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Variation in soil micro-organisms and nutrients underneath and outside the canopy of *Adesmia bedwellii* (Papilionaceae) shrubs in arid coastal Chile following drought and above average rainfall

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Micro-organisms and nutrients were determined in soil samples (top 20 cm) collected underneath and outside the canopy of *Adesmia bedwellii* in mid fall (April 1997), after 8 months of drought, and in mid spring (October 1997) after heavy winter rains associated with the 1997-ENSO in a Chilean coastal desert site. Bacteria and fungi were two orders of magnitude higher in mid spring, but no seasonal effects were detected for chemical parameters. Micro-organisms, organic matter and nitrogen content were significantly higher underneath shrub canopies, especially in mid fall. Micro-organism abundances were positively correlated with nitrogen levels and soil moisture.

Keywords: soil fertility; micro-organisms; micorrhizae; fertility island; arid zone; Chile

Introduction

In arid environments nutrients are scarce and patchily distributed, with greater amounts under shrub canopy generating 'fertility islands' (*sensu* García-Moya & McKell, 1970). Micro-organisms associated with these fertility islands are important for plant growth, since they favor the assimilation of nutrients (Davison, 1988), produce hormones that promote growth (Denarie *et al.*, 1992), fix nitrogen (Farnsworth *et al.*, 1977), suppress pathogens (Shippers *et al.*, 1987) and permit the dissolution of minerals (Nakas & Klein, 1980).

The abundance of micro-organisms in soil varies spatially as well as temporarily, and this pattern is related to temporal and spatial variations in the quantity and quality of nutrients (Nedwell & Gray, 1987; Wardle, 1992). Micro-organisms respond to nitrogen (Jenkins *et al.*, 1988; Wardle, 1992), organic matter (Hussey *et al.*, 1985; Jenkins *et al.*,

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1988; Lynch & Whipps, 1990) and soil moisture (Bottner, 1985; Jenkins *et al.*, 1988; Wardle, 1992). Despite the evident importance of micro-organisms associated with root systems of plants inhabiting arid regions, very few studies have been published to date (see references in Trappe, 1981; Dhillion & Zak, 1993), and the information on soil micro-organisms in the arid regions of South America is scarcer. In the Chilean fog-free desert, one of the driest desertic regions of the world (Evans & Ehleringer, 1994), only one study on soil micro-organisms associated with plants has been published. Dhillion *et al.* (1995) reported on the mycorrhizal status of annual, biennial weeds, and shrubs of this region, finding that over 90% of 38 species (19 families) studied had associations with vesicular arbuscular mycorrhizae (VAM). They hypothesized that temporally associated edaphic factors at the sites could influence the degree of colonization by VAM.

In Chilean arid environments, Gutiérrez *et al.* (1993) found higher levels of nutrients (nitrogen and phosphorus) underneath than outside the canopy of shrubs. They also suggested that decomposition activity would be favored by milder temperatures and greater soil moisture under shrubs in these environments. However, up to date, no data have been reported for the Chilean arid zone to test this hypothesis. Here, we examined whether the abundance of soil micro-organisms varies in two constrasting seasons (dry *vs.* wet) and microhabitats (underneath and outside the canopy of the woody thorn shrub *Adesmia bedwellii*).

Materials and methods

Species and study site

Adesmia bedwellii (Papilionaceae) is a dominant woody thorn shrub, with 2·6 to 5·7% plant cover at the study site, located in an interior valley (Quebrada de Las Vacas, 230 m elevation) in Parque Nacional Fray Jorge (71°40′ W, 30°38′ S) Chile, 5 km east of the Pacific Ocean (for more details see Gutiérrez *et al.*, 1993). Soils are loamy sand with 85% sand, 8% silt, and 6% clay (Gutiérrez *et al.*, 1993). The climate is arid mediterranean, with 90% of the 85 mm annual precipitation falling in winter months (May–September). The average relative air moisture is high (70–75%) all year round. Summer months are warm and dry. An important characteristic of this environment is the extreme inter-annual variation in rainfall due to episodic high rainfall associated with El Niño southern oscillation (ENSO) events every 3–7 years. For example, 1994, 1995, and 1996 were extremely dry (35, 56, 70 mm, respectively) whereas 1997—associated to an ENSO event—was a wet year (332 mm). In 1997, the first rains fell in May (16·4 mm) and the last ones in October (13·2 mm), reaching the maximum peak in June (173 mm) (Fig. 1). The total precipation between May and September 1997 was 295·4 mm, which is 4-fold higher than the average for that span.

