Differential modulation of host plant $\delta^{13}C$ and $\delta^{18}O$ by native and nonnative arbuscular mycorrhizal fungi in a semiarid environment

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Summary

• Native, drought-adapted arbuscular mycorrhizal fungi (AMF) often improve host-plant performance to a greater extent than nonnative AMF in dry environments. However, little is known about the physiological basis for this differential plant response.

• Seedlings of *Olea europaea* and *Rhamnus lycioides* were inoculated with either a mixture of eight native *Glomus* species or with the nonnative *Glomus claroideum* before field transplanting in a semiarid area.

• Inoculation with native AMF produced the greatest improvement in nutrient and water status as well as in long-term growth for both *Olea* and *Rhamnus*. Foliar $\delta^{18}O$ measurements indicated that native AMF enhanced stomatal conductance to a greater extent than nonnative AMF in *Olea* and *Rhamnus*. $\delta^{13}C$ data showed that intrinsic water-use efficiency in *Olea* was differentially stimulated by native AMF compared with nonnative AMF.

• Our results suggest that modulation of leaf gas exchange by native, drought-adapted AMF is critical to the long-term performance of host plants in semiarid environments. $\delta^{18}O$ can provide a time-integrated measure of the effect of mycorrhizal infection on host-plant water relations.

Key words: *Rhamnus lycioides*, *Olea europaea*, *Glomus* sp., stable isotopes, water-use efficiency (WUE), stomatal conductance, drought, semiarid environments.

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Introduction

Soil degradation limits the potential for re-establishment of native vegetation in semiarid areas affected by desertification processes (Agnew & Warren, 1996). In particular, desertification reduces the diversity and abundance of key mutualistic microbial symbionts such as arbuscular mycorrhizal fungi (AMF) which enhance the ability of plants to establish in the face of drought and low fertility conditions (Allen, 1989). The introduction of native shrub species in combination with a managed community of microbial symbionts can help guarantee the successful establishment of vegetation in degraded semiarid areas (Requena *et al.*, 2001; Caravaca *et al.*, 2002; Querejeta *et al.*, 2003). A critical step in this integral approach to ecosystem restoration is the selection of an appropriate AMF inoculum capable of maximizing plant survival and growth under drought conditions.

Despite their low host specificity (e.g. Klironomos, 2000), AMF are known to vary widely in their ability to take up phosphorus and stimulate the growth of host plants (Ravnskov & Jakobsen, 1995; Streitwolf-Engel *et al.*, 1997; van der Heijden *et al.*, 1998a,b; Helgason *et al.*, 2002; Klironomos, 2003; van der Heijden, 2004). Requena *et al.* (1996) reported that inoculation with a mixture of indigenous AMF

failed to stimulate the growth of the naturally co-occurring shrub *Anthyllis cytisoides* in AMF screening trials in the glasshouse, whereas inoculation with an exotic AMF (*Glomus intraradices*) significantly enhanced growth. In a subsequent field study, Requena *et al.* (2001) found that inoculation with the exotic AMF *Glomus intraradices* promoted faster *Anthyllis* growth than inoculation with a mixture of native AMF during the first year after seedling transplanting in a degraded semiarid area. However, 4 yr later the shrubs inoculated with the native AMF were much larger than the shrubs inoculated with the exotic AMF. These studies showed that the ability of nonnative AMF to stimulate plant growth in the glasshouse or during the early stages after outplanting may not be a reliable indicator of good long-term performance under semiarid field conditions. However, the nutritional and/or nonnutritional mechanisms behind the differential ability of native and nonnative AMF to promote host plant growth in the long term were not identified by Requena and coworkers.

Arbuscular mycorrhizal fungi have been shown to provide various nonnutritional benefits to the host plant (Newsham *et al.*, 1995; Allen *et al.*, 2003), although these roles are generally considered to be subsidiary roles of the AM symbiosis. In particular, AMF native to semiarid environments take up water and nutrients more efficiently in drying soil, therefore conferring better drought resistance to the host plant (Tobar *et al.*, 1994; Ruiz-Lozano & Azcón, 1995; Ruiz-Lozano *et al.*, 2001; Marulanda *et al.*, 2003; Porcel & Ruiz-Lozano, 2004; Ruiz-Lozano *et al.*, 1995a,b). Different AMF species have been shown to modulate the physiological response of host plants to drought differently, including stomatal conductance, photosynthetic rates or water use efficiency (Allen *et al.*, 1981; Allen & Boosalis, 1983; Dixon *et al.*, 1994; Mathur & Vyas, 1995; Ruiz-Lozano *et al.*, 1995a,b; Shrestha *et al.*, 1995).

