Plants Living on Gypsum: Beyond the Specialist Model

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• *Background and Aims* Plants from gypsum habitats are classified as gypsophiles and gypsovags. The former include both narrow endemics limited to small gypsum areas and regionally dominant gypsophiles growing in gypsum areas of large regions, whereas gypsovags are plants that can grow both in gypsum and non-gypsum soils. Factors controlling the distribution of gypsum plants are still not fully understood.
• *Methods* To assess how the different types of gypsum plants deal with the stressful conditions of gypsum substrates, comparisons were made of the leaf chemical composition of four gypsovags, five regionally dominant gypsophiles and four narrow gypsum endemics growing in two massive gypsum areas of the Iberian Peninsula.
• *Key Results* The chemical composition of gypsovags was clearly different from regionally dominant gypsophiles, while the chemical composition of narrow-gypsophile endemics was more similar to the chemical composition of gypsovags than to that of regionally dominant gypsophiles. Regionally dominant gypsophiles showed higher concentrations of ash, Ca, S, N, Mg P and Na, whereas gypsovags and local gypsophile endemics displayed higher concentrations of C and greater C : N ratios.
• *Conclusions* Such differences suggest that the three groups of gypsum plants follow diverse ecological strategies. It is suggested that regionally dominant gypsophiles might fit the 'specialist' model, being species specifically adapted to gypsum, whereas both gypsovags and narrow-gypsophile endemics might fit the 'refuge' model, being stress-tolerant species that find refuge on gypsum soils from competition. The analysis of the leaf chemical composition could be a good predictor of the degree of plants specialization to gypsum soils.

Key words: Gypsophily, gypsum-rich soils, leaf chemical composition, narrow-endemic gypsophytes, Mediterranean semi-arid environments, plant conservation, edaphic endemism.

INTRODUCTION

Gypsum soils spread over 100 million ha around the world (Verheye and Boyadgiev, 1997). They are confined to arid and semi-arid climates where low precipitation prevents gypsum from being removed by leaching (Parsons, 1976). Together with the arid conditions, gypsum soils have particularly stressful physical and chemical properties for plant life. Among the adverse physical features are the presence of a hard soil surface crust, which can restrict seedling establishment (Meyer, 1986; Escudero *et al.*, 1999, 2000*b*); the mechanical instability of the soil material due to its lack of plasticity, cohesion and aggregation (Bridges and Burnham, 1980); and, in certain areas, its low porosity, which might limit the penetration of plant roots (Guerrero Campo *et al.*, 1999*b*). In semi-arid regions, the low water retention of massive gypsum soils leads to a high infiltration of rainwater, which increases water deficit during drought periods (Guerrero Campo *et al.*, 1999*b*), although in some arid regions gypsum soils have been shown to display higher water availability during drought than adjacent soils (Meyer and García-Moya, 1989). Chemically adverse features of gypsum soils are mainly related to the intense nutritional impoverishment of the soil caused by the exchange of calcium for other ions retained

in the soil complex (Meyer *et al.*, 1992; Guerrero Campo *et al.*, 1999*b*), and by the high concentration of sulfate ions, which can be toxic for plants (Duvigneaud, 1968; Ruiz *et al.*, 2003). Such stressful conditions make gypsum soils largely unsuitable for the growth of trees (Rivas-Martínez and Costa, 1970), and thus vegetation is composed mainly of stress-tolerant sub-shrubs, some scattered shrubs, herbaceous perennials and annual plants (Parsons, 1976; Hodgson *et al.*, 1994).

Despite gypsum soils constituting extremely adverse habitats for plant life, they give rise to one of the most conspicuous and diversified set of endemic and rare plants in arid and semi-arid regions (Johnston, 1941; Parsons, 1976; Powell and Turner, 1977; Meyer, 1986; Meyer and García-Moya, 1989; Cerrillo *et al.*, 2002; Mota *et al.*, 2003). Most of these plants are seriously threatened, constituting a global biodiversity conservation priority (Meyer, 1986). In spite of being among the most threatened habitats in Europe and, specifically, in the Mediterranean Basin (Gómez-Campo, 1987; European Community, 1992; Mota *et al.*, 2003), gypsum environments have received remarkably little study (Meyer, 1986; Meyer *et al.*, 1992; Escudero *et al.*, 1999, 2000*a*, *b*).

Two different models have been proposed to explain the occurrence of edaphic endemics. In the 'refuge' model, edaphic endemics are stress-tolerant species that are not

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specifically adapted to the atypical soils in which they grow, but are able to tolerate the adverse and stressful conditions they impose. These species are out-competed from normal adjacent soils by dominant species and take refuge in marginal and unfertile soils, where interspecific competition is weaker (Gankin and Major, 1964). In the 'specialist' model, edaphic endemics are fit for the atypical soils in which they live, being more competitive on them, while becoming less competitive in normal and widely distributed habitats (Meyer, 1986).

Plants from gypsum habitats are classified as gypsophiles when they occur only in gypsum soils, and gypsovags when they can grow in gypsum soils but also in other non-gypsum soils (Duvigneaud, 1968; Meyer, 1986). Factors controlling the distribution and performance of gypsophiles and gypsovags are still not fully understood (Duvigneaud, 1968; Boukhris and Lossaint, 1970; Meyer, 1986; Escudero *et al.*, 1999, 2000*b*; Romao and Escudero, 2005). We hypothesize that gypsovags might be stress-tolerant plants that can occur in gypsum soils because competition with trees and shrubs is lower and their physiological abilities enable them to counteract the pernicious limitations of these soils. Therefore, our expectation is that they might fit the 'refuge' model. In contrast, gypsophiles, which include two groups of plants – those extremely narrow endemics limited to small gypsum areas, and those widely distributed in most gypsum areas of large regions, i.e. regionally dominant gypsophiles (Mota *et al.*, 2003) – might fit both models.

