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ScienceDirect
Applied Soil Ecology 34 (2006) 190–199
Applied Soil Ecology
www.elsevier.com/locate/apsoil

Lavandula species as accompanying plants in *Cupressus* replanting strategies: Effect on plant growth, mycorrhizal soil infectivity and soil microbial catabolic diversity

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Received 3 May 2005; received in revised form 10 January 2006; accepted 3 February 2006

Abstract

The general objective of this study was to measure the mycorrhizal dependencies of *Cupressus* and *Lavandula* species, to assess the contribution of *Lavandula* species to the mycorrhizal soil infectivity (MSI) and to determine the main effects of the association between a *Lavandula* species (*L. multifida*) and *Cupressus arizonica* on the soil microbial activities, on the growth and the mycorrhizal status of each plant species in experimental conditions. Three species of *Lavandula* (*L. stoechas* L., *L. dentata* L. and *L. multifida* L.) and three species of *Cupressus* (*C. atlantica*, *C. sempervirens* and *C. arizonica*) were tested. Plant species were inoculated with an arbuscular mycorrhizal fungus, *Glomus intraradices*, to determine their mycorrhizal dependencies in controlled conditions. The results showed that *L. multifida* and *L. dentata* were representatives of highly mycorrhizal dependent plant species as well *C. arizonica* and *C. sempervirens*. The contribution of *L. multifida* to the MSI was determined by the most probable number (MPN) method. In six different soils, *L. multifida* enhanced the mycorrhizal soil infectivity, even more if the soil was P deficient. When *C. arizonica* and *L. multifida* were grown together, the growth and mycorrhizal colonization of each species were higher than those recorded when each plant species grew alone. The impact of this dual cultivation on the growth and mycorrhizal colonization of *C. arizonica* was comparable to those recorded with *C. arizonica* seedlings previously inoculated with *G. intraradices*. In contrast, the microbial activities of the soil collected from each treatment were different. These results emphasize the role of ''resource islands'' and ''nurse plants'' of Lavender plants in the regeneration processes of tree species such as *Cupressus* spp. In addition, they confirm the role of AM fungi as a major factor contributing to the growth and co-existence of each of the plant species (*L. multifida* and *C. arizonica*).

Keywords: Arbuscular mycorrhizal fungi; *Cupressus*; *Lavandula*; Nurse plant; Microbial catabolic diversity

1. Introduction

While competition between plant species for resources has been widely studied and is known to play an important role in plant community structure (Goldberg and Barton, 1992; Sanchez and Peco, 2004), studies of

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doi:10.1016/j.apsoil.2006.02.002

mutualism are under-represented even in recent ecological and evolutionary works (Ferriere et al., 2002; Hoeksema and Schwartz, 2003). Mutualism is generally defined as an interaction in which two or more species benefit each other. It ranges from specific obligate symbiotic associations to facultative interactions among free-living species. It is well accepted that mutually beneficial interactions among species prevail in many ecological communities and play an important role in the functioning of plant communities (Boucher et al., 1982). Although ecological mechanisms by which plant composition and diversity are regulated and maintained are not well understood, it has been demonstrated that mycorrhizal symbiosis is an important determinant of plant diversity, ecosystem variability and productivity (Odum, 1959; Van der Hejden et al., 1998). The ability of the plant to uphold and to resist stress situations (nutrient deficiencies, drought, soil disturbance) could be enhanced by a well-developed and fully functional symbiosis (Odum, 1959; Barea et al., 1997; Schreiner et al., 1997; Bethlenfalvay and Linderman, 1992).

In the semiarid Mediterranean ecosystems desertification has occurred following several decades of particular climatic conditions (scarce and irregular rainfall, long dry period in summer) and over exploitation of natural resources (Francis and Thornes, 1990). The process of desertification usually results from degradation of natural plant communities (population structure, succession pattern and species diversity) and of physico-chemical and biological soil properties (nutrient availability, microbial activity, soil structure, etc.) (Garcia et al., 1997; Albaladejo et al., 1998; Requena et al., 2001). Among the biological processes involved desertification, the loss or reduction of the activity of mycorrhizal fungi was often detected (Bethlenfalvay and Schüepp, 1994; Degens et al., 1996).

In order to reverse this loss of biodiversity in Mediterranean ecosystems, some revegetation programmes have been undertaken, using drought-tolerant native species, to re-establish functional shrublands (Francis and Thornes, 1990).

