RESEARCH ARTICLE

Relationships among soil properties, plant nutrition and arbuscular mycorrhizal fungi–plant symbioses in a temperate grassland along hydrologic, saline and sodic gradients

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Abstract

Temporal variations in the relationships among plant nutrient concentrations, soil properties and arbuscular-mycorrhizal (AM) fungal dynamics were studied along a topographic and saline gradient in a temperate grassland soil. Soil and plant (*Lotus tenuis*, *Paspalum vaginatum*, *Stenotaphrum secundatum*) samples were collected on four seasonally based occasions. The morphology of AM root colonization had a similar pattern in the plants studied. Maximum arbuscular colonization occurred at the beginning of the growing season in late winter and was minimal in late summer, but maximal vesicular colonization occurred in summer and was minimal in winter, suggesting a preferential production of these morphological phases by the fungus with respect to season. The greatest arbuscular colonization was associated with the highest N and P concentrations in plant tissue, suggesting a correspondence with increases in the rate of nutrient transfer between the symbiotic partners. Water content, salinity and sodicity in soil were positively associated with AM root colonization and arbuscule colonization in *L. tenuis*, but negatively so in the grasses. There were distinct seasonally related effects with respect to both spore density and AM colonization, which were independent of particular combinations of plant species and soil sites.

Introduction

Water stress, salinity and low nutrient availability in soil are the major stress factors that depress plant yield (Szabolcs, 1991). One of the plant strategies to grow under adverse soil environment is the association with arbuscular-mycorrhizal (AM) fungi (Sylvia & Williams, 1992; Entry *et al.*, 2002). This symbiotic association is of particular importance in improving plant nutrition (Smith & Read, 1997), because of the ability of mycorrhizal fungal hyphae to acquire phosphorus (P) or other nutrients with low mobility such as Zn and Cu (Ruiz-Lozano *et al.*, 1996; Al-Karaki & Clark, 1998), beyond the limits of the rhizosphere depletion zone (Li *et al.*, 1991). Other benefits of the mycorrhizal symbioses are to reduce the detrimental effect of soil salinity (Jindal *et al.*, 1993; Ruiz-Lozano *et al.*, 1996) by improving nutrient uptake and water relations (Sylvia *et al.*, 1993; Ruiz-Lozano & Azcón, 1995; Al-Karaki & Clark, 1998). These effects may increase growth and lead to the subsequent dilution of toxic ion effects in plant tissue (Juniper & Abbott, 1993).

Natural grassland soils are commonly deficient in essential nutrients (N and P) to sustain maximum plant growth (Ginzo *et al.*, 1986; Lavado *et al.*, 1993). Field studies showed that grassland plants can be colonized by AM fungi under a wide range of soil conditions (Stutz *et al.*, 2000; Hartnett & Wilson, 2002; Muthukumar & Udaiyan, 2002; Escudero & Mendoza, 2005; Gai *et al.*, 2006).

The distribution of certain AM fungal species has been related to soil pH, soil P level, salinity (Abbott & Robson, 1991), vegetation (Johnson *et al.*, 1992) or the hydrologic condition of the soil (Miller & Bever, 1999; Escudero & Mendoza, 2005). In general, it was observed that high values of soil pH, nutrient status, moisture content and salinity are related to a decrease in either AM colonization in roots or the number of fungal propagules in soil (Abbott & Robson, 1991; Entry *et al.*, 2002). AM fungi are believed to require

well-aerated soils and are considered to be poorly adapted to conditions in flooded environments (Mosse *et al*., 1981), for instance some AM fungal species are relegated to the driest areas in wetlands (Miller & Bever, 1999). However, some reports have shown a higher AM spore density in either poorly drained (Troeh & Loynachan, 2003) or flooded soils (Escudero & Mendoza, 2005; Mendoza *et al*., 2005) or no relationship between soil moisture and AM root colonization in prairie wetlands (Wetzel & van der Valk, 1996). Escudero & Mendoza (2005) showed that seven months of flooding had no effect on spore density in the soil but it depressed AM colonization in *Lotus tenuis*, a mycotrophic flood-tolerant legume. Furthermore, Mendoza *et al*. (2005) found that AM fungi can survive in a waterlogged saline-sodic soil, and colonize roots in the next season by a strategy consisting of colonization reduction, the preferential production of resistance structures such as vesicles instead of nutrient transfer structures such as arbuscules and inhibition of spore germination.

AM fungal root colonization and spore density have shown seasonality associated with host phenology and climate variations (Bentivenga & Hetrick, 1992; Allen, 1996; Lugo *et al*., 2003; Escudero & Mendoza, 2005; García & Mendoza, 2007). Despite the importance of AM fungi in the physiology and nutrition of plants, little is known about the factors likely to influence the seasonal dynamics of AM fungi along a saline-sodic gradient. To better understand how soil properties and plant nutrient status (especially N and P) influence AM–plant symbioses requires the investigation of the association between soil properties and the temporal variations in AM colonization morphology under field conditions. Although relationships between P uptake and root colonization by AM fungi have been investigated under controlled conditions, several differences could be found in grassland plants under field conditions. Plant nutrient uptake mediated by AM fungi depends on how much of the soil can be exploited by the external hyphae, the rate at which hyphae take up available nutrients and how much of the internal fungus is active in transferring nutrients to the plant.

Greenhouse experiments showed that an increase of P concentration in soil or plant tissue was related to a decrease in the total length of colonized root and the proportion of root length colonized by arbuscules (Braunberger *et al*., 1991; Mendoza & Pagani, 1997; Cornwell *et al*., 2001). In grasslands, Sanders & Fitter (1992) observed that there was no relationship between AM colonization in roots and concentrations of P, Cu, Zn or Mn in shoot tissue, and only in a few cases was nutrient inflow related to colonization. However, high levels of P inflow do occur at certain periods during the growing season, suggesting that AM fungi may promote plant nutrient uptake during these periods. García & Mendoza (2007) showed that the arbuscular colonization fraction increased at the beginning of the growing season and was positively associated with an increase of P concentration in both shoot and root tissue of grassland plants, and proposed that the symbioses change the morphology of root colonization as a response to seasonality and P uptake by the host.