Some early studies on gypsum plants have reported that gypsophiles have higher concentrations of sulfur, calcium and total ash than gypsovags (Duvigneaud and Denaeyer-De Smet, 1966; Duvigneaud, 1968). Differences in leaf chemical composition may result from differences in the physiology and, ultimately, in the adaptation of plants to atypical substrates. In a recent study on congeneric serpentine and non-serpentine shrubs, serpentine species exhibited selective root-to-shoot Ca translocation and Mg exclusion mechanisms, which resulted in a greater tolerance of Ca deficiency and Mg toxicity, and higher leaf Ca : Mg ratios than their non-serpentine counterparts (O'Dell *et al.*, 2006). Consequently, high leaf Ca : Mg ratio was linked to the ability of plants to survive on serpentine soils and served as a classificatory trait for serpentine and non-serpentine species (O'Dell *et al.*, 2006). Similarly, many specialists of gypsum substrates have the ability to tolerate the accumulation of those elements found in excess in the soil, showing a distinctive leaf chemical composition (Duvigneaud and Denaeyer-De Smet, 1973). In the present study, use is made of those early findings to assess: (1) whether the chemical composition of gypsovags and regionally dominant gypsophiles differs; (2) whether the chemical composition of narrow-gypsophile endemics is more similar to that of gypsum specialists (i.e. regionally dominant gypsophiles) or to that of species that take refuge in gypsum soils (i.e. gypsovags); and (3) whether the variability in the chemical composition of plants growing in different gypsum outcrops may obscure the differences between gypsophiles and gypsovags.

MATERIALS AND METHODS

Species and study area

Thirteen species were selected for analysis: four gypsovags and nine gypsophiles. The latter comprised five widely distributed gypsophiles and four narrow gypsum endemics (Table 1). The distinction between the two types of gypsophiles was made according to the extent of their distribution area. Accordingly, widely distributed gypsophiles were species that showed a wide distribution range, growing in most gypsum outcrops of a large territory (the Iberian Peninsula), whereas narrow gypsum endemics were species that showed a limited number of populations, growing only in one gypsum area of the Iberian Peninsula. All study species were shrubs or sub-shrubs, which are the prevalent growth forms in gypsum outcrops (Parsons, 1976), and had a similar branch morphology and architecture.

Two different areas located in different biogeographical provinces of the Iberian Peninsula and more than 350 km apart were selected for study: central Spain (Middle Tajo Basin, near Madrid) and north-east Spain (Middle Ebro Basin, near Zaragoza). In both regions, samples were taken of gypsophiles growing in massive gypsum soils and gypsovags growing both in gypsum soils and calcareous soils. Populations were located as close as possible within each region to minimize environmental variability. The gypsophile *Gypsophila struthium* subsp. *hispanica* was collected only near Zaragoza, and the gypsovag *Teucrium polium* subsp. *capitatum* was sampled only near Madrid. Narrow gypsum endemics were collected exclusively in central Spain, as such types of endemics are much more frequent in this area than in the Middle Ebro Basin (Mota *et al.*, 1998). Study species and sampling sites are shown in Table 1, while climatic and edaphic features of each sampling site are given in Table 2.

Sampling of plant material

The species considered here bear two types of shoots, long and short, i.e. dolichoblasts and brachyblasts, respectively, which have different types of leaves (Orshan, 1972; Margaris, 1981). To obtain leaf samples that are comparable between different species it is important to avoid such leaf heterogeneity, which may affect leaf nutrient concentrations (Alonso and Herrera, 2001). To overcome such variability, only a single leaf type common to all selected species was sampled, namely the mature leaves of brachyblasts. Leaf phenological stage is also an important factor affecting the chemical composition of leaves (Aerts and Chapin, 2000); hence to obtain comparable samples it is necessary to collect leaves in similar phenological stages. In plants from Mediterranean semi-arid environments, brachyblasts start developing in spring and grow steadily until summer, when they arrest their elongation. Brachyblast growth resumes in autumn and stops again with the arrival of winter cold (Orshan, 1989; Palacio and Montserrat-Martí, 2005). In these species, brachyblast nutrient concentrations increase during autumn, but remain quite stable during winter (Palacio *et al.*, 2006). Thus, both the

TABLE 1. *Location and characteristics of study species and sites*

Species	Species type[a]	n[b]	Sampling sites[c]	UTM[d]	Altitude (m a.s.l.)	Date of sampling	Substratum[e]
Centaurea hyssopifolia Vahl.	LE	4	Chinchón (M)	VK6247	640	6-Feb-03	G
Gypsophila struthium L. subsp. *hispanica* (Wilk.) G. López	G	5	Villamayor 1 (Z)	XM8820	300	3-Feb-03	G
Helianthemum marifolium (L.) Mill. subsp. *conquense* Borja & Rivas Goday	LE	7	Tendilla (Cu)	WK4214	780	3-Mar-03	G
Helianthemum squamatum (L.) Pers.	G	5	Villamayor 1 (Z)	XM8718	290	21-Jan-03	G
			Chinchón (M)	VK6247	640	27-Jan-03	G
Herniaria fruticosa L.	G	C	Villamayor 1 (Z)	XM8820	300	20-Jan-03	G
				XM8718	290	21-Jan-03	G
			Chinchón (M)	VK6247	640	27-Jan-03	G
Lepidium subulatum L.	G	5	Villamayor 1 (Z)	XM8820	320	13-Jan-03	G
		4	Chinchón (M)	VK6247	640	27-Jan-03	G
Linum suffruticosum L.	GV	5	Villamayor 1 (Z)	XM8820	320	7-Jan-03	G
			Santos de la Humosa (M)	VK8451	850	20-Apr-03	Ca
			Chinchón (M)	VK6247	640	10-Apr-03	G
Ononis tridentata L.	G	5	Villamayor 1 (Z)	XM8820	300	20-Jan-03	G
		4	Chinchón (M)	VK6247	640	27-Jan-03	G
Rosmarinus officinalis L.	GV	5	Peñaflor (Z)	XM8626	300	24-Jan-03	G
			Murillo Gallego (Z)	XM8582	460	23-Jan-03	Ca
			Villarejo (M)	VK7541	700	6-Feb-03	Ca
			Valdarachas (M)	VK8223	760	9-Feb-03	G
Salvia lavandulifolia Vahl.	GV	5	Villamayor 1 (Z)	XM8820	320	21-Jan-03	G
			Villamayor 2 (Z)	XM8920	340	21-Jan-03	G + Ca
			Orés (Z)	XM6783	760	23-Jan-03	Ca
		C	Alcubierre (Hu)	YM0732	560	22-Jan-03	G + Ca
		5	Villarejo (M)	VK7541	700	6-Feb-03	Ca
			Chinchón (M)	VK8223	760	27-Jan-03	G
Teucrium polium L. subsp. *capitatum* (L.) Arcangelli	GV	3	Villarejo (M)	VK7541	700	6-Feb-03	Ca
		5	Chinchón (M)	VK6247	640	6-Feb-03	G
Teucrium pumilum L.	LE	6	Chinchón (M)	VK6247	640		G
Thymus lacaitae Pau	LE.	5	Alcalá de Henares (M)	VK8282	710	28-Jan-03	G

