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FOLIAR MINERAL ACCUMULATION PATTERNS OF GYPSOPHILES AND THEIR RELATIVES FROM THE USA AND SPAIN

A Thesis Submitted to the
Office of Graduate Studies
College of Arts & Sciences of
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In Partial Fulfillment of the Requirements
for the Degree of
Master of Science

By
Clare T. Muller
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SUMMARY

The origins and mechanisms enabling plant endemism, particularly in unique edaphic systems, is a growing area of interest in ecology. Gypsum endemism (gypsophily) is relatively understudied compared to other edaphic systems, despite the commonality of surface gypsum worldwide, including regions in Spain and North America. Because gypsum is chemically challenging for plants, previous studies investigating the functional ecology of gypsophiles (primarily conducted in Spain) have focused on the leaf mineral nutrition of gypsophiles. Results of these studies suggest the distribution extent (widely-distributed taxa versus narrowly-distributed taxa) of gypsophiles is correlated with their leaf nutritional patterns. In particular, widely-distributed gypsophiles accumulate elements in excess in gypsum soils (sulfur and calcium) and biomineralize gypsum in their leaves, but narrowly-distributed gypsophiles and non-endemic taxa do not. These patterns suggest some gypsophiles from Spain possess traits that may promote tolerance of the unique chemistry of gypsum. Our work focuses on the gypsum flora of the Chihuahuan Desert in the USA. We determined that leaf nutrient accumulation patterns from the gypsum flora of Spain are mirrored by patterns from taxa collected in the USA. We incorporated phylogenetic controls in our design to account for patterns due to shared evolutionary history among taxa and revealed trends that suggest phylogeny is important for delineating nutritional patterns for the gypsum floras from Spain and the USA. Finally, we present a first look at the whole-plant nutritional patterns of taxa from the Spanish gypsum flora, which suggests widely-distributed gypsophiles, narrowly-distributed gypsophiles, and non-endemics may differ in their nutrient accumulation patterns in multiple plant organ systems.

Chapter I: Phylogenetic patterns of foliar mineral nutrient accumulation among gypsophilic plants and their relatives in the Chihuahuan Desert

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ABSTRACT

Gypsum endemism in plants (gypsophily) is common on gypsum outcrops worldwide, but little is known about the functional ecology of Chihuahuan Desert gypsophiles. We investigated whether leaf chemistry of gypsophile lineages from the northern Chihuahuan Desert are similar to leaves of related non-endemic (gypsovag) species relative to their soil chemistry. We expected widely-distributed gypsophiles, hypothesized to be older lineages on gypsum, would have distinct leaf chemistry from narrowly-distributed, relatively younger lineages endemic to gypsum and gypsovags, reflecting adaptation to gypsum. We collected leaves from 23 gypsophiles and related non-endemic taxa growing on non-gypsum soils. Soils and leaves were analyzed for Ca, S, Mg, K, N, and P. Leaf gypsum was assessed using Fourier transform infrared spectroscopy. Most widespread gypsophilic lineages that are hypothesized to be relatively old accumulate foliar S, Ca and gypsum, but younger gypsophilic lineages and closely related gypsovags do not. Young, narrowly-distributed gypsophilic lineages have leaf chemical signatures similar to non-endemic congeners and confamilials. Our data suggest multiple adaptive mechanisms support life on gypsum in Chihuahuan Desert gypsophiles. Most widespread gypsophiles are specialized for life on gypsum, likely due to shared abilities to accumulate and assimilate S and Ca in leaves. In contrast, narrowly-distributed gypsophiles may have mechanisms to exclude excess S and Ca from their leaves, preventing toxicity. Future work will investigate the nutrient accumulation and exclusion patterns of other plant organs to determine at what level excess S and Ca uptake is restricted for young-lineage gypsophiles and gypsovags.

INTRODUCTION

Soil chemistry is an important environmental filter driving the ecology of plants (Laliberté et al., 2014). Soil conditions can restrict establishment and distribution of plant species, leading to strong phenotypic selection for edaphically endemic plants—species that only grow on specific soil types. Edaphic endemics are spatially limited to the distributions of a particular soil type and are often highly specialized to their habitats (Kruckeberg and Rabinowitz, 1985; Kruckeberg, 2004). Because unusual soils have patchy distributions and are host to specialized endemic floras, they often contribute to a significant portion of the world's plant biodiversity despite their limited distribution, and hence are often considered biodiversity hotspots and targets of conservation (Myers et al., 2000; Damschen et al., 2011; Escudero et al., 2014). Efforts to protect edaphic endemic plant communities are particularly important, since these communities may be more vulnerable to the effects of disturbance due to their specialization and limited distributions.

Soils rich in gypsum ($CaSO_4 \cdot 2H_2O$) host diverse, endemic plant communities around the world. Gypsum soils are almost completely restricted to arid and semiarid regions, for two principal reasons. First, evaporative demand creates capillary uplift of gypsum to surface soil layers, creating gypsum crusts; in more mesic or humid environments, water infiltration and percolation prevents gypsum crust development (Verheye and Boyadgiev, 1997). Second, mineral gypsum is relatively highly soluble (Herrero et al., 2009), and hence surface outcrops of mineral gypsum are much more likely to persist through evolutionarily meaningful time periods in arid and semiarid regions due to their much lower annual rainfall. Consequently, gypsum endemic floras

are strongly associated with outcrops of mineral gypsum in drier regions around the globe, particularly in the Mediterranean, the Middle East, the Horn of Africa, and southwestern North America (Escudero et al., 2014; Moore et al., 2014). Because gypsum soils have a less negative osmotic potential than saline soils, ion toxicity is not as significant in gypsum soils as saline or sodic soils (Herrero et al., 2009). In fact, gypsum may act as a dispersive agent in saline or sodic soils, minimizing ionic stress (Herrero and Porta, 2000). Gypsum has also been shown to increase the water retention capacity of soils (Moret-Fernández and Herrero, 2015). However, other characteristics of gypsum soils potentially limit plant nutrient availability. High SO_4^{2-} can induce plant toxicity (Ruiz et al., 2003) or nutrient deficiencies due to ion competition at the root surface (White, 2012), and high Ca:Mg may limit the availability of some macro- and micronutrients (Salmerón-Sánchez et al., 2014), due to precipitation and complexation with Ca^{2+} (e.g., precipitation of insoluble Ca-P phases). Additionally, high Ca^{2+} limits uptake of K^+ and Mg^{2+} due to similarity in ion size and charge (White, 2012). In soils that are high in gypsum concentration, cation exchange capacity decreases, further limiting nutrient availability (Castillejo et al., 2011; Escudero et al., 2014). The effects of the unique soil properties of gypsum on soil chemistry, compounded by limited soil nutrition and hydration in arid environments, challenge plant establishment and success.

Research aimed at understanding the specific drivers of gypsum endemism (henceforth, gypsophily) has been focused overwhelmingly on the Spanish gypsum flora (Palacio et al., 2007; Pueyo and Alados, 2007; Pueyo et al., 2007; Escudero et al., 2014; Salmerón-Sánchez et al., 2014), although some work has been undertaken in North America (Meyer, 1986; Borer et al., 2012) and Turkey (Bolukbasi et al., 2016). In North

America, early work from the Mojave Desert suggested soil physical factors, rather than differences in soil chemistry, drive patterns of distribution and abundance of plants on and off gypsum soils (Meyer, 1986). In this study, Mojave Desert species able to penetrate the gypsum surface crust could grow and establish in gypsum soils as well as non-gypsum soils. More current work from Europe suggests that gypsophiles are adapted to the unique chemistry of gypsum soils (Palacio et al., 2007; Palacio et al., 2014). This work has found that regionally dominant Spanish gypsophiles (those common on gypsum and occurring broadly on gypsum over a relatively wide geographic area, sometimes called "wide gypsophiles") have higher concentrations of total S and Ca, as well as other inorganic elements such as Mg, P, and Na, than narrowly distributed gypsophiles (sometimes called "narrow gypsophiles") or gypsovags (plants able to grow both on and off gypsum soils), and in some cases accumulate calcium oxalate and gypsum crystals in leaves (Palacio et al., 2007; Palacio et al., 2014). In contrast, evidence from both the Spanish (Palacio et al., 2007; Palacio et al., 2014) and Turkish (Bolukbasi et al., 2016) gypsum flora has shown that narrowly distributed gypsophiles possess leaf chemical signatures more similar to non-endemic species, suggesting there are multiple mechanisms that support gypsophily among gypsophiles of wide and narrow geographic distribution.

The Chihuahuan Desert gypsum flora provides an excellent study system for exploring questions regarding adaptation to gypsum soil. Gypsum outcrops of Permian through Triassic age are distributed throughout the Chihuahuan Desert region, creating an extensive "archipelago" of gypsum soils extending from San Luis Potosí in Mexico to northern New Mexico in the USA (Parsons, 1976; Powell and Turner, 1977; Turner and

Powell, 1979). These gypsum soils host the world's largest known gypsophilic flora, including over 230 species of gypsophiles in over 35 families (Moore et al., 2014). Extensive work on the systematics of the Chihuahuan Desert gypsum flora is ongoing (Moore and Jansen, 2007; Moore et al., 2014) and has revealed the existence of numerous clades of gypsophiles. Many such clades [e.g., the gypsophilic clades of *Acleisanthes* (Nyctaginaceae), *Nama* (Namaceae), *Nerisyrenia* (Brassicaceae), *Sartwellia* (Asteraceae), and *Tiquilia* (Ehretiaceae)] are hypothesized to be relatively old (on the order of 2–5 million years in age) based on molecular dating, their high morphological distinctiveness compared to non-gypsophilic congeners, the extent of speciation within these clades (with as many as 10 allopatric species of gypsophiles in a single clade), and the relatively wide total geographic distribution of each of these clades across the Chihuahuan Desert gypsum "archipelago" (with the total extent of many of these clades encompassing all or most of the Chihuahuan Desert) (Moore et al., 2014). In addition to these gypsophilic clades, there are numerous locally distributed gypsophilic taxa (narrow gypsophiles) that are hypothesized to be relatively young (< 2 million years in age) based on their limited geographic ranges, lack of speciation on gypsum, and high morphological similarity to their nearest congeners. These patterns suggest that the geographic extent of endemic lineages may be a good proxy for the relative age of a lineage of gypsophilic taxa. We expect hypothesized lineage age to be a better predictor of adaptive strategies for gypsophily than geographic extent, if evolutionary history affects the physiological adaptation mechanisms that support gypsophily. In all cases, the closest relatives of these gypsophilic lineages are gypsovag taxa, allowing for phylogenetic control in studies of physiological adaptation. In addition to this rich availability of gypsophilic taxa in the

Chihuahuan Desert, the strongly summer monsoon-driven climate of this region also provides a useful climatic contrast to ongoing studies of gypsum ecology in the primarily winter-wet Mediterranean, because the summer-wet climate of the former may reduce the severity of drought-induced nutrient limitation across soil types.

We sought to determine whether the chemical properties of gypsum soils are linked to unique leaf nutrient signatures in gypsophiles compared to non-endemic congeners or confamilials growing on non-gypsum soils. We expected gypsum soils to be enriched in total Ca and S compared to non-gypsum soils. We predicted that, if gypsophiles of the Chihuahuan Desert share physiological strategies with the gypsophilic flora of Spain, widespread, old-lineage gypsophiles would be enriched in both Ca and S in leaf tissue relative to congeners or confamilials growing on non-gypsum soils. We expected that old-lineage gypsophiles would also contain gypsum in their leaves. Additionally, we predicted that leaf concentrations of other nutrients (e.g., leaf N, P, K, and Mg) would be higher in all gypsophiles compared to close relatives growing on non-gypsum soils. Lastly, we expected to detect a phylogenetic pattern in leaf chemistry among gypsophiles and their non-endemic relatives, wherein congeners and confamilials would have more similar nutrient signatures compared to distantly related taxa.

MATERIALS AND METHODS

Primary sampling sites and taxon selection—The primary sampling of leaves and soils used in this study was conducted at five sites from September 4–6, 2014 (Appendix S1, see Supplemental Data with this article). We sampled from four sites in Eddy County, New Mexico in the northern Chihuahuan Desert: the Yeso Hills (32° 02′ 23″ N, -104° 27′

38″ W), Seven Rivers Hills (32° 33′ 18.4″ N, -104° 27′ 06.1″ W), near US Highway 285 (US 285) north of Carlsbad (32° 28′ 33.6″ N, -104° 17′ 31.5″ W), and along New Mexico Highway 128 (NM 128) east of Carlsbad (32° 18′ 36.4″ N, -103° 48′ 55.2″ W). The fifth primary sampling site was at the northern edge of Culberson County, Texas along Texas State Highway 54 (TX 54) north of Van Horn (31° 35′ 36.1″ N, -104° 51′ 19.3″ W). Mean annual temperature in Eddy County, NM is 16° C, and mean annual precipitation is 330 mm (averages represent 30 years of data obtained from Carlsbad NM station, National Climate Data Center, ncdc.noaa.gov). Our New Mexico sampling area encompasses large outcrops of Permian-aged gypsum, as well as limestone and alluvial soils. Soil complexes at NM sampling sites are primarily of the Reeves, Cottonwood, and Gypsum-Cottonwood series (Chugg et al., 1971). The Reeves and Cottonwood series have shallow gypsum soils, loamy textures, and little rock/gravel cover. Gypsum soils in Eddy County have gypsum bedrock, very shallow soils, and sometimes hard surface crusts (Chugg et al., 1971). The TX 54 gypsum soil site located in the Salt Basin of west Texas has Quaternary-aged, lacustrine-deposited gypsum. Soils in this region are well-weathered and of variable textures (Angle, 2001). Dominant plant species at our gypsum soil sites are perennial forbs rather than larger shrubs (Parsons, 1976) and often are endemic to gypsum. Gypsovag species were less common than gypsophiles at gypsum sites.

We aimed to include as many phylogenetic pairs of gypsophilic/non-gypsophilic taxa as possible, with the goal of encompassing a mix of gypsophilic taxa from various independent evolutionary origins, including taxa from widely distributed, morphologically divergent clades of gypsophiles (e.g., *Acleisanthes lanceolata*,

Sartwellia flaveriae, Tiquilia hispidissima; we will refer to these as "old-lineage" gypsophiles) as well as gypsophile taxa of more limited geographic extent that are less morphologically divergent from their congeners (e.g., *Linum allredii, Oenothera gayleana, Senecio warnockii;* we will refer to these as "young-lineage" gypsophiles) (Table 1). However, in some cases our ability to sample selected species was limited by plant health and availability at target sites. Sampling included 23 species in total, with members from fifteen genera and eight angiosperm families (Table 1). Eight old-lineage gypsophiles and seven young-lineage gypsophiles were sampled, along with eight gypsovag species. We collected congeners or confamilials growing on and off gypsum soils to account for phylogenetic patterns in the data, including six congener groupings, with at least one gypsophile lineage and one gypsovag per group (Table 1).

Sampling design—Soils were collected from all sampling sites from an area 1 m^2 around each plant replicate for eight of our target species (*Acleisanthes longiflora, A. lanceolata* var. *lanceolata, Anulocaulis leiosolenus* var. *gypsogenus, Tiquilia hispidissima, T. canescens* var. *canescens, Mentzelia strictissima, M. humilis* var. *humilis,* and *Nama carnosa*). We composited two soil subsamples from the plant canopy drip-line using soil corers up to 20 cm depth at each plot. Soils that were moist when collected were allowed to air dry prior to storage. Soils were then sieved (< 2 mm), and the gravel and fine soil fractions were weighed to determine gravel content.

We collected leaf samples from plants located at least 20 m from roadsides to limit the effects of disturbance on plant nutrition. However, due to site access limitations, *Acleisanthes lanceolata* individuals were collected within 20 m of roadside, but only in undisturbed gypsum. All gypsovags were sampled from non-gypsum soil sites. We

sampled at least five replicate plants for all species but *Senecio warnockii* (n = 2; Table 1). Replicate plants were randomly selected at each sampling location and were at least 10 m away from the nearest sampled individual of the same species. From each plant, we collected approximately 1–3 g of leaf tissue (dry weight) from the youngest, fully mature, green sun leaves for nutrient analysis.

Soil and plant nutrient analyses—Electrical conductivity (EC) and pH were determined from soil saturated paste extracts (Mosse et al., 2013). Saturated paste extracts were analyzed for soil soluble salts (Ca^{2+}, K^{+}, and Mg^{2+}) and S (representing SO_4^{2-}) using ICP-OES (Plasma 400; Perkin-Elmer). Total soil N was determined via micro Dumas combustion using a CN analyzer (ECS 4010; Costech Analytical). Olsen's extractable P was determined by the University of California Davis Analytical Laboratory.

All leaf tissues were rinsed briefly with deionized water to remove surface salts (< 15 s), dried in an oven for at least 24 hours at 60°C, finely ground (< 2 mm) using a ball mill, and prepared for cation analysis by microwave digestion using concentrated nitric acid. Digests were analyzed using ICP-OES for the elements P, S, Ca, K and Mg. Leaves were also measured for total N using the CN analyzer.

In addition to mineral nutrient analyses, the presence of gypsum was assessed in leaves using diamond attenuated total reflectance (DATR) Fourier transform infrared spectroscopy (FTIR) (Satellite spectrophotometer, Thermo Mattson; MKII Golden Gate DATR attachment, Specac). The spectrophotometer was fitted with a potassium bromide beam-splitter and a deuterated triglycine sulfate detector. Two hundred spectral scans were averaged over a range of 4000–400 cm^{-1} at 4 cm^{-1} resolution. A fresh background

was taken before each sample. Approximately 10 mg of dry, ground leaf tissue was placed directly onto the diamond window and dispersed evenly with a flat-tip powder press. Gypsum was identified in samples by O–H stretching peaks at 3547 and 3400 cm^{-1} and S–O bending at 669 and 599 cm^{-1} and compared to reference spectra of pure gypsum (Palacio et al., 2014). In combination with results from the mineral nutrient analyses, replicates were given one of three scores to be incorporated into multivariate analysis: gypsum present (2), potentially present (1), or absent (0). Samples were also analyzed for detection of calcium oxalate, but spectra were inconclusive for all samples.

Principal components analysis—Principal components analysis (PCA) was used to compare patterns in leaf chemistry between old-lineage gypsophiles, young-lineage gypsophiles, and gypsovags in Canoco v5 (Ter Braak and Šmilauer, 2012). Variables included in our PCA for leaf chemistry were S, Ca, Mg, K, N, P levels, and gypsum presence/absence. We created an additional PCA that excluded the gypsum spectral data in order to visualize the effect of the gypsum presence variable on sample clustering along principal components axes (Appendix S2). In these analyses, species means plotted as centroids, and those plotting closer to one another in multivariate space were more similar in their chemical signatures. We conducted a separate PCA to assess patterns in soil chemistry and gravel content among our sampling sites. In these analyses, soil centroids represented replicate plot means, in which plots were associated with individuals from six of our sampled species.

Phylogenetic MANOVA and ANOVA—Because this study incorporates interspecific comparisons of multivariate data, species non-independence was addressed using tests that control for the effect of phylogeny (Felsenstein, 1985). Gypsophile and

gypsovag groups from this study include members that span eight families in the angiosperm tree. Because scaled phylogenies of comparable resolution do not exist for all taxa in this study, we used simulation-based analysis to control for the effect of phylogeny using phylogenetic MANOVA and phylogenetic ANOVAs in R v3.3.1 with the package 'GEIGER' (Garland et al., 1993, 2005; Harmon et al., 2007; Revell, 2012; R Core Team, 2017). Phylogenetic ANOVA uses a proposed phylogeny to compare the variance of Monte Carlo-simulated continuous data plotted on the tree, computed under the assumption of Brownian motion, with the variance of our measured species means (Garland et al., 1993). We used a phylogeny constructed in Mesquite v3.2 (Maddison and Maddison, 2017) based on published phylogenies of Nyctaginaceae, Onagraceae, and angiosperms (Douglas and Manos, 2007; Johnson et al., 2009; Soltis et al., 2011; Panero et al., 2014) (Fig. 1). All tree branch lengths were set to one for phylogenetic analyses. The predictor variable for the phylogenetic MANOVA was gypsophilic "status" with three levels—old-lineage gypsophiles, young-lineage gypsophiles, and gypsovags. Because phylogenetic analysis requires the use of species means for interspecific comparisons, replication is at the level of species for all analyses (n = 8 for old-lineage gypsophiles, n = 7 for young-lineage gypsophiles, n = 8 for gypsovags). Response variables included in the MANOVA model were leaf S, Ca, Mg, N, P, and K. One thousand simulations were evaluated for each analysis. We calculated *P*-values for a model that incorporated phylogeny and a model that did not, as well as simulated model estimates of degrees of freedom. We also calculated Pillai's test statistic. Phylogenetic ANOVAs with Tukey's post-hoc tests comparing leaf Ca and S concentrations in old- and young-lineage gypsophiles and gypsovags were also conducted, and *P*-values for the

pairwise analyses were corrected for repeated tests using the Holm-Bonferroni method in the R package 'phytools' (Harmon et al., 2007; Revell, 2012).