There is little published literature relating to the temporal dynamics of AM root colonization morphology in grassland plants associated with changes in N and P plant status and soil properties in stressful environments. Even when it is accepted that grassland plants can be colonized by AM fungi along a wide range of soil conditions, associating changes in nutrient uptake with changes in AM root colonization are difficult to investigate. When soil conditions change, plant community structures also change, and hence cause–effect relationships are difficult to establish. An approach could be to study grassland plants with the ability to grow across a wide range of soil properties in the same location under the same climate conditions. These plants that occur across a wide soil gradient, as opposed to plants with a more narrow ecological distribution, may have a different response to mycorrhizas, as part of an overall more plastic strategy.

This study comprised an investigation into the temporal dynamics of AM fungal root colonization in grassland plants (legume and grasses) and propagules in soil, plant tissue nutrients and soil properties in a natural temperate grassland of the Argentinean Pampas along a topographic slope ranging from saline and/or sodic lowlands of high soil moisture content and soil pH to uplands of drier and neutral non saline soils. This was done in an attempt to explain whether seasonal variations in plant nutrient demands result in changes in AM root colonization morphology and to know whether these changes are associated with soil characteristics. The authors specifically aim to test the following hypotheses: (1) AM root colonization morphology is associated with period of growth of the plant species, specifically arbuscular colonization increases early in the growing season and vesicular colonization increases toward the end of the growing period; (2) spore density in soil and AM root colonization morphology varies seasonally; (3) seasonal differences will be expressed differently with host species and soil properties; and (4) increases in plant tissue nutrient concentrations are related to a decrease in the total length of colonized root and the proportion of root length colonized by arbuscules.

Materials and methods

Study sites

The research was performed in natural grassland of the Argentinean Pampas, located in San Vicente (35°S;

58°50′W), a province of Buenos Aires. The seasonal mean temperature and accumulated rainfall values at the site were, respectively: 22.7 °C and 536 mm in summer; 10.8 °C and 171 mm in autumn; 11.9 °C and 187 mm in winter; and 20.6 °C and 307 mm in spring. The experimental grassland extends over a topographic gradient that determines different soil characteristics and dynamic hydrologic and saline gradients. A transect 510 m long was laid across it, with the lowest end prescribed as a reference point for both distance and relative height for another three sampling sites; this was denoted Site 1, and distance and height co-ordinates set to (0, 0 m). Subsequent sites were set as follows: Site 2 at 170, 0.22 m; Site 3 at 400, 0.46 m); and Site 4 at 510, 0.65 m (i.e. at the opposite end of the transect). Sites 1 and 2 were each on a typic Natraqualf, and Sites 3 and 4 were on a typic Natraquoll (INTA-CIRN, 1990). Site 2 was the same site used previously by García & Mendoza (2007) and part of the data sets from plants, soil and AM fungal variables used in the present research were taken from that work.

Three forage species commonly present in the grassland were selected as test plants: *L. tenuis* Waldst. & Kit. and the codominant grasses *Paspalum vaginatum* Swartz and *Stenotaphrum secundatum* (Walt.) O.K. *Lotus tenuis* is a perennial herbaceous winter-spring-growing legume growing along a wide topographic gradient; *P. vaginatum* and *S. secundatum* are spring-summer-growing species. *Lotus tenuis* and *S. secundatum* were present along the topographic gradient; this allowed us to associate the variation of soil properties or plant nutrient uptake with AM fungal measures and propagule distribution studying the same host plants. In addition, *L. tenuis* is highly dependent on AM root colonization to grow under conditions of low phosphorus availability in soil (Mendoza & Pagani, 1997).

Experimental design and sampling procedure

A circular and permanent plot of 113 m^2 was placed in each one of the four sites. The wettest site was Site 1 and the driest one was Site 4. The plant communities of the Sites 1–4 were dominated by: *L. tenuis*, *P. vaginatum*, *Distichlis spicata* (L) Greene., *Eleocharis viridans* Kükenth and *Cynodon dactylon* (L) Pers. (Site 1); *L. tenuis*, *S. secundatum*, *Lolium multiflorum* Lam. and *Paspalum dilatatum* Poir. (Site 2); *L. tenuis*, *C. dactylon*, *S. secundatum* and *P. dilatatum* (Site 3); and by *L. tenuis*, *Bromus unioloides* H.B.K., *S. secundatum* and *L. multiflorum* (Site 4).

At each sampling site, two concentric circular plots of 8 and 12 m radius, respectively, were set up. Each 12 m-radius area was divided into five 72° angular-sector subplots (replicates). The outer subplot (the area of the ring defined by the 12- and 8-m plots) was used for seasonal sampling of soil chemical characteristics, spore density and AM root colonization. The inner subplot (the area of the ring defined by the 8-m plots and the centre point) was used for seasonal measures of floristic composition. 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 were 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 two portions to measure the soil properties and spore density. Soil samples were kept in plastic bags at 4 °C until processed. Soil samples and individuals of *L. tenuis* and a codominant grass species of the highest relative frequency in the community were collected at each site in summer (18 Mar 2003), autumn (17 Jun 2003), winter (18 Sep 2003) and spring (03 Dec 2003). In Site 1, the codominant plant species was *P. vaginatum*, and in Sites 2, 3 and 4 it was *S. secundatum*. Five replicates of each plant species (adult individuals of similar size) and rhizosphere soil samples (as indicated above) were randomly taken from the outer subplot. Even though the study was located on the same pasture ground, significant statistical differences among sites could not be determined because there were no replications of site subtypes. However, this design did allow analysis of data from an experiment in which several observations of the same variable were recorded on each of the experimental units over time (Rowell & Walters, 1976).

AM fungal root colonization morphology

Mycorrhizal root colonization was measured in fresh roots of *L. tenuis* and codominant plant species cleared in 10% KOH for 10 min at 90 °C and stained in 0.05% lactic–glycerol–Trypan Blue (Phillips & Hayman, 1970). Twenty-five root segments per plant sample were examined under a microscope at × 200 magnification. The assessment of AM fungal root colonization was followed by McGonigle *et al.* (1990). This method determined the total fraction of root length colonized and the fractions of root length containing arbuscules, vesicles and hyphae only.

Assessment of AM fungal spore density in soil

Spores of AM fungi were extracted from 30 g of rhizosphere soil by a modification of wet sieving, followed by a sucrose gradient centrifugation method (Daniels & 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 an 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 was expressed as the number of spores per gram of dry soil.