The $\delta^{13}C$ values in plant organic matter respond to the interplay among all aspects of plant carbon and water relations and are thereby more useful than single-time gas exchange measurements as integrators of whole plant function throughout the period the plant tissue was synthesized (Dawson *et al.*, 2002). The $\delta^{13}C$ signature of plants is linearly linked to the ratio between the partial pressure of CO_2 in the leaf intercellular spaces and that of the ambient air (Farquhar *et al.*, 1989). $\delta^{13}C$ reflects the relative magnitudes of net photosynthesis (A) and stomatal conductance (g_s) and therefore provides an estimate of mean growing season intrinsic water use efficiency (WUE; A/g_s) in C_3 plants (Farquhar *et al.*, 1989).

The amount of $\delta^{18}O$ in plant tissues is determined by the integrated leaf-to-air vapor pressure gradient during photosynthetic gas exchange (Farquhar *et al.*, 1998). This gradient is influenced by the plant physiological response to changes in environmental conditions such as atmospheric humidity or soil moisture. The simultaneous measurement of $\delta^{13}C$ and $\delta^{18}O$ in plant organic matter can help separate the independent effects of carbon fixation and stomatal conductance on $\delta^{13}C$, as $\delta^{18}O$ shares dependence on stomatal conductance with $\delta^{13}C$ but is not dependent on Rubisco activity (Scheidegger *et al.*, 2000; Keitel *et al.*, 2003). While $\delta^{13}C$ measurements have been successfully used to assess the physiological response of host plants to AM colonization (Di & Allen, 1991; Handley *et al.*, 1999; Querejeta *et al.*, 2003), mycorrhizal effects on plant $\delta^{18}O$ signature yet remain to be addressed.

Most published studies reporting differential effects of various AMF on host plant physiological response to drought were carried out in the glasshouse using agricultural plants species. We designed a field experiment in which seedlings of two native wild shrub species (*Olea europaea* and *Rhamnus lycioides*) belonging to different plant families were inoculated with either native or nonnative *Glomus* species before outplanting in an abandoned agricultural land. Our goal was to evaluate the nutritional and/or nonnutritional basis for any differential growth advantage conferred to the host plants by the drought-adapted native AMF under semiarid conditions. For that purpose, we measured the nutrient status, water content, growth, root AM colonization and shoot $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ of mycorrhizal *Olea* and *Rhamnus* seedlings 1 yr after transplanting in the field.

Materials and Methods

Study site

The experimental area was located in the foothills of the El Picarcho range in the province of Murcia, south-east Spain (1°10′ W, 38°23′ N, 400 m above sea level). The climate is semiarid Mediterranean, with an average annual rainfall of 290 mm, average annual potential evapotranspiration of 827 mm and a mean annual temperature of 15.9°C. The topography of the area is mostly flat, with slopes not exceeding 6%. The plant cover in the experimental area is sparse (< 20% canopy cover), degraded by secular logging, grazing and farming, and dominated by dwarf shrubs (< 1 m high) such as rosemary (*Rosmarinus officinalis*) and alpha grass (*Stipa tenacissima*). The soil is a Petrocalcic Xerosol (FAO, 1998) developed from limestone, with a silt loam texture. Some characteristics of the soil are shown in Table 1.

Plant material and mycorrhizal treatments

The shrub species used in the experiment are key components of the climax plant communities of semiarid south-east Spain. The shrub species were *O. europaea* L. ssp. *sylvestris* (Mill.) Lehr. (Oleaceae) and *R. lycioides* L. (Rhamnaceae). Both are well adapted to water stress conditions and are frequently used in revegetation of disturbed semiarid lands. The actinorhizal species *R. lycioides* forms symbiotic associations with nitrogen fixing microorganisms (*Frankia* spp.), while *O. europaea* is a nonfixer species.

