Mycorrhiza (2008) 19:47–60
DOI 10.1007/s00572-008-0203-4

ORIGINAL PAPER

High compatibility between arbuscular mycorrhizal fungal communities and seedlings of different land use types in a tropical dry ecosystem

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Received: 24 March 2008 / Accepted: 11 September 2008 / Published online: 26 September 2008

Abstract We conducted this study to explore limitations for the establishment of mycorrhizal associations in disturbed areas of the tropical dry ecosystem in the Chamela region of Jalisco, Mexico. Specifically, we: (1) assessed the diversity and composition of arbuscular mycorrhizal fungal (AMF) communities through spore morphospecies identification in three common land uses (primary forest, secondary forest, and pasture), (2) tested the inoculum potential of the AMF communities and the effect of water stress on the establishment of mycorrhizal associations in seedlings of various plant species, and (3) explored the importance of AMF community composition on early seedling development. Soil and root samples were taken from 15 random points in each of three plots established in two primary forests, two 26-year-old secondary forests, and two 26-year-old pastures. We expected that because of soil degradation and management, pastures would have the lowest and primary forests the highest AMF species richness. We found evidence for changes in AMF species composition due to land use and for higher morphospecies richness in primary forests than in secondary forests and pastures. We expected also that water stress limited plant and mycorrhizal development and that plants and AMF communities from secondary forests and pastures would be less affected by (better adapted to) water stress than those from the primary forest. We found that although all plant species showed biomass reductions under water stress, only some of the plant species had lower mycorrhizal development under water stress, and this was regardless of the AMF community inoculated. The third hypothesis was that plant species common to all land use types would respond similarly to all AMF communities, whereas plant species found mainly in one land use type would grow better when inoculated with the AMF community of that specific land use type. All plant species were however equally responsive to the three AMF communities inoculated, indicating that all plants established functionally compatible AMF in each community, with no preferences. The results suggest that early seedling growth and mycorrhizal development in secondary forests and pastures is not likely limited by diversity, quantity, or quality of mycorrhizal propagules but by the high temperature and water stress conditions prevailing at those sites.

Keywords Arbuscular · Land use · Mycorrhiza · Pasture · Tropical dry forest · Secondary forest · Water

Electronic supplementary material The online version of this article (doi:10.1007/s00572-008-0203-4) contains supplementary material, which is available to authorized users.

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Introduction

Tropical dry forests along the Pacific Coast of Mexico are being continuously converted by slash-and-burn into

cultivated land (Trejo and Dirzo 2000). Soil degradation after conversion results in low productivity and abandonment after some years of use, thereby allowing natural regeneration and establishment of secondary vegetation in abandoned plots (Burgos and Maass 2004). Vegetation, soils, and microclimates are therefore under continuous change, sometimes moving towards degradation and sometimes towards rehabilitation, in this highly disturbed ecosystem. Dry forest regeneration is slow, mainly due to the severe water limitation resulting from the loss of water retention mechanisms after the removal of forest cover (Maass 1995). Low-canopy secondary vegetation dominated by leguminous shrubs establishes in abandoned land, either immediately after opening or after some years of pasture management (Romero-Duque et al. 2007). Plant diversity is much lower in secondary forests (less than 100 tree species, Romero-Duque et al. 2007) than in primary forests (over 1,000 tree species, Lott 2002), despite the large chances for re-sprouting and propagule migration resulting from the unorganized arrangement of primary and secondary vegetation and agricultural fields (Kennard et al. 2002). The reasons for the low plant diversity and the predominance of leguminous bushes and trees in secondary forests even after decades of natural regeneration are unknown, but the few attempts to introduce seeds or seedlings to pasture plots in restoration efforts have shown that water stress and high temperatures strongly limit plant survival (Burgos 2004).

Besides water availability, soil quality, which is well documented to be reduced in cleared and disturbed sites (Maass et al. 2002), is likely an important factor for plant establishment. Mycorrhizal associations may also be crucial for seedling establishment in managed and abandoned sites. Mycorrhizal associations generally alleviate various kinds of stress in plants (Smith and Read 1997) and improve plant–water relations, especially under severe water limitation conditions (Augé 2001). Mycorrhizal associations established between native arbuscular mycorrhizal fungi (AMF) communities and plant species of the tropical dry ecosystem in the Chamela region of Jalisco (Mexico) have been studied in greenhouse experiments (Huante et al. 1993; Borrego-Kim 1999) and in undisturbed and disturbed field sites (Allen et al. 1998; Aguilar-Fernández 2000; Alvarez-Santiago 2002). The latter field studies have documented a loss of AMF species during the first years after slash-burn and up to 10 years after land conversion in various pasture plots of the same region. However, the significance of AMF species loss or of changes in AMF species composition on the functionality of mycorrhizal associations in managed or abandoned sites where harsh conditions limit plant establishment and growth remains unclear.

Guadarrama-Chávez et al. (2008) found highly diverse AMF communities, with similar infective potential, in plots 0–27 years after abandonment of agricultural practices in a tropical dry ecosystem in Oaxaca, Mexico. Allen et al. (2003) showed in another dry forest region in the Yucatan peninsula, Mexico that despite the reduction in AMF species after conversion of primary forest, the new fungal communities established had a good inoculum potential and contained species with higher plant-growth-promoting effects than the AMF communities of the primary forest. Similar results showing at least temporary high functional compatibility of AMF communities from disturbed sites with plant species from primary forest were reported also by Asbjornsen and Montagnini (1994) and Fischer et al. (1994) in other tropical forests. This evidence suggested that lack of functionally compatible AMF symbionts is not likely a factor hampering the recovery of vegetation in cleared areas as long as the loss of diversity is not severe. However, other local factors like plant species, soil quality, water, light, temperature, pathogens, etc. may play an important role in the establishment of functional mycorrhizal associations under field conditions (Allen et al. 2005). The interaction between water stress and the establishment of functional mycorrhizal associations, for example, which is highly relevant in this context, has not been explored. In addition, no work has been conducted in older pastures and secondary forests where soils have been severely degraded and dry and hot microclimate conditions have prevailed for a long time.

We conducted this study to understand potential limitations for plant establishment by natural regeneration or by restoration practices in disturbed areas of the tropical dry ecosystem region of Chamela, Jalisco, Mexico. Specifically, we: (1) described the AMF communities of three common land uses (primary forest, secondary forest, and pasture); (2) tested the inoculum potential of the AMF communities and the effect of water stress on the establishment of mycorrhizal associations in seedlings of various plant species; and (3) explored the importance of AMF community composition on early seedling development. We tested three hypotheses. The first hypothesis was that because of soil degradation and management, pastures would have the lowest and primary forests the highest AMF species richness. The second hypothesis was that water stress limits plant and mycorrhizal development and that the mycorrhizal associations established by the AMF communities from the primary forest would be more affected by water stress than the AMF communities from the disturbed sites (expected to be better adapted to water stress). The third hypothesis was that plant species common to all land use types would respond similarly to all AMF communities, whereas plant species found in one land use type would grow better when inoculated with the AMF community of the corresponding land use type.

