Reports

Sodium co-limits and catalyzes macronutrients in a prairie food web

MICHAEL KASPARI,[1,5] KARL A. ROEDER (iD),[1] BRITTANY BENSON,[1] MICHAEL D. WEISER[1] AND NATHAN J. SANDERS[2,3,4]

[1]*Graduate Program in EEB, Department of Biology, University of Oklahoma, Norman, Oklahoma 73019 USA*
[2]*Rocky Mountain Biological Laboratory, PO Box 519, Crested Butte, Colorado 81224 USA*
[3]*Center for Macroecology, Evolution, and Climate, Natural History Museum,*
University of Copenhagen, Copenhagen, 2100 Denmark

Abstract. Nitrogen and phosphorus frequently limit terrestrial plant production, but have a mixed record in regulating the abundance of terrestrial invertebrates. We contrasted four ways that Na could interact with an NP fertilizer to shape the plants and invertebrates of an inland prairie. We applied NP and Na to m^2 plots in a factorial design. Aboveground invertebrate abundance was independently co-limited by NaCl and NP, but with +NP plots supporting more individuals. We suggest the disparity arises because NP enhanced plant height by 35% (1 SD) over controls, providing both food and habitat, whereas NaCl provides only food. Belowground invertebrates showed evidence of serial co-limitation, where NaCl additions alone were ineffectual, but catalyzed access to NP. This suggests the increased belowground food availability in NP plots increased Na demand. Na and NP supply rates vary with climate, land use, and with inputs like urine. The co-limitation and catalysis of N and P by Na thus has the potential for predicting patterns of abundance and diversity across spatial scales.

Key words: grassland food webs; nitrogen; nutrient limitation; phosphorus; sodium.

INTRODUCTION

Experiments and comparative studies repeatedly indicate the importance of N and P availability in predicting terrestrial plant productivity (LeBauer and Treseder 2008, Cleveland et al. 2011), chemistry (Kaspari et al. 2008, Han et al. 2011) and diversity (Harpole et al. 2011, 2016, Fay et al. 2015). The same nutrients that constrain plant growth and diversity have the potential to constrain the abundance and diversity of consumers via increases in plant quantity (e.g., NPP, McNaughton et al. 1989, Siemann 1998, Kaspari 2001) or the nutrient density of plant tissues (Stiling and Moon 2005). However, invertebrate responses to gradients of biogeochemistry–natural or experimental–typically yield weaker and more variable responses compared to those of plants (Siemann 1998, Ritchie 2000, Kaspari et al. 2008, Kaspari and Yanoviak 2009, Joern et al. 2012, *unpublished manuscript*).

Evidence mounts that one element, sodium (Na), limits plant consumers, but not their food. If so, variation in Na availability would help account for the range of responses plant consumers show to NP fertilization. Sodium is a trace element in most plant tissue (Marschner 1995, Taiz and Zeiger 1998). Plant consumers with Na tissue levels 100–1000 fold higher than plants (Cromack et al. 1977, Frausto da Silva and Williams 2001) must find and accumulate Na from an Na-poor diet. Moreover, Na is metabolically expensive (ca. 1/3 of an animal cells' resting metabolism is invested in its Na-K pumps, Frausto da Silva and Williams 2001) and lacks a stable storage form in the body so that excretion must be followed by consumption or deviation from the Na set point will cause pathology (NRC 2005). For plant consumers, the costs of feeding and protecting their Na set point thus potentially constrains their abundance and vigor.

