Root colonisation by AM fungi differs between gypsum specialist and non-specialist plants: links to the gypsophile behaviour

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Abstract

Gypsum soils are among the most restrictive substrates for plant life, yet the mechanisms of plant adaptation to gypsum are still poorly understood. Arbuscular mycorrhizal fungi (AMF) can improve host plant nutrition and survival in stressful environments but little is known about the ubiquity and function of AMF in plants that grow in gypsum soils, both specialists and generalists. Previous studies indicate that most gypsophiles (specialists) show much higher concentration of nutrients than gypsovags (generalists), hence our hypothesis was that this would be related to increased mycorrhizal colonisation in gypsum specialists. We therefore quantified colonisation of the roots by mycorrhizal arbuscules (AC), vesicles (VC) and hyphae (HC) in six species of gypsophiles and six species of gypsovags growing in gypsum outcrops. Both groups of plants showed significant differences in AC, VC and HC but in contrast to our hypothesis, colonisation was greater in gypsovags than in gypsophiles. The extent of AMF colonisation does not seem to explain the distinctively high nutrient concentrations reported for gypsophiles. Our results indicate that increased AM colonisation could be a mechanism allowing non-specialist plants to cope with the restrictive conditions of gypsum.

Key words: arbuscular mycorrhizal fungi (AMF); gypsum soils; gypsophiles; Mediterranean semi-arid environments; edaphic endemism.

1. Introduction

Gypsum soils extend over 100 million ha in the world, and occur preferentially in arid and semiarid climates where low precipitation prevents gypsum from being leached (Parsons, 1976). Together with the arid conditions, gypsum soils have particularly stressful physical and chemical properties for plant life including the presence of hard soil crusts, high mechanical instability, low soil porosity, extreme nutritional deficits, high concentration of sulphates and moderate salinity (Guerrero Campo et al., 1999). As a consequence, they are among the most restrictive soils for plant life.

Such extremely adverse conditions contrast with the rich and specialized flora of gypsum soils, comprising one of the most diverse arrays of narrow endemic and rare plants in arid and semiarid regions, many of which are threatened or endangered (Meyer, 1986; Parsons, 1976). Consequently, gypsum soils constitute a global conservation biodiversity priority (Meyer, 1986). In spite of their huge ecological and geographical relevance, gypsum environments have received comparatively less attention than other restrictive substrates such as serpentines or saline soils.

Plants from gypsum habitats are classified as gypsophiles, when they are exclusive to gypsum soils, and gypsovags, which are found both in gypsum and other soil types (Duvigneaud, 1968; Meyer, 1986). Although efforts have been made in the last two decades to unravel the mechanisms behind plant performance in gypsum soils, factors controlling the distribution and occurrence of gypsophiles and gypsovags are still not fully understood (Duvigneaud, 1968; Meyer, 1986; Romao and Escudero, 2005).

Putative adaptative mechanisms of gypsophiles include the ability of seedlings to surpass the hard physical soil crust (Meyer, 1986; Romao and Escudero, 2005) and the ability of plants to accumulate certain elements such as sulphates, ashes and mineral nutrients such as N, P, Ca and Mg (Duvigneaud, 1968; Palacio et al., 2007, Table 1). Also, in some cases, gypsophiles show strikingly high tissue concentrations of aminoacids and proteins (Alvarado et al., 2000). The high nutrient concentrations of most gypsophiles are intriguing, not only for their magnitude (see *Lepidium subulatum* in Table 1), but also because gypsum soils are distinctively nutrient poor (Guerrero Campo et al., 1999). The physiological and ecological mechanisms behind the chemical composition of gypsophiles remain unexplored.

The majority of plant species form arbuscular mycorrhizas that improve the ability of plants to uptake nutrients and cope with water stress (Smith et al., 2010), and have been suggested as important factors for plant edaphic adaptation (Schechter and Bruns, 2008). In this sense, it is well known that most plant species from restrictive soils such as serpentine or saline soils form symbiotic associations with arbuscular mycorrhizal fungi (AMF; Hildebrandt et al., 2001; Schechter and Bruns, 2008 and references therein). Differences in the ability of gypsophiles and gypsovags to form symbioses with AMF may explain the observed nutritional differences between both groups of gypsum species. Spores of AMF (mainly related to *Glomus* sp.) are naturally present in gypsum soils (Alguacil et al., 2009b), and plants growing on gypsum soils inoculated with AMF showed enhanced nutrient uptake (Rao and Tak, 2001). More specifically, the AMF community composition of the roots of some gypsophile species has recently been described, showing that plants from well developed gypsum

communities are colonised by fungal types potentially specific to gypsum soils (Alguacil et al., 2009b, 2009a). Despite these previous findings, little is known about AMF symbiosis in the roots of plants with different specificity to gypsum soils, and the extent to which plant adaptation to gypsum substrates could be mediated by differential AMF colonisation remains unknown.

