HIGH NITROGEN AVAILABILITY DOES NOT IMPROVE SALINITY TOLERANCE IN SARCOBATUS VERMICULATUS

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ABSTRACT.—Natural and anthropogenic changes in basin lake levels in the western U.S. expose saline, alkaline substrates that are commonly colonized by shrubs in the Chenopodiaceae. On a chronosequence of recently exposed substrates at Mono Lake, California, *Sarcobatus vermiculatus* has greatest biomass accrual, seed production, seedling establishment, and leaf N at younger sites where soils are extremely saline and alkaline. These field observations and an understanding of the role of N-containing compatible solutes in salinity tolerance of halophytes led to our prediction that Na and N interactions stimulate *Sarcobatus* performance. To test this, we grew *Sarcobatus* juveniles for 2 years in the greenhouse at 4 levels of NaCl (5, 100, 300, and 450 mM) and 3 levels of N (0.04, 0.4, and 8 mM) in a randomized, complete-block design. Contrary to our expectations, high N availability did not induce salt-stimulated growth nor did it increase salinity tolerance in *Sarcobatus*. Increased N nutrition also had no significant effect on leaf cation ratios or selectivity. Plants grown at high salinity had significantly lower leaf K:Na, Ca:Na, and Mg:Na ratios than plants grown at lower salinity. However, plant selectivity for the macronutrient cations remained high, even at 450 mM NaCl. Without such high selectivity, the cation nutrition of *Sarcobatus* would decline to even lower levels, resulting in severe nutrient deficiencies. This study suggests that the ability of *Sarcobatus* to attain high leaf N, rather than an interaction between Na and N, enhances its performance at saline sites. In addition, the ability of *Sarcobatus* to maintain high macronutrient cation selectivity despite high salinity allows its distribution to extend to extremely saline and alkaline substrates in this arid system.

Key words: salinity, nitrogen, selectivity, cation, sodium, calcium, magnesium, halophyte, Sarcobatus vermiculatus, black greasewood.

Climate change–driven fluctuations over many millennia have caused large variation in water levels of basin lakes throughout the western U.S. Species in the Chenopodiaceae have been especially successful at colonizing substrates exposed by repeated recessions, and some apparently have adapted to extremely saline and alkaline substrates in this arid region (Trent et al. 1997 and references therein). In our model system at Mono Lake, California, rapid recession since 1941, caused by transfer of inflows to urban and agricultural uses, has created a chronosequence of dunes similar to chronosequences created over much longer times by natural climate change (Toft and Elliott-Fisk 2002). Shorelines and associated dunes closer to Mono Lake are more saline and alkaline than sites further from the lake (Donovan et al. 1997, Donovan and Richards 2000, Toft and Elliot-Fisk 2002). *Sarcobatus vermiculatus* (Hook.) Torrey (Chenopodiaceae; hereafter, *Sarcobatus*) colonizes the most saline sites in this system, but its recruitment extends throughout the salinity gradient to older, less saline dunes (Donovan and Richards 2000). At younger, highly saline shoreline dune complexes, *Sarcobatus* leaf N can be very high (3.3%–4.4% in June on sites exposed since 1941) despite accumulating Na (up to 14.5%) by late in the growing season (Donovan et al. 1997). Adult shrubs at these sites leaf out 2–4 weeks before plants on older, less saline dunes. In a common garden study, transplanted *Sarcobatus* juveniles produced the most biomass at younger, more saline sites (Donovan and Richards 2000), and natural seed production and seedling establishment were also highest at high leaf-N sites (Fort and Richards 1998). Based on these data, we hypothesized that increased N availability enhances *Sarcobatus* performance at high-salinity sites.

Improved N nutrition generally increases plant salinity tolerance, and this may result from effects on water relations in halophytes. To reduce plant water potential below that of salt-affected soil water potential, halophytes

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accumulate salts (predominantly Na^+, Cl^-, and organic anions) in their vacuoles and low molecular weight N compounds compatible with metabolic activity in their cytoplasm (Greenway and Munns 1980, Bohnert et al. 1995). Compatible solute synthesis may increase N demand in salt-accumulating species. For example, high-N supply increased salt tolerance in the salt marsh perennial *Halimione portulacoides* (Jensen 1985), and the above- and belowground biomass of 2 salt marsh C_4 grasses correlated strongly with N nutrition (Smart and Barko 1980). In addition, under high, but not low, N availability, growth in 2 annual *Atriplex* species was stimulated by salt (Rozema et al. 1983). Similarly, salt tolerance of 2 halophytic, annual *Spergularia* species increased as N availability increased, although their glycophytic congener's growth was not affected (Okusanya and Ungar 1984).

