UNIVERSITAT DE BARCELONA

The ecological significance of nutritional strategies in gypsum plant communities

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TESI DOCTORAL

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The ecological significance of nutritional strategies in gypsum plant communities

El significat ecològic de les estratègies nutricionals a les comunitats de plantes gipsícoles

Memòria presentada per Andreu Cera Rull per optar al grau de doctor per la Universitat de Barcelona.

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To Venus, mother of the race of Aeneas, delight of men and of gods:

It does not escape my mind that it is difficult to set forth clearly in Latin verse the obscure discoveries of the Greeks, especially since in discussing many of these I must make use of strange terms because of the poverty of our language and the novelty of my subject. Yet your worth and the hoped-for pleasure of sweet friendship persuade me to endure any hardship, and encourage me to spend the quiet nights without sleep as I seek the words and the poetry with which, at length, I may be able to spread before your mind clear lights by which you may see deeply into hidden matters.

(Book One on *Dē rērum natūra*, Lucretius, Translated by Russel M.Geer)

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Abstract

Gypsophile species are edaphic endemics of gypsum soils, and they are considered specialists of this stressful substrate. Gypsum endemics from different families and regions of the world tend to show a unique leaf elemental composition, similar to the chemical characteristics of gypsum soils. However, the ecological significance of their unique foliar composition remains unknown. The factors underlying the ecological amplitude of gypsophiles remain also poorly studied. The main literature is based on the distribution of gypsophiles linked to gypsum soils in drylands, although some studies suggest a broader physiological amplitude depending on soil type, and a positive influence of disturbance. Therefore, I have assumed that gypsophiles have evolved in disturbed drylands with gypsum soils. In order to adapt to this combination of factors, I hypothesised that gypsophiles have become soil specialists with high capacity of nutrient uptake to be more competitive than other species in gypsum soils. To test this, we conducted a germination trial and a common garden experiment to analyse the ecological restriction of gypsophiles to different substrates, and to analyse the effect of different substrates on the whole-plant elemental composition of plants with contrasting affinity for gypsum soils. In the field, we studied the assemblage of plant communities under different grazing intensities on high gypsum soils, and whether the assembly of plant communities is mediated by any trait related to gypsum specialisation or herbivory resistance. Next, a browsing simulation was conducted to assess individual plant responses in calcic and gypsum pots. In addition, the variation of foliar and rhizospheric soil nutrient contents, and AM fungal colonisation were analysed throughout a year in the field to study the nutrient acquisition strategies of gypsophiles. The results obtained in this PhD thesis show that the fundamental niche of gypsophiles is not only explained by edaphic factors unique to gypsum soils, but seems to be related to alkaline soils with high calcium availability. When analysed under herbivory pressure, species with high gypsum affinity and increased foliar S content (i.e. gypsophiles) were more likely to assemble than other species. These gypsophiles were foliar accumulators of gypsum excess elements, even in calcic pots. They also seem to be adapted to P-scarcity by being less dependent on AMF symbiosis, and adjusting their acquisition strategies to nutrient pulses. Therefore, it seems that gypsophiles are specialists of gypsum soils to be more competitive in disturbed drylands through a unique nutritional strategy.

Sinopsi

Les plantes gipsòfiles són endemismes edàfics del guixos, i són considerades especialistes d'aquest sòl estressant. Endemismes del guix de diferents famílies i regions del món tendeixen a mostrar una composició elemental foliar única, similar a les característiques químiques dels sòls guixencs. No obstant això, el significat ecològic de la seva composició foliar continua sent desconegut. Els factors que subjuguen l'amplitud ecològica de les gipsòfiles segueixen sent també poc estudiats. La majoria de la literatura es basa en una distribució lligada als sòls guixencs de les zones àrides, encara que alguns estudis suggereixen una amplitud fisiològica més àmplia segons el tipus de sòl, i una influència positiva de les pertorbacions. Per això, he assumit que les gipsòfiles han evolucionat en terres seques pertorbades amb sòls guixencs. Per adaptar-se a aquesta combinació de factors, he plantejat la hipòtesi que s'han convertit en especialistes edàfics amb alta capacitat d'absorció de nutrients per a ser més competitives que altres espècies en sòls de guix. Per comprovar-ho, vam dur a terme un experiment de germinació i un de cultiu per a analitzar la seva restricció ecològica en funció del tipus de sòl, i per analitzar la composició elemental de tota la planta. En el camp, vam estudiar l'assemblatge de les comunitats vegetals en sòls guixencs en diferents intensitats de pasturatge, i si l'assemblatge d'aquestes comunitats està mediat per algun tret relacionat amb l'especialització pels guixos o la resistència cap als herbívors. A continuació, vam realitzar una simulació de brosteig per a avaluar la resposta individual de les plantes en tests amb guix o sòl calcari. A més, vam analitzar durant un any la variació del contingut nutricional de les fulles, arrels i sòl rizosfèric, i la colonització dels fongs micorízics arbusculars, per estudiar les estratègies d'adquisició de les gipsòfiles en el camp. En conjunt, els resultats obtinguts en aquesta tesi doctoral demostren que el nínxol fonamental de les gipsòfiles no sols s'explica per factors singulars dels sòl guixencs, sinó més aviat per sòls alcalins amb alt contingut de calci. I quan afegim la pressió herbívora, les espècies amb alta afinitat pel guix i alt contingut de sofre foliar (és a dir, gipsòfiles) tenen més probabilitat d'assemblar-se que altres espècies. Hem comprovat també que aquestes gipsòfiles són acumuladores foliars dels elements en excés dels guixos, fins i tot quan creixen en tests amb sòl calcari. I que les gipsòfiles semblen estar adaptades a l'escassetat de fòsfor sent menys dependents de la simbiosi amb AMF, i probablement ajustant les estratègies d'adquisició als polsos nutricionals del sòl. Per tant,

sembla que les gipsòfiles s'han convertit en especialistes dels sòls guixencs per a ser més competitives en terres seques pertorbades amb sòls guixencs a través d'una estratègia nutricional singular.

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Gypsum plant communities in the Middle Ebro Basin. By Estephania Duplat

General introduction

Foreword

The restricted distribution of certain plants to unusual substrates has captivated many botanists and ecologists in the last centuries. One of such singular substrates are gypsum soils, which are distributed worldwide in drylands. Gypsum soils limit plant life due to their singular features. However, they harbour a singular flora with many edaphic endemics. These gypsum endemics are referred to in the literature as gypsophiles. Gypsophiles from different families and regions of the world share a unique leaf elemental composition, with high foliar S, Ca, and Mg and low foliar K, similar to the chemical composition of gypsum soils. Several questions about plant adaptation to gypsum environments remain unanswered. It is unknown what factors underlie the ecological restriction of gypsophiles to gypsum soils and their exclusion from other habitats. Gypsophiles, as gypsum-specialist species, might be more competitive (showing better performance and growth) under the harsh conditions of gypsum environments than other species with broader ecological distribution that also grow on gypsum, however this possibility has not been tested before. It is also not known up to what point the singular chemical composition of gypsophiles reflects a nutritional strategy, ecologically advantageous in this singular habitat. For the above reasons, this PhD thesis will seek to provide further insights into the mechanisms that underlie the adaptation of gypsophiles to gypsum environments.

Plant edaphism in gypsum soils

Unusual soils, edaphic-endemics and substrate-specific traits

The type of bedrock materials frequently conditions plant performance, distribution and, ultimately, the composition of plant communities (Mota et al., 2017). The weathering of rocks as serpentinites, halites, calcites, gypsum or dolomites generates soils with particular characteristics, frequently showing an atypical chemical composition, such as high concentrations of metals, salts and base cations, with important consequences for plant nutrition and survival (Kazakou et al., 2008; Kinzel, 1989; Mota et al., 2021; Tran et al., 2020). These particular chemical features alter soil pH and produce strong nutrient imbalances, which can lead to limited growth and even to the exclusion of plants (Rorison, 1960a). In addition, unusual soils often show singular physical features that limit plant life (e.g. Guerrero-Campo et al., 2008; Meyer, 1986; Romão and Escudero, 2005). These geologic phenomena linked to plant evolution result in the development of specialised floras associated to singular soils (Braun-Blanquet, 1932). This is the case of the calcicolous or calcifuge flora (Lee, 1998; Rorison, 1960b), but also of other floras associated with unusual soils like saline, serpentine, dolomite or gypsum soils, where edaphic endemics, i.e. plants that only (or almost only) appear on atypical soils (Kruckeberg and Rabinowitz, 1985), are frequently found (Mota et al., 2017). Moreover, in these soils there is usually an adaptive convergence of morphological or physiological characters between plants from different families growing on the same substrate (Mota et al., 2017), which should ultimately help them to optimise their performance and growth in such singular habitats (Cody, 1978).

Plants growing on gypsum soils

Plant communities in gypsum environments are composed by a highly diversified flora, with species distributed across several taxonomic orders and families (Moore et al., 2014), many of them endemics and specialist species (Mota et al., 2011; Musarella et al., 2018; Ochoterena et al., 2020; Pérez-García et al., 2017). The assembly of gypsum plant communities depends greatly on the affinity of species for gypsum soils (Luzuriaga et al., 2020, 2015). The existence of gypsum endemics is a worldwide phenomenon named “gypsophily” by Linnaeus (1753) in his *Species Plantarum*, and also used in other seminal works (Braun-Blanquet and Bolòs, 1958; Duvigneaud and Denaeyer-De Smet,

1966; Parsons, 1976; Rivas-Martínez and Costa, 1970). Nevertheless, the flora of gypsum environments is not only composed by edaphic endemics, but also by species with wide ecological amplitudes (in terms of soil affinity; Meyer 1980).

Many classifications have been proposed in the last decades to group plant species from gypsum soils according to their ecological amplitude or ecological strategies (Mota et al., 2016). A synthetic approximation was proposed by Meyer (1986), who described mainly two types of plants living on gypsum, depending on their affinity to this substrate: 1) gypsovags, species with wide ecological amplitude, which can grow both on and off gypsum soils; and 2) gypsophiles, edaphic endemics restricted to gypsum soils. In addition, two types of gypsophiles have been further described (Palacio et al., 2007): widely distributed gypsophiles (hereafter, wide gypsophiles) considered as gypsum specialists (*sensu* Gankin and Major, 1964), and narrowly distributed gypsophiles, which seem to show a lower degree of specialisation to gypsum and a closer ecological strategy to gypsovags.

The current ecological distribution of wide gypsophiles (hereafter, gypsophiles) is restricted to drylands with gypsum soils. This does not mean that gypsophiles require high concentrations of gypsum to survive, such as some halophytes or metallophytes (Lambers et al., 2008a). Accordingly, gypsophiles can experimentally grow in the absence of gypsum in marls (Ballesteros et al., 2014). However, no further studies have explored the differences in the performance and growth of gypsophiles on and off gypsum, and it is still unknown if they are able to complete their life cycle out of gypsum soils. If gypsophiles are truly gypsum specialist species (*sensu* Gankin and Major, 1964), they should perform and growth better in gypsum environments than other species with wider ecological distribution at some stage of the plant life cycle. For example, seed germination, which is one of the riskiest stages of the plant life cycle (Sánchez et al., 2014), and is largely affected by soil conditions (Baskin and Baskin 2014), would likely be higher in gypsophiles sown on *vs*. off gypsum soils. Experimentally, some gypsophiles germinate better in saturated gypsum solutions than in solutions with low gypsum content (Cañadas et al., 2014; Merlo et al., 1997), indicating a higher ability to germinate in gypsum than other plants. However, the response in natural soils is poorly studied (but see Romão and Escudero, 2005)), and gypsophiles may have high affinity for a particular feature of gypsum soils, in addition to gypsum content. Similarly, the seed germination of halophytes in saline soils depends on salt content (Orlovsky

et al., 2016; Pujol et al., 2000) and the germination of acidophilous and calcicolous plants depends on soil Ca concentrations (Anderson, 1982). Overall, the fundamental niche (or physiological amplitude) of gypsophiles based on soil type seems to be wider than their realised niche (or ecological amplitude) (Lambers et al., 2008a), although more experimental studies with natural soils are lacking. Further, other biotic and abiotic factors, rather than the type of soil, could be modifying the current ecological distribution of gypsophiles, but further studies are required.

Ecological constraints for plant life in gypsum ecosystems

Soil features

Gypsum are special soils with high gypsum (calcium sulphate dihydrate) content (Herrero and Porta, 2000), which frequently occur in drylands around the world (Eswaran and Zi-Tong, 1991), because they are easily weathered in humid climates (Poch et al., 2018). The high content of gypsum (above 40%) modifies the physical and chemical proprieties of soils (Herrero and Porta, 2000). For example, the moderate solubility of gypsum (about 2.4 g L^{-1}) produces very dynamic soil environments, with dissolution-precipitation sequences altering physical properties (Casby-Horton et al., 2015). In addition, it generates abnormally high ionic concentrations of calcium and sulphate in the soil solution, which saturate the cation exchange complex, leading to low nutrient retention and availability (FAO, 1990; Guerrero-Campo et al., 1999). Also, gypsum soils have a hard surface crust and low water holding capacity (Herrero et al., 2009). All these unique features of gypsum soils impose various restrictions on plant life, affecting plant performance and distribution (Escudero et al., 2015; Moore et al., 2014).

The effects of gypsum soils on plants have been explored in recent decades by several authors (i.e. Hernando Fernández et al., 1963; Meyer, 1986; Verheye and Boyadgiev, 1997). Various authors have found reduced seedling establishment and root penetration in gypsum soils (Olarieta et al., 2016; Romão and Escudero, 2005), as increasing gypsum content leads to the replacement of large substrate pores by smaller packing pores (Poch et al., 1998). On the other hand, the high concentration of calcium cation and sulphate in the soil solution produces a decrease in nutrient availability and subsequent plant K, P and Fe uptake (FAO, 1990), and increases the foliar concentration of Ca and S of most plants (Boukhris

and Lossaint, 1975; Palacio et al., 2007; Salmerón-Sánchez et al., 2014). Although the chemical conditions of gypsum soils do not produce osmotic stress in plants (Casby-Horton et al., 2015), they remarkably alter plant nutrition (Hernando Fernández et al., 1963) and ultimately limit plant growth (FAO, 1990). Therefore, gypsum-adapted species have evolved under nutritional stress, and they should have developed mechanisms and strategies to cope with the excess of Ca, S and Mg and low availability of N, K and P in gypsum soils (Casby-Horton et al., 2015; Duvigneaud and Denaeyer-De Smet, 1966).

Disturbance in gypsum environments

Gypsum plant communities are well represented in the Iberian Peninsula, the region in Europe with largest gypsum deposits and more plant species adapted to gypsum soils (Braun-Blanquet and Bolòs, 1958; Pérez-García et al., 2017). In this region, gypsum plant communities have historically been associated with disturbed environments, either by fauna or geomorphological processes (Braun-Blanquet and Bolòs, 1958; Guerrero-Campo et al., 1999). Indeed, they are usually open shrublands (Mota et al., 2017), which are community types associated with the effect of large herbivory and livestock grazing (Asner and Levick, 2012; Bakker et al., 2016), and with the effect of soil erosion (Guerrero-Campo et al., 2008). In the Middle Ebro Basin, where this thesis focusses, the Ass. *Lepidietum subulati* was described at the foot of vertical gypsum rock walls, frequently subjected to geomorphologic disturbances and the occurrence of the Ass. *Helianthemetum squamati* and *Ononidetum tridentatae* seems to be modulated by human disturbances, like fire or grazing (Braun-Blanquet and Bolòs, 1958). These disturbances prevent the accumulation of organic matter beneath the shrubs canopy, which would otherwise lead to the growth of plants associated with *Al. Quercion ilicis* and a decrease of the occurrence of gypsum plant communities in the steppes of the Middle Ebro Basin, especially Ass. *Helianthemetum squamati* (Braun-Blanquet and Bolòs, 1958). Thus, gypsum plant communities may have evolved under the selective forces of wild herbivores and livestock disturbance. Similarly, livestock grazing has been shown to influence performance and assembly of plant endemics in serpentine and saline soils (Beck et al., 2015; Bonis et al., 2005). Despite these evidences, the effect of herbivory on plant communities associated to unusual soils remains poorly understood, particularly on gypsum plant communities, despite extensive grazing practices prevail in most gypsum outcrops

worldwide (Akhani, 2015; Pueyo et al., 2008). Few previous studies analysed whether disturbance by herbivory modulates plant assembly on gypsum plant communities, favouring the assembly of gypsophiles in the most grazed conditions (see more on Pueyo et al. (2008)). Also, if herbivory has shaped plant adaptation to gypsum soils, gypsum-adapted species should have evolved grazing-related traits. Plants have evolved several traits to persist in grazed communities (Díaz et al., 2007), which can be divided into avoidance and tolerance mechanisms (Briske and Richards, 1995). Tolerance includes traits that increase growth after consumption to recover biomass loss. Contrastingly, avoidance mechanisms include traits that reduce plant accessibility and palatability to avoid consumption by herbivores. These traits would improve plant performance and growth under grazing pressure in gypsum environments. However, it is not known up to what point gypsum endemics show grazing tolerance or avoidance mechanisms to cope with grazing disturbance.

Gypsum-specific plant traits

The vegetation of gypsum soils in the Iberian Peninsula must withstand a markedly seasonal climate (Palacio and Montserrat-Martí, 2005), with summer drought (Palacio et al., 2017), and a soil with atypical and harsh physico-chemical characteristics (Escudero et al., 2015). For these reasons, gypsum plant communities are formed by species with predominantly stress-tolerant traits (Hodgson et al., 1994). This strategy of low growth rates due to the harshness of gypsum environments (Grime, 2006) seems to be shared by gypsovags, which are able to survive in gypsum and other stressful environments, and also by gypsophiles. In addition, gypsophiles have been described as gypsum specialists (Palacio et al., 2007), so they should have singular traits linked to the particular features of gypsum soils. Based on this idea, the literature on gypsum plants has mainly addressed whether there are traits to thrive in environments with gypsum soils shared only among gypsophiles from different families and not by gypsovags, which would pave the way to understanding the phenomenon of gypsophily (Alguacil et al., 2009; Alvarado, 1995; Alvarado et al., 2000; Ballesteros et al., 2014; Cañadas et al., 2014; Escudero et al., 1997; Llinares et al., 2015; Merlo et al., 1998, 2019; Meyer, 1986; Mota et al., 2016; Muller et al., 2017; Palacio et al., 2007, 2012, 2014a; Romão and Escudero, 2005; Torrecillas et al., 2014). Several

hypotheses have been proposed to explain the exclusion of certain species from gypsum environments and the restriction of gypsophiles based on abiotic and biotic factors (Meyer, 1980). Physical soil effects may operate to exclude some species, affecting phases of the life cycle as seedling establishment or root penetration (Meyer, 1986; Poch et al., 1998; Romão and Escudero, 2005). However, it remains unknown whether there are specific traits to overcome such physical limitations shared only by gypsophiles, and not by gypsovags (Moore et al., 2014; Mota et al., 2011).

The singular chemical features of gypsum soils can also exclude some species, as the presence of gypsum in excess can be toxic for plants (Reich et al., 2018; Ruiz et al., 2003). Further, the depletion of certain nutrients may alter plant nutrition, limiting plant growth and excluding some species (Meyer, 1980). Thus, plants need nutritional traits to cope with the strong ionic imbalance of gypsum soils (Merlo et al., 1998). Accordingly, wide gypsophiles, from different families and regions of the world, tend to have a unique leaf elemental composition, with high foliar S and Ca, low K and, sometimes, high foliar Mg (Alvarado, 1995; Duvigneaud and Denaeyer-De Smet, 1966; Merlo et al., 2019; Muller et al., 2017; Palacio et al., 2007). This unique foliar composition might be a result of a singular nutritional strategy of gypsophiles to grow on gypsum environments. However, it has never been tested whether gypsophiles are able to maintain foliar S, Ca and Mg accumulation out gypsum. Preserving the same leaf chemical composition off gypsum would imply that such foliar composition is independent of the substrate, which would be indicative of a metabolic specialisation of gypsophiles. Moreover, the ecological significance of S, Ca and Mg accumulation in gypsophiles remains unknown (Palacio et al., 2007). This unique leaf composition could be a reflection of an adaptation to the harsh soil conditions, but it could also be a result of another limiting factor of gypsum environments, such as herbivore disturbance. Ultimately, this trait shared by gypsophiles exemplifies the importance of studying mineral nutrition in gypsum plant communities in order to deepen our understanding of plant adaptation to unusual soils, where disturbance might have also played a significant role in species evolution.

Elemental composition of gypsum plants

Elemental composition summarises plant functioning

The elemental composition is used to understand plant adaptation to a given habitat (Peñuelas et al., 2019). Plant elemental composition has been analysed almost exclusively in leaves, and few studies have addressed concentration in roots or stems (Zhao et al., 2016). However, plants differ in nutrient concentration in their above- and below-ground organs (Lambers et al., 2008b), as the different role of organs in nutrient cycling define their elemental concentration (Zhao et al. 2016). Also, species differ in nutritional strategies (sets of traits) that affect nutrient cycling by plants, including nutrient acquisition, use, storage, remobilisation and recycling (Aerts and Chapin, 1999; Chapin, 1980; Millard and Grelet, 2010). Thus, studying the whole-plant elemental concentration can summarise how the different organs of plants interact nutritionally and how this relates to the environmental conditions where the plant grows (Marschner et al., 1996; Neugebauer et al., 2020; Zhao et al., 2016), and can provide useful information to identify the nutritional status and strategies of plants (Hogan et al., 2021).

Abiotic and biotic factors influence plant elemental composition

Several abiotic factors influence the elemental composition of plants. Mean annual precipitation and temperature affect plant elemental concentration (Jordi Sardans et al., 2016; Zhang et al., 2012). Similarly, soil composition strongly determines the elemental concentrations of plants (Lambers et al., 2008b). Nutrient availability affects plants growth (Chapin, 1980), and plants must couple to soil composition through nutritional strategies to thrive in their habitat (Aerts and Chapin, 1999), facing different nutrient conditions that range from nutrient excess to scarcity.

Some unusual soils have an excess of certain elements that surpasses plant requirements and may produce toxicity (Kabata-Pendias, 2010; Lambers et al., 2008b). Plants may cope with such excess elements by blocking their uptake at the root level or by tolerating them in high concentrations in leaves (Tran et al., 2020). Examples of unusual soils with excess elements are: calcicolous soils with Ca, serpentine with heavy metals or saline soils with Na and Cl (Kazakou et al., 2008; Moore et al., 2014; Munns and Tester, 2008; Rorison, 1960b). The whole-plant elemental composition may help to identify the nutritional mechanisms of plants to cope with specific excess elements in soil (Hogan et al., 2021).

For example, in unusual soils with high heavy metal availability, some grasses accumulate metals in roots at higher levels than in the surrounding soils (Almeida et al., 2011), while other grasses are metal hyper-accumulators in aboveground organs (Cambrollé et al., 2011). Similar responses have been suggested in other unusual soils. Halophytes show high foliar Na in saline soils (Matinzadeh et al., 2019), whereas other co-occurring species in saline environments must concentrate Na in roots to avoid toxicity to aboveground organs and maintain growth (Munns and Tester, 2008). In the case of gypsum soils, where S and Ca are found in excess (Casby-Horton et al., 2015), gypsophiles are accumulators of S, Ca and Mg at the leaf level, whereas gypsovags show lower concentrations in leaves (Duvigneaud & Denaeyer-De Smet 1968; Palacio et al., 2007; Merlo *et al.* 2019). It could be hypothesized that gypsovags may have elemental barriers at the root level to avoid translocation of excess S, Ca and Mg to leaves, but the concentrations of these elements in the different plant organs of gypsum plants remain unknown.

In soils with low nutrient availability, plants show traits to enhance nutrient acquisition, to improve nutrient retention (for example with slow tissue turnover rates), or to avoid biomass loss (such as the production of secondary compounds to deter herbivores; Aerts & Chapin 1999). Plants also differ in the way they promote nutrient acquisition, as they may: adjust their acquisition strategy to seasonal resource availability, show specialised root structures or functions, change rhizospheric conditions by the release of exudates, or use symbiosis with microorganisms (Richardson et al., 2009). In the case of perennial gypsum plants growing in Mediterranean drylands, their phenology is characterized by predominant shoot growth in spring, root growth mainly in autumn and flowering in spring and early summer (Orshan, 1989; Palacio and Montserrat-Martí, 2007). Shoot growth requires high N and P in leaves (Palacio et al., 2014), while flowering demands high P (Milla et al., 2005). However, the soil availability of P and N in Mediterranean drylands is higher in late summer (Magid and Nielsen, 1992) and autumn (Delgado-Baquerizo et al., 2011), respectively. Consequently, peak in plant demand for N and P may be decoupled from soil availability in these ecosystems. Unfortunately, seasonal studies linking nutrient acquisition strategies, plant nutrient status and soil nutrient availability in gypsum environments are very scarce (but see Palacio et al. 2014b).

An example of nutrient scarcity on gypsum environments is the low availability of P (Alvarado, 1995). Plants have several strategies to improve P nutrition in poor environments (Richardson et al., 2009) such as gypsum soils, but they are usually associated with arbuscular mycorrhizal fungi (AMF) (Johnson, 2010). However, an acquisition strategy based on AMF symbiosis is more carbon costy than others (Johnson, 2010), and usually a decrease in AMF colonisation would occur when P supply by roots is sufficient (Lambers et al., 2018). In the case of gypsum plants, gypsophiles show lower AMF colonisation and diversity within roots than gypsovags (Palacio et al., 2012; Torrecillas et al., 2014). According to these previous studies, gypsophiles seem to be more specialised to gypsum soils and to its P cycle and seasonal availability than gypsovags, likely adapting their growth to P availability. Conversely, the dependence of gypsovags on AMF symbiosis would indicate a generalist stress-tolerant strategy to cope with the limiting conditions in poor-nutrient environments (Palacio et al., 2012). Previous studies on AMF colonisation in gypsum environments were usually conducted in spring. However, root colonisation by AMF is seasonal, as is related to plant activity (Jakobsen et al., 2003) and soil nutrient availability (Hoeksema et al., 2010), which also varied seasonally. Thus, a seasonal study of AMF colonisation, and its relation to soil nutrient content and plant demands, would help to better identify the mechanisms of plants to cope with low and seasonal P availability in gypsum soils.

Biotic factors, and how herbivory influences plant elemental composition

Biotic factors such as plant-plant interactions or herbivory also influence plant elemental concentrations (Sardans et al., 2016). Plant facilitative interactions have been described in gypsum plant communities (Foronda et al., 2019). For example, it has been demonstrated that N is transferred from nurse to sink plants co-occurring in gypsum soils, allowing the coexistence of species with different elemental composition (Montesinos-Navarro et al., 2017), on soils where N is a scarce nutrient (FAO, 1990). Contrastingly, no previous studies have evaluated the effects of herbivory on the elemental composition of plants from gypsum environments. Gypsum-adapted species should have evolved grazing-related traits either for tolerance (such as a faster recovery of aboveground biomass), avoidance, or both. These traits would improve plant performance and growth under grazing pressure in gypsum environments. Edaphic-endemics are usually considered as stress-tolerant species with slow growth

rates (Rajakaruna, 2004), because they live on stressful habitats. Thus, they are assumed to have a high investment in grazing avoidance mechanisms (Rajakaruna, 2004). For example it has been suggested that the accumulation of gypsum and calcium oxalate crystals in the leaves of gypsophiles could play an anti-herbivore role (Palacio et al., 2014a). Increased foliar S accumulation has been related to herbivore-deterrent compounds in *Brassicales*, mainly in relation to the formation of glucosinolates (Tuominem et al., 2019). Also some species of *Acacia* have high concentration of Ca, Mg and S in leaves accumulated in the form of crystals (He et al., 2015), which may also play an anti-herbivore role (He et al., 2014). It is, however, unknown if this likely avoidance character (S-accumulation) is always present in gypsum plants (as a constitutive trait) or if it is produced in response to grazing (i.e. inductive trait) (Moreira et al., 2014). Herbivores could also play a major role in determining the elemental composition and adaptation of gypsum plants, but gypsum plant responses to herbivory have been poorly studied both at the individual and the population level.

Hypotheses

Several studies have proposed that wide-gypsophiles from Iberian Peninsula are gypsum endemic species that have evolved in drylands with singular soil limiting characteristics (Moore *et al.* 2014; Escudero *et al.* 2015), such as scarcity and seasonal availability of nitrogen and phosphorus, and excess of sulphur and calcium. Based on this assumption, the realised niche of gypsophiles may be explained solely by soil chemical features. However, some studies have suggested that disturbance like grazing also played a relevant role in the evolution of Iberian gypsum plants (Braun-Blanquet and Bolòs, 1958). Thus, I assumed that gypsophiles have evolved under edaphic constraints and grazing disturbance in drylands (hence in arid or semiarid conditions). In order to adapt to this combination of factors, I hypothesised that gypsophiles have become soil specialists, possessing a nutritional strategy linked to the features of gypsum soils. This unique nutritional strategy would consist on becoming permeable to gypsum soils, i.e. having more nutrient absorption capacity. Consequently, they may accumulate the excess elements of gypsum in leaves, and also they may adapt their nutrient uptake to the low and seasonal nutrient availability of gypsum soils. Contrastingly gypsovags, which have wider ecological

amplitude, would be mainly stress-tolerant species with elemental barriers at the root level to avoid ion imbalance, and with a high dependence of AM fungi to face nutrient limitation. The unique strategy of gypsophiles may impact on their ecology. Consequently, I expect gypsophiles to be more competitive than other species on gypsum environments, showing increased germination, growth and performance in gypsum than other soils, and having better resistance to herbivory than gypsovags when grown on gypsum.

Objectives

Objectives

The main aim of this PhD thesis was to analyse the ability of gypsophiles to cope with the harsh characteristics of gypsum environments. I also aimed to ascertain how various factors (soil composition and grazing) modulate the unique nutritional strategy of gypsophiles, and if this strategy provides an ecological advantage to gypsophiles over gypsovags. These main aims were divided into the following partial objectives:

- Analyse the relationship between the capacity to germinate, growth, produce viable seeds, accumulate nutrients in leaves of gypsum species growing on different substrates with varying gypsum content and pH.

- Evaluate the effect of herbivory, either natural or simulated by clipping, on gypsum plant communities and the performance of selected gypsophile and gypsovag species.

- Explore the role played by AMF fungi in the nutritional acquisition of gypsum plants and its linkage to the seasonality in nutrient availability and plant growth.

Structure of the PhD Thesis

The Thesis consists of an introductory chapter, five further chapters in article format, a general discussion, and some main conclusions. The main contents of these different chapters are summarized below.

The first and second chapters assess the high affinity of gypsophiles to gypsum soils, analysing whether gypsophiles can complete their life cycle in non-gypsum soils, and how the singular chemical features of gypsum soils affect plant performance and leaf chemical composition of selected gypsophile and gypsovag species.

Chapter 1 reports the results of an experiment to analyse the germination of gypsophiles and gypsovags from North America and Spain in field soils. The aim of this chapter was to analyse if plant restriction to gypsum soils is determined at the germination stage. Previous studies have observed that gypsophiles from the Iberian Peninsula and the Chihuahuan Desert show higher germination in saturated

solutions of gypsum, but there was no evidence that germination of gypsophiles increased in natural gypsum soils. We ran a germination experiment evaluating the percentage of germination of seeds of 10 gypsophiles and gypovags from the Iberian Peninsula and the Chihuahuan Desert, on acidic, calcic and gypsum field soils.

Chapter 2 includes results of a 29-month common garden experiment to analyse the growth, reproduction and production of viable seeds of gypsophiles and gypsovags in gypsum soils and in calcic soils (very low content of S but similar Ca concentrations). We also analysed the leaf elemental composition of both groups of gypsum species to test whether there was a nutritional requirement for any particular element.

Chapter 3 and 4 focus on the relationship between gypsum plants and herbivores to address the ecological significance of the unique nutritional strategy of gypsophiles. Nowadays and in the past, gypsum environments of the Middle Ebro Valley supported large herbivores and extensive grazing practices, and plants may have traits related to grazing resistance. Braun-Blanquet and Bolòs (1958) suggested that gypsophiles might be favoured by grazing disturbance. However, information on the effects of grazing on gypsum plants and their communities is very scarce.

Chapter 3 addresses a knowledge gap on the assembly of gypsum plant communities along grazing gradients. Following a trait-based approach, we assessed the assembly of species under different grazing intensities. We evaluated the species composition of perennial plant communities on gypsum outcrops of the Middle Ebro Basin (NE Spain), and whether species assembly was mediated by traits associated with adaptations to survive in gypsum soils (gypsum affinity and leaf S-accumulation) and by other classical functional traits sensitive to grazing.

Chapter 4 is the continuation of chapter 1, 2 and 3. Results from previous chapters show that gypsophiles are able to complete their life cycle out of gypsum soils (chapter 1 and 2), and to maintain the accumulation of S, Ca and Mg (chapter 2). In addition, grazing favours the assembly of gypsophiles and species with high S-accumulation (chapter 3). Thus, the aim of this study was to experimentally analyse the whole plant partitioning of S in gypsophiles and gypsovags in a clipping experiment where plants were cultivated on and off gypsum. This enabled testing for the interaction between substrate and response to herbivory, crucial to detect potential inducible defence mechanisms linked to gypsum soils.

The last article focuses on the acquisition strategies of selected gypsophile and gypsovag species in the nutrient deprived seasonal conditions typical of gypsum drylands. Chapter 5 is a field study to analyse the AMF colonisation of gypsum plants throughout a year. Gypsum soils are nutrient-deprived and lead to low leaf P concentration. Previous studies have described that gypsophiles have less AMF colonisation and diversity than gypsovags, indicating a possible different nutritional strategy related to gypsum affinity (Palacio et al., 2012; Torrecillas et al., 2014). However, these studies were performed in spring, but the plant activity and the soil nutrient availability of nitrogen and phosphorus are seasonal in drylands as gypsum environments (Delgado-Baquerizo et al., 2011; Palacio and Montserrat-Martí, 2007). The aim of this study was to assess the seasonality of AMF colonisation, and possible links with soil nutrient availability and plant activity, to improve knowledge on the ecological strategy of plants to thrive on nutrient-deprived habitats, such as gypsum soils.

Chapter 1

Seedlings of *Gypsophila struthium* Loefl. in gypsum soils. By Andreu Cera.

1 Do soil features condition seed germination of gypsum plants? An analysis of the effect of different natural soils

Nathaniel Heiden, Andreu Cera, Sara Palacio. Manuscript under second revision in *Journal of Arid Environment* (submitted in 30th March 2021)[1].

Abstract

Gypseous soils are widespread across arid and semiarid environments worldwide. They present remarkable challenges to plants and host a unique flora. We aimed to assess up to what point the specificity and distribution of species on gypsum might be driven by species-specific germination responses to soil gypsum availability. We analyzed the germination of six gypsum specialists and four closely related generalist plant species from the Iberian Peninsula and the Chihuahuan Desert in four different field soils with contrasting concentrations of gypsum, pH and soil texture. Plant restriction to gypsum was unrelated to the germinating ability of seeds on different substrates. Irrespective of their affinity for gypsum, most species germinated better on mixed gypsum-calcic soil and worse in the acidic soil treatment. Our data suggest soil pH and Ca availability were the main soil features driving seed germination, while the effect of gypsum content was generally not significant. The main exception was the Iberian gypsum specialist *Helianthemum squamatum* Pers., which showed increased germination on gypseous soils and higher germination in response to increased soil gypsum content. Except for this species, our findings indicate alkaline soils with high Ca availability favor the germination of most of the species analyzed, irrespectively of their gypsum content.

Keywords: edaphic endemism, seed germination, gypsophile, gypsovag, distribution

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1.1 Introduction

Gypseous soils are a type of alkaline soils containing gypsum (CaSO4•2H2O) as a main component (Herrero and Porta, 2000). They are spread over 100 million hectares globally, being prevalent in arid and semi-arid regions of the world (Eswaran and Zi-Tong, 1991). Gypseous soils present physical and chemical challenges for plants (Escudero et al., 2015; Verheye and Boyadgiev, 1997). The saturation of the soil solution with Ca2+ and sulfate ions (FAO, 1990), suggested to be toxic for some plants (Ernst, 1998), results in alkaline pH, moderate salinity, low nutrient retention capacity (Casby-Horton et al., 2015) and a remarkable nutrient impoverishment of the soil. Gypseous soils typically have hard soil surface crusts, which hamper root penetration and can restrict seedling establishment (Escudero et al., 2000, 1999; Meyer, 1986). They are mechanically unstable due to their lack of plasticity, cohesion and aggregation (Bridges and Burnham, 1980; FAO, 1990); and, in certain areas, they show a low porosity, which limits the penetration of some plant roots (Guerrero-Campo et al., 1999).

The limiting nature of gypseous soils contrasts with their remarkable floristic diversity, rich in endemic and specialized species, which has been defined as a conservation priority of international concern (European Community, 1992; Mota et al., 2011; Ochoterena et al., 2020). According to their specificity to gypsum, plants can be categorized into two general groups: gypsophiles, namely species growing only on gypseous soil; and gypsovags, which grow both on and off gypseous soil (Meyer, 1986). Information on the underlying factors determining gypsophile restriction to gypsum soils is scarce (Escudero et al., 2015). Previous studies have shown how widely distributed gypsophiles from Spain (Palacio et al., 2014a, 2007) and the Chihuahuan Desert (Muller et al., 2017) frequently share an ability to accumulate S and Ca in their leaves, which points at a convergent evolution towards similar adaptive traits in plants from these distantly related floras. This pattern could ultimately link to a nutritional specialization of gypsophiles to gypseous soils (Cera et al., 2021). Some gypsum endemic species have also been shown to have a higher ability than co-occurring gypsovags to surpass the hard physical crust typical of gypsum soils at the seedling stage (Romão and Escudero, 2005). The distribution of plants on gypsum soils could also be linked to a differential ability to germinate on gypsum (Romão and Escudero, 2005), since calcium and gypsum concentration can affect seed

germination, also in gypsophile species (Anderson, 1982; Cañadas et al., 2014; Merlo et al., 1997; Secor and Farhadnejad, 1978).

Seeds are responsive to their soil environment (Osuna et al., 2015), and germination is one of the riskiest parts of the plant life cycle (Sánchez et al., 2014). Seed germination is largely affected by soil moisture, temperature and light conditions, but soil chemical conditions may also play a role (Baskin and Baskin, 2014). Several studies have described reduced seed germination in response to soil salinity, as a result of negative osmotic potential and ionic toxicity (Pujol et al., 2000; Ungar, 1996 and references therein). High Ca concentrations also decreased the germination of species adapted to acidic soils and favored those of calcicolous species (Anderson, 1982). Soil pH differences also may lead to substantial changes in seed germination (Pierce et al., 1999), and some plant species have been shown to germinate preferentially in a specific pH range (Ma and Liang, 2007). Some widely distributed gypsophile species from Spain and the Chihuahuan Desert increased their germinating ability when treated with a gypsum saturated solution (Cañadas et al., 2014; Merlo et al., 1997; Secor and Farhadnejad, 1978), which some authors attributed to the effect of Ca (Merlo et al., 1997). Seeds of gypsum endemic species may be adapted to germinate in the stressful conditions of these soils, but experimental evidence on the effect of field gypseous soils is lacking (but see Romão and Escudero, 2005).

We analyzed the germinating ability of ten species (Table 1) showing different specificity to gypseous soils on four different natural substrates with contrasting gypsum content, soil texture and pH (Table 2). The species selected include representatives from the two best-known gypsum floras of the world: those of the Iberian Peninsula and the Chihuahuan Desert (Escudero et al., 2015; Moore et al., 2014). Field soils were chosen to cover a broad range in gypsum content and pH and included: gypseous, calcic, acidic and a 1:1 mixture of gypseous and calcic soils. We hypothesized that (1) the germination of gypsophiles would be higher in natural gypseous soil and lower in the acidic soil; (2) gypsovags would show similar germination on soil with or without gypsum; and (3) owing to the similar germination responses observed in gypsophiles from the Iberian Peninsula and the Chihuahuan Desert (e.g. Cañadas et al., 2014; Secor and Farhadnejad, 1978), we expect the aforementioned trends would hold true regardless of geographic origin.

1.2 Materials and methods

Study species and seed collection

Study species were two Iberian gypsophiles: *Helianthemum squamatum* and *Lepidium subulatum*; two Iberian gypsovags: *Helianthemum syriacum* and *Matthiola fruticulosa*; four gypsophiles native to the Chihuahuan Desert: *Gaillardia multiceps*, *Nama canescens*, *Nama carnosa* and *Nerisyrenia gracilis*; and two Chihuahuan Desert gypsovags: *Nerisyrenia camporum* and *Lepidium alyssoides* (Table 1.1). Species selection included widely distributed gypsophiles from the two regions of study plus closely related gypsovags for comparison.

Table 1.1: Species included in the study with indication of their taxonomic family, affinity to gypsum soils and geographical origin.

	Family	Gypsum affinity	Origin	Locality	Collection date
Gaillardia multiceps Greene	*Asteraceae*	Gypsophile	CD	Alkali Flat, New Mexico, US	August 2018
Helianthemum squamatum Pers.	*Cistaceae*	Gypsophile	Spain	Villamayor, Zaragoza, Spain	June 2015
Helianthemum syriacum (Jacq.) Dum.Cours.	*Cistaceae*	Gypsovag	Spain	Villamayor, Zaragoza, Spain	June 2015
Lepidium alyssoides A. Gray	*Brassicaceae*	Gypsovag	CD	Castillo Formation, Texas, US	August 2018
Lepidium subulatum L.	*Brassicaceae*	Gypsophile	Spain	Villamayor, Zaragoza, Spain	June 2015
Matthiola fruticulosa (Loefl. ex L.) Maire	*Brassicaceae*	Gypsovag	Spain	Villamayor, Zaragoza, Spain	July 2015
Nama canescens C.L.Hitchc.	*Namaceae*	Gypsophile	CD	Zaragoza, N.L. Mexico	August 2017
Nama carnosa C.L. Hitchc.	*Namaceae*	Gypsophile	CD	White Sands, Texas, US	September 2018
Nerisyrenia camporum (A. Gray) Greene	*Brassicaceae*	Gypsovag	CD	Socorro, New Mexico, US	August 2018
Nerisyrenia gracilis I.M. Johnst.	*Brassicaceae*	Gypsophile	CD	La Trinidad, N.L. Mexico	September 2017

Mature fruits were collected from one natural population per species in North America (U.S.A. and Mexico) or Spain. Source individuals were selected to represent the whole population, and included at least 10 different mature individuals separated more than 3 m from each other. Mature fruits from each species were pooled together and, once in the laboratory, they were sorted to collect filled seeds, remove

fruit remains and aborted seeds, and then stored at room conditions in paper envelopes from the date of sampling (Table 1.1) until germination tests were started.

Soil collection and analyses

Natural gypseous soil was collected from a gypsum outcrop in the Middle Ebro Basin (Villamayor del Gállego, Zaragoza, Spain, 41º41'44.5N, 0º44'26.7W), calcic soil was collected from the Iberic System (Ricla, Zaragoza, Spain, 41º30'45.8"N, 1º26'47.8"W), and acidic soil from Moncayo (Agramonte, Zaragoza, Spain, 41º49'40.94"N, 1º49'18.4"W). Soil was collected from top to 1 m depth by removing O horizons on unfertilized areas in talus slopes and sieved to pass a 1 cm mesh sieve, thoroughly mixed and used to fill pots. A 1:1 mixture of gypseous and calcic soils was obtained by thoroughly mixing equal volumes of both soils to produce a low gypsum content soil (Table 1.2). The gypsum soil included in our experiment was representative of natural gypsum soils where gypsum endemics frequently grow. Gypsum outcrops are frequently intermingled with alternating layers of calcic marls, limestone and clays (Quirantes, 1978). Consequently, calcic soils are the most readily available non-gypsum alternative for plants growing on gypseous soils in the wild, showing similar physicochemical features including similar Ca content and differing mainly in the higher S content of gypseous soils (FAO, 1990). Calcic and gypsum soils often form mixed soils such as the mixture included in our treatment, where gypsovags and, sometimes also gypsophiles, can be found. Although not representative of the soils where the selected species are normally found in nature, the inclusion of the acidic soil treatment responded to a need to increase the range in soil pH and Ca availability included in our study.

Three sub-samples were obtained from each soil for physical and chemical analyses. Soil samples were air dried during 2 months at room temperature prior to physical and chemical analyses and subsequently divided in two subsamples, one to be sieved to pass through a 2 mm sieve and the other to remain non-sieved. Sieved soils were used to measure the following variables: gypsum content, measured according to Artieda et al. (2006); soil texture, determined with a particle laser analyser (Mastersizer 2000 Hydro G, Malvern, UK); and soil pH and conductivity, measured with a pH/conductivity meter (Orio StarA215, Thermo Scientific, Waltham-MA, USA) by diluting samples

with distilled water to 1:2.5 (w/v) to measure pH and then 1:5 (w/v) to measure conductivity. A subsample of each sieved soil was finely ground using a ball mill (Retsch MM200, Restch GmbH, Haan, Germany) and subsequently used to analyse N and C elemental concentrations with an elemental analyzer (TruSpec CN, LECO, St. Joseph-MI, USA) by EEZ-CSIC Analytical Services. A second subsample of each sieved and finely grounded soil was used to account for bioavailable soil elements. This subsample was extracted following a modified protocol for special soil conditions (Ramos, 2002) using EDTA 0.05M at pH 7 with ammonium hydrate at 32%. Soil extracts were analysed for elemental composition (Ag, As, B, Ca, CU, Fe, K, Mg, Mn, Na, Ni, Pb, P, Si, S, Sr, Tg, Zn) with an ICP-OES at CEBAS-CSIC Analytical Services.

Table 1.2: Main physical and chemical features of the different soils included in the experiment. Values are means of N = 3 replicates ± SE.

	Acid	Gypsum	Mixed	Calcic
Sand (%)	37.36±3.79	62.58±5.75	71.63±2.69	83.39±0.78
Silt (%)	45.48±1.47	28.48±4.38	22.87±1.45	15.75±0.53
Clay (%)	17.16±2.44	8.94±1.47	5.50±1.24	0.86±0.33
Gypsum (%)	1.15±0.12	72.20±2.97	33.72±0.92	0.29±0.09
N (mg/g)	2.95±0.11	1.84±0.15	1.79±0.06	1.14±0.16
C (mg/g)	33.80±1.00	84.13±10.03	83.50±1.10	74.17±1.37
pH	4.31±0.01	8.42±0.02	8.47±0.01	9.06±0.07
EC	56.83±0.94	2201.67±13.62	2313.33±8.45	645.00±6.51
Ag (mg/g)	7.09±0.34	0.06±0.00	0.06±0.00	0.05±0.00
As (µg/g)	2.32±0.07	1.17±0.16	1.31±0.12	1.01±0.15
B (µg/g)	6.55±0.09	6.35±0.20	5.98±0.28	12.50±0.34
Ca (mg/g)	4.92±0.27	320.38±3.84	322.73±4.50	211.34±2.03
Cu (µg/g)	21.13±0.23	5.32±0.16	5.96±0.33	11.46±0.39
Fe (mg/g)	2.42±0.17	0.06±0.00	0.08±0.00	0.20±0.01
K (mg/g)	1.37±0.05	1.33±0.02	1.42±0.01	1.22±0.05
Mg (mg/g)	0.70±0.03	2.82±0.16	2.19±0.02	3.15±0.02
Mn (mg/g)	0.02±0.00	0.11±0.00	0.13±0.00	0.19±0.00
Na (mg/g)	0.32±0.01	0.73±0.03	2.16±0.03	4.30±0.08
Ni (µg/g)	2.54±0.04	1.68±0.02	1.25±0.01	1.04±0.03
Pb (µg/g)	25.71±1.33	5.02±0.15	4.88±0.20	4.13±0.06
P (µg/g)	13.00±0.73	19.93±2.21	26.03±1.15	41.82±0.62
Si (mg/g)	1.41±0.05	0.16±0.00	0.20±0.00	0.22±0.01
S (mg/g)	0.68±0.05	147.57±0.70	148.94±3.51	5.25±0.05
Sr (mg/g)	0.03±0.00	2.62±0.01	2.24±0.01	0.48±0.00
Tg (µg/g)	4.68±0.15	6.94±0.07	7.07±0.08	5.97±0.03
Zn (µg/g)	8.02±0.41	2.41±0.13	4.15±0.28	4.17±0.23

Germination experiment

Prior to germination tests, we applied species-specific seed treatments due to the different dormancy-breaking requirements of studied species (Baskin et al., 2006). Seeds from *Helianthemum* species were scarified mechanically with sand paper to break the seed coat that hampers seed re-hydration in these species (Pérez-García and González-Benito, 2006). The seeds of *G. multiceps*, *L. alyssoides*, *N. canescens*, *N. carnosa*, *N. camporum*, and *N. gracilis* were placed in the oven at 50ºC for two weeks prior to planting. Previous studies suggested heat shock treatment may increase germination for Chihuahuan desert species (Secor and Farhadnejad, 1978), a positive effect that was confirmed in pre-trials with *N. gracilis* and *N. canescens* (data not shown). No heat scarification is recommended for Iberian gypsophile and gypsovag species (Escudero et al., 1997). Similarly, *L. subulatum* and *M. fruticulosa* were not pre-treated before sowing, since no previous study indicated the need of dormancy breaking in these species. Seeds from all species were selected under the stereomicroscope to include filled firm seeds with signs of viability. Seeds were disinfected by soaking for 10 minutes in a 5 % bleach solution and then washed with distilled water twice. They were soaked in distilled water for a day before planting. All planting and sterilization were done with autoclaved, ethanol sprayed, and then UV sterilized materials.

Germination trials were set up in two different times in a growth chamber with identical settings, and monitored every two days for 60 days (Cañadas et al., 2014). Between February and April 2019, we analyzed *G. multiceps*, *L. alyssoides*, *L. subulatum*, *N. canescens*, *N. carnosa*, *N. gracilis*, *N. camporum*. Between July and September 2020, we analyzed *H. squamatum*, *H. syriacum* and *M. fruticulosa*. To evaluate seed germination responses to different soil types, 10 replicates of 10 seeds per pot per species were planted in each of the four soil treatments (except for *L. alyssoides* on mixed and acid soil and *H. squamatum* on gypseous soil, which had 9 replicates and *L. subulatum* on acid soil*, N. gracilis* on calcic soil and *N. camporum* on mixed soil, which had 11 replicates). A total of 400 seeds were planted per species by lightly covering the seeds with soil. Pots of 45 cm^3 (5 cm x 5 cm x 3 cm) were arranged randomly in a growth chamber with 16 hours of light (flux = 1743-1900 lm, CCT =4000 -6500 K, similar to Escudero et al., 1997) at 25 ºC and 8 hours of darkness at 15 ºC and 80 % humidity. Alternate 25ºC / 15ºC temperatures over daily cycles are suitable conditions to promote the germination of Iberian

gypsum plants (Escudero et al., 1997; Moruno et al., 2011; Sánchez et al., 2014). Soil surface in pots was kept moist at all times by regularly spraying distilled water. We considered a seed to have germinated once the cotyledons appeared above the soil surface (Wenk and Dawson, 2007). Once counted, emerged seedlings were removed from pots.

Calculations and statistical analyses

Germination percentage was calculated as the percentage of seeds that germinated within 60 days per pot, being pots our experimental units. Cumulative counts of germinated seeds were used as the response variable in statistical models evaluating the effects on plant germination. Data were not normal and variances increased with mean values, consequently, we used generalized linear mixed models (GLMMs) fixed to a Poisson distribution with log link function. "Affinity to gypsum", "soil treatment" and "geographical origin" were included as fixed effects, and "species" nested within "taxonomic family" as random effects, to test for differences in germination among plants. Similar GLMMs with "soil treatment" and "geographical origin" as fixed effects and "species" nested within "taxonomic family" as random effects were run separately for gypsovags and gypsophiles to account for soil effects on the germination of each group of gypsum affinity and geographical origin. Generalized linear models (GLMs) with "Soil treatment" as a fixed factor were run for each species separately. Post hoc Tukey tests were run after GLMMs and GLMs to account for differences among soil types.

To unravel the effect of different soil features on the germination ability of seeds we ran a Principal Component Analysis (PCA) with all soil features measured. This served both to explore the correlation between different soil variables and to summarize the multidimensional space of soil properties into two vectors explaining most of the variability of the dataset. Variables were scaled prior to analysis by subtracting the mean and dividing by the standard deviation, to avoid variation due to different units. The first two PCA axes, explaining 94 % of the variability (Appendix A, Table A.1) were not correlated ($t = -9.5 \cdot 10^{-17}$, $df = 10$, $P = 1$, $R^2 = 9.08 \cdot 10^{-34}$). Thus, they were subsequently included as covariates in GLMMs with "germination counts" as the response variable, "affinity to gypsum" and "geographical origin" as fixed effects and "species" nested within "taxonomic family" as random

effects. This aimed at exploring the effect of main soil features, geographical origin and affinity to gypsum soils on the germination ability of plants.

Table 1.3: Results of GLMMs testing: 1) the effect of soil type, plant affinity to gypsum soils (Class), geographical origin (Origin) and their interaction on the seed germination of study species (general model); and 2) the effect of soil type and geographical origin on the seed germination of gypsophiles and gypsovags separately. Models included "Family" and "Species" nested within "Family" as random effects. General model: N = 400; Gypsophiles: N = 241; Gypsovags: N = 159.

	General model			Gypsophiles			Gypsovags		
	DF	*Chisq*	*Pr(>Chisq)*	*DF*	*Chisq*	*Pr(>Chisq)*	*DF*	*Chisq*	*Pr(>Chisq)*
Soil Type	3	64.87	< 0.001	3	37.89	< 0.001	3	33.53	<0 .001
Class	1	8.88	0.003	-	-	-	-	-	-
Origin	1	24.38	< 0.001	1	22.25	< 0.001	1	6.60	0.010
Soil Type:Class	3	6.49	0.090	-	-	-	-	-	-
Soil Type:Origin	3	14.20	0.003	3	17.49	< 0.001	3	5.21	0.157
Class:Origin	1	9.62	0.002	-					
Soil Type:Class:Origin	3	8.41	0.038	-					

All statistical analyses were run in R 3.6.3. Shapiro Wilk normality tests were run with nortest (Gross and Ligges, 2015). GLMMs and GLMs were run with lme4 package version 1.1-15 (Bates et al., 2007). Multiple comparison Tukey tests were run with multcomp package (Hothorn et al., 2009). PCAs were run and visualized with vegan package version 2.5-6 (Oksanen et al., 2007), and ggplot2 package (Wickham, 2009), respectively.

1.3 Results

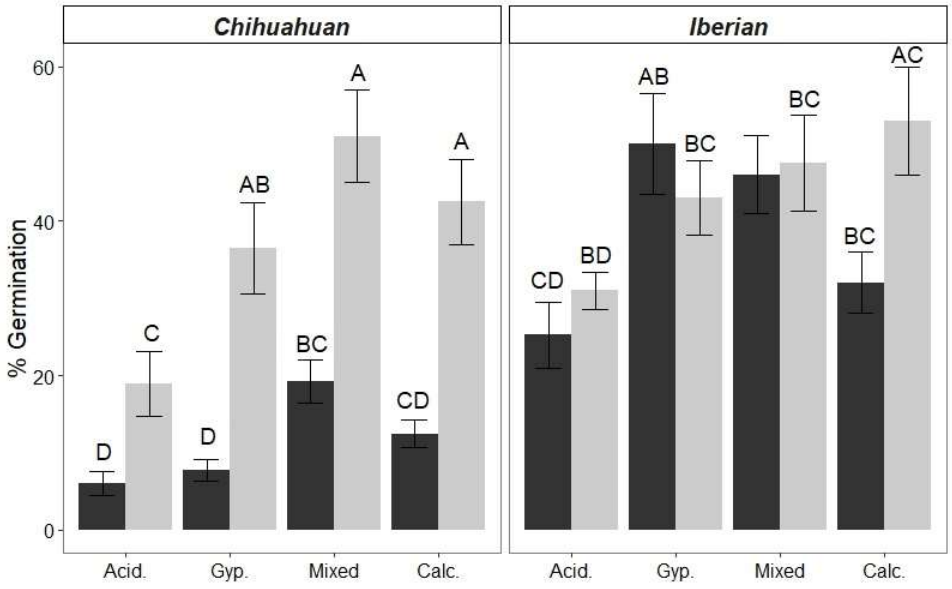
Gypsophiles and gypsovags showed similar germination but plants germinated better on mixed soils and worse on acidic soils

In general, gypsovags showed higher germination percentages than gypsophiles across substrates (Table 1.3, Fig. 1.1). However, the interaction between gypsum affinity and soil type was not significant, which indicates that the effect of soil type on germination did not differ between gypsophiles and gypsovags (Table 1.3). Plants germinated better on mixed (low gypsum content) soil than calcic (z = 3.03, P = 0.005) or acidic (z = 5.96, P < 0.001) soils. Also, plants germinated less on acidic soils than mixed, calcic (z = 2.85, P = 0.022), or gypseous (z = 3848, P < 0.001) soils. There was not a significant

difference in the germination of seeds planted in gypseous and mixed (z = 2.25, P = 0.110) or calcic (z = 1.05, P = 0.718) soils.

The analysis of results of each species separately indicated some species-specific responses. For example, the gypsum specialist *H. squamatum* showed a remarkable increase in germination in gypseous soils as compared to other substrates (Fig. 1.2). Most of the rest of species, either gypsophiles or gypsovags, showed trends consistent with the general response, germinating better in mixed soil and worse in acidic soil, whereas calcic and gypseous soils showed intermediate germination. Exceptions to this general trend were *G. multiceps* and *H. syriacum*, which showed no significant differences in the germination among substrates.

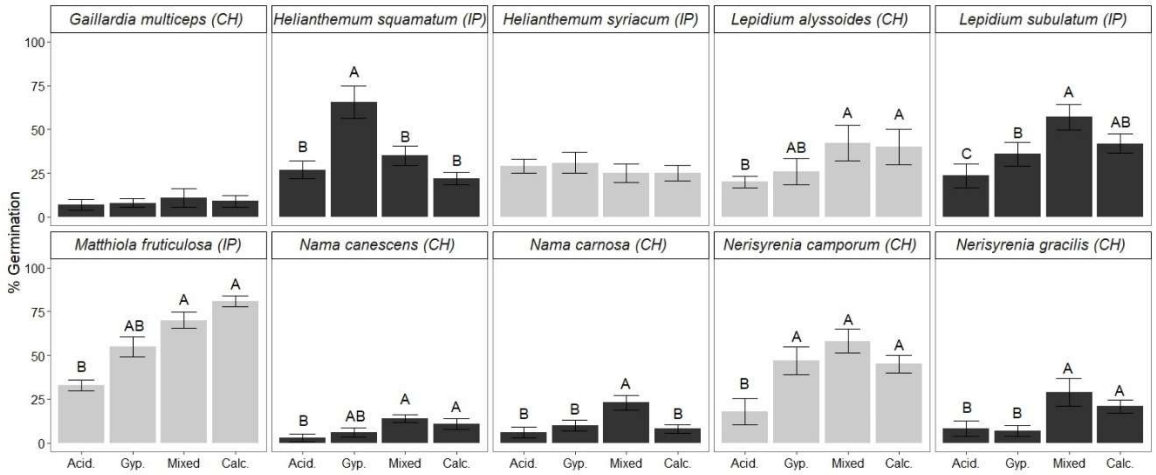
Figure 1.1: Germination of seeds of gypsophile (black bars) and gypsovag (light grey bars) species from the Iberian Peninsula and the Chihuahuan Desert sown on different substrates (Acid. = acidic; Calc. = calcic; Gyp. = gypseous; Mixed = 1:1 gypseous:calcic soils mixture). Different letters indicate significant differences among soil types within gypsum affinity groups after Tukey post hoc tests (α = 0.05). Values are means ± SE. N = 40 pots for gypsophile and N = 20 pots for gypsovag species from the Chihuahuan desert, except for gypsophiles on calcic soil and gypsovags on acid soil where N = 41 pots and 19 pots, respectively. N = 20 pots for Iberian species, except for gypsophiles on acid and gypsum soil where N = 21 pots and 19 pots, respectively.



Seeds from Iberian species germinated generally better than those from Chihuahuan plants (Table 1.3; Fig. 1.1), this was partly due to species like *N. canescens, N. carnosa, G. multiceps* or *N. gracilis,* which showed remarkably low germination in general, most values falling below 20 % (Fig. 1.2). This

effect was more important in gypsophiles than gypsovag species. Accordingly, the interaction between gypsum affinity and seed origin was also significant: Iberian gypsophiles showed higher germination percentages than those from Chihuahua (Table 1.3, Fig. 1.1). Finally, the interaction between seed origin and soil treatment was significant, since Iberian plants germinated generally better on gypseous soils than Chihuahuan plants. This was particularly due to the effect of *H. squamatum*. Indeed, the analysis of the effect of this interaction separately for gypsum specialists and generalists plants indicated that the interaction was only significant for gypsophiles, highlighting the effect of *H. squamatum* on the high germination of Iberian gypsophiles on gypsum. In accordance to these results, the triple interaction was highly significant (Table 1.3). Despite these differences due to the generally lower germination in seeds from Chihuahuan desert plants, patterns of variation in response to soil treatments were largely similar among different study species, regardless of the geographical origin, showing a increased germination on alkaline over acidic substrates (Fig. 1.2).

Figure 1.2: Box plots showing differences in the germination percentage (%) of study species species from the Iberian Peninsula (IP) and the Chihuahuan Desert (CH) cultivated on different substrates (Acid. = acidic; Calc. = calcic; Gyp. = gypseous; Mixed = 1:1 gypseous:calcic soils mixture). Different letters indicate significant differences among soil types after Tukey multiple comparisons tests ($\alpha = 0.05$). N = 10 pots, except for *L. alyssoides* on mixed and acid soil and *H. squamatum* on gypseous soil, where N = 9 pots, and *L. subulatum* on acid soil, *N. gracilis* on calcic soil and *N. camporum* on mixed soil, where N = 11 pots.



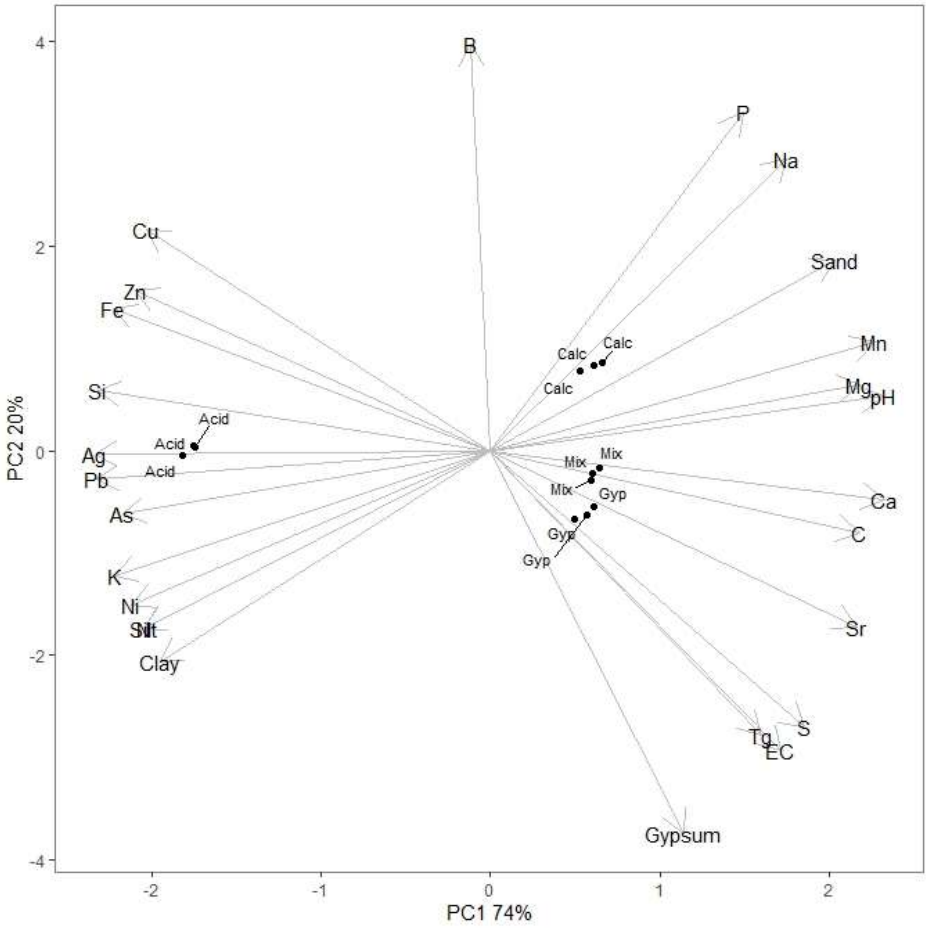
Soil pH and Ca^{2+} availability rather than gypsum content was the main soil feature driving seed germination

PCA of soil features indicated the variability in our soil treatments could be summarized in two main axes, which, together, explained 94 % of the variability (Appendix A, Table A.1). The first PC was related to soil pH, total C and N content, EC, soil texture and most of the bioavailable elements measured in soils, while the second PC was mainly driven by the gypsum content and, to a lower extent, EC (Appendix A, Table A.2). Consequently, the first PC separated acidic soil samples (with lower pH, higher total N, and higher bioavailable Ag, As, Cu, K, Fe, Ni, Si, Pb, Zn and a silty-clayish texture) from those of alkaline soils with a higher pH but also higher EC, total C (likely due to the carbonate content), and bioavailable Ca, Mn, Mg, Sr and a sandy texture (Fig. 1.3). This axis explained two thirds of the variation in the dataset (Appendix A, Table A.1). Contrastingly, PC2 segregated alkaline soils owing to their gypsum content, EC, and bioavailable B, Na, P, S, Tg, and explained 24.3 % of the variability. The inclusion of these two PCA components as covariates in GLMMs showed only PC1 was significant in the general model (Table 1.4), highlighting the effect of soil pH and ion content, rather than gypsum content and EC, on the germination of study species. The interaction between gypsum affinity of plants and PC2 was significant, indicating the germination of gypsophiles and gypsovags responded differently to gypsum content in the soil. Accordingly, while the effect of PC2 was not significant for gypsovags (Table 1.4), it became marginally significant when analyzed separately in gypsophiles (Table 1.4). This trend was largely due to the response of *H. squamatum*, and disappeared when this species was excluded from the analyses (data not shown). The geographical origin of plants also interacted with the effect of PC1 (Table 1.4), highlighting the overall higher germination percentages of seeds from Iberia, also in the acidic substrate (Fig. 1.2). Finally, the significant triple interaction between origin, affinity for gypsum and PC2 highlights the differential response of gypsophiles from different origins to soil gypsum content. Whereas the percentage of gypsum in the soil was unrelated to seed germination of Chihuahuan Desert gypsophiles, it significantly affected the germination of Iberian species, mainly through the response of *H. squamatum* (see above).

Table 1.4: Results of GLMM testing: 1) the effect of plant affinity to gypsum soils (Class), the geographical origin of seeds and the two main components (PC1 and PC2) of the PCA of main soil features on the germination of study species (general model); and 2) the effect of soil PCA components 1 and 2 on the germination of gypsophiles and gypsovags separately. "Family" and "Species" nested within "Family" were included as random effects. General model: N = 400 pots; Gypsophiles: N = 241 pots; Gypsovags: N = 159 pots.

	General model			Gypsophiles			Gypsovags		
	DF	*Chisq*	*Pr (>Chisq)*	*DF*	*Chisq*	*Pr(>Chisq)*	*DF*	*Chisq*	*Pr(>Chisq)*
Class	1	8.99	0.003	-	-	-	-	-	-
Origin	1	25.19	< 0.001	1	23.91	< 0.001	1	5.89	0.015
PC1	1	51.64	< 0.001	1	23.08	< 0.001	1	28.49	< 0.001
PC2	1	0.05	0.829	1	3.25	0.072	1	1.49	0.222
Class:Origin	1	9.49	0.002	-	-	-	-	-	-
Class:PC1	1	0.04	0.837	-	-	-	-	-	-
Class:PC2	1	4.54	0.033	-	-	-	-	-	-
Origin:PC1	1	4.31	0.038	1	1.23	0.267	1	3.18	0.074
Origin:PC2	1	0.85	0.356	1	5.47	0.019	1	0.52	0.471
Class:Origin:PC1	1	0.10	0.755	-	-	-	-	-	-
Class:Origin:PC2	1	5.06	0.025	-	-	-	-	-	-

Figure 1.3: Plot showing the results of PCA of the different soil types included in the experiment and their relation to the main soil features analyzed.



1.4 Discussion

Contrary to our expectations, the restriction of gypsophiles to gypsum observed in the wild did not alter their ability to germinate on a broad range of soils with different chemical and physical features. Similarly to the gypsovags analyzed, they germinated better on alkaline over acidic soils, irrespective of soil gypsum content. These results generally stood independently of seed origin. Most species analyzed showed the highest germination in mixed gypseous:calcic soil, with a low gypsum content and alkaline pH, whereas germination was decreased in acidic soils. The higher germination of most plants on the mixed soil treatment could be related to the relatively more favorable conditions (such as lower pH) in this soil type as compared to the rest of alkaline soil treatments. These results seem to indicate germination alone does not drive the restriction of gypsophiles to natural gypsum soils.

Exceptions to these general trends were the gypsophile *G. multiceps* and the gypsovag *H. syriacum* which showed no differences in the germination among substrates. The gypsum specialist *H. squamatum* also departed from the general trend, showing increased germination on gypseous soils compared to the rest of substrates. These results are in agreement with the sole previous study analyzing the effect of field soils on the germination of gypsophiles (Romão and Escudero, 2005). In their analysis, Romão and Escudero (2005) evaluated the germination of *Helianthemum squamatum* on natural gypseous and calcic soil and on a standard nurse substrate. They reported no significant differences on the seedling emergence on different types of substrates, but significantly higher survival of seedlings over 3 months on natural gypsum soils vs. commercial nursery substrate. Taken all together, these results point at a potential specialization of the seeds of *H. squamatum* to germinate and establish on soils with high gypsum content.

Similar to Romão and Excudero (2005), we found that the content of gypsum in the soil was unrelated to the germination ability of most of our study species. However, previous studies with the addition of $CaSO_4$ solutions to seeds of gypsophile and gypsovag species showed that increased $CaSO_4$ favored the germination of widely distributed gypsophiles (Merlo et al., 1997), including *Lepidium subulatum* (Cañadas et al., 2014) and *Gaillardia multiceps* (Secor and Farhadnejad, 1978), which was taken as evidence of the positive effect of gypsum on the germination of gypsophiles. These

discrepancies can be explained by the different experimental approaches in these studies. Instead of chemical solutions, our study and that of Romão and Escudero (2005) used field soils. This approach is closer to natural conditions (Ma et al., 2015), since it entails other physico-chemical factors such as soil texture, soil pH or EC. Although gypsophiles germinated generally better on mixed soils, their germination rates were also high on gypseous soils, in accordance to their occurrence on this type of substrates.

The analysis of the effect of different physico-chemical features of soils on the germination ability of seeds highlighted pH and Ca^{2+} availability as driving factors, with seeds of most species being favored by alkaline pH and high Ca^{2+} availability. Plant hormones, such as auxins and ethylene, play an important role in induction of germination, and the levels of these hormones have been shown to be affected by soil pH (Ribeiro et al., 2018). pH may also alter element toxicity, with subsequent effects on germination (Abedi et al., 2013). Most of the species included in this study grow frequently on alkaline soils, either gypseous (all gypsophiles), calcic (*N. camporum*, *L. alyssoides*, *H. syriacum*) or dolomites (H. syriacum). Consequently, it is not surprising that they show better performance on soils with relatively high pH. Similarly, cation availability, particularly Ca^{2+}, is a key factor affecting the germination ability of seeds of many plant species (Anderson, 1982). Merlo et al. (1997) suggested that the main effect of high $CaSO_4$ on gypsophile germination was through an increase in Ca^{2+} in the soil solution. Previous studies analyzed the separate effect of Ca^{2+} availability and pH on the germination ability of seeds, and concluded that the concentration of calcium compounds in the soil may be of primary importance at the germination stage due to their effects on pH, rather than their nutritional content (Pierce et al., 1999; Rorison, 1960a). However, gypsum endemic species are known to accumulate and show high Ca requirements (Cera et al., 2021). Further experiments disentangling the effect of soil pH from that of Ca^{2+} (and other base cations like Mg^{2+}) availability on the germination of gypsum plants would be required ascertain the individual relevance of each soil feature.

While soil pH and cation content (particularly Ca^{2+}) were the soil features showing highest loadings on the PC component explaining most of the variability in the germination of seeds, other factors correlated to pH, such as total C or soil texture, could also play a role. In our alkaline soils, total C may likely be the result of carbonate content, since the amount of organic matter in natural gypseous and

calcic soils is frequently low (Casby-Horton et al., 2015) and carbonates are a frequent component of gypseous soils in northeastern Spain (Palacio et al., 2007). As such, total C in our analysis may be a reflection of the alkaline nature of the soils and, consequently, highly correlated to pH.

In our analysis, soil texture (sand, silt and clay content) was a strong component of PC1, contributing to the separation of alkaline and acidic soils. The acidic soil included in our experiment had higher clay and silt content than alkaline soils, which were sandier. While soil texture is an important factor affecting the water holding capacity of soils and hence seed germination (Wenk and Dawson, 2007), it is unlikely that these factors played a role by themselves in our experiment. First, soils were kept constantly moist at all times throughout the development of the experiment. And second, silt and clay (which may favor soil water retention over sand) had negative loadings in PC1 and hence negatively affected germination. This result can only be explained by the circumstantial correlation between silt and clay and acidic pH derived from the design of the study.

Seeds from Iberia germinated generally better than those from the Chihuahuan desert. Pereira et al (2021) recently reported similarly low germination percentages in the American gypsum-associated species Arctomecon californica, which they attributed to the need of desert species to experience multi-year conditioning prior to germination, a trait related to the existence of permanent seed banks in perennial species from gypsum ecosystems (Caballero et al., 2003). We cannot rule out the possibility that germination conditions in our experiment might have been slightly cool for Chihuahuan Desert species. Secor and Farhadnejad (1978) germinated seeds at cycles of 23-29 ºC, slightly warmer than our 15 – 25 ºC. These authors also applied cold stratification to perennial seeds prior to germination, a pre-treatment not included in our experiment. However, despite the differences in overall seed germination, species from Iberia and the Chihuahuan Desert showed similar responses to different soil treatments, highlighting the higher germination of most species on alkaline vs. acidic soils, irrespectively of the gypsum content.

1.5 Conclusions

Our study did not find support for the hypothesis that plant specificity for gypsum is dependent, in part, on preferential germination in gypseous soils. This was true for most species both from Spain and from North America, except for the gypsum specialist *H. squamatum*, which showed increased germination on gypseous soils compatible with a selection at the germination stage. In the rest of species analyzed, however, we found a common trend among gypsophiles and gypsovags of increased germination in alkaline soils with high Ca^{2+} availability (both calcic and gypseous) and lower germination in acidic soils. According to this evidence, it appears unlikely that the distribution of most species on gypsum is largely driven by differential germination based on soil type. In contrast, soil variables other than gypsum content may be more important determinants of germination in most of our focal species. Our results are compatible with a potential origin of gypsophile lineages from calcicolous species.

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Author contributions

All authors designed the study. AC and SP set up the experiment. NH and AC ran the germination tests. All authors analyzed data and wrote the manuscript

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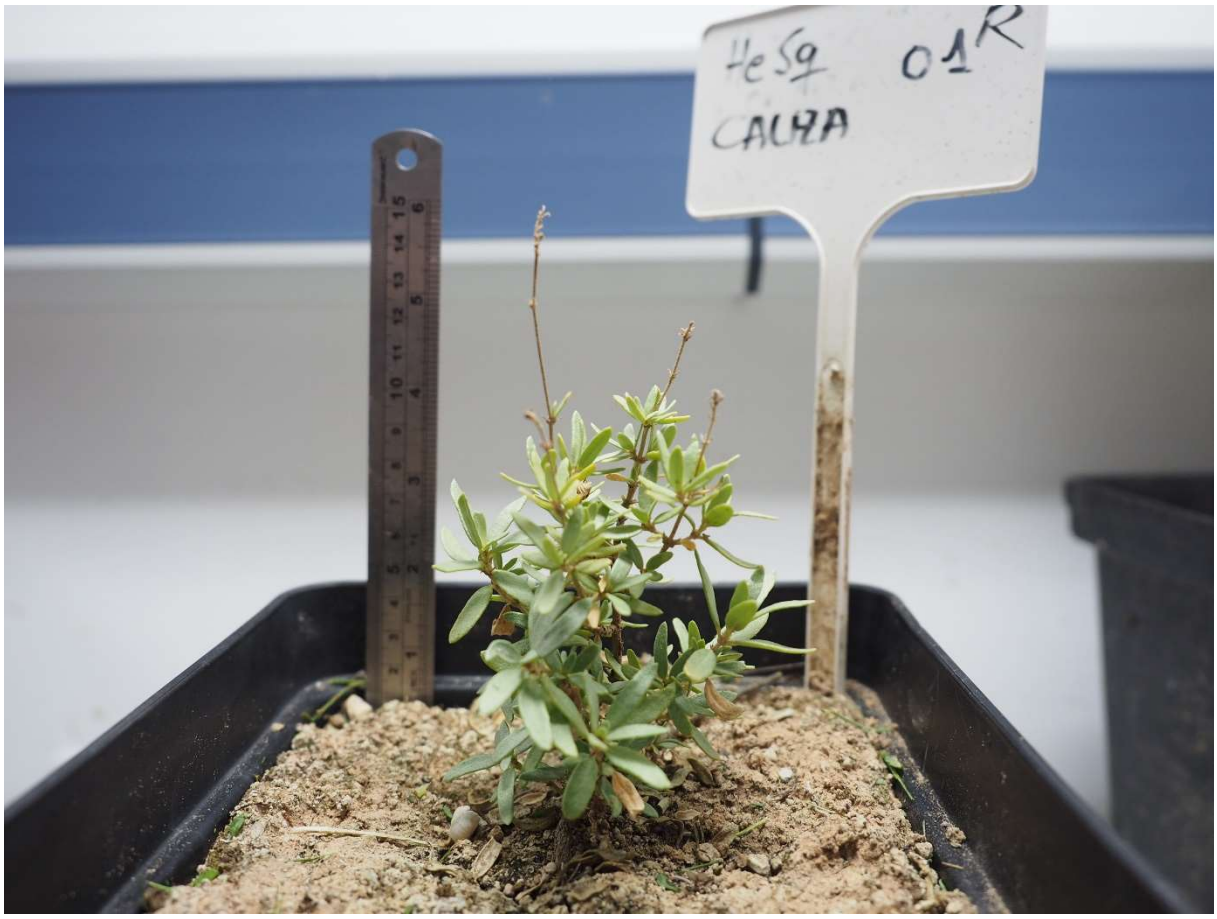
Chapter 2



Helianthemum squamatum Pers. grown in calcic pots. By Andreu Cera

2 Gypsum-exclusive plants accumulate more leaf S than non-exclusive species both in and off gypsum

Andreu Cera, Gabriel Montserrat-Martí, Juan Pedro Ferrio, Rebecca E. Drenovsky, Sara Palacio.

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Abstract

Gypsum-exclusive species (gypsophiles) are restricted to gypseous soils in natural environments. However, it is unclear why gypsophiles display greater affinity to gypseous soils than other soils. These plants are edaphic endemics, growing in alkaline soils with high Ca and S. Gypsophiles tend to show higher foliar Ca and S, lower K and, sometimes, higher Mg than non-exclusive gypsum species, named gypsovags. Our aim was to test if the unique leaf elemental signature of gypsophiles could be the result of special nutritional requirements linked to their specificity to gypseous soils. These nutritional requirements could hamper the completion of their life cycle and growth in other soil types. To test this hypothesis, we cultivated five gypsophiles and five gypsovags dominant in Spanish gypsum outcrops on gypseous and calcic (non-gypseous) field soil for 29 months. We regularly measured growth and phenology, and differences in leaf traits, final biomass, individual seed mass, seed viability, photosynthetic assimilation and leaf elemental composition. We found all the gypsophiles studied were able to complete their life cycle in non-gypseous soil, producing viable seeds, attaining greater biomass and displaying higher photosynthetic assimilation rates than in gypseous soil. The leaf elemental composition of some species (both gypsophiles and gypsovags) shifted depending on soil, although none of them showed leaf deficiency symptoms. Regardless of soil type, gypsophiles had higher leaf S, Mg, Fe, Al, Na, Mn, Cr and lower K than gypsovags. Consequently, gypsophiles have a unique leaf chemical signature compared to gypsovags of the same family, particularly due to their high leaf S regardless of soil conditions. However, these nutrient requirements are not sufficient to explain why gypsophiles are restricted to gypsum soil in natural conditions.

Keywords: gypsophile, semiarid, thiophores, leaf chemical signature, phenology, edaphism, gypsum

[2] Article published in Appendix F

2.1 Introduction

The effect of soil on plant performance and distribution has been studied by ecologists and botanists for decades, particularly in relation to the restriction of plants to certain types of soils with special physicochemical features. For example, serpentines and saline soils are special substrates that support singular floras (Mota et al., 2017) composed of species that tolerate the physicochemical challenges imposed by them (Kazakou et al., 2008; Munns and Tester, 2008).

Gypseous soils are also atypical substrates. These soils have high gypsum content (Casby-Horton et al., 2015) and normally develop in arid or semiarid environments, limiting plant life (Palacio et al., 2007). High gypsum content in soil impacts the physical and chemical properties of soils and their functions (Herrero and Porta, 2000). The moderate solubility of gypsum (about 2.4 g l^{-1}) leads to highly dynamic soil environments, with dissolution-precipitation sequences altering physical properties (Casby-Horton et al., 2015). Although the solubility of gypsum does not produce osmotic or ion-toxic stress for plants (Casby-Horton et al., 2015), the chemical conditions of gypseous soils influence plant nutrition (Boukhris and Lossaint, 1972; Palacio et al., 2007; Salmerón-Sánchez et al., 2014), ultimately limiting growth (FAO, 1990).

