LETTER

Seeking salt: herbivorous prairie insects can be co-limited by macronutrients and sodium

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Abstract

The canonical factors typically thought to determine herbivore community structure often explain only a small fraction of the variation in herbivore abundance and diversity. We tested how macronutrients and relatively understudied micronutrients interacted to influence the structure of insect herbivore (orthopteran) communities. We conducted a factorial fertilisation experiment manipulating macronutrients (N and P, added together) and micronutrients (Ca, Na and K) in large plots (30 × 30 m²) in a Texas coastal prairie. Although no single or combination of micronutrients affected herbivore communities in the absence of additional macronutrients, macronutrients and sodium added together increased herbivore abundance by 60%, richness by 15% and diversity by 20%. These results represent the first large-scale manipulation of single micronutrients and macronutrients in concert, and revealed an herbivore community co-limited by macronutrients and Na. Our work supports an emerging paradigm that Na may be important in limiting herbivore communities.

Keywords

Acrididae, grassland, micronutrient, nutrient limitation, prairie, sodium, tettigonidae.

INTRODUCTION

An understanding of the factors that determine herbivore abundance and diversity is of fundamental ecological interest. It is also necessary for the control of some herbivore species that cause damage (Hulme 1994; Branson et al. 2006) and to support those species that are important purveyors of ecosystem services (McNaughton et al. 1997; Prather et al. 2012). Several canonical factors are typically thought to largely regulate herbivore communities, including top-down consumers (Spiller & Schoener 1990; Dial & Roughgarden 1995) and bottom-up resources, typically characterised as plant biomass (Lawton 1983; McNaughton et al. 1989; Lewinsohn et al. 2005; Wimp et al. 2010), plant diversity (Hutchinson 1959; May 1990; Siemann et al. 1998; Pakeman & Stockan 2014), and plant nutritional quality (White 1984; Fagan et al. 2002; Huberty & Denno 2006; Joern et al. 2012). Although manipulations of any one these factors has usually resulted in some change to herbivore communities, our ability to explain herbivore abundance and diversity is still relatively weak. For example, plant richness only explains ~20% of the variation in herbivore richness across 320 studies (Castagneriol & Jacatel 2012). Similarly, other single factors like top-down consumers, plant productivity, and plant nutritional quality (often measured as the stoichiometry of nitrogen, N, and phosphorus, P) clearly influence herbivore communities, but considerable variation remains unexplained (e.g. Letourneau et al. 2009), even when more than one of these factors are considered together (Haddad et al. 2001; Joern et al. 2012). However, some of the intricacies of these factors have not been fully examined. For instance, most research examining the role of plant nutritional quality has only considered herbivore limitations by either N or P; N is a key component of amino acids (and proteins) while P is a defining element in nucleic acids and phospholipids. Following others (Kaspari & Powers 2016; Bonoan et al. 2018), here we refer to N and P as ‘macronutrients’, and elements that are rarer in living tissues as ‘micronutrients’. In the same way that multi-nutrient studies have shed new insight into limitations on plant diversity and abundance (Harpole et al. 2016), experiments that incorporate multiple nutrients to examine the possibility of multiple resource limitation (co-limitation) or that manipulate micronutrients may provide a better understanding of the relative importance of the factors that structure herbivore communities.

The historical focus on single factor limitation arose through the application of a parsimonious and logical concept: Leibig’s law of the minimum (van der Ploeg & Kirkham 1999). This law posits that one resource limits a population of organisms at any given time, and that the limiting resource is that which has the highest demand to supply ratio (but see Wilder & Eubanks 2010). For example, in the case of single nutrient limitation, the nutrient with the highest ratio of concentration in an organism’s body compared to the concentration of the nutrient in the organism’s food would be assumed to be the single limiting nutrient in that ecosystem. Nutrient limitation of herbivores, in particular, has largely focused on

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single nutrient limitation, with first a focus on nitrogen (Mattson 1980; White 1984), and later on phosphorus (Uraibe & Watanabe 1992; DeMott et al. 1998; Sterner & Schulz 1998), especially in cases when N was abundant (Elser et al. 2001; Makino et al. 2002; Huberty & Denno 2006; Tao & Hunter 2012). This focus on N and P was logical: both have relatively high demand to supply ratios in herbivores with much higher N and P concentrations in herbivore body tissues than plants (Sterner et al. 1992).

