Trophic differences regulate grassland food webs: herbivores track food quality and predators select for habitat volume

REBECCA M. PRATHER [iD],[1,2,4] ELLEN A. R. WELTI [iD],[1,3] AND MICHAEL KASPARI [iD][1]

[1]*Geographical Ecology Group, Department of Biology, University of Oklahoma, Norman, Oklahoma 73019 USA*
[2]*Department of Biological Science, Florida State University, Tallahassee, Florida 32306 USA*
[3]*Senckenberg Research Institute and Natural History Museum Frankfurt, Gelnhausen 63571 Germany*

Abstract. The impacts of altered biogeochemical cycles on ecological systems are likely to vary with trophic level. Predicting how these changes will affect ecological food webs is further complicated by human activities, which are simultaneously altering the availability of macronutrients like nitrogen (N) and phosphorus (P), and micronutrients such as sodium (Na). Here we contrast three hypotheses that predict how increasing nutrient availability will shape grassland food webs. We conducted a distributed factorial fertilization experiment (N and P crossed with NaCl) across four North American grasslands, quantifying the responses of aboveground plant biomass and volume, plant tissue and soil elemental concentrations, as well as the abundance of five arthropod functional groups. Fertilization with N and P increased plant biomass and foliar N and P concentrations in grasses but not forbs. Fertilization with Na had no effect on plant biomass but increased foliar Na concentrations. Consistent with the nutrient limitation hypothesis, we found strong evidence of nutrient limitation for insect herbivores across the four sites with sucking (phloem and xylem feeding) herbivores increasing in abundance with NP fertilization and chewing herbivores increasing in response to both Na and NP fertilization, and a trend for increased response of arthropods to lower plant nutrient availability. We found no evidence for an interaction of NaCl and NP on arthropod abundance as predicted by the serial colimitation hypothesis. Finally, consistent with the ecosystem size hypothesis, predator and parasitoid abundances increased with plant volume, but not fertilization. Our results suggest these functional group-specific responses to changes in plant nutrients and structure are key to predicting the future of grassland food webs in an era with increasing use of N and P fertilizers, and increasing terrestrial inputs of Na from road salt, saline irrigation water, and aerosols due to rising sea levels.

Key words: arthropod; fertilizer; functional group; habitat size; micronutrient; nitrogen; nutrient limitation; phosphorus; prairie; sodium.

INTRODUCTION

The availability of essential nutrients in terrestrial systems limits both plant primary productivity and arthropod abundance (Elser et al. 2007, Gruner et al. 2008, Butler et al. 2012, Joern et al. 2012, Lemoine et al. 2014, Sperfeld et al. 2016). However, the response of plants and herbivores to fertilization varies with the type of nutrient added. For example, although plant primary productivity does not respond strongly to micronutrient addition (Fay et al. 2015, Prather et al. 2020), there is growing evidence that micronutrients such as magnesium (Mg), potassium (K), and sodium (Na) play an important role in limiting abundance and activity of herbivorous insects (Joern et al. 2012, Kaspari and Powers 2016, Welti et al. 2020c). Na in particular has recently

been shown to improve animal performance, and it is relatively unique among the elements in that is it is required by animals but not most plants (Kaspari et al. 2017, Prather et al. 2018a, Welti et al. 2019, Kaspari 2020, Kaspari et al. 2020, Welti et al. 2020a, b). Human activity is increasing terrestrial Na availability via road salt application, saline irrigation, and increased aerosol deposition as a result of sea level rise (Ghassemi et al. 1995, Daliakopoulos et al. 2016). Additionally, plants are not just food to herbivorous insects, but habitat affecting how insects thermoregulate (Pincebourde and Casas 2019), mate (Bernays 1998), and hide from predators (Price et al. 1980). Here we use a distributed field experiment to explore how biogeochemistry regulates grassland food webs via the quantity and density of nutrients, as well as the volume of the habitat they produce. We contrast three hypotheses that predict the abundance of herbivores and their predators in grasslands.

The nutrient limitation hypothesis posits that when nutrients are both essential and in short supply to an organism relative to demand, then those nutrients should regulate abundance (White 1978, Sterner and Elser 2002). For example, the essential nutrients N, P, and Na often constrain the abundance and activity of terrestrial arthropods (Haddad et al. 2000, Ritchie 2000, Kaspari et al. 2017, Lind et al. 2017, Prather et al. 2018a, Welti et al. 2019, Kaspari 2020). The nutrient limitation hypothesis has two predictions. First, as a nutrient becomes more scarce, then its addition should have a larger positive effect on arthropod abundance (Lind et al. 2017, Welti et al. 2019). Second, arthropod trophic groups should respond differentially to nutrient limitation. Although most of the macro- and micronutrients needed by plants are also essential for their consumers, herbivores require higher nutrient concentrations than plants to maintain functioning. As nutrients tend to bioaccumulate trophically, fertilization of plants should also yield larger increases in abundance of herbivores than omnivores or predators (Sterner et al. 1992, Clay et al. 2014, 2017, Welti et al. 2019, Kaspari 2020). Finally, different insect herbivore feeding modes (i.e., piercing-sucking taxa such as aphids, vs. chewing taxa such as grasshoppers) may have different access to plant nutrients. We expect chewing herbivores will have stronger responses to nutrient fertilization, as they are generally more mobile, and as an adaptation to their reduced mobility, sucking herbivores have endosymbionts that can supply additional nutrients (McCutcheon and Moran 2012, Welti et al. 2020b).