Plants living on gypseous soils have to cope with alkaline soils saturated in calcium, sulphate and magnesium ions, and reduced in N, P and K availability (Moore et al., 2014). Gypseous soils have low nutrient retention (Casby-Horton et al., 2015) and high Ca cation activity due to the solubility of gypsum (FAO, 1990). The combination of high Ca, jointly with high sulphate, alters plant metabolism (Meyer, 1980) and decreases the availability and uptake of macronutrients like K and P (FAO, 1990; Stout et al., 1951). To overcome these chemical restrictions, plants growing on gypseous soils may have developed special mechanisms and strategies (Moore et al., 2014).

Species that thrive on special soils generally show different ecological amplitudes, ranging from tolerant species with a broad distribution, to highly specialized edaphic endemics restricted to them (Kruckeberg and Rabinowitz, 1985). In the case of gypseous soils, Meyer (1986) described mainly two types of plants living on gypsum, depending on their affinity to this substrate: 1) gypsovags, species with wide ecological amplitude, which can grow both on and off gypseous soils; and 2) gypsophiles,

edaphic endemics restricted to gypseous soils. Two types of gypsophiles have been further described (Palacio et al., 2007): widely distributed gypsophiles (hereafter, wide gypsophiles) considered as gypsum specialists (sensu Gankin & Major, 1964), and narrowly distributed gypsophiles, which, similar to gypsovags, would fit the refuge model, being stress tolerant species not specifically adapted to gypseous soils. Gypsovags seem to be stress tolerant plants that may display different mechanisms to cope with the limitations imposed by gypsum (Bolukbasi et al., 2016). Gypsophiles are usually restricted to gypseous soils (Mota et al., 2011), and their individual fitness may be compromised in non-gypseous soils (Ballesteros et al., 2014). However, it is unclear why gypsophiles display greater affinity to gypseous soils than other soil types.

Edaphic endemics often have substrate-specific physiological mechanisms or strategies to cope with the harsh conditions of special substrates (Mota et al., 2017). In soils with atypical chemical composition, the mineral nutrition of plants has been crucial to explain plant restriction or growth limitation (Rorison, 1960b). The concentration of elements in leaves (hereafter, leaf elemental composition) is used to understand plant mineral nutrition, since it links plant function (Aerts and Chapin, 1999) and soil chemistry. For example, halophytes require high concentrations of NaCl (100–200 mM) for optimal growth (Flowers et al., 1977) and show high leaf Na, Mg and low Ca, K, as compared to co-occurring non-specialized species (Matinzadeh et al., 2019). In serpentine soils, edaphic endemics maintain high leaf Ca:Mg molar ratios (O'Dell et al., 2006), indicating that they have a high selectivity for Ca at the root surface, maintaining sufficient Ca uptake despite a very low soil Ca:Mg ratio (Kazakou et al., 2008). Similarly, consistent chemical patterns have been found in wide gypsophiles, who display a common leaf elemental composition similar to that of the gypseous soils in which they grow (Duvigneaud and Denaeyer-De Smet, 1968).

Wide gypsophiles tend to have higher foliar S and Ca, lower K and, sometimes, higher foliar Mg as compared to co-existing gypsovags (Alvarado, 1995; Boukhris and Lossaint, 1975, 1972, 1970; Duvigneaud and Denaeyer-De Smet, 1968; Muller et al., 2017; Palacio et al., 2007). This unique leaf chemical composition was observed despite phylogenetic constraints in gypsophilic species from the Chihuahuan Desert (Muller et al., 2017). However, the ecological or adaptive implications of the atypical chemical composition of wide gypsophiles remain unexplored. It has been suggested that the

leaf elemental composition of wide gypsophiles could be a nutritional requirement to complete their life cycle and support growth or could confer some form of protection from competition or disturbances (Meyer, 1980). However, no previous studies have evaluated the nutrient composition of wide gypsophiles growing on non-gypseous soils.

Our aim was to test if wide gypsophiles are restricted to gypseous soils because they are not able to complete their life cycle off gypsum. We focused only on wide gypsophiles (hereafter, gypsophiles), since narrowly distributed gypsophiles are a less distinctive group. We also wanted to explore the extent to which the atypical chemical composition of gypsophiles is linked to chemical conditions of the substrate. To this end, we cultivated five widespread Iberian gypsophiles and five co-occurring gypsovags (some of them closely related phylogenetically) in gypsum and calcic (non-gypseous) soils. The selection of calcic soil as the non-gypsum treatment stemmed from the fact that gypsum outcrops are frequently intermingled with alternating layers of marls, limestone and clays (Quirantes, 1978). Consequently, calcic soils are the most readily available non-gypsum alternative for plants growing on gypseous soils in the wild, showing similar physicochemical features including similar Ca content and differing mainly in the higher S content of gypseous soils (FAO, 1990). We analysed plant survival and fitness and measured leaf elemental composition as a tool to understand plant mineral nutrition and its relationship with soil chemical features. We hypothesized that: 1) Gypsophiles would have lower growth and fitness in non-gypseous soils than in gypseous soils, in accordance with Ballesteros et al. (2014); 2) Gypsophiles would have substrate-specific physiological mechanisms or strategies linked with chemical features of gypseous soils (i.e., nutritional requirements), and as a result, they would accumulate higher S and Mg concentrations than gypsovags, irrespective of the substrate. However, such concentrations would be lower on calcic (non-gypseous) than on gypseous soil, owing to the lower S and Mg availability in the former.

2.2 Material and Methods

Study species

The selected species included a suite of ten dominant gypsophile and gypsovag sub-shrubs from gypsum environments in northeastern Spain (Table 2.1). Gypsophile species included *Gypsophila struthium* subsp. *hispanica* (Willk.) G.López., *Herniaria fruticosa* L., *Helianthemum squamatum* Pers., *Lepidium subulatum* L., *Ononis tridentata* L.; and gypsovag species included *Boleum asperum* Desv., *Helianthemum syriacum* (Jacq.) Dum.Cours., *Linum suffruticosum* DC., *Matthiola fruticulosa* (L.) Maire and *Rosmarinus officinalis* L. All the gypsophile species included in the study show high affinity for gypseous soils (Mota *et al.,*2011) and are widely distributed within the Iberian Peninsula (Palacio et al., 2007).

Table 2.1: Characteristics of study species

	Family	Gypsum affinity	Gypsophily*	Seed collection (Spain)
Boleum asperum Desv.	*Brassicaceae*	Gypsovag	3.03	Castelflorite
Gypsophila struthium subsp. *hispanica* (Willk.) G.López	*Caryophyllaceae*	Gypsophile	4.69	Villamayor de Gállego
Helianthemum squamatum Pers.	*Cistaceae*	Gypsophile	4.87	Villamayor de Gállego
Helianthemum syriacum (Jacq.) Dum.Cours.	*Cistaceae*	Gypsovag	-	Villamayor de Gállego
Herniaria fruticosa L.	*Caryophyllaceae*	Gypsophile	4.05	Villamayor de Gállego
Lepidium subulatum L.	*Brassicaceae*	Gypsophile	4.91	Villamayor de Gállego
Linum suffruticosum DC.	*Linaceae*	Gypsovag	-	Villamayor de Gállego
Matthiola fruticulosa (L.) Maire	*Brassicaceae*	Gypsovag	-	Sariñena
Ononis tridentata L.	*Fabaceae*	Gypsophile	4.43	Villamayor de Gállego
Rosmarinus officinalis L.	*Lamiaceae*	Gypsovag	-	Leciñena

*Exclusivity to gypseous soils in Spain from expert evaluation. Values of gypsophily range between 0 and 5. Extracted from Mota *et al.,* (2011)

Soil collection and analyses

Gypseous soil was collected from a gypsum outcrop in the Middle Ebro Basin (Villamayor de Gállego, Zaragoza, Spain, 41º41'44.5" N, 0º44'26.7" W) and calcic soil (non-gypseous, hereafter calcic) was collected from the Iberian System (Ricla, Zaragoza, Spain, 41º30'45.8"N, 1º26'47.8"W). Soil was collected by removing O horizons in unfertilized areas, sieved to 1 cm, and then thoroughly mixed and

used to fill pots. Physical and chemical properties were analysed from five replicates per experimental soil type (Appendix B, Table B.1).

Soils were air dried for 2 months prior to physical and chemical analyses and subsequently divided into two subsamples: one to be sieved to pass a 2 mm sieve, and the other to remain non-sieved. Sieved soils were used to measure the following variables: gypsum content, measured according to Artieda et al. (2006); carbonate content determined by Bernard calcimetry; soil texture, estimated with a particle laser analyser (Mastersizer 2000 Hydro G, Malvern, UK); and soil pH and conductivity, measured with a pH/conductivity meter (Orio StarA215, Thermo Scientific, Waltham-MA, USA) by diluting samples with distilled water to 1:2.5 (w/v) to measure pH and then 1:5 (w/v) to measure conductivity). A subsample of each sieved soil was finely ground using a ball mill (Retsch MM200, Restch GmbH, Haan, Germany) and subsequently used to analyse elemental concentrations. N and C concentrations were measured with an elemental analyzer (TruSpec CN, LECO, St. Joseph-MI, USA), whereas the elemental composition of Al, As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Si, Ti, V, Zn was measured by extracting samples with HNO_3-H_2O_2 (9:3) by microwave acid digestion (Speed Ave MWS-3^+, BERGHOF, Eningen, Germany), followed by inductively coupled plasma-optical emission spectrometry (Varian ICP 720-ES, Agilent Technologies, Santa Clara-CA, USA). All elemental analyses were performed by EEZ-CSIC Analytical Services.

Experimental design

For each species, seeds were collected from several individuals within the same population (Table 3.1). In April 2016, seeds were germinated in nursery trays with 0.06 L cells filled with a one-part gravel in the bottom of the cell and four-parts field soil on top of it. Half of the trays had calcic soil and the other half had gypseous soil (see Appendix B, Table B.1, B.2, for soil features). In November 2016, plants with high root volume (*G. hispanica*, *R. officinalis* and *O. tridentata*) and plants with shallow roots (the rest of species) were transplanted into 7 L and 5.6 L square pots, respectively. Five months after transplantation, pots were thinned to one plant per pot, with ten replicates per species and soil treatment. All plants were kept well-watered throughout the experiment and regularly de-weeded by hand, removing any potential competition and drought stress. Each year, throughout the duration of the

experiment, plants were housed in a greenhouse from November to March to avoid freezing damage. Five replicates per species and soil treatment were harvested in September 2019, 29 months after sowing.

Phenological patterns and growth

Phenological patterns were recorded for each plant every two weeks between 29 November 2017 and 7 Sept. 2019. Five phenophases were considered (adapted from Montserrat-Martí et al. (2009)): plant vegetative growth, flower bud formation, flowering, fruit set and leaf shedding. The incidence of each phenophase was estimated in the canopy of each individual as the percentage of stems displaying it. Canopy height, maximum shoot length (measured from the base of plant to the distant most leaf, hereafter canopy length), and the maximum canopy diameter and its perpendicular were measured monthly using a metallic millimetre straightedge. Mature fruits were collected at seeding and stored in a dry location at room temperature until seed viability tests.

Leaf gas exchange, plant biomass, functional traits and seed traits

Leaf gas exchange, including photosynthetic assimilation and stomatal conductance, were measured with a Portable Photosynthesis System coupled with a Chlorophyll Fluorescence Module (CIRAS-2, PP Systems, Amesbury-MA, USA), a LED light unit on the leaf cuvette (PLC6 U), and a circular bead plate of 18 mm diameter. Three plants of each soil treatment and species were measured after 9 am and before 1 pm on 11 July 2017, except for *M. fruticulosa* and *B. asperum*, which did not have enough green leaves for assessment.

At harvest in September 2019, plants were lifted from their pots and rinsed with tap water to remove soil. Plants were separated into green leaves, stems, fine roots (diameter < 2 mm), coarse roots (rest of roots), and seeds (if available). All plant fractions were subsequently dried to a constant weight at 50 °C and weighed in a precision scale (42 g / 0.00001 g, MS105DU, Mettler Toledo, Columbus-OH, USA).

Specific leaf area (SLA) was measured as the one-sided area of a fresh leaf divided by its oven-dry mass. Leaf dry matter content (LDMC) was measured as the oven-dry mass (mg) of a leaf, divided by its water-saturated fresh mass (g), expressed in mg g^{-1} (Pérez-Harguindeguy et al., 2013). To measure

leaf area, images of leaves were captured with a Dino-Lite Digital Microscope (AnMo Electronics, Taiwan) and processed with ImageJ (National Institutes of Health, Bethesda-MD, USA). SLA and LDMC were calculated for the final harvest from among 4-10 individual leaves of each plant with petioles included.

Individual seed mass was weighed on a precision scale (42 g / 0.00001 g, MS105DU, Mettler Toledo, Columbus-OH, USA) as total seed weight divided by number of seeds ($N = 20$). Seed viability was assessed by monitoring the emergence of 20 seeds per species over 30 days. Seeds were sown on filter paper inside Petri dishes, kept well-watered with distilled water, and placed in a growth chamber (ASL Aparatos Científicos, Madrid, Spain) with 16 hours of light (flux= 1743-1900 lm, CCT = 4000-6500 K) at 25 ºC and 8 hours of darkness at 15 ºC.

Leaf chemical analyses

To assess leaf elemental composition, we collected leaf tissue from three to five individuals per species and soil type during two sampling periods: October 2017 and September-November 2018; different replicates were assessed at the two sampling periods. Leaves were dried to a constant weight at 50 ºC and subsequently finely ground using a ball mill (Retsch MM200, Restch GmbH, Haan, Germany). N and C concentrations were analysed with an elemental analyzer (TruSpec CN, LECO, St. Joseph-MI, USA). The elemental composition of Al, As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Si, Ti, V, Zn was measured by extracting samples with HNO_3-H_2O_2 (8:2) by microwave acid digestion (Speed Ave MWS-3^+, BERGHOF, Eningen, Germany), followed by inductively coupled plasma-optical emission spectrometry (Varian ICP 720-ES, Agilent Technologies, Santa Clara-CA, USA). All elemental analyses were performed by EEZ-CSIC Analytical Services. Only elements with values above 0.025 ppm (the detection limit of the ICP-OES spectrometer) were included in the statistical analyses.

Calculations and statistics

All statistical analyses were run in R 3.6.0 .

To model the gradualness of growth and flowering patterns, changes in canopy length and in the percentage of shoots bearing flowers within the canopy over time were fitted to a Boltzmann sigmoid regression (Self-Starting Nls Four-Parameter Logistic Model function on R). In this analysis, the scale parameter indicates the steepness of the curve and, consequently, the gradualness of the change in growth or flowering (Palacio et al., 2013). Shoot growth rate (L-day, mm day^{-1}) was calculated as the difference in canopy length between two consecutive monthly measurements divided by the number of days elapsed between both measurements. The maximum value of shoot growth rate, the day with the maximum shoot growth rate, the day of first flowering, the day with the maximum percentage of stems with flowers (day of maximum flowering), and the maximum flowering (maximum percentage of stems with flowers) were selected as variables to study changes in phenological patterns. Water use efficiency (WUE) was calculated by dividing the photosynthetic assimilation (A) by stomatal conductance (g_s) (Pérez-Harguindeguy et al., 2013).

Differences between soils and gypsum affinity plant types (i.e. gypsophiles and gypsovags) for the variables canopy length and canopy area at harvest, gradualness of shoot growth (slope of the Boltzmann curve), maximum shoot growth rate, day of maximum shoot growth rate, photosynthetic assimilation (A), stomatal conductance (g_s), transpiration (E), instantaneous Water Use Efficiency (WUE), day of first flowering, day of maximum flowering, maximum percentage of flowering, individual seed mass, total biomass, root:shoot ratio and for each elemental concentration were analysed by generalized linear mixed models (hereafter GLMM) with "soil" (gypseous / calcic) and "gypsum affinity" (gypsophile / gypsovag) as fixed factors and "species" as a random factor. Species was included as a random factor to account for species-specific effects and avoid biases related to species selection. In the case of elemental concentrations, we also added taxonomic "family", and "species" nested within "family" and "year" as random factors to avoid biases related to phylogenetic effects on elemental concentration (Neugebauer et al., 2018) or different sampling dates. Analyses were assessed with function *glmm* on R (*lme4* package version 1.1-15 in R, Bates et al., 2012/2007)). The models were fitted to a Gamma distribution when there was not a normal distribution of residuals since, in most cases, data had a

constant coefficient of variation and variances increased with means (McCullagh and Nelder, 1989). Model link functions of the Gamma distribution were selected according to the lower AIC criterion and included in each table as sub-indexes. Similarly, differences between soil types within each gypsum affinity class and differences between soil types within each species were assessed by GLMM.

Principal Component Analysis (PCA, *vegan* package version 2.4-6 in R (Oksanen et al., 2007), and *ggplot2* package in R (Wickham, 2009)) was used to visualize relationships among elemental concentrations and taxa. We used elements with concentrations above the detection limit of the ICP-OES spectrometer and samples for which we also had N and C concentration data (N = 182). All elemental data were transformed to Center Log-Ratio coordinates (Aitchison, 1982) using CoDaPack (Comas-Cufí and Thió-Henestrosa, 2011), to maintain relationships between elements regardless of the concentration, which allows studying joint patterns among elements (Prater et al., 2019; Soriano-Disla et al., 2013). Redundancy Analysis (RDA, *vegan* package version 2.4-6 in R (Oksanen et al., 2007)) was performed with the same data set as the PCA, including "soil" (gypseous / calcic) and "gypsum affinity" (gypsophile / gypsovag) as fixed factors.

Differences in nutrient composition between soils and species were assessed using non-parametric contrasts based on distance (Adonis function on *vegan* package version 2.4-6 in R (Oksanen et al., 2007)) with "soil" (gypseous / calcic) and "species" as fixed factors and using the Euclidean as distance from Center Log-ratio coordinates. Significant interactions between soil and species on nutrient composition was analysed by multilevel pairwise comparisons with "interaction" as a fixed factor (*pairwiseAdonis* package version 0.3 in R (Martinez-Arbizu, 2019)).

A heat map (*ggplot2* package in R (Wickham, 2009)) was used to visualize the distances among soils and species jointly with a cladogram from an adapted phylogenetic tree. Distances were calculated using Euclidean as distance from Center Log-Ratio coordinates (vegdist function on *vegan* package version 2.4-6 in R (Oksanen et al., 2007)). Distances among branches of the cladogram were extracted from Tree of Life Web Project (Maddison and Schulz, 2007).

2.3 Results

Life cycle, growth and phenology

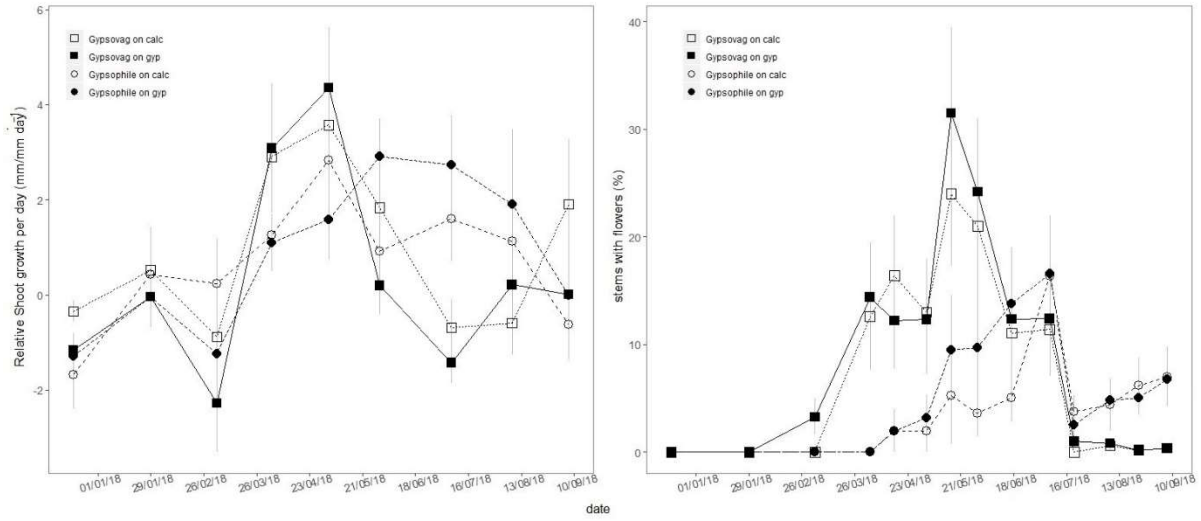
Contrary to our expectations, gypsophile species had similar growth, and similar maximum percentage of stems with flowers and individual seed mass, in both substrates (Table 2.2, and see F-ratios of GLMMs on Appendix B, Tables B.3, B.4). Also, they produced fruits which rendered viable seeds (data not shown). Similarly, and in agreement with our expectations, gypsovags completed their life cycle in both soil types, except for *R. officinalis,* which did not produce fruits in either substrate. Gypsovags had similar growth and lower individual seed mass in gypseous than calcic soils, and a similar maximum percentage of stems with flowers in both substrates ($P < 0.05$).

Table 2.2: Mean and standard deviation of leaf traits, seed traits, growth and phenological variables for each treatment. Upper case letters indicate significant differences between gypsophiles and gypsovags regardless of the soil type ($P < 0.05$). Lower case letters indicate significant differences between soil types ($P < 0.05$) within each gypsum affinity group.

	Gypsovags				Gypsophiles			
	Calcic		Gypseous		Calcic		Gypseous	
Final canopy area (dm^2)	10.71±7.30	A	8.73±4.57	A	18.42±11.03	Ba	15.27±9.42	Bb
Final length (mm)	189.28±64.37		200.17±68.29		176.24±88.65		165.71±76.22	
Gradualness	19.58±12.52		20.06±12.92		20.52±12.62		21.04±13.36	
Max. shoot growth rate ($mm \cdot day^{-1}$)	1.21±0.99		1.12±1.34		1.27±1.40		1.02±0.95	
Day max. shoot growth rate	372.44±49.63	A	388.54±63.54	A	425.40±44.03	Ba	398.04±56.15	Bb
E (mmol H_2O m^{-2} s^{-1})	5.77±2.37		6.12±3.33		8.07±3.56		6.47±4.08	
A (µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	12.01±1.94		10.01±3.18		12.87±5.49		9.69±4.19	
Gs ($mmol \cdot m\text{-}2 \cdot s^{-1}$)	507.31±292.08		563.49±375.79		599.43±362.60		475.69±313.17	
WUE	2.34±0.82		2.00±0.94		1.82±0.91		2.09±1.61	
SLA (cm^2/g)	63.36±33.78		55.02±20.81		84.63±45.46	a	66.46±38.28	b
LDMC ($mg \cdot g^{-1}$)	280.99±97.34	A	297.42±78.36	A	197.87±83.18	B	194.57±69.59	B
Leaf N (%)	1.72±0.98	a	1.66±0.87	b	1.56±0.97		1.59±0.84	
Day 1st Flower	340.25±30.25	Aa	360.18±28.80	Ab	402.40±41.83	B	411.80±32.05	B
Day Max Flowering	363.63±23.08	A	373.59±25.60	A	421.73±42.99	B	428.80±36.60	B
Max. Flowering (% stems)	63.13±27.68	A	68.82±17.64	A	42.00±26.17	B	29.33±23.74	B
Individual seed mass (mg)	0.77±0.73	a	0.91±0.62	b	1.38±2.19		2.83±3.33	
Total biomass (g)	12.85±11.96	a	9.40±6.39	b	13.13±10.52		12.54±9.40	
Root:Shoot	1.66±0.62	A	1.54±0.69	A	1.12±0.44	B	1.21±0.51	B

Gypsophiles and gypsovags differed in plant size, leaf traits, growth, and phenology (Table 2.2). Regardless of the soil type, gypsophiles had larger canopy areas than gypsovags, 1.4-fold lower Root:Shoot ratios and 1.5 fold lower LDMC ($P < 0.05$). Furthermore, the timing of phenological events was delayed in gypsophiles as compared to gypsovags, independent of soil type. Gypsophiles attained maximal shoot growth rate 31 days later on average than gypsovags in both soil types ($P < 0.05$, Fig. 2.1a). Gypsophiles also initiated flowering and reached maximal bloom almost two months later than gypsovags on average ($P < 0.05$ for both traits, Fig. 2.1b). Soil type had an effect on the flowering phenology of gypsovags: plants grown on calcic soil initiated flowering earlier than those grown on gypsum ($P < 0.05$).

Figure 2.1: A) Variation in relative shoot growth (mm/mm day^{-1}) and B) percentage of flowering stems (%) from December 2017 to September 2018. Centroids of each treatment mean were drawn (± *S.E.*). Circles indicate gypsophiles; squares, gypsovags. Filled symbols indicate plants grown on gypsiferous soils; empty symbols, calcic soils



Regardless of the species or gypsum affinity, plants grown in calcic soil had larger canopy area and total biomass ($P < 0.05$, Table 2.2). They also had 1.2-fold higher photosynthetic assimilation, 1.2-fold higher SLA, and started flowering ten days earlier on average than plants grown on gypseous soil ($P < 0.05$, Table 2.2). Considering each gypsum affinity separately, gypsophiles grown in calcic soil had larger canopy area and higher SLA than those grown on gypsum ($P < 0.05$, Table 2.2). Gypsophiles reached their maximal shoot growth rate 27 days later, on average ($P < 0.05$), when growing on calcic vs. gypseous soil. Gypsovags grown in calcic soil had higher total biomass and leaf N at harvest and

lower individual seed mass than those grown on gypsum ($P < 0.05$). Gypsovags also initiated flowering later on gypsum than calcic soil ($P < 0.05$, Table 2.2).

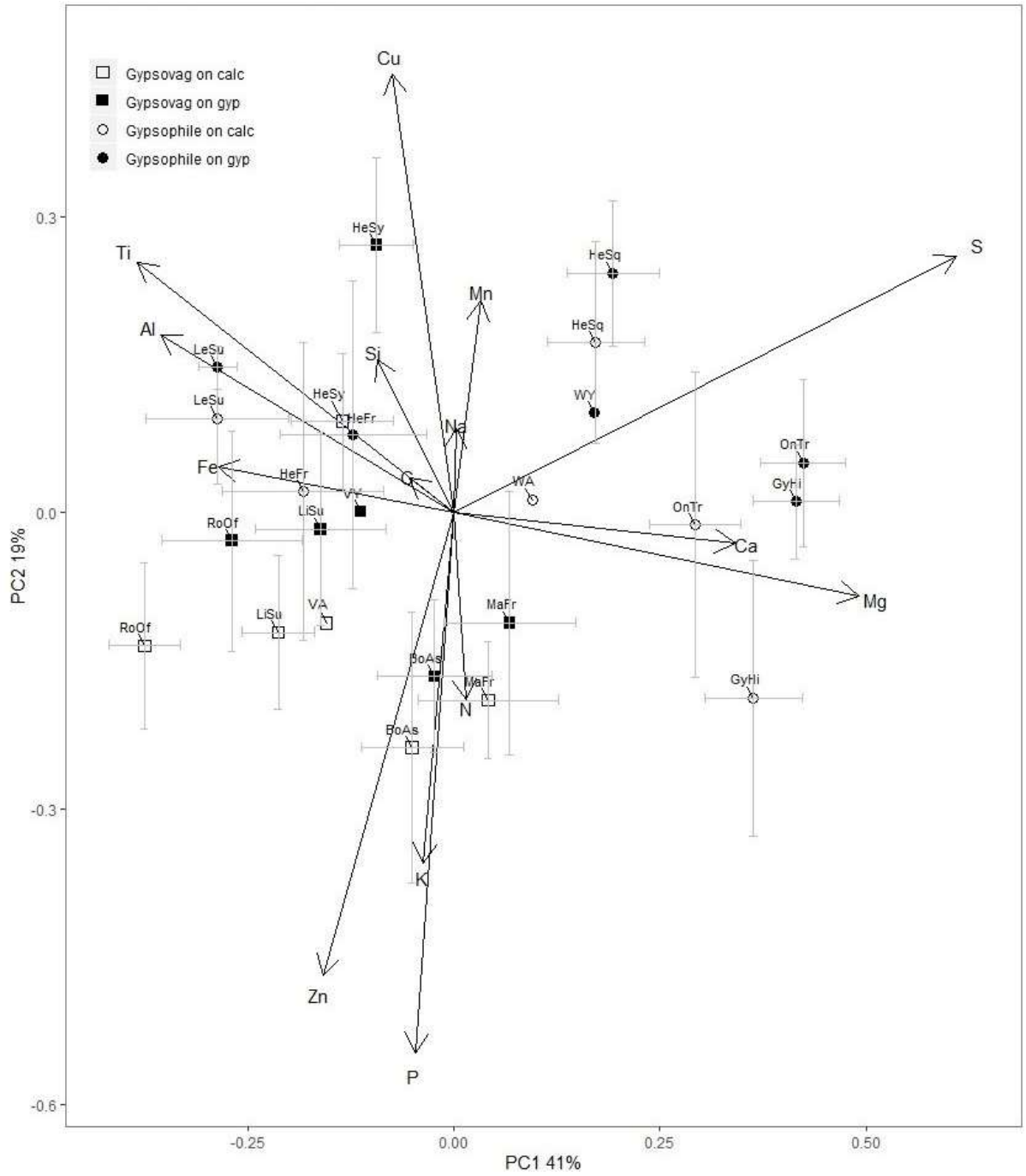
Table 2.3: *F*-ratios, *P*-value and Variability (SSfactor/SStotal) from non-parametric contrasts based on distances in which soil ($a = 2$), gypsum affinity types ($b = 2$) and species ($c = 10$) were fixed factors. Data set of $N = 180$.

Leaf elemental composition	Treatments				
	Soil	Gypsum affinity	Species	Soil*Gypsum affinity	Soil*Species
F-ratio	25.55	90.59	42.47	0.58	1.71
P-value	0.001	0.001	0.001	0.732	0.013
Variability (%)	4.04	14.33	53.75	0.09	2.17

Leaf elemental composition

Gypsophiles had a different leaf elemental composition compared to gypsovags that was independent of soil type ($P < 0.05$, Table 2.3), and these differences were maintained in both samplings (data not shown). Gypsophiles and gypsovags shifted their leaf elemental composition based on soil type ($P < 0.05$, Table 2.3). As indicated by the PCA biplot, plant leaf elemental composition was more strongly influenced by phylogenetic relationships than by soil type, with species of the same family plotting close to each other, regardless of the substrate (Fig. 2.2). The biplot of the first and second PCA axes indicated that gypsophiles showed a unique leaf elemental composition compared to gypsovags, irrespectively of the substrate. Gypsophiles growing on both soil types were located in the upper quadrants and associated with the vectors for S, Cu, Mg, Ti, Al, Fe, Mn. This pattern indicates they showed higher concentrations of these elements, regardless of soil type. In contrast, gypsovags were located in the bottom left quadrant, aligned with higher K, P, Zn and N concentrations. Furthermore, the biplot of first and second or second and third PCA axes (Figure 2.2, and Appendix B, Fig. B.1) indicated that plants from different soil types were distributed along the second component. Plants grown in gypseous soils had more positive values along the second component than those grown in calcic soils, and S vectors had positive values and K and P vectors had negative values. This pattern indicates plants grown on gypsum had high leaf S and low leaf K and P. In accordance with the PCA results, gypsum affinity and soil types were associated with different leaf elemental compositions based on the RDA analysis (*F*-ratio = 32.11 for gypsum affinity, $P < 0.05$; *F*-ratio = 8.72, $P < 0.05$, for soil type, respectively, *TVE*= 18.8 % for RDA model, and Appendix B, Figure B.2).

Figure 2.2: Biplot distance of first and second principal components based on Center Log-ratio transformation of leaf elemental composition. Centroids of each treatment mean were drawn (± *S.D.*). Circles indicate gypsophiles; squares, gypsovags. Filled symbols indicate plants grown on gypsiferous soils; empty symbols, calcic soils. BoAs: *B.asperum*; GyHi: *G.hispanica*; HeFr: *H.fruticosa*; HeSq: *H.squamatum*; HeSy: *H.syriacum*; LeSu: *L.subulatum*; LiSu: *L.suffruticosum*; MaFr: *M.fruticulosa*; OnTr: *O.tridentata*; RoOf: *R.officinalis*; WA: all gypsophiles on calcic soils; VA: all gypsovags on calcic soils; WY: all gypsophiles on gypsiferous soils; VY: all gypsovags on gypsiferous soils.



Assessing each element separately, gypsophiles had higher leaf Mg, S, Fe, Al, Na, Mn, Cu than gypsovags and lower K concentrations ($P < 0.05$, Table 2.4, see *F*-ratios of GLMMs in Appendix,

Tables B.5, B.6). Particularly large differences were observed for S and Mg. Leaf S of gypsophiles was triple that of gypsovags, and leaf Mg was 2.4-fold greater in gypsophiles than gypsovags. The leaf S concentration of gypsovags increased from 5.9 $mg \cdot g^{-1}$ in calcic soil to 7.7 $mg \cdot g^{-1}$ in gypsum ($P < 0.05$), whereas S concentrations in gypsophiles shifted from 15.6 $mg \cdot g^{-1}$ in calcic soil to 24.4 $mg \cdot g^{-1}$ in gypsum soil ($P < 0.05$). In contrast, leaf Mg of gypsovags and gypsophiles did not differ between soil types. Leaf Ca was similar between gypsophiles and gypsovags on gypsum, but gypsophiles had almost twice the leaf Ca concentrations of gypsovags when growing on calcic soil ($P < 0.05$). Gypsovags increased Ca concentrations up to 1.15-fold when growing on gypsum ($P < 0.05$), whereas gypsophiles had similar Ca concentrations on both substrates. For leaf Cr and Mo, gypsophiles had greater concentrations when grown on gypseous soil ($P < 0.05$), whereas gypsovags had similar concentrations in both soils. In general, plants grown on calcic soil had higher P, K and C and lower S, Mo, Li, Mn, Cu and Mg, than those cultivated on gypseous soils ($P < 0.05$).

Table 2.4: Means and standard deviation of leaf elemental concentration ($mg \cdot g^{-1}$) for each treatment. Upper case letters indicate significant differences between gypsophiles and gypsovags regardless of the soil type ($P < 0.05$) Lower case letters indicate significant differences between soil types ($P < 0.05$) within each gypsum affinity group.

Element ($mg \cdot g^{-1}$)	Gypsovags				Gypsophiles			
	Calcic		Gypseous		Calcic		Gypseous	
Al	0.3±0.1	A	0.3±0.1	A	0.5±0.6	B	0.4±0.4	B
C	429.0±47.7		427.7±47.7		389.7±47.7	a	378.8±56.7	b
Ca	26.4±12.3	a	30.3±14.1	b	46.9±22.4		46.0±21.8	
Cr	$1.3 \cdot 10^{-2} \pm 1.1 \cdot 10^{-2}$	A	$1.5 \cdot 10^{-2} \pm 1.7 \cdot 10^{-2}$	A	$3.4 \cdot 10^{-2} \pm 7.3 \cdot 10^{-2}$	Ba	$2.4 \cdot 10^{-2} \pm 4.7 \cdot 10^{-2}$	Bb
Cu	$1.0 \cdot 10^{-2} \pm 6.1 \cdot 10^{-3}$		$1.2 \cdot 10^{-2} \pm 9.4 \cdot 10^{-3}$		$1.2 \cdot 10^{-2} \pm 6.7 \cdot 10^{-3}$	a	$1.3 \cdot 10^{-2} \pm 5.6 \cdot 10^{-3}$	b
Fe	0.3±0.1	A	0.3±0.1	A	0.5±0.7	B	0.4±0.5	B
K	10.4±3.2	Aa	8.3±3.3	Ab	8.2±4.1	Ba	6.2±2.3	Bb
Li	$4.5 \cdot 10^{-3} \pm 4.5 \cdot 10^{-3}$	a	$6.8 \cdot 10^{-3} \pm 7.3 \cdot 10^{-3}$	b	$3.6 \cdot 10^{-3} \pm 3.0 \cdot 10^{-3}$	a	$5.4 \cdot 10^{-3} \pm 5.5 \cdot 10^{-3}$	b
Mg	4.1±2.1	A	4.1±2.0	A	8.8±5.3	B	10.8±7.4	B
Mn	$6.2 \cdot 10^{-2} \pm 2.8 \cdot 10^{-2}$	A	$5.7 \cdot 10^{-2} \pm 2.8 \cdot 10^{-2}$	A	$7.8 \cdot 10^{-2} \pm 2.8 \cdot 10^{-2}$	Ba	$6.4 \cdot 10^{-2} \pm 2.2 \cdot 10^{-2}$	Bb
Mo	$5.6 \cdot 10^{-3} \pm 5.6 \cdot 10^{-3}$		$7.8 \cdot 10^{-3} \pm 7.7 \cdot 10^{-3}$		$6.1 \cdot 10^{-3} \pm 4.4 \cdot 10^{-3}$	a	$1.7 \cdot 10^{-2} \pm 2.0 \cdot 10^{-2}$	b
N	17.3±8.8		15.9±8.9		15.3±8.1		14.5±7.4	
Na	$1.0 \cdot 10^{-1} \pm 6.5 \cdot 10^{-2}$	A	$8.7 \cdot 10^{-2} \pm 3.0 \cdot 10^{-2}$	A	$1.4 \cdot 10^{-1} \pm 6.1 \cdot 10^{-2}$	B	$1.3 \cdot 10^{-1} \pm 6.1 \cdot 10^{-2}$	B
P	2.3±1.5	a	1.1±0.6	b	2.4±2.6	a	1.0±0.7	b
S	5.9±4.2	Aa	7.7±4.7	Ab	15.6±7.6	Ba	24.4±16.4	Bb
Si	1.0±0.2		1.0±0.2		1.0±0.3		1.0±0.3	
Ti	$3.7 \cdot 10^{-3} \pm 1.3 \cdot 10^{-3}$		$4.2 \cdot 10^{-3} \pm 2.1 \cdot 10^{-3}$		$5.6 \cdot 10^{-3} \pm 5.8 \cdot 10^{-3}$		$5.5 \cdot 10^{-3} \pm 5.6 \cdot 10^{-3}$	
Zn	$5.1 \cdot 10^{-2} \pm 3.2 \cdot 10^{-2}$		$5.3 \cdot 10^{-2} \pm 2.9 \cdot 10^{-2}$		$5.1 \cdot 10^{-2} \pm 4.9 \cdot 10^{-2}$		$5.0 \cdot 10^{-2} \pm 3.8 \cdot 10^{-2}$	

Despite these general trends, some species-specific trends were observed. In accordance with the PCA results, the gypsophile *H. fruticosa* was closer to gypsovags than gypsophiles in both soils, whereas the opposite was true for the gypsovag *H. syriacum* (Fig. 2.2, and Appendix, Fig. B.2). Furthermore, some species of gypsophiles and gypsovags shifted their leaf elemental composition between soil types ($P < 0.05$, Appendix B, Table B.7), as observed in distance biplots (Fig. 2.2 and Appendix B, Fig. B.1) or the heatmap of distances (Appendix B, Fig.B.3). Shifts in leaf elemental composition were mainly related to leaf S, K, P, regardless of gypsum affinity (Appendix B, Tables B.8 and B.9).

2.4 Discussion

In contrast to our expectations, gypsophiles had equal or better growth and fitness when growing in calcic *vs*. gypseous soils. Gypsovags also had similar or higher growth and fitness in calcic soil than gypseous soil, which is not surprising owing to their widespread occurrence on both substrates. In support of our second hypothesis, gypsophiles showed higher S and Mg concentrations than gypsovags irrespective of the soil type. However, both groups of plants shifted their leaf elemental composition according to soil nutrient availability and had higher leaf S and Mg when growing on gypseous soils. Despite these general trends, species-specific responses were observed within gypsum affinities.

Gypsophiles completed their life cycle on calcic soil, being similarly or even more productive than on gypseous soil

Gypsum-exclusive species are restricted to gypseous soils in natural environments. However, we observed that gypsophiles were able to complete their life cycle, producing viable seeds in calcic soils in the greenhouse. This result demonstrates that soil chemistry alone is not a factor preventing the occurrence of gypsophiles off gypseous soils. This result is supported by field observations in Spain indicating that, even though gypsophiles are far more frequently found on gypseous soils, they are sometimes also found naturally off gypseous soils (Luzuriaga et al., 2015; Mota et al., 2011). Nevertheless, it is unclear if the few gypsophile individuals found growing off gypseous soils in nature could complete their life cycle, producing viable seeds and recruiting new individuals, since most data

were observations of presence / absence. In any case, care should be taken when extrapolating results from experimental studies to natural conditions (Wenk and Dawson, 2007). Our experiment involved regular de-weeding and watering, removing any potential competition from neighbouring plants or water stress, conditions that are far from those in natural environments. The combination of different stress factors (plant competition, drought and altered soil chemistry) could be the underlying mechanism explaining gypsophile restriction to gypseous soils, rather than soil chemistry alone, as demonstrated by our experiment. Further experiments on natural gypseous and calcic soils testing for the combined effects of soil chemistry plus plant competition and water availability are needed to shed light on these issues.

In contrast to our first hypothesis, some gypsophiles were more productive on calcic than on gypseous soil, showing higher photosynthetic assimilation rates, higher SLA and larger biomass. Ballesteros et al. (2014) found poorer plant performance on marls than gypseous soils. Our calcic soil had higher pH, N, P and K and lower conductivity, S and Mg concentrations, indicating better conditions than gypseous soil for standard plant growth. Both gypsum affinity groups showed a delay in the initiation of flowering when growing on gypseous soils, probably due to the more stressful conditions of gypsum for plant growth. However, we observed that gypsophiles showed a consistent phenological delay compared to gypsovags. Such a phenological delay has been described in the literature (Escudero et al., 2015), although the ecological and adaptive factors behind it remain unexplored. Furthermore, gypsophiles did not show any leaf deficiency symptoms and had similar maximum flower production and individual seed mass in both soils, similar to gypsovags. Similarly in Chapter 1, we observed that gypsophiles had low germination in acidic soils but germinated equally well on alkaline calcic and gypseous soils. Consequently, gypsophiles seem to require soils with high pH and high Ca availability to germinate and complete their life cycle, but do not have a requirement for high S or gypsum to grow and complete their life cycle under experimental conditions.

Gypsophiles displayed higher leaf S and Mg and lower leaf K than gypsovags both in and off gypsum

In accordance with our second hypothesis, gypsophiles had higher leaf S and Mg and lower K concentrations than gypsovags in both soil types. This pattern indicates a high preference of gypsophiles for these two elements, in accordance with previous studies of plants growing on gypseous soils, where the S and Mg concentrations of gypsophiles tended to be higher than those of gypsovags (Alvarado, 1995; Muller et al., 2017; Palacio et al., 2007).

The ability of gypsophiles to accumulate S was remarkable, reaching S foliar concentrations between 15 $mg \cdot g^{-1}$ and 25 $mg \cdot g^{-1}$, one order of magnitude higher than standard foliar S concentrations of non S-deprived plants (Kalra, 1997). Such high S-accumulation was maintained even when grown in calcic soil, which had 55-fold less S than the gypseous soil. Despite the lower S availability, gypsophile species managed to grow without any signs of deficiency and accumulated S to a higher extent than closely-related gypsovags on calcic soil. We cannot rule out the possibility that S-accumulation is a nutritional requirement of gypsophiles that may impede the completion of their life cycle or their competitive ability in natural conditions. The S content in calcic soils under greenhouse conditions could be sufficient, but the situation may be different in the field, with lower water availability and increased plant-plant competition. Finally, gypsophiles showed higher leaf S than gypsovags, although some gypsovags also had relatively high leaf S in both soil types. High leaf S concentrations are not exclusive of gypsophiles but also related to phylogenetic effects (Neugebauer et al., 2018). It has been suggested that the ability to accumulate S could be an ancient trait, evolved before the acquisition of gypsophily, that may serve as a pre-requisite to become a gypsophile (Moore et al., 2014).

The low leaf K (below 8 $mg \cdot g^{-1}$) of gypsophiles could be an adaptation to gypseous soils, since low K requirements may be advantageous in soils with low K availability (Alvarado, 1995), such as gypsum (Casby-Horton et al., 2015). Low K requirements are linked to high leaf Ca concentrations in the gypsophile species studied (Alvarado, 1995), since plants have a preference for using Ca ions over other cations such as K, Na or Mg as osmotic compounds (Kinzel, 1989). High leaf Ca has been considered a distinctive trait of gypsophiles (Muller et al., 2017; Palacio et al., 2007), although we did not observe differences between gypsum affinity types. However, gypsophiles showed high leaf Ca

concentrations irrespective of the soil type, whereas gypsovags increased Ca concentrations when growing on gypseous soil. This shift can be explained by the higher Ca activity of gypsum as compared to calcic soils (FAO, 1990). These results seem to indicate a higher ability to uptake Ca in gypsophiles than gypsovags, although further experiments are needed.

Gypsophiles species had higher leaf Mg than gypsovags, with increased Mg accumulation on gypsum, where it was highly available. However, neither group of plants shifted Mg concentrations in response to changes in the substrate. Mg accumulation is also a distinctive trait of gypsophile species (Merlo et al., 2019; Palacio et al., 2007). However, Mg concentrations are deeply affected by phylogenetic relationships (White et al., 2018), and some gypsophiles, such as *H. squamatum* and *L. subulatum,* did not show an accumulator pattern, as described by Merlo et al. (2019). It has been suggested that high Mg concentrations could be advantageous in gypseous soils, favouring foliar succulence (Merlo et al., 2019) or forming crystals with oxalate or sulphate to help detoxify excess S and Ca (He et al., 2012).

Finally, we observed higher leaf Fe, Al, Na, Mn, Cr and Mo concentrations in gypsophiles than gypsovags. Similar to our results, Alvarado et al. (1995) found higher leaf Fe and Mn in gypsophiles compared to gypsovags. Leaf Na was analysed only in few gypsum plant surveys (Bolukbasi et al., 2016; Merlo et al., 2019); where significant differences between gypsum affinities were not observed. Differences in Cr, Mo and Mn between gypsophiles and gypsovags are difficult to understand, although Mo and Mn are linked to S metabolism (Courbet et al., 2019; Maillard et al., 2016). Despite these general trends, species responded differently to each soil and had different leaf elemental concentration, indicating species-specific responses within gypsum affinities mainly related to S, K and Mg concentrations.

Gypseous soils affect the leaf elemental composition of plants

Plants grown in gypseous soils had higher leaf S, Mg, Li and lower P and K, mirroring their soil nutrient availability, and also higher Cu and Mn. Thus, gypseous soils affect the leaf elemental composition of plants (Palacio et al., 2007; Salmerón-Sánchez et al., 2014), leading mainly to high leaf S and low P regardless of the gypsum affinity of the species (FAO, 1990). Similarly, Boukhris &

Lossaint (1970) and Robson et al. (2017) observed that plants had high leaf Ca when cultivated on both gypsum and calcic soil, but less S when growing out of gypsum, according with soil nutrient availability. The mechanisms of P cycling in plants growing on gypsum deserve further study, due to the high relevance of this nutrient for plant growth and the remarkable P immobilization in gypseous soils (FAO, 1990).

2.5 Conclusions

Gypsophile species grew and were able to complete their life cycle in non-gypseous soils under experimental conditions and in the absence of competition, producing flowers and fruits which rendered viable seeds. Gypsum endemics had similar or higher growth on calcic than gypseous soil. Most species shifted their leaf elemental composition according to nutrient soil availability, displaying higher leaf S and lower P in gypseous soils. However, gypsophiles accumulated higher S and Mg and lower K concentrations than gypsovags, irrespective of the substrate. The remarkable ability of gypsophiles to accumulate S even in low S-availability conditions suggests a possible nutritional requirement for high S. However, our results indicate this nutritional requirement may not be the unique driver of the exclusion of gypsophiles from non-gypseous soils in natural environments, and the role of other biotic (plant-plant competition, herbivory) and abiotic (water stress) factors deserves further study.

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Author contributions

GM and SP designed and set up the experiment; AC, maintained the experiment, measured all variables, analysed data and led the manuscript writing; JPF measured and discussed photosynthesis and related traits; All authors discussed the results and wrote the manuscript.

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Chapter 3

Effect of animal disturbance on gypsum plant communities in the Middle Ebro Basin. By Gabriel Montserrat

3 When disturbances favour species adapted to stressful soils: grazing benefits soil specialists in gypsum plant communities

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Abstract

Question: What is the effect of grazing on plant communities rich in edaphic endemics growing on extreme soils?

Location: Plant communities on gypsum soils in the Ebro Valley (Spain)

Methods: We evaluated the effect of different grazing intensities on the assembly of perennial plant communities growing on gypsum soils. We considered the contribution of key functional traits of plants such as species gypsum affinity, traits related to gypsum specialisation (leaf S accumulation) or traits related to plant tolerance to herbivory such as leaf C and N concentrations. The effect of grazing intensity on plant community indices (i.e. richness, diversity, Community weighted-means (CWM) and functional diversity (FD) indices for each trait) were modelled using GLMMs. We analysed the relative contribution of interspecific and intraspecific trait variation (ITV) in shifts of community index values.

Results: Livestock grazing benefited gypsum plant specialists during community assembly, as species with high gypsophilic values, and high leaf S content, were more likely to assemble in the most grazed plots, especially at medium grazing intensities. Grazing also promoted species with traits related to herbivory tolerance, as species with a rapid-growth strategy (high leaf N, low leaf C) were selected under high grazing conditions. Species that eventually formed gypsum plant communities had sufficient functional variability among individuals to cope with different grazing intensities, as intraspecific variability was the main component of species assembly for CWM values.

Main conclusions: Livestock promotes edaphic specialisation of gypsum plants, pointing at herbivory as a driver of plant evolution on extreme soils. The positive effects of moderate grazing on plant

communities in gypsum soils indicate that livestock grazing may be a key tool for the conservation of these edaphic endemics.

Keywords: gypsophile, gypsovag, mineral nutrition, edaphism, functional diversity, plant–herbivore interactions, gypsophily, intraspecific variability

3.1 Introduction

Herbivory by domestic and wild ungulates is one of the main drivers of global vegetation dynamics. Grazing mammals affect plant performance by biomass removal (Huntly, 1991) and, accordingly, plants have developed a wide array of adaptations to cope with grazing disturbance throughout evolution (Díaz et al 2007). Grazing is usually considered to be a crucial biotic filter by restricting the range of trait values that allow species to survive and establish successfully (Violle et al., 2007). Grazing can exert contrasting effects on plant community properties. In productive environments, grazing may alleviate plant-plant competition through changes in competitive hierarchies (Louda et al., 1990; Noy-Meir et al., 1989). Trampling associated with grazing may create spatial heterogeneity (Moret-Fernández et al., 2011), and thus allow species coexistence due to niche differentiation (Rosemond et al., 1993). However, the selective removal of less grazing-tolerant species may result in a reduction in species richness and diversity (Milchunas et al., 1988). Overall, it is usually considered that grazing may restrict species richness when soil resources are scarce and plant biomass productivity is limited (Cingolani et al., 2005).

Extreme soils, such as saline, limestone, serpentine or gypsum, have particular physical and chemical characteristics that restrict plant growth and species distribution (Kazakou et al., 2008; Moore et al., 2014; Munns and Tester, 2008; Rorison, 1960a). Due to these constraints, atypical substrates are also major drivers of plant evolution (Hulshof and Spasojevic, 2020), leading to the development of specialised floras with numerous edaphic-endemics (Braun-Blanquet, 1932). Plants have developed different mechanisms to cope with the harsh conditions of extreme soils, and edaphic-endemics are usually soil specialists with substrate-specific strategies (Kruckeberg and Rabinowitz, 1985). These strategies allow them to optimise their performance and growth over other plants in their singular habitat (Cody, 1978), but may render them less competitive on standard soils, which would explain why edaphic-endemics are frequent in plant communities associated with extreme soils (Rajakaruna, 2004).

The assemblage of plant communities on extreme soils has traditionally been explained in relation to plant adaptation to the limiting conditions of these special substrates (Caçador et al., 2007; Kazakou et al., 2008; Luzuriaga et al., 2020, 2015). Plant communities developed on extreme soils are open

shrublands or grasslands (Brady et al., 2005; Mota et al., 2017), generally associated to large mammal herbivory and livestock grazing (Asner and Levick, 2012; Bakker et al., 2016). Consequently, the effect of grazing on plant community conformation may add to that of soil restrictions in these systems. Evidence of the effect of grazing on vegetation in extreme soils is controversial. Ballesteros et al. (2013) and (Pueyo et al., 2008) reported negative consequences on the abundance of two gypsum endemic species due to grazing, while other studies found that livestock grazing favoured edaphic-endemics over other plant species in extreme soils (Beck et al., 2015; Bonis et al., 2005).

Gypsum plant communities offer an excellent study system to evaluate the joint effect of extreme soils and herbivory on plant community assemblage. The weathering of gypsum rock generates an unusual soil with high Ca and S content that severely limits plant life (Casby-Horton et al., 2015; FAO, 1990). Gypsum soils occur worldwide in drylands, where extensive grazing frequently occurs (Akhani, 2015; Pueyo et al., 2008). The flora associated with gypsum is a unique endemic flora identified as an international conservation priority (Escudero et al., 2015; Moore et al., 2014), composed by edaphic-endemics, which are soil specialists (Palacio et al., 2007), and also species with wide ecological amplitudes (Meyer, 1980). Gypsum endemics show generally high affinity for gypsum soils (Luzuriaga et al., 2015), also referred as gypsophilic value (Mota et al., 2011), and share a singular foliar chemical composition linked to gypsum soils characterized by high foliar S-accumulation (Duvigneaud and Denaeyer-De Smet, 1966). Braun-Blanquet & Bolòs, (1958) suggested that plant communities rich in edaphic-endemics might be favoured by moderate grazing in gypsum soils. However, no previous studies have evaluated the effect of livestock grazing on the assembly of species with different affinity for gypsum soils, or its relationship to the foliar composition of gypsum plants.

In this context, it is timely to unveil the role that key functional traits play in the conformation of species assemblages on extreme soils with different intensities of herbivory. Plant functional traits that favour persistence under grazed conditions can be classified into avoidance and tolerance mechanisms (Briske and Richards, 1995). Avoidance mechanisms include traits that reduce plant accessibility and palatability, whereas tolerance traits lead to increased growth rate to compensate for biomass loss due to grazing. Leaf C and N content are known to be good estimators of species tolerance to herbivory (Capó et al., 2021), being high N content generally related to high growth rates (Pérez-Harguindeguy et

al., 2013) and thus, to species that are able to compensate the biomass lost by herbivory (Grime, 2006). Moreover, leaf S content is a functional trait related to plant specialisation to gypsum soils (Cera et al., 2021; Merlo et al., 2019), although its ecological significance remains unknown (Palacio et al., 2007). High leaf S content could play a significant role in grazing-resistance in gypsum environments (Palacio et al., 2014a), because foliar S accumulation has been related to herbivore-deterrent compounds in *Brassicales* (Ernst, 1990), and in some species of *Acacia* (He et al., 2015).

The aim of this study was to evaluate the extent to which the assembly of perennial plant communities under different grazing intensities on high gypsum soils is mediated by gypsum affinity of species, by species traits related to gypsum adaptation (leaf S concentration) or by traits related to plant tolerance to herbivory such as leaf C and N concentrations. If stressful conditions derived from atypical gypsum soils are the main selective force shaping plant communities on gypsum, we would expect gypsum-endemics to dominate over other species, independently of the grazing pressure. In this sense, the assembly of plant communities in gypsum environments would mainly depend on the gypsum affinity of plants (Luzuriaga et al., 2015, 2020) and species with high S leaf content would be always dominant in plant assemblages. However, if herbivory is the main factor modelling plant assembly on gypsum plant communities, species with a rapid-growth strategy (i.e. species with high leaf N content; tolerance mechanisms; Grime, 2006) and/or with deterrent traits (avoidance mechanisms, Briske & Richards, 1995) would be dominant under high grazing pressure in relation to non-grazed conditions. Finally, if as expected, herbivores have historically played an important role in shaping plant assembly in atypical soils of the Mediterranean region (Braun-Blanquet and Bolòs, 1958; Montserrat-Martí and Gómez-García, 2019), edaphic-endemics should have developed both mechanisms to tolerate or avoid grazing and mechanisms to persist in restrictive soils. In this context, species with high affinity for gypsum and traits to cope with restrictions typical of gypsum soils (i.e. leaf S-accumulation), also fitted with traits to tolerate (i.e. high growth rates) and/or avoid herbivory would be favoured under grazing conditions.

We scaled traits at the community level using two indices (Garnier *et al.*, 2016): the community-weighted mean (CWM), to quantify the mean contribution of species with different trait values to each species assemblage; and functional diversity (FD), to measure the dispersion of the trait in a given

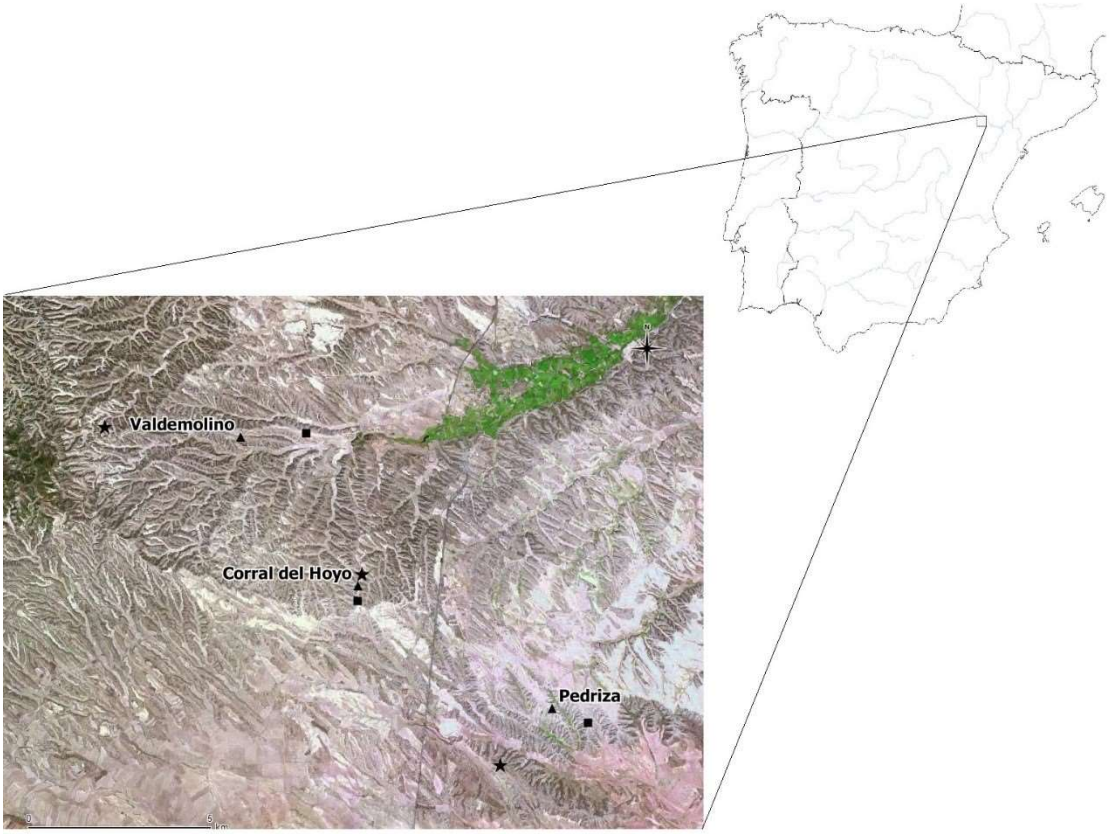
assemblage. We also evaluated the contribution of intraspecific trait variability to cope with different levels of herbivory during the species assembly process. For this purpose, we applied the method proposed by Lepš et al., (2011) and de Bello et al., (2011) to disentangle the effects of interspecific vs intraspecific trait variability on species assembly. In this context, if grazing acted as a biotic filter selecting the species better adapted to grazing conditions, then species assembly would be mainly due to species turnover. Conversely, if species have sufficient functional variability among individuals to cope with different intensity of grazing conditions, then species assembly would be mainly due to intraspecific trait variability.

3.2 Materials and Methods

Study site

This study was conducted in three sities in the Middle Ebro Valley (NE Spain): Pedriza (Mediana de Aragón 41°24'17"N, 0°41'20"W), Corral del Hoyo (Mediana de Aragón 41°25'38"N, 0°44'45"W) and Valdemolino (Mediana de Aragón 41°27'30"N, 0°45'31"W) (Fig. 3.1). All of them show gypsum soils as their main lithology and have a semi-arid Mediterranean climate with mean annual temperature of 14.9 ºC and mean total annual rainfall of 353.9 mm yr^{-1} (data from the nearest weather station at Farlete 41º50’56” N, 0º30’19” O). The landscape of this area consists mainly of low hills (480 m.a.s.l. average) and flat-bottomed valleys, which are currently cultivated (Foronda *et al.*, 2019). Above-ground vegetation in the three locations was predominantly composed of shrubs, forbs and grasses, like *Brachypodium retusum* (Pers.) P. Beauv., *Gypsophila struthium* subsp. *hispanica* (Willk.) G. López, *Helianthemum squamatum* Pers., *Herniaria fruticosa* L. and *Plantago albicans* L. The vegetation structure in our study sites was a matrix of plant patches and bare soil, with total vegetation cover of 25.45 % ± 12.97 on average.

Figure 3.1: Distribution of locations and their sites. Stars are low grazing sites. Triangles are medium grazing sites. Squares are high grazing sites.



Plant community surveys

We conducted vegetation surveys in June 2018 along three grazing gradients, one in each locality. We used three independent grazing gradients to reduce the influence of other environmental variables, however they are selected in the same region to avoid climatic biases. Gradients were established by selecting three flat hilltop sites with different grazing intensity in each locality (Fig. 3.1). Each gradient included one site with no grazing from several years ago (hereafter referred to as low grazing), one site with medium grazing and one site with high grazing pressure. Grazing intensity was estimated by counting pellets and interviewing local farmers (Pueyo *et al.*, 2013). Thirty-five 2 x 2 m plots were randomly established at each site in the three localities (N = 315 plots). In each plot, every species occurrence was registered and species cover was visually estimated. Total vegetation cover and maximum vegetation height were measured per plot. Species were identified in the field and revised

taxonomically in the laboratory using specific literature (Aizpuru *et al.*, 1999; Castroviejo, 1986). Nomenclature followed The International Plant Names Index (IPNI, 2021).

Soil sample collection and analyses

Three soil samples per site were collected from 5 to 15 cm depth, removing the surface crust (N = 27). Physical and chemical properties were analysed (Appendix C, Table C.1). All soil samples were air dried for 2 months and subsequently sieved through a 2 mm sieve before physical and chemical analyses. Gypsum content was measured according to Artieda *et al.* (2006). Soil texture was determined with a particle laser analyser (Mastersizer 2000 Hydro G, Malvern, UK). Soil pH and conductivity were measured with a pH/conductivity meter (Orio StarA215, Thermo Scientific, Waltham-MA, USA) by diluting samples with distilled water to 1:2.5 (w/v) and 1:5 (w/v), respectively. A subsample of each sieved soil was finely ground using a ball mill (Retsch MM200, Restch GmbH, Haan, Germany) and subsequently used to analyse the elemental concentrations of N and C with an elemental analyzer (TruSpec CN, LECO, St. Joseph-MI, USA), elemental analyses were performed by EEZ-CSIC Analytical Services.

Measures of plant traits

All perennial species recorded were classified by their affinity to gypsum soils, according to the gypsophilic value (hereinafter GV; Mota *et al.*, 2011), as performed in Luzuriaga *et al.* (2020). The GV was calculated by a group of experts on gypsum flora of the Iberian Peninsula, using the Delphi technique (Mota et al., 2009). The GV ranges from 1 (species that avoid gypsum soils) to 5 (species strictly linked to gypsum soils) and GV = 2 to gypsovag species not included in Mota *et al.* (2011), as all species found in our study were able to grow on gypsum soils.

Leaf traits were measured at three sites, covering a three-level gradient of grazing, in one locality (Valdemolino) and from 14 perennial species, which were present in the three sites in the locality and accounted for 75.6 % of total plant cover (Table 3.1). Leaf samples were collected from five different individuals per species at each site. Mature, non-senescent and undamaged leaves were collected. To assess the N, C and S concentrations in leaves, leaf samples were dried to a constant weight at 50 ºC

during five days and subsequently finely ground using a ball mill (Retsch MM200, Restch GmbH, Haan, Germany). N, C and S were analysed with an elemental analyser. N concentrations were analysed in EEZ-CSIC Analytical Services (TruSpec CN, LECO, St. Joseph-MI, USA), and C and S concentrations were measured in IPE-CSIC Analytical Services (TruSpec CNS, LECO, St. Joseph-MI, USA).

Table 3.1: Total cover (%) and standard error per plot of all the species with functional traits values.

	Low	Medium	High
Corral del Hoyo	59.9±4.7	82.4±5.2	100.0±3.2
Pedriza	67.2±2.4	67.2±4.4	56.5±4.2
Valdemolino	93.9±3.5	84.2±4.1	80.2±2.4
Averaged total	73.7±3.5	77.9±2.7	80.1±2.7

Community level indices and statistical analyses

All statistical analyses and graphics were performed using R version 4.0.2. To characterize gypsum affinity and leaf trait values (S, N and C leaf contents) at the community level, we calculated Community Weighted Means (CWM), and Functional Diversity indices (FD). The CWM quantifies the mean contribution of species with different traits to each species assemblage (Garnier *et al.*, 2016). It was calculated as:

$$CWM = \sum_{i=1}^{n} p_i trait_i \quad (1)$$

Where p_i represents the proportion of species i and $trait_i$ the value of that specific trait for the species i. We used the *dbFD* function in FD package version 1.0-12 (Laliberté and Shipley, 2011).

The FD evaluates the dispersion of trait values in the community. It was calculated as:

$$FD = \sum_{i=1}^{n} \sum_{j=1}^{n} p_i p_j d_{ij} \quad (2)$$

Where p_i and p_j represent the proportion of species i and j, respectively; d_{ij} the distance between both species in the functional space. We used the quadratic diversity of RaoQ (Botta-Dukát, 2005) using the *melodic* function (de Bello *et al.*, 2016) and Gower dissimilarity matrices of species-specific trait values. Also, we calculated Simpson index using the *melodic* function, as:

$$E = 1 - \sum_{i=1}^{n} p_i^2 \quad (3)$$

A PERMANOVA based on Bray-Curtis distances and type III Sum of Squares was performed to assess differences in species composition among the 315 plots using *adonis* function in vegan package

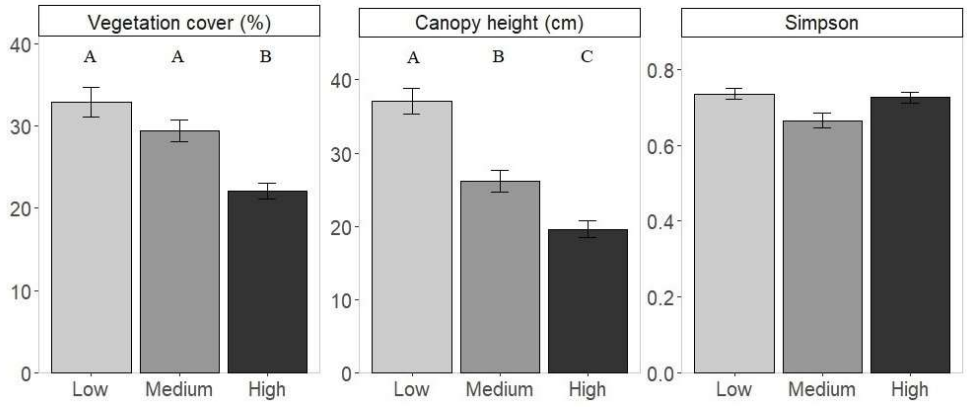
version 2.4-6 (Oksanen *et al.*, 2007). To prepare data for PERMANOVA, species that appeared in less than 5 % of the plots were removed, to avoid statistical biases of rare species, and cover data were square root-transformed. Grazing intensity was considered as a fixed factor with three levels, and locality as stratum. Soil properties were included in the model as a covariate. This covariate was the main ordination axis of a Principal Component Analysis (PCA) performed with the soil physicochemical features measured at each site after scaling all variables using the *rda* function in the vegan package version 2.4-6 (Oksanen *et al.*, 2007). Non-metric Multidimensional Scaling (NMDS) was used to represent relationships among species composition, environmental features (pH, conductivity, soil C, soil N, gypsum content, and sand, loam and clay proportions), and grazing intensity of plots. We used cover data of the 30 species from the 315 plots and *metaMDS* and *envfit* functions in vegan package version 2.4-6 (Oksanen *et al.*, 2007).

The effect of grazing intensity on plant community properties was evaluated using Generalised Linear Mixed Models (GLMM) with grazing intensity as a fixed factor, and plot nested into locality as random factors. We modelled eleven response variables at the community level: total vegetation cover, maximum canopy height, taxonomic diversity (Simpson index) and CWM and FD indices for gypsum affinity of species and three leaf functional traits (S, N and C contents). Over- and under-dispersion of model residuals were detected using *simulateResiduals* function in DHARMa package version 0.3.1 (Hartig, 2017). *lmer* function was applied when normality of residuals was fulfilled, otherwise the Gamma distribution was applied using the *glmer* function in the lme4 package version 1.1.5 (Bates *et al.*, 2012/2007) or, in case residuals were over- or under-dispersed, we used the *glmmTMB* function in the glmmTMB package version 1.1 (Magnusson *et al.*, 2019). When differences were statistically significant, we assessed multiple comparisons among levels of grazing intensity with the *glht* function in multcomp package version 1.4-13 in R (Hothorn *et al.*, 2009).

We used the method proposed by Lepš *et al.*, (2011) and de Bello *et al.*, (2011) to disentangle the effects of interspecific vs intraspecific trait variability on species assembly processes. We calculated three components for each index (CWM and FD) per plot: 1) The “Fixed” component: was calculated using the mean value of each trait measured over all 15 individuals of that species in the three grazing intensity levels of the study; 2) The “Specific” component: was calculated using the trait average values

of each species measured over all five individuals of that species at that particular level of grazing intensity; 3) The "Intra-specific" component: was calculated as the average difference between specific and fixed values. Sum of Squares was calculated for each component and trait using a parametric method (*lmer* function) with plot nested within locality as random factors when residuals fitted a normal distribution, and a non-parametric one (*adonis* function with Euclidean distance) with locality as strata, otherwise. Finally, we plotted the Sum of Squares decomposition of each CWM and FD values on grazing intensity to understand the relative contribution of interspecific variation, intraspecific trait variation and their covariation in community composition under different grazing intensities.

Figure 3.2: Differences in community properties among grazing intensity levels. Mean values ± standard errors are represented. Different letters indicate significant differences among levels of grazing intensity after multiple comparison tests ($P < 0.05$).



3.3 Results

Variation in species composition

Total plant cover and canopy height decreased from low to high grazing intensities (Fig. 3.2, Table 3.2). In this study, we found 52 perennial plant species 10 of which increased their cover in plots from low to high grazing levels, 17 decreased their cover, and 25 species did not change their cover significantly in response to grazing (Appendix C, Table C.2). Plant composition was significantly different among grazing levels (F-ratio = 18.23, P-value = 0.001), as well as in response to soil features (F-ratio = 4.45, P-value = 0.002; Appendix C, Fig. C.1, Table C.3). Specifically, Soil C ($R^2 = 0.41$) and gypsum content ($R^2 = 0.38$) explained the largest proportion of variability in plant species composition (Appendix C, Table C.3).

Table 3.2: Effect of grazing intensity on the main features of plant community properties after GLMMs. Chi-square values obtained by Wald tests based on generalised linear mixed models plus the family of error distributions and link functions assumed in the models are indicated. Id: identity link function; Log: logarithmic link function. Superscripts indicate GLMMs were run with the glmmTMB function to correct for dispersion of residuals.

	Family (link)	Chis-square	Pr(>Chisq)
Vegetation cover (%)	Gamma (Log)[1]	41.889	0.001
Canopy height (cm)	Gamma (Id)[1]	76.541	0.001
Simpson Index	Binomial[1]	1.506	0.471

Gypsum affinity and leaf traits (C, N, S-contents) at the community level

Gypsum specialists were favoured over non-specialists with increasing grazing. Our results show higher CWM of gypsophilic values (referred to GV) on the most heavily grazed plots than on the less grazed ones (Fig. 3.3, Table 3.3). Furthermore, the FD of gypsophilic value was lower in plots with lower grazing intensity (Fig. 3.3, Table 3.3), indicating a narrow range of gypsophilic values in the species assemblage. Plants with a rapid-growth strategy (high leaf N, low leaf C) and high leaf S were more likely to assemble in the most grazed plots, since CWM values of leaf C decreased and CWM values of leaf N and leaf S increased in higher grazing intensities (Fig. 3.3, Table 3.3). Medium grazing intensity was the most heavily filtered level on leaf C and leaf N, since plant communities showed the lowest FD values. Whereas, FD values of leaf S decreased with increasing grazing pressure (Fig. 3.3, Table 3.3). However, all plots along the gradient were heavily filtered, since FD values of leaf traits were generally lower than the Simpson Index (leaf C = 0.22 ± 0.01, leaf N = 0.25 ± 0.01, leaf S = 0.18 ± 0.01, GV = 0.34 ± 0.01), which was similar along the gradient (Mean = 0.78 ± 0.01).

Overall, the process of species assembly in different grazing intensities was mainly determined by intraspecific shifts in functional traits (Fig. 3.4), since CWMs of leaf traits along the grazing intensity gradient were largely explained by ITV. Contrastingly, the amplitude of the range of trait values that occur in different grazing intensities was explained by species turnover, since shifts in FDs of studied leaf traits were explained mainly by interspecific trait variability (Fig. 3.4). Further, the contribution of interspecific and ITV on CWM and FD values were positively correlated for leaf N and negatively for leaf S. Leaf C showed a positive correlation for FD and negative for CWM values (Fig. 3.4).

Figure 3.3: CWM and FD of gypsophilic values and leaf traits at the community level in different grazing intensities. Means ± standard errors are shown. Different letters indicate significant differences among levels of grazing intensity (low, medium and high) after multiple comparisons ($P < 0.05$).

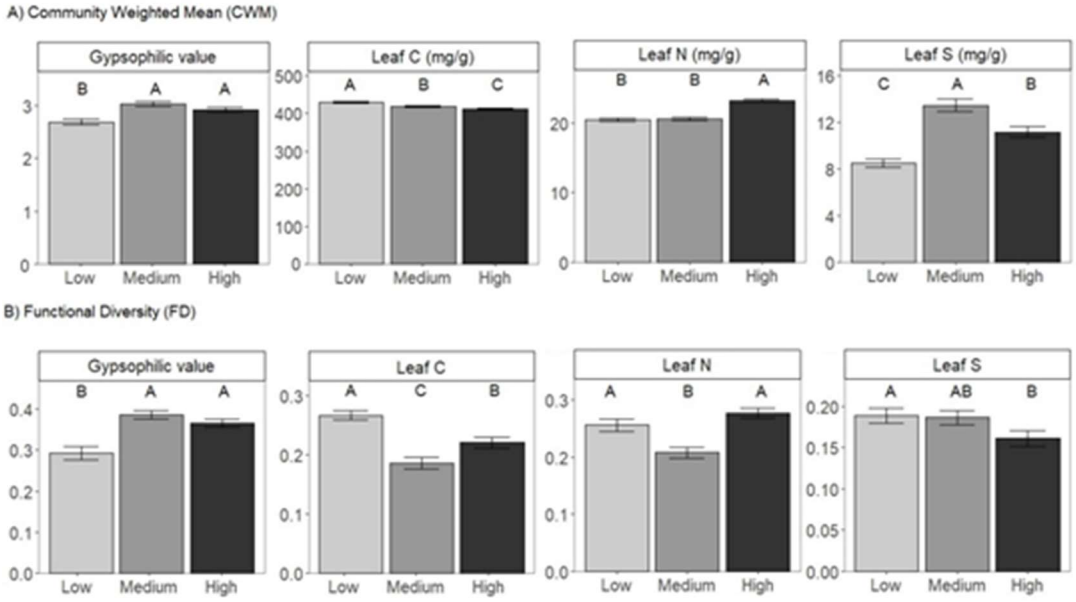


Table 3.3: Results of generalised linear mixed models (GLMMs) for community weighted mean (CWM) and functional diversity indices. Chi-square values were obtained by Wald test based on GLMMs. The family of error distributions and link functions assumed in each model are also indicated. Id: identity link function; Log: logarithmic link function.

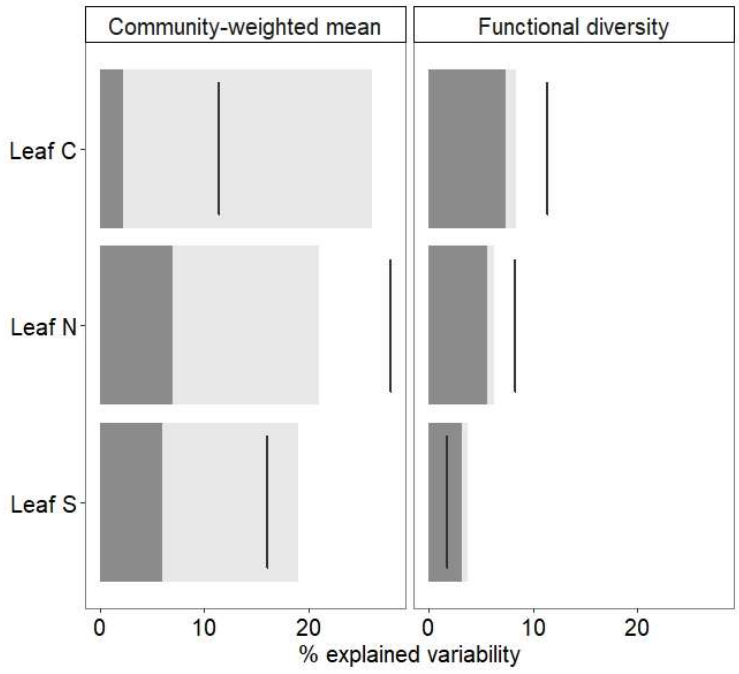
	Community-weighted mean		
	Family (link)	Chis-square	Pr(>Chisq)
Gypsophilic value	Gaussian	27.710	0.001
Leaf C	Gaussian	43.41	0.001
Leaf N	Gaussian	121.77	0.001
Leaf S	Gaussian	62.572	0.001
	Functional diversity		
	Family (link)	Chis-square	Pr(>Chisq)
Gypsophilic value	Gaussian	32.769	0.001
Leaf C	Gaussian	48.698	0.001
Leaf N	Gaussian	31.995	0.001
Leaf S	Gamma (Id)	6.7121	0.035

3.4 Discussion

Our results provide evidence that livestock grazing benefited soil specialist species during community assembly, at least in the range of grazing intensities evaluated in this study. Specifically, the CWM of the gypsophilic value and of the leaf S content of species increased in species assemblages

under medium and high grazing intensities. Although some studies found that certain soil specialists are more vulnerable to herbivory than their non-specialist relatives (Dechamps et al., 2008; Kay et al., 2011; Strauss & Boyd, 2011), our results highlight that under medium grazing pressure, species with higher affinity for gypsum soils were favoured. This is a remarkable result that aligns with previous studies of plant communities growing on extreme soils, such as white sands of tropical forests (Fine et al., 2004), serpentine grasslands (Beck et al., 2015) and saline soils (Bonis et al., 2005). All these studies found that the occurrence of edaphic specialists was dependent on herbivory, most likely because herbivores modified competitive plant-plant interactions (Grover and Holt, 1998; Louda et al., 1990) and may benefit the less competitive soil specialist species.

Figure 3.4: Decomposition of the variability in CWM and FD values explained by grazing intensity following Lepš et al. (2011). The dark grey portion of bars corresponds to the contribution of interspecific variability and the light grey portion to intraspecific effects. Black lines denote total variation. The ranges between the top of the bar and the black line correspond to the effect of covariation between inter and ITV; if the line is right to the bar the covariation is positive, and if the line crosses the bar, the covariation is negative.



Our study showed that species prone to accumulate S in their leaves were favoured in medium and highly grazed conditions (i.e. larger CWM values for the leaf-S trait). Leaf S-accumulation is usually related to gypsum specialist species (Merlo et al., 2019), but the specific ecological role of S-accumulation in gypsum plants remains unknown (Palacio et al., 2007). It has been described that a high translocation of the excess element in soil to plant tissues (i.e. S in gypsum soils) could be a strategy to optimise plant growth in extreme soils by avoiding interference of that specific element with plant metabolism (Kabata-Pendias, 2010; Tran et al., 2020). Other studies proposed that high leaf S content could be related to a herbivore-deterrent strategy to avoid biomass loss in nutrient-limited habitats (Ernst, 1990; Palacio et al., 2014a), as proposed for some species of *Brassicales* and *Acacia* (He et al., 2014; Tuominem et al., 2019). These results point at a selection of increased foliar S accumulation in plants growing on gypsum as a mechanism to deter herbivores (Boyd, 2007; Hoerger et al., 2013). Thus, herbivory may have acted as a selective force underlying the evolution of edaphic specialists (Fine et al., 2006; Lau et al., 2008), promoting the selection of foliar S accumulation in gypsum specialists.

Some authors suggested that when resource availability is scarce, the costs of losing plant tissues due to herbivory are high, and plants that invest in chemical defences would be selected (Coley et al., 1985), mainly because the ability to compensate biomass losses is dependent on resource availability (Strauss et al., 1999). Thus, tolerance to herbivory is expected to be low in plants from edaphically stressful substrates. Nevertheless, our results showed that species with higher leaf-N contents were selected in highly grazed areas compared to low grazed conditions (i.e. greater CWM of leaf-N and low leaf-C in highly grazed sites), suggesting that species with comparatively higher growth rates under the low resource availability of gypsum soils may have been favoured under grazing. These species could also be soil specialists. In the case of gypsum environments, soil specialists are expected to perform better in their atypical substrate than other soils (Cera et al., 2021). Also, they are more likely to assemble in gypsum soils than other soils (Luzuriaga et al., 2015), as we observed high CWM of the gypsophilic value along the gradient, regardless of grazing pressure. Consequently, our results indicate that traits of grazing tolerance (such as higher growth rates) are also selected for in gypsum plant communities subjected to grazing.