Plant nutrients, however, have complementary functions whereby some basic physiological function relies on more than one resource being at critical thresholds simultaneously; too little of any one of these complementary nutritional resources impacts physiological functioning, in particular the maintenance of cell and tissue homeostasis. For this reason, the applicability of Leibig’s law of the minimum to populations and communities has been questioned (e.g. Danger et al. 2008), and now multiple resource limitation is generally recognised to likely be common in plant communities (Harpole et al. 2011, 2017; Fay et al. 2015). Co-limitation may occur via several different pathways (Sperfeld et al. 2016): independent co-limitation occurs when two different nutrients separately enhance populations, and these effects are further enhanced when both nutrients are abundant: simultaneous limitation occurs when neither nutrient positively affects an organism, but together they produce a positive effect when a critical threshold of both is reached; and serial co-limitation in consumer communities, further explaining variation in herbivore community structure (plant biomass, diversity, and macronutrient limitation of plant biomass was alleviated. We derived inputs of Na and Ca, we expected that these two nutrients might become limiting when ambient levels may already be high. However, we hypothesised that these nutrients might become limiting when macronutrient limitation of plant biomass was alleviated. We also predicted that orthopterans that eat either a mixture of grass and forb or woody species (mixed plant feeders), or both plants and animal prey (omnivores) were less likely to be limited by these micronutrients than those that mainly eat grasses (grass feeders) because forbs and animals are much higher in micronutrients than grasses.

Recent research has shown that certain micronutrients may limit consumer communities, further explaining variation in herbivore abundance and diversity (Joern et al. 2012; Kaspari & Powers 2016). Besides macronutrients, around 23 other elements are also crucial to basic physiological functioning of all organisms (Kaspari & Powers 2016), including Cu, K and Na, which act as electrolytes and are crucial for multiple physiological processes. For example, Na, known to limit ants through a hypothesised increase in metabolism (Kaspari et al. 2017), is important for many physiological processes (Chown & Nicolson 2004): regulating gradients of ions across cell membranes (e.g. the sodium pump); generating electrical impulses in excitable cells, like neurons, via sodium channels; maintaining hydrologic homeostasis; and supporting neural and brain development (Snell-Rood et al. 2014). Na may also be important for reproduction in orthopterans, as suggested by changes in nymphal Na concentrations: newly hatched nymphs have the highest Na which steadily decreases over their development (Boswell et al. 2008). However, with a few exceptions (Beanland et al. 2003; Kaspari et al. 2008, 2010, 2014, 2017), studies showing the potential importance of micronutrients in animal communities have been correlative or laboratory experiments, and as such, are open to criticisms that they simply reflect unmeasured variables or do not reflect natural conditions.

We tested the overarching idea that micronutrients would alter the abundance and community structure of herbivores (orthopterans) using a factorial field experiment in a Texas prairie with large plots (30 × 30 m²). We chose to focus on orthopterans because they are relatively large (for insects), ecologically and economically important, generalist herbivores that are relatively easy to identify to genus or species. We first determined how well orthopteran communities at our site were predicted by the canonical variables described above vs. relatively understudied plant and soil micronutrients, using an observational approach. Based on these results, we then chose three micronutrients to manipulate (Ca, K and Na) that have been implicated in structuring insect (Kaspari et al. 2017) and orthopteran (Joern et al. 2012) communities in other studies. We then tested the hypotheses that micronutrients affect the abundance, diversity, and composition of common grassland insect herbivores (orthopterans), and that these effects depend on the availability of macronutrients (i.e. that micronutrients and macronutrients are co-limiting). We measured nutrient effects on plant biomass and chemistry, and also conducted feeding trials to determine if observed effects were due to changes in resource availability or nutritional quality. Because our coastal tallgrass prairie field site likely receives marine-derived inputs of Na and Ca, we expected that these two nutrients alone would not have strong effects on insects as ambient levels may already be high. However, we hypothesised that these nutrients might become limiting when macronutrient limitation of plant biomass was alleviated. We also predicted that orthopterans that eat either a mixture of grass and forb or woody species (mixed plant feeders), or both plants and animal prey (omnivores) were less likely to be limited by these micronutrients than those that mainly eat grasses (grass feeders) because forbs and animals are much higher in micronutrients than grasses.

