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Associations between arbuscular mycorrhizal fungi and grasses in the successional context of a two-phase mosaic in the Chihuahuan Desert

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Abstract The hypothesis that plant species are more responsive to mycorrhiza in late than in early successional stages was assessed in grasses from a successional process occurring in two-phase mosaics from the Mexican Chihuahuan Desert. We estimated the density of spores of arbuscular mycorrhizal (AM) fungi and the AM colonization of pioneer and late-successional grasses in the field. In growth chamber experiments, we tested the effect of the native AM fungal community on grasses growing in soils from different successional stages. Spore density was higher in late than in early successional stages. Late-successional species were more responsive to AM (positive AM responsiveness) whereas pioneer species were nondependent on mycorrhiza or if associated to AM fungi, the interaction showed a negative AM responsiveness for the seedling stage. Our findings showed that late successional species fitted the proposed models of mycorrhizal performance, but the two pioneer species differed in their AM condition and responsiveness. This further supports the idea that AM interactions are more complex along the successional processes than the predictions of the more widely cited hypotheses.

Keywords Arid environment · Mapimí Biosphere Reserve · Mycorrhizal responsiveness · Plant growth · Succession

Introduction

The importance of arbuscular mycorrhizal (AM) symbioses for plant nutrition was recognized in many arid and semiarid communities (Bethlenfalvay et al. 1984; Allen 1991; Carrillo-García et al. 1999; Fontenla et al. 2001; O'Connor et al. 2001; Titus et al. 2002; Camargo-Ricalde et al. 2003). These symbioses are generally referred to as mutualistic, although Johnson et al. (1997) and Klironomos (2003) showed from a phytocentric view that the outcome can range from parasitic to mutualistic, depending on the specific identities of plants and fungi. Some of the direct benefits that may be gained by the plants are improved nutrient acquisition (mainly phosphorus), protection of roots against pathogens and alleviation of water stress. As a consequence of these direct benefits, plants may enhance their competitive ability. In exchange, the fungi receive carbohydrates from their host plants (Smith and Read 1997). The cost/benefit balance for the plants and fungi will determine the position of each particular AM fungi-plant symbiosis in the mutualism–parasitism continuum. In the successional context, AM fungi were commonly found associated with plants in late-successional stages whereas pioneer plants tend to be facultative or nondependent on AM (Reeves et al. 1979; Janos 1980). However, there is contrasting evidence showing that plants from early successional stages benefit from the symbiosis with AM fungi (Allen and Allen 1992; Allen et al. 2003). This contradictory evidence suggests that AM prevalence in plant roots could be driven by plant intrinsic attributes, such as root morphology, rather than for their temporal occurrence during the successional process, although these factors could not be easily disentangled. In this respect, in a general study (i.e., not a successional study) in the Chihuahuan Desert (La Jornada, New México), Collier et al. (2003) found a clear-cut difference in root morphology and AM condition between annual species with fine roots and low levels of infection by AM fungi and perennial species with thick roots and high levels of infection by AM fungi.

In the Mapimí Biosphere Reserve (MBR) in the Mexican Chihuahuan Desert, an important successional process naturally occurs in two-phase banded mosaics. Here, the vegetation consists of patchily distributed vegetation bands (VB) within a matrix of almost bare soil (Cornet et al. 1988; Montaña 1992; Fig. 1). Due to the dynamics of the

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successional process, the VB are spatially structured (Montaña 1992; Vega and Montaña 2004) with a frontal zone facing the upslope (upslope fringe) that represents early successional stages where annual species are abundant although some short-lived perennials are common. In contrast, late-successional stages found toward the interior of the VB are constituted by tens of long-lived perennial species, which senesce toward the back of the VB because water is retained mostly in the interior of the VB.

In this successional process, we hypothesize that long-lived perennials in the interior of the VB (late successional stages) will be more responsive to AM whereas annuals and short-lived perennials in the upslope fringe (early successional stages) will be less or nonresponsive to AM. Species of grasses (*Poaceae*) are important components of the vegetation in MBR (62 grass species in a total of 403 plant species; García-Arévalo 2002) and concordantly, they are well represented in the VB, both in the upslope fringe and in the interior of the bands. In addition, recent evidence shows that typical grass species from the upslope fringe and the interior of the VB differ in some life history traits (time to reproduction, resource allocation to reproduction, and root/shoot ratio) and it was suggested that such differences are associated with the successional stage in which those grass species are found (Pezzani 2001). Therefore, grasses are a good system to investigate the role of AM fungi in the successional dynamics in the VB, an aspect that to our knowledge is not yet addressed for any banded two-phase mosaic (cf. Tongway et al. 2001). The role of AM fungi in the establishment of grasses is particularly interesting because in general, they tend to have small and poorly provisioned seeds and it was suggested that the establishment of plant species with such seeds may strongly depend on AM fungi associations (Allsopp and Stock 1992). In congruence with this, we will refer to plant-dependence on AM symbiosis exclusively associated to seedling establishment. In this study, we explore the potential roles that AM fungi could have on the growth of grass species from different successional stages within the VB. We aim to answer the following questions. Are there differences in the AM inoculum density between the upslope fringe and the interior of the VB? Are there differences in the level of root colonization by AM fungi between VB pioneer and late-successional species? Are there differential effects of AM interactions on the growth of grass species from different successional stages of the VB?

Materials and methods

Study site

The MBR (México) is located in the southern Chihuahuan Desert (26°29′–26°52′ N, 103°32′–103°58′ W) between 1,000 and 1,650 masl. The weather in the reserve is arid with cool winters, warm and rainy summers (71% of the annual rainfall falling between June and September), and dry for 8 to 9 months with a mean annual rainfall of 264 mm (Cornet 1988).

