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Seasonal variation of arbuscular mycorrhizal fungi in temperate grasslands along a wide hydrologic gradient

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Abstract We studied seasonal variation in population attributes of arbuscular mycorrhizal (AM) fungi over 2 years in four sites of temperate grasslands of the Argentinean Flooding Pampas. The sites represent a wide range of soil conditions, hydrologic gradients, and floristic composition. *Lotus glaber*, a perennial herbaceous legume naturalised in the Flooding Pampas, was dominant at the four plant community sites. Its roots were highly colonised by AM fungi. Temporal variations in spore density, spore type, AM root colonisation, floristic composition and soil chemical characteristics occurred in each site and were different among sites. The duration of flooding had no effect on spore density but depressed AM root colonisation. Eleven different types of spores were recognized and four were identified. Two species dominated at the four sites: *Glomus fasciculatum* and *Glomus intraradices*. Spore density was highest in summer (dry season) and lowest in winter (wet season) with intermediate values in autumn and spring. Colonisation of *L. glaber* roots was highest in summer or spring and lowest in winter or autumn. The relative density of *G. fasciculatum* and *G. intraradices* versus *Glomus* sp. and *Acaulospora* sp. had distinctive seasonal peaks. These seasonal peaks occurred at all four sites, suggesting differences among AM fungus species with respect to the seasonality of sporulation. Spore density and AM root colonisation when measured at any one time were poorly related to each other. However, spore density was significantly correlated with root colonisation 3 months before, suggesting that high colonisation in one season precedes high sporulation in the next season.

Keywords Arbuscular mycorrhizas · Seasonal variation · Grasslands · Hydrologic gradient · *Lotus glaber*

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Introduction

Soil characteristics, plant species, and climate may all regulate the arbuscular mycorrhizal (AM) fungi community in grasslands. The distribution of certain AM fungal species has been related to soil pH, phosphorus level, salinity, soil disturbance (Abbott and Robson 1991), vegetation (Johnson et al. 1992), or hydrologic condition of the soil (Ingham and Wilson 1999; Miller and Bever 1999). In general terms, increases in soil pH, nutrient status and salinity in soil are related to a decrease in AM root colonisation or spore density (Abbott and Robson 1991). Despite the importance of AM fungi in the physiology and nutrition of plants, as well as in shaping plant communities (Grime et al. 1987; Van der Heijden et al. 1998; Smith et al. 1999), factors affecting the presence, diversity, spore density, and root colonisation by AM fungi in soil are poorly understood. One reason is the difficulty of establishing causation from correlation of soil and plant factors with AM fungal populations. Another reason is that AM fungi can associate with a wide range of hosts present in a grassland community, but the sporulation rates of AM fungi have been found to be host dependent (Bever et al. 1996; Lugo and Cabello 2002). Host-dependence of AM fungal population growth rates in soil may play an important role in the maintenance of AM fungal species diversity in grasslands (Bever et al. 1996), and suppression of mycorrhizal symbioses may result in a decreasing of the dominant plant and an increase in species diversity (Hartnett and Wilson 1999). In addition, plant diversity may increase or decrease if the dominant plant competitors are more weakly or more strongly mycotrophic than their neighbours (Hartnett and Wilson 1999).

One difficulty when studying the relationship among soil characteristics, plant species, and climate with AM fungi, is the variable used to evaluate the magnitude of participation of AM fungi in plant nutrition. Neither spore counts in soil nor root colonisation are necessarily correlated with each other (Ingham and Wilson 1999; Mendoza et al. 2002), and the rate of extension of mycorrhiza formation is not always related to spore density (Abbott and Robson 1982).

An additional factor influencing populations of AM fungi in soil, which may in turn affect the performance of plant species relative to each other, is the hydrologic condition of the soil, which may vary seasonally. The hydrologic condition of the soil plays an important role in determining plant community structure, and this effect is even more important when soils are commonly subjected to periods of dryness and flooding (Chaneton et al. 1998). AM fungi have been found in the roots of many plants in wetlands (Ingham and Wilson 1999; Miller and Bever 1999), or salt marshes (Brown and Bledsoe 1996). This is relevant because the fungi are believed to require well-aerated soils, and are thought to have problems adapting to flooded conditions (Mosse et al. 1981). Nevertheless, little is known of AM fungi patterns in wetlands or of the influence of the hydrologic condition of the soil on populations of AM fungus species.

In this work we selected *Lotus glaber*—a perennial herbaceous legume naturalised in the Flooding Pampas of Argentina—as a test plant. It is highly appreciated by farmers because of its plasticity, productivity, ability to grow in nutrient-deficient soils, and its nutritional forage value for beef and dairy cattle farms (Mazzanti et al. 1988). During spring and summer it is the only co-dominant legume species in most plant communities of the Pampas grasslands (Collantes et al. 1988). Further, the seeds and plants of *L. glaber* seem to be tolerant of flooded conditions (Vignolio et al. 1996, 1999), which commonly occur in autumn, winter and part of the spring in the Pampas. *L. glaber* is highly dependant on root colonisation by AM fungi to grow at low phosphorus availability (Mendoza and Pagani 1997), and a high percentage of root length is colonised at different field sites (Mendoza et al. 2000). *L. glaber* is useful for the study of AM fungi patterns in wetlands because it grows along a wide temporal and spatial hydrologic soil gradient. We studied the effect of seasonal variation on populations of AM fungi in grasslands of the Argentinean Pampas that differ in soil conditions.