The effect of grazing on gypsum plant communities varied with grazing intensity. Low and medium grazed plots showed similar plant cover, but medium grazed plots displayed lower FD values (narrower range of trait values) than low and high grazed plots for leaf C and leaf N. Both leaf traits are linked to plants growth strategies (Grime et al., 1997; Pérez-Harguindeguy et al., 2016), indicating a narrow range of growth rate values of the species that assemble in medium grazed plots. These results can be explained by the effect of different grazing intensities on plant competiveness in the stressful conditions of gypsum soils. The higher FD in low grazed plots can be explained by the hypothesis of limiting similarity (Abrams, 1983). In these plots, there is high competition for resources due to the nutrient scarcity of gypsum soils (Boukhris and Lossaint, 1970). Species with different N requirements can coexist, because N acquisition niches do not overlap (Montesinos-Navarro et al., 2017), leading to higher FD values of traits related to growth strategy than medium grazed plots. Contrastingly, the top-down effect of sheep in high grazed plots seems to be due to disturbance associated to grazing that may reduce biomass of dominant species (Noy-Meir et al., 1989), alter the harsh physical crust typical of gypsum soils (Moret-Fernández et al., 2011) and eventually create new gaps for colonisation (Rosemond et al., 1993). These processes may allow species with contrasting growth rates to coexist in heavily grazed conditions, leading to higher FD values.

The relative contribution of species turnover and intraspecific trait variability on the species assembly process at different grazing intensities (Lepš et al., 2011) has been poorly analysed. Our results showed that intraspecific variability was the main component of species assembly for CWM values, while shifts in FD resulted from species turnover. The design of this study did not allow checking whether intraspecific variation was due to plastic responses of plants or heritable differences between genotypes (Bolnick et al., 2011). Nevertheless, the relevance of ITV would mean that the species that finally conformed gypsum plant communities had enough functional variability among individuals to cope with different grazing intensities. Considering that livestock has been a usual anthropogenic activity since the Neolithic era in the Iberian Peninsula (Balaguer et al., 2014), our results suggest that grazing has acted as an evolutionary driver over time, promoting a regional species pool fitted with successful strategies and enough functional variability among individuals to cope with herbivory. Consequently, in our study system, grazing did not act strictly as a biotic filter selecting certain species

and jeopardizing others, but it acted in a subtler way, modifying the range of values of each plant trait selected during plant community conformation. This is a remarkable result that aligns with previous studies on the effect of herbivory on plant community assembly in environments with a long evolutionary history of grazing, such as Tibetan alpine meadows (Niu et al. 2016), and Inner Mongolia grasslands (Zheng et al. 2015).

3.5 Conclusions

Grazing has likely been a powerful evolutionary driver in the conformation of plant assemblages on extreme soils. Our results indicate that herbivores most likely promoted the edaphic specialisation of plants in the extreme conditions of gypsum soils. Extensive livestock grazing, if tuned to adequate intensities to avoid over-grazing, may be a key tool to promote plant communities where soil specialists persist on extreme substrates, like gypsum soils.

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Author contributions

SP and GM conceived the study; SP, GM, AL and AC designed the methodology; YP identified the gradient; GM, SP and AC collected field data; AC measured plant and soil variables, supervised plant and soils analyses, analysed data and led the manuscript writing; SP, AL supervised statistical analysis. All authors discussed the results and wrote the manuscript.

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Chapter 4

Effect of browsing by sheep on *Gypsophila struthium* Loefl. in the Middle Ebro Basin. By Sara Palacio

4 Organ partitioning points at different nutritional strategies in gypsum endemic and non-endemic plants independent to clipping

Andreu Cera, Gabriel Montserrat-Martí, Sara Palacio. Manuscript in preparation to be submitted.

Abstract

Edaphic endemics are plants adapted to unusual soils, and they frequently show substrate-specific traits. Their adaptations have often been interpreted in relation to the harsh conditions of the soils they grow in. However, grazing also alters plant performance of edaphic endemics. Gypsum endemics are grazing-resistant species, and their unique foliar composition may confer better plant performance under grazing pressure. However, little research has been done on whether substrate-specific traits improve plants response to grazing. We studied the performance (plant biomass, height, root:shoot ratio) and the partitioning among different organs (leaves, stems, coarse roots, fine roots) of elements in five gypsum endemics and five non-gypsum endemics cultivated in gypsum and calcic soils and subjected to different levels of simulated browsing (unclipped controls and 75% shoot clipping). Clipping did not affect the height, root:shoot ratio or elemental composition of studied plants, but clipped plants of endemic and non-endemic species accumulated less biomass than control plants after one year of recovery. Gypsum endemics displayed generally higher concentration of S, Ca, Mg than non-endemics in all organs except fine roots. In addition, gypsum endemics showed higher pools of most elements in aboveground organs than non-endemics. Gypsum endemics and non-endemics did not compensate for biomass loss after clipping in gypsum and calcic soils. Endemics did not block excess elements in gypsum at the root level, tolerating high concentrations aboveground, while non-endemics accumulated them in roots. This unique elemental organ partitioning of endemics is maintained when plants grow off gypsum, but does not seem to be a grazing-induced mechanism.

Keywords: disturbance, mineral nutrition, soil specialist, functional ecology, drylands

4.1 Introduction

The weathering of rocks such as halites, calcites, serpentinites, dolomites or gypsum generates unusual soils with special features, which restrict plant growth and performance (Kazakou et al., 2008; Moore et al., 2014; Mota et al., 2021; Munns and Tester, 2008; Rorison, 1960b). Oftentimes the particular characteristics of unusual soils lead to strong nutrient imbalances, with important consequences for plant nutrition (Lambers et al., 2008b). For example, some elements are frequently found in excess of plant requirements, and may produce toxicity (Kabata-Pendias, 2010), yet other nutrients are found in low availability, limiting plant growth rates (Aerts and Chapin, 1999). The atypical characteristics of unusual soils linked to evolution lead to the development of floras enriched with edaphic-endemics (Hulshof and Spasojevic, 2020). These are species ecologically restricted to unusual soils (Kruckeberg and Rabinowitz, 1985) that often possess substrate-specific strategies to face the harsh conditions of these atypical soils (Mota et al., 2017). To cope with the strong ion imbalances in extreme soils, plants may accumulate excess elements in belowground organs, as a nutritional barrier (Tran et al., 2020). Alternatively, plants may show translocation of excess elements to shoots, but this requires the ability to tolerate high concentrations aboveground (Lux et al., 2021).

Herbivory affects plant performance by removing biomass (Huntly, 1991), and conditions the assemblage of plant communities (Jäschke et al., 2020). Grazing by domestic and wild ungulates is also an important factor in plant evolution (Díaz et al., 2007), and plants from frequently grazed ecosystems have evolved two main response strategies to grazing: avoidance or tolerance (Briske and Richards, 1995). These strategies depend on plant growth rates (van der Meijden et al., 1988): plants with rapid growth rates are usually grazing tolerant, with traits to compensate for biomass loss before recurrent disturbances (Grime, 2006). Examples of such compensating traits are increased photosynthetic activity or a rapid re- translocation of nutrients from roots to shoots when herbivory affects aboveground biomass (Hawkes & Sullivan, 2001). Conversely, plants with slow growth rates are often unable to compensate for biomass loss after herbivory (Grime, 2006), and usually follow an avoidance strategy, with high investment in plant defence (Briske and Richards, 1995). Further, plant responses to grazing

can be always present in the plant (constitutive traits) or produced only in response to grazing events (inductive traits; Moreira et al., 2014).

The ecological strategies of edaphic endemics have usually been studied in relation to their adaptation to the harsh conditions of unusual soils (Mota et al., 2017). However, grazing has also been shown to alter the ecology of edaphic-endemics, since atypical substrates are frequently open areas where herbivores thrive (Beck et al., 2015; Bonis et al., 2005). If herbivores contribute significantly to the performance of edaphic-endemics, they should possess some traits related to grazing resistance. Edaphic-endemics are often considered as stress-tolerant species with slow growth rates (Rajakaruna, 2004), because unusual soils are poor in essential nutrients for growth. They are, therefore, assumed to have a high investment in grazing avoidance mechanisms (Rajakaruna, 2004), although it is not known to what extent traits related to specialisation to unusual soils may mediate plant responses to herbivory.

Gypsum endemics are a good study case to analyse the functional responses of edaphic endemics to herbivory. They are soil specialists (Palacio et al., 2007), in a highly nutrient-limited soil with excess of Ca-Mg-S and scarcity of N-P-K (Casby-Horton et al., 2015; FAO, 1990). The high concentrations of Ca and S in the soil surpass plant nutrient requirements (Merlo et al., 1998), and could become toxic for some plants (Ernst, 1990). Furthermore, gypsum soils generally appear in drylands with severe water scarcity (Casby-Horton et al., 2015). Harsh edaphic and climatic conditions favour the presence of species with stress tolerant traits on gypsum soils (Hodgson et al., 1994), including slow growth rates (Grime, 2006). In addition, gypsum endemics show a remarkably higher leaf Ca, Mg, and S accumulation than coexisting non-gypsum endemics (Merlo et al., 2019; Muller et al., 2017; Palacio et al., 2007). The atypical leaf chemical composition of gypsum-endemics may point at an ability to tolerate excess elements by accumulating them in leaves, whereas non-gypsum endemics may block excess elements at the root level, accumulating them in roots. These nutritional strategies could be adaptations to the harsh soil conditions, but also to grazing resistance, as gypsum plant communities have historically been associated with disturbance by herbivores (Braun-Blanquet and Bolòs, 1958). Recent studies at the community level have shown that gypsum endemics are favoured under moderate grazing and that grazing promotes plants with increased foliar S concentrations (Chapter 4). At the same time, grazing also promoted species with rapid-growth rates in gypsum environments (Chapter 4). The

increased leaf S-accumulation of gypsum endemics may serve as an anti-herbivore mechanism (Palacio et al., 2014a). Similarly S-rich molecules like glucosinolates in *Brassicales*, or gypsum crystals in *Acacia* spp. have been suggested to play an anti-herbivore role (Ernst, 1990; He et al., 2014). However, no previous studies have evaluated the individual responses of gypsum plants to herbivory, and it is unknown up to what point the atypical foliar concentrations of gypsum endemics (in particular their remarkably high S concentrations) are a constitutive or inductive trait.

The objectives of this study were to analyse the plant performance (plant biomass, height, root:shoot ratio) and the whole plant partitioning of 15 elements among different organs (leaves, stems, fine roots, and coarse roots) in gypsum endemics and non-endemics cultivated in gypsum and calcic soils, and subjected to different levels of simulated browsing (removal of 66% aerial biomass). We hypothesised that: 1) the prevalence of gypsum endemics in grazed areas is due to avoidance rather than resistance mechanisms. Consequently, we expect them to be unable to compensate the biomass lost with clipping after one year. Owing to their specificity to gypsum soils, we expect gypsum endemics to have a higher ability to recover from clipping when grown on gypsum vs. calcic soils. 2) Gypsum endemic and non-endemic species will show differences in elemental partitioning across organs, particularly when grown on gypsum soils. Gypsum endemics will accumulate elements found in excess on gypsum (S, Ca and Mg) across the plant, but especially in leaves, while non-endemics will accumulate excess elements in fine roots as a nutritional barrier to avoid toxicity. 3) This nutritional strategy of gypsum endemics will respond to clipping by increasing the concentration and pool of S in leaves, as an induced mechanism of grazing-avoidance.

4.2 Materials and methods

Experimental design

Plants were germinated from seeds collected from natural populations on 0.06-L squared pots in April 2016. Half of the pots had calcic native soil and half had gypsum native soil (see Cera et al. (2021) or Chapter 2 for further details). Five months after transplantation, plants were cleared leaving one individual per pot. Seven months after germination (November 2016), plants were transplanted into 7-

L squared pots (large species) and 5.6-L squared pots (small species). Clipping treatments were applied in October 2017: five replicates of each species and soil were clipped and five were left unclipped as controls. The clipping treatment consisted in clipping 66 % of shoots with secateurs, leaving the apical stem undamaged and applying the same extent of leaf area removal to all replicates within a species. Plants were kept de-weeded and well-watered throughout the experiment by regular watering with tap water, and they were moved to a greenhouse between November and March to avoid freezing. All plants were harvested between September and November 2018, after a year of clipping and when plants were 29 month-old.

Study species

We selected representative gypsum endemics and non-endemics which are dominant in gypsum ecosystems of North Eastern Spain. All of them where sub-shrubs. Gypsophiles included *Gypsophila struthium* subsp. *hispanica* (Willk.) G. López., *Herniaria fruticosa* L., *Helianthemum squamatum* Pers., *Lepidium subulatum* L., *Ononis tridentata* L.; and gypsovag species were *Boleum asperum* Desv., *Helianthemum syriacum* (Jacq.) Dum. Cours., *Linum suffruticosum* DC., *Matthiola fruticulosa* (L.) Maire and *Rosmarinus officinalis* L. All gypsophile species selected show high affinity for gypsum soils in Spain (Mota et al., 2011).

Growth and plant biomass

Morphological measurements were taken before the application of the clipping treatment, and before harvest. Canopy height, maximum shoot length (measured from the base of the plant to the distant most leaf, hereafter canopy length), and the maximum canopy diameter and its perpendicular were measured using a millimetre ruler. At harvest, plants were fractionated in their main organs: leaves, stems, coarse roots and fine roots (< 2 mm of diameter). All clipped and harvested material was rinsed with water and dried in an oven at 50 ºC for 5 days. All dry plant fractions were weighted in a precision scale (42 g / 0.00001 g, MS105DU, Mettler Toledo, Columbus-OH, USA).

Elemental analyses

All dried organs were finely ground using a ball mill (Retsch MM200, Restch GmbH, Haan, Germany). N and C concentrations were analysed with an elemental analyzer (TruSpec CN, LECO, St. Joseph-MI, USA). The elemental concentration of Al, As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Si, Ti, V, Zn was measured by extracting samples with HNO_3-H_2O_2 (8:2) by microwave acid digestion (Speed Ave MWS-3+, BERGHOF, Eningen, Germany), followed by inductively coupled plasma-optical emission spectrometry (Varian ICP 720-ES, Agilent Technologies, Santa Clara-CA, USA). All elemental analyses were performed by EEZ-CSIC Analytical Services.

Calculations and statistics

All statistical and graphical analyses were carried out using R version 4.0.2. The graphs were designed with ggplot2 package 3.3.1 (Wickham, 2009), while the package used for each statistical analysis is specified below.

The effects of treatments on the growth of plants were assessed by analysing differences in canopy height, plant biomass and root: shoot ratio by generalised linear mixed models (GLMM) in lme4 package version 1.1-23 (Bates et al., 2007). Differences in growth variables were modelled with type of soil, group of gypsum affinity and clipping treatment as fixed factors, species as random factor and pre-clipping plant dimensions as a covariate. This covariate was the first component on a Principal Component Analysis (PCA) including canopy area, canopy height and canopy length of plants before the clipping treatment. The inclusion of this covariate in linear models allowed accounting for the variation between individuals caused by previous differences in the size and morphology of individuals, and not by treatments (Palacio et al. 2008). Principal Component Analysis (PCA) was performed using the *rda* function in vegan package (Oksanen et al., 2007). Also, we analysed intraspecific differences between treatments within each species by generalised linear models (GLM). In all models, Shapiro-Wilk and Bartlett's K-squared tests were performed to check for normality and homoscedasticity of residuals. When there was not a normal distribution of residuals, models were fitted to a negative binomial or a gamma distribution, according to the lower AIC. In addition, when differences were

statistically significant, multiple comparisons between the levels of each factor or interaction of factors were assessed with the *glht* function in multcomp package version 1.4-13 in R (Hothorn et al., 2009).

Effects on plant nutrition were analysed using the elemental concentrations and pools of different organs. Elemental concentrations were mass-based concentrations of the different elements analysed, while elemental pools were calculated as the proportion of the total elemental mass accounted by the weight of each element in each organ. To this end, elemental pools were calculated by multiplying the mass-based concentrations of the different organs by their biomass, and dividing it by total plant biomass. Also, we described the elemental nutrition using one-dimension data (the concentration or pool of each element) or multidimensional data (the composition of concentrations or pools). Compositional data comprise all elements and provide relative information among them (Aitchison, 1986). Compositional data was a vector of all elements for each replicate, transformed to Center Log Ratio to avoid scale invariance (Soriano-Disla et al. 2013) with composition package version 1.40-5 (van den Boogaart and Tolosana-Delgado, 2008).

We checked for differences among groups of gypsum affinity, clipping treatment and type of soil for both compositional and one-dimension elemental concentration and pool data. Differences in one-dimension data were assessed using GLMMs. We included taxonomic "family", and "species" nested within "family" as random factors. Models were fitted to a Gamma distribution when there was not a normal distribution of residuals since, in most cases, data had a constant coefficient of variation and variances increased with means (McCullagh and Nelder, 1989). Model link functions of the Gamma distribution were selected according to the lower AIC criterion. When differences were statistically significant, multiple comparisons among levels of each factor or interaction of factors were assessed. Differences in compositional data were assessed using PERMANOVA with Euclidean distances with *adonis* function in vegan package. Similarly, intraspecific differences between soil types and clipping treatment for each species were assessed for elemental concentrations and pools of one-dimension and compositional data. These models included clipping treatment and type of soil as fixed factors, and pre-clipping plant dimensions as a covariate. We performed a PCA with the compositional data of elemental concentrations to analyse the relationships among treatments and soil composition. The procedure followed was the same as described above for the PCA of pre-treatment size variables.

Table 4.1: ANOVA of generalised linear models of growth variables. Chi-squares values, and *P*-values in brackets. Significant effects are in bold type. Family of GLM's was Negative Binomial.

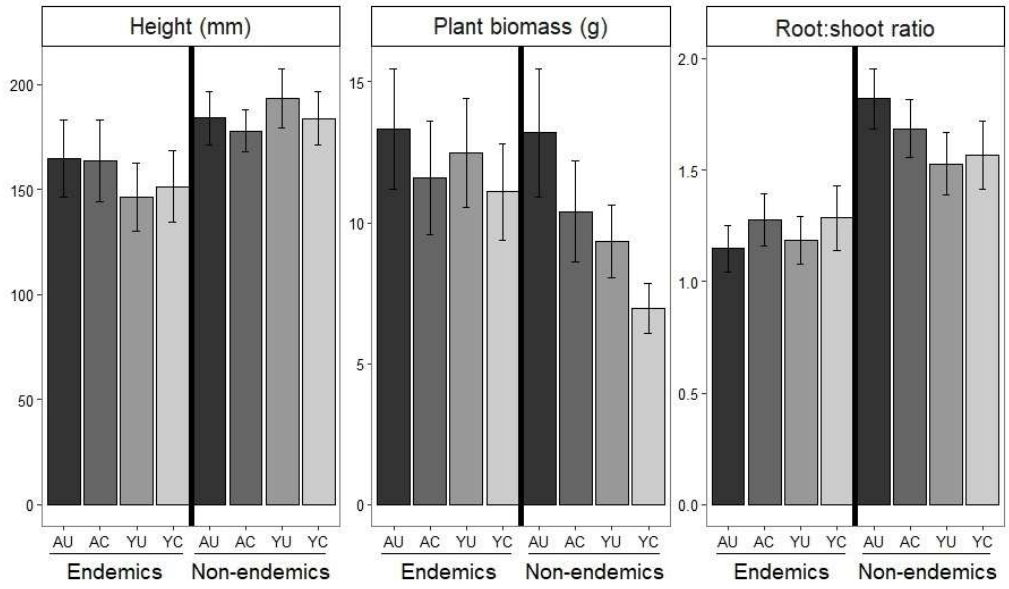
	Height	Plant biomass	Root:Shoot ratio
Gypsum affinity	1.16 (0.281)	0.15 (0.699)	3.60 (0.058)
Soil type	1.65 (0.199)	**13.18 (0.000)**	0.58 (0.448)
Clipping	0.55 (0.460)	**13.49 (0.000)**	0.02 (0.902)
PC1	1.42 (0.233)	**10.29 (0.001)**	0.49 (0.485)
Gyp. x Soil	**4.19 (0.041)**	**9.05 (0.003)**	0.64 (0.424)
Gyp. x Clip.	1.59 (0.208)	1.57 (0.210)	0.52 (0.471)
Soil x Clip.	0.05 (0.821)	0.06 (0.800)	0.19 (0.661)
Gyp. x Soil x Clip.	0.30 (0.586)	0.63 (0.429)	0.35 (0.556)

4.3 Results

Effects on growth and biomass allocation of plants

Clipped plants accumulated less biomass than control plants one year after clipping ($P < 0.05$, Fig. 4.1, Table 4.1). However, clipping did not affect the height or root: shoot ratio of plants. Soil type was also a significant factor affecting the growth of plants: plants grown in calcic soil showed higher biomass than those grown on gypsum ($P < 0.05$). Contrastingly, gypsum affinity had only a slight effect on the root:shoot ratio of plants ($P = 0.058$), as non-endemics had a higher ratio than gypsum endemics. In addition, the interaction between type of soil and gypsum affinity was the only significant interaction found. Non-endemics grown in calcic soils accumulated more biomass than those grown in gypsum soil, while gypsum endemics showed no differences in biomass between soil treatments. The interaction among clipping, gypsum affinity and substrate was not significant, indicating that, contrary to our expectation, gypsum endemics and non-endemics showed comparable responses to clipping on both types of substrates.

Figure 4.1: Means and standard errors of height, plant biomass and, root:shoot ratio of unclipped and clipped gypsum plants in calcic and gypsum soils. AU: unclipped plants grown in calcic (darker bars). AC: clipped plants grown in calcic. YU: unclipped plants grown in gypsum. YC: clipped plants grown in gypsum (brighter bars).



The responses of certain species, however, differed from these general trends (Appendix D, Table D.1). For example, control *L. subulatum* plants grown in calcic soil showed higher biomass than those in gypsum soil ($P < 0.05$), but these differences disappeared with clipping indicating a higher compensating ability of this species when grown on gypsum soil. The gypsum endemic *Gypsophila struthium* showed greater height in calcic than gypsum soils ($P < 0.05$), while *Helianthemum squamatum* increased plant biomass and root: shoot ratio ($P < 0.05$) on calcic soil. The non-endemics *Matthiola fruticulosa* had higher height ($P < 0.05$) and *Rosmarinus officinalis* had higher root: shoot ratio ($P<0.05$) when grown in calcic than gypsum soils.

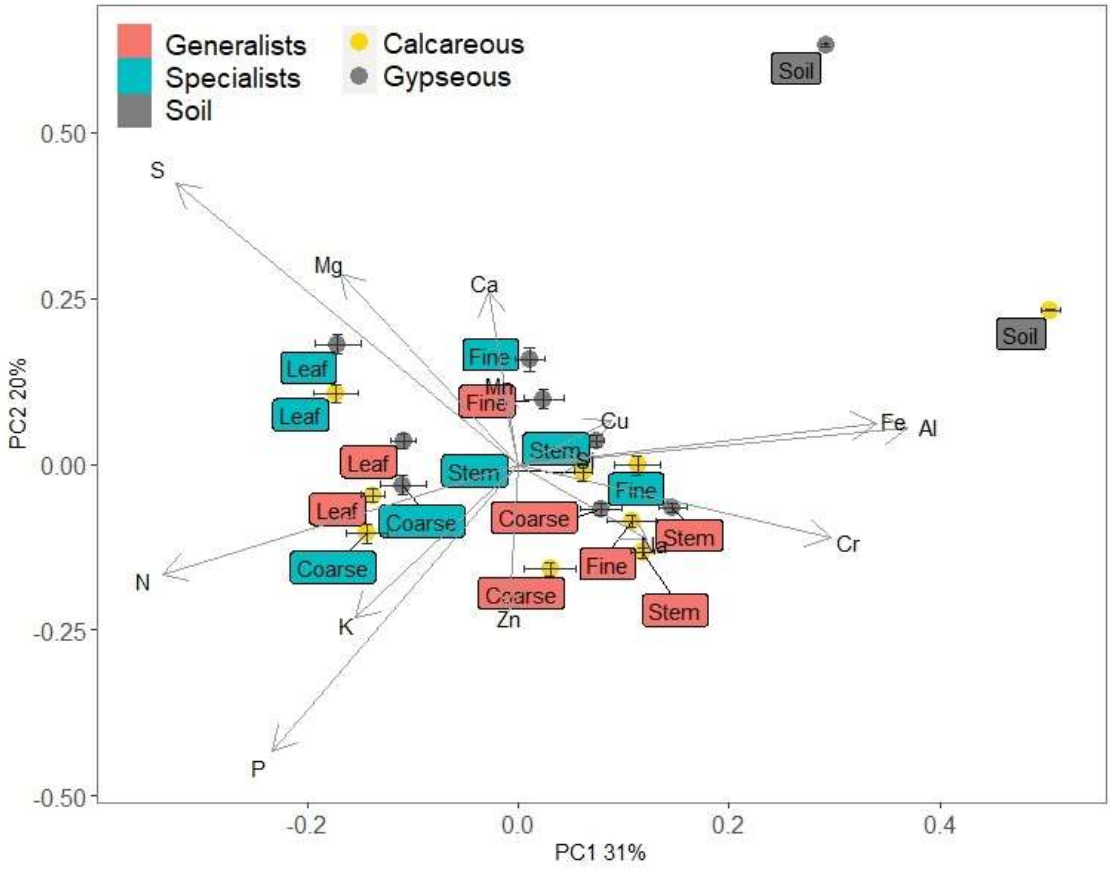
Table 4.2: PERMANOVA testing the effect of organ, affinity to gypsum soils, soil type, clipping and their interaction on the elemental composition of plants. *F*-ratios and *P*-values are shown. Bold type indicates significant effects at $\alpha = 0.05$.

	F-ratio	*P*-value
Organ	**75.76**	**0.001**
Gypsum affinity	**35.28**	**0.001**
Soil type	**31.45**	**0.001**
Clipping	0.34	0.817
Organ x Gyp. aff.	**9.59**	**0.001**
Organ x Soil	**5.70**	**0.001**
Gyp. aff. x Soil	0.84	0.365
Organ x Clip.	0.11	1.000
Gyp. aff. x Clip.	0.45	0.713
Soil x Clipping	0.21	0.952
Organ x Gyp. x Soil	0.42	0.923
Organ x Gyp. x Clip.	0.08	1.000
Organ x Soil x Clipp.	0.08	1.000
Gyp. x Soil x Clipp.	0.26	0.928
Organ x Gyp. x Soil x Clipp.	0.13	1.000

Effects of treatments on the elemental concentrations across plant organs

The multivariate elemental composition (also referred to as ionome o elementome, (Baxter et al., 2008; Peñuelas et al., 2019)) of plants was different between organs, between endemic and non-endemic species, and also between plants grown on gypsum and calcic soils ($P < 0.05$, Table 4.2). However, clipping did not alter the elemental composition of plants. From a compositional point of view, the elemental concentrations in plant organs clearly differed from that of the soil (Fig. 4.2). Generally, leaves and fine roots were the organs with the highest concentrations of S, Ca, Mg, Al, Fe, while coarse roots showed generally higher concentrations of K, P and Zn ($P < 0.05$, Fig. 4.2). Furthermore, all organs showed different elemental composition when plants were grown in gypsum (towards higher concentrations of S, Mg and Ca) or calcic soils (towards higher concentrations of K and P). Likewise, there were different patterns in endemics (with higher concentrations of S, Mg and Ca) and non-endemics. In addition, leaves, stems and coarse roots were more similar among plants in the same gypsum affinity group than in plants cultivated on the same soil type, while fine roots were more similar between plants in the same soil type than by gypsum affinity (Fig. 4.2).

Figure 4.2: Distance biplot of principal components analysis from elemental concentration data. Points with standard error show the elemental composition of organs and soils separated between gypsum soils (black points) and calcic soils (yellow points). Labels were organs (leaf, stem, coarse roots, fine roots) and soil, while the group of gypsum affinity is indicated by the label colour.



Clipping did not affect the concentrations of individual elements, and no significant interactions were observed between clipping and the rest of factors analysed. Soil type had a significant effect on the elemental concentration of some of the different elements analysed, but the effect varied depending on each organ (Fig. 4.3, Appendix D, Table D.2 and D.3). Plants grown on gypsum soils tended to show higher S and lower P concentrations in all organs, lower K and Zn in leaves, lower K and Mn in stems, higher Al, Mn and Si and lower Na in coarse roots and lower Al, Cr, K, Mn, Si, Zn in fine roots than plants grown in calcic soils. On the other hand, the differences for each element between endemic and non-endemic plants also depended on each organ. Endemics tended to show higher concentrations of Al, Ca, Cr, Cu, Mg, Mn, S, Si and Zn and lower concentration of C, K in leaves; higher Al, Ca, Cu, K, Mg, Mn, N, P, S and Zn and lower C in stems; higher C, Ca, Cu, Mg, Mn, N, P, S and Zn and lower Al,

Cr, K, Na in coarse roots; and higher Ca, Cr, Mg, N, and Si and lower C, K, Na and Zn in fine roots than non-endemics. In addition, there was a significant interaction among soil type, gypsum affinity and organ in the concentration of Na, P and S. The concentration of S of each organ, except in fine roots, was highest in endemics grown on gypsum, decreased in endemics grown in calcic soil, then in non-endemics grown on gypsum, and finally the lowest was found in non-endemics grown in calcic soil. Contrastingly, in fine roots, the highest concentrations of S were found in plants grown on gypsum, and the lowest in plants grown in calcic, irrespective of their affinity for gypsum soils.

Some species showed different responses to these general trends in the elemental concentrations of certain elements between clipping and soil treatments (Appendix D, Table D.4 and D.5). For example, the effect of soil on the elemental composition of plants differed among organs in all species but *Lepidium subulatum* and *Linum suffruticosum*, which responded similarly across the plant. In addition, contrary to the general trend, some species changed their elemental composition after clipping. For example, *Helianthemum syriacum* and *H. squamatum* showed higher P in clipped than unclipped plants, while *Herniaria fruticosa* showed higher Mg in unclipped than clipped plants.

Figure 4.3: Means and standard errors of elemental concentrations grouped by macronutrients (A), oligoelements (B), and microelements (C). Calc.soil were plants grown in calcic soils. Gyp.soil were plants grown in gypsum soils. VC: clipped non-endemics (darker bars). VU: unclipped non-endemics. WC: clipped endemics. WU: unclipped endemics (brighter bars)

A) Macronutrients ($mg \cdot g^{-1}$)

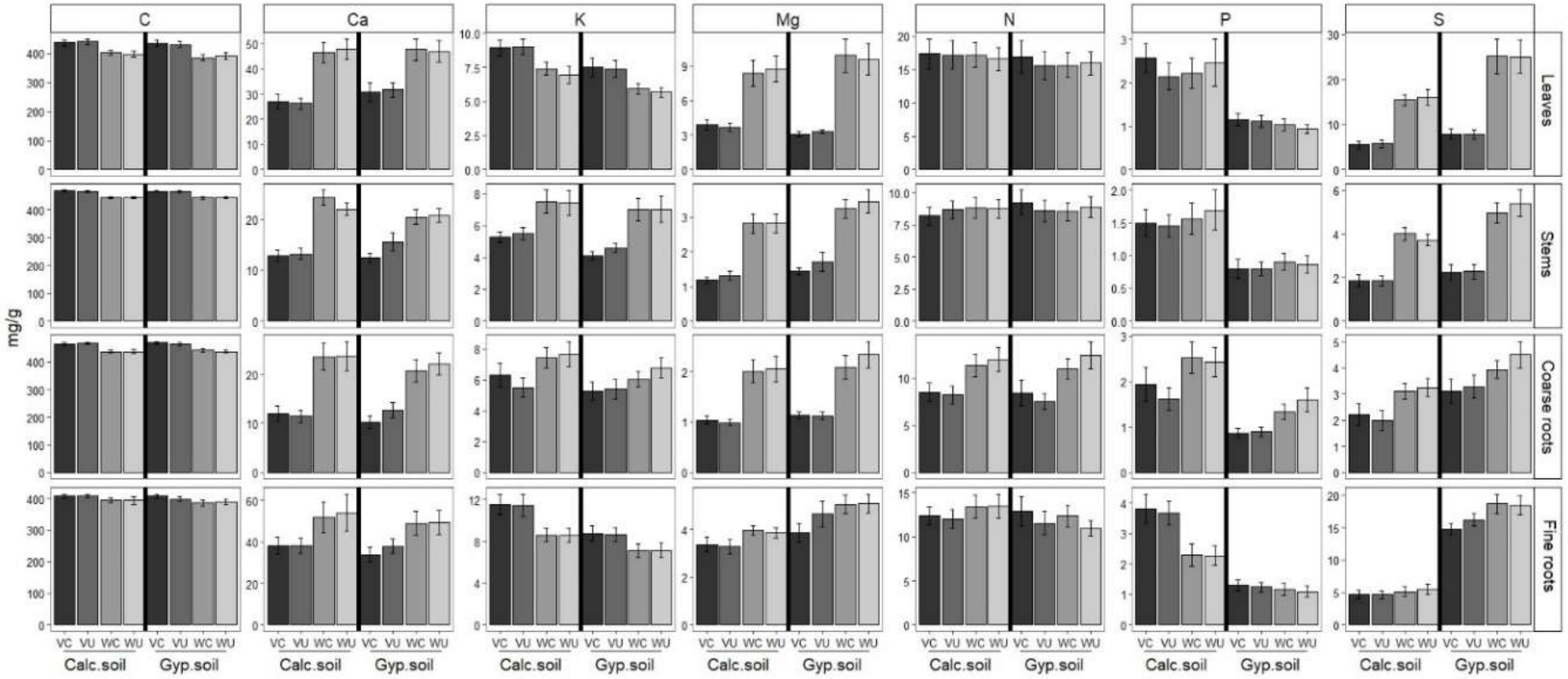
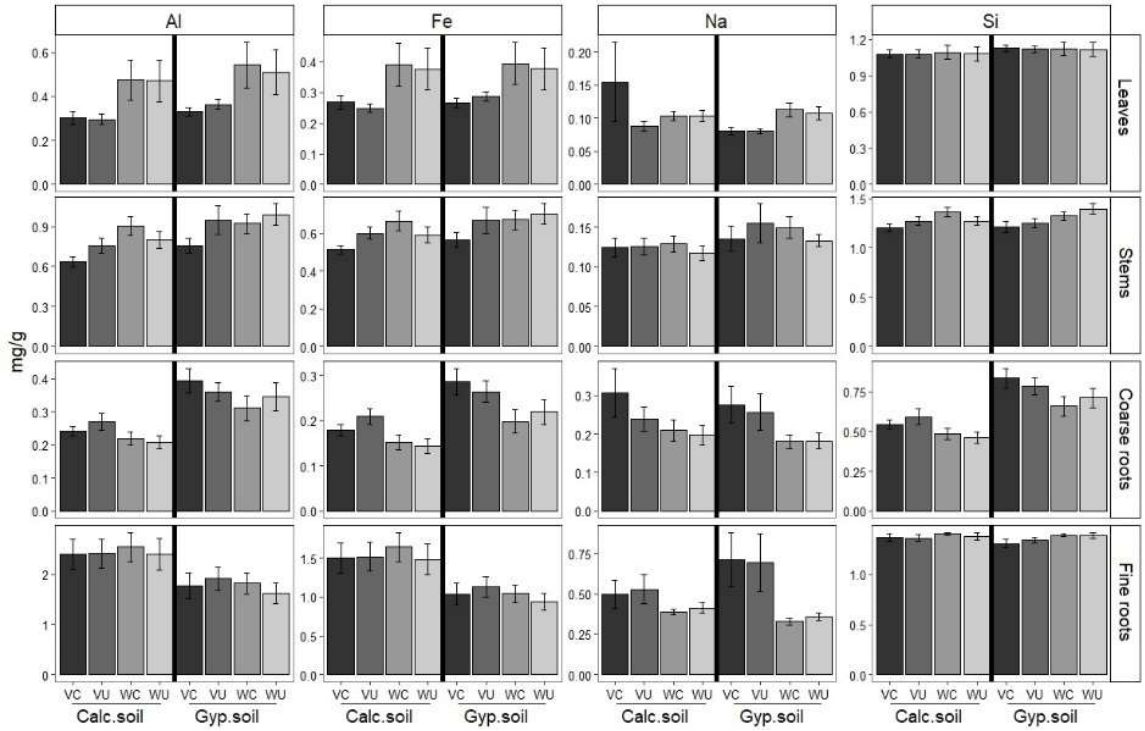
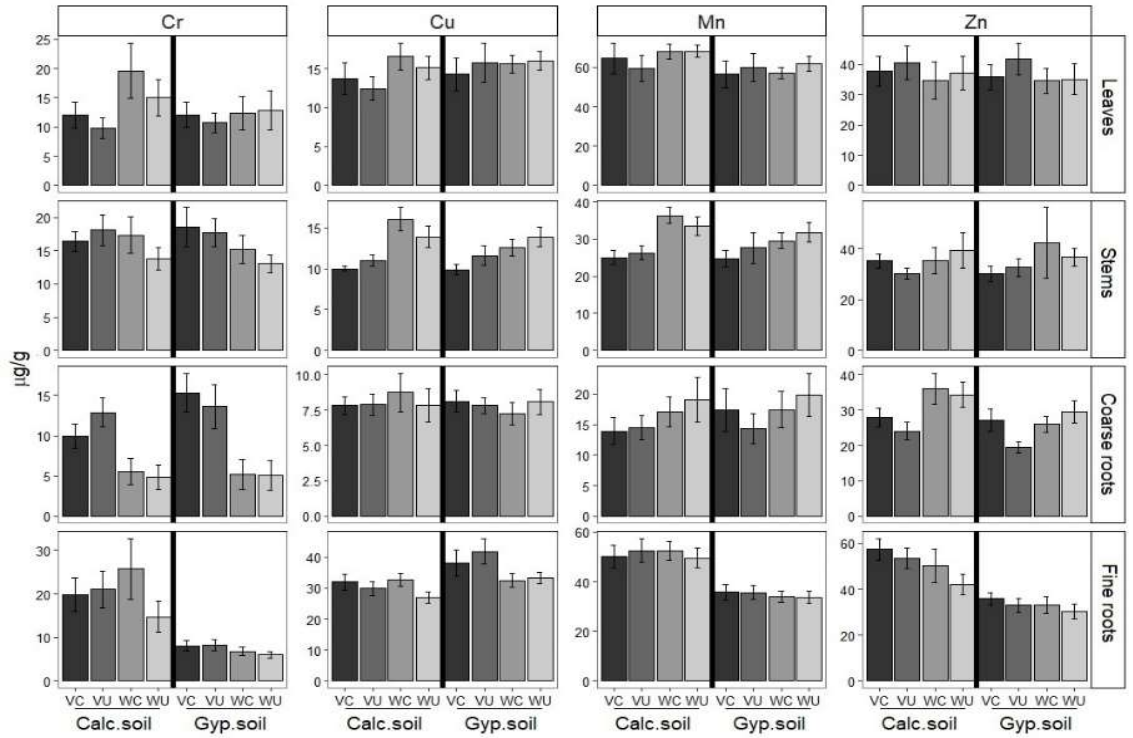


Figure 4.3 (Continued)

B) Oligoelements ($mg \cdot g^{-1}$)



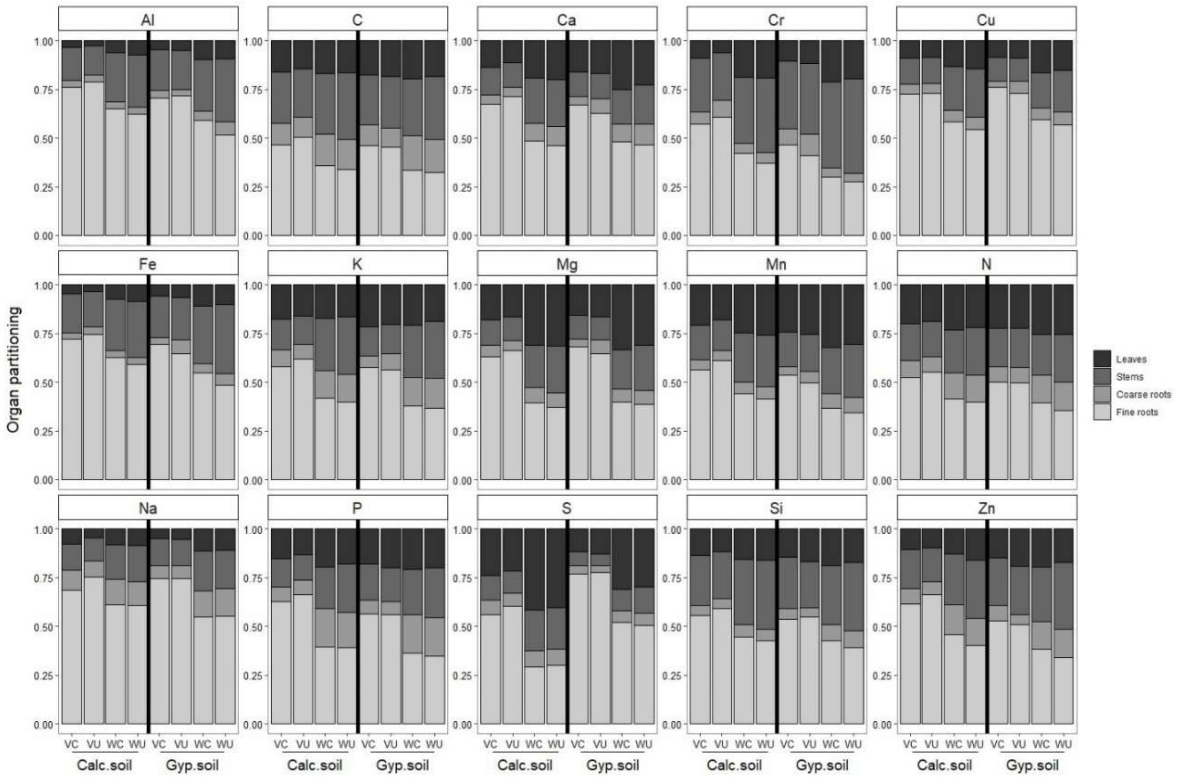
B) Microelements ($\mu g \cdot g^{-1}$)



Elemental pools in plant organs

The multivariate elemental pool was different among organs, soil treatments and between endemic and non-endemic species ($P < 0.05$, Appendix D, Table D.6). However, clipping did not affect the allocation of elements among different organs within the plant. Affinity to gypsum soils and the type of soil used for cultivation significantly affected the elemental pools in different organs (Fig. 4.4, Appendix D, Table D.7 and D.8). Plants grown in gypsum soil showed higher pools of some metals like Al, Fe and Cr in leaves, stems or coarse roots, and lower pools of Cr in fine roots than those from calcic soil ($P < 0.05$). Further, plants cultivated on gypsum showed lower S pools in leaves, stems and coarse roots, but accumulated proportionally higher S pools in fine roots than those grown in calcic soils ($P < 0.05$), when generally biomass of fine roots was also lower in gypsum than calcic soils. On the other hand, gypsum endemics tended to have generally higher elemental pools in leaves, stems and coarse roots than non-endemics, while non-endemics tended to have higher pools of most elements in fine roots (Fig. 4.4, Appendix D, Table D.7 and D:8).

Figure 4.4: Bar plot of organ partitioning for elemental pool data. From top to bottom: Leaves are the darkest bars, followed by stems, coarse roots and fine roots (light grey). Calc.soil are plants grown in calcic soils. Gyp.soil are plants grown in gypsum soils. VC: clipped non-endemics. VU: unclipped non-endemics. WC: clipped endemics. WU: unclipped endemics



Overall, clipping did not affect the elemental pools in any plant organ, but some species modified their elemental pool in certain organs in response to clipping (Appendix D, Table D.9 and D.10). For example, clipped *Gypsophila struthium* plants showed reduced Al, Fe, K, Mg, Mn, and Si pools in leaves, stems and coarse roots, but not in fine roots, in contrast to control plants ($P < 0.05$). In addition, clipped *Lepidium subulatum* plants accumulated Al, Ca, Cu, K, Mg, Mn, N, P, S and Zn ($P < 0.05$) into roots and decreased allocation to shoots as compared to controls. Clipped *Boleum asperum* plants only modified the pool of Cr towards a higher accumulation in leaves and stems, and reduced it in coarse roots ($P < 0.05$). *Matthiola fruticulosa* showed a similar pattern with N, accumulating higher pools in leaves and lower in fine roots after clipping ($P < 0.05$). Finally, *Linum suffruticosum* modified several elemental pools after clipping, accumulating higher Al, C, Ca, Fe, Mg, N, P, S, Si and Zn pools in leaves, stems and coarse roots and lower pools in fine roots ($P < 0.05$).

4.4 Discussion

Plants were unable to compensate biomass losses in any soil after clipping

In accordance to our expectations, studied plants were negatively affected by clipping. One year after treatments, all species accumulated less biomass in clipped than control plants. Further, none of the studied species showed higher leaf N in clipped plants, which would be an indicator of higher leaf activity and a trait related to grazing tolerance (Capó et al., 2021). Compensation varies depending on resource availability and on how well adapted plants are to low or high resource availability, because this ultimately affects growth rates (Wise & Abrahamson 2005). Gypsum endemics seem to have evolved under grazing pressure, as gypsum plant communities are usually shrublands or grasslands (Mota et al., 2017), which are community types associated with the effect of large herbivores and livestock grazing (Asner and Levick, 2012; Bakker et al., 2016). Indeed, a recent study found increased dominance of gypsum endemics on moderately grazed areas as compared to areas of low grazing (Chapter 4). Consequently, we expected gypsum endemics, as plants adapted to gypsum soils, to compensate better biomass removal due to grazing in gypsum than calcic soils. This behaviour was only shown by *Lepidium subulatum*, which showed smaller differences between clipped and control plants

when on gypsum vs. calcic soils. Consequently, the response of most gypsum endemics to clipping was not improved when grown on gypsum soils. These results seem to indicate that gypsum endemics are stress-tolerant plants which follow disturbance avoidance strategies rather than tolerance ones, having high investments in plant defense rather than increased growth rates to compensate for biomass loss after consumption. However, a note of caution should be introduced when interpreting our results, since only short-term effects were considered, which may be very different to those observed in the long-term, after repeated and sustained grazing (Palacio et al., 2008).

Gypsum endemics accumulated elements aboveground, while non-endemics accumulated them in fine-roots

Plants differed in the nutrient concentration of their above- and below-ground organs, and these organs were compositionally different from soil (Lambers et al., 2008b; Zhao et al., 2016). The different functions of organs determine their nutrient concentration (Aerts and Chapin, 1999). In our experiment, leaves and fine roots were the organs with the highest concentrations of S, Ca, Mg, Al, Fe, which are the elements with highest content in soils. A function of leaves and fine roots could be to accumulate excess elements. While coarse roots showed generally higher concentrations of K, P and Zn, because probably they are reserve organs of elements with lower content in soils. Elemental composition of organs also shifted according to soil type. Gypsum soils are slightly less alkaline, more saline, and show higher content of S, Mg and Ca in the soil solution than calcic soils (Casby-Horton et al., 2015). Such features modulate nutrient concentration of plants (FAO, 1990). Accordingly, plants grown on gypsum showed higher concentrations of S, Mg and Ca, whereas plants grown in calcic soils showed higher concentrations of K and P. These results agree with previous studies on gypsum plant nutrition (Boukhris and Lossaint, 1970; Cera et al., 2021; Mota et al., 2016).

Affinity for gypsum soils also affected the elemental composition of plants. In accordance with previous studies, gypsum endemics showed higher leaf S and Mg than non-endemic species (Merlo et al., 2019; Muller et al., 2017; Palacio et al., 2007). Furthermore, our results are the first evidence that non-endemics and endemics differ in their elemental composition across organs. Gypsum endemics tended to have generally higher elemental pools in leaves, stems and coarse roots than non-endemics,

while non-endemics tended to have higher pools of most elements in fine roots. This behaviour was especially clear with S, the most discriminating element between calcic and gypsum soils (Cera et al., 2021), and between gypsum endemics and non-endemics foliar content (Merlo et al., 2019). Plants adapt to excess elements in soils (in our case S) by accumulating them in roots to maintain growth, as a nutritional barrier, or by tolerating them in leaves (Tran et al. 2020). Gypsum endemic species are hence leaf accumulators, while non-endemics seem to show mechanisms that block S uptake at the fine root level.

The differential nutritional strategy between gypsum endemics and non-endemics may influence plant nutrition and ultimately, plant performance on gypsum soils. Non-endemic species may block excess elements in roots (especially cations), probably developing apoplastic barriers in the endodermis (Sattelmacher, 2001), such as Casparian band strips and suberin lamellae formation, similar to plants in saline, calcic and metalliferous environments (Barberon, 2017; Lux et al., 2021; White and Broadley, 2003). Endodermal barriers reduce permeability of elements from the rhizosphere to the plant, leading to decreases in foliar contents of Ca, Mn, and Zn (Courbet et al., 2019). The blockage of S at the root level in non-endemics may be achieved by decreased expression of S transporters, because aboveground organs may have low demand in contrast to high content in soils (Davidian and Kopriva, 2010; Lappartient and Touraine, 1996), since sulfate uptake predominantly follows the symplastic route (Hawkesford et al., 2012). Such reduced S uptake may in turn interfere with other nutrients such as Mo and Se, which can also share transporters with sulfate (Courbet et al., 2019), although we could not detect them with ICP-OES. Contrastingly, gypsum endemics may be more permeable to nutrients in general, through reduced apoplastic barriers and likely enhanced symplastic uptake through regulated expression of sulfate transporters (Davidian and Kopriva, 2010), particularly when grown in soils with lower S availability, like calcic soils. Similarly, the S accumulator *Brassica napa* upregulated sulfate transporters expression when cultivated on low S media (Koralewska et al., 2007). Gypsum is considered a very nutrient-limited soil, especially for P, N, K, Fe and some micronutrients (FAO, 1990). If gypsum endemics show fewer endodermal barriers and improved S uptake, they could be more efficient in uptake of these scarce nutrients. However, more research on acquisition and growth of gypsum-adapted species is needed to fully understand the implications of the different nutritional

strategies of gypsum endemics and non-endemics, since we did not observe better growth on gypsum soils by endemics relative to non-endemics.

In any case, gypsum endemics need mechanisms to tolerate gypsum excess elements (S, Ca, Mg) in aboveground organs. A common strategy is to accumulate them in the shoot epidermis (Lux et al., 2021), which may be achieved, for example, by the biomineralization of elements (He et al., 2014) or by the assimilation of excess elements in organic molecules like secondary metabolites (Ruiz et al., 2005). Gypsum endemic plants accumulate high contents of sulphate and calcium in leaf vacuoles (Ernst, 1990; Kinzel, 1989). Some of these species form oxalate calcium or gypsum crystals (Palacio et al., 2014), while others accumulate excess S as organic compounds (Ruiz et al., 2005). S-accumulation, either as biocrystals or as S-rich organic compounds like glucosinolates can play an anti-herbivore role (He et al., 2014; Ernst, 1990), with potential implications on the resistance of gypsum endemics to grazing.

Grazing did not alter S accumulation in gypsum endemics

Livestock grazing promotes leaf S-accumulation in gypsum plant communities, enhancing the assembly of species with this trait, but also by intraspecific shifts of populations promoting the assembly of individuals with higher leaf S in the most grazed communities (Chapter 4). We, consequently, hypothesized that S-accumulation could be an induced mechanism in response to grazing. However, clipping did not alter the elemental composition and S-accumulation of studied plants. It could well be that our short-term experiment with a single clipping event is not enough to induce this mechanism and longer-term experiments, with repeated and sustained grazing are required to induce a response in plants (Canadell and López-Soria, 1998). Alternatively, our results seem to suggest that grazing could filter plants at the population level with constitutively higher leaf S concentration rather than producing an induced mechanism (Bolnick et al., 2011).

4.5 Conclusions

Studied gypsum endemics and non-endemics did not fully compensate for biomass loss in neither gypsum nor calcic soils, indicating an avoidance rather than a tolerance strategy to resist grazing. Gypsum endemics are permeable to excess elements in gypsum soils, while non-endemics block them at the root level, accumulating them in fine roots. Such allocation patterns were maintained irrespectively of clipping or soil type. The permeability to the substrate shown by gypsum endemics may confer them a better ability to uptake scarce elements in nutrient deprived gypsum environments. The high foliar S accumulation resulting from such nutritional strategy does not seem to be a grazing-induced mechanism. Further, longer-term studies are needed to ascertain the functional mechanisms underlying the responses of gypsum endemics to herbivory and the potential role of foliar S accumulation as a constitutive defense.

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Author contributions

GM and SP designed and set up the experiment; AC, maintained the experiment, measured all variables, analysed data and led the manuscript writing; All authors discussed the results and wrote the manuscript.

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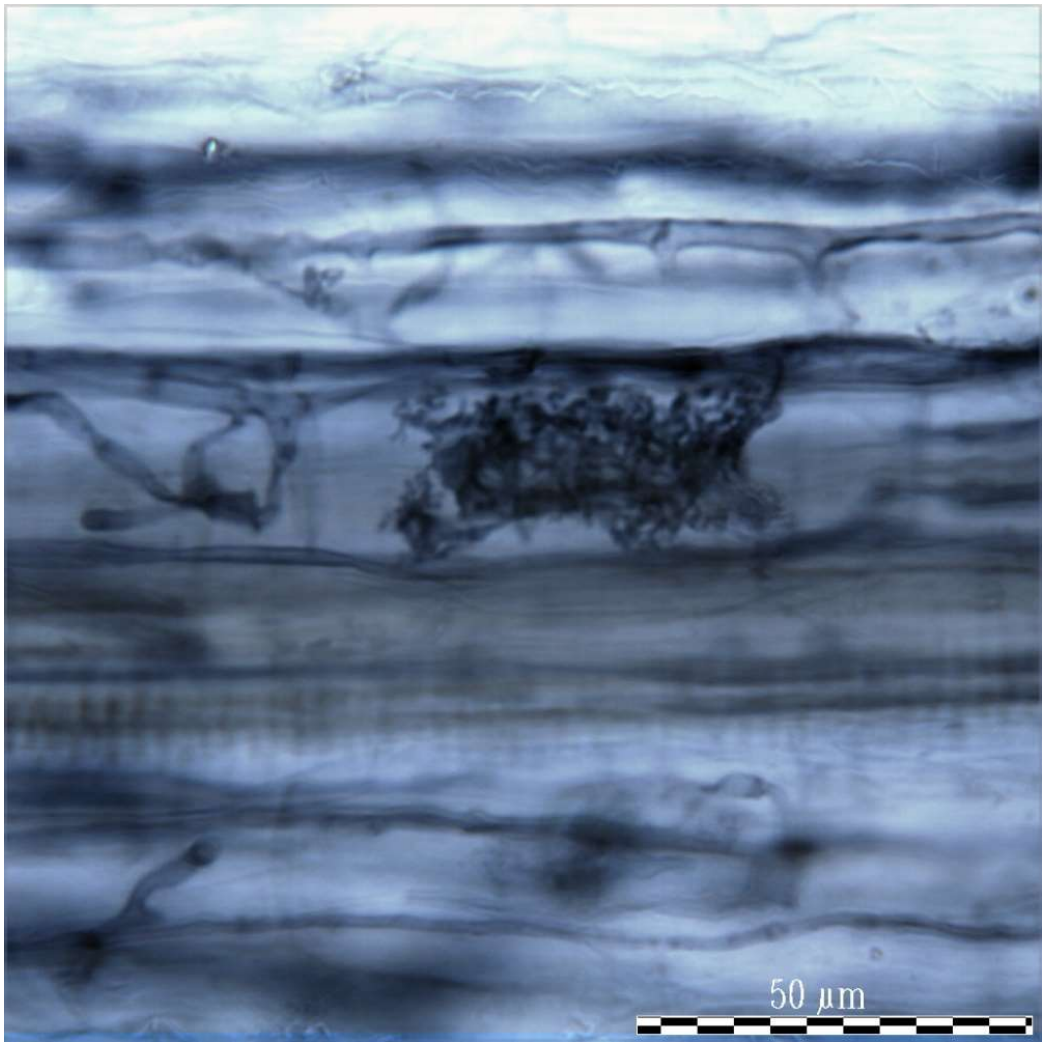
Chapter 5



Arbuscule on fine roots of *Lepidium subulatum* L. . By Estephania Duplat and Antonio Gómez-Bolea

5 Seasonal variation in AMF colonisation, soil and plant nutrient content in gypsum specialist and generalist species growing in P-impoverished soils

Andreu Cera, Estephania Duplat, Gabriel Montserrat-Martí, Antonio Gómez-Bolea, Susana Rodríguez-Echeverría, Sara Palacio. Manuscript submitted under second revision in *Plant and Soil* (11th June 2021).

Abstract

Aims Gypsum soils are special substrates harsh for plant life due, among other characteristics, to their low phosphorus (P) availability. They frequently occur in drylands, where plant activity and soil nutrient availability are seasonal. Despite the importance of P nutrition in gypseous soils and the crucial role arbuscular mycorrhiza fungi (AMF) play in P nutrition, no previous studies have analysed the seasonality of AMF colonisation in gypsum plants. Our aim was to evaluate the seasonal changes in plant nutrient status, AMF colonisation and rhizospheric soil nutrient availability in gypsum specialist and generalist species.

Methods We evaluated seasonal variation in the proportion of root length colonised by AMF structures (hyphae, vesicules and arbuscules), plant nutrient status (leaf C, N and P and fine root C and N) and rhizospheric soil content (P_{Olsen}, organic matter, nitrate and ammonium) of three gypsum specialists and two generalists throughout a year.

Results All species showed arbuscules within roots, including species of *Caryophyllaceae* and *Brassicaceae*. Root colonisation by arbuscules (AC) was higher in spring than in other seasons, when plants showed high leaf P-requirements. Higher AC was decoupled from inorganic N and P availability in rhizospheric soil, and foliar nutrient content. Generalists showed higher AC than specialists, but only in spring.

Conclusions Seasonality was found in AMF colonisation, rhizospheric soil content and plant nutrient status. The mutualism with AMF peaks in spring, when P-requirements are higher for plants, especially

in generalists. However, AMF decouple from plant demands in autumn, when nutrient availability increases in rhizospheric soil.

Keywords: Mediterranean, semiarid and arid environments, functional ecology, gypsophiles, gypsovags, leaf elemental concentration

5.1 Introduction

Nitrogen (N) and phosphorus (P) are the most common limiting nutrients in a wide variety of terrestrial ecosystems (Vitousek et al., 2010). Nutrient availability underlies the nutritional strategy of plants (Chapin, 1980). In the case of nutrient-poor environments as drylands, plants have frequently evolved a retention strategy *versus* a rapid growth strategy, affecting acquisition, use, storage and resorption of nutrients (Aerts and Chapin, 1999). These nutritional strategies are reflected in plant nutrient concentration (Grime et al., 1997), which summarises the functioning of plants in relation to their environment (Peñuelas et al., 2019).

Plant nutrient concentrations vary throughout the year due to shifts in nutrient availability and plant activity imposed by climate seasonality (Chapin, 1980). Plant phenology of perennial species in Mediterranean drylands is characterized by predominant shoot growth in spring, root growth mainly in autumn and flowering in spring and early summer (Orshan, 1989; Palacio and Montserrat-Martí, 2007). Shoot growth requires high N and P in leaves (Palacio et al., 2014b), while flowering demands high P (Milla et al., 2005). However, the availability of inorganic P is high in late summer (Magid and Nielsen, 1992), and inorganic N is high in autumn in soils from Mediterranean drylands (Delgado-Baquerizo et al., 2011). Consequently, peak plant demands for N and P may be decoupled from soil availability in Mediterranean drylands. Unfortunately, seasonal studies linking nutrient acquisition strategies, plant nutrient status and soil nutrient availability in Mediterranean drylands are scarce (but see Palacio et al., 2014).

Plant strategies for nutrient acquisition in soils vary depending on the structural and functional features of roots, and the association of roots with microorganisms (Richardson et al., 2009). Plant symbiotic interactions with soil microorganisms have been broadly explored as a strategy to enhance N and P acquisition in nutrient poor-environments (Aerts and Chapin, 1999). Plants may be associated with symbionts to improve N uptake, as N-fixing bacteria or ectomycorrhizal fungi (Chalot and Brun, 1998; Miller and Cramer, 2005), and with arbuscular mycorrhiza fungi (AMF) to improve N and P acquisition (Vance et al., 2003). AMF symbiosis generally improves plant growth in P-limited soils (Johnson, 2010), providing plants with access to low-mobility P inorganic forms, such as phosphates

(Hawkesford et al., 2012). Root colonisation by AMF is seasonal, as it relates to plant activity (Jakobsen et al., 2003) and soil nutrient availability (Hoeksema et al., 2010). However, few studies have demonstrated a relationship between seasonal AMF colonisation and soil P concentration in natural populations of wild plants (i.e. Mullen and Schmidt, 1993). Consequently, shifts in AMF colonisation may be determined by the interaction of soil nutrient availability and plant demands, which ultimately define carbon supply by plants to the fungi (Johnson, 2010).

The analysis of AMF structures within roots allows us to understand fungal activity in relation to plant activity (Jakobsen et al., 2003). Arbuscules appear when nutrient plant requirements and nutrient exchanges rates between fungi and plants are high, whereas at other times they may be absent (Allen, 1983; Mullen and Schmidt, 1993). Contrastingly, vesicles are storage structures, which appear in periods without high nutrient plant acquisition (Abbott et al., 1984). Seasonal shifts in AMF colonisation within roots have been described in drylands (Fakhech et al., 2019; Roldan and Albaladejo, 1993; Varela-Cervero et al., 2016), and have been related to plant activity (López-Sánchez and Honrubia, 1992). Previous studies found high AMF colonisation in spring (Roldan and Albaladejo, 1993) generally when plants sprouted or flowered, and slightly high in autumn (López-Sánchez and Honrubia, 1992). Most of the studies on AMF seasonality on drylands only provided hyphal colonisation (hereafter, HC). However, seasonal studies on arbuscular colonisation (hereafter, AC) and vesicular colonisation (hereafter, VC) are required to improve knowledge on AMF activity in nutrient acquisition (Jakobsen et al., 2003).

Nutrient limitation increases in soils with minimal content of clay and organic matter, such as gypseous soils (Casby-Horton et al., 2015). Gypseous soils are special substrates with high gypsum (calcium sulphate dihydrate) content (Herrero and Porta, 2000), which frequently occur in drylands around the world (Verheye and Boyadgiev, 1997). The high gypsum content of gypseous soils modifies the physical and chemical proprieties of soils (Herrero et al., 2009). For example, the high solubility of gypsum produces high Ca^{2+} activity in the soil solution (Casby-Horton et al., 2015), leading to a decrease in macronutrient availability and plant acquisition, particularly P (Stout et al., 1951). These features severely limit plant life on gypseous soils (FAO, 1990). Despite these limitations, gypsum environments

host a unique flora, identified as an international conservation priority (Escudero et al., 2015; Ochoterena et al., 2020).

Gypsum plants are adapted to a harsh substrate (Moore et al., 2014), where there is a strong seasonality in water and nutrient availability (Delgado-Baquerizo et al., 2011; Palacio et al., 2017). There are two types of gypsum plants according to their gypsum affinity (Meyer, 1986): specialist species (also referred as gypsophiles), and generalist species (gypsovags). Gypsum specialist species are considered edaphic endemics with specific features related to gypseous soils (Duvigneaud and Denaeyer-De Smet, 1968). Gypsum specialist species differ from generalist species in their foliar S, Ca and Mg concentrations (Palacio et al., 2007; Merlo et al, 2019), but not in their leaf P and N (Muller et al., 2017; Sánchez-Martín et al., 2021). In addition, plants growing on gypseous soils show decreased foliar P concentrations, owing to the remarkably low P availability in this type of soil (Cera et al., 2021). Previous studies analysed the differences in AMF colonisation between gypsum specialist and generalist species. They found higher AMF colonisation and higher phylogenetic diversity of AMF in roots of gypsum generalist *vs.* specialist species (Palacio et al., 2012; Torrecillas et al., 2014). However, these studies were usually performed in spring, and no previous studies have evaluated the seasonality in AMF colonisation in gypsum plants, or the possible links with soil nutrient availability and plant activity and nutrient demands which are seasonal in these ecosystems (Delgado-Baquerizo et al., 2011; Palacio and Montserrat-Martí, 2005).

The aim of this study was to evaluate the seasonal changes in plant nutrient status, AMF colonisation and rhizospheric soil nutrient availability and their interaction in five studied plant species, which included both gypsum specialists and generalists. Root colonisation by AMF (accounting for hyphae, vesicles and arbuscules separately), concentration of C, N and P in leaves and of C, N in fine roots and P_{Olsen}, organic matter content, and concentration of nitrate and ammonium in the rhizospheric soil were analysed four times throughout a year. We hypothesised that: 1) All species will display AMF structures (hyphae, vesicles and arbuscules) indicative of AMF colonisation/symbiosis throughout the year, because gypseous soils are remarkably P-improvished; 2) The degree of AMF colonisation will vary seasonally, according to previous studies in semiarid environments (Varela-Cervero et al., 2016); 3) The seasonality of AMF colonisation will follow plant nutrient content and rhizospheric soil nutrient

concentration (especially P), displaying the highest HC and AC in autumn and spring, when nutrient plant concentration will be high, and the highest VC in summer, when both plants and fungi have to cope with the harshest environmental conditions. 4) Generalist gypsum species will show higher HC and AC than specialist gypsum species according to previous studies (Palacio et al., 2012).

5.2 Materials and methodology

Study site

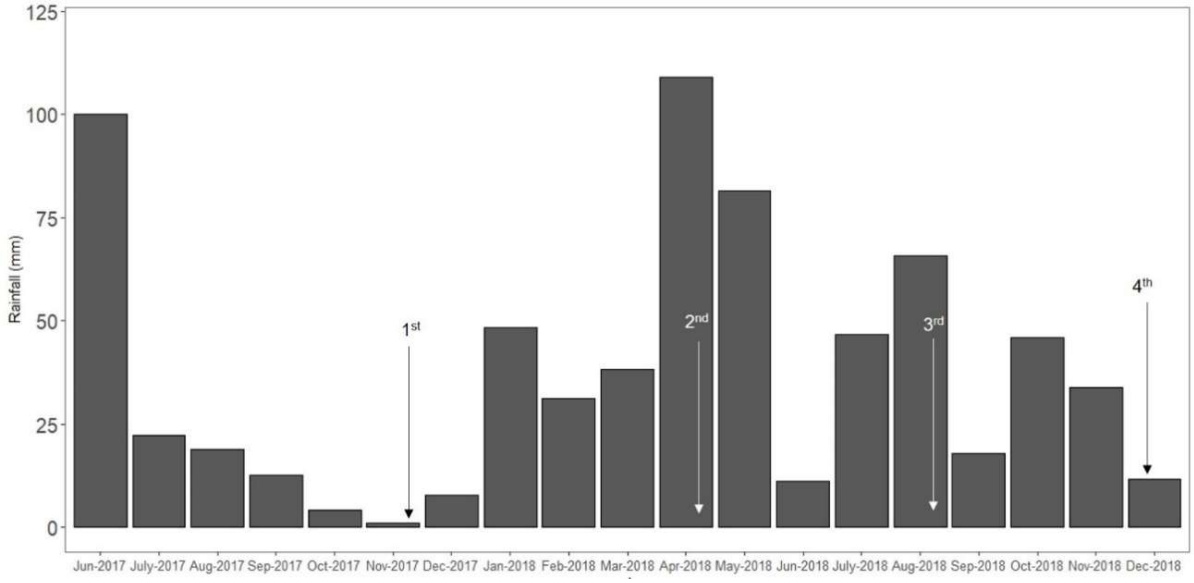
This study was conducted at one locality in the Middle Ebro Basin (Villamayor, Zaragoza, NE Spain, 41°42'39.2"N 0°44'22.8"W; 295 m a.s.l), within a sampling area of approximately 3000 m^2.The main lithology is an extensive area of massive gypsum deposits and gypseous soils with high contents of gypsum (Palacio et al., 2012), with few thin outcrops of marls and clays inserted (Quirantes, 1978, Appendix E, Table E.1). The locality has a semi-arid Mediterranean climate, with an annual average rainfall of 322 mm and a mean annual temperature of 15.5 °C (data from the nearest weather station at Zaragoza 41°37'15"N, 0°56'6"W, between 1981-2010). Vegetation was composed predominantly of shrubs, forbs and grasses, like, *Gypsophila struthium subsp. hispanica* (Willk.) G. López, *Helianthemum squamatum* Pers., *Helianthemum syriacum* (Jacq.) Dum. Cours., *Herniaria fruticosa* L., *Lepidium subulatum* L., *Rosmarinus officinalis* L., *Thymus vulgaris* L., *Plantago albicans* L., *Brachypodium retusum* (Pers.) P. Beauv., *Stipellula parviflora* (Desf.) Röser & Hamasha.

Sampling design

Five plant species were selected for analysis. All of them were sub-shrubs, which are prevalent growth forms in gypsum outcrops (Martínez-Hernández et al., 2011; Parsons, 1976). They included two Cistaceae: a specialist (*Helianthemum squamatum* Pers.) and its congener generalist (*Helianthemum syriacum* (Jacq.) Dum.Cours.); two Brassicaeae: a specialist (*Lepidium subulatum* L.) and a con-familial generalist (*Matthiola fruticulosa* (L.) Maire); and a Caryophyllaceae specialist (*Gypsophila struthium* Loefl.).

Five specimens of each species were collected in the same locality at four different times: late autumn (28th November 2017), spring (26th April 2018), summer (21st August 2018) and late autumn (13th December 2018). We chose isolated individuals located at least five meters apart from each other. Selected individuals were healthy adult plants with their foliage exposed to full sunlight. We selected spring as the main period of growth, summer as the period of arrested shoot growth (Palacio and Montserrat-Martí, 2005), and autumn as the period with high soil nutrient availability (Delgado-Baquerizo et al., 2011). The autumn harvest in 2017 followed a dry summer (with 79.9 mm of rainfall) and a dry autumn (with only 14.3 mm precipitation; Fig. 5.1). Contrastingly, the autumn harvest in 2018 followed a wet summer (128.6 mm) and autumn (93.1 mm). We collected complete specimens, with rhizospheric soil attached, placed them individually in polyethylene bags and transported them to the laboratory, where plant tissues were separated from the soil and processed.

Figure 5.1: Diagram of rainfall near the sampling location with indication of sampling times throughout the period of study.



Soil analyses

Physical and chemical soil properties were analysed from five replicates per species and sampling (N = 100). Rhizospheric soil, here considered as soil adhered to the root system, was gently separated from the fine roots using dissection forceps, and subsequently divided into two subsamples: one to be

sieved at 2 mm and air dried for 2 months at room temperature prior to physical and chemical analyses, and another one to be stored at 4 ºC prior to extraction with KCl for nitrate and ammonium analyses. Relative humidity was measured in all soil samples before drying and storage. Dried soils were used to measure the following variables: gypsum content, measured according to Artieda et al. (2006); carbonate content, measured by Bernard calcimetry (Muller and Gatsner, 1971); soil texture, determined with a particle laser analyser (Mastersizer 2000 Hydro G, Malvern, UK); soil pH and conductivity, measured with a pH/conductivity meter (Orio StarA215, Thermo Scientific, Waltham-MA, USA) by diluting samples with distilled water to 1:2.5 (w/v) and 1:5 (w/v), respectively; and available Olsen-P (P_{Olsen}) determined following standard methods (Anderson and Ingram, 1989). A subsample of each dried and sieved soil was finely ground using a ball mill (Retsch MM200, Restch GmbH, Haan, Germany) and subsequently used to analyse organic matter following standard methods (Anderson and Ingram, 1989). For nitrate and ammonium analyses, 10 g of fresh soil were extracted with 50 mL KCl (1M). Extracts were shaken and filtered through a filter (7-9 µm pore, 0.160 mm thickness). Ammonium concentration in the extracts was estimated by colorimetry (salicylate method, Kempers and Zweers, 1986). Nitrate concentration was analysed according to Kaneko et al. (2010) as the difference in absorbance between 260 nm and 220 nm.

Plant analyses

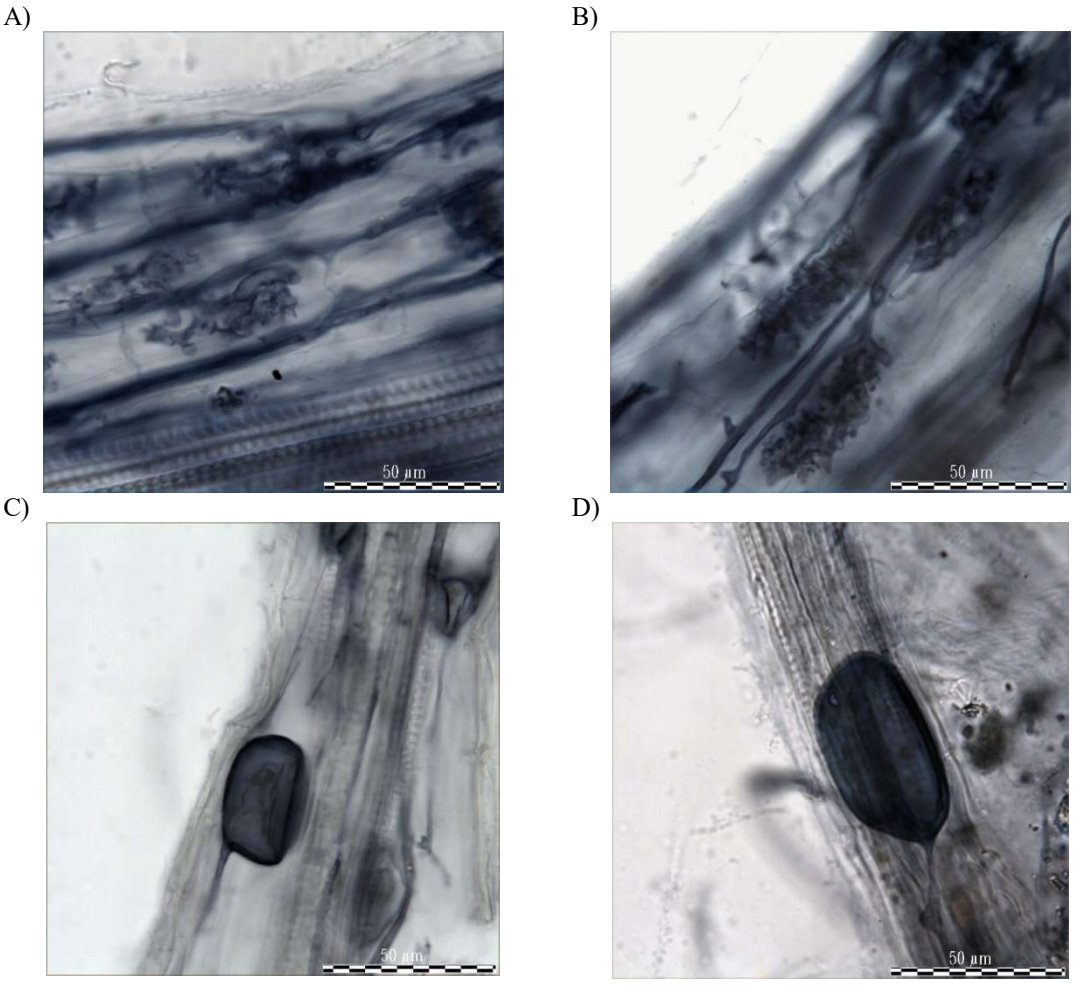
Leaves and a subsample of fine roots were collected at each harvest, washed and dried to a constant weight at 50 ºC for 5 days and subsequently finely ground using a ball mill (Retsch MM200, Restch GmbH, Haan, Germany) to measure P, N and C concentrations. P concentrations were determined by vanado-molybdate colorimetry (Becker, 1961). N and C concentrations were measured with an elemental analyzer (TruSpec CN, LECO, St. Joseph-MI, USA). N, C analyses were performed by EEZ-CSIC Analytical Services, and P analyses by IPE-CSIC Analytical Services.

Mycorrhizal colonisation

A subsample of fine roots was separated from each plant, washed in distilled water to remove soil and stored in 50 % ethanol at 4 ºC. Mycorrhizal colonisation was analysed by cutting the roots into

approx.1 cm fragments and rinsing them in distilled water. Dead and old fine roots were removed under a stereo microscope. Root samples were cleared in 10 % KOH for 20 min at 120 °C (5 min longer for some species with very dark roots) as in Brundrett et al. (1996) and stained with trypan blue in lactoglycerol as in Phillips and Hayman (1970). Later, the roots were mounted on glass slides with Hoyer's medium (Cunningham, 1972) for examination under the microscope. The proportion of root length containing arbuscules, vesicles and hyphae (i.e arbuscular (AC), vesicle (VC) and hyphal colonisation (HC)) was calculated under an optical microscope following the magnified intersections method (McGonigle et al., 1990). The average number of intersections observed ranged between 332 and 405 per specimen.

Figure 5.2: Arbuscules in *Gypsophila struthium* (Caryophyllaceae) (A) and *Lepidium subulatum* (Brassicaceae) (B). Vesicules in *Helianthemum syriacum* (Cistaceae) (C) and in *Gypsophila struthium* (D). All structures were recorded in autumn 2017.



Statistical analyses

All statistical analyses and graphics were performed using R version 4.0.2. The effect of season and gypsum affinity on mycorrhizal colonisation, plant nutrient concentrations and rhizospheric soil characteristics was evaluated using generalised linear mixed models (GLMMs) with season and gypsum affinity as fixed factors and family and species nested within family as random factors. We also analysed the effect of season within each species on mycorrhizal colonisation, plant nutrient concentrations and rhizospheric soil characteristics using generalised linear models (GLMs) with season as a fixed factor. Shapiro-Wilk and Bartlett's K-squared tests were performed to check for normality and homoscedasticity of residuals. Models were run with the *glm* or *glmer* functions (Bates et al., 2007). When residuals were normally distributed, models were fitted to a Gaussian distribution. While when not normally distributed, models were fitted to a: Gamma distribution if data were continuous, had a constant coefficient of variation and variances increased with means (McCullagh and Nelder, 1989); Binomial distribution if dealing with mycorrhizal colonisation (Alvarez-Santiago et al., 1996); and Negative binomial distribution if data were proportions (McCullagh and Nelder, 1989). Dispersion of residuals for data without normal distribution was checked using *simulateResiduals* function in DHARMa package version 0.3.1 (Hartig, 2017). If residuals were dispersed, we ran analyses with a Quasibinomial distribution or Binomial distribution weighted by total of intercepts for mycorrhizal colonisation data (Hartig, 2017), and with *glmmTMB* (Magnusson et al., 2019) for other variables. When differences were statistically significant, multiple comparisons among levels of fixed factors were assessed with the *glht* function in multcomp package version 1.4-13 in R (Hothorn et al., 2009)

To analyse the relationships among soil features, we performed a Principal Component Analysis (PCA) with the rhizospheric soil features measured underneath each plant using the *rda* function in the vegan package version 2.4-6 (Oksanen et al., 2007).

Table 5.1: Generalised linear models of mycorrhizal colonisation with gypsum affinity and season as fixed factors, and family and species nested within family as random factors. [a] Models were fitted to a Gaussian distribution. [b] Models were fitted to a Binomial distribution and weighted by total counts per individuals.

	Hyphal colonisation[a]		Arbuscular colonisation[b]		Vesicular colonisation[a]	
	Chisq	Pr(>Chisq)	Chisq	Pr(>Chisq)	Chisq	Pr(>Chisq)
Gypsum affinity	0.077	0.779	0.619	0.432	0.006	0.937
Season	10.423	0.015	284.962	<0.001	72.755	<0.001
Gypsum affinity x Season	2.588	0.460	104.096	<0.001	1.630	0.653

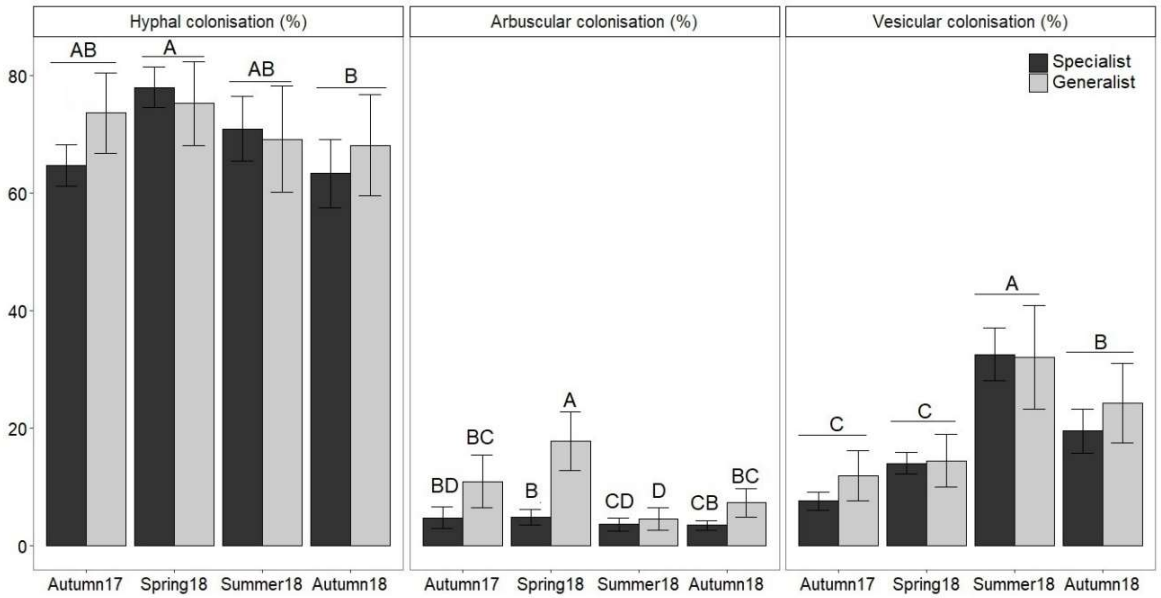
5.3 Results

AMF colonisation

All species displayed arbuscular mycorrhizal fungi in their fine roots, showing typical structures of arbuscular mycorrhizas (hyphae, vesicles and arbuscules) in all samples throughout the year studied (Fig. 5.2). The main differences in AMF colonisation were between different families, while individuals from the same species showed similar colonisation (data not shown): *Cistaceae* species showed the highest colonisation, then *Caryophyllace*, and finally *Brassicaceae* species. A significant effect of sampling time (season) was found for hyphal, arbuscular and vesicular colonisation (Table 5.2, Fig. 5.3). Gypsum affinity was not a significant factor affecting AMF colonisation, although we found a significant interaction between gypsum affinity and season for arbuscular colonisation (Table 5.2, Fig. 5.3). The highest HC was observed in spring and the lowest in autumn 2018, while the highest VC was in summer. The highest AC was in spring, when gypsum generalists also showed higher AC than specialist species, and the lowest AC was in summer (Fig. 5.3).