This study was conducted in the west "bajada" (piedmonts leading to endorrheic basins, 0.5 to 2% slopes) on a hill called San Ignacio. Yermosols and xerosols are formed on the top of alluvia and colluvia substrata (FAO/UNESCO 1974; Delhoume 1988) and the typical vegetation is a two-phase mosaic (Fig. 1). Similar to most banded-

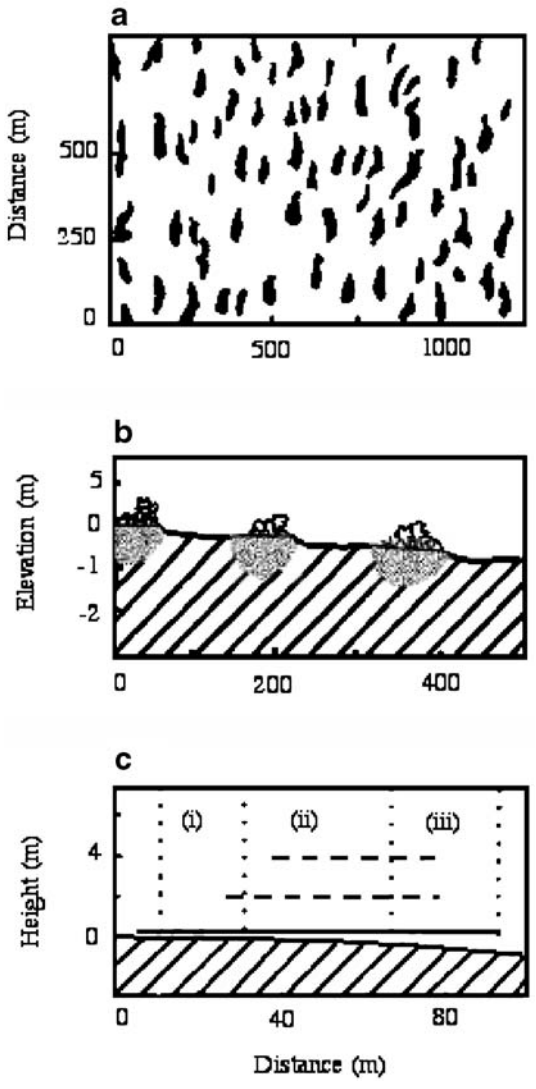

Fig. 1 Schematic representation of two-phase mosaics commonly found in the Mapimí Biosphere Reserve, southern Chihuahuan Desert, México. In all three diagrams, the slope descends from left to right. **a** Aerial view at 1:25,000 scale showing the alternation of vegetation bands (in *black*) and almost bare areas. **b** Idealized cross-section of the landscape showing the distribution of vegetation in a step-like microtopographical profile and the distribution of soil moisture (indicated by a swarm of points below each vegetation band) immediately after rain. **c** Idealized cross-section of a vegetation band. *Horizontal straight lines* indicate the range vegetated and the height of herbaceous species (*continuous line*), shrubs (*lower discontinuous line*), and small trees (*upper discontinuous line*). *Discontinuous vertical lines* indicate the three subdivisions of the bands: (i) upslope (colonization zone), (ii) interior, and (iii) downslope (senescence zone). (Taken from Fig. 1 in Montaña 1992. Permission courtesy of Blackwell Science)

patterns, the system in MBR is mainly water-driven. The bare soil phase acts as a microwater-catchment area that facilitates water run-off into the vegetated phase where the water flow is slowed down and water infiltrates into the soil. As a consequence, the vegetated phase receives 1.5 to 2.5 times the annual rainfall due to run-off with the highest amount of water infiltrating into the interior of the bands. This gradient in water availability correlates with changes in the physical and chemical properties of the soil (Cornet et al. 1992) and plant community structure is mediated by a successional process in which pioneer species of the upslope fringe are typically replaced by perennial grasses and seedlings of woody species (Cornet et al. 1992; Montaña 1992). In the upslope fringe, the dominant grass species are *Dasyochloa pulchella* (Kunth) Willd ex Rybd. (synonym *Tridens pulchellus*), *Bouteloua barbata* Lag., *Chloris virgata* Sw. and *Scleropogon brevifolius* Phil., whereas the common grass species in the interior of the bands are *Pleuraphis mutica* Buckley (synonym *Hilaria mutica*) and *Trichloris crinita* (Lag.) Parodi. Dominant shrubs are *Prosopis glandulosa* Torr. var. *torreyana* (L. D. Benson) M. C. Johnst., *Flourensia cernua* DC., *Lippia graveolens* Kunth., and *Larrea tridentata* (Sessé and Moc. ex DC.) Coville (Montaña et al. 2001).

Study species

For this study, we chose four grass species: two from the upslope fringe, *D. pulchella* and *C. virgata* (pioneer species), and two from the interior, *P. mutica* and *T. crinita* (late-successional species). These four species belong to the *Chloridoideae* subfamily (Clayton and Renvoize 1986), all of them have Kranz anatomy (C_4), and they show different attributes that may be related to AM fungal dependency (Table 1).

Mycorrhizal inoculum density and root colonization under field conditions

To assess whether there are differences in AM inoculum density between the zones of the two-phase mosaic, we collected ten soil samples (100 g) up to 5 cm in depth in the upslope fringe and in the interior of the bands in the dry season (May 2003). Spores of AM fungi were extracted from soil samples following the sugar solution density gradient method (Daniels and Skipper 1982). Spores were counted under a stereomicroscope and mounted in polyvinil alcohol with and without Melzer's reagent (Schenck and Pérez 1990) for further identification based on morphological characters.