[a]LE = Local endemism; G = gypsophile; GV = gypsovag.
[b]*n* = number of sampled individuals; C = composite sample.
[c]M = Madrid (central Spain, Middle Tajo Basin); Cu = Cuenca (central Spain, Middle Tajo Basin); Z = Zaragoza (north-east Spain, Middle Ebro Basin) Hu = Huesca (north-east Spain, Middle Ebro Basin).
[d]UTM = Universal Transverse Mercator coordinates.
[e]Type of substratum: G = gypsum soil; Ca = calcareous soil; G + Ca = calcareous soils with small amounts of gypsum.

growth and the chemical composition of brachyblast are steady during winter. For this reason, all plant material was collected between January and early February. In order to minimize other known sources of variation, such as topography (Guerrero Campo *et al.*, 1999*a*) and age (Aerts and Chapin, 2000), only mature individuals in similar positions within gypsum landscapes were collected, excluding piedmonds, summits and other atypical sites where the study species are rarer.

Three to five adult individuals in each studied population were harvested (Table 1). Once in the laboratory, a 15-g sample of the brachyblast leaves of each individual was collected and oven-dried to a constant weight at 60 °C. Dry and damaged leaves were excluded from the analyses. For *Herniaria fruticosa* and *Salvia lavandulifolia* (in the Sierra de Alcubierre, Middle Ebro Basin) we could only obtain a single composite sample per population, as the small leaf biomass of the plant individuals prevented

the collection of one 15-g leaf sample per individual (Table 1).

Chemical analyses

Samples were ground in a mill (IKA MF10, IKA-Werke, Staufen, Denmark) to a fine powder. N and C concentrations were analysed with an elemental analyzer (Elementar VarioMax N/CN, Hanau, Germany). Subsamples were burnt at 550 °C for 4 h and ash was dissolved in HNO_3–HCl–H_2O (1 : 3 : 9) and filtered. Concentrations of Na and K were measured in the soluble (silica-free) ash by flame photometry, Ca and Mg concentrations were determined by complexometry (Allen, 1989) and P concentration was assessed by vanado-molybdate colorimetry (Becker, 1961). Total sulfur was analysed using a turbidimetric method with barium chloride (Allen, 1989).

TABLE 2. *Climatic and edaphic characteristics of study sites*

Study sites	Area[a]	Climate		Soil				
		T (°C)	P (mm)	pH	SOM (%)	N (%)	CaCO₃ (%)	Gypsum (%)
Villamayor 1 (Z)	Z	14·2	370	7·7	1·9	0·14	20·0	55·5
Villamayor 2 (Z)	Z	14·2	370	7·7	4·7	0·25	44·8	2·0
Murillo de Gállego (Z)	Z	12·8	680	7·6	3·9	0·13	38·4	0·0
Orés (Z)	Z	12·4	780	8·5	3·2	0·13	32·2	0·0
Alcalá de Henares (M)	M	13·2	350	7·8	1·8	0·15	20·6	32·0
Chinchón (M)	M	13·8	422	7·7	2·1	0·12	9·7	59·3
Santos de la Humosa (M)	M	13·0	450	8·3	5·2	0·22	38·8	0·0
Villarejo (M)	M	13·5	445	8·2	4·7	0·18	35·4	0·0
Valdarachas (M)	M	13·3	440	7·9	2·8	0·11	19·3	40·4
Tendilla (Cu)	M	13·0	410	7·8	2·4	0·17	22·3	29·3

T = Mean annual temperature; P = total annual rainfall; SOM = soil organic matter; N = nitrogen.
[a]M = Madrid (central Spain, Middle Tajo Basin); Z = Zaragoza (north-east Spain, Middle Ebro Basin).

Statistical analyses

Constrained ordinations were used to examine the multivariate relationships between the nutrient content of each individual and a complete set of classificatory dummy variables (Legendre and Anderson, 1999). The evaluation of the effect of a set of classificatory variables on this multivariate data set can be viewed as a problem of covariation. This type of covariation can efficiently be approached by means of constrained ordinations considering the dummy variables as variables upon which statistical analyses are performed. As suggested by McCune (1997), these techniques (ter Braak, 1986; ter Braak and Prentice, 1988) can be used as tools for hypothesis testing (Borcard *et al.*, 1992). Classificatory predictors were the type of soil (gysum/calcareous), the region of origin (Madrid/Zaragoza), the type of gypsum plant (gypsophile/gypsovag) and, finally, the distribution range (wide/narrow). A nutrient-content data matrix was constructed with all individual plants and their chemical content (see Appendix). The null hypothesis was that the influence of these classificatory variables on the multivariate nutrient-content data matrix was not significantly different from random. This means that variables are not able to explain significant fractions of the total variation in the nutrient matrix. With this in mind, a Detrended Correspondence Analysis (Hill and Gauch, 1980) was conducted with the complete nutrient-content data set by detrending by segments and non-linear rescaling of the axes, which has the property that the extracted axes are scaled in units of average standard deviation (Gauch, 1982). This technique is exclusively used for measuring the length of the extracted axes. Values of the extracted gradients above 3 s.d. units suggest the use of techniques assuming unimodal responses, such as Canonical Correspondence Analysis (CCA) or other related techniques (ter Braak, 1986; Legendre and Anderson, 1999). Since the extracted gradients of this data set were relatively short (s.d. <2), we conducted a Redundancy Analysis (hereafter RDA), which is a constraining ordination technique that assumes linear responses of the species with the extracted axes. Total variation explained (TVE) by the classificatory variables was calculated as the sum of all extracted canonical axes (Borcard *et al.*, 1992). A Monte Carlo permutation test (1000 randomizations) was performed to determine the accuracy of the relationship between the two data sets, using the sum of all canonical eigenvalues or trace to build the *F*-ratio statistic (ter Braak, 1990; Verdonschot and ter Braak, 1994; Legendre and Anderson, 1999).

If the RDA model with all the dummy predictors and selected interactions was significant, a forward stepwise procedure was carried out to select a reduced model including only significant variables. Explanatory variables were incorporated one at a time and step by step in the order of their decreasing eigenvalues after eliminating the variation accounted for by the already-included variables. The process stopped when the new variable was not significant ($P > 0·01$ after being adjusted for multiple comparison with the Holm's method; Legendre and Legendre, 1998). Improvement of the reduced model with each new selected variable was determined by a Monte Carlo permutation test with 1000 randomizations.