The mycorrhizal inoculum used was either a nonnative isolate of *Glomus claroideum* Schenk & Smith (EEZ 24), or a mixture of native AMF isolated from a nearby semiarid area

Table 1 Some characteristics of the soil in the experimental area

Parameter	
pH (H_2O)	7.6 ± 0.0
Electrical conductivity (1 : 5) (μS cm^{-1})	144 ± 3
Total organic carbon (g kg^{-1})	20.8 ± 0.9
Water soluble carbon (μg g^{-1})	134 ± 6
Total carbohydrates (μg g^{-1})	1956 ± 82
Water-soluble carbohydrates (μg g^{-1})	1 ± 0
Total nitrogen (g kg^{-1})	0.7 ± 0.1
Available P (μg g^{-1})	20 ± 4
Extractable K (μg g^{-1})	177 ± 47
Aggregate stability (%)	19.5 ± 3.0
AM infective propagules (MPN g^{-1} dry soil)	0.24 ± 0.01

MPN, most probable number; AM, arbuscular mycorrhiza.
Each value is the mean of five soil samples (± SE).

where the target plants grow naturally. The mixture of native endophytes included *Glomus geosporum* (Nico. and Gerd.) Walker (EEZ 31), *Glomus albidum* Walker and Rhodes (EEZ 39), *Glomus microaggregatum* Koske, Genma & Olexia (EEZ 40), *Glomus constrictum* Trappe (EEZ 42), *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe (EEZ 43), *Glomus coronatum* Giovannetti (EEZ 44), *G. intraradices* Schenk & Smith (EEZ 45), and an unidentified *Glomus* sp. (EEZ 46). The acronym EEZ refers to the AMF collection of the Estación Experimental Zaidín-CSIC in Granada, Spain.

The AMF inoculum consisted of a mixture of rhizospheric soil from trap cultures (using *Sorghum* sp.), containing spores, hyphae and mycorrhizal root fragments. Once germinated, seedlings were transplanted into the growing substrate, consisting of peat and cocopeat (1 : 1, v : v, Paisajes del Sur, Granada). The corresponding AMF inoculum was applied at a rate of 5% (v : v). The same amount of autoclaved inoculum was added to control plants, supplemented with a filtrate (< 20 μm) of the inoculum to provide the microbial populations accompanying the mycorrhizal fungi. Inoculated and noninoculated seedlings were grown for 8 months under nursery conditions without any fertilizer addition.

Experimental design and layout

The experiment was a two-factor factorial design with five replicate blocks. The first factor was shrub species (two levels). The second factor was mycorrhizal treatment, including three levels: no inoculation (control), inoculation with *G. claroideum* and inoculation with the mixture of eight native *Glomus* species. In January 2000, an area of 1200 m^2 was mechanically prepared with a subsoiler. Three rows per plant species (1 m wide, 3 m apart) were established in each block, and mycorrhizal treatments were randomly assigned to them. In early November 2000, seedlings were planted in individual holes 1 m apart from each other. Fifteen seedlings per mycorrhizal treatment per replicate block were planted for each shrub species.

The field experiment was carried out under strictly natural conditions, without any watering or fertilizer treatments. Total rainfall during the 18 month duration of the field experiment was 466 mm.

Sampling and laboratory procedures

Five plants of each shrub species × mycorrhizal treatment combination were harvested just before field transplanting, and again 12 months and 18 months after outplanting (one seedling per replication block). During seedling harvesting in the field, we tried to minimize damage to roots by carefully excavating a soil rhizosphere volume of 40 × 40 × 40 cm. Shoot fresh weights were measured in the laboratory within 2 h of seedling harvesting in order to estimate shoot water contents. Dry (65°C, 24 h) weights of shoots were measured afterwards. Oven-dried plant tissues were finely ground before chemical analysis. Only new shoots and leaves produced after field transplanting were used for nutrient and isotopic analyses. Foliar concentrations of phosphorus and potassium were determined after digestion in nitric–perchloric acid for 6 h at 210°C. Foliar phosphorus was determined colorimetrically, nitrogen was determined by the Kjeldahl method and potassium was estimated by flame photometry as described in Caravaca *et al.* (2002).

Three subsamples from the upper, middle and lower root system of each seedling were taken, and the percentage of root length colonized by AMF was calculated by the gridline intersect method (Giovannetti & Mosse, 1980) after staining with Trypan blue (Philips & Hayman, 1970).

The $\delta^{13}C$ and $\delta^{15}N$ analyses were conducted at the University of California Davis Stable Isotope Facility, using a continuous flow, isotope ratio mass spectrometer (CF-IRMS; Europa Scientific, Crewe, UK) in the dual-isotope mode, interfaced with a CN sample converter. Concentrations of ^{13}C and ^{15}N are reported using differential notation, showing differences between the observed concentration and that of a common standard. The standard for $\delta^{13}C$ was Pee Dee Belemnite, and for $\delta^{15}N$, atmospheric nitrogen (N_2).

