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Effects of drought and water pulses on microbial functionality and the role of Cyanoprokaryota in the rhizospheres of gypsophytes

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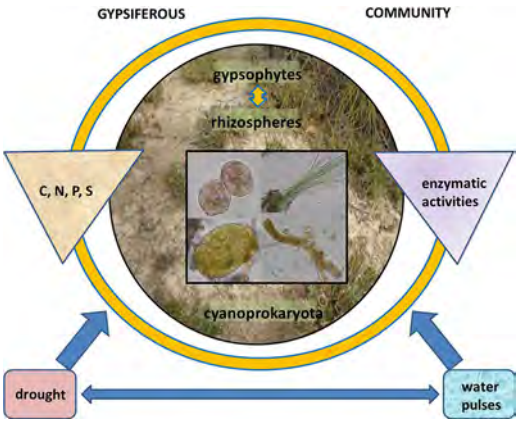
HIGHLIGHTS

- Cyanoprokaryota had a relevant role in the rhizospheres of gypsophytes in drought conditions.
- Three types of adaptation mechanisms of the gypsophytes were observed, depending of the cyanoprokaryota.
- Gypsiferous communities were activated by pulses of water.
- Under drought the nitrogen cycle was activated and in spring the carbon cycle.

GRAPHICAL ABSTRACT



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ABSTRACT

In the rhizospheres of three gypsophytes and in non-rhizospheric soil, two samplings were carried out - the first during a summer drought and the second during spring - to detect the responses to the availability of water in the soil. Urease and protease showed higher values after the drought whereas β-glucosidase was highest in the spring. This pattern was the same for all the rhizospheres tested. However, the arylsulfatase and alkaline phosphatase did not change. Surprising results were obtained when water retention and water loss were studied, with the highest values being obtained for the dry season due to the association of Cyanoprokaryota with the rhizospheres. The results are also explained by two water pulses that occurred before the samplings. Several parameters, whose values changed markedly due to the microbiological activation just after the drought and water pulses, are proposed as indicators of this activation: microbial biomass carbon and basal respiration rate, together with urease and protease. However, it was the dehydrogenase activity in spring that best reflected the microbiology associated with the carbon cycle, together with β-glucosidase. The interrelationships between carbon and nitrogen were shown through the indices: water soluble nitrogen and water soluble carbon. We propose three functional adaptation mechanisms of these plants associated with the Cyanoprokaryota in their rhizospheres and related to the water availability as determined by drought and water pulse effects. *Herniaria fruticosa* is a pioneer with the greatest diversity of Cyanoprokaryota, in both summer and spring (10 species and 11 species, respectively), and with high-medium abundance (5–30%). *Teucrium balthazaris* exhibits an intermediate strategy, with greater diversity of Cyanoprokaryota in spring (7 species) and predominance of high-medium abundance

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(5–30%). Finally, *Helianthemum squamatum* has lower diversity, with one species in summer (with low abundance, <5%) and no species in spring.

1. Introduction

The habitat of the gypsiferous outcrops in the Southeastern Iberian Peninsula is characterized by endemic and threatened plants (Escudero, 2009), which tend to be closely linked to the substrate distribution (Meyer, 1986) that is confined to arid and semiarid climates. The importance of the protection of these areas is manifested by the EU Habitats Directive (European Community, 1992). The Mediterranean vegetation of the gypsiferous steppes (1520* Gypsophiletalia) or *yesares* consists of formations of species of woody, small or medium sized plants, with high diversity and richness. This type of plant community usually appears as highly fragmented fertility islands, which alternate with bare soil surfaces where the biological crust supports a high coverage of species. Here, the lichen and moss communities have been widely studied and identified (Guerra et al., 1995; Egea and Alonso, 1996), while very little is known about the cyanobacterial communities (Domínguez and Asencio, 2011), although they are widely distributed in gypsiferous environments. The processes within vegetated patches and soil crusts were assumed to be relatively independent (Schlesinger et al., 1990), but evidence is now accumulating to suggest that these patches may be interconnected by networks of fungal hyphae (Collins et al., 2008).

It is considered that the Cyanoprokaryota of the arid and semiarid zones that grow in soils with high concentrations of salts are of great importance in soil stabilization and nutrient enrichment, due to their nitrogen (N) fixation and organic carbon (C) production. Cyanoprokaryota are capable of photosynthesis, respiration, decomposition, and mineralization (Mager, 2010) producing various growth promoting substances, like gibberellins, auxins, vitamin B12, free amino acids, and polysaccharides. Such substances have beneficial effects on soil structure, the growth of crop plants as well as useful bacteria (El-Enany and Issa, 2000). Baran et al. (2011) showed that the interactions with primary producers and N_2 fixers (many of them Cyanoprokaryota) and other bacteria are key for the long-term viability of N-limited environments, through a high level of microbial substrate specialization (exametabolites or exopolysaccharides).

Gypsiferous soils from semiarid environments are subjected to continuous cycles of drying and wetting due to the episodic nature of the rains, which occur during spring and autumn. The water retention in soils and reservoirs will be increasingly important under the climate change projections characterized by a decrease in precipitation and increased droughts and torrential rains in semiarid and arid areas (Eekhout et al., 2018). Native gypsophytes are generally well prepared to cope with water limitations, although changes in the timing of drought, particularly advances in the onset, can be detrimental (Matesanz et al., 2008). These plants do not seem to have a common adaptation, but rather several strategies - including the loss of leaves during the summer, a life cycle adapted to moisture sufficiency, deep roots, and even a dew collection system (Mota et al., 2011). However, the plants growing on gypsum may not be affected by water limitation since it has been seen that *Helianthemum squamatum* can extract water from structural gypsum in the summer in these environments (Palacio et al., 2014). *Helianthemum* is one of the most studied genera in gypsiferous environments, much more so than others such as *Teucrium* and *Herniaria*.

Whitham et al. (2006) concluded that, in non-cultivated ecosystems, the plant community diversity and the genotypes of individual plants can influence the composition of their associated communities, both aboveground and belowground. In this sense, plants can influence the net ecosystem changes through exudates released into the rhizosphere that attract, or inhibit the growth of, specific microorganisms. This rhizodeposition is vital, not only for the plant–microbial C and N pathways (Winkler et al., 2010). Thus, the N returns to the soil (Wichern et al., 2008) and plays a significant role in N cycling (Scandellari et al., 2010), but this transfer to the soil by rhizodeposition in N-limited systems (Dijkstra et al., 2013) has received little attention (Wichern et al., 2008).

Ecological plant-microbe interactions in the rhizosphere are responsible for a number of intrinsic processes such as carbon sequestration, ecosystem functioning, and nutrient cycling (Singh et al., 2004). The composition and quantity of microbes in the soil influence the ability of a plant to obtain N and other nutrients. Cyanoprokaryota in N-limited soils of the arid and semiarid zones seem to preserve the water in the soil in summer and appear to be activated by the humidity resulting from dew and scarce rain (Lázaro et al., 2008); however, nothing is known about the participation of Cyanoprokaryota in the rhizospheres of gypsophytes. The interest in investigating the role of Cyanoprokaryota in rhizospheres arises from the fact that they are components of the biological crust and colonize gyprocks. It is known that Cyanoprokaryota photosynthesize, although a small number of strains can also use hydrogen sulfide (H_2S) and convert it to elemental sulfur (Cohen et al., 1986); in general, they can tolerate low-oxygen conditions and concentrations of H_2S that are toxic to other microorganisms. Cyanoprokaryota establish endosymbioses in Cycads, in highly specialized lateral roots (in complete darkness); for instance, *Nostoc* transfers fixed nitrogen to the cycad cells, and it is expected to have a heterotrophic mode of carbon nutrition (Lindblad, 2008). Singh (2014) conducted a review of agricultural soils, with regard to the role of possible molecules that induce Cyanoprokaryota to improve plant growth and provide tolerance against biotic or abiotic stress. In this sense, phytohormones, polysaccharides, vitamins, amino acids and peptides- considered crucial for the growth and development of plants- can be taken up by plants at the rhizosphere level through the established symbiosis.

Some studies have demonstrated that soil moisture controls both soil biological activity (Carbone et al., 2011) and nutrient availability for plant uptake and growth (Sardans and Peñuelas, 2004). Soil microbial activity has been assessed frequently through biological and biochemical parameters, such as biomass C and enzyme activities. Soil extracellular enzyme activities are sensitive and respond rapidly to environmental stresses (Sanaullah et al., 2011). Although enzyme synthesis requires that both C and N be available, it has been shown that neither resource alone stimulates enzyme production or vigorous microbial activity (Allison and Vitousek, 2005). C and N have been used as indicators of the health and sustainability of ecosystems. Water soluble C (WSC), as a component of the labile C pool, may also be sensitive to perturbation and stress in soil-plant ecosystems (Ghani et al., 2003) and, in a similar way to water soluble N (WSN); therefore, they could be used as a sensitive indicators of soil quality. We hypothesized that the microbial functionality is dependent on the type of gypsophyte colonizing these environments, as well as being strongly regulated by environmental factors such as seasonal drought and water pulses. To test this, we determined soil moisture and soil microbiological and biochemical properties, especially enzymatic activities, related to the metabolic activity of the microbiota in the rhizosphere of three gypsophyte species - *Helianthemum squamatum*, *Teucrium balthazaris*, and *Herniaria fruticosa* - before and after a drought period. In addition, we also ascertained the unknown role of Cyanoprokaryota in the ecosystem functioning of the rhizospheres.