We suggest four likely scenarios for the interactions of Na with macronutrients like NP (Harpole et al. 2011, Fay et al. 2015, Sperfeld et al. 2015, Kaspari and Powers 2016; Fig. 1). In classical Single (or Liebig) Limitation only one nutrient—the one with maximum demand relative to supply–limits abundance. A second scenario is that Na is ineffectual by itself but catalyzes access to additional NP (Serial Co-limitation). A third scenario is that Na and NP both enhance fitness when supplemented, but by different mechanisms (Independent Co-limitation). Finally, Simultaneous Co-limitation results if Na and NP
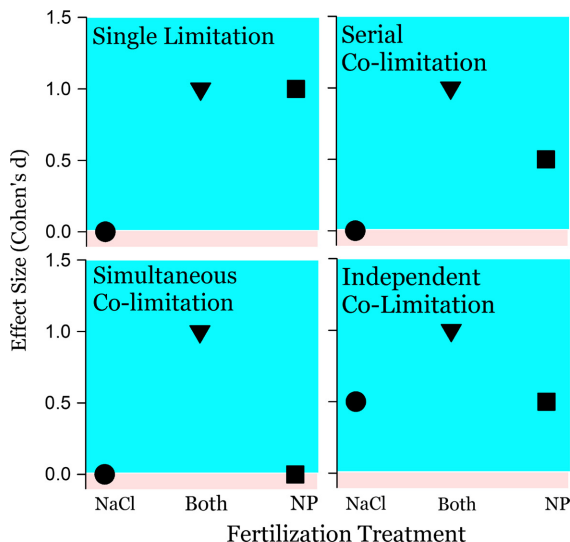
Fig. 1. When two fertilizers, NaCl and NP, are applied in a factorial design, they can interact in at least four ways. In single (or Liebig) limitation, only NP increases abundance; in Serial Co-limitation (or catalysis), NaCl alone has no effect but increases the efficacy of NP; in Simultaneous Co-limitation both nutrients are ineffective except in the other's presence; and in Independent Co-limitation, both NaCl and NP promote abundance by different mechanisms.

contribute in different ways to the same mechanism that enhances fitness. Support for any of the four patterns in Fig. 1 makes concrete suggestions as to the physiological mechanisms that produce them.

Here we report on a factorial fertilization of an inland prairie with NaCl and NP, contrasting four different hypotheses of nutrient limitation (Fig. 1). We test how these fertilizers shape plant quantity and quality, and how they ramify to shape the abundance of above- and below-ground invertebrate assemblages.

Materials and Methods

We set up 60 m^2 plots, arrayed in 10 rows of 6, 5 m apart on the Sam Noble Museum prairie (35.194° N, 97.449° W) in Norman Oklahoma. The prairie is ca. 7.7 ha of sandy loam dominated by bunch grasses *Schizachyrium scoparium*, *Sorghastrum nutans*, and *Andropogon gerardii*. The prairie is mowed once a year in November but otherwise undisturbed. Key dates in the experiment were its start on 21 May 2015, sampling of the above and belowground invertebrates on 16 July, and sampling of soils and plants on 24 August 2015.

Each plot received one of four treatments in a stratified random design. The treatments were a factorial application of Control: 0.5 L of water every 14 d; +NaCl: 0.5 L of 1% NaCl solution by weight every 14 d (ca. the concentration found in ungulate urine, Steinauer and Collins 1995); +NP: a one time application on 21 May of Nitrogen (10 g elemental mass as Urea) and Phosphorus (10 g

elemental mass as supertriplephosphate) matching that of the NutNet experiment (Borer et al. 2014), followed by 0.5 L of water every 14 days; +Both: the +NP and +NaCl treatment on Day 1, followed by +NaCl for the rest of the experiment.

Sampling invertebrates

We sampled above and belowground invertebrates from each plot on 16 July, near the peak of the growing season. Aboveground invertebrates were sampled by passing the nozzle of a modified leaf blower (Stewart and Wright 1995) through the vegetation for 30 s, transferring the catch to a plastic bag for later processing in the lab. We also sampled belowground invertebrates with a 10-cm diameter, 5-cm deep soil core, and used salt flotation (see Appendix S1; Moldenke 1994) to separate invertebrates from the soil. We collected a diversity of invertebrate groups (Appendix S1: Table S1), quantified the total abundance of individuals with the aid of a dissecting microscope, and stored samples in 95% EtOH where they are part of MK's collections at the University of Oklahoma.