Materials and methods

Study site

The study area is located at the Pacific Coast in the State of Jalisco, Mexico, (19°29′ N, 105°01′ W) and has a mean annual temperature of 25°C and mean annual rainfall of 746 mm, concentrated between June and October (García-Oliva et al. 2002). Soils are Eutric Regosols with slightly acid pH (Cotler et al. 2002). The primary dry forest located in the Chamela-Cuixmala Biosphere Reserve is a highly diverse tropical dry forest where the majority of plant species are leafless half of the year (Lott 1993). It is dominated by deciduous trees 4–15 m in height. Common tree species include *Bursera* spp., *Jatropha simpetala* Standl. & Blake (J. Standleyi Steyerm.), *Caesalpinia eriostachys* Benth, *Caesalpinia coriaria* (Jacq.) Willd, *Cordia alliodora* (Ruiz & Pav.) Oken, and *Lonchocarpus constrictus* Pitt. (Lott 2002). The pastures include the introduced forage grasses *Panicum maximum* Jacq. (Guinea grass) and *Cenchrus ciliaris* L. (Buffel grass), herbaceous weeds, and thorny leguminous shrubs. The secondary forests are dominated by thorny legumes and in our sites specifically by *Mimosa arenosa*, (Willd.) Poir. var. *leiocarpa* (DC.) Barneby, which is a non-native species not found in primary forest (Romero-Duque et al. 2007) and associated with the introduction of cattle to the new pastures. Secondary forests with more than 20 years of natural regeneration in this area may be diverse in plant species (Romero-Duque et al. 2007), but the soil is still largely exposed to erosion, desiccation, and solar radiation due to a scarce litter layer.

Primary forest, secondary forest, and pasture sites with the same management history, landscape position, and soil type were selected within the region. Because of the high variation in management history, we found only two sites of secondary forest and pasture that met the selection criteria. The management history of the study sites is summarized in Table 1. The primary forest sites were located 0.45 km NE (Búho, PF1) and 0.87 km N (Tejón, PF2), respectively, of the main buildings of Chamela Biology Station (ChBS) within the Chamela-Cuixmala Biosphere Reserve. The pasture sites were located at the Ejido of San Mateo, 8 km NW (Cerrito, P1) and 9 km N (Estanque, P2) of ChBS. Pastures had been in use for 26 years, with intensive foraging, clearing fires, and herbicide weed control. Secondary forest sites were located in the Ejido of Quémaro, 18 km (Guayabilloso, SF1) and 22 km N (Abuela, SF2) of ChBS. Secondary forests developed after 6 years of use as pastures and 26 years of natural regeneration, with occasional cattle grazing and wood extraction. Three parallel 10-×15-m plots at least 10 m apart were established at each site in south-facing hillsides with the long side of the plot along the slope.

AMF community characterization

Soils were collected in February, July and October 2005, May and October 2006, and May and September 2007 from 15 random points (0–20 cm depth) located along the slope within each plot and mixed to form a composite soil sample per plot. These samples were used for spore extraction and spore propagation. Soil samples for spore extraction and identification were refrigerated until processing. AMF trap cultures for spore propagation were established in 2 kg soil pots and 300 g soil cylindrical containers. Pots and containers were planted with seedlings of the species used for the experiments (selected herbaceous and shrubs species of the tropical forest) and maize as host trap plants. Plants were grown in greenhouses of the Chamela Biological Station and Centro de Investigaciones en Ecosistemas, in Jalisco and Michoacan, Mexico, respectively, for at least 6 months before examining spore propagation.

AMF spores were extracted from 100 g soil by wet sieving and decanting (Gerdemann and Nicolson 1963) and centrifugation in 50% sucrose. Spores were mounted on slides with polyvinyl alcohol-lactic acid-glycerol (PVLG) with and without Melzer's reagent (Brundrett et al. 1994). They were identified using original species descriptions summarized in Schenck and Pérez (1990) and represented on-line at http://www.lrz-muenchen.de/~schuessler/amphylo/amphylo_species.html and isolate descriptions of

Table 1 Management history of the field sites

	Primary forest		Secondary forest		Pasture	
	1	2	1	2	1	2
Conversion	–	–	1973	1973	1979	1979
Disturbance	–	–	Clear-cut	Clear-cut	Slash-burn	Slash-burn
Years in use	–	–	6	6	26	26
Land use	Reserve	Reserve	Cattle	Cattle	Cattle	Cattle
Abandonment	–	–	1979	1979	–	–
Regeneration	–	–	26 years	26 years	0	0

(–) does not apply to the land use in question

reference cultures and vouchers for many species presented on-line at http://invam.caf.wvu.edu/index.html. Most of the identifications correspond to spores isolated directly from field samples, as spore reproduction in the trap cultures with soils from several sampling dates and established with several of the plant species of the study area, and maize repeatedly failed in most cases. Field spores were often too damaged to allow proper wall structure evaluations; many spores had to be assigned to *Glomus*-, *Acaulospora*-, or *Gigaspora*-like groups. A new morphospecies was assigned when at least ten spores of the same type, clearly different from the others found, were recovered from the sievings. A final list with the morphospecies found in the entire set of field samples and propagation pots was used to calculate Sørensen's similarity index (Magurran 1988), based on presence/absence criteria: The number of morphospecies shared by sites a and b was multiplied by two and divided by the sum of morphospecies present in site a and site b.