The intensity of root colonization by AM fungi was assessed in the fine roots of ten randomly selected adult plants of each species. Roots were washed and fixed in 50% ethanol, cleared with KOH solution, and stained with trypan blue as proposed by Koske and Gemma (1989). We scanned 30 root segments (about 1 cm long) per individual, covering a total of 90 observation fields in which structures of AM fungi (hyphae, vesicles, arbuscules, and spores) were recorded. The observations were made with a Nikon E600 microscope and we calculated the ratio of colonization as the quotient of the number of fields bearing AM fungi structures and the total number of observed fields.

Seedling establishment under controlled conditions

To evaluate the potential role of AM fungi in the establishment of the four grass species, we grew plants in sterilized and nonsterilized soils from the upslope fringe and the interior of the VB. For this experiment, we used seedlings of about 2 cm of foliar length. All seedlings were obtained from seeds collected in the study site (from five randomly selected mother plants) in September 2003. After mechanical scarification, seeds were germinated in a growth chamber (Luminiteck 2K) for 14 h with light at 32°C and 10 h in the dark at 28°C, using vermiculite as a substrate. When seedlings were transplanted to the experimental pots, we recorded the foliar length (measured to the nearest millimeter) for all species except *D. pulchella*, which was very sensitive to manipulation. This species was sown directly in the experimental pots. Seedlings were transplanted into four treatments arising from the combination of the two levels of soil origin (upslope fringe and interior of the VB) and of the two levels of soil condition (sterilized and nonsterilized). Sterilization was accomplished by steaming for 2 h at 100°C and 15 psi for 3 days in a row. To avoid any potential phytotoxic effect, sterilized soil samples were allowed to cool in a flow chamber for 24 h and then stored at 4°C until the experiment was set up. To improve the porosity of the soil, we mixed the soil with sterilized vermiculite (10:1). We used 55 seedlings of each species in each of the four treatments, which gives a total of 880 seedlings (220 per species). All seedlings were transplanted to individual pots (5×5×7 cm) with 250 g of soil-vermiculite mix. Transplanted seedlings were kept under the same conditions described above for 30 days and then all plants were harvested.

Table 1 Main characteristics of the four grass species

	Dasyochloa pulchella	*Chloris virgata*	*Pleuraphis mutica*	*Trichloris crinita*
Successional stage	Pioneer	Pioneer	Late-successional	Late-successional
Life cycle	Short-lived perennial	Annual	Perennial	Perennial
Growth habit	Stoloniferous	Stoloniferous	Tufted	Tufted
Root morphology	Thick	Fine	Thick	Thick

The response variables for this experiment were survivorship, biomass, root/shoot ratio, root colonization, and AM responsiveness (calculated as the difference between the biomass in nonsterilized soil and sterilized soil divided by the biomass in nonsterilized soil; Planchette et al. 1983; Wilson and Hartnett 1998). We estimated survivorship as the percentage of seedlings that were still alive after 30 days. Root colonization was measured (with the methodology already described in the last section) in five randomly selected plants for each species in each treatment and we separately recorded colonization by AM fungi and by other fungi. For the remaining plants of each treatment, the above-ground and the below-ground structures were separated and oven-dried for 3 days at 60°C. The dry biomass was then weighed using an analytical scale to the nearest hundredth of a milligram. From this, we estimated total biomass production, carbon allocation as root/shoot ratio (below ground biomass/above ground biomass), and AM responsiveness.

Soil analyses

Before and after the sterilization process, we carried out chemical analyses in five randomly selected soil samples from each of the two soil origins to assess potential changes due to the sterilization process. Soil pH was measured in a standard 1:2 solution of soil in distilled water. Organic matter was estimated with the method of Walkley and Black (Nelson and Sommers 1982). Total nitrogen was estimated using the micro-Kjeldahl method (Bremmer 1965; Bremmer and Mulvaney 1982). Readily available phosphorus was extracted using $NaHCO_3$ and assayed using the ammonium-molybdate method (Olsen and Sommers 1982). Potassium was extracted from the soil in 1 N ammonium acetate and analyzed by flame photometry (Knudsen et al. 1982). In addition, we compared the number of spores of AM fungi in 100 g of sterilized soil from each of the two soil origins with the field inoculum density using the same technique as previously.

Statistical analyses

Comparison of AM inoculum density (number of spores per soil weight) between zones (upslope fringe vs interior of the VB) was done with a Kruskall–Wallis test (Sokal and Rohlf 1995) using the statistical package STATVIEW 5.0.1 (SAS Institute 1999).

We used a one-way ANOVA to assess for differences of root AM colonization among species under field conditions. To evaluate the effect of steam sterilization on soil mineral nutrient concentrations, we used a two-way ANOVA for each soil parameter. In the model, soil origin (upslope fringe and interior) and soil treatment (sterilized and nonsterilized) were the main factors. We used a similar model but included the foliar length as a covariate to analyze the effect of soil origin and soil treatment on seedling biomass production. In all cases for the regression lines, we tested the homogeneity of slopes between the covariate and the biomass for each level of the factors (Winer et al. 1991) as a prerequisite to conduct the analysis of covariance. We carried out this analysis separately for each species because there are marked inherent differences in size among them. We implemented the same two-way ANOVA used when analyzing the variability of the soil parameters to address the effect of soil origin on root colonization in seedlings of the four grass species. We separately analyzed colonization by AM fungi and other fungi. We used a three-way ANOVA (i.e., adding species as a third factor) to investigate the effects of soil origin and soil treatment on root/shoot ratio among seedlings of the grass species.