Concentrations of N and P in plant tissue

Oven-dried (75 °C for 48 h) shoot and root tissues of *L. tenuis*, *P. vaginatum* and *S. secundatum* were digested separately in sulphuric acid to determine N by the Kjeldahl method, and in a nitric–perchloric acid mixture to determine P by the molybdovanadophosphoric acid method (Jackson, 1958).

Soil characteristics

Soil pH (soil : solution ratio of 1 : 2.5 in water), moisture content, electrical conductivity at saturation (Jackson, 1958), exchangeable Na (Jackson, 1958), exchangeable sodium percentage, total carbon (Richter & von Wistinghausen, 1981), total nitrogen (Bremmer & Mulvaney, 1982) and phosphorus availability (Bray & Kurtz, 1945) were measured in each season.

Statistical analysis

AM fungal variables, N and P concentrations in plant tissue and soil variables measured at each site were tested for normality and homogeneity of variance. The equality of seasonal means for each variable was tested with ANOVA. Multiple comparisons among the season means for each variable were tested with the Tukey test. In those cases were the log transformation did not normalize the data of a variable, seasonal effects were tested with the Kruskal–Wallis nonparametric test.

Implications of soil properties and nutrients in plant tissue on AM fungal variables

The canonical correspondence analyses (CCA) ordination technique as performed by the CANOCO algorithm (Ter Braak, 1987–1992) was used to identify the best linear combinations of soil chemical properties and concentrations of N and P in shoot and root tissues that influence AM fungal measurements. Firstly, 16 seasonal sampling points (4 sites × 4 replicates per site) of seven AM fungal variables: total spore density in soil; total fraction of root length colonized in *L. tenuis* (MCLt); total fraction of root length colonized in roots of the grasses *P. vaginatum* in Site 1 and *S. secundatum* in Site 2–4 (MCGs); fraction of root containing arbuscules in *L. tenuis* (ACLt) or in either *P. vaginatum* or *S. secundatum* (ACGs); fraction of root containing vesicles in *L. tenuis* (VCLt) or in either *P. vaginatum* or *S. secundatum* (VCGs), were included in the main matrix. Similarly, the second matrix was constituted by the measured seven soil properties (pH, electrical conductivity, exchangeable Na, P, Ct, Nt and water content). A second CCA included the main matrix used in the preceding test, but the second matrix was comprised 16 seasonal sampling points (4 sites × 4 replicates per site) for the eight variables representing concentrations of N and P in plant tissue: %P in shoots of *L. tenuis* (PSLt); %P in roots of *L. tenuis* (PRLt); %P in shoots of the grasses (*P. vaginatum* in Site 1 and *S. secundatum* in Site 2–4) (PSGs); %P in roots of the grasses (*P. vaginatum* in Site 1 and *S. secundatum* in Site 2–4) (PRGs); %N in shoots of *L. tenuis* (NSLt); %N in roots in roots of *L. tenuis* (NRLt); %N in shoots of the grasses (*P. vaginatum* in Site 1 and *S. secundatum* in Site 2–4) (NSGs); and %N in roots of the grasses (*P. vaginatum* in Site 1 and *S. secundatum* in Site 2–4) (NRGs). To assess the significance in the CCA axes, the Monte Carlo simulation was used to test the hypothesis that there was no correlation between the main (AM fungal) and secondary (soil or nutrient) matrices. The *P* values were based on the proportion of 1000 Monte Carlo simulations with an Eigen value greater than the Eigen value observed.

Results

Soil sites

With the exception of Site 1 and soil pH, soil properties varied significantly among sites and seasons (Table 1). The annual mean value of soil moisture content always decreased from Site 1 to Site 4. Moisture content values were 31%, 26%, 22% and 21% for Sites 1–4, respectively, in the summer sampling. The lowest moisture content values were 25%, 23%, 19% and 20% for Sites 1–4, respectively, in the spring sampling. Sites 1 and 2 have saline-sodic soils with electrical conductivity and exchangeable sodium percentage values higher than 4 dS m^{-1} and 15%, respectively, and Sites 3 and 4 have nonsaline-sodic soils with electrical conductivity values lower than 4 dS m^{-1} and exchangeable sodium percentage values higher than 15% (Richards, 1974). In Site 2, electrical conductivity, exchangeable Na and P availability increased in spring (Table 1). In contrast, Site 4 did not show changes in the saline or sodic conditions during the year. In Sites 3 and 4 total carbon and total nitrogen increased in spring, but not in Sites 1 and 2 (Table 1). In spring, P availability increased in Sites 1 and 2, but decreased in Site 4 (Table 1).

AM fungal root colonization

AM fungal root length colonization indexes (total fraction of root length colonized, arbuscular colonization and vesicular colonization) were consistently higher in *L. tenuis* roots than in the codominant grass roots (*P. vaginatum* in Site 1 and *S. secundatum* in Sites 2–4) at all sites and seasons. In contrast, the hyphae-only fraction was higher in the roots of the grasses than in *L. tenuis* (Fig. 1). The overall mean values over sites and seasons for *L. tenuis* were total colonization 89%, arbuscule fraction 47%, vesicle fraction 23% and hyphae-only fraction 27%; for the codominant

Table 1. Seasonal variation in soil properties

		Season			
Site	Soil properties	Summer	Autumn	Winter	Spring
1	pH *kw*	9.49 b	9.53 b	9.28 a	9.20 a
	EC (dS m^{-1})	9.01 a	10.10 a	9.14 a	8.56 a
	Na+($cmol_c$ kg^{-1})	17.92 a	19.45 a	17.94 a	16.51 a
	P Bray I (mg kg^{-1})	6.86 a	6.50 a	6.70 a	8.72 a
	Ct (g kg^{-1}) *kw*	9.78 ab	9.10 a	12.70 b	12.41 b
	Nt (g kg^{-1}) *ln*	1.23 a	1.02 a	1.18 a	1.13 a
2	pH	9.18 a	9.23 a	9.15 a	9.30 a
	EC (dS m^{-1})	5.75 ab	5.15 a	5.75 ab	8.17 b
	Na^+ ($cmol_c$ kg^{-1})	9.74 a	10.92 ab	10.88 ab	14.76 b
	P Bray I (mg kg^{-1})	8.28 b	6.74 a	6.64 a	9.42 b
	Ct (g kg^{-1})	13.21 a	11.24 a	11.73 a	12.61 a
	Nt (g kg^{-1})	1.35 a	1.07 a	1.04 a	1.07 a
3	pH	7.36 a	8.21 b	8.18 ab	7.75 ab
	EC (dS m^{-1}) *ln*	1.61 a	1.40 a	1.81 a	0.92 a
	Na^+ ($cmol_c$ kg^{-1}) *ln*	2.42 a	3.04 a	7.26 b	7.60 b
	P Bray I (mg kg^{-1})	5.75 b	4.28 a	5.12 ab	5.38 ab
	Ct (g kg^{-1}) *ln*	13.26 ab	10.76 a	13.43 ab	16.70 b
	Nt (g kg^{-1})	1.20 ab	0.95 a	1.24 ab	1.45 b
4	pH	6.24 a	7.19 a	6.95 a	6.61 a
	EC (dS m^{-1})	1.49 a	2.69 a	1.67 a	0.88 a
	Na^+ ($cmol_c$ kg^{-1})	3.28 a	6.45 a	4.06 a	4.58 a
	P Bray I (mg kg^{-1})	5.70 a	5.48 a	3.98 a	3.92 a
	Ct (g kg^{-1})	18.85 a	18.80 a	21.16 a	24.24 a
	Nt (g kg^{-1})	1.94 a	1.63 a	1.98 a	2.22 a