This study aims to explore the differences in the colonisation by AMF of the roots of gypsophiles and gypsovags. We hypothesized that both groups of plants will show differences in the proportion of root length colonised by AMF, being higher in those species specific to gypsum soils. This is based on the observation that gypsophile species can have symbiosis with fungal types specific to gypsum (Alguacil et al., 2009b, 2009a), and hence presumably more chances for successful root colonisation than gypsovags. Also, we expected the high nutrient concentrations observed in most gypsophiles to be related to increased mycorrhizal infection.

2. Materials and methods

2.1 Study site, species and sampling design

Twelve species were selected for analysis: six gypsovags and six gypsophiles (Table 1), the latter include only widely distributed gypsum endemisms, common to most gypsum outcrops of the Iberian Peninsula. All study species are shrubs or sub-shrubs, which are prevalent growth forms in gypsum outcrops (Parsons, 1976) and have similar branch morphology and architecture, except for the gypsophile *Reseda stricta* and the gypsovag *Launaea pumila* that are biannual and perenial herbaceous plants, respectively (Table 1).

The study was conducted in the gypsum outcrops of the Middle Ebro Basin (Villamayor, near Zaragoza, NE Spain), an extensive area of massive gypsum deposits and gypsum soils with high contents of gypsum. We selected twelve locations located at least 2 km apart (table 2). Locations were selected according to the presence and abundance of each species in the local assemblage. Five individuals of each species (three in the case of *Helianthemum* sp. and *Ononis tridentata*) were harvested in total, one from each location (see table 1), between the May 18th and June 4th, 2009, when fungal activity of semi-arid Mediterranean communities is supposed to be the highest (e.g. Alguacil et al., 2009a).

Soil samples (5 cm diameter and 10-12 cm deep) were collected with an auger corer from each location to characterize soil properties: pH, conductivity, % gypsum, % carbonates, N, C:N ratio, % organic matter (see Table 2). Organic matter content (% OM) was estimated by chromic acid digestion following Heanes (1984), carbonate content was determined by Bernard calcimetry, total N was analysed with an elementar analyser (VarioMax C/N), and the percentage of gypsum in soil was estimated by measuring total S with an elemental analyser (LECO).

2.2 Mycorrhizal colonisation

The percentage of root length colonised by AMF was estimated by visual observation of diagnostic structures after clearing washed roots in 10% KOH at 90ºC for 10 min (longer for some species with very dark roots) followed by acidification in 2% HCl for 15-20 minutes and staining with boiling 0.05% trypan blue in lactoglycerol (*v/v/v*) as in Phillips and Hayman (1970). The proportion of

root length containing arbuscules, vesicles and hyphae (i.e arbuscular (AC), vesicular (VC) and hyphal colonisation (HC)) was calculated under a microscope (Olympus, BH2) following the magnified intersections method (McGonigle et al., 1990). The number of intercepts observed per root averaged 188 and ranged between 94 and 711.

2.3 Statistical analyses

Differences between gypsophiles and gypsovags in the extent of mycorrhizal infection (i.e. AC, VC and HC) were analysed by a generalised linear model (hereafter GLM) with "gypsum type" (i.e gypsophile / gypsovag) as a fixed factor and "species" as a nested factor within "gypsum type". The gypsum content of soils was included as a covariate in the analyses to account for the geologic inherent variability. Percentage data were angularly transformed to meet a Gaussian response and hence be treated with an identity log link. The relationship between AMF infection and nutrient status of study plants was assessed by Pearson correlation analyses between AC, VC and HC and the N and P concentrations reported for study plants (Table 1). Statistical analyses were performed in SPSS 17.0.