2. Materials and methods

2.1. Study site

The study was performed in a gypsum outcrop, with N90°E direction and an average slope of 10%, at the botanical microreserve "Yesos del Rincón" (Lorca, SE Spain, 37° 51′N, 1° 52′W. Fig. 1S, supplementary material), at 739 m a.s.l. The predominant soils are Lithic Leptosols as well as Petric and Hypergypsic Gypsisols (IUSS, 2015). The dominant geological unit is the Triassic. The main characteristics of the soil are shown in Table 1. The climate type is semiarid Mediterranean, with an annual average potential evapotranspiration (ETP) of 1293 mm and an annual average rainfall of 268 mm. The mean annual temperature is 15 °C, with an average minimum temperature in January of 0.4 °C and an average maximum of 30 °C in August. Table 2 shows the meteorological data (at the La Paca station; 37° 51′N, 1° 49′W. Altitude: 713 m) corresponding to the intervals previous to each of the two sampling dates. The rainfall between the two sampling dates was 81.3 mm, with the following distribution: 1.9 mm until the end of September, 7.6 mm in October, 44.7 mm in November, 7.6 mm in December (all 2011), 19.4 mm in January 2012 (10.2 mm on 17/01/12), and 2.0 mm in February 2012.

The dwarf scrub community has been named *Teucrio balthazaris-Santolinetum viscosae* (Alcaraz et al., 2008). Specifically, in the study area the target habitat is characterized by gypsophytes: *Teucrium balthazaris* Sennen, *Herniaria fruticosa* L. subsp. *fruticosa*, and *Helianthemum squamatum* (L.) Pers. and gypsovags: *Frankenia thymifolia* Desf., *Senecio auricula* Bourg. ex Coss. subsp. *auricola*, and *Chaenorhinum rupestre* (Guss.) Maire. The bare zone is covered by a biological crust and some gypsum crystals. The soil biological crust is dominated by *Diploschistes diacapsis* (Ach.) Lumbsch, *Squamarina cartilaginea* (Huds.) Poelt, *Acarospora placodiiformis* Magnusson, and *Toninia coeruleonigricans* (Light.) Th. Fr. (Egea, 1985, Egea and Alonso, 1996), but other species also occur. Among the Cyanoprokaryota found in the biocrust, *Gloeocapsa rupicola*, *Tolypothrix elenkinii*, *Leptolyngbya* sp., *Microcoleus chthonoplastes*, *Nostoc microscopicum*, *Phormidium* sp., *Schizothrix* cf. *calcicola*, and *Scytonema* sp. predominate, but other species with chasmoendolithic growth also occur (Asencio, unpublished data). Also, the Streptophyte *Klebsormidium* sp. has been found in the biocrust.

Three gypsophytes were selected based on their distribution and abundance: those with an habitual and exclusive presence, as is the case of *Herniaria fruticosa* (H) and *Helianthemum squamatum* (HS), or with a diagnostic presence, as for HS and *Teucrium balthazaris* (T). *Herniaria fruticosa* is a typical gypsum bush with a nitrophilous tendency. *Helianthemum squamatum* is a widely studied dwarf chamaephyte in gypsum outcrops of the Iberian Peninsula (Escudero et al., 2005; Eugenio et al., 2012), and may be a good indicator of saline soils. *Teucrium balthazaris* is endemic in Almeria and Murcia, and grows in compacted soil where crystals of gypsum are often visible. At the study site it is a vulnerable species and it is a near-threatened species in the red list of the Spanish Vascular Flora (Moreno, 2008).

Table 1
Physical, chemical, physico-chemical and biochemical values of non-rhizospheric soil for the different sampling dates.

	Sep 2011	May 2012
pH	8.30 ± 0.04	7.84 ± 0.01
EC (μS cm^{-1})	2447 ± 10	2437 ± 3
Cl^- (mg l^{-1})	13 ± 1	27 ± 8
SO_4^{-2} (mg l^{-1})	1637 ± 33	1703 ± 24
NO3- (mg l^{-1})	28 ± 2	14 ± 1
Ca^{+2} (mg l^{-1})	634 ± 14	591 ± 8
Mg^{+2} (mg l^{-1})	15 ± 2	21 ± 4
Na^+ (mg l^{-1})	8.7 ± 0.8	11.7 ± 2.9
K^+ (mg l^{-1})	4.8 ± 0.8	6.2 ± 1.2
Gypsum (%)	87 ± 2	89 ± 2
$CaCO_3$ + $MgCO_3$ (%)	9.6 ± 1.9	6.9 ± 1.1
Total P (μg g^{-1})	36 ± 3	24 ± 3
Total S (g kg^{-1})	73 ± 7	48 ± 2
Water soluble C (μg g^{-1})	27 ± 1	22 ± 1
Water soluble N (μg g^{-1})	4.5 ± 0.4	8.3 ± 0.3
Total organic C (g kg^{-1})	5.2 ± 0.3	4.0 ± 0.3
Total C (g kg^{-1})	20 ± 3	15 ± 2
Total N (g kg^{-1})	0.5 ± 0.1	0.8 ± 0.1
Water retention 1/3 atm (%)	11.44 ± 0.05	9.89 ± 0.26
Water retention 15 atm (%)	1.98 ± 0.05	1.57 ± 0.13
Available water content (%)	9.45 ± 0.04	8.32 ± 0.35
Microbial biomass carbon (μgC g^{-1})	342.6 ± 10.8	321.2 ± 5.2
Basal respiration rate (mg CO_2 g^{-1} h^{-1})	1.388 ± 0.038	0.323 ± 0.074
Dehydrogenase (μmolPNFh^{-1} g^{-1})	0.125 ± 0.016	0.251 ± 0.015
β-Glucosidase (μmolPNPh^{-1} g^{-1})	0.272 ± 0.031	1.438 ± 0.186
Alkaline phosphatase (μmolPNP h^{-1} g^{-1})	8.61 ± 1.21	15.57 ± 1.72
Arylsulphatase (μmolPNFh^{-1} g^{-1})	0.638 ± 0.100	0.323 ± 0.074
Urease (μmolNH_3h^{-1} g^{-1})	0.757 ± 0.145	0.434 ± 0.075
Protease (μmolNH_3h^{-1} g^{-1})	0.413 ± 0.028	0.155 ± 0.019

Table 2
Meteorological data corresponding to La Paca (Murcia, Spain).

	01-07/21-09-2011	01-03/30-04-2012
Mean temperature (°C)	23.3	10.5
Maximum temperature (°C)	31.2	17.7
Minimum temperature (°C)	15.4	4.0
Mean humidity (%)	55.9	59.1
Maximum humidity (%)	82.6	86.3
Minimum humidity (%)	30.9	32.2
Mean radiation (MJ m^{-2})	28.5	20.0
Maximum radiation (MJ m^{-2})	30.8	27.7
Minimum radiation (MJ m^{-2})	14.7	3.2
Hours of sun (mean)	11.3	10.1
Rainfall (mm)	31.2	57.3

2.2. Sampling procedures

A field sampling survey was carried out in September 2011 (considered as summer drought) and May 2012 (spring) in a homogenous area, measuring approximately 3300 m^2. For this survey, 18 individual plants for each of the three target species, similar in size, were randomly chosen. Six rhizosphere soil samples of each plant species were collected. Each sample consisted of three subsamples taken in the rhizosphere of three individual plants. The rhizosphere was considered as the soil adhering to the plant root system. The non-rhizospheric soil (Table 1) was sampled in a zone without plant roots or biological crust, at a depth (0–30 cm) similar to that used in the rhizospheric sampling. The soil samples were placed in plastic bags for transport to the laboratory. Field-moist soil samples were divided into two subsamples: one was sieved at 2 mm and stored at 4 °C for microbiological and biochemical analyses and the other was allowed to dry at room temperature before being sieved at 2 mm for the rest of the soil analyses detailed in the next section (Section 2.3).

2.3. Soil analyses

The mineralogy of the rhizospheres was studied by X-ray diffraction. After being ground in an agate mortar, a sample was deposited in an aluminum sample holder, without favoring any preferential orientation. The X-ray diffractograms were obtained with a Philips PW1700 model. The samples were rolled with values of 2 θ between 3 and 80°, at a rate of 5° per minute.

Micromorphological analysis was performed by scanning electron microscopy (SEM), with an energy dispersive system (EDS). Identification of the chemical composition of minerals was carried out using EDX analysis.

Soil pH and electrical conductivity were measured in a 1:5 (*w*/*v*) aqueous extract. In this extract were measured the anions (chloride, sulfate, and nitrate, using a Dionex ICS-2100 ion chromatograph) and the cations (calcium, magnesium, sodium, and potassium, by ICP plasma).

Gypsum was determined by thermogravimetric analysis, using TGA-DTA and Differential Scanning Calorimetry (DSC) in a TA Instruments SDT 2960 simultaneous analyzer and a TA Instruments DSC 2920 model.

The ground samples were subjected to an air atmosphere with a flow of 30 $cm^3\ min^{-1}$, heating at 10 °C min^{-1} until reaching 300 °C. The water contents were measured gravimetrically at 1/3 (Field Capacity Point) and 15 (Permanent Wilting Point) atm using a pressure plate extractor (Soil Moisture Equipment Corp., Santa Barbara, CA) as described by Dirksen (1999). The difference between the values of the two parameters was the available water content. Carbonates were estimated by the volumetric method of the Bernard calcimeter. Total phosphorus (TP) and total sulfur (TS) were quantified using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Thermo Electron Corporation Mod. IRIS Intrepid II XDL). In soil aqueous extracts, water soluble carbon (WSC) and water soluble nitrogen (WSN) were determined with an automatic Carbon Analyzer for liquid samples (Shimadzu TOC-5050A). Total organic carbon (TOC), total carbon (TC), and total nitrogen (TN) were determined with an automatic Nitrogen and Carbon Analyzer after pre-treatment with HCl to eliminate carbonates and combustion at 1020 °C. Soil moisture content was measured by gravimetry, considering that the maximum temperature of the oven was below 50 °C (Porta, 1998) until a constant weight was obtained.