Materials and methods

Study sites

This research was undertaken in the Flooding Pampas, a grassland of 90,000 km^2, which extends along the Atlantic coast, northwest to southeast, in the Buenos Aires Province of Argentina (34°50 to 37°40S; 56°40' to 61°00'W). From a total of 18 grassland sites initially sampled, 4 were selected to contrast different soil characteristics such as pH, P status, salinity, hydrologic condition throughout the year, and the frequency of *L. glaber* in plant community (Table 1). The soil sites were named after the nearest locality; in the text we will refer to them with the corresponding number according to the initial identification. Site 2 was an abandoned disturbed pasture where *L. glaber* Mill., was sown in the autumn of 1997. *L. glaber*, *Phyla canescens* (H.B.K.) Greene, *Eryngium ebracteum* Lam. and *Piptochaetium bicolor* (Vahl) Desv., dominated the plant community. Sites 5, 9 and 16 were natural grasslands frequently grazed. Site 5 was dominated by *L. glaber*, *Ambrosia tenuifolia* Spreng., *Cyperus laetus* C. Presl. and *Juncus microcephalus* H.B.K., Site 9 by *L. glaber*, *Distichlis spicata* (L) Greene., *Eleocharis viridans* Kükenth and *Cynodon dactylon* (L) Pers., Site 16 by *L. glaber*, *Stenotaphrum secundatum* (Walt.) O.K., *Lolium multiflorum* Lam., *Stipa paposa* Nees., and *Paspalum dilatatum* Poir. The distance between sites ranged from 60 to 140 km. All sites were subjected to different flooding during the autumn-winter-spring period and became dry during part of the summer. For each study site and period of flood we calculated a flooding index (*FI*) by relating the number of days each site was subjected to flooding during the season to the total number of days of the season:

$$FI = \text{flooding period} / \text{total period} \quad (1)$$

Table 1 Soil site, soil classification, environment, dominant plant species and period of flooding of the studied sites

Soil site	Location and soil classification	Environment and dominant plant species	Period of flooding	Days[a]
2	Verónica	Disturbed grassland of	1998: mid December–end December	10
	Aquic Hapludert	*Lotus glaber-Phyla canescens*	1999: early March–mid July	152
			2000: early April–early August	120
5	Monte	Natural grassland of	1999: end April–end May	30
	Entic Hapludoll	*Lotus glaber-Ambrosia tenuifolia*	2000: early May–end July	76
9	San Vicente	Natural grassland of	1999: early January–mid August	219
	Typic Natraqualf	*Lotus glaber-Distichlis spicata*	2000: mid April–mid August	124
16	Samborombon	Natural grassland of	1999: non-flooding	0
	Typic Natraquoll	*Lotus glaber-Stenotaphrum secundatum*	2000: mid May–early June	23

[a]Days when standing water was present above the soil surface

where total period represents the number of days of each season (90 days) and the flooding period the number of days subjected to flooding during that time. Thus, *FI* ranges from 0 to 1. A value of *FI* equal to 1 means that the site was flooded over the 90 days of the seasonal period. In contrast, a *FI* of 0 means that no flooding occurs. *FI* values permitted quantification of the hydrologic condition of the sites during the experimental period, and were used to relate that variable to spore density, floristic composition and soil chemical properties using a correlation matrix. We defined "flooding" as occurring when standing water above the soil surface was present. In addition, mean air temperature, *T* (°C) and rainfall were recorded at each site.

Experimental design

A 2-year experiment was conducted within two permanent and concentric circular non-replicated plots of 8 and 12 m radius, respectively. The plots represented the typical plant community of each site and were chosen based on the presence of *L. glaber*. The plots were established in January 1999 and sampled each season (early in January, April, July and October) until October 2000. We refer to Jan, Apr, Jul and Oct respectively, followed by 99 (1999) or 00 (2000) according to the year. Because of flooding, two plant and soil seasonal samples could not be taken during the experiment (site 5, Oct00 and site 9, Jan99).

The circular plot was divided in five angular subplots where the internal plot (8 m) was used to measure the seasonal changes in floristic composition, each time on the same experimental unit. The remaining 4 m of external plot was used for soil and plant sampling to analyse seasonal changes in soil chemical characteristics, spore density, and *L. glaber* AM root colonisation. These samples were collected from three of the five subplots. This design permits analysis of data from an experiment in which several independent observations of the same variable were recorded on each of the experimental units over time (Rowell and Walters 1978).

Fungal spore extraction and analyses

For differentiation of fungal communities by monitoring spore density over time, four soil core samples per replicate were taken and mixed homogeneously to form a composite sample. The soil core sample was 12 cm deep. The top 2 cm was removed to eliminate part of the leaf litter. Each soil core contained approximately 125 g soil. The three composite soil samples were thoroughly mixed and divided into three portions to measure soil moisture, chemical characteristics and spore density. After sampling, soils were stored at 4°C until processing.

Spores were extracted from 5 g soil using a modification of the sucrose centrifugation technique (Daniels and Skipper 1982). First, a 250 μm sieve was used to separate the organic matter and a 37 μm sieve to collect the spores. The supernatant, both water and sucrose, was then filtered and the spores collected on the filter paper. All spores present on the filter paper were removed with a fine brush using a binocular stereomicroscope (40×). A spore of each type was mounted in polyvinyl-lacto-glycerol (PVLG) and Melzer's reagent and crushed under a glass coverslip for identification of species. Prepared slides were examined using a light microscope (100–1,000×) and spores identified (where possible) using current taxonomic criteria as described by the International Collection of Vescular and Arbuscular Mycorrhizal Fungi (INVAM; http://www.invam.caf.wvu.edu/). Spores that could not be identified at the species level were given a designation by genus. A spore reference collection of permanent slides is stored at the Centro de Estudios Farmacológicos y Botánicos (CEFYBO).

The relative density of each of the species in each composite sample was calculated as:

$$D = (n_i/N_t) \times 100 \quad (2)$$

where D is the relative density (%), n_i is the number of spores from the i^{th} species present, and N_t the total number of spores examined in the sample. Spore density (*Nt*) was expressed per gram of dry soil.

Mycorrhizal root colonisation

Measurements of mycorrhizal root colonisation were carried out in fresh roots of *L. glaber* and of a co-dominant plant species, cleared in 10% KOH for 30 min at 90°C, and stained in lactic acid-glycerol Trypan blue. Root length was determined by the line intercept method (Giovannetti and Mosse 1980) and expressed as percentage of root length colonised.