3. Results and Discussion

Mycorrhizal colonisation could be estimated in the roots of all study species except the gypsovag *Thymelaea tinctoria*, which were too dark to be properly stained. Longer digestion times with KOH or HCl proved ineffective and additional bleaching resulted in the destruction of AMF structures. Consequently,

although presence of fungi was detected in the roots of this species, AC, VC and HC could not be measured and it was not included in the analyses.

Roots of gypsophiles and gypsovags showed significant differences in the proportion of their roots containing arbuscules, vesicules and hyphae (Fig. 1). However, contrary to our hypothesis, the extent of AMF infection was higher in gypsovags than in gypsophiles ($P < 0.001$). Gypsovags showed on average 13%, 10% and 68% of their root length colonised by arbuscules, vesicles and hyphae, respectively, while AC, VC and HC in gypsophiles accounted for only 4%, 3% and 41% of root length. When differences were evaluated at the species level general trends for both groups were maintained, but important species-specific differences arose (Fig. 1). For example, the gypsovag *Artemisia herba-alba* showed higher root mycorrhizal infection (AC = 40%, VC = 14% and HC = 97%) than other gypsovags such as *Salsola vermiculata* (AC = 0.3%, VC = 3% and HC = 26%) (Fig. 1), which showed even lower AMF colonisation than some gypsophiles such as *Ononis tridentata* (AC = 8%, VC = 3% and HC = 64%). Differences in the root mycorrhizal infection of gypsophiles and gypsovags stood when *Artemisia herba-alba* was not included in the analyses ($P = 0.021$ for AC, $P < 0.001$ for VC and $P = 0.007$ for HC, data not shown). The absence of arbuscules in the roots of the gypsophile *Reseda stricta* indicates that this species may not form mycorrhizas. The few hyphae and vesicles observed could be produced by non-mycorrhizal fungi colonising the roots of this and the rest of study species (McGonigle et al., 1990). Similarly to other species of Cistaceae, both *Helianthemum* species showed ectomycorrhizas (ECM) in their root tips.

Our results indicate that gypsovags tend to be more heavily colonised with AMF than gypsophiles, although differences between species are remarkable.

Gypsovags are frequently considered as stress tolerant species that find refuge from competition in gypsum soils (Palacio et al., 2007) . Contrary to gypsophiles, gypsovags do not seem to show distinct physiological adaptations to counteract the atypical chemical composition of gypsum soils (Palacio et al., 2007). The enhanced ability of most gypsovags to form mycorrhizas could be a mechanism for non-specialized species to tolerate the nutrient and water deficiencies typical of gypsum soils. Increased plant dependence on AMF colonisation with increased soil nutrient stress has long been acknowledged (Habte and Manjunath, 1987), and the beneficial features of AMF in alleviating water and nutrient stress and ameliorating soil structure seem to be particularly relevant in arid environments (Smith et al., 2010). However, since we only analysed AMF colonisation of gypsovags growing on gypsum soils, and not in and out of gypsum, we can not go further to assess the relevance of AMF symbiosis as a mechanism for gypsovag adaptation to gypsum soils.

Although previous studies have shown that gypsophiles can form symbioses with AMF potentially exclusive to gypsum soils (Alguacil et al., 2009b, 2009a), this did not translate into enhanced AMF colonisation of the roots of gypsophiles. The question remains if the community composition and function of AMF colonising the roots of gypsovags growing on gypsum will markedly differ from those of gypsophiles. An evaluation of the differences in the AMF community composition of gypsophiles and gypsovags growing in a similar area will shed light on the role of AMF assemblages for plant adaptation to edaphic extremes.

Correlations between AC, VC and HC and the N and P concentrations reported for study species (Table 1) were not significant in any case ($P > 0.05$,

data not shown). Consequently, the extent of AMF colonisation can not explain the high nutrient concentrations reported for most of the species of gypsophiles studied (Alvarado et al., 2000; Duvigneaud, 1968; Palacio et al., 2007). High AC values have been interpreted as indicative of active AMF symbiosis, since arbuscules are sites of resource exchange between plant and fungi (McGonigle et al., 1990). However, different species of AMF show different efficiency in supplying plants with nutrients (Cavagnaro et al., 2005). Consequently, the efficiency of the AM symbiosis may be determined by the identity of the plant and the fungi, rather than by just the amount of exchanging sites (i.e. arbuscules). One explanation to our results could, hence, be that gypsophile species form symbioses with more efficient AMF species than gypsovags. Indeed, gypsophiles have been reported to form symbioses with AMF potentially exclusive to gypsum soils (Alguacil et al., 2009b, 2009a). These AMF could also be more efficient than more widespread AMF species under the particular conditions of gypsum soils. Field and laboratory experiments analyzing the nutrient transfer efficiency of different species of fungi forming AM symbiosis with gypsum plants are needed to explore this hypothesis.