Work on arthropod nutrient limitation has historically focused on the macronutrients N and P because of their importance in arthropod growth, survival, and reproductive rates (Fischer and Fiedler 2000, Huberty and Denno 2006, Rode et al. 2017), but evidence mounts in ecological communities for limitation by multiple nutrients (nutrient colimitation) (Elser et al. 2007, Gruner et al. 2008, Harpole et al. 2011, Sperfeld et al. 2016, Prather et al. 2018a). The serial colimitation hypothesis builds on nutrient limitation to posit positive synergistic interactions among nutrients (i.e., positive interactions [Harpole et al. 2011, Kaspari et al. 2017]). For example, in an Oklahoma prairie, fertilization with NaCl had no effect on detritivore abundance, but when added with N and P, enhanced abundance greater than NP alone (Kaspari et al. 2017).

Although nutrient availability regulates herbivore abundance, another effect of fertilization—increasing plant biomass and volume—can shape the relative abundance of both herbivores and predators. The ecosystem size hypothesis predicts that predators are more limited by habitat volume than herbivores (Power 1992, Post et al. 2000, Donoso et al. 2013, Welti et al. 2020b), as predator habitat requirements scale more steeply than their prey (Peters 1983, Post et al. 2000), and/or increasing structural means to catch prey (e.g., in grasslands, more opportunities for web-building spiders or

sit-and-wait predators on flowers [Gómez et al. 2016]). Although herbivores may also be attracted to higher habitat volume (Langellotto and Denno 2004, Prather and Kaspari 2019, Welti et al. 2020b), the ecosystem size hypothesis predicts a stronger effect on predators.

Here we use a factorial Na and NP fertilization experiment distributed across four grasslands encompassing a gradient of Na availability to explore how biogeochemistry shapes arthropod food webs in both bottom-up and top-down fashion. Guided by the above three hypotheses, we examine (1) whether insect herbivore nutrient limitation increases as plant nutrient content decreases, (2) the ubiquity and nature of Na limitation with the expectation that Na will more strongly limit arthropods in lower trophic levels, (3) whether Na addition catalyzes access to NP, and (4) if habitat volume has stronger effects on predator and parasitoid abundance relative to herbivore abundance. By simultaneously evaluating these hypotheses regarding different arthropod trophic levels, we aim to understand the consequences of nutrient addition for whole grassland arthropod green food webs.

METHODS

Site description

We studied the plant and arthropod communities from May through September 2018 at four grassland sites (from south to north): Selman Living Lab (SLL, 36.69° N, −99.28° W), Konza Prairie (KNZ, 36.09° N, −96.56° W), Platte River Prairie (PRP, 40.73° N, −98.58° W), and Niobrara Valley Preserve (NVP, 42.78° N, −100.04° W). These sites vary across many ecological gradients. Annual precipitation increases from west to east; thus, the two more western sites, SLL and NVP, are mixed-grass prairies, and the two more eastern sites, KNZ and PRP, are tallgrass prairies. Sites also vary in soil type, with the northern sites, PRP and NVP, having sandier soil while the southern sites, SLL and KNZ, have clay soil. Soil N, P, and Na decrease with latitude across the four sites (Appendix S1: Table S1).

Experimental design

We set up 20 plots at each site, each 7×7 m and separated by 7 m. Ten plots at every site were assigned to a Na addition (+Na) and treated with NaCl dissolved in water, whereas the remaining 10 control plots were treated with water only. Treatments were applied monthly from May to September, with +Na plots receiving 10 g $NaCl/m^2$ by dissolving NaCl in 1 L water and control plots receiving 1 L of water applied over plants using watering cans. Na amount was chosen to mimic the level of Na available via bison urine (Steinauer and Collins 1995). Nested within these treatments, each plot contained two 4-m^2 subplots, separated by 1 m (+NP or control; Appendix S1: Fig. S1). The +NP subplot

treatment was applied once in May at a rate of 10 g/m^2 of each element. N was added as time-released urea, and P was added as nonsulfur coated super-triple phosphate.