**MATERIALS AND METHODS**

We worked in a coastal tallgrass prairie in Texas, about 45 kilometers south of Houston and 20 kilometers north of the Gulf Coast at the University of Houston’s Coastal Center (UHCC; 29°23′26.96″ N; 95°1′51.95″ W). UHCC prairies are managed using fire (on average, every 8 years) or mowing (every winter) to prevent woody encroachment and the spread of invasive species. Around 1070 mm of rain falls throughout the year, and the average temperature is around 20.9 °C. This
prairie is dominated by graminoids (early summer – sedges, e.g. *Rhyncospora cauduca* Elliot, later summer – tall grasses, e.g. *Andropogon gerardii* Vitman and *Schizachyrium scoparium* (Michx) Nash). Common forbs are largely in the aster (e.g. *Liatris pycnostachya* Michx and *Helianthus angustifolius* L.) and carrot families (e.g. *Centella erecta* (L. f.) Fernald and *Eryngium yuccafolium* Michx), and several woody plant species are common (e.g. *Myrica cerifera* L., *Baccharis halimifolia* L., and *Rubus riograndis* Bailey; Siemann et al. 2007). Around 16 species of orthopterans (excluding crickets) occur at the site, which can be classified into four feeding guilds based on preliminary feeding trials at our site (Prather and Pennings, unpublished): grass feeders (e.g. *Orphulella speciose* Scudder), forb feeders (e.g. *Hesperotettix speciosus* Scudder), mixed feeders (which feed on both grasses and forbs or woody plants, e.g. *Melanoplus femurrubrum* De Geer), and omnivores (which eat both plants and animal prey, e.g. *Orthelium vulgare* Scudder).

We conducted a field survey at 12 locations in prairies at UHCC in 2011 (detailed methodology in Appendix S1) to determine correlational relationships between orthopteran communities and canonical factors as well as micronutrients. We measured orthopteran abundance and diversity, soil characteristics, plant biomass and diversity along with soil and plant nutrients at each location. We used step-wise multiple regression to determine what factor or combination of factors best predicted herbivore abundance and diversity. The resulting relationships suggested the importance of certain micronutrients (Ca, Na and K), leading to their inclusion as treatments in the subsequent fertilisation experiment.

We conducted a large fertilisation experiment in which we manipulated macronutrients (N and P combined) and three micronutrients (Ca, K and Na, each manipulated individually) beginning in 2015. Although it would have been interesting to include N and P as separate treatments, the resulting experiment would have been twice as large and logistically infeasible, and our interest was primarily in the understudied role of micronutrients. We fertilised large (30 m²) plots using a fully-crossed, factorial design: 2 macronutrient levels (ambient vs. fertilised) × 2 Ca levels (ambient vs. fertilised) × 2 K levels (ambient vs. fertilised) × 2 Na levels (ambient vs. fertilised) × 8 replicates of each treatment combination for a total of 16 treatments and 128 plots. Plots were located in eight blocks that spanned a 2 cm gradient in elevation, with each block containing a single replicate of each treatment. Although 2 cm is not a large elevational difference, it was sufficient to cause differences in soil moisture at our site, which has a flat topography. The large size of the plots acted to minimize artifacts caused by transient arthropods, and allowed us to sample plants and arthropods without markedly affecting the densities of either in the plots.

