John Carroll University
Carroll Collected

10-2017

Phylogenetic patterns of foliar mineral nutrient accumulation among gypsophiles and their relatives in the Chihuahuan Desert

Clare Muller
John Carroll University, cmuller15@jcu.edu

Michael J. Moore
Oberlin College

Zoe Feder
Oberlin College

Helene Tiley
Oberlin College

Rebecca E. Drenovsky
John Carroll University, rdrenovsky@jcu.edu

Follow this and additional works at: https://collected.jcu.edu/fac_bib_2017

Part of the Biology Commons, Botany Commons, and the Plant Biology Commons

Recommended Citation

Phylogenetic patterns of foliar mineral nutrient accumulation among gypsophiles and their relatives in the Chihuahuan Desert[1]

Clare T. Muller[2], Michael J. Moore[3], Zoë Feder[3], Helene Tiley[3], and Rebecca E. Drenovsky[2,4]

PREMISE OF THE STUDY: 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 nonendemic (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.

METHODS: We collected leaves from 23 gypsophiles and related nonendemic taxa growing on nongypsum soils. Soils and leaves were analyzed for Ca, S, Mg, K, N, and P. Leaf gypsum was assessed using Fourier transform infrared spectroscopy.

KEY RESULTS: Most widespread gypsophile lineages that are hypothesized to be relatively old accumulate foliar S, Ca, and gypsum, but younger gypsophile lineages and closely related gypsovags do not. Young, narrowly distributed gypsophile lineages have leaf chemical signatures similar to nonendemic congeners and confamilials.

CONCLUSIONS: 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.

KEY WORDS assimilation; biomineralization; calcium; gypsum endemism; phylogeny; sulfur

Soil chemistry is an important driver of plant ecology (Laliberté et al., 2014). Soil conditions can restrict establishment and distribution of plant species, leading to strong phenotypic selection for edaphically endemic plants, i.e., 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, because 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 because of 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+} because of 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 nongypsum 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). Among plants found on gypsum, there are three ecologically distinct groups: regionally dominant gypsophiles that are common and have broad distributions on gypsum ("wide gypsophiles"), locally-endemic gypsophiles ("narrow gypsophiles"), and plants able to grow on or off gypsum (gypsovags). Work from Spain has found that wide gypsophiles have higher concentrations of total S and Ca, as well as Mg, P, and Na, than narrowly distributed gypsophiles or gypsovags, 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 narrow gypsophiles possess leaf chemical signatures more similar to gypsovags, 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 ages 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 gypsophile 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 gypsophile 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 four factors: (1) molecular dating, (2) their high morphological distinctiveness compared to nongypsophile congeners, (3) the extent of speciation within these clades (with as many as 10 allopatric species of gypsophiles in a single clade), and (4) 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 gypsophile clades, there are numerous locally distributed gypsophile taxa known from only a small number of nearby sites (narrow gypsophiles), which have been shown to be relatively young (< 2 million years in age; e.g., *Tiquilia turneri,* Moore and Jansen (2007)) or are hypothesized to be relatively young based on their limited geographic ranges, lack of speciation on gypsum, and high morphological similarity to their nearest congeners. We hypothesize that lineages in this group may have limited distributions because of insufficient time to disperse extensively across the Chihuahuan Desert gypsum archipelago, although dispersal rates also may depend on other factors such as dispersal mechanism (Schenk, 2013). These patterns suggest that the geographic extent of endemic lineages may be a good proxy for the relative age of a lineage of gypsophile taxa. We expect that hypothesized lineage age will 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 gypsophile lineages are gypsovag taxa, allowing for phylogenetic control in studies of physiological adaptation. In addition to this rich availability of gypsophile 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. The summer-wet climate of the Chihuahuan Desert 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 nonendemic congeners or confamilials growing on nongypsum soils. We expected gypsum soils to be enriched in total Ca and S compared to nongypsum soils. We predicted that if gypsophiles of the Chihuahuan Desert share physiological strategies with the gypsophile 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 nongypsum 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 nongypsum soils. Lastly, we expected to detect a phylogenetic pattern in leaf chemistry among gypsophiles and their nonendemic 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 is 16°C, and mean annual precipitation is 330 mm (averages represent 30 yr of data obtained from the Carlsbad 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 the New Mexico 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). The nongypsum site along NM 128 has soils of the Kermit-Berino complex, with deep, fine sandy loams. Seven Rivers Hills soils are of the Reagan-Upton association, with gravelly alluvial loams. The US 285 site has Ector stony loam with shallow limestone soils and rocky gravel (Chugg et al., 1971). 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 attempted to include as many phylogenetic pairs of gypsophiles/nongypsophiles as possible, with the goal of encompassing a mix of gypsophile taxa from various independent evolutionary origins, including taxa from widely distributed, morphologically divergent clades of gypsophiles (e.g., *Acleisanthes lanceolata, Sartwellia flaveriae, Tiquilia hispidissima*; referred to as "old-lineage" gypsophiles hereafter). We also included gypsophile taxa of more limited geographic extent that are less morphologically divergent from their congeners (e.g., *Linum allredii, Oenothera gayleana, Senecio warnockii*; referred to as "young-lineage" gypsophiles hereafter) (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 15 genera and eight angiosperm families (Table 1). Eight old-lineage gypsophiles and seven young-lineage gypsophiles were sampled, along with