Sampling plant and soil

We quantified plant and soil responses to fertilization on 24 August. Maximum plant height was measured twice with a meter stick from the four corners and center of the plot, and averaged. Three investigators worked together to tally the number of plant and forb species in each plot. A 20 × 20 cm quadrat placed in the center of the plot was clipped to the soil surface, and then weighed fresh and after drying at 60°C to constant mass. Four 2-cm soil cores were taken down to 5 cm and homogenized. Both soil and plant tissue was tested for N using a Lachat Flow injection autoanalyzer, P using Mehlich 3 and a Spectro ICP Spectrometer, and total Na using the Spectro ICP by the OSU soil, water and forage lab (2012).

Statistics

To describe the responses of soils, plants, and invertebrates to our factorial fertilization, we used a mix of effect sizes, general linearized models, and post hoc comparisons. As we measured a variety of response variables with a variety of units, we sought to standardize our description of their responses using Cohen's d effect size (Cohen 1988). Cohen's d standardized the direction and magnitude of each of the three fertilizer treatments relative to the control by dividing the means of (Treatment-Control) by the pooled standard deviation of each. The units of d are Standard Deviations of difference and, *sensu* Cohen, we define a large effect as $d > |1.0|$, where the mean effect of the fertilizer treatment is at the 84th percentile of the control.

Next we used inferential statistics to assign *P* values to the two fertilizer's main effects and their interaction. We

used a factorial generalized linear model (GLIMMIX in SAS V 9.2, SAS 2009) matched to the distribution (e.g., normal, log-normal, and gamma) of the dataset. The four limitation scenarios each predict a different combination of how treatment responses compare to control and to each other (Fig. 1). We thus used the PDIFF option, with a threshold of $P < 0.05$, to compare least square means of treatment versus control, and among treatments. We further explored relationships between plants and invertebrate abundance using stepwise regression, identifying the best model using Mallow's C(p) criteria.

RESULTS

Soil N did not vary with NaCl or NP fertilization (GLM P's = 0.76, 0.95) though it trended ca. 0.5 SD lower in fertilized plots (Fig. 2). Background levels of soil P were 15 times higher than N (Appendix S1: Table S1),
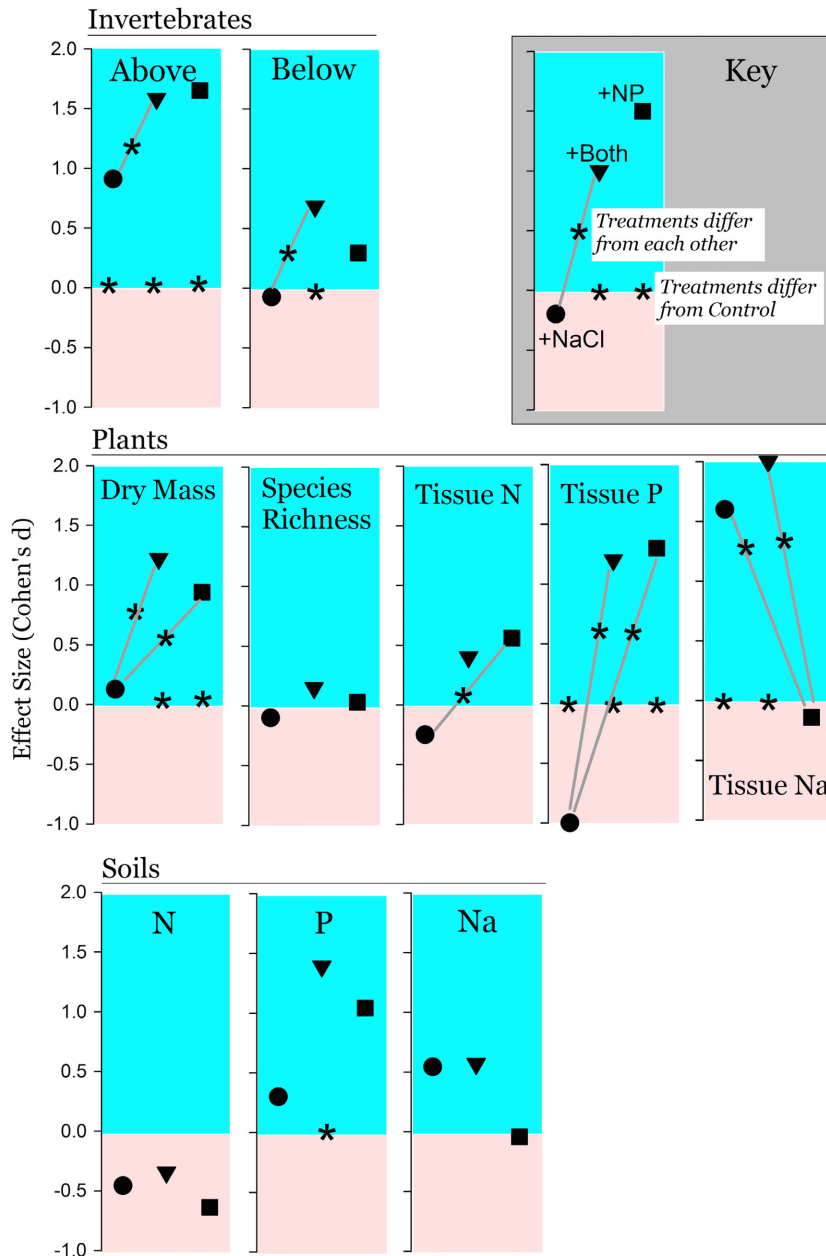