Floristic composition

The 8 m radius internal plot was used to estimate the frequency of each plant species by a modified point-transect method (Daget and Poissonet 1971). A randomly located transect in each internal plot, containing pins 1 m apart (8 pins per transect, 40 pins per plot), was used to estimate plant cover. Every contact of the aboveground structures of each plant species with each pin was recorded. From the pin-contact data, the relative frequency (*F*=number of pin-contacts made by individuals of species/total number of pin-contacts of all species ×100) was estimated for each plant species. We recorded the relative frequency of *L. glaber* (*FLt*), the relative frequency of a co-dominant plant species of highest frequency (*FCd*) and relative frequency of other plants present in the community (*FOPl*). In addition, we calculated plant species richness (*R*), as the mean number of relative species per plot, and diversity (Shannon's index, *H'*).

Soil analyses

Soil pH (soil:solution ratio of 1:2.5 in water) (Peech 1965), electrical conductivity (EC) (Bower and Wilcox 1965), phosphorus (P) (Bray and Kurtz 1945), cation exchange capacity (CEC), and exchangeable Ca, Mg, Na and K (Chapman 1965) were analysed seasonally.

Statistical analyses

Within each site, soil chemical data, floristic composition, and AM fungi variables were analysed to determine whether the data were normally distributed and the variances homogeneous. Spore data with non-normal distributions were corrected with ln transformations. Analysis of variance was used to determine if seasonal differences existed within each site. Mean separation was performed by the Tukey test. In cases where the non-normal distribution of a variable persisted, data were analysed by Kruskal-Wallis non-parametric test. Statgraphic 5.0 Plus software was used for statistical analyses, and Graph Pad Prism 2.01 was used to determine correlations (Pearson coefficient) between variables.

To establish how the AM fungi measures are related to the measured soil chemical (pH, P and Na), floristic (*FLt*, *FCd*, *H*) and environmental (*FI*, *T*) variables, we used the canonical correspondence analyses (CCA) ordination technique by CANOCO program (Ter Braak 1987–1992). Five AM fungi variables: total spore density (*Spores*), relative spore density of *G. fasciculatum* and *G. intraradices* (*DF+I*), relative spore density of *Glomus* sp. and *Acaulospora* sp (*DG+A*), relative density of other spore types (*DOt*) and colonisation of *L. glaber* root (*RCLt*), were included in the main matrix, corresponding to the 5×30 seasonal sampling points. The second matrix constituted the soil, floristic and environmental variables (3, 3 and 2, respectively) corresponding to the 8×30 seasonal sampling points. CCA permits identification of the best linear combinations of variables that influence the AM fungi variables. The significance of the two first canonical axes was analysed by the Monte Carlo permutation test.

Table 2 Mean (± sttandard error) of three replicates of some chemical and physical characteristics of the soil sites before the experiment. C_t Total Carbon,N_t total nitrogen,*P* phosphorus (Bray and Kurtz 1945), *EC* electrical conductivity, *CEC* cation exchange capacity

Soil property	Soil site			
	2	5	9	16
pH (1:2.5; H_2O)	5.63±013	7.38±1.10	8.89±0.43	7.64±0.45
C_t (g/kg)[a]	55.3	26.6	32.7	89.7
N_t (g/kg)[a]	4.53	2.34	2.76	5.22
P Bray I (mg/kg)	1.38±0.31	6.23±3.31	10.93±2.96	6.88±3.22
EC (dSl/m)	0.51±0.28	1.93±1.60	5.03±2.43	1.52±0.61
CEC (cmol/kg)	17.93±0.99	12.98±2.29	13.60±1.16	24.75±2.08
Ca (cmol/kg)	8.12±0.72	9.42±2.17	4.27±0.95	11.63±1.65
Mg (cmol/kg)	3.81±0.39	2.96±0.55	2.16±0.46	6.99±0.77
Na (cmol/kg)	1.31±0.13	3.17±2.22	11.59±2.49	6.89±1.19
K (cmol/kg)	0.60±0.12	0.39±0.14	1.54±0.24	1.80±0.18
Clay (%)[a]	38.7	3.7	15.9	26.7
Loam (%)[a]	52.4	9.6	61.6	60.2
Sand (%)[a]	8.9	86.7	22.5	13.1

[a]Mean of two measures (less than 5% of variation) from one composite sample

Results

Soil sites

The soil sites selected differed widely in their physical and chemical characteristics (Table 2). Such differences are typical of the Flooding Pampas. Soil 2 is heavy and P-deficient for plant growth. Soil 9 has high pH and high exchangeable sodium. Soil 5 has a high sand content, and consequently a low water retention capacity during the