Experiment 1. Inoculum potential and water stress

The experiment was a completely randomized factorial with three factors: soil (six levels), plant species (six levels), and watering regime (three levels) and five replicates of each treatment combination. In February 2005, during the dry season, soil was collected from the area surrounding the three plots marked in each site, at 0–20 cm depth, for the experiment. The soil collected at each site was mixed; stones were removed by passing it through a 1-cm mesh sieve and stored at room temperature. Pots were filled with 500 g soil from each site. The soils from the two sites of each land use type were kept separate, and the six sites were considered independent levels of the site factor. Plant species for the experiments were selected depending on seed availability, seed germination, and main habitat distribution to include species from different families and with both narrow and wide distribution range within the three land use types (Table 2). Seeds were collected from at least ten individuals of each plant species. Plant species used for this experiment were *C. eriostachys*, *Caesalpinia platyloba*, *Ipomoea wolcottiana*, *Tabebuia rosea*, *Acacia farnesiana*, and *Enterolobium cyclocarpum*. A non-mycorrhizal species, *E. cyclocarpum*, was included as a reference to test for other soil effects on plant growth, which were not attributable to AMF. Seeds were surface-sterilized for 10 min with 5% with sodium hypochlorite, rinsed with water, scarified chemically or mechanically when needed, rinsed with water, and sown on wet sand trays. Two germinated seedlings were planted in each pot and thinned to one after establishment. Extra pots were prepared for all treatments to ensure survival of at least five replicates, as many species are prone to fungal attack as early seedlings, and to make periodical evaluations without disturbing the experimental pots. Pots were placed in a greenhouse located at Chamela Biology Station inside the Reserve. The plants were placed on partially shaded greenhouse benches to reduce excessive heat and radiation. Average non-controlled growing conditions were minimum temperature, 25°C, maximum temperature 41°C, up to 1,200 microeinsteins per square meter per second photosynthetic photon flux density, and 12 h natural photoperiod. Daily regimes of photoperiod, light intensity, temperature, and relative humidity are almost constant during the dry season in this region, and the growing conditions were therefore uniform during the experiment. Plants were watered by weight to field capacity for 2 weeks to ensure seedling establishment before starting the watering treatments. Watering afterwards was done every second day to either 100%, 80%, or 60% of the measured soil water holding capacity. Plants were maintained under these conditions for four more weeks before evaluating shoot and root development in some of the extra pots. All plant species tested are slow-growing at the early stages and had not developed enough lateral roots to determine mycorrhizal colonization. Plants were allowed to grow three more weeks before being harvested. All pots were watered to 100% of the water holding capacity the night before harvesting. Stem water potential was measured with a Scholander pressure pump PMS-670 (Instrument Co., Oregon, USA), immediately after cutting the shoot at the base. All measurements were carried out before dawn of the harvest day. Leaves were scanned between two transparent acetate sheets, and the images obtained were processed with the software ImageJ version 1.37 to calculate leaf area. It was not possible to measure leaf area for the legume *E. cyclocarpum* because the leaves began to fold immediately after cutting. The shoot was dried and weighed. Roots were washed from the soil, weighed and divided in two subsamples. One sample was dried and weighed, and the other one was stained in trypan blue, mounted on slides, and scored for mycorrhizal colonization as in McGonigle et al. (1990).

Experiment 2. AMF communities and seedling growth

The experiment was a completely randomized factorial with two factors: inoculation (four levels) and plant species (eight levels) and six replicates of each treatment combination. The four inoculation levels were the AMF communities of one of the primary forest sites (Búho, PF1), one of the secondary forest sites (Guayabilloso, SF1), one of the pastures (Estanque, P2), and an autoclaved mixture of the inocula (control).

Soil used as substrate was collected from the area surrounding the three plots marked in one of the primary

Table 2 Scientific names, families, abbreviations used in text and figures, and main habitats of the plant species studied

Species	Family	Abbreviation	Habitat
Acacia farnesiana (L.) Willd.	Leguminosae	A. far	SF, P
Amphytperigium adstringens (Schlecht.) Schiede	Julianaceae	A. ads	PF, SF
Caesalpinia eriostachys Benth.	Leguminosae	C. eri	PF, SF, P
Caesalpinia platyloba S. Wats.	Leguminosae	C. pla	PF, SF, P
Cochlospermum vitifolium (Willd.) Spreng.	Cochlospermaceae	C. vit	PF
Enterolobium cyclocarpum (Jacq.) Griseb.	Leguminosae	E. cyc	PF
Hintonia latiflora (Sessé & Moc. ex DC.) Bullock	Rubiaceae	H. lat	PF, SF
Ipomoea wolcottiana Rose	Convolvulaceae	I. wol	SF, P
Mimosa arenosa (Willd.) Poir. var. *leiocarpa* (DC.) Barneby	Leguminosae	M. are	SF
Ruprechtia fusca Fern. [R. standleyana Cocucci]	Polygonaceae	R. fus	PF, SF
Tabebuia rosea (Bertol.) DC.	Bignoniaceae	T. ros	PF

The only non-native species is *Mimosa arenosa* (Romero-Duque et al. 2007). Habitats: *PF* primary forest, *SF* secondary forest, P pasture

forest sites (Búho, PF1), 0–20 cm depth, for the experiment. The soil was mixed and stones were removed by passing soils through a 1-cm mesh sieve. Mycorrhizal propagules were eliminated from the soil by heating for two 24-h periods in an electric soil heater and allowing aeration between and after the heating periods. Three kilograms disinfected soil were placed in black plastic bags and mixed with 100 g of the corresponding inoculum, which consisted of roots and soil recently collected from the selected sampling plots. The plant species used for this experiment were *T. rosea*, *Hintonia latiflora*, *C. platyloba*, *M. arenosa*, *C. eriostachys*, *Amphypterigium adstringens*, *Cochlospermum vitifolium*, and *Ruprechtia fusca*. *R. fusca* was used as the nonmycorrhizal reference plant species in this experiment. Three germinated seeds were transplanted to each pot and thinned to one after establishment. Pots were placed in the greenhouse at Chamela Biological Station under the same conditions as in experiment 1 and rotated regularly on the greenhouse benches. Plants were maintained well watered for 16 weeks (average duration of the rainy season, therefore also of the growth season in the region) before harvesting. All biomass, water potential, and mycorrhizal colonization measurements were performed as explained in experiment 1. Leaf area measurements were made from photographs, instead of scanned images, with an image analyzer (ΔT-Devices Burwell Cambridge, UK).

Statistical analysis

Data from experiment 1 were analyzed by means of a three-way analysis of variance (ANOVA) with plant species, site of origin for the soil, and watering as main factors. The soils from the six sites were not grouped according to land use. A two-way ANOVA with plant species and inoculation treatment as main factors was used for the results of experiment 2. Tukey post hoc tests were conducted to separate differences among means. Data sets for the analyses were transformed as required to meet normal distribution and homogeneity of variances assumptions of ANOVA. All variables were log-transformed except for mycorrhizal colonization proportions, which were angle-transformed. Differences were considered significant at $P<0.05$. Statistical analysis were performed with Statistix 7.0 software.

Results

AMF communities

We found 39 morphospecies of AMF in the study sites (Table 3). Morphospecies not matching a published description are described in the Supplementary Electronic Material (Table S1). The largest morphospecies richness was found in the primary forest, but this was mainly due to one of the primary forest sites as the other five sites had similar richness. The AMF communities of the six sites shared at least three morphospecies: *Acaulospora* aff. *tuberculata*, *Gigaspora ramisporophora*, and *Glomus geosporum*. Besides these morphospecies, the three land use types shared as well *Acaulospora scrobiculata*, *Acaulospora* sp. 3, *Ambispora appendicula*, and *Glomus* aff. *fasciculatum* in one of their sites. Similarity indices between sites under different land use had the highest value of 0.48. The highest similarity between sites under the same land use was found in the pasture sites (0.67) and the lowest in the primary forest sites (0.51, Table 4). Secondary forest was in an intermediate state having the same similarity index (0.53) with primary forest and pasture, whose AMF communities were the least similar (0.42). AMF communities of the three land use types were therefore diverse but quite distinct, as they shared less than 30% of their morphospecies.