We added macro- and micronutrient fertilisers to the plots in late winter (in early March before the growing season began after plots were mowed) in 2016 and 2017. We chose forms of nutrients that were (1) simple, so as to not add complex molecules or other non-target nutrients into the plot, (2) relatively water soluble, (3) granular instead of powder for ease of spreading and more precise application, and (4) not overly cost prohibitive (Table 1). Macronutrients were each added at 10 g m⁻² as is common in fertilisation experiments (e.g. Nutrient Network: Borer et al. 2014). Micronutrients were added at levels needed to create soil concentrations of each that were ~30% higher than average ambient levels (~1 standard deviation above the average) found in the top 10 cm of soil our site (Table 1), and all final levels were concentrations that naturally occur at the site. We measured plant biomass via vegetation clipping (0.1 m² strips; 5 per plot), and measured the nutrient content of 4 common plant species on which we have witnessed abundant herbivory (2 graminoids: *Rhyncospora cauduca* and *Schizachyrium scoparium*; 2 forbs: *Liatris pycnostachya* and *Centella erecta*) on at least three plant individuals per plot and nutrient concentrations in the soil using a pooled sample of three soil cores (n = 8 plots per treatment). To assess the response of orthopterans to nutrient treatments, we measured the abundance and diversity of grasshoppers each summer (June) in each plot using sweep net samples (100 sweeps per plot), and identified all orthopterans to species (unless nymphs were too small to identify to species, in which case they were identified to genus). We also conducted feeding trials with seven orthopteran species from three feeding guilds to determine if feeding preferences matched results from our fertilisation experiment (i.e. if orthopterans chose leaves from treatments where orthopteran abundances were high over those with low abundances; methods in Appendix S2).

We report data from the second year of the fertilisation experiment (2017) as effects did not fully manifest until the second year, and so the intermediate results are of less interest. To determine the effect of macro- and micronutrients and their interactions on plant biomass and orthopteran communities (abundance, richness, and diversity calculated as the

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Average concentration (mg kg⁻¹)</th>
<th>Range of concentration (mg kg⁻¹)</th>
<th>Form of fertiliser used in experiment</th>
<th>Chemical formula</th>
<th>% above average ambient</th>
<th>Rate of nutrient applied (g m⁻² year⁻¹)</th>
<th>% element in fertiliser</th>
<th>Amount of fertiliser applied annually (g m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>0.70 (NH₄)</td>
<td>0.6–1.06</td>
<td>Granular MAP + granular urea</td>
<td>NH₄H₂PO₄ + N₂H₄CO</td>
<td>53%</td>
<td>10</td>
<td>46</td>
<td>192 + 36</td>
</tr>
<tr>
<td>P</td>
<td>2.52</td>
<td>0.86–5.01</td>
<td>Granular MAP</td>
<td>NH₄H₂PO₄</td>
<td>63%</td>
<td>10</td>
<td>32</td>
<td>192</td>
</tr>
<tr>
<td>Ca</td>
<td>2597</td>
<td>1530–3380</td>
<td>Granular calcium carbonate</td>
<td>CaCO₃</td>
<td>28%</td>
<td>46.5</td>
<td>41</td>
<td>1134</td>
</tr>
<tr>
<td>K</td>
<td>156</td>
<td>84–238</td>
<td>Granular potassium chloride</td>
<td>KCl</td>
<td>32%</td>
<td>3.1</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>Na</td>
<td>296</td>
<td>200–409</td>
<td>Granular soda ash</td>
<td>Na₂CO₃</td>
<td>33%</td>
<td>6.2</td>
<td>43</td>
<td>144</td>
</tr>
</tbody>
</table>

Table 1  Naturally occurring concentrations of macro- and micronutrients in the top 10 cm of soil at the study site were obtained from a correlative study that was 2 years before starting the fertilisation experiment, and these values were used to calculate the amounts of fertilisers used in the field experiment.
inversely Simpson’s index) and on individual orthopteran species (for those that were abundant enough throughout the treatments to perform statistical analyses), we used GLMs with macronutrients, Ca, K and Na as fixed factors (each with 2 levels – ambient or addition). For orthopteran total abundance and species abundance, we used Poisson log-linear models, and for the other variables (richness and diversity) we used linear models. To determine the effect of nutrient treatments on nutrient chemistry and stoichiometry, we used GLMs with nutrient treatments (macro, Ca, K and Na) and plant species (4 species) as factors for several plant chemistry variables and soil chemistry variables (log Ca, log K, log Na, log N, log P, N : Na and N : P), and performed Bonferroni corrections as these concentrations and ratios were not independent of one another ($P = 0.006$ for significance). We used Cohen’s $d$ (the difference between the means of treatments and controls divided by the pooled standard deviation) to determine the effect sizes of different treatments on orthopteran abundance, richness, and diversity. We examined how orthopteran community structure changed using PCA to simplify the six most common orthopteran species found in our sampling into two major axes, and visualised these differences by plotting the average and standard error of each of the 16 treatment combination for each principle component on a scatterplot.

