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Arbuscular mycorrhizal fungi and plant symbiosis in a saline-sodic soil

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Abstract The seasonality of arbuscular mycorrhizal (AM) fungi–plant symbiosis in *Lotus glaber* Mill. and *Stenotaphrum secundatum* (Walt.) O.K. and the association with phosphorus (P) plant nutrition were studied in a saline-sodic soil at the four seasons during a year. Plant roots of both species were densely colonized by AM fungi (90 and 73%, respectively in *L. glaber* and *S. secundatum*) at high values of soil pH (9.2) and exchangeable sodium percentage (65%). The percentage of colonized root length differed between species and showed seasonality. The morphology of root colonization had a similar pattern in both species. The arbuscular colonization fraction increased at the beginning of the growing season and was positively associated with increased P concentration in both shoot and root tissue. The vesicular colonization fraction was high in summer when plants suffer from stress imposed by high temperatures and drought periods, and negatively associated with P in plant tissue. Spore and hyphal densities in soil were not associated with AM root colonization and did not show seasonality. Our results suggest that AM fungi can survive and colonize *L. glaber* and *S. secundatum* roots adapted to extreme saline-sodic soil condition. The symbiosis responds to seasonality and P uptake by the host altering the morphology of root colonization.

Keywords Arbuscular mycorrhizas · *Lotus glaber* · *Stenotaphrum secundatum* · Saline-sodic soil · Seasonal variation · P nutrition

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Introduction

The symbiosis between plant and arbuscular mycorrhizal (AM) fungi is one of the plant strategies for growing under a variety of stress conditions (Entry et al. 2002). The distribution of certain AM fungal species has been related to soil pH, phosphorus (P) level, salinity, soil disturbance (Abbott and Robson 1991), vegetation (Johnson et al. 1992) or hydrologic condition of the soil (Miller and Bever 1999; Ingham and Wilson 1999; Escudero and Mendoza 2005). In general, increases in soil pH, nutrient status and salinity in soil are related to a decrease in AM root colonization or in spore density (Abbott and Robson 1991).

AM fungi in saline soil may improve early plant salinity tolerance and growth (Jain et al. 1989). Several studies have reported the ability of plants to be colonized by AM fungi in saline conditions (Juniper and Abbott 1993), salt marshes (Carvalho et al. 2004), and at neutral (Yano-Melo et al. 2003) or moderate alkaline soil pH (Wang et al. 2004). However, little information exists regarding extreme soil salinity and sodicity in grasslands (Landwehr et al. 2002; Escudero and Mendoza 2005; Mendoza et al. 2005).

Adverse environmental conditions can negatively affect the infectivity and survival of AM fungal propagules from one period of root growth to the next (Juniper and Abbott 1993). However, previous contributions suggest that AM fungi could survive in soil and the roots of some forage species tolerant to saline-sodic soils (Escudero and Mendoza 2005; Mendoza et al. 2005). Improved salt tolerance of mycorrhizal plants can be mainly related to enhanced mineral nutrition, particularly N or P (Graham 1986), and changes in physiological processes such as increased carbon dioxide exchangeable rate, transpiration, stomatal conductance and water efficiency (Ruiz-Lozano and Azcón 2000).

It is known that AM fungal root colonization has seasonal dynamics, and these seasonal patterns are closely related to host phenology and climate variations (Bentivenga and Hetrick 1992; Sanders and Fitter 1992; DeMars and Boerner 1995; Allen 1996). Seasonal changes in AM fungus diversity were studied in several systems (Singüeza et al. 1996; Blaszkowski et al. 1998; Guadarrama and Álvarez-Sánchez 1999; Vestberg 1995; Lugo and Cabello 2003). Some studies were carried out in saline-sodic grassland systems (Escudero and Mendoza 2005), but the relation between temporal patterns of the symbiosis and changes in P uptake by the host were not analysed.

Lowlands in the Pampas grasslands of the Province of Buenos Aires (Argentina) occupy a significant proportion of the total production area for dairy cattle. These grasslands are subjected to periodic droughts or floods, and their soils show extreme values of salinity and sodicity. These stress conditions represent a particular situation to understand seasonal patterns of AM colonization and plant nutrient demands.