Foliar in $\delta^{18}O$ tissues was determined at the Earth and Planetary Sciences Department, University of New Mexico. Finely ground leaf material was placed in silver capsules and dropped into a high-temperature (1450°C) reduction furnace (MAT TC-EA; Finnigan, Bremen, Germany) in a helium flow. The pyrolysis unit was interfaced with a Finnigan Mat Delta Plus XL mass spectrometer. The $\delta^{18}O$ values are given relative to VSMOW (Vienna standard mean oceanic water).

Statistical analyses

Plant species and mycorrhizal inoculation effects on measured variables were tested by a two-way analysis of variance. Comparisons among means within plant species were made using the least significant difference (LSD) multiple range test.

Statistical procedures were carried out with the software package SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Glasshouse conditions

Before field transplanting, M (mixed native *Glomus*)- and G (*G. claroideum*)-inoculated seedlings showed very similar levels of AM root colonization in *Olea*, whereas M-inoculated *Rhamnus* plants had higher percentage colonization than G-inoculated ones (Table 2).

Both M- and G-inoculation enhanced seedling growth in *Olea* compared with the uninoculated controls (Table 2). The M- and G-inoculated plants had similar size in *Olea*. Inoculation with *G. claroideum* failed to stimulate growth compared with the uninoculated controls in *Rhamnus*. Only the native *Glomus* inoculum enhanced *Rhamnus* growth in the glasshouse.

Both AMF inoculation treatments improved the nutrient status of *Olea* and *Rhamnus* seedlings under glasshouse conditions (Table 2). The G-inoculated seedlings showed similar phosphorus concentrations but higher nitrogen concentrations than M-inoculated ones in *Olea*. Foliar nitrogen and phosphorus concentrations were similar in M- and G-inoculated *Rhamnus*. The G-inoculated plants had higher potassium concentrations than M-inoculated ones in *Rhamnus*.

Mycorrhizal inoculation treatments did not affect the shoot water content of *Olea* or *Rhamnus* seedlings under glasshouse conditions (Table 2).

Twelve months after field transplanting

Control seedlings showed low AM root colonization levels (< 10%) in both *Olea* and *Rhamnus* (Table 3). The M- and G-inoculated seedlings had much higher AM colonization than their respective controls in both shrub species, although M-inoculated seedlings showed higher colonization than G-inoculated ones.

The M- and G-inoculated seedlings were significantly larger than control ones for both *Olea* and *Rhamnus* (Table 3). Shoot dry biomass of M- and G-inoculated plants was similar in *Olea,* whereas M-inoculated plants were larger than G-inoculated ones in *Rhamnus*.

The M-inoculated seedlings had higher nitrogen foliar concentrations than their controls in both *Olea* and *Rhamnus*, while G-inoculated plants showed higher nitrogen concentrations than their controls in *Olea* only. The M- and G-inoculated seedlings showed very similar nitrogen foliar concentrations in *Olea*. Inoculation with the mixture of native *Glomus* species improved nitrogen status in *Rhamnus* over inoculation with *Glomus claroideum*.

The M-inoculated plants (but not G-inoculated plants) showed significantly higher phosphorus foliar concentrations than their controls in *Olea* and *Rhamnus* (Table 3). Phosphorus concentrations in M- and G-inoculated plants were not significantly different for *Rhamnus*, while for *Olea* the M-inoculated plants had higher values.

Potassium foliar concentration was not affected by the inoculation treatments in *Olea* (Table 3). M- and G-inoculated seedlings showed lower potassium concentrations than their controls in *Rhamnus*. M- and G-inoculated seedlings showed similar potassium foliar concentrations for both shrub species.

The M-inoculated seedlings had higher shoot water content than their controls for both *Olea* and *Rhamnus* (Table 3). The G-inoculated plants had higher water content than their controls in *Olea* only. Inoculation with the mix of native *Glomus* species improved shoot water content to a greater extent than inoculation with *Glomus claroideum* in both *Olea* and *Rhamnus*.

The M-inoculated *Olea* plants used water more efficiently than their uninoculated controls during the first year under field conditions, as shown by $\delta^{13}C$ data (Table 3). Intrinsic WUE was also higher in M- than in G-inoculated plants, as indicated by higher $\delta^{13}C$ values. By contrast, mycorrizal treatments did not affect shoot $\delta^{13}C$ significantly in *Rhamnus*.