Microbial biomass C was assayed, by substrate-induced respiration, after glucose was mixed into the soil (at 60% of its field capacity) at a rate of 0.5% (*w*/w): the CO_2 production was monitored for 24 h, using the μ-Trac 4200 system (SY-LAB GmbH, P.O. Box 47, A-3002 Pukersdorf, Austria). This system is based on the variation of the electrical impedance of a 0.2% aqueous KOH solution (Fernández et al., 2004). Respiration rates were calculated in the linear phase of the respiration curves. Basal soil respiration was assessed with the same system described for microbial biomass C but in the absence of glucose. Dehydrogenase activity was determined using INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) as oxidizing agent (García et al., 1997). Urease activity (EC 3.5.1.5) and N-α-benzoyl-L-argininamide (BAA) hydrolyzing protease activities were assayed according to the method of Nannipieri et al. (1980), using 1 M urea and 0.03 M BAA urea as substrates, respectively. Alkaline phosphatase (EC 3.1.3.1), arylsulfatase (EC 3.1.6.1), and β-glucosidase (EC 3.2.1.21) activities were determined using *p*-nitrophenyl phosphate (disodium) (PNPP, 0.115 M), *p*-nitrophenyl sulfate (PNS, 0.05 M), and *p*-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M) as substrates, respectively. These assays are based on the release and detection of p-nitrophenol (PNP) and were performed according to Tabatabai (1994).

2.4. Cyanoprokaryota analyses

Cyanoprokaryota were collected in biocrust (for reference purposes), where cyanobacterial growth was visible as colored patina, and in rhizospheric and non-rhizospheric soils. The material was kept dry in plastic and paper bags in a cooler at 4 °C before examination. Cultivation is usually necessary for detailed taxonomic studies of subaerial cyanobacteria, since the thalli of the microorganisms occurring in the patina are usually covered by large amounts of inorganic material in native preparations. The morphology of the species was therefore studied for both field-collected material and cultivated specimens. Part of the scraped field material was spread aseptically over the surface of Petri dishes containing agarized BG 11 medium (Rippka et al., 1979) and kept at 25.0 °C with a light intensity of 70.0 μE $m^{-2}\ s^{-1}$ and a 16-h photoperiod. Microscopic examinations were made with a LAN OPTICS stereomicroscope and an Olympus BX4 l compound microscope.

The following publications were used for the morphological identification of Cyanoprokaryota: Geitler (1932), Starmach (1966), Komárek and Anagnostidis (1998), Komárek and Anagnostidis (2005), and Komárek (2013).

In the Cyanoprokaryota results (Table 6) the diversity or richness of species of Cyanoprokaryota has been considered as the number of species. The abundance is indicated as low, medium, or high: + low (<5%), ++ medium (5–15%), +++ (15–30%) high presence.

2.5. Statistical analyses

The normality and the homogeneity of variance of the dependent variables were tested by means of the Kolmogorov-Smirnov and Levene tests, respectively. The data were ln transformed to achieve normality. We used the non-parametric Kruskal-Wallis test for those variables with a non-normal distribution. The effects of the gypsophyte species (S), the date of sampling (D), and their interaction (S × D) on the measured variables were tested by a two-way analysis of variance. Correlation analysis among all the soil parameters measured was carried out using Spearman's rank correlation coefficients. The statistical procedures were carried out with the software package IBM SPSS 22.0 for Windows.

3. Results

The comparison of the results of non-rhizospheric soils (shown in Table 1) with those of rhizospheres has been excluded from the statistical analyses, because if we consider them in relation to the non-rhizospheric soil, statistically significant differences are obtained for each of the rhizospheres, but not between the rhizospheres of the different gypsophytes.

3.1. Gypsum

In the study area the outcrop of primary gypsum occurs in crystalline forms of great development (massive, acicular, or fibrous) that are versicolor (with dominance of grayish and ocher tonalities) and have obvious signs of dissolution.

Microcrystalline gypsum, mainly due to gyprock weathering, can form lenticular gypsum by dissolution and reprecipitation; these gypsum forms are secondary and can be observed as whitish, powdery or crystalline, soft masses, silt-sized with a flour-like consistency or diffused in the soil matrix. The topographic setting strongly influences the type of gypsum in the soil: thus, in the high parts there is a predominance of pedogenic gypsum and in the areas on the lower slopes the diagenic gypsum predominates.

We observed the micromorphology and estimated the in situ chemical composition of selected samples by SEM. In the microscopic observations, globular gypsum crystals predominated, some tabular and cluster of gypsum crystal. In terms of chemical composition, the predominant elements - in accordance with the gypsiferous nature - were S and Ca, while Al, Fe, and Si appeared in minor amounts. In the non-rhizospheric samples (Fig. 1a) there was a lower amount of all the elements than in the rhizospheric samples (Fig. 1b), which indicates greater physical and chemical alteration of the substrate by both microorganisms and the roots of the plants. The XR diffractograms of non-rhizospheric and rhizospheric soil are shown in Fig. 2. The most intense reflection at 7.56 Å is diagnostic for gypsum (diagnostic peaks at 7.56, 3.66, 4.27, and 2.53 Å). Gypsum was the dominant mineral in all samples, in accordance with the electron microscopy observations.

Along with gypsum, the X-ray diffractograms showed the main diagnostic reflections of calcite (3.04, 1.87, 2.10 Å) and dolomite (2.88, 2.19, 1.78 Å).

It can be seen that all soil samples, both non-rhizospheric (Fig. 2a) and rhizospheric (Fig. 2b), corresponded to gypsum (analytical gypsum and brushite were used as reference standards for all samples).

The percentage of gypsum relative to non-rhizospheric samples is shown in Table 1. In the rhizospheric samples the percentage of gypsum was between 75 and 95%, without statistically significant differences.

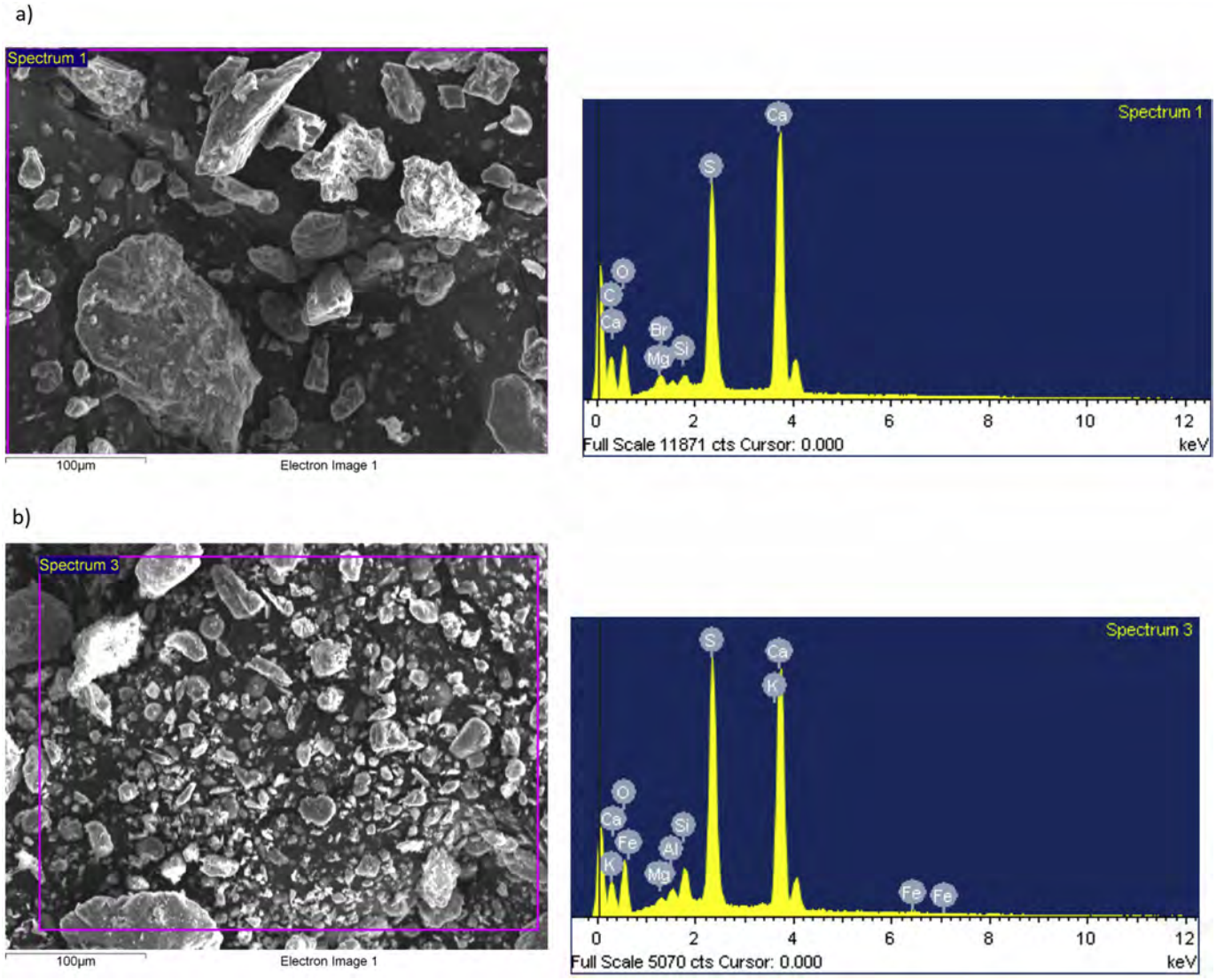

Fig. 1. Back scattered electron microscopy images of the crystal forms of gypsum, together with Energy-Dispersive X-ray (EDX) spectra of gypsum crystals: (a) Non-rhizospheric soil, (b) Rhizospheric soil.