Soil sampling

In mid fall (April) 1997 before rainfalls, one soil sample from the top 20 cm (*c.* 1 kg), which is the region with the highest mycorrhiza incidence (Davidson & Christensen, 1977; Schwab & Reeves, 1981; Allen, 1983), was collected under the canopy of each of 12 randomly chosen *A. bedwellii* shrubs. A similar number of soil samples were taken in the open, 5 m away from *A. bedwellii* canopy. The same procedure was repeated in mid spring (October) 1997, after winter rainfalls. Hence, a total of 48 soil samples were collected and each treatment (site and season) had 12 replicates for all the analyses (chemical and microbial) described below. Soil samples were stored in plastic bags and transported to the laboratory within 6 h for chemical and microbial analyses.

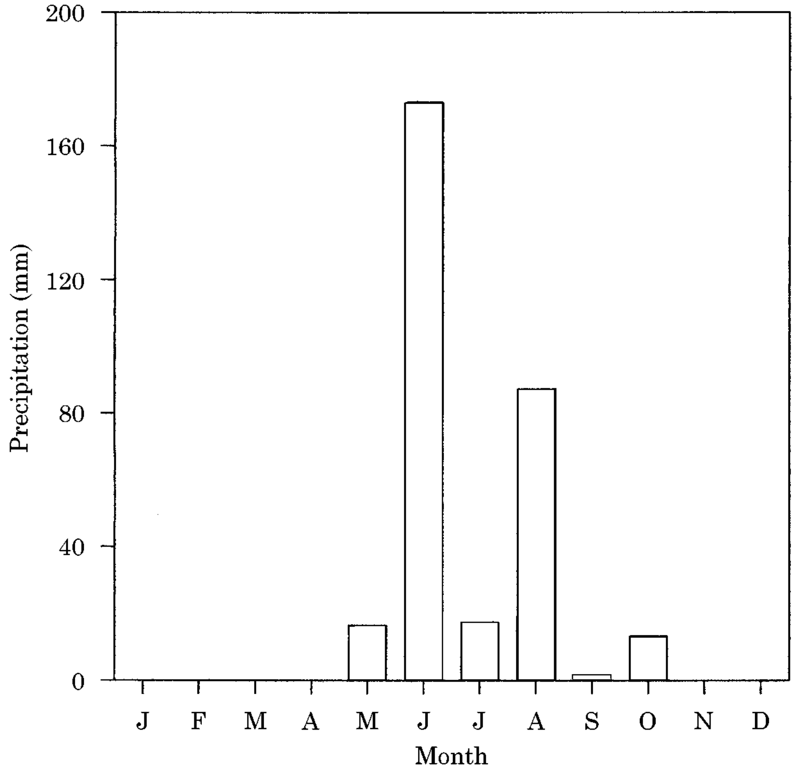

Figure 1. Monthly rains distribution in Fray Jorge, Chile during 1997.

Chemical analyses

pH was determined in a 1:5 (w/v) suspension of soil in water. Organic matter was calculated from the percent organic carbon estimated by oxidization with dichromate in presence of H_2SO_4, without application of external heat. Electrical conductivity was determined by a saturated-paste method (Dewis & Freitas, 1984). Available nitrogen (ammonium, nitrite and nitrate) was extracted with 2M KCl. Available phosphorus was extracted with 2 M ammonium acetate at pH 7·0, and soil moisture was measured gravimetricaly (Dewis & Freitas, 1984).

Microbial analysis

Bacteria and fungi were counted by the dilution plate count method (Parkinson *et al.*, 1971). One gram of air-dried soil from each soil sample was aseptically weighed and transferred to dilution bottles containing 100 ml of sterile deionized water. Bottles were allowed to stand on a magnetic stirrer for 15 min and then the soil was dispersed with the magnetic stirrer bar (2·5 × 0·8 cm) at about 2800 rpm for 15 min. Immediately following dispersion, we made four series of 10-fold dilutions of the suspension by pipetting 1 ml aliquots into tubes containing 9 ml of sterile deionized water. Final dilution was 10^6-fold. To count total aerobic–mesophillic–heterotrophic bacteria, 1 ml alicuota of three last dilutions were transfered to 9 cm diameter Petri dishes (replicated twice), and then 20 ml of molten medium Plate Count Agar at 45°C was added. Plates were put in an incubator at 25°C. Colonies were counted after 7 days. To count total yeast and filamentous fungi a similar procedure was followed but the medium used was Potato Dextrose Agar.