Up to four independent ordinations were conducted with every group of dummy variables in order to know whether each group of variables was able to explain significant fractions of variation. When the RDA model with the gypsum plant type was conducted, the interaction between wide distribution and gypsophile was included to account for the variability within gypsophiles. A variance partitioning was performed with RDA to evaluate the relative importance of some predictors after adjusting the variability of other variables. This last data set was considered as a covariable data set (Borcard *et al.*, 1992). This procedure has been called partial RDA because it determines the variation explained by the explanatory variables after removing the variation accounted for by the covariable data set. More specifically, we wanted to know if the remaining information after adjusting our main data set to the covariable data set could be significantly explained by the resulting matrix. If not, both explanatory data sets were concomitant. All multivariate analyses were conducted with CANOCO for Windows v. 4.5 (ter Braak and Smilauer, 1997).

Generalized linear models (McCullagh and Nelder, 1989) were constructed for nutrient concentration of gypsovags using S-PLUS. GLMs allow the handling of larger

distribution types for the response variable than standard linear regressions. Type of soil (1. d.f.), site location (1 d.f.) and the corresponding interaction were included as fixed variables. Chi-square tests were conducted to evaluate whether or not selected predictors explained a significant fraction of the total variance (Guisan *et al.*, 2002).

Some populations of *Salvia lavandulifolia* occured in soils that were calcareous but slightly covered with gypsum materials. Individuals from these sites were included in our RDA models as '1' for both dummy soil variables, 'Calcareous soil' and 'Gypsum'. In the corresponding GLMs, these plants were not included. Only five plants per site and soil (genuine gypsum and calcareous soils) were considered.

RESULTS

All the classificatory variables considered, i.e. type of soil (gysum/calcareous), region of origin (Madrid/Zaragoza), type of gypsum plant (gypsophile/gypsovag), distribution range (wide/narrow) and the interaction between type of gypsum plant and distribution range (gypsophile wide/ gypsophile narrow), had a significant effect on the multi-variate nutrient-content data matrix when analysed together ($F = 26.051$, $P = 0.002$) and accounted for 51·2 % of the total variance explained (TVE). Nevertheless, only 'gypso-phile wide' and 'calcareous soil' were selected as significant explanatory variables ($P = 0.002$ and 0.03, respectively, after the *F*-ratio test) when a forward stepwise procedure was conducted (Fig. 1). Altogether, these two variables explained 49 % of the TVE, which corresponds to 98 % of the variance explained by the saturated RDA model. However, of the two significant variables, 'gypsophile wide' explained most of the variability whereas 'calcareous soil' explained a small but significant fraction of the TVE.

The analysis of the effect of the gypsum plant type (i.e. gypsovags, wide gypsophiles and narrow gypsophiles) sep-arately from the rest of classificatory variables, showed that: (1) the chemical composition of gypsovags was clearly different from that of regionally dominant gypsophiles (wide gypsophiles; Fig. 2); and (2) the chemical compo-sition of local gypsophile endemics (narrow gypsophiles) was more similar to the chemical composition of gypsovags than to the composition of regionally dominant gypsophiles. This model was also highly significant ($F = 64.102$, $P = 0.002$, TVE 50·2 %). Regionally dominant gypsophiles showed higher concentrations of ash, calcium and sulfur, while gypsovags and local gypsophile endemics displayed higher concentrations of carbon and greater C : N ratios, as shown in the corresponding biplots. Regionally dominant gypsophiles also tended to show higher N, Mg, P and Na concentrations than gypsovags (Fig. 2).

Soil type also explained a significant fraction of the TVE when analysed separately from the rest of the dummy variables (TVE = 10·8 %, $F = 7.719$, $P = 0.002$; Fig. 3). Nevertheless, such effects disappeared when a partial RDA analysis was carried out and the variability explained by the gypsum plant type was removed ($F = 0.800$, $P = 0.578$). Finally, the type of origin and the type of distribution had no significant effect on the
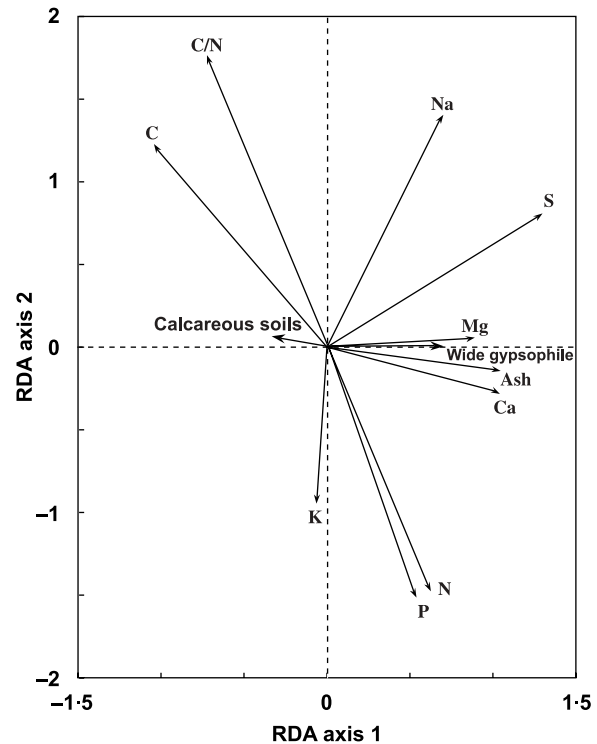

FIG. 1. Biplot for the RDA model with all the dummy predictors and selected interactions. Only 'gypsophile wide' and 'calcareous soils' are represented as they were the only factors selected as significant explana-tory variables after a forward stepwise procedure. $P = 0.002$ and 0.03, for 'gypsophile wide' and 'calcareous soils', respectively after an *F*-ratio test. See text for further details of calculations.

chemical composition of the study species when analysed separately from the rest of the dummy variables ($F = 2.954$, $P = 0.055$ and $F = 2.456$, $P = 0.068$, for the type of origin and the type of distribution, respectively).