Alternatively, the significantly higher AC of gypsovags may indicate that gypsophiles use other mechanisms apart from AM to achieve an efficient nutrient uptake. Long root hairs, highly branched root systems, highly specialized root clusters, or the ability to produce exudates to mobilize nutrients retained in the soil are well-known mechanisms to enhance nutrient acquisition in plants growing in nutrient limited soils (Lambers et al., 2008). Since gypsophiles do not seem to show any of these morphological adaptations in their roots (Palacio and Montserrat-Martí, personal observation), other mechanisms related to nutrient

uptake could be operating. Interactions with other non-AM soil microorganisms such as dark septate fungi have been shown to mediate N and C transfers between plants and N-fixing organisms in arid ecosystems (Green et al, 2008), although such interactions have never been assessed in gypsum ecosystems. Also, in soils where nutrients are scarce such as gypsum soils, temporally and spatially heterogeneous pulses of nutrient availability may account for a significant proportion of the annual nutrient budget of plants (Bilbrough and Caldwell, 1997). Under these circumstances, rapid root proliferation in response to ephemeral nutrient pulses has been described as an important mechanism for plant nutrient exploitation (Jackson and Caldwell, 1989). Temporal and spatial differences in the ability to exploit nutrient availability between gypsophiles and gypsovags may also partly account for the observed differences in plant nutrition. Accordingly, an analysis of the seasonal N dynamics of the gypsophile *Lepidium subulatum* and the gypsovag *Linum suffruticosum* in this same study area, suggested that the gypsophile may have a higher ability to uptake N in autumn than the gypsovag (Palacio and Montserrat-Martí, unpublished results).

In conclusion, our results indicate that specialist and non-specialist gypsum plants differ in their ability to form symbioses with AMF, with colonisation being greater in most species of the gypsovags analysed. Increased colonisation by AMF could be a mechanism of non-specialist stress-tolerant plants that have not exclusively adapted to gypsum to cope with the restrictive conditions of these soils. Given the central role of AMF in mediating resource exchange between plants and soils, our results indicate the need to undertake further studies investigating the functional importance of the observed differences in colonisation.

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References

Alguacil, M.M., Roldan, A., Torres, M.P., 2009a. Assessing the diversity of AM fungi in arid gypsophilous plant communities. Environmental Microbiology 11, 2649-2659.

Alguacil, M.M., Roldan, A., Torres, M.P., 2009b. Complexity of semiarid gypsophilous shrub communities mediates the AMF biodiversity at the plant species level. Microbial Ecology 57, 718-727.

Alvarado, J.J., Ruiz, J.M., López-Cantarero, I., Molero, J., Romero, L., 2000. Nitrogen metabolism in five plant species characteristic of Gypsiferous soils. Journal of Plant Physiology 156, 612-616.

Bilbrough, C.J., Caldwell, M.M., 1997. Exploitation of springtime ephemeral N pulses by six Great Basin plant species. Ecology 78, 231-243.

Cavagnaro, T.R., Smith, F.A., Smith, S.E., Jakobsen, I., 2005. Functional diversity in arbuscular mycorrhizas: exploitation of soil patches with different phosphate enrichment differs among fungal species. Plant Cell and Environment 28, 642-650.

Duvigneaud, P., 1968. Essai de classification chimique (éléments minéraux) des plantes gypsicoles du bassin de l'Ebre. Bulletin de la Société Royale de Botanique de Belgique 101, 279-291.

Green, L.E., Porras-Alfaro, A., Sinsabaugh, R.L. 2008. Translocation of nitrogen and carbon integrates biotic crust and grass production in desert grasslands. Journal of Ecology 96, 1076-1085.

Guerrero Campo, J., Alberto, F., Maestro Martínez, M., Hodgson, J., Montserrat Martí, G., 1999. Plant community patterns in a gypsum area of NE Spain. II.- Effects of ion washing on topographic distribution of vegetation. Journal of Arid Environments 41, 411-419.