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 subsp. *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. subsp. *filifolia* (Eastw.) W.L.Wagner & Hoch	M. J. Moore et al., 2285	Young-lineage gypsophile	Yeso Hills	OEHA-F	5
Onagraceae	*Oenothera hartwegii* Benth. subsp. *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

eight gypsovag species. We collected congeners or confamilials growing on and off gypsum soils to account for phylogenetic patterns in the data, including five congeneric 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 m² around each plant replicate for eight of our target species (*Acleisanthes longiflora*, *Acleisanthes 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, because of site access limitations, *Acleisanthes lanceolata* individuals were collected within 20 m of roadside, but only in undisturbed gypsum. Gypsovags were sampled to investigate the leaf chemistry of nonspecialist outgroups in more typical soil environments, especially in relation to potential shared preadaptive traits, so all gypsovags were sampled from nongypsum soil sites. We sampled at least five replicate plants for all species except *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 soil SO_4^{2-}) using ICP-OES (Plasma 400; Perkin-Elmer, Waltham, Massachusetts, USA). Total soil N was determined via micro Dumas combustion using a CN analyzer (ECS 4010; Costech Analytical, Valencia, California, USA). 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 h at 60°C, finely ground (<2 mm) using a ball mill, and prepared for 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 Scientific, Waltham, Massachusetts, USA; MKII Golden Gate DATR attachment, Specac, Fort Washington, Pennsylvania, USA). Gypsum was detected as a means of identifying which taxa are able to biomineralize gypsum in their leaves. 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 were 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 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 nonindependence 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 specificity to gypsum 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 posthoc tests comparing leaf Ca and S concentrations in old- and young-lineage gypsophiles and gypsovags were also conducted; *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).
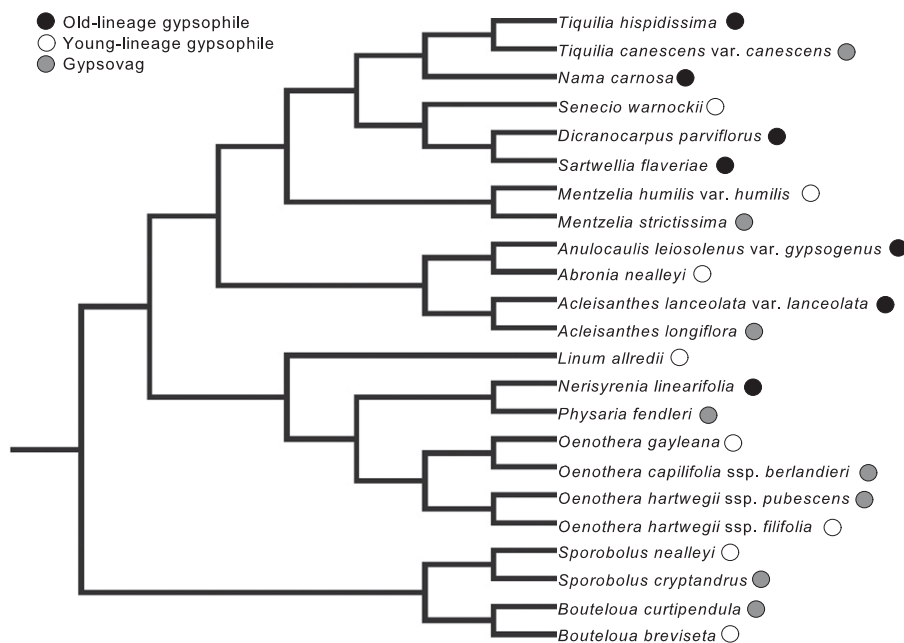
FIGURE 1 Phylogeny of the taxa included in our primary sampling, based on published work (see Materials and Methods). For phylogenetic statistical analyses, branch lengths were all set to 1.