The four studied species of gypsovags showed different behaviour when growing on gypsum and calcareous soils (Tables 3 and 4). Thus, while no significant differences were found in the nutrient concentrations of plants of *Teucrium polium* subsp. *capitatum* growing on both soil types, the remaining species displayed different nutrient concentrations depending on the type of substrate they were growing on (Table 4). For example, concentrations of Mg where significantly higher in plants of *Salvia lavandu-lifolia*, *Linum suffruticosum* and *Rosmarinus officinalis* growing on gypsum soils. However, even within those species of gypsovags that changed their nutrient concen-trations depending on the type of soil, metabolic strategies were diverse. Hence, whereas S concentrations were higher in plants of *S. lavandulifolia* and *L. suffruticosum* growing on gypsum soils, plants of *R. officinalis* had similar S concentrations irrespective of the type of soil on which they were growing (Tables 3 and 4).

DISCUSSION

It is widely known that harsh and unusual soils constitute important havens of richness of native species and
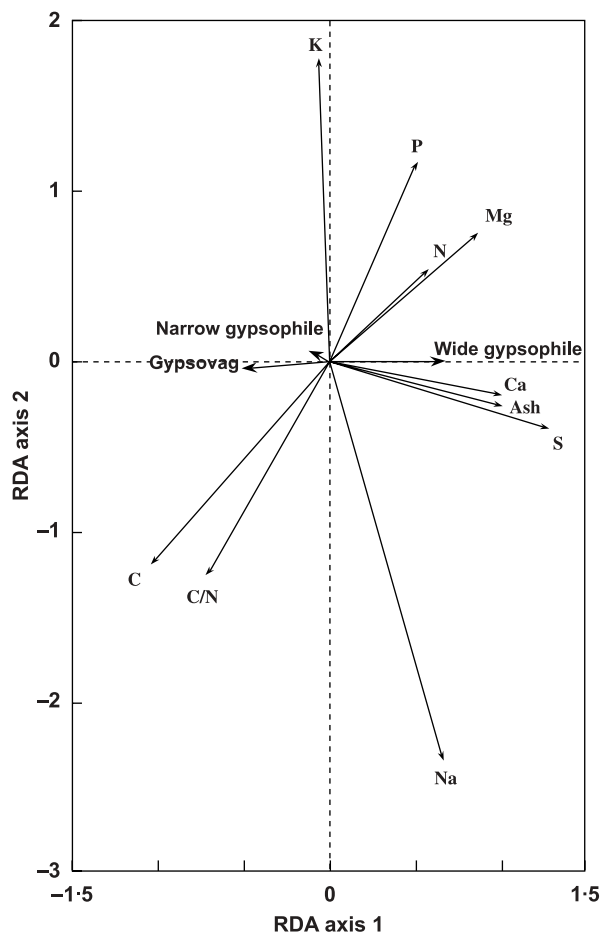
FIG. 2. Biplot for the RDA model analysing the effect of the 'gypsum plant type' separately from the rest of classificatory variables. $P = 0.002$, $F = 64.102$, TVE $= 50.2\%$. See text for further details of calculations.
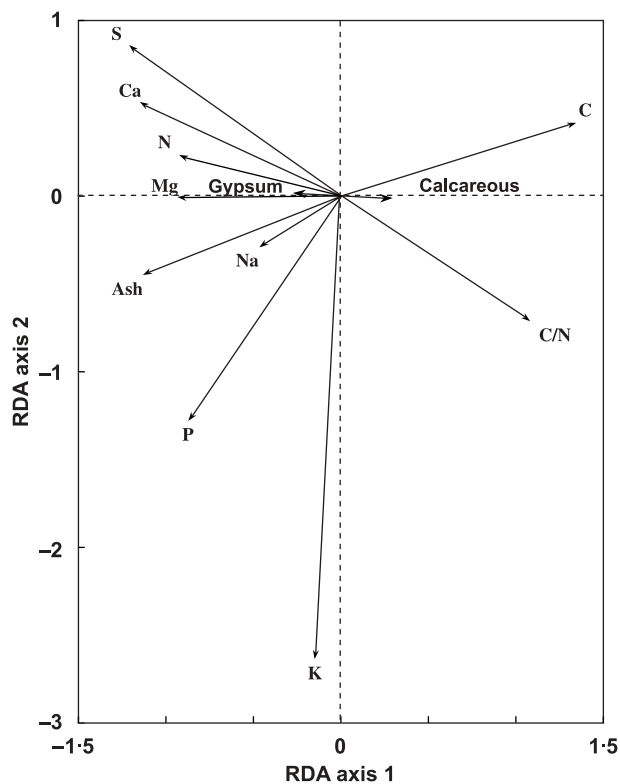

FIG. 3. Biplot for the RDA model analysing the effect of the 'type of soil' separately from the rest of classificatory variables. $P = 0.002$, $F = 7.718$, TVE $= 10.8\%$. See text for further details of calculations.

endemics (Anderson *et al.*, 1999). Despite a great amount of work that has been devoted to serpentine endemics (Kruckeberg, 1992; Harrison *et al.*, 2006*a*, *b*), knowledge about the factors controlling the existence of other edaphic specialists remains significantly lower. In a recent work, we showed that the physical properties of gypsum soils – i.e. the need for seedlings to be able to surpass the hard gypsum surface crust after germination – may explain, at least partially, the different distribution of gypsophiles and gypsovags (Romao and Escudero, 2005). However, widely distributed gypsophiles, such as *Helianthemum squamatum*, showed a better performance after emergence on gypsum than on normal soils, which also suggests that the chemical characteristics of gypsum soils may play an important role in the distribution of these species (Romao and Escudero, 2005). The results reported here help complete our understanding of gypsophily, especially on the chemical adaptation and flexibility of gypsum plants.

