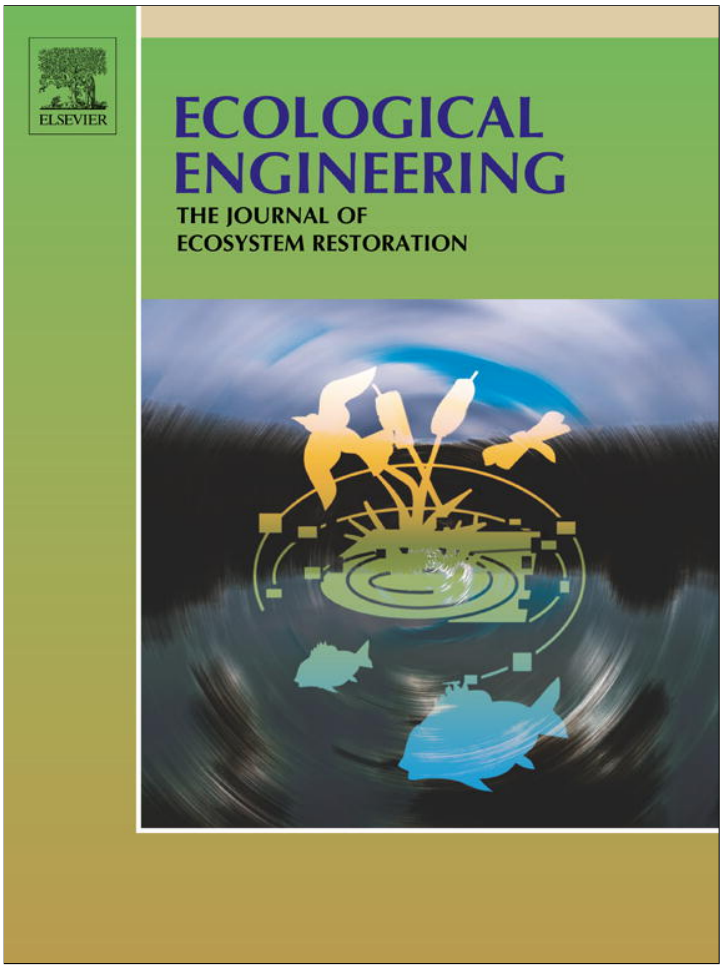

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Research paper

Managing Mediterranean nurse plants-mediated effects on soil microbial functions to improve rock phosphate solubilization processes and early growth of *Cupressus atlantica* G

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ABSTRACT

The main objective was to evaluate the impact of nurse plant species commonly found in Mediterranean areas (*Lavandula dentata* and *Thymus satureoides*) on microbial soil functions, on the native inoculum potential of AM fungi involved in the rock phosphate weathering and to measure the potential benefits to the growth of Atlas Cypress (*Cupressus atlantica* G.), an endemic Cupressacea of Morocco. Soils collected from an old *C. atlantica* forest and pre-cultivated with each of the target plant species (*L. dentata* and *T. satureoides*). After 5 months of cultivation, they were uprooted and the treated substrate was amended or not with Khouribga Rock Phosphate (KRP). Then pots were filled with the soil mixtures and planted with one pre-germinated seed of *C. atlantica*. The results show that pre-cultivation step with native mycotrophic plant species improves the mycorrhizal soil infectivity, modifies soil microbial functionalities and increases the impact of rock phosphate amendment on the *C. atlantica* growth. This low cost cultivation practice by improving forest plant development and cultural soil quality constitutes a promising ecological engineering tool to improve the performances of ecosystem restoration.

1. Introduction

In Mediterranean areas, deforestation has been a common practice for more than 2000 years leading to the loss of most primeval forests (Bauer, 1991; Blondel and Aronson, 1999). It has been estimated that only 9–10% of the Mediterranean area is currently forested (Marchand, 1990). These man-mediated degradative activities (overgrazing, non-regulated cultivation techniques, deforestation, etc.) associated with irregularity of rainfall distribution, long dry and hot summer have accelerated the land desertification processes. The consequences are a lost in biodiversity and a decrease of productive capacity of forest and agricultural lands. Following the degradation of forest mature and agricultural land abandon, the surface covered by shrublands has significantly increased (Grove and Rackham, 2001) leading to a failure of the ecological succession process (Pickett et al., 2001) and, consequently, to limitations in restoring the structure and complexity of original mature community (Blondel and Aronson, 1999). Hence human intervention is necessary to assist secondary succession at the shrubland stage and to facilitate recovery of woodlands. In this context, attempt to replant tree species is a common practice to accelerate and recover forest ecosystems but reforestation performances remain very low (Garcia-Salmeron, 1995), and shrub communities are generally discarded since they are considered as heavy competitors to the newly planted trees (Savill et al., 1997). Yet studies during the nineties, it has been reported that certain shrubs act positively on the survival and growth of other neighboring plant species by creating a better environmental habitat with low stresses from high radiation and temperature as well as from soil nutrient and moisture deficiencies (Callaway and Walker,

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1997) referred to as "the nurse-plant syndrome" (Niering et al., 1963). This ecological facilitation between plant species provides the patchy distribution of the vegetation commonly observed in Mediterranean basin, especially in degraded ecosystems (Callaway and Pugnaire, 1999). Hence it has been suggested that the use of nurse plants as planting microhabitats in Mediterranean degraded ecosystems could promote the survival and development of native tree species and constitute an alternative reforestation technique compared to the standard practices (Gomez-Aparicio et al., 2004; Ouahmane et al., 2006b; Padilla and Pugnaire, 2006; Duponnois et al., 2011).