Mycorrhizal analyses

From each soil sample, VAM spores were isolated employing a wet-sieving density gradient procedure (Anderson & Liberta, 1989) and intact spores with filled cytoplasms were counted and identified following Schenck & Perez (1990). Additionally, 20 cm of roots 1–2 mm in diameter were collected from each soil sample. In each root sample, the VAM infection was determined by the Phillips & Hayman's method (Phillips & Hayman, 1970).

Statistical analyses

Two-way ANOVAs, with site and season as independent variables, were used to compare soil chemical and microbial parameters. Prior to the analyses, percentage data were arcsine square-root transformed. Microbial parameters were related to soil chemical characteristics by multiple regression analysis using PC SAS program. Data on figures are displayed as means ± 1 S.E Groups were considered significantly different when $p < 0{\cdot}05$.

Results

Physical–chemical characteristics of soil samples

Soil moisture and pH were the only physical–chemical parameters that changed significantly between the two sampling periods (Table 1, Fig. 2(b,c)), although there was a general tendency for higher organic matter, nitrogen and phosphorus in the wet season (Fig. 3(a–c)). On average, soil moisture increased two-fold (2·24% *vs.* 4·76%) during spring (Fig. 2(c)). Organic matter and nitrogen content were significantly higher underneath than outside the canopy of *A. bedwellii* and these differences were larger (two-fold) in the wet season (Table 1, Fig. 3(a,b)). The electrical conductivity was higher underneath shrubs (Fig. 2(a)) and showed a marginally significant ($F_{(1,44)} = 3{\cdot}27$; $p = 0{\cdot}0776$) decrease during the wet period in both sampling environments, probably as a result of dilution and/or leaching of salts. The slightly acid nature of soils (pH $\sim 6{\cdot}7$) would be given by the loamy sand texture of soils, which would favor salt leaching.

Microbial responses to season a microhabitat

The number of bacteria and fungi increased by two orders of magnitude in the wet period in relation to the dry one (Fig. 4(a,b)). The abundance of VAM spores also increased significantly in the wet period, but the VAM root infection showed a non-significant decreasing trend (Table 1, Fig. 4(c,d)). Similarly, the number of bacteria, VAM spores and VAM root infection were significantly greater in soil samples collected under the canopy of *A. bedwellii* than between shrubs (Table 1, Fig. 4(a,c,d)). These differences were higher in the dry season. For all microbiological parameters studied, only bacteria and fungi were correlated with soil chemical parameters. The number of fungi was positively correlated with nitrogen ($t = 2{\cdot}245$, $p < 0{\cdot}05$, df. $= 40$) and soil moisture ($t = 2{\cdot}553$, $p < 0{\cdot}05$, df. $= 40$), and the abundance of bacteria was marginally positively correlated with nitrogen ($t = 1{\cdot}941$, $p = 0{\cdot}0594$, df. $= 40$).

Table 1. F *values for two-way ANOVAs of several soil parameters in Fray Jorge, Chile during 1997*

Source	Bacteria log CFU/g	Fungi log CFU/g	VAM spores (N°/25g)	VAM infection (%)	Humidity (%)	Electrical conductivity (mV)	pH	Organic matter (%)	N (p.p.m.)	P (p.p.m.)	K (p.p.m.)
Time (T)	123·5***	113·5***	4·41*	0·00	19·21***	3·27	15·77***	2·50	2·71	1·40	0·44
Site(S)	10·3***	1·59	13·67***	5·29*	0·12	8·64**	3·61	14·29***	10·44***	1·32	1·26
T × S	4·16*	0·15	0·30	2·02	0·03	0·04	3·61	1·52	1·64	0·84	1·18

$^{*}p < 0·05$; $^{**}p < 0·01$; $^{***}p < 0·005$; df. = 1,44.