FIG. 2. Responses of soil, plants, and invertebrates to factorial fertilization with NaCl and NP, measured as effect sizes (in standard deviations from control). LS Mean differences at $P < 0.05$ indicated by asterisk in two ways: when treatments differ from control, asterisks are on the baseline; when they differ from each other, asterisks are associated with a thin grey line connecting the two treatments.

and were further enhanced with NP fertilization (GLM $P = 0.02$) by 1SD or more (Fig. 2). Soil Na increased by 0.5 SD with NaCl fertilization (GLM $P = 0.02$). In no cases did the fertilization interact to shape soil nutrient stocks (GLM interaction P's > 0.19). In sum, by August, a May application of NP fertilizers yielded 1 SD more soil P, but no more N; biweekly fertilization with NaCl solution enhanced soil Na by 0.5 SD, but had no effect on N or P.

NP and NaCl fertilization increased those nutrients in plant tissue

Plant height and biomass were weakly correlated across the 60 plots ($r = 0.36$). NP fertilization increased plant height and biomass by ca. 1 SD (Appendix S1: Table S2; Fig. 2), and plots fertilized with NP averaged 35% taller plants and 80% more biomass (GLM NP effect P's = 0.0001). NP fertilization increased N in plant tissue ca. 6% and 0.5 SD (GLM $P = 0.02$) and increased P in plant tissue by ca. 70% and 1.2 SD (GLM $P < 0.0001$). NP fertilization had no discernible effect on plant diversity in the m² plots (Fig. 2). NaCl fertilization increased Na in plant tissue 37-fold (1.8 SD, GLM $P = 0.0001$), but otherwise had no effect, direct or indirect, on plant height, biomass, N, P or richness (Appendix S1: Table S2; Fig. 2).

Aboveground and belowground invertebrates both responded, but differently

Both above- and belowground invertebrate abundance ranged 2–3 o.m. across the 60 plots. However, their abundances across this experiment were uncorrelated (Pearson $r = 0.21$, $P = 0.10$; Appendix S1: Fig. S1). For aboveground abundance, Na, NP, and Both treatments generated higher aboveground abundance than controls (Fig. 2; Appendix S1: Table S2, d's = 0.9–1.7 SD) in a pattern consistent with Independent Co-limitation in which NP was a more limiting nutrient. The biweekly NaCl fertilization alone increased aboveground abundance by 80% and 0.9 SD over controls (GLM NaCl effect $P = 0.02$). Plots receiving the one-time May fertilization yielded abundances ca. 2–3 times and 1.6 SDs higher than controls (GLM NP effect: $P < 0.0001$).