In Morocco, there is a rich biodiversity with a large number of ecosystems and rich flora (over 4500 species of higher plants with about 200 of them considered as rare or threatened). Unfortunately, overgrazing, deforestation caused by demographic pressure, irregularity of rainfall distribution and changes in cultural practices have resulted in the reduction of this natural diversity. In particular, the area of natural and introduced cypress stands (one natural species *Cupressus atlantica* and two introduced species: *C. sempervirens* and *C. arizonica*) has declined and observations indicate a complete absence of natural regeneration (Ouahmane, personal communication). Attempts have been made to replant these species, but the rate of success was very low.

Although the mycorrhizal symbiosis is considered as a key factor to sustain a vegetation cover in natural habitats, there are no previous assessments of the mycorrhizal dependencies of *Cupressus* species. However, the mycorrhizal potential contribution of small woody members of the shrubland community, such as Lavender plants, frequently observed in *Cupressus* stands in Morocco, has been assessed (Azcon and Barea, 1997).

The general objective of this study was to (i) evaluate the mycorrhizal dependencies of *Cupressus* and *Lavandula* species, (ii) determine the contribution of *Lavandula* species to the mycorrhizal soil infectivity and (iii) determine the main effects of the presence of a *Lavandula* species (*L. multifida*) on the soil microbial activities and on the growth and the mycorrhizal status of *C. arizonica* in experimental conditions.

2. Materials and methods

2.1. Experiment 1: Mycorrhizal dependencies of Lavandula and Cupressus species

2.1.1. Fungal inoculum

The arbuscular mycorrhizal (AM) fungus *Glomus intraradices* (Schenk & Smith, DAOM 181602, Ottawa Agricultural Herbarium) was propagated on leek (*Allium porrum* L.) for 12 weeks under greenhouse conditions on Terragreen^{TM} substrate. This calcinated clay (average particule size 5 mm), Oil-Dri US-special Ty/IIIR (Oil-Dri Company, Chicago, USA), is an attapulgite from Georgia used as substrate for propagation of AM fungi (Plenchette et al., 1996). The mycorrhizal inoculum consisted of infected leek root pieces (around 250 vesicles cm^{-1}, average length 0.5 cm). Non-mycorrhizal leek roots were used for the control treatment.

2.1.2. Inoculation and plant culture

The sandy soil used in this experiment was crushed, passed through a 2-mm sieve and autoclaved (140 °C, 40 min) to eliminate native microorganisms. 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}.

Seeds of *L. stoechas* (L.), *L. dentata* (L.) and *L. multifida* (L.) collected from the field were germinated on moistened disinfected (140 °C, 40 min) sand. Eight-day-old seedlings were individually transplanted to the pots,

each one containing 1 kg of soil. One-half of the pots was inoculated with *G. intraradices* and the other was not. Seeds of *C. atlantica*, *C. sempervirens* and *C. arizonica* 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.

One hole (1 cm × 5 cm) was made in the soil of each pot and filled with 1 g of fresh leek root. Half of the pots received AM roots (experimental group), and the other half received non-inoculated roots (control group). The holes were then covered with the same autoclaved soil.

The pots were arranged in a randomized, complete block design with eight replicates per treatment. They were placed in a greenhouse under natural light (daylight approximately 12 h, mean temperature 30 °C) and watered daily.

2.1.3. Measurements

Three months after planting, the plants were harvested. Shoot dry weight was recorded after drying at 70 °C for 1 week. The roots were washed with tap water, cleared and stained according to the method of Phillips and Hayman (1970). They were 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/number of non-mycorrhizal root pieces × 100. Then the dry weight of roots was measured (65 °C, 1 week). The mycorrhizal dependency (MD) of each plant species was determined by expressing the difference between the total dry weight of a mycorrhizal plant and the total dry weight of a non-mycorrhizal plant as a percentage of the total dry weight of the mycorrhizal plant (Plenchette et al., 1983).

2.2. Experiment 2: Potential contribution of *L. multifida* to the mycorrhizal soil infectivity

The mycorrhizal soil infectivity (MSI) was determined by the most probable number (MPN) of infective propagules method according to Sieverding (1991). Six different sandy soils were used and their chemical characteristics are indicated in Table 1. Eight-day-old seedlings of Lavender plants were individually grown in 0.5-l polythene bags filled with each of the sandy soils. The same bags filled with each of the sandy soils, but without plants, were in the control group. The bags were arranged in a randomized, complete block design with 10 replicates per treatment. They were placed in a glasshouse under natural light (daylight approximately 12 h, mean temperature 30 °C) and watered daily. After 5 months culturing, Lavender plants were uprooted and, for each treatment, the 10 polythene bags were emptied and the collected soil was mixed. Six dilutions were made of each soil treatment by thoroughly mixing the original soil in 1:4 proportions with the same autoclaved soil (140 °C, 40 min). 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. One germinated seed was then transplanted into each of the 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 40 days of growth, the entire root system of each seedling was collected, washed under tap water, cleared and stained as described above. The 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 number of AM propagules in 100 g of dry soil and the confidence limits were assigned according to Fisher and Yates (1970).