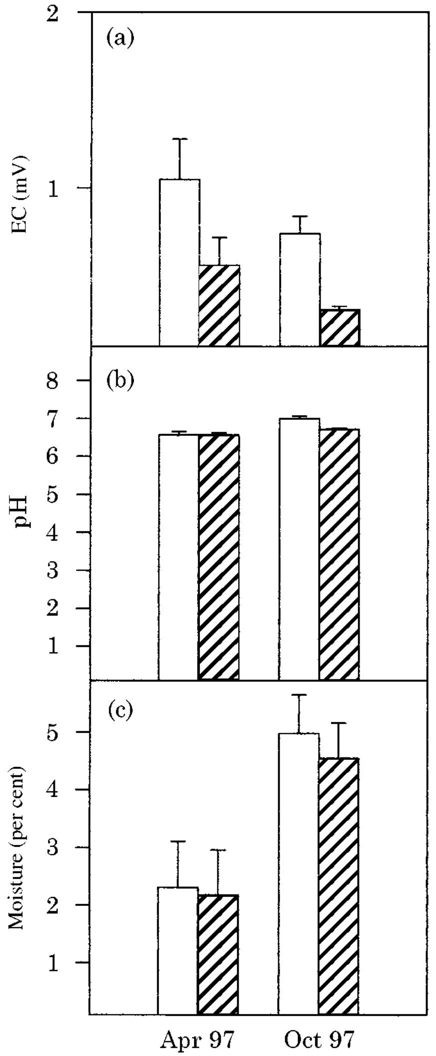

Figure 2. Chemical characteristics (mean ± S.E.) of soil samples taken underneath and outside of canopy of *Adesmia bedwellii* shrubs at Fray Jorge National Park. (a) Electrical conductivity; (b) pH; (c) soil moisture; (□) underneath canopy; (▨) outside canopy.

Discussion

The availability of nutrients for plants is tightly related to microbial activity under shrubs, which at the same time is regulated by the soil moisture (Whitford & Freckman, 1988). The presence, abundance and trophic relationships of symbiotic micro-organisms and/or those that decompose organic matter and bacteria that participate in the cycle of essential elements in plant nutrition, are crucial when the plant is in full development and their demands surpass the rates of mineralization of each of these microbial components (Ingham *et al.*, 1985).

In our study the abundance of micro-organisms in the soil varied spatially as well as temporarily and was related to the amount of nutrients and soil moisture. The number of bacteria and fungi increased by two orders of magnitude in the wet season relative to the

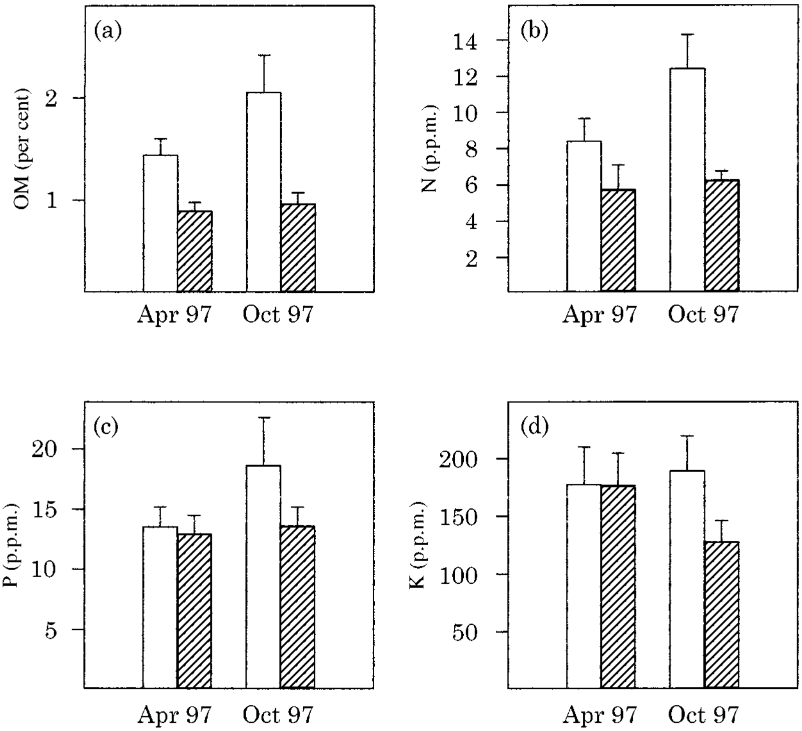

Figure 3. Chemical characteristics (mean $\pm$ S.E.) of soil samples taken underneath and outside the canopy of *Adesmia bedwellii* shrubs at Fray Jorge National Park. (a) Organic matter; (b) nitrogen; (c) phosphorus; (d) potassium; (□) underneath canopy; (▨) outside canopy.