Table 3 List of the AMF spore morphospecies found in field samples and propagation pots from two primary forests (PF), two secondary forests (SF), and two pastures (P) of the tropical dry ecosystem in the Chamela region of Jalisco, Mexico

		Site					
		PF1	PF2	SF1	SF2	P1	P2
	Archaeosporales						
	Ambisporaceae						
1	*Ambispora appendicula* (Spain, Sieverd. & N.C. Schenck) C. Walker	X		X		X	X
	Diversisporales						
	Acaulosporaceae						
2	*Acaulospora* aff. *tuberculata* Janos & Trappe	X	X	X	X	X	X
3	*Acaulospora delicata* C. Walker, C.M. Pfeiff. & Bloss	X					
4	*Acaulospora rehmi* Sieverd. & S. Toro	X	X				
5	*Acaulospora scrobiculata* Trappe	X		X		X	
6	*Acaulospora* sp. 1	X					
7	*Acaulospora* sp. 2			X	X		
8	*Acaulospora* sp. 3	X		X	X		X
9	*Acaulospora* sp. 4					X	
10	*Acaulospora* sp. 5	X					
	Diversisporaceae						
11	*Diversispora spurca* (C.M. Pfeiff., C.Walker & Bloss) C. Walker & A. Schüβler	X				X	X
	Gigasporaceae						
12	*Gigaspora decipiens* I.R. Hall & L.K. Abbott	X					
13	*Gigaspora gigantea* (T.H. Nicolson & Gerd.) Gerd. & Trappe	X	X	X	X		
14	*Gigaspora ramisporophora* Spain, Sieverd. & N.C. Schenck	X	X	X	X	X	X
15	*Gigaspora* sp. 1	X					
	Glomerales						
	Glomeraceae						
16	*Glomus aff. diaphanum* J.B. Morton & C. Walker				X	X	
17	*Glomus aff. fasciculatum* (Thaxt.) Gerd. & Trappe, emend. C. Walker & R. Koske	X			X	X	X
18	*Glomus aff. intraradices* N.C. Schenck & G.S. Sm.						X
19	*Glomus clarum* T.H. Nicolson & N.C. Schenck		X				
20	*Glomus geosporum* (T.H. Nicolson & Gerd.) C. Walker	X	X	X	X	X	X
21	*Glomus glomerulatum* Sieverd.					X	X
22	*Glomus microaggregatum* Koske, Gemma & P.D. Olexia	X	X			X	X
23	*Glomus monosporum* Gerd. & Trappe	X	X				
24	*Glomus sinuosum* R.T. Almeida & N.C. Schenck	X	X		X		
25	*Glomus tortuosum* N.C. Schenck & G. S. Sm.	X	X	X			
26	*Glomus* sp. 1			X	X		
27	*Glomus* sp. 2	X					
28	*Glomus* sp. 3	X					
29	*Glomus* sp. 4		X				
30	*Glomus* sp. 5					X	
31	*Glomus* sp. 6				X	X	
32	*Glomus* sp. 7	X					
33	*Glomus* sp. 8					X	X
34	*Glomus* sp. 9						X
35	*Glomus* sp. 10		X				
36	*Glomus* sp. 11	X					
37	*Glomus* sp. 12	X	X		X		
38	*Glomus* sp. 13		X	X			
39	*Glomus* sp. 14	X				X	
	Total morphotypes per site	25	14	11	12	15	12
	Total morphotypes per land use	29		16		18	

Table 4 Similarity index between sites with different land use, between land use types, and within each land use, calculated from the number of spore morphotypes identified from two primary forests (PF1, PF2), two secondary forests (SF1, SF2), and two pastures (P1, P2)

Between sites	S. I.	Between land uses	S. I.	Within land use	S. I.
PF1-SF1	0.44	PF-SF	0.53	PF1-PF2	0.51
PF1-SF2	0.43	PF-P	0.42	SF1-SF2	0.61
PF2-SF1	0.48	SF-P	0.53	P1-P2	0.67
PF2-SF2	0.46				
PF1-P1	0.45				
PF1-P2	0.43				
PF2-P1	0.28				
PF2-P2	0.31				
SF1-P1	0.38				
SF1-P2	0.43				
SF2-P1	0.44				
SF2-P2	0.42				

Experiment 1. Inoculum potential and water stress

There were significant main effects and two-factor interactions, but no three-factor interactions, in the variables measured (Table 5). The plant species×water interaction was the most significant of the two-factor interactions in all cases. There were also a few site×plant species or site×water interactions, but given their low significance and lack of interpretable patterns, only site main effects and plant species×water interactions, which showed consistent trends, will be presented and discussed in this paper.

Total plant biomass was significantly higher (5–10% on average) in seedlings grown in soils from the two secondary forest soils than in soils from primary forests or pastures (F=36.3, P<0.0001). Mycorrhizal colonization percentage was above 60% of root length on average in all sites, and the significant differences in colonization between some sites (F=8.51, P<0.0001) did not indicate limitations in inoculum potential or lack of compatible AMF species with the plant species studied in any site. Data are not shown for site main effects.

Total biomass and leaf area decreased in all plant species as water availability decreased (Fig. 1a,b). The biomass decrease was less pronounced in *C. platyloba* and *E. cyclocarpum* than in the other four species, which showed significant reductions already with watering at 80% water holding capacity (Fig. 1a). Leaf area was markedly reduced in all plant species with 80% and 60% watering (Fig. 1b). Plant species differed in their water potentials at full watering, but most of them were able to maintain their water potential in the reduced watering treatments (Fig. 1c). The four leguminous species had more negative water potentials than the two non-leguminous species *I. wolcottiana* and *T. rosea*. Mycorrhizal colonization decreased with reduced watering in all mycorrhizal species except for *I. wolcottiana* (Fig. 1d). Colonization was significantly reduced in *A. farnesiana*, *C. platyloba*, and *T. rosea* when watering was reduced to 60% of the full watering value and in *C. eriostachys* already when watering was reduced to 80%.

Experiment 2. AMF communities and seedling growth

There was a significant inoculation×plant species interaction in all variables measured (Table 6). Total biomass was higher in plants inoculated with the three AMF communities than in nonmycorrhizal controls in all plant species but the nonmycorrhizal species *R. fusca* (Fig. 2a). There were no differences in plant-growth responsiveness to the three AMF communities. Leaf area followed the same pattern as total biomass (Fig. 2b). Shoot/root ratio was higher in the three mycorrhizal treatments than in the nonmycorrhizal treatment in *C. eriostachys*, *C. vitifolium*, and *M. arenosa* (Fig. 2c). There were no differences in shoot/root ratio among inoculation treatments for the other five plant species. The only plant species in which mycorrhizal plants had a higher water potential than nonmycorrhizal plants was *A. adstringens* (Fig. 2d). This species and *C. vitifolium* had also higher stem water potentials than the other six species. Inoculation with the three AMF communities resulted in similar mycorrhizal colonization percentages within each plant species (Fig. 2e).