***Mexico sampling and analysis*—**In preparation for the primary sampling reported in this study, leaves were also collected from an additional suite of gypsophilic taxa and congeners from the USA (New Mexico and Texas) and Mexico (Chihuahua, Coahuila, Durango, and Nuevo León) from August 15 to September 10, 2013. The youngest fully mature green sun leaves were collected for 54 species of gypsophiles and gypsovags (Appendix S3). The primary purpose of this 2013 field expedition was molecular systematics, so replication in nutrient sampling was much more limited than for taxa collected in 2014 (see later). Nevertheless, mineral nutrient analysis of these samples revealed highly similar patterns to those observed in the 2014 sampling, and hence these results are reported here. To investigate the potential for strong patterns of leaf nutrition in a broader suite of the gypsum endemic taxa, we conducted a separate PCA including both 2013 and 2014 collection taxa (Appendix S4). The variables included in the PCA were leaf S, Ca, Mg, N, P, K, and gypsum. Rather than classify them into "old" and "young" lineages, gypsophile taxa from the 2013 field sampling were treated as wide vs. narrow gypsophiles based on the extent of their geographic distributions (i.e., relatively broadly distributed vs. narrowly endemic at one or a few adjacent sites) because good estimates of lineage ages are not available for many of the 2013 taxa (Appendix S3). Nutrient analyses and FTIR spectral analyses were conducted in the same manner as described for the primary 2014 sampling. Due to limited replication, no additional statistical analyses of the 2013 taxa were performed.

RESULTS

***Soil chemistry*—**Soil chemistry differed between gypsum and non-gypsum soils, primarily due to concentrations of the elements associated with gypsum, Ca and S (Fig. 2). Gypsum soils had almost four times higher Ca and seven times higher S than non-gypsum soils (Appendix S5). Gypsum soils also had four times higher EC than non-gypsum soils, reflecting greater concentrations of charged ions. Extractable Mg, K, and total N did not drive separation between soil types (Fig. 2). Mean Mg in gypsum soils was half the concentration of non-gypsum soils. Extractable P varied among non-gypsum soil sites, but P concentrations in all gypsum soil samples were below detectable limits (< 1 ppm). Soil total N was three times higher in non-gypsum soils compared to gypsum (Appendix S5).

***Leaf chemistry*—**Our primary finding, corroborated by both PCA and phylogenetic MANOVA, is that leaf chemical signatures of old-lineage gypsophiles differed significantly from young-lineage gypsophiles and gypsovags (Table 2, Fig. 3). The primary drivers of separation between gypsophile groups were leaf S, Ca, and the presence of gypsum. There was an effect of phylogeny on leaf chemical signatures, as MANOVA and ANOVA tests were more significant when phylogeny was taken into account in the models (Table 2).

Tukey's tests revealed that old-lineage gypsophiles had significantly higher leaf S compared to young-lineage gypsophiles (Tukey's test, $P = 0.004$) and gypsovags (Tukey's test, $P = 0.003$) (Table 2, Appendix S6a). Mean leaf S in old-lineage gypsophiles was three times higher than leaf S in young-lineage gypsophiles and

gypsovags on average (Fig. 4). In contrast, leaf S between young-lineage gypsophiles and gypsovags was not significantly different (Tukey's test, $P = 0.767$).

While leaf Ca significantly differed among species based on gypsophilic status, when phylogeny was taken into account in the ANOVA model (Table 2), old-lineage gypsophiles were only marginally distinct from young-lineage gypsophiles and gypsovags based on a Tukey's post hoc test ($P = 0.06$). Young-lineage gypsophiles and gypsovags did not differ in leaf Ca (Tukey's test, $P = 0.875$). Mean leaf Ca among young-lineage gypsophiles and gypsovags was about 1.5 times lower than leaf Ca in wide gypsophiles (Fig. 4, Appendix S6a).

All old-lineage gypsophile FTIR spectra strongly indicated the presence of gypsum, with the notable exception of *Nerisyrenia linearifolia*, which had a weakly present gypsum peak. The only young-lineage gypsophile that may have contained gypsum in leaf tissue was *Abronia nealleyi* (Appendix S6a). *Abronia nealleyi* also contained high leaf S and Ca compared to most young-lineage gypsophiles. Leaf S in *A. nealleyi* was three times higher and leaf Ca was 2.5 times higher than in other young-lineage gypsophiles on average. Gypsovag taxa did not contain detectable gypsum in almost all cases, with the possible exception of *Tiquilia canescens* var. *canescens*, which had weak possible gypsum signatures in some replicates.

Leaf Mg was also a partial driver of separation on the PCA between old-lineage gypsophiles and other taxa (Fig. 3); however, gypsovags had particularly high mean leaf Mg due to the concentration observed in *Acleisanthes longiflora,* which was six times higher than the other species on average (Appendix S6a). Leaf N, P, and K were not strong drivers of separation in leaf chemical signatures (Fig. 3).

***Mexico collection leaf chemistry*—**The leaf chemical signatures of taxa collected in 2013 largely mirrored the nutrient trends observed for the 2014 taxa (Appendix S4). In general, wide gypsophiles had high concentrations of S and Ca compared to gypsovags and narrow gypsophiles (Appendix S6b). Leaf S, Ca, and gypsum drove separation of leaf chemical signatures among wide gypsophiles and other taxa along the first principal components axis (Appendix S7). Leaf Mg, N, P, and K were all drivers of separation along the second principal components axis, in which some gypsovag species tended to have higher concentrations of all macronutrients than other gypsovags (Appendix S7). Gypsophiles varied less in foliar concentrations of Mg, N, P, and K compared to gypsovags. Gypsum accumulation varied more for taxa collected in 2013 compared to those collected in 2014. Most 2013 collections of wide gypsophiles were found to have elevated S and Ca and the presence gypsum in leaves, with some exceptions. Notably, wide gypsophile species with a large shrub habit (*Leucophyllum alejandrae, L. coahuilense,* and *Fouquieria shrevei*) did not contain detectable gypsum, and had lower leaf S and Ca (Appendix S6b, Appendix S7). Additionally, some gypsovags with wide gypsophile congeners (e.g., *Tiquilia canescens* and *Nerisyrenia camporum*) that were collected on gypsum soils contained gypsum in their leaves, and some gypsovags collected on non-gypsum soils (e.g., *Acleisanthes longiflora*) had a weak signal for gypsum.

DISCUSSION

As predicted, widespread, old-lineage gypsophiles had distinct leaf chemical signatures compared to narrowly-distributed, young-lineage gypsophiles and gypsovags

growing off gypsum. Leaf concentrations of S and Ca were higher in old-lineage gypsophiles compared to young-lineage gypsophiles and gypsovags, and almost all old-lineage gypsophiles contained gypsum in their leaves. Our results are consistent with the findings of studies conducted on the mineral nutrition of gypsophiles in Spain and Turkey (Palacio et al., 2007, 2014; Bolukbasi et al., 2016) and suggest there are multiple mechanisms supporting gypsum adaptation in endemic species.

One strategy, employed by widely distributed, older gypsophilic lineages, appears to be the accumulation of foliar S and Ca in the form of gypsum and occasionally calcium oxalate (although not measured in this study). Gypsum and oxalate production in leaf tissues may prevent toxic concentrations of Ca and sulfate ions from accumulating in the cytosol, which could impact leaf physiology (He et al., 2014, 2015). Formation of crystal compounds from excess ions in leaves can prevent physiological stress (Munns, 2002; Parida and Das, 2004), and previous work suggests that storage of calcium sulfate or gypsum crystals in leaf vacuoles may be a strategy for excess ion sequestration in the woody species *Pinus palustris* (Pritchard et al., 2000) *Acacia robeorum* (He et al., 2014, 2015), and *Tamarix aphylla* (Storey and Thomson, 1994), as well as in herbaceous, widespread gypsophiles in Spain (Palacio et al., 2014). For old-lineage gypsophiles that accumulate high concentrations of foliar S but may not accumulate gypsum (e.g., *N. linearifolia*), secondary compounds rich in S are produced to prevent sulfate ion toxicity (Palacio et al., 2014). Leaf S concentrations observed in our wide gypsophiles were 24 g kg^{-1} on average, whereas typical concentrations of leaf S are 1–5 g kg^{-1} (Römheld, 2012). In a previous study from Spain, widespread gypsophiles accumulated leaf S, but very little in the form of sulfate ions, indicating that formation of assimilated compounds is a

potential strategy for tolerating excess S in the leaves of Spanish widespread gypsophiles (Ruiz et al., 2003). Analysis of the forms of foliar Ca in Chihuahuan Desert gypsovags has been conducted (Borer et al., 2012), in which some species accumulate high concentrations of physiologically unavailable Ca in leaves compared to labile Ca forms. However, the forms of leaf S beyond gypsum are not fully explained. We hypothesize that for species in the Brassicaceae, such as *N. linearifolia*, with only weak indicators of gypsum, glucosinolate compounds rich in S and N may account for high leaf S and N. Other organic molecules, including amino acids, may be produced in other groups to account for high concentrations of leaf S not in the form of gypsum or sulfate.

We hypothesized that wide gypsophiles would have higher concentrations of other ions in their leaves, especially N, P, K, and Mg compared to gypsovags. Although leaf N, P, K, and Mg did not drive separation in leaf chemical signatures among old and narrow gypsophiles and gypsovags, leaf N, P, and K concentrations tended to be higher in narrowly and widely distributed gypsophiles in the Asteraceae and Brassicaceae compared to other taxa (Fig. 3). This is of particular note because gypsum soils were relatively nutrient poor (Fig. 2) and were extremely low in extractable P (Table 2).

In contrast to the other nutrients, high leaf Mg was associated with taxa that had the highest concentrations of leaf Ca, especially in the Nyctaginaceae (Fig. 3). Many species in the Nyctaginaceae are known to produce calcium oxalate crystals (Kubitzki et al., 1993), and this may be a key mechanism to accumulate excess Ca for members of this family. Due to the similar size and charge of Mg and Ca ions, it is interesting that gypsophiles on substrates high in Ca can also accumulate high leaf Mg despite potential ion competition at the root surface (George et al., 2012). Other studies conclude that

selectivity for ions with reduced concentrations in soils indicates adaptation. For example, Sambatti and Rice (2006) found that serpentine ecotypes of the sunflower *Helianthus exilis* successfully excluded excess Mg uptake at the root surface to maintain favorable leaf Ca:Mg in serpentine soils, while non-serpentine ecotypes lacked this ability. As a consequence, biomass production was higher for serpentine than non-serpentine ecotypes on serpentine soils, suggesting they are adapted to serpentine soils. Similarly, the salt-tolerant shrub *Sarcobatus vermiculatus* possesses increased selectivity for uptake of leaf Mg throughout the growing season to compensate for increasing soil and leaf Na over time, suggesting that *S. vermiculatus* is adapted to select for essential nutrients during saline toxic stress (Donovan et al., 1997). Some old-lineage gypsophiles, hypothesized to be highly specialized to gypsum soils, may have more selective Mg transporters to compensate for the high Ca:Mg ratio observed in gypsum soils.

Interestingly, some gypsovag congeners and confamilials of old-lineage, widely distributed gypsophiles, particularly *Physaria fendleri*, *Acleisanthes longiflora*, and *Tiquilia canescens* var. *canescens*, had high concentrations of leaf S and Ca compared to most other gypsovags, suggesting a phylogenetic effect on leaf chemistry. In addition, statistical models that incorporated phylogeny in this study yielded results with stronger statistical significance than models that did not account for evolutionary history. The phylogenetic trends observed in some families, like the Brassicaceae, Namaceae, Nyctaginaceae, and Ehretiaceae, and the fact that the preponderance of gypsophilic plant taxa worldwide fall into just a few larger angiosperm clades, such as Caryophyllales, Brassicales, and asterids (Moore et al., 2014), lead us to suggest that the ancestors of many gypsophile lineages may have inherited certain preadaptive traits (perhaps

including Ca oxalate and gypsum synthesis) that have facilitated their survival on gypsum. Hypotheses regarding potential shared physiological traits of old-lineage gypsophiles and closely related gypsovags should be tested with reciprocal transplant experiments. If widely distributed, old-lineage gypsophiles are from groups preadapted for life on gypsum, congener gypsovag relatives with high Ca and S may be capable of accumulating gypsum when grown in gypsum soils. Furthermore, understanding the plasticity of the leaf chemistry of gypsophiles and gypsovags in response to substrate chemistry is critical for investigating the degree to which evolutionary history has influenced gypsophily. For some taxa sampled from multiple populations in 2013 in this study (in particular, *Tiquilia hispidissima*), leaf S varied substantially between sites (sd = 9.59 g kg^{-1}), suggesting leaf chemistry may depend on soil chemistry for some taxa. More rigorous sampling of gypsophilic lineages and related gypsovags can also enable more powerful statistical analysis of the phylogenetic impact on plant mineral nutrition.

While our results provide strong evidence for accumulation of foliar S, Ca and gypsum as a strategy for gypsum tolerance in wide gypsophiles, the mechanisms of S and Ca exclusion from the leaves of narrowly distributed, young-lineage gypsophiles are still unclear. Although almost all young-lineage gypsophiles have much lower foliar concentrations of leaf S and Ca compared to old-lineage gypsophiles, it is unknown whether young-lineage gypsophiles are excluding excess ions from their leaves, or preventing some uptake in other organs. In serpentine ecosystems, O'Dell et al. (2006) found that serpentine endemic species controlled transport of Mg from roots to shoots, but did not inhibit uptake at the root level, while non-endemic congeners did not regulate Mg translocation to the same extent. Regulation of Mg translocation to aboveground

tissues enabled serpentine endemics to maintain higher Ca:Mg than non-endemic species (O'Dell et al., 2006). Characterization of the mineral nutrition of multiple organ systems in gypsophiles and related gypsovags may clarify how young-lineage gypsophiles tolerate the chemistry of gypsum differently from old-lineage gypsophiles. This work is currently being investigated by our research group.

The gypsophilic flora of North America is particularly diverse, and phylogeny potentially plays a key role in determining the nutritional physiology of taxa growing on chemically restrictive soils. By sampling within a phylogenetic context and accounting for shared evolutionary history in statistical models, we have begun to unravel the specific role of phylogeny in shaping the adaptive strategies of the gypsophilic flora of the Chihuahuan Desert. We have shown that leaf chemical signatures are distinct between widely distributed, old-lineage gypsophiles and narrowly distributed, young-lineage gypsophiles and gypsovags in the Chihuahuan Desert of Texas and New Mexico. We have also observed that hypothesized lineage ages of endemic taxa predict foliar nutrient accumulation strategies, strongly supporting the idea that geographic extent of gypsophilic lineages is a proxy for their relative age.

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Table 1. Taxa collected in September 2014 for leaf nutrient analysis. Under the Status column, assignment to old-lineage vs. young-lineage gypsophile is based on references within Moore et al. (2014). Site refers to the sampling site where species were collected; Abbr. indicates the abbreviation for each taxon as it is shown in figures; "n" indicates the number of individual replicates. Vouchers were deposited in the herbarium of Oberlin College (OC).

Family	Species	Voucher	Status	Site	Abbr.	n
Asteraceae	*Dicranocarpus parviflorus* A.Gray	M.J. Moore 1756	Old-lineage gypsophile	Yeso Hills	DIPA	5
Asteraceae	*Sartwellia flaveriae* A.Gray	M.J. Moore et al. 652	Old-lineage gypsophile	Yeso Hills	SAFL	5
Asteraceae	*Senecio warnockii* Shinners	M.J. Moore et al. 2916	Young-lineage gypsophile	Yeso Hills	SEWA	2
Brassicaceae	*Nerisyrenia linearifolia* (S.Watson) Greene	M.J. Moore et al. 2929	Old-lineage gypsophile	Yeso Hills	NELI	5
Brassicaceae	*Physaria fendleri* (A.Gray) O'Kane & Al-Shehbaz	M.J. Moore et al. 2926	Gypsovag	Seven Rivers	PHFE	4
Ehretiaceae	*Tiquilia canescens* (A.DC.) A.T.Richardson var. *canescens*	M.J. Moore et al. 2925	Gypsovag	Seven Rivers	TICA	5
Ehretiaceae	*Tiquilia hispidissima* (Torr. & A.Gray) A.T.Richardson	M.J. Moore et al. 2928	Old-lineage gypsophile	Yeso Hills	TIHI	5
Linaceae	*Linum allredii* Sivinski & M.O.Howard	M.J. Moore et al. 2917	Young-lineage gypsophile	Yeso Hills	LIAL	5
Loasaceae	*Mentzelia humilis* (Urb. & Gilg) J.Darl. var. *humilis*	M.J. Moore et al. 2915	Young-lineage gypsophile	Yeso Hills	MEHU	5
Loasaceae	*Mentzelia strictissima* (Wooton & Standl.) J.Darl.	M.J. Moore et al. 2934	Gypsovag	NM 128	MEST	5

Namaceae	*Nama carnosa* (Wooton) C.L.Hitchc.	M.J. Moore et al. 651	Old-lineage gypsophile	Yeso Hills	NACAR	5
Nyctaginaceae	*Abronia nealleyi* Standl.	M.J. Moore et al. 2287	Young-lineage gypsophile	Yeso Hills	ABNE	5
Nyctaginaceae	*Acleisanthes lanceolata* (Wooton) R.A.Levin var. *lanceolata*	M.J. Moore et al. 2912	Old-lineage gypsophile	TX 54	ACLA-L	5
Nyctaginaceae	*Acleisanthes longiflora* A.Gray	M.J. Moore et al. 2922	Gypsovag	US 285	ACLO	5
Nyctaginaceae	*Anulocaulis leiosolenus* (Torr.) Standl.) var. *gypsogenus* (Waterf.) Spellenb. & T.Wootten	M.J. Moore et al. 648	Old-lineage gypsophile	Yeso Hills	ANLE-G	5
Onagraceae	*Oenothera capillifolia* Scheele ssp. *berlandieri* (Spach) W.L.Wagner & Hoch	M.J. Moore et al. 2933	Gypsovag	NM 128	OECA	5
Onagraceae	*Oenothera gayleana* B.L.Turner & M.J.Moore	M.J. Moore et al. 2286	Young-lineage gypsophile	Yeso Hills	OEGA	5
Onagraceae	*Oenothera hartwegii* Benth. ssp. *filifolia* (Eastw.) W.L.Wagner & Hoch	M.J. Moore et al. 2285	Young-lineage gypsophile	Yeso Hills	OEHA-F	5
Onagraceae	*Oenothera hartwegii* Benth. ssp. *pubescens* (A.Gray) W.L.Wagner & Hoch	M.J. Moore et al. 2923	Gypsovag	US 285	OEHA-P	8
Poaceae	*Bouteloua breviseta* Vasey	R.D. Worthington 34991	Young-lineage gypsophile	Yeso Hills	BOBR	5
Poaceae	*Bouteloua curtipendula* (Michx.) Torr.	M.J. Moore et al. 2927	Gypsovag	Seven Rivers	BOCU	5

Poaceae	*Sporobolus cryptandrus* (Torr.) A.Gray	M.J. Moore et al. 2935	Gypsovag	NM 128	SPCR	5
Poaceae	*Sporobolus nealleyi* Vasey	M.J. Moore et al. 2920	Young-lineage gypsophile	Yeso Hills	SPNE	10

Table 2. Results of phylogenetic MANOVA and ANOVAs. Pillai's test statistic is reported for the phylogenetic MANOVA. Degrees of freedom for the MANOVA represent estimates for the model given phylogeny.

Test	**dfn, dfd**	**Estimated F**	**P-value**	**P-value given phylogeny**	**Pillai's test**
Leaf nutrients (MANOVA)	14, 30	2.28	0.0296	0.003	1.03
Leaf S (ANOVA)	2, 20	10.26	0.0009	0.001	NA
Leaf Ca (ANOVA)	2, 20	2.49	0.11	0.03	NA

FIGURE LEGENDS

Figure 1. Phylogeny of the taxa included in our primary sampling, based on published work (see Materials and Methods). For phylogenetic statistical analyses, branch lengths were all set to 1.

Figure 2. Principal Components Analysis (PCA) of soil properties. Centroids are mean soil samples ± standard deviation (n = 6). Replicate plots were associated with individuals from eight of the sampled taxa. Gypsum soils are black circles, non-gypsum soils are white circles. Vectors indicate the direction of increase for each measured variable.

Figure 3. Principal Components Analysis (PCA) of leaf tissue chemistry. Centroids are species means ± standard deviation (refer to Table 1 for replication). Black circles represent old-lineage gypsophiles, white circles represent young-lineage gypsophiles, and gray circles are gypsovags. Vectors represent measured variables and indicate the direction of increase for each element.

Figure 4. Mean leaf sulfur and calcium for sampled taxa categorized as old-lineage gypsophiles, young-lineage gypsophiles, and gypsovags. Error bars represent standard deviation (refer to Table 1 for replication). Letters correspond to the results of Tukey's post hoc tests for phylogenetic ANOVA of leaf S ($\alpha = 0.05$).

Figure 1.