Mean of five replicates are shown.

EC, electrical conductivity; Na^+, exchangeable sodium; P Bray I, phosphorus available; Ct, total carbon; Nt, total nitrogen. Means followed by different letters are significantly different ($P < 0.05$) by the Tukey test. *ln*, log transformation data; *kw*, Kruskal–Wallis nonparametric test.

roots, the respective values were 68%, 19%, 7% and 43% (Fig. 1).

The temporal patterns of the total fraction of root colonization were different among plant species in all sites. The total AM colonization in *L. tenuis* roots was the highest in spring and the summer and lowest in autumn and winter in all sites (Fig. 1a, c, e and g). The total AM colonization in grass roots was generally lowest in spring, and fairly similar in the other seasons (Fig. 1d, f and h), with the exception of its value for *P. vaginatum* in Site 1 (Fig. 1b).

The arbuscular colonization and vesicular colonization showed a similar seasonal pattern in the roots of both the legume and the grasses, but the maximum and minimum values of each index occurred in different seasons. The maximum arbuscular colonization occurred in winter and the minimum in summer, but maximum vesicular colonization occurred in summer and the minimum in winter (Fig. 1). In Site 1, the maximum arbuscular colonization in roots of *P. vaginatum* occurred in spring and the maximum vesicular colonization occurred in late summer, with small changes in the other seasons. The hyphae-only colonization was higher in grass than in legume roots, and they were the highest component of the AM colonization compared with arbuscular or vesicular colonization in grass roots (Fig. 1).

AM fungal spore density in soil

AM fungal spore density in soil seasonally varied along the topographic gradient, with the exception of Site 2, where it did not change much during the year (Fig. 2). The mean spore values were significantly higher in summer, ranging from 209 in Site 1 to 88 spores g^{-1} dry soil in Site 2, and decreased from autumn to spring (Fig. 2).

Concentrations of N and P in plant tissue

The concentration of N in plant tissue (shoots and roots) was almost double in *L. tenuis* compared with the grasses (*P. vaginatum* and *S. secundatum* in Sites 1 and 2–4, respectively; Table 2). The seasonal pattern of plant-N showed similar trends among the experimental plants. In general, N concentrations in roots were less variable than N in shoots. There were few changes in root-N from all sites (except for *P. vaginatum* in Site 1). The nitrogen concentration in shoots was significantly the highest in late winter and lowest in summer (except for *L. tenuis,* where shoot-N did not change in Sites 2 and 3, and for *P. vaginatum*, where shoot-N in Site 1 increased in winter and spring) (Table 2).

The concentration of P in plant tissue (shoots and roots) was higher in *L. tenuis* than in the grasses in all sites (Table 3). As for N, the seasonal pattern of plant-P was also similar among the experimental plants; high concentrations were observed in autumn but mainly in late winter, and low concentrations were found in spring and summer (Table 3).

Neither N nor P in shoots or roots showed significant correlations ($P > 0.05$) with available N or P measured in soil.

Implications of soil properties on AM fungal variables

The first three axes of the CCA study explained 67.8% of the total accounted variance: Axis I: 40.4%, Axis II: 19.9% and Axis III: 7.5% (Fig. 3). In addition, the Monte Carlo test indicated that the overall effect of the soil properties included in the second matrix and the first three canonical axes were statistically significant at a probability (P) of 0.234, 0.002 and 0.016 for Axis I, II and Axis III, respectively.

Axis I was positively correlated with spore density ($P < 0.001$) and vesicular colonization ($P < 0.050$), and negatively so with arbuscular colonization for *L. tenuis* roots ($P < 0.001$). Axis II was positively correlated with total AM colonization ($P < 0.050$) and vesicular colonization ($P < 0.050$) for *L. tenuis* and grass roots. Axis III was positively correlated with total AM colonization ($P < 0.010$)