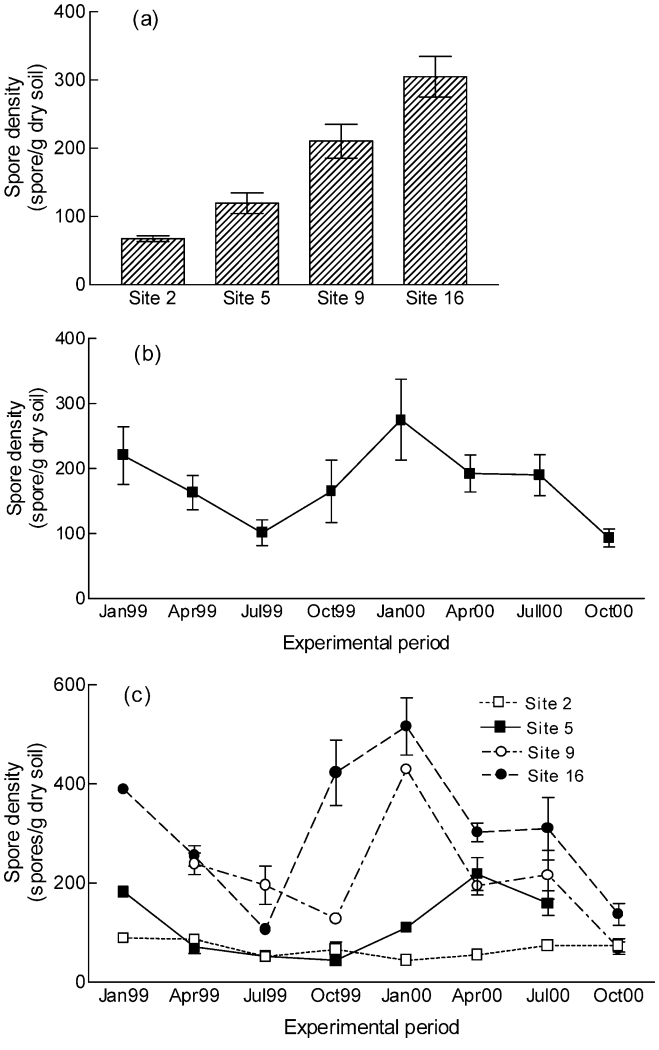

Fig. 1 Total spore density at each site (**a**) and seasonal variation during the two experimental years for four sites together (**b**) and separately by site (**c**). Data are mean values of 24 (**a**), 12 (**b**) and 3 (**c**) observations. *Bars* Standard error of mean (not transformed data)

summer drought. Soil 16 seems to be relatively well balanced for plant growth. Even though we are not able to determine significant statistical differences among sites because we have no replication by grassland subtype, marked differences among sites are obvious.

AM fungal spore communities and AM fungal root colonisation

The total spore density across different seasons varied among sites (Fig. 1a). There were also differences between seasons when averaged over all sites (Fig. 1b) and individual differences for each site (Fig. 1c). The number of spores per gram of dry soil ranged from 44 for site 2 in Jan00 to 516 for site 16 in Jan00 (Fig. 1c). Site 2 had both the lowest spore density and no significant ($P>0.05$) seasonal variation (Fig. 1c). For the other three sites, spore density was significantly ($P<0.05$) influenced by the season (Fig. 1c). The overall trend was higher spore density in summer and lower in winter, with intermediate values in autumn and spring (Fig. 1b). In contrast to site 2, site 16 had highest spore density and significant (ln transformed, $P<0.001$) fluctuation throughout the experimental period (Fig. 1c).

From the spore characteristics we grouped the spores into 15 types. We identified four of these as: *Glomus fasciculatum*, *Glomus intraradices*, *Glomus* sp. (resembling *G. diaphanum*), *Acaulospora* sp. The other 11 types of spores were grouped as "Others". Two species, *G. fasciculatum* and *G. intraradices*, dominated the AM spore communities and were found in high relative density at all four sites. We distinguished *G. fasciculatum* from *G. intraradices* with a compound microscope, but hundreds of field-collected spores are difficult to separate by a dissecting microscope. In addition, many species reported in the literature as *G. fasciculatum* appear now to be synonymous with *G. intraradices* (INVAM; http://www.invam.caf.wvu.edu/). Accordingly, we analysed *G. fasciculatum* and *G. intraradices* together as one species.

The relative density (D) of the *G. fasciculatum-G. intraradices* complex differed seasonally within sites having the highest relative spore density at all sites and dates sampled (Fig. 2), with two exceptions (Site 2, Jul00; and Site 9, Apr99). These types of spore were most abundant in Site 16 with maximum values ranging from 75% (Apr99, Jul00 and Oct00) to 96% (Jan00) and a mean value of 82% of the total spore count. The lowest relative values were in Site 2 with 46% (Jul00) and a mean value of 71% (Fig. 2).

The relative density of spores of *Glomus* sp. and *Acaulospora* sp. changed with time. For all sites and dates, the relative densities of these two types of spore were strongly correlated ($r=0.650$; $P<0.001$). They were most abundant in autumn 1999 (Apr99) at all four sites (Fig. 2), suggesting the occurrence of a special seasonal condition that may promote sporulation of these two AM species. Apart from this peak, there were few other changes with time in the spores of *Glomus* sp. and of *Acaulospora* sp. (Fig. 2).

Figure 2 shows distinct seasonal peaks between the relative densities of *G. fasciculatum* and *G. intraradices* versus *Glomus* sp. and *Acaulospora* sp. The relative densities of these two groups of spores were negatively correlated ($r=0.601$; $P<0.001$). These seasonal peaks occurred at all four sites.

The overall mean values of AM spores grouped as "Others" ranged from 11% in sites 9 and 5 to 18% in site 2 (Fig. 2).

The percentage root length of *L. glaber* colonised varied among sites from 85 (site 9) to 95% (site 5) (Fig. 3a). It also varied seasonally within sites (Fig. 3b, c). The effect of season was significant in sites 2, 9 and 16 (P values