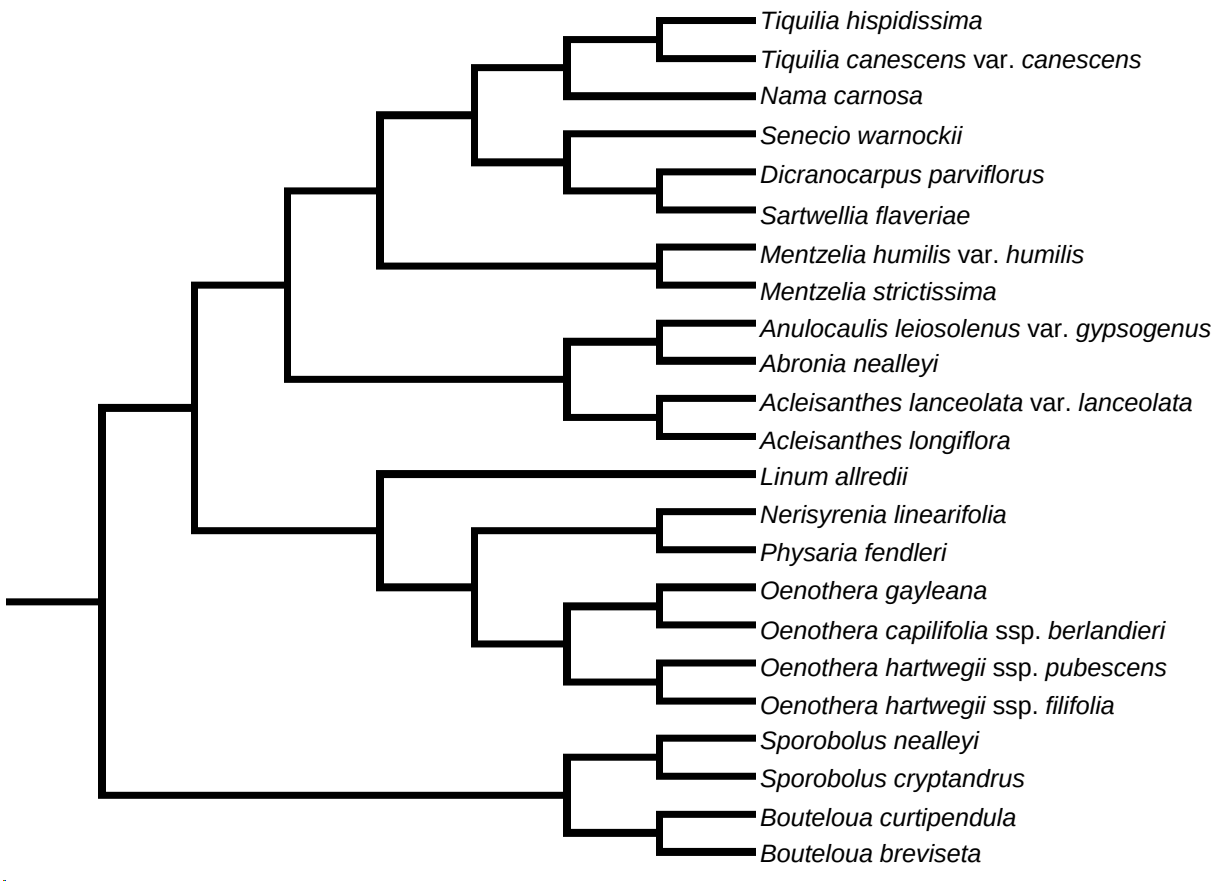

Tiquilia hispidissima
Tiquilia canescens var. *canescens*
Nama carnosa
Senecio warnockii
Dicranocarpus parviflorus
Sartwellia flaveriae
Mentzelia humilis var. *humilis*
Mentzelia strictissima
Anulocaulis leiosolenus var. *gypsogenus*
Abronia nealleyi
Acleisanthes lanceolata var. *lanceolata*
Acleisanthes longiflora
Linum allredii
Nerisyrenia linearifolia
Physaria fendleri
Oenothera gayleana
Oenothera capilifolia ssp. *berlandieri*
Oenothera hartwegii ssp. *pubescens*
Oenothera hartwegii ssp. *filifolia*
Sporobolus nealleyi
Sporobolus cryptandrus
Bouteloua curtipendula
Bouteloua breviseta

Figure 2.

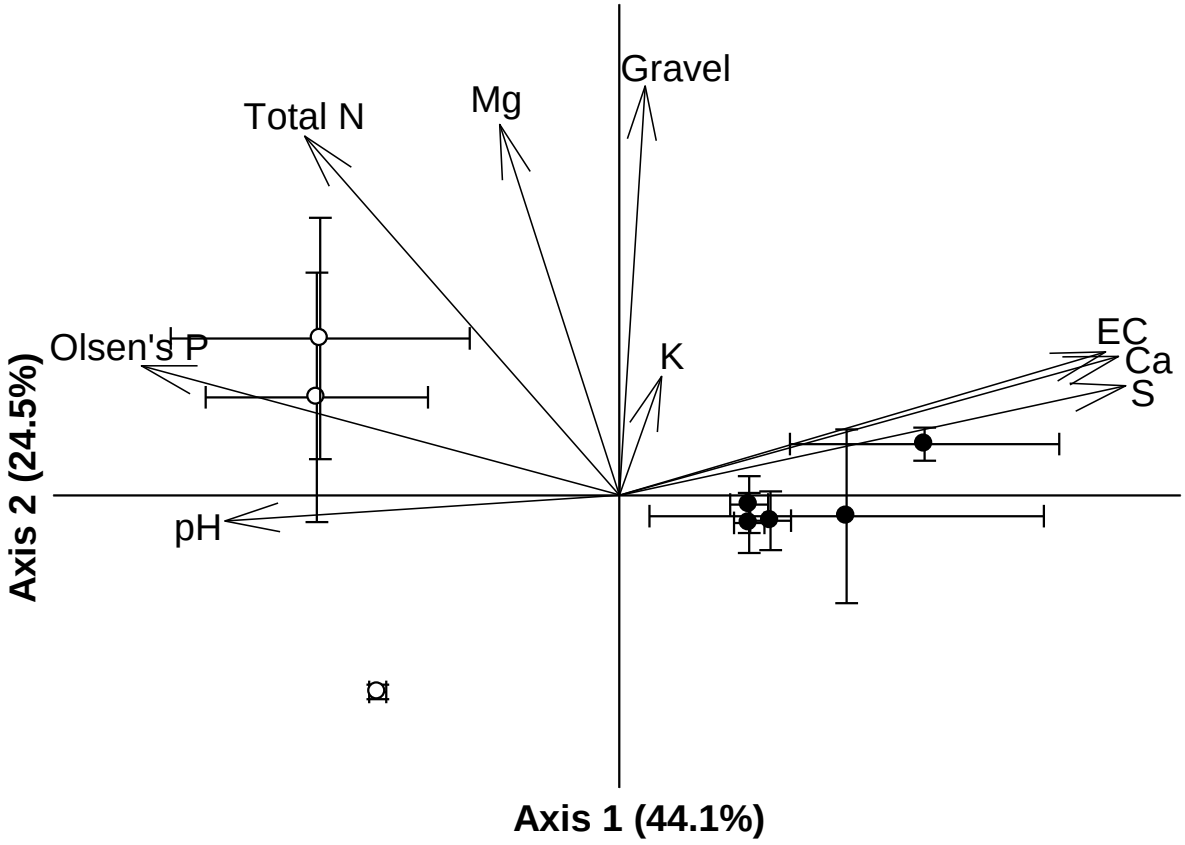


Figure 3.

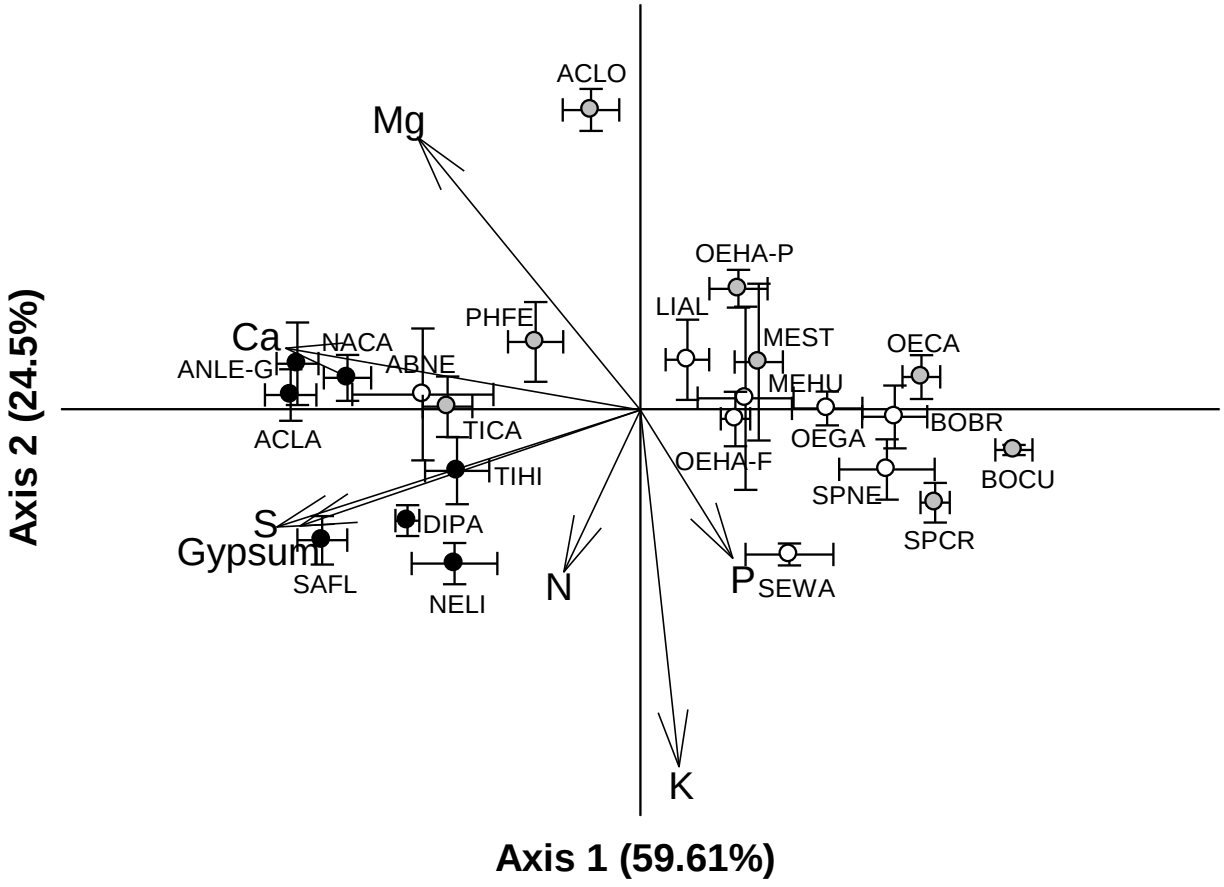
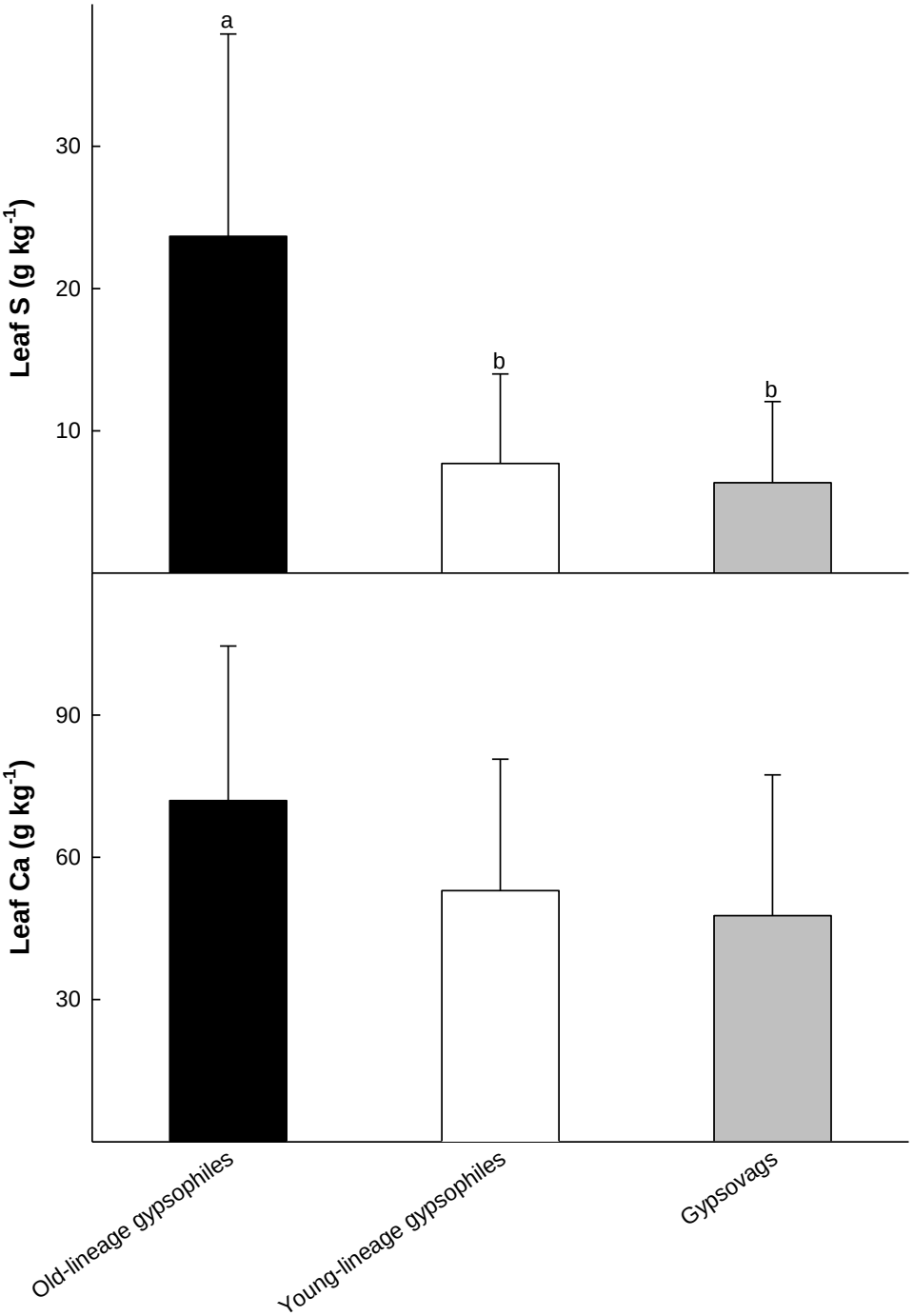

ACLO
Mg
OEHA-P
LIAL
PHFE
Ca
NACA
MEST
OECA
ANLE-G
ABNE
MEHU
BOBR
ACLA
TICA
OEGA
OEHA-F
BOCU
TIHI
SPNE
S
DIPA
SPCR
Gypsum
SAFL
N
P
SEWA
NELI
K
Axis 2 (24.5%)
Axis 1 (59.61%)

Figure 4.


a
b
b
30
20
10
Leaf S (g kg-1)
90
60
30
Leaf Ca (g kg-1)
Old-lineage gypsophiles
Young-lineage gypsophiles
Gypsovags

Supplemental Table 1. Information for taxa collected in 2013. Soil characterization is not available, but soil descriptions indicate whether leaves were sampled from gypsum soil, non-gypsum soil, or if samples from both gypsum and non-gypsum soils were collected. We do not provide hypotheses for endemic lineage ages for the taxa unique to this collection year. Vouchers were deposited in the herbarium of Oberlin College (OC).

Family	Taxon	Voucher	Status	Location	Soil	n	Abbr.
Asteraceae	*Dicranocarpus parviflorus* A.Gray	M.J. Moore et al. 2262, 2323, 2398, 2612	Wide gypsophile	New Mexico	Gypsum	4	DIPA
Asteraceae	*Gaillardia henricksonii* B.L.Turner	M.J. Moore et al. 2575	Wide gypsophile	Coahuila	Gypsum	1	GAHE
Asteraceae	*Gaillardia* sp. nov.	M.J. Moore et al. 2613	Wide gypsophile	Coahuila	Gypsum	1	GASP
Asteraceae	*Gaillardia suavis* (A.Gray & Engelm.) Britton & Rusby	M.J. Moore et al. 2584	Gypsovag	Coahuila	Non-gypsum	1	GASU
Asteraceae	*Gaillardia turneri* Averett & A.M.Powell	M.J. Moore et al. 2400, 2419	Wide gypsophile	Chihuahua	Gypsum	2	GATU
Asteraceae	*Haploësthes greggii* A.Gray	M.J. Moore et al. 2480	Wide gypsophile	Coahuila	Gypsum	1	HAGR
Asteraceae	*Haploësthes greggii* A.Gray var. *multiflora* I.M.Johnst.	M.J. Moore et al. 2630	Wide gypsophile	Nuevo León	Gypsum	1	HAGR-M

Asteraceae	*Sartwellia gypsophila* A.M.Powell & B.L.Turner	M.J. Moore et al. 2376	Wide gypsophile	Chihuahua	Gypsum	1	SAGY
Asteraceae	*Sartwellia puberula* Rydb.	M.J. Moore et al. 2469, 2513, 2582	Wide gypsophile	Coahuila, Durango	Gypsum	3	SAPU
Brassicaceae	*Nerisyrenia camporum* (A.Gray) Greene	M.J. Moore et al. 2318, 2367, 2459, 2330	Gypsovag	New Mexico, Texas, Chihuahua	Gypsum	4	NECA
Brassicaceae	*Nerisyrenia gracilis* I.M.Johnst.	M.J. Moore et al. 2477	Wide gypsophile	Coahuila	Gypsum	1	NEGR
Brassicaceae	*Nerisyrenia gypsophila* J.D.Bacon	M.J. Moore et al. 2396, 2421, 2506	Wide gypsophile	Chihuahua, Durango	Gypsum	3	NEGY
Brassicaceae	*Nerisyrenia incana* Rollins	M.J. Moore et al. 2552, 2580	Wide gypsophile	Coahuila	Gypsum	2	NEIN
Caryophyllaceae	*Nerisyrenia linearifolia* (S.Watson) Greene	M.J. Moore et al. 2317	Wide gypsophile	New Mexico	Gypsum	1	NELI
Commelinaceae	*Drymaria subumbellata* I.M.Johnst.	M.J. Moore et al. 2503	Wide gypsophile	Durango	Gypsum	1	DRSU
Ehretiaceae	*Tiquilia canescens* (A.DC.) A.T.Richardson var. *canescens*	M.J. Moore et al. 2432, 2562	Gypsovag	Chihuahua, Coahuila	Non-gypsum	2	TICA

Ehretiaceae	*Tiquilia gossypina* (Wooton & Standl.) A.T.Richardson	M.J. Moore et al. 2368	Gypsovag	Chihuahua	Gypsum	1	TIGO
Ehretiaceae	*Tiquilia greggii* (Torr. & A.Gray) A.T.Richardson	M.J. Moore et al. 2357, 2378	Gypsovag	Texas, Chihuahua	Both	2	TIGR
Ehretiaceae	*Tiquilia hispidissima* (Torr. & A.Gray) A.T.Richardson	M.J. Moore et al. 2370, 2478, 2512	Wide gypsophile	Chihuahua, Coahuila, Durango	Gypsum	2	TIHI
Fabaceae	*Tiquilia mexicana* (S.Watson) A.T.Richardson	M.J. Moore et al. 2490	Gypsovag	Coahuila	Gypsum	1	TIME
Fouquieriaceae	*Dermatophyllum gypsophilum* (B.L.Turner & A.M.Powell) Vincent	M.J. Moore et al. 2417	Narrow gypsophile	Chihuahua	Gypsum	1	DEGY
Fouquieriaceae	*Fouquieria shrevei* I.M.Johnst.	M.J. Moore et al. 2468, 2555	Wide gypsophile	Coahuila	Gypsum	2	FOSH
Hydrophyllaceae	*Fouquieria splendens* Engelm.	M.J. Moore et al. 2499	Gypsovag	Coahuila	Gypsum	1	FOSP
Hydrophyllaceae	*Phacelia gypsogenia* I.M.Johnst.	M.J. Moore et al. 2414	Wide gypsophile	Chihuahua	Gypsum	1	PHGY
Loasaceae	*Phacelia sivinskii* N.D.Atwood, P.J.Knight, & Lowrey	M.J. Moore et al. 2213, 2250	Narrow gypsophile	New Mexico	Gypsum	2	PHSI

Namaceae	*Mentzelia todiltoensis* N.D.Atwood & S.L.Welsh	M.J. Moore et al. 2208, 2321	Wide gypsophile	New Mexico	Gypsum	2	METO
Namaceae	*Nama canescens* C.L.Hitchc.	M.J. Moore et al. 2640	Wide gypsophile	Nuevo León	Gypsum	1	NACAN
Namaceae	*Nama carnosa* (Wooton) C.L.Hitchc.	M.J. Moore et al. 2334, 2460	Wide gypsophile	Texas, Chihuahua	Gypsum	2	NACAR
Namaceae	*Nama constancei* J.D.Bacon	M.J. Moore et al. 2516, 2554	Wide gypsophile	Durango, Coahuila	Gypsum	1	NACO
Namaceae	*Nama flavescens* Brandegee	M.J. Moore et al. 2479	Wide gypsophile	Coahuila	Gypsum	1	NAFL
Namaceae	*Nama havardii* A.Gray	M.J. Moore et al. 2372	Wide gypsophile	Chihuahua	Gypsum	1	NAHA
Nyctaginaceae	*Nama stewartii* I.M.Johnst.	M.J. Moore et al. 2412	Wide gypsophile	Chihuahua	Gypsum	1	NAST
Nyctaginaceae	*Acleisanthes acutifolia* Standl.	M.J. Moore et al. 2447	Gypsovag	New Mexico	Gypsum	1	ACAC
Nyctaginaceae	*Acleisanthes chenopodioides* (A.Gray) R.A.Levin	M.J. Moore et al. 2246	Gypsovag	New Mexico	Non-gypsum	1	ACCH
Nyctaginaceae	*Acleisanthes diffusa* (A.Gray) R.A.Levin	M.J. Moore et al. 2258	Gypsovag	New Mexico	Non-gypsum	1	ACDI