Table 5 *F* values and probabilities of significance from the three-way ANOVA performed on variables measured at harvest in experiment 1

Variable	Plant	Site	Water	P×S	S×W	P×W	P×S×W
Total biomass	149***	36***	714***	1.5 ns	3.0**	20***	1.2 ns
Leaf area	159***	42***	809***	3.1***	5.8***	27***	1.2 ns
Water potential	213***	3.0*	16***	1.4 ns	0.9 ns	4.1***	0.7ns
Colonization	161***	8.5***	143***	1.2 ns	2.6**	18***	1.4 ns

Plant species, site, and watering regime as main effects, two- and three-factor interactions
ns not significant
*P<0.05, **P<0.01, ***P<0.001

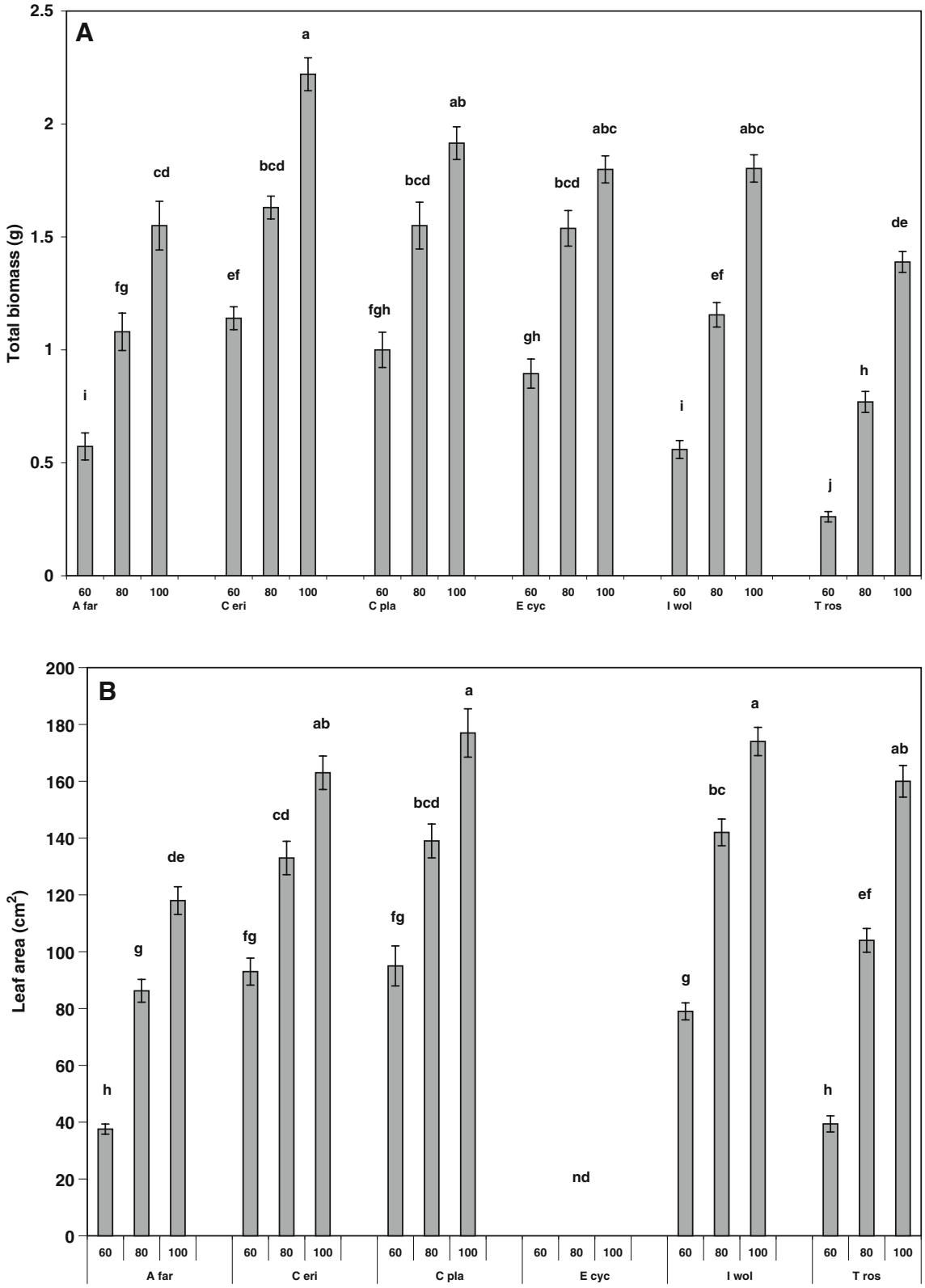

Fig. 1 Experiment 1. Total biomass (**a**), leaf area (**b**), water potential (**c**), and mycorrhizal colonization (**d**) at harvest in seedlings of several plant species from the Chamela dry ecosystem (Mexico). Plant species×watering regime interaction is depicted as the most significant factor interaction after ANOVA. Means (±SE, n=30 pooling values for the six sites) accompanied by the same letter do not differ significantly at $P<0.05$. *nd* not determined