All ANOVAs mentioned above were done with STATVIEW 5.0.1 (SAS Institute 1999). When required, we normalized the data using the Box–Cox transformation procedure and for all cases when it was necessary, we used the Fisher's protected least significant differences post hoc test (Sokal and Rohlf 1995).

We used a bootstrap resampling method (in R 2.0.1; Venables and Smith 2004) to generate a probability distribution for the AM responsiveness of each grass species in each soil origin. Because the number of surviving plants differed for each soil treatment (sterilized and nonsterilized), the number of randomly generated pairs for each run of the bootstrap analysis was equal to the number of plants in the treatment with the lowest survivorship. Pairs were generated without replacement and for each set of pairs, we calculated the average AM responsiveness and we ran 10,000 iterations to work out the 95% confidence intervals of the distribution of average AM responsiveness.

Seedling survivorship was evaluated through a generalized linear model in which the response variable was the number of surviving plants at the end of the experiment. The independent variables were species (four species), soil origin (upslope fringe and interior), and soil treatment (sterilized and nonsterilized). We fitted the model with Poisson error and log-link function. This analysis was performed in GLMSTAT 5.7.7 (Beath 2004). In addition, we performed a second linear model with the same parameters described above but foliar length was included as a covariate. *D. pulchella* was excluded from this analysis because the foliar length for this species was not recorded (see "Seedling establishment under controlled conditions").

Results

Mycorrhizal inoculum density and root colonization under field conditions

We found that the abundance of spores of AM fungi in the interior of the VB is significantly higher (Hc=14.29, $P<0.001$) than in the upslope fringe (Table 2). Also, species richness of AM fungi spores was higher in the interior

Table 2 Soil attributes in the zones of the vegetation bands (VB)

	Upslope fringe of the VB	Interior of the VB
Mycorrhizal inoculum density [median (1st–3rd quartile)]	31 (23.5–38)[a]	193.5 (171.25–243.25)[b]
Soil pH		
Before sterilization	8.45±0.03[a]	8.39±0.04[a]
After sterilization	8.79±0.03***	8.79±0.01***
Total soil organic matter (%)		
Before sterilization	1.07±0.04[a]	1.44±0.07[b]
After sterilization	0.09±0.03**	1.27±0.07**
Total soil nitrogen (%)		
Before sterilization	0.088±0.004[a]	0.128±0.004[b]
After sterilization	0.074±0.003***	0.109±0.004***
Extractable soil phosphorus (mg kg^{-1})		
Before sterilization	4.17±0.22[a]	3.95±0.19[a]
After sterilization	4.75±0.06**	4.52±0.18**
Extractable soil potassium (mg kg^{-1})		
Before sterilization	1.45±0[a]	2.25±0[b]
After sterilization	1.48±0.08[a]***	1.82±0.02[b]***

Mycorrhizal inoculum density measured as the number of spores in 100 g of soil. Soil mineral nutrient content before and after sterilization. For the mycorrhizal inoculum density (which was analyzed through a nonparametric test) the median and quartile values are shown. All other parameters are reported as means±SE

Superscript identical letters indicate homogeneous groups between the zones in the VB whereas ** and *** indicate effects due to sterilization process and stand for $P<0.01$ and $P<0.001$, respectively. The interaction for main effects was only significant for potassium

(20 species) than in the upslope fringe (11 species) with seven shared species (Table 3).

Percentage of root colonization by AM fungi varied significantly among species ($F=17.2$; $df=3, 36$; $P<0.0001$; Table 4). Most of the observed fungal structures corresponded to hyphae and vesicles. All individuals of *C. virgata*, *P. mutica*, and *T. crinita* were colonized, whereas for *D. pulchella* only three out of ten sampled individuals were colonized and the multiple comparison test showed that colonization of this later species differed significantly from the rest ($t=0.4703$, $df=18$, $P<0.0001$).

Soil analyses

Soils in the VB both in the upslope fringe and in the interior are alkaline (pH>8) with a low content of organic matter (less than 2%) and low nitrogen and phosphorus (Table 2). The process of sterilization modified the soil properties but the variance explained by the ANOVA models was mainly attributed to the origin of the soil (upslope fringe vs interior of the VB) for organic matter (83%), nitrogen (87%), and potassium (77%), whereas steaming explained most of the observed variance in pH (99%) and phosphorus (77%). The content of organic matter ($F=50.4$; $df=1, 16$; $P<0.0001$) and nitrogen ($F=107.8$; $df=1, 16$; $P<0.0001$) were higher for about 35 and 46%, respectively, in the interior of the VB than in the upslope fringe, whereas there were no

Table 3 Species of AM fungi (spores) found in the soil samples of the upslope fringe and the interior of the vegetated bands in the Mapimí Biosphere Reserve in the dry season (May 2003)

	Upslope fringe	Interior		Upslope fringe	Interior
Acaulospora delicata	X	X	*Glomus cerebriforme*	X	X
Acaulospora denticulata	X	X	*Glomus claroideum*	X	X
Acaulospora morrowiae	X		*Glomus constrictum*		X
Acaulospora sp. 2	X		*Glomus etunicatum*	X	
Acaulospora sp. 3		X	*Glomus geosporum*		X
Acaulospora sp. 4		X	*Glomus microaggregatum*		X
Acaulospora sp. 5		X	*Glomus mosseae*		X
Acaulospora sp. 6		X	*Glomus* sp. 1	X	X
Acaulospora sp. 7		X	*Glomus* sp. 2	X	
Acaulospora sporocarpia	X	X	*Glomus* sp. 3		X
Entrophospora infrequens	X	X	*Glomus* sp. 4		X
Gigaspora decipiens		X	*Glomus* sp. 5		X