Nyctaginaceae	*Acleisanthes lanceolata* (Wooton) R.A. Levin var. *lanceolata*	M.J. Moore et al. 2209, 2251	Wide gypsophile	New Mexico	Gypsum	2	ACLA-L
Nyctaginaceae	*Acleisanthes lanceolata* (Wooton) R.A. Levin var. *megaphylla* (B.A.Fowler & B.L.Turner) Spellenb. & J.Poole	M.J. Moore et al. 2374, 2328	Wide gypsophile	Texas, Chihuahua	Gypsum	3	ACLA-M
Nyctaginaceae	*Acleisanthes longiflora* A.Gray	M.J. Moore et al. 2328, 2359, 2386, 2434, 2439, 2561	Gypsovag	Texas, Chihuahua, Coahuila	Non-gypsum	6	ACLO
Nyctaginaceae	*Acleisanthes parvifolia* (Torr.) R.A.Levin	M.J. Moore et al. 2360	Wide gypsophile	Texas	Gypsum	1	ACPA
Nyctaginaceae	*Anulocaulis eriosolenus* (A.Gray) Standl.	M.J. Moore et al. 2362, 2471, 2565	Gypsovag	Chihuahua, Coahuila	Both	3	ANER
Nyctaginaceae	*Anulocaulis leiosolenus* (Torr.) Standl. var. *lasianthus* I.M.Johnst.	M.J. Moore et al. 2366, 2406	Wide gypsophile	Chihuahua	Gypsum	2	ANLE-LA
Nyctaginaceae	*Anulocaulis leiosolenus* (Torr.) Standl. var. *leiosolenus*	M.J. Moore et al. 2341	Wide gypsophile	Texas	Gypsum	1	ANLE-LE
Nyctaginaceae	*Anulocaulis reflexus* I.M.Johnst.	M.J. Moore et al. 2361, 2387, 2457	Wide gypsophile	Texas, Chihuahua	Gypsum	3	ANRE
Onagraceae	*Nyctaginia capitata* Choisy	M.J. Moore et al. 2585	Gypsovag	Coahuila	Non-gypsum	1	NYCA

Onagraceae	*Oenothera hartwegii* Benth. ssp. *filifolia* (Eastw.) W.L.Wagner & Hoch	M.J. Moore et al. 2333	Wide gypsophile	Texas	Gypsum	1	OEHA-F
Papaveraceae	*Oenothera hartwegii* Benth. ssp. *hartwegii*	M.J. Moore et al. 2563	Gypsovag	Coahuila	Non-gypsum	1	OEHA-H
Plantaginaceae	*Argemone turnerae* A.M.Powell	M.J. Moore et al. 2380, 2411	Wide gypsophile	Chihuahua	Gypsum	2	ARTU
Rubiaceae	*Mabrya erecta* (Hemsl.) Elisens	M.J. Moore et al. 2502	Gypsovag	Durango	Gypsum	1	MAER
Scrophulariaceae	*Hedyotis teretifolia* (Terrell) G.L.Nesom	M.J. Moore et al. 2550	Wide gypsophile	Coahuila	Gypsum	1	HETE
Scrophulariaceae	*Leucophyllum alejandrae* G.L.Nesom	M.J. Moore et al. 2631	Wide gypsophile	Nuevo León	Gypsum	1	LEAL
Scrophulariaceae	*Leucophyllum candidum* I.M.Johnst.	M.J. Moore et al. 2356	Gypsovag	Texas	Non-gypsum	1	LECA
Scrophulariaceae	*Leucophyllum coahuilense* J.Henrickson	M.J. Moore et al. 2515	Wide gypsophile	Durango	Gypsum	1	LECO
Scrophulariaceae	*Leucophyllum frutescens* (Berl.) I.M.Johnst.	M.J. Moore et al. 2586	Gypsovag	Coahuila	Non-gypsum	1	LEFR

Supplemental Table 2. Mean values for soil chemistry ± standard deviation (n = 5 for all sites except TX 54, for which n = 4) for each sampling site in 2014.

Site	Soil type	S (ppm)	Ca (ppm)	Mg (ppm)	K (ppm)	P (ppm)	C (ppm)	N (ppm)	pH	EC (mS m^{-1})
Seven Rivers	Calcareous	0.062 ± 0.098	0.14 ± 0.13	0.0095 ± 0.0080	0.0036 ± 0.0017	3.38 ± 0.99	60.66 ± 5.67	1.91 ± 0.23	7.86 ± 0.37	1.02 ± 0.93
Yeso Hills	Gypsum	0.20 ± 0.023	0.30 ± 0.022	0.0029 ± 0.0016	0.0030 ± 0.0036	< 1.00	9.76 ± 7.28	0.44 ± 0.13	6.43 ± 1.86	3.33 ± 1.73
NM 128	Red sand	0.0024 ± 0.0006	0.021 ± 0.0019	0.0012 ± 0.0001	0.0026 ± 0.0006	2.08 ± 0.44	2.00 ± 0.62	0.19 ± 0.039	7.62 ± 0.27	0.12 ± 0.066
US 285	Limestone	0.026 ± 0.038	0.071 ± 0.043	0.0084 ± 0.0054	0.0029 ± 0.0010	2.80 ± 1.64	13.89 ± 2.74	1.27 ± 0.12	8.29 ± 0.085	1.12 ± 0.82
TX 54	Gypsum	0.19 ± 0.020	0.26 ± 0.0033	0.0049 ± 0.0011	0.0037 ± 0.0007	< 1.00	7.01 ± 0.69	0.32 ± 0.093	7.21 ± 0.34	2.81 ± 0.28

Supplemental Table 3. Leaf nutrition data for each taxon collected in 2014. Means are presented with standard deviation (see Table 1 for replication). For gypsum detection, leaves from each replicate were scored as either 2 (gypsum present), 1 (gypsum maybe present), or 0 (gypsum absent); averages of all replicates are presented here.

Taxon	**Ca (g kg^{-1})**	**S (g kg^{-1})**	**Mg (g kg^{-1})**	**N (g kg^{-1})**	**P (g kg^{-1})**	**K (g kg^{-1})**	**Gypsum**
Abronia nealleyi	97.56 ± 12.79	18.08 ± 8.72	10.45 ± 2.06	26.32 ± 3.84	1.09 ± 0.32	11.47 ± 7.67	2
Acleisanthes lanceolata var. *lanceolata*	89.12 ± 5.56	31.76 ± 3.36	16.18 ± 2.20	27.43 ± 3.31	0.79 ± 0.06	9.72 ± 2.77	2
Acleisanthes longiflora	71.80 ± 12.62	4.39 ± 1.40	29.60 ± 1.56	18.41 ± 1.76	0.55± 0.04	1.50 ± 0.29	0
Anulocaulis leiosolenus var. *gypsogenus*	93.19 ± 0.76	35.85 ± 6.85	10.72 ± 3.37	14.30 ± 2.20	0.65 ± 0.08	4.84 ± 1.38	2
Bouteloua breviseta	26.17 ± 2.50	3.11 ± 0.44	1.91 ± 0.73	14.49 ± 2.02	0.79 ± 0.21	8.66 ± 3.33	0
Bouteloua curtipendula	15.28 ± 1.77	1.78 ± 0.15	1.41 ± 0.22	14.94 ± 1.25	0.91 ± 0.04	11.46 ± 1.25	0
Dicranocarpus parviflorus	92.79 ± 0.56	28.183 ± 1.96	2.32 ± 0.32	31.69 ± 4.13	1.23 ± 0.35	9.29 ± 1.86	2

Linum allredii	70.14 ± 7.07	7.04 ± 1.20	2.64 ± 0.92	11.43 ± 1.73	0.60 ± 0.03	4.92 ± 2.54	0
Mentzelia humilis var. *humilis*	50.73 ± 7.48	5.48 ± 0.93	2.87 ± 0.85	19.16 ± 0.72	0.86 ± 0.08	9.96 ± 8.14	0
Mentzelia strictissima	54.36 ± 10.78	3.81 ± 0.26	4.03 ± 0.86	25.26 ± 3.88	1.27 ± 0.21	8.21 ± 4.91	0
Nama carnosa	92.58 ± 2.18	29.93 ± 3.48	6.50 ± 1.11	13.63 ± 1.11	0.65 ± 0.03	4.20 ± 1.51	2
Nerisyrenia linearifolia	71.48 ± 12.48	28.99 ± 5.96	3.14 ± 0.57	31.06 ± 3.28	1.01 ± 0.16	20.37 ± 2.93	1
Oenothera capillifolia ssp. *berlandieri*	22.79 ± 2.27	2.53 ± 0.18	2.00 ± 0.16	16.94 ± 0.96	1.33 ± 0.12	5.22 ± 1.41	0
Oenothera gayleana	28.31 ± 3.05	4.84 ± 1.04	2.27 ± 0.40	20.10 ± 2.85	0.91 ± 0.15	6.57 ± 1.20	0
Oenothera hartwegii ssp. *filifolia*	46.48 ± 4.34	6.45 ± 0.49	2.68 ± 0.53	23.73 ± 0.45	1.04 ± 0.05	7.82 ± 1.62	0
Oenothera hartwegii ssp. *pubescens*	38.30 ± 7.57	4.74 ± 0.62	7.08 ± 1.36	18.45 ± 2.69	1.20 ± 0.13	3.65 ± 0.60	0
Physaria fendleri	89.41 ± 9.49	12.53 ± 1.26	7.50 ± 0.59	16.35 ± 2.67	1.09 ± 0.37	6.59 ± 2.96	0

Sartwellia flaveriae	102.43 ± 10.99	46.75 ± 6.27	3.01 ± 0.52	28.49 ± 3.46	1.17 ± 0.14	11.91 ± 4.27	2
Senecio warnockii	32.89 ± 3.89	7.55 ± 1.24	1.33 ± 0.54	28.77 ± 0.84	1.26 ± 0.15	21.46 ± 6.52	0
Sporobolus cryptandrus	13.58 ± 1.01	3.60 ± 0.37	2.38 ± 0.19	26.38 ± 0.24	1.30 ± 0.07	19.25 ± 3.21	0
Sporobolus nealleyi	16.70 ± 2.84	5.21 ± 1.21	1.94 ± 0.94	17.27 ± 3.02	1.00 ± 0.26	11.14 ± 2.55	0
Tiquilia canescens var. *canescens*	88.17 ± 10.55	19.27 ± 1.99	6.07 ± 1.08	14.91 ± 2.76	0.77 ± 0.09	7.93 ± 1.75	0.8
Tiquilia hispidissima	98.22 ± 14.40	18.39 ± 5.04	2.89 ± 0.85	15.35 ± 1.66	0.78 ± 0.14	9.20 ± 2.75	2

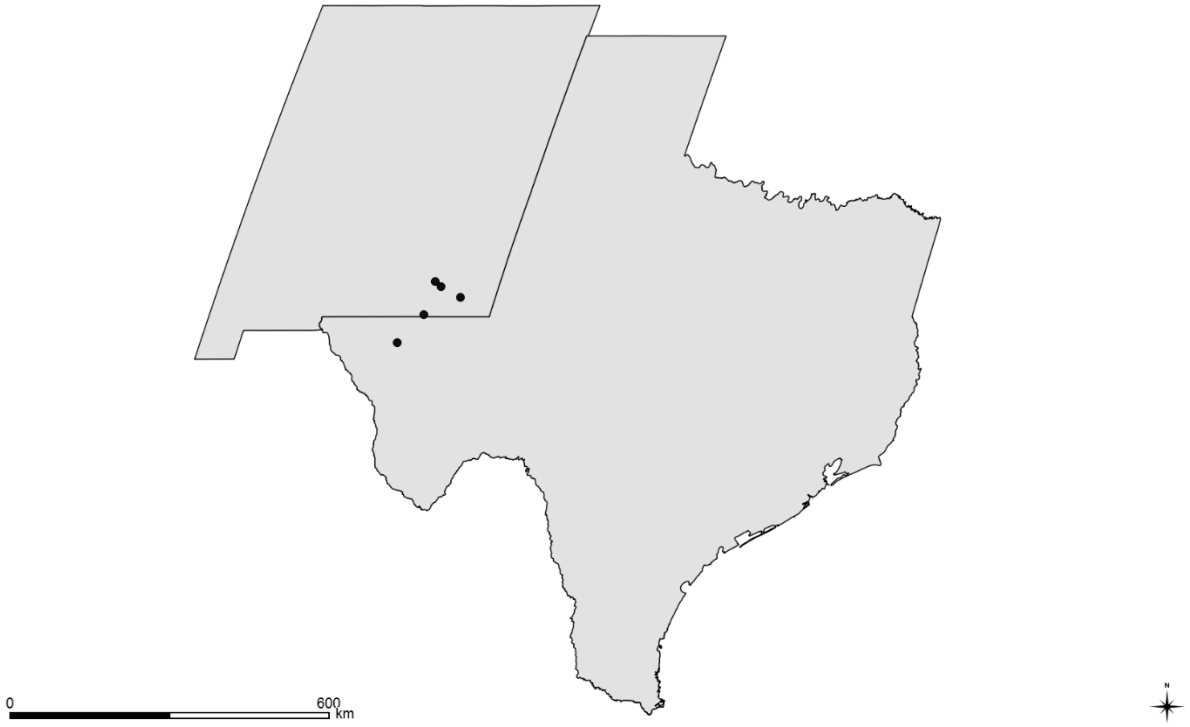
Supplemental Table 4. Mean leaf nutrition for the taxa collected in 2013 ± standard deviation (see Suppl. Table 1 for replication). For gypsum detection, leaves from each replicate were scored as either 2 (gypsum present), 1 (gypsum maybe present), or 0 (gypsum absent); averages of all replicates are presented here.

Taxon	**Ca (g kg^{-1})**	**S (g kg^{-1})**	**Mg (g kg^{-1})**	**N (g kg^{-1})**	**P (g kg^{-1})**	**K (g kg^{-1})**	**Gypsum**
Acleisanthes acutifolia	44.93	4.67	9.54	25.50	0.50	8.27	1
Acleisanthes chenopodioides	29.64	4.55	8.89	45.36	1.48	38.93	1
Acleisanthes diffusa	29.78	8.84	9.04	43.56	1.47	28.21	1
Acleisanthes lanceolata var. *lanceolata*	71.31 ± 12.46	37.91 ± 2.69	8.51 ± 0.11	32.03 ± 6.86	0.75 ± 0.22	20.26 ± 5.67	2
Acleisanthes lanceolata var. *megaphylla*	54.89 ± 12.42	41.35 ± 4.90	19.31 ± 1.16	30.54 ± 2.46	0.73 ± 0.06	16.34 ± 8.51	2
Acleisanthes longiflora	35.34 ± 12.14	3.82 ± 0.83	14.29 ± 3.92	39.45 ± 3.63	0.79 ± 0.16	18.81 ± 5.63	0.83
Acleisanthes parvifolia	23.59	14.00	14.69	35.02	1.38	29.07	1
Anulocaulis eriosolenus	78.10 ± 2.99	36.23 ± 12.37	5.07 ± 2.03	37.28 ± 8.68	1.05 ± 0.22	21.05 ± 4.84	2
Anulocaulis leiosolenus var. *lasianthus*	67.67 ± 13.59	43.98 ± 9.80	5.09 ± 1.63	29.54 ± 1.42	1.02 ± 0.05	13.55 ± 8.92	1.5
Anulocaulis leiosolenus var. *leiosolenus*	78.93	58.22	4.95	31.84	0.98	5.10	2

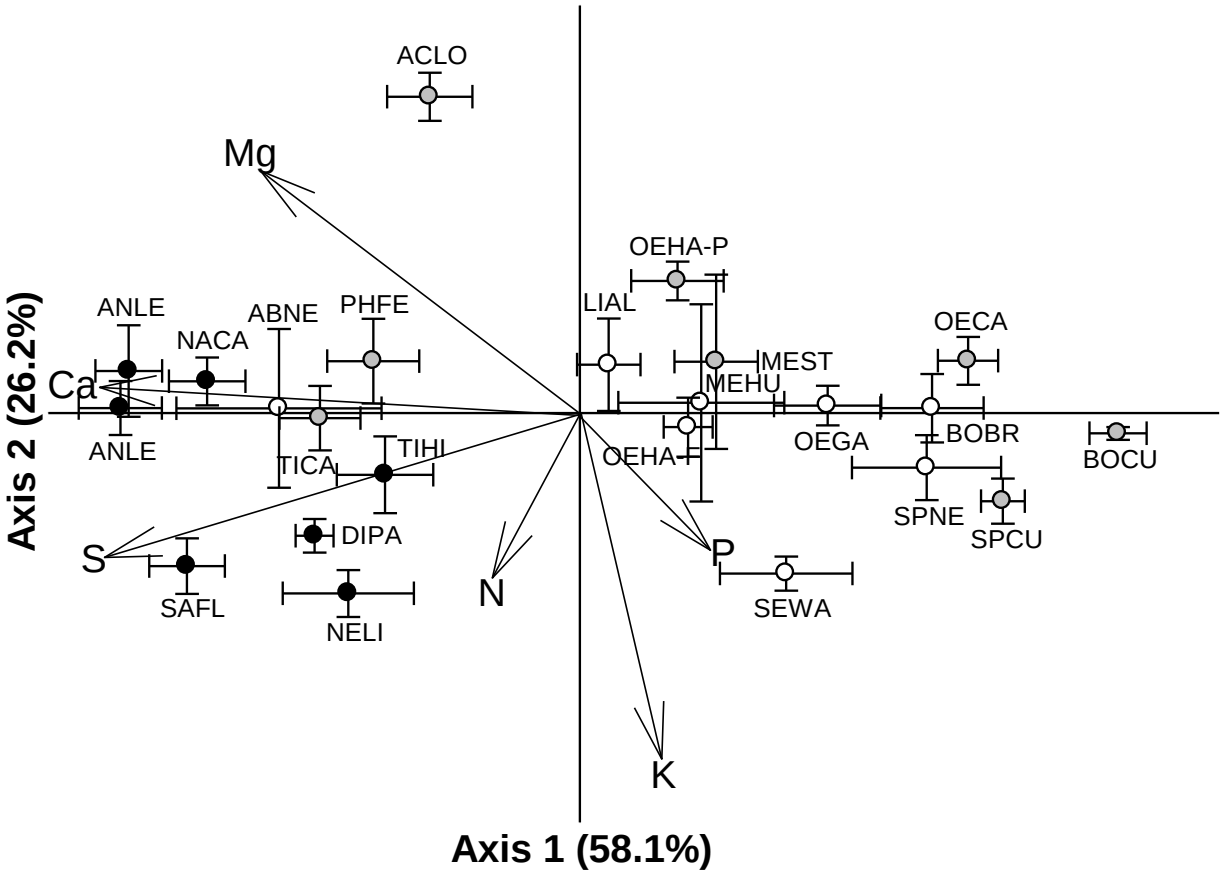
Anulocaulis reflexus	63.37 ± 14.74	36.85 ± 7.156	5.86 ± 2.16	31.88 ± 2.60	1.10 ± 0.45	18.13±10.28	2
Argemone turnerae	21.85 ± 13.34	11.60 ± 1.48	5.27 ± 4.05	24.04 ± 1.11	1.12 ± 0.19	14.44 ± 0.36	0
Dermatophyllum gypsophilum	5.87	2.00	4.31	34.52	0.83	8.64	0
Dicranocarpus parviflorus	83.67 ± 9.23	70.41 ± 8.49	2.79 ± 0.38	32.73 ± 8.55	0.97 ± 0.36	14.74 ± 4.32	2
Drymaria subumbellata	19.88	6.27	9.80	25.82	0.52	26.49	0
Fouquieria shrevei	35.84 ± 5.45	7.81 ± 0.35	3.93 ± 4.77	13.65 ± 0.16	0.41 ± 0.01	9.23 ± 1.53	0
Fouquieria splendens	53.08	15.18	3.06	16.13	0.67	10.84	0
Gaillardia henricksonii	60.38	34.67	4.95	21.76	0.81	10.13	2
Gaillardia sp. nov.	57.20	42.34	6.73	23.95	0.75	11.09	2
Gaillardia suavis	31.90	6.03	3.23	24.78	1.67	25.92	0
Gaillardia turneri	43.30 ± 3.52	20.78 ± 2.02	6.60 ± 6.38	29.39 ± 5.19	0.99 ± 0.27	22.56±16.49	1
Haploësthes greggii	78.51	87.16	1.76	19.85	0.60	7.90	2
Haploësthes greggii var. *multiflora*	54.52	65.58	2.01	23.22	0.94	21.63	2
Hedyotis teretifolia	58.26	51.49	7.76	14.85	0.47	9.93	2
Leucophyllum alejandrae	15.88	3.59	1.42	14.21	0.72	7.81	0
Leucophyllum candidum	7.36	2.80	1.24	13.93	0.53	7.64	0
Leucophyllum coahuilense	7.46	2.70	2.68	13.13	0.35	8.60	0
Leucophyllum frutescens	16.11	3.11	6.51	25.11	0.90	16.40	0
Mabrya erecta	79.66	75.25	8.20	28.29	1.16	11.97	2
Mentzelia todiltoensis	35.67 ± 0.36	14.18 ± 2.83	6.65 ± 3.19	27.59 ± 2.59	0.83 ± 0.25	21.09 ± 4.12	0