Table 1
Physico-chemical characteristics of the sandy soils tested in Experiment 2

Parameters	Soils					
	1	2	3	4	5	6
pH (H_2O)	5.8	4.9	5.4	5.7	6.1	5.7
Total C (g C kg^{-1})	3.99	2.85	6.62	3.17	7.29	3.09
Organic matter (%)	0.7	0.5	1.1	0.5	1.3	0.5
Total N (g kg^{-1})	0.26	0.19	0.46	0.12	0.60	0.47
Total P (mg kg^{-1})	68.5	78.7	119.2	48.4	200.7	90.0

2.3. Experiment 3: Influence of *L. multifida* on the growth and on AM colonization of *C. arizonica*

2.3.1. Experimental design

Germinated seeds of *C. arizonica* were individually grown in 0.5-l polythene bags filled with the same autoclaved sandy soil (140 °C, 40 min) used in Experiment 1. The soil was inoculated with leek roots colonized by *G. intraradices* in one-half of the bags and with non-mycorrhizal leek roots (for the treatment without fungus) in the other half as described above (Experiment 1). Eight-day-old seedlings of *L. multifida* were individually planted in 0.5-l polythene bags filled

with the same autoclaved sandy soil but not inoculated with *G. intraradices*. The bags were arranged in a randomized, complete block design with 12 replicates per treatment (with or without *G. intraradices*) for *C. arizonica* and 12 replicates for *L. multifida*. They were placed in a glasshouse under natural light (daylight approximately 12 h, mean temperature 30 °C) and watered daily during 5 months.

After 5 months culturing, six plants of *C. arizonica* were randomly chosen from both treatments. They were uprooted and the roots gently washed. The AM colonization was assessed as described above.

To study the effects of preinoculation (*G. intraradices*), native fungal inoculum, and vicinity of *L. multifida* on *C. arizonica* growth the following treatments were done with six replicates: 5 months old *C. arizonica*, inoculated or not, were transferred into 20-l pots filled with the same sandy soil but not autoclaved. Two other treatments were made one by placing in the same pot, 10 cm apart, a non-mycorrhized *C. arizonica* plant and a randomly chosen *L. multifida* plant, the other being *L. multifida* alone.

There were four treatments (*C. arizonica* alone, CA; *C. arizonica* + *G. intraradices*, CG; *C. arizonica* + *L. multifida*, CL; *L. multifida* alone, LM). The pots were arranged in a randomized, complete block design with six replicates per treatment. Plants were grown in nursery conditions at ambient temperature from 25 to 35 °C with daily watering.

2.3.2. Measurements

The height of the plants was measured every 2 weeks for 4 months. For *C. arizonica*, number of branches per plant was also determined every 2 weeks. Then, all the plants were uprooted and the root systems gently washed. For the CL treatment, the roots of each species were carefully separated. The height and the oven-dried weight (1 week at 65 °C) of the shoots were measured. Five hundred milligrams of fresh root was randomly collected along the root system of each plant to evaluate the intensity of AM symbiosis. They were cleared and stained and the AM colonization was determined and expressed as described above.

Microbial functional diversity in soil treatments was assessed by measuring the patterns of in situ catabolic potential (ISCP) of microbial communities (Degens and Harris, 1997). Eleven substrates, comprising a range of organic acids (glutamic, ketobutyric, citric, fumaric, oxalic, gluconic, tartaric, malic, malonic and succinic acids) and glucose, were screened for differences in substrate induced respiration (SIR) responsiveness between soil treatments. The substrate concentrations providing optimum SIR responses were 100 and 75 mM for the organic acids and glucose, respectively (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 analyzer (IRGA) (Polytron IR CO_2, Dräger^{TM}) in combination with a thermal flow meter (Heinemeyer et al., 1989). Results were expressed as $\mu g\ CO_2\ g^{-1}$ soil h^{-1}.

2.4. Statistical analysis

Data were treated with one-way analysis of variance. Means were compared using PLSD Fisher test ($p < 0.05$). Mycorrhizal indexes were transformed by arcsin (sqrt) before statistical analysis.