**RESULTS**

In 2011, prior to starting the fertilisation experiment, total orthopteran abundance at the site was correlated with a combination of macronutrients (soil nitrate concentration) and micronutrients (the concentration of foliar K) (Table S1). The abundance of different feeding guilds were similarly predicted by various combinations of macronutrients (soil and foliar N and P, and foliar C : N : P) and micronutrients (Ca, Na and K), except for grass feeders, which were only related to plant species richness (Table S1). Based on these results, we chose to manipulate the three micronutrients (Ca, Na and K) that were correlated with aspects of the orthopteran community at the site in the fertilisation experiment. We did not consider crickets further, other than herbivorous tree crickets, because our sampling methods were not optimised to collect them and because our focus was on the taxa that eat significant amounts of living plant material.

In the fertilisation experiment, plant biomass was enhanced with any addition of macronutrients ($P < 0.001$), but was not affected by micronutrients and the positive effect of macronutrients did not change when any micronutrient or combination thereof was added (all micronutrients & all interactions: $P > 0.05$; Fig. 1). Soil nutrient concentrations of Na, N and P each increased when their respective nutrient was applied, and there was a trend for Ca and K concentrations to be higher in the soil when each of these nutrients was added (Figs S1 and S2). Nutrient treatment effects on leaf nutrient concentrations were more complicated, and differed by nutrient or ratio examined and plant species (Figs S1 and S2). Of note was that leaf Na was not higher for any plant species when Na was added, but was higher in both forb species when macronutrients were added (Fig. S3). Additionally, foliar N : Na ratios in all plants were lower in the macronutrient plus Na treatments (Fig. 2a), and foliar P was higher in all plants when macronutrients were added (Table S3).

Orthopterans collected from the fertilisation experiment represented 15 species: 6 tettigoniids, 2 gryllids and 7 acridids (Table S4). Overall orthopteran abundance was serially co-limited by macronutrients and Na (Figs 2c and 3a,b). Total numbers increased slightly with macronutrients (GLM macro effect: $< 0.001$), but Na enhanced the positive effect that macronutrients had on orthopteran abundance (Macro $\times$ Na: $P = 0.005$; Table 2, Fig. 3a) in a pattern consistent with serial co-limitation, with over 1.5 times more individuals where both macronutrients and Na were added than when neither was added (Figs 2c and 3). However, no single micronutrient, including Na, or combination of micronutrients affected orthopteran abundance in the absence of macronutrients (GLM Ca, Na, and K effects & their interactions all had $P > 0.05$). Effect sizes for all treatments that including both Na and macronutrients were higher than when macronutrients were added alone or when macronutrients were added with micronutrients other than Na (Fig. 3b).

Despite our prediction that mixed plant-feeders and omnivores were less likely to be limited by Na, the orthopteran species found in the plots were all mixed feeders and omnivores that were indeed co-limited by macronutrients and Na. Within the orthopteran community, different species responded in different ways to the treatments (Appendix S3, Table S2, Fig. S4). Of note was that the most strongly responding species, Scudderia texensis Saussure and Pictet was positively affected by macronutrients, and this effect was enhanced by the addition of Na. Males of this species

![Figure 1](image-url)
were actually only found in plots with both macronutrients and Na.