The aim of the present work was to study the relationship between seasonal changes in root colonization morphology and changes in P uptake generated by growth stages of two forage species commonly present in a saline-sodic lowlands of Depresión del Salado (Buenos Aires, Argentina).

Materials and methods

Study site and sample collection

This research was carried out in a natural grassland located in San Vicente (35°S, 58°50′W) Province of Buenos Aires, Argentina. The soil site was classified as a Salic Solonetz (FAO classification) affected by salinity (5.5 dS m^{-1} of electrical conductivity), exchangeable sodium percentage (65%) and pulses of water table position during the year. The plant community was dominated by *Lotus glaber* Mill., *Stenotaphrum secundatum* (Walt.) O.K., *Lolium multiflorum* Lam. and *Paspalum dilatatum* Poir.

Soil samples and individuals of *L. glaber* and *S. secundatum* adult plants were collected in summer (18-03-03), autumn (17-06-03), winter (18-09-03) and spring (3-12-03). *L. glaber* is a winter–spring growing legume with a taproot, and *S. secundatum* is a spring–summer growing grass with a shallow and fibrous root system. The study area consisted of two permanent and concentric circular plots of 8- and 12-m radius, respectively. The 12-m radius circular area was divided into five 72° sector subplots. In each of one of these five subplots, the inner 8-m radius sector was used to measure the seasonal changes in floristic composition, and the segment of annulus external to it (i.e. the area delimited by the 8- and 12 m-radius sector) was used for soil and plant sampling to analyse seasonal changes of soil chemical characteristics, AM fungal spore density and root colonization. This design permitted analysis of data from an experiment in which several independent observations of the same variable were recorded on each of the experimental units through time (Rowell and Walters 1976). Ten soil core samples per replicate were taken and mixed homogeneously to form a composite sample. Each soil core sample was 12 cm deep. The top 2 cm was removed to eliminate part of the top leaf litter. Each soil core contained 185 g of soil. The five composite soil samples were thoroughly mixed and divided into three portions to measure soil properties, spore and hyphae densities. Soil samples were kept in plastic bags at 4°C until processed.

Soil characteristics

The following measurements were performed on soil samples: moisture content, pH (soil/solution ratio of 1:2.5 in water), electrical conductivity at saturation (EC) (Jackson 1958), exchangeable Na and Ca (Jackson 1958), total C (Ct; Richter and von Wistinghausen 1981) and total N (Nt; Bremmer and Mulvaney 1982). Extractable P (Bray I) was determined colorimetrically with ammonium molybdate and stannous chloride reagents (Bray and Kurtz 1945).

AM root colonization

Roots were washed in tap water and cleared with 10% KOH for 10 min at 90°C, placed in 10% HCl for 2 min and then stained with 0.05% lactic-glycerol-Trypan Blue for 20 min at 90°C by a modification of the method of Phillips and Hayman (1970), omitting phenol from the reagents. Twenty-five root segments per plant were examined under microscope at ×200 magnification. AM root colonization was assessed with the method of McGonigle et al. (1990); the total colonized roots (MC), and the fraction of root length containing arbuscules (AC), vesicles (VC) and hyphae only (HO) were determined.

The number of entry points (EP) was measured at 3-mm intervals along root fragments observed under ×200 magnification (Amijee et al. 1989). EP was expressed as the number of entry points per millimetre of colonized root.

Recovery and counting of AM spores

AM fungal spores were isolated from 30 g of rhizospheric soil by a modification of wet sieving followed by a sucrose gradient centrifugation method (Daniels and Skipper 1982). Water was added to a soil sample, and the solution was passed through a 500-μm sieve followed by a 35-μm sieve.

The fraction collected in the last sieve was centrifuged in 80% sucrose solution. Spores were collected from the water–sucrose interface, poured through a sieve, rinsed with distilled water and counted under ×35 magnification in a dissecting microscope. Spore density (SD) was expressed as the number of spores per gram of dry soil.