Nama canescens	82.71	53.87	3.34	17.67	0.37	10.72	2
Nama carnosa	81.35 ± 3.00	40.79 ± 4.84	3.37 ± 2.27	13.97 ± 1.39	0.43 ± 0.08	8.51 ± 2.13	2
Nama constancei	79.86	61.00	4.51	9.35	0.31	10.99	2
Nama flavescens	78.27	92.76	1.58	17.77	0.62	9.77	2
Nama havardii	80.07	88.80	4.90	26.05	0.80	6.77	2
Nama stewartii	100.72	71.46	3.39	23.08	0.89	7.32	2
Nerisyrenia camporum	79.75 ± 1.36	47.04 ± 12.48	3.50 ± 1.19	28.78 ± 3.79	0.68 ± 0.16	14.03 ± 1.96	2
Nerisyrenia gracilis	81.24	51.23	8.26	33.36	0.44	10.50	2
Nerisyrenia gypsophila	75.21± 5.57	57.35 ± 7.68	6.59 ± 2.65	26.14 ± 3.35	0.70 ± 0.17	8.25 ± 1.72	2
Nerisyrenia incana	79.92± 1.20	58.02 ± 3.52	5.49 ± 3.14	25.21 ± 2.93	0.44 ± 0.21	8.03 ± 0.23	2
Nerisyrenia linearifolia	75.86	45.72	3.07	33.35	0.81	8.89	2
Nyctaginia capitata	64.67	19.82	4.96	54.94	1.26	21.67	NA
Oenothera hartwegii. ssp. *filifolia*	29.74	8.67	3.29	24.31	0.94	10.86	0
Oenothera hartwegii. ssp. *hartwegii*	60.23	26.46	7.24	28.07	0.88	14.54	2
Phacelia gypsogenia	34.95	12.16	8.40	25.96	0.51	11.08	0
Phacelia sivinskii	44.51± 5.11	20.44 ± 2.00	4.87 ± 3.00	39.13 ± 3.93	1.56 ± 0.97	16.75 ± 3.94	2
Sartwellia gypsophila	80.34	65.80	1.78	32.54	0.83	18.60	2
Sartwellia puberula	73.44 ± 11.60	65.76 ± 22.83	3.69 ± 1.69	23.09 ± 1.51	0.79 ± 0.23	26.66 ± 7.80	2

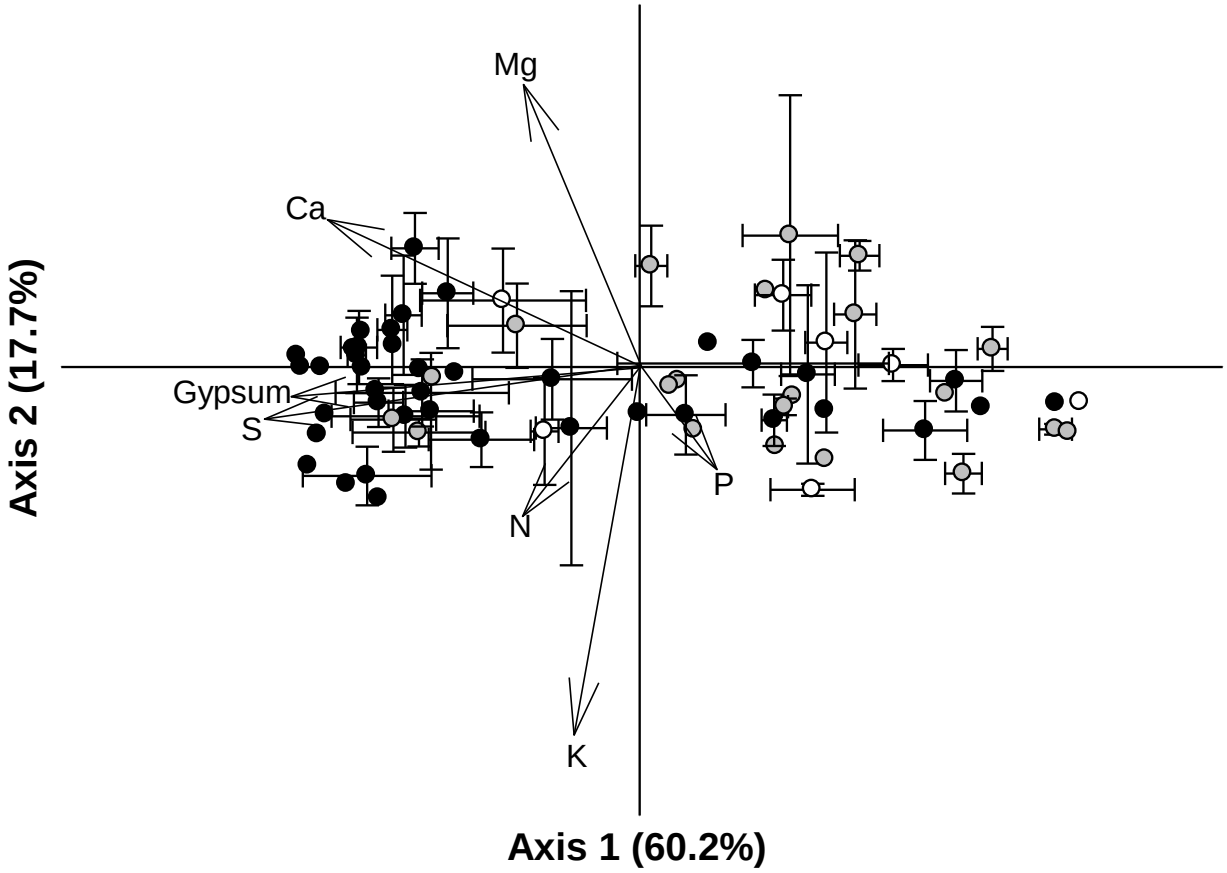
Tiquilia canescens var. *canescens*	80.09 ± 0.02	39.78 ± 3.69	3.65 ± 0.66	21.51 ± 1.15	0.61 ± 0.01	9.40 ± 2.27	2
Tiquilia gossypina	70.46	33.88	6.60	24.39	0.74	14.13	2
Tiquilia greggii	37.59 ± 12.12	7.75 ± 0.97	3.79 ± 1.59	25.45 ± 3.23	0.87 ± 0.02	15.00 ± 0.21	0
Tiquilia hispidissima	53.19 ± 6.61	19.51 ± 9.59	4.23 ± 0.41	22.13 ± 4.19	0.72 ± 0.08	18.24± 3.56	1.33
Tiquilia mexicana	68.36	13.00	3.83	15.14	0.60	16.19	0



Supplemental Figure 1. A map of sampling site locations in New Mexico and Texas for 2014 plant and soil collection.

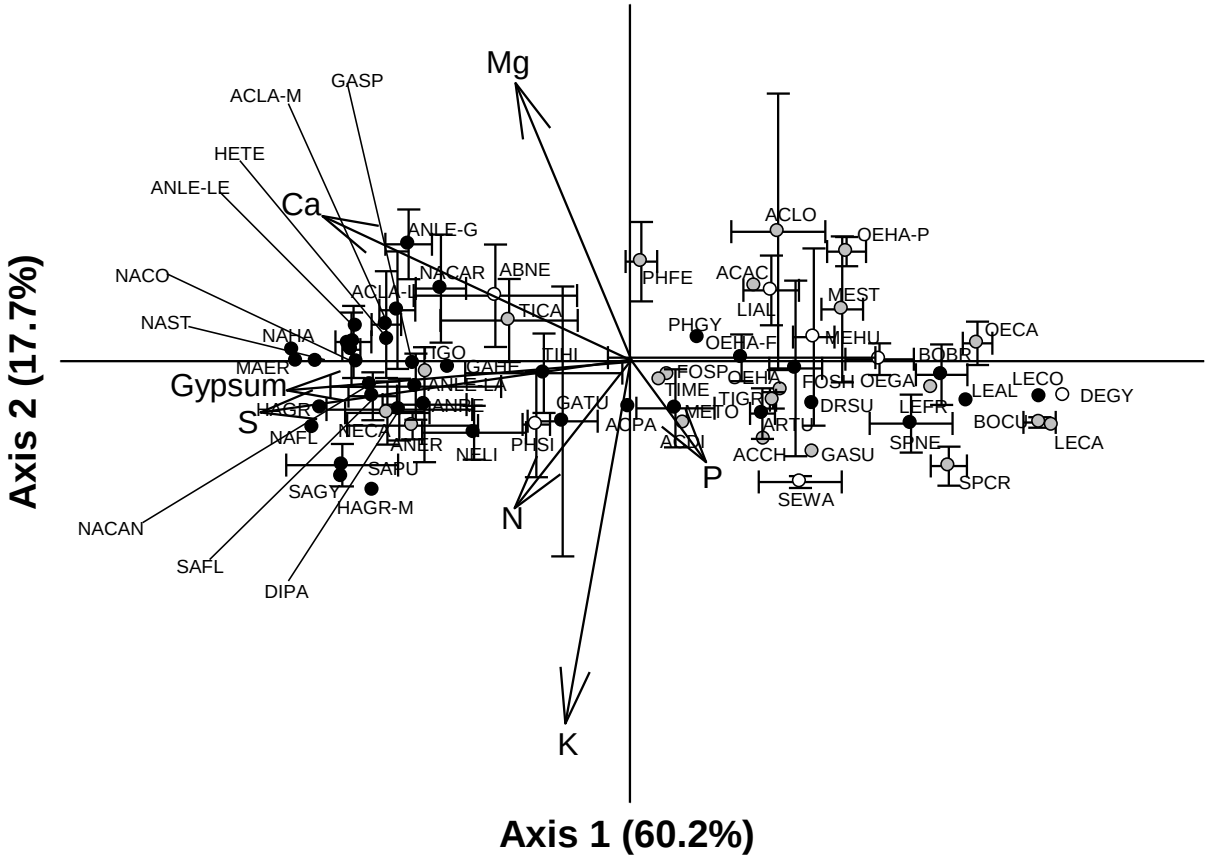


Supplemental Figure 2. Principal Components Analysis (PCA) for the leaf mineral nutrition of the taxa collected in 2014, excluding gypsum spectral data. Centroids represent species means ± standard deviation (refer to Table 1 for replication) colored according to lineage age (black centroids are old-lineage gypsophiles, white centroids are young-lineage gypsophiles, and gray centroids are gypsovags). The plot is remarkably similar to the PCA that includes gypsum presence as a response variable, suggesting gypsum accumulation is highly linked to the accumulation of Ca and S in the leaves of old-lineage gypsophiles.

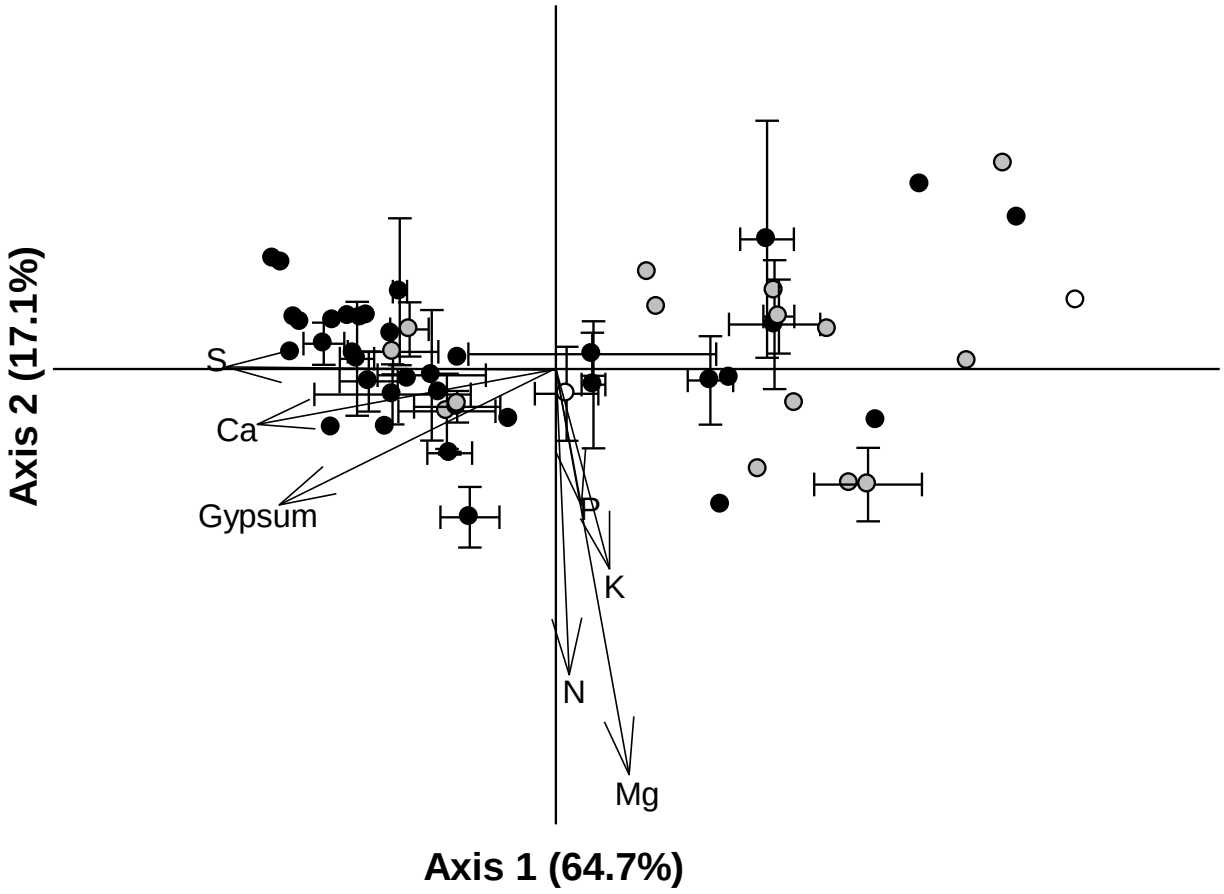


(a)

Supplemental Figure 3. Principal Components Analysis **(**PCA) for the leaf mineral nutrition of taxa collected both in 2013 and 2014. Centroids [unlabeled in panel (a), labeled in panel (b)] are species means ± standard deviation (see Table 1 and Suppl. Table 1 for replication). Gypsophiles from a widely distributed lineage are represented by black centroids (n = 40), and gypsophiles from a narrowly distributed lineage are represented by white centroids (n = 7), while gypsovags are represented by grey centroids (n = 21). Vectors indicate direction of increase for each response variable, including leaf S, Ca, Mg, N, P, K, and gypsum.

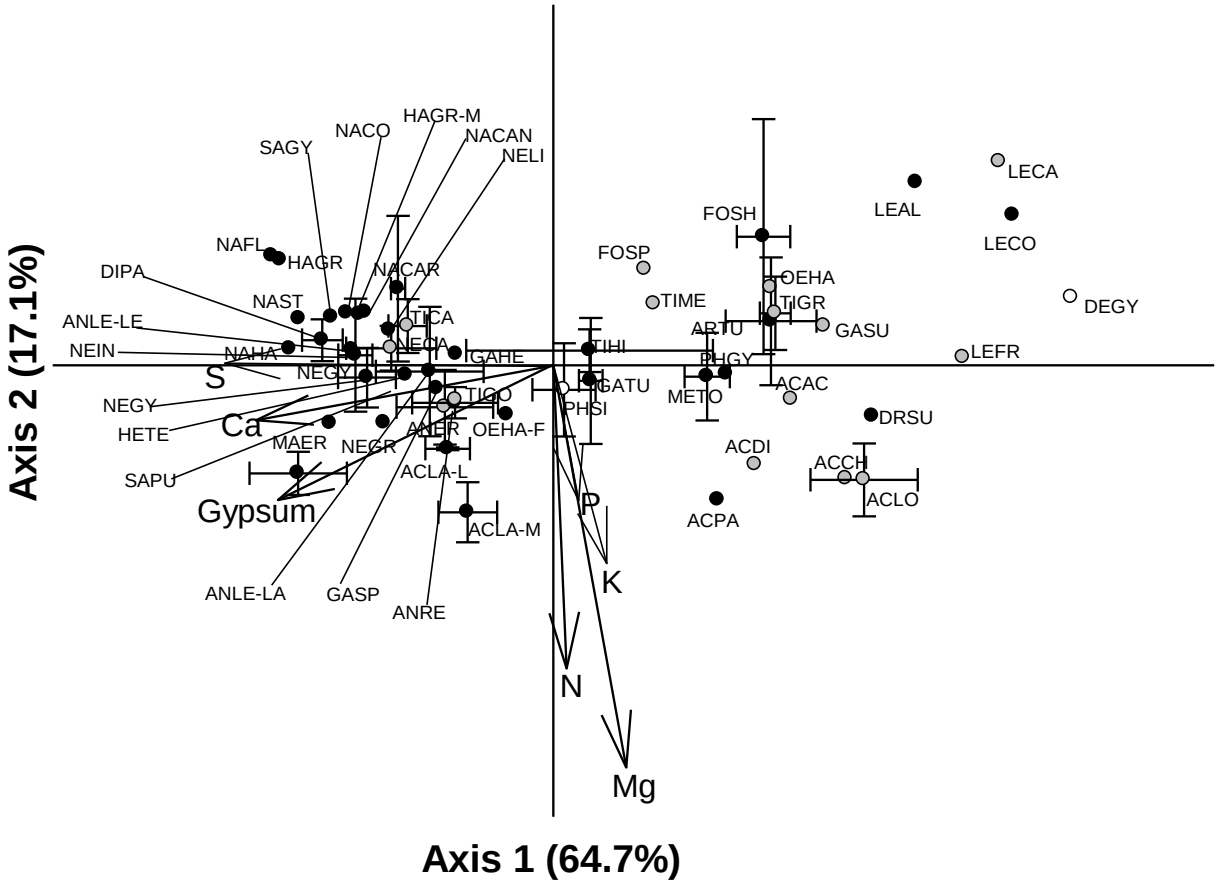

Mg
GASP
ACLA-M
HETE
ANLE-LE
Ca
ANLE-G
NACO
NACAR
ABNE
ACLA-L
TICA
NAST
NAHA
TIGO
TIHI
MAER
GAHE
Gypsum
ANLE-LA
HAGR
ANHE
S
GATU
NAFL
NECA
ANER
NELI
PHSI
ACPA
SAPU
SAGT
HAGR-M
NACAN
SAFL
DIPA
N
K
PHFE
ACLO
OEHA-P
ACAC
MEST
LIAL
PHGY
OEHA-F
NIEHU
QECA
BOBR1
FOSP
TIME
OEHA
FOSH
OEGA
LECO
DEGY
LEAL
TIGR
DRSU
LEFR
METO
ARTU
BOCU
SPNE
LECA
ACCH
GASU
SPCR
SEWA
P
Axis 2 (17.7%)
Axis 1 (60.2%)

(b)



(a)

Supplemental Figure 4. Principal Components Analysis (PCA) for the leaf mineral nutrition of taxa collected in 2013. Centroids [unlabeled in panel (a), labeled in panel (b)] are species means ± standard deviation (see Suppl. Table 1 for replication). Gypsophiles from a widely distributed lineage are represented by black centroids (n = 40), and gypsophiles from a narrowly distributed lineage are represented by white centroids (n = 2), while gypsovags are represented by grey centroids (n = 15).


Axis 2 (17.1%)
Axis 1 (64.7%)
HAGR-M
NACO
NACAN
SAGY
NELI
LECA
LEAL
FOSH
LECO
NAFL
HAGR
FOSP
DIPA
NACAR
OEHA
TIME
NAST
TIGR
DEGY
TICA
ARTU
GASU
ANLE-LE
NEIN
NECA
LEFR
NAHA
IGAHE
TIHI
PHGY
NEGY
S
IGATU
ACAC
METO
NEGY
TIGO
PHSI
DRSU
HETE
Ca
AMER
OEHA-F
ACDI
MAER
NEGR
ACCH
SAPU
ACLA-L
ACLO
P
Gypsum
ACPA
ACLA-M
K
ANLE-LA
GASP
ANRE
N
Mg

(b)

Chapter II: Foliar and whole-plant nutrition of gypsum floras from the USA and Spain

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ABSTRACT

Gypsum endemism (gypsophily) is common in the Chihuahuan Desert and Spain, but the physiology of gypsophiles has been poorly studied in relation to the evolutionary history of endemic taxa. Much of what is known about gypsophile physiology comes from work conducted in Spain, in which the leaf chemistry of gypsophiles and non-endemic taxa (gypsovags) was compared to the unique chemistry of gypsum soils. These studies have suggested that assimilation of excess S and Ca as biomineralized gypsum in the leaves of widely-distributed gypsophiles is an important mechanism supporting life on gypsum for those taxa. However, few phylogenetic studies have been conducted on the gypsum flora from Spain. In contrast, the gypsum flora of the Chihuahuan Desert has been examined by molecular phylogeneticists for years, but little is known of their physiology. In this study, we compare the physiological trends in leaf nutrition from the Chihuahuan Desert gypsum flora with trends observed for the Spanish gypsum flora when sampled with respect to phylogenic relationships among taxa. We observed that there are global trends in leaf nutrition of widely-distributed gypsophiles, characterized by accumulation and assimilation of S and Ca, and that phylogeny is important for understanding plant nutrition among gypsophiles and gypsovags from both floras. We also observed some trends in the whole-plant nutrition of taxa from Spain that suggest widely-distributed gypsophiles, narrowly-distributed gypsophiles, and gypsovags are mechanistically different in multiple organ systems.