Mexico sampling and analysis—In preparation for the primary sampling reported in this study, leaves were also collected from an additional suite of gypsophile 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 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. Because of limited replication, no statistical models comparing wide gypsophiles, narrow gypsophiles, and gypsovags were analyzed for the 2013 taxa.

RESULTS

Soil chemistry—Soil chemistry differed between gypsum and nongypsum soils, primarily because of concentrations of the elements associated with gypsum, i.e., Ca and S (Fig. 2). Gypsum soils had almost four times higher Ca and seven times higher S than nongypsum soils (Appendix S5). Gypsum soils also had four times higher EC than nongypsum soils, reflecting greater concentrations of charged ions. Mean Mg in gypsum soils was half the concentration of nongypsum soils. Extractable P and K varied among nongypsum soil sites, but P concentrations in all gypsum soil samples were below detectable limits (<1 ppm). Soil total N was three times higher in nongypsum 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 the three groups of taxa 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 on average leaf Ca was 2.5 times higher, than in other young-lineage gypsophiles. 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 because of 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).
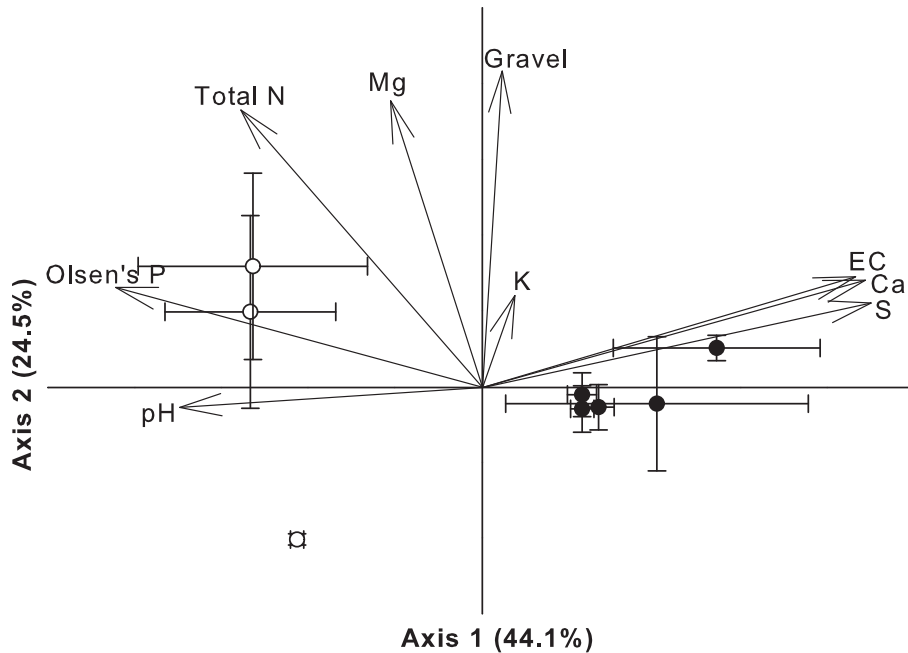
FIGURE 2 Principal Components Analysis (PCA) of soil properties. Centroids are mean soil samples ±SD (*n* = 6). Replicate plots were associated with individuals from eight of the sampled taxa. Gypsum soils are black circles, nongypsum soils are white circles. Vectors indicate the direction of increase for each measured variable.

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 (lineages whose distributions are known from gypsum exposures throughout the Chihuahuan Desert) had high concentrations of S and Ca compared to gypsovags and narrow gypsophiles (gypsophile lineages that occur in only small regions of the Chihuahuan Desert) (Bolukbasi et al., 2016) (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 of 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, S7). Additionally, some gypsovags with wide gypsophile congeners (e.g., *Tiquilia canescens*, *Nerisyrenia camporum*) that were collected

on gypsum soils contained gypsum in their leaves, and some gypsovags collected on nongypsum 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 gypsophile species.