Hyphal extraction and measurement

Hyphal density (HD) was measured in summer and winter. The length of external hyphae was determined after they were extracted with a procedure modified from Abbott et al. (1984). A sample of 2.5 g of rhizospheric soil was blended with deionized water for 30 s. The suspension was then poured through a 250-μm sieve and washed with water through a 35-μm sieve to collect hyphae. The recovered material was resuspended in water, transferred to a beaker, shaken for several seconds and allowed to settle for 1 min. The supernatant (containing hyphae) was filtered (pore size 2 μm) under vacuum. The procedure was repeated three times to insure the thorough extraction of soil hyphae. The filter (while still in the filter holder) was covered with 0.05% lactic-glycerol-Trypan Blue for 5 min. The excess of stain in the filter was removed by washing with deionized water and vacuum filtration. The filter was cut into halves and placed on microscope slides to dry off. Dry filters were mounted on polyvinyl alcohol lacto-glycerol under a cover slip. The length of the recovered hyphae was measured on 150 fields per filter using the modified grid line intersect method (Giovannetti and Mosse 1980) under ×200 magnification. HD was expressed as centimetre of hyphae per cubic centimetre of dry soil.

Concentration of P in plant tissue

Shoots and roots of *L. glaber* and *S. secundatum* were oven-dried at 75°C for 48 h, separately ground and digested in a nitric–perchloric acid mixture to determine P by the molybdovanadophosphoric acid method (Jackson 1958).

Statistical analysis

Analysis of variance was used to compare the season means of each studied variable. Mean separation was performed by the Tukey test. Correlation coefficients were calculated to quantify the association between selected pairs of variables (P in plant tissue vs MC, AC and VC; EP vs MC and SD). Soil chemical data and AM fungi variables were tested for normality and homogeneity of variances. In case of non-normality, data sets were compared with a Kruskal–Wallis non-parametric test.

Influence of the environment and season on the AM symbiosis

Canonical correspondence analyses (CCA) ordination technique by CANOCO algorithm (Ter Braak 1987–1992) was used to identify the best linear combinations of soil chemical properties, concentration of P in root tissue and environmental variables that influence the AM fungal measurements. Successive runs of CCA with different combinations of variables were made before selecting the final set of variables. An extra variable was included only if its inclusion significantly increased the total accounted variance. The combination of variables with the least number of them and the highest value of the total accounted variance was finally chosen.

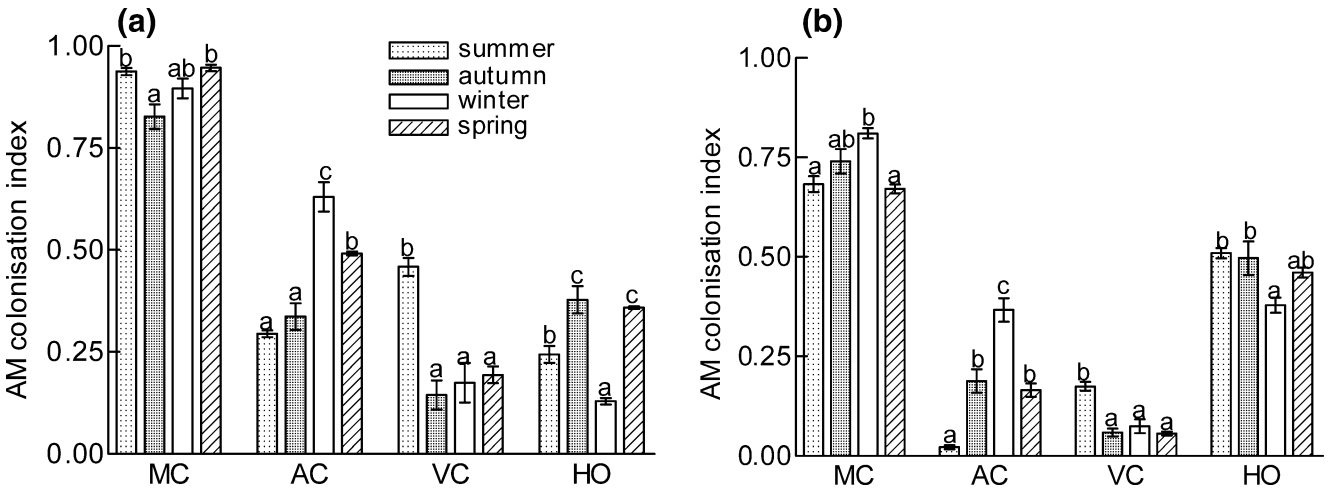

Fig. 1 Seasonal variation of AM fungal colonization in roots of *Lotus glaber* (**a**) and *Stenotaprhum secundatum* (**b**). Mean values of five replicates ± standard errors are shown. *MC* total colonized root, *AC* arbuscular colonization, *VC* vesicle colonization and *HO* hyphae only colonization. Means followed by *different letters* are significantly different ($P<0.05$) by the Tukey test