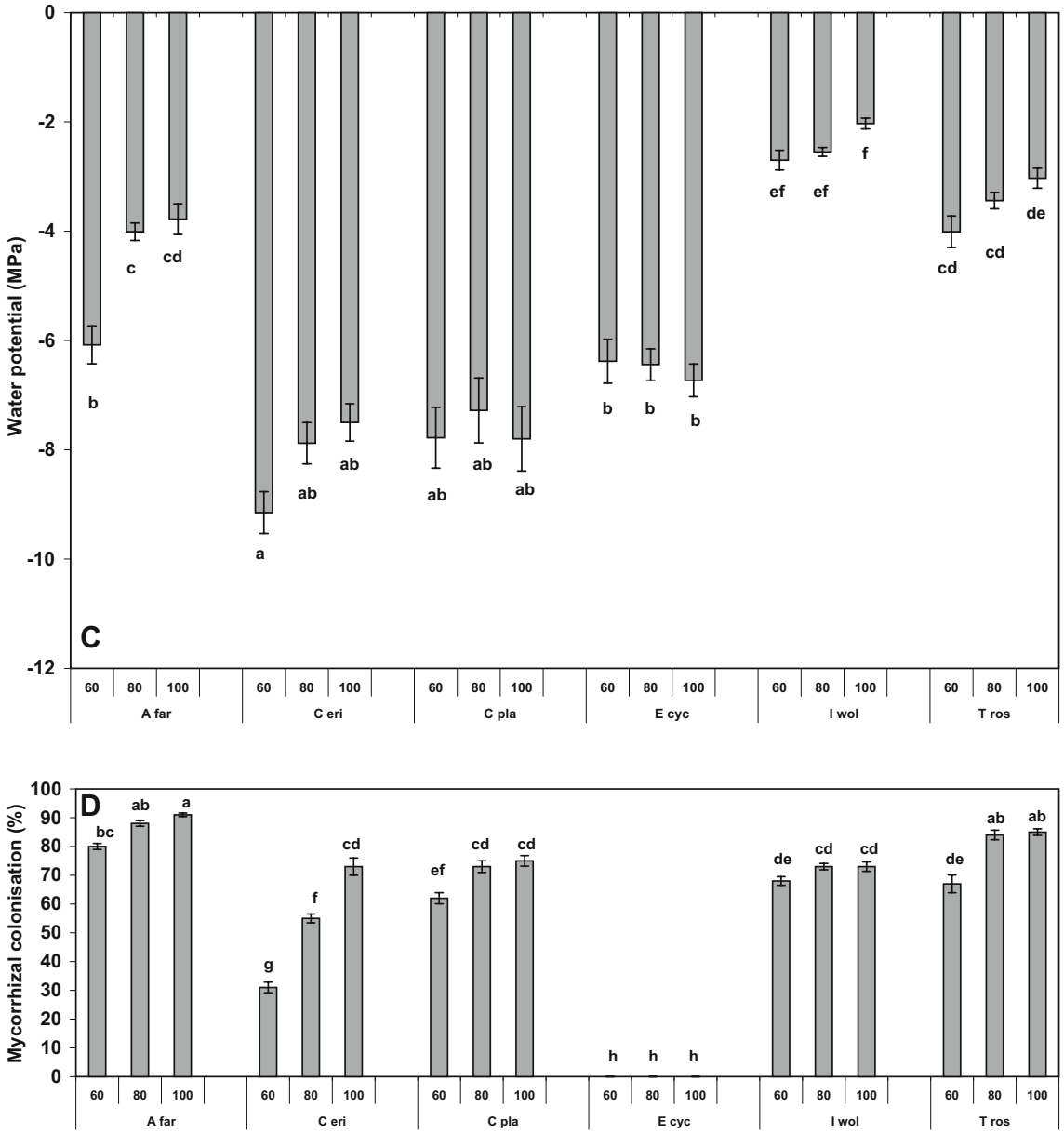

Fig. 1 (continued)

Discussion

This was the first survey examining long-term effects of pasture management or natural regeneration in abandoned plots with secondary vegetation in the tropical dry region of Chamela. Our results suggested that the study sites had quite distinct AMF communities as a result of the long time under different land use. The first hypothesis expecting the highest AMF morphospecies richness in primary forest and the lowest in pastures was only partially supported because of the large variation from site to site. Despite having, as predicted, the highest averaged morphospecies richness, the two primary forest sites were very different, and richness in one of them was as low as in secondary forest and pasture sites. Therefore, no clear separation due to land use could be made based on the number of morphospeciess found, but there was an indication for higher diversity in primary forest, which possibly could be demonstrated by increasing sampling efforts and number of sites. Such a thorough characterization of AMF communities was beyond the objectives of this study, but our results suggested that highly degraded 26-year-old pastures and 26-year-old secondary vegetation have similar morphospecies richness. Therefore, the natural regeneration time in secondary vegetation seems not to help increase AMF diversity. However, changes in species composition suggested that AMF communities from the three land uses placed them in the hypothesized order: primary forest and pasture as

Table 6 *F* values and probabilities of significance from the two-way ANOVA performed on variables measured at harvest in experiment 2

	Inoculation	Plant species	I x P
Total biomass	224*	22*	14*
Leaf area	346*	57*	24*
Shoot:root ratio	48*	74*	5.4*
Water potential	7.7*	228*	4.5*
Root colonization	178*	53*	6.7*

ns not significant
*$P<0.001$

opposite extremes. The similarity of the AMF communities from secondary forests with those from primary forests and pastures indicated that AMF communities in secondary forests were in an intermediate transition state. Therefore, although morphospecies richness measurements were not clear enough to separate land uses, changes in morphospecies composition confirmed land use groups and documented the differences in the AMF communities of the six sites compared. Allen et al. (1998) and Alvarez-Santiago (2002) reported a reduction in species richness after forest conversion in young pastures of the Chamela region. Cuenca et al. (1998) have also observed a reduction in AMF diversity in highly disturbed and revegetated sites, when compared to natural communities in Venezuela. Our results support nevertheless the observations of Guadarrama-Chávez et al. (2007) in Oaxaca, Mexico, Johnson and Wedin (1997) in tropical dry forest converted to grassland in Costa Rica, and Picone (2000) in tropical rain forests and old pastures in Nicaragua and Costa Rica where, despite the observed changes in species richness and composition, the AMF communities of forests and pastures were still highly diverse and shared many species. A remarkable finding of our AMF community characterization study was the absence of *Scutellospora* morphospecies in all our samples, given that several species were reported in previous studies (Allen et al. 1998); Aguilar-Fernández 2000; Alvarez-Santiago 2002) in primary forests and pastures of the Chamela region and by Guadarrama-Chávez et al. (2007) in Oaxaca.

The inoculum potential of the different AMF communities was high and similar in all plant species tested, so it seems that in terms of root colonization, the changes in species richness or composition were not important. Water stress limited, as expected, plant development in all plant species tested and mycorrhizal development in some of them as well. There was, however, no indication for adaptation to water stress in any of the plant species or AMF communities. All plants grown in all soils were equally susceptible to the reduction in water availability. Uninoculated plants may have experienced lower water stress, as they were smaller and had lower leaf area. This could mean that although pastures and secondary forests had shown adequate soil quality (in terms of capacity to support plant growth), good inoculum potential, and diverse AMF communities to establish functional mycorrhizal associations, harsh and dry field conditions might be hampering the development and function of mycorrhizal associations in disturbed sites. Plants in this region may experience weeks without rain and with high temperatures that burn newly established seedlings under field conditions in pastures (Burgos 2004). These conditions prevail also in secondary forests despite the presence of bushes and small trees because of the large areas with no plant cover and no litter layer where the soil is exposed. We reduced water availability in two of our treatments, but we watered regularly to maintain the treatments. It is therefore likely that plants and mycorrhizal fungi experience much stronger water stress when they grow in pastures and secondary forests and that the effects we observed are more pronounced under field conditions with the large variations of natural rain. Such a reduction in mycorrhizal colonization by water stress has been observed by Ryan and Ash (1996) and Al-Karaki et al. (2004) in field-grown wheat. We found no reports of AMF development under water stress conditions for other native plants presumably possessing adaptations to grow in water limiting environments. The mechanism behind the reduction in plant and mycorrhizal colonization development in water-stressed plants is unknown but may be associated to a reduction in photosynthesis as a consequence of stomatal closure to reduce water loss (Ryan and Ash 1996). A reduction in photosynthesis might explain the biomass reductions observed in all plant species but does not explain why mycorrhizal colonization was reduced in some, not all, plant species. The extent and relevance of mycorrhizal development reductions under water stress remains to be explored further and under field conditions.

The protection conferred by mycorrhizal associations against water stress has been widely documented in numerous plant species, most of them crops or ornamentals (Augé 2001) with commercial value. Native plants inoculated with native AMF communities had the same stem water potential as nonmycorrhizal controls in experiment 2 and were equally water stressed when growing with the AMF communities from the six sites in experiment 1. There was therefore no indication for mycorrhizal plants being more hydrated than nonmycorrhizal plants or for plants inoculated with a specific AMF community being more tolerant to water stress.