One strategy, employed by widely distributed, older gypsophile 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 affect 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

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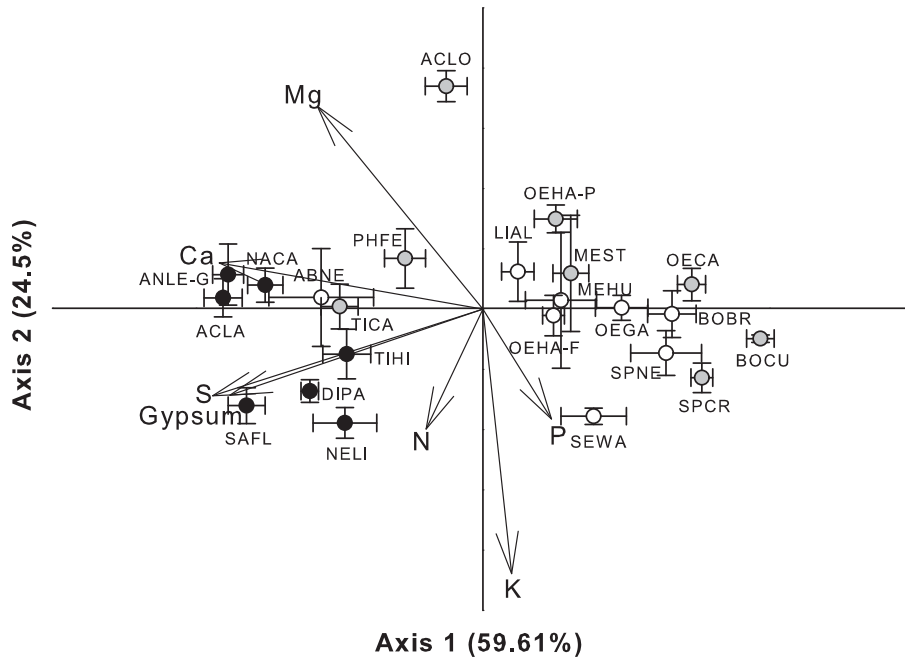
FIGURE 3 Principal Components Analysis (PCA) of leaf tissue chemistry. Centroids are species means ±SD (refer to Table 1 for replication). Black circles represent old-lineage gypsophiles, white circles represent young-lineage gypsophiles, and gray circles are gypsovags. Vectors represent measured variables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

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 wide 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. Because of 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 nonserpentine ecotypes lacked this ability. As a consequence, biomass production

was higher for serpentine than nonserpentine 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 gypsophile 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 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 gypsophile lineages and related gypsovags can also enable more powerful statistical analysis of the phylogenetic effect 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
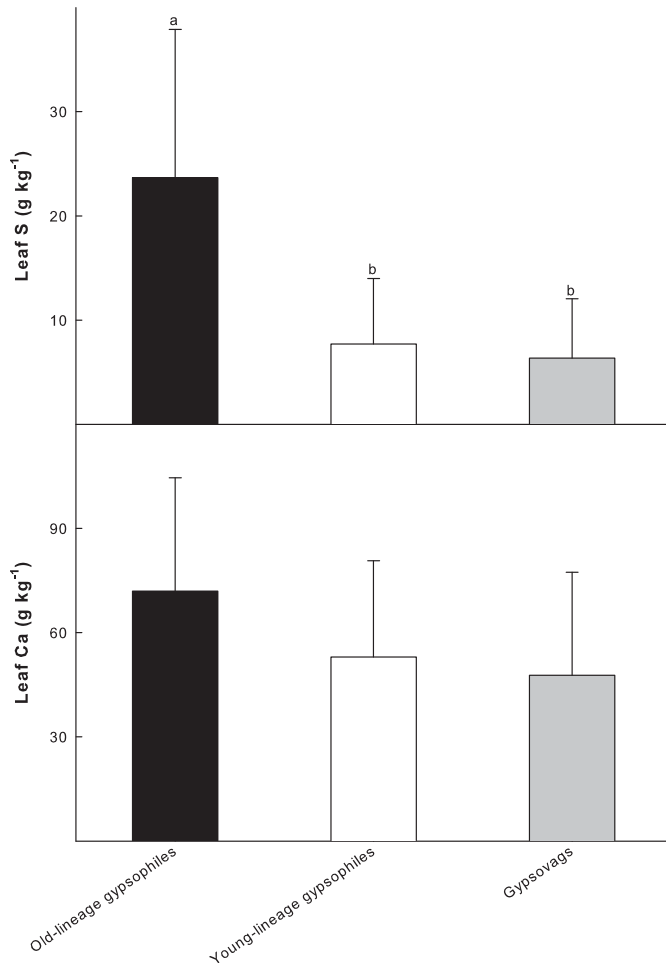
FIGURE 4 Mean leaf sulfur and calcium for sampled taxa categorized as old-lineage gypsophiles, young-lineage gypsophiles, and gypsovags. Error bars represent standard deviation (refer to Table 1 for replication). Letters correspond to the results of Tukey's post hoc tests for phylogenetic ANOVA of leaf S ($\alpha = 0.05$).