Habte, M., Manjunath, A., 1987. Soil solution phosphorus status and mycorrhizal dependency in *Leucaena leucocephala*. Applied and Environmental Microbiology 53, 797-801.

Heanes, D.L., 1984. Determination of total organic-C in soils by an improved chromic acid digestion and spectrophotometric procedure. Communications in Soil Sciences and Plant Analysis 15,1191-1213.

Hildebrandt, U., Janetta, K., Ouziad, F., Renne, B., Nawrath, K., Bothe, H., 2001. Arbuscular mycorrhizal colonisation of halophytes in Central European salt marshes. Mycorrhiza 10, 175-183.

Jackson, R.B., Caldwell, M.M., 1989. The timing and degree of root proliferation in fertile-soil microsites for 3 cold-desert perennials. Oecologia 81, 149-153.

Lambers, H., Raven, J.A., Shaver, G.R., Smith, S.E., 2008. Plant nutrient-acquisition strategies change with soil age. Trends in Ecology and Evolution 23, 95-103.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, J.A., 1990. A new method which gives an objective-measure of colonisation of roots by vesicular arbuscular mycorrhizal fungi. New Phytologist 115, 495-501.

Meyer, S.E., 1986. The ecology of gypsophile endemism in the Eastern Mojave desert. Ecology 67, 1303-1313.

Palacio, S., Escudero, A., Montserrat-Marti, G., Maestro, M., Milla, R., Albert, M.J., 2007. Plants living on gypsum: beyond the specialist model. Annals of Botany 99, 333-343.

Parsons, R.F., 1976. Gypsophily in plants. A review. American Midland Naturalist 96, 1-20.

Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55, 158-&.

Rao, A.V., Tak, R., 2001. Influence of mycorrhizal fungi on the growth of different tree species and their nutrient uptake in gypsum mine spoil in India. Applied Soil Ecology 17, 279-284.

Romao, R.L., Escudero, A., 2005. Gypsum physical soil crusts and the existence of gypsophytes in semi-arid central Spain. Plant Ecology 181, 127-137.

Schechter, S.P., Bruns, T.D., 2008. Serpentine and non-serpentine ecotypes of *Collinsia sparsiflora* associate with distinct arbuscular mycorrhizal fungal assemblages. Molecular Ecology 17, 3198-3210.

Smith, S.E., Facelli, E., Pope, S., Smith, F.A., 2010. Plant performance in stressful environments: interpreting new and established knowledge of the roles of arbuscular mycorrhizas. Plant and Soil 326, 3-20.

Figure captions

Fig. 1. Percentage of arbuscular (AC), vesicular (VC) and hyphal colonisation (HC) in the roots of gypsophiles (white bars) and gypsovags (grey bars) (left pannel) and study species (right pannel). Ah = *Artemisia herba-alba*, Hsy = *Helianthemum syriacum*, Lp = *Launaea pumila*, Li = *Linum suffruticosum*, Sv = *Salsola vermiculata*, Gh = *Gypsophila struthium* subsp. *hispanica*, Hsq = *Helianthemum squamatum*, Hf = *Herniaria fruticosa*, Le = *Lepidium subulatum*, Ot = *Ononis tridentata*, Rs = *Reseda stricta*. Values are means + SE. Differences between groups were significant at $\alpha = 0.05$ for all variables analysed. Comparison between gypsophiles and gypsovags: AC: $P < 0.001$, $F = 32.29$; VC: $P < 0.001$, $F = 22.40$; HC: $P < 0.001$, $F = 23.20$. Comparison between species: AC: $P < 0.001$, $F = 16.03$; VC: $P = 0.011$, $F = 2.90$; HC: $P < 0.001$, $F = 5.80$.

Table 1. Name, taxonomic family, specificity to gypsum soils, growth form, nutrient content* (N, P, Mg and S concentrations) and sampling locations (see details in Table 2) of study species.