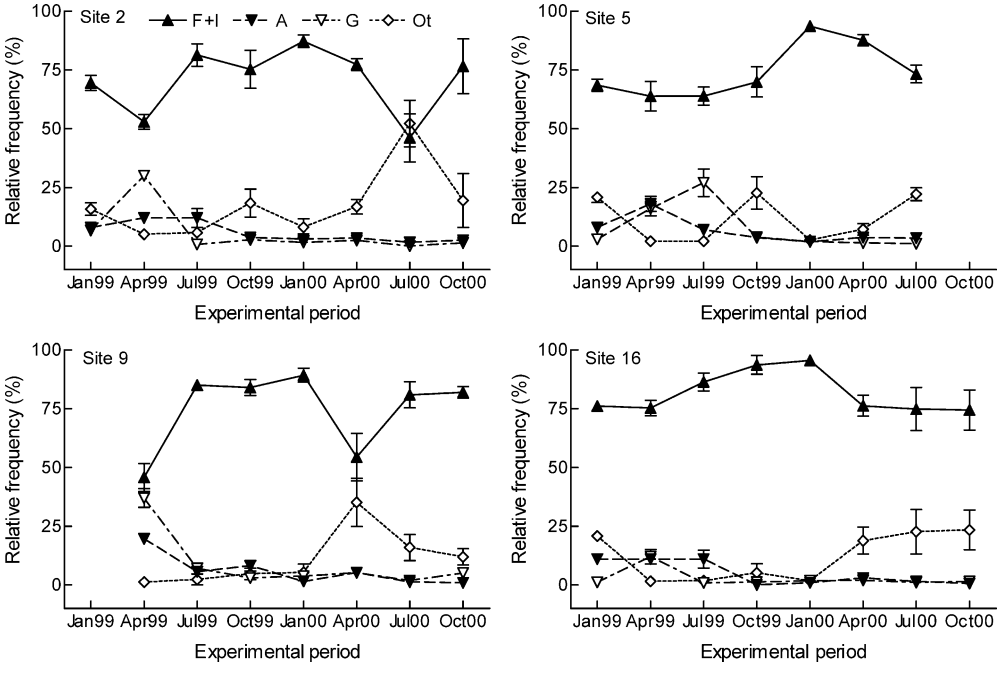

Fig. 2 Seasonal changes of the relative spore density of *Glomus fasciculatum* and *Glomus intraradices* (*F+I*), *Acaulospora* sp. (*A*), *Glomus* sp. (*G*), and other spores (*Ot*), at the four studied sites. *Bars* Standard error of mean (not transformed)

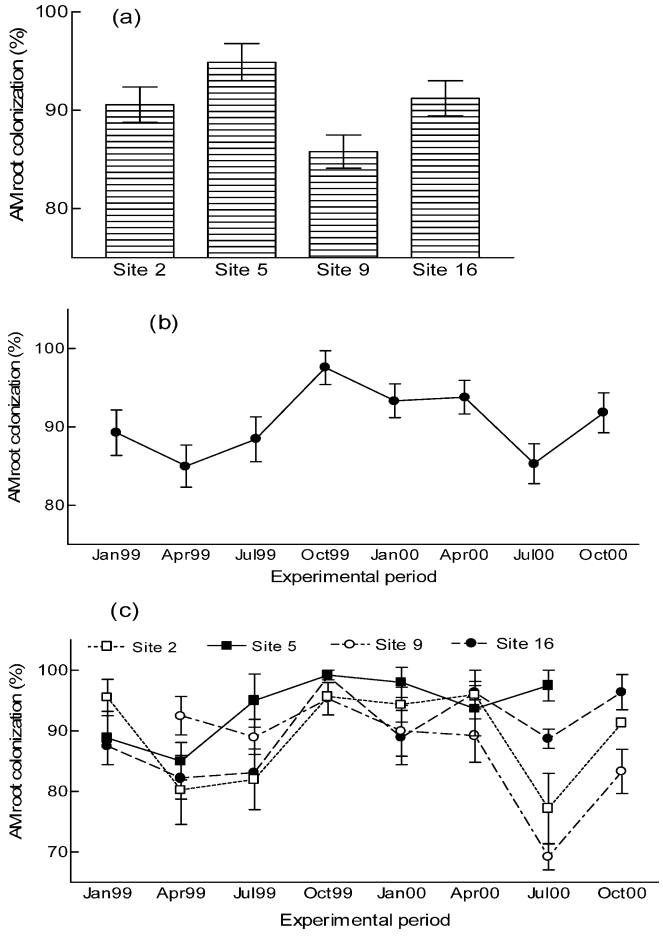

Fig. 3 Percentage of total root length colonised in *Lotus glaber* by arbuscular mycorrhizal (AM) fungi at each site (**a**) and seasonal variation during the two experimental years for all sites together (**b**) and separately by site (**c**). Data are mean values of 24 (**a**), 12 (**b**) and 3 (**c**) observations. *Bars* Standard error of mean (not transformed)

0.031, 0.010, and 0.051, respectively). The roots of *L. glaber* in site 5 were most highly colonised and varied least ($P<0.079$) during the experimental period (Fig. 3c). The lowest percentage of root length colonised was 69% in winter for site 9 (Jul00), and the highest was 99% in spring for sites 5 and 16 (Oct99). In the 1st year, highest rates of root colonisation occurred in summer or spring and lowest in winter or autumn (Fig. 3b). In the 2nd year there was a marked decrease in root colonisation in sites 2 and 9 in July 2000 to 72 and 68% respectively (Fig. 3c).

There was no significant relationship ($r=0.027$, $P<0.981$, $n=30$) between spore density in one season and *L. glaber* root colonisation in that season (Fig. 4a), but when we plotted the observations of spore density measured in one season against the percentage of root length colonised in the preceding season (Fig. 4b), the relationship improved ($r=0.531$, $P<0.0509$, $n=26$).

Influence of the environment and season on AM fungus structure

Figure 5 displays the seasonal samples on the two first ordination axes of the CCA study. The two first axes, indicators of the amount of AM fungi community variation, explained 46% of the total variance (axis I: 32.8% and axis II: 13.2%). The Monte Carlo test indicated that both the overall effect of the variables (which includes soil, vegetation and environment) and the two first canonical axes were significant (axis I, $P=0.0477$ and axis II, $P=0.0111$). The ordination placed observations of sites 2 and 5 on the negative side and those of sites 9 and 16 on the positive side of axis I. With some exceptions, along the two axes the observations for each of the sites are separated from each other, suggesting seasonality, and confirming the results already shown in Table 2 and Figs. 1, 2, and 3. Axis I was positively correlated ($P<0.001$) with *Spores*, while axis II was negatively correlated ($P<0.001$) with *DG+A* but positively correlated ($P<0.01$) with *DF+I*. Figure 5 also reveals the ordination of soil, floristic and environmental variables that significantly correlated with the axes. The length of the vector representing variables describes the relative significance of the correlation of that variable with the axes. Axis I positively correlated with soil pH ($r=0.67$, $P<0.001$), exchangeable Na ($r=0.76$, $P<0.001$) and available P ($r=0.57$, $P<0.01$). Axis II negatively correlated with *FLt* ($r=-0.74$, $P<0.001$) and *T* ($r=-0.47$, $P<0.01$). Axis I, representing the increment in spore density, towards the right side of the diagram, is associated with higher values of soil pH, Na and P. The negative scale of axis II represents the increment of *DG+A* and is related to higher *FLt* and air temperature (*T*), while the positive scale of axis II is associated with an