that serpentine endemic species controlled transport of Mg from roots to shoots, but did not inhibit uptake at the root level, while nonendemic 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 nonendemic 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.

The gypsophile 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 gypsophile 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 gypsophile lineages is a proxy for their relative age.

ACKNOWLEDGEMENTS

LITERATURE CITED

Angle, E. S. 2001. Hydrogeology of the Salt Basin. *Aquifers of West Texas: Texas Water Development Board Report* 356: 232–247.

Bolukbasi, A., L. Kurt, and S. Palacio. 2016. Unravelling the mechanisms for plant survival on gypsum soils: an analysis of the chemical composition of gypsum plants from Turkey. *Plant Biology)* 18: 271–279.

Borer, C. H., M. N. Hamby, and L. H. Hutchinson. 2012. Plant tolerance of a high calcium environment via foliar partitioning and sequestration. *Journal of Arid Environments* 85: 128–131.

Castillejo, J. M., R. Castelló, A. G. San Cristobal, and S. Abad. 2011. Soil-plant relationship along a semiarid gypsum gradient (Rio de Aguas, SE Spain). *Plant Ecology* 212: 1287–1297.

Chugg, J. C., G. W. Anderson, D. L. King, and L. H. Jones. 1971. Soil survey of Eddy area, New Mexico. United States Department of Agriculture, Soil Conservation Service, Washington DC, USA.

Damschen, E. I., B. M. Going, and B. L. Anacker. 2011. Climate change and plant communities on unusual soils. *In* S. Harrison, and N. Rajakaruna [eds.], Serpentine, The Evolution and Ecology of a Model System, 359–382. University of California Press, Berkeley, California, USA.

Donovan, L. A., J. H. Richards, and E. Joy Schaber. 1997. Nutrient relations of the halophytic shrub, *Sarcobatus vermiculatus*, along a soil salinity gradient. *Plant and Soil* 190: 105–117.

Douglas, N. A., and P. S. Manos. 2007. Molecular phylogeny of Nyctaginaceae: Taxonomy, biogeography, and characters associated with a radiation of xerophytic genera in North America. *American Journal of Botany* 94: 856–872.

Escudero, A., S. Palacio, F. T. Maestre, and A. L. Luzuriaga. 2014. Plant life on gypsum: a review of its multiple facets. *Biological Reviews of the Cambridge Philosophical Society* 90: 1–18.

Felsenstein, J. 1985. Phylogenies and the comparative method. *American Naturalist* 125: 1–15.

Garland, T., A. F. Bennett, and E. L. Rezende. 2005. Phylogenetic approaches in comparative physiology. *The Journal of Experimental Biology* 208: 3015–3035.

Garland, T., A. W. Dickerman, C. M. Janis, and J. A. Jones. 1993. Phylogenetic analysis of covariance by computer simulation. *Systematic Biology* 42: 265–292.

George, E., W. J. Horst, and E. Neumann. 2012. Adaptation of plants to adverse chemical soil conditions. *In* P. Marschner [ed.], Marschner's Mineral Nutrition of Higher Plants, 409–472. Elsevier, Boston, Massachusetts, USA.

Harmon, L. J., J. T. Weir, C. D. Brock, R. E. Glor, and W. Challenger. 2007. GEIGER: investigating evolutionary radiations. *Bioinformatics)* 24: 129–131.