3.2. Soil moisture and biochemical analysis

The values of the physical, chemical, physico-chemical, and biochemical parameters for the non-rhizospheric soil are shown in Table 1. Table 3 shows the water retention capacity (Field Capacity Point, Permanent Wilting Point, and Available Water Content) in the rhizosphere soil of the three gypsophytes under study (H, HS, and T), as well as analytical compounds (brushite and gypsum). For Field Capacity Point (1/3 atm) the highest values were for species T and H, and the lowest was for HS, the differences being statistically significant for the factor S and its interaction with D. However, at the Permanent Wilting Point (15 atm) the differences were statistically significant for the factor D, and the lowest values were in spring 2012. The Available Water Content seemed to respond to the trend found with regard to retention at Field Capacity Point.

Statistically significant differences were observed in Field Capacity Point among the species studied (Table 3): H and T had higher values than HS. However, Permanent Wilting Point showed statistically significant differences in terms of D, being higher in the dry season (Table 3). The Available Water Content differed significantly in terms of the species and their interaction with the date, the highest values being for H and T (Table 3).

The thermograms indicated, in all cases (non-rhizospheric and rhizospheric soils), multistage decomposition with relatively stable intermediates, corresponding to the pattern of the analytical compound, gypsum. In a more detailed description, thermograms were amplified in the 30–60 °C range, to see the corresponding dehydration. In all the rhizospheric samples, the dehydration was greater than in the non-rhizospheric samples. Fig. 3a shows the minimum dehydration that occurred in a non-rhizospheric soil sample, and Fig. 3b the maximum dehydration that occurred in a sample of rhizospheric soil belonging to species H; both samples belonged to the first sampling after the drought.

The type of gypsophyte species (S) had no effect on the concentrations of total P and S, water soluble C and N, total organic C and total C, and carbonates. Only the total N differed significantly among species (Table 4). Except for total organic C, total C, and carbonates, the soil chemical parameters measured varied significantly between the two sampling dates (D). The highest values of such parameters were detected in samples of the rhizosphere soil of species H, HS, and T taken in September 2011, except for WSN and total N. There was a significant interaction between the type of plant species and the sampling date (S × D) with respect to the concentration of total N. For all three gypsophytes the values of WSC were higher in September than in

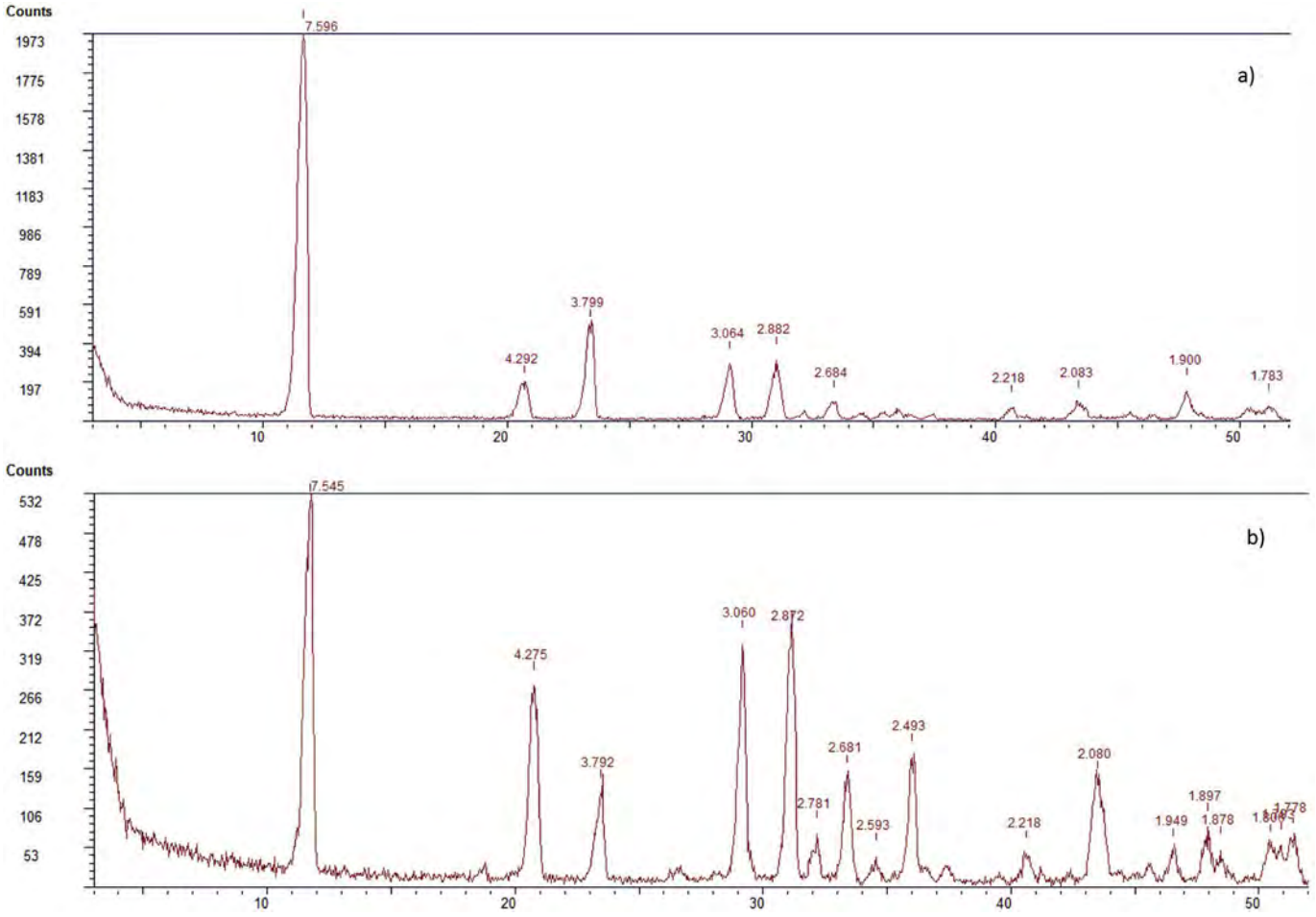

Fig. 2. XR diffractograms: (a) Non-rhizospheric soil, (b) Rhizospheric soil.

May. In particular, the decline from September to May was more pronounced in H and T (about 38% and 44%, respectively). By contrast, the values of WSN were lower in September than in May, and the most striking change in this case was for HS, with an increase of 150%.

The lowest values of microbial biomass C and basal respiration were recorded in the samples of May, regardless of the plant species, as shown in Fig. 4. For all three gypsophytes, the urease and protease activities were highest in the sampling of September (Fig. 5). However, the dehydrogenase and β-glucosidase activities were greater in spring than in summer. Neither the type of gypsophyte nor the sampling date had a significant effect on the alkaline phosphatase and arylsulfatase activities.

In general, the values of the soil chemical parameters were significantly correlated with those of some of the biochemical parameters (Table 5). Excepting dehydrogenase activity, WSN, and TN, the values of the parameters in the rhizosphere of the gypsophyte plants correlated positively with the basal respiration rate. In the case of β-glucosidase, the correlation was negative. Biomass C showed positive correlations with the urease and protease activities, WSC, TOC, TP, and TS. The highest correlations for both basal respiration rate and microbial biomass C were with the protease and urease activities. Also notable was the correlation between basal respiration and TP (0.826***).

The dehydrogenase and β-glucosidase activities were correlated positively with WSN and negatively with TS, while β-glucosidase exhibited some negative correlations (with urease and protease activities, TP, and TS) and dehydrogenase was correlated negatively only with TS. However, the arylsulfatase activities showed no significant correlations, and phosphatase showed positive correlations with protease activity. The urease and protease activities showed positive correlations with WSC, TP, TS, TC, and carbonates.

The WSC was positively correlated with basal respiration, biomass C, urease and protease activities, TP, and TS. However, WSN presented positive correlations with dehydrogenase and β-glucosidase, and a negative correlation with TS. The TOC was correlated positively with basal respiration, biomass C, TP, and TS. Positive correlations existed between TC and basal respiration, the urease and protease activities, TOC, and TP. However, TN was correlated with TP and TS but not with basal

Table 3
Water retention (FCP: Field Capacity Point, 1/3 atm, PWP: Permanent Wilting Point, 15 atm, and AWC: Available Water Content:) in the rhizosphere soil of the three gypsophytes (H: *H. fruticosa*, HS: *H. squamatum*, and T: *T. balthazaris*) and brushite and gypsum, for the different sampling dates.