INTRODUCTION

Gypsum soils (>60% $CaSO_4 \cdot 2H_2O$) pose physiological challenges to plants due to their unique chemistry, as excess SO_4^{2-} and Ca^{2+} in soils can alter plant biochemical processes and limit plant performance (Engels et al., 2012). Consequently, effective mechanisms to assimilate, sequester, or exclude these ions are critical for gypsum adaptation (Palacio et al., 2007, 2014). Despite the potential difficulties of life on gypsum, gypsophilic (i.e., gypsum endemic) plants are common where gypsum soils occur, with numerous independent origins yielding a highly diverse flora (Powell and Turner, 1977; Moore et al., 2014). Most of what we know about the physiological strategies supporting gypsophile adaptation to gypsum soils comes from Spain and has focused on foliar nutrition (Escudero et al., 2014).

Previous work in Spain revealed high leaf S and Ca accumulation patterns in some gypsophiles relative to non-gypsophiles (Palacio et al., 2007). Despite high Ca:Mg in soil and high Ca in leaves, some gypsophiles have been shown to maintain adequate Mg concentrations (Palacio et al., 2007). Similarly, serpentine-adapted plants growing on serpentine soils (i.e., soils with low Ca:Mg) accumulate Mg and Ca in roots, but selectively translocate Ca into shoots (O'Dell and Claassen, 2006). Combined, these data suggest that the ability of gypsophiles to accumulate S and Ca in tissues, but also to maintain nutrient balance, is reflective of gypsum specialization. Furthermore, distinction between gypsophiles of regionally wide geographic distribution and narrow geographic distribution is important for understanding particular strategies for coping with gypsum soil chemistry (Palacio et al., 2007; Escudero et al., 2014). Studies from Spain (Palacio et al., 2007), the USA (Muller et al., 2015), and Turkey (Bolukbasi et al., 2016) have

described the ability of many widely-distributed gypsophiles to accumulate S and Ca in leaves, whereas narrowly-distributed gypsophiles typically have leaf chemical signatures more similar to non-endemic taxa (i.e., lower leaf S, Ca, Mg, and N). This difference in accumulation pattern based on biogeographic distribution indicates that among gypsophiles, there are likely multiple mechanisms supporting gypsum adaptation. Two such mechanisms are assimilation or exclusion of minerals in excess.

Assimilation of excess leaf Ca ions is known to occur in multiple plant families (He et al., 2015). Sequestration of Ca as Ca-oxalate crystals in leaf vacuoles prevents high concentrations of Ca in the cytoplasm, which can negatively impact cell metabolism (Borer et al., 2012; He et al., 2015). Previous data (Feder et al., 2016) revealed that some widely-distributed gypsophiles, hypothesized to be from older gypsophile lineages, may contain oxalate in their leaves, suggesting some taxa (e.g., Nyctaginaceae) may use this strategy to cope with high soil Ca concentrations found in gypsum soils. In addition, many old-lineage gypsophiles contained gypsum within their leaves as precipitated crystals, which do not affect cytoplasmic stability and can be sequestered in vacuoles of succulent leaves (George et al., 2012).

Assimilation of excess S as components of organic compounds other than gypsum may be a mechanism employed by some gypsophilic taxa. In particular, old-lineage gypsophiles in Brassicaceae may assimilate excess S as glucosinolate compounds, rich in S and N. Families with mechanisms that allow for assimilation and sequestration of excess S and Ca may be pre-adapted for life on gypsum soils. Though these strategies may explain the leaf chemistry of widely-distributed, old-lineage gypsophiles, other

mechanisms utilized by these taxa, as well as the mechanisms of narrowly-distributed, young-lineage gypsophiles, are still poorly understood.

Although it has not been examined for gypsum floras, exclusion of minerals in excess is a mechanism by which plants on serpentine and saline soils tolerate unusual soil chemistry. In serpentine systems under low Ca:Mg soil conditions, plant Ca:Mg balance is important for maintaining tissue nutrient stoichiometry (O'Dell and Claassen, 2006). In one study, the serpentine ecotype of *Achillea millefolium* was able to maintain higher Ca:Mg in stems than the granite ecotype by selectively translocating Ca into aboveground parts (O'Dell and Claaseen, 2006). Similarly, in saline soil systems, some drought-tolerant plants exclude excess Na ions from leaves by selectively transporting similarly charged K ions in stems (Wang et al., 2004). Many halophytes have high selectivity for K compared to Na, including species that accumulate Na in leaves (Flowers and Colmer, 2008). Similar exclusion mechanisms to those observed for the serpentine and saline floras may exist for taxa in the gypsum flora, particularly for narrowly-distributed, young-lineage gypsophiles observed to have low leaf S and Ca relative to other gypsophiles. We propose that young-lineage gypsophiles may exclude excess S and Ca from leaves, but maintain higher concentrations in belowground tissues.

In North America, gypsum soils are primarily restricted to the arid regions of the Chihuahuan and Mojave Deserts (Parsons, 1976). The Chihuahuan Desert contains extensive gypsum deposits and is host to an exceptionally diverse gypsophilic flora with over 230 known endemic taxa (Moore et al., 2014). In addition, the geographic extent of gypsophile lineages in the Chihuahuan Desert is hypothesized to be positively correlated with lineage age: widely distributed, regionally-dominant gypsophiles are hypothesized

to represent older lineages relative to narrowly-distributed gypsophiles (Moore et al., 2014; Muller et al., 2015). Despite its optimal conditions as a study system for gypsum endemism, very little previous work has investigated how gypsum soil chemistry influences gypsophilic physiology of the Chihuahuan Desert flora (Meyer et al., 1992; Muller et al., 2015).

Our previous work in the Chihuahuan Desert, specifically in southeastern New Mexico, is the only study that has investigated gypsophile leaf nutrient chemistry for taxa in this region (Muller et al., 2015). In September 2014, we conducted a field survey of a suite of endemic species growing on gypsum soils (gypsophiles), paired with congener or confamilial non-endemic taxa growing on non-gypsum soils (gypsovags), to compare their leaf chemical signatures with statistical models that control for the effects of phylogeny. Our data revealed patterns of foliar accumulation similar to previous observations from Spain (Palacio et al., 2007). Almost all widely-distributed gypsophiles were found to possess leaf chemistry enriched in S, Ca, and gypsum, whereas almost all narrowly-distributed gypsophiles were more similar to non-gypsophilic taxa, which lack specialized adaptation to gypsum. Statistical models that incorporated phylogeny were able to detect differences among old-lineage gypsophiles, young-lineage gypsophiles, and gypsovags better than tests that did not incorporate phylogeny, suggesting evolutionary history impacts foliar nutritional patterns for our taxa of interest. These data indicate that widely-distributed, old-lineage gypsophiles and narrowly-distributed, young-lineage gypsophiles differ in their physiological mechanisms supporting adaptation to gypsum soils.

In order to place the physiological strategies of the Chihuahuan Desert flora in a broader context, we compared their leaf chemical with the leaf chemistry of taxa in confamilial groups of widely-distributed and narrowly-distributed gypsophiles from the Spanish gypsophilic flora. Because lineage age is hypothesized to correlate positively with geographic distribution for gypsophiles in North America, our prediction was that widely-distributed gypsophiles from Spain would share similar strategies for ion accumulation or exclusion with widely-distributed, old-lineage gypsophiles from the Chihuahuan Desert, and narrowly-distributed Spanish gypsophiles would reflect patterns observed in narrowly-distributed, young-lineage gypsophiles in the Chihuahuan Desert. We also aimed to describe the nutrient patterns of accumulation and exclusion for gypsophilic lineages in Spain at the whole plant level. We hypothesized that narrowly-distributed gypsophiles from Spain exclude excess S and Ca from their leaves, but retain higher S and Ca concentrations in roots compared to other taxa.

MATERIALS AND METHODS

Spain study sites and taxa selection—Collections took place in the regions of Andalusia (southeast Spain) and Zaragoza (northeast Spain). Similar to the Chihuahaun Desert, soils in Spain are a mosaic of calcareous and gypsum substrates (Palacio et al., 2007). The Iberian Peninsula has a semi-arid Mediterranean climate, with wet, cool winters, and dry, hot summers. Gypsophiles in the Iberian Peninsula are typically sub-shrubs and shrubs, but are commonly less dominant than plants found on both gypsum and non-gypsum soils (gypsovags) at gypsum sites (Palacio et al., 2007, Escudero et al., 2014). Taxa from Spain were from the families Brassicaceae, Caryophyllaceae,

Cistaceae, and Fabaceae, and were selected because they included a mix of gypsovags as well as narrowly- and widely-distributed gypsophiles within the same family (Table 1).

USA study sites and taxa selection—Plant collections were conducted in Eddy County, New Mexico (32.0387°N -104.4727°W; 32.5551°N -104.4516°W; 32.476°N -104.2920°W; 32.3101°N -103.8153°W), and Culberson County, Texas (31.5933°N -104.8553°W), USA in the northern Chihuahuan Desert. The Chihuahuan Desert has an arid to semi-arid climate, characterized by a monsoonal rainfall pattern with relatively low mean annual winter precipitation (e.g., 88 mm) and mean annual summer precipitation that peaks in July through September (e.g., 143 mm). Mean annual winter temperature is 9.3°C and mean annual summer temperature is 25°C (Munson, 2013). Sampling sites were selected primarily based on known populations of sampling taxa. Gypsum sites in New Mexico have a USDA Natural Resources Conservation Service ecological site description of Gyp Upland and are characterized by Cottonwood and Reeves soil series (Sylvester and Bestelmeyer, 2011). The Reeves and Cottonwood series have shallow gypsum soils, loamy textures, and little rock/gravel cover (Chugg et al., 1971). The gypsum site in Texas is part of the Salt Basin and soils are Quaternary-aged, lacustrine-deposited gypsum, heavily weathered and of variable texture (Angle, 2001). We collected non-endemic plants (primarily congeners of gypsophiles) near gypsum sites from areas dominated by calcareous or sandy soils. Plant assemblages on gypsum soils in the Chihuahuan Desert are dominated by gypsophile perennial forbs concentrated in three major plant clades: the asterids, Caryophyllales, and Brassicaceae (Moore and Jansen, 2007; Moore et al. 2014) as well as numerous grass species. Sampling focused on groups that include regionally dominant gypsophiles from the families Asteraceae, Brassicaceae,

Nyctaginaceae, Poaceae, Ehretiaceae, Namaceae, Loasaceae, and Onagraceae (Table 2). To help control for the effect of phylogeny on leaf chemical patterns, confamilials or congeners were sampled to include taxa from each of three groups: (1) old-lineage gypsophiles; (2) young-lineage gypsophiles; and (3) gypsovags growing on non-gypsum soils.

***Field sampling design*—**In Spain, leaves, stems, coarse roots (> 1 cm diameter), and fine roots (< 1 cm diameter) were collected from at least 5 plant replicates per species (except *Ononis tridentata* ssp. *tridentata*, which did not have accessible fine roots). Collections included five wide gypsophiles, one narrow gypsophile, and two gypsovags sampled on gypsum soils (Table 2). Sampling replicates were selected randomly from within an area approximately 50 m x 50 m and at least 20 m from roadsides to minimize the effects of disturbance. Each replicate was at least 10 m away from other sampled replicates of the same species. All plant tissues were stored in silica gel after collection.

In the USA, leaves were collected from 23 taxa including ten widely distributed gypsophiles, five narrowly distributed gypsophiles, and eight gypsovags sampled on non-gypsum soil (Table 1). Collections were conducted for Muller et al. (2015) using the same protocol described for collections in Spain.

Plant chemical analyses—Plant tissues were briefly rinsed with deionized water (< 10 s), oven-dried, and finely ground using a ball mill or Thomas Wiley Mini Mill until tissue passed through a 40-mesh screen (< 2 mm). Ground tissues were prepared for analysis by microwave digestion using concentrated trace metal grade HNO_3 and analyzed for total S, Ca, Mg, P, and K with ICP-OES. Total N for each plant sample was

analyzed using micro-Dumas combustion on a CN analyzer (EDS 4010; Costech Analytical).

Statistical analyses—To understand leaf nutritional patterns in gypsophiles and non-gypsophiles from a more global perspective, we compared leaf chemical signatures of species from the Chihuahuan Desert and Spain with Principal Components Analysis (PCA) in Canovo v 5 for Windows (Ter Braak and Šmilauer, 2012). PCA is a linear, multivariate method used for understanding and visualizing variance in data. Variables are graphed as vectors, indicating the direction and magnitude of increase for each measured element. Species means are plotted as centroids on orthogonal axes, and the first axis explains the greatest amount of variance in the data. Additional PCAs for Spanish taxa were conducted to visualize differences in stem, coarse root, and fine root nutrition. Response variables for all PCAs were tissue S, Ca, Mg, N, P, and K.

We also tested how nutrient accumulation and exclusion patterns are reflected specifically in tissue S and Ca, the components of gypsum. For Spanish taxa, sampling limitations required that narrow gypsophiles and gypsovags be considered as one group to compare with wide gypsophiles, because replication is at the species level in all models (i.e., samples within plant organ and species were averaged). Previous work has shown that narrow gypsophiles and gypsovags are statistically similar in leaf chemistry (Palacio et al., 2007; Muller et al., 2015; Bolukbasi et al., 2016). Two-sample, right-tailed t-tests for S and Ca were conducted to test our hypothesis that narrow gypsophiles and gypsovags would have lower leaf S and Ca compared to wide gypsophiles. We also used two-tailed t-tests in R v 3.3.2 (R Core Team, 2016) to analyze the difference in tissue S and Ca means of narrow gypsophiles and gypsovags compared to wide gypsophiles for

stems, coarse roots, and fine roots. Data were tested for equal variance using Levene's test prior to analysis.

RESULTS

***Global comparison of leaf mineral nutrition*—**Patterns in the leaf chemical signatures for Spanish gypsophiles and gypsovags reflect patterns observed for taxa collected in the USA. Leaves of wide gypsophiles from Spain clustered along PC1 with old-lineage gypsophiles from the Chihuahuan Desert (Figure 1), reflecting the distinct leaf chemical signatures of wide gypsophiles from narrow gypsophiles and gypsovags. This distinction in leaf chemistry was driven primarily by high concentrations of S and Ca in the leaves of wide gypsophiles. Narrow gypsophiles and gypsovags from Spain had lower leaf S than wide gypsophiles ($t = -1.975$; $df = 6$; $P = 0.0478$), but did not have significantly lower leaf Ca ($t = -1.260$, $df = 6$, $P = 0.127$). *Helianthemum alypoides*, a narrow gypsophile from Spain, had similar leaf chemistry to young-lineage gypsophiles from the USA, which were characterized by reduced concentrations of S, Ca, and Mg (Figure 1). Gypsovags collected in Spain also had similar leaf chemistry to gypsovags collected in the USA, and clustered with narrow gypsophiles along PC1 (Figure 1). The PCA for leaves of the Spanish taxa differed from the PCA for leaves of the USA taxa in the importance of some elemental variables in differentiating samples (Figures 2 and 3). Vectors for S and Ca were less closely associated with PC1 in the PCA for Spanish taxa (Figure 2). In addition, Mg was highly associated with PC1, indicating its importance for differentiating taxa collected in Spain. Leaf N was also more important for separation of

taxa along PC2 for the Spanish taxa (Figure 2). Leaf K and P were similar in their importance for both analyses.

Comparison of Spanish gypsophile and gypsovag tissue nutrition—Overall, low species-level replication limited our ability to detect statistically significant patterns in the data, with differences in leaf S being the only statistically significant pattern. However, some interesting trends may be important to investigate with greater replication. At the leaf level, wide gypsophiles tended to have higher Ca, Mg, and S than narrow gypsophiles or gypsovags (Table 1, Figure 4). However, leaf N, P, and K tended to be similar across all taxa (Figure 5). Stems of narrow gypsophiles and gypsovags also tended to have lower S concentrations than wide gypsophiles (Table 2, Figure 6). Some wide gypsophiles had high concentrations of Ca in coarse roots (even higher than in leaves for *Gypsophila struthium* ssp. *hispanica*), particularly for taxa in the Caryophyllaceae (Table 5, Figure 7). Taxa in the Brassicaceae tended to have higher fine root S concentrations (Table 6, Figure 8). Any trends in tissue N, P, and K were primarily driven by species-specific differences (e.g., high leaf K in *Matthiola fruticulosa*) (Tables 3–6, Figure 2).

Whole-plant patterns in S and Ca accumulation—Leaves of wide gypsophiles possessed nearly 4-fold higher S concentrations than stems and coarse roots, and nearly 3-fold higher than fine roots (Tables 3–6, Figure 4). In contrast, leaves of narrow gypsophiles and gypsovags from Spain possessed only 3.5-fold higher S concentrations than stems and coarse roots, and were similar to fine roots in S concentrations.

Accumulation patterns for Ca in wide gypsophiles reflected patterns of tissue S, except for in coarse roots. Coarse root concentrations of Ca for wide gypsophiles were

1.5-fold higher than in fine roots and stems (Tables 5–6, Figure 4). Leaves of narrow gypsophiles and gypsovags possessed about 2-fold higher Ca concentrations than their other tissues, which were similar in Ca (Tables 3–6).

DISCUSSION

Global patterns in gypsophile leaf chemistry—As hypothesized, the leaf chemical signatures of widely distributed, old-lineage gypsophiles were distinct from narrowly distributed, young-lineage gypsophiles and gypsovags for the Chihuahuan Desert and Spanish taxa. This distinction was driven by high concentrations of S and Ca in the leaves of wide gypsophiles relative to narrow gypsophile and gypsovag taxa. For the Chihuahuan Desert flora, old-lineage gypsophiles with foliar concentrations of S greater than 18 g kg^{-1} have been observed to contain gypsum in their leaves (Feder et al., 2016). Likewise, for many of the Spanish wide gypsophiles sampled here, high leaf S was associated with the presence of gypsum in previous work (Palacio et al., 2014). This study provides further support for the hypothesis that assimilation of excess S and Ca as gypsum is a shared mechanism for wide, old-lineage gypsophiles from the USA and Spain, given the strong trend for high leaf Ca and S in wide gypsophiles.

Whole-plant patterns of S and Ca accumulation—The results of this study suggest that wide gypsophiles may also be capable of maintaining higher concentrations of S in stems and roots compared to narrow gypsophiles and gypsovags. In addition, some wide gypsophiles tended to have high Ca coarse root concentrations relative to other tissues. We hypothesize that wide gypsophiles in the Caryophyllaceae with the highest Ca concentrations may biomineralize excess Ca in roots, as they are able to do in

leaves (White and Broadley, 2003; Palacio et al., 2014). Confamilials were more similar in fine root S and Ca than taxa grouped based on gypsum specificity, according to PCA assessment (Figure 8). This trend suggests that uptake mechanisms at the root-soil interface may be conserved for the taxa in this study and could be related to the ability of some groups to supply tissues with concentrations of S and Ca required for assimilate production.

For the only collected narrow gypsophile, *H. alypodies,* Ca concentrations among tissue types were nearly equal to each other, and leaf S concentrations were extremely low compared to its wide gypsophile congener (Tables 3–6). More sampling is needed to clarify the tissue accumulation patterns of narrow gypsophiles as a group, but based on these preliminary results, it may be that narrow gypsophiles exclude uptake of excess S and Ca at the root level. There is little to suggest that *H. alypoides,* or its gypsovag congener *H. syriacum,* are selectively translocating Mg into shoots to mediate excess leaf Ca, similar to what has been observed for some serpentine taxa (O'Dell and Claassen, 2006). It is possible that instead, uptake at the root-soil interface is limited. More narrow gypsophiles in comparison with gypsovag relatives need to be analyzed to further understand where and how exclusion is occurring in roots.