He, H., Y. Kirilak, J. Kuo, and H. Lambers. 2015. Accumulation and precipitation of magnesium, calcium, and sulfur in two *Acacia* (Leguminosae; Mimosoideae) species grown in different substrates proposed for mine-site rehabilitation. *American Journal of Botany* 102: 290–301.

He, H., E. J. Veneklaas, J. Kuo, and H. Lambers. 2014. Physiological and ecological significance of biomineralization in plants. *Trends in Plant Science* 19: 166–174.

Herrero, J., O. Artieda, and W. H. Hudnall. 2009. Gypsum, a tricky material. *Soil Science Society of America Journal* 73: 1757–1763.

Herrero, J., and J. Porta. 2000. The terminology and the concepts of gypsum-rich soils. *Geoderma* 96: 47–61.

Johnson, M. T. J., S. D. Smith, and M. D. Rausher. 2009. Plant sex and the evolution of plant defenses against herbivores. *Proceedings of the National Academy of Sciences, USA* 106: 18079–18084.

Kruckeberg, A. R. 2004. Geology and Plant Life: The Effects of Landforms and Rock Types on Plants. University of Washington Press, Seattle, Washington, USA.

Kruckeberg, A. R., and D. Rabinowitz. 1985. Biological aspects of endemism in higher plants. *Annual Review of Ecology Evolution and Systematics* 16: 447–479.

Kubitzki, K., J. G. Rohwer, and V. Bittrich. 1993. Flowering Plants, Dicotyledons: Magnoliid, Hamamelid, and Caryophyllid Families. Springer-Verlag, New York, USA.

Laliberté, E., G. Zemunik, and B. L. Turner. 2014. Environmental filtering explains variation in plant diversity along resource gradients. *Science* 345: 1602–1605.

Maddison, W. P., and D. R. Maddison. 2017. Mesquite: a modular system for evolutionary analysis. URL: http://mesquiteproject.org.

Meyer, S. E. 1986. The ecology of gypsophile endemism in the Eastern Mojave Desert. *Ecology* 67: 1303–1313.

Moore, M. J., and R. K. Jansen. 2007. Origins and biogeography of gypsophily in the Chihuahuan Desert plant group *Tiquilia* subg. *Eddya* (Boraginaceae). *Systematic Botany* 32: 392–414.

Moore, M. J., J. F. Mota, N. A. Douglas, H. Flores-Olvera, and H. Ochoterena. 2014. The ecology, assembly, and evolution of gypsophile floras. *In* N. Rajakaruna, R. Boyd, and T. Harris [eds.], Plant Ecology and Evolution in Harsh Environments, 97–128. Nova Science Publishers, Hauppauge, New York, USA.

Moret-Fernández, D., and J. Herrero. 2015. Effect of gypsum content on soil water retention. *Journal of Hydrology (Amsterdam)* 528: 122–126.

Mosse, K. P. M., J. Lee, B. T. Leachman, S. J. Parikh, T. R. Cavagnaro, A. F. Patti, and K. L. Steenwerth. 2013. Irrigation of an established vineyard with winery cleaning agent solution (simulated winery wastewater): vine growth, berry quality, and soil chemistry. *Agricultural Water Management* 123: 93–102.

Munns, R. 2002. Comparative physiology of salt and water stress. *Plant, Cell & Environment* 25: 239–250.

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403: 853–858.

O'Dell, R. E., J. J. James, and J. H. Richards. 2006. Congeneric serpentine and nonserpentine shrubs differ more in leaf Ca:Mg than in tolerance of low N, low P, or heavy metals. *Plant and Soil* 280: 49–64.

Palacio, S., M. Aitkenhead, A. Escudero, G. Montserrat-Martí, M. Maestro, and A. H. J. Robertson. 2014. Gypsophile chemistry unveiled: Fourier transform infrared (FTIR) spectroscopy provides new insight into plant adaptations to gypsum soils. *PLoS One* 9: e107285.

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

Panero, J. L., S. E. Freire, L. Ariza Espinar, B. S. Crozier, G. E. Barboza, and J. J. Cantero. 2014. Resolution of deep nodes yields an improved backbone phylogeny and a new basal lineage to study early evolution of Asteraceae. *Molecular Phylogenetics and Evolution* 80: 43–53.