In addition to interacting with N nutrition, salinity reduces K, Ca, and Mg uptake in both glycophytic and halophytic species (Rozema et al. 1983, Lynch and Läuchli 1985, Zhong and Läuchli 1994, Marschner 1995). Under field conditions *Sarcobatus* leaf K, Ca, Mg, and P concentrations decreased as soil salinity increased (Donovan et al. 1997). In many halophytes soil salinity also decreases selectivity for the nutrient cations (where selectivity is the ratio of nutrient:Na in leaves versus nutrient:Na in soil). In contrast, under field conditions *Sarcobatus* Mg selectivity increased 54-fold, maintaining sufficient internal Mg levels as soil salinity increased (Donovan et al. 1997). Similar nutrient:salt interactions are expected under greenhouse conditions. However, since N plays an important role in both nutrient uptake and metabolism, increased N nutrition may have a positive influence on cation uptake and use under saline conditions.

Literature results and our observations suggest N nutrition influences salinity tolerance of *Sarcobatus*. Because of this interaction, we predicted that (1) *Sarcobatus* salt tolerance would be greater under high-N conditions than under low-N conditions, resulting in enhanced growth at low salinity and less reduction in growth at high salinity; (2) under increased salinity *Sarcobatus* would maintain leaf K:Na, Ca:Na, and Mg:Na ratios, and selectivity for the nutrient cations would increase with higher N availability.

MATERIALS AND METHODS

Seedling Collection and Growth

On 29 June 1998 we collected small *Sarcobatus* plants in at least their 2nd season of growth from the northern shore of Mono Lake, CA (38°5'N, 118°58'W; elevation 1957 m). At this site the soil is both saline and highly alkaline. Saturated soil paste electrical conductivity is approximately 5 dS m^{-1}, and pH is 9.9 ± 0.1 (see description of site in Donovan et al. 1997, Donovan and Richards 2000). In these previous studies this site is referred to as "Transverse Dunes" and was exposed between 1940 and 1950 as Mono Lake receded (Toft and Elliott-Fisk 2002).

Juvenile plants were transplanted into a 1:1 mixture of sand and fritted clay in 4-L, 35-cm-deep Tree Pots™ (Stuewe and Sons, Corvallis, OR) and were grown in an unheated University of California–Davis greenhouse with natural light. Plants were assigned randomly to 5 blocks and a factorial combination of 4 NaCl levels (5, 100, 300, and 450 mM) and 3 N levels (0.04, 0.4, and 8 mM). Nitrogen was supplied as both NO_3^- and NH_4^+ in a 7:1 ratio in all treatments. We supplied other nutrients at one-half strength in a modified Hoagland's solution (Epstein 1972), adding supplemental salts (K_3PO_4, $CaCl_2$) to the medium- and low-N treatment solutions. Following leaf senescence in October 1998, the plants were pruned (removing all new shoot material) and allowed to overwinter with all N and NaCl treatments continued.

In January 1999 we raised the watering solutions to pH 10 with K_2CO_3 and KOH to better replicate the alkaline soil conditions at the field site. This modification was made before the plants started to form new shoots. At each twice-weekly watering, the plants were saturated with their respective pH-adjusted NaCl solution and then given 100 mL of the appropriate pH-adjusted nutrient solution. The plants were grown under high-pH conditions from January 1999 until the May 1999 harvest.

Growth Assessment and Shoot Harvest

Prior to harvest, plant growth was assessed as the number of new shoots produced and the length of each new shoot. During 12–14 May

1999 all aboveground plant organs were harvested. We dried all stem material at 60°C and weighed it, and leaf material was lyophilized and weighed.

Leaf-nutrient Determinations

Leaf material from the 5-, 300-, and 450-mM NaCl treatments was ground with an agate mortar and pestle. A subsample of leaf tissue was dry-ashed, suspended in 1 N HCl, and analyzed on an ICP-AES (Thermo Jarrell Ash, Franklin, MA) to determine leaf K, Ca, Mg, P, and Na concentrations. Leaf N concentration was determined by micro-Dumas combustion using a CN analyzer (Carlo Erba, Milan, Italy).