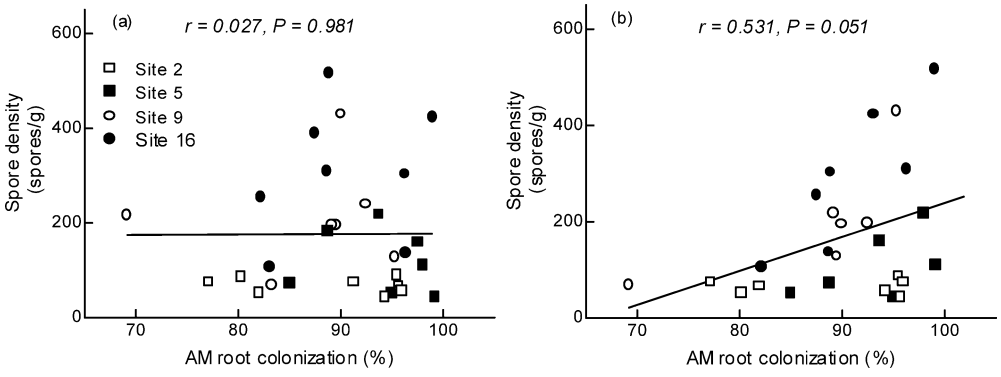

Fig. 4 Relationship between seasonal observations of spore density and percentage of *L. glaber* root length colonisation (**a**), and the relationship with spore density measured 3 months after the root measurements, during the next season (**b**)

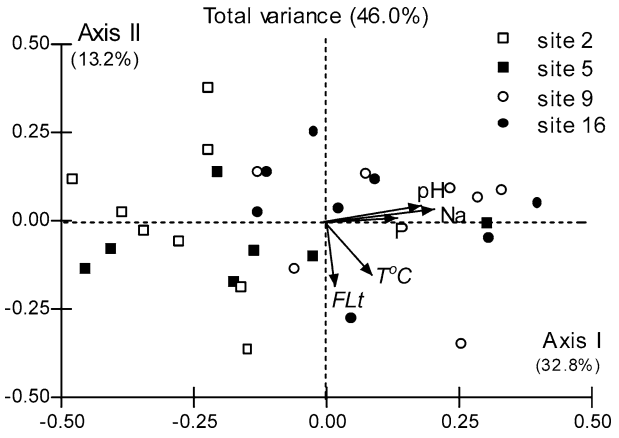

Fig. 5 Ordination diagram (axes I and II) of seasonal observations based on AM fungi variables (main matrix) and, soil, vegetation and environment variables (second matrix) by canonical correspondence analyses (CCA)

increment in *DF+I*. *FI* had no correlation with either axis I (r=−0.24), or axis II (r=−0.318) suggesting no association between *FI* and AM fungal structure. Nevertheless, simple regressions between *FI* and the AM fungi variables indicated that *FI* was negatively correlated with *RCLt* (r=−0.43, P=0.0178).

Discussion

We observed significant seasonal variation in the composition of AM fungal communities in temperate grasslands from the Argentinean Flooding Pampas. The selected sites represent a wide range in soil type; physical and chemical soil properties, floristic composition of vegetation and the period over which the soils are subjected to flooding. Some soil sites had seasonal variations both in spore density and AM root colonisation, others had little variation. There were high values for spore density and for colonisation of *L. glaber* roots even when the period over which the soil was subjected to flooding ranged from 8% (site 5 in 1999) to 60% of the year (site 9 in 1999). This is important because the fungi are believed to require well-aerated soils, and are thought to have problems adapting to flood conditions (Mosse et al. 1981). Other authors studying spore density variability (Ingham and Wilson 1999), and root colonisation (Turner et al. 2000) in wetlands found results similar to ours. Spore density was highest during the summer (dry season) and lowest in winter (wet season) with intermediate values in autumn or spring. This seasonal pattern in spore density has been observed in salt marsh soils (Carvalho et al. 2001). In xeric Mediterranean grasslands, the variation in total spore density appears to be controlled mainly by dry and wet periods (Lugo and Cabello 2002), coinciding with our results.

In the present work, the mean value of spore density varied 5-fold among soil sites, from 305 per gram of dry soil in the nutritionally well balanced soil (Site 16) to 68 per gram of dry soil in the clay, P-deficient, soil (site 2). Previous work from cool-dry region grasslands showed that spore density was positively correlated with N, P, and soil pH (Mendoza et al. 2002). Thus, our previous and present results are inconsistent with the general hypothesis that spore density decreases with increasing soil pH, nutrient status or salinity. The soil sites differed widely in many soil chemical properties and it is unlikely that a single property would explain the AM fungi dynamics. Our conclusion is that the relationship between spore density, and other measures of AM fungi, with chemical soil properties is a result of the interactions of many soil, floristic, and climate parameters, and could be specific for each particular case. It has been reported that in field soils, spore density appears to reach a maximum value in conditions where P status in soil is less than that required for maximum shoot growth (Abbott and Robson 1991), and may then decrease with increasing P status. In our case, P status in soil (1.38–10.93 ppm P) was always less than that required for maximum plant growth. An increase in P level in these P-deficient soils could increase spore count before the expected decrease occurs. In addition, the P required to reach maximum plant growth generally differs between soils and this could be another reason to justify no direct relationship between soil P and AM fungi measurements. Soil pH has an effect on spore density similar to that of P status. Some AM fungal species are restricted to either acid or alkaline soils; others occur in both (Young et al. 1985; Porter et al. 1978; Robson and Abbott 1989). With this natural selection of species adapted to different soil conditions, it is not surprising that there was no marked trend or limiting value found for soil pH, exchangeable Na or P availability to increase or decrease spore density.