Table 2 Percentage arbuscular mycorrhizal (AM) fungal colonization of roots, shoot dry biomass, shoot nutrient concentrations and shoot water content of 8-month-old nursery grown *Olea* and *Rhamnus* seedlings

	Olea europaea ssp. *sylvestris*			*Rhamnus lycioides*		
	C	M	G	C	M	G
AM root colonization (%)	0a	74 ± 1b	69 ± 2b	0a	66 ± 2c	43 ± 1b
Shoot dry biomass (g)	0.29 ± 0.02a	0.74 ± 0.11b	0.93 ± 0.08b	0.14 ± 0.04a	0.35 ± 0.03b	0.13 ± 0.04a
Nitrogen (mg g^{-1})	7.7 ± 0.3a	10.4 ± 0.4b	14.6 ± 0.8c	2.9 ± 0.3a	14.6 ± 0.2b	12.4 ± 0.7b
Phosphorus (mg g^{-1})	0.23 ± 0.04a	0.75 ± 0.02b	0.73 ± 0.03b	0.38 ± 0.03a	0.75 ± 0.04b	0.63 ± 0.05b
Potassium (mg g^{-1})	5.9 ± 0.3a	8.2 ± 0.1b	7.5 ± 0.3ab	5.4 ± 0.5a	10.3 ± 0.3b	13.8 ± 0.8c
Shoot water content (%)	54.1 ± 1.3a	56 ± 3.2a	55.3 ± 1.3a	58.2 ± 1.2a	56.6 ± 1.3a	58.5 ± 1.8a

C, Uninoculated plants; M, plants inoculated with a mixture of eight native *Glomus* species; G, plants inoculated with *Glomus claroideum*. Within plant species, values sharing the same letter are not significantly different ($P < 0.05$) according to the LSD test.

Table 3 Percentage arbuscular mycorrhizal (AM) fungal colonization of roots, shoot dry biomass, shoot nutrient concentrations, shoot water content and carbon, nitrogen and oxygen stable isotope compositions of *Olea* and *Rhamnus* seedlings 1 yr after field transplanting

	Olea europaea ssp. *sylvestris*			*Rhamnus lycioides*					
	C	M	G	C	M	G			
AM root colonization (%)	9 ± 2a	72 ± 10c	48 ± 7b	6 ± 5a	68 ± 7c	31 ± 6b			
Shoot dry biomass (g)	0.64 ± 0.05a	4.92 ± 0.56b	4.42 ± 0.89b	0.56 ± 0.05a	2.22 ± 0.16c	0.81 ± 0.12b			
Nitrogen (mg g^{-1})	6.8 ± 1.2a	14 ± 0.9b	14.5 ± 0.6b	8.5 ± 0.8a	15.2 ± 1.1b	9.1 ± 1.1a			
Phosphorus (mg g^{-1})	0.60 ± 0.05a	0.82 ± 0.03b	0.67 ± 0.06a	0.45 ± 0.08a	0.62 ± 0.05b	0.54 ± 0.02ab			
Potassium (mg g^{-1})	5.4 ± 0.4a	6.8 ± 0.3a	6.2 ± 0.5a	3.8 ± 0.1b	2.9 ± 0.1a	2.9 ± 0.1a			
Shoot water content (%)	41.3 ± 0.2a	49 ± 1.7c	45.6 ± 1.1b	35.6 ± 0.9a	41.9 ± 0.7b	36.6 ± 0.8a			
$\delta^{13}C$ (‰)	–29.3 ± 0.1a	–28.5 ± 0.4b	–29.2 ± 0.3a	–29.1 ± 0.2a	–29.6 ± 0.1a	–29.3 ± 0.2a			
$\delta^{15}N$ (‰)	2.1 ± 0.2a	3.3 ± 0.4b	2.8 ± 0.3ab	–0.4 ± 0.3a	0.2 ± 0.2a	0.1 ± 0.4a			
$\delta^{18}O$ (‰)	28 ± 0.5b	26.8 ± 0.5a	28.3 ± 0.7b	28.2 ± 0.5b	26.7 ± 0.4a	29.4 ± 0.3b*			
	AM colonization	Shoot biomass	Nitrogen	Phosphorus	Potassium	Water content	$\delta^{13}C$	$\delta^{15}N$	$\delta^{18}O$
Shrub species	NS	0.000	0.000	0.000	0.000	0.000	NS	0.000	NS
AMF	0.000	0.000	0.000	0.001	NS	0.000	NS	0.003	0.009
Shrub × AMF	NS	0.001	0.000	NS	NS	NS	0.024	NS	NS

C, Uninoculated plants; M, plants inoculated with a mixture of eight native *Glomus* species; G, plants inoculated with *Glomus claroideum*. Within plant species, values sharing the same letter are not significantly different ($P < 0.05$) according to the LSD test. *C and G are different at $P < 0.073$. Significance of effects of shrub species and mycorrhizal treatments on the measured variables are also shown. NS, not significant; AMF, mycorrhizal inoculation treatment.