In the third hypothesis, we predicted that plant species common to all land use types would respond similarly to all AMF communities, whereas plant species found mainly in one land use type would grow better when inoculated with

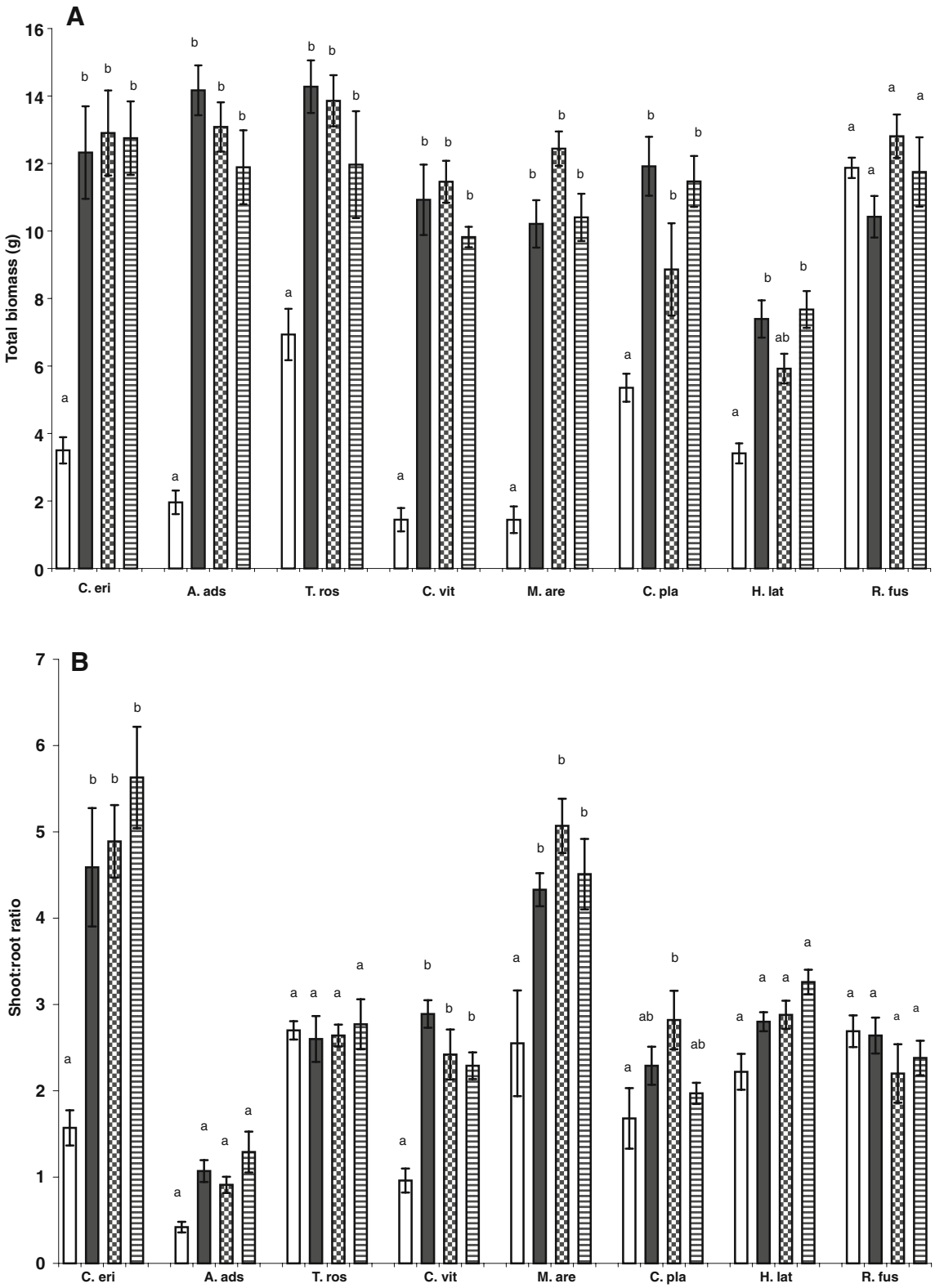

Fig. 2 Experiment 2. Total biomass (**a**), shoot/root ratio (**b**), leaf area (**c**), water potential (**d**), and mycorrhizal colonization (**e**), at harvest in seedlings of several plant species from the Chamela dry ecosystem (Mexico). Means (±SE, $n=6$) accompanied by the *same letter* do not differ significantly at $P<0.05$. *White bars* Nonmycorrhizal treatment, *dark bars* mycorrhizal with pasture AMF community, *crossed bars* mycorrhizal with secondary forest AMF community, *horizontal line bars* mycorrhizal with primary forest AMF community. *Letters* indicate differences among means only within each plant species and not among plant species, to avoid saturation of the figures showing the plant species×inoculation interaction. Relevant differences among plant species are explained in text