Statistical Analyses

Comparisons between treatments were made using 1- and 2-factor ANOVA models. Due to treatment-specific effects, many plants showed low or no growth, causing most measured variables to be skewed and heteroscedastic. Transformations failed to meet the ANOVA assumptions. Therefore, nonparametric ANOVA on ranks was used to analyze the data, except where otherwise noted (Zar 1999). Any nonsignificant effects (except for block) were removed from our models. When a significant Na*N interaction ($P < 0.05$) was identified, we combined the main effects of Na and N as a single "treatment" variable and ran the model as a 2-way ANOVA with treatment and block as the 2 main effects. Differences between treatments were determined using the Ryan-Einot-Gabriel-Welsch multiple comparisons of means test. All data were analyzed using SAS (SAS Institute 1997).

RESULTS

Growth Measurements

As hypothesized, there was a statistically significant Na*N interaction for new shoot biomass production ($P < 0.0001$). However, the pattern was not as predicted. Although we expected the threshold for salinity tolerance to depend on the level of supplied N, there was no evidence to support this prediction. Instead, the threshold for salinity tolerance was somewhere between 300 mM and 450 mM NaCl for all 3 N treatments, with new shoot biomass decreasing to near-zero values at 450 mM NaCl in all N treatments (Fig. 1A). In the high-N treatment, biomass consistently decreased
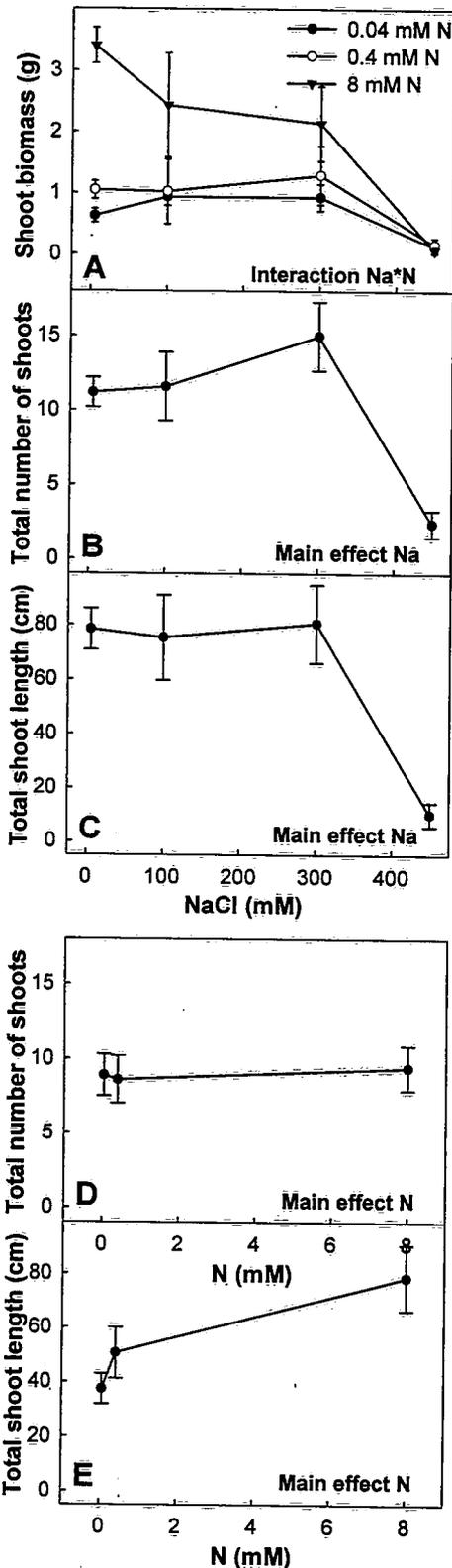
as Na increased, whereas low- and medium-N treated plants produced little shoot biomass in the low- and 2 moderate-salinity treatments before declining to near-zero values at 450 mM NaCl. In all treatments leaf biomass accounted for 67% ± 1.2% (mean ± $s_{\bar{x}}$) of shoot biomass.