Sodium and macronutrients added together increased orthopteran richness and diversity, and changed composition. Neither single micronutrients nor macronutrients alone affected orthopteran richness or diversity (Table 2, Fig. 3; GLM for all main effects > 0.05). In fact, species richness and diversity tended to be lower with the addition of each micronutrients alone than in control plots (Fig. 3e,f; 0.67, respectively). However, the addition of macronutrients plus Na increased richness and diversity (Table 2, Fig. 3; GLM macro × Na interaction P = 0.007 and 0.004, respectively). As for abundance, effect sizes for richness and diversity for all treatment combinations including both macronutrients and Na were greater than or equal to effect sizes for macronutrients alone, or any other treatment combinations. Orthopterans preferred to eat leaves from NP × Na treatments: on average, around one-third of total leaf consumption consisted of leaves from the NP × Na treatment, whereas with no preference each leaf would make up an equal amount of total consumption (i.e. 25% for the 4 leaves in each trial; Fig. 3b, all plant and orthopteran species pooled, P = 0.01). Orthopterans did not prefer leaves from other treatments.

PCA reduced the six most abundant species, which represented ~80% of all the orthopterans, to two principle components (PC1 and PC2; Fig. 4) that collectively explained 43% of the variance in the samples. PC1 represented three tettigoniid species (O. vulgare, −0.50; Orchelimum concinnum Scudder, 0.51; and Conocephalus strictus Scudder, 0.54), and one acridid species (Paroxya atlantica Scudder, 0.64). PC2 represented two tettigoniid species (Neoconocephalus sp., 0.59; and S. texensis, 0.67). The macronutrient and micronutrient treatments had different effects on orthopteran community composition. Orthopterans in the control treatment and in treatments with micronutrients alone (open circles) or in the macronutrients alone treatment (filled circle without label) clustered in the middle of the figure. Communities in either the macronutrients plus Na treatment or the NaK treatment fell in the upper right quadrant of the figure. In contrast, treatment combinations including macronutrients plus Ca fell in the upper left quadrant of the figure.

DISCUSSION

With this first manipulation of single micronutrients and macronutrients in concert in plots of large size (30 × 30 m²), we show a grassland herbivore community limited by macronutrients and Na. In a pattern consistent with serial co-limitation, the increases in abundance with these co-limiting nutrients (~60% more individuals) were much greater than for Na alone (which had no significant effect, but tended to suppress abundance), or for macronutrients alone, which slightly increased orthopteran abundance. Here, we discuss the potential reasons for Na having a strong effect, propose mechanisms for co-limitation, and put our results in perspective in light of biogeographic and anthropogenically-altered distributions of these nutrients.

Anecdotally, natural historians have long known that Na is important to animals. Mammals and birds sometimes use sodium rich salt licks or wood with little nutritional value besides high concentrations of Na (gorillas – Rothman et al. 2006; parrots – Lee et al. 2010). Lepidopterans often puddle at salty soil, carcasses, tears and perspiration (Arms et al. 2007). Salty human urine has been used as insect bait, both to catch insect pests (Martín & Piñeiro 2004) and in ecological studies (e.g. Gordon 2012). Sodium is known to be crucial for the intact functioning of many physiological processes, such as the sodium pump, cell signalling, the maintenance of hydrologic homeostasis, and neural and brain development (Chown & Nicolson 2004; Snell-Rood et al. 2014).

Despite this extensive anecdotal information and the known importance of Na in animal physiology, it was only recently that the first terrestrial small-scale (1 m²) field studies were done experimentally manipulating Na (Kaspari et al. 2014).
These studies showed that Na played an important role in determining the structure of termites (Kaspari et al. 2014), ants (Clay et al. 2014), and more recently, prairie insect communities (Kaspari et al. 2017). In the most recent study with prairie insect communities, above-ground insect abundance increased by over 50% when macronutrients plus Na were added (Kaspari et al. 2017), which is qualitatively very similar to how orthopterans responded in our larger-scale study.

