. satureioides* rhizosphere soils than in the soils of other origins. Soluble organic acids are involved in plant nutrient acquisition and they particularly act as biological weathering of minerals in soils. They could be of high molecular weight (HMW) (i.e. humic substances) to low molecular weight (LMW) produced by plant roots and soil microorganisms (Ochs 1996). It has been assessed that dissolution rates in the rhizosphere were fast in comparison with rates in the bulk soil, due to the exudation of simple organic ligands by soil microorganisms and, in particular, by mycorrhizal roots. On the other hand, the influence of humidified soil organic matter seemed to be smaller than generally expected (Shibata and Yano 2003). Among LMW organic acids involved in mineral dissolution, oxalate, malate and citrate are considered to be the strongest chelators of trivalent metals (Gadd 1999). They are produced by many different fungal species, in particular, by mycorrhizal fungi (Dutton and Evans 1996; Smith and Read 1997). In the *C. atlantica* and *T. satureioides* rhizosphere soils, AM fungal communities probably produced large quantities of carboxylic acids, such as oxalic, fumaric and tartric, which exerted a selected influence on soil microbial communities through a multiplication of oxalate, tartrate and fumarate catabolizing microorganisms (higher SIR responses to these carboxylic acids). This mycorrhizal effect could not explain the ISCP patterns of microbial communities in *Lavandula* rhizosphere soils. However, other biological processes could be involved in P acquisition from non-labile sources in soil. For example, it has been shown that acid phosphatase activity was associated with the roots of Lavender plants (Azcon et al. 1982; Azcon and Barea 1997). This root acid phosphatase activity was usually increased in P-deficient plants (Khalil et al. 1994).

As the mycorrhizal potential of the rhizosphere soils was higher than that of the bare soil, the growth of the *C. atlantica* seedlings was significantly higher in the *C. atlantica* soil and in the soils originating from shrub species than in the bare soil. This result suggests that *C. atlantica* has to be mycorrhizal in order to reach its optimal growth, and that it is 'highly dependent on mycorrhizas' (Habte and Manjunath 1991). The mycorrhizal colonization extent ranged from *T. satureoides* > *C. atlantica* > *Lavandula* spp. > bare soil and was significantly linked with the phosphorus and nitrogen contents in plant ($R^2 = 0.77$ and $R^2 = 0.72$, respectively). The effect of AM fungi in improving N uptake from soil corroborates previous findings (Tobar et al. 1994a, b; Azcon and Barea 1997). The impact of AM fungi on P plant nutrition is well documented and it is generally admitted that AM symbiosis increases plant uptake of low mobility minerals such as phosphorus (Plenchette and Fardeau 1988). Moreover, it has been demonstrated that the increase of AM fungal diversity led to an increased phosphorus content in plant material and, consequently, to a more efficient exploitation of soil phosphorus (Van der

Hejden et al. 1998). Therefore, *T. satureioides* could be associated with a larger number of AM fungal species, including more compatible and effective AM fungal partners (Hart et al. 2003).

It has been suggested that AM fungi might also be important agents in promoting plant-coexistence (Janos 1980; Allen and Allen 1990). The presence of AM fungi is known to have a strong effect on the direction of succession (Medve 1984). This fungal effect is mainly important in early successional ecosystems where plant and soil have been severely disturbed and where AM fungi are absent or are in low abundance and patchily distributed (Hart et al. 2003). Although the AM inoculum potential is not sufficient to ensure the development of forest trees in Mediterranean ecosystems, the use of plant nurses such as *T. satureioides* or *Lavandula* spp. could be of great interest in restoring a self-sustaining vegetation cover in order to act against desertification.

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