Two AM species, *G. fasciculatum* and *G. intraradices*, dominated the spore communities at all four sites. The relative densities of *G. fasciculatum* and *G. intraradices* versus *Glomus* sp. and *Acaulospora* sp. were negatively correlated (P<0.001). This relationship occurred in spite of the broad differences among the four sites, and may suggest niche differentiation among AM species with respect to the seasonality of sporulation. The spores of *G. fasciculatum* and *G. intraradices* were relatively more abundant in summer than in winter, when flooding is most frequent in the Argentinean Pampas. The temporal variation in spore density is a consequence of many interacting factors such as plant communities, soil site characteristics, and climate. The spore density is a standing crop and reflects the net effect of sporulation versus spore disappearance (leaching, dispersal, germination, mortality, predation, etc). These two processes are difficult to separate, and changes in spore density may not necessarily reflect net differences in sporulation.

Soil moisture or flood factors appear to significantly affect total spore density, but there have been conflicting reports in the literature suggesting that the total spore density may increase, decrease, or show no change with soil moisture or flooding (Carvalho et al. 2001; Entry et al. 2002). Some reports suggest that because AM fungi are obligately aerobic, flooding would reduce sporulation (Aziz et al. 1995), and total spore density would be negatively correlated with soil moisture or redox potential (Anderson et al. 1984; Khan 1993). Others have found

higher values of total spore density in wet soils than in dry soils (Rickerl et al. 1994), and have suggested that high sporulation is a stress response of the fungi to adverse weather conditions. In the present work the duration of flooding, reflected in the *FI*, had no association with spore density.

We found no relationship between spore density and colonisation of *L. glaber* roots when comparing measures in the same season. Similar results were reported in a previous work (Ingham and Wilson 1999). Miller and Bever (1999) suggested that it is possible that the pattern observed in spore density may reflect not the activity of these AM fungi within roots, but rather their propensity to sporulate along a hydrologic gradient. We have proposed an additional explanation for the relationship between root colonisation and spore density. When we plotted the values of colonisation of *L. glaber* roots against the values of spore density measured 3 months later, in the next season, the relationship improved (from r=0.027, P=0.981 to; r=0.531, P=0.0509). We think that colonisation in one season foreshadows sporulation in the next season. This explanation may not necessarily contradict the explanation of Miller and Bever (1999), but may rather complement it.

Even when flooding was prolonged during the year in some of the soils, colonisation of *L. glaber* roots was never lower than 70%, and colonisation of accompanying species was never lower than 50% of total root length. Some legumes of economic importance can grow while flooded. This fact is related to the ability to develop a continuous network tissue (aerenchyma) of air pathways from the aerial stem to the roots (Kleiman et al. 1992; Vignolio et al. 1996; James and Crawford 1998) and *L. glaber* has shown the presence of aerenchyma tissue in both stems and roots (Vignolio et al. 1996). It was found that the number of spores in soil was not affected by extended flooding, indicating that mycorrhizal formation is related to plant growth (Miller 2000; Miller and Sharits 2000). Thus, AM fungal species can tolerate and persist in roots under flooded conditions and justify the high values of root length colonisation, even when we found evidence that the duration of flooding may depress colonisation of *L. glaber* roots.

It is also difficult to separate the influences of host plant species and soil characteristics on spore density or any other measure of AM fungi. Some authors suggest that host plant factors are more important than soil factors (Koomenn et al. 1987; Mendoza et al. 2002). Others suggest that plant and soil factors are equally important in regulating sporulation (Johnson et al. 1992). The present work has shown that some soil factors (pH, Na, and P) are much more associated with total spore density, while the relative frequency of *L. glaber*, and mean air temperature were much more associated with changes in the relative spore density. However, soil pH and Na are auto-correlated (P<0.001) and, together with the relative frequency of *L. glaber*, exhibit seasonal changes during the experiment. Hence, it is difficult to establish specific causation.

The total spore counts we measured in the present work at each soil site are a consequence of the influence of many plant community and soil variables on the AM fungal community rather than of the influence of one specific dominant plant species or soil property. Under field conditions, differences in sporulation in the rhizosphere of each plant species are impossible to separate because plants grow in close proximity and their root systems overlap. Accordingly, we were not able to associate the relative frequency of a host plant (*L. glaber* or accompanying species) with total or relative spore density. The plant community interacts with the soil and can modify edaphic properties relatively quickly, but edaphic factors also interact with the plant community and can modify floristic composition as well. Consequently, there cannot be a clear separation between plant and soil factors on AM fungal sporulation in these temperate grasslands.

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References

Abbott L, Robson A (1982) Infectivity of vesicular-arbuscular mycorrhizal fungi in agricultural soil. Aust J Agric Res 33:1049

Abbott L, Robson A (1991) Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. Agric Ecosyst Environ 35: 121–150

Anderson R, Liberta A, Dickman L (1984) Interaction of vascular plants and vesicular-arbuscular mycorrhizal fungi across a soil moisture-nutrient gradient. Oecologia 64:111–117

Aziz T, Sylvia D, Doren R (1995) Activity and species composition of arbuscular mycorrhizal fungi following soil removal. Ecol Appl 5:776–784

Bever J, Morton J, Antonovics J, Schultz P (1996) Host-dependent sporulation and diversity of arbuscular mycorrhizal fungi in a mown grassland. J Ecol 84:71–82

Bower CA, Wilcox LW (1965) Soluble salts. In: Black CA (ed) Methods of soil analysis, part 2, 2nd edn. American Society of Agronomy, Madison, Wis., pp 933–951

Bray RH, Kurtz LT (1945) Determination of total organic and available forms of phosphorus in soils. Soil Sci 59:39–45

Brown A, Bledsoe C (1996) Spatial and temporal dynamics of mycorrhizas in *Jaumea carnosa*, a tidal saltmarsh halophyte. J Ecol 84:703–715

Carvalho LM, Cacados I, Martiris-Loucao MA (2001) Temporal and spatial variation of arbuscular mycorrhizas in salt marsh plants of Tagus estuary (Portugal). Mycorrhiza 11:303–309

Chaneton E, Facelli J, León R (1998) Floristic changes induced by flooding on grazed lowland grasslands in Argentina. J Range Manage 41:495–499

Chapman HD (1965) Cation exchange capacity. In: Black CA (ed) Methods of soil analysis, part 2, 2nd edn. American Society of Agronomy, Madison, Wis., pp 891–901