The "fertility islands" (Garner and Steinberger, 1989) or "resource islands" (Schlesinger et al., 1996) resulting from the establishment of these nurse plants show a higher arbuscular mycorrhizal (AM) soil infectivity compared to the adjacent soil away from plant influence (Ouahmane et al., 2006a; Duponnois et al., 2011), which can improve plant growth and survival in arid conditions, by increasing the supply of nutrient to the plants (especially for soil P uptake) (Ouahmane et al., 2007), enhancing soil aggregation in eroded soils (Caravaca et al., 2002) and reducing water stress (Augé, 2001).

Phosphorus uptake is one of the major constraints to plant growth due to its insolubility and high sorption capacity in soil resulting to low contents in P readily available for plant development (Marschner, 1995). Nevertheless, P biodisponibility can be improved through plant exudation and soil microbial activities by gaining access to previously unavailable inorganic and organic soil P reserves (Jones, 1998; Cardoso and Kuyper, 2006). More particularly, phosphate solubilization activity of AM fungi was shown to highly contribute to plant P nutrition from inorganic phosphates (Caravaca et al., 2004; Duponnois et al., 2005).

To reduce P deficiencies and ensure plant productivity, the use of rock phosphate as an alternative source of P fertilizer has been largely studied and more particularly in Morocco, the third-largest producer of rock phosphate (behind the United States and China). Unfortunately rock phosphate solubilization rarely occurs in non-acidic soils frequently recorded in Mediterranean areas (Caravaca et al., 2004). In order to improve this solubilization process in such environmental conditions, the use of appropriate mycorrhizal technologies could reinforce or replace the native inoculum potential of AM fungi in the soil (Caravaca et al., 2003).

Although it has been shown that rhizosphere soils of some Mediterranean shrub species are highly colonized by AM fungi (Requena et al., 2001; Azcon-Aguilar et al., 2003; Ouahmane et al., 2006b), their influence on the rock phosphate solubilization has been rarely assessed. Hence, the main objective of this study was to determine the impact of shrub species commonly found in Mediterranean areas (*Lavandula dentata* and *Thymus satureoides*) on microbial soil functions and on the native inoculum potential of AM fungi involved in the rock phosphate weathering and to measure the potential benefits to the growth of Atlas Cypress (*Cupressus atlantica* G.), an endemic Cupressacea of Morocco.

2. Materials and methods

2.1. Soil sampling

The experimental site (1 ha) was located in the N'Fis valley (Haut Atlas, Morocco) at the Idni station (8°17′02″ W, 31°54′34″, 1700 m above sea level). At the research site, the vegetation is an old *C. atlantica* forest with mainly old *C. atlantica* trees. A sparse and degraded understorey plant cover develops under Cypress canopy included various shrub species and more particularly of *L. dentata* and *T. satureioides*. Soil samples were randomly collected from bare soil sites, from the 10 to 20 cm layer, away from plant influence (at least 2 m from any established *L. dentata* and *T. satureoides* and at least 20 m from any established *C. atlantica* tree). Before use, soil samples were pooled together and the mixture was crushed and passed through a 2 mm sieve. Its chemical characteristics were as follows: pH (H_2O) 7.6; total carbon (%) 1.60; total nitrogen (%) 0.1; soluble P (mg kg^{-1}) 19.8.

2.2. Plant cultivation conditions

2.2.1. Soil preparation by shrub cultivation

Seeds of *L. dentata* and *T. satureoides* (provenance Idni, Morocco) were germinated on moistened autoclaved sand (140 °C, 40 min). Pots (0.5 l) filled with the bare soil mixture were individually planted with eight-day old seedlings (or remained unplanted for the control treatment). The plants were arranged in a randomized, complete block design with 20 replicates per treatment. They were screened from the rain and grown under natural light (day length approximately 10 h, mean temperatures 22 °C) and watered daily with tap water during 5 months of growth.