As for the differences in AMF colonisation between seasons for each plant species (Fig. 5.4), *L. subulatum* did not show seasonality in any AMF structures, whereas the rest of species showed seasonality in some of the structures (Appendix E, Tables E.2 and E.3). Significant differences in HC were found for *G. struthium,* showing higher values in spring. VC varied significantly among seasons in *G. struthium* and both *Helianthemum* species. Seasonal shifts in AC were significant in *M. fruticulosa* and *H.squamatum.* However, while the trend was to show an increase in AC in spring, *H.squamatum* showed also a peak in AC in autumn 2017.

Figure 5.3: Arbuscular mycorrhizal colonisation in plants with different affinity to gypseous soils between autumn 2017 and autumn 2018. Data are means ± *SE*. Different letters indicate significant differences among gypsum affinity and seasons after multiple comparisons.



Plant nutrient content

Leaf C, N, P, N:P ratio and fine root N concentration showed significant seasonal variability (Table 5.2). Gypsum affinity was not a significant factor affecting plant nutrient content, although a significant interaction between gypsum affinity and season was found for leaf P and fine root N (Table 5.2). Overall, the highest leaf C and N concentrations were found in autumn, and the lowest in summer (Table 5.3, Fig. 5.4). Similarly, the highest P was observed in autumn and the lowest in summer, but specialist species showed higher leaf P than generalist species in autumn 2017 and spring (Table 5.3). The highest fine root N concentrations were observed in autumn and the lowest in summer (Table 5.3). Generalist species showed higher fine root N than specialist species in both autumns, and lower values in spring and summer. The highest leaf N:P ratio was in spring, and the lowest in autumn 2017 (Table 5.3).

When we analysed each species separately (Fig. 5.4, see GLMs and means with SE for each species in Appendix E, Tables E.4 and E.5), leaf N, C and P concentrations varied seasonally in all species ($P < 0.05$). Species showed similar patterns of seasonal variation for leaf C, N and P, except for *H. syriacum*, with highest leaf N and leaf P in spring, and *Helianthemum* species, with the highest leaf C in summer and the lowest in autumn 2017. In the case of fine roots, *M. fruticulosa* and *L. subulatum* showed significant seasonal differences for fine root C ($P < 0.05$), with the lowest C concentration in

spring and the highest in autumn. *M. fruticulosa* also displayed seasonality for fine root N ($P < 0.05$), following general trends. Season was a significant effect for leaf N:P ratio only in *Helianthemum* species ($P < 0.05$).

Table 5.2: Generalised linear models of plant organ concentrations of C, N and P with gypsum affinity and season as fixed factors and family and species nested within family as random factors. a Models were fitted to a Gaussian distribution. b Models were fitted to a Negative Binomial distribution.

	Fine root N[b]		Fine root C[b]	
	Chisq	Pr(>Chisq)	Chisq	Pr(>Chisq)
Gypsum affinity	0.040	0.841	2.012	0.156
Season	18.497	0.004	6.410	0.093
Gypsum affinity x Season	14.242	0.003	1.660	0.646
	Leaf N[a]		Leaf C[b]	
	Chisq	Pr(>Chisq)	Chisq	Pr(>Chisq)
Gypsum affinity	0.919	0.338	2.589	0.108
Season	62.377	<0.001	9.382	0.025
Gypsum affinity x Season	6.958	0.073	2.710	0.439
	Leaf P[a]		Leaf N:P[b]	
	Chisq	Pr(>Chisq)	Chisq	Pr(>Chisq)
Gypsum affinity	0.730	0.393	0.947	0.331
Season	60.377	<0.001	8.049	0.045
Gypsum affinity x Season	9.036	0.029	1.378	0.711

Rhizospheric soil chemical characteristics

Season was a significant factor affecting all variables measured in the rhizospheric soil, except for organic matter ($P = 0.312$). Gypsum affinity had only a marginally significant effect ($P = 0.072$) for ammonium concentration (Table 5.4). In general, the highest soil relative humidity was found in spring and autumn 2018, and the lowest in autumn 2017 and summer (Table 5.5). The highest soil nitrate and ammonium concentrations were in autumn 2018, whereas the lowest nitrate was in autumn 2017 and summer (Table 5.5). We recorded the highest P_{olsen} in summer, while other seasons displayed similar concentrations (Table 5.5, Fig. 5.4). However, the rhizospheric soil collected underneath generalist species showed higher P content than that of specialist species in all seasons, except in autumn 2018 (Tables 5.4 and 5.5).

Rhizospheric soil underneath each species showed different ranges in gypsum content, conductivity, carbonate content and pH (Appendix E, Tables E.6 and E.7). Season was also a significant factor affecting the relative humidity of the rhizospheric soil of all species ($P < 0.05$, see Appendix E, Tables E.6 and E.7). All species had different P_{Olsen} in their rhizosphere in different sampling dates

following the general trend (Fig. 5.3), except for *M. fruticulosa*, with highest P_{Olsen} content in autumn 2017, since they were collected in very low gypsum content (Appendix E, Tables E.6 and E.7). The only species displaying significant seasonal changes for ammonium and nitrate were *G.struthium* and *M. fruticulosa,* respectively (Fig. 5.4).

Figure 5.4: Differences in leaf P, soil P_{Olsen} and mycorrhizal colonisation in different sampling dates for each study species. Bars are means with standard errors. Different letters indicate significant differences among seasons within each species (see GLM in Appendix E). Lines are means for all species. Soil P_{Olsen} values of *M. fruticulosa* were out of scale and hence they were divided by 10 for presentation purposes. HC: Hyphal colonisation. AC: Arbuscular colonisation. VC: Vesicular colonisation.

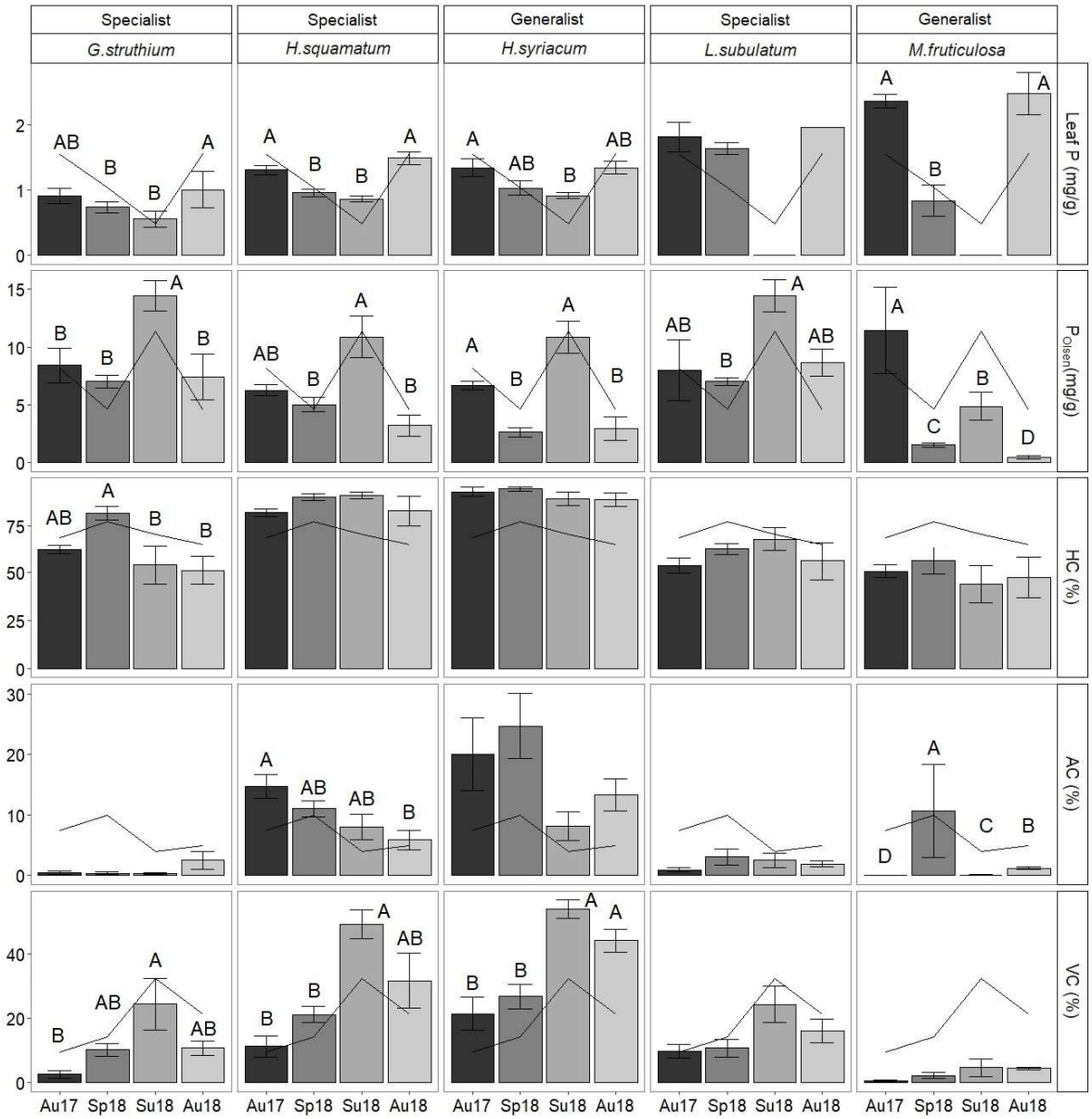


Table 5.3: Means and standard errors of plant organ concentrations. Different letters indicate significant differences among gypsum affinity and seasons after multiple comparisons on generalised models with family and species nested within family as random factors. [a] Models were fitted to a Gaussian distribution. [b] Models were fitted to a Negative binomial distribution.

	Autumn 2017		Spring 2018		Summer 2018		Autumn 2018	
	Specialist	Generalist	Specialist	Generalist	Specialist	Generalist	Specialist	Generalist
Fine root C (mg g^{-1})[b]	466.77±5.53	466.44±3.59	442.50±6.61	454.56±7.22	456.58±6.56	460.50±7.14	451.54±7.76	463.13±3.32
Fine root N (mg g^{-1})[b]	11.20±1.37 AC	12.81±2.86 A	11.12±1.28 AC	8.79±0.85 BC	10.89±1.62 AC	8.67±1.05 C	13.61±1.30 AB	15.97±3.77 A
Leaf C (mg g^{-1})[b]	377.20±11.29	409.73±4.06	385.07±13.21	406.70±9.41	362.60±22.76	439.80±1.02	399.38±10.75	419.40±5.78
Leaf N (mg g^{-1})[a]	24.65±3.46 A	27.22±3.92 A	22.02±2.11 B	19.21±1.20 B	12.05±0.60 C	13.53±0.76 C	28.64±3.24 A	30.66±4.23 A
Leaf N:P ratio[a]	18.61±1.94	14.72±1.05	20.81±1.77	19.23±1.37	19.52±2.74	14.93±0.86	16.55±1.15	16.09±1.40
Leaf P (mg g^{-1})[a]	1.33±0.13 A	1.80±0.18 AC	1.11±0.11 B	1.04±0.08 BC	0.71±0.08 BC	0.91±0.05 BC	1.44±0.10 A	1.84±0.24 A

Table 5.4: Generalised linear model of rhizospheric soil features with gypsum affinity and season as fixed factors and species as a random factor. [a] Models were fitted to a Gaussian distribution. [b] Models were fitted to a Negative binomial distribution.

	Ammonium[a]		Nitrate[b]		Organic matter[a]		P_{Olsen}[b]		Relative humidity[b]	
	Chisq	Pr(>Chisq)	Chisq	Pr(>Chisq)	Chisq	Pr(>Chisq)	Chisq	Pr(>Chisq)	Chisq	Pr(>Chisq)
Gypsum affinity	3.23	0.072	0.01	0.913	0.32	0.571	0.95	0.331	1.11	0.293
Season	14.60	0.002	20.57	<0.001	3.57	0.312	97.92	<0.001	148.15	<0.001
Gypsum affinity x Season	5.25	0.154	4.38	0.223	6.04	0.110	54.33	<0.001	2.03	0.565

Table 5.5: Means and standard errors of rhizospheric soil concentrations. Different letters indicate significant differences among gypsum affinity and seasons after multiple comparisons on generalised models with species as a random factor. [a] Models were fitted to a Gaussian distribution. [b] Models were fitted to a Negative binomial distribution.

	Autumn 2017		Spring 2018		Summer 2018		Autumn 2018	
	Specialist	Generalist	Specialist	Generalist	Specialist	Generalist	Specialist	Generalist
Ammonium (mg kg^{-1})[a]	11.07±0.93 B	11.88±0.91 B	9.37±0.84 B	12.45±0.34 B	10.56±0.80 B	12.12±0.48 B	13.65±0.65 A	13.20±0.37 A
Nitrate (mg kg^{-1})[b]	34.40±5.66 B	35.50±6.02 B	59.69±12.72 AB	40.59±7.10 AB	38.86±6.33 B	40.60±8.43 B	59.74±7.34 A	92.77±18.58 A
Organic matter (mg g^{-1})[a]	11.35±1.16	11.26±1.40	12.14±1.06	12.92±2.05	10.40±1.19	13.21±2.44	8.05±1.12	13.60±2.53
P_{Olsen} (mg g^{-1})[b]	7.60±0.98 BCD	64.45±23.10 AB	6.33±0.37 CD	8.10±2.19 DE	13.25±0.93 AE	26.36±7.53 AC	5.71±1.03 CD	3.60±0.80 DE
Relative humidity (%)[b]	1.07±0.13 B	1.42±0.17 B	6.04±0.82 A	6.19±1.07 A	1.19±0.20 B	1.27±0.22 B	7.13±1.56 A	10.86±1.27 A

5.4 Discussion

According to our first hypothesis, all gypsum species studied displayed AMF in their fine roots, showing typical structures of arbuscular mycorrhizae (hyphae, vesicles and arbuscules) in all samples throughout the year. In support to our second hypothesis, AMF colonisation was seasonal, since the highest VC was in summer and the highest AC was in spring. Contrary to our third hypothesis, the highest AMF root colonisation did not concur with the highest foliar or lowest rhizospheric soil P content, but with the time of maximum P demand for plant growth (i.e. the time when leaf N:P ratios were lowest). Finally, in partial support of our last hypothesis, gypsum generalist species showed higher AMF colonisation than specialist species, although only for AC in spring.

Gypsum species showed seasonal differences in AMF colonisation

All five gypsum plant species analysed displayed AMF, with the formation of arbuscules throughout the year. They included *Brassicaceae* and *Caryophyllace* species, which are usually cited as non-mycorrhizal families (Brundrett, 2009). Colonisation by arbuscules had already been found in *L. subutatum* and *G. struthium* on gypsum (Palacio et al., 2012) and in other taxa of *Lepidium*, *Matthiola* and *Gypsophila* from other environments (Hempel et al., 2013), which calls for caution when assuming the inability of *Brassicaceae* and *Caryophyllaceae* to interact with AMF. Studied species of *Cistaceae* showed the highest hyphal colonisation and *Brassicaceae* showed the lowest, independently of their affinity to gypsum soils. Apart from AMF, we observed Hartig nets typical of ectomycorrhiza fungi in both *Helianthemum* species, although we did not quantify their root colonisation. Gypsum plants in our study also had colonisation of dark septate endophytes, such as those described by Porras-Alfaro et al. (2014) in plants growing on gypseous soils from the Chihuahuan Desert.

Previous studies had reported AMF colonisation in plants from gypseous soils (Alguacil et al., 2009; Hernández y Hernández et al., 2020; Palacio et al., 2012; Torrecillas et al., 2014), but seasonality was neglected and most of these studies were conducted only in spring, when plants show high growth activity (Alguacil et al., 2009).Our results confirm that arbuscular mycorrhizal colonisation in gypsum species varies seasonally, similar to previous studies in other drylands (Fakhech et al., 2019; Roldan and

Albaladejo, 1993; Varela-Cervero et al., 2016). Most of these previous studies measured the highest hyphal colonisation in spring, but did not account for vesicular or arbuscular colonisation. Our results for arbuscular colonisation agree with those for hyphal colonisation of previous studies. However, these results are not fully comparable, since arbuscules and hyphae differ in functionality. Arbuscules are the unique structures involved directly in nutrient transfer to the plant (Allen, 1983; Mullen and Schmidt, 1993), whereas hyphae are the vegetative structures of fungi (Brundrett, 2009), and vesicules are storage structures (Jakobsen et al., 2003). We observed seasonality in arbuscular (AC) and vesicular colonisation (VC), but not in hyphal colonisation (HC). AC was high in spring, when the highest AM fungal activity is expected in the Mediterranean climate (Alguacil et al., 2009), and low in summer, when plants showed reduced growth activity in our study system (Palacio and Montserrat-Martí, 2005). In addition, VC was high in summer, since vesicles appear at later stages of fungal colonisation (Jakobsen et al., 2003) and during arbuscule senescence (Brundrett, 2009). AM fungi are not the unique root-associated fungi with seasonal colonisation (Mandyam and Jumpponen, 2008), and consequently we also found seasonal colonisation of dark septate fungi (DSF). DSF showed higher root colonisation in *Helianthemum squamatum* in spring than autumn 2017, and inversely in *Matthiola fruticulosa* (data not shown). While the beneficial role of AMF fungi on plant nutrition is well-established (Johnson, 2010), the effect of DSE on plant nutrition remains equivocal (Newsham et al., 2009).

Both gypsum specialist and generalist species showed increased root colonisation by arbuscules during high P-requirements in spring

All plants analysed showed the highest foliar P and N concentrations in autumn, after the peak of P_{Olsen} rhizospheric soil concentration in summer, and concurring with maximum nitrate and ammonium concentrations in the soil. Such increased nutrient foliar concentrations were decoupled from arbuscular colonisation, since we observed low root colonisation by arbuscules in summer and autumn. We expected a high arbuscular colonisation when plants demanded P, either autumn or spring, since gypsum soils are remarkably P-impoverished (FAO, 1990). For example, gypseous soils led to lower plant growth and lesser P accumulation on leaves than other similar calcic soils (Cera et al., 2021). Similar to our results, Hernández and Hernández et al., (2020) found a negative correlation between AMF root

colonisation, dissolved organic nutrients in soil and microbial N and P in gypseous soils from the Chihuahua Desert. These results may indicate that, despite the low N and P concentration in gypseous soils, gypsum plants use other acquisition strategies, different to AMF, to uptake P and N, especially when nutrient availability in the soil is high (for example in autumn with high relative humidity). Symbiosis with AM fungi may benefit plants when P demand by the plant exceeds the capacity of the root system to uptake nutrients independently of AMF (Fitter, 1991).

In the seasonal environment analysed, studied species arrested growth in summer and some species, like *Lepidium subulatum* and *Matthiola fruticulosa* are summer deciduous. Gypsum plants restart their growth at the end of summer (Palacio and Montserrat-Martí, 2005), probably absorbing nutrients with acquisition strategies not only related to AMF symbiosis, but to phosphatase and organic acid exudation, or enhanced expression of P_i transporters (Lambers et al., 2018; Vance et al., 2003). All study species but *G.struthium* have shallow roots and reduced root development (Guerrero-Campo et al., 2006), thus without specialised root architecture to enhance P-mining (Palacio et al., 2012). However, the main root growth in these plants is in autumn (Palacio and Montserrat-Martí, 2007), which can favour nutrient uptake. For example, *Lepidium subulatum* shows an opportunistic growth to exploit sporadic N pulses in autumn (Palacio et al., 2014b), probably with rapid root proliferation to enhance nutrient acquisition in seasonal environments (Jackson and Caldwell, 1989; Palacio and Montserrat-Martí, 2007). A decrease in AMF colonisation may occur when P supply by roots is high and plants limit the symbiosis with fungi to reduce associated carbon costs (Lambers et al., 2008c). Accordingly, we found that N content in fine roots was high in autumn, indicating high fine root activity (Roumet et al., 2016). In addition, during the wet autumn (2018), species showed higher vesicular colonisation than in autumn 2017 (dry), probably because AMF may not be providing nutrients to the host plants, but keeping them to support future growth or storage (Johnson, 1993; Koyama et al., 2017).

In spring, the studied species showed high leaf N:P ratio, which indicates high P requirements in leaves (Güsewell, 2004). At this time of the year, most study species showed a peak in shoot growth rate (Palacio and Montserrat-Martí, 2005), flowering in spring and early summer (data not shown), with increased demand for P (Milla et al., 2005). However, such increased demand concurred with decreased P-inorganic (measured as P_{Olsen}) availability in rhizospheric soils. It is, hence, not surprising that the

highest arbuscular colonisation (AC) was recorded in spring, when plants can benefit from AMF getting extra P than that available to their roots alone.

The gypsum generalist species studied displayed higher arbuscular colonisation than specialist species, although only in spring. This result was similar to another previous study on gypsum outcrops (Palacio et al., 2012), indicating that spring is the most discriminating season to analyse responses in AMF between gypsum generalist and specialist species. According to Palacio et al., (2012) and Torrecillas et al. (2014), specialist species seem to be more specialised to gypseous soils, and to its P cycle and seasonal availability, likely displaying other mechanisms of nutrient acquisition, because they displayed reduced AMF-symbiosis. On the other hand, the dependence of generalist species on AMF symbiosis would indicate a stress-tolerant strategy to cope with the limiting conditions in gypsum environments (Palacio et al., 2012).

5.5 Conclusions

Studied gypsum species showed seasonal AMF colonisation, decoupled from seasonal shifts in foliar N and P content and from shifts in N and P rhizospheric soil availability. Arbuscular colonisation was higher in spring, when P demand by the plant may exceed the capacity of the root system to uptake sufficient nutrients due to low soil availability. These trends were particularly marked in studied generalist species. Our results exemplify the need to study seasonal changes in plant-AMF-soil interactions to gain insight into P-acquisition strategies in plants growing in nutrient-limited environments.

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Author contributions

AC and SP conceived the study and designed the methodology; SD, GM, SP and AC collected field data; SD, AG, AC conducted analyses. SD, AC analysed data. AC led the manuscript writing. All authors discussed the results and wrote the manuscript.

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General discussion

General discussion

This PhD thesis provides evidence that widely distributed Iberian gypsophile shrubs (hereafter, gypsophiles) are soil specialists adapted to gypsum environments, owing to their edaphic factors, but also to other factors affecting the evolution of plants, like grazing. According to the results presented here, the fundamental niche of gypsophiles is not only constrained to gypsum soils, but mainly to alkaline soils with high calcium availability. This would identify soil pH as a limiting factor for the establishment of gypsum endemics. Results from Chapter 1 show how a set of six gypsophiles from the Iberian Peninsula and North America were able to germinate in calcic, but not in acidic soils. This result is in line with the fact that five dominant gypsophiles from the Iberian Peninsula were able to complete their life cycle also in calcic soils (Chapter 2). Importantly, this PhD thesis shows that herbivory plays a crucial role in the assembly of plant communities in gypsum drylands (Chapter 3). In accordance with the main hypothesis that gypsophiles are soil specialists with strategies linked to gypsum soils, the results obtained show that gypsophiles have a whole-plant elemental concentration linked to features of gypsum soils, which is also maintained when grown in calcic soil (Chapter 2). Indeed, gypsophiles show high capacity to uptake nutrients in gypsum soils, being foliar accumulators of gypsum excess elements (S, Mg, Ca), whereas gypsovags accumulate them at root level (Chapter 4). Gypsophiles also adapt to the phosphorus scarcity of gypsum environments by being less dependent on AMF symbiosis, and probably adjusting their uptake to seasonal nutrient pulses (Chapter 5). Finally, our data do not support the hypothesis that gypsophiles have better performance than gypsovags only in gypsum environments because they are gypsum specialists with gypsum-specific strategies. According to our results, gypsophiles showed similar germination, and similar or lower biomass in gypsum than calcic pots in the two experiments conducted, and similar trends to gypsovags (chapter 1,2,4). However, species with high affinity to gypsum and high leaf S content (i.e. gypsophiles) were more likely to assemble than other species under high or moderate herbivory pressure in gypsum soils (Chapter 3). These results point at a relevant role of grazing in providing selective advantage to gypsophiles when growing on gypsum, although the underlying mechanisms and the individual responses of gypsophiles to herbivory remain poorly resolved (Chapter 4).

Gypsophiles have high affinity to alkaline soils with Ca

Iberian gypsophile shrubs are largely restricted to gypsum soils in natural environments (Mota et al., 2011), and are broadly considered as edaphic endemics (Escudero et al., 2015; Moore et al., 2014; Parsons, 1976), indicating edaphic factors mediate prominently on the ecological amplitude of gypsophiles (Guerrero-Campo et al., 1999; Meyer, 1986; Romão and Escudero, 2005). However, Ballesteros et al. (2014) showed experimentally that some Iberian gypsophiles were able to grow on marls, and this PhD thesis contributed that gypsophiles were able to complete their life cycle, producing viable seeds in calcic soils in a common garden experiment (Chapter 2). Further, most gypsophiles germinated better in alkaline than in acidic soils, regardless of the gypsum content of soils, in a germination trial with natural soils including both gypsum, acidic and calcic soils (Chapter 1). Consequently, it seems soil pH and soil Ca content may be two factors constraining the fundamental niche (or physiological amplitude) of gypsophiles, but they are not the only ones modulating their realised niche (or ecological amplitude; Lambers et al., 2008). The gypsum and calcic soils used in our common garden experiment shared high Ca^{2+} availability, high carbonate content and alkaline pH (Chapter 1). Ca^{2+} availability is a key factor affecting seed germination ability of many plant species (Anderson, 1982), and could be the main soil effect on germination of gypsophiles (Merlo et al., 1997). Moreover, North American gypsophiles showed similar trends in the same germination trial (Chapter 1). Field observations in Spain also support these experimental results indicating that, even though gypsophiles are much more frequently found in gypsum soils, they are also sometimes found naturally off gypsum (Luzuriaga et al., 2015; Mota et al., 2011). The soils where gypsophiles grow off gypsum are frequently calcic or other alkaline soils, as gypsum outcrops are commonly intermixed with alternating layers of marls, limestone and clays (Quirantes, 1978). Nevertheless, it is unclear whether the few gypsophile individuals found growing off gypseous soils in nature could complete their life cycle, producing viable seeds and recruiting new individuals, since most data were observations of presence / absence. In any case, care should be taken when extrapolating results from experimental studies to natural conditions (Wenk and Dawson, 2007). In both experiments (Chapter 1, 2), weeding and watering were carried out regularly, eliminating any potential competition from neighbouring plants or water stress, conditions that are far from those in natural environments. The combination of different

factors (plant competition, drought, altered soil chemistry) could be the underlying mechanisms explaining gypsophile restriction to gypsum soils, rather than soil chemistry alone, as demonstrated by both experiments. Further experiments on natural gypsum and calcic soils testing for the combined effects of soil chemistry plus plant competition and water availability are needed to shed light on these issues. Despite the need for caution, these results would support the notion of a possible origin of Iberian gypsophile lineages from a calcicolous flora (Chapter 2, 3).

Herbivores play a role in determining plant assemblages in gypsum drylands

Plant community assembly on gypsum soils has traditionally been explained in relation to plant adaptation to the limiting soil conditions of gypsum (Luzuriaga et al., 2020, 2015). Similarly, the literature on plant functional traits to thrive in gypsum environments focused mainly on soil constraints (Escudero et al., 2015; Moore et al., 2014). However, gypsum plant communities have also been associated with disturbed environments, either by fauna or geomorphology (Braun-Blanquet and Bolòs, 1958; Guerrero-Campo et al., 1999). Braun-Blanquet and Bolòs (1958) even suggested that grazing disturbance may favour gypsophiles in gypsum plant communities of the Iberian Peninsula. Even, livestock practice in the Iberian Peninsula could have modulated the populations of gypsophiles (such as *Lepidium sublatum* in Blanco-Sánchez et al., 2021). Contrastingly, Pueyo et al., (2008) found that grazing negatively affected the annual gypsum specialist *Campanula fastigiata* Dufour ex Schult, while perennial gypsophiles had similar cover in grazed and ungrazed conditions in the Middle Ebro Basin. This PhD thesis remarkably contributes to this topic, by providing evidence on the major role played by herbivory in the ecology of gypsophiles, together with edaphic factors (Chapter 3). The assembly of gypsum plant communities under high or moderate grazing intensities is mediated by the affinity of species for gypsum, promoting gypsophiles over other species with low gypsum affinity (Chapter 3).

Positive effects of grazing on plant communities in resource-poor environments, like gypsum environments, are usually observed in communities with a long evolutionary history of grazing (Cingolani et al., 2005). The effect of large herbivores and livestock grazing have usually been associated with open shrublands (Asner and Levick, 2012; Bakker et al., 2016), similar to the vegetation found in gypsum soils (Mota et al., 2017). Thus, gypsophiles likely evolved under grazing pressure

mediated by large herbivores, with sufficient functional variability among individuals to cope with different grazing intensities (Chapter 3). Similar results were observed in other edaphic endemic species associated to unusual soils. For example, moderate grazing favoured native serpentine grasslands over exotic plants (Beck et al., 2015) and halophytes or subhalophytes over glycophytes (Bonis et al., 2005). In both cases, edaphic-endemics appear to be more ecologically successful when grazing releases competition and promotes the harsh conditions of unusual soils (Bonis et al., 2005).

Gypsophiles have a unique nutritional strategy related to gypsum environments

If herbivory has been a driver in the evolution of gypsophiles, they should have resistance mechanisms to tolerate or avoid grazing in order to not be excluded or to perform better than other plants. The results presented here show that gypsophiles are specialists of gypsum soils, tolerating high concentration of elements found in excess in their leaves, and they are less dependent to AMF symbiosis. I suggest that both mechanisms are part of a gypsum-specific nutritional strategy and that such strategy is related to their ability to tolerate grazing on gypsum soils. This PhD thesis evidences that gypsophiles had higher S and Mg and lower K concentrations in leaves, stems and coarse roots than gypsovags irrespective of the substrate (Chapter 2,4). These results are in accordance with previous studies of plants growing on gypsum soils, where the leaf S and Mg concentrations of gypsophiles tended to be higher than those of gypsovags (Alvarado, 1995, Palacio *et al.*, 2007, Muller *et al.*, 2017). Gypsophiles and gypsovags differed in the elemental composition of all vegetative organs when grown on gypsum soils (Chapter 4). However, gypsophiles maintained the same ability to accumulate S and Mg in calcic soils without gypsum (Chapter 2,4), indicating a metabolic specialisation of gypsophiles to gypsum soils (Kruckeberg & Rabinowitz, 1985; Mota et al., 2017; Palacio et al., 2007). Gypsophiles also had generally higher elemental pools in leaves, stems and coarse roots than gypsovags, while gypsovags tended to have higher pools of most elements in fine roots (Chapter 4). This behaviour was especially clear for S, which is the main discriminant element between calcic and gypsum soils (Chapter 2), and between gypsophiles and gypsovags foliar composition (Merlo et al., 2019). Consequently, these results confirm that gypsophiles show high capacity to uptake excess element in gypsum, resulting in a

remarkable leaf accumulation of S and Mg (Merlo et al. 2019), while gypsovags accumulate these elements in fine roots, possibly blocking translocation to aboveground organs.

Scarcity of nutrients is a common feature of gypsum soils (Alvarado, 1995), often considered a limiting factor for plant life (FAO, 1990). Previous studies on gypsum outcrops showed that Iberian gypsovags displayed higher colonisation of arbuscules than gypsophiles (Muries Berenguer, 2017; Palacio et al., 2012), which was interpreted as an strategy to cope with the low availability of phosphorus in gypsum soils. These studies focused only in spring, the period with high activity of AM fungi in gypsum environments (Alguacil et al., 2009). However, the peak of phosphorus and nitrogen in Mediterranean gypsum soils is in late summer and autumn (Delgado-Baquerizo et al., 2011; Magid and Nielsen, 1992), and root colonisation by AMF is also associated with soil nutrient availability (Hoeksema et al., 2010). This PhD thesis evidences that gypsovags showed higher colonisation of arbuscules than gypsophiles only in spring, and that gypsophiles always showed low AMF dependence regardless of season. The dependence of gypsovags on AMF symbiosis would indicate a stress-tolerant strategy to cope with the limiting conditions in gypsum environments (Palacio et al., 2012), because AMF-symbiosis is a high C- costly strategy to enhance P-acquisition (Johnson, 2010; Lambers et al., 2018). Whereas gypsophiles seem to be more specialised to gypsum soils, and to its P cycle and seasonal availability (Palacio et al., 2012; Torrecillas et al., 2014), probably adapting their P uptake to nutrient pulses (Palacio et al., 2014), or with other low C-costly strategies to improve P-uptake such as specialized root architecture (see more on Lambers et al., 2018; Vance et al., 2003).

Ecological significance of the unique nutritional strategy of gypsophiles

Gypsophiles would have become soil specialists to perform better under grazing pressure on gypsum soils, and not just to perform or grow better on gypsum soils. Gypsum environments are stressful, with water stress and marked soil ion imbalances (Guerrero-Campo et al., 1999). Both gypsovags and gypsophiles seem to be adapted to stressful environments. Hodgson et al. (1994) found mainly traits related to stress tolerance strategy in gypsum plant communities in the Iberian Peninsula. Thus, adaptation to stressful conditions (climatic and edaphic) would not explain the different realised niche between gypsophiles and gypsovags. Our results indicate that gypsophiles grow similarly in

gypsum soils and in more nutrient rich substrates, such as the calcic soil used in our common garden experiments (Chapter 1, 2, 4). However, gypsophiles responded positively to moderate and high grazing pressure in the field (Chapter 3). This positive response would only occur under "gypsophilic" conditions, i.e. with soil stress caused by high gypsum content in soil. Further, "gypsophilic" conditions usually only occur in drylands, as gypsum is easily weathered in humid climates (Poch et al., 2018). Thus, gypsophiles appear to be adapted to stressful gypsum environments with some degree of disturbance, where many stress-tolerant species would be excluded. This argument would explain why gypsovags currently dominate species in gypsum plant communities in the Middle Ebro Basin where disturbance frequency and intensity are low (Cera, Palacio, Montserrat-Martí, personal observation), or why gypsophiles are excluded from gypsum soils in more humid climates (Pérez-García et al., 2017). We suggest that gypsophiles should become soil specialists to overcome the harsh conditions in gypsum soils, but also acquire traits related to disturbance resistance, such as geomorphological factors (Guerrero-Campo et al., 2008) or herbivory.

One of these traits could be to become more permeable to gypsum soils, which has consequences on the mineral nutrition of gypsophiles (Chapter 4). The permeability of gypsophiles to gypsum soils could underline, at last partly, the better performance of gypsophiles over gypsovags in disturbed gypsum environments (Chapter 3). Gypsovags could have endodermal barriers at the root level as part of their stress-tolerant strategy, which would reduce ionic permeability and limit cation acquisition (Courbet et al., 2019), like plants adapted to saline soils or to soils with high heavy metal availability (Barberon, 2017). Whereas gypsophiles might be more permeable to excess gypsum cations, and may also have mechanism to promote sulphate acquisition, probably by enhancing demand in aboveground organs (Lappartient and Touraine, 1996). This likely strategy of gypsophiles could be more efficient in nutrient acquisition in an ion- imbalanced soil as gypsum soils, potentially leading to higher growth. However, we did not observe better germination nor performance of gypsophiles over gypsovags in any experiment in gypsum soils (Chapter 1,2,4). We observed a better assembly of species with high gypsum affinity and high leaf S content (gypsophiles) than other species (perennial gypsovags) in gypsum drylands under grazing pressure (Chapter 3). Thus, the unique nutritional strategy of gypsophiles could be also a mechanism to confer resistance to grazing. Plant resistance to grazing can be achieved through

two different strategies: tolerance or avoidance (Briske and Richards, 1995). Tolerance means the ability to persist after disturbance, being able to compensate with new growth for the biomass lost. Contrastingly, avoidance is related to mechanisms enabling plants to escape disturbance, such as, for example, thorns or spikes in plants subjected to grazing (Briske and Richards, 1995). The clipping experiment described in Chapter 4, did not show evidence of compensatory growth in any species one year after a grazing event, irrespectively of their affinity for gypsum soils or the substrate where they grew. However, we observed higher leaf N content in some gypsophiles living in areas subjected to long-term high grazing in the field (data not shown). In either case, more precise measurements of plant growth rates are needed to demonstrate if gypsophiles grow better after grazing in gypsum than other soils. The S and Mg accumulating ability of gypsophiles may also be related to a high investment in plant defence leading to avoidance mechanisms. Their permeability to gypsum soils requires mechanisms to tolerate gypsum excess elements (S, Ca, Mg) in aerial organs, and accumulation in the shoot epidermis is a plausible mechanism (Lux et al., 2021). This strategy has been associated to plant defence in other plant species, such as heavy metal hyperaccumulation (Boyd, 2007), accumulation in the form of crystals by *Acacia* (He et al., 2014), or glucosinolate accumulation by *Brassicales* (Ernst, 1990). Our results show such avoidance mechanisms are not induced by grazing, at least after an isolated grazing event (Chapter 4). Nevertheless, more studies are needed to evaluate the individual responses of gypsum plants to herbivory, and to unveil the underlying role that the unique nutritional strategies of gypsophiles may play.

Conclusions

Main conclusions

- The adaptation of gypsophiles to gypsum environments has been traditionally associated with edaphic features, although this PhD thesis provided further results that indicate herbivory also played a major role in defyining the ecology of gypsophiles from the Iberian Peninsula.
- The ability of gypsophiles to germinate and grow in calcic soils could indicate a possible origin of Iberian gypsophile lineages from calcicolous species.
- Gypsophiles are gypsum specialists with a singular nutritional strategy not shared by gypsovags, linked to the characteristics of gypsum soils, and even maintained when growing off gypsum. This singular nutritional strategy involves being permeable to the excess elements in gypsum soils, but also adapting root nutrient uptake to the low and seasonal nutrient availability of P in gypsum environments.
- As a consequence of their unique nutritional strategy, gypsophiles are foliar accumulators of S, Ca, Mg, and have less dependence on AMF symbiosis. Whereas gypsovags accumulate S, Ca, Mg at the root level, and have high dependence on AMF symbiosis to face P scarcity.
- Gypsophiles are soil specialists that perform better under grazing on gypsum soils. They, hence, seem to be adapted to both constraints: gypsum soils and disturbance, because they have similar germination and growth in gypsum soils than gypsovags, and appear to be more ecologically successful when grazing increases on gypsum.
- The unique nutritional strategy of gypsophiles would confer better performance in disturbance conditions only in gypsum soils. However, this PhD thesis only provides evidences after long-term effects in the field, because species with high leaf S content were more likely to assemble under moderate and high grazing.
- More studies are needed to evaluate plant responses to gypsum environments (including edaphic factors, climatic factors, disturbances and plant-plant interactions), especially focusing on plants growth rate and fitness. It is particularly relevant to unveil to what extent the unique nutritional strategies of gypsophiles mediates their responses to disturbance.

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Supporting information

Appendix A. Supplementary information of chapter 1

Table A.1. Eigenvalues and their contribution to the three PCA components of different soil features.

	PC1	PC2	PC3
Eigenvalue	19.3310	5.1637	0.78662
Proportion Explained	0.7435	0.1986	0.03025
Cumulative Proportion	0.7435	0.9421	0.97236

Table A.2. Loadings of different soil variables after PCA.

	PC1	PC2	PC3
Sand	0.81216262	0.74742263	-0.63035516
Silt	-0.81979579	-0.69603774	0.63570697
Clay	-0.78049907	-0.82403258	0.60681641
Gypsum	0.45792349	-1.49481653	1.06165979
N	-0.81973022	-0.6983762	-0.70418734
C	0.8711098	-0.32190382	0.07435113
pH	0.92710277	0.21442372	0.1864288
EC	0.6860122	-1.17419175	-0.90880439
Ag	-0.93431942	-0.01267348	0.0034175
As	-0.86805307	-0.24858742	-0.49291883
B	-0.04683177	1.5891217	2.13940186
Ca	0.92949281	-0.18988606	0.0652658
Cu	-0.81214226	0.86473665	0.46641504
Fe	-0.88907602	0.55701829	0.11247929
K	-0.88860049	-0.49079509	0.4448465
Mg	0.87277194	0.2598964	1.35888703
Mn	0.90666248	0.42404762	0.19107025
Na	0.69708902	1.14290731	-0.86596146
Ni	-0.84657452	-0.60380213	1.12106367
Pb	-0.93075734	-0.11298284	0.12901004
P	0.59568485	1.32305527	0.45531298
Si	-0.92655958	0.23439107	-0.06576585
S	0.73964353	-1.08197612	-0.58859987
Sr	0.86437176	-0.68857866	-0.12821485
Tg	0.63861159	-1.11403242	1.59361653
Zn	-0.8364914	0.62800259	-0.98981175

Appendix B. Supplementary information of chapter 2

Table B.1: Means and standard deviation of elemental composition of native soils

Element ($mg \cdot g^{-1}$)	Calcic soil	Gypseous soil
Al	9.02±1.78	6.84±0.54
C	38.27±11.82	14.1±7.01
Ca	142.04±5.84	144.38±2.17
Cr	1.5E-2.0±2.0E-3.0	1.1E-2.0±3.2E-4.0
Cu	1.5E-2.0±1.6E-3.0	1.1E-2.0±9.6E-4.0
Fe	8.09±0.16	4.91±0.12
K	3.49±0.84	2.18±0.30
Li	1.0E-2.0±6.9E-4.0	1.6E-2.0±1.1E-3.0
Mg	4.76±0.16	14.1±0.62
Mn	1.5E-1.0±2.2E-3.0	1.2E-1.0±6.1E-3.0
Mo	0	0
N	0.50±0.06	0.52±0.02
Na	0.26±0.02	0.39±0.03
P	0.63±0.02	0.18±0.00
S	3.00±0.11	167.93±3.79
Si	5.97±0.80	4.96±0.29
Ti	0.13±0.05	0.11±0.01
Zn	5.7E-2.0±1.2E-2.0	2.0E-2.0±1.4E-3.0

Table B.2: Means and standard deviation of physicochemical features of native soils

Soils	Chemical propierties		Texture			Minerals	
	pH	Conductivity (µS)	Sand (%)	Lime (%)	Clay (%)	Gypsum (%)	CaCO3 (%)
Gypseous	8.10±0.02	2330.60±15.03	54.11±1.76	32.51±0.98	13.39±0.96	70.68±4.48	13.54±0.35
Calcic	8.60±0.07	232.18±128.72	68.59±3.63	25.35±2.21	6.06±1.46	1.28±1.25	48.98±3.65

Table B.3: F-ratios of GLMMs in which soil (calcic and gypseous) and gypsum affinity (gypsovag and gypsophile) were fixed factors and species was a random factor. Bold type indicates $P < 0.05$.

Variables		Treatments		
	N	Soil	Gypsum affinity	Soil* Gypsum affinity
Final canopy area[2]	98	**6.55**	**6.96**	0.03
Final length[2]	98	0.00	2.81	1.39
Gradualness[2]	96	0.62	0.03	0.07
Max. Length per day[2]	99	0.19	0.02	0.05
Day max. Length per day[3]	99	0.27	**7.74**	**4.08**
E[3]	50	1.44	0.45	1.79
A[3]	50	**6.03**	0.04	0.31
Gs[1]	50	1.10	0.00	1.48
WUE[1]	50	0.03	0.16	0.80
SLA[2]	98	**6.83**	1.99	1.37
LDMC[3]	98	0.18	**4.28**	0.65
Leaf N[3]	93	2.16	0.05	2.48
Day 1st Flower[1]	82	**8.87**	**33.36**	1.54
Day Max Flowering[2]	82	2.48	**32.66**	0.16
Max. Flowering	80	1.03	**5.22**	1.43
Individual seed mass[2]	66	1.66	0.38	2.46
Total biomass[2]	98	**6.00**	0.20	1.33
Root:Shoot[2]	98	0.36	**5.46**	1.79

All differences were assessed with Normal distribution, whether residuals did not fit a normal distribution: [1]Differences were assessed with Gamma distribution with identity as link function. [2]Differences were assessed with Gamma distribution with log as link function.

Table B.4: F-ratios of GLMMs within gypsum affinities in which soil (calcic and gypseous) was a fixed factor and species was a random factor. Bold type indicates $P < 0.05$.

Variables	Gypsovags	Gypsophiles
Final canopy area	2.40[2] (*N*=49)	**4.60[2]** (*N*=49)
Final length	0.59[2] (*N*=49)	1.68 (*N*=49)
Gradualness	0.02[2] (*N*=39)	1.13[2] (*N*=45)
Max. Length per day	0.02[2] (*N*=49)	0.20[2] (*N*=49)
Day max. Length per day	1.03[2] (*N*=49)	**4.51[1]** (*N*=49)
E	0.12 (*N*=19)	2.85 (*N*=31)
A	2.67 (*N*=19)	3.92 (*N*=31)
Gs	0.18 (*N*=19)	2.25[1] (*N*=31)
WUE	0.86 (*N*=19)	0.26[1] (*N*=31)
SLA	1.62[2] (*N*=49)	**5.27[2]** (*N*=49)
LDMC	0.93 (*N*=49)	0.06 (*N*=49)
Leaf N	**4.38** (*N*=44)	0.00 (*N*=49)
Day 1st Flower	**7.19**[1] (*N*=42)	1.13 (*N*=40)
Day Max Flowering	3.64[1] (*N*=42)	0.45 (*N*=40)
Max. Flowering	0.00 (*N*=42)	1.89 (*N*=40)
Individual seed mass	**4.27[2]** (*N*=33)	0.02[2] (*N*=33)
Total biomass	**5.35[2]** (*N*=49)	1.09[2] (*N*=49)
Root:Shoot	1.70[1] (*N*=49)	0.78 (*N*=49)

All differences were assessed with Normal distribution, whether residuals did not fit a normal distribution:[1]Differences were assessed with Gamma distribution with identity as link function. [2]Differences were assessed with Gamma distribution with log as link function.

Table B.5: F-ratios of GLMMs with soil (calcic and gypseous) and gypsum affinity (gypsovag and gypsophile) as fixed factors and time, family and species nested within family as random factors. Bold type indicates P <0.05.

Elements N=192	Treatments		
	Soil	Gypsum affinity	Soil*Gypsum affinity
Al[1]	0.39	**10.28**	0.00
C (N=182)	**16.29**	2.52	2.04
Ca	2.19	3.22	**7.47**
Cr(N=186)[2]	3.10	**4.23**	**8.77**
Cu[1]	**4.43**	1.54	2.23
Fe[1]	0.58	**15.00**	0.76
K[2]	**28.53**	**27.39**	0.00
Li (N=185)[2]	**19.72**	2.72	1.40
Mg[1]	**9.06**	**49.89**	0.08
Mn[2]	**9.51**	**7.16**	1.01
Mo (N=178)[2]	**46.90**	3.07	**5.67**
N (N=182)[2]	1.42	1.17	0.91
Na[1]	1.18	**9.37**	0.18
P[1]	**45.66**	0.06	0.11
S[2]	**82.77**	**48.54**	0.09
Si	0.30	0.09	0.12
Ti (N=190)[2]	0.85	0.21	0.79
Zn[2]	0.33	0.28	0.03

All differences were assessed with Normal distribution, whether residuals did not fit a normal distribution:[1]Differences were assessed with Gamma distribution with identity as link function. [2]Differences were assessed with Gamma distribution with log as link function.

Table B.6: F-ratios of GLMMs within gypsum affinity types in which soil (calcic and gypseous) was a fixed factors and time, family and species nested within family were random factors. Bold type indicates P <0.05.

Elements	Gypsovag (N=93)	Gypsophile (N=99)
Al	0.16[1]	0.27[1]
C	2.36(N=90)	**11.64[1]**(N=92)
Ca	**11.15**	0.53[2]
Cr	1.35[1](N=92)	**5.44[2]**(N=94)
Cu	**4.02**	2.54[2]
Fe	0.14[1]	1.18[1]
K	**19.95[2]**	**11.56[1]**
Li	**13.67[2]**(N=91)	**5.92[2]**(N=94)
Mg	0.02[1]	1.97
Mn	1.94[1]	**8.70[2]**
Mo	0.49[1](N=83)	**16.11[1]**(N=96)
N	2.58[1](N=90)	0.00[2](N=92)
Na	0.43[2]	0.941
P	**63.93[1]**	**15.54[2]**
S	**34.83[2]**	**48.33**
Si	0.02	0.48
Ti	0.78[2]	0.00[1](N=97)
Zn	0.28[1]	0.00[2]

All differences were assessed with Normal distribution, whether residuals did not fit a normal distribution:[1]Differences were assessed with Gamma distribution with identity as link function. [2]Differences were assessed with Gamma distribution with log as link function.

Table B.7: Means and standard deviation of the Euclidean distance between the chemical composition of each species in gypseous vs. calcic soil, plus differences between soil types on each species based on Center Log-ratio transformed elements. F-ratios. P-value from multiple comparisons of species*soil interaction. P-values were adjusted by Bonferroni. Bold type indicates the three most variable elements on each species between soil types.

Species	Gypsum affinity	Distance	F-ratio	P-value	P-adjusted
Boleum asperum	Gypsovag	1.55±0.45	0.972	0.428	1.000
Gypsophila hispanica	Gypsophile	2.15±0.65	8.1987	0.002	0.380
Herniaria fruticosa	Gypsophile	1.83±0.72	1.2709	0.268	1.000
Helianthemum squamatum	Gypsophile	1.56±0.43	1.5726	0.173	1.000
Helianthemum syriacum	Gypsovag	1.68±0.31	11.102	0.001	0.190
Lepidium subulatum	Gypsophile	1.17±0.35	0.9161	0.459	1.000
Linum suffruticosum	Gypsovag	1.61±0.48	4.1974	0.002	0.380
Matthiola fruticulosa	Gypsovag	1.82±0.49	1.9173	0.084	1.000
Ononis tridentata	Gypsophile	1.86±0.42	7.5144	0.001	0.190
Rosmarinus officinalis	Gypsovag	1.80±0.42	7.4364	0.001	0.190

Table B.8: F-ratios of GLMM within species in which soil (calcic and gypseous) was a fixed factor and time was a random factor (b=2). Bold type indicates $P < 0.05$.

Species	Gypsum affinity	Al	C	Ca	Cr	Cu	Fe	K	Li	Mg
Boleum asperum (*N*=16)	Gypsovag	0.17	0.01(*N*=15)	1.67	**5.09**	0.43	2.80	0.22	0.05[1]	0.05
Gypsophila hispánica (*N*=20)	Gypsophile	0.10	1.89	0.05	1.29[1](*N*=19)	0.00	0.01	**10.00**	4.18(*N*=16)	4.18
Herniaria fruticosa(*N*=20)	Gypsophile	0.65[2]	0.79	0.23	1.75[1]	0.07	1.35[1]	0.38[1]	**7.82**(*N*=19)	**7.82**
Helianthemum squamatum (*N*=20)	Gypsophile	0.19	0.02	0.13	1.27(*N*=17)	1.28	0.33[1]	1.77[1]	**4.79**	**4.79**
Helianthemum syriacum (*N*=19)	Gypsovag	0.04	**8.94**	2.20	**4.88**(*N*=19)	**8.09**	0.06	**23.61[2]**	2.45(*N*=19)	2.45
Lepidium subulatum (*N*=19)	Gypsophile	1.07	0.65(*N*=12)	1.40	0.77[2]	1.17	1.83	0.03	**9.94**	**9.94**
Linum suffruticosum (*N*=20)	Gypsovag	0.90	1.33	**13.22**	0.21	**4.63**	0.83	**9.32[2]**	**17.19**	**17.19**
Matthiola fruticulosa (*N*=18)	Gypsovag	1.02	3.45(*N*=16)	3.98	0.52	**5.21**	1.48	0.96[1]	0.24(*N*=17)	0.24
Ononis tridentata (*N*=20)	Gypsophile	**5.97[1]**	**33.51**	3.36	**5.19[1]**(*N*=19)	**31.47**	**6.21**	3.30[2]	**16.13[1]**	**16.13**
Rosmarinus officinalis (*N*=20)	Gypsovag	0.24	0.21	1.99	0.48	1.05	0.06[1]	**22.76**	**22.32[2]**	**22.32**

All differences were assessed with Normal distribution, whether residuals did not fit a normal distribution:[1]Differences were assessed with Gamma distribution with identity as link function. [2]Differences were assessed with Gamma distribution with log as link function

Table B.8 (continued):

Species	Gypsum affinity	Mn	Mo	N	Na	P	S	Si	Ti	Zn
Boleum asperum (*N*=16)	Gypsovag	1.55[1]	**7.21[1]**	0.03(*N*=15)	0.25	4.04[1]	**5.12**	0.70	0.95	0.04
Gypsophila hispánica (*N*=20)	Gypsophile	1.12[1]	**11.45**(*N*=19)	0.10	1.44	**17.63[1]**	**30.70**	4.00	3.21(*N*=19)	0.21
Herniaria fruticosa (*N*=20)	Gypsophile	1.87	**17.21[1]**	0.21[1]	0.34	3.00[1]	**6.50**	0.64	0.12	0.49[1]
Helianthemum squamatum (*N*=20)	Gypsophile	1.93	**11.73**(*N*=18)	0.00	0.16	**8.63[1]**	**4.52**	1.61	0.03(*N*=19)	0.18[1]
Helianthemum syriacum (*N*=19)	Gypsovag	0.00	**4.58**	2.01	2.02[1]	**62.94**	**11.08**	0.00	1.53	0.25
Lepidium subulatum (*N*=19)	Gypsophile	**9.71**	0.06	0.09(*N*=12)	0.49	2.90	**5.93**	4.12	0.00	0.29
Linum suffruticosum (*N*=20)	Gypsovag	2.08	0.00(*N*=13)	0.17	0.22[1]	**7.53**	**26.69**	1.32	1.97	0.89
Matthiola fruticulosa (*N*=18)	Gypsovag	**7.60**	0.38(*N*=17)	1.28(*N*=16)	3.79[1]	**19.25**	**5.59**	0.35	0.07	1.18
Ononis tridentata (*N*=20)	Gypsophile	4.30	**47.67[1]**	0.63	3.46[2]	2.61[1]	**48.40[1]**	2.87	1.00	**6.38[2]**
Rosmarinus officinalis (*N*=20)	Gypsovag	2.99	0.75[1](*N*=17)	1.33	**7.70[2]**	**14.38**	**11.21[1]**	0.00	0.33	0.79

All differences were assessed with Normal distribution, whether residuals did not fit a normal distribution:[1]Differences were assessed with Gamma distribution with identity as link function. [2]Differences were assessed with Gamma distribution with log as link function

Table B.9: Means and standard deviation of elemental concentrations of study species grown on calcic and gypseous soil.

Species	Gypsum affinity	Soil	Al	C	Ca	Cr	Cu	Fe	K
Boleum asperum	Gypsovag	Calcic	0.3±0.1	402.3±16.5	29.9±5.8	2.7E-2±9.4E-3	7.0E-3±2.3E-3	0.4±0.1	12.0±2.2
		Gypsiferous	0.3±0.1	403.7±11.4	33.0±3.4	4.2E-2±2.4E-2	7.7E-3±1.6E-3	0.5±0.2	11.6±1.6
Gypsophila hispanica	Gypsophile	Calcic	0.1±0.0	328.2±15.2	80.9±12.0	6.0E-3±5.3E-3	7.0E-3±2.1E-3	0.1±0.0	1.21±0.54
		Gypsiferous	0.1±0.0	321.3±13.6	81.9±9.2	4.2E-3±1.9E-3	7.0E-3±1.6E-3	0.1±0.0	6.5±2.2
Herniaria fruticosa	Gypsophile	Calcic	0.7±0.3	440.7±16.3	31.7±6.6	1.9E-2±1.8E-2	1.5E-2±6.0E-3	0.5±0.3	8.4±2.4
		Gypsiferous	0.6±0.2	436.2±14.3	32.9±7.9	1.2E-2±7.7E-3	1.6E-2±7.0E-3	0.5±0.1	7.7±2.6
Helianthemum squamatum	Gypsophile	Calcic	0.1±0.1	422.8±13.6	24.4±6.1	4.2E-3±2.8E-3	1.5E-2±6.0E-3	0.1±0.1	5.2±2.1
		Gypsiferous	0.2±0.0	422.0±17.2	25.1±2.9	1.5E-2±1.8E-2	1.7E-2±3.6E-3	0.1±0.0	4.4±1.1
Helianthemum syriacum	Gypsovag	Calcic	0.4±0.1	427.6±7.8	23.5±4.4	3.1E-3±1.6E-3	1.9E-2±6.3E-3	0.3±0.1	7.8±1.0
		Gypsiferous	0.4±0.1	421.9±3.8	25.9±4.2	4.4E-3±2.1E-3	2.7E-2±1.1E-2	0.3±0.1	5.8±0.9
Lepidium subulatum	Gypsophile	Calcic	1.5±0.6	414.9±13.0	37.1±7.4	1.3E-1±1.2E-1	1.8E-2±6.0E-3	1.6±1.0	6.5±1.2
		Gypsiferous	1.2±0.2	420.0±6.5	34.0±3.2	9.8E-2±6.9E-2	1.6E-2±4.6E-3	1.3±0.4	6.6±1.6
Linum suffruticosum	Gypsovag	Calcic	0.3±0.1	406.3±9.4	22.4±4.5	1.5E-2±6.7E-3	1.1E-2±3.7E-3	0.3±0.1	9.3±4.7
		Gypsiferous	0.3±0.1	401.3±10.0	28.1±5.3	1.6E-2±5.8E-3	1.4E-2±4.7E-3	0.3±0.1	5.5±1.6
Matthiola fruticulosa	Gypsovag	Calcic	0.3±0.1	386.6±12.3	465.±6.6	2.0E-2±8.2E-3	6.5E-3±1.4E-3	0.3±0.1	10.5±2.2
		Gypsiferous	0.2±0.1	378.7±10.6	53.5±8.2	1.7E-2±7.4E-3	5.4E-3±2.0E-3	0.3±0.1	9.3±4.1
Ononis tridentata	Gypsophile	Calcic	0.2±0.1	349.4±15.5	60.3±6.9	7.2E-3±7.6E-3	6.1E-3±1.7E-3	0.2±0.1	8.6±4.7
		Gypsiferous	0.2±0.0	315.3±14.1	55.0±6.0	3.0E-3.0±7.7E-4.0	1.1E-2±2.6E-3	0.2±0.0	5.9±2.5
Rosmarinus officinalis	Gypsovag	Calcic	0.3±0.0	512.7±18.1	12.3±1.6	1.9E-3±3.7E-4	6.3E-3±1.2E-3	0.2±0.0	12.9±1.5
		Gypsiferous	0.3±0.1	510.3±10.8	13.5±2.3	1.8E-3±5.4E-4	7.0E-3±2.3E-3	0.2±0.1	9.7±2.2

Table B.9 (continued)

Species	Soil	Li	Mg	Mn	Mo	N	Na	P	S	Si	Ti	Zn
Boleum asperum	Calcic	4.0E-3±8.4E-4	6.9±1.7	3.9E-2±1.1E-2	4.5E-3±1.9E-3	29.5±4.7	7.6E-2±2.0E-2	3.4±3.1	8.8±1.9	1.0±0.1	3.8E-3±1.2E-3	9.1E-2±4.1E-2
	Gypsiferous	5.7E-3±3.7E-3	6.9±2.9	5.2E-2±2.5E-2	1.4E-2±1.2E-2	29.6±6.1	7.2E-2±9.4E-3	1.7±0.5	11.0±1.9	1.0±0.2	4.6E-3±2.6E-3	8.8E-2±2.3E-2
Gypsophila hispanica	Calcic	6.4E-4±3.7E-4	15.1±1.9	8.1E-2±1.6E-2	6.3E-4±6.1E-4	9.6±2.5	9.4E-2±5.2E-2	4.9±3.7	14.8±2.9	0.6±0.1	1.2E-3±1.9E-4	2.8E-2±1.7E-2
	Gypsiferous	5.2E-4±5.3E-4	16.4±4.5	7.2E-2±3.1E-2	9.0E-3±2.8E-3	9.3±2.1	7.7E-2±2.1E-2	0.9±0.4	22.5±3.6	0.7±0.1	1.4E-3±3.7E-4	2.6E-2±1.0E-2
Herniaria fruticosa	Calcic	6.3E-4±6.1E-4	6.6±1.4	7.9E-2±3.9E-2	3.4E-3±1.6E-3	13.3±2.5	1.7E-1±5.9E-2	2.9±3.3	6.6±1.0	1.3±0.1	6.9E-3±2.3E-3	6.9E-2±4.0E-2
	Gypsiferous	9.5E-4±1.0E-3	7.7±1.1	6.0E-2±1.8E-2	7.3E-3±2.3E-3	14.0±4.9	1.8E-1±4.0E-2	1.2±1.4	8.0±1.7	1.2±0.3	7.3E-3±3.4E-3	5.9E-2±1.6E-2
Helianthemum squamatum	Calcic	5.0E-3±2.0E-3	4.5±0.5	8.1E-2±1.6E-2	3.5E-3±1.9E-3	8.9±1.0	1.3E-1±6.4E-2	1.3±0.9	14.9±4.3	1.0±0.1	4.9E-3±4.0E-3	9.9E-3±3.2E-3
	Gypsiferous	6.8E-3±3.1E-3	4.6±1.0	7.2E-2±1.3E-2	6.2E-3±1.5E-3	8.9±1.3	1.3E-1±7.4E-2	0.7±0.1	18.5±3.2	1.0±0.1	1.9E-3±6.6E-4	1.2E-2±5.5E-3
Helianthemum syriacum	Calcic	6.7E-4±6.3E-4	2.9±0.5	9.3E-2±2.2E-2	7.5E-3±1.3E-3	9.8±1.3	9.1E-2±3.2E-2	1.8±0.5	6.3±1.1	1.1±0.1	4.1E-3±1.7E-3	2.1E-2±5.8E-3
	Gypsiferous	8.7E-4±8.6E-4	3.2±0.4	9.4E-2±2.3E-2	9.8E-3±3.2E-3	8.9±1.7	7.7E-2±9.5E-3	0.6±0.2	8.8±2.5	1.1±0.1	5.0E-3±2.3E-3	2.2E-2±8.4E-3
Lepidium subulatum	Calcic	3.9E-3±2.0E-3	3.1±0.3	1.0E-1±2.6E-2	1.1E-2±5.8E-3	31.1±4.8	1.6E-1±4.0E-2	1.8±0.4	15.2±2.8	1.4±0.1	1.5E-2±5.3E-3	1.1E-1±6.5E-2
	Gypsiferous	6.1E-3±3.0E-3	3.4±0.7	7.8E-2±9.6E-3	1.2E-2±5.2E-3	30.7±5.3	1.8E-1±4.9E-2	1.6±0.3	18.1±2.2	1.5±0.1	1.5E-2±3.0E-3	1.0E-1±4.2E-2
Linum suffruticosum	Calcic	1.1E-2±2.4E-3	4.2±0.9	7.7E-2±2.3E-2	1.4E-3±2.4E-4	17.1±2.1	1.2E-1±2.1E-2	1.6±0.6	2.0±0.4	1.2±0.1	3.0E-3±7.0E-4	6.0E-2±1.9E-2
	Gypsiferous	1.9E-2±4.9E-3	4.4±0.8	6.3E-2±2.0E-2	1.8E-3±8.1E-4	16.7±2.7	1.1E-1±4.1E-2	1.0±0.4	3.5±0.8	1.2±0.1	3.9E-3±2.1E-3	6.8E-2±2.0E-2
Matthiola fruticulosa	Calcic	5.5E-3±2.9E-3	5.7±1.1	5.7E-2±2.1E-2	9.4E-3±1.0E-2	25.2±4.0	1.6E-1±1.2E-1	2.9±0.6	12.0±2.0	0.9±0.2	3.3E-3±1.6E-3	6.0E-2±2.1E-2
	Gypsiferous	5.7E-3±3.0E-3	4.1±1.5	4.1E-2±1.1E-2	1.1E-2±8.1E-3	22.6±5.2	8.8E-2±3.1E-2	1.6±0.7	13.9±1.6	0.8±0.3	3.1E-3±1.7E-3	5.5E-2±2.9E-2
Ononis tridentata	Calcic	6.5E-3±2.9E-3	14.7±1.5	4.9E-2±7.3E-3	7.0E-3±3.7E-3	18.6±3.5	1.3E-1±6.4E-2	1.0±0.8	26.4±7.6	0.9±0.2	2.5E-3±7.9E-4	3.9E-2±2.1E-2
	Gypsiferous	1.2E-2±5.8E-3	21.1±3.5	4.2E-2±7.8E-3	5.0E-2±2.4E-2	17.7±1.4	1.0E-1±2.3E-2	0.6±0.1	54.0±6.5	0.8±0.2	2.2E-3±7.3E-4	5.6E-2±2.3E-2
Rosmarinus officinalis	Calcic	3.7E-4±3.6E-4	1.6±0.4	4.1E-2±1.0E-2	2.3E-3±5.2E-4	7.9±1.6	6.4E-2±2.0E-2	1.9±1.0	1.5±0.3	0.9±0.2	4.1E-3±8.6E-4	3.2E-2±1.6E-2
	Gypsiferous	2.3E-3±2.0E-3	2.5±0.5	3.4E-2±7.5E-3	3.2E-3±1.5E-3	7.3±1.9	8.5E-2±3.3E-2	0.7±0.3	2.6±1.2	0.9±0.3	4.3E-3±1.7E-3	3.7E-2±1.1E-2

Figure B.1: Biplot distance of second and third principal components based on Center Log-Ratio transformed elements. Centroids of each treatment mean were drawn (±S.D.). Circles indicate gypsophiles; squares, gypsovags. Filled symbols indicate plants grown on gypsiferous soils; empty symbols, calcic soils. BoAs: *B.asperum*; GyHi: *G.hispanica*; HeFr: *H.fruticosa*; HeSq: *H.squamtum*; HeSy: *H.syriacum*; LeSu: *L.subulatum*; LiSu: *L.suffruticosum*; MaFr: *M.fruticulosa*; OnTr: *Ononis tridentata*; RoOf: *R.officinalis*; WA: all gypsophiles on calcic soils; VA: all gypsovags on calcic soils; WY: all gypsophiles on gypsiferous soils; VY: all gypsovags on gypsiferous soils.

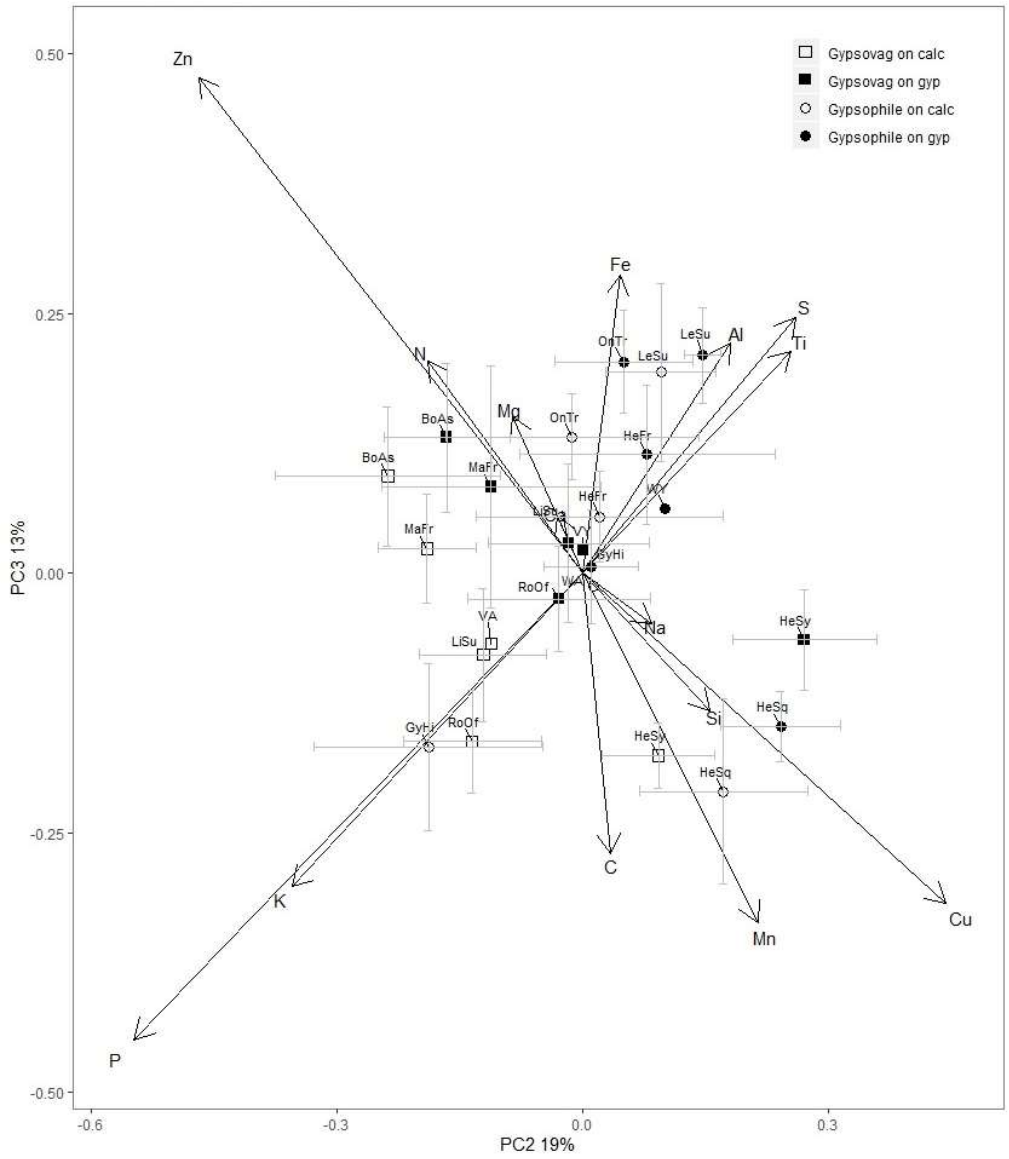


Figure B.2: Biplot distance of first and second redundancy components based on Center Log-Ratio transformed elements. Centroids of each treatment mean were drawn (± S.D.). Circles indicate

gypsophiles; squares, gypsovags. Filled symbols indicate plants grown on gypsiferous soils; empty symbols, calcic soils. BoAs: *B.asperum*; GyHi: *G.hispanica*; HeFr: *H.fruticosa*; HeSq: *H.squamatum*; HeSy: *H.syriacum*; LeSu: *L.subulatum*; LiSu: *L.suffruticosum*; MaFr: *M.fruticulosa*; OnTr: *Ononis tridentata*; RoOf: *R.officinalis*; WA: all gypsophiles on calcic soils; VA: all gypsovags on calcic soils; WY: all gypsophiles on gypsiferous soils; VY: all gypsovags on gypsiferous soils.

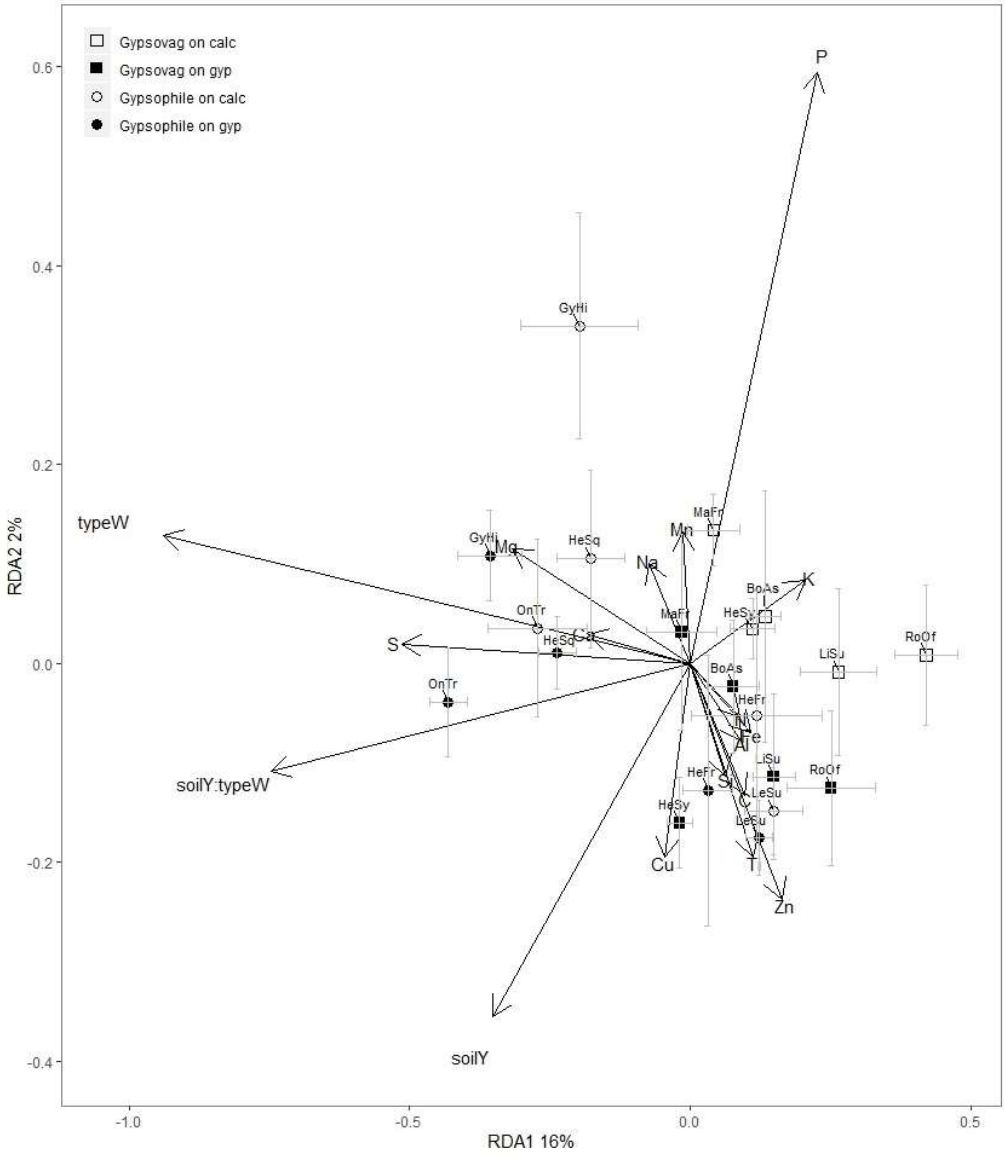
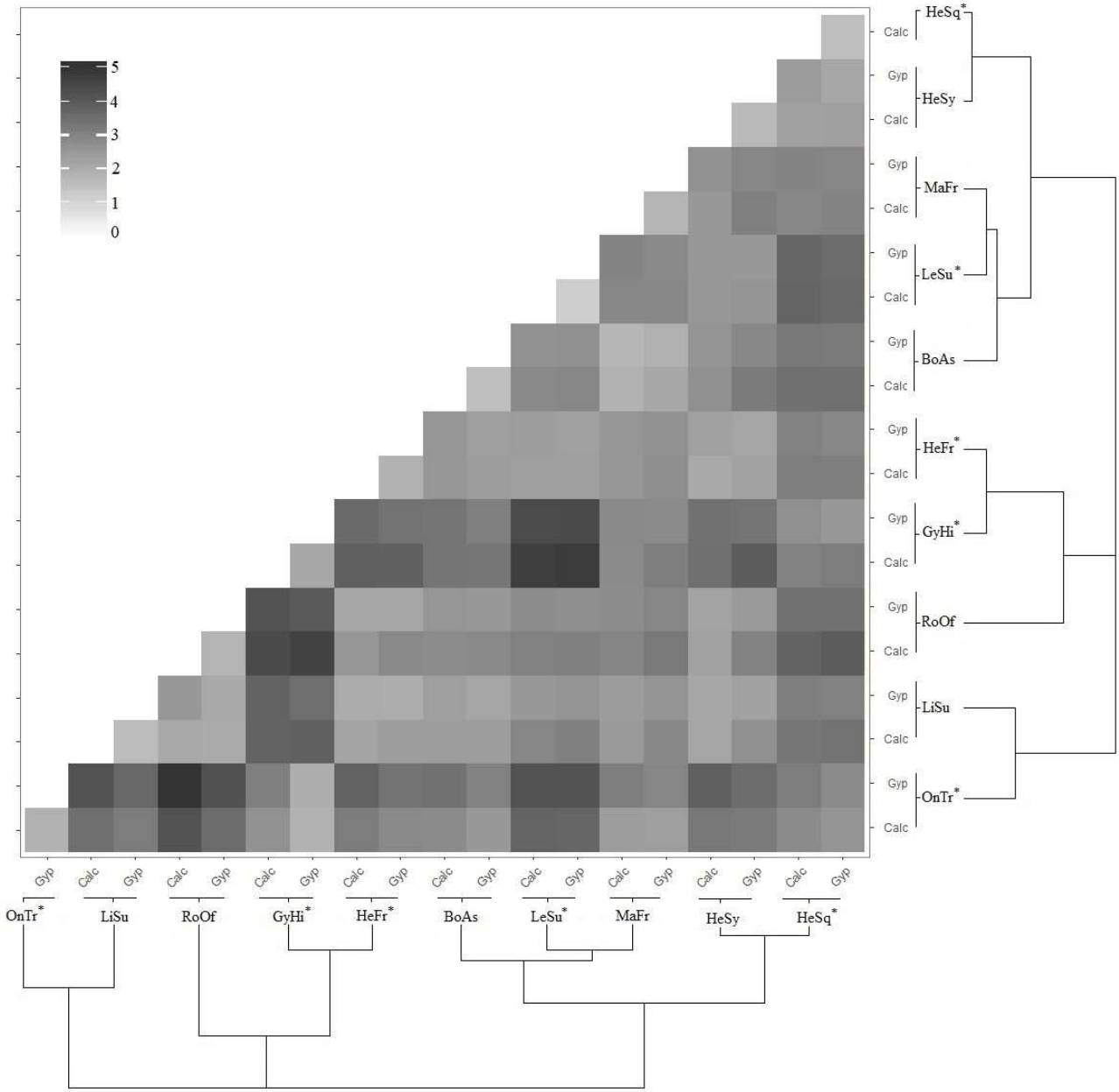


Figure B.3: HeatMap of Euclidean distance based on Center Log-ratio transformed foliar concentrations of elements. Centroids of each treatment mean were drawn. Greyer colours indicate furthest and whitish grey shows plants with more similar leaf elemental composition. BoAs: *B.asperum*; GyHi: *G.hispanica*; HeFr: *H.fruticosa*; HeSq: *H.squamatum*; HeSy: *H.syriacum*; LeSu: *L.subulatum*; LiSu: *L.suffruticosum*; MaFr: *M.fruticulosa*; OnTr: *Ononis tridentata*; RoOf: *R.officinalis*; Calc: on calcic soils; Gyp: on gypsiferous soils. Species with asterisk are gypsophiles. The cladogram is an adapted phylogenetic tree.



Appendix C. Supplementary information of chapter 3

Table C.1. Percentage cover and standard error per grazing intensity in each locality for soil features

Locality	Grazing intensity	Gypsum (%)	pH	Conductivity (µm/s)	N (mg/g)	C (mg/g)	Clay (%)	Lime (%)	Sand(%)
Corral del Hoyo									
	High	83.39±6.71	7.94±0.10	2231.67±28.75	0.86±0.26	25.70±3.22	7.70±2.04	34.82±2.87	57.48±2.68
	Medium	68.83±31.99	5.64±4.06	2232.00±97.25	1.16±0.46	19.97±9.36	8.38±2.85	31.03±7.41	60.59±9.61
	Low	56.06±20.68	8.01±0.03	2286.00±47.32	0.95±0.10	26.30±3.47	11.44±2.69	35.35±4.85	53.21±7.49
Pedriza									
	High	64.25±15.55	8.02±0.07	2276.00±20.78	0.86±0.22	17.93±3.99	8.09±2.25	32.81±5.39	59.10±6.55
	Medium	87.77±22.58	8.01±0.05	2231.33±64.24	0.76±0.27	12.68±5.53	11.19±5.72	36.50±7.32	52.31±12.95
	Low	80.60±2.42	8.03±0.06	2239.33±10.07	0.71±0.23	20.40±4.16	9.65±1.65	35.10±2.41	55.24±3.87
Valdemolino									
	High	83.71±8.44	8.06±0.06	2357.33±176.53	0.70±0.27	11.17±4.64	7.12±0.65	32.94±3.61	59.94±4.25
	Medium	73.55±22.48	8.00±0.02	2274.67±56.62	0.64±0.07	28.37±12.19	11.86±4.64	38.11±6.69	50.04±10.95
	Low	77.46±9.66	7.98±0.05	2235.00±46.57	1.00±0.27	25.03±6.90	9.02±1.85	35.63±2.19	55.35±3.85

Table C.2: Perennial species included in the study with indication of their life form and gypsophilic value (GV) according to (Mota et al., 2011). Gypsophilic value range from 2 (generalist species) to 5 (the highest affinity to gypsum soils, specialist species). The direction of shifts in species cover from low to high levels of grazing intensity is indicated along with the F-ratio and P-value of GLMMs among levels based on each species cover.