Seedlings inoculated with the mix of native *Glomus* species showed lower shoot $\delta^{18}O$ signatures than control or G-inoculated seedlings for both *Olea* and *Rhamnus* (Table 3). M-inoculated shrubs showed higher $\delta^{15}N$ values than their respective controls, although differences were significant for *Olea* only (Table 3).

Eighteen months after field transplanting

The survival rates of M- and G-inoculated *Olea* seedlings were higher than that of their uninoculated controls (Table 4). Survivorship was not affected by mycorrhizal inoculation treatments in *Rhamnus*. Survival rates of M- and G-inoculated plants were nearly identical for both *Olea* and *Rhamnus*.

Uninoculated control plants still showed negligible levels (8–10%) of AM root colonization in *Olea* and *Rhamnus* 18 months after field transplanting (Table 4). By contrast, M- and G-inoculated plants showed similarly high (67–88%) levels of AM root colonization in both *Olea* and *Rhamnus*.

Shoot biomass of M-inoculated shrubs was between 12 (*Olea*) and 16 (*Rhamnus*) times larger than that of their respective uninoculated controls (Table 4). The G-inoculated plants were also significantly larger than control plants in *Olea* and *Rhamnus*. The M-inoculated plants had much greater shoot biomass than their G-inoculated counterparts in both *Olea* and *Rhamnus*.

Over the entire 18-month period in the field, plants inoculated with the mixture of native *Glomus* fungi showed by far the highest relative growth rates in both shrub species (Table 4).

Discussion

Large differences in AM percentage colonization between control and nursery-inoculated seedlings persisted throughout the 18-month field experiment. The local AMF community showed little capacity to colonize shrub roots, likely because of low fungal species diversity (Azcón-Aguilar *et al.*, 2003) and low abundance of mycorrhizal propagules in the soil (Table 1).

The local AMF community was much less effective than the added (native or nonnative) *Glomus* inoculum at stimulating host plant growth for both *Olea* and *Rhamnus*. Inoculation with the mixture of native *Glomus* species conferred a clear growth advantage to *Rhamnus* over inoculation with *G. claroideum*. This growth advantage increased after field transplanting, indicating strong specificity of response to different *Glomus* species in *Rhamnus*. Inoculation with a mixture of eight AMF species increases the probability of plant–fungus matches that stimulate optimal plant growth compared with inoculation with a single AMF species (van der Heijden *et al.*, 1999). Moreover, coevolution and co-occurrence of the symbiotic partners, better adaptation of native AMF to local

Table 4 Percentage arbuscular mycorrhizal (AM) fungal colonization of roots, shoot dry biomass, relative growth rate and survival rate of *Olea* and *Rhamnus* seedlings 18 months after field transplanting

	Olea europaea ssp. *sylvestris*			*Rhamnus lycioides*		
	C	M	G	C	M	G
AM root colonization (%)	10 ± 1a	85 ± 2b	88 ± 3b	8 ± 1a	67 ± 2b	74 ± 6b
Shoot dry biomass (g)	1.4 ± 0.1a	16.5 ± 2.7c	6.7 ± 0.2b	0.5 ± 0.1a	8.1 ± 2.1c	1.8 ± 0.2b
Relative growth rate (g g^{-1})	3.9	21.2	6.2	2.9	22.2	12.5
Survival rate (%)	45a	95b	92b	55a	62a	64a

C, Uninoculated plants; M, plants inoculated with a mixture of eight native *Glomus* species; G, plants inoculated with *Glomus claroideum*. Within plant species, values sharing the same letter are not significantly different ($P < 0.05$) according to the LSD test.

semiarid conditions and potential functional complementarity among AMF species would all be expected to provide a growth advantage to the seedlings inoculated with the mixture of native *Glomus* fungi (Klironomos, 2003).