Table 4 Field mycorrhizal status of the four grass species and root colonization of seedlings in pot experiments (mean±SE)

	Dasyochloa pulchella	*Chloris virgata*	*Pleuraphis mutica*	*Trichloris crinita*
Adults under field condition				
Root colonization by AM fungi (%)	2.6±1.8^{a} *n*=10	27.8±3.6^{bc} *n*=10	37.8±7.1^{b} *n*=10	22.3±5.1^{c} *n*=10
Seedling under controlled conditions in nonsterilized soil from the upslope				
Root colonization by AM fungi (%)	13.3 ± 13.3 *n*=4	51.7 ± 9.6 *n*=5	29.0 ± 12.2 *n*=5	43.3 ± 18.0 *n*=5
Root colonization by fungi other than AM (%)	30.0±14.0 *n*=4	1.3±1.3 *n*=5	25.3±4.9 *n*=5	13.3±8.2 *n*=5
Seedling under controlled conditions in nonsterilized soil from the interior				
Root colonization by AM fungi (%)	5.0±5.0 *n*=4	40.5±8.6 *n*=5	36.1±9.5 *n*=5	54.8±8.6 *n*=5
Root colonization by fungi other than AM (%)	25.0±10.8 *n*=4	11.6±6.6 *n*=5	23.6±8.6 *n*=5	54.4±5.1 *n*=5
Seedling under controlled conditions in nonsterilized soil (mean±SE of the results from the two soil origins)				
AM fungi (%)	9.2±6.7^{a}	46.1±6.3^{b}	32.5±7.4^{b}	49.1±9.6^{b}
Other fungi than AM (%)	27.5±8.2^{a}	6.5±3.6^{b}	24.5±4.7^{a}	33.9±4.0^{a}

Identical letters in the same row indicate nonsignificant differences (P>0.05)

significant differences in soil pH and phosphorus content. Sterilization had significant effects both on the mineral nutrient concentrations and on the AM inoculum density of soils. Soil pH and readily available phosphorous increased after sterilization by 4.3% (F=212; df=1, 16; P<0.0001) and 14.2% (F=10.9; df=1, 16; P=0.0045), respectively. On the other hand, nitrogen and organic matter decreased as a consequence of sterilization; nitrogen content was reduced by 14% (F=18.6; df=1, 16; P=0.0005) and the organic matter decreased by 11% in the upslope fringe and 18% in the interior of the VB (F=11.6; df=1, 16; P=0.0036). Potassium content was 55% higher in the interior of the VB than in the upslope. Sterilization decreased by 19% the K content in the soil samples from the interior of the VB, whereas K content in soil samples from the upslope showed almost no change (2% increase) after sterilization. The sterilization process greatly reduced the AM inoculum density of the soil. After sterilization, we found five and 12 AM fungi spores in 100 g of soil from the upslope fringe and of the interior of the VB, respectively, which contrast with the median count of spores under field conditions (31 and 193 spores/100 g soil in the upslope fringe and in the interior of the VB).

Seedling establishment

Survivorship

The linear model fitted was highly significant and explained 46.5% (χ^2=165.6, df=7, P<0.0001) of the observed deviance. Species was the main factor and accounted for 36.3% of the observed deviance (χ^2=136.4, df=3, P<0.0001, Fig. 2). The soil treatment (sterilization) had a significant effect (χ^2=10.7, df=1, P=0.0011) but only accounted for 3% of the observed deviance. The interactions of these two factors also showed a significant effect (χ^2=25.5, df=3, P<0.0001) and accounted for 7.2% of the observed deviance. Soil origin had no significant influence on plant survival (P>0.05) so it was not included in the final model. All 220 seedlings of *C. virgata* survived to the end of the experiment whereas only

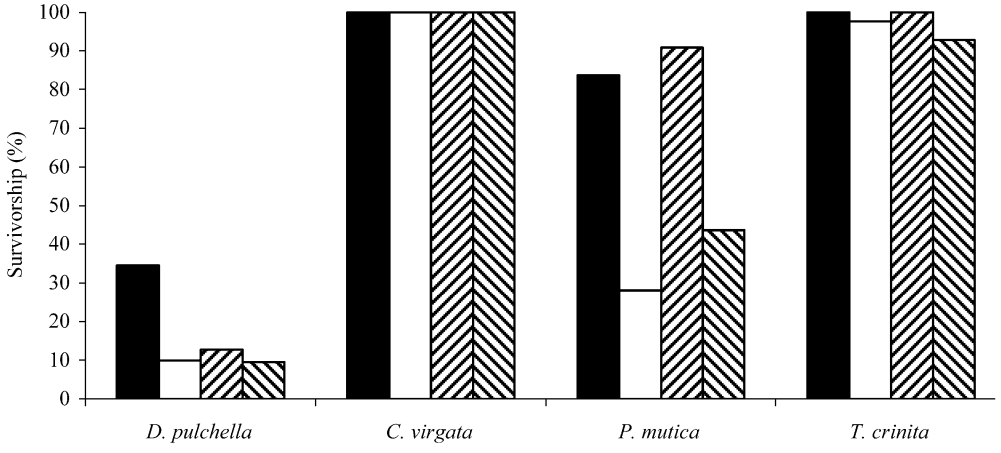

Fig. 2 Survivorship of grass seedlings (%) in sterilized and nonsterilized soils from the upslope fringe and the interior of the VB in Mapimí Biosphere Reserve. Nonsterilized soil from the upslope fringe of the VB (*closed bars*); sterilized soil from the upslope fringe of the VB (*open bars*); nonsterilized soil from the interior of the VB (*slashed bars*); sterilized soil from the interior of the VB (*back slashed bars*). *Chloris virgata* and *Dasyochloa pulchella*: species from the upslope fringe of the VB. *Pleuraphis mutica* and *Trichloris crinita*: species from the interior of the VB