Seven AM fungi variables were included in the main matrix: total spore density in soil (SD), colonization of *L. glaber* roots (MCLt), AC (ACLt), VC (VCLt) and colonization of *S. secundatum* roots (MCSt), AC (ACSt), VC (VCSt). The second matrix contained seven variables: soil properties (P available, exchangeable Ca and Na), P concentration in root tissue of *L. glaber* (PLt) and *S. secundatum* (PSt), and air temperature (*T*°C) and rainfall (Rf) as environmental variables. The statistical significance of the three first canonical axes was analysed by the Monte Carlo permutation test.

Results

AM fungal structure

The roots of the two plant species were extensively colonized by AM fungi. The percentage of colonized root length in *L. glaber* (90%) was higher ($P<0.001$) than in *S. secundatum* (73%). The root colonization index (MC) showed both seasonal changes and different patterns in each plant species (Fig. 1a and b). In *L. glaber* (Fig. 1a), the highest mean values of MC were in summer (0.95) and spring (0.94), and the lowest in autumn (0.83). However, in *S. secundatum* (Fig. 1b), the highest values of MC were in winter (0.81), and the lowest in spring (0.67) and summer (0.68).

The mean values of AC, VC and HO were, respectively, 0.44, 0.24 and 0.28 in *L. glaber*, and respectively, 0.18, 0.09 and 0.46 in *S. secundatum* (Fig. 1a and b). The AC and VC fractions showed a similar level of seasonality for maximum and minimum in both plant species, but they took place in different seasons. The maximum AC value occurred in winter and the minimum in summer, but the maximum VC values occurred in summer and the minimum in winter. Therefore, AC and VC were negatively correlated ($P<0.05$) in the roots of the studied plants. With the exception of HO, the magnitude of the colonization structures (MC, AC and VC) in *L. glaber* was always significantly higher ($P<0.001$) than in *S. secundatum* (Fig. 1a and b).

Spore density in soil (SD) did not change during the year (Table 1). The overall mean of AM fungal spores was about 81 spores per gram of dry soil. Hyphal density (HD) in soil was 15.73 m cm^{-3} of dry soil; this is an average of summer and winter samplings, whose mean values did not differ ($P>0.05$) from each other. The number of entry points per millimetre of colonized root (EP) showed a similar yearly variation in the two species. Maximum values were in spring, and the values for the other seasons did not differ ($P>0.05$) from each other (Table 1). SD, MCLt and MCSt were not correlated with EP ($P>0.05$). The average EP for the legume was 1.94 times ($P<0.001$) the average for the grass (Table 1).

Concentration of P in plant tissue

The concentration of P in plant tissue (shoots and roots) was higher in *L. glaber* than in *S. secundatum*. The seasonal pattern of P in shoots and roots was also similar in both species; the concentration of P was high in autumn and winter and low in spring and summer (Fig. 2a and b). The concentration of P in plant tissue was negatively correlated with soil P availability (*L. glaber*: shoot, $r=-0.5863$, $P=0.0083$; root, $r=-0.5646$, $P=0.0095$. *S. secundatum*: shoot, $r=-0.7532$, $P=0.0002$; root, $r=-0.5958$, $P=0.0056$). The concentration of P in shoot and root tissues was always positively associated with AC index (*L. glaber*: shoot, $r=0.4502$, $P=0.0531$; root, $r=0.1536$, $P=0.5178$. *S. secundatum*: shoot, $r=0.7578$, $P=0.0002$; root, $r=0.3059$, $P=0.1897$), but negatively associated with VC index (*L. glaber*: shoot, $r=-0.7161$, $P=0.0006$; root, $r=-0.5639$, $P=0.0096$. *S. secundatum*: shoot, $r=-0.5181$, $P=0.0231$; root, $r=-0.3199$, $P=0.1692$).