Parida, A. K., and A. B. Das. 2004. Effects of NaCl stress on nitrogen and phosphorous metabolism in a true mangrove *Bruguiera parviflora* grown under hydroponic culture. *Journal of Plant Physiology* 161: 921–928.

Parsons, R. F. 1976. Gypsophily in plants. *American Midland Naturalist* 96: 1–20.

Powell, A. M., and B. L. Turner. 1977. Aspects of the plant biology of the gypsum outcrops of the Chihuahuan Desert. *In* R. H. Wauer & D. H. Riskind [eds.], Transactions of the symposium on the biological resources of the Chihuahuan Desert region, United States and Mexico, Sul Ross State University, Alpine, Texas, 17–18 October 1974, 315–325. U.S. Department of the Interior, National Park Service Transactions and Proceedings Series, Number 3.

Pritchard, S. G., S. A. Prior, H. H. Rogers, and C. M. Peterson. 2000. Calcium sulfate deposits associated with needle substomatal cavities of container-grown longleaf pine (*Pinus palustris*) seedlings. *International Journal of Plant Sciences* 161: 917–923.

Pueyo, Y., and C. L. Alados. 2007. Abiotic factors determining vegetation patterns in a semi-arid Mediterranean landscape: Different responses on gypsum and non-gypsum substrates. *Journal of Arid Environments* 69: 490–505.

Pueyo, Y., C. L. Alados, M. Maestro, and B. Komac. 2007. Gypsophile vegetation patterns under a range of soil properties induced by topographical position. *Plant Ecology* 189: 301–311.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria.

Revell, L. J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* 3: 217–223.

Römheld, V. 2012. Diagnosis of deficiency and toxicity of nutrients. *In* P. Marschner [ed.], Marschner's Mineral Nutrition of Higher Plants, 299–312. Elsevier, Boston, Massachusetts, USA.

Ruiz, J. M., I. López-Cantarero, R. M. Rivero, and L. Romero. 2003. Sulphur phytoaccumulation in plant species characteristic of gypsiferous soils. *International Journal of Phytoremediation* 5: 203–210.

Salmerón-Sánchez, E., M. I. Martínez-Nieto, F. Martínez-Hernández, J. A. Garrido-Becerra, A. J. Mendoza-Fernández, C. G. de Carrasco, J. J. Ramos-Miras, et al. 2014. Ecology, genetic diversity and phylogeography of the Iberian endemic plant *Jurinea pinnata* (Lag.) DC. (Compositae) on two special edaphic substrates: dolomite and gypsum. *Plant and Soil* 374: 233–250.

Sambatti, J. B. M., and K. J. Rice. 2006. Local adaptation, patterns of selection, and gene flow in the Californian serpentine sunflower (*Helianthus exilis*). *Evolution* 60: 696–710.

Schenk, J. J. 2013. Evolution of limited seed dispersal ability on gypsum islands. *American Journal of Botany* 100: 1811–1822.

Soltis, D. E., S. A. Smith, N. Cellinese, K. J. Wurdack, D. C. Tank, S. F. Brockington, N. F. Refulio-Rodriguez, et al. 2011. Angiosperm phylogeny: 17 genes, 640 taxa. *American Journal of Botany* 98: 704–730.

Storey, R., and W. W. Thomson. 1994. An x-ray microanalysis study of the salt glands and intracellular calcium crystals of *Tamarix. Annals of Botany* 73: 307–313.

Ter Braak, C. J. F., and P. Šmilauer. 2012. Canoco reference manual and user's guide: software for ordination. Microcomputer Power. Ithaca, New York, USA.

Turner, B. L., and A. M. Powell. 1979. Deserts, gypsum, and endemism. *In* J. R. Goodin and D. K. Northington [eds.], Arid land plant resources: Proceedings of the International Arid Lands Conference on Plant Resources, Texas Tech University, 96–116. International Center for Arid and Semi-Arid Land Studies, Lubbock, Texas, USA.

Verheye, W. H., and T. G. Boyadgiev. 1997. Evaluating the land use potential of gypsiferous soils from field pedogenic characteristics. *Soil Use and Management* 13: 97–103.

White, P. J. 2012. Ion uptake mechanisms of individual cells and roots: short-distance transport. *In* P. Marschner [ed.], Marschner's Mineral Nutrition of Higher Plants, 7–47. Elsevier, Boston, Massachusetts, USA.