Plant and soil sampling

Each month, we sampled plant volume using a disc pasture meter (Bransby and Tainton 1977). A disc pasture meter measures plant volume by recording the resting height at which a disc is supported by the vegetation when dropped from a set height. We took one disc pasture meter reading per 1 m^2 in each subplot, totaling four readings for each control and +NP subplot and eight readings per plot. We analyzed the concentration of N, P, and Na in plants and soil collected from each site. Plants were sampled from a 0.1 × 1 m clip strip in each subplot in July, prior to monthly Na addition. Plants were then sorted into grasses or forbs, followed by drying for 48 h at 60°C. Once dried, grass and forb samples were weighed to estimate biomass of each functional group in each plot. Soils were collected from each plot (outside of the subplots) in June. Grasses, forbs, and soil were analyzed for %N using combustion analysis and for P and Na concentrations using hot plate digestion at the Cornell Nutrient Analysis Laboratory.[5]

Arthropod sampling

We sampled arthropods monthly across the growing season from June through September at each site, 48 h after +Na and water treatments were applied. To collect arthropods, we used a vacuum sampler for 30 s per plot (Stewart and Wright 1995). Arthropods were put on ice in the field and kept frozen until sorted. We counted and identified all arthropods to family and assigned them to functional trophic groups (chewing herbivore, sucking herbivore, pollinator, omnivore, predator, parasitoid, detritivore, and unknown; Appendix S2: Table S1). Because of equipment failure, we did not sample arthropods in August at Selman Living Lab. We did not sample seven plots (four +Na and three control plots) at Platte River Prairie from July–September because of excessive poison ivy presence.

Data analysis

To examine how grass and forb concentrations of N, P, and Na changed with NP, Na, and NaNP fertilization across our four sites, we used linear mixed-effect models with plot nested within site as a random factor to account for multiple observations on the same plot during different months (Bolker et al. 2009). We used Akaike's information criterion (AIC$_c$) to perform model selection. We log-transformed grass N, P, and Na and forb N and Na concentrations to meet normality assumptions. To examine whether soil Na concentration

[5] https://cnal.cals.cornell.edu/

changed with Na fertilization, we used a two-sample t-test to compare soil Na from control and +Na plots.

To test the effects of Na, NP, their interaction (NaNP), and plant volume on the abundance of arthropod trophic groups across sites, we again used AIC$_c$ selection of linear mixed-effect models with plot nested within site as the random factor. We analyzed six models total, one for total arthropods and for each of five arthropod trophic groups (chewing herbivore, sucking herbivore, omnivore, predator, and parasitoid). Arthropod abundances and plant volume were log-transformed to meet normality assumptions. We did not analyze detritivores and pollinators separately because of their low abundances across sites (Appendix S1: Table S2).

To visualize the magnitude of the response of arthropod functional group abundance to +Na, +NP, and +NaNP treatments, we calculated average effect sizes (Cohen's d) by trophic group. To examine the variation in the response based on plant Na, N, and P availability across sites, we regressed invertebrate response to weighted plant Na, N, and P concentration. Weighted plant nutrient concentrations were calculated by multiplying the proportion of forb and grass per plot by the concentration of Na, N, and P in forb and grass, respectively, and then summing these two values. We fitted linear mixed models using the R package lme4 (Bates et al. 2014) and the MuMIn package to calculate AIC$_c$ model comparisons (Burnham and Anderson 2003, Bolker et al. 2009, Bárton 2016). All analyses were conducted in R version 3.6.1. (R Core Team 2019).

RESULTS

We collected 11,400 arthropods across the four sites with abundance varying from 0 to 253 arthropods per 1-m^2 sample (mean: 17.87 arthropods ± 1.18 SE; Appendix S1: Table S2). As predicted by the nutrient limitation hypothesis, all herbivores increased with NP addition, and chewing herbivores also increased with Na addition. We did not find evidence to support the serial colimitation hypothesis in the form of Na catalysis by any arthropod functional group. Although chewing herbivores increased with both Na and NP addition, the lack of a significant interaction suggests they did so additively (Fig. 1, Table 1). Predators and parasitoids, as predicted by the ecosystem size hypothesis, increased with habitat volume.

Soil chemistry varied among the four sites

Soil Na in control plots varied fivefold from 19 ppm in the sandy soils of Platte River Prairie to 96 ppm in the clay-rich soils of Selman Living Lab (Appendix S1: Table S1). Soil N in control plots varied about threefold (0.06 ppm to 0.22 ppm; Appendix S1: Table S1), as did soil P, from 188 ppm at northern Niobrara Valley Preserve to 582 ppm at southern Selman Living Lab (Appendix S1: Table S1). Soil Na increased with Na
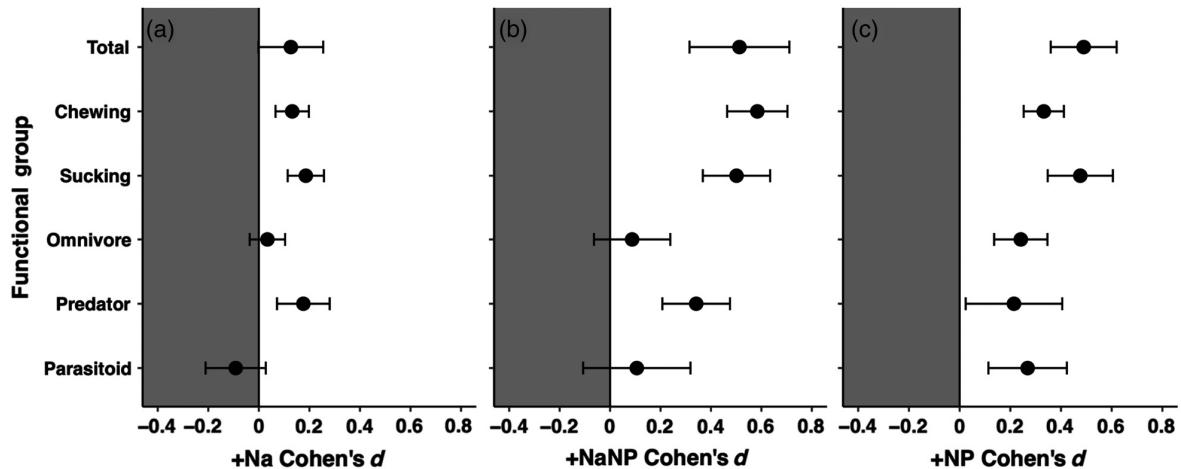
FIG. 1. Mean effect sizes (and standard error) show attraction of invertebrate functional groups across sites to (a) Na, (b) NaNP, and (c) NP. Positive effects sizes indicate attraction whereas negative effect sizes indicate avoidance. Cohen's *d* was calculated by comparing the three treatments to the control.