Table 1 Seasonal variation in spore and hyphal densities in soil and entry points in *L. glaber* and *S. secundatum* roots

Variable	Season			
	Summer	Autumn	Winter	Spring
SD (spore g^{-1} dry soil)	88.19±7.96 a	73.03±7.32 a	88.65±16.56 a	72.89±6.61 a
HD (cm cm^{-3} dry soil)	1542.97±177.37 a	ND	1602±224.83 a	ND
EPLt (*number*, mm^{-1} colonized root) *kw*	5.44±0.77 a	6.14±0.09 ab	6.75±0.45 b	7.96±0.32 c
EPSt (*number*, mm^{-1} colonized root)	2.63±0.28 a	3.10±0.25 a	3.45±0.23 a	4.46±0.22 b

Mean values of five replicates ± standard errors are shown. In each row, means followed by different letters are significantly different ($P<0.05$) by Tukey or Kruskal–Wallis tests.

kw Kruskal–Wallis, *SD* spore density, *HD* hyphal density, *EPLt* entry points in *L. glaber* roots, *EPSt* entry points in *S. secundatum* roots, *ND* no determination

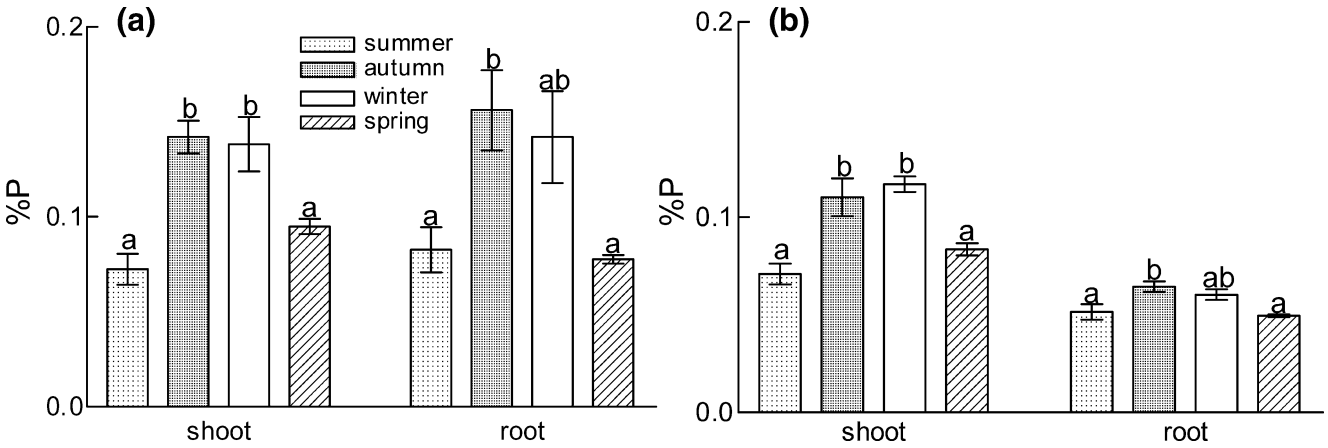

Fig. 2 Seasonal variation of P concentration in shoots and roots of *L. glaber* (**a**) and *S. secundatum* (**b**). Mean values of five replicates ± standard errors are shown. Means followed by *different letters* are significantly different ($P<0.05$) by the Tukey test

Soil properties

Some of the soil properties showed changes during the year (Table 2). Soil P availability increased in summer and spring (8.28 and 9.42 mg kg^{-1}) and significantly decreased in autumn and winter (6.74 and 6.64 mg kg^{-1}). Exchangeable Na was high in spring (14.8 cmol kg^{-1}) and low in summer (9.7 cmol kg^{-1}) with intermediate values in autumn and winter. In contrast, exchangeable Ca was high in winter (4.7 cmol kg^{-1}) and low in spring (2.5 cmol kg^{-1}). Soil pH, EC, Ct and Nt did not show seasonality during the year (Table 2).

Influence of the environment and season on the AM symbiosis

The coordinates from the first three ordination axes of the CCA study are shown in Fig. 3a and b. The three first axes, as indicators of the size of the AM root colonization morphology and fungal propagules variation, explained 51.5% of the total accounted variance (axis I, 35.9%; axis II, 14.0% and axis III, 1.6%).