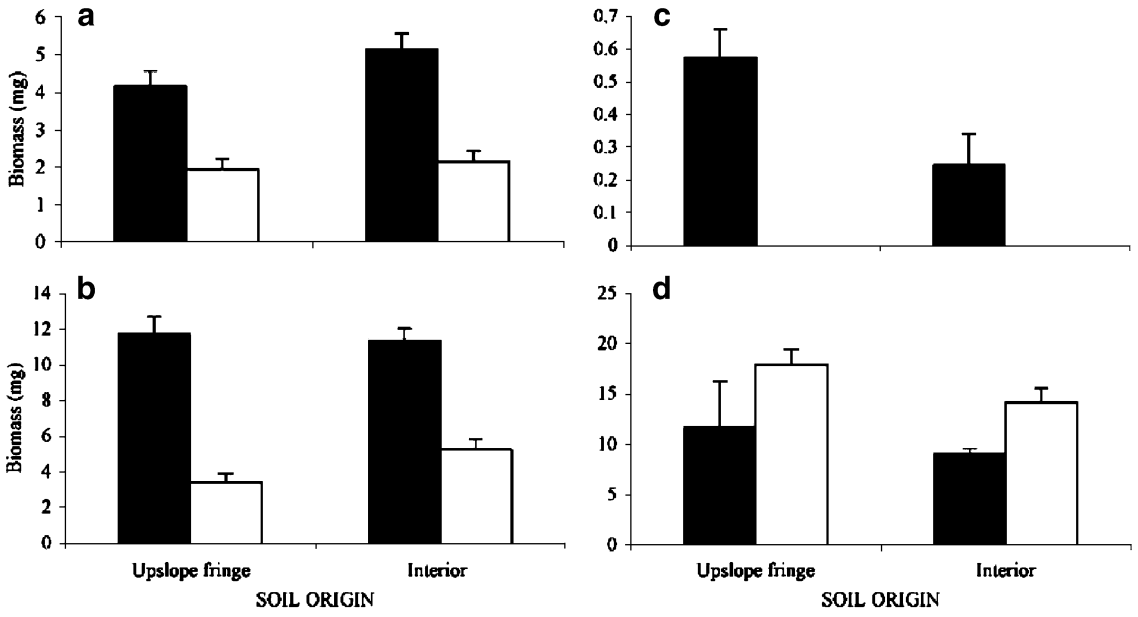

Fig. 3 Biomass production of surviving grass seedlings (mean±SE) 30 days after transplantation in nonsterilized soil (*closed bars*) and in sterilized soil (*open bars*). Species from the interior of the VB: **a** *Pleuraphis mutica* and **b** *Trichloris crinita*. Species from the upslope fringe: **c** *Dasyochloa pulchella* and **d** *Chloris virgata*

32 seedlings of *D. pulchella* survived. Most of the surviving seedlings of *D. pulchella* (19 seedlings representing 59% of them) grew on nonsterilized soil from the upslope fringe. The two species of the interior of the VB (*P. mutica* and *T. crinita*) showed a consistent pattern in nonsterilized soil: a large number of seedlings survived regardless of soil origin. However, it must be quoted that far more *T. crinita* than *P. mutica* seedlings survived in the experiment after sterilization, independent of soil origin.

Similar results were obtained analyzing a second model that excluded *D. pulchella* but evaluated the effect of the foliar length as a covariate (which showed no significant effect on survivorship).

Biomass production and AM responsiveness

The two species from the interior of the VB showed a consistent pattern in the production of biomass (Fig. 3a,b). Plants of *P. mutica* (F=7.4; df=1, 92; P=0.0077) and plants of *T. crinita* (F=20.1; df=1, 156; P<0.0001) were larger in nonsterilized soil than in sterilized soil, regardless of soil origin. Overall, the AM responsiveness for these species was positive (Fig. 4). However, the responsiveness of *T. crinita* in the two soil origins was different, whereas in *P. mutica*, the soil origin did not matter.

The covariate foliar length of the seedlings at the beginning of the experiment also had a significant effect on the final biomass of *P. mutica* (F=7.4; df=1, 92; P=0.0076) and *T. crinita* (F=9.3; df=1, 156; P=0.0026). Only for *P. mutica* did the interaction between soil origin and the covariate had a significant effect on biomass production (F=4.6; df=1, 92; P=0.0034): Initially larger plants in the soil of the interior of the VB tended to have a larger final biomass, whereas for those plants in soil from the upslope fringe the slope did not differ from zero.