2.2.2. Rock phosphate amendment and C. atlantica cultivation

After 5 months of culturing, *L. dentata* and *T. satureoides* seedlings were uprooted and the soils collected from each treatment were carefully mixed and passed through a 2 mm sieve to remove any root fragments. Then each soil origin was divided into two equal volumes and one was mixed with Khouribga Rock Phosphate (KRP) (0.1%, w/v; insoluble rock phosphate powder) whereas the other part remained unamended. Khouribga Rock Phosphate (Morocco) was ground with pestle and mortar and passed through a 90 μm sieve. Its chemical characteristics were as follows (%): SiO_2 3.1; Al_2O_3 0.5; Fe_2O_3 0.27; P_2O_5 33.4; MgO 0.5; CaO 54; K_2O 0.06; Na_2O 0.76; CaP 2.64; CO_2 2.1 (Hafidi, 1996). Then the pots (0.5 l) were filled with the soil mixtures and planted with one pre-germinated seed of *C. atlantica*. Before planting, seeds of *C. atlantica* (Provenance Idni, Morocco) were surface disinfected with 30% hydrogen peroxide for 5 min. They were rinsed for 24 h and at 4 °C in sterile distilled water and transferred aseptically to Petri dishes filled with 1% (w/v) agar/water medium. After 8 days of incubation at 20 °C in the dark, the germinating seeds were used when rootlets were 1–2 cm long.

Pots were arranged in a randomized complete block design with 10 replicates per treatment (soil origin and KRP amendment). They were placed in a glasshouse under natural conditions (approximately 10 h of daylight, daily average temperature 20 °C) and were daily watered with tap water during 12 months of growth.

2.3. Assessment of C. atlantica growth and mycorrhizal status

After 12 months of culturing, the height and the stem diameter of *C. atlantica* seedlings were measured. Then the seedlings were uprooted and their root systems gently washed. For each plant, the entire root system was gently washed, cleared and stained according to the method of Phillips and Hayman (1970). Roots were cut into 1-cm pieces, mixed and placed on slides for microscopic observations at 250× magnification (Brundrett et al., 1985). About 100 root pieces were observed per plant. The extent of AM colonization was expressed as a percentage mycorrhizal root pieces. Then the dry weights of shoots and roots were measured (60 °C, 1 week). After drying, sub-samples of ground shoot and root tissues were ashed (500 °C), digested in 2 ml HCl 6 M and 10 ml HNO_3 1 M and analyzed by colorimetry for P (John, 1970). Then soil sampled (100 g) collected from each pot was kept at 4 °C for further measurements.

2.4. Soil chemical and biological analysis in C. atlantica growth experiments

2.4.1. Soil analysis

All soils sampled from each pot were analyzed by measuring pH, total soil organic C after dichromate oxidation (Aubert, 1978), total organic N by the Kjeldahl method and soluble P by the Olsen method (Olsen et al., 1954).

2.4.2. Mycorrhizal soil potential assessment

AM hyphal length was measured on membrane filters according to Jakobsen and Rosendahl (1990). The mycorrhizal soil infectivity (MSI) was determined for each soil treatment. A bioassay based on a dose (quantity of a non disinfected soil) – response (mycorrhizal status of test plants) was used according to the biological assay principle (Plenchette et al., 1989). The method is based on the cultivation of a population of mycotrophic plantlets on a range of concentrations of natural soil diluted with the same disinfected soil. Six dilutions of each soil samples were realized by mixing the original soil in various quantities (100, 48, 24, 12, 6 and 3%, w:w) with the same autoclaved soil (140 °C, 40 min) to give a range of concentrations. There were 5 replicates per dilution. Seeds of millet (*Pennisetum typhoides* L.) were pre-germinated for two days in Petri dishes on humid filter paper. Ten germinated seeds were transplanted into plastic pots (5.5 cm diameter; 6 cm high) filled with 100 g of each dilution. Pots were placed in a glasshouse (30 °C day, 20 °C night, 10-h photoperiod) and watered daily with tap water. After 2 weeks of culturing, the entire root system of each seedling was collected, gently washed under tap water, clarified in 10% KOH for 30 min at 90 °C and stained for 15 min with acid fuchsin (0.05% in lactoglycerol). Each root system was mounted on a microscope slide and observed at a 250× magnification to detect the presence of mycorrhizal structures. A single AM hyphal entry was considered as a record of mycorrhizal infection to give an all or nothing quantitative response. The infected plants were counted and the results were expressed as percentages of mycorrhizal plants per pot.

For each soil treatment, the percentage of mycorrhizal plants was plotted against the logarithm of undisinfected soil concentration. Regression curves (model $Y = BX + A$) were calculated for each soil treatment and variance analysis was performed to test the non-equality of their slope. The mycorrhizal soil infectivity (MSI) unit was calculated using a regression line equation (Duvert et al., 1990) and defined as the minimum dry weight (g) of soil required to infect 50% (MSI_{50}) of a plant population under the bioassay conditions and calculated for $Y = 50\%$.