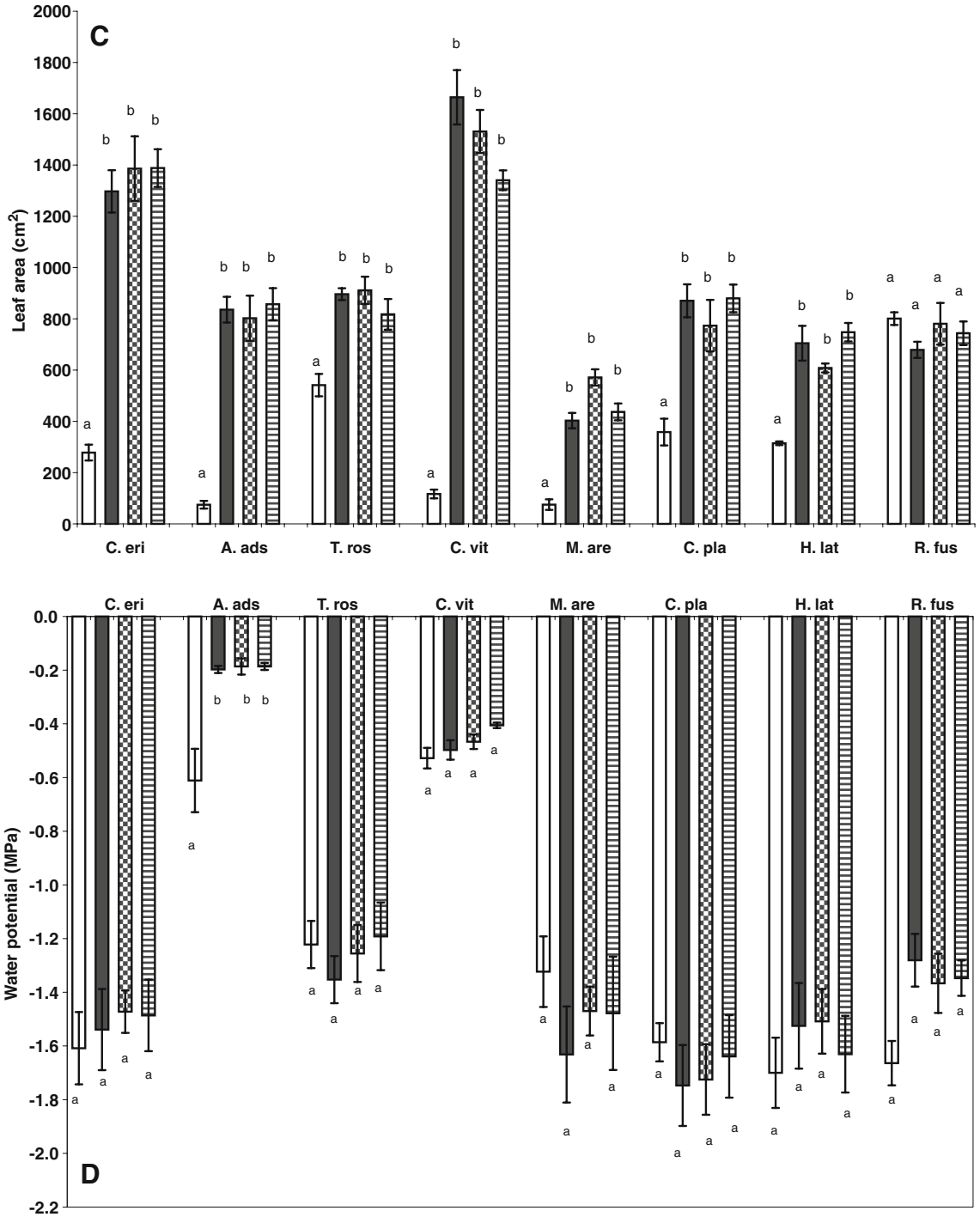

Fig. 2 (continued)

the AMF community of that specific land use type. The results showed though almost identical growth responses with the three AMF communities, indicating that all plant species found functionally compatible AMF species in each inoculum type and the three inocula were equally good at least at the seedling stage. The soil from the secondary forest sites seemed to be a better substrate for plant growth than primary forest and pasture soils because all plant species, including the nonmycorrhizal species, grew better in secondary forest soils in experiment 1. There were no differences when all plants grew on the same soil and received only a small portion of roots and soil with the three inocula in experiment 2. The fact that in both experiments the nonmycorrhizal species included did not

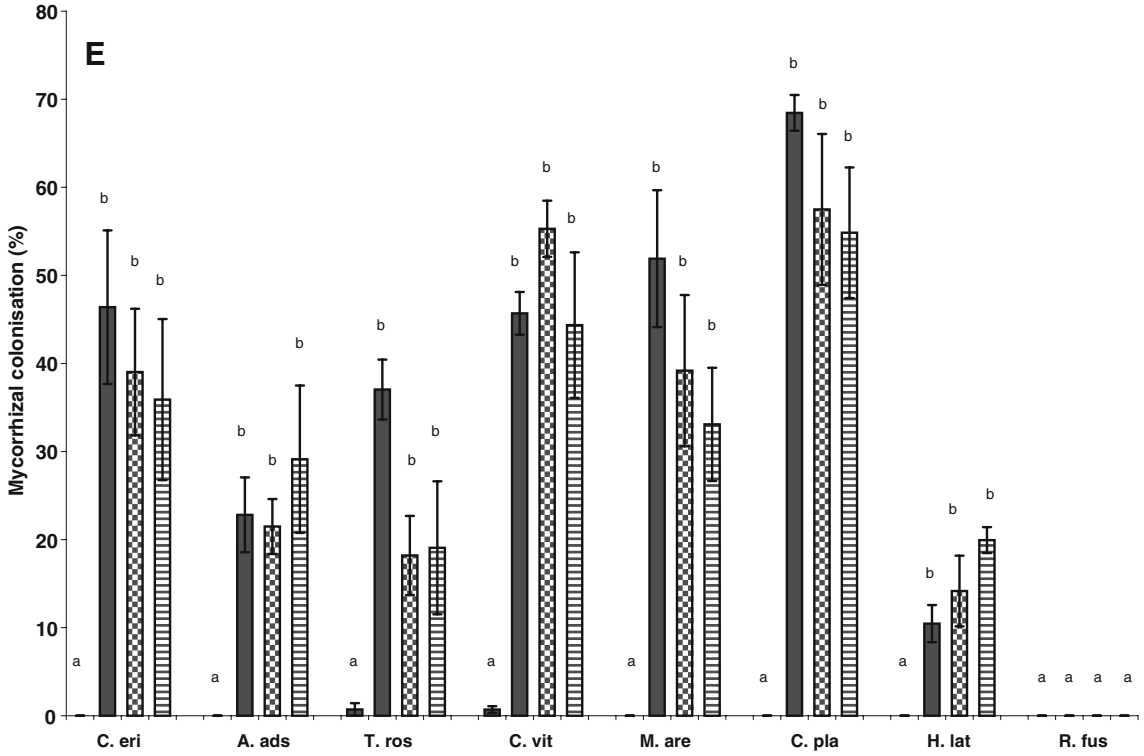

Fig. 2 (continued)

show distinct patterns from the mycorrhizal species in either growth or water relations suggested therefore no confounding effects from the microflora associated to each inoculum.

Asbjornsen and Montagnini (1994), Fischer et al. (1994), Allen et al. (2003), and Allen et al. (2005) have reported preference of certain plant species for inoculation with a specific AMF community in other tropical forests. It was sometimes a preference for the AMF communities from primary forest and sometimes for those from disturbed plots, but indicating some degree of specificity between the plant and fungal combinations tested. It seems that in our context, at least in these early growth stages, the main determinants of early plant and mycorrhizal development were the plant species in question and the availability of water, not the AMF communities. It is more likely that what limits seedling establishment in secondary forests and pastures are the microclimatic conditions that worsen drought and high temperature stress due to the absence of full plant cover and water retention mechanisms in those sites. This was nevertheless a first attempt to explore plant and AMF community compatibility at the seedling stage, and much more work is needed to finally understand how complex mycorrhizal associations evolve under secondary succession in the tropical dry ecosystem. Theoretical models have been proposed for secondary succession in the humid tropics (Janos 1980) where other limitations prevail and more information is available. Water is clearly a critical factor to explore further before similar models can be suggested for the dry tropics.

Acknowledgments We are grateful to Arturo Jiménez Martínez, Salvador Araiza, Eloy Araiza, Abel Verduzco, Gustavo Verduzco, and Ana Lidia Sandoval Pérez for valuable technical and field assistance. Mr. Ramiro Peña and Araiza family kindly allowed us to work in their pastures and secondary forests. This research was supported by Programa de Apoyo a Proyectos de Investigación Científica e Innovación Tecnológica, Universidad Nacional Autónoma de México (PAPIIT- IN222005).

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