Co-inertia analysis (COIA) was used to analyze the relationships between *C. arizonica* shoots growth, mycorrhizal colonization and the SIR responses. Co-inertia analysis (Dray et al., 2003) is a multivariate analysis technique that describes the relationship between two data tables. Classical methods like principal components analysis (PCA) or correspondence analysis (CA), aim at summarizing a table by searching orthogonal axes on which the projection of the sampling points (rows of the table) have the highest possible variance. This characteristic ensures that the associated graphs (factor maps) will best represent the initial data. To extract information common to two tables, canonical correlation analysis (CANCOR) searches successive pairs of axes (one for each table) with a maximum correlation. The problem is that this analysis often leads to axes with a very high correlation, but with very low percentages of explained variance. To overcome this difficulty, COIA searches pairs of axes with maximum covariance (instead of correlation). Computations are based on the cross-table between SIR responses and plant variables. The importance of each axis is given by the percentage of total co-inertia, which is similar to the percentage of explained variance for each canonical axis.

Another problem of CANCOR and also of canonical correspondence analysis (CCA, Ter Braak, 1986), is that the number of rows of both tables must be high compared to the number of columns of the independent variables table. When the number of rows is low,

CANCOR cannot be used, and CCA is reduced to a plain CA (see, for example, Dray et al., 2003). COIA does not suffer from this problem, and it can be used even if the number of rows is lower than the number of columns, which is the case in this paper. A simple PCA-like standardization was applied to both data tables before computing COIA.

Monte-Carlo tests can be used to check the significance of the relationship between the two tables. This method consists in performing a random permutation of the rows of one or both tables many times, followed by the re-computation of the total co-inertia. By comparing the total co-inertia obtained in the normal analysis with the co-inertia obtained after randomization, we get an estimation of the probability to encounter a situation similar to the observed situation, without relationship between the two tables (i.e., a significance test of the relationship). Computations and graphical displays were made with the free ADE-4 software (Thioulouse et al., 1997), available on Internet at http://pbil.univ-lyon1.fr/ADE-4/.

3. Results

3.1. Experiment 1

Mycorrhizal inoculation with *G. intraradices* significantly increased the shoot and root growth of *Lavandula* species (Table 2). Mycorrhizal dependency of *L. stoechas* (33.1%) was significantly lower than those recorded for other *Lavandula* species, 62.5 and 57.5%, for *L. dentata* and *L. multifida*, respectively. No significant differences were detected for mycorrhizal colonization between the *Lavandula* species tested (Table 2). This positive effect of *G. intraradices* inoculation was also recorded on the growth of *Cupressus* species (Table 2). Mycorrhizal dependency of *C. atlantica* (37.6%) was significantly lower than that calculated for *C. arizonica* and *C. sempervirens* (54.1 and 51.3%, respectively) (Table 2). Mycorrhizal colonization was not significantly different between *Cupressus* species (Table 2).

3.2. Experiment 2

The development of *L. multifida* seedlings significantly enhanced the number of AM infective potential propagules per 100 g of soil in three soils (soils 1, 2 and 4) whereas no significant effect of this *Lavandula* species was recorded on the mycorrhizal soil infectivity in the soils 3, 5 and 6 (Table 3). The increase of MSI by *L. multifida* seedlings was correlated to the total P content ($y = 211{,}763x^{-1.4734}$, $R = 0.73$, $p < 0.05$).

3.3. Experiment 3

After 5 months growth, the root colonization index of mycorrhizal *C. arizonica* seedlings was about 40% whereas no mycorrhizal structures were detected in the treatment without *G. intraradices*. There was already a significant effect of inoculation with *G. intraradices* on the height of *C. arizonica* seedlings at the time of

Table 2
Growth responses and mycorrhizal colonization of *Lavandula* and *Cupressus* species to the *G. intraradices* inoculation after 3 months growth in glasshouse conditions on an autoclaved soil (Experiment 1)

Plant species	Treatments	SB (mg)	RB (mg)	Mycorrhizal colonization (%)
L. multifida	Control	122 a[a]	59 a	0
	+*G. intraradices*	302 b	124 b	52
L. dentata	Control	110 a	45 a	0
	+*G. intraradices*	211 b	96 b	48
L. stoechas	Control	172 a	65 a	0
	+*G. intraradices*	236 b	118 b	55
C. atlantica	Control	100 a	36 a	0
	+*G. intraradices*	153 b	65 b	32
C. arizonica	Control	112 a	43 a	0
	+*G. intraradices*	253 b	85 b	45
C. sempervirens	Control	62 a	31 a	0
	+*G. intraradices*	132 b	59 b	41

SB: shoot biomass (mg dry weight). RB: root biomass (mg dry weight).
[a] For each plant species, data in the same column followed by the same letter (a and b) are not significantly different according to one-way analysis of variance ($p < 0.05$).