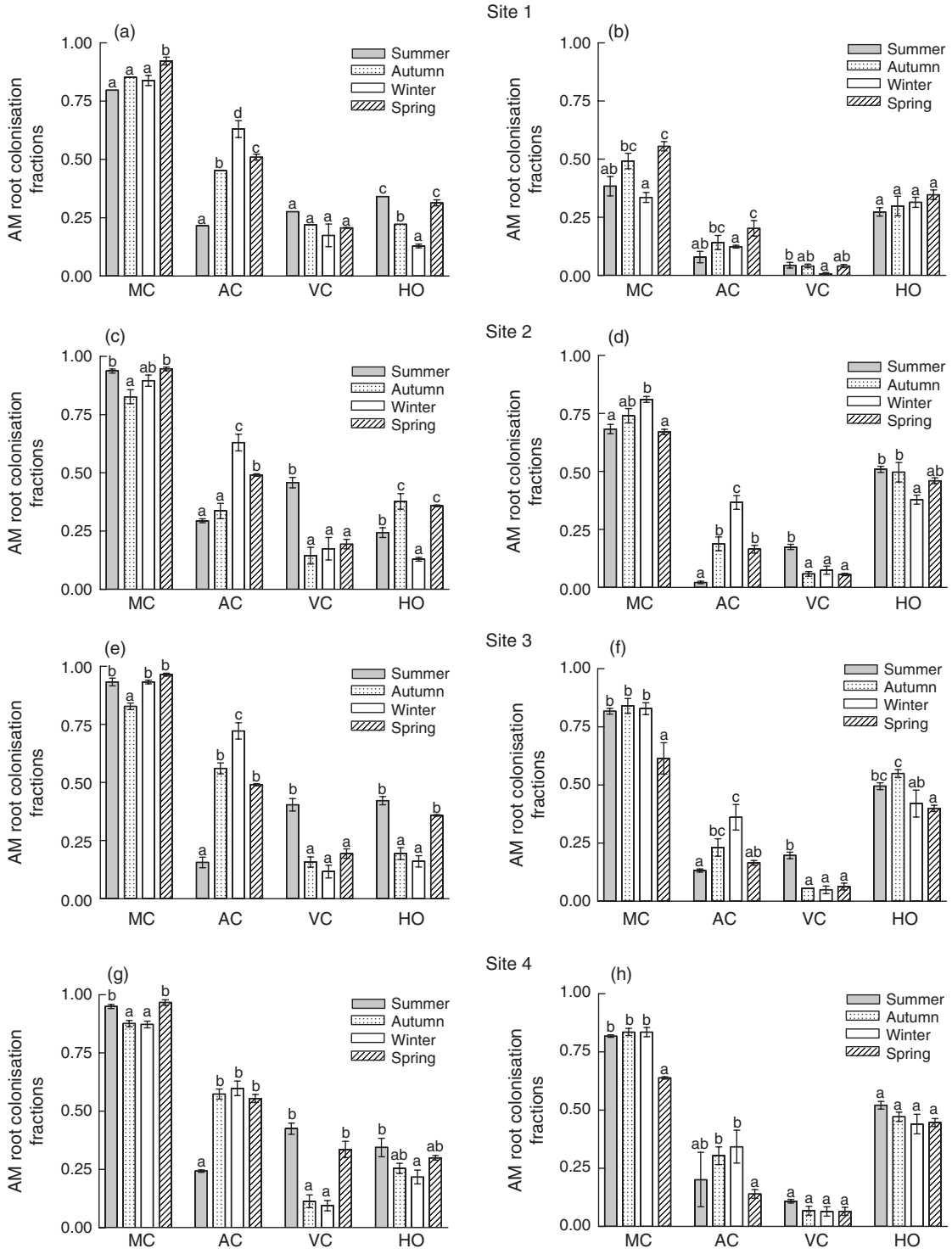

Fig. 1. Seasonal and spatial variations of AM fungal colonization in roots of *Lotus tenuis* (a, c, e and g), *Paspalum vaginatum* (b) and *Stenotaprhum secundatum* (d, f and h). Mean ± SE of the mean of five replicates are shown. MC, total colonized root; AC, arbuscular colonization fraction; VC, vesicle colonization fraction; HO, hyphae only colonization fraction. Means followed by different letters are significantly different ($P < 0.05$) by the Tukey test. AC in *S. secundatum* roots (Sites 3 and 4) were transformed to *ln*. HO data in grass roots (Site 3) was analysed by the Kruskal–Wallis nonparametric test.

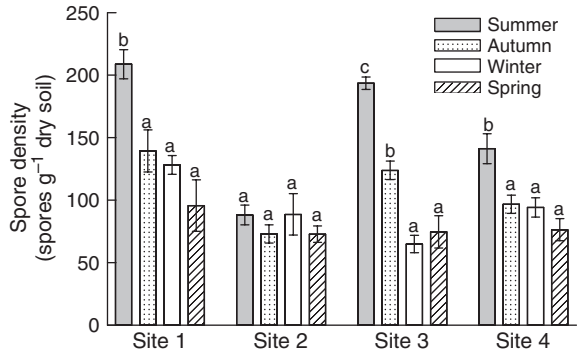

Fig. 2. Seasonal and spatial variation of spore density in soil. Mean ± SE of five replicates are shown. In each site, means followed by different letters are significantly different ($P < 0.05$) by the Tukey test.

Table 2. Seasonal and spatial variations of N concentration in shoots and roots of *Lotus tenuis*, *Paspalum vaginatum* and *Stenotaprhum secundatum*

			Season			
Site	Plant		Summer	Autumn	Winter	Spring
1	*L. tenuis*	Shoot *kw*	1.75 a	2.11 b	2.24 b	1.53 a
		Root	1.68 a	1.75 a	1.85 a	1.32 a
	P. vaginatum	Shoot *ln*	0.61 a	0.61 a	0.74 b	0.82 b
		Root	0.60 bc	0.56 b	0.37 a	0.72 c
2	*L. tenuis*	Shoot	1.88 a	2.01 a	1.88 a	1.96 a
		Root	1.61 a	1.76 a	1.67 a	1.82 a
	S. secundatum	Shoot	0.77 a	0.95 ab	1.20 b	1.01 ab
		Root	0.91 a	0.72 a	0.76 a	0.85 a
3	*L. tenuis*	Shoot	1.80 a	1.95 a	2.07 a	2.10 a
		Root	1.97 a	1.85 a	1.73 a	1.69 a
	S. secundatum	Shoot *ln*	0.90 a	0.93 a	1.18 b	0.85 a
		Root	0.65 a	0.68 a	0.81 a	0.77 a
4	*L. tenuis*	Shoot	2.00 a	2.32 b	2.18 ab	2.00 a
		Root	2.05 a	1.95 a	1.87 a	1.91 a
	S. secundatum	Shoot *ln*	0.93 a	1.22 ab	1.32 b	0.96 a
		Root	0.63 a	0.66 a	0.79 a	0.66 a

Mean of five replicates are shown. In each site, means followed by different letters are significantly different ($P < 0.05$) by the Tukey test. *ln*, log transformation data; *kw*, Kruskal–Wallis nonparametric test.

and arbuscular colonization ($P < 0.050$) for the codominant plant roots.