In contrast, belowground abundance responded only to NP, and then with effect sizes one-third of NP effects aboveground (Fig. 2). Plots receiving +NaCl alone were nearly identical to Controls (−0.1 SD). However, consistent with Serial (or Catalytic) Co-limitation, when NP was added with NaCl, the resulting average densities doubled that of controls ($d = 0.7$ SD), were significantly higher than +NaCl plots (Fig. 2), and had 50% more individuals than plots in which NP was added alone.

To explore the role that the six plant responses may have had in driving invertebrate responses, we used stepwise regression with Mallow's C(p) criteria (Appendix S1: Table S3). Above and belowground models varied in both plant drivers and efficacy. The best model for aboveground abundance had it increasing with plant height, Na, mass and N, and accounted for 53% of the observed variance. In contrast, belowground abundance increased with plant height and plant P, and decreased with plant N and plant richness, in a model accounted for only 19% of variation across the 60 plots. In both cases, plant height was the single best predictor of abundance, accounting individually for 35% and 9% of the variation, respectively.

DISCUSSION

Gradients of the macronutrients N and P in the soil often dictate the distribution of terrestrial plant biomass, chemical composition, and diversity (Ritchie 2000, Harpole et al. 2011, Fay et al. 2015). Nitrogen, in particular, has long been a suspected driver of terrestrial herbivore populations (Rossi and Strong 1991, White 1993, Huberty and Denno 2006) because it tends to increase the quantity and quality of leaf tissues. In this inland prairie, a mix of N and P enhanced plant biomass and volume, as well as N and P content. But we also show that the mineral Na has a potentially large role to play in shaping the abundance of terrestrial grassland invertebrates. For aboveground invertebrates NaCl independently increased abundance ca. 1 SD over control plots consistent with Independent Co-limitation. Belowground Na played a different role, neutral when supplemented by itself, but boosting the effect of NP in a fashion consistent with Serial Co-limitation, or catalysis. In both systems, NaCl combined with NP to help make sense of the 2–3 o.m. patchiness found in herbivores and detritivores.

While gradients of N, P, and Na occur at multiple spatial scales (Walker and Syers 1976, Schlesinger 1997), the dosage and grain of this experiment best simulates a widespread form of fertilization: excretion by large herbivores (McNaughton et al. 1997, Steinauer and Collins 2001, Clay et al. 2014). Urine is a common and pervasive way that N and Na are added together to terrestrial food webs (ungulate urine is 12:1 N:Na, Steinauer and Collins 1995). Our results are consistent with experiments on two prairies that showed urine enhancing plant abundance and grazing pressure (Steinauer and Collins 1995, 2001) and road salt enhancing the development of two species of butterflies (Snell-Rood et al. 2014). Here we extend those results from bison and butterflies to communities of invertebrates.

In the aboveground invertebrates there was no obvious synergy between NaCl and NP additions, with both increasing abundance in a pattern consistent with Independent Co-limitation. We suggest a simple mechanism for the disparity of effect sizes: NP plots enhanced abundance 2–3 times more than Na because it enhanced both food quality and quantity. Like the +NaCl plots, +NP plots provided essential nutrients, in this case by enhancing N and P in plant tissue. Unlike the +NaCl plots, the increased plant biomass and height on NP plots provided both more food and more habitat volume. If

our working hypothesis is valid, then adding a clipping treatment that eliminates the 35% increase in height and 80% increase in plant biomass should reduce the disparity of Na and NP effects. Likewise, repeating our experiment in a system grazed by large herbivores should attenuate the arthropod increase on +Both plots if grazers remove plant biomass on +NaCl plots.

In contrast—and consistent with the pattern of Serial Co-Limitation, or Catalysis–NaCl alone never increased the abundance of belowground invertebrates but did increase it above control levels when it was added with NP. This would in turn suggest that the Na supplies in this prairie soil are adequate to support the ambient abundance of invertebrates, but that the increased plant production brought about by NP taxes the ability of invertebrates to respond without a Na subsidy. If true, we would predict that (1) NP did in fact enhance belowground production; and (2) adding alternate forms of soil carbon like cellulose or glucose would increase the likelihood of Na limitation of the belowground fauna.