Species	**Family**	**Type**	**Growth form**	**N (mg g^{-1})**	**P (mg g^{-1})**	**K (mg g^{-1})**	**Mg (mg g^{-1})**	**S (%)**	**Ash (%)**	**Sampling locations ID**
Artemisia herba-alba Asso	Asteraceae	G_V	S	38.1 (1.3)	2.5 (0.2)	13.2 (0.4)	3.0 (0.3)	0.2 (0.02)	7.8 (0.1)	1, 2, 3, 5, 6
Gypsophila struthium L. subsp. *hispanica* (Wilk.) G. López	Caryophyllaceae	G	S	24.9 (1.3)	1.9 (0.2)	11.8 (1.6)	12.1 (2.3)	3.0 (0.5)	26.7 (1.5)	1, 2, 4, 5, 6
Helianthemum squamatum (L.) Pers	Cistaceae	G	S	16.8 (2.7)	1.2 (0.3)	6.4 (1.6)	6.1 (1.8)	3.0 (0.2)	14.1 (0.9)	2, 3, 4
Helianthemum syriacum (Jacq.) Dum. Cours.	Cistaceae	G_V	S	17.6 (0.9)	2.5 (0.1)	5.0 (0.3)	3.1 (0.3)	1.0 (0.03)	10.9 (0.1)	1, 3, 4
Herniaria fruticosa L.	Caryophyllaceae	G	S	25.3 (1.7)	1.1 (0.1)	9.2 (1.2)	7.7 (0.6)	1.1 (0.1)	12.2 (0.04)	1, 2, 3, 4, 5
Launaea pumila (Cav.) O. Kuntze	Asteraceae	G_V	H	-	-	-	-	-	-	2, 4, 5, 7, 9
Lepidium subulatum L.	Brassicaceae	G	S	50.0 (4.6)	2.5 (0.1)	13.2 (2.2)	2.0 (1.3)	2.7 (1.1)	12.8 (0.5)	1, 2, 3, 5, 6
Linum suffruticosum L.	Linaceae	G_V	S	32.9 (1.5)	1.9 (0.1)	6.6 (2.5)	2.2 (0.5)	0.02 (0.01)	14.4 (1.4)	1, 2, 3, 4, 5
Ononis tridentata L.	Fabaceae	G	S	24.3 (1.9)	1.0 (0.1)	3.1 (0.9)	23.8 (3.4)	4.9 (0.4)	23.5 (0.7)	1, 4, 12
Reseda stricta Pers. subsp. *stricta*	Resedaceae	G	H	-	-	-	-	-	-	1, 2, 3, 4, 5
Salsola vermiculata L.	Chenopodiaceae	G_V	S	37.6 (1.0)	1.7 (0.05)	14.2 (1.6)	12.5 (0.6)	1.4 (0.1)	15.2 (0.7)	4, 5, 8, 9, 10
Thymelaea tinctoria (Pourr.) Endl. subsp. *Tinctoria*	Thymelaeaceae	G_V	S	13.3 (0.3)	0.9 (0.05)	5.7 (0.6)	2.9 (0.2)	0.14 (0.01)	5.9 (0.2)	1, 2, 3, 4, 5

* Nutrient content values as reported in the literature for plants growing in the same study area (Palacio et al., 2007) or analysed by the authors following the methods in Palacio et al. (2007).