Total number of shoots and total shoot length were affected negatively by NaCl ($P < 0.0001$ for both variables). The magnitude of both variables, although similar between 5, 100, and 300 mM, decreased by over 80% at 450 mM NaCl (Figs. 1B,C). Although N treatment had no significant effect on total number of new shoots (Fig. 1D; $P \equiv 0.47$), high-N plants had 35% and 50% greater total shoot length than medium- or low-N plants, respectively (Fig. 1E; $P = 0.003$). Contrary to our expectations and unlike the biomass values, there was not a statistically significant Na*N interaction for either total number of shoots or total shoot length ($P = 0.95$ and $P = 0.24$, respectively).

Leaf Nutrients

Salinity alone had a significant effect on leaf cation concentrations. High salinity decreased leaf K, Ca, and Mg concentrations ($P < 0.0001$; Figs. 2A–C) and increased leaf Na concentrations ($P \equiv 0.0004$; Fig. 2F). Leaf molar ratios K:Na and Ca:Na decreased similarly at all N levels as NaCl increased (salinity main effect: $P < 0.0001$; Fig. 3A). Although the Mg:Na leaf molar ratio decreased more precipitously in the low-N treatment than in the medium- and high-N treatments as salinity increased (Na*N interaction: $P < 0.0001$), leaf Mg:Na decreased to very low values in all N treatments (Fig. 3B). Leaf P did not vary by Na treatment ($P = 0.75$; Fig. 2D), but leaf N was greater in high-N plants ($P = 0.0004$; Fig. 2E).

Selectivity coefficients ($S_{K/Na}$, $S_{Ca/Na}$, $S_{Mg/Na}$, $S_{Ca/Mg}$) were calculated for each treatment, where $S =$ (nutrient/Na)$_{leaf}$ / (nutrient/Na)$_{soil\ solution}$ with all data as molar concentrations. These coefficients integrate plant acquisition of potentially interfering cations with the relative availability of those ions in the soil solution. Since the growing medium in our experiment had very low cation exchange capacity and nutrients were replenished at each watering, the nutrient levels supplied in the watering solutions were used as the measure of soil nutrient availability. Selectivity$_{cation/Na}$ increased as the applied salinity was raised

Figs. 1A–E. Average dry shoot biomass, total number of new shoots, and total shoot length of *Sarcobatus* grown at different levels of N and Na. Data are means ± $s_{\bar{x}}$ (n = 5–30).

from 5 mM to 300 mM NaCl. However, cation selectivity plateaued at 450 mM NaCl, near the plant's physiological limit for salinity tolerance (Fig. 3C). In contrast, N alone affected $S_{Ca/Mg}$, with low-N plants having significantly lower $S_{Ca/Mg}$ than medium- and high-N plants ($S_{Ca/Mg}$ = 0.19, 0.27, and 0.29, respectively; P = 0.002).