With the exception of Site 1, which was clearly discriminated from the other sites in both subplots, the ordination placed the observations disaggregated mainly by season rather than by site (Fig. 3a and b). In the Axes I vs. II plot, the observations taken in summer were displaced to the positive scale of the Axis I, and those taken in winter were so to the negative scale (Fig. 3a). Similarly, the Axes II vs. III plot shows the summer observations segregated to the positive scale of Axis II, and the winter ones to the negative scale of the same axis (Fig. 3b). The spring and autumn points were close to the origin of Axis II (Fig. 3b). Site 1 was

Table 3. Seasonal and spatial variations of P concentration in shoots and roots of *Lotus tenuis*, *Paspalum vaginatum* and *Stenotaprhum secundatum*

			Season			
Site	Plant		Summer	Autumn	Winter	Spring
1	*L. tenuis*	Shoot	0.08 a	0.21 b	0.20 b	0.11 a
		Root	0.11 a	0.31 b	0.28 b	0.10 a
	P. vaginatum	Shoot	0.01 a	0.12 ab	0.14 b	0.16 b
		Root	0.06 ab	0.09 c	0.07 b	0.05 a
2	*L. tenuis*	Shoot	0.07 a	0.14 b	0.14 b	0.09 a
		Root	0.08 a	0.16 b	0.14 ab	0.08 a
	S. secundatum	Shoot	0.07 a	0.11 b	0.12 b	0.08 a
		Root	0.05 a	0.06 b	0.06 ab	0.05 a
3	*L. tenuis*	Shoot *ln*	0.09 a	0.16 bc	0.19 c	0.11 ab
		Root *kw*	0.12 a	0.18 b	0.21 b	0.10 a
	S. secundatum	Shoot *kw*	0.08 a	0.11 b	0.14 b	0.08 a
		Root	0.05 a	0.06 a	0.06 a	0.05 a
4	*L. tenuis*	Shoot *kw*	0.12 a	0.19 b	0.24 b	0.13 a
		Root *ln*	0.17 ab	0.22 b	0.39 c	0.12 a
	S. secundatum	Shoot	0.10 a	0.13 a	0.17 b	0.12 a
		Root	0.05 a	0.07 bc	0.07 c	0.06 ab

Mean of replicates are shown. In each site, means followed by different letters are significantly different ($P < 0.05$) by the Tukey test. *ln*, log transformation data; *kw*, Kruskal–Wallis nonparametric test.

situated at the bottom right of the plot in Fig. 3a, and at the bottom left quadrant in Fig. 3b. The biplots in Fig. 3a and b show the ordination of the soil properties (electrical conductivity, exchangeable Na and water content) of the second matrix that were significantly correlated with the axes. The arrows representing water content ($P < 0.050$) and exchangeable Na ($P < 0.050$), which was positively correlated with Axis II, are pointing in the direction of Site 1, thus indicating that the largest values of these soil properties were in this site, and they were positively associated with spore density (Fig. 3). In the Axis II vs. III plot, water content, electrical conductivity and exchangeable Na are also pointing to Site 1. This plot also showed that electrical and exchangeable Na were positively associated with total AM colonization and arbuscular colonization for *L. tenuis* roots, and water content was positively associated with spore density (Fig. 3a).

The values of arbuscular colonization for legume and grasses were displaced to the bottom left quadrant, which is associated with winter observations, and in an opposite position, to the right top quadrant associated with summer observations, are the vesicular colonization for *L. tenuis* and grasses (Fig. 3a). In addition, Axis III discriminated the AM fungal variables of *L. tenuis* segregated to the positive scale from those of the grasses segregated to the negative scale (Fig. 3b). Figure 3b also shows that the highest values of total AM colonization were associated with the spring for *L. tenuis* and were so with the summer for the grasses.

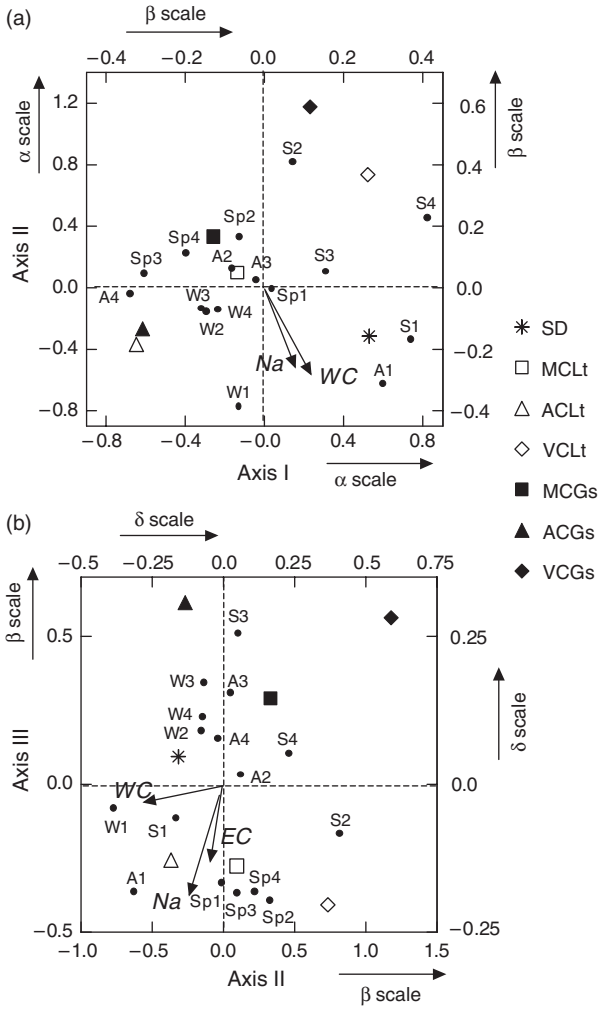

Fig. 3. Ordination diagram from the CCA of seasonal and spatial observations based on AM fungal variables (main matrix) and soil properties (second matrix). Lt, *Lotus tenuis*; Gs, grasses; MC, total colonized root; AC, arbuscular colonization fraction; VC, vesicle colonization fraction; SD, spore density in soil; Na, exchangeable sodium; WC, water content; EC, electrical conductivity; S, summer; A, autumn; W, winter; Sp, spring. The number following each season indicates a site.

Implications of nutrient in plant tissue on AM fungal variables

In the second CCA study, the three first axes explained 82.2% of the total accounted variance: Axis I: 55.0%, Axis II: 19.65% and Axis III: 7.6% (Fig. 4). In addition, the Monte Carlo test indicated that the overall effect of the plant nutrient variables included in the second matrix and the three first canonical axes were statistically significant at a probability (P) of 0.008, 0.012 and 0.090 for Axes I, II and III, respectively. These results show that Axes II and I are more relevant for explaining the associations between plant nutrient and AM fungal variables than Axis III.