Table 3
Mycorrhizal soil infectivity (assessed by the MPN method) of six sandy soils planted or not with *L. multifida* after 5 months growth

Soils	Treatment	AM infective propagules per 100 g soil (95% confidence limits)
1	−*L. multifida*	127.1 (59.6–271.1)
	+*L. multifida*	660.4 (309.9–1409.3)
2	−*L. multifida*	187.8 (75.9–345.1)
	+*L. multifida*	1309.7 (613.7–2792.5)
3	−*L. multifida*	187.8 (75.9–345.1)
	+*L. multifida*	332.7 (155.9–709.6)
4	−*L. multifida*	127.1 (59.6–261.1)
	+*L. multifida*	570.1 (267.2–1215.9)
5	−*L. multifida*	165.1 (77.4–352.1)
	+*L. multifida*	252.3 (118.4–538.3)
6	−*L. multifida*	333.1 (156.1–710.4)
	+*L. multifida*	464.4 (217.7–990.7)

Table 5
Time course changes in ramification number of *C. arizonica* seedlings in the different treatments (*C. arizonica* alone, CA; *C. arizonica* + *G. intraradices*, CG; *C. arizonica* + *L. multifida*, CL)

Time (week)	Treatments		
	CA	CG	CL
0	14.8 a[a]	16.8 a	12.4 a
3	21.8 a	27.2 a	21.2 a
5	30.8 a	36.6 a	30.0 a
7	39.2 a	47.6 a	43.8 a
9	46.0 a	57.4 b	59.0 b
11	57.0 a	69.6 b	75.0 b
13	69.6 a	81.4 b	88.8 b
14	82.4 a	95.2 b	106.6 c
16	94.0 a	113.8 b	120.2 b

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

outplanting together or not with a L. multifida plantlet. A significant positive effect of *G. intraradices* (CG treatment) inoculation or of *L. multifida* vicinity (CL treatment), on *C. arizonica* height was recorded along the 16 weeks growth compared to the control (CA treatment) (Table 4). However, the positive effect of *L. multifida* (CL treatment) was significantly lower than the one recorded with the CG treatment, except at the end of the experiment where no significant difference was recorded between treatments (Table 4). The number of branches per plant was also significantly enhanced in the CG and CL treatments after 9 weeks culturing without significant differences between treatments (Table 5). In the presence of *C. arizonica* seedling (CL treatment), the height of *L. multifida* became significantly higher than in the control (LM treatment) after 5 weeks culturing and remained higher until the end of the experiment (Table 6).

After 16 weeks growth, shoot and root growths of *C. arizonica* seedlings of the CG treatment were significantly higher than the ones recorded in the control (Table 7). In the CL treatment, this positive effect was only recorded on the root growth (Table 7). In the CG and CL treatments, the extent of root mycorrhizal colonization was not significantly different, but it was significantly higher than the one recorded in the control (CA treatment) (Table 7). For *L. multifida* seedlings, root

Table 4
Time course changes in plant height (expressed in cm) of *C. arizonica* seedlings in the different treatments (*C. arizonica* alone, CA; *C. arizonica* + *G. intraradices*, CG; *C. arizonica* + *L. multifida*, CL) (Experiment 3)

Time (week)	Treatments		
	CA	CG	CL
0	10.2 a[a]	18.4 b	12.5 a
3	11.5 a	20.1 c	16.3 b
5	11.8 a	21.1 c	17.9 b
7	13.8 a	22.5 c	19.6 b
9	16.3 a	24.7 c	21.4 b
11	19.1 a	26.7 c	23.5 b
12	22.0 a	29.0 c	25.5 b
14	24.4 a	31.3 c	27.8 b
16	26.9 a	34.5 b	32.5 b

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

Table 6
Time course changes in plant height (expressed in cm) of *L. multifida* seedlings in the different treatments (*L. multifida* alone, LM; *C. arizonica* + *L. multifida*, CL)

Time (week)	Treatments	
	LM	CL
0	1.4 a[a]	1.6 a
3	3.6 a	6.1 a
5	3.6 a	13.6 b
7	19.5 a	27.6 b
9	22.5 a	47.7 b
11	33.8 a	55.2 b
13	41.1 a	59.2 b
14	47.5 a	64.4 b
16	56.1 a	67.8 b