Collantes M, Kade M, Myacszynski C, Santanatoglia O (1988) Distribución de especies en función de factores edáficos en un pastizal natural de la Depresión del Río Salado (Provincia de Buenos Aires). Studia Oecologica 5:77–93

Daget P, Poissonet J (1971) Uné méthode d'analyse phytologique des prairies. Critéres d'application. Ann Agron 22:5–41

Daniels N, Skipper H (1982) Methods for the recovery and quantitative estimation of propagules from soil. In: Methods and principles of mycorrhizal research. American Phytopathology Society, Washington DC, pp 29–35

Entry JA, Rygiewicz PT, Watrud LS, Donnelly PK (2002) Influence of adverse soil conditions on the formation and function of *Arbuscular mycorrhizas*. Adv Environ Res 7:123–138

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol 84:489–500

Grime JP, Mackey JM, Hillier SM, Read DJ (1987) Floristic diversity in a model system using experimental microcosms. Nature 328:420–422

Hartnett D, Wilson G (1999) Mycorrhizae influence plant community structure and diversity in tallgrass prairie. Ecology 80:1187–1195

Ingham E, Wilson M (1999) The mycorrhizal colonization of six wetland species at sites differing in land use history. Mycorrhiza 9:233–235

James EK, Crawford RMM (1998) Effect of oxygen availability on nitrogen fixation by two *Lotus* especies under flooded conditions. J Exp Bot 49:599–609

Johnson NC, Tilman D, Wedin D (1992) Plant and soil controls on mycorrhizal fungal communities. Ecology 73:2034–2042

Khan AG (1993) Occurrence and importance of mycorrhizae (VAM) in aquatic trees of New South Wales, Australia. Mycorrhiza 3:31–38

Kleiman ID, Cogliatti DH, Santa María GE (1992) Efecto de la hipoxia sobre el crecimiento y adquisición de nutrimentos en *Lolium multiflorum*. Turrialba 42:210–219

Koomenn I, Grace C, Hayman DS (1987) Effectiveness of single and multiple mycorrhizal inocula on growth or clover and strawberry plants at two soil pHs. Soil Biol Biochem 16:539–544

Lugo MA, Cabello MN (2002) Native arbuscular mycorrhizal (AMF) from mountain grassland (Córdoba, Argentina) I. Seasonal variation of fungal spore diversity. Mycologia 94:579–586

Mazzanti A, Montes L, Minon D, Sarlangue H, Chepi C (1988) Utilización de *Lotus tenuis* en la Pampa Deprimida: resultado de una encuesta. Rev Agric Prod Anim 8:301–305

Mendoza R, Pagani E (1997) Influence of phosphorus nutrition on mycorrhizal growth response and morphology of mycorrhizae in *Lotus tenuis*. J Plant Nutr 20:625–639

Mendoza R, Pagani E, Pomar MC (2000) Variabilidad poblacional de *Lotus glaber* en relación con la absorción de fósforo en suelo. Ecol Austr 10:3–14

Mendoza R, Goldmann V, Rivas J, Escudero V, Pagani E, Collantes M, Marbán L (2002) Poblaciones de hongos micorrízicos arbusculares en relación con propiedades del suelo y planta hospedante en pastizales de Tierra del Fuego. Ecol Austr 12:9–20

Miller S (2000) Arbuscular mycorrhizal colonization of semi-aquatic grasses along a wide hydrologic gradient. New Phytol 145:145–155

Miller S, Bever J (1999) Distribution of arbuscular mycorrhizal fungi in stands of the wetland grass *Panicum hemitomon* along a wide hydrologic gradient. Oecologia 119:586–592

Miller S, Sharitz RR (2000) Manipulation of flooding and arbuscular mycorrhizal formation influences growth and nutrition of two semi-aquatic species. Funct Ecol 14:738–748

Mosse B, Stribley D, Le Tacon F (1981) Ecology of mycorrhizas and mycorrhizal fungi. In: Alexander M (ed) Advances in microbial ecology. Plenun Press, New York, pp 137–210

Peech M (1965) Hydrogen-ion activity, In: Black CA (ed) Methods of soil analysis, part 2, 2nd edn. American Society of Agronomy, Madison, Wis., pp 914–926

Porter WM, Abbott LK, Robson AD (1978) Field survey of the distributions of VA mycorrhizal fungi in relation to soil pH. J Appl Ecol 24:659–662

Rickerl DH, Sancho FO, Ananth S (1994) Vesicular-arbuscular endomycorrhizal colonization of wetland plants. J Environ Qual 23:913–916

Robson AD, Abbott LK (1989) The effect of soil acidity on mycrobial activity in soils. In: Robson AD (ed) Soil acidity and plant growth. Academic Press, Sydney, 99:139–165

Rowell JG, Walters DE (1978) Analysing data with repeated observations on each experimental unit. J Agric Sci 87:423–432

Smith M, Hartnett D, Wilson G (1999) Interacting influence of mycorrhizal symbiosis and competition on plant diversity in tallgrass prairie. Oecologia 121:574–582

Ter Braak CJF (1987–1992) CANOCO—a FORTRAN program for canonical community ordination. Microcomputer Power, Ithaca, N.Y.

Turner SD, Amon JP, Schneble RM, Friese CF (2000) Mycorrhizal fungi associated with plants in ground-water fed wetlands. Wetlands 20:200–204

Van der Heijden MGA, Kilronomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72

Vignolio O, Fernández O, Maceira N (1996) Respuestas de *Lotus tenuis* y *Lotus corniculatus* (Leguminosae) al anegamiento en plantas de distintas edades. Rev Fac Agron La Plata 101:57–66

Vignolio O, Fernández O, Maceira N (1999) Flooding tolerance in five populations of *Lotus glaber* Mill. (Syn. *Lotus tenuis* Waldst. Et. Kit.). Aust J Agric Res 50:555–559

Young JL, Davis EA, Rose SL (1985) Endomycorrhizal fungi in breeder wheats and *Triticale* cultivars field grown on a fertile soil. Agron J 77:219–224