The Pearson correlation coefficient between the scores of the first three axes derived from the AM fungal data sets and the sample scores that are combined linearly with the variables were statistically significant: 0.68, 0.88 and 0.76 for axes I, II and III, respectively. The Monte Carlo test indicated that the overall effect of the variables included in the second matrix (soil chemical properties, P content in roots and environmental variables) and two (axis II and axis III) of the three first canonical axes were also significant (axis I, $P=0.3100$, axis II, $P=0.0067$ and axis III, $P=0.0033$). These results indicated that axes II and III are more important than axis I to explain the relationships between the AM fungal variables and these axes. Axis II was positively correlated with MCLt ($P<0.001$), VCLt ($P<0.001$) and VCSt ($P<0.05$) but negatively correlated with MCSt ($P<0.001$) and ACSt ($P<0.01$). Axis III was positively correlated with ACLt and ACSt ($P<0.01$), and axis I was with SD ($P<0.01$).

Table 2 Seasonal variation in soil properties

Soil properties	Season			
	Summer	Autumn	Winter	Spring
Moisture (%)	26.09±0.91 ab	26.56±0.73 ab	27.48±0.70 b	23.84±0.61 a
pH (1:2.5, water)	9.18±0.09 a	9.23±0.08 a	9.15±0.07 a	9.30±0.05 a
EC (dS m^{-1})	5.75±0.66 a	5.15±0.35 a	5.42±0.68 a	5.70±0.60 a
Na (cmol kg^{-1})	9.74±1.13 a	10.92±0.66 ab	10.88±1.03 ab	14.76±1.05 b
Ca (cmol kg^{-1}) *kw*	4.44±0.97 bc	3.94±0.39 b	4.72±0.26 c	2.50±0.14 a
P Bray I (mg kg^{-1})	8.28±0.54 b	6.74±0.58 a	6.74±0.12	9.42±0.46 b
Ct (%)	1.32±0.13 a	1.12±0.06 a	1.17±0.09 a	1.26±0.09 a
Nt (%)	0.13±0.014 a	0.11±0.009 a	0.10±0.008 a	0.11±0.003 a

Mean values of 5 replicates ± standard errors are shown. In each row, means followed by different letter are significantly different ($P<0.05$) by the Tukey or Kruskal–Wallis tests.

kw Kruskal–Wallis, *EC* electrical conductivity, *Na* exchangeable sodium, *Ca* exchangeable calcium, *P Bray I* phosphorus available, *Ct* total carbon, *Nt* total nitrogen