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

Table 7
Growth and mycorrhizal colonization of *C. arizonica* seedlings in the different treatments after 4 months culturing in a non-disinfected soil

Treatments	Shoot biomass (mg dry weight)	Root biomass (mg dry weight)	Mycorrhizal colonization (%)
CA[a]	3393 a[b]	735 a	4 a
CG	6909 b	1650 b	70 b
CL	3456 a	1499 b	92 b

[a] For legend, see Table 4.
[b] Data in the same column followed by the same letter (a and b) are not significantly different according to the one-way analysis of variance ($p < 0.05$).

biomass and mycorrhizal colonization were significantly higher in the CL treatment than those in the LM treatment (Table 8).

The permutation test of the COIA between SIR responses, *C. arizonica* shoot and root growth and mycorrhizal colonization was highly significant ($p < 0.001$), which means that there were strong relationships between these variables. The results of COIA (Fig. 1) showed that the increase of shoot biomass of *C. arizonica* (SB) was positively linked to the inoculation of *G. intraradices*, but not to the presence of Lavender seedlings. Root biomass and mycorrhization colonization level were positively linked to both *G. intraradices* inoculation and Lavender presence (Fig. 1B and D). On the SIR responses figures (Fig. 1A and C), the substrates corresponding to high root biomass and mycorrhization rate were tartric and oxalic acids, as opposed to glucose, glutamic and malonic acids that corresponded to low root biomass and mycorrhization rate. Shoot biomass increase was positively linked to gluconic, citric and fumaric acids, and negatively linked to keto acids. Positive effect of Lavender seedlings was therefore visible only on root biomass. Differences between SIR responses of soil samples from different treatments (Table 9) confirmed the conclusions of the co-inertia analysis.

Table 8
Growth and mycorrhizal colonization of *L. multifida* seedlings in the different treatments after 4 months culturing in a non-disinfected soil

Treatments	Shoot biomass (mg dry weight)	Root biomass (mg dry weight)	Mycorrhizal colonization (%)
LM[a]	10.2 a[b]	1.1 a	10 a
CL	10.3 a	2.1 b	50 b

[a] For legend, see Table 6.
[b] Data in the same column followed by the same letter (a and b) are not significantly different according to the one-way analysis of variance ($p < 0.05$).

Table 9
SIR responses of soil samples from the different treatments expressed in $\mu g\ CO_2\ g^{-1}$ soil h^{-1} after 4 months culture in a non-disinfected soil

Substrates	Treatments			
	CA[a]	CG	CL	LM
Glucose	10.8 c[b]	13.4 d	4.9 a	7.4 b
Glutamic acid	2.7 bc	2.4 b	1.5 a	3.8 c
Ketobutyric acid	14.5 b	9.9 a	27.3 c	16.8 b
Citric acid	23.9 b	37.2 c	20.9 ab	16.8 a
Fumaric acid	22.8 b	31.3 c	18.3 a	20.6 ab
Oxalic acid	11.9 a	19.4 d	17.7 c	13.4 b
Gluconic acid	3.9 a	19.7 b	3.8 a	4.8 a
Tartric acid	1.3 a	3.7 ab	12.6 c	6.2 b
Malic acid	12.9 a	11.3 a	21.1 b	22.8 b
Malonic acid	37.6 b	45.8 c	16.9 a	17.5 a
Succinic acid	22.3 ab	25.5 b	20.3 a	19.3 a

[a] For legend, see Tables 4 and 6.
[b] Data in the same line followed by the same letter (a–d) are not significantly different according to the one-way analysis of variance ($p < 0.05$).

4. Discussion

It is well established that mycorrhizal symbiosis is a key component of revegetation processes in degraded soils. In Mediterranean ecosystems, the use of drought-tolerant, native plant species has been proposed to accelerate the natural successions and to re-establish functional shrublands (Francis and Thornes, 1990; Herrera et al., 1993). In addition, it has been advised to describe the mycorrhizal status of these native plant species before initiating revegetation programmes (Jasper, 1994).