	FCP (%)	PWP (%)	AWC (%)
Sep. 2011			
H	16.97 ± 0.62	3.44 ± 0.29	13.52 ± 0.66
HS	15.79 ± 0.76	2.89 ± 0.44	12.90 ± 0.62
T	21.26 ± 0.92	3.40 ± 0.21	17.86 ± 0.74
May 2012			
H	20.33 ± 2.06	1.95 ± 0.27	18.38 ± 2.22
HS	11.67 ± 1.09	2.70 ± 0.11	8.97 ± 1.02
T	20.60 ± 1.72	2.72 ± 0.10	17.88 ± 1.67
ANOVA *P*-values			
Species (S)	<0.001	NS	<0.001
Date (D)	NS	<0.001	NS
S × D	<0.001	NS	<0.001
Brushite	45.07 ± 0.25	12.10 ± 0.32	32.98 ± 0.08
Gypsum	59.38 ± 0.97	4.05 ± 1.02	55.33 ± 1.99

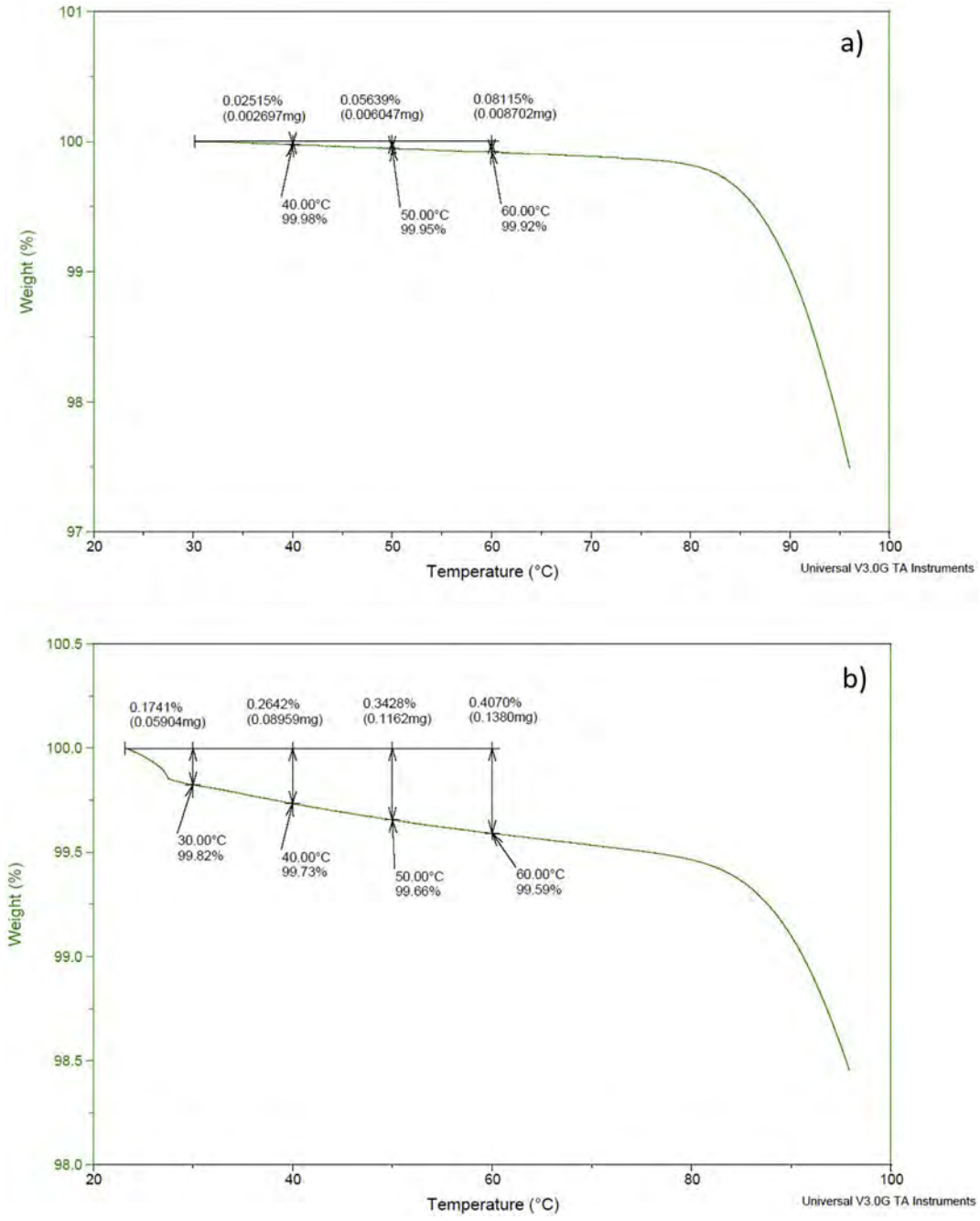

Fig. 3. Thermograms amplified between 30 and 60 °C, corresponding to summer data of (a) Non-rhizospheric soil, (b). Rhizospheric soil of *Herniaria fruticosa*.

respiration, biomass C, or any of the enzymatic activities studied. The TP and TS showed positive correlations with basal respiration, urease and protease activities, WSC, and TN, and negative ones with β-glucosidase. Specifically, positive correlations between TP and microbial biomass C were established, and negative ones between TS and dehydrogenase and WSN. Finally, the carbonate values were correlated positively with basal respiration, protease and urease activities, TP, and TC.

3.3. Cyanoprokaryota

A total of 14 species were identified in both the rhizospheric and non-rhizospheric soils of the study area (Table 6). All these species were present in the biocrust samples analyzed for reference purposes, with the exception of *Leptolyngbya* and *Phormidium*. Of the Cyanoprokaryota studied, coccoid species predominated over filamentous species (9,5); the former are included in the order Chroococcales, which exhibited most diversity (64.3%), followed by the Nostocales (21.4%) and the Oscillatoriales (14.3%).

The most diverse genus was *Gloeocapsa*, with five species, whereas the rest of the genera were represented by only one species each. The most abundant species were *Microcoleus chthonoplastes*, *Nostoc microscopicum*, *Schizothrix* cf. *calcicola*, and *Scytonema* sp. (Fig. 6), whereas the least plentiful were *Asterocapsa salina*, *Gloeocapsa rupestris*, and *Pseudocapsa dubia*.

There were more Cyanoprokaryota species in rhizospheric soils than in non-rhizospheric soils. Within the rhizospheres, the cyanobacteria were most abundant in that of gypsophyte H (12 species in spring and 11 in summer), followed by the rhizosphere of T (8 species in spring and 3 in summer) and, finally, the rhizosphere of HS (2 species, only in summer).

In spring the presence and abundance of Cyanoprokaryota species were very similar in the rhizospheres of H and T but lower in that of

Table 4
Chemical properties of the rhizosphere soil of the three gypsophytes (H: *H. fruticosa*, HS: *H. squamatum* and T: *T. balthazaris*) for the different sampling dates.

	TP ($\mu g\ g^{-1}$)	TS ($g\ kg^{-1}$)	WSC ($\mu g\ g^{-1}$)	WSN ($\mu g\ g^{-1}$)	TOC ($g\ kg^{-1}$)	TN ($g\ kg^{-1}$)	TC ($g\ kg^{-1}$)	Carbonates (%)
Sep 2011								
H	48 ± 4	71 ± 3	50 ± 1	11 ± 2	8.0 ± 0.8	0.8 ± 0.1	21 ± 3	8.0 ± 1.4
HS	50 ± 4	76 ± 3	46 ± 1	6 ± 4	8.2 ± 0.8	0.8 ± 0.1	16 ± 2	4.7 ± 0.3
T	49 ± 2	75 ± 6	60 ± 1	10 ± 1	9.3 ± 0.6	0.9 ± 0.1	21 ± 2	7.6 ± 1.0
May 2012								
H	30 ± 2	57 ± 5	31 ± 1	21 ± 1	7.8 ± 0.2	1.6 ± 0.1	18 ± 3	6.1 ± 2.1
HS	37 ± 2	45 ± 2	34 ± 2	15 ± 1	6.8 ± 0.3	0.6 ± 0.1	16 ± 1	4.8 ± 0.6
T	29 ± 2	47 ± 3	34 ± 1	21 ± 1	7.9 ± 0.3	0.8 ± 0.1	15 ± 2	3.9 ± 0.9
ANOVA *P*-values								
Species (S)	NS	NS	NS	NS	NS	<0.001	NS	NS
Date (D)	<0.001	<0.001	<0.001	<0.05	NS	<0.05	NS	NS
S × D	NS	NS	NS	NS	NS	<0.001	NS	NS

HS. However, in summer there were many differences among H, T, and HS.

Gloeocapsa developed in the rhizospheres of H and T, its presence being more constant over time in H, while in T it was more abundant in spring. *Microcoleus chthonoplastes* (Fig. 6) was the most abundant species in the gypsophytes, except in the rhizosphere of HS. *Chroococcopsis* cf. *fluviatilis* and *Tolypothrix elenkinii* (Fig. 6) had similar behavior; they appeared in the rhizosphere of H in summer and spring and in the rhizosphere of T in spring. *Schizothrix* cf. *calcicola* only appeared in spring in the rhizospheres of H and T and in summer only in that of H. Less abundant were *Asterocapsa salina* and *Pseudocapsa dubia*, appearing only in the rhizosphere of H. Only *M. chthonoplastes* was detected in the rhizosphere of HS in summer, whereas in spring no Cyanoprokaryota were found in the rhizosphere of HS.

In non-rhizospheric soils, *Nostoc microscopicum* and *Scytonema* sp. (Fig. 6) were very abundant in spring and summer, without being affected by seasonality.

In addition to the Cyanoprokaryota, the presence of the green alga *Klebsormidium* (Table 6) was detected in the rhizospheres of HS and H in summer and in those of H and T in spring.