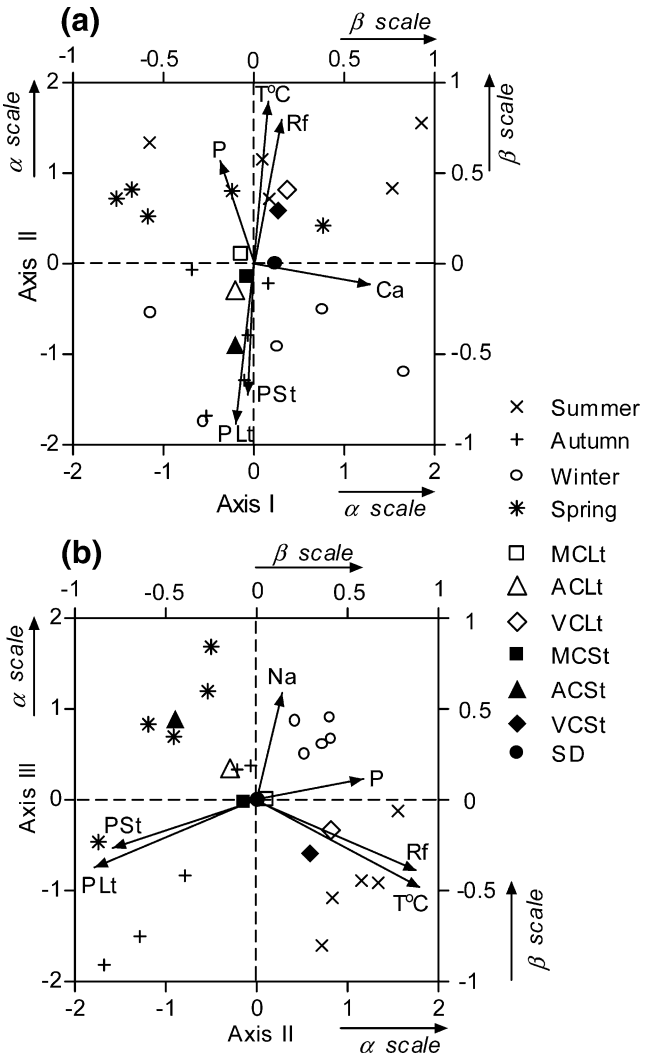

Fig. 3 Ordination diagram from the CCA of seasonal observations based on AM fungal variables (*main matrix*), and soil properties, P concentration in root tissue and environmental variables (*second matrix*). In *L. glaber* (*Lt*) and *S. secundatum* (*St*). *MC* Total colonized root, *AC* arbuscular colonization, *VC* vesicle colonization, *PLt* and *PSt* phosphorus concentration in root tissue, *SD* spore density, *P* phosphorus availability in soil, *Ca* exchangeable calcium, *Na* exchangeable sodium, *T°C* = air temperature, *Rf* rainfall

The ordination placed the observations disaggregated by season, especially when axis II vs axis III were plotted (Fig. 3b). With some exceptions along the axes II and III, the distribution of the observations suggested seasonality. The biplots in Fig. 3a and b show the ordination of the variables of the second matrix (soil, P in root tissue and environmental variables) that were significantly correlated with the axes. The length of the vector describes the relative significance of the correlation of that variable with the axes, and the angles between the vectors reflect the inter-correlation between the variables (Fig. 3a and b). The angle between a vector and any axis is a measure of the degree of correlation of the variable with that axis. Thus, on axis II, PLt ($P<0.001$), PSt ($P<0.001$) and the climate variables *T*°C ($P<0.001$) and Rf ($P<0.001$), were the most significant variables determining the variation of AM fungi in *L. glaber* and *S. secundatum*; availability of P in soil followed in importance ($P<0.010$). On the other two axes, exchangeable Ca ($P<0.010$) and Na ($P<0.010$) positively correlated with axis I and III, respectively (Fig. 3a and b).

Different associations emerged clearly in separate parts in the diagrams of Fig. 3. More concentration of P in roots (PLt, PSt) is displayed on the negative scale of the axis II and was associated with AC in roots of the two species (ACLt, ACSt) and MCSt (Fig. 3a and b). High temperature and rainfall (*T*°C, Rf) are on the positive scale of the axis II and were associated with a higher fraction of VC (VCLt, VCSt) and MCLt. In axis I, increments in SD were associated with an increment of exchangeable Ca during the year (Fig. 3a), and in axis III, AC (ACLt, ACSt) was associated with an increment in exchangeable Na observed in spring (Table 2).

Discussion

Roots of *L. glaber* and *S. secundatum* plants growing in saline-sodic soil conditions were highly colonized by AM fungi. The capability of becoming densely colonized is an important trait of these saline-tolerant plants because high levels of soil pH, nutrient status and salinity have been reported to decrease AM root colonization and spore density in soil (Abbott and Robson 1991; Mendoza et al. 2000; Escudero and Mendoza 2005). Thus, AM fungal species can tolerate and persist in plant roots under adverse soil conditions and may explain the high values of colonized root length measured in *L. glaber* and *S. secundatum*.

The percentage of colonized root length was higher in *L. glaber* than in *S. secundatum*, and it showed different seasonal pattern between the two plant species. The growth rate of fungi within the root, the growth rate of root within the soil and the number of entry points forming new colonization units are some of the main factors controlling the percentage of colonized root length. *L. glaber* is a winter–spring growing legume, which begins to grow earlier than *S. secundatum*, a spring–summer growing grass. Differences in length and seasonal pattern of colonized root between the two plants may be ascribed to interactions among the growth rates of both fungi and roots. Then, one may not necessarily expect a similar seasonal pattern of colonized root length in the two plant species.