DISCUSSION

Salinity and nitrogen stresses influenced growth and development of *Sarcobatus*. Nitrogen-limited plants produced few shoots and those grew very little, regardless of salinity treatment, whereas high-N plants were only growth-limited at higher salinity levels. The inland salt marsh annual *Atriplex triangularis* responded similarly to *Sarcobatus* when raised under varying Na and N availabilities (Drake and Ungar 1989). It is unclear why some halophytes are salt-stimulated under high N and others, such as *Sarcobatus* and *A. triangularis*, are not. It is unlikely this difference is related to life form or phylogenetic relationships. Some perennials and annuals are salt-stimulated under high-N conditions while others are not, and species within the Chenopodiaceae respond differently to Na and N availability. Contrary to our expectations, high N did not increase salt tolerance of *Sarcobatus*, as evidenced by enhanced growth, in this greenhouse study. In fact, at high N, growth strongly decreased as salinity in the watering solution increased. In the field we observe better plant performance at saline, high leaf-N sites compared to non-saline, low leaf-N sites (e.g., Donovan and Richards 2000). The current study's results suggest increased plant performance at saline sites is related more closely to plant N status than to an interaction between Na and N. In support, N-fertilized *Sarcobatus* adults at non-saline sites in the field initiated leaf expansion at the same time as control plants from high-N but saline sites, and end-of-season growth was not significantly different between these 2 treatments (Drenovsky 2002).
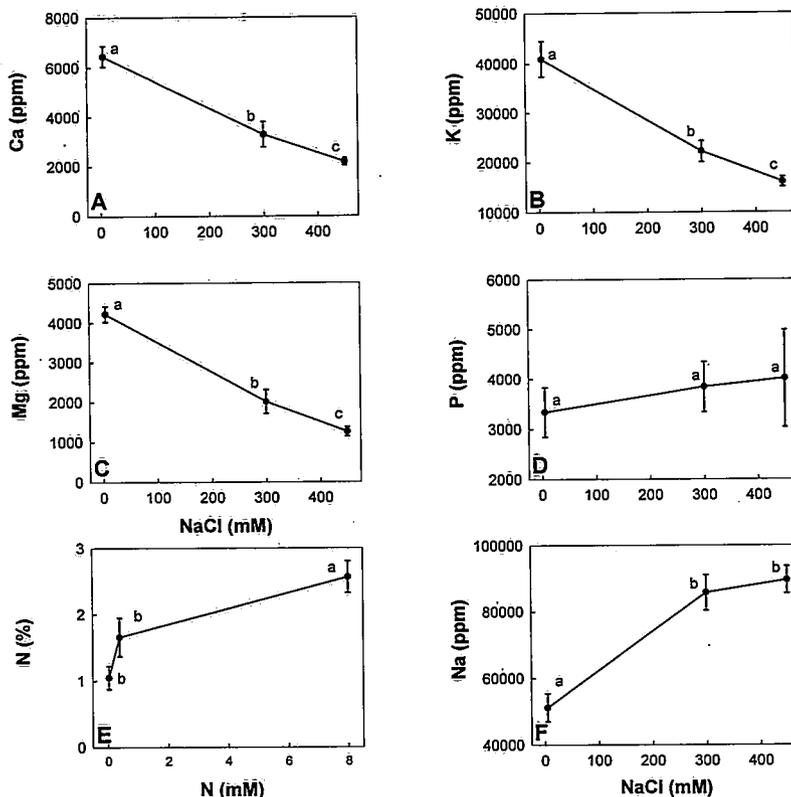
Figs. 2 A–F. Elemental composition of mature *Sarcobatus* leaves from plants grown at different levels of N and Na. Only main effects are shown (see text). Leaf-N values were averaged over all NaCl levels. In all other figures values were averaged over all N levels. There was no significant interaction. Data are means $\pm s_{\bar{x}}$ ($n = 6$–15). Letters indicate significant differences ($P < 0.05$) between treatments.

Similar to salinity's detrimental growth effects in juvenile plants, *Sarcobatus* seed germination was highest at low salinity in 2 studies (Romo and Eddleman 1985; Dodd and Donovan 1999), and increasing salinity slowed *Sarcobatus* seed germination (Romo and Haferkamp 1987). Also consistent with our results, increasing salinity up to 200 mM NaCl at 4 mM N did not reduce *Sarcobatus* seedling relative growth rate for the first 1.5 months of growth (Dodd and Donovan 1999).

Leaf nutrient concentrations reported for this greenhouse study are similar to previous greenhouse and field results for *Sarcobatus*, with increasing salinity depressing leaf cation nutrition (Richards 1994, Donovan et al. 1997). Sodium competes with and/or reduces K, Ca, and Mg uptake in both halophytic and glycophytic plants (Rozema et al. 1983, Lynch and Läuchli 1985, Zhong and Läuchli 1994, Mar-

schner 1995). Contrary to our predictions, higher N availability did not significantly improve plant cation nutrient status.