Species	Family	Life form	GV	Pattern	F-ratio	P-value
Allium sphaerocephalon L.	Amaryllidaceae	Geophyte	2	Constant	1.71	0.183
Artemisia herba-alba Asso	Asteraceae	Chamaephyte	2	Increase	3.83	0.023
Asperula aristata L.f.	Rubiaceae	Hemicryptophyte	2	Constant	2.03	0.133
Asphodelus fistulosus L.	Asphodelaceae	Geophyte	2	Increase	3.81	0.023
Astragalus alopecuroides L.	Fabaceae	Hemicryptophyte	2	Decrease	6.85	0.001
Astragalus incanus L.	Fabaceae	Hemicryptophyte	2	Decrease	20.03	0.000
Astragalus monspessulanus L.	Fabaceae	Hemicryptophyte	2	Increase	3.07	0.048
Atractylis humilis L.	Asteraceae	Chamaephyte	2	Constant	2.84	0.060
Brachypodium retusum (Pers.) P.Beauv.	Poaceae	Chamaephyte	2	Constant	0.17	0.843
Bromus erectus Huds.	Poaceae	Hemicryptophyte	2	Constant	1.00	0.369
Carduus tenuiflorus Curtis	Asteraceae	Hemicryptophyte	2	Constant	1.00	0.369
Caroxylon vermiculatum (L.) Akhani & Roalson	Amaranthaceae	Chamaephyte	2	Constant	0.86	0.425
Centaurea aspera L.	Asteraceae	Chamaephyte	2	Constant	1.71	0.183
Convolvulus arvensis L.	Convolvulaceae	Hemicryptophyte	2	Constant	1.00	0.369
Coris monspeliensis L.	Primulaceae	Chamaephyte	2	Constant	1.00	0.369
Crepis vesicaria subsp. taraxacifolia (Thuill.) Thell.	Asteraceae	Hemicryptophyte	2	Constant	1.36	0.258

Dactylis glomerata subsp. hispanica (Roth) Nyman	Poaceae	Hemicryptophyte	2	Constant	1.45	0.235
Dipcadi serotinum (L.) Medik.	Asparagaceae	Geophyte	2	Decrease	5.50	0.005
Echinops ritro L.	Asteraceae	Hemicryptophyte	2	Constant	1.62	0.199
Genista scorpius (L.) DC	Fabaceae	Chamaephyte	2	Decrease	4.08	0.018
Gypsophila struthium subsp. hispanica (Willk.) G.López	Caryophyllaceae	Chamaephyte	4.69	Constant	0.02	0.981
Hedysarum boveanum subsp. europaeum Guitt. & Kerguélen	Fabaceae	Chamaephyte	2	Decrease	4.13	0.017
Helianthemum marifolium (L.) Mill.	Cistaceae	Chamaephyte	2	Decrease	7.67	0.001
Helianthemum squamatum Pers.	Cistaceae	Chamaephyte	4.87	Decrease	12.35	0.000
Helianthemum syriacum (Jacq.) Dum.Cours.	Cistaceae	Chamaephyte	2	Decrease	14.28	0.000
Helianthemum violaceum (Cav.) Pers.	Cistaceae	Chamaephyte	2	Decrease	40.92	0.000
Helichrysum stoechas (L.) Moench	Asteraceae	Chamaephyte	2	Decrease	9.62	0.000
Helictochloa bromoides (Gouan) Romero Zarco	Poaceae	Hemicryptophyte	2	Constant	0.08	0.919
Herniaria fruticosa L.	Caryophyllaceae	Chamaephyte	4.05	Increase	7.60	0.001
Koeleria vallesiana (Honck.) Gaudin	Poaceae	Hemicryptophyte	2	Decrease	4.44	0.013
Launaea fragilis (Asso) Pau	Asteraceae	Chamaephyte	3.1	Constant	2.00	0.137
Launaea pumila (Cav.) Kuntze	Asteraceae	Hemicryptophyte	3.22	Increase	12.65	0.000
Linum suffruticosum L.	Linaceae	Chamaephyte	2	Constant	1.24	0.290
Lygeum spartum Loefl. ex L.	Poaceae	Hemicrytophyte	2	Increase	14.56	0.000
Matthiola fruticulosa (L.) Maire	Brassicaceae	Chamaephyte	2	Constant	1.62	0.200
Ononis tridentata L.	Fabaceae	Chamaephyte	4.43	Constant	1.00	0.369
Onopordum nervosum Boiss. Cf	Asteraceae	Hemicrytophyte	2	Constant	1.35	0.261

Orobanche cernua Loefl.	Orobanchaceae	Geophyte	2	Constant	1.00	0.369
Petrosedum sediforme (Jacq.) Grulich	Crassulaceae	Chamaephyte	2	Decrease	7.77	0.001
Plantago albicans L.	Plantaginaceae	Chamaephyte	2	Increase	14.63	0.000
Poa bulbosa L.	Poaceae	Hemicryptophyte	2	Constant	2.03	0.133
Polygala rupestris Pourr.	Polygalaceae	Chamaephyte	2	Constant	2.03	0.133
Reseda stricta Pers.	Resedaceae	Hemicryptophyte	3.97	Increase	3.07	0.048
Rosmarinus officinalis L.	Lamiaceae	Chamaephyte	2	Decrease	27.50	0.000
Sideritis fruticulosa Pourr.	Lamiaceae	Chamaephyte	2	Increase	15.20	0.000
Sonchus tenerrimus L.	Asteraceae	Chamaephyte	2	Constant	0.47	0.624
Stipa barbata Desf.	Poaceae	Hemicryptophyte	2	Constant	1.39	0.250
Stipa lagascae Roem. & Schult.	Poaceae	Hemicryptophyte	2	Decrease	20.77	0.000
Stipellula parviflora (Desf.) Röser & Hamasha	Poaceae	Hemicryptophyte	2	Increase	10.90	0.000
Teucrium capitatum L.	Lamiaceae	Chamaephyte	2	Decrease	3.70	0.026
Thymus vulgaris L.	Lamiaceae	Chamaephyte	2	Decrease	12.24	0.000
Thymus zygis L.	Lamiaceae	Chamaephyte	2	Decrease	6.95	0.001

Table C.3: Coordinates in NMDS of environmental variables showing the effect of different soil features on gypsum plant communities. R-square coefficients and their p-values are also shown.

	NMDS1	NMDS2	R^2	P-value
Gypsum	0.53	-0.84	0.38	0.001
pH	-0.03	-0.99	0.02	0.018
Conductivity	-0.22	0.97	0.04	0.004
Nitrogen	-0.62	0.78	0.14	0.001
Carbon	-0.99	0.05	0.41	0.001
Clay	-0.95	-0.30	0.04	0.003
Lime	-0.44	-0.90	0.09	0.001
Sand	0.64	0.77	0.06	0.001

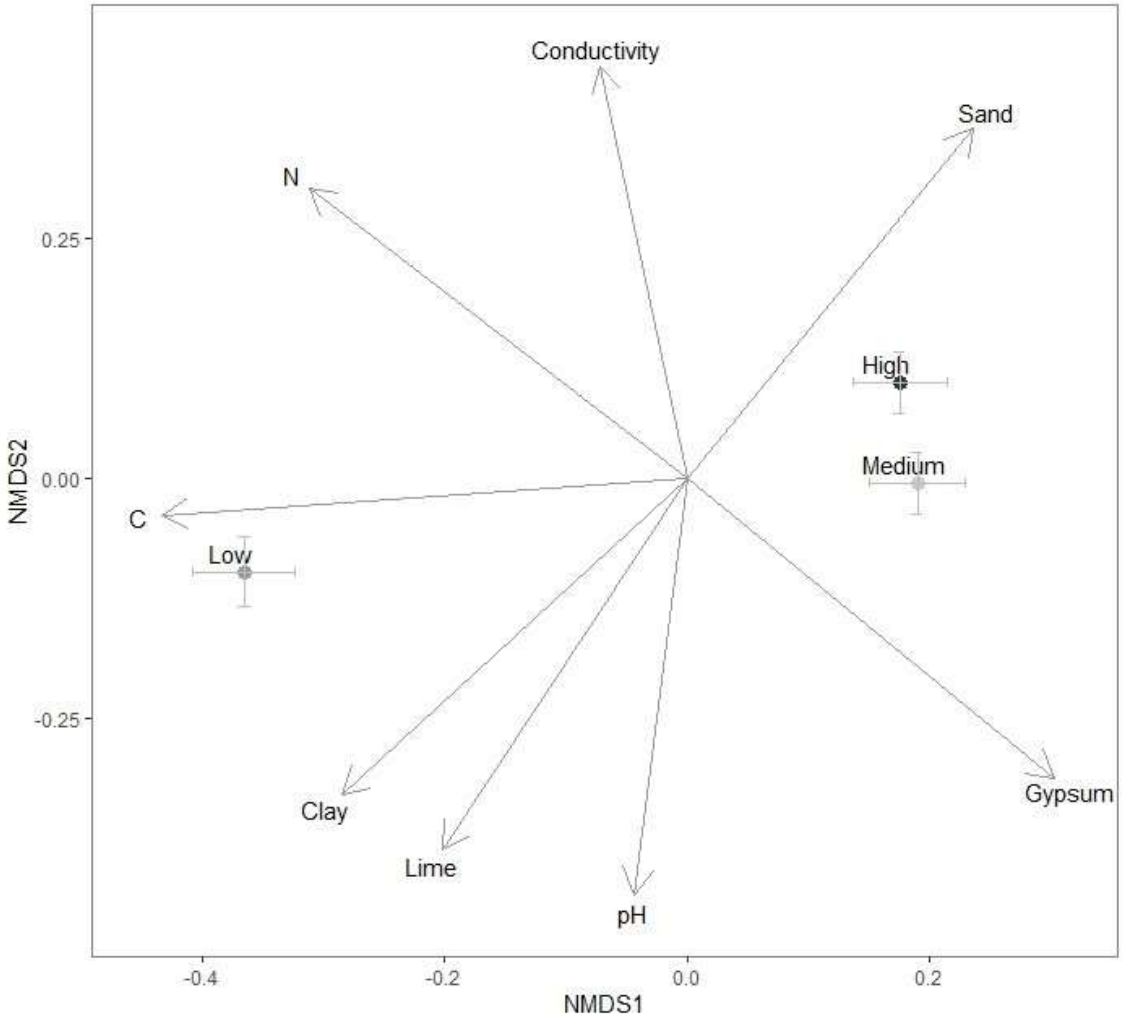
Table C.4. Means and standard errors of functional trait values of each species for each grazing intensity from a subset of 14 species in Valdemolino.

Species	Grazing intensity	Leaf C (mg/g)	Leaf N (mg/g)	Leaf S (mg/g)
Brachypodium retusum	High	428.84±1.38	16.19±0.29	4.82±0.31
	Medium	450.08±2.27	16.41±0.52	4.49±0.12
	Low	442.78±2.56	14.02±0.60	3.91±0.34
Genista scorpius	High	470.73±3.88	27.64±1.37	2.59±0.39
	Medium	467.06±2.38	32.60±2.32	3.15±0.66
	Low	471.16±3.46	30.84±1.00	2.79±0.16
Gypsophila hispanica	High	338.73±6.06	23.88±0.64	13.65±1.53
	Medium	349.42±5.01	26.31±1.26	14.90±2.64
	Low	386.50±17.28	26.42±2.01	10.68±2.47
Helianthemum squamatum	High	396.17±2.76	18.79±1.04	28.01±1.79
	Medium	403.18±2.77	17.66±0.94	24.05±0.27
	Low	363.66±15.67	17.57±1.11	21.03±4.66
Helychrisum stoeches	High	436.83±8.10	21.12±0.31	6.20±0.14
	Medium	450.58±1.31	17.65±0.75	4.67±0.99
	Low	466.30±4.24	16.70±0.85	3.58±0.36
Helianthemum syriacum	High	436.30±2.62	19.67±0.73	9.41±0.49
	Medium	437.50±4.07	19.24±1.09	9.01±1.07
	Low	432.14±1.67	15.19±0.56	7.37±0.84
Helianthemum violaceum	High	453.67±10.29	21.54±0.21	3.44±0.21
	Medium	451.04±1.76	23.66±0.88	3.00±0.06
	Low	453.06±2.61	22.98±0.95	3.04±0.07
Herniaria fruticosa	High	424.14±3.65	27.06±0.55	8.49±1.04
	Medium	419.04±2.37	20.69±0.74	7.63±1.04
	Low	440.24±1.61	18.68±0.88	6.18±0.38
Koelleria vallesiana	High	426.30±4.47	19.12±0.35	3.98±0.26
	Medium	434.48±2.66	18.58±1.03	3.52±0.19
	Low	430.94±1.95	16.67±0.64	2.77±0.18
Linum suffruticosum	High	423.00±4.76	32.09±0.94	3.73±0.13
	Medium	431.30±2.41	25.86±0.87	2.95±0.09
	Low	427.40±3.26	30.20±1.98	3.17±0.22
Ononis tridentata	High	317.53±7.31	24.97±0.55	50.67±3.06
	Medium	337.74±9.24	25.46±0.44	47.05±4.48
	Low	347.56±3.04	25.63±1.50	32.78±2.29
Sideritis cavallensis	High	439.13±1.85	30.61±0.88	3.14±0.19
	Medium	438.64±5.58	29.42±1.47	3.04±0.09
	Low	429.10±2.88	26.22±0.64	2.41±0.07
Teucrium captitatum	High	492.10±1.90	27.59±1.00	4.90±0.37
	Medium	509.54±3.21	27.17±1.91	3.79±0.27
	Low	506.62±2.42	22.72±0.66	3.68±0.21
Thymus vulgaris	High	481.90±6.45	21.48±1.10	3.43±0.53
	Medium	494.98±2.44	18.56±0.49	3.54±0.28
	Low	504.84±3.07	18.47±0.57	3.28±0.19

Table C.5. Means and standard errors of CWM and FD values. Indices of canopy and leaf traits obtained from a single value per species (Fixed average), a value of each level of grazing per species (Specific average) and differences between specific and fixed averages (Intraspecific)

	Fixed average			Intraspecific variability			Specific average		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
CWM									
Leaf C (mg/g)	403.83±3.90	409.40±1.86	412.27±2.19	26.75±4.14	9.04±1.02	-0.76±1.48	430.59±2.16	418.44±2.08	411.51±2.22
Leaf N (mg/g)	21.57±0.21	20.57±0.19	22.12±0.19	-1.08±0.14	0.02±0.04	1.13±0.11	20.48±0.24	20.59±0.20	23.24±0.16
Leaf S (mg/g)	11.99±0.43	13.49±0.48	10.48±0.39	-3.50±0.28	-0.05±0.09	0.69±0.12	8.49±0.36	13.44±0.52	11.17±0.46
FD									
Leaf C	0.14±0.01	0.08±0.00	0.09±0.00	0.12±0.01	0.10±0.01	0.13±0.01	0.27±0.01	0.19±0.01	0.22±0.01
Leaf N	0.28±0.01	0.23±0.01	0.28±0.01	-0.02±0.01	-0.02±0.00	-0.01±0.00	0.26±0.01	0.21±0.01	0.28±0.01
Leaf S	0.22±0.01	0.21±0.01	0.18±0.01	-0.04±0.01	-0.02±0.00	-0.02±0.00	0.19±0.01	0.19±0.01	0.16±0.01

Figure C.1: NMDS with environmental variables. All environmental variables were scaled prior to analyses. N = 315 plots of 2 x 2 m. N: Nitrogen content. C: Carbon content



Appendix D. Supplementary information of chapter 4

Table D.1: Means and standard errors of growth and leaf traits of unclipped and clipped for each studied species of gypsum exclusive (A) and non-exclusive (B) species in each soil. Capital letters indicate significant differences between types of soil, and minuscule letters indicate significant differences between defoliation. Unclip.: Unclipped plants. Clipped: Clipped plants. Calc. Plants grown in calcic soil. Gyp.: plants grown in gypseous soil.

A)

	Plant Biomass (g)	Root:Shoot ratio	Height (mm)
G.struthium			
Unclip. Calc.	21.54±3.11	1.89±0.25	210.40±24.34 A
Unclip. Gyp.	20.45±2.26	2.10±0.09	181.40±8.94 B
Clipped Calc.	20.38±1.73	2.30±0.15	219.80±27.69 A
Clipped Gyp.	20.29±2.38	2.62±0.18	166.00±8.72 B
H.fruticosa			
Unclip. Calc.	5.99±0.67	0.78±0.14	78.80±5.34
Unclip. Gyp.	6.48±1.00	1.03±0.15	75.00±6.33
Clipped Calc.	5.27±0.23	0.99±0.11	68.60±2.60
Clipped Gyp.	5.20±0.58	0.98±0.08	83.40±7.11
H.squamatum			
Unclip. Calc.	6.33±0.47 A	1.20±0.17 A	98.40±3.82
Unclip. Gyp.	5.20±0.59 B	0.86±0.03 B	97.40±7.30
Clipped Calc.	5.15±0.38 A	0.93±0.07 A	107.40±4.61
Clipped Gyp.	3.98±0.32 B	0.75±0.09 B	103.40±6.21
L.subulatum			
Unclip. Calc.	5.66±0.40 A	0.92±0.08	123.40±10.96
Unclip. Gyp.	4.23±0.26 B	0.86±0.04	103.00±12.90
Clipped Calc.	5.47±0.71 AB	1.29±0.08	122.00±6.83
Clipped Gyp.	4.79±0.50 AB	1.16±0.08	100.00±9.22
O.tridentata			
Unclip. Calc.	27.09±4.84	0.94±0.16	312.40±8.93
Unclip. Gyp.	24.37±2.86	1.00±0.11	265.40±27.44
Clipped Calc.	21.71±6.45	0.87±0.09	300.80±39.08
Clipped Gyp.	21.22±1.88	0.92±0.07	303.80±17.61

Table D.1 (Continued)

B)

	Plant Biomass (g)	Root:Shoot ratio	Height (mm)
B.asperum			
Unclip. Calc.	5.76±1.41 A	2.11±0.28	141.60±13.57
Unclip. Gyp.	3.94±0.31 B	1.60±0.15	143.60±7.10
Clipped Calc.	4.25±0.35 A	2.29±0.36	133.60±13.24
Clipped Gyp.	3.39±0.13 B	2.08±0.29	119.20±13.64
H.syriacum			
Unclip. Calc.	10.58±2.52	1.72±0.20	161.00±14.87
Unclip. Gyp.	5.45±0.91	1.33±0.23	134.80±9.81
Clipped Calc.	7.83±0.86	1.88±0.17	146.80±10.43
Clipped Gyp.	4.70±0.81	1.64±0.42	126.50±9.43
L.suffruticosum			
Unclip. Calc.	11.25±0.63	2.43±0.22	172.20±9.21
Unclip. Gyp.	10.50±0.82	2.45±0.29	189.60±14.99
Clipped Calc.	7.76±0.87	1.64±0.28	176.00±6.60
Clipped Gyp.	7.75±0.86	2.03±0.14	186.00±15.33
M.fruticulosa			
Unclip. Calc.	5.99±1.05	0.88±0.13	171.00±23.47 B
Unclip. Gyp.	6.07±0.96	0.78±0.16	259.50±52.47 A
Clipped Calc.	5.02±0.43	0.89±0.07	183.20±9.21 B
Clipped Gyp.	4.31±0.69	0.71±0.09	233.00±26.77 A
R.officinalis			
Unclip. Calc.	32.38±5.01 A	1.96±0.14 A	274.20±33.19
Unclip. Gyp.	20.00±1.25 B	1.33±0.11 B	252.20±17.87
Clipped Calc.	27.10±1.97 A	1.73±0.15 A	249.20±22.81
Clipped Gyp.	14.16±0.91 B	1.39±0.35 B	242.60±10.30

Table D.2: ANOVA of generalised linear models of elemental concentration data for each organ Chi-squares, and P-values in brackets.

Element	Family (link)	Organ	Gyp.affinity	Soil	Clipping	Organ x Gyp.	Organ x Soil	Gyp x Soil	Organ x Clip.
Al	Gamma(log)	**1702.36 (0.000)**	0.45 (0.505)	**4.49 (0.034)**	0.11 (0.737)	**19.55 (0.000)**	**54.93 (0.000)**	0.50 (0.481)	1.09 (0.778)
C	Gamma(Id)	**502.01 (0.000)**	1.36 (0.244)	3.81 (0.051)	0.03 (0.870)	**25.57 (0.000)**	4.03 (0.258)	0.07 (0.799)	0.60 (0.996)
Ca	Gamma(Id)	**767.70 (0.000)**	0.54 (0.461)	0.88 (0.348)	3.60 (0.058)	**31.34 (0.000)**	3.25 (0.355)	2.99 (0.084)	0.32 (0.957)
Cr	Gamma(Id)	**135.04 (0.000)**	0.14 (0.706)	**26.31 (0.000)**	2.15 (0.142)	**90.21 (0.000)**	**48.05 (0.000)**	3.79 (0.052)	0.87 (0.831)
Cu	Gamma(id)	**908.64 (0.000)**	2.46 (0.116)	0.17 (0.679)	0.02 (0.895)	**39.23 (0.000)**	**12.60 (0.006)**	1.12 (0.290)	1.13 (0.770)
Fe	Gamma(id)	**708.10 (0.000)**	0.22 (0.640)	**6.50 (0.011)**	0.17 (0.680)	2.63 (0.451)	**38.28 (0.000)**	1.27 (0.260)	1.45 (0.694)
K	Gamma(id)	**217.51 (0.000)**	0.01 (0.919)	**59.59 (0.000)**	0.01 (0.922)	**130.00 (0.000)**	**12.33 (0.006)**	0.24 (0.627)	0.45 (0.930)
Mg	Gamma(id)	**785.90 (0.000)**	**6.76 (0.009)**	**14.04 (0.000)**	0.38 (0.539)	**151.44 (0.000)**	**21.44 (0.000)**	0.02 (0.889)	0.67 (0.880)
Mn	Gamma(log)	**1115.34 (0.000)**	0.32 (0.571)	**25.37 (0.000)**	0.12 (0.733)	**8.77 (0.032)**	**33.81 (0.000)**	2.09 (0.148)	0.27 (0.966)
N	Gamma(log)	**1029.82 (0.000)**	0.21 (0.648)	**6.71 (0.010)**	0.16 (0.693)	**41.06 (0.000)**	6.63 (0.085)	0.20 (0.653)	3.38 (0.336)
Na	Gamma(id)	**342.34 (0.000)**	1.12 (0.289)	0.08 (0.780)	1.89 (0.169)	**7.93 (0.048)**	5.74 (0.125)	1.94 (0.163)	1.58 (0.664)
P	Gamma(id)	**37.54 (0.000)**	3.17 (0.075)	**147.79 (0.000)**	0.49 (0.485)	**24.56 (0.000)**	**43.98 (0.000)**	**5.36 (0.021)**	0.27 (0.966)
S	Gamma(log)	**1921.25 (0.000)**	**10.24 (0.000)**	**504.81 (0.000)**	0.51 (0.477)	**207.84 (0.000)**	**309.61 (0.000)**	3.54 (0.060)	0.25 (0.969)
Si	Gamma(log)	**1586.67 (0.000)**	0.01 (0.915)	**31.89 (0.000)**	0.12 (0.730)	**36.80 (0.000)**	**88.82 (0.000)**	0.43 (0.512)	0.35 (0.951)
Zn	Gamma(id)	**44.71 (0.000)**	0.14 (0.708)	**15.44 (0.000)**	1.38 (0.241)	**16.53 (0.001)**	**27.58 (0.000)**	0.04 (0.838)	2.49 (0.476)

Table D.2 (Continued)

Element	Family (link)	Gyp x Clip	Soil x Clip	Organ x Gyp x Soil	Organ x Gyp x Clip	Organ x Soil x Clip	Gyp x Soil x Clip	Organ x Gyp x Soil x Clip
Al	Gamma(log)	1.56 (0.212)	0.30 (0.583)	0.31 (0.958)	1.84 (0.605)	0.49 (0.922)	0.03 (0.887)	1.88 (0.598)
C	Gamma(Id)	0.20 (0.655)	0.01 (0.976)	0.53 (0.912)	0.22 (0.974)	0.35 (0.950)	1.21 (0.272)	0.84 (0.840)
Ca	Gamma(Id)	0.54 (0.464)	2.43 (0.119)	2.71 (0.439)	0.19 (0.979)	0.46 (0.928)	0.03 (0.869)	0.45 (0.930)
Cr	Gamma(Id)	1.47 (0.226)	0.56 (0.454)	2.36 (0.501)	1.42 (0.701)	2.28 (0.517)	3.68 (0.055)	1.27 (0.737)
Cu	Gamma(id)	0.39 (0.532)	3.09 (0.051)	2.34 (0.504)	1.90 (0.594)	2.98 (0.395)	1.18 (0.277)	0.98 (0.807)
Fe	Gamma(id)	0.22 (0.639)	0.08 (0.783)	0.79 (0.852)	3.34 (0.342)	0.98 (0.805)	0.08 (0.775)	1.39 (0.707)
K	Gamma(id)	0.54 (0.462)	2.74 (0.098)	2.00 (0.572)	3.38 (0.337)	0.65 (0.884)	0.78 (0.376)	0.24 (0.972)
Mg	Gamma(id)	0.15 (0.699)	1.22 (0.269)	4.91 (0.179)	1.59 (0.661)	1.08 (0.782)	0.00 (0.959)	1.66 (0.645)
Mn	Gamma(log)	0.00 (0.946)	0.47 (0.495)	0.54 (0.910)	2.77 (0.428)	1.90 (0.594)	0.52 (0.469)	1.13 (0.769)
N	Gamma(log)	2.64 (0.104)	0.76 (0.382)	0.79 (0.852)	1.32 (0.724)	0.76 (0.858)	2.05 (0.152)	1.45 (0.693)
Na	Gamma(id)	2.40 (0.624)	0.86 (0.354)	**10.91 (0.012)**	2.69 (0.441)	0.77 (0.856)	1.61 (0.204)	0.95 (0.814)
P	Gamma(id)	0.19 (0.664)	1.35 (0.245)	**24.91 (0.000)**	0.79 (0.851)	0.05 (0.997)	0.19 (0.667)	0.33 (0.953)
S	Gamma(log)	0.12 (0.730)	0.83 (0.363)	**7.87 (0.049)**	0.58 (0.901)	0.43 (0.935)	0.00 (0.991)	0.83 (0.842)
Si	Gamma(log)	0.49 (0.482)	0.40 (0.530)	0.37 (0.946)	0.56 (0.906)	0.41 (0.938)	2.16 (0.141)	2.94 (0.401)
Zn	Gamma(id)	0.67 (0.414)	0.04 (0.838)	5.74 (0.125)	2.75 (0.432)	0.28 (0.963)	0.52 (0.470)	2.53 (0.470)

Table D.3: Means and standard errors of concentration data (mg/g) for each organ and treatment

A) Leaves

	Gypsovag Clipped Calc	Gypsovag Clipped Gyp	Gypsovag Unclipped Calc	Gypsovag Unclipped Gyp	Gypsophile Clipped Calc	Gypsophile Clipped Gyp	Gypsophile Unclipped Calc	Gypsophile Unclipped Gyp
Al	0.30±0.03	0.33±0.02	0.30±0.02	0.36±0.02	0.47±0.09	0.54±0.10	0.47±0.10	0.51±0.10
C	437.32±10.62	435.80±12.01	439.82±10.50	430.50±10.79	402.68±9.11	386.12±11.52	397.52±9.66	390.04±11.17
Ca	27.06±2.79	30.76±3.74	26.15±2.32	31.57±2.91	46.18±4.04	47.50±4.27	47.53±4.02	46.78±4.19
Cr	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.02±0.00	0.01±0.00	0.02±0.00	0.01±0.00
Cu	0.01±0.00	0.01±0.00	0.01±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
Fe	0.27±0.02	0.27±0.02	0.25±0.01	0.29±0.02	0.39±0.07	0.40±0.07	0.38±0.07	0.38±0.07
K	8.93±0.57	7.51±0.67	9.04±0.56	7.38±0.62	7.41±0.49	5.94±0.37	6.95±0.65	5.66±0.35
Mg	3.89±0.45	3.11±0.22	3.68±0.36	3.29±0.15	8.38±1.11	9.91±1.46	8.75±1.10	9.59±1.37
Mn	0.06±0.01	0.06±0.01	0.06±0.01	0.06±0.01	0.07±0.00	0.06±0.00	0.07±0.00	0.06±0.00
N	17.37±2.24	16.90±2.40	17.18±2.10	15.63±2.07	17.20±1.90	15.64±1.89	16.59±1.74	15.95±1.72
Na	0.15±0.06	0.08±0.01	0.09±0.01	0.08±0.00	0.10±0.01	0.11±0.01	0.10±0.01	0.11±0.01
P	2.57±0.34	1.15±0.15	2.15±0.31	1.11±0.14	2.21±0.36	1.03±0.13	2.46±0.55	0.93±0.10
S	5.52±0.78	7.90±1.20	5.72±0.89	7.86±1.02	15.43±1.26	25.13±3.90	16.03±1.69	25.07±3.65
Si	1.08±0.04	1.13±0.03	1.08±0.04	1.12±0.03	1.09±0.06	1.12±0.05	1.08±0.06	1.12±0.06
Zn	0.04±0.00	0.04±0.00	0.04±0.01	0.04±0.01	0.03±0.01	0.03±0.00	0.04±0.01	0.04±0.01

B) Stems

	Gypsovag Clipped Calc	Gypsovag Clipped Gyp	Gypsovag Unclipped Calc	Gypsovag Unclipped Gyp	Gypsophile Clipped Calc	Gypsophile Clipped Gyp	Gypsophile Unclipped Calc	Gypsophile Unclipped Gyp
Al	0.63±0.04	0.76±0.06	0.76±0.06	0.95±0.11	0.90±0.07	0.92±0.07	0.80±0.06	0.99±0.08
C	468.24±2.78	467.42±2.14	466.20±2.46	466.00±2.44	443.92±3.13	443.92±4.12	446.04±3.03	444.25±3.02
Ca	12.91±1.08	12.47±0.96	13.25±1.06	15.60±1.75	24.29±1.51	20.52±1.49	22.06±1.21	20.86±1.34
Cr	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.01±0.00	0.01±0.00
Cu	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.02±0.00	0.01±0.00	0.01±0.00	0.01±0.00
Fe	0.51±0.02	0.56±0.04	0.60±0.03	0.67±0.07	0.66±0.05	0.67±0.05	0.59±0.04	0.70±0.05
K	5.30±0.33	4.10±0.28	5.50±0.40	4.61±0.31	7.51±0.72	7.00±0.71	7.43±0.79	7.02±0.83
Mg	1.18±0.08	1.43±0.10	1.31±0.11	1.70±0.27	2.81±0.28	3.24±0.27	2.83±0.27	3.44±0.33
Mn	0.03±0.00	0.02±0.00	0.03±0.00	0.03±0.00	0.04±0.00	0.03±0.00	0.03±0.00	0.03±0.00
N	8.17±0.69	9.21±0.96	8.66±0.70	8.58±0.85	8.81±0.80	8.52±0.71	8.74±0.72	8.85±0.80
Na	0.12±0.01	0.14±0.02	0.13±0.01	0.15±0.02	0.13±0.01	0.15±0.01	0.12±0.01	0.13±0.01
P	1.50±0.21	0.80±0.14	1.46±0.17	0.80±0.10	1.57±0.24	0.90±0.13	1.70±0.30	0.87±0.13
S	1.86±0.26	2.25±0.36	1.86±0.25	2.28±0.34	4.02±0.30	4.97±0.46	3.72±0.25	5.41±0.61
Si	1.20±0.04	1.21±0.05	1.27±0.04	1.25±0.05	1.36±0.05	1.32±0.04	1.27±0.04	1.39±0.05
Zn	0.04±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.04±0.01	0.04±0.01	0.04±0.01	0.04±0.00

Table D.3 (Continued)

C) Coarse roots

	Gypsovag Clipped Calc	Gypsovag Clipped Gyp	Gypsovag Unclipped Calc	Gypsovag Unclipped Gyp	Gypsophile Clipped Calc	Gypsophile Clipped Gyp	Gypsophile Unclipped Calc	Gypsophile Unclipped Gyp
Al	0.24±0.01	0.39±0.04	0.27±0.02	0.36±0.03	0.22±0.02	0.31±0.04	0.21±0.02	0.35±0.04
C	464.24±5.36	469.79±4.22	468.00±4.33	464.80±4.86	438.40±6.11	442.26±5.57	438.30±6.57	438.37±4.66
Ca	12.01±1.58	10.27±1.24	11.40±1.24	12.75±1.58	23.58±2.77	20.77±2.24	23.69±2.95	22.15±2.31
Cr	0.01±0.00	0.02±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.00±0.00	0.01±0.00
Cu	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00
Fe	0.18±0.01	0.29±0.03	0.21±0.02	0.26±0.02	0.15±0.02	0.20±0.03	0.14±0.02	0.22±0.03
K	6.30±0.76	5.28±0.61	5.50±0.64	5.41±0.65	7.43±0.66	6.01±0.50	7.68±0.80	6.77±0.65
Mg	1.04±0.07	1.13±0.07	0.99±0.07	1.12±0.08	2.01±0.23	2.09±0.25	2.06±0.25	2.34±0.26
Mn	0.01±0.00	0.02±0.00	0.01±0.00	0.01±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
N	8.51±0.99	8.44±1.39	8.22±0.94	7.54±0.85	11.34±1.19	11.00±1.06	11.99±1.24	12.43±1.46
Na	0.31±0.06	0.28±0.05	0.24±0.03	0.26±0.05	0.21±0.03	0.18±0.02	0.20±0.03	0.18±0.02
P	1.94±0.38	0.87±0.11	1.62±0.25	0.90±0.10	2.53±0.35	1.34±0.17	2.43±0.31	1.60±0.27
S	2.23±0.42	3.11±0.45	1.99±0.39	3.28±0.45	3.10±0.30	3.94±0.35	3.25±0.34	4.51±0.50
Si	0.55±0.03	0.83±0.06	0.59±0.05	0.78±0.05	0.48±0.04	0.66±0.06	0.46±0.03	0.71±0.06
Zn	0.03±0.00	0.03±0.00	0.02±0.00	0.02±0.00	0.04±0.00	0.03±0.00	0.03±0.00	0.03±0.00

D) Fine roots

	Gypsovag Clipped Calc	Gypsovag Clipped Gyp	Gypsovag Unclipped Calc	Gypsovag Unclipped Gyp	Gypsophile Clipped Calc	Gypsophile Clipped Gyp	Gypsophile Unclipped Calc	Gypsophile Unclipped Gyp
Al	2.41±0.30	1.77±0.25	2.41±0.28	1.91±0.23	2.54±0.29	1.82±0.21	2.40±0.31	1.62±0.20
C	407.48±6.03	407.88±6.40	409.24±5.78	399.04±6.65	395.00±9.04	385.80±9.16	393.76±11.47	389.21±8.80
Ca	38.14±4.07	33.78±3.52	38.36±3.77	38.00±3.66	51.86±7.57	48.97±5.86	54.13±9.06	49.43±5.70
Cr	0.02±0.00	0.01±0.00	0.02±0.00	0.01±0.00	0.03±0.01	0.01±0.00	0.01±0.00	0.01±0.00
Cu	0.03±0.00	0.04±0.00	0.03±0.00	0.04±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00
Fe	1.51±0.19	1.04±0.14	1.52±0.19	1.13±0.14	1.64±0.19	1.04±0.12	1.48±0.20	0.94±0.11
K	11.54±0.99	8.76±0.69	11.49±1.06	8.69±0.66	8.61±0.64	7.10±0.65	8.58±0.69	7.13±0.73
Mg	3.36±0.30	3.87±0.40	3.27±0.31	4.65±0.54	3.95±0.20	5.04±0.39	3.86±0.23	5.09±0.40
Mn	0.05±0.00	0.04±0.00	0.05±0.00	0.04±0.00	0.05±0.00	0.03±0.00	0.05±0.00	0.03±0.00
N	12.36±1.05	12.89±1.62	11.99±1.05	11.57±1.35	13.40±1.30	12.36±1.18	13.47±1.40	10.96±0.91
Na	0.50±0.09	0.71±0.17	0.53±0.09	0.69±0.18	0.39±0.02	0.33±0.02	0.41±0.03	0.36±0.02
P	3.81±0.49	1.29±0.18	3.66±0.39	1.24±0.15	2.30±0.37	1.15±0.20	2.26±0.32	1.09±0.19
S	4.66±0.61	14.76±0.88	4.62±0.64	16.15±0.92	5.13±0.72	18.70±1.52	5.47±0.84	18.47±1.43
Si	1.37±0.04	1.30±0.04	1.36±0.03	1.34±0.03	1.40±0.02	1.39±0.01	1.38±0.04	1.39±0.03
Zn	0.06±0.00	0.04±0.00	0.05±0.00	0.03±0.00	0.05±0.01	0.03±0.00	0.04±0.00	0.03±0.00

Table D.4: ANOVA of generalised linear models of elemental concentration data for each species. Chi-squares, and P-values in brackets.

A) Gypsophila struthium

	Organ	Soil	Clipping	Organ x Soil	Organ x Clipp	Soil x Clip	Organ x Soil x Clipp
Al	**883.15 (0.000)**	**12.33 (0.000)**	**4.47 (0.034)**	**21.88 (0.000)**	6.32 (0.097)	0.23 (0.631)	1.20 (0.754)
C	**881.93 (0.000)**	0.03 (0.868)	1.45 (0.228)	**13.79 (0.003)**	4.50 (0.212)	2.21 (0.137)	2.26 (0.521)
Ca	**572.94 (0.000)**	0.74 (0.390)	0.66 (0.418)	**25.45 (0.000)**	3.18 (0.365)	0.22 (0.641)	1.28 (0.734)
Cr	**83.14 (0.000)**	1.42 (0.233)	0.17 (0.678)	**20.02 (0.000)**	3.01 (0.390)	0.03 (0.867)	6.86 (0.076)
Cu	**1143.43 (0.000)**	0.02 (0.888)	2.65 (0.103)	**16.54 (0.001)**	4.84 (0.184)	4.47 (0.034)	1.70 (0.637)
Fe	**808.39 (0.000)**	**19.81 (0.000)**	**5.89 (0.015)**	**13.97 (0.003)**	4.36 (0.225)	0.21 (0.650)	1.13 (0.771)
K	**55.73 (0.000)**	**32.84 (0.000)**	2.50 (0.114)	**25.34 (0.000)**	0.55 (0.909)	**0.00 (0.944)**	1.88 (0.598)
Mg	**2895.84 (0.000)**	0.37 (0.541)	1.24 (0.265)	**32.63 (0.000)**	6.48 (0.090)	2.75 (0.097)	4.60 (0.204)
Mn	**393.19 (0.000)**	**30.35 (0.000)**	1.35 (0.245)	**27.42 (0.000)**	6.20 (0.102)	0.23 (0.628)	0.59 (0.898)
N	**280.37 (0.000)**	2.74 (0.098)	0.06 (0.809)	3.06 (0.383)	0.56 (0.905)	0.74 (0.388)	2.35 (0.503)
Na	**517.00 (0.000)**	**7.29 (0.007)**	0.06 (0.801)	**14.71 (0.002)**	3.16 (0.368)	0.07 (0.797)	0.17 (0.982)
P	**11.63 (0.009)**	**114.79 (0.000)**	2.28 (0.131)	1.82 (0.611)	0.38 (0.945)	3.65 (0.056)	0.63 (0.890)
S	**1127.37 (0.000)**	**106.55 (0.000)**	0.98 (0.323)	**282.91 (0.000)**	4.99 (0.173)	1.30 (0.254)	0.76 (0.859)
Si	**568.33 (0.000)**	0.81 (0.368)	0.36 (0.551)	8.16 (0.043)	6.37 (0.095)	0.13 (0.719)	0.93 (0.818)
Zn	**26.26 (0.000)**	**82.13 (0.000)**	3.41 (0.065)	**13.77 (0.003)**	0.93 (0.818)	2.28 (0.131)	2.13 (0.545)

B) Herniaria fruticosa

	Organ	Soil	Clipping	Organ x Soil	Organ x Clipp	Soil x Clip	Organ x Soil x Clipp
Al	**346.39 (0.000)**	3.43 (0.064)	2.27 (0.132)	**45.00 (0.000)**	1.25 (0.742)	1.84 (0.175)	1.40 (0.705)
C	**74.76 (0.000)**	**5.83 (0.016)**	0.40 (0.527)	3.71 (0.294)	1.66 (0.645)	0.46 (0.497)	0.77 (0.856)
Ca	**28.99 (0.000)**	0.10 (0.755)	1.67 (0.197)	5.71 (0.127)	0.59 (0.899)	1.54 (0.214)	2.30 (0.512)
Cr	**148.11 (0.000)**	0.47 (0.494)	1.62 (0.203)	5.33 (0.149)	4.41 (0.221)	0.89 (0.345)	1.44 (0.697)
Cu	**318.20 (0.000)**	2.35 (0.125)	0.03 (0.863)	5.24 (0.155)	0.56 (0.905)	2.18 (0.140)	3.38 (0.336)
Fe	**440.53 (0.000)**	2.05 (0.152)	2.67 (0.102)	**30.74 (0.000)**	2.03 (0.566)	1.51 (0.219)	0.77 (0.857)
K	**110.63 (0.000)**	**12.63 (0.000)**	0.02 (0.884)	3.46 (0.326)	**10.45 (0.015)**	1.26 (0.262)	0.85 (0.837)
Mg	**104.19 (0.000)**	**26.94 (0.000)**	**10.23 (0.001)**	4.82 (0.185)	2.96 (0.398)	2.69 (0.101)	2.17 (0.539)
Mn	**32.59 (0.000)**	0.10 (0.757)	**8.38 (0.004)**	7.36 (0.061)	2.21 (0.530)	0.40 (0.528)	1.78 (0.619)
N	**89.71 (0.000)**	3.54 (0.060)	2.18 (0.140)	3.02 (0.388)	0.79 (0.853)	5.23 (0.022)	1.98 (0.576)
Na	**389.84 (0.000)**	1.86 (0.172)	0.03 (0.874)	**18.30 (0.000)**	0.20 (0.978)	3.01 (0.083)	2.80 (0.423)
P	**24.83 (0.000)**	**21.31 (0.000)**	0.48 (0.488)	8.55 (0.036)	2.52 (0.472)	3.34 (0.068)	0.26 (0.968)
S	**249.38 (0.000)**	**102.33 (0.000)**	0.24 (0.627)	**144.61 (0.000)**	0.18 (0.981)	2.52 (0.112)	0.87 (0.833)
Si	**420.51 (0.000)**	**8.36 (0.004)**	0.34 (0.562)	**87.70 (0.000)**	1.85 (0.605)	**0.00 (0.949)**	0.79 (0.851)
Zn	**39.48 (0.000)**	1.05 (0.304)	1.55 (0.213)	4.14 (0.246)	7.20 (0.066)	0.73 (0.394)	3.09 (0.378)

C) Helianthemum squamatum

	Organ	Soil	Clipping	Organ x Soil	Organ x Clipp	Soil x Clip	Organ x Soil x Clipp
Al	**987.80 (0.000)**	3.30 (0.069)	0.14 (0.712)	**26.18 (0.000)**	1.48 (0.687)	0.55 (0.458)	0.94 (0.815)
C	**449.69 (0.000)**	0.79 (0.375)	2.25 (0.134)	3.00 (0.223)	0.36 (0.834)	0.06 (0.801)	0.33 (0.848)
Ca	**746.55 (0.000)**	1.31 (0.252)	0.06 (0.806)	0.96 (0.810)	2.98 (0.395)	0.12 (0.729)	0.85 (0.838)
Cr	**143.69 (0.000)**	**27.00 (0.000)**	**7.47 (0.006)**	**25.74 (0.000)**	0.78 (0.854)	0.87 (0.351)	0.85 (0.838)
Cu	**137.99 (0.000)**	1.08 (0.299)	**6.33 (0.012)**	**11.64 (0.009)**	2.09 (0.554)	3.21 (0.073)	1.64 (0.650)
Fe	**766.25 (0.000)**	**8.89 (0.003)**	0.64 (0.425)	**14.55 (0.002)**	1.04 (0.791)	0.53 (0.466)	0.97 (0.809)
K	**17.62 (0.001)**	**17.55 (0.000)**	3.59 (0.058)	6.11 (0.107)	0.27 (0.966)	0.51 (0.477)	0.10 (0.991)
Mg	**457.22 (0.000)**	**7.98 (0.005)**	0.04 (0.847)	**28.57 (0.000)**	2.84 (0.416)	0.13 (0.720)	1.26 (0.738)
Mn	**182.19 (0.000)**	**8.37 (0.004)**	0.18 (0.670)	5.80 (0.122)	1.42 (0.701)	3.73 (0.053)	0.38 (0.944)
N	**259.80 (0.000)**	3.45 (0.063)	0.02 (0.876)	1.76 (0.415)	1.94 (0.379)	0.96 (0.328)	1.78 (0.411)
Na	**485.14 (0.000)**	3.43 (0.064)	0.31 (0.578)	**17.39 (0.001)**	0.58 (0.902)	**0.00 (0.987)**	0.39 (0.942)
P	**24.39 (0.000)**	**63.61 (0.000)**	**12.67 (0.000)**	**7.85 (0.049)**	1.31 (0.726)	**5.11 (0.024)**	0.50 (0.919)
S	**524.80 (0.000)**	**127.76 (0.000)**	0.01 (0.904)	**165.55 (0.000)**	1.40 (0.705)	**0.00 (0.952)**	1.18 (0.758)
Si	**140.80 (0.000)**	**10.36 (0.001)**	0.28 (0.599)	**44.83 (0.000)**	0.38 (0.944)	0.29 (0.593)	5.19 (0.158)
Zn	**141.83 (0.000)**	2.46 (0.116)	**6.27 (0.012)**	**9.38 (0.025)**	0.60 (0.897)	0.07 (0.796)	2.68 (0.444)

D) Lepidium subulatum

	Organ	Soil	Clipping	Organ x Soil	Organ x Clipp	Soil x Clip	Organ x Soil x Clipp
Al	**623.01 (0.000)**	1.17 (0.280)	0.00 (0.977)	1.95 (0.583)	3.54 (0.316)	3.43 (0.064)	4.66 (0.198)
C	**392.16 (0.000)**	1.16 (0.282)	1.64 (0.201)	3.42 (0.332)	2.07 (0.558)	0.08 (0.781)	3.67 (0.300)
Ca	**1396.67 (0.000)**	0.12 (0.730)	0.01 (0.923)	**14.15 (0.003)**	**8.33 (0.040)**	2.50 (0.114)	**10.31 (0.016)**
Cr	**880.62 (0.000)**	3.16 (0.075)	0.72 (0.395)	**9.19 (0.027)**	5.62 (0.132)	**4.93 (0.026)**	5.23 (0.156)
Cu	**659.80 (0.000)**	**5.14 (0.023)**	0.59 (0.442)	3.29 (0.349)	0.28 (0.964)	**11.42 (0.001)**	**7.91 (0.048)**
Fe	**736.51 (0.000)**	0.15 (0.696)	0.07 (0.793)	4.37 (0.224)	5.02 (0.171)	2.93 (0.087)	6.90 (0.075)
K	**133.31 (0.000)**	0.79 (0.375)	0.31 (0.579)	1.84 (0.606)	3.84 (0.279)	0.82 (0.365)	3.16 (0.368)
Mg	**154.68 (0.000)**	0.17 (0.677)	1.08 (0.299)	6.38 (0.095)	4.30 (0.231)	0.90 (0.342)	6.71 (0.082)
Mn	**776.84 (0.000)**	**8.03 (0.005)**	0.35 (0.556)	**11.73 (0.008)**	**12.01 (0.007)**	3.47 (0.062)	3.62 (0.306)
N	**384.85 (0.000)**	0.68 (0.409)	0.01 (0.913)	2.80 (0.423)	3.92 (0.270)	0.03 (0.859)	5.43 (0.143)
Na	**232.19 (0.000)**	0.00 (0.967)	0.00 (0.968)	4.16 (0.245)	3.78 (0.287)	**5.56 (0.018)**	4.28 (0.232)
P	**35.44 (0.000)**	**8.79 (0.003)**	0.12 (0.732)	0.27 (0.965)	1.47 (0.688)	1.35 (0.245)	0.87 (0.833)
S	**767.86 (0.000)**	**25.31 (0.000)**	0.06 (0.811)	**12.04 (0.007)**	**8.62 (0.035)**	0.45 (0.503)	2.22 (0.527)
Si	**760.47 (0.000)**	1.96 (0.162)	1.68 (0.195)	5.37 (0.147)	3.86 (0.277)	3.52 (0.061)	**8.43 (0.038)**
Zn	**20.15 (0.000)**	**10.18 (0.001)**	0.79 (0.375)	3.54 (0.316)	1.17 (0.761)	1.51 (0.219)	0.78 (0.854)

E) Ononis tridentata

	Organ	Soil	Clipping	Organ x Soil	Organ x Clipp	Soil x Clip	Organ x Soil x Clipp
Al	**1620.41 (0.000)**	**12.09 (0.001)**	0.00 (0.962)	**20.43 (0.000)**	1.51 (0.681)	0.71 (0.400)	**11.92 (0.008)**
C	**254.34 (0.000)**	1.59 (0.207)	0.10 (0.748)	5.92 (0.116)	0.91 (0.824)	0.66 (0.415)	3.23 (0.357)
Ca	**615.04 (0.000)**	**6.30 (0.012)**	0.01 (0.929)	**17.58 (0.001)**	1.76 (0.624)	1.26 (0.262)	1.39 (0.707)
Cr	**264.56 (0.000)**	**33.14 (0.000)**	6.92 (0.009)	**23.54 (0.000)**	2.74 (0.433)	1.11 (0.293)	0.78 (0.855)
Cu	**261.68 (0.000)**	**14.32 (0.000)**	1.55 (0.213)	**11.00 (0.012)**	1.53 (0.675)	0.34 (0.558)	5.91 (0.116)
Fe	**1352.82 (0.000)**	**18.08 (0.000)**	0.00 (0.991)	**22.17 (0.000)**	0.89 (0.828)	0.08 (0.782)	**12.25 (0.007)**
K	**23.98 (0.000)**	**11.42 (0.001)**	0.00 (0.977)	0.66 (0.884)	1.57 (0.665)	0.24 (0.625)	0.11 (0.990)
Mg	**1211.95 (0.000)**	**24.82 (0.000)**	1.05 (0.306)	**29.01 (0.000)**	1.50 (0.683)	0.25 (0.616)	**11.56 (0.009)**
Mn	**1019.46 (0.000)**	**25.46 (0.000)**	0.04 (0.843)	**9.62 (0.022)**	1.18 (0.757)	0.04 (0.837)	6.91 (0.075)
N	**76.57 (0.000)**	0.11 (0.737)	0.04 (0.832)	5.29 (0.152)	1.37 (0.712)	0.00 (0.951)	2.37 (0.499)
Na	**453.56 (0.000)**	3.10 (0.078)	1.02 (0.313)	1.53 (0.676)	2.42 (0.489)	0.00 (0.953)	5.07 (0.167)
P	5.08 (0.166)	**7.09 (0.008)**	0.90 (0.343)	1.25 (0.740)	0.41 (0.939)	1.34 (0.246)	0.27 (0.966)
S	**933.66 (0.000)**	**174.40 (0.000)**	0.03 (0.858)	**139.52 (0.000)**	0.19 (0.979)	1.68 (0.195)	5.56 (0.135)
Si	**474.76 (0.000)**	1.69 (0.194)	0.14 (0.708)	**31.51 (0.000)**	1.15 (0.765)	**4.44 (0.035)**	4.56 (0.207)
Zn	**34.36 (0.000)**	**7.10 (0.008)**	1.93 (0.165)	**16.70 (0.001)**	1.13 (0.770)	0.72 (0.398)	5.06 (0.167)

F) Boleum asperum

	Organ	Soil	Clipping	Organ x Soil	Organ x Clipp	Soil x Clip	Organ x Soil x Clipp
Al	**139.12 (0.000)**	1.38 (0.240)	0.01 (0.910)	**11.87 (0.008)**	0.30 (0.960)	0.13 (0.715)	3.18 (0.364)
C	**382.47 (0.000)**	3.49 (0.062)	0.09 (0.761)	0.45 (0.929)	0.77 (0.857)	0.29 (0.589)	3.36 (0.339)
Ca	**196.43 (0.000)**	0.56 (0.456)	0.19 (0.665)	2.82 (0.420)	3.10 (0.376)	0.00 (0.981)	0.70 (0.872)
Cr	**237.11 (0.000)**	0.51 (0.477)	0.39 (0.531)	**15.52 (0.001)**	7.01 (0.071)	**4.39 (0.036)**	0.75 (0.863)
Cu	**94.42 (0.000)**	0.25 (0.619)	0.35 (0.555)	5.97 (0.113)	1.14 (0.767)	0.21 (0.649)	2.74 (0.434)
Fe	**103.48 (0.000)**	2.56 (0.109)	0.01 (0.937)	**11.03 (0.012)**	1.20 (0.754)	0.68 (0.409)	3.21 (0.360)
K	**182.59 (0.000)**	2.92 (0.088)	0.91 (0.339)	**10.70 (0.013)**	0.92 (0.821)	0.03 (0.854)	1.54 (0.672)
Mg	**609.44 (0.000)**	0.14 (0.707)	2.44 (0.119)	**16.58 (0.001)**	1.46 (0.692)	1.19 (0.276)	1.97 (0.579)
Mn	**26.54 (0.000)**	**6.00 (0.014)**	**6.33 (0.012)**	0.17 (0.983)	0.33 (0.955)	1.21 (0.272)	2.29 (0.515)
N	**347.84 (0.000)**	**14.72 (0.000)**	**5.46 (0.019)**	**10.80 (0.013)**	4.86 (0.182)	1.41 (0.236)	0.98 (0.807)
Na	**126.77 (0.000)**	1.70 (0.192)	1.20 (0.273)	1.08 (0.782)	0.94 (0.816)	1.01 (0.316)	1.97 (0.580)
P	**10.94 (0.012)**	2.13 (0.145)	0.00 (0.980)	1.35 (0.717)	0.21 (0.976)	1.69 (0.193)	1.09 (0.780)
S	**315.23 (0.000)**	**74.55 (0.000)**	0.04 (0.847)	2.71 (0.438)	1.42 (0.702)	0.02 (0.898)	1.32 (0.725)
Si	**143.80 (0.000)**	**6.40 (0.011)**	0.11 (0.737)	**20.57 (0.000)**	0.65 (0.885)	0.01 (0.926)	2.55 (0.467)
Zn	**46.62 (0.000)**	0.63 (0.427)	0.03 (0.854)	0.33 (0.953)	6.39 (0.094)	1.18 (0.278)	0.70 (0.873)

G) Helianthemum syriacum

	Organ	Soil	Clipping	Organ x Soil	Organ x Clipp	Soil x Clip	Organ x Soil x Clipp
Al	**1463.63 (0.000)**	0.61 (0.436)	0.09 (0.760)	**15.45 (0.001)**	6.01 (0.111)	0.35 (0.552)	2.49 (0.478)
C	**586.50 (0.000)**	2.00 (0.157)	0.05 (0.816)	1.37 (0.505)	0.10 (0.950)	0.23 (0.633)	0.50 (0.779)
Ca	**1352.80 (0.000)**	0.01 (0.930)	0.09 (0.762)	**9.31 (0.025)**	1.05 (0.789)	1.67 (0.197)	6.36 (0.095)
Cr	**210.35 (0.000)**	**26.43 (0.000)**	0.10 (0.755)	**17.78 (0.000)**	0.77 (0.857)	0.00 (0.976)	0.53 (0.911)
Cu	**574.40 (0.000)**	**26.70 (0.000)**	0.13 (0.716)	**7.90 (0.048)**	0.47 (0.925)	**6.47 (0.011)**	0.28 (0.964)
Fe	**1294.76 (0.000)**	**6.70 (0.010)**	0.00 (0.957)	**9.78 (0.021)**	4.01 (0.260)	0.88 (0.349)	2.66 (0.447)
K	**672.90 (0.000)**	**77.44 (0.000)**	0.04 (0.834)	6.33 (0.097)	1.54 (0.673)	1.88 (0.171)	2.34 (0.506)
Mg	**1129.97 (0.000)**	**58.84 (0.000)**	0.66 (0.418)	**24.93 (0.000)**	1.28 (0.734)	**7.49 (0.006)**	0.68 (0.877)
Mn	**550.43 (0.000)**	**5.23 (0.022)**	0.01 (0.922)	5.91 (0.116)	0.16 (0.983)	0.00 (0.958)	1.97 (0.579)
N	**212.34 (0.000)**	**19.50 (0.000)**	1.77 (0.183)	0.88 (0.644)	0.38 (0.829)	0.26 (0.609)	0.56 (0.756)
Na	**383.84 (0.000)**	2.90 (0.089)	0.52 (0.470)	7.00 (0.072)	0.22 (0.974)	0.02 (0.902)	0.84 (0.839)
P	**138.19 (0.000)**	**274.54 (0.000)**	**4.48 (0.034)**	**13.95 (0.003)**	0.62 (0.892)	3.79 (0.051)	1.41 (0.703)
S	**1758.50 (0.000)**	**443.90 (0.000)**	0.04 (0.843)	**235.55 (0.000)**	1.36 (0.714)	1.89 (0.170)	0.12 (0.989)
Si	**513.03 (0.000)**	1.39 (0.238)	0.08 (0.783)	**31.31 (0.000)**	5.04 (0.169)	1.02 (0.312)	0.16 (0.983)
Zn	**237.47 (0.000)**	**18.58 (0.000)**	2.21 (0.137)	7.37 (0.061)	1.79 (0.618)	1.02 (0.311)	1.78 (0.619)

H) Linum suffruticosum

	Organ	Soil	Clipping	Organ x Soil	Organ x Clipp	Soil x Clip	Organ x Soil x Clipp
Al	**408.87 (0.000)**	0.13 (0.723)	**4.71 (0.030)**	**32.44 (0.000)**	3.35 (0.341)	0.90 (0.343)	2.89 (0.409)
C	**272.05 (0.000)**	2.56 (0.109)	**8.60 (0.003)**	1.41 (0.703)	4.35 (0.226)	1.97 (0.161)	2.10 (0.552)
Ca	**95.23 (0.000)**	2.87 (0.090)	**6.54 (0.011)**	7.11 (0.068)	2.68 (0.444)	1.40 (0.236)	1.81 (0.612)
Cr	**11.71 (0.008)**	0.57 (0.452)	0.75 (0.386)	**26.21 (0.000)**	1.01 (0.798)	0.00 (0.999)	3.22 (0.358)
Cu	**684.26 (0.000)**	**11.09 (0.001)**	0.03 (0.858)	7.10 (0.069)	4.55 (0.208)	0.40 (0.525)	1.43 (0.700)
Fe	**368.20 (0.000)**	0.40 (0.529)	**7.11 (0.008)**	**28.86 (0.000)**	2.36 (0.502)	1.26 (0.261)	4.72 (0.193)
K	**307.21 (0.000)**	**4.10 (0.043)**	0.18 (0.673)	**9.42 (0.024)**	5.65 (0.130)	1.98 (0.159)	5.22 (0.157)
Mg	**246.07 (0.000)**	0.03 (0.855)	**7.17 (0.007)**	2.97 (0.396)	6.10 (0.107)	2.02 (0.155)	0.23 (0.973)
Mn	**179.78 (0.000)**	0.10 (0.746)	0.85 (0.355)	**15.16 (0.002)**	3.07 (0.381)	0.16 (0.691)	3.28 (0.351)
N	**210.29 (0.000)**	3.66 (0.056)	1.45 (0.229)	5.67 (0.129)	3.46 (0.325)	0.67 (0.414)	1.10 (0.776)
Na	**69.62 (0.000)**	**5.34 (0.021)**	2.43 (0.119)	1.04 (0.790)	0.70 (0.874)	**6.26 (0.012)**	4.73 (0.193)
P	**203.65 (0.000)**	**141.41 (0.000)**	1.83 (0.176)	3.26 (0.353)	**10.30 (0.016)**	1.56 (0.212)	2.61 (0.456)
S	**596.12 (0.000)**	**287.49 (0.000)**	**4.66 (0.031)**	**17.16 (0.001)**	1.20 (0.753)	0.56 (0.454)	1.10 (0.777)
Si	**176.03 (0.000)**	**12.37 (0.000)**	0.16 (0.688)	**79.88 (0.000)**	1.34 (0.719)	0.83 (0.362)	1.43 (0.698)
Zn	**69.64 (0.000)**	**11.51 (0.001)**	0.01 (0.924)	**26.96 (0.000)**	0.94 (0.815)	1.30 (0.254)	3.75 (0.290)

I) Matthiola fruticulosa

	Organ	Soil	Clipping	Organ x Soil	Organ x Clipp	Soil x Clip	Organ x Soil x Clipp
Al	**242.69 (0.000)**	**7.23 (0.007)**	0.41 (0.522)	**9.20 (0.027)**	4.51 (0.211)	0.98 (0.323)	0.98 (0.807)
C	**1216.56 (0.000)**	0.16 (0.689)	0.89 (0.345)	**26.99 (0.000)**	**11.81 (0.008)**	**6.68 (0.010)**	2.61 (0.455)
Ca	**1183.24 (0.000)**	3.08 (0.079)	0.41 (0.520)	6.24 (0.101)	4.63 (0.201)	0.79 (0.375)	3.64 (0.304)
Cr	**90.00 (0.000)**	0.49 (0.485)	1.71 (0.191)	**21.47 (0.000)**	1.84 (0.606)	0.01 (0.914)	1.61 (0.658)
Cu	**522.53 (0.000)**	**20.69 (0.000)**	0.04 (0.835)	6.53 (0.089)	1.34 (0.719)	3.44 (0.064)	2.24 (0.524)
Fe	**203.45 (0.000)**	**10.78 (0.001)**	0.15 (0.697)	**13.07 (0.004)**	4.26 (0.235)	1.48 (0.223)	0.31 (0.959)
K	**211.21 (0.000)**	**44.87 (0.000)**	2.31 (0.128)	1.36 (0.716)	6.63 (0.085)	**14.13 (0.000)**	5.74 (0.125)
Mg	**999.78 (0.000)**	**63.51 (0.000)**	1.36 (0.243)	4.65 (0.199)	5.53 (0.137)	2.22 (0.136)	1.12 (0.771)
Mn	**606.77 (0.000)**	**25.54 (0.000)**	0.00 (0.961)	**18.31 (0.000)**	5.34 (0.148)	2.45 (0.118)	0.84 (0.840)
N	**431.88 (0.000)**	**11.22 (0.001)**	1.08 (0.299)	0.58 (0.901)	1.12 (0.772)	0.03 (0.870)	0.14 (0.987)
Na	**60.01 (0.000)**	**6.77 (0.009)**	3.76 (0.052)	**7.83 (0.050)**	**8.82 (0.032)**	1.16 (0.282)	1.53 (0.675)
P	**37.37 (0.000)**	**230.21 (0.000)**	0.40 (0.526)	**30.94 (0.000)**	1.74 (0.628)	**9.67 (0.002)**	2.48 (0.479)
S	**700.42 (0.000)**	**24.63 (0.000)**	0.25 (0.615)	6.40 (0.094)	0.84 (0.840)	0.39 (0.535)	2.93 (0.402)
Si	**326.60 (0.000)**	0.14 (0.707)	0.01 (0.911)	**29.11 (0.000)**	4.37 (0.224)	0.98 (0.323)	2.24 (0.524)
Zn	**87.28 (0.000)**	**10.61 (0.001)**	1.69 (0.194)	**24.81 (0.000)**	**8.84 (0.032)**	0.00 (0.957)	0.24 (0.970)

J) Rosmarinus officinalis

	Organ	Soil	Clipping	Organ x Soil	Organ x Clipp	Soil x Clip	Organ x Soil x Clipp
Al	**1106.76 (0.000)**	3.25 (0.071)	0.11 (0.739)	**18.66 (0.000)**	**9.68 (0.021)**	0.17 (0.681)	3.31 (0.347)
C	**1228.13 (0.000)**	2.20 (0.138)	0.34 (0.559)	5.68 (0.128)	3.93 (0.270)	0.06 (0.804)	0.22 (0.974)
Ca	**568.39 (0.000)**	0.01 (0.915)	0.34 (0.558)	**10.90 (0.012)**	6.98 (0.073)	0.20 (0.659)	1.93 (0.587)
Cr	**401.21 (0.000)**	**12.07 (0.001)**	0.24 (0.627)	5.13 (0.163)	1.52 (0.677)	1.03 (0.309)	7.74 (0.052)
Cu	**1089.36 (0.000)**	**35.22 (0.000)**	0.56 (0.454)	**20.85 (0.000)**	5.56 (0.135)	0.48 (0.490)	3.73 (0.293)
Fe	**1044.56 (0.000)**	**8.32 (0.004)**	0.01 (0.927)	4.95 (0.175)	5.94 (0.114)	0.07 (0.787)	5.53 (0.137)
K	**426.36 (0.000)**	**66.50 (0.000)**	0.79 (0.374)	**26.05 (0.000)**	3.05 (0.384)	**8.95 (0.003)**	3.40 (0.334)
Mg	**856.34 (0.000)**	**117.16 (0.000)**	1.41 (0.236)	**22.53 (0.000)**	2.62 (0.454)	0.01 (0.911)	3.27 (0.351)
Mn	**763.79 (0.000)**	**35.25 (0.000)**	0.34 (0.561)	**9.18 (0.027)**	**9.44 (0.024)**	0.00 (0.970)	2.30 (0.513)
N	**153.35 (0.000)**	1.06 (0.303)	2.85 (0.091)	0.25 (0.969)	3.20 (0.361)	**9.99 (0.002)**	2.60 (0.458)
Na	**1037.64 (0.000)**	**34.82 (0.000)**	0.60 (0.437)	**15.25 (0.002)**	4.04 (0.257)	0.00 (0.987)	4.05 (0.256)
P	**116.80 (0.000)**	**123.42 (0.000)**	0.09 (0.770)	5.64 (0.131)	2.83 (0.419)	**3.99 (0.046)**	1.84 (0.607)
S	**1264.68 (0.000)**	**454.57 (0.000)**	0.25 (0.621)	**60.46 (0.000)**	2.65 (0.449)	0.21 (0.649)	0.64 (0.886)
Si	**191.72 (0.000)**	0.11 (0.743)	3.45 (0.063)	6.55 (0.088)	2.16 (0.540)	0.08 (0.779)	7.01 (0.072)
Zn	**33.94 (0.000)**	**12.64 (0.000)**	0.76 (0.382)	**14.81 (0.002)**	4.78 (0.189)	0.02 (0.887)	**9.39 (0.024)**

Table D.5: Means and standard errors of biomass and elemental concentration for each treatment and species.

Species	Organ	Soil	Clipping	Biomass	Al (mg/g)	C (mg/g)	Ca (mg/g)	Cr (µg/g)	Cu (µg/g)	Fe (mg/g)	K (mg/g)
Boleum asperum	Coarse roots	Calc	Clipped	0.54±0.10	0.28±0.02	475.00±4.93	6.65±0.21	1.35±0.46	0.92±0.03	0.22±0.03	5.11±0.47
Boleum asperum	Coarse roots	Calc	Unclipped	2.08±1.60	0.28±0.05	475.00±2.08	7.55±1.07	1.79±0.55	0.74±0.11	0.24±0.05	4.86±0.55
Boleum asperum	Coarse roots	Gyp	Clipped	0.28±0.12	0.48±0.03	0.00±0.00	6.47±0.16	2.31±0.72	0.58±0.03	0.38±0.05	4.02±0.25
Boleum asperum	Coarse roots	Gyp	Unclipped	0.33±0.11	0.46±0.07	0.00±0.00	9.50±1.80	3.44±0.92	0.58±0.03	0.43±0.02	3.83±0.36
Boleum asperum	Fine roots	Calc	Clipped	2.45±0.39	0.67±0.15	435.80±6.76	21.35±4.22	0.50±0.10	1.59±0.27	0.38±0.09	9.10±0.57
Boleum asperum	Fine roots	Calc	Unclipped	2.68±0.23	0.62±0.10	437.60±6.21	22.63±5.50	0.50±0.04	1.74±0.34	0.34±0.05	9.34±0.35
Boleum asperum	Fine roots	Gyp	Clipped	1.95±0.08	0.53±0.08	436.00±5.59	21.53±3.77	0.33±0.04	1.62±0.27	0.32±0.05	7.45±0.78
Boleum asperum	Fine roots	Gyp	Unclipped	2.14±0.18	0.64±0.07	432.00±4.88	24.30±4.05	0.33±0.04	1.90±0.47	0.38±0.04	7.49±0.61
Boleum asperum	Leaves	Calc	Clipped	0.31±0.06	0.21±0.03	416.00±7.77	27.46±3.09	2.72±0.46	0.59±0.04	0.27±0.04	9.93±0.89
Boleum asperum	Leaves	Calc	Unclipped	0.41±0.12	0.23±0.03	416.67±2.33	28.16±1.07	2.15±0.50	0.76±0.17	0.27±0.03	9.86±0.35
Boleum asperum	Leaves	Gyp	Clipped	0.43±0.10	0.29±0.06	410.00±2.08	35.19±1.85	2.29±0.66	0.88±0.07	0.30±0.05	10.17±0.89
Boleum asperum	Leaves	Gyp	Unclipped	0.56±0.13	0.24±0.02	411.00±1.53	32.30±1.35	1.50±0.35	0.70±0.04	0.24±0.02	12.58±1.19
Boleum asperum	Stems	Calc	Clipped	1.06±0.13	0.67±0.03	480.20±3.64	12.39±0.57	2.67±0.27	0.86±0.05	0.56±0.03	5.25±0.43
Boleum asperum	Stems	Calc	Unclipped	1.41±0.22	0.77±0.06	476.00±2.85	12.01±0.82	3.36±0.69	0.99±0.15	0.64±0.05	6.00±0.63
Boleum asperum	Stems	Gyp	Clipped	0.77±0.10	0.85±0.09	465.20±3.25	10.94±0.55	4.05±0.79	1.10±0.06	0.73±0.08	5.23±0.63
Boleum asperum	Stems	Gyp	Unclipped	1.06±0.05	0.74±0.03	477.40±4.69	10.09±0.64	2.37±0.29	1.00±0.06	0.60±0.03	5.19±0.54
Gypsophila struthium	Coarse roots	Calc	Clipped	4.16±0.48	0.17±0.03	407.40±5.20	38.59±3.48	0.18±0.04	0.60±0.05	0.11±0.02	9.10±0.90
Gypsophila struthium	Coarse roots	Calc	Unclipped	4.65±0.75	0.22±0.04	397.80±2.15	42.46±1.81	0.26±0.08	0.57±0.02	0.15±0.02	9.59±0.80
Gypsophila struthium	Coarse roots	Gyp	Clipped	5.75±1.21	0.21±0.02	413.40±0.93	31.27±0.91	0.06±0.01	0.53±0.02	0.13±0.01	6.93±0.33
Gypsophila struthium	Coarse roots	Gyp	Unclipped	6.63±1.39	0.23±0.03	418.00±3.99	29.70±2.71	0.08±0.02	0.57±0.06	0.15±0.01	7.62±0.55
Gypsophila struthium	Fine roots	Calc	Clipped	9.96±1.00	2.60±0.42	400.80±9.21	46.46±3.03	0.55±0.10	3.81±0.51	1.72±0.31	9.86±0.31
Gypsophila struthium	Fine roots	Calc	Unclipped	9.21±1.89	1.98±0.23	412.60±4.23	41.09±2.49	0.42±0.04	2.87±0.34	1.23±0.13	11.12±0.74
Gypsophila struthium	Fine roots	Gyp	Clipped	8.88±1.01	1.33±0.19	401.60±6.95	38.79±3.76	0.34±0.03	3.50±0.15	0.81±0.12	7.79±0.29
Gypsophila struthium	Fine roots	Gyp	Unclipped	7.13±0.89	1.06±0.11	408.40±6.00	37.27±2.15	0.63±0.30	3.92±0.17	0.66±0.06	7.88±0.41
Gypsophila struthium	Leaves	Calc	Clipped	2.42±0.38	0.13±0.01	343.20±6.11	75.60±4.90	0.42±0.09	0.77±0.08	0.13±0.01	9.33±0.93
Gypsophila struthium	Leaves	Calc	Unclipped	1.93±0.45	0.12±0.01	336.80±3.90	76.65±4.17	0.75±0.32	0.74±0.05	0.13±0.02	9.91±2.38
Gypsophila struthium	Leaves	Gyp	Clipped	2.08±0.63	0.18±0.04	328.60±4.11	83.52±1.85	0.56±0.19	0.84±0.09	0.17±0.03	4.93±0.46
Gypsophila struthium	Leaves	Gyp	Unclipped	1.68±0.31	0.12±0.01	330.60±2.25	81.32±4.22	0.41±0.07	0.75±0.05	0.13±0.01	6.04±0.56
Gypsophila struthium	Stems	Calc	Clipped	3.83±0.30	0.53±0.08	423.80±3.40	25.73±1.67	0.79±0.11	1.29±0.15	0.39±0.06	11.58±0.51
Gypsophila struthium	Stems	Calc	Unclipped	5.75±0.82	0.46±0.04	425.40±4.26	24.03±2.26	0.69±0.05	1.01±0.09	0.36±0.02	12.58±0.69
Gypsophila struthium	Stems	Gyp	Clipped	3.57±0.29	0.74±0.05	415.80±2.13	30.33±1.21	0.77±0.11	0.91±0.06	0.48±0.04	11.41±0.17
Gypsophila struthium	Stems	Gyp	Unclipped	5.01±0.79	0.65±0.06	428.20±4.13	26.61±2.85	0.62±0.09	0.72±0.07	0.40±0.03	11.49±0.63
Helianthemum squamatum	Coarse roots	Calc	Clipped	0.24±0.02	0.40±0.05		29.87±0.47	2.43±0.40	2.35±0.34	0.33±0.04	4.00±0.23
Helianthemum squamatum	Coarse roots	Calc	Unclipped	0.53±0.03	0.35±0.09		27.67±3.41	2.08±0.73	1.97±0.50	0.28±0.08	3.81±0.34
Helianthemum squamatum	Coarse roots	Gyp	Clipped	0.21±0.05	0.62±0.10		29.60±1.17	2.71±0.43	1.56±0.13	0.44±0.07	3.71±0.19
Helianthemum squamatum	Coarse roots	Gyp	Unclipped	0.31±0.06	0.78±0.08		29.48±0.99	2.41±0.70	1.76±0.13	0.52±0.03	3.57±0.15
Helianthemum squamatum	Fine roots	Calc	Clipped	2.29±0.35	3.87±0.68	375.00±10.07	67.02±2.15	7.40±1.69	3.93±0.40	2.43±0.53	5.04±0.31
Helianthemum squamatum	Fine roots	Calc	Unclipped	3.09±0.69	3.46±0.64	375.33±12.72	68.74±4.58	4.12±1.05	2.74±0.48	2.03±0.37	5.27±1.09
Helianthemum squamatum	Fine roots	Gyp	Clipped	1.69±0.28	3.04±0.10	360.67±4.70	60.92±2.99	1.45±0.13	4.57±0.38	1.62±0.05	3.77±0.14
Helianthemum squamatum	Fine roots	Gyp	Unclipped	2.28±0.32	3.09±0.24	362.67±6.69	63.72±3.08	0.92±0.11	4.02±0.23	1.66±0.12	3.34±0.23

Helianthemum squamatum	Leaves	Calc	Clipped	1.61±0.12	0.16±0.01	428.20±5.80	26.54±1.94	0.57±0.06	2.25±0.12	0.15±0.01	5.38±1.16
Helianthemum squamatum	Leaves	Calc	Unclipped	1.73±0.21	0.15±0.04	429.60±5.94	27.05±3.23	0.56±0.14	1.78±0.19	0.14±0.03	4.83±0.43
Helianthemum squamatum	Leaves	Gyp	Clipped	1.45±0.11	0.22±0.03	428.40±6.21	27.03±2.41	0.46±0.06	1.99±0.14	0.17±0.02	4.77±0.56
Helianthemum squamatum	Leaves	Gyp	Unclipped	1.61±0.26	0.19±0.02	435.80±4.78	25.24±1.61	0.39±0.07	1.93±0.05	0.14±0.01	3.95±0.24
Helianthemum squamatum	Roots	Calc	Clipped	2.38±0.27		372.00±5.00					
Helianthemum squamatum	Roots	Calc	Unclipped	3.14±0.69		385.00±12.00					
Helianthemum squamatum	Roots	Gyp	Clipped	1.38±0.12		367.00±24.00					
Helianthemum squamatum	Roots	Gyp	Unclipped	2.15±0.67		383.50±2.50					
Helianthemum squamatum	Stems	Calc	Clipped	1.07±0.13	0.66±0.09	454.20±2.89	19.09±1.02	3.00±0.98	2.20±0.15	0.51±0.09	3.97±0.45
Helianthemum squamatum	Stems	Calc	Unclipped	1.17±0.14	0.69±0.06	460.20±1.77	17.39±1.50	2.35±0.51	2.23±0.16	0.50±0.05	3.81±0.28
Helianthemum squamatum	Stems	Gyp	Clipped	0.85±0.10	0.77±0.07	453.20±1.83	19.71±1.73	2.65±0.80	2.14±0.14	0.55±0.07	3.54±0.37
Helianthemum squamatum	Stems	Gyp	Unclipped	1.18±0.08	0.83±0.06	455.60±2.64	17.52±0.69	1.72±0.33	2.07±0.24	0.56±0.05	3.17±0.25
Helianthemum syriacum	Coarse roots	Calc	Clipped	0.36±0.01	0.24±0.03		24.22±1.18	1.41±0.33	1.18±0.20	0.19±0.01	3.02±0.09
Helianthemum syriacum	Coarse roots	Calc	Unclipped	1.00±0.77	0.22±0.03		20.22±3.31	1.45±0.72	1.13±0.21	0.20±0.06	2.96±0.14
Helianthemum syriacum	Coarse roots	Gyp	Clipped	0.19±0.01	0.36±0.01		22.66±0.01	1.42±0.20	0.89±0.09	0.25±0.01	2.41±0.15
Helianthemum syriacum	Coarse roots	Gyp	Unclipped	0.36±0.18	0.31±0.04		26.77±1.17	1.17±0.32	0.91±0.04	0.22±0.03	2.60±0.14
Helianthemum syriacum	Fine roots	Calc	Clipped	4.13±0.36	4.46±0.54	355.25±5.25	72.27±2.99	5.27±0.88	3.98±0.33	2.87±0.29	4.40±0.10
Helianthemum syriacum	Fine roots	Calc	Unclipped	6.77±1.65	4.66±0.34	346.67±2.85	77.59±1.93	5.67±0.74	3.42±0.47	3.14±0.10	5.23±0.23
Helianthemum syriacum	Fine roots	Gyp	Clipped	2.61±0.77	3.29±0.09	364.00±14.00	59.88±3.84	1.55±0.13	5.23±0.56	1.88±0.07	3.32±0.10
Helianthemum syriacum	Fine roots	Gyp	Unclipped	2.78±0.52	3.21±0.26	358.33±8.25	64.70±2.47	1.73±0.43	6.42±0.64	1.74±0.15	3.10±0.22
Helianthemum syriacum	Leaves	Calc	Clipped	1.66±0.18	0.34±0.05	432.00±0.71	26.20±0.36	0.52±0.08	2.93±0.24	0.25±0.03	8.48±0.63
Helianthemum syriacum	Leaves	Calc	Unclipped	2.22±0.38	0.43±0.05	433.60±2.16	25.76±1.60	0.43±0.04	2.38±0.12	0.30±0.03	7.77±0.32
Helianthemum syriacum	Leaves	Gyp	Clipped	1.19±0.40	0.43±0.02	425.75±3.77	29.34±1.03	0.62±0.10	3.09±0.24	0.31±0.02	5.73±0.17
Helianthemum syriacum	Leaves	Gyp	Unclipped	1.54±0.20	0.47±0.04	424.00±1.67	28.97±0.89	0.57±0.08	3.49±0.31	0.32±0.03	6.19±0.47
Helianthemum syriacum	Roots	Calc	Clipped	7.52±0.00		356.00±0.00					
Helianthemum syriacum	Roots	Calc	Unclipped	4.78±0.12		383.50±12.50					
Helianthemum syriacum	Roots	Gyp	Clipped	2.62±0.10		342.00±10.00					
Helianthemum syriacum	Roots	Gyp	Unclipped	3.09±1.85		339.00±21.00					
Helianthemum syriacum	Stems	Calc	Clipped	1.07±0.10	0.85±0.07	451.60±2.94	19.43±0.79	1.53±0.25	1.13±0.06	0.60±0.05	3.51±0.25
Helianthemum syriacum	Stems	Calc	Unclipped	1.78±0.65	1.10±0.10	451.20±2.33	21.19±1.26	1.66±0.26	1.13±0.14	0.78±0.07	3.18±0.13
Helianthemum syriacum	Stems	Gyp	Clipped	0.79±0.15	1.15±0.07	450.75±2.78	19.95±0.19	1.72±0.26	1.21±0.04	0.78±0.04	2.81±0.04
Helianthemum syriacum	Stems	Gyp	Unclipped	0.79±0.14	1.16±0.12	452.00±3.54	19.94±0.54	1.74±0.25	1.34±0.05	0.77±0.07	2.88±0.14
Herniaria fruticosa	Coarse roots	Calc	Clipped	0.60±0.08	0.23±0.03	421.60±3.72	30.03±2.19	0.42±0.13	0.87±0.13	0.16±0.02	11.84±0.23
Herniaria fruticosa	Coarse roots	Calc	Unclipped	0.81±0.11	0.22±0.02	427.00±3.73	29.05±2.69	0.23±0.04	0.85±0.12	0.16±0.02	12.92±1.09
Herniaria fruticosa	Coarse roots	Gyp	Clipped	0.62±0.13	0.42±0.05	0.00±0.00	25.52±1.13	0.30±0.10	0.75±0.02	0.24±0.03	9.62±0.52
Herniaria fruticosa	Coarse roots	Gyp	Unclipped	0.67±0.10	0.39±0.03	423.20±2.44	30.39±0.68	0.24±0.03	0.84±0.04	0.22±0.01	10.67±1.12
Herniaria fruticosa	Fine roots	Calc	Clipped	1.98±0.18	1.44±0.22	428.40±3.44	26.22±2.19	0.60±0.10	3.69±0.50	0.93±0.13	11.99±0.42
Herniaria fruticosa	Fine roots	Calc	Unclipped	1.72±0.32	1.40±0.11	433.60±2.42	27.84±0.80	0.55±0.09	3.69±0.45	0.85±0.10	11.89±0.72
Herniaria fruticosa	Fine roots	Gyp	Clipped	1.94±0.25	1.31±0.17	417.40±7.61	30.26±3.24	0.43±0.03	3.47±0.34	0.80±0.10	10.50±0.21
Herniaria fruticosa	Fine roots	Gyp	Unclipped	2.59±0.49	0.98±0.13	420.40±7.46	30.62±2.86	0.34±0.03	3.63±0.36	0.59±0.08	10.92±0.62
Herniaria fruticosa	Leaves	Calc	Clipped	1.05±0.13	0.57±0.08	455.80±2.52	33.40±2.92	1.55±0.10	2.12±0.35	0.42±0.05	8.93±0.68
Herniaria fruticosa	Leaves	Calc	Unclipped	1.39±0.14	0.66±0.09	451.40±6.43	35.47±2.88	1.19±0.07	2.00±0.19	0.46±0.04	6.77±0.84
Herniaria fruticosa	Leaves	Gyp	Clipped	1.23±0.15	0.90±0.09	448.00±6.38	34.93±2.68	1.19±0.12	1.85±0.18	0.57±0.05	7.16±0.50