Remarkably, *Olea* shrubs inoculated with a single nonnative *Glomus* species had comparable size to those inoculated with a mixture of eight native *Glomus* species, both in the glasshouse and 12 months after field transplanting. Only in the longer term (18 months) did the native *Glomus* inoculum provide a clear growth advantage over *Glomus claroideum* for *Olea*. Overall, these results support the idea that plant growth response to different AMF is highly dependent on environmental conditions (Johnson *et al.*, 1997). However, as illustrated by *Olea*, diverging plant growth responses to different AMF may only become evident in the long term under the wide range of extreme environmental conditions that characterize semiarid ecosystems.

The putative mechanisms underlying the growth advantage provided in the longer term by the mixture of native *Glomus* sp. varied between shrub species. Inoculation with native AMF enhanced shoot concentrations of either nitrogen (*Rhamnus*) or phosphorus (*Olea*) to a greater extent than inoculation with nonnative AMF during the first year after field outplanting. Functional complementarity among the native *Glomus* species added as inoculum may have contributed to increased nutrient uptake in M-inoculated plants compared with G-inoculated ones (Koide, 2000). Differing spatial abilities to acquire nutrients has been cited as one possible reason why colonization by multiple AMF can be more beneficial than colonization by a single species (Smith *et al.*, 2000). However, it is interesting to note that, under glasshouse conditions, *Olea* and *Rhamnus* seedlings inoculated with *G. claroideum* had similar or even higher nutrient concentrations than those inoculated with the mixture of eight native *Glomus*. Greater enhancement of nutrient uptake by the native compared with the nonnative AMF occurred only after field transplanting, indicating that the former were more efficient at absorbing nutrients in a xeric natural environment, but not under more mesic glasshouse conditions. Drought-adapted native *Glomus* species have been shown to be particularly effective at scavenging for nutrients in dry soil (Tobar *et al.*, 1994; Ruiz-Lozano *et al.*, 1995a).

Lower shoot $\delta^{18}O$ values in *Olea* and *Rhamnus* seedlings inoculated with the native *Glomus* species than in their respective uninoculated controls could just reflect a plant size effect. Evaporative isotopic enrichment of soil water near the surface is very pronounced in semiarid environments (Barnes & Allison, 1983; Allison & Hughes, 1983), creating sharp gradients in soil water $\delta^{18}O$ with depth. Larger M-inoculated plants likely had access to deeper, less isotopically enriched soil water than control plants. Since the oxygen isotope ratio of plant cellulose reflects the signature of source water (with an enrichment of 27‰; Sternberg *et al.*, 1986), uptake of water with dissimilar $\delta^{18}O$ signatures would lead to differences in shoot $\delta^{18}O$ among mycorrhizal treatments (Barbour *et al.*, 2002). However, this cannot explain the large difference in shoot $\delta^{18}O$ found between M- and G-inoculated *Olea* seedlings, as they had similar size and presumably were extracting water from similar depths. Moreover, control and G-inoculated *Olea* seedlings showed similar shoot $\delta^{18}O$ values despite large size differences between them, suggesting that depth of water uptake was not the major determinant of $\delta^{18}O$ variability among mycorrhizal treatments.

The source water signal can be modified by large variability in evaporative enrichment in drought-adapted Mediterranean species with tight stomatal regulation of transpiration (Ferrio & Voltas, 2005). The ratio of water vapor pressure of the air outside and inside the leaf is the parameter controlling evaporative enrichment of O^{18} in leaf water and therefore in plant organic matter (Farquhar *et al.*, 1998; Keitel *et al.*, 2003). Since greater stomatal conductance cools the leaf and reduces internal water pressure, the $\delta^{18}O$ signature of plant tissues is a useful tool to characterize g_s, independent from effects of carbon fixation (Keitel *et al.*, 2003). Lower $\delta^{18}O$ values indicated enhanced g_s in seedlings inoculated with the native *Glomus* species compared with those inoculated with *G. claroideum*, as the $\delta^{18}O$ signature of plant organic matter decreases in response to increased g_s (Scheidegger *et al.*, 2000; Barbour *et al.*, 2002). More efficient hyphal water uptake and transport in dry soil by drought-adapted, native AMF may have

contributed to greater g_s in their host plants (Augé, 2001; Marulanda *et al.*, 2003). Higher shoot water content in plants inoculated with the native *Glomus* inoculum is consistent with and supports the aforementioned interpretation of $\delta^{18}O$ differences among mycorrhizal treatments. Shoot water content was negatively correlated with $\delta^{18}O$ in both *Olea* (Pearson correlation coefficient = –0.589; $P < 0.05$) and *Rhamnus* (Pearson correlation coefficient = –0.655; $P < 0.01$). To our knowledge, this is the first study showing evidence of a mycorrhizal effect on the $\delta^{18}O$ signature of host plants. Our results indicate that $\delta^{18}O$ can provide an integrated measure of the effect of mycorrhizal infection on host plant water relations over the period when the plant tissue was formed.