2.4.3. Measurement of microbial functional diversity

Microbial functional diversity in the soils from each treatment was examined by measuring the patterns of *in situ* catabolic potential (ISCP) of microbial communities as described in Ouahmane et al. (2009). This method is based on the measurement of short-term respiration responses of soils mixed with a range of simple organic compounds (amino-acids, carbohydrates, organic acids and amides) (Degens and Harris, 1997). Thirty two substrates were used for the analysis, (i) ten amino acids (L-arginine, L-serine, L-glutamic acid, L-phenylalanine, L-asparagine, L-lysine, L-cysteine, L-tyrosine, L-glutamine and L-histidine), (ii) three amides (D-glucosamine, *N*-methyl-D-glucamine and L-succinamide), (iv) three carbohydrates (D-mannose, D-sucrose, and D-glucose), and (v) 16 carboxylic acids (α-ketoglutaric acid, α-ketobutyric acid, fumaric acid, oxalic acid, tartaric acid, gluconic acid, ascorbic acid, DL-malic acid, malonic acid, quinic acid, DL-α-hydroxybutyric acid, formic acid, gallic acid, succinic acid, trisodium citric acid, and uric acid). The amides and amino acids were added at 10 mM, whereas the carbohydrates were added at 75 mM and the carboxylic acids at 100 mM (Degens and Vojvodic-Vukovic, 1999). Catabolic evenness (a measure of relative variability in the catabolic functions) microbial biomass C (MBC), substrate induced respiration (SIR) and the metabolic quotient (qCO_2) were calculated as described in Ouahmane et al. (2009).

2.5. Statistical analysis

Data were treated with one-way analysis of variance. Means were compared using the Newman–Keuls test ($P < 0.05$). The percentages of mycorrhization were transformed by arcsin(sqrt) before statistical analysis. Between-Group Analysis (BGA, Culhane et al., 2002) was used to analyze the relationships between SIR responses and the three soil origins: *L. dentata* (Lde), *T. satureioides* (Tsa) and bare-soil (BS) amended or not with Khouribga Rock Phosphate (KRP). After BGA analysis, the Monte-Carlo test (multivariate permutation test) was used to check the significance of the differences between groups. Computations and graphical displays were realized with the free ade4 package for R (http://pbil.univ-lyon1.fr/ade4/) (R Development Core Team, 2007).

3. Results

3.1. Plant growth

After 12 months of cultivation, soil treatments with *L. dentata* and *T. satureoides* significantly improved the growth of *C. atlantica* seedlings (height, shoot and root biomass, shoot and root P contents) compared to the control (unplanted soil treatment) (Table 1). KRP amendment effect on plant growth was dependent to the soil treatments (Table 1). For the control treatment, a depressive effect has been recorded for the shoot and the total biomass whereas shoot and root P contents were increased after KRP amendment (Table 1). In contrast, for the *L. dentata* soil treatment, KRP amendment has increased all the growth parameters excepted for the stem diameter, shoot and total biomass (Table 1). The same positive effects have been recorded in the *T. satureoides* treatment excepted for the seedling height (Table 1).

3.2. Chemical, mycorrhizal and microbial functionalities of soil treatments

No significant differences have been found for the pH, total carbon and total nitrogen within all the treatments (Table 2). Highest soluble P contents were recorded in the control with or without KRP amendment whereas the lowest were found in the *L. dentata* and *T. satureoides* treatments without KRP addition (Table 2). For these two treatments, KRP amendment enhanced soil soluble P contents (Table 2).

AM colonization of *C. atlantica* seedlings was significantly higher in all the nurse plant soil treatments with or without KRP addition than in the control and the highest value was found in the *T. satureoides* treatment with KRP (Table 3). The hyphal network was more important in the soils collected from *L. dentata* and *T. satureoides* treatments compared to the control (Table 3). KRP addition has significantly improved the extrametrical mycelium extend in both target plant treatments (Table 3). The MSI_{50} was significantly lower in the *L. dentata* and *T. satureoides* treatments than those measured in the control soil amended or not with KRP (Table 3). The positive effect of pre-cultivation with both target plant species on the mycorrhizal propagule abundance was significantly reinforced with KRP amendment (Table 3).

Table 1
Growth of *C. atlantica* seedlings in the *L. dentata*, *T. satureioides* and unplanted soil treatments, amended or not with Khouribga Rock Phosphate (KRP) after 12 months of plantation in glasshouse conditions.