Although our 2011 correlational sampling indicated that micronutrients were important, it did not predict orthopteran responses to particular treatments in the manipulative experiment, especially the importance of Na. In the observational study, although soil nitrate was correlated with total orthopteran abundance, Na was not. Na was correlated with the abundance of forb feeders (not collected in the fertilisation experiment) and crickets (less than 0.01% of the sampled individuals in the experiment). However, the inconsistencies in these results might point out the unreliability of correlations between soil and foliar nutrient concentrations and the abundance of consumers that might arise because of unmeasured variables, and also indicate the importance of testing putative relationships with manipulative experiments. Because both the survey and manipulative experiment were conducted in field plots rather than enclosures, we do not know whether positive responses of organisms in some treatments reflect rates of movement among plots, with organisms moving to or remaining in preferred plots, or reflect increased population growth or survival rates. Understanding whether these differences are a result of orthopteran movement or changes to reproductive rates requires further experiments that we are currently attempting. Moreover, grasshopper densities were lower at our site than at other grasslands (e.g. Batáry et al. 2007; O’Neill et al. 2010), perhaps indicating that additional factors beyond those we studied limit grasshopper densities at our site.

Figure 3 Orthopteran community measurements were all highest in treatments with both macronutrients and Na added: (a) orthopteran abundance ($P = 0.005$), (b) effect size on abundance, (c) richness ($P = 0.007$), (d) effect size on richness, (e) diversity ($P = 0.004$), and (f) effect size on diversity. Each bar (averages ± 1 SE) and point (Cohen’s $d$) represents one micronutrient treatment (labelled on $x$-axis) and macronutrient treatment (white bars/points represent ambient macronutrients; grey bars/points represent added macronutrients). These data suggest that this orthopteran community is serially co-limited by macronutrients and Na.
Our results are consistent with a serial co-limitation pathway: orthopteran abundance responded positively but weakly to macronutrients, did not respond to sodium addition, and strongly increased when both macronutrients and sodium were added (Table 2, Figs 2c and 3). Serial limitation such as this has previously been indicated in ‘limitation cascades’ whereby ample amounts of one nutrient could cause others to become limiting. Sodium has been hypothesised to be crucial in limitation cascades in herbivores (Kaspari & Powers 2016) because Na concentrations that are 100–1000 times higher in herbivore bodies than plant tissue, and insect herbivores have no stable storage mechanism for Na.

This serial co-limitation may be occurring via direct or indirect mechanisms, as has been shown for other orthopteran species (tettigonids – Mormon crickets, Simpson et al. 2006). We hypothesise that co-limitation is mainly occurring via direct mechanisms, not indirect changes in plant composition. Macronutrients increased the amount of food available to these generalist herbivores, but increased orthopteran abundance was not due solely to a direct increase in plant biomass (which only responded to macronutrients), but instead likely occurred through some change in plant palatability. Although we need to conduct experiments with artificial diets to confirm this, the most obvious trend in the data is that the N : Na ratio decreased in treatments with added Na (Fig. 2c), so Na was likely more bioavailable. Additionally, foliar P was higher in these leaves. An alternative hypothesis is that plant composition changed; however, our feeding experiments where plant composition was held constant showed that a change in plant quality alone is likely sufficient to explain our results (however, we are currently doing experiments that explore this potential mechanism). Additionally, differences in the orthopteran community were not seen until the second year of fertilisation, likely because...
treatments did not induce wide-spread changes in plant stoichiometry until the second year.

One notable feature of the orthopteran community at this site is that it is dominated by tettigoniids (katydids made up 80% of the individuals and 7 out of 15 species) that are often omnivorous, eating not only leaves, but seeds and pollen as well as small animals. Accordingly, this increase in plant biomass likely also resulted in increases in seed or flower abundance, or increases in prey availability for omnivorous species, and these increases in resource availability could have increased performance of the tettigoniid members of the community.

The combination of macronutrients and Na altered leaf chemistry in ways that could affect herbivore populations directly through altered physiological functioning, as is suggested by our leaf chemistry and feeding trial data (Fig. 2b,c). Na has been suggested to be important for reproduction in other orthopterans (Boswell et al. 2008). *Scudderia texensis*, the species that responded mostly strongly to macronutrient plus Na treatments uses nuptial gifts in its mating system. Although females were spread across treatments, male *S. texensis* were collected only in macronutrient plus Na plots, suggesting that this treatment was especially attractive to males. In most lepidopteran species, it is males that most commonly exhibit puddling behaviour, especially those that offer nuptial gifts (Sculley & Boggs 1996; Molleman et al. 2005). Male diet also affects egg nutrients: females that receive these nuptial gifts from males that have consumed Na-rich diets lay eggs that are higher in sodium (Smedley & Eisner 1996). Egg nutrients may benefit larvae that have little opportunity for sodium acquisition in their diets. In katydids, better nutritional condition in males (determined by feeding them high protein diets) increases the size of nuptial gifts (Jia et al. 2000), but nothing is known about the importance of Na in nuptial gifts in katydids. If similar relationships between sodium and nuptial gifts exist in katydids as in lepidoptera, this suggests that future studies of Na limitation should look for differential effects in males, which may seek out Na in order to enhance the quality of nuptial gifts or other reasons.