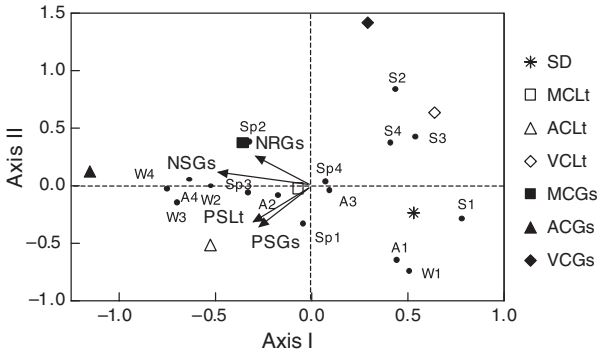

Fig. 4. Ordination diagram from CCA of seasonal and spatial observations based on AM fungal variables (main matrix) and N and P concentrations measures (second matrix). Lt, *Lotus tenuis*; Gs, grasses; MC, total colonized root; AC, arbuscular colonization fraction; VC, vesicle colonization fraction; SD, spore density in soil; NS, shoot N concentration; NR, root N concentration; PS, shoot P concentration; PR, root P concentration; S, summer; A, autumn; W, winter; Sp, spring. The number following each season indicates a site.

Axis I was positively correlated with spore density ($P < 0.001$), arbuscular colonization ($P < 0.001$) and vesicular colonization ($P < 0.001$) for *L. tenuis*, but negatively so with total AM colonization ($P < 0.050$) and arbuscular colonization ($P < 0.001$) for grasses. Axis II was positively correlated with total AM colonization ($P < 0.050$) and vesicular colonization ($P < 0.050$) for the legume, total AM colonization ($P < 0.010$) and vesicular colonization ($P < 0.001$) for the grasses. Axis III did not correlate either with the fungal variables or with the concentration of N or P in plant tissue.

With the exception of Site 1, because it was discriminated from the other sites, the ordination disaggregated the observations mainly by season rather than by site (Fig. 4). The seasonal observations from Site 1 were displaced to the bottom right quadrant (Fig. 4), but for Sites 2 to 4 the ordination disaggregated the observations by season rather than by site (Fig. 4). The observations taken in summer were displaced to the positive scale of the Axis I and those taken in winter to the negative scale of that axis (Fig. 4). The autumn points are discriminated along the Axis I, but the spring points approached the axes origin and did not show any special distribution (Fig. 4). The biplots show the ordination of the variables of the second matrix representing the concentration of N and P in plant tissue (shoots and roots) (N in shoots and roots of grasses, P in shoots of *L. tenuis* and grasses) that were significantly correlated with the first two axes (Fig. 4). The arrows representing shoot ($P < 0.001$) and root ($P < 0.050$) N concentration in grass tissue, P in shoots of the legume ($P < 0.050$) and grasses ($P < 0.050$), which correlated negatively with Axis I, are pointing to the winter observations and opposite to both Site 1 and the summer observations (Fig. 4). The arrows from N in roots of grasses

($P < 0.010$) correlated positively with Axis II, and those from P in shoots of *L. tenuis* ($P < 0.050$) and grasses ($P < 0.010$) correlated negatively with this axis (Fig. 4). The other N or P concentrations in shoots or roots did not correlate significantly ($P > 0.050$) with the axes but showed a trend similar to those that did. In addition, the N measures were displaced to the positive scale and the P measures to the negative scale of Axis II (Fig. 4). The plot also shows that P in shoots of *L. tenuis* was positively associated with arbuscular and mycorrhizal colonization, and that N in the grass tissues were associated with arbuscular and mycorrhizal colonization.

Discussion

This study showed that AM fungi were present along hydrologic, saline and/or sodic gradients in a temperate grassland of the Argentinean Pampas; root colonization in forage plants was extensive and propagules in soil were abundant, and both showed noticeable seasonal trends. The capability of plants to be densely colonized by AM fungi is an important fact in plant nutrition because it means that the AM fungi–plant symbioses can persist in roots under a wide range of soil moisture saturation levels, nutrient status, salinity and sodicity in space and time.

The temporal variation of mycorrhizal colonization morphology, particularly arbuscular colonization, found in *L. tenuis*, *P. vaginatum* and *S. secundatum* roots suggests that these plant species are functionally mycorrhizal for a diversity of soil conditions ranging from lowland sites of high pH, moisture content, salinity and sodicity to much drier, neutral and fertile upland nonsaline soil sites. These results are consistent with previous field studies in that soil moisture and salinity have little or no depressive effect on AM fungi survival and colonization in roots (Brown & Bledsoe, 1996; Mendoza *et al.*, 2005). It is important to point out that without the concomitant analysis of the AM fungal community in each site, it cannot be concluded that the results of this work represent a wide ecological flexibility of the fungus or a shift of fungal species composition along the topographic gradient.

The mycorrhizal colonization index was consistently larger in *L. tenuis* than in the codominant grasses (*P. vaginatum* in Site 1 and *S. secundatum* in Sites 2–4) as well as seasonally different at all sites or seasons. The principal factors that directly or indirectly influence the extent of mycorrhizal root colonization include soil variables and plant characteristics (Baylis, 1975). Van der Heijden *et al.* (1998) found that plants respond differentially to specific AM fungal species. Therefore, one should not necessarily expect a similar seasonal pattern of mycorrhizal colonization index from the experimental plants along the topographic gradient, particularly when they differ to root systems, growing period and dependence on mycorrhizas to grow in nutrient-deficient soils. Differences in the length and seasonal pattern of colonized root among *L. tenuis*, *P. vaginatum* and *S. secundatum* plants may be ascribed to interactions among the growth rates of both the fungi within the roots and roots within the soil.

It is well documented that the morphology and architecture of roots are important factors in determining mycorrhizal responsiveness (Pate, 1994). Grasses are species with relatively fine and highly branched roots. These characteristics constitute an alternative absorption strategy for the acquisition of soil resources, which resulted in smaller responsiveness to mycorrhizal colonization than in *L. tenuis*. Based on the results of the present work, *L. tenuis* is more mycotrophic than the codominant grasses: *S. secundatum* and *P. vaginatum*.