	Soil treatments					
	Control		*L. dentata*		*T. satureioides*	
	−KRP	+KRP	−KRP	+KRP	−KRP	+KRP
Height (cm)	11.7 (0.33)[1] a[2]	13.0 (0.6) a	25.7 (0.7) b	29.7 (0.9) c	28.2 (1.2) b	29.7 (1.2) b
Stem diameter (mm)	4.0 (0.0) a	4.0 (0.0) a	5.3 (0.33) a	5.3 (0.33) a	5.0 (0.0) a	6.0 (0.0) b
Shoot biomass (g dry weight)	1.5 (0.05) b	1.2 (0.08) a	2.6 (0.07) c	2.7 (0.05) c	2.1 (0.08) c	3.3 (0.14) d
Root biomass (g dry weight)	0.84 (0.08) a	0.76 (0.04) a	1.13 (0.02) b	1.27 (0.02) c	1.16 (0.05) b	1.70 (0.07) d
Total biomass (g dry weight)	2.38 (0.08) b	1.96 (0.12) a	3.74 (0.07) c	3.99 (0.07) c	3.25 (0.13) c	5.04 (0.21) d
Shoot P content (mg per plant)	0.33 (0.02) a	0.43 (0.02) b	0.74 (0.02) c	1.0 (0.02) d	0.56 (0.02) b	0.97 (0.04) d
Root P content (mg per plant)	0.03 (0.003) a	0.11 (0.006) c	0.06 (0.001) b	0.35 (0.004) d	0.08 (0.003) b	0.10 (0.004) c
Total P content (mg per plant)	0.36 (0.03) a	0.54 (0.029) b	0.79 (0.02) d	1.35 (0.02) f	0.64 (0.02) c	1.09 (0.04) e

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

Table 2
Soil chemical characteristics of the *L. dentata*, *T. satureioides* and unplanted soil treatments, amended or not with Khouribga Rock Phosphate (KRP) after 12 months of *C. atlantica* plantation in glasshouse conditions.

	Soil treatments					
	Control		*L. dentata*		*T. satureioides*	
	−KRP	+KRP	−KRP	+KRP	−KRP	+KRP
pH (H_2O)	7.6 (0.28)[1] a[2]	7.2 (0.23) a	7.0 (0.17) a	7.1 (0.19) a	7.5 (0.11) a	7.2 (0.23) a
Total carbon (%)	1.60 (0.21) a	1.61 (0.09) a	1.60 (0.06) a	1.64 (0.14) a	1.67 (0.12) a	1.65 (0.15) a
Total nitrogen (%)	0.10 (0.01) a	0.11 (0.02) a	0.13 (0.03) a	0.12 (0.04) a	0.09 (0.02) a	0.11 (0.03) a
Soluble P (mg kg^{-1})	19.8 (0.62) c	20.1 (0.58) c	7.9 (0.87) a	12.3 (0.51) b	9.8 (0.47) a	12.8 (0.58) b

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

The analysis of the table of SIR results showed the strong influence of both pre-cultivation treatments with *L. dentata* and *T. satureoides* and of the KRP amendment on the SIR profiles (Fig. 1). The control samples exhibited an intermediate position between both pre-cultivation treatments. The pre-cultivation plant effect was highly significant ($P < 0.001$). The KRP effect (after removing the target plant effect) was also significant ($P < 0.01$) showing that the bacterial functional diversity was strongly affected by both treatments (plant species and KRP amendment). However, the effect of KRP amendment was inverted in the control treatments and in the pre-cultivation treatments. *T. satureoides* increased the use of malonic, tartaric, oxalic, succinic, malic, ketoglutaric and fumaric acids. Conversely, *L. dentata* decreased the use of organic acids and increased the use of succinamide. KRP amendment decreased the use of organic acids in soils sampled from the pre-cultivation treatment with targeted plant species, while it increased the use of these acids in the control soils.

The lowest soil microbial biomass was found in the *T. satureoides* treatment without KRP amendment whereas the highest was recorded in the control with KRP amendment (Table 4). The specific respiration rate of the soil microbial biomass was significantly higher in the *T. satureoides* treatment with or without KRP amendment compared to the other treatments (Table 4). The soil catabolic evenness was significantly higher in the *L. dentata* treatment with or without KRP addition than in the other treatments whereas the lowest was recorded for the *T. satureoides* treatment without KRP amendment (Table 4). The highest average respiration (SIRs) to amino-acids and carboxylic acids was recorded in the soils collected from the control treatment amended or not with KRP (Table 4). The highest SIR responses with amides were found with *L. dentata* and *T. satureoides* soil treatments amended or not with KRP (Table 4). The lowest SIR response with carbohydrates was recorded in the *T. satureoides* treatment without KRP and the highest in the control treatment with KRP (Table 4).

Table 3
Mycorrhizal soil potentials, hyphal lengths and arbuscuslar mycorrhizal colonization of *C. atlantica* in the *L. dentata*, *T. satureioides* and unplanted soil treatments, amended or not with Khouribga Rock Phosphate (KRP) after 12 months of plantation in glasshouse conditions.