4. Discussion

4.1. Effects of drought and water pulses on soil properties in gypsiferous soils

It is interesting to know at a mineralogical level the composition of gypsiferous soil (non-rhizospheric and rhizospheric), since gypsum has the ability to eliminate dissolved phosphate by brushite precipitation (Pinto et al., 2009). The X-ray diffractograms (Fig. 2) show that all the samples were gypsum, and all were analyzed regarding their patterns of gypsum and brushite. The micromorphology of the gypsum crystals, in both non-rhizospheric and rhizospheric soils, had a predominance of globular, tabular, and cluster-like characteristics, typical of an aridic-xeric moisture regime, according to Hashemi et al. (2011).

The differences obtained among the rhizospheres of the gypsophytes in the study area in terms of water retention (Table 3), and the thermograms show differences in the amount of water retained in the rhizospheres (Fig. 3) in the drought period. These values can be attributed to the presence and differences in composition of Cyanoprokaryota, in terms of both species and date of sampling (Table 6), possible due to either exopolysaccharides production (Otero and Vincenzini, 2003) and/or differences in the stimulation caused by rhizodeposition.

Most Cyanoprokaryota can resist long periods of drought, tolerating large fluctuations of salinity and temperature and high radiation stress in a vegetative state, and metabolic activity revives soon after rewetting (Adhikary, 2004). Cyanoprokaryota are characterized by the presence of mucilaginous sheaths whose volumes may vary considerably as they act as water reservoirs, thus avoiding desiccation and allowing activity to persist, even in drought conditions (Caiola et al., 1996; Asencio and Aboal, 2003). In adaptation to desiccation, proteins play an important role (Wright et al., 2005), as indicated by the influence of drought on scytonemin production, at least in some cases (Asencio and Hoffmann, 2013). Exopolysaccharides may retain large amounts of water and form a gel that stabilizes the macromolecular components and the cell structure of the Cyanoprokaryota and other organisms that produce them. This allows them to overcome long periods of drought by the formation of hydrogen bonds with proteins, membrane lipids, and DNA, thereby replacing the water shell surrounding these cell constituents

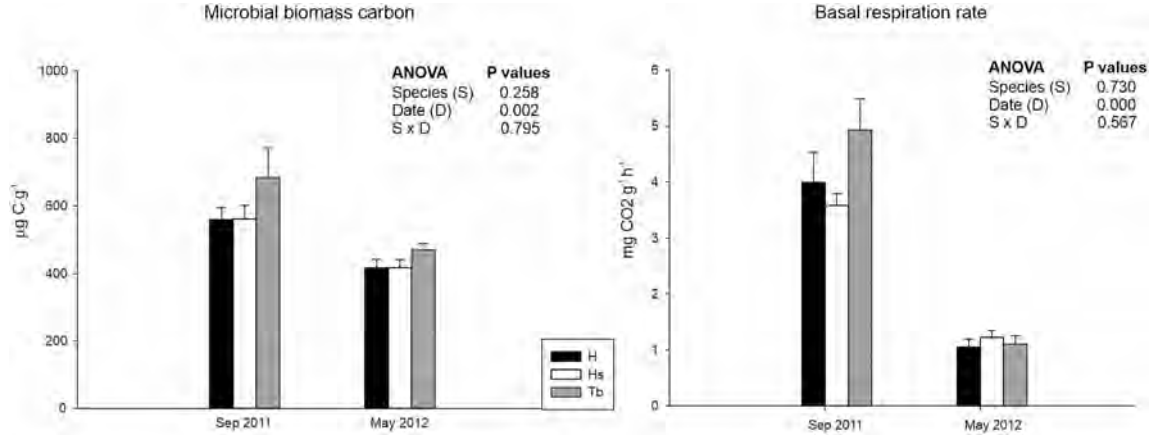

Fig. 4. Microbial biomass C and basal respiration rate in the rhizosphere soil of the three gypsophytes (H: *H. fruticosa*, HS: *H. squamatum*, and T: *T. balthazaris*) for the different sampling dates.

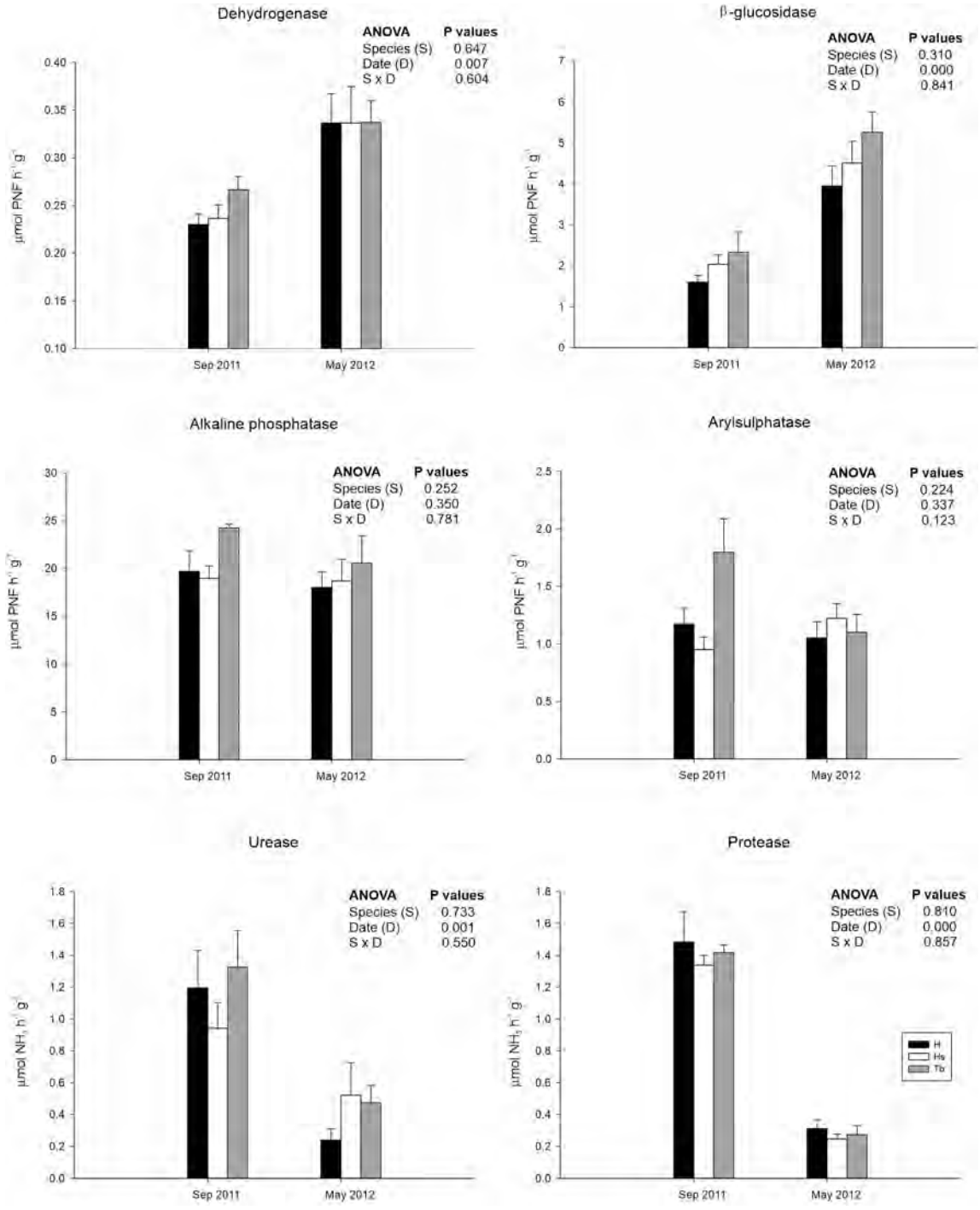

Fig. 5. Enzyme activities in the rhizosphere soil of the three gypsophytes (H: *H. fruticosa*, HS: *H. squamatum*, and T: *T. balthazaris*) for the different sampling dates.

(Caiola et al., 1996; Potts, 1999). This could explain the differences in water retention and dehydration that we found in rhizospheric soils, with seasonal variations, and particularly in *Herniaria*.

Soil organic matter mineralization is very sensitive to drought in Mediterranean ecosystems (Sardans and Peñuelas, 2013). Decreases in soil enzyme activity (Sardans et al., 2007; Hueso et al., 2011), and soil respiration (Emmett et al., 2004; Asensio et al., 2007) have been widely observed under drought conditions. During summer the soil becomes dry but upon subsequent rewetting by a rain pulse there is a burst of decomposition, mineralization, and release of inorganic nitrogen and CO_2 in Mediterranean environments, named the Birch effect (Jarvis et al., 2007) - this is based on the conceptual paradigm of pulse-reserve (Noy-Meir, 1973). Such bursts of microbiological activation are dependent on the reserves of carbon (WSC), total phosphorus, and total sulfur (Table 4).

In the semiarid area at Yesos del Rincón, after the summer drought (60 days with only 3.9 mm of rainfall), on 2/09/11 rainfall (27.3 mm out of a total of 31.2 mm shown in Table 2) triggered a pulse pattern of biological activities and biogeochemical cycles (Austin et al., 2004; Huxman et al., 2004; Collins et al., 2008) at the rhizosphere level. After 20 days of rain, the soils and rhizospheres were sampled. The microbial biomass decreased during the drought stage but was stimulated

Table 5
Spearman's coefficients of correlation between soil chemical and biochemical properties.