Herniaria fruticosa	Leaves	Gyp	Unclipped	1.42±0.28	0.83±0.05	445.80±5.96	38.72±2.23	1.38±0.45	2.16±0.14	0.56±0.03	6.53±0.29
Herniaria fruticosa	Stems	Calc	Clipped	1.64±0.12	1.01±0.07	431.80±3.35	30.74±2.49	1.11±0.09	2.35±0.37	0.71±0.04	11.18±0.57
Herniaria fruticosa	Stems	Calc	Unclipped	2.07±0.38	0.92±0.08	438.20±3.62	28.07±1.54	0.92±0.24	1.78±0.23	0.67±0.06	11.09±0.53
Herniaria fruticosa	Stems	Gyp	Clipped	1.41±0.18	1.23±0.08	429.20±3.38	25.32±1.91	1.18±0.13	1.37±0.14	0.90±0.05	10.29±0.64
Herniaria fruticosa	Stems	Gyp	Unclipped	1.81±0.35	1.12±0.09	431.40±5.57	27.84±1.70	1.06±0.20	1.68±0.14	0.81±0.06	11.13±0.70
Lepidium subulatum	Coarse roots	Calc	Clipped	2.30±0.09	0.29±0.01	938.20±2.77	8.44±0.34	0.35±0.06	0.60±0.05	0.20±0.01	10.86±0.62
Lepidium subulatum	Coarse roots	Calc	Unclipped	0.84±0.31	0.13±0.02	468.00±2.71	4.25±0.27	0.36±0.04	0.47±0.03	0.09±0.02	5.29±0.61
Lepidium subulatum	Coarse roots	Gyp	Clipped	1.67±0.16	0.35±0.02	935.50±2.39	7.42±0.17	0.25±0.05	0.39±0.01	0.23±0.01	11.16±0.47
Lepidium subulatum	Coarse roots	Gyp	Unclipped	0.54±0.22	0.20±0.02	462.50±2.40	4.18±0.25	0.45±0.09	0.51±0.04	0.13±0.02	6.28±1.07
Lepidium subulatum	Fine roots	Calc	Clipped	3.49±0.21	1.38±0.06	864.60±3.35	35.29±1.03	0.63±0.05	2.80±0.15	0.90±0.03	21.17±0.50
Lepidium subulatum	Fine roots	Calc	Unclipped	1.85±0.25	0.59±0.09	435.20±4.55	14.54±2.45	0.47±0.03	2.27±0.19	0.36±0.07	10.28±0.90
Lepidium subulatum	Fine roots	Gyp	Clipped	2.83±0.17	1.31±0.02	857.25±3.27	40.08±1.49	0.34±0.02	1.95±0.11	0.77±0.01	21.03±0.74
Lepidium subulatum	Fine roots	Gyp	Unclipped	1.42±0.11	0.61±0.02	427.25±3.71	20.71±1.14	0.40±0.07	2.87±0.42	0.37±0.01	10.79±0.97
Lepidium subulatum	Leaves	Calc	Clipped	1.03±0.09	2.47±0.24	844.80±5.23	71.37±2.99	6.07±1.08	2.37±0.23	1.94±0.17	12.91±0.18
Lepidium subulatum	Leaves	Calc	Unclipped	0.56±0.04	1.18±0.05	419.80±4.14	36.49±1.95	4.37±0.33	2.31±0.21	0.92±0.07	6.21±0.28
Lepidium subulatum	Leaves	Gyp	Clipped	1.10±0.07	2.66±0.26	831.90±5.46	72.53±2.28	3.68±0.65	1.89±0.23	1.91±0.17	14.62±0.49
Lepidium subulatum	Leaves	Gyp	Unclipped	0.50±0.04	1.41±0.03	417.50±1.89	36.25±0.56	4.63±0.19	2.05±0.10	1.01±0.02	6.54±0.39
Lepidium subulatum	Stems	Calc	Clipped	4.30±0.23	2.56±0.14	906.00±4.37	34.22±1.81	1.64±0.16	1.14±0.13	1.71±0.10	12.45±0.65
Lepidium subulatum	Stems	Calc	Unclipped	2.41±0.21	1.23±0.07	449.80±2.84	17.37±0.68	1.40±0.23	1.10±0.16	0.84±0.04	6.05±0.57
Lepidium subulatum	Stems	Gyp	Clipped	3.42±0.22	2.96±0.20	911.75±5.30	27.49±1.35	1.70±0.28	0.94±0.08	2.01±0.14	13.05±0.34
Lepidium subulatum	Stems	Gyp	Unclipped	1.77±0.12	1.66±0.15	454.75±3.07	15.23±1.40	1.58±0.14	1.12±0.11	1.09±0.08	6.51±0.55
Linum suffruticosum	Coarse roots	Calc	Clipped	0.80±0.27	0.22±0.03	0.00±0.00	14.27±3.28	1.26±0.44	0.64±0.04	0.17±0.03	3.25±0.06
Linum suffruticosum	Coarse roots	Calc	Unclipped	0.52±0.08	0.27±0.05	0.00±0.00	13.64±1.68	1.76±0.28	0.67±0.06	0.22±0.03	2.41±0.18
Linum suffruticosum	Coarse roots	Gyp	Clipped	0.50±0.08	0.51±0.10	0.00±0.00	13.59±0.72	2.41±0.48	0.84±0.05	0.38±0.06	2.72±0.11
Linum suffruticosum	Coarse roots	Gyp	Unclipped	0.54±0.08	0.50±0.02	0.00±0.00	16.70±0.87	1.90±0.35	0.80±0.05	0.35±0.02	2.84±0.19
Linum suffruticosum	Fine roots	Calc	Clipped	3.97±0.99	2.35±0.14	428.00±3.39	28.67±1.24	1.59±0.27	4.52±0.67	1.42±0.10	11.31±0.66
Linum suffruticosum	Fine roots	Calc	Unclipped	7.42±0.51	2.73±0.34	419.40±5.16	35.38±2.09	1.67±0.24	4.23±0.33	1.69±0.21	10.04±0.77
Linum suffruticosum	Fine roots	Gyp	Clipped	4.69±0.53	1.70±0.20	428.00±3.73	25.33±1.61	0.61±0.10	5.61±0.48	1.04±0.11	10.84±0.47
Linum suffruticosum	Fine roots	Gyp	Unclipped	6.86±0.62	2.31±0.42	398.80±16.92	34.16±6.58	0.65±0.12	5.53±0.23	1.48±0.29	10.15±0.58
Linum suffruticosum	Leaves	Calc	Clipped	0.73±0.03	0.29±0.03	412.60±1.08	24.75±1.67	1.29±0.20	1.42±0.12	0.24±0.03	7.04±1.16
Linum suffruticosum	Leaves	Calc	Unclipped	0.82±0.11	0.24±0.02	409.80±4.62	25.48±1.94	1.09±0.13	1.29±0.18	0.21±0.01	6.52±1.18
Linum suffruticosum	Leaves	Gyp	Clipped	0.68±0.09	0.30±0.03	414.60±5.80	29.48±2.36	1.44±0.22	1.52±0.12	0.25±0.02	4.52±0.37
Linum suffruticosum	Leaves	Gyp	Unclipped	0.69±0.15	0.39±0.06	402.20±4.97	31.71±1.92	1.78±0.32	1.64±0.21	0.35±0.03	4.71±0.84
Linum suffruticosum	Stems	Calc	Clipped	2.26±0.27	0.54±0.07	472.00±5.90	14.85±2.64	1.37±0.33	1.04±0.04	0.46±0.05	4.44±0.46
Linum suffruticosum	Stems	Calc	Unclipped	2.49±0.18	0.63±0.04	468.00±5.94	15.12±0.95	1.47±0.27	1.12±0.11	0.53±0.02	4.47±0.24
Linum suffruticosum	Stems	Gyp	Clipped	1.88±0.22	0.65±0.07	472.20±2.96	16.10±1.08	1.45±0.16	1.22±0.14	0.50±0.05	3.03±0.08
Linum suffruticosum	Stems	Gyp	Unclipped	2.41±0.26	1.19±0.50	464.40±3.52	25.28±6.64	2.23±0.92	1.74±0.46	0.88±0.33	4.77±1.18
Matthiola fruticulosa	Coarse roots	Calc	Clipped	1.01±0.16	0.19±0.02	433.20±2.56	7.07±0.58	0.37±0.04	0.50±0.05	0.12±0.01	11.93±0.72
Matthiola fruticulosa	Coarse roots	Calc	Unclipped	1.27±0.21	0.15±0.02	444.25±2.95	5.78±0.49	0.39±0.15	0.40±0.07	0.10±0.01	8.94±0.74
Matthiola fruticulosa	Coarse roots	Gyp	Clipped	0.61±0.26	0.25±0.04	0.00±0.00	7.36±0.57	0.84±0.23	0.45±0.06	0.18±0.03	7.06±0.87
Matthiola fruticulosa	Coarse roots	Gyp	Unclipped	0.77±0.16	0.23±0.03	0.00±0.00	7.69±0.68	0.50±0.14	0.47±0.04	0.16±0.03	9.15±1.21
Matthiola fruticulosa	Fine roots	Calc	Clipped	1.34±0.17	1.15±0.11	409.00±4.69	23.55±2.07	0.87±0.12	2.88±0.35	0.74±0.08	13.92±0.53
Matthiola fruticulosa	Fine roots	Calc	Unclipped	1.83±0.48	1.33±0.21	410.00±2.02	24.42±2.28	0.86±0.16	2.50±0.31	0.80±0.13	12.81±0.80

Matthiola fruticulosa	Fine roots	Gyp	Clipped	1.12±0.10	0.70±0.08	412.80±1.74	21.21±1.29	0.39±0.08	1.64±0.11	0.42±0.05	7.91±0.54
Matthiola fruticulosa	Fine roots	Gyp	Unclipped	1.77±0.36	0.85±0.11	407.40±1.50	23.24±1.78	0.43±0.09	1.95±0.24	0.51±0.07	10.09±0.77
Matthiola fruticulosa	Leaves	Calc	Clipped	0.39±0.07	0.36±0.14	385.25±2.63	50.49±0.86	2.08±0.46	0.78±0.08	0.35±0.10	7.12±0.71
Matthiola fruticulosa	Leaves	Calc	Unclipped	0.45±0.08	0.25±0.05	397.25±2.75	43.66±2.80	1.65±0.45	0.78±0.05	0.26±0.04	9.35±1.19
Matthiola fruticulosa	Leaves	Gyp	Clipped	0.32±0.03	0.32±0.06	370.00±8.74	62.02±7.27	2.28±0.34	0.77±0.08	0.30±0.06	6.20±0.30
Matthiola fruticulosa	Leaves	Gyp	Unclipped	0.52±0.09	0.29±0.05	380.50±2.90	55.39±2.01	1.61±0.31	0.71±0.08	0.26±0.05	7.28±0.81
Matthiola fruticulosa	Stems	Calc	Clipped	2.30±0.25	0.54±0.08	458.40±3.70	10.08±1.35	1.06±0.17	0.84±0.03	0.47±0.07	5.60±0.09
Matthiola fruticulosa	Stems	Calc	Unclipped	2.69±0.31	0.53±0.06	460.20±3.35	10.04±1.12	0.97±0.18	0.80±0.01	0.47±0.05	5.52±0.48
Matthiola fruticulosa	Stems	Gyp	Clipped	2.32±0.58	0.43±0.03	467.00±2.79	8.71±0.72	0.84±0.06	0.51±0.05	0.33±0.02	3.84±0.26
Matthiola fruticulosa	Stems	Gyp	Unclipped	2.72±0.48	0.51±0.04	459.40±2.23	11.07±0.95	0.93±0.10	0.63±0.10	0.41±0.03	5.13±0.23
Ononis tridentata	Coarse roots	Calc	Clipped	3.87±1.61	0.20±0.03	454.40±5.73	17.72±1.23	0.15±0.03	0.55±0.08	0.12±0.02	5.26±0.68
Ononis tridentata	Coarse roots	Calc	Unclipped	4.70±1.41	0.17±0.02	460.40±3.23	16.61±1.43	0.13±0.02	0.55±0.04	0.10±0.01	5.23±1.06
Ononis tridentata	Coarse roots	Gyp	Clipped	3.44±0.54	0.28±0.04	452.40±2.56	17.78±0.57	0.13±0.02	0.73±0.08	0.18±0.02	4.00±0.32
Ononis tridentata	Coarse roots	Gyp	Unclipped	4.80±0.75	0.27±0.03	454.60±1.78	16.34±1.57	0.11±0.01	0.68±0.03	0.17±0.02	4.35±0.61
Ononis tridentata	Fine roots	Calc	Clipped	6.08±1.34	3.84±0.24	342.60±30.32	99.65±25.67	3.68±1.65	2.16±0.14	2.46±0.18	4.84±0.76
Ononis tridentata	Fine roots	Calc	Unclipped	7.74±1.08	4.51±0.41	308.20±32.30	121.20±25.99	1.80±0.34	1.93±0.22	2.84±0.29	4.55±0.69
Ononis tridentata	Fine roots	Gyp	Clipped	6.70±0.77	3.16±0.40	316.80±14.68	98.42±5.64	0.85±0.06	2.77±0.46	1.80±0.22	3.34±0.20
Ononis tridentata	Fine roots	Gyp	Unclipped	7.41±1.50	2.56±0.37	326.60±17.57	90.49±11.12	0.66±0.08	2.17±0.08	1.49±0.20	3.57±0.40
Ononis tridentata	Leaves	Calc	Clipped	2.71±0.75	0.22±0.03	361.20±9.38	60.47±3.50	1.19±0.33	0.77±0.09	0.23±0.03	6.70±1.31
Ononis tridentata	Leaves	Calc	Unclipped	3.52±0.41	0.25±0.03	350.00±9.28	61.98±2.05	0.64±0.16	0.72±0.08	0.23±0.02	7.03±1.63
Ononis tridentata	Leaves	Gyp	Clipped	3.74±0.41	0.16±0.01	311.20±4.53	55.74±2.67	0.31±0.04	1.21±0.14	0.16±0.01	4.79±0.82
Ononis tridentata	Leaves	Gyp	Unclipped	3.72±0.35	0.18±0.02	326.00±4.07	50.27±1.74	0.30±0.03	1.18±0.08	0.18±0.02	5.43±1.28
Ononis tridentata	Stems	Calc	Clipped	9.05±3.00	0.99±0.15	453.60±5.42	29.02±4.54	2.14±0.66	1.05±0.07	0.85±0.15	4.39±1.12
Ononis tridentata	Stems	Calc	Unclipped	11.14±3.18	0.69±0.08	456.60±4.19	23.45±3.05	1.53±0.36	0.82±0.20	0.58±0.07	3.60±0.25
Ononis tridentata	Stems	Gyp	Clipped	7.35±0.69	0.57±0.07	464.40±6.47	15.00±1.41	1.32±0.24	0.92±0.11	0.52±0.06	3.21±0.32
Ononis tridentata	Stems	Gyp	Unclipped	8.44±1.25	0.82±0.10	453.40±3.28	15.95±1.13	1.61±0.30	1.30±0.11	0.73±0.09	2.70±0.38
Rosmarinus officinalis	Coarse roots	Calc	Clipped	1.90±0.27	0.29±0.03	485.40±1.60	8.15±0.51	0.82±0.09	0.80±0.05	0.20±0.03	7.08±0.34
Rosmarinus officinalis	Coarse roots	Calc	Unclipped	2.20±0.83	0.39±0.03	486.20±2.97	10.66±0.97	1.14±0.16	1.04±0.11	0.26±0.02	7.74±0.42
Rosmarinus officinalis	Coarse roots	Gyp	Clipped	1.05±0.18	0.35±0.05	486.60±2.16	6.62±0.67	0.79±0.20	1.19±0.13	0.24±0.04	8.33±0.48
Rosmarinus officinalis	Coarse roots	Gyp	Unclipped	1.43±0.20	0.31±0.05	486.60±1.94	7.41±1.14	0.55±0.07	1.13±0.04	0.21±0.03	6.85±0.31
Rosmarinus officinalis	Fine roots	Calc	Clipped	15.07±1.47	3.52±0.21	409.20±3.18	43.79±1.70	1.66±0.12	3.04±0.11	2.19±0.08	18.76±0.51
Rosmarinus officinalis	Fine roots	Calc	Unclipped	18.92±2.10	3.35±0.27	417.80±3.92	41.03±3.65	1.79±0.29	3.07±0.40	2.08±0.16	20.25±0.81
Rosmarinus officinalis	Fine roots	Gyp	Clipped	6.56±0.53	2.58±0.26	398.60±9.93	42.50±3.57	1.30±0.21	5.30±0.54	1.56±0.14	13.07±0.16
Rosmarinus officinalis	Fine roots	Gyp	Unclipped	9.89±0.62	2.35±0.16	406.40±6.30	41.14±1.60	0.96±0.10	5.14±0.25	1.40±0.09	12.28±0.54
Rosmarinus officinalis	Leaves	Calc	Clipped	6.52±0.59	0.30±0.04	521.80±5.35	11.25±0.50	0.19±0.04	0.70±0.07	0.25±0.04	12.12±0.95
Rosmarinus officinalis	Leaves	Calc	Unclipped	6.75±1.12	0.30±0.01	524.00±8.05	12.02±0.77	0.20±0.01	0.72±0.05	0.21±0.02	12.08±0.65
Rosmarinus officinalis	Leaves	Gyp	Clipped	3.63±0.81	0.31±0.03	520.00±4.24	11.76±1.41	0.17±0.03	0.74±0.07	0.21±0.03	11.12±0.87
Rosmarinus officinalis	Leaves	Gyp	Unclipped	5.74±0.61	0.36±0.01	517.00±4.09	14.56±0.65	0.17±0.02	0.81±0.05	0.24±0.01	8.18±0.87
Rosmarinus officinalis	Stems	Calc	Clipped	3.61±0.48	0.57±0.07	479.00±0.71	7.78±2.28	1.57±0.15	1.12±0.07	0.48±0.03	7.71±0.63
Rosmarinus officinalis	Stems	Calc	Unclipped	4.51±1.07	0.74±0.18	475.60±2.98	7.87±1.59	1.59±0.31	1.44±0.15	0.58±0.08	8.36±0.53
Rosmarinus officinalis	Stems	Gyp	Clipped	2.93±0.48	0.79±0.08	478.60±0.81	8.13±0.42	1.19±0.11	0.94±0.07	0.52±0.08	5.31±0.62
Rosmarinus officinalis	Stems	Gyp	Unclipped	2.93±0.27	1.15±0.05	476.80±2.03	11.59±1.02	1.61±0.07	1.09±0.06	0.69±0.02	5.08±0.42

Table D.5 (Continued)

Species	Organ	Soil	Clipping	Mg (mg/g)	Mn (µg/g)	N (mg/g)	Na (mg/g)	P (mg/g)	S (mg/g)	Si (mg/g)	Zn (µg/g)
Boleum asperum	Coarse roots	Calc	Clipped	1.00±0.14	3.72±0.53	15.21±1.37	0.10±0.00	2.18±0.70	3.50±0.33	0.64±0.05	3.93±1.15
Boleum asperum	Coarse roots	Calc	Unclipped	0.96±0.09	3.16±0.30	13.12±1.98	0.16±0.08	2.08±0.84	2.99±0.44	0.64±0.09	3.41±0.82
Boleum asperum	Coarse roots	Gyp	Clipped	1.24±0.07	4.63±0.33	0.00±0.00	0.11±0.02	1.75±0.23	4.53±0.66	1.04±0.05	4.72±0.40
Boleum asperum	Coarse roots	Gyp	Unclipped	1.22±0.06	3.84±0.19	0.00±0.00	0.11±0.00	1.64±0.19	4.11±0.25	0.98±0.12	3.07±0.25
Boleum asperum	Fine roots	Calc	Clipped	1.78±0.27	2.72±0.47	20.50±0.96	0.20±0.03	2.34±0.68	7.41±0.29	1.18±0.16	4.86±0.95
Boleum asperum	Fine roots	Calc	Unclipped	1.76±0.09	2.38±0.09	19.77±0.99	0.23±0.04	2.89±0.66	7.00±0.43	1.14±0.10	4.82±0.55
Boleum asperum	Fine roots	Gyp	Clipped	1.93±0.18	2.87±0.26	27.00±2.00	0.17±0.03	2.51±0.48	11.74±0.85	1.04±0.09	5.22±0.60
Boleum asperum	Fine roots	Gyp	Unclipped	1.91±0.14	2.67±0.12	23.57±0.55	0.19±0.04	2.08±0.43	12.66±0.46	1.19±0.10	5.12±1.04
Boleum asperum	Leaves	Calc	Clipped	5.90±0.26	3.13±0.77	36.57±3.43	0.07±0.00	3.59±1.76	7.64±0.59	0.84±0.06	4.68±0.85
Boleum asperum	Leaves	Calc	Unclipped	5.52±0.48	3.39±0.30	34.38±2.08	0.07±0.01	3.83±2.00	8.01±1.22	0.91±0.07	7.73±2.27
Boleum asperum	Leaves	Gyp	Clipped	4.96±0.25	4.46±0.63	38.75±1.42	0.07±0.00	2.38±0.40	12.31±0.53	1.04±0.11	5.76±0.57
Boleum asperum	Leaves	Gyp	Unclipped	4.07±0.12	3.27±0.74	34.37±2.74	0.07±0.00	2.03±0.17	11.38±1.40	0.93±0.06	6.85±0.73
Boleum asperum	Stems	Calc	Clipped	1.20±0.15	2.71±0.20	12.91±1.39	0.07±0.00	1.27±0.35	3.62±0.40	1.45±0.03	2.89±0.16
Boleum asperum	Stems	Calc	Unclipped	1.23±0.08	2.61±0.19	13.64±0.69	0.08±0.01	1.90±0.56	3.79±0.27	1.52±0.04	2.89±0.37
Boleum asperum	Stems	Gyp	Clipped	1.68±0.16	3.48±0.45	16.95±1.26	0.09±0.00	1.86±0.43	5.26±0.47	1.63±0.03	3.59±0.43
Boleum asperum	Stems	Gyp	Unclipped	1.29±0.02	2.57±0.19	15.73±0.71	0.08±0.00	1.36±0.35	5.04±0.61	1.63±0.03	2.96±0.18
Gypsophila struthium	Coarse roots	Calc	Clipped	0.84±0.07	1.52±0.16	4.38±0.63	0.26±0.03	2.48±0.52	1.69±0.10	0.40±0.06	3.68±0.57
Gypsophila struthium	Coarse roots	Calc	Unclipped	0.97±0.04	1.63±0.11	4.77±0.70	0.27±0.08	3.39±0.56	1.79±0.10	0.49±0.06	4.35±0.58
Gypsophila struthium	Coarse roots	Gyp	Clipped	1.04±0.07	1.13±0.11	4.31±0.77	0.19±0.01	1.13±0.32	2.02±0.09	0.48±0.04	1.63±0.25
Gypsophila struthium	Coarse roots	Gyp	Unclipped	0.98±0.07	1.33±0.22	3.65±0.29	0.18±0.02	0.84±0.12	2.34±0.27	0.52±0.06	1.44±0.10
Gypsophila struthium	Fine roots	Calc	Clipped	2.96±0.15	6.64±0.86	6.64±0.60	0.51±0.02	2.13±0.57	2.12±0.09	1.47±0.02	4.06±0.82
Gypsophila struthium	Fine roots	Calc	Unclipped	3.10±0.15	6.49±0.69	7.48±0.23	0.55±0.08	3.07±0.55	2.06±0.07	1.50±0.02	5.31±0.78
Gypsophila struthium	Fine roots	Gyp	Clipped	4.00±0.46	2.98±0.37	7.30±0.37	0.39±0.03	0.87±0.10	16.27±2.89	1.42±0.03	2.06±0.17
Gypsophila struthium	Fine roots	Gyp	Unclipped	3.45±0.15	3.01±0.33	7.00±0.24	0.40±0.04	0.82±0.09	16.17±1.13	1.46±0.03	2.35±0.19
Gypsophila struthium	Leaves	Calc	Clipped	14.58±0.74	7.03±0.50	11.40±0.36	0.08±0.01	4.01±1.30	15.89±2.68	0.71±0.02	1.76±0.29
Gypsophila struthium	Leaves	Calc	Unclipped	15.10±1.07	7.03±0.75	11.73±0.61	0.06±0.01	5.85±1.75	15.51±1.25	0.66±0.04	2.57±0.61
Gypsophila struthium	Leaves	Gyp	Clipped	14.72±0.29	6.42±0.23	9.74±0.51	0.07±0.01	1.17±0.36	18.89±1.54	0.89±0.08	1.87±0.19
Gypsophila struthium	Leaves	Gyp	Unclipped	13.05±1.28	5.58±1.06	10.10±0.80	0.06±0.00	0.96±0.12	24.39±1.63	0.77±0.04	1.73±0.22
Gypsophila struthium	Stems	Calc	Clipped	2.01±0.21	3.47±0.42	4.98±0.21	0.10±0.01	2.49±0.72	3.32±0.27	1.41±0.11	2.80±0.65
Gypsophila struthium	Stems	Calc	Unclipped	1.97±0.18	2.66±0.10	4.83±0.22	0.08±0.02	3.13±0.80	3.04±0.09	1.30±0.08	3.64±0.82
Gypsophila struthium	Stems	Gyp	Clipped	3.27±0.24	3.20±0.26	5.07±0.44	0.14±0.02	0.86±0.23	3.73±0.27	1.40±0.09	1.42±0.19
Gypsophila struthium	Stems	Gyp	Unclipped	2.37±0.18	2.40±0.20	4.88±0.23	0.11±0.01	0.57±0.08	3.00±0.56	1.28±0.07	1.13±0.10
Helianthemum squamatum	Coarse roots	Calc	Clipped	2.00±0.10	1.87±0.21		0.15±0.02	1.38±0.10	5.23±0.35	0.79±0.07	3.17±0.93
Helianthemum squamatum	Coarse roots	Calc	Unclipped	1.81±0.13	1.72±0.22		0.14±0.01	1.31±0.09	5.04±0.11	0.70±0.16	2.90±0.53
Helianthemum squamatum	Coarse roots	Gyp	Clipped	2.36±0.13	2.02±0.15		0.26±0.02	1.12±0.03	6.53±0.58	1.11±0.10	3.88±0.41
Helianthemum squamatum	Coarse roots	Gyp	Unclipped	2.43±0.10	2.29±0.18		0.23±0.02	0.82±0.04	7.91±1.47	1.27±0.03	3.97±0.19
Helianthemum squamatum	Fine roots	Calc	Clipped	4.17±0.08	6.05±0.89	7.53±0.17	0.35±0.04	1.42±0.03	5.80±0.42	1.42±0.03	9.07±2.27
Helianthemum squamatum	Fine roots	Calc	Unclipped	4.04±0.37	4.78±0.73	7.55±0.70	0.32±0.04	1.48±0.68	5.83±0.11	1.41±0.01	4.86±1.16
Helianthemum squamatum	Fine roots	Gyp	Clipped	5.55±0.20	3.94±0.31	6.78±0.47	0.33±0.02	0.75±0.19	23.43±2.11	1.42±0.00	6.05±0.78
Helianthemum squamatum	Fine roots	Gyp	Unclipped	6.03±0.93	4.28±0.62	6.62±0.82	0.31±0.03	0.47±0.05	23.08±2.05	1.41±0.02	4.97±0.38

Helianthemum squamatum	Leaves	Calc	Clipped	4.81±0.16	7.66±0.55	9.45±0.81	0.08±0.01	2.55±0.57	16.57±2.20	1.06±0.03	0.82±0.09
Helianthemum squamatum	Leaves	Calc	Unclipped	4.54±0.20	7.49±0.54	9.72±0.22	0.08±0.02	1.63±0.40	15.98±2.15	1.01±0.04	0.83±0.16
Helianthemum squamatum	Leaves	Gyp	Clipped	4.23±0.38	5.84±0.57	9.24±0.27	0.08±0.01	1.10±0.26	19.35±1.80	1.02±0.03	0.97±0.11
Helianthemum squamatum	Leaves	Gyp	Unclipped	3.89±0.10	7.25±0.64	9.48±0.30	0.08±0.01	0.67±0.03	17.96±1.66	1.08±0.03	0.75±0.08
Helianthemum squamatum	Roots	Calc	Clipped			8.67±0.70					
Helianthemum squamatum	Roots	Calc	Unclipped			7.96±0.43					
Helianthemum squamatum	Roots	Gyp	Clipped			8.38±0.50					
Helianthemum squamatum	Roots	Gyp	Unclipped			6.95±0.64					
Helianthemum squamatum	Stems	Calc	Clipped	2.16±0.07	4.11±0.55	5.08±0.22	0.08±0.01	1.05±0.05	5.00±0.24	1.26±0.06	3.46±0.84
Helianthemum squamatum	Stems	Calc	Unclipped	2.31±0.19	4.10±0.39	5.61±0.16	0.07±0.00	0.95±0.11	5.32±0.18	1.36±0.03	3.99±0.76
Helianthemum squamatum	Stems	Gyp	Clipped	2.44±0.12	2.88±0.38	5.21±0.24	0.10±0.01	0.81±0.06	5.46±0.32	1.40±0.06	10.68±6.59
Helianthemum squamatum	Stems	Gyp	Unclipped	2.51±0.14	3.41±0.54	4.90±0.28	0.10±0.00	0.60±0.05	5.45±0.50	1.39±0.09	5.17±0.68
Helianthemum syriacum	Coarse roots	Calc	Clipped	0.78±0.04	1.71±0.15		0.14±0.01	1.16±0.06	1.11±0.07	0.55±0.06	2.12±0.25
Helianthemum syriacum	Coarse roots	Calc	Unclipped	0.72±0.02	1.76±0.29		0.16±0.02	1.06±0.09	1.05±0.06	0.50±0.07	1.45±0.18
Helianthemum syriacum	Coarse roots	Gyp	Clipped	0.92±0.05	1.67±0.01		0.19±0.03	0.70±0.13	1.53±0.14	0.77±0.01	1.49±0.43
Helianthemum syriacum	Coarse roots	Gyp	Unclipped	0.87±0.06	1.42±0.22		0.18±0.01	0.82±0.04	1.54±0.05	0.68±0.08	1.62±0.61
Helianthemum syriacum	Fine roots	Calc	Clipped	4.23±0.16	7.00±0.40	7.79±0.49	0.26±0.01	1.64±0.18	2.44±0.11	1.40±0.04	7.11±0.63
Helianthemum syriacum	Fine roots	Calc	Unclipped	4.05±0.27	7.46±0.62	7.37±0.30	0.30±0.02	1.60±0.13	2.59±0.13	1.39±0.00	6.00±0.77
Helianthemum syriacum	Fine roots	Gyp	Clipped	5.95±0.54	5.67±0.45	6.03±0.25	0.26±0.00	0.54±0.06	18.43±2.32	1.41±0.01	3.52±0.43
Helianthemum syriacum	Fine roots	Gyp	Unclipped	7.75±2.03	5.18±0.48	6.35±0.85	0.28±0.04	0.56±0.05	17.06±1.63	1.41±0.03	3.76±0.19
Helianthemum syriacum	Leaves	Calc	Clipped	2.90±0.11	11.22±1.37	11.47±0.86	0.10±0.01	2.62±0.34	7.27±0.53	1.16±0.02	1.83±0.13
Helianthemum syriacum	Leaves	Calc	Unclipped	2.73±0.21	10.34±1.10	10.63±0.30	0.10±0.01	1.94±0.24	6.51±0.25	1.19±0.04	1.76±0.14
Helianthemum syriacum	Leaves	Gyp	Clipped	2.64±0.17	9.84±0.90	9.02±0.54	0.08±0.00	0.70±0.04	9.81±1.07	1.19±0.03	1.61±0.07
Helianthemum syriacum	Leaves	Gyp	Unclipped	3.15±0.09	10.52±1.20	9.00±0.87	0.08±0.00	0.72±0.08	10.82±0.46	1.16±0.02	1.62±0.14
Helianthemum syriacum	Roots	Calc	Clipped			9.02±0.00					
Helianthemum syriacum	Roots	Calc	Unclipped			6.71±0.44					
Helianthemum syriacum	Roots	Gyp	Clipped			6.38±0.50					
Helianthemum syriacum	Roots	Gyp	Unclipped			5.62±0.19					
Helianthemum syriacum	Stems	Calc	Clipped	1.11±0.09	3.73±0.37	5.31±0.24	0.12±0.00	0.88±0.08	1.13±0.09	1.05±0.03	3.80±0.20
Helianthemum syriacum	Stems	Calc	Unclipped	1.12±0.08	3.92±0.31	5.27±0.54	0.11±0.01	0.80±0.06	1.16±0.11	1.13±0.05	3.29±0.54
Helianthemum syriacum	Stems	Gyp	Clipped	1.67±0.09	3.50±0.19	5.00±0.26	0.13±0.01	0.44±0.03	1.30±0.10	1.10±0.02	3.66±0.19
Helianthemum syriacum	Stems	Gyp	Unclipped	1.66±0.12	3.41±0.44	4.69±0.26	0.13±0.02	0.54±0.06	1.40±0.15	1.11±0.03	3.24±0.44
Herniaria fruticosa	Coarse roots	Calc	Clipped	3.36±0.37	3.57±0.26	16.80±2.03	0.15±0.02	4.13±0.57	2.26±0.08	0.54±0.06	5.89±0.95
Herniaria fruticosa	Coarse roots	Calc	Unclipped	4.11±0.15	4.86±0.66	17.12±1.96	0.17±0.02	2.93±0.49	2.21±0.23	0.50±0.03	4.41±0.69
Herniaria fruticosa	Coarse roots	Gyp	Clipped	3.78±0.15	4.24±0.24	0.00±0.00	0.15±0.01	1.65±0.33	3.06±0.24	0.90±0.08	3.44±0.23
Herniaria fruticosa	Coarse roots	Gyp	Unclipped	4.02±0.29	4.72±0.26	18.33±2.25	0.13±0.01	2.52±0.38	3.14±0.21	0.85±0.05	4.30±0.75
Herniaria fruticosa	Fine roots	Calc	Clipped	5.36±0.24	5.11±0.35	14.29±1.06	0.38±0.03	2.31±0.64	2.02±0.05	1.43±0.04	3.21±0.65
Herniaria fruticosa	Fine roots	Calc	Unclipped	5.48±0.30	5.70±0.39	14.21±1.50	0.40±0.01	2.28±0.52	2.10±0.08	1.53±0.03	3.03±0.51
Herniaria fruticosa	Fine roots	Gyp	Clipped	6.25±0.24	4.43±0.23	11.54±0.69	0.32±0.03	0.83±0.14	10.13±2.08	1.43±0.02	3.22±0.55
Herniaria fruticosa	Fine roots	Gyp	Unclipped	6.64±0.21	4.21±0.17	11.68±0.79	0.30±0.01	1.18±0.23	10.02±2.03	1.45±0.06	2.42±0.22
Herniaria fruticosa	Leaves	Calc	Clipped	4.98±0.37	5.21±0.87	12.95±0.85	0.15±0.01	1.37±0.53	7.65±0.54	1.30±0.09	3.93±0.48
Herniaria fruticosa	Leaves	Calc	Unclipped	6.09±0.75	6.10±0.65	12.21±0.65	0.14±0.01	2.17±1.33	6.01±0.40	1.35±0.04	5.46±0.88
Herniaria fruticosa	Leaves	Gyp	Clipped	6.57±0.73	5.15±0.77	10.90±0.34	0.17±0.01	0.54±0.07	6.65±0.59	1.39±0.06	3.86±0.58

Herniaria fruticosa	Leaves	Gyp	Unclipped	7.02±0.45	6.48±0.48	12.83±0.55	0.17±0.01	0.73±0.12	7.34±0.75	1.42±0.03	5.38±1.00
Herniaria fruticosa	Stems	Calc	Clipped	3.74±0.16	3.70±0.30	10.03±0.68	0.17±0.01	1.42±0.52	2.73±0.15	1.15±0.03	2.38±0.17
Herniaria fruticosa	Stems	Calc	Unclipped	4.96±0.50	3.99±0.36	9.12±0.52	0.18±0.01	1.55±0.57	2.52±0.17	1.14±0.05	2.76±0.28
Herniaria fruticosa	Stems	Gyp	Clipped	5.54±0.29	3.97±0.32	8.62±0.48	0.21±0.01	0.59±0.13	3.49±0.22	1.09±0.05	2.58±0.24
Herniaria fruticosa	Stems	Gyp	Unclipped	6.19±0.29	4.49±0.36	10.47±0.89	0.19±0.01	0.96±0.28	3.61±0.43	1.15±0.04	3.55±0.57
Lepidium subulatum	Coarse roots	Calc	Clipped	2.42±0.14	1.17±0.19	26.83±1.70	0.14±0.01	7.04±0.49	10.16±0.38	0.66±0.03	3.74±0.78
Lepidium subulatum	Coarse roots	Calc	Unclipped	1.18±0.17	0.84±0.04	13.68±0.78	0.07±0.00	3.51±0.69	5.48±0.39	0.30±0.04	4.19±0.49
Lepidium subulatum	Coarse roots	Gyp	Clipped	2.20±0.07	0.94±0.04	28.68±1.16	0.14±0.01	5.72±0.14	12.49±0.58	0.80±0.04	2.57±0.14
Lepidium subulatum	Coarse roots	Gyp	Unclipped	1.35±0.15	0.98±0.11	15.97±1.07	0.07±0.01	3.45±0.30	7.18±0.53	0.46±0.05	3.33±0.67
Lepidium subulatum	Fine roots	Calc	Clipped	5.62±0.31	3.17±0.80	45.90±2.44	0.66±0.03	8.38±0.84	24.65±0.74	2.36±0.08	5.73±1.75
Lepidium subulatum	Fine roots	Calc	Unclipped	2.54±0.25	1.91±0.18	23.53±1.43	0.29±0.03	3.90±1.17	13.07±0.51	1.06±0.04	5.14±1.54
Lepidium subulatum	Fine roots	Gyp	Clipped	4.70±0.12	1.74±0.06	40.67±1.48	0.59±0.03	5.78±0.42	33.57±0.85	2.48±0.02	2.79±0.32
Lepidium subulatum	Fine roots	Gyp	Unclipped	2.38±0.18	1.56±0.11	18.40±0.63	0.37±0.13	2.90±0.16	16.08±0.20	1.18±0.03	4.12±0.91
Lepidium subulatum	Leaves	Calc	Clipped	5.63±0.18	8.97±0.53	66.17±2.38	0.27±0.01	4.22±0.16	31.05±1.49	2.79±0.02	7.63±1.91
Lepidium subulatum	Leaves	Calc	Unclipped	3.10±0.15	8.24±0.41	32.11±1.25	0.14±0.01	2.02±0.15	15.22±1.82	1.37±0.08	7.08±1.09
Lepidium subulatum	Leaves	Gyp	Clipped	5.69±0.35	6.96±0.39	65.50±2.82	0.31±0.02	3.71±0.16	37.25±1.97	2.90±0.04	6.00±0.49
Lepidium subulatum	Leaves	Gyp	Unclipped	2.92±0.12	7.91±0.52	32.76±1.58	0.15±0.01	1.85±0.06	17.69±0.84	1.47±0.06	6.49±0.79
Lepidium subulatum	Stems	Calc	Clipped	3.44±0.32	4.38±0.28	29.76±0.62	0.31±0.01	4.79±0.74	6.82±0.37	2.64±0.17	6.31±1.96
Lepidium subulatum	Stems	Calc	Unclipped	1.74±0.19	4.15±0.59	14.44±0.74	0.14±0.01	2.48±0.47	3.36±0.34	1.24±0.04	7.44±2.92
Lepidium subulatum	Stems	Gyp	Clipped	4.17±0.25	3.30±0.29	28.57±1.00	0.35±0.05	3.91±0.30	9.38±0.44	2.78±0.05	3.31±0.25
Lepidium subulatum	Stems	Gyp	Unclipped	2.25±0.28	4.09±0.33	14.85±0.52	0.16±0.01	2.05±0.09	5.02±0.33	1.43±0.09	4.60±0.51
Linum suffruticosum	Coarse roots	Calc	Clipped	1.37±0.14	0.89±0.08	0.00±0.00	0.29±0.07	1.45±0.13	0.98±0.09	0.50±0.06	3.15±0.40
Linum suffruticosum	Coarse roots	Calc	Unclipped	1.37±0.15	1.25±0.15	0.00±0.00	0.17±0.02	1.33±0.09	0.96±0.06	0.58±0.09	2.56±0.42
Linum suffruticosum	Coarse roots	Gyp	Clipped	1.37±0.14	1.86±0.62	0.00±0.00	0.16±0.02	0.66±0.06	2.05±0.20	1.00±0.14	2.17±0.47
Linum suffruticosum	Coarse roots	Gyp	Unclipped	1.53±0.04	1.56±0.23	0.00±0.00	0.19±0.01	0.73±0.09	2.23±0.14	1.03±0.04	2.15±0.28
Linum suffruticosum	Fine roots	Calc	Clipped	5.47±0.50	5.33±0.47	13.32±0.55	0.23±0.03	6.35±0.75	2.65±0.11	1.43±0.04	7.72±0.92
Linum suffruticosum	Fine roots	Calc	Unclipped	5.88±0.37	6.83±0.76	11.67±0.64	0.21±0.01	5.84±0.49	2.41±0.13	1.42±0.01	7.38±1.51
Linum suffruticosum	Fine roots	Gyp	Clipped	4.62±0.40	3.88±0.35	11.22±0.45	0.20±0.02	1.44±0.16	12.26±0.70	1.46±0.02	3.45±0.31
Linum suffruticosum	Fine roots	Gyp	Unclipped	6.15±0.65	4.32±0.47	9.78±0.48	0.21±0.01	1.39±0.19	17.49±3.50	1.43±0.02	2.55±0.25
Linum suffruticosum	Leaves	Calc	Clipped	3.88±0.33	7.85±1.06	16.69±1.03	0.12±0.01	2.40±0.76	2.11±0.26	1.21±0.01	6.41±1.21
Linum suffruticosum	Leaves	Calc	Unclipped	4.01±0.46	6.71±0.86	16.59±1.16	0.12±0.01	1.20±0.21	2.21±0.18	1.25±0.02	5.92±0.77
Linum suffruticosum	Leaves	Gyp	Clipped	3.15±0.24	6.96±1.26	16.55±0.84	0.11±0.01	1.04±0.14	3.65±0.33	1.19±0.02	5.11±0.80
Linum suffruticosum	Leaves	Gyp	Unclipped	3.93±0.17	7.37±0.80	16.28±1.42	0.10±0.00	0.90±0.11	3.47±0.42	1.20±0.02	6.82±0.98
Linum suffruticosum	Stems	Calc	Clipped	1.78±0.15	2.56±0.30	9.72±0.73	0.17±0.03	1.36±0.06	1.08±0.09	1.12±0.05	5.05±0.79
Linum suffruticosum	Stems	Calc	Unclipped	2.33±0.11	2.82±0.20	10.52±0.58	0.13±0.01	1.17±0.12	1.09±0.06	1.18±0.07	4.22±0.63
Linum suffruticosum	Stems	Gyp	Clipped	1.83±0.27	2.85±0.10	10.45±0.29	0.12±0.01	0.74±0.09	1.53±0.21	1.26±0.05	4.51±0.70
Linum suffruticosum	Stems	Gyp	Unclipped	3.18±1.18	4.37±1.88	9.68±0.64	0.14±0.03	0.98±0.21	1.89±0.44	1.20±0.04	5.66±0.81
Matthiola fruticulosa	Coarse roots	Calc	Clipped	1.28±0.04	0.62±0.05	8.18±0.85	0.45±0.25	4.30±1.03	5.26±0.49	0.41±0.04	1.57±0.15
Matthiola fruticulosa	Coarse roots	Calc	Unclipped	0.98±0.05	0.56±0.05	8.14±1.29	0.22±0.06	3.28±0.23	4.90±0.54	0.32±0.05	1.23±0.23
Matthiola fruticulosa	Coarse roots	Gyp	Clipped	0.88±0.14	0.56±0.08	0.00±0.00	0.22±0.05	1.01±0.16	5.97±0.67	0.56±0.08	2.57±0.55
Matthiola fruticulosa	Coarse roots	Gyp	Unclipped	0.84±0.10	0.57±0.07	0.00±0.00	0.15±0.01	1.18±0.17	6.33±0.69	0.54±0.06	1.60±0.23
Matthiola fruticulosa	Fine roots	Calc	Clipped	2.16±0.09	2.50±0.12	13.22±1.32	0.68±0.25	5.73±1.20	8.97±0.64	1.43±0.02	2.98±0.31
Matthiola fruticulosa	Fine roots	Calc	Unclipped	2.12±0.14	2.91±0.29	14.96±0.69	0.73±0.22	5.39±0.44	9.51±0.33	1.44±0.03	3.27±0.31

Matthiola fruticulosa	Fine roots	Gyp	Clipped	1.71±0.09	1.44±0.13	11.32±0.34	0.64±0.21	1.01±0.13	12.99±0.43	1.19±0.08	2.17±0.21
Matthiola fruticulosa	Fine roots	Gyp	Unclipped	1.91±0.16	1.66±0.20	12.38±1.41	0.34±0.08	1.47±0.30	14.39±1.07	1.30±0.08	2.32±0.17
Matthiola fruticulosa	Leaves	Calc	Clipped	6.85±0.62	4.93±0.62	25.24±1.96	0.45±0.32	3.65±0.31	10.78±0.41	1.08±0.11	4.34±0.35
Matthiola fruticulosa	Leaves	Calc	Unclipped	5.66±0.32	3.80±0.47	26.10±1.39	0.10±0.01	2.78±0.15	12.42±1.08	0.93±0.09	3.88±0.46
Matthiola fruticulosa	Leaves	Gyp	Clipped	3.28±0.08	3.60±0.49	21.16±0.90	0.09±0.00	1.37±0.16	16.08±2.70	1.16±0.03	3.16±0.25
Matthiola fruticulosa	Leaves	Gyp	Unclipped	3.32±0.20	3.65±0.35	21.19±1.02	0.08±0.01	1.84±0.26	13.64±0.76	1.07±0.10	2.96±0.23
Matthiola fruticulosa	Stems	Calc	Clipped	1.04±0.06	1.74±0.23	8.39±0.60	0.13±0.04	2.84±0.68	2.85±0.41	1.18±0.09	2.41±0.25
Matthiola fruticulosa	Stems	Calc	Unclipped	1.07±0.10	1.71±0.16	8.89±0.61	0.14±0.04	2.50±0.15	2.63±0.23	1.23±0.06	2.20±0.15
Matthiola fruticulosa	Stems	Gyp	Clipped	0.80±0.09	1.15±0.06	7.87±0.14	0.10±0.01	0.45±0.05	2.40±0.27	1.04±0.09	1.56±0.27
Matthiola fruticulosa	Stems	Gyp	Unclipped	0.93±0.05	1.42±0.10	8.03±0.20	0.09±0.01	0.72±0.08	2.54±0.18	1.12±0.10	1.57±0.14
Ononis tridentata	Coarse roots	Calc	Clipped	2.61±0.30	0.45±0.05	11.02±1.20	0.38±0.06	0.67±0.26	2.49±0.05	0.46±0.06	1.36±0.12
Ononis tridentata	Coarse roots	Calc	Unclipped	2.11±0.17	0.39±0.02	12.38±1.20	0.31±0.03	0.55±0.20	2.43±0.12	0.41±0.03	1.13±0.16
Ononis tridentata	Coarse roots	Gyp	Clipped	2.53±0.23	0.49±0.05	13.47±0.45	0.26±0.04	0.43±0.14	3.83±0.30	0.64±0.07	2.01±0.60
Ononis tridentata	Coarse roots	Gyp	Unclipped	2.75±0.21	0.51±0.04	12.47±0.54	0.29±0.05	0.41±0.04	3.85±0.18	0.64±0.06	2.25±0.38
Ononis tridentata	Fine roots	Calc	Clipped	4.24±0.21	5.27±0.31	15.70±2.63	0.35±0.04	0.85±0.33	4.25±0.22	1.39±0.02	3.09±0.44
Ononis tridentata	Fine roots	Calc	Unclipped	4.00±0.23	5.88±0.47	14.43±2.76	0.51±0.09	0.70±0.23	4.61±0.13	1.39±0.02	2.65±0.36
Ononis tridentata	Fine roots	Gyp	Clipped	7.47±0.42	3.91±0.39	13.27±1.08	0.39±0.06	0.39±0.08	27.06±3.52	1.38±0.02	2.41±0.58
Ononis tridentata	Fine roots	Gyp	Unclipped	6.71±0.60	3.47±0.46	12.46±1.23	0.41±0.04	0.43±0.03	26.41±2.78	1.40±0.03	1.47±0.19
Ononis tridentata	Leaves	Calc	Clipped	15.02±0.93	5.17±0.40	18.17±2.39	0.08±0.00	0.93±0.32	21.19±2.90	0.97±0.06	3.17±0.64
Ononis tridentata	Leaves	Calc	Unclipped	14.94±0.67	5.24±0.36	17.18±1.31	0.09±0.00	0.64±0.10	27.45±4.39	1.01±0.04	2.56±0.68
Ononis tridentata	Leaves	Gyp	Clipped	21.26±1.32	4.17±0.51	15.59±0.85	0.08±0.01	0.50±0.05	61.19±3.07	0.88±0.05	4.57±0.46
Ononis tridentata	Leaves	Gyp	Unclipped	19.75±1.50	4.07±0.24	17.94±0.68	0.08±0.00	0.62±0.04	56.47±3.57	0.92±0.07	3.75±0.50
Ononis tridentata	Stems	Calc	Clipped	4.43±0.87	2.48±0.41	8.66±0.30	0.13±0.02	0.56±0.29	5.60±0.94	1.58±0.16	2.71±0.62
Ononis tridentata	Stems	Calc	Unclipped	3.16±0.32	1.83±0.24	9.73±0.24	0.11±0.01	0.37±0.11	4.36±0.67	1.31±0.08	1.83±0.28
Ononis tridentata	Stems	Gyp	Clipped	3.01±0.19	1.40±0.12	9.98±0.76	0.10±0.01	0.40±0.07	7.82±1.64	1.37±0.11	3.19±0.61
Ononis tridentata	Stems	Gyp	Unclipped	3.66±0.26	1.67±0.20	10.37±0.57	0.11±0.01	0.40±0.05	9.89±1.47	1.72±0.07	4.10±0.48
Rosmarinus officinalis	Coarse roots	Calc	Clipped	0.70±0.04	1.02±0.10	4.12±0.22	0.43±0.03	0.52±0.07	0.58±0.02	0.66±0.05	3.51±0.55
Rosmarinus officinalis	Coarse roots	Calc	Unclipped	0.80±0.04	1.14±0.04	4.31±0.25	0.42±0.05	0.63±0.09	0.66±0.03	0.85±0.06	3.14±0.29
Rosmarinus officinalis	Coarse roots	Gyp	Clipped	1.11±0.09	0.86±0.07	4.41±0.13	0.56±0.08	0.51±0.07	1.64±0.22	0.79±0.10	2.65±0.61
Rosmarinus officinalis	Coarse roots	Gyp	Unclipped	1.08±0.19	0.76±0.07	4.32±0.64	0.57±0.12	0.38±0.05	1.85±0.37	0.73±0.09	1.64±0.22
Rosmarinus officinalis	Fine roots	Calc	Clipped	3.05±0.07	7.55±0.32	6.72±0.26	1.10±0.08	2.95±0.45	1.84±0.07	1.41±0.01	6.02±1.01
Rosmarinus officinalis	Fine roots	Calc	Unclipped	2.92±0.09	6.74±0.40	6.44±0.45	1.17±0.12	2.74±0.36	1.80±0.06	1.42±0.01	5.29±0.83
Rosmarinus officinalis	Fine roots	Gyp	Clipped	5.41±0.40	4.46±0.35	7.38±0.48	2.16±0.18	0.79±0.12	17.87±1.92	1.44±0.02	3.54±0.49
Rosmarinus officinalis	Fine roots	Gyp	Unclipped	5.81±0.35	3.96±0.30	6.06±0.16	2.39±0.20	0.65±0.08	16.93±1.03	1.45±0.01	2.78±0.41
Rosmarinus officinalis	Leaves	Calc	Clipped	1.33±0.08	3.51±0.45	6.15±0.19	0.06±0.01	1.23±0.34	1.71±0.15	0.99±0.07	2.09±0.21
Rosmarinus officinalis	Leaves	Calc	Unclipped	1.63±0.16	4.07±0.34	6.88±0.50	0.05±0.00	1.79±0.36	1.68±0.16	1.02±0.04	2.38±0.37
Rosmarinus officinalis	Leaves	Gyp	Clipped	2.22±0.22	2.91±0.43	7.88±0.66	0.05±0.00	0.75±0.13	3.08±0.48	1.03±0.06	2.55±0.33
Rosmarinus officinalis	Leaves	Gyp	Unclipped	2.31±0.14	3.65±0.39	5.91±0.42	0.07±0.01	0.59±0.06	2.54±0.36	1.15±0.04	3.46±0.58
Rosmarinus officinalis	Stems	Calc	Clipped	0.77±0.07	1.79±0.12	4.54±0.10	0.13±0.02	1.14±0.28	0.63±0.06	1.21±0.08	3.48±0.68
Rosmarinus officinalis	Stems	Calc	Unclipped	0.81±0.10	2.09±0.28	5.00±0.19	0.17±0.02	0.93±0.04	0.64±0.06	1.32±0.09	2.45±0.15
Rosmarinus officinalis	Stems	Gyp	Clipped	1.21±0.07	1.60±0.15	4.93±0.18	0.24±0.05	0.44±0.06	0.56±0.05	1.01±0.07	1.92±0.26
Rosmarinus officinalis	Stems	Gyp	Unclipped	1.43±0.09	2.05±0.12	4.77±0.17	0.33±0.08	0.40±0.04	0.52±0.04	1.17±0.05	2.90±0.53

Table S6: PERMANOVA of differences among treatments on the composition of elemental pools. F-ratios and P-values.

	F-ratio	*P*-value
Organ	75.76	0.001
Gypsum affinity	35.28	0.001
Soil type	31.45	0.001
Clipping	0.34	0.817
Organ x Gyp. aff.	9.59	0.001
Organ x Soil	5.70	0.001
Gyp. aff. x Soil	0.84	0.365
Organ x Clip.	0.11	1.000
Gyp. aff. x Clip.	0.45	0.713
Soil x Clipping	0.21	0.952
Organ x Gyp. x Soil	0.42	0.923
Organ x Gyp. x Clip.	0.08	1.000
Organ x Soil x Clipp.	0.08	1.000
Gyp. x Soil x Clipp.	0.26	0.928
Organ x Gyp. x Soil x Clipp.	0.13	1.000

Table S7: ANOVA of generalised linear mixed models of elemental pools data. Chi-squares, and P-values in brackets.

	Organ	Gypsum affinity	Soil type	Clipping	Organ x Gyp. Aff.	Organ x Soil	Gyp. x Soil	Organ x Clipp.
Al	**2073.98 (0.000)**	**3.91 (0.048)**	**20.21 (0.000)**	0.05 (0.828)	**35.37 (0.000)**	**17.19 (0.001)**	0.10 (0.749)	0.36 (0.948)
C	**472.42 (0.000)**	3.03 (0.082)	0.50 (0.481)	0.14 (0.704)	**54.75 (0.000)**	3.94 (0.268)	0.01 (0.933)	1.25 (0.741)
Ca	**1076.32 (0.000)**	1.30 (0.254)	1.47 (0.226)	0.58 (0.447)	**75.11 (0.000)**	**9.57 (0.023)**	1.61 (0.204)	4.30 (0.230)
Cr	**1128.92 (0.000)**	0.28 (0.598)	2.94 (0.086)	0.09 (0.770)	**133.43 (0.000)**	**28.90 (0.000)**	**7.61 (0.006)**	3.89 (0.274)
Cu	**1410.65 (0.000)**	**7.59 (0.006)**	0.13 (0.721)	0.90 (0.342)	**49.61 (0.000)**	3.85 (0.278)	0.16 (0.693)	2.69 (0.441)
Fe	**1745.28 (0.000)**	2.02 (0.155)	**19.75 (0.000)**	1.59 (0.207)	**30.58 (0.000)**	**21.53 (0.000)**	0.19 (0.660)	6.84 (0.077)
K	**613.70 (0.000)**	**3.97 (0.046)**	0.36 (0.546)	0.03 (0.860)	**126.56 (0.000)**	5.16 (0.160)	0.00 (0.959)	0.73 (0.867)
Mg	**1234.31 (0.000)**	2.07 (0.150)	0.06 (0.812)	0.27 (0.602)	**143.35 (0.000)**	0.25 (0.969)	0.07 (0.785)	1.48 (0.687)
Mn	**1115.95 (0.000)**	0.35 (0.551)	3.05 (0.081)	0.28 (0.597)	**53.38 (0.000)**	**11.75 (0.008)**	0.12 (0.728)	1.52 (0.677)
N	**875.24 (0.000)**	3.11 (0.078)	0.81 (0.367)	0.00 (0.962)	**83.12 (0.000)**	5.11 (0.164)	0.15 (0.699)	1.30 (0.729)
Na	**1468.39 (0.000)**	**3.87 (0.049)**	0.29 (0.590)	1.15 (0.283)	**75.87 (0.000)**	4.75 (0.191)	3.78 (0.052)	0.79 (0.852)
P	**567.15 (0.000)**	**4.63 (0.032)**	2.48 (0.115)	0.43 (0.512)	**191.50 (0.000)**	**9.05 (0.029)**	1.46 (0.227)	0.78 (0.853)
S	**1432.64 (0.000)**	2.80 (0.094)	**47.07 (0.000)**	0.41 (0.523)	**178.89 (0.000)**	**105.92 (0.000)**	**13.33 (0.000)**	0.64 (0.887)
Si	**1872.37 (0.000)**	**4.76 (0.029)**	2.96 (0.085)	0.27 (0.604)	**52.73 (0.000)**	**8.90 (0.031)**	0.50 (0.479)	0.34 (0.953)
Zn	**833.54 (0.000)**	3.14 (0.076)	**4.59 (0.032)**	0.31 (0.575)	**100.86 (0.000)**	**31.32 (0.000)**	1.18 (0.277)	5.43 (0.143)

	Gyp. x Clipp.	Soil x Clipp.	Organ x Gyp. x Soil	Organ x Gyp. x Clipp.	Organ x Soil x Clipp.	Gyp. x Soil x Clipp.	Organ x Gyp. x Soil x Clipp.
Al	2.76 (0.096)	0.43 (0.511)	5.87 (0.118)	2.87 (0.412)	1.03 (0.795)	0.02 (0.875)	4.81 (0.186)
C	0.01 (0.927)	0.09 (0.764)	1.05 (0.790)	0.94 (0.815)	0.22 (0.974)	0.11 (0.741)	0.53 (0.913)
Ca	0.03 (0.868)	1.58 (0.209)	1.77 (0.622)	1.86 (0.602)	2.84 (0.417)	1.23 (0.267)	2.41 (0.492)
Cr	0.01 (0.925)	0.16 (0.687)	4.63 (0.201)	2.15 (0.542)	0.98 (0.807)	1.08 (0.298)	2.21 (0.530)
Cu	0.00 (0.956)	0.47 (0.495)	0.50 (0.918)	2.03 (0.567)	2.12 (0.549)	0.83 (0.361)	2.58 (0.461)
Fe	0.05 (0.818)	1.70 (0.192)	0.23 (0.972)	2.28 (0.516)	2.99 (0.394)	1.29 (0.256)	1.69 (0.640)
K	0.02 (0.902)	0.41 (0.522)	0.65 (0.884)	0.89 (0.827)	2.07 (0.557)	0.44 (0.508)	1.35 (0.717)
Mg	0.01 (0.906)	1.73 (0.188)	1.10 (0.778)	2.66 (0.447)	5.10 (0.165)	1.06 (0.304)	3.84 (0.280)
Mn	0.00 (0.989)	0.46 (0.500)	1.25 (0.741)	1.03 (0.795)	1.29 (0.731)	0.42 (0.515)	1.16 (0.763)
N	0.18 (0.668)	0.17 (0.678)	0.97 (0.810)	1.85 (0.605)	0.54 (0.910)	0.12 (0.727)	0.34 (0.953)
Na	1.84 (0.175)	0.85 (0.358)	7.56 (0.056)	1.35 (0.717)	1.58 (0.663)	1.22 (0.270)	2.99 (0.393)
P	0.43 (0.511)	0.21 (0.645)	3.02 (0.389)	1.81 (0.613)	0.70 (0.874)	0.18 (0.669)	0.40 (0.940)
S	1.97 (0.161)	0.09 (0.767)	0.60 (0.897)	2.89 (0.409)	0.59 (0.898)	0.09 (0.764)	1.04 (0.791)
Si	0.42 (0.517)	0.02 (0.889)	6.42 (0.093)	2.62 (0.454)	0.45 (0.930)	0.02 (0.899)	2.60 (0.458)
Zn	1.18 (0.277)	0.00 (0.964)	3.46 (0.327)	5.28 (0.152)	0.64 (0.888)	0.22 (0.641)	4.94 (0.176)

Table S8: Means and standard errors of elemental pool data (%) for each organ and treatment.

A) Leaves

Gyp. Affinity Clipping Soil	Gypsovag Clipped Calc	Gypsovag Clipped Gyp	Gypsovag Unclipped Calc	Gypsovag Unclipped Gyp	Gypsophile Clipped Calc	Gypsophile Clipped Gyp	Gypsophile Unclipped Calc	Gypsophile Unclipped Gyp
Al	3.5±0.7	4.5±0.5	2.5±0.3	5.2±0.6	6.2±1.2	9.9±1.7	7.5±1.7	9.3±2.0
C	15.9±2.0	17.7±2.6	14.4±2.0	18.9±3.0	17.0±2.0	19.5±2.4	16.6±2.1	18.5±2.7
Ca	13.7±1.5	16.2±1.9	11.3±1.0	16.8±2.1	19.3±1.0	25.2±1.5	20.1±1.6	23.0±2.3
Cr	9.0±1.6	10.7±1.4	6.4±1.0	11.7±1.1	18.7±3.0	21.1±2.8	19.3±3.1	19.8±2.9
Cu	8.8±1.4	8.8±1.2	8.6±1.6	8.8±1.8	13.3±1.6	16.5±1.6	14.7±1.8	15.4±2.3
Fe	4.6±0.7	6.0±0.6	3.5±0.4	6.4±0.8	7.4±1.3	11.0±1.6	8.6±1.7	10.3±1.9
K	17.2±2.5	21.7±3.2	16.2±2.6	20.4±3.4	17.1±2.0	20.7±2.8	16.4±2.2	19.0±3.0
Mg	18.0±1.8	15.5±1.8	16.5±1.5	16.2±2.3	31.0±2.9	33.3±2.6	31.2±2.6	31.0±3.1
Mn	20.3±2.5	24.4±3.0	18.0±2.6	25.7±3.6	24.6±2.0	32.1±2.4	26.0±2.4	30.5±3.1
N	19.8±1.5	22.1±2.6	18.8±1.8	22.8±3.0	23.0±1.8	25.6±2.2	21.9±1.8	25.6±2.9
Na	7.8±2.3	5.0±0.8	4.5±0.7	5.5±1.0	8.0±1.0	11.4±1.4	8.6±1.4	10.9±2.2
P	15.2±2.3	18.0±2.6	13.0±2.4	19.8±2.8	19.7±2.8	20.8±2.8	18.0±2.4	19.7±2.8
S	23.7±3.3	11.8±1.8	21.4±3.4	12.9±2.3	41.7±3.1	31.0±2.8	40.5±3.2	29.9±3.2
Si	13.5±1.5	14.5±2.0	11.6±1.5	16.8±2.6	15.5±1.7	18.7±1.8	15.9±1.8	17.3±2.5
Zn	10.3±0.8	14.9±1.6	9.7±0.8	19.2±2.3	12.8±1.5	19.4±2.2	15.8±2.5	17.3±2.7

B) Stems

Gyp. Affinity Clipping Soil	Gypsovag Clipped Calc	Gypsovag Clipped Gyp	Gypsovag Unclipped Calc	Gypsovag Unclipped Gyp	Gypsophile Clipped Calc	Gypsophile Clipped Gyp	Gypsophile Unclipped Calc	Gypsophile Unclipped Gyp
Al	17.0±3.5	20.9±3.7	15.1±3.1	20.1±3.1	25.3±3.8	26.1±3.1	26.7±4.1	32.4±3.4
C	26.4±2.8	25.6±2.9	24.9±2.7	26.5±2.9	30.9±1.9	29.2±1.8	34.2±2.1	32.4±1.8
Ca	14.1±2.5	12.7±2.2	12.6±2.2	13.2±2.1	23.0±2.7	17.5±1.6	23.8±3.0	20.0±1.8
Cr	27.4±4.2	34.5±3.8	24.4±3.9	36.4±4.0	34.0±3.5	44.2±1.8	38.0±3.1	48.2±2.7
Cu	13.5±2.4	11.9±2.8	13.4±2.1	11.8±2.4	22.4±1.9	17.9±1.5	24.5±2.0	21.3±1.9
Fe	20.1±4.1	21.1±3.9	18.0±3.6	21.8±3.7	26.5±3.7	29.3±3.1	28.9±4.0	35.2±3.4
K	15.5±1.9	14.7±2.3	14.5±1.8	14.9±2.0	26.7±1.6	27.0±1.3	29.6±2.0	29.1±1.3
Mg	12.8±2.4	12.4±2.4	12.3±2.1	12.2±2.2	21.6±2.2	20.3±1.8	24.1±2.4	23.4±2.1
Mn	17.6±3.0	17.7±3.0	15.8±2.8	18.7±3.2	25.2±2.6	23.7±2.0	26.2±3.0	27.4±2.5
N	18.6±2.3	19.7±2.5	18.1±2.0	20.2±2.3	22.0±1.5	20.8±1.3	24.5±1.8	24.4±1.4
Na	13.1±2.1	14.0±2.2	12.0±1.8	13.4±2.0	17.7±2.0	20.3±2.4	18.2±2.4	19.7±1.9
P	14.3±2.3	18.3±2.2	13.3±2.3	17.4±1.8	21.2±1.6	23.0±1.8	24.5±2.4	25.7±1.5
S	12.9±1.9	7.1±1.8	11.6±1.5	5.7±1.3	20.6±2.1	11.0±1.0	21.1±1.7	13.3±1.4
Si	25.7±3.3	26.4±3.5	24.2±3.1	23.8±2.9	33.5±2.6	30.3±1.9	35.3±2.7	34.7±2.4
Zn	20.1±2.7	24.2±2.6	17.5±2.7	24.8±2.8	26.1±2.6	28.2±3.3	29.9±2.7	34.2±3.1

C) Coarse roots

Gyp. Affinity Clipping Soil	Gypsovag Clipped Calc	Gypsovag Clipped Gyp	Gypsovag Unclipped Calc	Gypsovag Unclipped Gyp	Gypsophile Clipped Calc	Gypsophile Clipped Gyp	Gypsophile Unclipped Calc	Gypsophile Unclipped Gyp
Al	3.4±0.8	4.1±0.7	3.6±1.5	3.1±0.6	3.4±0.5	5.0±0.6	3.5±0.5	6.7±1.2
C	11.1±1.7	10.7±3.1	10.2±2.2	9.9±2.2	16.4±1.8	17.7±2.1	15.2±1.2	16.7±2.3
Ca	4.8±1.0	4.0±0.6	4.7±1.2	7.4±3.4	9.3±1.1	9.4±1.6	10.0±1.6	10.7±2.2
Cr	6.5±1.1	8.3±1.0	8.4±2.9	11.0±3.2	5.2±0.7	4.6±0.3	5.8±0.8	4.6±0.5
Cu	4.8±1.0	3.4±0.7	5.0±1.9	6.6±3.4	6.1±0.7	6.2±0.7	6.5±0.6	6.5±1.1
Fe	3.3±0.8	3.8±0.6	4.1±1.8	7.3±3.9	3.4±0.6	4.7±0.6	3.6±0.5	6.2±1.2
K	8.9±2.1	6.1±1.1	7.5±2.1	8.3±2.1	14.5±1.5	14.6±1.7	14.1±1.5	15.3±2.3
Mg	5.9±1.4	3.8±0.7	5.2±1.5	7.0±3.4	7.9±1.0	6.7±0.7	7.4±0.7	7.0±0.9
Mn	5.0±1.4	4.3±0.9	5.3±2.3	5.8±2.2	5.8±0.7	7.4±1.0	6.2±1.0	7.9±1.5
N	8.6±1.4	8.1±2.4	7.6±1.8	7.9±2.0	13.4±1.2	14.3±1.3	13.8±1.2	14.5±1.7
Na	10.3±2.3	6.5±1.0	8.0±1.8	6.4±1.0	13.1±1.8	13.3±1.6	12.5±1.5	14.2±2.3
P	7.8±1.6	7.1±1.3	7.2±2.0	6.9±1.2	19.8±2.4	20.0±2.2	18.5±2.1	19.8±2.4
S	7.3±1.6	4.2±1.2	6.5±1.8	3.6±1.0	8.6±1.1	6.0±0.8	8.3±0.7	6.1±0.9
Si	5.2±0.8	5.3±0.6	5.0±1.5	4.5±0.4	6.2±0.6	8.4±0.8	6.1±0.5	8.7±1.1
Zn	7.8±1.4	8.1±2.1	6.4±1.8	5.1±0.8	15.1±1.6	14.1±1.8	13.9±1.4	14.4±2.1

D) Fine roots

Gyp. Affinity Clipping Soil	Gypsovag Clipped Calc	Gypsovag Clipped Gyp	Gypsovag Unclipped Calc	Gypsovag Unclipped Gyp	Gypsophile Clipped Calc	Gypsophile Clipped Gyp	Gypsophile Unclipped Calc	Gypsophile Unclipped Gyp
Al	76.1±4.5	70.5±4.3	78.8±4.0	71.5±3.4	65.1±4.9	59.0±4.5	62.3±5.2	51.7±4.7
C	46.3±3.2	45.9±3.7	50.5±3.0	46.0±2.8	35.7±2.0	33.6±1.7	33.9±2.3	32.5±2.1
Ca	67.2±4.3	67.2±3.6	71.4±3.6	62.6±4.0	48.3±3.3	47.9±2.6	46.1±4.2	46.4±3.6
Cr	56.9±6.2	46.5±5.1	60.8±5.7	40.9±5.0	42.2±5.0	30.1±3.0	36.8±4.5	27.4±3.3
Cu	72.0±3.2	75.9±3.3	73.0±2.9	72.7±3.9	58.2±2.7	59.4±2.6	54.4±3.1	56.8±3.4
Fe	71.9±5.0	69.2±4.5	74.5±4.6	64.4±4.8	62.7±4.9	54.9±4.4	59.0±5.2	48.4±4.6
K	57.0±3.2	57.4±4.1	61.8±3.4	56.3±3.5	41.6±1.8	37.7±1.6	39.8±2.5	36.6±2.5
Mg	62.5±4.6	68.3±3.9	66.1±3.9	64.6±4.4	39.5±2.2	39.7±1.9	37.3±2.8	38.6±2.9
Mn	55.7±3.6	53.6±3.2	60.9±3.8	49.8±3.3	44.3±3.0	36.8±2.6	41.5±3.7	34.2±3.0
N	52.2±3.3	50.0±3.7	55.2±2.5	50.6±2.7	41.6±1.6	39.3±1.9	39.7±2.2	35.6±2.3
Na	68.8±4.6	74.6±3.3	75.5±3.1	74.7±2.9	61.3±2.7	55.0±2.5	60.7±3.0	55.2±3.0
P	62.6±3.9	56.6±3.5	66.5±4.0	56.0±3.1	39.4±1.5	36.2±2.0	38.9±2.4	34.7±2.1
S	56.2±3.4	76.9±3.8	60.5±3.3	77.7±3.3	29.1±2.4	52.0±2.8	30.1±2.9	50.7±3.6
Si	55.5±3.3	53.7±3.2	59.2±3.0	55.0±2.7	44.8±2.5	42.6±1.9	42.7±2.8	39.2±2.6
Zn	61.7±4.2	52.8±3.5	66.4±3.6	50.9±2.7	46.0±3.8	38.3±3.8	40.3±3.8	34.1±3.8

Table S9: ANOVA of generalised linear models of elemental pools data for each species. Chi-squares, and P-values in brackets.