Mycorrhizal parameters	Soil treatments					
	Control		*L. dentata*		*T. satureioides*	
	−KRP	+KRP	−KRP	+KRP	−KRP	+KRP
AM colonization (%)	35 (4.5)[1] a[2]	49 (1.6) b	46 (2.9) b	53 (4.6) b	48.3 (5.2) b	65.8 (6.2) c
Hyphal length (m g^{-1} soil)	1.2 (0.06) a	1.4 (0.02) a	2.4 (0.11) b	3.3 (0.08) c	2.5 (50.09) b	3.5 (0.14) c
Mycorrhizal soil potential						
Y-intercept	0.142 a	0.211 b	0.332 b	0.416 b	0.345 b	0.351 b
Regression coefficient	0.61	0.71	0.75	0.78	0.73	0.81
MSI_{50} units (g per 100 g)	>100 c	72.8 c	32.6 b	11.1 a	29.4 b	12.5 a

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

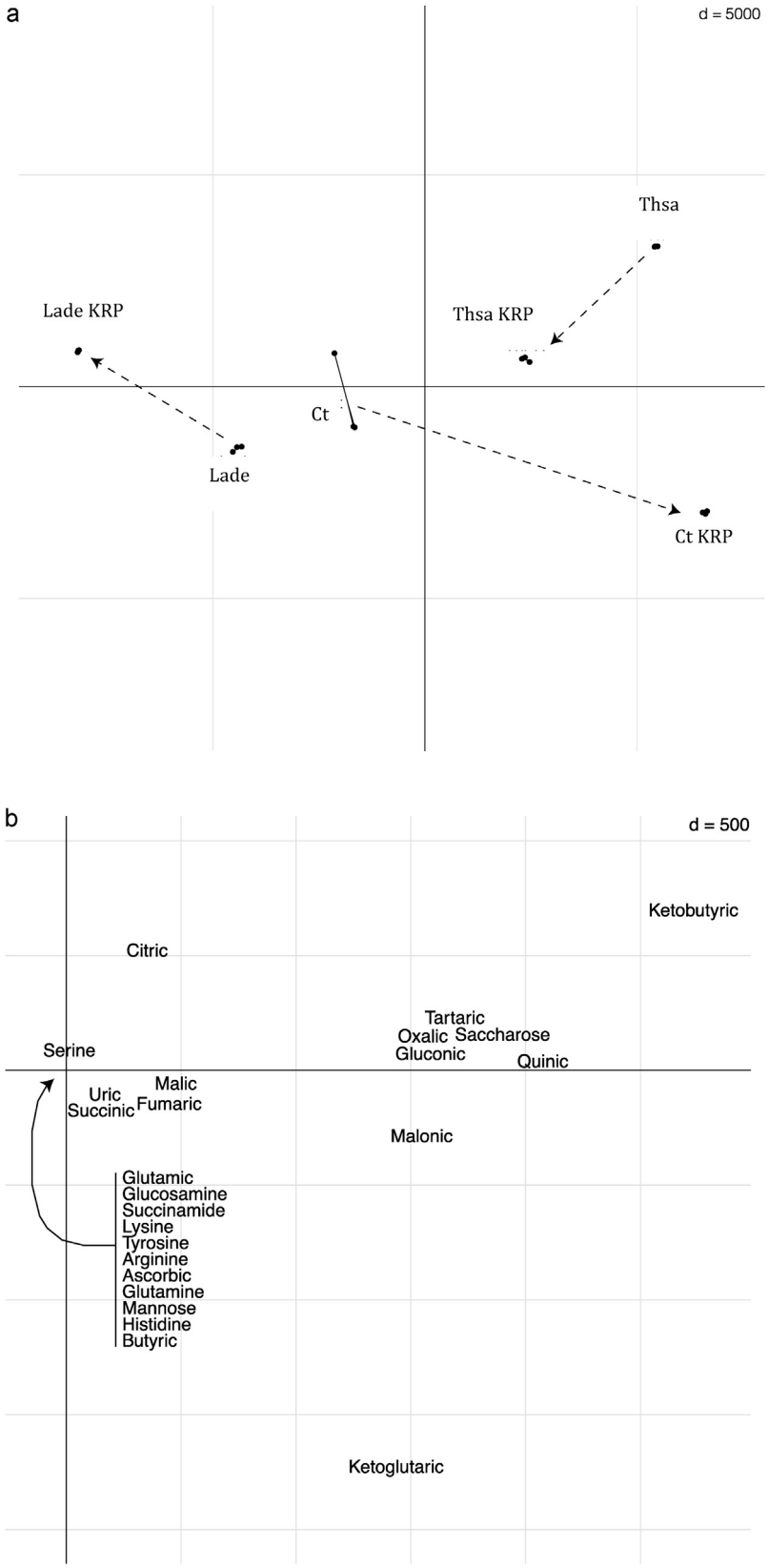

Fig. 1. Principal component analysis of the table of SIR results. The percentages of explained variance for the first two axes are F1 (horizontal axis) = 85%, F2 (vertical axis) = 12%. (A) Factor map of the sample scores (three repetitions for each treatment). The treatment codes are as follows: Lade = *Lavandula dentata*, Thsa = *Thymus satureioides*, C = Control, KRP = Khourigba Rock Phosphate amendment. Each soil sample is represented by a small black dot, and for each treatment, repetitions are linked by a line. The dotted arrows show the effect of KRP amendment in the three cases (Control, Lade, and Thsa). (B) Factor map of the SIR substrates. The substrates located far from the origin are the most heavily used. The ones located near the origin are the least used. Substrates which location on the factor map is in the same direction as a given soil sample are the ones that are the most used in this sample. For example, on the first axis, Malonic acid (on the right) is heavily used in samples with treatments Thsa and Ct + KRP (on the right). Its use is intermediate in Control samples, low in Lade samples, and the lowest in Lade + KRP samples (on the left).