	BR	BC	Des.	β-Glu.	Phos.	Aryls.	Ur.	Prot.	WSC	WSN	TOC	TN	TP	TS	TC	Carbonates
BR	1	0.602***	NS	−0.384*	0.398*	0.460**	0.626***	0.832***	0.709***	NS	0.371*	NS	0.826***	0.498**	0.430**	0.464*
BC		1	NS	NS	NS	NS	0.534***	0.659***	0.484**	NS	0.374*	NS	0.473**	NS	NS	NS
Des.			1	0.396*	NS	NS	NS	NS	NS	0.664***	NS	NS	NS	−0.371*	NS	NS
β-Glu.				1	NS	NS	−0.418*	−0.465**	NS	0.350*	NS	NS	−0.366*	−0.595***	NS	NS
Phos.					1	0.463**	NS	0.365*	NS	NS	NS	NS	NS	NS	NS	NS
Aryls.						1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Ur.							1	0.554***	0.370*	NS	NS	NS	0.580***	0.490**	0.518***	0.509**
Prot.								1	0.644***	NS	NS	NS	0.682***	0.544***	0.366*	0.368*
WSC									1	NS	NS	NS	0.594***	0.368*	NS	NS
WSN										1	NS	NS	NS	−0.347*	NS	NS
TOC											1	0.613***	0.399**	NS	0.446**	NS
TN												1	0.687***	0.692***	NS	NS
TP													1	0.451**	0.630***	0647***
TS														1	NS	NS
TC															1	0.955***
carbonates																1

*, **, *** significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. Ns = not significant. WSC: water soluble C; TOC: total organic C.

after wetting, and the wet-dry cycle itself resulted in higher net N and C mineralization when compared to continuously moist soils (Austin et al., 2004).

The Cyanoprokaryota of the rhizospheres at Yesos del Rincón (Table 6) and the microorganisms of this system can be accelerated and were measured as the microbial biomass C and basal respiration rate (Fig. 4). The high pulse of CO_2 produced can affect plants, causing rhizodeposition (Phillips et al., 2011) and/or exopolysaccharides production by Cyanoprokaryota, as shown above. Much, but not all, of the C in exudates can be taken up without extracellular enzymatic decomposition (Weintraub et al., 2007). This fraction contains WSC and nutrients (Table 4): WSC was increased significantly by the effects of drought, and however WSN was decreased (Table 4). Steinweg et al. (2013) showed that WSC was greater under drought than under ambient or wet experimental conditions. McDaniel et al. (2013) also found higher values of WSC in summer, compared to spring, under a humid continental climate, with rather severe winters and warm summers (in experiments on global warming, it appears that warming increases the availability of C more than that of N). Prolonged drought can result in a decline in the N_2 fixation capacity of Cyanoprokaryota, whereas persistent moisture results in an increase (Gundale et al., 2009). This probably explains the frequent reports of decreased N_2 fixation rates in midsummer, when conditions are at their driest (DeLuca et al., 2002; Zackrisson et al., 2004).

The 27.3 mm pulse of rainfall is sufficient, in the rhizosphere system of Yesos del Rincón, to produce the connectivity of the soil pores. Cyanoprokaryota fix N_2 biologically and the availability of this limiting factor in these environments promotes the activation of extracellular enzymes produced by other microorganisms. This water pulse into the rhizospheres activated the N cycle and, consequently, increased the protease and urease activities (Fig. 5) since water pulses are intimately related to the N cycle (Austin et al., 2004). High correlations of WSC with the basal respiration rate, microbial biomass C, urease, and protease were found (Table 5). However, WSN was correlated only with dehydrogenase and β-glucosidase, all of these parameters having higher values in the spring.

Enzyme activities related to N cycling were increased by the effect of drought, for all three gypsophytes (Fig. 5); Sanaullah et al. (2011) showed similar results. Despite the great richness of arbuscular mycorrhizal fungi at Yesos del Rincón (Alguacil et al., 2012), when seasonal variations occur and with respect to the decomposition and N transformation processes, these fungi seem to need associations with primary producers (States et al., 2001; Porras-Alfaro et al., 2008) and this could happen with respect to their uptake of phosphorus forms (Singh and

Table 6
Cyanoprokaryota species in the non-rhizospheric soil and in the rhizosphere soil of the three gypsophytes (H: *H. fruticosa*, HS: *H. squamatum*, and T: *T. balthazaris*) for the different sampling dates.

Species	September 2011				May 2012			
	Non-rhizospheric	Rhizospheres			Non-rhizospheric	Rhizospheres		
	Soil	HS	H	T	Soil	HS	H	T
Cyanophyta								
Chroococcopsis cf. *fluviatilis*			++				++	+
Gloeocapsa biformis			++	+			+	
Gloeocapsa rupestris				+				
Gloeocapsa rupicola			++				++	++
Gloeocapsa salina			+				++	++
Gloeocapsa violascea							+	+
Myxosarcina sp.			++				+	
Microcoleus chthonoplastes		+	+++	+++			++	++
Nostoc microscopicum	+++				+++		++	
Pseudocapsa dubia							+	
Schizothrix cf. *calcicola*			+++				+++	+++
Scytonema sp.	+++		+		+++			
Tolypothrix elenkinii			+				++	++
Streptophyta								
Klebsormidium sp.		+	++				+	+

+ low (<5%), ++ medium (5–15%) and +++ (15–30%) high presence. The lack of signs indicates absence.

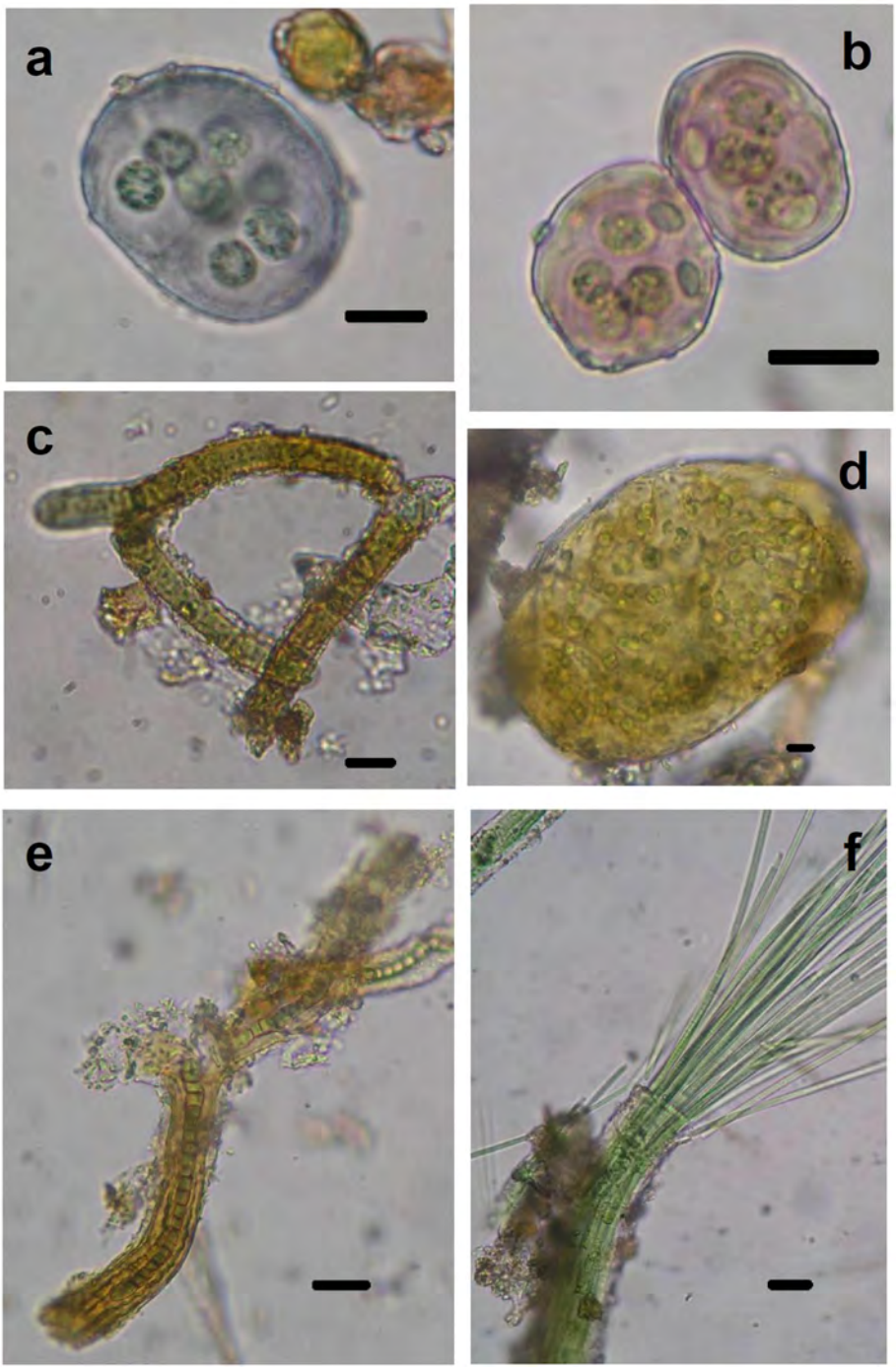

Fig. 6. a–f. Light micrographs [scale bar:10 μm] of a. *Gloeocapsa violascea*, b. *Gloeocapsa rupicola*, c. *Tolypothrix elenkinii*, d. *Nostoc microscopicum*, e. *Scytonema* sp., and f. *Microcoleus chthonoplastes*.

Kapoor, 1999). It is believed that certain amounts of phosphorus (De Nobel et al., 1997; Whitton et al., 2005) and sulfur (Mus et al., 2016) are required for optimal N_2 fixation. In Yesos del Rincón, after the drought there were reserves of total phosphorus and sulfur sufficient for this (Table 4).