This experiment, while showing how NaCl and N and/ or P ramify through a prairie food web, also highlights our uncertainty as to mechanism. For example, the role of population interactions among the plants, plant consumers, and their predators (Denno et al. 1995, Schmitz 2010) leaves many of our conclusions about nutrient limitation as working hypotheses. As we have outlined, future experiments are necessary to describe the processes underlying the varying patterns of nutrient limitation in Fig. 1. Understanding these dynamics (and their spatial and temporal scale dependence) are important not just to understanding the prairie populations, but to the processes they regulate, from decomposition (Kaspari et al. 2009, Clay et al. 2014) to herbivory (Steinauer and Collins 1995, 2001).

LITERATURE CITED

Borer, E. T., W. S. Harpole, P. B. Adler, E. M. Lind, J. L. Orrock, E. W. Seabloom, and M. D. Smith. 2014. Finding generality in ecology: a model for globally distributed experiments. Methods in Ecology and Evolution 5:65–73.

Clay, N. A., D. A. Donoso, and M. Kaspari. 2014. Urine as an important source of sodium increases decomposition in an inland but not coastal tropical forest. Oecologia 2014: 1–9.

Cleveland, C. C., A. R. Townsend, P. Taylor, S. Alvarez-Clare, M. Bustamante, G. Chuyong, S. Z. Dobrowski, P. Grierson, K. E. Harms, and B. Z. Houlton. 2011. Relationships among net primary productivity, nutrients and climate in tropical rain forest: a pan-tropical analysis. Ecology Letters 14: 939–947.

Cohen, J. 1988. Statistical power analysis for the behavioral sciences. Second edition. L. Erlbaum, Hillsdale, New Jersey, USA.

Cromack, F. J., P. Sollins, R. L. Todd, D. A. J. Crossley, W. M. Fender, R. Fogel, and A. W. Todd. 1977. Soil microorganism-arthropod interactions: fungi as major calcium and sodium sources. Pages 78–84 in W. J. Mattson, editor. The role of arthropods in forest ecosystems. Springer Verlag, New York, New York, USA.

Denno, R. F., M. S. McClure, and J. R. Ott. 1995. Interspecific interactions in phytophagous insects: competition reexamined and resurrected. Annual Review of Entomology 40:297–331.

Fay, P. A., et al. 2015. Grassland productivity limited by multiple nutrients. Nature Plants 1:15080.

Frausto da Silva, J. J. R., and R. J. P. Williams. 2001. The biological chemistry of the elements: the inorganic chemistry of life. Second edition. Oxford University Press, Oxford, UK.

Han, W. X., J. Y. Fang, P. B. Reich, F. Ian Woodward, and Z. H. Wang. 2011. Biogeography and variability of eleven mineral elements in plant leaves across gradients of climate, soil and plant functional type in China. Ecology Letters 14:788–796.

Harpole, W. S., et al. 2011. Nutrient co-limitation of primary producer communities. Ecology Letters 14:852–862.

Harpole, W. S., L. L. Sullivan, E. M. Lind, J. Firn, P. B. Adler, E. T. Borer, J. Chase, P. A. Fay, Y. Hautier, and H. Hillebrand. 2016. Addition of multiple limiting resources reduces grassland diversity. Nature 537:93–96.

Huberty, A. F., and R. F. Denno. 2006. Consequences of nitrogen and phosphorus limitation for the performance of two planthoppers with divergent life-history strategies. Oecologia 149:444–455.

Joern, A., T. Provin, and S. T. Behmer. 2012. Not just the usual suspects: insect herbivore populations and communities are associated with multiple plant nutrients. Ecology 93:1002–1015.

Kaspari, M. 2001. Taxonomic level, trophic biology, and the regulation of local abundance. Global Ecology and Biogeography 10:229–244.

Kaspari, M., and J. S. Powers. 2016. Biogeochemistry and geographical ecology: embracing all twenty-five elements required to build organisms. American Naturalist 188:S62–S73.

Kaspari, M., and S. P. Yanoviak. 2009. Biogeochemistry and the structure of tropical brown food webs. Ecology 90: 3342–3351.