A) Gypsophila struthium

	Organ	Soil type	Clipping	Organ x Soil	Organ x Clipp.	Soil x Clipp.	Organ x Soil x Clipp.
Al	**780.50 (0.000)**	**26.27 (0.000)**	**4.09 (0.043)**	**24.58 (0.000)**	**11.06 (0.011)**	1.00 (0.318)	0.28 (0.964)
C	**297.13 (0.000)**	0.01 (0.920)	0.03 (0.869)	**10.88 (0.012)**	**11.12 (0.011)**	0.05 (0.825)	0.66 (0.883)
Ca	**141.56 (0.000)**	0.72 (0.395)	0.58 (0.447)	4.07 (0.254)	6.56 (0.087)	0.08 (0.772)	0.24 (0.971)
Cr	**415.12 (0.000)**	1.38 (0.241)	0.49 (0.484)	**8.81 (0.032)**	4.98 (0.173)	0.36 (0.547)	3.88 (0.274)
Cu	**630.60 (0.000)**	0.27 (0.605)	2.39 (0.122)	**9.62 (0.022)**	3.89 (0.273)	0.74 (0.389)	1.18 (0.758)
Fe	**818.40 (0.000)**	**28.02 (0.000)**	**6.52 (0.011)**	**28.43 (0.000)**	**12.74 (0.005)**	1.20 (0.274)	0.38 (0.944)
K	**301.49 (0.000)**	0.61 (0.436)	0.02 (0.889)	**22.02 (0.000)**	**8.35 (0.039)**	0.15 (0.703)	2.06 (0.559)
Mg	**426.16 (0.000)**	**3.93 (0.048)**	2.84 (0.092)	**14.39 (0.002)**	**8.54 (0.036)**	0.08 (0.782)	1.23 (0.747)
Mn	**324.52 (0.000)**	**11.46 (0.001)**	0.74 (0.390)	**28.79 (0.000)**	**10.53 (0.015)**	0.01 (0.931)	0.90 (0.825)
N	**189.93 (0.000)**	0.03 (0.868)	0.37 (0.541)	6.40 (0.094)	6.72 (0.081)	0.12 (0.726)	1.05 (0.788)
Na	**646.12 (0.000)**	**3.92 (0.048)**	0.06 (0.801)	**9.88 (0.020)**	2.69 (0.442)	0.00 (0.962)	0.13 (0.988)
P	**106.70 (0.000)**	0.64 (0.424)	0.00 (0.988)	**10.08 (0.018)**	3.78 (0.286)	0.00 (0.973)	0.28 (0.963)
S	**271.26 (0.000)**	**14.43 (0.000)**	1.88 (0.170)	**74.82 (0.000)**	3.41 (0.333)	0.20 (0.653)	3.24 (0.356)
Si	**400.11 (0.000)**	2.16 (0.142)	0.04 (0.844)	**9.28 (0.026)**	**8.04 (0.045)**	0.01 (0.932)	0.12 (0.990)
Zn	**161.72 (0.000)**	0.50 (0.479)	0.01 (0.916)	4.34 (0.227)	1.79 (0.618)	0.17 (0.677)	1.12 (0.773)

B) Herniaria fruticosa

	Organ	Soil type	Clipping	Organ x Soil	Organ x Clipp.	Soil x Clipp.	Organ x Soil x Clipp.
Al	**452.56 (0.000)**	**3.98 (0.046)**	0.93 (0.334)	**9.91 (0.019)**	2.70 (0.441)	0.72 (0.397)	1.65 (0.648)
C	**176.42 (0.000)**	0.06 (0.802)	0.01 (0.905)	4.45 (0.217)	1.19 (0.755)	0.37 (0.544)	4.72 (0.194)
Ca	**135.38 (0.000)**	0.29 (0.591)	0.04 (0.847)	**10.54 (0.014)**	1.37 (0.711)	0.05 (0.819)	2.16 (0.540)
Cr	**1231.34 (0.000)**	0.53 (0.468)	0.52 (0.470)	2.25 (0.522)	2.61 (0.456)	0.24 (0.623)	1.87 (0.601)
Cu	**495.76 (0.000)**	0.99 (0.319)	0.34 (0.560)	**8.38 (0.039)**	0.86 (0.836)	0.62 (0.432)	2.39 (0.495)
Fe	**502.32 (0.000)**	1.81 (0.179)	1.11 (0.291)	4.69 (0.196)	4.06 (0.255)	0.82 (0.366)	1.88 (0.597)
K	**209.09 (0.000)**	0.26 (0.613)	0.01 (0.930)	5.63 (0.131)	3.61 (0.306)	0.36 (0.547)	4.17 (0.243)
Mg	**309.74 (0.000)**	0.60 (0.438)	0.33 (0.564)	6.04 (0.110)	4.48 (0.214)	0.83 (0.362)	7.05 (0.070)
Mn	**133.25 (0.000)**	0.01 (0.908)	0.28 (0.596)	0.87 (0.833)	3.58 (0.311)	0.49 (0.486)	3.58 (0.310)
N	**142.19 (0.000)**	0.04 (0.845)	0.47 (0.492)	2.65 (0.449)	5.08 (0.166)	0.23 (0.632)	3.33 (0.344)
Na	**411.89 (0.000)**	0.02 (0.899)	0.73 (0.392)	7.45 (0.059)	1.88 (0.598)	2.05 (0.152)	5.57 (0.134)
P	**59.14 (0.000)**	0.00 (0.962)	0.11 (0.746)	0.85 (0.837)	1.22 (0.749)	0.20 (0.655)	2.37 (0.500)
S	**229.40 (0.000)**	**4.40 (0.036)**	0.00 (0.990)	**64.77 (0.000)**	0.46 (0.928)	0.16 (0.692)	1.71 (0.635)
Si	**373.77 (0.000)**	1.14 (0.286)	0.02 (0.881)	**12.67 (0.005)**	0.87 (0.833)	0.66 (0.417)	3.50 (0.321)
Zn	**47.19 (0.000)**	0.29 (0.588)	0.01 (0.930)	7.63 (0.054)	**8.25 (0.041)**	0.02 (0.897)	0.87 (0.831)

C) Helianthemum squamatum

	Organ	Soil type	Clipping	Organ x Soil	Organ x Clipp.	Soil x Clipp.	Organ x Soil x Clipp.
Al	**263.22 (0.000)**	**9.52 (0.002)**	0.55 (0.460)	**11.26 (0.010)**	1.05 (0.789)	0.35 (0.553)	0.42 (0.936)
C	**520.39 (0.000)**	0.20 (0.655)	0.95 (0.329)	**11.14 (0.011)**	5.50 (0.138)	0.04 (0.847)	2.93 (0.403)
Ca	**205.26 (0.000)**	0.16 (0.689)	0.05 (0.818)	5.63 (0.131)	0.73 (0.866)	0.20 (0.656)	0.20 (0.977)
Cr	**427.12 (0.000)**	**10.89 (0.001)**	0.84 (0.360)	**69.50 (0.000)**	3.04 (0.386)	1.17 (0.279)	1.37 (0.713)
Cu	**182.35 (0.000)**	0.36 (0.550)	0.67 (0.413)	2.58 (0.460)	1.67 (0.643)	0.00 (0.980)	0.21 (0.976)
Fe	**232.28 (0.000)**	**6.01 (0.014)**	0.30 (0.582)	**10.94 (0.012)**	0.77 (0.856)	0.15 (0.699)	0.24 (0.972)
K	**441.11 (0.000)**	0.29 (0.591)	0.21 (0.648)	**18.55 (0.000)**	1.14 (0.768)	0.02 (0.902)	1.26 (0.738)
Mg	**242.69 (0.000)**	0.08 (0.778)	0.20 (0.656)	2.17 (0.538)	0.90 (0.826)	0.03 (0.857)	0.28 (0.963)
Mn	**496.77 (0.000)**	0.33 (0.568)	0.08 (0.777)	5.17 (0.160)	0.69 (0.876)	0.15 (0.700)	1.27 (0.736)
N	**677.69 (0.000)**	0.14 (0.712)	1.05 (0.306)	**14.50 (0.002)**	3.96 (0.266)	0.03 (0.869)	2.27 (0.518)
Na	**312.46 (0.000)**	**4.66 (0.031)**	0.30 (0.585)	6.71 (0.082)	0.84 (0.840)	0.21 (0.644)	1.20 (0.753)
P	**239.37 (0.000)**	0.46 (0.496)	1.12 (0.291)	**13.33 (0.004)**	5.35 (0.148)	0.17 (0.683)	2.34 (0.506)
S	**528.75 (0.000)**	0.30 (0.585)	2.64 (0.104)	**21.24 (0.000)**	5.24 (0.155)	0.00 (0.946)	0.65 (0.885)
Si	**521.37 (0.000)**	1.12 (0.291)	0.56 (0.453)	**8.40 (0.038)**	3.83 (0.281)	0.06 (0.807)	1.49 (0.684)
Zn	**221.78 (0.000)**	0.16 (0.686)	1.73 (0.189)	**10.94 (0.012)**	2.75 (0.431)	0.34 (0.561)	0.80 (0.850)

D) Lepidium subulatum

	Organ	Soil type	Clipping	Organ x Soil	Organ x Clipp.	Soil x Clipp.	Organ x Soil x Clipp.
Al	**632.76 (0.000)**	0.01 (0.920)	**8.08 (0.004)**	3.62 (0.306)	**20.48 (0.000)**	0.04 (0.836)	1.02 (0.797)
C	**284.99 (0.000)**	0.24 (0.622)	0.92 (0.337)	6.68 (0.083)	**31.58 (0.000)**	0.20 (0.658)	0.47 (0.925)
Ca	**417.74 (0.000)**	0.22 (0.641)	3.22 (0.073)	**11.68 (0.009)**	**17.89 (0.000)**	0.00 (0.967)	2.71 (0.439)
Cr	**694.13 (0.000)**	**4.08 (0.043)**	1.69 (0.193)	**10.88 (0.012)**	7.14 (0.068)	0.12 (0.731)	0.64 (0.888)
Cu	**348.68 (0.000)**	0.19 (0.665)	3.57 (0.059)	4.09 (0.252)	**19.22 (0.000)**	0.54 (0.464)	2.39 (0.496)
Fe	**633.33 (0.000)**	0.25 (0.619)	**8.25 (0.004)**	4.49 (0.213)	**19.71 (0.000)**	0.02 (0.878)	0.99 (0.803)
K	**345.12 (0.000)**	0.25 (0.619)	2.67 (0.102)	**9.12 (0.028)**	**19.74 (0.000)**	0.26 (0.610)	2.23 (0.526)
Mg	**324.13 (0.000)**	0.00 (0.993)	1.21 (0.272)	**13.49 (0.004)**	**20.73 (0.000)**	0.23 (0.635)	5.43 (0.143)
Mn	**390.00 (0.000)**	0.07 (0.787)	**7.52 (0.006)**	7.08 (0.069)	**27.64 (0.000)**	0.01 (0.940)	0.84 (0.839)
N	**170.75 (0.000)**	0.09 (0.765)	1.54 (0.214)	**8.30 (0.040)**	**21.92 (0.000)**	0.02 (0.895)	1.09 (0.780)
Na	**352.12 (0.000)**	0.60 (0.439)	2.46 (0.117)	**8.14 (0.043)**	**15.87 (0.001)**	1.89 (0.169)	5.19 (0.158)
P	**244.43 (0.000)**	0.63 (0.427)	0.37 (0.543)	6.85 (0.077)	**13.67 (0.003)**	0.11 (0.737)	0.64 (0.887)
S	**286.92 (0.000)**	0.37 (0.546)	0.95 (0.329)	6.77 (0.080)	**14.73 (0.002)**	0.05 (0.817)	0.95 (0.814)
Si	**445.96 (0.000)**	0.04 (0.840)	3.29 (0.070)	3.19 (0.363)	**16.19 (0.001)**	0.02 (0.888)	1.40 (0.706)
Zn	**100.48 (0.000)**	0.15 (0.702)	1.08 (0.298)	**8.25 (0.041)**	**11.35 (0.010)**	0.21 (0.645)	2.47 (0.482)

E) Ononis tridentata

	Organ	Soil type	Clipping	Organ x Soil	Organ x Clipp.	Soil x Clipp.	Organ x Soil x Clipp.
Al	**696.98 (0.000)**	**4.64 (0.031)**	0.43 (0.511)	**10.32 (0.016)**	0.39 (0.942)	2.06 (0.151)	4.59 (0.205)
C	**297.69 (0.000)**	0.46 (0.498)	0.03 (0.872)	2.75 (0.432)	1.84 (0.606)	0.00 (0.976)	0.71 (0.870)
Ca	**242.90 (0.000)**	0.04 (0.834)	0.04 (0.848)	**10.66 (0.014)**	0.44 (0.932)	0.59 (0.444)	2.19 (0.534)
Cr	**804.62 (0.000)**	1.44 (0.230)	0.02 (0.880)	**23.51 (0.000)**	4.79 (0.188)	1.04 (0.308)	2.30 (0.513)
Cu	**413.50 (0.000)**	1.39 (0.238)	0.74 (0.391)	**8.45 (0.038)**	2.18 (0.537)	0.00 (0.960)	5.37 (0.147)
Fe	**657.48 (0.000)**	**7.73 (0.005)**	0.06 (0.813)	**13.09 (0.004)**	0.26 (0.967)	0.97 (0.325)	4.14 (0.247)
K	**31.24 (0.000)**	0.09 (0.767)	0.06 (0.802)	3.19 (0.363)	2.26 (0.521)	0.00 (0.974)	0.63 (0.891)
Mg	**242.78 (0.000)**	0.89 (0.344)	0.46 (0.497)	**16.19 (0.001)**	0.72 (0.869)	0.81 (0.369)	4.53 (0.210)
Mn	**504.22 (0.000)**	3.14 (0.076)	0.16 (0.694)	**13.05 (0.005)**	0.62 (0.893)	0.81 (0.367)	4.81 (0.187)
N	**102.55 (0.000)**	0.35 (0.554)	0.10 (0.746)	5.58 (0.134)	2.85 (0.415)	0.00 (0.988)	0.11 (0.990)
Na	**429.61 (0.000)**	0.10 (0.747)	0.39 (0.530)	3.16 (0.367)	1.43 (0.698)	0.68 (0.409)	4.76 (0.191)
P	**53.52 (0.000)**	0.10 (0.748)	0.02 (0.902)	2.98 (0.395)	0.76 (0.859)	0.01 (0.918)	0.20 (0.978)
S	**268.95 (0.000)**	3.25 (0.071)	0.33 (0.567)	**32.67 (0.000)**	0.39 (0.943)	0.81 (0.368)	4.44 (0.218)
Si	**435.03 (0.000)**	2.47 (0.116)	0.02 (0.887)	**9.72 (0.021)**	0.13 (0.988)	0.19 (0.662)	2.83 (0.418)
Zn	**110.10 (0.000)**	0.41 (0.520)	0.01 (0.913)	**18.70 (0.000)**	3.74 (0.291)	0.09 (0.764)	5.16 (0.161)

F) Boleum asperum

	Organ	Soil type	Clipping	Organ x Soil	Organ x Clipp.	Soil x Clipp.	Organ x Soil x Clipp.
Al	**233.37 (0.000)**	0.21 (0.647)	0.13 (0.717)	**12.72 (0.005)**	1.43 (0.699)	0.72 (0.396)	2.87 (0.412)
C	**125.34 (0.000)**	0.03 (0.867)	0.00 (0.944)	**19.38 (0.000)**	1.01 (0.800)	0.00 (0.971)	1.88 (0.598)
Ca	**149.94 (0.000)**	1.69 (0.194)	0.03 (0.854)	**16.94 (0.001)**	4.17 (0.243)	0.15 (0.701)	0.88 (0.831)
Cr	**57.82 (0.000)**	0.19 (0.666)	0.10 (0.751)	3.70 (0.296)	**8.24 (0.041)**	0.20 (0.654)	0.30 (0.959)
Cu	**191.04 (0.000)**	0.64 (0.422)	0.11 (0.745)	**26.88 (0.000)**	1.00 (0.801)	0.57 (0.452)	1.23 (0.747)
Fe	**162.92 (0.000)**	0.01 (0.913)	0.00 (0.980)	**8.73 (0.033)**	3.77 (0.287)	0.24 (0.624)	1.92 (0.590)
K	**132.73 (0.000)**	0.25 (0.614)	0.00 (0.988)	**24.57 (0.000)**	0.42 (0.937)	0.05 (0.829)	2.17 (0.537)
Mg	**120.79 (0.000)**	1.98 (0.160)	0.00 (0.956)	**11.94 (0.008)**	2.39 (0.495)	0.21 (0.645)	1.56 (0.667)
Mn	**91.70 (0.000)**	0.00 (0.974)	0.01 (0.909)	**17.76 (0.000)**	0.71 (0.870)	0.04 (0.832)	0.74 (0.863)
N	**124.33 (0.000)**	1.21 (0.271)	0.05 (0.822)	**15.00 (0.002)**	0.54 (0.910)	0.06 (0.810)	2.12 (0.548)
Na	**243.16 (0.000)**	0.05 (0.823)	0.06 (0.812)	**11.53 (0.009)**	1.53 (0.675)	0.04 (0.841)	1.44 (0.697)
P	**117.49 (0.000)**	0.55 (0.458)	0.06 (0.809)	**8.47 (0.037)**	0.96 (0.810)	0.00 (0.961)	0.78 (0.855)
S	**150.60 (0.000)**	2.27 (0.132)	0.03 (0.867)	**17.09 (0.001)**	0.64 (0.887)	0.60 (0.439)	1.67 (0.643)
Si	**174.91 (0.000)**	0.03 (0.862)	0.02 (0.883)	**13.05 (0.005)**	1.10 (0.778)	0.39 (0.530)	2.83 (0.418)
Zn	**95.74 (0.000)**	0.07 (0.786)	0.00 (0.975)	**13.61 (0.003)**	0.48 (0.922)	0.34 (0.560)	1.06 (0.786)

G) Helianthemum syriacum

	Organ	Soil type	Clipping	Organ x Soil	Organ x Clipp.	Soil x Clipp.	Organ x Soil x Clipp.
Al	**306.17 (0.000)**	**5.89 (0.015)**	1.28 (0.257)	3.09 (0.377)	0.99 (0.804)	0.79 (0.373)	1.48 (0.688)
C	**141.17 (0.000)**	1.73 (0.189)	0.86 (0.355)	5.45 (0.142)	5.00 (0.172)	0.58 (0.445)	5.78 (0.123)
Ca	**147.86 (0.000)**	**5.59 (0.018)**	1.78 (0.182)	**19.15 (0.000)**	5.82 (0.121)	0.01 (0.934)	1.52 (0.678)
Cr	**277.50 (0.000)**	**35.00 (0.000)**	1.06 (0.303)	**27.53 (0.000)**	5.06 (0.167)	0.54 (0.464)	3.13 (0.372)
Cu	**146.54 (0.000)**	1.87 (0.171)	1.89 (0.169)	**19.07 (0.000)**	6.55 (0.088)	0.01 (0.921)	1.80 (0.615)
Fe	**177.91 (0.000)**	**10.24 (0.001)**	3.04 (0.081)	**12.99 (0.005)**	4.79 (0.188)	0.00 (0.985)	2.35 (0.504)
K	**214.22 (0.000)**	**7.02 (0.008)**	0.66 (0.415)	**26.00 (0.000)**	4.53 (0.209)	0.01 (0.912)	3.17 (0.366)
Mg	**147.50 (0.000)**	1.66 (0.197)	1.55 (0.213)	**17.22 (0.001)**	4.57 (0.206)	0.00 (0.952)	2.67 (0.445)
Mn	**170.11 (0.000)**	**4.61 (0.032)**	0.79 (0.376)	**18.40 (0.000)**	4.25 (0.236)	0.03 (0.873)	3.81 (0.283)
N	**187.32 (0.000)**	2.27 (0.132)	1.27 (0.260)	4.29 (0.232)	3.86 (0.277)	0.33 (0.566)	4.96 (0.175)
Na	**741.14 (0.000)**	2.87 (0.090)	0.10 (0.754)	2.94 (0.401)	0.31 (0.958)	0.00 (0.983)	0.95 (0.814)
P	**529.01 (0.000)**	**5.83 (0.016)**	0.40 (0.526)	**10.54 (0.015)**	0.76 (0.859)	0.00 (0.987)	1.39 (0.707)
S	**490.01 (0.000)**	**5.44 (0.020)**	0.00 (0.987)	**40.48 (0.000)**	1.76 (0.624)	0.44 (0.509)	3.02 (0.389)
Si	**766.58 (0.000)**	2.62 (0.106)	0.01 (0.916)	4.81 (0.186)	1.79 (0.616)	0.67 (0.413)	3.07 (0.381)
Zn	**525.47 (0.000)**	**10.52 (0.001)**	0.07 (0.790)	**11.13 (0.011)**	0.99 (0.803)	0.34 (0.562)	0.92 (0.820)

H) Matthiola fruticulosa

	Organ	Soil type	Clipping	Organ x Soil	Organ x Clipp.	Soil x Clipp.	Organ x Soil x Clipp.
Al	**306.61 (0.000)**	1.09 (0.297)	1.61 (0.204)	4.37 (0.224)	3.27 (0.352)	0.82 (0.365)	1.75 (0.626)
C	**117.27 (0.000)**	0.20 (0.655)	0.47 (0.494)	3.03 (0.388)	5.81 (0.121)	0.10 (0.756)	4.61 (0.203)
Ca	**133.17 (0.000)**	0.00 (0.961)	0.07 (0.789)	2.07 (0.558)	0.49 (0.921)	0.11 (0.740)	0.90 (0.826)
Cr	**183.92 (0.000)**	0.13 (0.722)	0.17 (0.678)	2.57 (0.464)	2.25 (0.522)	0.22 (0.640)	6.58 (0.087)
Cu	**247.20 (0.000)**	1.11 (0.291)	0.18 (0.675)	5.04 (0.169)	1.22 (0.748)	0.02 (0.895)	0.65 (0.885)
Fe	**286.22 (0.000)**	0.49 (0.482)	0.63 (0.426)	1.90 (0.594)	2.31 (0.511)	0.56 (0.454)	1.60 (0.660)
K	**116.87 (0.000)**	0.56 (0.454)	0.14 (0.713)	**11.73 (0.008)**	2.06 (0.561)	0.60 (0.439)	**8.19 (0.042)**
Mg	**84.78 (0.000)**	1.00 (0.317)	0.02 (0.899)	6.43 (0.092)	0.25 (0.969)	0.37 (0.543)	2.35 (0.502)
Mn	**169.23 (0.000)**	0.01 (0.911)	0.18 (0.674)	7.21 (0.066)	3.07 (0.381)	0.25 (0.620)	0.70 (0.874)
N	**58.86 (0.000)**	0.03 (0.869)	0.59 (0.441)	2.72 (0.437)	8.20 (0.042)	0.11 (0.743)	4.97 (0.174)
Na	**94.81 (0.000)**	0.26 (0.608)	0.28 (0.596)	3.52 (0.318)	4.15 (0.246)	1.33 (0.248)	**10.54 (0.014)**
P	**172.78 (0.000)**	1.68 (0.195)	1.15 (0.283)	**15.83 (0.001)**	5.39 (0.145)	0.31 (0.578)	4.06 (0.255)
S	**176.48 (0.000)**	0.56 (0.454)	0.96 (0.326)	5.34 (0.148)	2.72 (0.437)	0.27 (0.600)	1.18 (0.757)
Si	**395.22 (0.000)**	0.30 (0.585)	0.49 (0.484)	3.58 (0.311)	2.66 (0.447)	1.06 (0.302)	2.33 (0.506)
Zn	**126.33 (0.000)**	0.15 (0.697)	0.90 (0.343)	2.86 (0.413)	**10.02 (0.018)**	0.07 (0.797)	3.86 (0.277)

I) Linum suffruticosum

	Organ	Soil type	Clipping	Organ x Soil	Organ x Clipp.	Soil x Clipp.	Organ x Soil x Clipp.
Al	**415.58 (0.000)**	1.32 (0.251)	**9.59 (0.002)**	0.62 (0.892)	7.69 (0.053)	**4.92 (0.027)**	2.60 (0.458)
C	**442.28 (0.000)**	0.47 (0.491)	3.60 (0.058)	2.07 (0.557)	**10.40 (0.015)**	2.24 (0.134)	5.31 (0.150)
Ca	**340.47 (0.000)**	0.23 (0.633)	**5.58 (0.018)**	1.69 (0.638)	**6.27 (0.099)**	1.82 (0.178)	3.29 (0.350)
Cr	**249.23 (0.000)**	3.62 (0.057)	2.97 (0.085)	**8.79 (0.032)**	4.53 (0.209)	0.23 (0.634)	2.12 (0.549)
Cu	**258.54 (0.000)**	3.00 (0.083)	3.32 (0.069)	2.42 (0.491)	3.62 (0.305)	1.54 (0.214)	1.68 (0.640)
Fe	**338.36 (0.000)**	0.03 (0.869)	**5.83 (0.016)**	0.25 (0.970)	4.71 (0.194)	1.01 (0.314)	0.89 (0.828)
K	**132.47 (0.000)**	1.45 (0.229)	1.04 (0.308)	1.65 (0.649)	2.48 (0.479)	1.41 (0.235)	2.24 (0.524)
Mg	**360.85 (0.000)**	1.84 (0.175)	7.45 (0.006)	2.04 (0.565)	**8.87 (0.031)**	1.76 (0.184)	3.34 (0.343)
Mn	**189.97 (0.000)**	0.18 (0.675)	**3.31 (0.069)**	0.71 (0.871)	4.93 (0.177)	0.66 (0.418)	1.28 (0.734)
N	**346.94 (0.000)**	0.01 (0.925)	**3.52 (0.061)**	0.94 (0.817)	**10.48 (0.015)**	3.07 (0.080)	5.38 (0.146)
Na	**493.38 (0.000)**	1.86 (0.173)	**8.52 (0.004)**	4.33 (0.228)	**13.57 (0.004)**	2.77 (0.096)	**12.41 (0.006)**
P	**395.98 (0.000)**	**3.87 (0.049)**	**12.69 (0.000)**	4.44 (0.217)	**9.65 (0.022)**	**8.64 (0.003)**	5.18 (0.159)
S	**547.81 (0.000)**	**45.35 (0.000)**	**8.75 (0.003)**	**28.05 (0.000)**	**7.99 (0.046)**	0.72 (0.397)	2.55 (0.466)
Si	**466.56 (0.000)**	0.00 (0.978)	**5.72 (0.017)**	3.01 (0.390)	7.05 (0.070)	1.71 (0.191)	3.15 (0.368)
Zn	**288.81 (0.000)**	1.35 (0.246)	**3.89 (0.049)**	7.31 (0.063)	6.59 (0.086)	**4.32 (0.038)**	6.80 (0.078)

J) Rosmarinus officinalis

	Organ	Soil type	Clipping	Organ x Soil	Organ x Clipp.	Soil x Clipp.	Organ x Soil x Clipp.
Al	**1254.00 (0.000)**	**32.09 (0.000)**	0.06 (0.805)	**23.64 (0.000)**	0.77 (0.857)	0.08 (0.784)	2.63 (0.451)
C	**423.44 (0.000)**	1.67 (0.197)	0.75 (0.387)	7.65 (0.054)	2.48 (0.479)	0.00 (0.994)	3.74 (0.291)
Ca	**967.63 (0.000)**	**5.10 (0.024)**	0.07 (0.794)	**12.38 (0.006)**	0.34 (0.953)	0.02 (0.894)	1.36 (0.714)
Cr	**1098.89 (0.000)**	**8.11 (0.004)**	0.00 (0.985)	**18.23 (0.000)**	1.77 (0.622)	1.27 (0.260)	5.12 (0.163)
Cu	**1177.11 (0.000)**	1.61 (0.204)	0.05 (0.823)	2.72 (0.437)	0.04 (0.998)	0.06 (0.808)	4.15 (0.246)
Fe	**1259.36 (0.000)**	**25.31 (0.000)**	0.01 (0.927)	**16.94 (0.001)**	0.57 (0.904)	0.33 (0.564)	4.57 (0.206)
K	**784.81 (0.000)**	**12.09 (0.001)**	1.46 (0.226)	**13.90 (0.003)**	1.66 (0.647)	0.01 (0.934)	1.91 (0.592)
Mg	**1341.35 (0.000)**	1.61 (0.205)	0.61 (0.434)	3.85 (0.278)	1.82 (0.611)	0.32 (0.573)	1.28 (0.733)
Mn	**1329.02 (0.000)**	**19.33 (0.000)**	0.00 (0.963)	**23.79 (0.000)**	3.80 (0.284)	0.17 (0.679)	3.67 (0.299)
N	**580.75 (0.000)**	2.65 (0.104)	0.13 (0.715)	5.72 (0.126)	0.25 (0.970)	0.00 (0.972)	2.16 (0.539)
Na	**1177.65 (0.000)**	0.02 (0.878)	2.14 (0.143)	**7.80 (0.050)**	1.34 (0.719)	0.16 (0.688)	5.10 (0.164)
P	**916.29 (0.000)**	**47.89 (0.000)**	0.61 (0.434)	**54.39 (0.000)**	3.59 (0.309)	0.17 (0.676)	0.21 (0.975)
S	**1414.85 (0.000)**	**94.50 (0.000)**	1.43 (0.232)	**83.72 (0.000)**	1.62 (0.655)	0.39 (0.531)	2.69 (0.442)
Si	**833.25 (0.000)**	2.50 (0.114)	0.39 (0.534)	5.56 (0.135)	0.73 (0.866)	0.06 (0.801)	4.11 (0.250)
Zn	**301.13 (0.000)**	**5.30 (0.021)**	0.39 (0.531)	**18.37 (0.000)**	5.12 (0.163)	0.03 (0.866)	2.71 (0.439)

Table D.10: Means and standard errors of biomass and elemental pools for each treatment and species

Species	Organ	Soil	Clipping	Biomass	Al	C	Ca	Cr	Cu	Fe	K
Boleum asperum	Coarse roots	Calc	Clipped	0.54±0.10	7.45±2.01	14.40±3.05	5.72±0.93	13.38±4.69	10.33±1.19	8.33±2.53	9.59±2.35
Boleum asperum	Coarse roots	Calc	Unclipped	2.08±1.60	13.56±9.92	22.75±12.74	12.08±7.47	28.24±19.91	17.25±12.85	16.71±12.44	15.21±9.89
Boleum asperum	Coarse roots	Gyp	Clipped	0.28±0.12	6.91±2.21	8.33±3.34	2.93±1.35	9.62±1.65	3.67±1.67	6.87±1.55	5.01±2.04
Boleum asperum	Coarse roots	Gyp	Unclipped	0.33±0.11	5.00±1.33	7.87±2.44	3.60±1.51	19.88±8.14	3.04±1.12	7.05±2.04	4.15±1.67
Boleum asperum	Fine roots	Calc	Clipped	2.45±0.39	54.44±4.34	48.54±6.39	59.42±2.04	20.68±4.29	66.25±3.21	45.22±4.74	60.03±6.26
Boleum asperum	Fine roots	Calc	Unclipped	2.68±0.23	51.91±10.89	45.12±8.95	59.47±12.11	17.58±4.53	61.23±11.39	42.42±10.80	57.18±9.47
Boleum asperum	Fine roots	Gyp	Clipped	1.95±0.08	50.56±4.43	55.70±5.34	59.81±8.97	12.44±3.09	67.33±8.32	41.06±3.38	57.96±4.97
Boleum asperum	Fine roots	Gyp	Unclipped	2.14±0.18	60.67±1.14	52.15±0.81	66.15±4.92	14.72±2.40	73.94±5.87	51.50±1.02	56.53±4.64
Boleum asperum	Leaves	Calc	Clipped	0.31±0.06	2.95±0.40	7.10±1.32	13.15±1.23	15.56±1.09	3.76±0.26	5.38±0.57	10.08±0.85
Boleum asperum	Leaves	Calc	Unclipped	0.41±0.12	2.41±0.52	5.56±0.46	10.28±2.39	9.17±2.89	3.39±0.23	4.09±0.77	7.67±0.53
Boleum asperum	Leaves	Gyp	Clipped	0.43±0.10	6.39±0.34	11.76±2.34	23.43±6.30	18.05±4.90	8.21±1.84	9.19±0.95	19.27±2.01
Boleum asperum	Leaves	Gyp	Unclipped	0.56±0.13	4.93±1.25	12.20±2.13	18.63±4.65	13.62±3.15	5.99±1.88	6.92±1.55	21.38±3.77
Boleum asperum	Stems	Calc	Clipped	1.06±0.13	35.17±4.11	29.96±3.69	21.71±3.62	50.38±6.06	19.67±3.43	41.07±4.41	20.29±4.50
Boleum asperum	Stems	Calc	Unclipped	1.41±0.22	32.12±4.78	26.57±4.16	18.17±3.43	45.01±12.58	18.12±2.43	36.78±5.12	19.94±2.83
Boleum asperum	Stems	Gyp	Clipped	0.77±0.10	36.15±2.48	24.21±4.18	13.82±3.10	59.89±7.68	20.79±5.72	42.89±3.04	17.75±3.02
Boleum asperum	Stems	Gyp	Unclipped	1.06±0.05	29.41±1.48	27.78±2.34	11.62±1.07	51.78±3.06	17.03±3.28	34.54±1.14	17.95±1.14
Gypsophila struthium	Coarse roots	Calc	Clipped	4.16±0.48	2.62±0.41	21.19±2.71	17.66±1.09	7.98±1.96	5.51±0.74	2.54±0.41	18.95±2.84
Gypsophila struthium	Coarse roots	Calc	Unclipped	4.65±0.75	4.82±0.88	20.86±1.89	22.39±2.12	10.46±2.28	7.79±1.83	4.84±0.90	18.59±2.46
Gypsophila struthium	Coarse roots	Gyp	Clipped	5.75±1.21	7.82±1.67	28.82±3.57	22.17±2.56	5.02±0.60	7.90±1.20	7.76±1.54	24.49±3.00
Gypsophila struthium	Coarse roots	Gyp	Unclipped	6.63±1.39	12.72±3.21	32.00±4.37	26.51±5.60	6.67±1.71	11.17±3.02	13.26±3.37	28.56±5.04
Gypsophila struthium	Fine roots	Calc	Clipped	9.96±1.00	88.62±1.09	48.79±2.65	51.34±1.61	51.97±2.38	79.61±1.65	87.07±1.46	48.05±2.28
Gypsophila struthium	Fine roots	Calc	Unclipped	9.21±1.89	80.45±3.31	43.42±5.23	44.45±6.84	38.28±5.63	70.11±7.55	77.23±3.86	42.80±5.14
Gypsophila struthium	Fine roots	Gyp	Clipped	8.88±1.01	72.29±4.80	44.36±3.14	43.05±2.75	43.69±3.12	79.52±1.44	70.91±4.13	43.53±2.94
Gypsophila struthium	Fine roots	Gyp	Unclipped	7.13±0.89	59.77±6.86	36.07±5.12	37.63±6.05	44.94±6.75	75.78±3.97	59.23±6.64	33.49±5.30
Gypsophila struthium	Leaves	Calc	Clipped	2.42±0.38	1.12±0.14	10.09±1.32	19.66±1.24	9.27±0.41	3.97±0.55	1.71±0.18	11.00±1.63
Gypsophila struthium	Leaves	Calc	Unclipped	1.93±0.45	1.21±0.46	7.31±1.59	17.26±4.56	10.70±1.77	4.87±2.15	1.97±0.62	7.82±2.14
Gypsophila struthium	Leaves	Gyp	Clipped	2.08±0.63	2.14±0.59	7.95±1.96	20.43±4.82	11.39±1.28	3.96±0.67	3.13±0.69	5.73±1.00
Gypsophila struthium	Leaves	Gyp	Unclipped	1.68±0.31	1.63±0.32	6.69±1.23	18.51±3.21	8.59±1.10	3.37±0.48	2.69±0.51	5.52±0.48
Gypsophila struthium	Stems	Calc	Clipped	3.83±0.30	7.64±1.24	19.93±0.27	11.34±1.30	30.78±3.55	10.92±1.31	8.67±1.53	22.00±1.58
Gypsophila struthium	Stems	Calc	Unclipped	5.75±0.82	13.52±2.44	28.40±2.24	15.89±1.31	40.56±5.47	17.22±3.73	15.97±2.80	30.78±2.00
Gypsophila struthium	Stems	Gyp	Clipped	3.57±0.29	17.76±3.41	18.87±1.51	14.35±1.96	39.91±2.79	8.62±0.96	18.20±2.91	26.25±2.08
Gypsophila struthium	Stems	Gyp	Unclipped	5.01±0.79	25.88±3.66	25.25±1.56	17.35±0.70	39.80±4.75	9.68±0.97	24.81±2.84	32.43±2.02
Helianthemum squamatum	Coarse roots	Calc	Clipped	0.24±0.02	2.57±0.30	11.15±1.54	11.82±1.93	4.55±0.74	3.82±0.64	2.62±0.38	12.24±1.66
Helianthemum squamatum	Coarse roots	Calc	Unclipped	0.53±0.03	3.42±0.48	13.72±1.88	13.45±1.71	3.91±0.27	5.04±0.71	3.72±0.52	17.47±2.73
Helianthemum squamatum	Coarse roots	Gyp	Clipped	0.21±0.05	4.71±0.95	11.95±2.41	10.59±2.27	3.83±0.46	4.16±0.84	4.04±0.79	12.04±2.25
Helianthemum squamatum	Coarse roots	Gyp	Unclipped	0.31±0.06	4.75±1.05	10.37±1.25	10.42±1.81	3.71±0.29	3.69±0.58	4.10±0.87	11.32±1.82
Helianthemum squamatum	Fine roots	Calc	Clipped	2.29±0.35	53.22±5.83	37.04±2.52	33.49±3.53	24.14±2.59	52.40±3.47	50.89±5.73	40.51±2.77
Helianthemum squamatum	Fine roots	Calc	Unclipped	3.09±0.69	43.82±6.23	28.19±4.39	26.50±3.83	19.66±2.05	46.48±8.39	39.39±5.21	31.61±4.40
Helianthemum squamatum	Fine roots	Gyp	Clipped	1.69±0.28	44.43±3.20	36.02±0.90	37.95±3.05	20.63±1.73	58.35±3.17	41.77±3.25	40.97±1.22
Helianthemum squamatum	Fine roots	Gyp	Unclipped	2.28±0.32	41.49±4.99	38.48±2.80	38.34±4.74	20.21±2.60	57.57±3.50	38.22±4.53	42.93±2.92

Helianthemum squamatum	Leaves	Calc	Clipped	1.61±0.12	12.04±2.97	21.03±2.39	22.38±2.24	33.80±1.76	16.60±3.11	13.16±2.99	16.27±2.58
Helianthemum squamatum	Leaves	Calc	Unclipped	1.73±0.21	17.80±2.93	24.15±1.87	28.13±2.80	38.05±3.16	21.65±4.36	18.79±2.57	14.93±1.48
Helianthemum squamatum	Leaves	Gyp	Clipped	1.45±0.11	19.98±2.86	24.90±1.89	28.00±1.91	35.51±1.33	20.44±2.55	19.63±2.58	17.68±0.90
Helianthemum squamatum	Leaves	Gyp	Unclipped	1.61±0.26	20.45±5.03	23.44±4.97	26.92±5.88	35.04±1.67	19.88±4.61	20.60±4.78	15.16±3.37
Helianthemum squamatum	Roots	Calc	Clipped	2.38±0.27	32.17±3.93	30.78±1.41	32.31±2.52	37.51±1.23	27.18±1.47	33.33±3.63	30.98±1.03
Helianthemum squamatum	Roots	Calc	Unclipped	3.14±0.69	34.97±3.96	33.94±3.69	31.93±2.92	38.38±3.04	26.83±4.47	38.10±3.59	35.98±4.02
Helianthemum squamatum	Roots	Gyp	Clipped	1.38±0.12	30.88±2.34	27.13±1.78	23.46±2.44	40.03±1.12	17.05±2.07	34.57±2.49	29.31±2.25
Helianthemum squamatum	Roots	Gyp	Unclipped	2.15±0.67	33.32±2.88	27.72±2.22	24.33±2.35	41.05±3.06	18.86±1.74	37.08±3.52	30.59±2.00
Helianthemum squamatum	Stems	Calc	Clipped	1.07±0.13	4.82±2.39	4.61±0.31	4.63±1.48	5.89±1.73	4.08±1.12	4.72±2.31	4.36±0.84
Helianthemum squamatum	Stems	Calc	Unclipped	1.17±0.14	5.21±2.35	6.92±1.09	5.25±1.69	8.53±1.64	5.86±1.52	5.28±2.26	5.21±1.12
Helianthemum squamatum	Stems	Gyp	Clipped	0.85±0.10	4.77±1.74	4.26±0.55	2.98±1.56	6.55±0.39	2.90±1.44	4.17±1.78	3.16±0.36
Helianthemum squamatum	Stems	Gyp	Unclipped	1.18±0.08	7.86±3.51	5.03±0.80	4.10±2.34	6.04±0.47	3.61±1.93	5.87±3.32	3.37±0.69
Helianthemum syriacum	Coarse roots	Calc	Clipped	0.36±0.01	85.74±2.73	39.18±1.66	66.40±3.64	74.56±2.42	56.32±4.02	83.44±2.83	47.19±3.16
Helianthemum syriacum	Coarse roots	Calc	Unclipped	1.00±0.77	84.75±2.44	42.47±3.60	69.57±2.27	68.54±4.09	52.95±5.65	82.55±2.17	49.24±5.96
Helianthemum syriacum	Coarse roots	Gyp	Clipped	0.19±0.01	74.88±4.75	33.34±3.11	58.37±4.27	42.93±4.55	56.51±5.85	71.02±4.86	34.72±4.44
Helianthemum syriacum	Coarse roots	Gyp	Unclipped	0.36±0.18	66.37±11.24	30.32±6.50	56.29±11.51	33.37±7.62	49.94±10.43	63.54±11.55	32.90±7.30
Helianthemum syriacum	Fine roots	Calc	Clipped	4.13±0.36	2.49±0.15	32.99±1.71	19.50±1.59	5.05±0.97	23.95±1.60	3.63±0.31	32.12±4.17
Helianthemum syriacum	Fine roots	Calc	Unclipped	6.77±1.65	2.29±0.57	29.53±4.36	17.04±1.95	6.29±1.55	21.64±3.26	3.39±0.71	30.07±6.22
Helianthemum syriacum	Fine roots	Gyp	Clipped	2.61±0.77	6.98±1.48	38.73±3.00	26.68±2.00	12.98±0.84	24.66±2.92	8.99±1.70	43.69±4.83
Helianthemum syriacum	Fine roots	Gyp	Unclipped	2.78±0.52	7.42±3.95	36.37±5.95	26.83±8.65	13.81±2.25	26.31±6.63	8.98±4.22	39.97±6.50
Helianthemum syriacum	Leaves	Calc	Clipped	1.66±0.18	6.95±1.09	23.23±2.18	9.48±1.18	14.50±2.62	15.66±1.96	8.21±1.41	16.34±1.64
Helianthemum syriacum	Leaves	Calc	Unclipped	2.22±0.38	7.75±2.25	21.08±2.40	8.14±2.14	16.64±2.28	19.55±4.88	8.79±2.13	15.48±2.52
Helianthemum syriacum	Leaves	Gyp	Clipped	1.19±0.40	13.38±1.98	23.67±2.15	11.98±2.67	37.54±4.83	15.93±3.33	15.82±1.80	18.43±1.10
Helianthemum syriacum	Leaves	Gyp	Unclipped	1.54±0.20	19.04±4.59	28.32±1.69	12.85±1.67	46.67±6.30	20.21±3.09	22.02±4.65	23.74±1.47
Helianthemum syriacum	Roots	Calc	Clipped	7.52±0.00	1.41±0.93	4.54±0.46	1.11±0.92	2.15±0.73	1.11±0.80	1.31±0.95	1.69±0.46
Helianthemum syriacum	Roots	Calc	Unclipped	4.78±0.12	2.45±1.25	4.74±1.60	2.29±1.11	2.80±1.19	2.30±1.01	2.32±1.28	2.03±0.64
Helianthemum syriacum	Roots	Gyp	Clipped	2.62±0.10	4.39±2.17	4.46±0.68	5.12±1.74	5.77±0.52	4.19±1.92	4.40±2.06	4.80±1.10
Helianthemum syriacum	Roots	Gyp	Unclipped	3.09±1.85	3.07±1.82	14.25±9.62	19.88±14.78	18.30±12.84	18.19±15.00	20.54±16.93	12.11±7.54
Helianthemum syriacum	Stems	Calc	Clipped	1.07±0.10	91.54±1.34	55.23±2.18	82.85±1.34	88.04±2.23	74.40±0.81	91.03±1.25	53.82±3.85
Helianthemum syriacum	Stems	Calc	Unclipped	1.78±0.65	86.92±3.12	53.10±3.05	79.00±3.25	87.65±2.20	71.35±3.32	86.46±3.26	55.21±3.37
Helianthemum syriacum	Stems	Gyp	Clipped	0.79±0.15	82.83±0.88	49.93±4.67	74.08±2.94	65.28±0.55	76.12±2.16	80.57±0.80	50.07±6.18
Helianthemum syriacum	Stems	Gyp	Unclipped	0.79±0.14	80.93±4.24	40.65±5.74	57.66±11.84	53.11±10.91	60.56±12.01	63.32±15.02	43.09±4.92
Herniaria fruticosa	Coarse roots	Calc	Clipped	0.60±0.08	2.69±0.60	23.13±1.43	10.05±1.36	2.82±0.80	15.52±4.00	2.50±0.81	28.78±5.92
Herniaria fruticosa	Coarse roots	Calc	Unclipped	0.81±0.11	3.86±0.80	24.31±1.32	11.85±1.59	2.65±0.36	20.09±2.64	4.03±0.81	33.02±2.24
Herniaria fruticosa	Coarse roots	Gyp	Clipped	0.62±0.13	4.65±1.44	25.96±5.53	14.04±3.42	8.76±1.64	14.77±3.11	5.78±1.67	33.01±7.03
Herniaria fruticosa	Coarse roots	Gyp	Unclipped	0.67±0.10	7.26±1.61	30.52±6.49	17.49±5.27	12.92±1.50	17.88±5.96	8.56±1.95	36.43±8.37
Herniaria fruticosa	Fine roots	Calc	Clipped	1.98±0.18	4.36±0.71	16.21±0.65	5.16±0.49	6.54±1.49	4.96±0.32	4.66±0.64	9.42±0.83
Herniaria fruticosa	Fine roots	Calc	Unclipped	1.72±0.32	6.77±1.22	17.85±2.07	6.86±1.07	6.90±0.95	6.26±0.44	7.19±1.32	9.74±1.47
Herniaria fruticosa	Fine roots	Gyp	Clipped	1.94±0.25	8.13±0.81	20.89±1.17	6.77±0.47	20.19±1.46	4.92±0.45	9.25±0.59	12.12±0.50
Herniaria fruticosa	Fine roots	Gyp	Unclipped	2.59±0.49	8.74±1.84	16.74±3.06	4.97±1.25	15.67±2.73	3.38±0.80	7.58±1.91	8.36±1.71
Herniaria fruticosa	Leaves	Calc	Clipped	1.05±0.13	5.07±0.95	27.40±2.91	6.93±1.26	6.68±1.42	10.08±1.82	5.11±1.12	19.61±3.02
Herniaria fruticosa	Leaves	Calc	Unclipped	1.39±0.14	2.24±0.28	15.73±1.55	3.76±0.18	4.42±0.64	5.03±1.33	2.20±0.23	10.70±1.32
Herniaria fruticosa	Leaves	Gyp	Clipped	1.23±0.15	4.32±0.71	24.97±3.00	5.25±0.91	4.98±0.72	7.92±1.30	4.08±0.64	15.46±2.18

Herniaria fruticosa	Leaves	Gyp	Unclipped	1.42±0.28	2.19±0.72	12.64±4.39	2.98±1.17	3.60±0.97	3.75±1.54	2.23±0.69	9.26±2.83
Herniaria fruticosa	Stems	Calc	Clipped	1.64±0.12	27.62±4.32	28.59±2.74	37.75±5.49	14.11±1.92	51.84±3.99	27.90±5.02	42.67±3.40
Herniaria fruticosa	Stems	Calc	Unclipped	2.07±0.38	24.22±4.77	32.03±2.86	30.33±5.14	13.31±2.20	49.02±5.57	21.89±4.22	45.87±4.16
Herniaria fruticosa	Stems	Gyp	Clipped	1.41±0.18	25.37±1.65	28.60±3.15	37.38±2.60	8.86±1.01	46.81±1.41	21.43±1.29	40.39±2.61
Herniaria fruticosa	Stems	Gyp	Unclipped	1.81±0.35	19.07±1.77	32.96±3.56	38.54±3.22	9.39±0.81	54.80±3.00	17.44±1.42	46.16±3.44
Lepidium subulatum	Coarse roots	Calc	Clipped	2.30±0.09	13.33±1.15	8.45±0.67	19.12±2.34	37.76±2.94	13.15±1.40	15.48±1.83	8.21±1.14
Lepidium subulatum	Coarse roots	Calc	Unclipped	0.84±0.31	13.91±3.62	9.36±1.28	21.70±3.11	35.07±3.99	15.22±2.61	15.83±3.68	8.75±1.75
Lepidium subulatum	Coarse roots	Gyp	Clipped	1.67±0.16	18.08±1.08	11.65±0.38	30.32±1.95	38.55±1.80	19.09±0.75	19.60±0.85	13.69±0.50
Lepidium subulatum	Coarse roots	Gyp	Unclipped	0.54±0.22	15.44±1.37	11.30±1.34	23.63±1.45	39.44±1.52	14.23±1.11	16.84±1.69	9.98±1.17
Lepidium subulatum	Fine roots	Calc	Clipped	3.49±0.21	53.98±3.16	35.56±1.12	36.20±2.80	41.45±0.91	24.94±2.63	51.51±3.19	29.52±0.98
Lepidium subulatum	Fine roots	Calc	Unclipped	1.85±0.25	59.64±2.30	42.88±2.31	44.21±3.19	47.21±4.19	30.73±3.60	60.08±2.26	34.68±3.26
Lepidium subulatum	Fine roots	Gyp	Clipped	2.83±0.17	52.23±1.23	34.78±1.61	27.05±1.07	47.62±1.65	26.19±0.58	54.89±0.99	30.46±2.60
Lepidium subulatum	Fine roots	Gyp	Unclipped	1.42±0.11	63.30±0.51	43.09±1.09	34.84±1.03	47.57±2.38	27.23±1.68	63.50±0.86	34.60±1.29
Lepidium subulatum	Leaves	Calc	Clipped	1.03±0.09	3.22±2.23	12.11±5.19	7.62±2.95	7.70±1.96	4.88±3.24	2.97±1.62	8.52±4.54
Lepidium subulatum	Leaves	Calc	Unclipped	0.56±0.04	0.71±0.21	4.83±0.55	3.15±0.64	6.07±0.63	1.28±0.20	1.09±0.15	2.56±1.00
Lepidium subulatum	Leaves	Gyp	Clipped	1.10±0.07	2.73±0.66	6.58±0.67	4.19±0.49	12.67±2.97	1.51±0.26	2.85±0.76	3.03±0.90
Lepidium subulatum	Leaves	Gyp	Unclipped	0.50±0.04	1.50±0.26	5.53±0.71	3.36±0.65	7.96±1.99	1.10±0.15	1.49±0.30	2.78±0.95
Lepidium subulatum	Stems	Calc	Clipped	4.30±0.23	77.54±10.86	46.93±10.41	59.58±10.96	54.41±11.67	72.08±12.50	72.13±11.06	56.14±10.45
Lepidium subulatum	Stems	Calc	Unclipped	2.41±0.21	90.52±1.97	64.00±2.26	77.23±2.29	68.10±3.85	84.76±2.35	85.83±2.52	72.47±7.56
Lepidium subulatum	Stems	Gyp	Clipped	3.42±0.22	81.85±2.65	58.85±1.89	68.39±1.86	42.87±6.73	85.42±2.31	78.42±2.50	75.70±8.14
Lepidium subulatum	Stems	Gyp	Unclipped	1.77±0.12	81.93±4.30	62.27±1.78	71.01±5.15	46.60±8.66	86.30±2.94	80.73±3.85	73.67±7.72
Linum suffruticosum	Coarse roots	Calc	Clipped	0.80±0.27	2.99±1.39	9.08±0.96	14.17±3.04	10.32±2.43	8.60±2.96	4.95±1.66	16.54±5.73
Linum suffruticosum	Coarse roots	Calc	Unclipped	0.52±0.08	0.88±0.11	6.91±0.87	8.62±2.50	6.24±1.12	5.48±2.51	2.66±1.40	12.26±6.80
Linum suffruticosum	Coarse roots	Gyp	Clipped	0.50±0.08	2.22±0.31	8.32±0.62	12.65±2.06	11.68±1.28	5.78±2.44	3.82±1.15	11.82±6.75
Linum suffruticosum	Coarse roots	Gyp	Unclipped	0.54±0.08	1.93±0.76	6.63±1.81	8.03±2.80	9.61±1.21	4.49±2.76	2.96±1.39	10.10±7.18
Linum suffruticosum	Fine roots	Calc	Clipped	3.97±0.99	16.24±7.27	31.19±5.02	18.63±5.82	27.57±8.27	14.45±7.35	19.95±8.27	18.79±4.61
Linum suffruticosum	Fine roots	Calc	Unclipped	7.42±0.51	7.88±1.87	23.93±2.28	11.01±1.40	19.58±2.82	8.48±1.83	10.41±2.04	12.71±0.97
Linum suffruticosum	Fine roots	Gyp	Clipped	4.69±0.53	13.21±1.87	23.99±1.59	14.76±2.13	32.78±3.87	7.30±1.51	14.90±2.12	9.45±0.70
Linum suffruticosum	Fine roots	Gyp	Unclipped	6.86±0.62	14.64±3.99	29.16±3.75	17.59±4.76	35.83±8.69	8.12±2.39	14.82±3.84	13.45±1.87
Linum suffruticosum	Leaves	Calc	Clipped	0.73±0.03	5.93±1.83	19.64±3.06	8.22±2.07	7.25±0.58	7.32±2.10	5.11±1.34	24.50±3.53
Linum suffruticosum	Leaves	Calc	Unclipped	0.82±0.11	4.39±1.18	18.76±2.50	7.12±1.61	8.28±1.31	6.37±1.59	4.05±0.80	20.36±3.95
Linum suffruticosum	Leaves	Gyp	Clipped	0.68±0.09	6.88±1.70	32.04±17.65	6.64±2.20	7.27±0.88	6.21±3.73	4.24±1.85	14.40±4.92
Linum suffruticosum	Leaves	Gyp	Unclipped	0.69±0.15	5.83±1.32	14.29±2.67	6.51±1.76	7.07±1.17	6.64±1.31	5.35±1.09	17.27±3.38
Linum suffruticosum	Stems	Calc	Clipped	2.26±0.27	51.98±6.58	27.26±1.61	41.39±3.62	26.70±7.40	61.84±5.71	45.79±7.15	43.37±3.20
Linum suffruticosum	Stems	Calc	Unclipped	2.49±0.18	58.18±7.60	28.33±3.73	46.40±6.12	30.89±4.19	59.18±5.50	49.79±7.75	44.00±5.17
Linum suffruticosum	Stems	Gyp	Clipped	1.88±0.22	36.44±5.58	16.20±8.16	42.96±13.74	32.74±17.13	55.49±13.93	47.36±17.51	35.78±8.16
Linum suffruticosum	Stems	Gyp	Unclipped	2.41±0.26	44.74±1.43	29.99±4.71	38.99±5.00	15.29±2.61	55.70±5.62	37.32±1.25	43.21±5.93
Matthiola fruticulosa	Coarse roots	Calc	Clipped	1.01±0.16	5.23±2.97	7.05±1.23	23.28±4.09	16.65±4.82	4.55±1.19	6.11±2.82	5.85±0.34
Matthiola fruticulosa	Coarse roots	Calc	Unclipped	1.27±0.21	2.41±0.52	5.95±0.67	17.83±1.42	13.20±1.94	4.12±0.35	3.50±0.65	7.43±1.18
Matthiola fruticulosa	Coarse roots	Gyp	Clipped	0.61±0.26	4.63±0.53	11.08±5.21	24.80±6.12	15.73±3.18	9.74±3.21	7.00±0.70	19.05±10.03
Matthiola fruticulosa	Coarse roots	Gyp	Unclipped	0.77±0.16	4.48±0.88	7.62±1.55	26.70±4.89	18.04±2.54	6.35±1.52	5.72±1.13	8.07±1.67
Matthiola fruticulosa	Fine roots	Calc	Clipped	1.34±0.17	36.87±4.48	46.05±1.79	27.11±3.30	49.40±4.61	26.29±3.34	42.99±5.42	26.28±1.48
Matthiola fruticulosa	Fine roots	Calc	Unclipped	1.83±0.48	35.02±6.35	46.97±3.34	28.65±5.67	47.64±3.90	30.34±4.75	42.66±6.88	28.21±3.70

Matthiola fruticulosa	Fine roots	Gyp	Clipped	1.12±0.10	52.04±4.32	41.75±17.24	25.60±11.35	44.25±14.32	28.57±13.64	41.40±16.47	30.77±11.48
Matthiola fruticulosa	Fine roots	Gyp	Unclipped	1.77±0.36	44.95±2.12	48.10±4.52	27.80±1.04	59.59±4.87	31.31±4.67	51.61±2.14	31.45±3.14
Matthiola fruticulosa	Leaves	Calc	Clipped	0.39±0.07	2.10±0.50	17.72±1.49	5.69±1.20	1.07±0.11	6.92±1.22	1.81±0.45	17.43±2.12
Matthiola fruticulosa	Leaves	Calc	Unclipped	0.45±0.08	1.90±0.46	18.90±1.76	5.38±1.44	1.79±0.44	8.55±1.16	1.72±0.43	18.71±3.78
Matthiola fruticulosa	Leaves	Gyp	Clipped	0.32±0.03	3.57±0.53	18.69±2.03	5.86±0.91	2.63±0.21	7.94±1.43	3.64±0.54	17.97±3.14
Matthiola fruticulosa	Leaves	Gyp	Unclipped	0.52±0.09	5.20±1.11	22.47±1.64	7.89±1.84	2.64±0.12	9.65±1.61	4.71±0.90	22.93±3.39
Matthiola fruticulosa	Stems	Calc	Clipped	2.30±0.25	70.17±5.70	24.98±1.74	52.42±9.61	45.97±12.58	50.95±4.47	64.31±7.16	29.73±2.61
Matthiola fruticulosa	Stems	Calc	Unclipped	2.69±0.31	78.39±5.13	23.58±3.19	59.58±10.10	44.44±8.12	53.29±2.19	73.78±6.04	29.57±3.71
Matthiola fruticulosa	Stems	Gyp	Clipped	2.32±0.58	77.83±2.82	25.71±1.59	62.89±4.94	34.55±1.31	55.82±3.73	69.47±3.57	29.13±3.21
Matthiola fruticulosa	Stems	Gyp	Unclipped	2.72±0.48	65.37±6.93	24.71±3.42	59.65±6.25	25.49±4.14	45.71±5.87	57.19±7.36	29.30±5.09
Ononis tridentata	Coarse roots	Calc	Clipped	3.87±1.61	2.07±0.69	12.30±1.37	16.08±2.87	7.43±1.50	8.63±1.91	3.00±0.98	18.12±1.59
Ononis tridentata	Coarse roots	Calc	Unclipped	4.70±1.41	2.19±0.55	12.77±2.26	16.42±3.78	6.47±0.81	9.94±2.08	2.94±0.64	20.47±3.18
Ononis tridentata	Coarse roots	Gyp	Clipped	3.44±0.54	2.36±0.43	14.19±0.86	20.44±3.05	6.83±0.81	14.52±1.75	3.82±0.64	22.58±3.11
Ononis tridentata	Coarse roots	Gyp	Unclipped	4.80±0.75	2.75±0.69	13.17±1.65	19.08±2.97	5.91±0.64	12.96±1.09	3.89±0.75	22.45±4.43
Ononis tridentata	Fine roots	Calc	Clipped	6.08±1.34	25.66±5.33	44.99±1.60	25.80±7.31	45.53±13.76	33.51±3.44	30.88±6.95	34.72±3.90
Ononis tridentata	Fine roots	Calc	Unclipped	7.74±1.08	17.53±4.63	44.75±3.16	18.62±6.06	47.30±8.29	28.22±3.50	21.55±5.50	31.26±4.52
Ononis tridentata	Fine roots	Gyp	Clipped	6.70±0.77	16.23±2.29	41.41±1.20	10.80±1.66	55.99±1.51	21.72±2.83	23.07±2.95	30.32±3.00
Ononis tridentata	Fine roots	Gyp	Unclipped	7.41±1.50	26.68±5.57	39.65±4.15	13.38±2.80	65.96±4.41	31.68±4.45	34.20±6.22	25.32±4.09
Ononis tridentata	Leaves	Calc	Clipped	2.71±0.75	1.08±0.30	7.89±1.44	2.17±0.51	5.05±1.24	2.99±0.74	1.20±0.37	3.47±0.62
Ononis tridentata	Leaves	Calc	Unclipped	3.52±0.41	1.11±0.25	6.60±1.32	2.36±0.64	4.71±0.38	2.98±0.66	1.17±0.23	2.90±0.51
Ononis tridentata	Leaves	Gyp	Clipped	3.74±0.41	1.81±0.43	8.36±1.90	2.08±0.51	5.71±0.88	2.88±0.30	1.98±0.51	5.82±1.08
Ononis tridentata	Leaves	Gyp	Unclipped	3.72±0.35	1.59±0.32	7.73±1.10	2.00±0.39	4.77±0.49	2.75±0.50	1.72±0.31	5.35±1.21
Ononis tridentata	Stems	Calc	Clipped	9.05±3.00	91.63±1.67	50.42±2.79	84.62±1.91	74.29±2.58	81.20±3.19	89.35±1.82	69.96±3.25
Ononis tridentata	Stems	Calc	Unclipped	11.14±3.18	91.55±1.22	55.29±2.49	85.04±2.07	76.45±3.21	81.01±3.05	90.02±1.08	74.67±1.57
Ononis tridentata	Stems	Gyp	Clipped	7.35±0.69	81.89±2.03	41.80±4.95	79.30±2.79	63.64±2.99	83.63±2.14	80.70±2.45	57.76±5.98
Ononis tridentata	Stems	Gyp	Unclipped	8.44±1.25	79.67±0.97	44.38±1.91	75.83±1.67	59.15±2.97	84.19±0.63	78.67±0.95	62.69±1.63
Rosmarinus officinalis	Coarse roots	Calc	Clipped	1.90±0.27	3.75±1.14	27.79±1.32	9.69±1.26	3.83±0.99	8.61±1.85	4.82±1.35	19.84±2.48
Rosmarinus officinalis	Coarse roots	Calc	Unclipped	2.20±0.83	2.90±0.35	24.02±1.42	8.95±1.16	3.15±0.37	6.87±0.82	3.31±0.39	15.47±1.00
Rosmarinus officinalis	Coarse roots	Gyp	Clipped	1.05±0.18	5.50±1.26	27.86±5.25	11.80±2.63	4.02±0.60	6.71±1.55	5.86±1.28	25.71±5.28
Rosmarinus officinalis	Coarse roots	Gyp	Unclipped	1.43±0.20	7.17±0.84	32.53±2.44	15.80±2.07	6.27±0.78	7.82±0.99	8.11±1.13	24.14±3.16
Rosmarinus officinalis	Fine roots	Calc	Clipped	15.07±1.47	3.54±0.51	13.90±1.19	3.52±0.98	16.83±2.10	7.19±0.98	4.63±0.45	6.74±0.69
Rosmarinus officinalis	Fine roots	Calc	Unclipped	18.92±2.10	4.44±0.91	14.09±1.19	3.65±0.75	15.69±2.73	9.14±1.81	5.51±0.65	6.96±0.76
Rosmarinus officinalis	Fine roots	Gyp	Clipped	6.56±0.53	10.81±0.77	21.98±3.45	6.82±0.88	26.63±2.96	6.79±1.09	11.47±1.59	10.71±2.57
Rosmarinus officinalis	Fine roots	Gyp	Unclipped	9.89±0.62	11.57±0.68	15.36±1.04	6.37±0.83	29.81±2.20	5.24±0.27	11.50±0.66	7.83±1.10
Rosmarinus officinalis	Leaves	Calc	Clipped	6.52±0.59	0.30±0.04	521.80±5.35	11.25±0.50	0.00±0.00	0.01±0.00	0.25±0.04	12.12±0.95
Rosmarinus officinalis	Leaves	Calc	Unclipped	6.75±1.12	0.30±0.01	524.00±8.05	12.02±0.77	0.00±0.00	0.01±0.00	0.21±0.02	12.08±0.65
Rosmarinus officinalis	Leaves	Gyp	Clipped	3.63±0.81	0.31±0.03	520.00±4.24	11.76±1.41	0.00±0.00	0.01±0.00	0.21±0.03	11.12±0.87
Rosmarinus officinalis	Leaves	Gyp	Unclipped	5.74±0.61	0.36±0.01	517.00±4.09	14.56±0.65	0.00±0.00	0.01±0.00	0.24±0.01	8.18±0.87
Rosmarinus officinalis	Stems	Calc	Clipped	3.61±0.48	0.57±0.07	479.00±0.71	7.78±2.28	0.02±0.00	0.01±0.00	0.48±0.03	7.71±0.63
Rosmarinus officinalis	Stems	Calc	Unclipped	4.51±1.07	0.74±0.18	475.60±2.98	7.87±1.59	0.02±0.00	0.01±0.00	0.58±0.08	8.36±0.53
Rosmarinus officinalis	Stems	Gyp	Clipped	2.93±0.48	0.79±0.08	478.60±0.81	8.13±0.42	0.01±0.00	0.01±0.00	0.52±0.08	5.31±0.62
Rosmarinus officinalis	Stems	Gyp	Unclipped	2.93±0.27	1.15±0.05	476.80±2.03	11.59±1.02	0.02±0.00	0.01±0.00	0.69±0.02	5.08±0.42

Table D.10 (continued)

Species	Organ	Soil	Clipping	Mg	Mn	N	Na	P	S	Si	Zn
Boleum asperum	Coarse roots	Calc	Clipped	8.49±1.94	18.50±4.24	10.82±3.09	10.54±2.89	2.18±0.70	8.23±2.16	8.14±1.87	12.86±4.50
Boleum asperum	Coarse roots	Calc	Unclipped	13.90±8.86	25.02±12.74	17.64±11.51	16.01±9.15	2.08±0.84	14.29±9.61	14.59±10.19	18.05±11.59
Boleum asperum	Coarse roots	Gyp	Clipped	4.74±1.92	10.82±3.72	6.24±2.45	6.17±2.46	1.75±0.23	4.21±2.23	7.06±2.67	7.49±2.79
Boleum asperum	Coarse roots	Gyp	Unclipped	4.45±1.38	10.36±3.56	5.35±1.20	6.26±2.38	1.64±0.19	3.27±1.16	5.44±1.41	5.48±2.86
Boleum asperum	Fine roots	Calc	Clipped	44.85±3.67	46.86±0.96	55.32±6.16	69.62±6.07	2.34±0.68	62.70±7.22	48.38±0.40	60.67±4.30
Boleum asperum	Fine roots	Calc	Unclipped	47.97±8.44	43.20±9.00	50.53±8.76	68.02±10.54	2.89±0.66	58.22±9.45	47.64±10.03	55.65±10.55
Boleum asperum	Fine roots	Gyp	Clipped	47.22±3.27	48.96±0.67	60.38±5.10	73.03±2.80	2.51±0.48	66.33±6.83	49.60±4.38	57.49±8.67
Boleum asperum	Fine roots	Gyp	Unclipped	54.49±3.20	51.53±3.10	57.87±1.86	73.63±4.64	2.08±0.43	71.76±1.59	53.32±1.19	60.58±6.34
Boleum asperum	Leaves	Calc	Clipped	27.72±2.75	8.63±2.38	13.74±1.52	4.53±1.35	3.59±1.76	9.99±2.34	5.78±0.21	7.85±0.70
Boleum asperum	Leaves	Calc	Unclipped	19.31±1.16	7.88±0.70	11.50±0.22	2.82±0.60	3.83±2.00	9.12±1.08	4.76±0.27	9.87±1.90
Boleum asperum	Leaves	Gyp	Clipped	29.25±3.35	16.71±2.40	17.69±3.58	6.00±0.28	2.38±0.40	16.05±4.15	10.95±0.89	16.27±5.27
Boleum asperum	Leaves	Gyp	Unclipped	25.10±3.84	13.99±3.00	19.90±2.74	6.06±1.58	2.03±0.17	14.55±2.26	9.30±2.03	17.55±2.15
Boleum asperum	Stems	Calc	Clipped	18.94±1.47	26.01±3.02	20.13±2.07	15.31±3.33	1.27±0.35	19.08±3.16	37.70±2.47	18.62±0.42
Boleum asperum	Stems	Calc	Unclipped	18.82±2.38	23.91±3.16	20.33±3.89	13.15±3.61	1.90±0.56	18.36±2.40	33.01±4.98	16.44±1.74
Boleum asperum	Stems	Gyp	Clipped	18.80±2.53	23.51±1.53	15.69±3.26	14.79±2.09	1.86±0.43	13.41±3.44	32.39±3.79	18.74±5.02
Boleum asperum	Stems	Gyp	Unclipped	15.96±1.36	24.11±2.52	16.88±0.73	14.06±1.52	1.36±0.35	10.42±0.62	31.94±1.60	16.39±4.00
Gypsophila struthium	Coarse roots	Calc	Clipped	4.78±0.66	6.31±0.68	14.30±2.84	16.53±2.50	2.48±0.52	9.32±1.38	7.54±1.76	22.85±4.03
Gypsophila struthium	Coarse roots	Calc	Unclipped	6.19±0.51	8.12±1.19	15.65±2.93	17.30±2.54	3.39±0.56	10.81±0.24	9.05±1.50	21.24±1.35
Gypsophila struthium	Coarse roots	Gyp	Clipped	7.26±1.05	11.20±1.51	18.79±2.74	21.63±3.91	1.13±0.32	5.71±0.76	12.40±2.03	24.68±4.36
Gypsophila struthium	Coarse roots	Gyp	Unclipped	10.09±2.15	17.76±4.22	21.04±4.39	25.01±5.06	0.84±0.12	8.89±2.62	15.90±3.23	27.48±5.58
Gypsophila struthium	Fine roots	Calc	Clipped	39.44±2.73	63.62±2.56	50.29±3.37	75.29±2.08	2.13±0.57	27.89±2.99	62.06±1.58	55.90±4.72
Gypsophila struthium	Fine roots	Calc	Unclipped	40.20±5.73	61.08±5.37	48.99±6.66	73.46±4.10	3.07±0.55	27.30±5.21	54.78±5.79	50.40±4.61
Gypsophila struthium	Fine roots	Gyp	Clipped	43.93±5.25	46.02±4.18	52.05±4.82	65.77±4.64	0.87±0.10	69.01±3.20	56.89±2.48	49.92±4.90
Gypsophila struthium	Fine roots	Gyp	Unclipped	39.42±6.58	42.26±4.24	43.98±5.23	60.59±5.13	0.82±0.09	61.28±7.53	48.98±5.34	48.68±6.07
Gypsophila struthium	Leaves	Calc	Clipped	45.49±3.58	16.82±2.38	20.66±2.11	2.65±0.40	4.01±1.30	46.28±4.77	7.28±0.99	6.41±1.61
Gypsophila struthium	Leaves	Calc	Unclipped	37.94±5.32	13.89±2.87	15.57±3.33	2.19±0.87	5.85±1.75	38.11±6.35	5.34±1.62	6.44±3.16
Gypsophila struthium	Leaves	Gyp	Clipped	34.09±6.17	22.06±5.09	14.51±2.68	2.59±0.81	1.17±0.36	18.04±4.08	7.60±1.77	10.84±4.12
Gypsophila struthium	Leaves	Gyp	Unclipped	32.56±5.17	16.84±1.92	14.29±2.32	2.23±0.45	0.96±0.12	22.18±5.40	5.97±1.11	8.15±1.45
Gypsophila struthium	Stems	Calc	Clipped	10.30±0.83	13.26±1.16	14.75±0.88	5.53±0.80	2.49±0.72	16.51±1.33	23.12±1.90	14.85±1.41
Gypsophila struthium	Stems	Calc	Unclipped	15.67±0.96	16.90±2.01	19.79±1.43	7.06±0.79	3.13±0.80	23.78±1.39	30.82±3.74	21.92±2.28
Gypsophila struthium	Stems	Gyp	Clipped	14.73±1.53	20.72±2.38	14.65±1.37	10.01±1.61	0.86±0.23	7.23±1.33	23.11±2.21	14.56±2.82
Gypsophila struthium	Stems	Gyp	Unclipped	17.92±1.79	23.15±1.69	20.69±1.39	12.17±0.96	0.57±0.08	7.64±1.19	29.15±2.13	15.68±0.77
Helianthemum squamatum	Coarse roots	Calc	Clipped	8.07±0.57	9.24±1.43	14.36±1.38	6.76±0.69	1.38±0.10	7.62±0.90	4.85±0.64	19.46±1.68
Helianthemum squamatum	Coarse roots	Calc	Unclipped	11.08±1.48	13.56±2.64	19.26±2.70	11.14±2.61	1.31±0.09	9.63±1.40	5.74±0.71	17.86±3.52
Helianthemum squamatum	Coarse roots	Gyp	Clipped	7.54±1.00	11.85±2.91	14.90±2.36	7.23±0.73	1.12±0.03	6.07±1.68	8.64±1.87	12.36±1.83
Helianthemum squamatum	Coarse roots	Gyp	Unclipped	6.90±1.46	10.57±1.90	15.41±1.86	6.24±1.08	0.82±0.04	5.31±1.29	7.08±1.27	12.71±2.51
Helianthemum squamatum	Fine roots	Calc	Clipped	44.45±3.54	42.66±1.65	41.50±2.57	58.12±4.08	1.42±0.03	22.89±2.57	44.25±3.80	34.26±2.19
Helianthemum squamatum	Fine roots	Calc	Unclipped	29.91±4.73	31.63±4.56	31.77±3.63	47.25±5.86	1.48±0.68	18.88±2.91	36.07±5.71	23.03±4.51
Helianthemum squamatum	Fine roots	Gyp	Clipped	40.08±2.03	36.93±2.47	39.51±0.74	50.75±1.28	0.75±0.19	54.69±5.89	41.92±0.73	36.66±5.33
Helianthemum squamatum	Fine roots	Gyp	Unclipped	41.27±3.67	34.67±4.08	37.85±3.59	52.79±4.28	0.47±0.05	55.77±7.67	44.09±3.88	28.18±5.01

Helianthemum squamatum	Leaves	Calc	Clipped	22.00±2.66	22.51±3.07	20.05±1.96	12.65±1.94	2.55±0.57	44.45±2.86	21.55±3.20	23.73±2.93
Helianthemum squamatum	Leaves	Calc	Unclipped	26.91±1.84	28.38±3.78	23.55±1.29	14.60±1.95	1.63±0.40	44.14±2.36	26.18±2.39	33.80±4.13
Helianthemum squamatum	Leaves	Gyp	Clipped	26.39±1.68	27.21±3.55	23.93±0.94	17.67±1.37	1.10±0.26	24.27±2.99	26.17±1.97	28.56±4.61
Helianthemum squamatum	Leaves	Gyp	Unclipped	25.17±5.84	29.64±6.39	23.37±4.81	17.65±4.58	0.67±0.03	24.07±6.58	24.40±5.22	32.68±8.09
Helianthemum squamatum	Roots	Calc	Clipped	25.47±0.98	25.59±1.23	24.09±1.04	22.47±2.19	2.18±0.26	25.04±1.31	29.35±1.92	22.55±1.11
Helianthemum squamatum	Roots	Calc	Unclipped	32.11±3.40	26.44±2.58	25.42±2.50	27.02±3.12	1.20±0.04	27.35±3.81	32.01±3.92	25.31±3.31
Helianthemum squamatum	Roots	Gyp	Clipped	25.99±2.10	24.01±1.99	21.66±1.56	24.34±1.91	0.83±0.18	14.97±2.42	23.28±1.25	22.42±3.37
Helianthemum squamatum	Roots	Gyp	Unclipped	26.66±1.70	25.12±1.73	23.36±1.41	23.33±2.52	0.52±0.04	14.84±2.25	24.43±1.59	26.43±1.30
Helianthemum squamatum	Stems	Calc	Clipped	3.34±0.98	2.97±0.89	5.11±0.31	5.79±1.38	1.05±0.05	3.00±0.13	4.13±0.71	6.39±1.63
Helianthemum squamatum	Stems	Calc	Unclipped	4.08±1.28	3.64±0.92	7.23±1.01	7.32±1.10	0.95±0.11	4.74±0.90	5.27±0.77	8.16±1.89
Helianthemum squamatum	Stems	Gyp	Clipped	2.51±1.15	2.92±0.45	4.63±0.49	7.31±1.23	0.81±0.06	3.46±1.07	4.62±0.53	4.51±1.02
Helianthemum squamatum	Stems	Gyp	Unclipped	3.01±1.52	2.51±0.72	5.24±0.84	8.65±1.50	0.60±0.05	5.06±1.45	5.78±0.80	6.45±0.89
Helianthemum syriacum	Coarse roots	Calc	Clipped	45.84±3.02	42.48±3.51	43.59±2.35	72.56±3.65	1.16±0.06	28.12±3.95	48.33±2.51	74.04±1.68
Helianthemum syriacum	Coarse roots	Calc	Unclipped	50.46±5.40	41.32±5.31	45.00±4.29	72.60±4.36	1.06±0.09	31.03±2.53	51.57±3.14	60.83±9.03
Helianthemum syriacum	Coarse roots	Gyp	Clipped	46.68±4.97	34.08±3.10	36.02±1.95	63.57±5.27	0.70±0.13	48.79±3.31	41.87±2.78	54.81±12.13
Helianthemum syriacum	Coarse roots	Gyp	Unclipped	47.01±10.61	30.95±7.09	32.13±7.19	57.01±11.34	0.82±0.04	47.44±10.27	36.65±7.81	46.40±11.18
Helianthemum syriacum	Fine roots	Calc	Clipped	39.09±2.06	40.47±2.03	37.72±2.97	12.94±2.22	1.64±0.18	57.07±4.63	26.57±1.53	5.57±0.87
Helianthemum syriacum	Fine roots	Calc	Unclipped	34.13±5.73	40.25±5.20	34.38±4.05	12.99±4.44	1.60±0.13	51.97±3.29	22.43±2.91	8.36±3.72
Helianthemum syriacum	Fine roots	Gyp	Clipped	38.21±4.43	49.17±2.45	44.80±2.30	17.29±3.57	0.54±0.06	40.99±2.46	30.11±2.61	7.48±1.52
Helianthemum syriacum	Fine roots	Gyp	Unclipped	34.41±9.29	49.59±7.44	45.28±7.03	19.16±7.82	0.56±0.05	38.97±8.92	29.88±6.22	7.43±1.63
Helianthemum syriacum	Leaves	Calc	Clipped	11.73±1.25	14.08±1.41	13.58±1.58	8.71±1.29	2.62±0.34	11.81±1.24	20.97±2.06	14.00±1.50
Helianthemum syriacum	Leaves	Calc	Unclipped	11.33±1.58	14.79±2.08	13.38±1.68	7.08±1.23	1.94±0.24	12.26±1.49	20.73±3.26	22.65±6.92
Helianthemum syriacum	Leaves	Gyp	Clipped	12.60±1.10	13.83±0.89	14.56±0.99	11.83±2.11	0.70±0.04	6.76±0.54	23.40±1.51	33.20±13.94
Helianthemum syriacum	Leaves	Gyp	Unclipped	15.59±1.53	16.95±1.16	17.39±0.89	15.17±2.56	0.72±0.08	8.62±0.99	27.70±1.96	39.69±9.17
Helianthemum syriacum	Roots	Calc	Clipped	1.34±0.81	1.37±0.50	4.69±0.44	4.03±0.38	1.86±0.00	2.07±0.39	2.65±0.45	2.74±0.57
Helianthemum syriacum	Roots	Calc	Unclipped	2.06±0.99	1.67±0.66	4.90±1.75	4.63±1.10	1.24±0.10	2.31±0.63	2.93±0.67	2.93±0.88
Helianthemum syriacum	Roots	Gyp	Clipped	4.22±2.04	3.50±1.28	4.56±0.70	5.97±1.20	0.60±0.10	4.14±2.11	4.33±1.19	4.26±1.40
Helianthemum syriacum	Roots	Gyp	Unclipped	17.69±14.89	11.80±9.50	13.27±8.47	5.69±1.51	0.63±0.12	2.33±1.17	3.15±0.53	3.68±1.09
Helianthemum syriacum	Stems	Calc	Clipped	75.80±1.85	58.85±1.31	57.35±2.94	78.05±1.65	0.88±0.08	45.58±4.06	65.90±1.96	79.36±1.89
Helianthemum syriacum	Stems	Calc	Unclipped	71.41±3.44	58.77±5.27	53.87±2.79	77.71±1.89	0.80±0.06	44.90±3.95	62.91±2.50	76.52±3.21
Helianthemum syriacum	Stems	Gyp	Clipped	78.32±2.52	52.66±5.53	51.24±5.45	74.31±3.48	0.44±0.03	76.82±3.52	59.33±4.47	61.64±4.68
Helianthemum syriacum	Stems	Gyp	Unclipped	62.68±13.47	42.02±3.94	44.09±6.65	72.76±4.24	0.54±0.06	70.29±6.97	54.68±6.45	63.06±4.09
Herniaria fruticosa	Coarse roots	Calc	Clipped	14.68±3.09	26.66±6.73	26.17±3.03	10.16±1.50	4.13±0.57	47.58±4.42	19.88±1.65	7.78±1.30
Herniaria fruticosa	Coarse roots	Calc	Unclipped	20.61±2.35	31.28±4.04	30.84±2.00	9.87±1.21	2.93±0.49	46.91±3.50	20.48±1.50	9.08±1.35
Herniaria fruticosa	Coarse roots	Gyp	Clipped	12.20±3.43	34.63±7.21	31.52±6.30	9.57±2.71	1.65±0.33	17.44±4.72	22.08±5.09	13.49±4.01
Herniaria fruticosa	Coarse roots	Gyp	Unclipped	16.08±6.75	40.02±9.29	33.70±9.49	11.92±1.75	2.52±0.38	25.82±5.87	29.07±5.95	16.91±3.42
Herniaria fruticosa	Fine roots	Calc	Clipped	4.56±0.67	7.15±0.24	8.80±0.58	7.76±0.84	2.31±0.64	4.77±0.43	11.57±0.64	10.12±1.09
Herniaria fruticosa	Fine roots	Calc	Unclipped	5.91±0.74	8.28±0.98	10.39±0.78	7.79±0.99	2.28±0.52	5.88±0.81	13.68±1.72	11.47±1.65
Herniaria fruticosa	Fine roots	Gyp	Clipped	5.26±0.64	9.20±0.83	12.82±0.55	10.15±1.42	0.83±0.14	1.60±0.16	14.26±0.54	20.62±2.46
Herniaria fruticosa	Fine roots	Gyp	Unclipped	3.55±1.02	6.15±1.23	11.48±1.75	9.62±2.39	1.18±0.23	1.56±0.23	13.09±0.80	16.35±2.62
Herniaria fruticosa	Leaves	Calc	Clipped	15.70±2.31	8.56±1.59	18.66±2.24	10.23±1.66	1.37±0.53	17.03±2.63	8.78±1.68	18.27±2.30
Herniaria fruticosa	Leaves	Calc	Unclipped	8.46±0.74	3.88±0.54	10.70±1.30	5.97±1.21	2.17±1.33	10.39±1.40	4.49±0.95	12.39±2.75
Herniaria fruticosa	Leaves	Gyp	Clipped	10.85±1.52	8.16±0.92	16.10±1.79	10.76±2.39	0.54±0.07	11.93±1.60	7.36±1.11	18.73±2.67

Herniaria fruticosa	Leaves	Gyp	Unclipped	7.35±2.40	3.79±1.32	10.12±3.14	4.35±1.68	0.73±0.12	8.00±2.69	4.37±1.30	9.16±3.50
Herniaria fruticosa	Stems	Calc	Clipped	44.46±3.91	25.55±5.56	35.94±3.39	54.55±2.89	1.42±0.52	47.14±3.90	35.34±3.29	29.91±6.25
Herniaria fruticosa	Stems	Calc	Unclipped	40.71±4.99	19.86±3.61	39.88±2.83	52.45±6.52	1.55±0.57	52.92±2.54	33.52±5.00	28.42±5.72
Herniaria fruticosa	Stems	Gyp	Clipped	36.65±2.35	18.79±1.33	35.87±4.02	38.11±3.07	0.59±0.13	49.59±3.75	34.94±3.64	24.29±1.67
Herniaria fruticosa	Stems	Gyp	Unclipped	35.79±2.33	15.97±0.64	34.36±3.99	53.56±7.83	0.96±0.28	51.73±3.70	32.44±2.45	30.03±4.64
Lepidium subulatum	Coarse roots	Calc	Clipped	11.09±1.05	22.45±1.86	16.44±1.36	6.07±0.77	7.04±0.49	19.51±3.01	11.45±1.10	12.59±0.85
Lepidium subulatum	Coarse roots	Calc	Unclipped	15.56±2.66	24.77±3.63	16.33±1.04	8.34±2.00	3.51±0.69	18.25±1.49	13.27±2.32	13.36±3.00
Lepidium subulatum	Coarse roots	Gyp	Clipped	18.35±0.44	32.22±1.25	22.64±2.43	12.59±1.22	5.72±0.14	24.02±2.92	16.32±1.04	22.90±1.41
Lepidium subulatum	Coarse roots	Gyp	Unclipped	15.37±0.66	28.31±1.13	21.51±2.89	8.98±1.30	3.45±0.30	20.19±2.69	14.25±1.38	17.47±2.61
Lepidium subulatum	Fine roots	Calc	Clipped	28.75±1.51	43.44±3.51	28.96±1.20	29.15±1.44	8.38±0.84	16.32±0.88	44.43±2.59	39.23±4.10
Lepidium subulatum	Fine roots	Calc	Unclipped	35.27±3.90	51.49±4.00	33.09±3.27	33.24±4.44	3.90±1.17	18.43±2.65	48.71±5.25	45.83±6.37
Lepidium subulatum	Fine roots	Gyp	Clipped	34.14±1.48	40.83±1.31	25.38±1.73	38.55±4.54	5.78±0.42	14.46±1.64	41.39±2.33	34.08±1.87
Lepidium subulatum	Fine roots	Gyp	Unclipped	41.49±0.78	51.92±1.13	34.01±0.67	33.11±5.52	2.90±0.16	20.08±1.58	48.94±1.82	43.35±2.72
Lepidium subulatum	Leaves	Calc	Clipped	5.91±3.64	3.25±1.52	10.07±4.85	17.37±8.60	4.22±0.16	7.28±3.90	5.38±2.71	7.37±3.80
Lepidium subulatum	Leaves	Calc	Unclipped	1.35±0.17	1.27±0.12	3.25±0.86	4.12±0.39	2.02±0.15	2.24±0.39	2.09±0.41	2.06±0.54
Lepidium subulatum	Leaves	Gyp	Clipped	2.26±0.43	3.18±1.30	5.34±0.71	5.89±0.58	3.71±0.16	1.57±0.18	4.65±0.70	3.83±1.13
Lepidium subulatum	Leaves	Gyp	Unclipped	1.44±0.11	1.88±0.50	4.54±0.43	5.31±0.73	1.85±0.06	1.05±0.23	3.89±0.41	3.28±0.71
Lepidium subulatum	Stems	Calc	Clipped	67.04±12.65	50.64±7.45	51.56±10.63	52.17±11.68	4.79±0.74	62.37±11.63	55.08±11.24	56.04±10.77
Lepidium subulatum	Stems	Calc	Unclipped	81.65±0.69	70.20±7.85	66.24±1.83	75.41±1.33	2.48±0.47	77.78±2.08	70.82±2.35	75.37±3.32
Lepidium subulatum	Stems	Gyp	Clipped	78.19±2.14	58.15±4.86	59.45±1.72	70.81±1.32	3.91±0.30	89.71±1.30	64.89±1.75	55.15±3.48
Lepidium subulatum	Stems	Gyp	Unclipped	81.59±3.67	62.95±8.41	63.02±2.94	73.60±3.43	2.05±0.09	92.48±1.55	69.33±2.62	47.28±5.47
Linum suffruticosum	Coarse roots	Calc	Clipped	11.07±1.91	26.50±5.97	13.85±1.94	6.08±1.33	1.45±0.13	11.83±2.91	10.14±1.63	10.12±1.47
Linum suffruticosum	Coarse roots	Calc	Unclipped	7.20±1.32	17.21±8.14	10.23±0.93	4.58±0.54	1.33±0.09	7.79±0.82	6.97±1.04	7.13±1.20
Linum suffruticosum	Coarse roots	Gyp	Clipped	8.94±1.20	21.90±7.18	12.76±0.90	5.68±0.52	0.66±0.06	4.06±0.64	7.77±0.67	12.03±1.96
Linum suffruticosum	Coarse roots	Gyp	Unclipped	5.62±1.72	16.38±8.39	10.56±2.45	3.62±0.98	0.73±0.09	2.57±1.09	6.19±1.78	13.00±3.80
Linum suffruticosum	Fine roots	Calc	Clipped	15.99±7.49	19.61±5.15	23.72±4.53	24.37±3.52	6.35±0.75	18.52±4.96	29.40±7.09	26.47±6.12
Linum suffruticosum	Fine roots	Calc	Unclipped	9.80±1.97	11.32±2.14	19.38±2.13	15.89±1.50	5.84±0.49	12.19±1.60	20.12±2.57	15.44±2.14
Linum suffruticosum	Fine roots	Gyp	Clipped	10.62±2.31	16.77±2.41	21.06±1.51	17.63±1.29	1.44±0.16	4.66±0.83	22.69±1.27	28.99±4.00
Linum suffruticosum	Fine roots	Gyp	Unclipped	11.34±4.04	18.79±6.75	25.98±4.51	17.46±3.00	1.39±0.19	3.90±0.73	20.59±1.26	36.45±4.65
Linum suffruticosum	Leaves	Calc	Clipped	13.87±2.92	5.97±1.51	14.53±2.72	15.79±3.59	2.40±0.76	18.47±3.51	7.52±1.80	13.01±3.13
Linum suffruticosum	Leaves	Calc	Unclipped	11.06±1.58	5.25±0.97	13.12±1.94	16.69±4.92	1.20±0.21	16.18±2.75	5.61±1.47	9.18±1.79
Linum suffruticosum	Leaves	Gyp	Clipped	6.92±3.34	4.09±1.79	24.16±13.88	12.77±5.47	1.04±0.14	13.37±4.05	6.97±1.82	21.94±10.66
Linum suffruticosum	Leaves	Gyp	Unclipped	8.49±2.03	4.91±1.13	10.17±1.64	12.76±3.38	0.90±0.11	11.56±2.96	6.62±1.48	9.82±1.94
Linum suffruticosum	Stems	Calc	Clipped	33.66±1.96	36.50±5.02	33.15±1.93	50.54±10.41	1.36±0.06	45.19±3.62	38.38±3.56	34.47±1.68
Linum suffruticosum	Stems	Calc	Unclipped	38.56±3.37	44.92±6.35	38.19±3.49	57.79±8.37	1.17±0.12	49.03±3.61	40.12±5.45	40.60±5.16
Linum suffruticosum	Stems	Gyp	Clipped	49.19±17.79	30.75±7.22	19.89±9.38	57.96±17.30	0.74±0.09	45.94±4.07	26.75±4.94	24.57±7.19
Linum suffruticosum	Stems	Gyp	Unclipped	40.76±5.01	31.32±3.32	35.59±3.25	58.10±6.67	0.98±0.21	57.78±4.95	36.92±2.53	38.68±5.35
Matthiola fruticulosa	Coarse roots	Calc	Clipped	27.50±3.05	18.98±4.56	18.10±1.85	16.40±12.50	4.30±1.03	15.60±2.10	8.01±1.94	13.83±2.34
Matthiola fruticulosa	Coarse roots	Calc	Unclipped	22.56±2.98	12.20±1.85	14.92±0.82	2.80±0.63	3.28±0.23	14.27±1.33	5.46±0.51	10.32±1.45
Matthiola fruticulosa	Coarse roots	Gyp	Clipped	17.00±2.99	28.31±10.57	19.41±5.06	2.52±0.76	1.01±0.16	18.93±3.77	7.98±1.71	10.65±2.17
Matthiola fruticulosa	Coarse roots	Gyp	Unclipped	21.24±4.86	21.10±4.53	17.97±3.55	4.19±1.11	1.18±0.17	15.00±2.61	8.64±1.84	13.44±3.37
Matthiola fruticulosa	Fine roots	Calc	Clipped	24.97±1.87	38.55±4.95	34.22±1.53	17.27±4.06	5.73±1.20	20.74±1.52	46.10±1.94	38.69±1.38
Matthiola fruticulosa	Fine roots	Calc	Unclipped	27.82±4.50	37.63±6.61	33.77±3.50	22.72±5.11	5.39±0.44	20.51±2.52	48.81±4.75	39.89±5.90