TABLE 1. Results of linear mixed-effects models testing the effects of Na, NP, NaNP interaction, and plant volume on abundance of total arthropods and five arthropod guilds across four sites.

Functional group	Model variables	df	LL	AIC_c	ΔAIC_c	w_i	R^2
All arthropods	Subplot (+NP)	5	−846.58	1,703.3	0	0.47	0.20
	Plant volume, subplot (+NP)	6	−845.99	1,704.1	0.88	0.30	0.19
Chewing	Subplot (+NP)	5	−607.54	1,225.2	0	0.54	0.09
	Subplot (+NP), treatment (+Na)	6	−606.66	1,225.5	0.28	0.46	0.09
Sucking	Subplot (+NP)	5	−818.92	1,647.9	0	1.00	0.16
Omnivore	Null	4	−630.58	1,269.2	0	0.56	0.05
	Plant volume	5	−630.48	1,271.1	1.83	0.23	0.06
Predator	Plant volume	5	−578.72	1,167.5	0	0.77	0.13
Parasitoid	Plant volume	5	−490.90	991.9	0	0.80	0.18

Notes: Akaike's information criterion (: AIC) statistics include: df, degrees of freedom; LL, log likelihood; AIC_c, AIC statistic; ΔAIC_c, AIC_c minus top model AIC_c; w_i, model weight; R^2, adjusted regression coefficient.

fertilization ($t = -2.52$, df = 66.03, $P = 0.014$; Appendix S1: Fig. S2).

Grass and forb chemistry and volume responded differently to NP and NaCl fertilization

Grass N and P concentrations increased on average onefold with NP fertilization ($P = 0.001$ and $P = 0.002$, respectively), but forb N and P concentrations did not (null AIC_c models; Appendix S1: Table S3). Both grass and forb Na concentrations increased with Na fertilization, 9-fold and 17-fold, respectively (both $P < 0.001$; Appendix S1: Table S3).

Plant volume, measured using a disc pasture meter, increased with NP fertilization across sites ($P < 0.001$; Appendix S1: Fig. S3, Table S4). This increase was driven by forb biomass, which increased about threefold with NP fertilization ($P = 0.011$; Appendix S1: Fig. S3, Table S4). Grass biomass, on the other hand, did not

change with fertilization (null AIC_c model; Appendix S1: Fig. S3, Table S4).

All herbivores increased with NP, and chewing herbivores increased with Na fertilization

Our results supported the hypothesis of nutrient limitation of herbivore abundance across the four sites (Fig. 1; Table 1; Appendix S1: Table S5). Both chewing and sucking herbivores increased on +NP plots, about onefold and twofold, respectively (both $P < 0.001$; Fig. 1; Table 1). Chewing herbivore abundance was also about onefold higher on +Na plots ($P = 0.02$; Fig. 1; Table 1). The increase in herbivores with NP fertilization resulted in an overall increase in total arthropod abundance, whereas treatment effects of neither +NP nor +Na fertilization affected the abundance of omnivores, predators, or parasitoids (Table 1).

Habitat volume, not nutrient density, regulated parasitoid and predator numbers

Predator and parasitoid abundances across the four sites increased with habitat volume as measured by disc pasture meter plant volume (both $P < 0.001$; Table 1; Appendix S1: Figs. S4, S5). On average across the four sites, for a doubling of aboveground plant volume, both predator and parasitoid abundance increased by about twofold. Variation in omnivore abundance was not well explained by the tested drivers, as the top model was a null model containing no predictor variables, but plant volume was included in the second model predicting omnivore abundance (Table 1). Nutrient addition treatments never appeared in top models predicting abundance of these higher trophic levels across the sites (Table 1).