At Yesos del Rincón, in spring, another 25.5 mm of rain fell in a single day (20/03/2012), out of a total of 57.3 mm for this period (Table 2), and after 10 days of rain the soil and rhizospheres were sampled. Another type of pulse may have occurred, activating the microbiological activity related to the C cycle, with increases in the activities of dehydrogenase (an indicator of microbiological activity) and β-glucosidase (Fig. 5). It seems that the dehydrogenase enzyme does not accumulate extracellularly in the soil and depends on intact cells (Das and Varma, 2011). On this date the highest values of WSN were recorded (Table 4).

Dehydrogenase activity was extremely high in the rhizospheres of all three gypsophytes tested in Yesos del Rincón, even higher than in the Ah horizons of Galician agricultural soils and similar to those found in soils of Tabernes (Miralles et al., 2012). Dehydrogenase and β-glucosidase activities were highest during spring, whereas urease and protease peaked during late summer (Fig. 5). Under drought stress, the activity of β-glucosidase was decreased in the rhizosphere of all three gypsophytes (Fig. 5).

No changes in alkaline phosphatase and arylsulfatase activities between dates were found (Fig. 5). Drought had no significant effects on

the phosphatase and arylsulfatase activities at Yesos del Rincón; similarly, Sardans et al. (2006) obtained no changes in soil phosphatase activities in response to a drought treatment under semiarid Mediterranean conditions. Phosphatase enzymes are predominantly secreted by plant roots and associated mycorrhiza and other fungi, as pointed out by Joner et al. (2000).

However, the role of Cyanoprokaryota is that of primary colonizers and many species have been shown to possess the property of tricalcium phosphate solubilization. Rock phosphate is abundant but, being insoluble, is unavailable to crop plants. Some cyanobacteria - like *Tolypothrix*, *Scytonema*, and *Hapalosiphon*, among others - have been reported to solubilize rock phosphate. Moreover, extracellular phosphatase activities were detected in different cyanobacterial strains (Whitton et al., 1991). It is generally considered that such activities are related to the maintenance of the phosphate supply (Mateo et al., 2010), but there is still a lot of uncertainty and questions pertaining to enzymatic control remain unanswered. The organic P increased in a drought experiment in a Mediterranean area when alkaline phosphatase activity declined (Sardans et al., 2008). Perhaps, plants obtain P from their interrelationships with microorganisms, in some cases through mycorrhizal associations and in others through Cyanoprokaryota.

At Yesos del Rincón, no changes in arylsulfatase activity between the sampling dates were found (Fig. 5), probably because in this gypsiferous environment, with very abundant sulfates, sulfur will not be limiting. With respect to this element, it is known that Cyanoprokaryota incorporate it into their sheath by compartmentalization, in hypersaline environments (Canfora et al., 2016), and that some excrete it in the form of sulfonated exopolysaccharides (Sudo et al., 1995) and also sulfur it is an essential component of nitrogenase (Mus et al., 2016). Additionally many algae are able to use sulfur surplus to produce sulfonium compounds with different functions related with abiotic and biotic stresses (Ratti and Giordano, 2008), it can be thought that the presence of *Klebsormidium* in rhizospheres may be involved in these functions.

4.2. Role of Cyanoprokaryota in gypsiferous soils

The gypsiferous soils of Yesos del Rincón support a high degree of Cyanoprokaryota diversity and rarity. In the previous section we have seen how these organisms can be stimulated by the water pulse that occur at the end of drought periods and can produce exopolysaccharides, which, along with the rhizodeposition of to the gypsophytes, activate the extracellular enzyme of the N cycle; Cyanoprokaryota also help to maintain an optimal moisture content for these enzymes. Moreover, these Cyanoprokaryota may be an important source of N for plants and soils in this area since the majority of the species can fix atmospheric N_2. It is considered that the species that act as N-fixing agents are those that produce heterocytes, such as *Nostoc microscopicum*, *Scytonema* sp., and *Tolypothrix elenkinii*. However, it has been found that some species of the genus *Gloeocapsa* (Asencio and Aboal, 2009), which lack heterocytes, can also fix N_2 when they are not performing photosynthesis, so that the nitrogenase enzyme responsible for N_2 fixation is not inhibited by the oxygen released in photosynthesis. Hence, the 14 species identified can improve soil fertility, which, in turn, influences vascular plant nutrition. Cyanobacterial communities can also influence the germination and establishment of vascular plants in gypsiferous soils, perhaps acting as plant growth promotors (Gayathri et al., 2017).

The presence of *Klebsormidium* in Yesos del Rincón is in accordance with studies of succession on newly exposed surfaces where conditions are unfavorable for rapid invasion by rooted plants; these have provided many examples of Cyanoprokaryota having an important role during the early stages of succession. In the case of gypsum rocks in SE Spain, succession seems to start with domination by Cyanoprokaryota, followed by green algae (Dana and Mota, 2006). However, this is the first time that the genus *Klebsormidium* a charophyte green algae has been cited in gypsum environments, despite the fact that it was detected in Los Cabecicos de Villena (Asencio, com. pers.).

Cyanoprokaryota species appeared in the rhizospheres of *Helianthemum squamatum*, *Herniaria fruticosa*, and *Teucrium balthazaris* in the gypsiferous soils of Yesos del Rincón, where coccoids predominated over filamentous species; this coincides with the findings of Domínguez and Asencio (2011) in gypsum environments.

Of the 10 genera identified, *Gloeocapsa* was the one with the highest number of different species, five. The abundance of this genus in the study area indicates that soil colonization by blue-green algae is at an initial stage since, according to Fritsch (1907), Pentecost (1992), and Domínguez and Asencio (2011), these blue-green algae are pioneers of the colonization of rocks.

Nostoc microscopicum is a very frequent species in the gypsum area of Los Cabezos in Villena (Domínguez and Asencio, 2011), due to its capacity to tolerate the osmotic stress that is a result of the desiccation and concentration of salts (Büdel et al., 1994).

These three studied gypsophytes are in a well conserved community. In previous work in the study area, Muries (2017) observed that they exhibited different percentages of mycorrhization: *Teucrium balthazaris* had the highest percentage (exceeding 70%), *Helianthemum squamatum* had 40%, and *Herniaria fruticosa* around 35%. Furthermore, these three species have differing degrees of affinity for gypsum (Mota et al., 2011): it is greatest for *Helianthemum squamatum*, moderate for *Teucrium balthazaris*, and lowest for *Herniaria fruticosa*. Marked differences among the three gypsophyte species studied were found with regard to the Cyanoprokaryota in their rhizospheres.

Three functional strategies or adaptation mechanisms with regard to water availability could be discerned in these gypsophytes. On the one hand, *H. fruticosa* - that can be considered as a pioneer woody species (Mota et al., 2011) - had rhizospheres with a high association of Cyanoprokaryota on both sampling dates (Table 6). An intermediate strategy is that of *T. balthazaris*, which showed the presence of some Cyanoprokaryota, with marked differences in the spring season (Table 6). Finally, for *H. squamatum* it is striking that Cyanoprokaryota were not present in its rhizosphere in spring and that at the end of the drought period only two species were detected (Table 6). These adaptation mechanisms can explain the greater amount of water in the samples of rhizosphere soil after drought in *H. fruticosa*, because they contain more Cyanoprokaryota. Since *H. fruticosa* is the species with least mycorrhization, together with a lower percentage affinity for gypsum, and is considered one of the pioneer species, it may need to be associated with Cyanoprokaryota.

5. Conclusions

The microbial functionality at a particular site is dependent on the type of gypsophyte colonizing it, as well as being strongly regulated by environmental factors such as seasonal drought and water pulses. The differences in soil moisture found in the rhizospheres of gypsophytes during drought, as a result of a water pulse, were associated with the presence and abundance of Cyanoprokaryota.

The three gypsophyte species studied seemed to have the same behavior in many of the biochemical parameters studied in their rhizospheres, with the exception of the total N that had its highest value in the rhizosphere of *Herniaria fruticosa* in spring. The pulse of water seemed to trigger the activity of soil organisms, producing an activation of the N cycle through extracellular enzymes such as urease and protease regardless of the type of gypsophyte.

The microbial biomass C and basal respiration rate appeared to be the ideal indicators of the microbiological processes that were activated after drought. In spring the water pulse boosted the C cycle. Dehydrogenase was a good indicator of the changes that occurred in the soil moisture in spring. The increase of soil moisture produced an activation of the C cycle through β-glucosidase, regardless of the type of gypsophyte.

This work demonstrates the tight linkage between the N and C inputs into rhizospheres and the nature, magnitude, and occurrence of water pulses. The WSC, WSN, and extracellular enzymes are good indicators of the responses of these communities.

The alkaline phosphatase and arylsulfatase activities were not influenced by the gypsophyte species or date.

In this regard, we suggest three functional strategies or adaptation mechanisms related to water availability, as determined by drought and water pulse effects, in gypsophytes: *Herniaria fruticosa*, a pioneer species, had the greatest diversity and abundance of Cyanoprokaryota, *Teucrium balthazaris* exhibited an intermediate strategy with greater diversity and abundance of Cyanoprokaryota in spring, and, finally, *Helianthemum squamatum* had lower diversity and abundance.

In this context, future research on the role of Cyanoprokaryota in rhizospheres of gypsophytes could be necessary to confirm the adaptation mechanisms. This would show the potential of Cyanoprokaryota to thrive and to help other organisms develop under future, climate-induced changes.

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