The two species from the upslope fringe showed marked differences in biomass production. *D. pulchella* was about 2.4 times larger in the soil from the upslope fringe than in the soil from the interior (Fig. 3c). In sterilized soil, only three seedlings survived with $0.16\pm5.49\times10^{-5}$ mg of biomass. The mean value of *D. pulchella* biomass grown in nonsterilized soil ($0.386\pm6.34\times10^{-5}$ mg) doubled that of sterilized soil, although we could not statistically test this difference due to the low survival of the species in sterilized soil. For *C. virgata* the sterilization treatment affected biomass production (Fig. 3d). Plants in the sterilized soil were about 29% larger than those on nonsterilized soil (F=5.9; df=1, 191; P=0.0016). Overall, the AM responsiveness for this species was negative and this effect was stronger in the soil from the upslope fringe

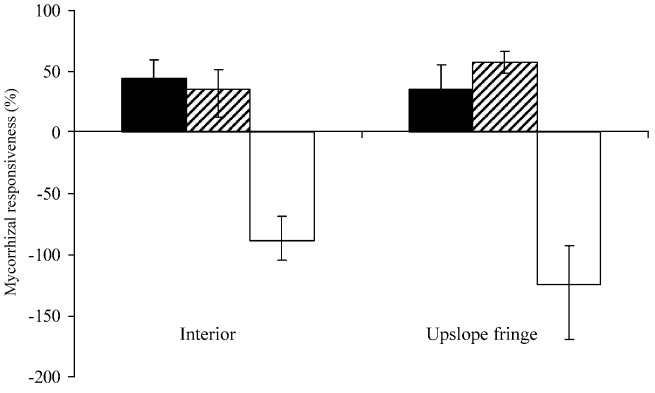

Fig. 4 Mycorrhizal responsiveness (%) of grass seedlings (mean± 95% confidence intervals from the bootstrap analysis). *Pleuraphis mutica* (*filled bars*), *Trichloris crinita* (*hatched bars*), and *Chloris virgata* (*white bars*)

than in the soil from the interior of the VB. Similar to the species of the interior, initially large seedlings of *C. virgata* resulted in large seedlings at the end of the experiment (F=9.1; df=1, 191; P=0.003).

Root/shoot ratio

There were significant differences among species in root/shoot ratio (F=1,162.9; df=2, 451; P<0.0001). *C. virgata* had the lowest root/shoot ratio (22.4±1.06) whereas *P. mutica* had the highest value (34.74±1.81) and *T. crinita* was intermediate (29.91±1.46) but significantly different from the other two species (t=86.9, df=361, P<0.0001; t=69.4, df=262, P<0.0001). Overall, the seedlings produced a larger root/shoot ratio (due to a larger root system) when growing in sterilized soil (31.15±4.01) than in nonsterilized soil (26.88±2.81; F=5.9, df=1, 451; P=0.0148). Only the species by origin interaction was significant (F=5.284; df=2, 451; P=0.0054). The significance of this interaction is given by the differential effect on *T. crinita* of soil origin; in the soil of the interior, the root/shoot ratio was smaller (25.06±2.44) than in the upslope fringe (34.75±3.98) for this species.

Root colonization

AM fungi colonized seedlings of all four species that grew in nonsterilized soil and there were significant differences between species (F=7.2; df=3, 32; P=0.0008; Table 4). *D. pulchella* had the lowest level of colonization whereas the other three species did not differ significantly. Although soil origin had no significant effect (P>0.05) on the colonization by AM fungi in any of the grass species, the suggested pattern is that grasses tend to have more extensive colonization when they grew in the soil from the same zone of the VB where they naturally occur (Table 4). Overall, the root colonization by AM fungi in seedlings growing in sterilized soil was 2.4±1.26% (mean±SE). Only four individuals out of the 32 analyzed (five individuals of each species in each soil origin but for *D. pulchella*, only two individuals survived) were colonized and the four of them grew in sterilized soil from the interior of the VB, which represents 25% of the plants under this combination. In addition, we estimated whether AM fungi had colonized roots of seedling that died through the experiment. For *D. pulchella* 33% of the dead seedlings that grew in soil from the upslope fringe were colonized by AM fungi, whereas none of the dead seedlings that grew in soil from the interior were colonized. For *P. mutica*, none of the dead seedlings analyzed were colonized by AM fungi regardless of the soil origin.

Root colonization by fungi other than AM was represented by thick and dark cenocytic hyphae most certainly of *Zygomycetes*. This kind of hypha was observed in all four species (Table 4) but was lowest in *C. virgata* (F=4.33; df=3, 32; P=0.0119).

Discussion

The AM inoculum density in the interior of the VB (202±22.7 spores/100 g soil, mean±SE) in MBR is higher than those reported for other arid environments in the same season: from the Tehuacán Valley, on average 152 spores/100 g soil, below legume shrubs canopies (Camargo-Ricalde and Dhillion 2003) and for the Sonoran Desert, 28 spores/100 g soil under shrubs and arborescent cacti canopies (Carrillo-García et al. 1999). This suggests that the plant community in the VB may be more responsive and/or dependent on mycorrhiza than other arid communities. However, an alternative explanation could be that the set of species of AM fungi occurring in the VB has a higher rate of sporulation than those in Tehuacán and the Sonoran Desert. Unfortunately, a more detailed comparison is not possible because Camargo-Ricalde and Dhillion (2003) and Carrillo-García et al. (1999) did not provide a list of the AM fungi species.

The model proposed by Reeves et al. (1979) and Janos (1980) predicts that plant species in late successional stages will be highly dependent on AM, whereas a greater proportion of plants in early successional stages will be slightly responsive or not responsive to AM. In accordance with this model, the two grass species from the interior of the VB (late successional stage) had high levels of colonization under field conditions, whereas *D. pulchella*, a species from the upslope fringe, had low levels of colonization by AM fungi. On the other hand, *C. virgata*, a species from the upslope fringe with high levels of colonization, did not fit the prediction of this model. Furthermore, the high levels of AM colonization in *C. virgata* also oppose the findings of Collier et al. (2003) because this species has a fine and highly branched root system.