***Importance of phylogenetic sampling*—**Although more extensive species-level sampling in Spain was not possible, trends in our data suggest that phylogeny is likely playing a large role that cannot be fully accounted for by the limited design. This caveat underlies each of the previous sections, but we provide some key examples in which a phylogenetic lens is needed to understand unresolved patterns in this dataset. The foremost example is that there are key shared traits related to assimilation of excess S and

Ca in some families, including foliar biomineralization of gypsum and Ca-oxalate (Palacio et al., 2014; Muller et al., 2015; Feder et al., 2015) and potentially the ability to retain high concentrations of Ca in coarse roots for some wide gypsophiles. In addition, phylogeny seems to be important for leaf chemical patterns in gypsovags. Some gypsovags appear to possess the ability to accumulate and assimilate excess S and Ca in leaves like their wide gypsophile relatives (e.g., *Tiquilia canescens* var. *canescens* and *M. fruticulosa*), but others do not (e.g., *H. syriacum*). Taxa in the Brassicaceae from the USA and Spain tended to have higher leaf S and N, regardless of their specificity to gypsum, which may be related to their ability to accumulate and assimilate S via formation of S and N-rich glucosinolate compounds (Palacio et al., 2014; Muller et al., 2015). Deeper taxonomic sampling and manipulative experiments can better investigate these trends and potentially resolve putative adaptive mechanisms.

In addition to phylogenetic sampling, this dataset underlines the importance of having information about the relative lineage ages of the taxa. Previous study on the Chihuahuan Desert flora suggests that lineage age may be a key factor distinguishing gypsum adaptation patterns (Moore et al., 2014; Muller et al., 2015). For example, the wide gypsophiles *Oenothera hartwegii* ssp. *filifolia* and *Mentzelia humilis* var. *humilis* from the USA did not accumulate S and Ca in leaves. These taxa are also hypothesized to be relatively younger than most wide gypsophiles. Similarly, the wide gypsophile *Herniaria fruticosa* had low leaf S and Ca compared to other wide gypsophiles. Information about the ages of independent lineages of gypsophiles from Spain could clarify inconsistencies in their leaf nutrient patterns.

***Conclusions and Future Directions*—**We have described a more global perspective of gypsophile physiology than in any previous work by providing a multivariate assessment of the leaf chemistry of gypsophiles and their relatives from both Spain and the USA. We have shown that there are statistically consistent trends in the foliar accumulation of S and Ca in widely distributed, old-lineage gypsophiles from both floras, and there are strong phylogenetic patterns in S and Ca accumulation, particularly for gypsovags that are congeners or confamilials of wide gypsophiles. This study also provides a preliminary first look at the whole-plant tissue chemistry of the Spanish gypsum flora in a phylogenetic context.

Our current aims are to increase sampling of Spanish taxa to include additional narrow gypsophiles and complete confamilial groups, to finish analyses of whole-plant tissue chemistry for the Chihuahuan Desert gypsum flora, and to provide more rigorous support for phylogenetic trends in tissue accumulation patterns for both USA and Spanish gypsum floras. In the future, manipulative studies that test the effect of soil chemistry on plant tissue nutrition will be important for clarifying trends we observed from the Spanish flora. Manipulative experiments in the greenhouse will also be important for testing additional mechanisms of excess S and Ca assimilation other than gypsum in the leaves of wide gypsophiles.

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Table 1. Taxa collected from Spain. Column 'Abbr.' indicates the abbreviations used to represent taxa in figures. Column 'n' indicates the number of replicates for each taxon.

Family	Taxon	Status	n	Abbr.
Brassicaceae	*Lepidium subulatum* L.	Wide gypsophile	4	LESU
Brassicaceae	*Matthiola fruticulosa* (L.) Maire	Gypsovag	5	MAFR
Caryophyllaceae	*Gypsophila struthium* ssp. *hispanica* (Willk.) G. López	Wide gypsophile	5	GYHI
Caryophyllaceae	*Herniaria fruticosa* L.	Wide gypsophile	5	HEFR
Cistaceae	*Helianthemum alypoides* Losa Espana & Rivas Goday	Narrow gypsophile	5	HEAL
Cistaceae	*Helianthemum squamatum* (L.) Pers.	Wide gypsophile	5	HESQ
Cistaceae	*Helianthemum syriacum* Dum. Cours.	Gypsovag	5	HESY
Fabaceae	*Ononis tridentata* L. ssp. *tridentata*	Wide gypsophile	5	ONTR

Table 2. Taxa collected from the USA (Muller et al., 2015). Column 'Abbr.' indicates the abbreviations used to represent taxa in figures. Column 'n' indicates the number of replicates for each taxon.

Family	Taxon	Status	n	Abbr.
Asteraceae	*Dicranocarpus parviflorus* A.Gray	Old-lineage gypsophile	5	DIPA
Asteraceae	*Sartwellia flaveriae* A.Gray	Old-lineage gypsophile	5	SAFL
Asteraceae	*Senecio warnockii* Shinners	Young-lineage gypsophile	2	SEWA
Brassicaceae	*Nerisyrenia linearifolia* (S.Watson) Greene	Old-lineage gypsophile	5	NELI
Brassicaceae	*Physaria fendleri* (A.Gray) O'Kane & Al-Shehbaz	Gypsovag	4	PHFE
Ehretiaceae	*Tiquilia canescens* (A.DC.) A.T.Richardson var. *canescens*	Gypsovag	5	TICA
Ehretiaceae	*Tiquilia hispidissima* (Torr. & A.Gray) A.T.Richardson	Old-lineage gypsophile	5	TIHI
Linaceae	*Linum allredii* Sivinski & M.O.Howard	Young-lineage gypsophile	5	LIAL
Loasaceae	*Mentzelia humilis* (Urb. & Gilg) J.Darl. var. *humilis*	Young-lineage gypsophile	5	MEHU
Loasaceae	*Mentzelia strictissima* (Wooton & Standl.) J.Darl.	Gypsovag	5	MEST
Namaceae	*Nama carnosa* (Wooton) C.L.Hitchc.	Old-lineage gypsophile	5	NACA
Nyctaginaceae	*Abronia nealleyi* Standl.	Young-lineage gypsophile	5	ABNE

Nyctaginaceae	*Acleisanthes lanceolata* (Wooton) R.A.Levin var. *lanceolata*	Old-lineage gypsophile	5	ACLA
Nyctaginaceae	*Acleisanthes longiflora* A.Gray	Gypsovag	5	ACLO
Nyctaginaceae	*Anulocaulis leiosolenus* (Torr.) Standl. var. *gypsogenus* (Waterf.) Spellenb. & T.Wootten	Old-lineage gypsophile	5	ANLE-G
Onagraceae	*Oenothera capillifolia* Scheele ssp. *berlandieri* (Spach) W.L.Wagner & Hoch	Gypsovag	5	OECA
Onagraceae	*Oenothera gayleana* B.L.Turner & M.J.Moore	Young-lineage gypsophile	5	OEGA
Onagraceae	*Oenothera hartwegii* Benth. ssp. *filifolia* (Eastw.) W.L.Wagner & Hoch	Young-lineage gypsophile	5	OEHA-F
Onagraceae	*Oenothera hartwegii* Benth. ssp. *pubescens* (A.Gray) W.L.Wagner & Hoch	Gypsovag	8	OEHA-P
Poaceae	*Bouteloua breviseta* Vasey	Young-lineage gypsophile	5	BOBR
Poaceae	*Bouteloua curtipendula* (Michx.) Torr.	Gypsovag	5	BOCU
Poaceae	*Sporobolus cryptandrus* (Torr.) A.Gray	Gypsovag	5	SPCR
Poaceae	*Sporobolus nealleyi* Vasey	Young-lineage gypsophile	10	SPNE

Table 3. Leaf nutrition for the gypsum flora from Spain. Means and standard deviation are presented for each element analyzed (see Table 1 for replication).

Taxon	S (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})
GYHI	18.51 ± 2.06	58.43 ± 5.30	5.58 ± 1.76	30.24 ± 2.97	1.70 ± 0.14	12.56 ± 1.27
HEAL	7.41 ± 1.54	16.15 ± 1.49	2.48 ± 0.56	15.90 ± 3.48	1.14 ± 0.16	6.08 ± 1.22
HEFR	8.60 ± 1.55	28.41 ± 4.24	6.42 ± 0.74	20.89 ± 4.39	0.68 ± 0.09	7.48 ± 2.07
HESQ	26.09 ± 2.55	32.44 ± 3.95	5.10 ± 0.93	17.30 ± 2.75	0.84 ± 0.14	5.65 ± 1.08
HESY	8.32 ± 1.77	25.72 ± 2.97	1.88 ± 0.45	19.95 ± 1.60	1.02 ± 0.22	8.04 ± 1.84
LESU	31.56 ± 5.50	22.63 ± 6.97	2.08 ± 0.47	44.23 ± 4.43	1.25 ± 0.14	6.57 ± 1.08
MAFR	17.07 ± 1.35	34.83 ± 5.90	2.31 ± 0.54	37.11 ± 3.63	1.26 ± 0.20	20.16 ± 4.15
ONTR	29.81 ± 2.37	45.57 ± 10.74	16.61 ± 3.22	22.01 ± 2.07	0.99 ± 0.20	4.30 ± 0.83

Table 4. Stem nutrition for the gypsum flora from Spain. Means and standard deviation are presented for each element analyzed (see Table 1 for replication).

Taxon	S (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})
GYHI	2.49 ± 0.89	20.45 ± 15.34	0.53 ± 0.19	6.14 ± 1.38	0.31 ± 0.10	5.75 ± 1.38
HEAL	3.23 ± 0.52	11.04 ± 3.23	1.46± 0.17	5.44 ± 1.40	0.67 ± 0.16	5.60 ± 1.67
HEFR	3.66 ± 0.86	27.48 ± 5.40	6.38 ± 2.11	17.50 ± 3.96	0.56 ± 0.16	14.00 ± 2.80
HESQ	8.47 ± 3.74	17.29 ± 4.94	2.00 ± 1.00	6.83 ± 1.20	0.50 ± 0.12	3.72 ± 1.86
HESY	1.68 ± 0.43	20.41 ± 5.61	0.50 ± 0.08	7.58 ± 1.10	0.40 ± 0.10	1.86 ± 0.31
LESU	6.29 ± 1.93	5.08 ± 0.60	0.94 ± 0.24	18.09 ± 6.06	0.68 ± 0.24	6.07 ± 2.98
MAFR	4.26 ± 1.12	6.44 ± 1.12	0.53 ± 0.24	11.30 ± 1.74	0.36 ± 0.09	6.63 ± 1.35
ONTR	5.166 ± 0.81	18.07 ± 3.19	1.99 ± 0.57	6.94 ± 1.04	0.18 ± 0.05	1.39 ± 0.34

Table 5. Coarse root nutrition for the gypsum flora from Spain. Means and standard deviation are presented for each element analyzed (see Table 1 for replication).

Taxon	S (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})
GYHI	3.21 ± 0.50	62.44 ± 1.81	0.62 ± 0.12	13.00 ± 3.44	0.81 ± 0.50	6.21 ± 1.32
HEAL	2.71 ± 0.70	14.30 ± 3.78	1.07 ± 0.31	3.24 ± 0.69	0.45 ± 0.16	2.41 ± 0.82
HEFR	4.61 ± 0.65	20.88 ± 6.41	3.52 ± 0.87	21.02 ± 3.60	0.65 ± 0.30	9.86 ± 1.08
HESQ	5.79 ± 0.90	24.80 ± 6.28	1.00 ± 0.23	6.46 ± 1.17	0.53 ± 0.22	4.14 ± 2.89
HESY	1.41 ± 0.48	13.92 ± 6.55	0.47 ± 0.15	6.70 ± 1.70	0.43 ± 0.24	1.89 ± 0.74
LESU	11.69 ± 3.04	5.35 ± 0.66	0.91 ± 0.08	20.78 ± 4.03	0.67 ± 0.25	5.02 ± 0.95
MAFR	5.45 ± 2.88	6.09 ± 1.75	0.62 ± 0.08	11.56 ± 4.39	0.35 ± 0.12	8.30 ± 1.37
ONTR	7.51 ± 2.42	29.12 ± 9.22	2.89 ± 1.83	12.66 ± 3.23	0.24 ± 0.07	1.34 ± 0.81

Table 6. Fine root nutrition for the gypsum flora from Spain. Means and standard deviation are presented for each element analyzed (see Table 1 for replication). No fine roots were collected for *O. tridentata* ssp. *tridentata.*

Taxon	S (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})
GYHI	3.66 ± 0.39	31.45 ± 6.07	1.28 ± 0.74	14.36 ± 2.67	0.78 ± 0.47	9.18 ± 1.78
HEAL	3.69 ± 0.81	13.20 ± 4.05	1.42 ± 0.08	4.91 ± 0.42	0.65 ± 0.08	3.58 ± 0.96
HEFR	3.65 ± 0.67	19.82 ± 5.58	5.00 ± 1.51	18.60 ± 2.16	0.49 ± 0.17	12.18 ± 2.27
HESQ	6.93 ± 1.11	18.83 ± 4.91	0.73 ± 0.27	6.14 ± 1.19	0.37 ± 0.11	2.61 ± 1.76
HESY	2.51 ± 0.77	15.07 ± 3.71	0.49 ± 0.10	8.12 ± 1.33	0.41 ± 0.18	2.68 ± 0.51
LESU	18.87 ± 4.19	8.61 ± 2.40	0.97 ± 0.20	24.50 ± 6.90	0.69 ± 0.13	7.63 ± 1.84
MAFR	15.35 ± 2.99	10.69 ± 5.40	1.25 ± 0.71	22.03 ± 9.95	0.68 ± 0.41	16.42 ± 5.74

FIGURE LEGENDS

Figure 1. Principal Components Analysis of leaf tissue chemistry for taxa collected in Spain (indicated with asterisks) and in the USA. Centroids are species means ± standard deviation (see Tables 1 and 2 for replication). Black centroids are wide gypsophiles, white are narrow gypsophiles, and gray are gypsovags. Vectors represent measured leaf element concentrations.

Figure 2. Principal Components Analysis of leaf tissue chemistry for taxa collected in Spain. Centroids are species means ± standard deviation (see Table 1 for replication). Black centroids are wide gypsophiles, white are narrow gypsophiles, and gray are gypsovags. Vectors represent measured leaf element concentrations.

Figure 3. Principal Components Analysis of leaf tissue chemistry for taxa collected in the USA. Centroids are species means ± standard deviation (see Table 2 for replication). Black centroids are wide gypsophiles, white are narrow gypsophiles, and gray are gypsovags. Vectors represent measured leaf element concentrations.

Figure 4. Mean tissue concentrations of sulfur, calcium, and magnesium for wide gypsophiles, narrow gypsophiles, and gypsovags from Spain. Error bars represent standard deviation (see Table 1 for replication).

Figure 5. Mean tissue concentrations of nitrogen, phosphorus, and potassium for wide gypsophiles, narrow gypsophiles, and gypsovags from Spain. Error bars represent standard deviation (see Table 1 for replication).

Figure 6. Principal Components Analysis of stem tissue chemistry for taxa collected in Spain. Centroids are species means ± standard deviation (see Table 1 for replication). Black centroids are wide gypsophiles, white are narrow gypsophiles, and gray are gypsovags. Vectors represent measured leaf element concentrations.

Figure 7. Principal Components Analysis of coarse root tissue chemistry for taxa collected in Spain. Centroids are species means ± standard deviation (see Table 1 for replication). Black centroids are wide gypsophiles, white are narrow gypsophiles, and gray are gypsovags. Vectors represent measured leaf element concentrations.

Figure 8. Principal Components Analysis of fine root tissue chemistry for taxa collected in Spain. Vectors represent measured leaf element concentrations. Centroids are species means ± standard deviation (see Table 1 for replication). Black centroids are wide gypsophiles, white are narrow gypsophiles, and gray are gypsovags. Confamilial taxa are circled.

Figure 1.

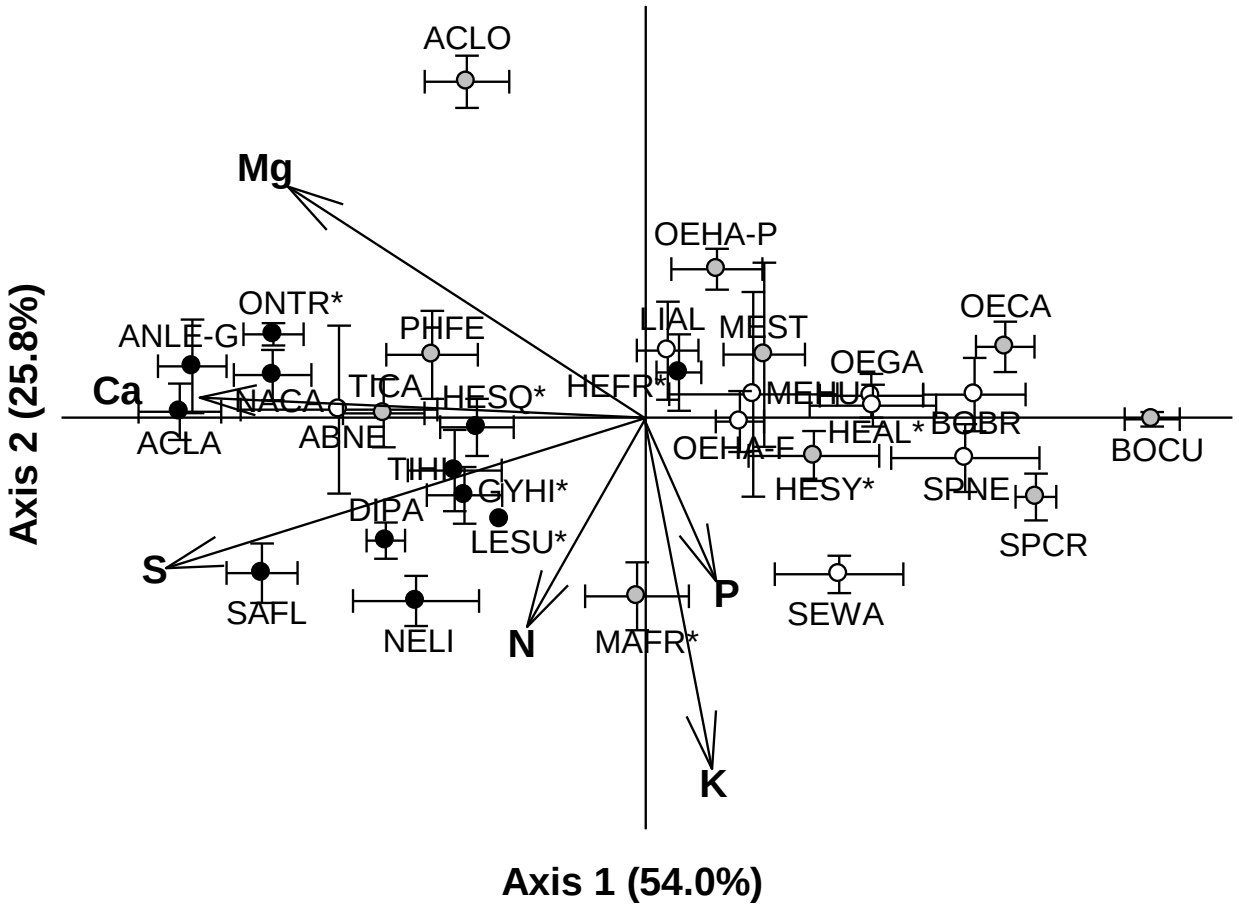

ACLO
Mg
OEHA-P
ONTR*
ANLE-G
PHFE
LIAL
MEST
OECA
OEGA
Ca
TRCA
HESO*
HEFR*
MEHU
NACA
ACLA
ABNE
HEAL*
BOBR
OEHA-F
BOCU
THHE
GYHI*
HESY*
SPNE
DIPA
LESU*
SPCR
S
P
SAFL
SEWA
NELI
N
MAFR*
K
Axis 2 (25.8%)
Axis 1 (54.0%)

Figure 2.