In accordance with Hypothesis 1, the seasonal pattern of the arbuscular colonization was similar in roots of *L. tenuis* and the grasses. Larger arbuscular colonization fractions in roots were found at the beginning of the growing season in late winter at all the sites, and this might have been related to the period of active nutrient uptake by the host plant (Allen, 1983; Sigüenza *et al.*, 1996; Gai *et al.*, 2006). The seasonal pattern of vesicular colonization was similar in the roots of both *L. tenuis* and the grasses. Larger vesicle colonization fractions were found at all the sites and plant species in summer, when plant growth rate decreases because of the frequency of dry periods and high temperature spells. This fact suggests the presence of a seasonal effect on vesicle formation in roots. Vesicles produced by AM fungi are considered to function as temporary storage organs (Barker *et al.*, 1998; Hirsch & Kapulnik, 1998), and are signs that the fungi speed up their life cycles in annual plants towards the end of the growing season (Gavito & Varela, 1993). From the present work, it is clear that maximum arbuscular and vesicular formation in roots took place at different seasons. These seasonally related effects suggest the preferential production of one kind of morphological colonization forms by the fungus during a specific season.

Soil fertility may be associated with the status of C, N and/or P, while adverse soil conditions for plant growth may be associated with high values of pH, electrical conductivity and exchangeable Na. In the lowest (Site 1) and the highest (Site 4) sites, soil properties changed little with time, but at the intermediate altitude sites, especially in Site 2, electrical conductivity and exchangeable Na increased in spring, driven by the soil salinization process facilitated by the ascent and descent of the shallow and the saline water table that occurs as pulses in late spring and summer (Lavado *et al.*, 1992). In spite of the high levels and the seasonal increases of electrical conductivity and exchangeable Na in spring in Sites 1 and 2, the CCA analysis (Fig. 3b) shows a positive association between these soil variables and total

AM colonization and arbuscular colonization in *L. tenuis* roots, but a negative association with these indexes in the roots of the grasses. In these, the values of total root colonization and arbuscular colonization in Sites 1 and 2 were lower compared with the other two sites, suggesting that electrical conductivity and exchangeable Na may affect the symbiosis more in the grasses than in the legume even when the symbioses is functional for any combination between plant and site. Along the topographic gradient, the levels of C, N and P in soil did not show any association with AM fungal variables, presumably because they were low in availability and deficient for plant growth. However, in a broader gradient in soil fertility one may have a different conclusion. Despite the differences between the legume and the grasses, spore density in soil and AM colonization morphology in plants vary seasonally, which is in agreement with Hypothesis 2. These seasonal effects on AM fungal variables were independent of a particular combination between plant species and soil sites, suggesting that seasonality was an important factor in regulating both spore density in soil and changes in AM root colonization morphology in different studied plants. This is in disagreement with hypothesis 3, which had indicated that the seasonal differences in both spore density and AM root colonization morphology will be expressed differently with host species and soil properties.

Concentrations of N and P in plant tissue were always higher in *L. tenuis* than in the grasses, reflecting the differences in nutrient requirements between plant species. The changes of both nutrients in shoots were more marked than in roots. These results were the outcome of two effects. Firstly, the changes in plant nutrients partly occurred because plant growth can either dilute or concentrate nutrients in plant tissue, and also because N and P in soil are not sufficiently available to sustain the demand by growth, in which case the nutrient concentration in plants can decrease. Secondly, the other effect is related to the operation of a physiological plant mechanism when a deficiency exists. At a deficient nutrient stage, the plant satisfies firstly the nutrient requirements demanded by the roots, and afterwards the demand from shoots' growth (Loneragan & Asher, 1967). Even when N was characterized as deficient in soil for plant growth, there likely was an additional part of plant-N contributed by atmospheric N_2 fixed by *Rhizobium* spp. in *L. tenuis* roots that could have also been taken up by the grasses and alleviated their deficiency in N.

The CCA biplots show that arrows denoting N and P in plant tissue are oriented to arbuscular colonization and are opposite to vesicular colonization in the roots of the studied plants (Fig. 4). Several studies of plants with differing phenologies have indicated that mycorrhizal fungus activity is tied more closely to host metabolism than to host phenology (Allen *et al.*, 1984; Bentivenga & Hetrick, 1992). Allen (1983) found more arbuscules during periods of active nutrient uptake, and Mullen & Schmidt (1993) reported that AM root colonization closely followed nutrient demands generated by growth. Sanders & Fitter (1992) reported a significant nutritional benefit to the host during the growing period. In the present study, the higher arbuscular colonization was found at the beginning of the growing season, when the rate of growth and nutrient uptake increases were associated with the highest concentrations of N and P in plant tissue. This suggests the existence of some correspondence between symbioses partners and the rate of nutrients' transfer. Contrary to Hypothesis 4, these results suggest that increases of nutrient concentration in plant tissue, especially P, do not necessarily depress the arbuscular colonization in the roots. The authors think that increases of P in plant tissues may be associated with increases, decreases or no changes in the arbuscular colonization for roots, and that the sensitivity and the direction of this process depends on the initial level of available P for plant growth. In the present study, available P was always deficient for plant growth along the topographic slope, increases of P in plant tissue were not sufficient to alleviate the deficiency of P and the arbuscular colonization index increased. This interpretation was already proposed by Bolan *et al.* (1984), Mendoza & Gigli (1995), Mendoza & Pagani (1997) and Escudero & Mendoza (2005).

Higher densities of AM fungal spores were found in summer, suggesting that this storage structure was mainly formed at high temperatures and evapotranspiration rates and when the rate of plant growth decreased. This process was independent of soil characteristics or plant community, because it was observed at all the sites studied. Other authors studying spore density variability in several ecosystems found similar results (Carvalho *et al.*, 2001; Lugo & Cabello, 2002; Escudero & Mendoza, 2005). Sporulation of AM fungi depends on the season, and the seasonality of spore formation is an expression of AM fungi to survive when environmental conditions are unfavourable in soil. This is in agreement with Rickerl *et al.* (1994), who found twice more spores in wet than in drier soil; they suggested that higher sporulation in wet soil was a stress response of the fungi to the wet condition.

Soil characteristics, plant species and climate factors are known to influence AM fungal communities in grasslands (Johnson *et al.*, 1992; Allen *et al.*, 1995; Muthukumar & Udaiyan, 2002; Escudero & Mendoza, 2005). It is difficult to conclude which one of those factors is more important in controlling changes in AM fungal variables. One reason is the difficulty of establishing causation from simple correlations among soils, plants and climate factors with AM fungal variables. Another reason is that AM fungi may be associated with a wide range of hosts present in a grassland

community, and changes in a soil environment may promote the development of AM fungi at one point in time for one plant species and inhibit it for another plant species at another time.

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