Fertilization effects on invertebrate abundance trended higher in the low nutrient sites

Although we had only four sites and thus too few locations for a robust geographical test, we conducted a preliminary examination of regional differences in attraction to fertilization across gradients of plant nutrient availability. We found some evidence for reduced arthropod attraction to nutrients as plant nutrient supply increased, especially for plant P concentrations (Fig. 2) although this trend did not hold for herbivores alone (Appendix S1: Fig. S6). Additionally, the trend was less strong across gradients of plant concentrations of Na and N, with SLL being a potential outlier in having strong arthropod responses to fertilization as plant

tissue concentrations of N and Na in control plots were high compared to other sites (Fig. 2).

DISCUSSION

Across four grasslands in the North American Great Plains, arthropod abundance responded strongly to changes in plant nutrient content and plant productivity, but responses varied by trophic level. Consistent with the nutrient limitation hypothesis, all herbivores increased with added NP, and chewing herbivores, such as grasshoppers and caterpillars, increased with Na fertilization. Although our study lacked enough regional replication for a robust test, arthropod effect sizes in response to fertilization trended stronger when plants were more deficient in that nutrient. Consistent with the ecosystem size hypothesis, habitat volume, not nutrient supply, constrained predator and parasitoid abundance. Despite both Na and NP fertilization increasing chewing herbivore abundance, we saw no evidence of interaction as predicted by the serial colimitation hypothesis.

Fertilization changed plant N, P, and Na concentrations

Soil nutrients varied from three- to fivefold with latitude, but this variation was not reflected in plant N, P, and Na concentrations. Specifically, in control plots the two northern sites of PRP and NVP had the lowest soil P concentrations but the highest plant P concentrations. Additionally, these two sites had the lowest soil Na and N levels, but plant Na and N did not differ much in control plots across the four sites. These anomalies are likely due to either nutrient leaching from the sandy soils at
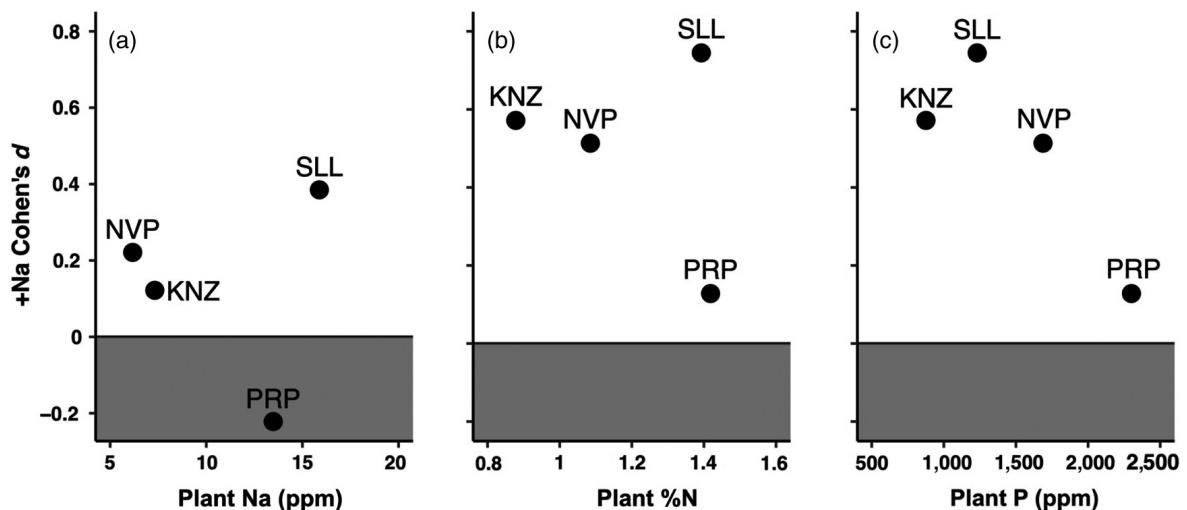

FIG. 2. Mean effect sizes (and standard error) show Na, NP, and NaNP attraction of all invertebrate functional groups across sites. (a), (b), and (c) show Na and NP attraction based on plant sodium (ppm), nitrogen (%N), and phosphorus (ppm), respectively. The Cohen's *d* effect sizes in (a) are calculated by comparing +Na to control plots, and the effect sizes in (b) and (c) are calculated by comparing +NP to control plots. SLL = Selman Living Lab; KNZ = Konza Prairie; PRP = Platte River Prairie; NVP = Niobrara Valley Preserve.

Platte River Preserve and Niobrara Valley Prairie and/or better nutrient uptake by plants at those sites. Interestingly, plant nutrient uptake in treatment plots differed by functional group. Grass N, P, and Na concentrations increased with fertilization across sites; forbs only responded to Na fertilization. The lack of increased N and P concentrations in foliar tissue with fertilization may be a dilution response in forbs, as forb biomass increased with fertilization while grass biomass did not show that same increase (Appendix S1: Fig. S3). Dilution of both macro- and micronutrients can occur with increases in plant aboveground biomass (Loladze 2014, Welti et al. 2020c). As we show, plant quality can constrain arthropod abundance, so future studies should further explore the differential response of grasses and forbs to nutrient addition.