Lavander plants are representative plant species in Mediterranean shrublands and belong to the natural succession in some semiarid Mediterranean ecosystems (Barea et al., 1992). In addition, they have been classified as ‘‘obligatory mycorrhizal’’ (Brundrett, 1991) or as ‘‘highly dependent on mycorrhiza’’ (Habte and Manjunath, 1991). More recent studies have confirmed the high mycorrhizal dependency of these plant species (Azcon and Barea, 1997). In the present study, mycorrhizal dependencies of three Lavender species tested were not so high as those indicated in previous studies. For instance, mycorrhizal dependencies of *Lavandula spica* ranged from 80 to 92.7% in four different soils (Azcon and Barea, 1997). However, that experiment has been performed with other soils, other AM fungus (*G. mosseae*) which was inoculated at a different rate (10 g per pot). As in this study the amount of mycorrhizal inoculum was 10 times lower, it could be assumed by the magnitude of *L. multifida* and *L. dentata* mycorrhizal dependencies that they are representatives of highly

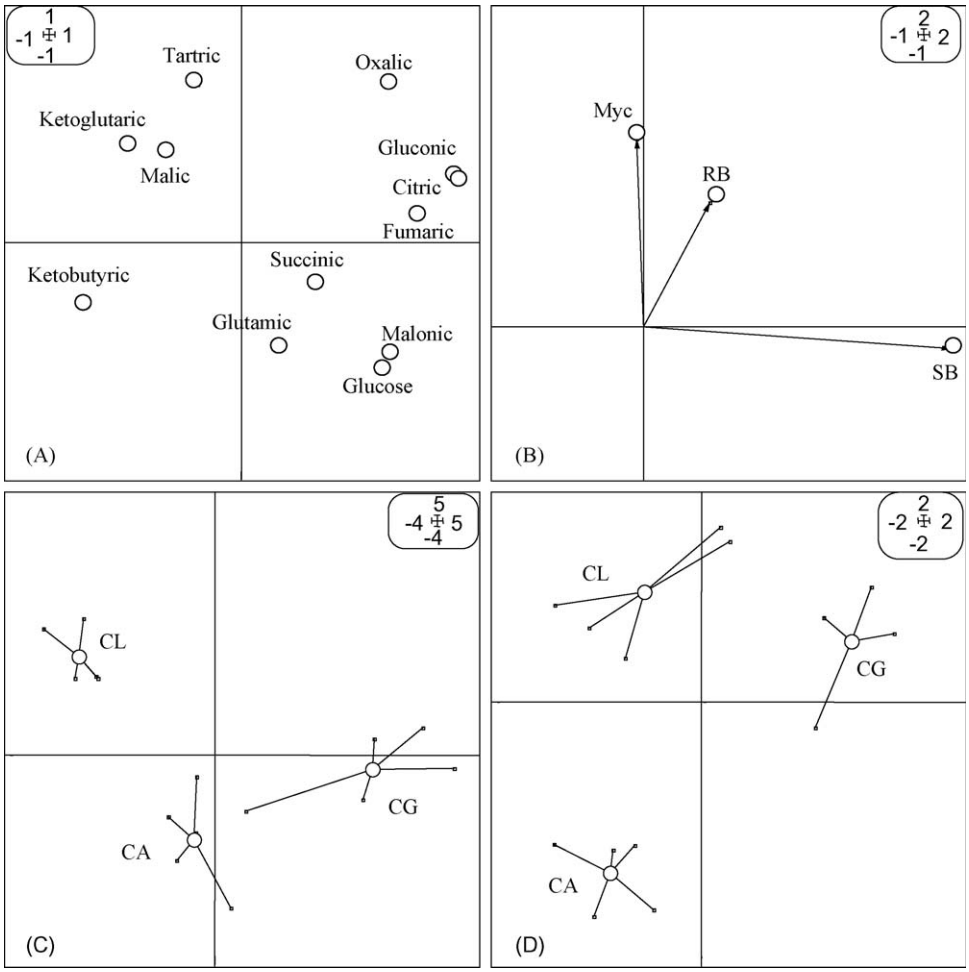

Fig. 1. Co-inertia analysis of the SIR responses of the soil planted with *C. arizonica* (mycorrhized or not with *G. intraradices*), *C. arizonica* and *L. multifida*, plant growth and mycorrhizal colonization. (A) Factor map of SIR responses; (B) factor map of plant growth and mycorrhizal colonization (Myc: Mycorrhizal colonization, RB: Root biomass; SB: Shoot Biomass); (C) factor map of SIR responses soil samples (CA: *C. arizonica* alone; CG: *C. arizonica* inoculated with the AM fungus *G. intraradices*, CL: *C. arizonica* + *L. multifida*); (D) factor map of plant growth and mycorrhizal colonization (for legend, see part C).

mycotrophic plant species. Some studies report AM colonization of *Cupressus* sp. (Michelsen et al., 1993) but to our knowledge, no references were available for the responses of *Cupressus* species to the AM inoculation. Mycorrhizal dependencies of *C. arizonica* and *C. sempervirens* were comparable to those recorded with *L. multifida* and *L. dentata* and, consequently, these *Cupressus* species should also be listed as ''highly dependent on mycorrhiza'' (Habte and Manjunath, 1991).