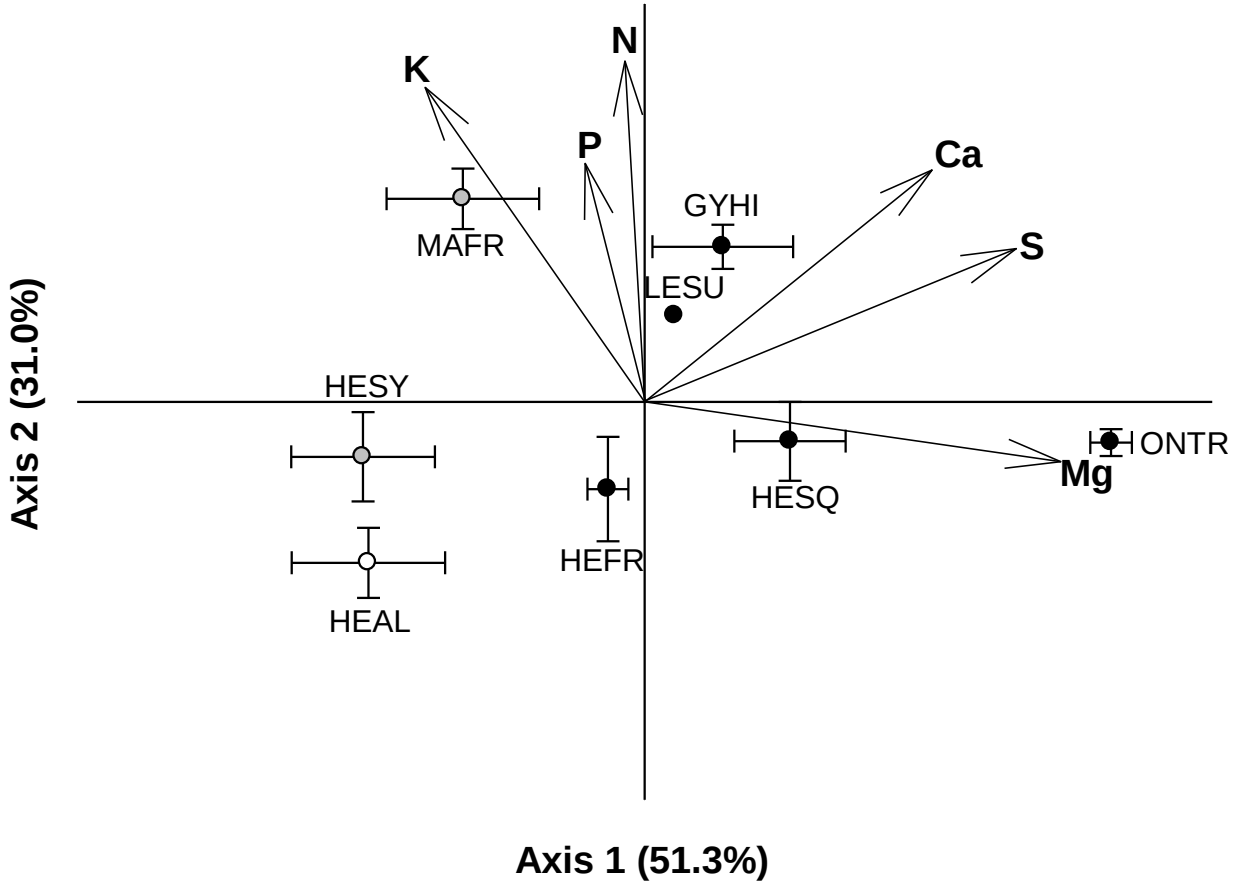

N
K
P
Ca
S
GYHI
MAFR
LESU
HESY
ONTR
Mg
HESQ
HEFR
HEAL
Axis 2 (31.0%)
Axis 1 (51.3%)

Figure 3.

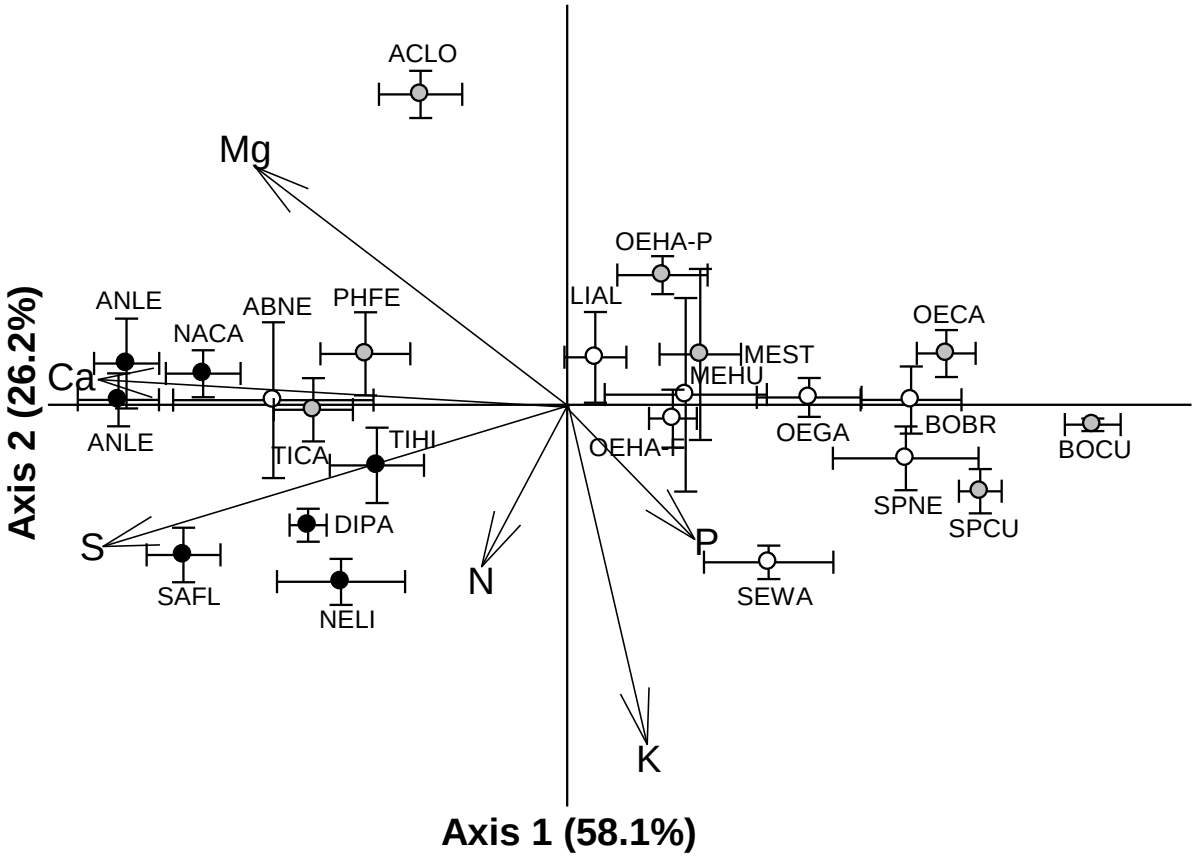


Figure 4.

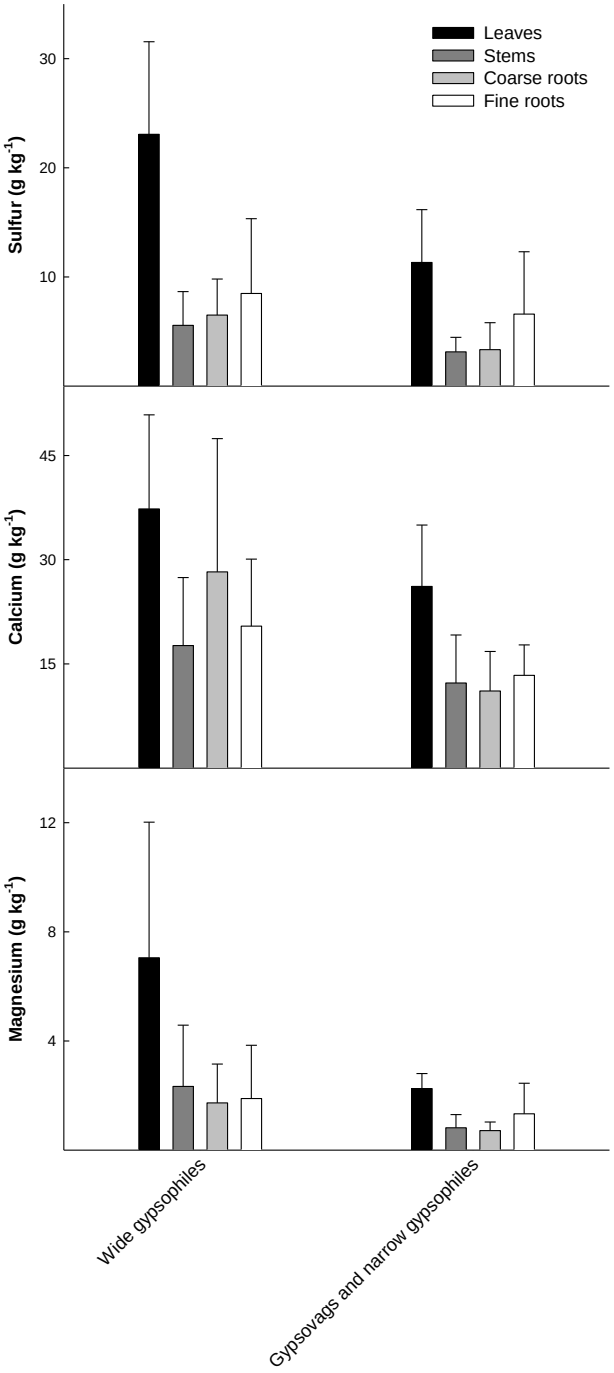

Leaves
Stems
Coarse roots
Fine roots
Sulfur (g kg-1)
30
20
10
Calcium (g kg-1)
45
30
15
Magnesium (g kg-1)
12
8
4
Wide gypsophiles
Gypsovags and narrow gypsophiles

Figure 5.

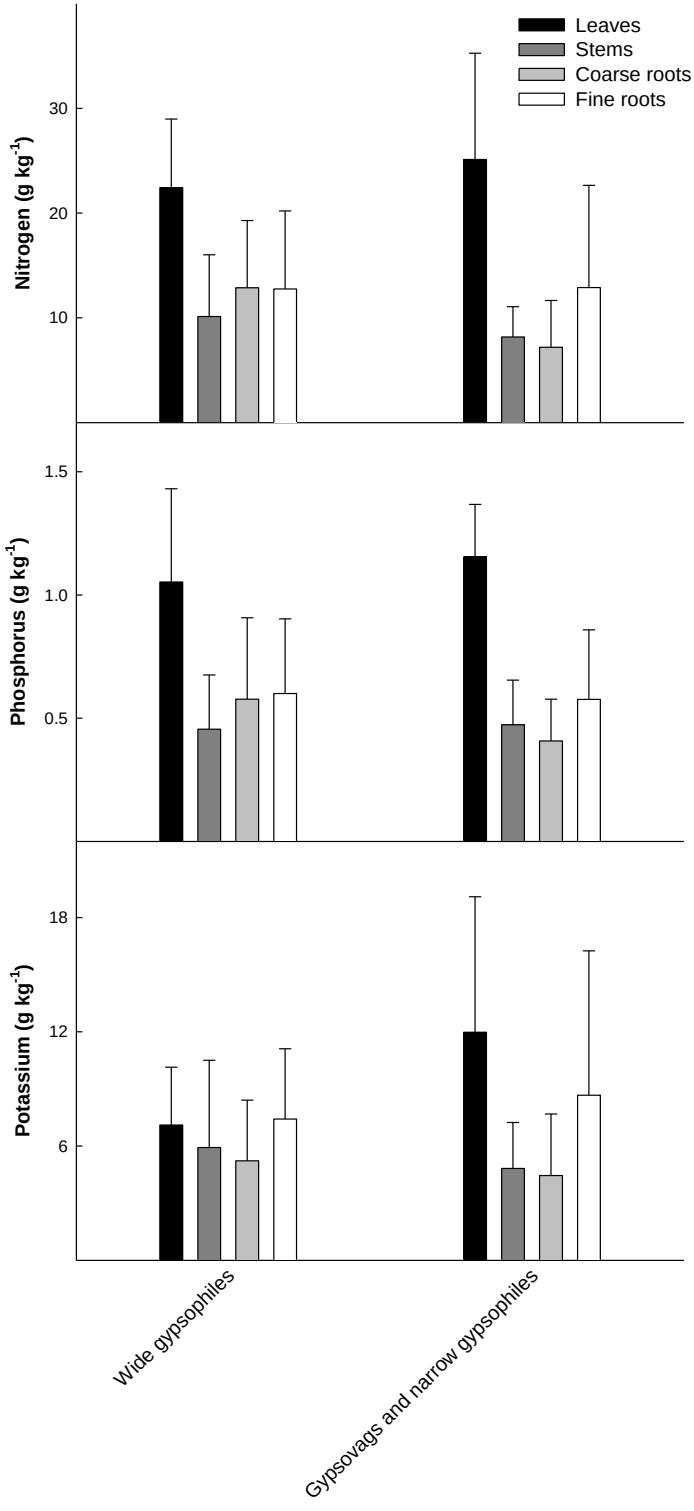

Leaves
Stems
Coarse roots
Fine roots
Nitrogen (g kg-1)
30
20
10
Phosphorus (g kg-1)
1.5
1.0
0.5
Potassium (g kg-1)
18
12
6
Wide gypsophiles
Gypsovags and narrow gypsophiles

Figure 6.

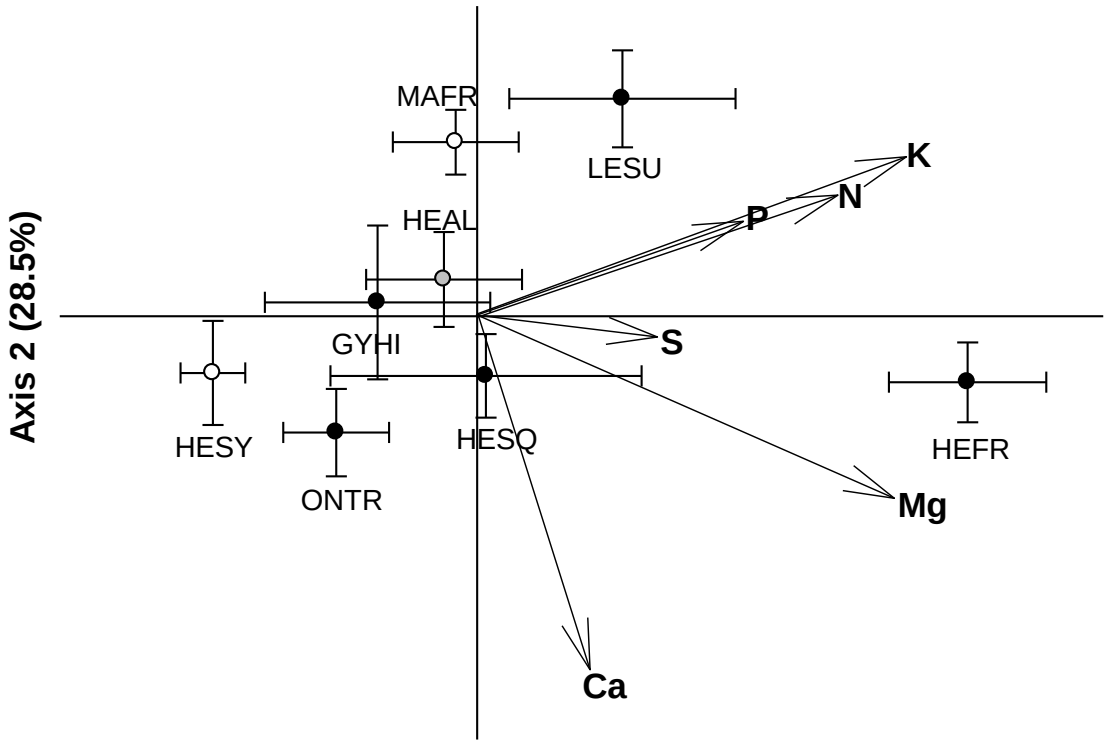

MAFR
LESU
K
N
P
HEAL
GYHI
S
HESY
ONTR
HESQ
HEFR
Mg
Ca
Axis 2 (28.5%)
Axis 1 (43.1%)

Figure 7.

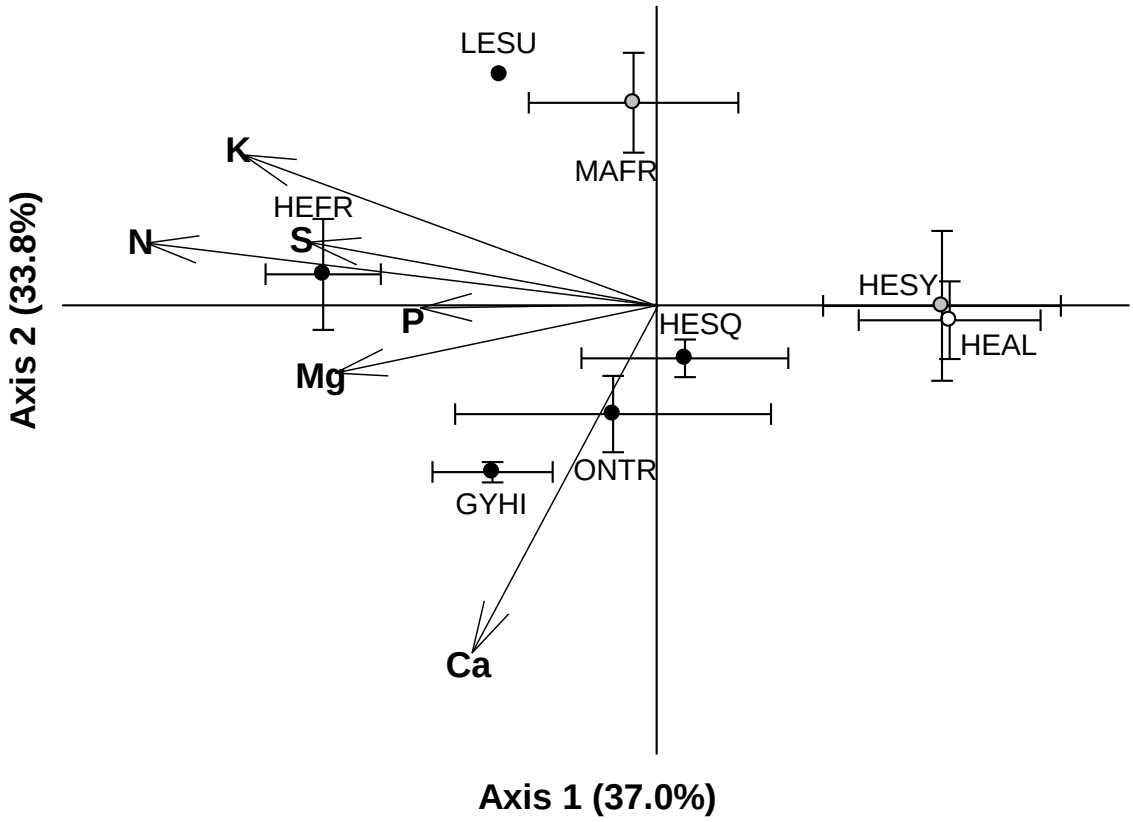
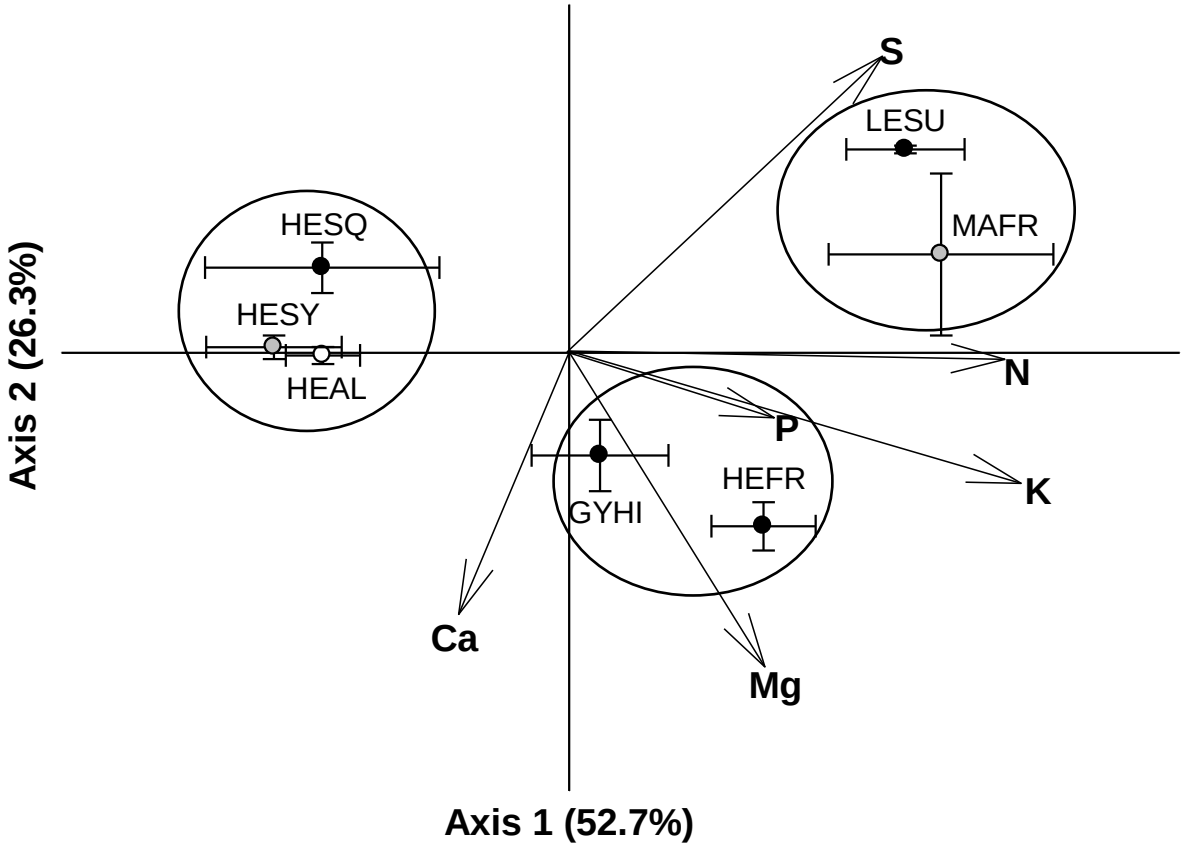

LESU
MAFR
K
HEFR
N
S
P
Mg
HESY
HESQ
HEAL
ONTR
GYHI
Ca
Axis 2 (33.8%)
Axis 1 (37.0%)

Figure 8.


S
LESU
MAFR
HESQ
HESY
HEAL
N
P
K
HEFR
GYHI
Ca
Mg
Axis 2 (26.3%)
Axis 1 (52.7%)