Herbivores are nutrient limited

Across four sites, herbivore abundance was regulated by plant quality rather than plant biomass. Consistent with other studies, we found that both chewing and sucking herbivores were NP limited (Huberty and Denno 2006, Lemoine et al. 2014, Rode et al. 2017, Welti et al. 2020c). NP fertilization often increases plant biomass (LeBauer and Treseder 2008, Harpole et al. 2011, Fay et al. 2015) as was the case for forbs in our study. Additionally, NP fertilization increased grass foliar nutrient concentrations (Appendix S1: Table S3), suggesting fertilization differentially affected plant functional groups, which then mediated arthropod responses to fertilization.

We find further evidence that NP availability constrains the local abundance of grassland invertebrate herbivores, in two common functional groups: the xylem and phloem feeders like aphids and leafhoppers (see also Huberty and Denno [2006], Butler et al. [2012]), as well as the chewers, like grasshoppers (Huberty and Denno 2006, Rode et al. 2017, Welti et al. 2020c). Although our results support the growing evidence that chewing herbivores accumulate at sodium sources (Joern et al. 2012, Welti et al. 2019, Kaspari 2020), we found no such evidence for sucking herbivores. This may be because fluid feeders have easier access to existing amounts of sodium being transported in the xylem, even on control plots. Alternatively, as we added NaCl from above and plants can absorb sodium directly from their leaves (Benes et al. 1996), it is possible that sodium accumulated only in the leaves and levels did not appreciably increase in the xylem. Finally, Na fertilization could result in other changes in plants that offset any benefit of Na fertilization such as altering plant chemistry via displacing K ions (Wolf and Jeschke 1987). Regardless, such differences in nutritional ecology of common herbivore functional groups has implications for their co-regulation and interactions.

Finally, contrary to earlier speculation and consistent with an earlier factorial study in a southern Great Plains

grassland (Kaspari et al. 2017), we found no evidence that higher levels of Na increased the attraction of NP-rich tissue to chewing herbivores like grasshoppers. For leaf-chewing insects, enhancing sodium may foster the development of muscle and neural tissue (Snell-Rood et al. 2014) and promote the ability to escape from predators (Peterson et al. 2021), but its effect on abundance and fitness seems orthogonal to the macronutrients N and P.

Predators and parasitoids are limited by ecosystem size

At all four sites, plant biomass varied considerably, even on control plots, allowing us to explore it as a covariate driving arthropod abundance. Consistent with the ecosystem size hypothesis, we found that predator and parasitoid abundance was constrained by an index of habitat volume. In further support of the nutrient limitation hypothesis, predators and parasitoids that eat N, P, and Na-rich flesh did not respond to our fertilization treatments, but rather were most abundant on plots with the greatest plant biomass (Appendix S1: Figs. S4, S5).

Most of the evidence for the ecosystem size hypothesis has come from aquatic systems and not terrestrial green food webs (but see Donoso et al. [2013] for evidence from a terrestrial detrital, or brown food web). The mechanisms, however, even in aquatic systems (Post 2000) where it was first investigated, remain unclear. We propose four, testable, nonexclusive, working hypotheses for how the ecosystem size hypothesis affects terrestrial grassland food webs. (1) Habitat volume is proportional to the standing detritus, and living plants may be a proxy for primary productivity, which in turn governs food chain length (Power 1992, Post et al. 2000, Donoso et al. 2013, Gómez et al. 2016, Welti et al. 2020b). (2) Habitat volume is positively correlated with niche dimensionality (Power 1992, Post et al. 2000, Gómez et al. 2016, Welti et al. 2020b): Our high-volume plots frequently contained a larger mix of broadleaf forbs, with more volume for spiders to hang their webs, and more flowers to feed predacious and parasitoid wasps. (3) Higher habitat volume increases shade and increases humidity (Prather and Kaspari 2019), potentially benefiting water-stressed predators like spiders and their hydraulically controlled limbs (Leinbach et al 2019). Finally, (4) although high-volume plots attract both predators and their prey (Siemann 1998, Rzanny et al. 2013), herbivores may subsequently be consumed, or frightened away at higher rates.

Nutrient limitation increases as plant supply decreases

Across our four sites distributed north and south through the Great Plains, we found some support for increased arthropod nutrient limitation—that is, increased accumulation on fertilized plots—as plant nutrient supply decreased. Specifically, arthropods tended to respond more strongly to our nutrient treatments at lower levels of plant P, and potentially at lower

levels of plant N and Na apart from SLL (Fig. 2). This is consistent with a study of 54 Na pulse experiments across North America, where increases in arthropod abundance on +Na plots was proportional to that grassland's plant Na supplies (Welti et al. 2019). The 10–1,000-fold variation in essential nutrient densities in both plant species and entire grasslands (Han et al. 2011) is a potentially useful template for predicting the geography of nutrient limitation in grassland food webs.