Table 4
Catabolic evenness and average SIR responses (μg CO_2 g^{-1} soil h^{-1}) with each substrate group (carboxylic acids, amino-acids, Amides and carbohydrates) of the soils collected from the *L. dentata*, *T. satureioides* and unplanted soil treatments, amended or not with Khouribga Rock Phosphate (KRP) after 12 months of plantation in glasshouse conditions.

	Soil treatments					
	Control		*L. dentata*		*T. satureioides*	
	−KRP	+KRP	−KRP	+KRP	−KRP	+KRP
Microbial biomass (μg C g^{-1} soil) (MBC)	622 (16.8)[1] b[2]	924 (60.6) c	672 (33.6) b	756 (100.8) bc	286 (44.4) a	538 (16.8) b
qCO_2 (μg-C-CO_2 g^{-1} MBC h^{-1})	0.27 (0.014) a	0.22 (0.015) a	0.27 (0.031) a	0.29 (0.043) a	0.77 (0.010) b	0.43 (0.028) b
Catabolic evenness	11.7 (0.05) b	11.6 (0.04) b	11.9 (0.03) c	12.1 (0.03) d	11.3 (0.02) a	11.5 (0.02) b
Amino-acids	116.7 (4.69) a	202.9 (14.38) b	151.4 (11.16) a	158.6 (15.73) a	122.9 (2.18) a	137.6 (5.49) a
Amides	70.1 (2.88) a	86.67 (4.41) b	130.1 (2.89) c	128.3 (3.33) c	123.3 (3.33) c	125.1 (5.00) c
Carbohydrates	88.3 (4.41) b	183.3 (7.27) c	115.1 (2.89) b	120.1 (10.41) b	55.2 (7.64) a	121.7 (4.41) c
Carboxylic acids	342.6 (2.95) b	486.3 (1.12) e	314.4 (1.23) b	249.1 (0.12) a	457.9 (0.61) d	410.4 (0.89) c

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

4. Discussion

The aim of the study was to setup pre-cultivation step with native mycotrophic plant species, based on the nurse plant concept, to (i) improves the mycorrhizal soil infectivity and a further plant cultivation performance, (ii) modifies soil microbial functionalities and (iii) increases the impact of rock phosphate amendment on the *C. atlantica* growth.

It is well admitted that reforestation practices in Mediterranean ecosystems are crucial due to the increase of desertification processes. However their efficiency is highly dependent of soil biological parameters such as mycorrhizal soil infectivity and soil microbial functionalities. A well-known strategy is the mycorrhizal inoculation of tree seedlings but results are strongly dependent of plant-mycorrhiza associations, climatic conditions and soil characteristics (Hoeksema et al., 2010). An alternative strategy for successful reforestation practices could be the use of the nurse plant syndrome to improve tree seedlings growth and survival by enhancing soil biological quality (Gomez-Aparicio et al., 2004; Ouahmane et al., 2006b; Padilla and Pugnaire, 2006; Duponnois et al., 2011). Nevertheless, soil inorganic P content remains one the major limiting factor for plant productivity, and over time organic P content builds up at the expense of inorganic P (Richardson et al., 2004). In Morocco, efficient rock phosphate solubilization appears as one of the solution to maintain soil inorganic P content and plant productivity, but it rarely occurs in non-acidic soils frequently recorded in Mediterranean areas.