Different chemical compositions of regionally dominant gypsophiles and gypsovags

Our results agree with the observations of Duvigneaud and Denaeyer-De Smet (Duvigneaud and Denaeyer-De Smet, 1966, 1973; Duvigneaud, 1968), suggesting that gypsophiles and gypsovags can be statistically separated in terms of the chemical composition of their leaves. According to our results, regionally dominant gypsophiles show greater S, Ca, Mg, N, P and ash concentrations whereas gypsovags display greater C concentrations and C : N ratios. These differences were constant across the different provenances of plant samples. These results suggest that regionally dominant gypsophiles and gypsovags show distinct physiological adaptations to counteract the atypical chemical composition of gypsum soils. Accordingly, regionally dominant gypsophiles seem to selectively accumulate nutrients such as N and P, which are scarce on gypsum soils (Guerrero Campo *et al.*, 1999*b*), whereas gypsovags show normal N and P concentrations. Indeed, some regionally dominant gypsophiles such as *Lepidium subulatum* showed a N concentration ($50-52$ mg g^{-1}) up to 3-fold that of other Mediterranean woody species such as *Olea europaea* ($15-25$ mg g^{-1}), while the leaf N concentrations of gypsovags were within the ranges reported for fruit cultivars and forest trees (Jones *et al.*, 1991). In addition, regionally dominant gypsophiles seem to show a greater tolerance for the accumulation of Mg and S – elements found in excess in gypsum soils that can reduce the growth of non-gypsum species (Verheye and Boyadgiev, 1997). For example, the concentrations of S (49 mg g^{-1}) and Mg ($24-13$ mg g^{-1}) in the regionally

TABLE 3. *Gaussian models for the two gypsovags occurring in gypsum and calcareous soils at both sites. The* P-*value of the corresponding analysis of deviance and the mean value of each variable in each class are indicated*

Variable	Salvia lavandulifolia			Rosmarinus officinalis		
	Gy/Ca[a]	Site[b]	Interaction term[c]	Gy/Ca[a]	Site[b]	Interaction term[c]
Ash	0·037 Ca = 6·36; Gy = 7·23	ns	ns	0·007 Ca = 5·43; Gy = 6·04	ns	ns
Ca	0·001 Ca = 1·50; Gy = 1·94	ns	ns	0·019 Ca = 1·20; Gy = 1·43	ns	0·020 Z-Ca = 1·17; Z-Gy = 1·64; M-Ca = 1·22; M-Gy = 1·22
C	0·001 Ca = 56·37; Gy = 54·94	0·001 Z = 54·7; M = 56·6	ns	0·032 Ca = 56·35; Gy = 55·96	ns	0·011 Z-Ca = 56·21; Z-Gy = 56·30; M-Ca = 56·49; M-Gy = 55·62
C/N	0·0001 Ca = 37·53; Gy = 31·31	ns	ns	ns	ns	ns
Mg	0·003 Ca = 3·03; Gy = 3·28	0·003 Z = 2·34; M = 3·97	0·003 Z-Ca = 3·04; Z-Gy = 1·64; M-Ca = 3·01; M-Gy= 4·92	0·001 Ca = 1·69; Gy = 2·77	ns	ns
N	0·0001 Ca = 15·08; Gy = 17·64	ns	ns	ns	ns	ns
Na	ns	0·0001 Z = 0·52; M = 0·35	ns	ns	ns	ns
P	0·006 Ca = 0·85; Gy = 1·03	0·003 Z = 1·03; M = 0·85	0·003 Z-Ca = 0·85; Z-Gy = 1·21; M-Ca = 0·85; M-Gy = 0·84	0·004 Ca = 0·85; Gy = 0·68	ns	ns
K	ns	0·005 Z = 6·80; M = 4·22	ns	0·017 Ca = 10·04; Gy = 8·05	ns	ns
S	0·001 Ca = 0·05; Gy = 0·11	ns	ns	ns	ns	ns

[a]Ca = calcareous soil, Gy = gypsum soil.
[b]M = Madrid (central Spain, Middle Tajo Basin); Z = Zaragoza (north-east Spain, Middle Ebro Basin).
[c]Z-Ca = calcareous soils in Zaragoza; Z-Gy = gypsum soils in Zaragoza; Ca-M = calcareous soils in Madrid; Gy-M = gypsum soils in Madrid.
ns indicates that predictors do not explain a significant fraction of deviance. See text for details on statistics. $n = 5$.

dominant gypsophile *Ononis tridentata* were up to 10-fold those of common fruit trees and shrubs such as *Malus* sp. (2–4 mg g^{-1}) or *Vaccinium corymbosum* (2–4 mg g^{-1}; Jones *et al.*, 1991). Similarly, previous studies have reported high S (Ruiz *et al.*, 2003) and N (Alvarado *et al.*, 2000) concentrations in regionally dominant gypsophiles from the Iberian Peninsula such as *L. subulatum*, *Gypsophila struthium* subsp. *hispanica* and *H. squamatum*, although, in accordance with our results, *H. squamatum* showed relatively lower concentrations of N. Nevertheless, in an analysis of the chemical composition of gypsum plants in Tunisia, Boukhris and Lossaint (1970, 1975) found that although most gypsophiles showed higher concentrations of sulfur than gypsovags, some gypsophiles had sulfur concentrations similar to those of non-gypsum plants. One possible explanation for these results might be the existence of contrasting metabolic strategies within gypsophiles, as suggested by the authors, or the inclusion of narrow endemics in the set of gypsophile species analysed (see below).

The physiological differences between gypsophiles and gypsovags might entail different ecological strategies in both groups of species. In particular, regionally dominant gypsophiles seem to fit the 'specialist' model, being species specifically adapted to gypsum substrates that accumulate in their leaves those elements found in excess in gypsum soils, while gypsovags seem to be stress-tolerant species fitting the 'refuge' model. Indeed, the greater C : N ratio of gypsovags seems to be indicative of their stress-tolerant nature as compared to the more competitive regionally dominant gypsophiles (Grime, 2001).

Our results demonstrate that gypsovags exhibit different strategies to cope with the atypical chemical composition of gypsum soils. Among the gypsovags studied, *T. capitatum* followed an exclusion strategy, avoiding the accumulation of toxic elements in its leaves and hence displaying similar leaf chemical compositions in gypsum and non-gypsum soils (Duvigneaud and Denaeyer-De Smet, 1973). On contrast, *S. lavandulifolia* and *L. suffruticosum* showed a certain tolerance to the accumulation of toxic elements such as Mg or S in their leaves, whereas *R. officinalis* followed a mixed strategy, accumulating some toxic elements (Mg) and excluding others (S). These results are in agreement with previous observations by Duvigneau and Denaeyer-de Smet on similar gypsum

TABLE 4. *Gaussian models for the gypsovags collected from both gypsum and calcareous soils exclusively in Madrid. The* P-*value of the corresponding analysis of deviance and the mean value of each variable in each class are indicated*

Variable	*Teucrium polium* subsp. *capitatum*	*Linum suffruticosum*[a]
Ash	ns	ns
Ca	ns	ns
C	ns	ns
C/N	ns	0·001
		Ca = 17·84; Gy = 16·39
Mg	ns	0·001
		Ca = 2·71; Gy = 2·63
N	ns	0·0004
		Ca = 24·43; Gy = 27·9
Na	ns	ns
P	ns	0·004
		Ca = 1·44; Gy = 1·66
K	ns	ns
S	ns	0·0032
		Ca = 0·06; Gy = 0·07

[a] Ca = calcareous soils; Gy = Gypsum soils.

ns indicates that predictors do not explain a significant fraction of deviance. See text for details on statistics. $n = 5$.

outcrops from north-east and central Spain (Duvigneaud and Denaeyer-De Smet, 1966, 1973; Duvigneaud, 1968).