Conclusions and future directions

Here we use a distributed factorial experiment across the Great Plains to reveal how nutrient limitation and habitat volume combine to shape the abundance of arthropods in grassland food webs. Across four grasslands, the effects of increased availability of N, P, and Na on arthropod abundance vary with trophic group and are likely mediated by plant responses to fertilization. As nutrient cycles are increasingly altered in the Anthropocene (Vitousek 1994, Jackson and Jobbagy 2005) our research design (four sites, 7 × 7 m plots with and without NaCl plus embedded 2 × 2 m plots with and without NP), points to the challenges of designing still larger experimental manipulations (e.g., Prather et al. [2018a]) that run over longer time spans than 2-d snapshots (e.g., Welti et al. [2019]) or single growing seasons (Kaspari et al. 2017) toward simulating long-term nutrient deposition effects on grassland food webs.

Additionally, as ca. 25 elements are essential to build a grassland of microbes, plants, and animals (Kaspari and Powers 2016), more complex experiments, including adding multiple nutrient combinations, are needed to address the predicted simultaneous changes of many biogeochemical cycles. The highly significant, catalytic effects of micronutrient combinations on arthropod, but not plant abundance (Fay et al. 2015, Prather et al. 2020), suggests we have not yet found the "active element(s)" that drive the serial colimitation hypothesis. The answer is likely linked to the different ionomic compositions of the plant and animal functional groups (e.g., forbs vs. grasses, chewing vs. sucking herbivores, herbivores vs. predators and parasitoids), and suggests the need for a deeper understanding of the physiological mechanisms driving their abundance. In the end, understanding how and why functional groups change in response to changing biogeochemistry will improve our ability to forecast the future of grassland food webs in a world that is simultaneously eutrophying some ecosystems and impoverishing others.

Literature Cited

Bárton, K. 2016. MuMIn: model selection and model averaging based on information criteria (AICc and alike). R package version 1.15.6. https://cran.r-project.org/web/packages/MuMIn/MuMIn.pdf

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2014. Fitting linear mixed-effects models using lme4. arXiv preprint arXiv:1406.5823.

Benes, S. E., R. Aragüés, S. R. Grattan, and R. B. Austin. 1996. Foliar and root absorption of Na+ and Cl− in maize and barley: Implications for salt tolerance screening and the use of saline sprinkler irrigation. Plant and Soil 180:75–86.

Bernays, E. A. 1998. Evolution of feeding behavior in insect herbivores. BioScience 48:35–44.

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J.-S.-S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology & Evolution 24:127–135.

Bransby, D., and N. Tainton. 1977. The disc pasture meter: possible applications in grazing management. Proceedings of the Annual Congresses of the Grassland Society of Southern Africa 12:115–118.

Burnham, K. P., and D. R. Anderson. 2003. Model selection and multimodel inference: a practical information-theoretic approach. Springer Science & Business Media, New York, New York, USA.

Butler, J., M. Garratt, and S. Leather. 2012. Fertilisers and insect herbivores: a meta-analysis. Annals of Applied Biology 161:223–233.

Clay, N. A., R. J. Lehrter, and M. Kaspari. 2017. Towards a geography of omnivory: Omnivores increase carnivory when sodium is limiting. Journal of Animal Ecology 86:1523–1531.

Clay, N. A., S. P. Yanoviak, and M. Kaspari. 2014. Short-term sodium inputs attract microbi-detritivores and their predators. Soil Biology and Biochemistry 75:248–253.

Daliakopoulos, I. N., I. K. Tsanis, A. Koutroulis, N. N. Kourgialas, A. E. Varouchakis, G. P. Karatzas, and C. J. Ritsema. 2016. The threat of soil salinity: A European scale review. Science of the Total Environment 573:727–739.

Donoso, D. A., M. K. Johnston, N. A. Clay, and M. E. Kaspari. 2013. Trees as templates for trophic structure of tropical litter arthropod fauna. Soil Biology and Biochemistry 61:45–51.

Elser, J. J., M. E. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters 10:1135–1142.

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

Fischer, K., and K. Fiedler. 2000. Response of the copper butterfly Lycaena tityrus to increased leaf nitrogen in natural food plants: evidence against the nitrogen limitation hypothesis. Oecologia 124:235–241.

Ghassemi, F., A. J. Jakeman, and H. A. Nix. 1995. Salinisation of land and water resources: human causes, extent, management and case studies. UNSW Press; CAB International, Sydney, Australia.

Gómez, J. E., J. Lohmiller, and A. Joern. 2016. Importance of vegetation structure to the assembly of an aerial web-building spider community in North American open grassland. Journal of Arachnology 44:28–35.

Gruner, D. S., et al. 2008. A cross-system synthesis of consumer and nutrient resource control on producer biomass. Ecology Letters 11:740–755.

Haddad, N. M., J. Haarstad, and D. Tilman. 2000. The effects of long-term nitrogen loading on grassland insect communities. Oecologia 124:73–84.

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.

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.

Jackson, R. B., and E. G. Jobbagy. 2005. From icy roads to salty streams. Proceedings of the National Academy of Science of the United States of America 102:14487–14488.