dry one, and the number of bacteria was significantly higher under the canopy of *A. bedwellii* than in soils between shrubs. However, during the wet season the magnitude of the site-specific differences was lower. Soil moisture under the canopy of *A. bedwellii* would stimulate the growth and activity of micro-organisms, which in turn may affects the mineralization rate of organic matter favoring plant mineral nutrition. Similar patterns have been observed for other arid regions of the world. For example, Parker *et al.* (1984) found that simulated rains quickly triggered high activity levels in the soil biota associated with the litter in the Chihuahuan Desert. Herman *et al.* (1994) found that temporary soil moisture fluctuations was the only abiotic variable accounting for the numerical fluctuation of free-living nitrogen-fixing micro-organisms in the same desert.

With respect to symbiotic micro-organisms, Dhillion & Zak (1993) postulated that when plants have a high availability of nutrients, a negative response of VAM fungi and low infection levels should be expected. In our study, the number of VAM spores was higher under shrubs and in the wet season. However, the percentage of infection was significantly greater in roots collected under shrubs, but a lower infection was found in the wet period, when the organic matter and nitrogen increased as well. Positive effects of mycorrhiza are associated with decreased availability of nutrients, especially phosphorus and nitrogen, and root infection levels by VAM fungi are generally inversely correlated with the availability of nutrients and soil moisture (Miller *et al.*, 1986; Dhillion & Ampornpan, 1992; Dhillion & Zak, 1993). Our results support these hypotheses. During periods of nutrient and water scarcity (i.e. droughts), trophic relationships between shrubs with VAM fungi should gain importance, allowing plants

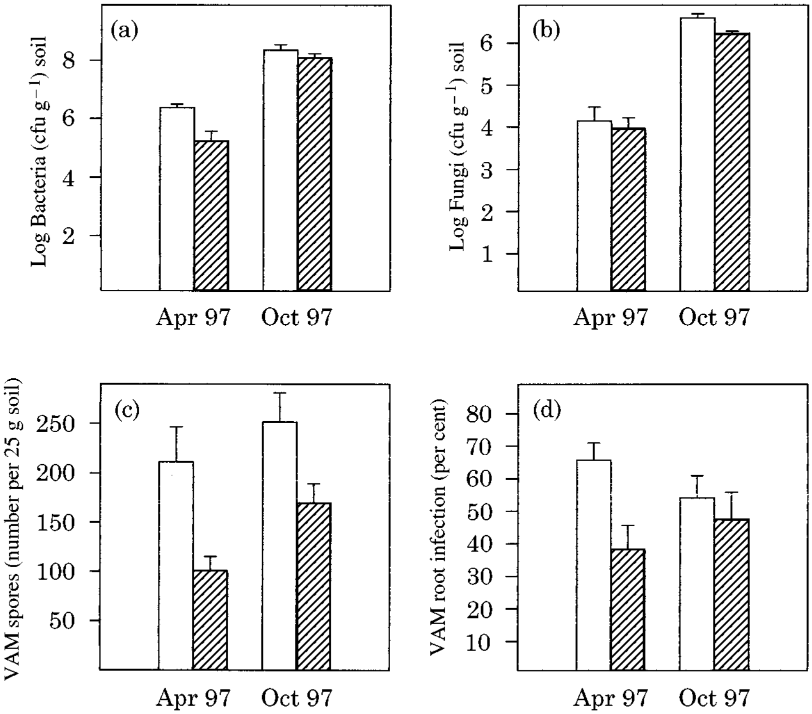

Figure 4. Microbial parameters (mean ± S.E.) of soil samples taken underneath and outside the canopy of *Adesmia bedwelli* shrubs at Fray Jorge National Park. cfu = colony forming units. (a) Bacteria; (b) fungi; (c) VAM spores; (d) VAM root infection; (□) underneath canopy; (▨) outside canopy.

to overcome environmental stress generated in the dry period. In contrast, during wet periods, microbial decomposition activity associated with organic resources under the canopy increases, thus releasing nutrients for plants, whose demand is greater during vegetative growth.

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