Chemical composition of dominant gypsophiles, narrow-gypsophile endemics and gypsovags

According to the results, the narrow-gypsophile endemics studied here seem to follow a rather similar physiological strategy to gypsovags, showing a poor ability to counteract the low availability of N and P and the high S, Ca and Mg concentrations of gypsum soils. Therefore, it is suggested that these species also fit the 'refuge' model. Their restriction to gypsum soils could then be related to their low competitive ability linked to their nature as stress-tolerant plants, and not to their special adaptation to gypsum soils. According to this hypothesis, harsh and unusual soils might serve as havens of rare, low-competitive and highly stress-tolerant plants, sheltering them from competition with trees, shrubs and grass species, which perform better in more productive soils (Gankin and Major, 1964). Further studies assessing the competitive ability and stress tolerance of this type of gypsum species are needed to verify such a hypothesis.

Iberian forests reached a maximum expansion in the climatic optimum of the Atlantic period, dated approx. 8000–5000 BP (years before present; Carrión and Dupré, 1996). During this period, gypsum outcrops could have served as important refuges for stress-tolerant species of open areas, providing places away from the competition of trees and tall shrubs. The adverse physical and chemical features of gypsum outcrops could have impeded the development of forest communities in these areas (Guerrero Campo *et al.*, 1999*a*, *b*). The distribution range and regional abundance of narrow-gypsophile endemics could have been further affected by a human presence

since the Neolitic period, which had an extreme impact on the vegetation of the Iberian Peninsula (Burjachs *et al.*, 1997). Stress-tolerant species have low growth and organ turnover rates, which makes them highly vulnerable to severe disturbances (Grime, 2001). Human impact has been particularly intense in the Ebro Depression since the establishment of Ibero-Roman populations (2500 BP; Peña *et al.*, 2001), which might have accounted for the much lower occurrence of narrow-gypsophile endemics in this area as compared with central Spain.

The results of this study seem to indicate the different ecological strategies of gypsum species, and hence might have important implications for their conservation and management. If narrow-gypsophile endemics are stress-tolerant refugee species on gypsum soils, their conservation could be favoured by the reduction of disturbances such as ploughing, off-road traffic, overgrazing and trampling (Nelson and Harper, 1991; Guerra *et al.*, 1995), and by avoiding the re-afforestation and the expansion of forest and dense shrub communities into these areas. In contrast, regionally dominant gypsophiles are competitive on gypsum soils and some species, such as *L. subulatum* and *H. squamatum*, even show a great ability to survive disturbances (Braun-Blanquet and Bolòs, 1957; Mota *et al.*, 2003).

The different chemical composition of gypsovags and regionally dominant gypsophiles suggests that leaf chemistry is a useful tool to distinguish species specifically adapted to gypsum soils from those that take refuge in gypsum soils. Leaf chemical composition seems to be informative of the specialization of plants to gypsum substrates. However, further information on the physiological mechanisms that cause the distinct chemical composition of regionally dominant gypsophiles may be crucial for the understanding of their adaptation to gypsum soils. In addition, more information on the stress tolerance and competitive ability of the different types of gypsum plants would be required to test the hypotheses proposed in this study. In this regard, basic experiments with different types of soils and/or competition levels, and the quantification of some key functional traits of gypsum plants such as maximum relative growth rate, specific leaf area or leaf N concentration (Reich *et al.*, 1992; Wilson *et al.*, 1999), would be particularly useful.

CONCLUSIONS

Gypsum plants show differences in their leaf chemical composition that are suggestive of their different ecological strategies. Regionally dominant gypsophiles seem to be specifically adapted to gypsum soils whereas gypsovags seem to be stress-tolerant species that follow diverse strategies to counteract the chemical constraints of gypsum soils. The restriction of narrow-gypsophile endemics to gypsum soils could be due to their nature as stress-tolerant plants and not to their special adaptation to gypsum soils. The procedure used here seems to be a useful tool to identify plants' specialization to gypsum soils, provided comparable organs in similar phenological and developmental stages are considered.

ACKNOWLEDGEMENTS

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APPENDIX

Chemical composition of study species. Species in bold are gypsovags. Mean values (and s.d.) are given