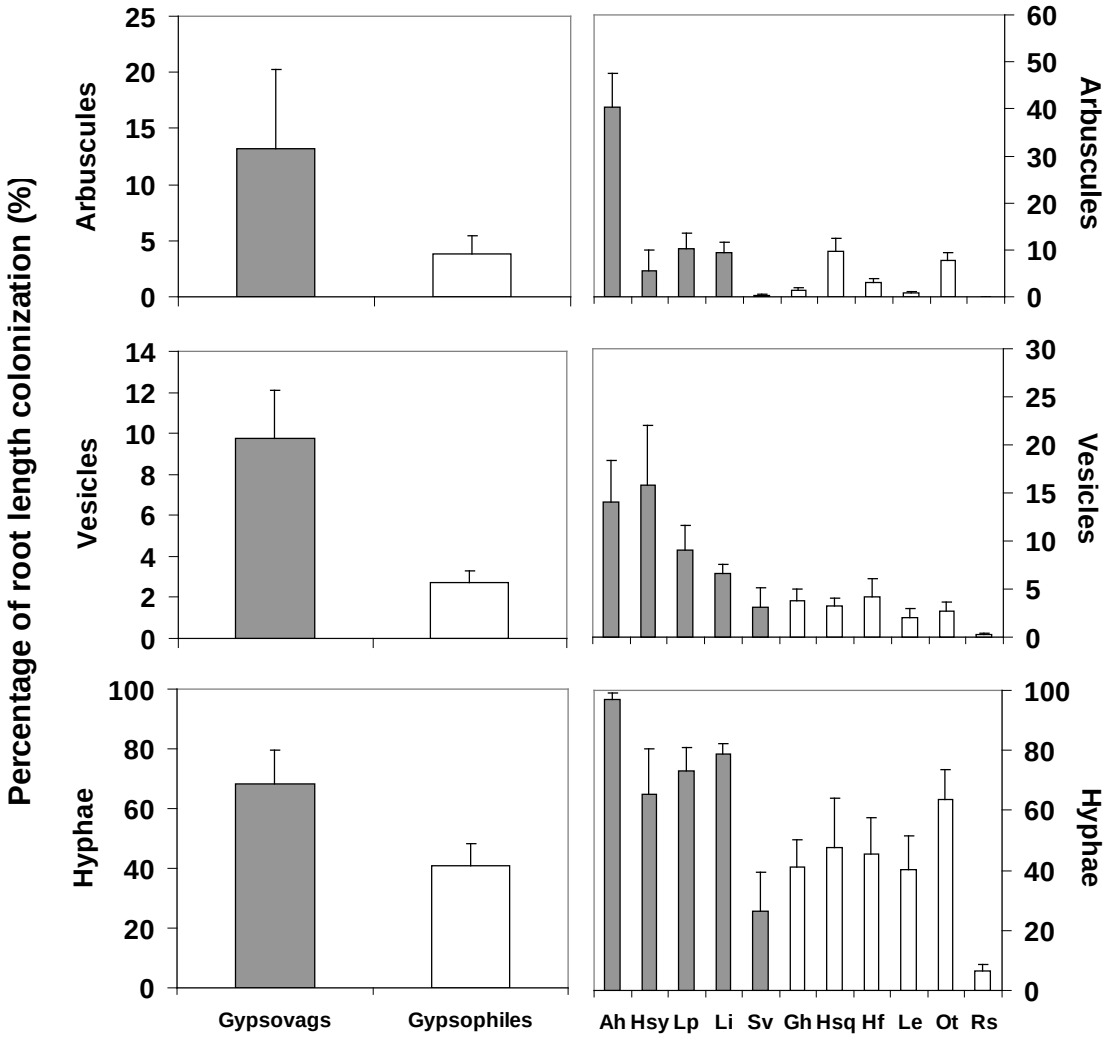
G_V = Gypsovag, G = Gypsophile, H = Herb, S = Shrub.

Table 2. Coordinates and main mean chemical characteristics of sampling locations.

Location ID	Coordinates	pH	EC ($\mu S\ cm^{-1}$)	% OM	% $CaCO_3$	% N	C:N	% Gypsum
1	0º 44' 8.3"E							
	41º 42' 20.5"N	8.04	2.09	1.15	21.80	0.07	10.09	69.04
2	0º 43' 57.2"E							
	41º 42' 48.6"N	8.15	1.87	1.42	26.01	0.09	9.61	50.84
3	0º 43' 55.2"E							
	41º 42' 6.8"N	8.32	2.16	0.81	23.95	0.05	9.89	62.29
4	0º 42' 34.7"E							
	41º 42' 15.6"N	8.13	2.40	0.83	26.05	0.05	10.56	50.56
5	0º 44' 39.1"E							
	41º 40' 44.2"N	8.11	2.11	0.81	17.93	0.05	10.77	64.37
6	0º 45' 27.3"E							
	41º 41' 2.9"N	8.09	2.12	1.14	10.74	0.08	8.57	71.16
7	0º 43' 47.2"E							
	41º 41' 47.6"N	8.22	1.81	0.28	38.05	0.01	12.17	25.27
8	0º 44' 4.0"E							
	41º 42' 38.8"N	8.09	1.89	1.08	33.56	0.06	9.75	40.79
9	0º 42' 59.3"E							
	41º 42' 42.6"N	8.15	1.84	0.90	53.23	0.04	11.54	7.79
10	0º 42' 54.1"E							
	41º 42' 19.6"N	8.13	1.43	1.36	41.77	0.09	9.09	28.67

EC = Electric conductivity, OM = Organic Matter.

Fig 1


Percentage of root length colonization (%)
Arbuscules
Vesicles
Hyphae
Gypsovags
Gypsophiles
Ah Hsy Lp Li Sv Gh Hsq Hf Le Ot Rs