The high levels of colonization of the two species from the interior of the VB may be simply a consequence of the high AM inoculum density found in this zone. However, seedlings of these two species in the experiment were also highly colonized in soils from the upslope fringe containing only 30.6±3.3 AM fungi spores per 100 g of soil. Furthermore, all dead seedlings of *P. mutica* that grew in nonsterilized soil showed no sign of colonization by AM fungi, a fact that suggests *P. mutica* depends on the interaction with AM fungi for establishment. In addition, the biomass production of seedlings of the two species from the interior decreased significantly when grown on sterilized soil. Consequently, they had a strong AM responsiveness, which may be slightly overestimated because sterilized soils also had a lower concentration of N than control soil. The root/shoot ratio of these species also changed significantly in response to soil sterilization. More carbon was allocated to underground structures when AM fungi were removed. All this is in accordance to the perennial life cycle of these species and their root morphology (thick roots) attributes that are usually associated with plants that benefit form the symbiosis with AM (Hetrick et al. 1990; Smith and Smith 1996; Wilson and Hartnett 1998; Collier et al. 2003). Therefore,

the overall evidence suggests that *P. mutica* and *T. crinita* are in fact dependent and responsive to AM. The AM responsiveness for these two species may be underestimated in this study because about 25% of the analyzed individuals that grew in sterilized soil from the interior of the VB were colonized (at low levels) by AM fungi. Therefore, it is very likely that we would find a stronger AM responsiveness if the sterilization process was entirely effective.

For the species of the upslope fringe, *C. virgata* and *D. pulchella*, there is no clear pattern. *D. pulchella* is a short-lived perennial species having relatively thick roots, a characteristic that is usually present in AM-dependent species. Nevertheless, *D. pulchella* showed low levels of colonization. This is consistent with previous reports of the colonization of *D. pulchella* (synonym *Erioneuron pulchellum*) and *Tridens muticus* (a close relative species) by AM fungi in the Mojave Desert (Titus et al. 2002). However, other relatively close species, *S. brevifolius* (also commonly found in the upslope fringe of the VB in MBR) that shares some life history attributes (perennial, small size, and stoloniferous) with *D. pulchella* is highly colonized by AM fungi (personal observation). The low levels of colonization and survivorship of *D. pulchella* in all soil conditions suggest that factors other than AM symbiosis could be affecting the establishment of this species, an aspect that deserves further investigation.

In contrast to *D. pulchella*, *C. virgata* is an annual species with a fine and highly branched root system, attributes that are usually associated with species that are slightly dependent or nondependent on AM fungi (Hetrick et al. 1990; Smith and Smith 1996; Wilson and Hartnett 1998; Collier et al. 2003). However, despite a negative effect of the AM fungi symbiosis for this species suggested by a negative AM responsiveness, we observed that adults of *C. virgata* under field conditions and seedlings of the same species in growth chamber experiments had high levels of colonization. These facts suggest that an unfavorable cost/benefit balance results from the interaction between *C. virgata* and AM fungi. Alternatively, interactions with parasitic organisms (also eliminated by sterilization) could also explain these results although *C. virgata* had the lowest level of colonization by fungi other than AM (Table 4) and we found no signs of necrosis in any of the four species.

Bearing in mind that the P concentrations in the VB both in the upslope fringe and the interior are low (around 4 mg kg^{-1}), our hypothesis that the interaction of *C. virgata* with AM fungi is parasitic is in accordance with the observations of Bethlenfalvay et al. (1983) and Bethlenfalvay and Dakessian (1984) who established experimentally that the benefits of the interaction between plants and AM fungi occur in a narrow range of P concentration (4–12 mg kg^{-1}). Under field conditions, adult plants of *C. virgata* showed medium levels of colonization (27.8±3.64%) by AM fungi, but we have no data to infer about the cost/benefit balance of these symbiosis in *C. virgata* in adult stage. Potential changes in the outcome of AM symbiosis as a function of plant phenology are worth investigating and *C. virgata* may be an interesting system to study such changes.

The effects of sterilization on plant performance cannot be strictly attributed to the elimination of AM fungi because the sterilization procedure is not AM fungi-specific and consequently, other soil organisms were surely affected. Also, there were significant changes in N and P concentrations. However, we strongly believe that the changes in soil mineral nutrient concentrations and in the microbiota due to sterilization are not sufficient to explain the observed plant responses especially because there are inconsistent results among species.

The overall evidence suggests that AM fungi may play important roles in the successional dynamics of the two-phase mosaic plant community in MBR. Pioneer grass species are not dependent to AM or, if associated to AM fungi at least for the seedling stage, the interaction seems to be parasitic as suggested by the negative AM responsiveness. More detailed experiments are needed to draw conclusive evidence about this possible parasitic relationship. On the other hand, late-successional grass species are strongly dependent and responsive to AM.

Furthermore, there are differences in the species richness of AM fungi between the upslope fringe (11 species, four of them exclusive to this zone) and the interior of the band (20 species, 13 of them exclusive). Such differences could be correlated with plant species richness that is almost twice in the interior of the VB than in the upslope fringe (C. Montaña, unpublished observation). This would be in accordance with the proposals of Bever (2003) who found for temperate grasslands in North America, asymmetric associations and changes in host specificity between plants and AM fungi and a positive correlation between plant species diversity and the diversity of the AM fungi community.

Further investigations aiming to gain insights into the successional dynamics of the VB should consider the AM interactions of nongrass species, such as forbs and bushes, and to record the dynamics of the AM fungi interactions in different seasons.

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