In this study $S_{K/Na}$, $S_{Ca/Na}$, and $S_{Mg/Na}$ increased as salinity increased, suggesting that root surface ion competition is not responsible for declining plant nutrition. Typically, $S_{K/Na}$ is reduced by salinity (e.g., Zhong and Läuchli 1994, Marschner 1995), whereas in *Sarcobatus* we found $S_{K/Na}$, $S_{Mg/Na}$, and $S_{Ca/Na}$ increased by 9.5-, 11.5-, and 3.5-fold, respectively, as salinity increased from 5 mM NaCl to 300 mM NaCl. Although selectivity remained high at 450 mM NaCl, these levels were insufficient to maintain adequate cation uptake, causing overall cation nutrition to decrease. Contrary to our predictions, additional N did not promote increases in selectivity or ameliorate the effects of salinity on cation nutrition in *Sarcobatus*. Across a salinity gradient in the
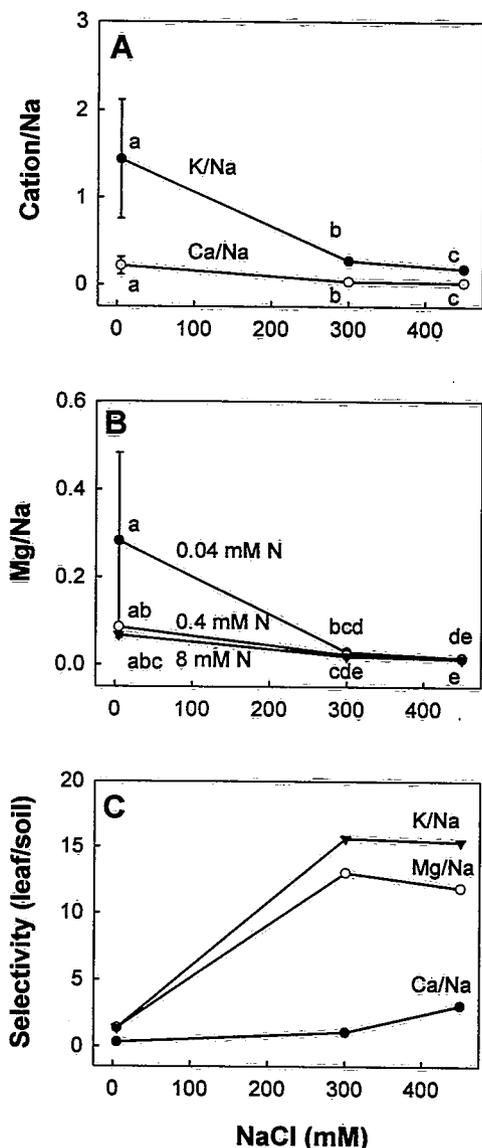
Figs. 3 A–C. Effect of salinity on *Sarcobatus* leaf molar ratios of K:Na and Ca:Na averaged over all N treatments (A) and on Mg:Na at each N level (B). Selectivity coefficients relative to Na for *Sarcobatus* as watering solution salinity increased are also shown, averaged over all N treatments (C). Data are means $\pm s_{\bar{x}}$ ($n = 6$–15). Letters indicate significant differences ($P < 0.05$) between treatments.

field, $S_{K/Na}$ and $S_{Ca/Na}$ in *Sarcobatus* were not affected, whereas $S_{Mg/Na}$ increased 54-fold as salinity increased (Donovan et al. 1997). More detailed studies are required to determine the cause of these discrepancies between field and greenhouse data. Experiments should be con-

ducted in the field and greenhouse to determine possible intra-population genetic differences in selectivity responses. Also, hydroponic studies would allow for more controlled experimental conditions and permit observations of individual plant responses to both low and high salinity. Nevertheless, increased or maintained selectivity is essential for plant nutrition at increasing salinity. The halophyte *Sarcobatus* maintains high selectivity even when nearing its physiological limit for salinity tolerance. Without such high selectivity, its cation nutrition would decline to even lower levels under high salinity, causing severe nutrient deficiencies. Other researchers have related salinity tolerance in yeast and other plants to selectivity maintenance (Alfocea et al. 1993, Reimann and Breckle 1993, Zhong and Läuchli 1994, Zhu 2000). Nitrogen did increase $S_{Ca/Mg}$. Selectivity values from this study were comparable to other literature values for $S_{Ca/Mg}$ (Zarate 2000) and suggest that *Sarcobatus* is able to maintain its Ca:Mg ratio, regardless of applied salinity. Likewise, leaf Ca:Mg was not affected by salinity in *Cucumis melo* plants grown under varying levels of NaCl (Navarro et al. 2000).

The strong plant performance of *Sarcobatus* at saline field sites likely is related to its ability to attain high leaf-N levels and to respond with rapid growth under high-N availability, rather than to interactions between Na and N. In addition, the ability of *Sarcobatus* to sustain high selectivity for plant nutrient cations allows its distribution to extend to the youngest, most saline regions along the chronosequence despite low availability of Mg, K, and/or Ca in alkaline soils.

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