Mycorrhizal treatments had contrasting effects on shoot $\delta^{13}C$ depending on the specific plant–fungus combination. $\delta^{13}C$ data showed differential ability of native and nonnative *Glomus* fungi to modulate intrinsic water use efficiency in *Olea*. Greater WUE in M-inoculated *Olea* seedlings compared with G-inoculated seedlings was likely the result of better phosphorus status in the former, as improved nutrition can lead to specific stimulation of photosynthetic capacity over stomatal conductance (Koide, 1993; Querejeta *et al.*, 2003). Higher $\delta^{13}C$ but lower $\delta^{18}O$ in M-inoculated *Olea* seedlings compared with G-inoculated seedlings suggests a very strong enhancement of photosynthetic rate capable of increasing plant WUE despite increased stomatal conductance (Scheidegger *et al.*, 2000). Nonnutritional mechanisms may have also played a role in the differential modulation of host plant photosynthetic capacity by different AMF species in *Olea* (e.g. differences in carbon sink strength; Wright *et al.*, 1998).

Foliar $\delta^{13}C$ was not significantly affected by the mycorrhizal treatments in *Rhamnus*, although inoculation with the native AMF tended to decrease $\delta^{13}C$ compared with the uninoculated controls ($P = 0.08$). Lower $\delta^{18}O$ values in seedlings inoculated with the native AMF further suggest enhancement of stomatal conductance over photosynthetic capacity in *Rhamnus* (Scheidegger *et al.*, 2000).

Differences in $\delta^{13}C$ between *Olea* and *Rhamnus* were largest when comparing seedlings inoculated with the native AMF, which supports a previous study indicating that much of the interspecific variability in foliar $\delta^{13}C$ found in semiarid plant communities results from dissimilar patterns of physiological response to AMF infection (Querejeta *et al.*, 2003).

Greater enhancement of shoot nitrogen concentration by native than by nonnative AMF was found in *Rhamnus* but not in *Olea*, suggesting specific stimulation of atmospheric nitrogen fixation rather than of soil nitrogen uptake in the former. *Rhamnus* showed $\delta^{15}N$ values around 0‰ which reflected substantial atmospheric nitrogen biological fixation, as soil nitrogen is typically enriched in ^{15}N compared with atmospheric nitrogen ($\delta^{15}N$ = 0‰; Shearer *et al.*, 1983). Ruiz-Lozano *et al.* (2001) showed that the AM symbiosis enhanced the nitrogen status of legume plants by protecting them against drought-induced nodule senescence, and partially attributed this protective effect to better hydration of AM plants. Arbuscular mycorrhizal symbiosis may exert a similar protective effect against drought in actinorhizal plants. Improved water status in *Rhamnus* seedlings inoculated with native AMF may have favoured the activity of their associated nitrogen-fixing *Frankia* symbionts, thus leading to much higher shoot nitrogen concentration than in those inoculated with the nonnative AMF.

The interpretation of natural abundance $\delta^{15}N$ in plants in the field is complex as it reflects the net effect of a wide range of processes, including soil nitrogen sources, mycorrhizal infection, internal fractionations and rooting depth (Evans, 2001). The ^{15}N isotopic enrichment in M-inoculated *Olea* plants could reflect greater uptake of soil nitrogen in the field, as the $\delta^{15}N$ values of mycorrhizal shrubs were closer to that of the surface soil layer ($\delta^{15}N = 4.6 \pm 0.3$‰ at 0–20 cm depth, $n = 5$). This ^{15}N enrichment might also reflect the larger size and presumably greater rooting depth of M-inoculated seedlings, since there is usually a steep gradient of increasing $\delta^{15}N$ down the soil profile (Handley *et al.*, 2001).

In conclusion, inoculation with a mixture of native AMF greatly improved the nutrient and water status as well as the long-term growth of *Olea* and *Rhamnus* seedlings in a semiarid environment. Oxygen and carbon isotopic measurements showed that native AMF enhanced stomatal conductance in both *Olea* and *Rhamnus*, and stimulated photosynthetic capacity and WUE in *Olea*. These results suggest that modulation of leaf gas exchange parameters by drought-adapted, native AMF is critical to the long-term performance of host plants in semiarid environments.

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