Species	Soil[a]	Site[b]	Ca (%)	C (%)	C/N	Mg (mg g^{-1})	N (mg g^{-1})	Na (mg g^{-1})	P (mg g^{-1})	K (mg g^{-1})	S (%)	Ash (%)
Centaurea hyssopifolia	G (n = 4)	M	2·9 (0·7)	44·7 (0·9)	11·1 (0·7)	4·9 (1·3)	40·2 (2·3)	0·6 (0·03)	2·7 (0·3)	23·3 (1·5)	0·8 (0·2)	14·47 (1·29)
Gypsophila struthium subsp. *hispanica*	G (n = 5)	Z	7·4 (0·5)	37·0 (0·7)	14·9 (0·79)	12·1 (2·3)	24·9 (1·3)	0·6 (0·04)	1·9 (0·2)	11·8 (1·6)	3·0 (0·5)	26·67 (1·50)
Helianthemum marifolium subsp. *conquense*	G (n = 7)	M	1·9 (0·1)	49·1 (0·3)	29·4 (2·4)	2·6 (0·7)	16·8 (1·5)	0·3 (0·2)	1·1 (0·2)	3·9 (0·5)	0·1 (0·03)	6·49 (0·24)
Helianthemum squamatum	G (n = 7)	Z	3·4 (0·3)	43·7 (0·4)	26·7 (4·7)	6·1 (1·8)	16·8 (2·7)	0·8 (0·2)	1·2 (0·3)	6·4 (1·6)	3·0 (0·2)	14·1 (0·9)
	G (n = 5)	M	3·5 (0·5)	43·4 (0·9)	27·8 (2·9)	7·6 (2·1)	15·7 (1·7)	0·6 (0·1)	1·1 (0·3)	5·6 (0·7)	2·6 (0·2)	15·0 (0·9)
Herniaria fruticosa	G (n = 2)	Z	2·9 (0·1)	47·4 (0·3)	18·8 (1·4)	7·7 (0·6)	25·3 (1·7)	0·5 (0·04)	1·1 (0·1)	9·2 (1·2)	1·1 (0·1)	12·2 (0·04)
Lepidium subulatum	G (n = 5)	Z	2·7 (0·2)	44·9 (0·1)	9·0 (0·8)	2·0 (1·3)	50·0 (4·6)	0·6 (0·1)	2·5 (0·1)	13·2 (2·2)	2·7 (1·1)	12·8 (0·5)
	G (n = 4)	M	2·7 (0·3)	45·4 (0·6)	8·7 (0·4)	5·5 (1·1)	52·3 (3·0)	0·5 (0·03)	2·5 (0·2)	6·3 (0·5)	1·9 (0·1)	12·2 (0·9)
Linum suffruticosum	G (n = 5)	M	2·4 (0·3)	44·2 (0·5)	13·5 (0·5)	2·2 (0·5)	32·9 (1·5)	0·6 (0·1)	1·9 (0·1)	11·7 (2·7)	0·02 (0·01)	12·3 (0·9)
	G (n = 5)	Z	2·9 (0·4)	44·2 (0·9)	19·3 (1·8)	2·7 (0·8)	23·1 (2·2)	0·6 (0·1)	1·5 (0·3)	6·6 (2·5)	0·1 (0·04)	14·4 (1·4)
	C (n = 5)	M	2·7 (0·3)	43·1 (1·2)	17·8 (2·0)	4·0 (0·7)	24·4 (2·9)	0·6 (0·1)	1·4 (0·2)	8·5 (2·7)	0·1 (0·01)	14·8 (2·3)
Ononis tridentata	G (n = 5)	Z	5·0 (0·8)	35·6 (0·9)	14·7 (0·9)	23·8 (3·4)	24·3 (1·9)	0·7 (0·1)	1·0 (0·1)	3·1 (0·9)	4·9 (0·4)	23·5 (0·7)
	G (n = 4)	M	6·5 (0·6)	35·8 (1·9)	15·0 (1·4)	12·9 (3·5)	24·1 (3·4)	1·0 (0·3)	1·3 (0·2)	2·5 (0·7)	4·1 (0·5)	24·7 (2·0)
Rosmarinus officinalis	C (n = 5)	Z	1·2 (0·2)	56·2 (0·1)	43·3 (7·1)	1·3 (0·4)	13·3 (2·3)	0·6 (0·04)	0·9 (0·1)	10·3 (2·2)	0·1 (0·02)	5·4 (0·4)
	C (n = 5)	M	1·2 (0·1)	56·5 (0·4)	56·5 (5·7)	2·1 (0·4)	10·1 (1·1)	0·5 (0·04)	0·8 (0·2)	9·8 (1·3)	0·1 (0·01)	5·4 (0·2)
	G (n = 5)	Z	1·6 (0·2)	56·3 (0·5)	45·6 (2·0)	2·2 (0·5)	12·4 (0·5)	0·6 (0·1)	0·7 (0·1)	8·5 (1·0)	0·1 (0·04)	6·5 (0·5)
	G (n = 5)	M	1·2 (0·3)	55·6 (0·5)	60·1 (5·8)	3·3 (0·5)	9·3 (0·1)	0·5 (0·1)	0·7 (0·1)	7·6 (2·0)	0·1 (0·1)	5·6 (0·6)
Salvia lavandulifolia	C (n = 5)	Z	1·3 (0·3)	55·6 (0·6)	37·1 (3·1)	3·0 (1·4)	15·1 (1·2)	0·5 (0·03)	0·9 (0·1)	6·7 (2·5)	0·1 (0·01)	6·3 (1·0)
	C (n = 5)	M	1·7 (0·2)	57·2 (1·4)	38·0 (2·1)	3·0 (0·4)	15·1 (0·8)	0·4 (0·1)	0·9 (0·2)	5·0 (0·8)	0·03 (0·01)	6·4 (0·9)
	G + C (n = 5)	Z	1·5 (0·1)	55·7 (0·4)	37·0 (3·8)	1·9 (0·6)	15·3 (1·5)	0·5 (0·1)	0·8 (0·1)	5·0 (1·4)	0·05 (0·01)	5·7 (0·4)
	G (n = 5)	Z	2·0 (0·3)	53·8 (0·6)	29·8 (2·2)	1·6 (1·2)	18·2 (1·3)	0·5 (0·02)	1·2 (0·1)	6·9 (2·0)	0·1 (0·02)	7·3 (0·6)
	G (n = 5)	M	1·9 (0·3)	56·0 (0·6)	32·9 (2·2)	4·9 (1·1)	17·1 (1·4)	0·4 (0·1)	0·8 (0·1)	3·4 (1·2)	0·1 (0·06)	7·1 (1·0)
Teucrium polium subsp. ***capitatum***	C (n = 4)	M	1·8 (0·2)	51·3 (1·0)	22·4 (1·4)	2·6 (1·2)	23·0 (1·3)	0·5 (0·1)	1·5 (0·1)	6·3 (1·6)	0·05 (0·01)	7·3 (1·3)
	G (n = 4)	M	1·9 (0·4)	52·1 (0·8)	19·9 (2·3)	2·4 (0·9)	26·5 (3·2)	0·45 (0·1)	1·3 (0·1)	7·2 (1·9)	0·06 (0·02)	7·2 (0·8)
Teucrium pumilum	G (n = 6)	M	2·0 (0·1)	52·3 (0·4)	31·8 (4·4)	6·1 (1·7)	16·7 (2·3)	0·5 (0·03)	0·7 (0·3)	7·6 (1·4)	0·6 (0·2)	8·1 (0·4)
Thymus lacaitae	G (n = 5)	M	1·6 (0·2)	51·7 (0·9)	37·4 (6·7)	4·0 (0·6)	14·2 (2·8)	0·5 (0·1)	1·1 (0·1)	5·6 (1·0)	0·04 (0·02)	8·6 (1·0)

[a] G = gypsum soils; C = calcareous soils; G + C = mixed limestone and gypsum soils.
[b] M = Madrid (Central Spain, Middle Tajo Basin); Z = Zaragoza (north-east Spain, Middle Ebro Basin).

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