Lavender plants are very mycotrophic and they also enrich their cultural soils in AM fungal propagules. This positive contribution is linked with the total soil P contents. Arid and semiarid ecosystems are generally characterized by a patchy distribution of individual plant species (Halvorson et al., 1994). The vegetation patches commonly create ''fertility islands'' (Garner and Steinberger, 1989), or ''resource islands'' (Schlesinger et al., 1996). As Lavender plants have a patchy distribution in *Cupressus* stands in Haut Atlas Mountains and as *L. multifida* can stimulate root growth and mycorrhizal root colonization of *C. arizonica* seedlings, this *Lavandula* species could act as a ''nurse plant'' (Carillo-Garcia et al., 1999) by (i) enhancing the mycorrhizal soil infectivity, even more if the soil is P deficient and (ii) stimulating the mycorrhizal colonization of *C. arizonica*.

In addition, although the ''plant nurse'' effect was not significantly different than that of AM inoculation on mycorrhizal colonization and on the shoot growth, strong modifications have been recorded in the patterns of ISCP of microbial communities in both treatments.

Mycorrhizal symbiosis modifies root functions and microbial equilibrium in the rhizosphere (Rambelli, 1973; Leyval and Berthelin, 1993). This zone influenced by both the roots and the mycorrhizal fungus has been named ''mycorrhizosphere'' by Linderman (1988) and included the more specific term ''hyphosphere'' which referred only to the zone surrounding individual fungal hyphae (Johansson et al., 2004). AM fungi can exude substances that have selective effects on the microbial community in the rhizosphere and in the soil (Hobbie, 1992; Söderström, 1992). For instance, mycorrhizal fungi can solubilize surrounding weatherable minerals through excretion of organic acids (Landeweert et al., 2001). Furthermore, these fungal exudations could favour or alter some hyphosphere microorganisms. In this study, although the mycorrhizal colonization was not significantly different between the CG and CL treatments, it appears that the functionalities of microbial communities to catabolize organic acids are different and it suggests that the plants and their fungal symbionts select different microbial communities. In addition, the exudates between *Cupressus* and Lavender mycorhizosphere should be probably different.

AM fungi have usually been considered to be generalists with the plant hosts they colonize, to be functionally equivalent in their effects on a host and on the soil microflora (Hart et al., 2003). However, it has been recently demonstrated that AM fungal growth and species composition is host specific when different plant species and fungi are grown together (Van der Hejden et al., 1998). In addition, the growth of the host plant was also largely dependent on the AM fungal diversity in the cultural soil (Van der Hejden et al., 1998). Our results are partially in accordance with these conclusions as root biomass of *L. multifida* and *C. arizonica*, when they were planted in the same pot, was higher than that measured when each plant species was singly cultured. The same effect was recorded with the mycorrhizal colonization of the plants. Moreover, it has been also demonstrated that the functions of AM fungi with regard to the host they infect were also modified when different plant species and fungi are grown together. From our results, this conclusion could be broadened to the ''mycorhizosphere'' effect of AM fungal communities.

These results emphasize the role of ''resource islands'' and ''nurse plants'' of Lavender plants in the regeneration processes of tree species such as *Cupressus* spp. In addition, they confirm the role of AM fungi as a major factor contributing to the growth and co-existence of each of the plant species (*L. multifida* and *C. arizonica*). The beneficial effect of the association of these plants is reciproqual since *C. arizonica* also stimulate the height of *L. multifida*. The impact of such associations between a tree and a shrub on mycorrhizal fungal diversity and, consequently, on ecosystem functions such as plant biodiversity, productivity and variability remains unclear and has to be studied.

Native AM fungi seemed to have a better capacity than *G. intraradices* to colonized roots. But it is noticeable that inoculation with *G. intraradices* is the only treatment that gave a significant growth increase. Thus, from a practical point of view, the impact of Lavender plants on the growth of young seedlings of *Cupressus* has to be taken in account together with inoculation of the seedlings in nurseries with selected AM fungal strain in order to ensure the success of revegetation programmes in degraded areas.

Acknowledgement

This study is supported by the PAI (Program me d'Action Incitative) ''Volubilis'', Ministère des Affaires Etrangères, France.

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