Kaspari, M., J. Wright, J. Yavitt, K. Harms, M. Garcia, and M. Santana. 2008. Multiple nutrients regulate litterfall and decomposition in a tropical forest. Ecology Letters 11:35–43.

Kaspari, M., S. P. Yanoviak, R. Dudley, M. Yuan, and N. A. Clay. 2009. Sodium shortage as a constraint on the carbon cycle in an inland tropical forest. Proceedings of the National Academy of Sciences USA 106:19405–19409.

LeBauer, D. S., and K. K. Treseder. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89:371–379.

Marschner, H. 1995. Mineral nutrition in higher plants. Academic Press, San Diego, California, USA.

McNaughton, S., M. Oesterheld, D. Frank, and K. Williams. 1989. Ecosystem-level patterns of primary productivity and herbivory in terrestrial habitats. Nature 341:142–144.

McNaughton, S. J., F. F. Banyikwa, and M. M. McNaughton. 1997. Promotion of the cycling of diet-enhancing nutrients by African grazers. Science 278:1798–1800.

Moldenke, A. R. 1994. Pages 517-542 in P. S. Bottomley, J. S. Angle, and R. W. Weaver, editors. Methods of Soil Analysis:

Reports

Part 2—Microbiological and Biochemical Properties. Soil Science Society of America, Madison, Wisconsin, USA.

NRC. 2005. Mineral tolerance of animals. National Research Council, The National Academies Press, Washington, D.C., USA.

OSU. 2012. Soil, water and forage analytical laboratory. http://www.soiltesting.okstate.edu/

Ritchie, M. E. 2000. Nitrogen limitation and trophic vs. abiotic influences on insect herbivores in a temperate grassland. Ecology 81:1601–1612.

Rossi, A., and D. Strong. 1991. Effects of host-plant nitrogen on the preference and performance of laboratory populations of Carneocephala floridana (Homoptera: Cicadellidae). Environmental Entomology (USA) 20:1349–1355.

SAS. 2009. SAS/STAT user's guide. Version 9.2. SAS Institute Inc., Cary, North Carolina, USA.

Schlesinger, W. H. 1997. Biogeochemistry: an analysis of global change. Academic Press, San Diego, California, USA.

Schmitz, O. J. 2010. Resolving ecosystem complexity. Princeton University Press, Princeton, New Jersey, USA.

Siemann, E. 1998. Experimental tests of effects of plant productivity and diversity on grassland arthropod diversity. Ecology 79:2057–2070.

Snell-Rood, E. C., A. Espeset, C. J. Boser, W. A. White, and R. Smykalski. 2014. Anthropogenic changes in sodium affect neural and muscle development in butterflies. Proceedings of the National Academy of Sciences 111: 10221–10226.

Sperfeld, E., D. Raubenheimer, and A. Wacker. 2015. Bridging factorial and gradient concepts of resource co-limitation: towards a general framework applied to consumers. Ecology Letters 19:201–215.

Steinauer, E. M., and S. L. Collins. 1995. Effects of urine deposition on small-scale patch structure in prairie vegetation. Ecology 76:1195–1205.

Steinauer, E. M., and S. L. Collins. 2001. Feedback loops in ecological hierarchies following urine deposition in tallgrass prairie. Ecology 82:1319–1329.

Stewart, A. J., and A. F. Wright. 1995. A new inexpensive suction apparatus for sampling arthropods in grassland. Ecological Entomology 20:98–102.

Stiling, P., and D. C. Moon. 2005. Quality or quantity: the direct and indirect effects of host plants on herbivores and their natural enemies. Oecologia 142:413–420.

Taiz, L., and E. Zeiger. 1998. Plant physiology. Second edition. Sinauer, Sunderland, Massachusetts, USA.

Walker, T. W., and J. K. Syers. 1976. The fate of phosphorus during pedogenesis. Geoderma 15:1–19.

White, T. C. R. 1993. The inadequate environment: nitrogen and the abundance of animals. Springer, Berlin, Germany.

Supporting Information

Reports