Matthiola fruticulosa	Fine roots	Gyp	Clipped	26.89±12.66	36.85±15.46	38.34±11.61	26.75±11.13	1.01±0.13	21.76±4.08	58.31±4.74	42.84±2.96
Matthiola fruticulosa	Fine roots	Gyp	Unclipped	29.51±2.64	42.66±1.89	36.27±2.20	24.95±4.80	1.47±0.30	15.66±2.23	47.82±2.43	38.06±4.75
Matthiola fruticulosa	Leaves	Calc	Clipped	7.66±1.59	2.15±0.39	14.48±2.11	26.15±3.82	3.65±0.31	5.90±1.00	5.76±1.05	8.56±1.08
Matthiola fruticulosa	Leaves	Calc	Unclipped	7.29±1.47	2.04±0.38	16.37±2.00	20.85±3.70	2.78±0.15	5.76±1.17	5.86±0.91	9.80±2.58
Matthiola fruticulosa	Leaves	Gyp	Clipped	5.46±0.92	3.08±0.48	17.28±1.92	19.53±3.00	1.37±0.16	2.70±0.42	8.89±1.50	10.27±2.15
Matthiola fruticulosa	Leaves	Gyp	Unclipped	7.91±1.17	4.25±0.61	19.60±2.26	24.94±3.81	1.84±0.26	3.74±0.74	9.62±1.02	15.23±2.68
Matthiola fruticulosa	Stems	Calc	Clipped	23.35±3.18	47.12±5.37	36.63±1.80	45.80±5.31	2.84±0.68	19.46±4.13	33.95±5.10	35.88±4.69
Matthiola fruticulosa	Stems	Calc	Unclipped	25.06±4.30	53.44±6.19	32.95±1.74	57.65±3.42	2.50±0.15	20.50±4.68	37.39±5.57	39.01±5.50
Matthiola fruticulosa	Stems	Gyp	Clipped	31.28±2.63	48.26±5.96	33.17±2.07	56.68±1.65	0.45±0.05	38.00±6.34	37.33±2.43	25.78±6.74
Matthiola fruticulosa	Stems	Gyp	Unclipped	28.85±4.48	43.28±7.32	29.35±4.26	51.52±4.95	0.72±0.08	37.39±6.89	32.68±4.50	16.49±4.19
Ononis tridentata	Coarse roots	Calc	Clipped	37.13±4.99	20.91±2.83	20.05±2.76	5.58±1.23	0.67±0.26	41.34±6.22	10.66±1.81	15.92±1.58
Ononis tridentata	Coarse roots	Calc	Unclipped	41.70±5.62	22.95±4.61	19.64±2.38	4.99±0.89	0.55±0.20	50.15±7.40	12.49±2.17	17.19±4.15
Ononis tridentata	Coarse roots	Gyp	Clipped	49.44±2.83	29.64±5.32	22.04±1.60	6.97±1.06	0.43±0.14	47.71±5.24	13.40±1.23	27.24±2.92
Ononis tridentata	Coarse roots	Gyp	Unclipped	44.32±4.42	27.90±4.32	22.70±2.74	6.15±1.18	0.41±0.04	42.27±5.48	11.38±1.70	20.64±3.77
Ononis tridentata	Fine roots	Calc	Clipped	31.87±6.07	29.82±5.08	28.84±1.24	22.47±2.44	0.85±0.33	33.31±7.09	49.63±4.64	39.64±4.58
Ononis tridentata	Fine roots	Calc	Unclipped	25.94±5.69	21.58±3.84	31.04±2.07	16.51±0.93	0.70±0.23	23.59±5.03	44.26±5.35	34.00±3.84
Ononis tridentata	Fine roots	Gyp	Clipped	13.83±1.09	19.02±2.13	27.51±1.78	16.82±2.20	0.39±0.08	11.59±2.09	40.38±1.94	36.71±4.80
Ononis tridentata	Fine roots	Gyp	Unclipped	18.91±3.60	24.56±3.83	28.34±3.17	17.38±2.86	0.43±0.03	16.60±3.98	46.32±5.67	47.64±3.07
Ononis tridentata	Leaves	Calc	Clipped	2.45±0.56	1.48±0.38	5.10±1.16	4.80±0.98	0.93±0.32	2.85±0.64	4.01±0.91	6.30±2.23
Ononis tridentata	Leaves	Calc	Unclipped	2.21±0.46	1.35±0.28	4.25±0.87	3.47±0.68	0.64±0.10	2.58±0.47	3.92±0.63	5.05±1.52
Ononis tridentata	Leaves	Gyp	Clipped	2.55±0.59	2.12±0.47	5.27±1.36	3.78±0.80	0.50±0.05	1.42±0.40	4.88±0.91	7.56±2.55
Ononis tridentata	Leaves	Gyp	Unclipped	2.07±0.52	1.68±0.32	5.54±1.21	3.14±0.81	0.62±0.04	1.39±0.25	4.10±0.70	4.32±1.04
Ononis tridentata	Stems	Calc	Clipped	78.14±1.60	78.33±2.03	60.84±3.04	90.31±0.96	0.56±0.29	65.56±1.89	63.65±3.34	72.10±3.21
Ononis tridentata	Stems	Calc	Unclipped	78.00±1.82	77.29±1.07	62.05±1.78	92.24±1.12	0.37±0.11	69.43±3.27	65.99±2.02	74.46±4.23
Ononis tridentata	Stems	Gyp	Clipped	74.49±2.41	66.45±3.67	51.31±5.35	89.71±2.04	0.40±0.07	89.06±2.14	56.62±4.38	57.52±4.74
Ononis tridentata	Stems	Gyp	Unclipped	74.78±2.50	58.21±3.82	52.51±2.23	91.74±1.34	0.40±0.05	90.04±0.92	56.38±1.40	46.42±2.78
Rosmarinus officinalis	Coarse roots	Calc	Clipped	14.81±1.09	15.81±1.79	24.37±2.04	2.38±0.47	0.52±0.07	26.21±1.27	19.58±2.34	11.70±1.70
Rosmarinus officinalis	Coarse roots	Calc	Unclipped	15.07±1.27	16.08±0.64	22.87±0.54	1.35±0.21	0.63±0.09	22.49±2.47	16.44±1.17	12.18±2.12
Rosmarinus officinalis	Coarse roots	Gyp	Clipped	15.65±2.85	21.11±3.83	28.32±5.94	1.36±0.34	0.51±0.07	8.19±2.02	21.26±4.23	20.57±3.79
Rosmarinus officinalis	Coarse roots	Gyp	Unclipped	17.69±2.66	31.25±4.53	29.83±3.79	1.52±0.20	0.38±0.05	7.76±0.93	26.01±2.29	33.30±3.94
Rosmarinus officinalis	Fine roots	Calc	Clipped	4.60±0.35	4.38±0.40	9.69±0.78	2.51±0.34	2.95±0.45	5.38±0.79	12.76±1.10	9.90±1.19
Rosmarinus officinalis	Fine roots	Calc	Unclipped	4.72±0.39	5.27±0.56	10.83±0.75	2.94±0.38	2.74±0.36	5.51±0.62	13.65±1.17	8.30±1.35
Rosmarinus officinalis	Fine roots	Gyp	Clipped	7.31±0.70	10.32±0.93	15.11±2.12	5.15±2.10	0.79±0.12	1.33±0.27	17.24±1.94	14.34±2.82
Rosmarinus officinalis	Fine roots	Gyp	Unclipped	5.45±0.39	8.85±0.47	12.12±0.65	3.59±0.71	0.65±0.08	0.81±0.07	13.51±1.02	15.96±4.41
Rosmarinus officinalis	Leaves	Calc	Clipped	1.33±0.08	0.04±0.00	6.15±0.19	0.06±0.01	1.23±0.34	1.71±0.15	0.99±0.07	0.02±0.00
Rosmarinus officinalis	Leaves	Calc	Unclipped	1.63±0.16	0.04±0.00	6.88±0.50	0.05±0.00	1.79±0.36	1.68±0.16	1.02±0.04	0.02±0.00
Rosmarinus officinalis	Leaves	Gyp	Clipped	2.22±0.22	0.03±0.00	7.88±0.66	0.05±0.00	0.75±0.13	3.08±0.48	1.03±0.06	0.03±0.00
Rosmarinus officinalis	Leaves	Gyp	Unclipped	2.31±0.14	0.04±0.00	5.91±0.42	0.07±0.01	0.59±0.06	2.54±0.36	1.15±0.04	0.03±0.01
Rosmarinus officinalis	Stems	Calc	Clipped	0.77±0.07	0.02±0.00	4.54±0.10	0.13±0.02	1.14±0.28	0.63±0.06	1.21±0.08	0.03±0.01
Rosmarinus officinalis	Stems	Calc	Unclipped	0.81±0.10	0.02±0.00	5.00±0.19	0.17±0.02	0.93±0.04	0.64±0.06	1.32±0.09	0.02±0.00
Rosmarinus officinalis	Stems	Gyp	Clipped	1.21±0.07	0.02±0.00	4.93±0.18	0.24±0.05	0.44±0.06	0.56±0.05	1.01±0.07	0.02±0.00
Rosmarinus officinalis	Stems	Gyp	Unclipped	1.43±0.09	0.02±0.00	4.77±0.17	0.33±0.08	0.40±0.04	0.52±0.04	1.17±0.05	0.03±0.01

Appendix E. Supplementary information of chapter 5

Table E.1. Means and standard errors of rhizospheric soil proprieties.

	Gypsophila struthium	*Helianthemum squamatum*	*Helianthemum syriacum*	*Lepidium subulatum*	*Matthiola fruticulosa*
pH	7.98±0.03	8.00±0.02	7.93±0.02	7.96±0.02	8.15±0.05
Conductivity (mS/m)	2.14±0.02	2.14±0.02	2.11±0.02	2.15±0.02	1.43±0.23
Gypsum (%)	46.03±2.87	57.59±4.93	53.00±2.44	64.06±3.17	19.81±4.37
Carbonate (%)	25.96±2.92	13.63±3.50	23.59±2.79	17.17±2.89	39.65±3.45
Clay (%)	9.26±1.20	8.47±1.45	5.00±0.83	9.31±1.57	9.92±1.75
Lime (%)	25.74±0.80	26.66±1.80	23.77±1.10	26.90±1.41	25.86±1.66
Sand (%)	65.00±1.96	64.87±2.97	71.23±1.81	63.80±2.78	64.23±3.35

Table E.2. Analyses of variance by generalised linear models (GLMs) evaluating changes in mycorrhizal colonisation within each species and among seasons. The distribution family used in GLMs is indicated.

	Family of GLM	Chisq	Pr(>Chisq)
Hyphal colonisation			
Gypsophila struthium	Gaussian	4.17	0.023
Helianthemum squamatum	Quasibinomial	1.14	0.365
Helianthemum syriacum	Gaussian	0.92	0.454
Lepidium subulatum	Gaussian	1.01	0.415
Matthiola fruticulosa	Gaussian	0.41	0.745
Vesicular colonisation			
Gypsophila struthium	Quasibinomial	14.96	0.002
Helianthemum squamatum	Gaussian	8.44	0.002
Helianthemum syriacum	Gaussian	13.58	<0.001
Lepidium subulatum	Gaussian	3.15	0.054
Matthiola fruticulosa	Quasibinomial	7.145	0.067
Arbuscular colonisation			
Gypsophila struthium	Quasibinomial	8.80	0.032
Helianthemum squamatum	Quasibinomial	11.57	0.009
Helianthemum syriacum	Quasibinomial	7.31	0.063
Lepidium subulatum	Gaussian	1.01	0.413
Matthiola fruticulosa	Binomial	85.67	<0.001

Table E.3. Means and standard errors of mycorrhizal colonisation for each species. Different letters indicate significant differences among gypsum affinity and seasons after multiple comparisons (P<0.05) in generalised linear models (GLM).

	Autumn 2017	Spring 2018	Summer 2018	Autumn 2018
Hyphal colonisation (%)				
Gypsophila struthium	62.16±2.45 AB	81.37±3.53 A	54.12±10.29 B	51.18±7.28 B
Helianthemum squamatum	81.68±2.07	89.91±1.74	90.78±1.84	82.58±7.81
Helianthemum syriacum	92.67±2.41	94.11±1.11	89.12±3.53	88.60±3.43
Lepidium subulatum	53.64±3.78	62.48±3.14	67.75±6.19	56.22±9.87
Matthiola fruticulosa	50.66±3.36	56.36±7.04	44.04±9.77	47.51±10.48
Vesicular colonisation (%)				
Gypsophila struthium	2.57±1.23 B	10.21±1.99 AB	24.38±8.02 A	10.79±2.23 AB
Helianthemum squamatum	11.19±3.30 B	21.13±2.54 B	49.01±4.49 A	31.59±8.59 AB
Helianthemum syriacum	21.41±5.15 B	26.73±3.85 B	53.79±2.80 A	44.01±3.58 A
Lepidium subulatum	9.72±2.15	10.71±2.73	24.24±5.59	16.03±3.57
Matthiola fruticulosa	0.57±0.29	2.20±0.96	4.69±2.69	4.46±0.41
Arbuscular colonisation (%)				
Gypsophila struthium	0.45±0.28	0.35±0.22	0.33±0.18	2.53±1.48
Helianthemum squamatum	14.74±1.98 A	11.08±1.34 AB	8.03±2.07 AB	5.89±1.60 B
Helianthemum syriacum	20.03±5.99	24.72±5.44	8.19±2.36	13.33±2.71
Lepidium subulatum	0.95±0.32	3.11±1.33	2.54±1.15	1.94±0.45
Matthiola fruticulosa	0.00±0.00 D	10.70±7.65 A	0.07±0.07 C	1.20±0.21 B

Table E.4. Analyses of variance of generalised linear models (GLMs) evaluating differences in plant nutrient content within each species among seasons. The distribution family used in GLMs is indicated.

	Family of GLM	F-ratio	P-value
Leaf N			
Gypsophila struthium	Gaussian	6.30	0.006
Helianthemum squamatum	Gaussian	22.30	<0.001
Helianthemum syriacum	Gaussian	6.35	0.004
Lepidium subulatum	Gaussian	300.58	<0.001
Matthiola fruticulosa	Gaussian	166.55	<0.001
Fine root N			
Gypsophila struthium	Gaussian	4.18	0.033
Helianthemum squamatum	Gaussian	1.99	0.159
Helianthemum syriacum	Gaussian	1.69	0.211
Lepidium subulatum	Gaussian	0.76	0.534
Matthiola fruticulosa	Gaussian	5.97	0.011
Leaf C			
Gypsophila struthium	Gaussian	9.1554	0.001
Helianthemum squamatum	Gaussian	24.764	<0.001
Helianthemum syriacum	Gaussian	14.986	<0.001
Lepidium subulatum	Gaussian	4125.7	<0.001
Matthiola fruticulosa	Negative binomial	2169.9	<0.001
Fine root C			
Gypsophila struthium	Gaussian	2.41	0.122
Helianthemum squamatum	Gaussian	1.01	0.415
Helianthemum syriacum	Gamma	1.58	0.237
Lepidium subulatum	Gaussian	9.85	<0.001
Matthiola fruticulosa	Gamma	17.88	<0.001
Leaf P			
Gypsophila struthium	Gaussian	5.60	0.009
Helianthemum squamatum	Gaussian	15.58	<0.001
Helianthemum syriacum	Gaussian	4.11	0.023
Lepidium subulatum			
Matthiola fruticulosa	Gaussian	14.79	0.001
Leaf N:P ratio			
Gypsophila struthium	Negative binomial	2.05	0.105
Helianthemum squamatum	Gaussian	7.37	0.003
Helianthemum syriacum	Gaussian	3.26	0.047
Lepidium subulatum			
Matthiola fruticulosa	Gaussian	1.18	0.347

Table E.5. Means and standard errors of tissue N, C and P concentrations for each species. Different letters indicate significant differences among gypsum affinity groups and seasons after multiple comparisons in generalised linear models (GLM).

	Autumn 2017	Spring 2018	Summer 2018	Autumn 2018
Leaf N (mg g^{-1})				
Gypsophila struthium	16.58±1.88 AB	17.13±1.57 AB	12.26±1.20 B	21.54±0.57 A
Helianthemum squamatum	14.82±0.68 B	16.57±0.99 B	11.83±0.41 C	21.00±1.02 A
Helianthemum syriacum	16.03±0.67 AB	17.64±1.03 A	13.53±0.76 B	18.39±0.88 A
Lepidium subulatum	42.56±1.05 A	32.34±1.85 B	0.00 C	45.30±1.05 A
Matthiola fruticulosa	40.66±1.03 A	20.78±2.05 B	0.00 C	42.93±2.09 A
Fine root N (mg g^{-1})				
Gypsophila struthium	12.66±2.97 AB	10.31±0.61 AB	7.36±0.41 B	13.40±0.51 A
Helianthemum squamatum	6.87±0.44	7.06±0.26	7.15±0.74	8.68±0.71
Helianthemum syriacum	7.27±0.28	7.17±0.10	6.88±0.0.76	8.21±0.47
Lepidium subulatum	14.67±1.95	17.19±2.15	18.21±1.10	17.73±1.89
Matthiola fruticulosa	23.91±2.30 AB	10.82±1.36 B	10.46±1.56 B	23.72±5.12 A
Leaf C (mg g^{-1})				
Gypsophila struthium	321.80±6.89 AB	316.80±4.04 BC	295.60±8.39 C	345.75±5.81 A
Helianthemum squamatum	390.00±3.35 C	410.40±3.17 B	429.60±4.01 A	414.80±2.42 B
Helianthemum syriacum	420.83±1.72 C	428.80±1.77 BC	439.80±1.02 A	435.60±3.59AB
Lepidium subulatum	419.80±4.31 A	428.00±4.28 A	0.00 B	433.75±2.53 A
Matthiola fruticulosa	396.40±2.16 A	384.60±12.28 A	0.00 B	403.20±2.46 A
Fine root C (mg g^{-1})				
Gypsophila struthium	435.00±4.36	414.60±5.08	422.00±7.81	413.50±6.64
Helianthemum squamatum	472.00±4.73	466.20±1.71	472.40±3.50	466.75±1.55
Helianthemum syriacum	473.17±1.89	472.20±2.91	455.00±13.88	468.50±4.63
Lepidium subulatum	480.6±2.16 A	447.75±6.81 B	462.75±2.53 AB	469.80±4.81 A
Matthiola fruticulosa	453.00±1.15 A	432.50±2.60 B	466.00±5.02 A	457.75±3.28 A
Leaf P (mg g^{-1})				
Gypsophila struthium	0.91±0.12 AB	0.74±0.09 B	0.55±0.12 B	1.25±0.16 A
Helianthemum squamatum	1.26±0.07 A	0.96±0.06 B	0.86±0.04 B	1.49±0.10 A
Helianthemum syriacum	1.34±0.14 A	1.03±0.11 AB	0.91±0.05 B	1.34±0.10 AB
Lepidium subulatum	1.81±0.23	1.64±0.09	0	1.96
Matthiola fruticulosa	2.36±0.11 A	1.04±0.15 B	0 C	2.47±0.32 A
Leaf N:P ratio				
Gypsophila struthium	18.45±0.61	25.18±4.84	25.19±4.14	17.83±1.70
Helianthemum squamatum	11.96±0.89 B	17.41±0.87 A	13.84±0.67 B	14.31±0.88 AB
Helianthemum syriacum	12.50±1.19 B	17.61±1.48 A	14.93±0.86 AB	14.01±1.17 AB
Lepidium subulatum	25.40±3.99	19.86±0.99	0	22.65
Matthiola fruticulosa	17.38±0.85	21.24±2.29	0	18.69±2.32

Table E.6 (a) Analyses of variance of generalised linear models (GLMs) evaluating differences in rhizospheric soil features among seasons, with species as random factors. The distribution family used in GLMs is indicated.

	GLM distribution	F-ratio	P-value
Organic matter			
Gypsophila struthium	Gaussian	0.34	0.796
Helianthemum squamatum	Gaussian	0.51	0.681
Helianthemum syriacum	Gaussian	0.62	0.614
Lepidium subulatum	Gaussian	0.65	0.593
Matthiola fruticulosa	Gamma	1.93	0.166
Nitrate			
Gypsophila struthium	Gaussian	0.54	0.663
Helianthemum squamatum	Gaussian	2.07	0.145
Helianthemum syriacum	Gaussian	2.06	0.146
Lepidium subulatum	Gaussian	1.57	0.238
Matthiola fruticulosa	Gaussian	9.56	<0.001
Ammonium			
Gypsophila struthium	Gaussian	4.33	0.022
Helianthemum squamatum	Gaussian	1.48	0.257
Helianthemum syriacum	Gaussian	0.53	0.671
Lepidium subulatum	Gaussian	0.58	0.638
Matthiola fruticulosa	Quasibinomial	0.43	0.738
P_{Olsen}			
Gypsophila struthium	Gaussian	8.07	0.002
Helianthemum squamatum	Gamma	7.88	0.002
Helianthemum syriacum	Gamma	11.82	<0.001
Lepidium subulatum	Gamma	2.95	0.066
Matthiola fruticulosa	Gamma	31.51	<0.001

Table E.6 (b). Analyses of variance of generalised linear models (GLMs) evaluating differences in rhizospheric soil features among seasons, with species as random factors. The distribution family used in GLMs is indicated.

	Family of GLM	F-ratio	P-value
Gypsum			
Gypsophila struthium	Guassian	9.92	<0.001
Helianthemum squamatum	Gaussian	5.35	0.010
Helianthemum syriacum	Gaussian	4.71	0.014
Lepidium subulatum	Gaussian	0.43	0.733
Matthiola fruticulosa	Binomial	28.60	<0.001
Conductivity			
Gypsophila struthium	Gaussian	3.85	0.032
Helianthemum squamatum	Gaussian	1.13	0.366
Helianthemum syriacum	Gamma	0.64	0.602
Lepidium subulatum	Gaussian	11.95	<0.001
Matthiola fruticulosa	Gamma	25.29	<0.001
Relative humidity			
Gypsophila struthium	Gaussian	4.19	0.024
Helianthemum squamatum	Gaussian	7.72	0.002
Helianthemum syriacum	Negative binomial	13.07	<0.001
Lepidium subulatum	Negative binomial	13.18	<0.001
Matthiola fruticulosa	Gaussian	244.36	<0.001
pH			
Gypsophila struthium	Gaussian	17.63	<0.001
Helianthemum squamatum	Gaussian	7.14	0.003
Helianthemum syriacum	Negative binomial	0.01	0.999
Lepidium subulatum	Gaussian	3.11	0.058
Matthiola fruticulosa	Gaussian	37.92	<0.001

Table E.7 (a). Means and standard errors of rhizospheric soil features for each species. Different letters indicate significant differences among gypsum affinity and seasons after multiple comparisons in generalised linear models (GLM).

	Autumn 2017	Spring 2018	Summer 2018	Autumn 2018
Organic matter (%)				
Gypsophila struthium	0.98±0.22	1.23±0.14	1.06±0.22	1.05±0.12
Helianthemum squamatum	1.14±0.19	1.13±0.19	1.01±0.18	0.87±0.15
Helianthemum syriacum	0.83±0.09	0.71±0.11	0.68±0.09	0.66±0.10
Lepidium subulatum	1.29±0.22	1.29±0.25	1.05±0.25	0.88±0.19
Matthiola fruticulosa	1.48±0.20	1.87±0.08	1.96±0.24	2.06±0.19
Nitrate (mg/kg)				
Gypsophila struthium	45.10±10.47	59.23±27.69	31.93±12.53	62.83±21.49
Helianthemum squamatum	18.89±2.77	40.24±11.52	41.04±13.57	55.43±10.68
Helianthemum syriacum	22.66±3.77	28.30±8.88	26.78±8.67	48.21±10.14
Lepidium subulatum	39.21±11.15	79.61±24.63	43.63±7.78	62.04±5.96
Matthiola fruticulosa	50.90±8.31 B	52.89±8.53 B	54.41±12.21 B	137.33±21.41 A
Ammonium (mg/kg)				
Gypsophila struthium	11.63±1.88 AB	6.91±0.94 B	10.22±1.09 AB	13.68±1.19 A
Helianthemum squamatum	11.76±1.31	11.55±1.26	10.83±1.85	14.78±1.23
Helianthemum syriacum	11.90±0.64	12.08±0.52	12.11±0.74	12.86±0.27
Lepidium subulatum	9.81±1.78	9.65±1.49	10.64±1.43	12.21±0.58
Matthiola fruticulosa	11.87±1.98	12.82±0.42	12.14±0.71	13.55±0.69
P_{Olsen} (%)				
Gypsophila struthium	0.84±0.15 B	0.70±0.05 B	1.44±0.13 A	0.92±0.10 B
Helianthemum squamatum	0.64±0.04 AB	0.50±0.06 B	1.09±0.18 A	0.32±0.09 B
Helianthemum syriacum	0.67±0.03 A	0.26±0.04 B	1.09±0.14 A	0.29±0.10 B
Lepidium subulatum	0.80±0.27 AB	0.70±0.03 B	1.44±0.14 A	0.82±0.14 AB
Matthiola fruticulosa	13.38±2.70 A	1.36±0.25 C	4.19±1.15 B	0.43±0.13 D

Table E.7 (b). Means and standard errors of rhizospheric soil features for each species. Different letters indicate significant differences among gypsum affinity and seasons after multiple comparisons in generalised linear models (GLM).

	Autumn 2017	Spring 2018	Summer 2018	Autumn 2018
Gypsum (%)				
Gypsophila struthium	36.03±2.22 B	61.97±3.11 A	42.91±5.28 B	42.51±2.79 B
Helianthemum squamatum	55.81±6.34 AB	55.99±6.27 AB	80.66±4.48 A	37.90±11.46 B
Helianthemum syriacum	60.92±3.63 A	57.09±4.23 AB	50.81±3.19 AB	41.59±4.53 B
Lepidium subulatum	59.66±4.51	69.78±4.94	64.21±8.97	62.25±7.42
Matthiola fruticulosa	0.66±0.33	32.14±5.11	4.09±3.29	42.35±2.54
Conductivity (µS/m)				
Gypsophila struthium	2220.20±12.13 A	2088.20±41.44 B	2094.60±29.30 AB	2150.50±39.27 AB
Helianthemum squamatum	2186.40±35.50	2102.00±42.21	2135.20±26.01	2132.40±24.74
Helianthemum syriacum	2138.67±30.97	2095.80±22.94	2069.00±58.15	2116.60±31.02
Lepidium subulatum	2238.60±24.50 A	2207.80±7.23 A	2075.80±35.38 B	2062.25±28.46 B
Matthiola fruticulosa	156.74±15.17 B	2189.40±20.57 A	1132.68±554.33 A	2184.00±8.50 A
pH				
Gypsophila struthium	7.84±0.02 B	7.92±0.03 B	8.09±0.02 A	8.09±0.04 A
Helianthemum squamatum	7.94±0.04 B	7.90±0.03 C	8.10±0.04 A	8.06±0.03 AB
Helianthemum syriacum	7.92±0.02	7.84±0.03	7.90±0.02	8.09±0.02
Lepidium subulatum	7.90±0.02	7.96±0.02	7.97±0.04	8.02±0.03
Matthiola fruticulosa	8.27±0.03 A	7.93±0.01 B	8.41±0.07 A	8.04±0.02 B
Relative humidity (%)				
Gypsophila struthium	1.07±0.08 B	6.02±1.71 A	1.47±0.53 AB	4.75±1.81 AB
Helianthemum squamatum	1.29±0.35 B	4.19±1.21 AB	0.88±0.21 B	7.43±1.77 A
Helianthemum syriacum	1.17±0.18 B	5.02±2.03 A	0.71±0.06 B	7.17±0.61 A
Lepidium subulatum	0.84±0.11 B	7.90±0.98 A	1.21±0.25 B	9.13±4.47 A
Matthiola fruticulosa	1.73±0.28 C	7.36±0.58 B	1.83±0.25 C	14.54±0.35 A

Appendix F. Published article of Chapter 2

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Gypsum-exclusive plants accumulate more leaf S than non-exclusive species both in and off gypsum

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ABSTRACT

Gypsum-exclusive species (gypsophiles), are restricted to gypseous soils in natural environments. However, it is unclear why gypsophiles display greater affinity to gyspeous soils than other soils. These plants are edaphic endemics, growing in alkaline soils with high Ca and S. Gypsophiles tend to show higher foliar Ca and S, lower K and, sometimes, higher Mg than non-exclusive gypsum species, named gypsovags. Our aim was to test if the unique leaf elemental signature of gypsophiles could be the result of special nutritional requirements linked to their specificity to gypseous soils. These nutritional requirements could hamper the completion of their life cycle and growth in other soil types. To test this hypothesis, we cultivated five gypsophiles and five gypsovags dominant in Spanish gypsum outcrops on gypseous and calcareous (non-gypseous) field soil for 29 months. We regularly measured growth and phenology, and differences in leaf traits, final biomass, individual seed mass, seed viability, photosynthetic assimilation and leaf elemental composition. We found all the gypsophiles studied were able to complete their life cycle in non-gypseous soil, producing viable seeds, attaining greater biomass and displaying higher photosynthetic assimilation rates than in gypseous soil. The leaf elemental composition of some species (both gypsophiles and gypsovags) shifted depending on soil, although none of them showed leaf deficiency symptoms. Regardless of soil type, gypsophiles had higher leaf S, Mg, Fe, Al, Na, Mn, Cr and lower K than gypsovags. Consequently, gypsophiles have a unique leaf chemical signature compared to gypsovags of the same family, particularly due to their high leaf S regardless of soil conditions. However, these nutrient requirements are not sufficient to explain why gypsophiles are restricted to gypsum soil in natural conditions.

1. Introduction

The effect of soil on plant performance and distribution has been studied by ecologists and botanists for decades, particularly in relation to the restriction of plants to certain types of soils with special physicochemical features. For example, serpentines and saline soils are special substrates that support singular floras (Mota et al., 2017) composed of species that tolerate the physicochemical challenges imposed by them (Kazakou et al., 2008; Munns and Tester, 2008).

Gypseous soils are also atypical substrates. These soils have high gypsum content (Casby-Horton et al., 2015) and normally develop in arid or semiarid environments, limiting plant life (Palacio et al., 2017). High gypsum content in soil impacts the physical and chemical properties of soils and their functions (Herrero and Porta, 2000). The moderate solubility of gypsum (about 2.4 g l^{-1}) leads to highly dynamic soil environments, with dissolution-precipitation sequences altering

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physical properties (Casby-Horton et al., 2015). Although the solubility of gypsum does not produce osmotic or ion-toxic stress for plants (Casby-Horton et al., 2015), the chemical conditions of gypseous soils influence plant nutrition (Boukhris and Lossaint, 1972; Palacio et al., 2007; Salmerón-Sánchez et al., 2014), ultimately limiting growth (Food and Agriculture Organization of the United Nations and Soil Resources, and Conservation Service (FAO), 1990).

Plants living on gypseous soils have to cope with alkaline soils saturated in calcium, sulphate and magnesium ions, and reduced in N, P and K availability (Moore et al., 2014). Gypseous soils have low nutrient retention (Casby-Horton et al., 2015) and high Ca cation activity due to the solubility of gypsum (Food and Agriculture Organization of the United Nations and Soil Resources, and Conservation Service (FAO), 1990). The combination of high Ca, jointly with high sulphate, alters plant metabolism (Meyer, 1980) and decreases the availability and uptake of macronutrients like K and P (Stout et al., 1951; Food and Agriculture Organization of the United Nations and Soil Resources, and Conservation Service (FAO), 1990). To overcome these chemical restrictions, plants growing on gypseous soils may have developed special mechanisms and strategies (Moore et al., 2014).

Species that thrive on special soils generally show different ecological amplitudes, ranging from tolerant species with a broad distribution, to highly specialized edaphic endemics restricted to them (Kruckeberg and Rabinowitz, 1985). In the case of gypseous soils, Meyer (1986) described mainly two types of plants living on gypsum, depending on their affinity to this substrate: 1) gypsovags, species with wide ecological amplitude, which can grow both on and off gypseous soils; and 2) gypsophiles, edaphic endemics restricted to gypseous soils. Two types of gypsophiles have been further described (Palacio et al., 2007): widely distributed gypsophiles (hereafter, wide gypsophiles) considered as gypsum specialists (sensu Gankin and Major, 1964), and narrowly distributed gypsophiles, which, similar to gypsovags, would fit the refuge model, being stress tolerant species not specifically adapted to gypseous soils. Gypsovags seem to be stress tolerant plants that may display different mechanisms to cope with the limitations imposed by gypsum (Bolukbasi et al., 2016). Gypsophiles are usually restricted to gypseous soils (Mota et al., 2011), and their individual fitness may be compromised in non-gypseous soils (Ballesteros et al., 2014). However, it is unclear why gypsophiles display greater affinity to gypseous soils than other soil types.

Edaphic endemics often have substrate-specific physiological mechanisms or strategies to cope with the harsh conditions of special substrates (Mota et al., 2017). In soils with atypical chemical composition, the mineral nutrition of plants has been crucial to explain plant restriction or growth limitation (Rorison, 1960). The concentration of elements in leaves (hereafter, leaf elemental composition) is used to understand plant mineral nutrition, since it links plant function (Aerts and Chapin, 1999) and soil chemistry. For example, halophytes require high concentrations of NaCl (100–200 mM) for optimal growth (Flowers et al., 1977) and show high leaf Na, Mg and low Ca, K, as compared to co-occurring non-specialized species (Matinzadeh et al., 2019). In serpentine soils, edaphic endemics maintain high leaf Ca:Mg molar ratios (O'Dell et al., 2006), indicating that they have a high selectivity for Ca at the root surface, maintaining sufficient Ca uptake despite a very low soil Ca:Mg ratio (Kazakou et al., 2008). While, consistent chemical patterns have been found in wide gypsophiles, who display a common leaf elemental composition similar to that of the gypseous soils in which they grow (Duvigneaud and Denaeyer-De Smet, 1968).

Wide gypsophiles tend to have higher foliar S and Ca, lower K and, sometimes, higher foliar Mg as compared to co-existing gypsovags (Duvigneaud and Denaeyer-De Smet, 1968; Boukhris and Lossaint, 1970, 1972, 1975; Alvarado, 1995; Palacio et al., 2007; Muller et al., 2017). This unique leaf chemical composition was observed despite phylogenetic constraints in gypsophilic species from the Chihuahuan Desert (Muller et al., 2017). However, the ecological or adaptive implications of the atypical chemical composition of wide gypsophiles remain unexplored (Palacio et al., 2014). It has been suggested that the leaf elemental composition of wide gypsophiles could be a nutritional requirement to complete their life cycle and support growth or could confer some form of protection from competition or disturbances (Meyer, 1980). However, no previous studies have evaluated the nutrient composition of wide gypsophiles growing on non-gypseous soils.

Our aim was to test if wide gypsophiles are restricted to gypseous soils because they are not able to complete their life cycle off gypsum. We focused only on wide gypsophiles (hereafter, gypsophiles), since narrowly distributed gypsophiles are a less distinctive group. We also wanted to explore the extent to which the atypical chemical composition of gypsophiles is linked to chemical conditions of the substrate. To this end, we cultivated five widespread Iberian gypsophiles and five co-occurring gypsovags (some of them closely related phylogenetically) in gypsum and calcareous (non-gypseous) soils. The selection of calcareous soil as the non-gypsum treatment stemmed from the fact that gypsum outcrops are frequently intermingled with alternating layers of marls, limestone and clays (Quirantes, 1978). Consequently, calcareous soils are the most readily available non-gypsum alternative for plants growing on gypseous soils in the wild, showing similar physicochemical features including similar Ca content and differing mainly in the higher S content of gypseous soils (Food and Agriculture Organization of the United Nations and Soil Resources, and Conservation Service (FAO), 1990). We analysed plant survival and fitness and measured leaf elemental composition as a tool to understand plant mineral nutrition and its relationship with soil chemical features. We hypothesized that: 1) Gypsophiles would have lower growth and fitness in non-gypseous soils than in gypseous soils, in accordance with Ballesteros et al. (2014); 2) Gypsophiles would have substrate-specific physiological mechanisms or strategies linked with chemical features of gypseous soils (i.e., nutritional requirements), and as a result, they would accumulate higher S and Mg concentrations than gypsovags, irrespective of the substrate. However, such concentrations would be lower on calcareous (non-gypseous) than on gypseous soil, owing to the lower S and Mg availability in the former.

2. Material and methods

2.1. Study species

The selected species included a suite of five dominant gypsophile and gypsovag sub-shrubs from gypsum environments in northeastern Spain (Table 1). Gypsophile species included *Gypsophila struthium* subsp. *hispanica* (Willk.) G.López., *Herniaria fruticosa* L., *Helianthemum squamatum* Pers., *Lepidium subulatum* L., *Ononis tridentata* L.; and gypsovag species included *Boleum asperum* Desv., *Helianthemum syriacum* (Jacq.) Dum. Cours., *Linum suffruticosum* DC., *Matthiola fruticulosa* (L.) Maire and *Salvia officinalis* Spenn. All the gypsophile species included in the study show high affinity for gypseous soils (Mota et al., 2011) and are widely distributed within the Iberian Peninsula (Palacio et al., 2007).

2.2. Soil collection and analyses

Gypseous soil was collected from a gypsum outcrop in the Middle Ebro Basin (Villamayor del Gállego, Zaragoza, Spain, 41°41′44.5″ N, 0°44′26.7″ W) and calcareous soil (non-gypseous, hereafter calcareous) was collected from the Iberian System (Ricla, Zaragoza, Spain, 41°30′45.8″N, 1°26′47.8″W). Soil was collected by removing O horizons in unfertilized areas, sieved to 1 cm, and then thoroughly mixed and used to fill pots. Physical and chemical properties were analysed from five replicates per experimental soil type (Table A.1).

Soils were air dried for 2 months prior to physical and chemical analyses and subsequently divided into two subsamples: one to be sieved to pass a 2 mm sieve, and the other to remain non-sieved. Sieved soils were used to measure the following variables: gypsum content,

Table 1
Characteristics of study species.

Species	Family	Gypsum affinity	Gypsophily *	Seed collection (Spain)
Boleum asperum Desv.	Brassicaceae	Gypsovag	3.03	Castelflorite
Gypsophila struthium subsp. *hispanica* (Willk.) G. López	Caryophyllaceae	Gypsophile	4.69	Villamayor de Gállego
Helianthemum squamatum Pers.	Cistaceae	Gypsophile	4.87	Villamayor de Gállego
Helianthemum syriacum (Jacq.) Dum. Cours.	Cistaceae	Gypsovag	–	Villamayor de Gállego
Herniaria fruticosa L.	Caryophyllaceae	Gypsophile	4.05	Villamayor de Gállego
Lepidium subulatum L.	Brassicaceae	Gypsophile	4.91	Villamayor de Gállego
Linum suffruticosum DC.	Linaceae	Gypsovag	–	Villamayor de Gállego
Matthiola fruticulosa (L.) Maire	Brassicaceae	Gypsovag	–	Sariñena
Ononis tridentata L.	Fabaceae	Gypsophile	4.43	Villamayor de Gállego
Rosmarinus officinalis L.	Lamiaceae	Gypsovag	–	Leciñena

* Exclusivity to gypseous soils in Spain from expert evaluation. Values of gypsophily range between 0 and 5. Extracted from Mota et al (2011).

measured according to Artieda et al. (2006); carbonate content determined by Bernard calcimetry; soil texture, estimated with a particle laser analyser (Mastersizer 2000 Hydro G, Malvern, UK); and soil pH and conductivity, measured with a pH/conductivity meter (Orio StarA215, Thermo Scientific, Waltham-MA, USA) by diluting samples with distilled water to 1:2.5 (w/v) to measure pH and then 1:5 (w/v) to measure conductivity). A subsample of each sieved soil was finely ground using a ball mill (Retsch MM200, Restch GmbH, Haan, Germany) and subsequently used to analyse elemental concentrations. N and C concentrations were measured with an elemental analyzer (TruSpec CN, LECO, St. Joseph-MI, USA), whereas the elemental composition of Al, As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Si, Ti, V, Zn was measured by extracting samples with HNO_3-H_2O_2 (9:3) by microwave acid digestion (Speed Ave MWS-3$^+$, BERGHOF, Eningen, Germany), followed by inductively coupled plasma-optical emission spectrometry (Varian ICP 720-ES, Agilent Technologies, Santa Clara-CA, USA). All elemental analyses were performed by EEZ-CSIC Analytical Services.

2.3. Experimental design

For each species, seeds were collected from several individuals within the same population (Table 1). In April 2016, seeds were germinated in nursery trays with 0.06 L cells filled with a one-part gravel in the bottom of the cell and four-parts field soil on top of it. Half of the trays had calcareous soil and the other half had gypseous soil (see Table A.1, A.2, for soil features). In November 2016, plants with high root volume (*G. hispanica*, *R. officinalis* and *O. tridentata*) and plants with shallow roots (the rest of species) were transplanted into 7 L and 5.6 L square pots, respectively. Five months after transplantation, pots were thinned to one plant per pot, with ten replicates per species and soil treatment. All plants were kept well-watered throughout the experiment and regularly de-weeded by hand, removing any potential competition and drought stress. Each year, throughout the duration of the experiment, plants were housed in a greenhouse from November to March to avoid freezing damage. Five replicates per species and soil treatment were harvested in September 2019, 29 months after sowing.

2.4. Phenological patterns and growth

Phenological patterns were recorded for each plant every two weeks between 29 November 2017 and 7 Sept. 2019. Five phenophases were considered (adapted from Montserrat-Marti et al., 2009): plant vegetative growth, flower bud formation, flowering, fruit set and leaf shedding. The incidence of each phenophase was estimated in the canopy of each individual as the percentage of stems displaying it. Canopy height, maximum shoot length (measured from the base of plant to the distant most leaf, hereafter canopy length), and the maximum and their perpendicular canopy diameters were measured monthly using a metallic millimetre straightedge. Mature fruits were collected at seeding and stored in a dry location at room temperature until seed viability tests.

2.5. Leaf gas exchange, plant biomass, functional traits and seed traits

Leaf gas exchange, including photosynthetic assimilation and stomatal conductance, were measured with a Portable Photosynthesis System coupled with a Chlorophyll Fluorescence Module (CIRAS-2, PP Systems, Amesbury-MA, USA), a LED light unit on the leaf cuvette (PLC6 U), and a circular bead plate of 18 mm diameter. Three plants of each soil treatment and species were measured after 9 a.m. and before 1 p.m. on 11 July 2017, except for *M. fruticulosa* and *B. asperum*, which did not have enough green leaves for assessment.

At harvest in September 2019, plants were lifted from their pots and rinsed with tap water to remove soil. Plants were separated into green leaves, stems, fine roots (diameter < 2 mm), coarse roots (rest of roots), and seeds (if available). All plant fractions were subsequently dried to a constant weight at 50 °C and weighed in a precision scale (42 g/0.00001 g, MS105DU, Mettler Toledo, Columbus-OH, USA).

Specific leaf area (SLA) was measured as the one-sided area of a fresh leaf divided by its oven-dry mass. Leaf dry matter content (LDMC) was measured as the oven-dry mass (mg) of a leaf, divided by its water-saturated fresh mass (g), expressed in mg g^{-1} (Pérez-Harguindeguy et al., 2013). To measure leaf area, images of leaves were captured with a Dino-Lite Digital Microscope (AnMo Electronics, Taiwan) and processed with ImageJ (National Institutes of Health, Bethesda-MD, USA). SLA and LDMC were calculated for the final harvest from among 4–10 individual leaves of each plant with petioles included.

Individual seed mass was weighed on a precision scale (42 g/0.00001 g, MS105DU, Mettler Toledo, Columbus-OH, USA) as total seed weight divided by number of seeds ($N = 20$). Seed viability was assessed by monitoring the emergence of 20 seeds per species over 30 days. Seeds were sown on filter paper inside Petri dishes, kept well-watered with distilled water, and placed in a growth chamber (ASL Aparatos Científicos, Madrid, Spain) with 16 h of light (flux = 1743−1900 lm, CCT = 4000−6500 K) at 25 °C and 8 h of darkness at 15 °C.

2.6. Leaf chemical analyses

To assess leaf elemental composition, we collected leaf tissue from three to five individuals per species and soil type during two sampling periods: October 2017 and September-November 2018; different replicates were assessed at the two sampling periods. Leaves were dried to a constant weight at 50 °C and subsequently finely ground using a ball mill (Retsch MM200, Restch GmbH, Haan, Germany). N and C concentrations were analysed with an elemental analyzer (TruSpec CN, LECO, St. Joseph-MI, USA). The elemental composition of Al, As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Si, Ti, V, Zn was measured by extracting samples with HNO_3-H_2O_2 (8:2) by microwave acid digestion (Speed Ave MWS-3$^+$, BERGHOF, Eningen, Germany),

followed by inductively coupled plasma-optical emission spectrometry (Varian ICP 720-ES, Agilent Technologies, Santa Clara-CA, USA). All elemental analyses were performed by EEZ-CSIC Analytical Services. Only elements with values above 0.025 ppm (the detection limit of the ICP-OES spectrometer) were included in the statistical analyses.

2.7. Calculations and statistics

All statistical analyses were run in R 3.6.0 (R Core Team, 2020).

To model the gradualness of growth and flowering patterns, changes in canopy length and in the percentage of shoots bearing flowers within the canopy over time were fitted to a Boltzmann sigmoid regression (Self-Starting Nls Four-Parameter Logistic Model function on R). In this analysis, the scale parameter indicates the steepness of the curve and, consequently, the gradualness of the change in growth or flowering (Palacio et al., 2013). Shoot growth rate (L-day, mm day^{-1}) was calculated as the difference in canopy length between two consecutive monthly measurements divided by the number of days elapsed between both measurements. The maximum value of shoot growth rate, the day with the maximum shoot growth rate, the day of first flowering, the day with the maximum percentage of stems with flowers (day of maximum flowering), and the maximum flowering (maximum percentage of stems with flowers) were selected as variables to study changes in phenological patterns. Water use efficiency (WUE) was calculated by dividing the photosynthetic assimilation (A) by stomatal conductance (g_s) (Pérez-Harguindeguy et al., 2013).

Differences between soils and gypsum affinity plant types (i.e. gypsophiles and gypsovags) for the variables canopy length and canopy area at harvest, gradualness of shoot growth (slope of the Boltzmann curve), maximum shoot growth rate, day of maximum shoot growth rate, photosynthetic assimilation (A), stomatal conductance (g_s), transpiration (E), instantaneous Water Use Efficiency (WUE), day of first flowering, day of maximum flowering, maximum percentage of flowering, individual seed mass, total biomass, root:shoot ratio and for each elemental concentration were analysed by generalized linear mixed models (hereafter GLMM) with "soil" (gypseous / calcareous) and "gypsum affinity" (gypsophile / gypsovag) as fixed factors and "species" as a random factor. Species was included as a random factor to account for species-specific effects and avoid biases related to species selection. In the case of elemental concentrations, we also added taxonomic "family", and "species" nested within "family" and "year" as random factors to avoid biases related to phylogenetic effects on elemental concentration (Neugebauer et al., 2018) or different sampling dates. Analyses were assessed with function *glmm* on R (*lme4* package version 1.1–15 in R, Bates et al., 2007). The models were fitted to a Gamma distribution when there was not a normal distribution of residuals since, in most cases, data had a constant coefficient of variation and variances increased with means (McCullagh and Nelder, 1989). Model link functions of the Gamma distribution were selected according to the lower AIC criterion and included in each table as sub-indexes. Similarly, differences between soil types within each gypsum affinity class and differences between soil types within each species were assessed by GLMM.

Principal Component Analysis (PCA, *vegan* package version 2.4–6 in R, Oksanen et al., 2007, and *ggplot2* package in R, Wickham, 2016) was used to visualize relationships among elemental concentrations and taxa. We used elements with concentrations above the detection limit of the ICP-OES spectrometer and samples for which we also had N and C concentration data ($N = 182$). All elemental data were transformed to Center Log-Ratio coordinates (Aitchison, 1982) using CoDaPack (Comas-Cufí and Thió-Henestrosa, 2011), to maintain relationships between elements regardless of the concentration, which allows studying joint patterns among elements (Soriano-Disla et al., 2013, Prater et al., 2019). Redundancy Analysis (RDA, *vegan* package version 2.4–6 in R, Oksanen et al., 2007) was performed with the same data set as the PCA, including "soil" (gypseous / calcareous) and "gypsum affinity" (gypsophile / gypsovag) as fixed factors.

Differences in nutrient composition between soils and species were assessed using non-parametric contrasts based on distance (Adonis function on *vegan* package version 2.4-6 in R, Oksanen et al., 2007) with "soil" (gypseous / calcareous) and "species" as fixed factors and using the Euclidean as distance from Center Log-ratio coordinates. Significant interactions between soil and species on nutrient composition was analysed by multilevel pairwise comparisons with "interaction" as a fixed factor (*pairwiseAdonis* package version 0.3 in R, Martinez Arbizu, 2019).

A heat map (*ggplot2* package in R, Wickham, 2016) was used to visualize the distances among soils and species jointly with a cladogram from an adapted phylogenetic tree. Distances were calculated using Euclidean as distance from Center Log-Ratio coordinates (vegdist function on *vegan* package version 2.4-6 in R, Oksanen et al., 2007). Distances among branches of the cladogram were extracted from Tree of Life Web Project (Maddison and Schulz, 2007).

3. Results

3.1. Life cycle, growth and phenology

Contrary to our expectations, gypsophile species had similar growth, and similar maximum percentage of stems with flowers and individual seed mass, in both substrates (Table 2, and see F-ratios of GLMMs on Tables A.3, A.4). Also, they produced fruits which rendered viable seeds. Similarly, and in agreement with our expectations, gypsovags completed their life cycle in both soil types, except for *R. officinalis,* which did not produce fruits in either substrate. Gypsovags had similar growth and lower individual seed mass in gypseous than calcareous soils, and a similar maximum percentage of stems with flowers in both substrates ($P < 0.05$).

Gypsophiles and gypsovags differed in plant size, leaf traits, growth, and phenology (Table 2). Regardless of the soil type, gypsophiles had larger canopy areas than gypsovags, 1.4-fold lower Root:Shoot ratios and 1.5 fold lower LDMC ($P < 0.05$). Furthermore, the timing of phenological events was delayed in gypsophiles as compared to gypsovags, independent of soil type. Gypsophiles attained maximal shoot growth rate 31 days later on average than gypsovags in both soil types ($P < 0.05$, Fig. 1a). Gypsophiles also initiated flowering and reached maximal bloom almost two months later than gypsovags on average ($P < 0.05$ for both traits, Fig. 1b). Soil type had an effect on the flowering phenology of gypsovags: plants grown on calcareous soil initiated flowering earlier than those grown on gypsum ($P < 0.05$).

Regardless of the species or gypsum affinity, plants grown in calcareous soil had larger canopy area and total biomass ($P < 0.05$, Table 2). They also had 1.2-fold higher photosynthetic assimilation, 1.2-fold higher SLA, and started flowering ten days earlier on average than plants grown on gypseous soil ($P < 0.05$, Table 2). Considering each gypsum affinity separately, gypsophiles grown in calcareous soil had larger canopy area and higher SLA than those grown on gypsum ($P < 0.05$, Table 2). Gypsophiles reached their maximal shoot growth rate 27 days later, on average ($P < 0.05$), when growing on calcareous vs. gypseous soil. Gypsovags grown in calcareous soil had higher total biomass and leaf N at harvest and lower individual seed mass than those grown on gypsum ($P < 0.05$). Gypsovags also initiated flowering later on gypsum than calcareous soil ($P < 0.05$, Table 2).

3.2. Leaf elemental composition

Gypsophiles had a different leaf elemental composition compared to gypsovags that was independent of soil type ($P < 0.05$, Table 3), and these differences were maintained in both samplings (data not shown). Gypsophiles and gypsovags shifted their leaf elemental composition based on soil type ($P < 0.05$, Table 3). As indicated by the PCA biplot, plant leaf elemental composition was more strongly influenced by phylogenetic relationships than by soil type, with species of the same

Table 2
Means and standard deviation of leaf traits, seed traits, growth and phenological variables for each treatment. Major letters indicate significant differences between gypsophiles and gypsovags regardless of the soil type ($P < 0.05$). Minor letters indicate significant differences between soil types ($P < 0.05$) within each gypsum affinity group.

Variables	Gypsovags				Gypsophiles			
	Calcareous		Gypseous		Calcareous		Gypseous	
Final canopy area (dm^2)	10.71 ± 7.30	A	8.73 ± 4.57	A	18.42 ± 11.03	Ba	15.27 ± 9.42	Bb
Final length (mm)	189.28 ± 64.37		200.17 ± 68.29		176.24 ± 88.65		165.71 ± 76.22	
Gradualness	19.58 ± 12.52		20.06 ± 12.92		20.52 ± 12.62		21.04 ± 13.36	
Max. shoot growth rate ($mm{\cdot}day^{-1}$)	1.21 ± 0.99		1.12 ± 1.34		1.27 ± 1.40		1.02 ± 0.95	
Day max. shoot growth rate	372.44 ± 49.63	A	388.54 ± 63.54	A	425.40 ± 44.03	Ba	398.04 ± 56.15	Bb
E (mmol H_2O m^{-2} s^{-1})	5.77 ± 2.37		6.12 ± 3.33		8.07 ± 3.56		6.47 ± 4.08	
A (μmol CO_2 $m^{-2}{\cdot}s^{-1}$)	12.01 ± 1.94		10.01 ± 3.18		12.87 ± 5.49		9.69 ± 4.19	
Gs (mmol m-2·s^{-1})	507.31 ± 292.08		563.49 ± 375.79		599.43 ± 362.60		475.69 ± 313.17	
WUE	2.34 ± 0.82		2.00 ± 0.94		1.82 ± 0.91		2.09 ± 1.61	
SLA(cm^2/g)	63.36 ± 33.78		55.02 ± 20.81		84.63 ± 45.46	a	66.46 ± 38.28	b
LDMC (mg g^{-1})	280.99 ± 97.34	A	297.42 ± 78.36	A	197.87 ± 83.18	B	194.57 ± 69.59	B
Leaf N (%)	1.72 ± 0.98	a	1.66 ± 0.87	b	1.56 ± 0.97		1.59 ± 0.84	
Day 1 st Flower	340.25 ± 30.25	Aa	360.18 ± 28.80	Ab	402.40 ± 41.83	B	411.80 ± 32.05	B
Day Max Flowering	363.63 ± 23.08	A	373.59 ± 25.60	A	421.73 ± 42.99	B	428.80 ± 36.60	B
Max. Flowering (% stems)	63.13 ± 27.68	A	68.82 ± 17.64	A	42.00 ± 26.17	B	29.33 ± 23.74	B
Individual seed mass (mg)	0.77 ± 0.73	a	0.91 ± 0.62	b	1.38 ± 2.19		2.83 ± 3.33	
Total biomass (g)	12.85 ± 11.96	a	9.40 ± 6.39	b	13.13 ± 10.52		12.54 ± 9.40	
Root:Shoot	1.66 ± 0.62	A	1.54 ± 0.69	A	1.12 ± 0.44	B	1.21 ± 0.51	B

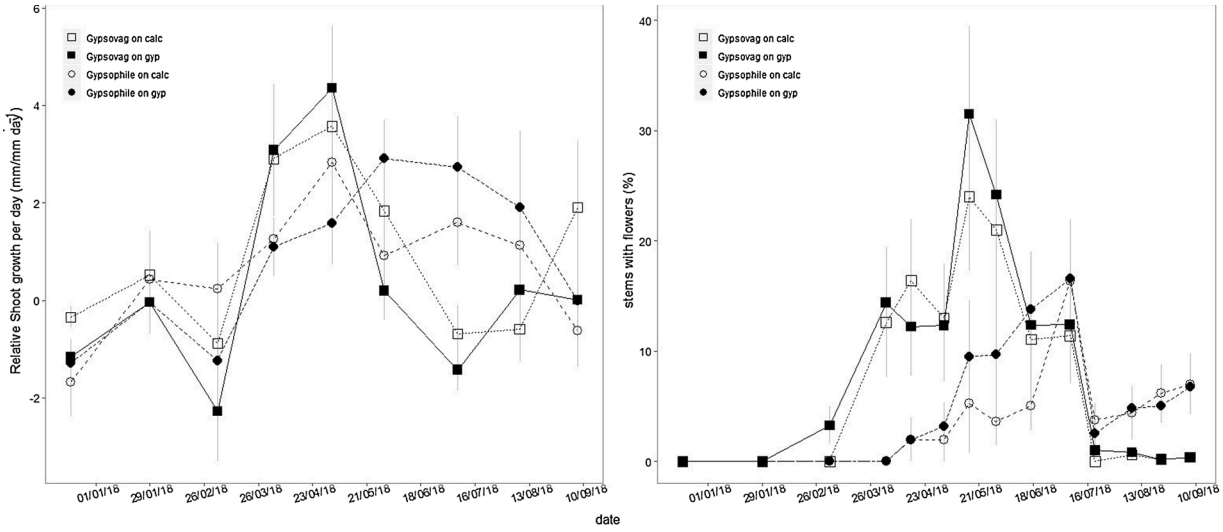

Fig. 1. A) Variation in relative shoot growth (mm/mm day-1) and B) percentage of flowering stems (%) from December 2017 to September 2018. Centroids of each treatment mean were drawn (±S.E.). Circles indicate gypsophiles; squares, gypsovags. Filled symbols indicate plants grown on gypsiferous soils; empty symbols, calcareous soils.

Table 3
F-ratios, P-value and Variability (SS_{factor}/SS_{total}) from non-parametric contrasts based on distances in which soil (a = 2), gypsum affinity types (b = 2) and species (c = 10) were fixed factors. Data set of $N = 180$.

Leaf elemental composition	Treatments				
	Soil	Gypsum affinity	Species	Soil*Gypsum affinity	Soil*Species
F-ratio	25.55	90.59	42.47	0.58	1.71
P-value	0.001	0.001	0.001	0.732	0.013
Variability (%)	4.04	14.33	53.75	0.09	2.17

family plotting close to each other, regardless of soil type (Fig. 2). The biplot of the first and second PCA axes indicated that gypsophiles showed a unique leaf elemental composition compared to gypsovags, irrespectively of the substrate. Gypsophiles growing on both soil types were located in the upper quadrants and associated with the vectors for S, Cu, Mg, Ti, Al, Fe, Mn. This pattern indicates they showed higher concentrations of these elements, regardless of soil type. In contrast, gypsovags were located in the bottom left quadrant, aligned with higher K, P, Zn and N concentrations. Furthermore, the biplot of first and second or second and third PCA axes (Figs. 2, and B.1) indicated that plants from different soil type were distributed along the second component. Plants grown in gypseous soils had more positive values along the second component than those grown in calcareous soils, and S vectors had positive values and K and P vectors had negative values. This pattern indicates plants grown on gypsum had high leaf S and low leaf K and P. In accordance with the PCA results, gypsum affinity and soil types were associated with different leaf elemental compositions based on the RDA analysis (*F-ratio* = 32.11 for gypsum affinity, $P < 0.05$; F-ratio = 8.72, $P < 0.05$, for soil type, respectively, *TVE* = 18.8 % for RDA model, Fig. B.2).

Assessing each element separately, gypsophiles had higher leaf Mg, S, Fe, Al, Na, Mn, Cu than gypsovags and lower K concentrations ($P < 0.05$, Table 4, see F-ratios of GLMMs on Tables A.5, A.6). Particularly

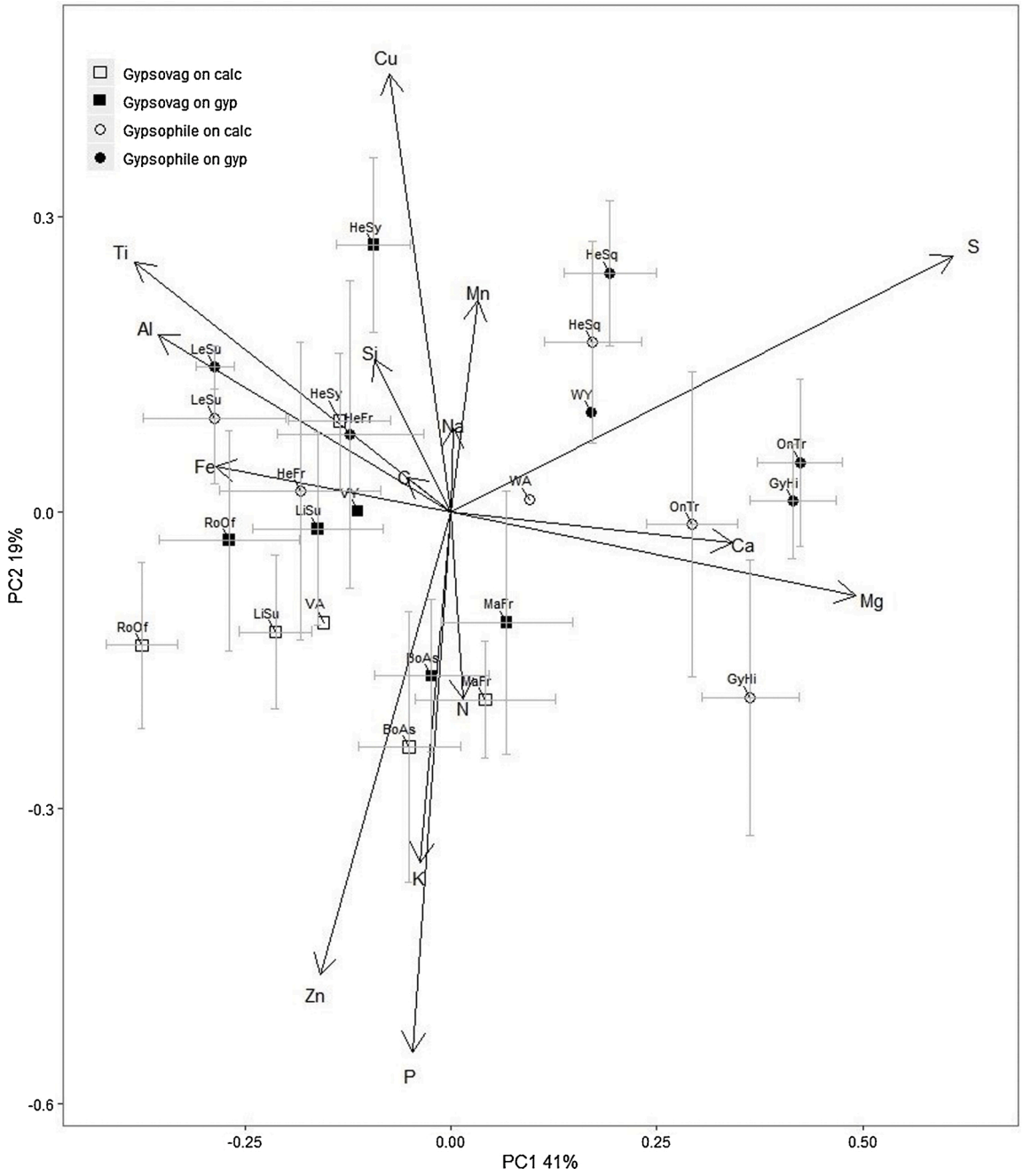

Fig. 2. Biplot distance of first and second principal components based on Center Log-ratio transformation of leaf elemental composition. Centroids of each treatment mean were drawn (± S.D.). Circles indicate gypsophiles; squares, gypsovags. Filled symbols indicate plants grown on gypsiferous soils; empty symbols, calcareous soils. BoAs: *B.asperum*; GyHi: *G.hispanica*; HeFr: *H.fruticosa*; HeSq: *H.squamatum*; HeSy: *H.syriacum*; LeSu: L.*subulatum*; LiSu: L.*suffruticosum*; MaFr: *M.fruticulosa*; OnTr: *Ononis tridentata*; RoOf: *R.officinalis*; WA: all gypsophiles on calcareous soils; VA: all gypsovags on calcareous soils; WY: all gypsophiles on gypsiferous soils; VY: all gypsovags on gypsiferous soils.

large differences were observed for S and Mg. Leaf S of gypsophiles was triple that of gypsovags, and leaf Mg was 2.4-fold greater in gypsophiles than gypsovags. The leaf S concentration of gypsovags increased from 5.9 mg g^{-1} in calcareous soil to 7.7 mg g^{-1} in gypsum ($P < 0.05$), whereas S concentrations in gypsophiles shifted from 15.6 mg g^{-1} in calcareous soil to 24.4 mg g^{-1} in gypsum soil ($P < 0.05$). In contrast, leaf Mg of gypsovags and gypsophiles did not differ between soil types. Leaf Ca was similar between gypsophiles and gypsovags on gypsum, but gypsophiles had almost twice the leaf Ca concentrations of gypsovags when growing on calcareous soil ($P < 0.05$). Gypsovags increased Ca concentrations up to 1.15-fold when growing on gypsum ($P < 0.05$), whereas gypsophiles had similar Ca concentrations on both substrates. For leaf Cr and Mo, gypsophiles had greater concentrations when grown on gypseous soil ($P < 0.05$), whereas gypsovags had similar concentrations in both soils. In general, plants grown on calcareous soil had higher P, K and C and lower S, Mo, Li, Mn, Cu and Mg, than those cultivated on gypseous soils ($P < 0.05$).

Despite these general trends, some species-specific trends were observed. In accordance with the PCA results, the gypsophile *H. fruticosa* was closer to gypsovags than gypsophiles in both soils, whereas the opposite was true for the gypsovag *H. syriacum* (Figs. 2, and B.2). Furthermore, some species of gypsophiles and gypsovags shifted their leaf elemental composition between soil types ($P < 0.05$, Table A.7), as observed in distance biplots (Figs. 2 and B.1) or the heatmap of distances

Table 4
Means and standard deviation of leaf elemental concentration ($mg \cdot g^{-1}$) for each treatment. Major letters indicate significant differences between gypsophiles and gypsovags regardless of the soil type ($P < 0.05$) Minor letters indicate significant differences between soil types ($P < 0.05$) within each gypsum affinity group.

Element ($mg\ g^{-1}$)	Gypsovags				Gypsophiles			
	Calcareous		Gypseous		Calcareous		Gypseous	
Al	0.3 ± 0.1	A	0.3 ± 0.1	A	0.5 ± 0.6	B	0.4 ± 0.4	B
C	429.0 ± 47.7		427.7 ± 47.7		389.7 ± 47.7	a	378.8 ± 56.7	b
Ca	26.4 ± 12.3	a	30.3 ± 14.1	b	46.9 ± 22.4		46.0 ± 21.8	
Cr	1.3E-2 ± 1.1E-2	A	1.5E-2 ± 1.7E-2	A	3.4E-2 ± 7.3E-2	Ba	2.4E-2 ± 4.7E-2	Bb
Cu	1.0E-2 ± 6.1E-3		1.2E-2 ± 9.4E-3		1.2E-2 ± 6.7E-3	a	1.3E-2 ± 5.6E-3	b
Fe	0.3 ± 0.1	A	0.3 ± 0.1	A	0.5 ± 0.7	B	0.4 ± 0.5	B
K	10.4 ± 3.2	Aa	8.3 ± 3.3	Ab	8.2 ± 4.1	Ba	6.2 ± 2.3	Bb
Li	4.5E-3 ± 4.5E-3	a	6.8E-3 ± 7.3E-3	b	3.6E-3 ± 3.0E-3	a	5.4E-3 ± 5.5E-3	b
Mg	4.1 ± 2.1	A	4.1 ± 2.0	A	8.8 ± 5.3	B	10.8 ± 7.4	B
Mn	6.2E-2 ± 2.8E-2	A	5.7E-2 ± 2.8E-2	A	7.8E-2 ± 2.8E-2	Ba	6.4E-2 ± 2.2E-2	Bb
Mo	5.6E-3 ± 5.6E-3		7.8E-3 ± 7.7E-3		6.1E-3 ± 4.4E-3	a	1.7E-2 ± 2.0E-2	b
N	17.3 ± 8.8		15.9 ± 8.9		15.3 ± 8.1		14.5 ± 7.4	
Na	1.0E-1 ± 6.5E-2	A	8.7E-2 ± 3.0E-2	A	1.4E-1 ± 6.1E-2	B	1.3E-1 ± 6.1E-2	B
P	2.3 ± 1.5	a	1.1 ± 0.6	b	2.4 ± 2.6	a	1.0 ± 0.7	b
S	5.9 ± 4.2	Aa	7.7 ± 4.7	Ab	15.6 ± 7.6	Ba	24.4 ± 16.4	Bb
Si	1.0 ± 0.2		1.0 ± 0.2		1.0 ± 0.3		1.0 ± 0.3	
Ti	3.7E-3 ± 1.3E-3		4.2E-3 ± 2.1E-3		5.6E-3 ± 5.8E-3		5.5E-3 ± 5.6E-3	
Zn	5.1E-2 ± 3.2E-2		5.3E-2 ± 2.9E-2		5.1E-2 ± 4.9E-2		5.0E-2 ± 3.8E-2	

(Fig. B.3). Shifts in leaf elemental composition were mainly related to leaf S, K, P, regardless of gypsum affinity (Tables A.8 and A.9).

4. Discussion

In contrast to our expectations, gypsophiles had equal or better growth and fitness when growing in calcareous soils. Gypsovags also had similar or higher growth and fitness in calcareous soil than gypseous soil, which is not surprising owing to their widespread occurrence on both substrates. In support of our second hypothesis, gypsophiles showed higher S and Mg concentrations than gypsovags irrespective of the soil type. However, both groups of plants shifted their leaf elemental composition according to soil nutrient availability and had higher leaf S and Mg when growing on gypseous soils. Despite these general trends, species-specific responses were observed within gypsum affinities.

4.1. Gypsophiles completed their life cycle on calcareous soil, being similarly or even more productive than on gypseous soil

Gypsum-exclusive species are restricted to gypseous soils in natural environments. However, we observed that gypsophiles were able to complete their life cycle, producing viable seeds in calcareous soils in the greenhouse. This result demonstrates that soil chemistry alone is not a factor preventing the occurrence of gypsophiles off of gypseous soils. This result is supported by field observations in Spain indicating that, even though gypsophiles are far more frequently found on gypseous soils, they are sometimes also found naturally off gypseous soils (Mota et al., 2011, Luzuriaga et al., 2015). Nevertheless, it is unclear if the few gypsophile individuals found growing off gypseous soils in nature could complete their life cycle, producing viable seeds and recruiting new individuals, since most data were observations of presence / absence. In any case, care should be taken when extrapolating results from experimental studies to natural conditions (Wenk and Dawson, 2007). Our experiment involved regular de-weeding and watering, removing any potential competition from neighbouring plants or water stress, conditions that are far from those in natural environments. The combination of different stress factors (plant competition, drought and altered soil chemistry) could be the underlying mechanism explaining gypsophile restriction to gypseous soils, rather than soil chemistry alone, as demonstrated by our experiment. Further experiments on natural gypseous and calcareous soils testing for the combined effects of soil chemistry plus plant competition and water availability are needed to shed light on these issues.

In contrast to our first hypothesis, some gypsophiles were more productive on calcareous than on gypseous soil, showing higher photosynthetic assimilation rates, higher SLA and larger biomass. Ballesteros et al. (2014) found poorer plant performance on marls than gypseous soils. Our calcareous soil had higher pH, N, P and K and lower conductivity, S and Mg concentrations, indicating better conditions than gypseous soil for standard plant growth. Both gypsum affinity groups showed a delay in the initiation of flowering when growing on gypseous soils, probably due to the more stressful conditions of gypsum for plant growth. However, we observed that gypsophiles showed a consistent phenological delay compared to gypsovags. Such a phenological delay has been described in the literature (Escudero et al., 2014), although the ecological and adaptive factors behind it remain unexplored. Furthermore, gypsophiles did not show any leaf deficiency symptoms and had similar maximum flower production and individual seed mass in both soils, similar to gypsovags. Similarly, Heiden et al., (unpubl. res.) observed that gypsophiles had low germination in acidic soils but germinated equally well on alkaline calcareous and gypseous soils. Consequently, gypsophiles seem to require soils with high pH and high Ca availability to germinate and complete their life cycle, but do not have a requirement for high S or gypsum to grow and complete their life cycle under experimental conditions.

4.2. Gypsophiles displayed higher leaf S and Mg and lower leaf K than gypsovags both in and off gypsum

In accordance with our second hypothesis, gypsophiles had higher leaf S and Mg and lower K concentrations than gypsovags in both soil types. This pattern indicates a high preference of gypsophiles for these two elements, in accordance with previous studies of plants growing on gypseous soils, where the S and Mg concentrations of gypsophiles tended to be higher than those of gypsovags (Alvarado, 1995; Palacio et al., 2007; Muller et al., 2017).

The ability of gypsophiles to accumulate S was remarkable, reaching S foliar concentrations between 15 $mg\ g^{-1}$ and 25 $mg\ g^{-1}$, one order of magnitude higher than standard foliar S concentrations of non S-deprived plants (Kalra, 1997). Such high S-accumulation was maintained even when grown in calcareous soil, which had 55-fold less S than the gypseous soil. Despite the lower S availability, gypsophile species managed to grow without any signs of deficiency and accumulated S to a higher extent than closely-related gypsovags on calcareous soil. We cannot rule out the possibility that S-accumulation is a nutritional requirement of gypsophiles that may impede the completion of their life

cycle or their competitive ability in natural conditions. The S content in calcareous soils under greenhouse conditions could be sufficient, but the situation may be different in the field, with lower water availability and increased plant-plant competition. Finally, gypsophiles showed higher leaf S than gypsovags, although some gypsovags also had relatively high leaf S in both soil types. High leaf S concentrations are not exclusive of gypsophiles but also related to phylogenetic effects (Neugebauer et al., 2018). It has been suggested that the ability to accumulate S could be an ancient trait, evolved before the acquisition of gypsophily, that may serve as a pre-requisite to become a gypsophile (Moore et al., 2014).

The low leaf K (below 8 mg g^{-1}) of gypsophiles could be an adaptation to gypseous soils, since low K requirements may be advantageous in soils with low K availability (Alvarado, 1995), such as gypsum (Casby-Horton et al., 2015). Low K requirements are linked to high leaf Ca concentrations in the gypsophile species studied (Alvarado, 1995), since plants have a preference for using Ca ions over other cations such as K, Na or Mg as osmotic compounds (Kinzel, 1989). High leaf Ca has been considered a distinctive trait of gypsophiles (Palacio et al., 2007; Muller et al., 2017), although we did not observe differences between gypsum affinity types. However, gypsophiles showed high leaf Ca concentrations irrespective of the soil type, whereas gypsovags increased Ca concentrations when growing on gypseous soil. This shift can be explained by the higher Ca activity of gypsum as compared to calcareous soils (Food and Agriculture Organization of the United Nations and Soil Resources, and Conservation Service (FAO), 1990). These results seem to indicate a higher ability to uptake Ca in gypsophiles than gypsovags, although further experiments are needed.

Gypsophiles species had higher leaf Mg than gypsovags, with increased Mg accumulation on gypsum, where it was highly available. However, neither group of plants shifted Mg concentrations in response to changes in the substrate. Mg accumulation is also a distinctive trait of gypsophile species (Palacio et al., 2007; Merlo et al., 2019). However, Mg concentrations are deeply affected by phylogenetic relationships (White et al., 2018), and some gypsophiles, such as *H. squamatum* and *L. subulatum,* did not show an accumulator pattern, as described by Merlo et al. (2019). It has been suggested that high Mg concentrations could be advantageous in gypseous soils, favouring foliar succulence (Merlo et al., 2019) or forming crystals with oxalate or sulphate (He et al., 2012) to help detoxify excess S and Ca.

Finally, we observed higher leaf Fe, Al, Na, Mn, Cr and Mo concentrations in gypsophiles than gypsovags. Similar to our results, Alvarado et al. (1995) found higher leaf Fe and Mn in gypsophiles compared to gypsovags. Leaf Na was analysed only in few gypsum plant surveys (Bolukbasi et al., 2016; Merlo et al., 2019); where significant differences between gypsum affinities were not observed. Differences in Cr, Mo and Mn between gypsophiles and gypsovags are difficult to understand, although Mo and Mn are linked to S metabolism (Maillard et al., 2016, Courbet et al., 2019). Despite these general trends, species responded differently to each soil and had different leaf elemental concentration, indicating species-specific responses within gypsum affinities mainly related to S, K and Mg concentrations.

4.3. Gypseous soils affect the leaf elemental composition of plants

Plants grown in gypseous soils had higher leaf S, Mg, Li and lower P and K, mirroring their soil nutrient availability, and also higher Cu and Mn (despite total leaf concentrations that were similar to those in calcareous soils). Thus, gypseous soils affect the leaf elemental composition of plants (Palacio et al., 2007; Salmerón-Sánchez et al., 2014), leading mainly to high leaf S and low P regardless of the gypsum affinity of the species (Food and Agriculture Organization of the United Nations and Soil Resources, and Conservation Service (FAO), 1990). Similarly, Boukhris and Lossaint (1970) and Robson et al. (2017) observed that plants had high leaf Ca when cultivated on both gypsum and calcareous soil, but less S when growing out of gypsum, according with soil nutrient availability. The mechanisms of P cycling in plants growing on gypsum deserve further study, due to the high relevance of this nutrient for plant growth and the remarkable P immobilization in gypseous soils (Food and Agriculture Organization of the United Nations and Soil Resources, and Conservation Service (FAO), 1990).

5. Conclusions

Gypsophile species grew and were able to complete their life cycle in non-gypseous soils under experimental conditions and in the absence of competition, producing flowers and fruits which rendered viable seeds. Gypsum endemics had similar or higher growth on calcareous than gypseous soil. Most species shifted their leaf elemental composition according to nutrient soil availability, displaying higher leaf S and lower P in gypseous soils. However, gypsophiles accumulated higher S and Mg and lower K concentrations than gypsovags, irrespective of the substrate. The remarkable ability of gypsophiles to accumulate S even in low S-availability conditions suggests a possible nutritional requirement for high S. However, our results indicate this nutritional requirement may not be the unique driver of the exclusion of gypsophiles from non-gypseous soils in natural environments, and the role of other biotic (plant-plant competition, herbivory) and abiotic (water stress) factors deserves further study.

Author contributions

GM and SP designed and set up the experiment; AC, maintained the experiment, measured all variables, analysed data and led the manuscript writing; JPF measured and discussed photosynthesis and related traits; All authors discussed the results and wrote the manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.envexpbot.2020.104294.

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A wonderful tale of passion for the substrate, avidity for sulphur and phosphorus, and struggle for survival!

Andreu Cera Rull
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