The biogeography of nutrients influences whether organisms are limited by a particular nutrient. For instance, ant communities have been shown to be limited by Na inland but not in coastal areas where soil Na concentrations are high from marine-derived sources (Kaspari et al. 2008). *Scudderia texensis*, the species that responded mostly strongly to macronutrient plus Na treatments uses nuptial gifts in its mating system. Although females were spread across treatments, male *S. texensis* were collected only in macronutrient plus Na plots, suggesting that this treatment was especially attractive to males. In most lepidopteran species, it is males that most commonly exhibit puddling behaviour, especially those that offer nuptial gifts (Sculley & Boggs 1996; Molleman et al. 2005). Male diet also affects egg nutrients: females that receive these nuptial gifts from males that have consumed Na-rich diets lay eggs that are higher in sodium (Smedley & Eisner 1996). Egg nutrients may benefit larvae that have little opportunity for sodium acquisition in their diets. In katydids, better nutritional condition in males (determined by feeding them high protein diets) increases the size of nuptial gifts (Jia et al. 2000), but nothing is known about the importance of Na in nuptial gifts in katydids. If similar relationships between sodium and nuptial gifts exist in katydids as in lepidoptera, this suggests that future studies of Na limitation should look for differential effects in males, which may seek out Na in order to enhance the quality of nuptial gifts or other reasons.

The biogeography of nutrients influences whether organisms are limited by a particular nutrient. For instance, ant communities have been shown to be limited by Na inland but not in coastal areas where soil Na concentrations are high from marine-derived sources (Kaspari et al. 2008). Additionally, the assumed high Na inputs at our coastal tallgrass prairie site may explain the lack of a direct effect of Na. If Na limitation of orthopterans occurs on a wider geographic scale, these results may have important implications in light of the wide-ranging anthropogenic effects on global Na distribution. The use of salt for de-icing purposes has tripled since the early 1990s, which has consequently increased sodium availability in a variety of terrestrial habitats (Findlay & Kelly 2011), with potentially unintended consequences for grassland herbivore communities. Additionally, agricultural soils are not only often higher in N and P availability from fertiliser additions, but also higher in Na: irrigation of agricultural areas and rangelands often uses water with more Na than rainwater, leading to high salinity in at least 20% of irrigated lands globally (Ghassemi et al. 1995). Therefore, current agricultural management practices may unintentionally be benefiting orthopteran herbivores that cost ~$1.25 billion in agricultural plant production annually in the US alone (Branson et al. 2006).

CONCLUSIONS

The canonical explanations for patterns in herbivore community structure have largely ignored the possible role of micronutrients. We provide further evidence for an alternative possibility: certain herbivore communities are co-limited by macronutrients and micronutrients, especially Na. Given our still rather limited ability to predict the structure of herbivore communities, a better understanding of possible herbivore co-limitation by macro- and micronutrients is necessary. This understanding is especially important with human disruption of ambient biogeography of almost all the macro- and micronutrients that are crucial to life. This study highlights that altered distributions and availability of macronutrients and Na could potentially have large, long-term effects on insect herbivore communities. Considering these factors in future research may lead to a better understanding of herbivore community structure and have wide-ranging implications for herbivore pest management and the promotion of beneficial herbivore groups.

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AUTHORSHIP

CP and SP conceived of this project; CP, AL, JC, KG, and RR collected the data; CP performed statistical analyses, and wrote the first draft, and all authors contributed substantially to revisions.

REFERENCES


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