The temporal pattern of the root fraction containing arbuscules differed from that containing vesicles. These two seasonal patterns were similar in the studied plants. Maximum AC and VC in roots of the two plant species take place at different seasons, suggesting a preference for

the morphological forms by the fungus in a specific season. Allen (1983) found more arbuscules during periods of active nutrient uptake, and Mullen and Schmidt (1993) reported that AM root colonization closely followed nutrient demands generated by growth stages of plants. In our study, P concentration in plant tissue also followed a similar seasonal pattern in the two plant species, and it was positively correlated with AC and negatively with VC indexes. Sanders and Fitter (1992) reported a significant nutritional benefit to the host during the growing period. In this study, the higher AC fraction found at the beginning of the growing period (late winter) and during the greatest rate of plant growth (spring) was associated with high values of nutrient transfer between symbiosis partners.

In this study, we observed arbuscules at all sampling times, suggesting that host plants and AM fungi may establish a functional symbiosis in this saline-sodic soil, as reported by Oliveira et al. (2005) in highly alkaline sediments.

During the summer, the plant community in the studied site is frequently exposed to high temperature and dry periods, but also to an alternating sequence of dry and wet pulses originated by rainfall and high evaporation rates. Furthermore, salinization occurs as pulses in late spring and summer and is facilitated by a shallow and saline water table. Salts move upward from deep horizons and reach the soil surface in periods of high evapotranspiration rate (Lavado et al. 1992). These climate and soil factors subject plants to different types of stress conditions. Ours results showed that the VC index increased at the end of the summer in roots of both plant species. It has been reported that the mobilization of carbohydrates and lipids stored in vesicles to form over-wintering structures such as spores (Bentivenga and Hetrick 1992), and that vesicle formation is a sign of the fungi speeding up their life cycles in annual plants towards the end of the growing season (Gavito and Varela 1993). Furthermore, we have often observed thick-walled vesicles resembling spores, suggesting that they could function as propagules when isolated from roots (Biermann and Linderman 1983) or supporting the re-growth of intercellular hyphae when appropriate conditions occur (Smith and Read 1997). The higher VC fraction that we have observed late in summer can be associated with the formation of storage structures, such as vesicles.

Entry points per millimetre of colonized root were higher in *L. glaber* than in *S. secundatum*, and the number increased late in spring for both plant species. Maximum EP occurred in the growing season when exchangeable Na and P in soil also increased, which is another evidence of AM fungus adaptability to colonize roots in the adverse soil conditions.

Spore and hyphal densities in soil did not change during the year. We do not know the relative proportion of infection units derived from extraradical hyphae or spores germination. But it is reasonable to suggest that the infectivity of these two fungal propagules was sufficient to colonize *L. glaber* and *S. secundatum* roots. Spore and hyphal densities are controlled by both soil and host factors (Johnson et al. 1992; Mendoza et al. 2002), but also reflect the net effect of sporulation or hyphae growth vs spore or hyphae disappearance (predation, leaching, dispersal, mortality, germination, degradation, etc). Thus, it is difficult to determine if soil or host factors are more important controlling spore and hyphal densities in soil. In our study, pH, EC, Nt and Ct did not change, and that fact could partly explain why spore and hyphal densities did not vary during the year.

The associations between soil chemicals, P in plant tissue and environmental variables with AM fungi measurements emerging from the CCA analyses explained the most significant variables determining AM fungal seasonal variation. The AC fraction was positively associated with variation of P in plant tissue of both plants, and VC fraction was positively associated with temperature and rainfall. These results are consistent with the seasonal patterns observed in plant tissue P and root colonization morphology of both plants. In addition, the CCA analysis also indicated that AC was not affected by increments in exchangeable Na, suggesting that the symbiosis was functional even when an increased Na occurred in spring. An increase of exchangeable Na in soil does not necessarily indicate an increase of Na in plant tissue. Mycorrhizal plants have lower Na concentration in shoots than non-mycorrhizal plants, suggesting the potential of AM fungi colonization for protecting plants against salt stress (Al-Karaki 2000).

Our results suggest that AM fungi can survive and colonize *L. glaber* and *S. secundatum* roots, adapting to extreme saline-sodic soil conditions imposed by the environment. The high levels of root colonization observed suggest that either the plants respond with slow root growth, the fungi colonize roots more completely or the interaction enables considerable root colonization. The symbiosis responds to seasonality and P uptake by the host, altering the morphology of colonized root.

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