Both target plants examined used in the pre-cultivation step are known to be highly infected by AM fungi (Requena et al., 1997, 2001; Azcon and Barea, 1997; Ouahmane et al., 2006a,b). In the case of *Lavandula* species, they have even been classified as 'obligatory mycorrhizal' (Brundrett, 1991) or as 'highly dependent on mycorrhiza' (Habte and Manjunath, 1991). Pre-cultivations with both plant species led to a higher mycorrhizal soil potential and a larger hyphal network measured at the end of the experiment as well as a higher *C. atlantica* development. In field conditions, it has been showed that *L. dentata* and *T. satureoides* behaved as a source of mycorrhizal inoculum for the surrounding area and create "fertility islands" that will be positively involved in the natural succession process (Ouahmane et al., 2006b; Duponnois et al., 2011; Barea et al., 2011). In particular, this plant nurse effect acts positively on the mycorrhizal soil potential (Requena et al., 2001; Azcon-Aguilar et al., 2003). These benefits resulting from the pre-cultivation with both target plant species have been recorded at the end of the present experiment for the soil mycorrhizal potential and *C. atlantica* growth. In soils AM fungi are found as spores, hyphae or infected root pieces (Duponnois et al., 2001). All these propagules are sources of AM inoculum. Previous studies have attested that the AM mycelial network was the main source of AM inoculum in semi-arid and arid ecosystems (Brundrett and Kendrick, 1991; Bashan et al., 2000). It is also known that a better establishment of the mycelial network allows a more efficient exploitation of soil nutrients (particularly for mineral nutrients with poor mobility or in low concentration in the soil solution such as phosphate and ammonium) and water (Barea et al., 2005), which may explain the better growth and nutrient uptake of *C. atlantica* seedling recorded in the present study. After 12 months of plantation, Cypress seedlings growing in the different soil treatments were probably colonized by different AM communities. It has been previously assessed that AM fungal communities of different composition colonized species of *Lavandula* (*i.e. L. latifolia*) and of *Thymus* (*T. zygis*, *T. mastichina*) (Sanchez-Castro et al., 2008). It has been also reported that the development of mycorrhizal propagules was influenced differently according to the plant species (Azcon-Aguilar et al., 2003). All these data suggest that different shrub species generate and support different AM fungal communities. In the present study and in the soil treatments without KRP addition, P contents of *C. atlantica* seedlings were different according to the target plant used during the pre-cultivation step. Although it is largely admitted that almost all plants can be colonized simultaneously by several species of AM fungi, it has been also demonstrated that some different mycorrhizal ecotypes could be more beneficial for the host-plant than others (Sanders, 2002) and that every fungal symbionts cannot infect every plant species (Barea et al., 2008). This information could also explain the differences in Cypress P contents after 12 months of plantation. It suggests that the AM community associated with Cypress seedlings in the *L. dentata* treatment provides a more efficient exploitation of soil P resources than the AM community in the *T. satureoides* treatment.

Differences in the composition of AM communities could also induce modifications in soil microbial functionalities. Numerous studies have showed that AM symbiosis enhances root exudation (Grayston et al., 1996), alters carbohydrate metabolism of the host-plant (Shachar-Hill et al., 1995), and modifies microbial community equilibrium in the rhizosphere (Johansson et al., 2004). These AM effects lead to a microbial compartment commonly named "mycorrhizosphere" (Linderman, 1988) and included the more specific term "hyphosphere" that only referred to the zone surrounding individual fungal hyphae (Johansson et al., 2004). It can thus be hypothesized that in the current study the functional diversity changes of the soil microbiota (as assessed by the differences recorded between the SIR responses of the unplanted soil (control) and the *L. dentata* and *T. satureoides* treatments, qCO_2 and catabolic evenness) could result from the enhancement of the soil mycorrhizal potential. These different patterns could also result from differences in the composition of AM communities (Marshner

and Baumann, 2003; Marshner and Timonen, 2005) attested in particular by the differences in the SIR profiles recorded from the *L. dentata* and *T. satureoides* soil treatments.

Changes in soil microbial functionalities are also strongly observed by rock phosphate amendment. These changes are probably due to a response from microorganisms producing organic acids, phenolic compounds, protons and siderophores that can induce a biological weathering or biochemical weathering (Drever and Vance, 1994). In addition, plant-secreted compounds are also known to be involved in such processes (Ochs, 1996). In the present study, the changes in the abundance of infective AM propagules and probably, the composition of AM communities were linked to changes in functional characteristics of soil microbial communities potentially involved in rock phosphate weathering. Indeed, SIR responses with organic acids could reflect the amount of organic acids excreted into the soil and able to solubilize the rock phosphate. These organic compounds could also exert a selective influence on soil microbial communities through a multiplication of microorganisms that catabolize organic acids resulting in high value of carboxylic acid SIR responses (Ouahmane et al., 2007). These authors suggested that AM fungi and their associated hyphosphere microflora excreted higher amounts of such organic acids. In the present study, these microorganisms are also present in the control treatment amended with rock phosphate (highest SIR responses with carboxylic acids) but these systems did not induce a better rock phosphate use by the host plant. Hence, we conclude that the enhancement of P mobilization from rock phosphate results in closed interactions between the AM fungal communty, the mycorrhizosphere microbiota and the host plant.

From a practical point of view, this low-cost cultural practice, based on the nurse plant syndrome, and combining of the use of native plant species and natural mineral resources (rock phosphate) could be of great relevance to enhance the performances of afforestation programs in Mediterranean areas. It improves the efficiencies of microbial functions (such as rock phosphate solubilization) and enhances the mycorrhizal soil potential (abundance of infective propagules and extensive hyphal network) resulting to a better growth of forest plant species as *C. atlantica* in nurseries. This cultural strategy aims both improvement of forest plant development and cultural soil quality by a better quality of tree seedlings produced (better growth, high mycorrhizal colonization), and constitute thus a promising ecological engineering tool to improve the performances of ecosystem restoration.

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