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. 2020. The seventh macronutrient: how sodium shortfall ramifies through populations, food webs and ecosystems. Ecology Letters 23:1153–1168.

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., K. A. Roeder, B. Benson, M. D. Weiser, and N. J. Sanders. 2017. Sodium co-limits and catalyzes macronutrients in a prairie food web. Ecology 98:315–320.

Kaspari, M., E. A. Welti, and K. M. de Beurs. 2020. The nutritional geography of ants: Gradients of sodium and sugar limitation across North American grasslands. Journal of Animal Ecology 89:276–284.

Langellotto, G. A., and R. F. Denno. 2004. Responses of invertebrate natural enemies to complex-structured habitats: a meta-analytical synthesis. Oecologia 139:1–10.

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

Leinbach, I. L., K. E. McCluney, and J. L. Sabo. 2019. Predator water balance alters intraguild predation in a streamside food web. Ecology 100:e02635.

Lemoine, N. P., S. T. Giery, and D. E. Burkepile. 2014. Differing nutritional constraints of consumers across ecosystems. Oecologia 174:1367–1376.

Lind, E. M., et al. 2017. Increased grassland arthropod production with mammalian herbivory and eutrophication: a test of mediation pathways. Ecology 98:3022–3033.

Loladze, I. 2014. Hidden shift of the ionome of plants exposed to elevated CO_2 depletes minerals at the base of human nutrition. eLife 3:e02245.

McCutcheon, J. P., and N. A. Moran. 2012. Extreme genome reduction in symbiotic bacteria. Nature Reviews Microbiology 10:13–26.

Peters, R. H. 1983. The ecological implications of body size. Cambridge University Press, Cambridge, UK.

Peterson, T., E. A. R. Welti, and M. Kaspari. 2021. Dietary sodium levels affect grasshopper growth and performance. Ecosphere 12:e03392.

Pincebourde, S., and J. Casas. 2019. Narrow safety margin in the phyllosphere during thermal extremes. Proceedings of the National Academy of Science of the United States of America 116:5588–5596.

Post, D. M., M. L. Pace, and N. G. Hairston, Jr. 2000. Ecosystem size determines food-chain length in lakes. Nature 405:1047.

Power, M. E. 1992. Top-down and bottom-up forces in food webs: do plants have primacy. Ecology 73:733–746.

Prather, C. M., A. N. Laws, J. F. Cuellar, R. W. Reihart, K. M. Gawkins, and S. C. Pennings. 2018a. Seeking salt: herbivorous prairie insects can be co-limited by macronutrients and sodium. Ecology Letters 21:1467–1476.

Prather, R. M., K. A. Roeder, N. J. Sanders, and M. Kaspari. 2018b. Using metabolic and thermal ecology to predict temperature dependent ecosystem activity: a test with prairie ants. Ecology 99:2113–2121.

Prather, R. M., K. Castillioni, M. Kaspari, L. Souza, C. M. Prather, R. W. Reihart, and E. A. R. Welti. 2020. Micronutrients enhance macronutrient effects in a meta-analysis of grassland arthropod abundances. Global Ecology and Biogeography 29:2273–2288.

Prather, R. M., and M. Kaspari. 2019. Plants regulate grassland arthropod communities through biomass, quality, and habitat heterogeneity. Ecosphere 10:e02909.

Prather, R., E. Welti, and M. Kaspari. 2021. Trophic differences regulate grassland food webs: herbivores track food quality and predators select for habitat volume. Dryad, data set. https://doi.org/10.5061/dryad.qnk98sfgr

Price, P. W., C. E. Bouton, P. Gross, B. A. McPheron, J. N. Thompson, and A. E. Weis. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. Annual Review of Ecology and Systematics 11:41–65.

R Core Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.r-project.org

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

Rode, M., N. P. Lemoine, and M. D. Smith. 2017. Prospective evidence for independent nitrogen and phosphorus limitation of grasshopper (Chorthippus curtipennis) growth in a tallgrass prairie. PLoS One 12:e0177754.

Rzanny, M., A. Kuu, and W. Voigt. 2013. Bottom–up and top–down forces structuring consumer communities in an experimental grassland. Oikos 122:967–976.

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 of the United States of America 111:10221–10226.

Sperfeld, E., D. Raubenheimer, and A. Wacker. 2016. Bridging factorial and gradient concepts of resource co-limitation: towards a general framework applied to consumers. Ecology Letters 19:201–215.

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

Sterner, R. W., and J. J. Elser. 2002. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, Princeton, New Jersey, USA.

Sterner, R. W., J. J. Elser, and D. O. Hessen. 1992. Stoichiometric relationships among producers, consumers and nutrient cycling in pelagic ecosystems. Biogeochemistry 17:49–67.

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

Vitousek, P. 1994. Beyond global warming: ecology and global change. Ecology 75:1861–1876.

Welti, E. A. R., L. Kuczynski, K. Marske, N. J. Sanders, K. M. de Beurs, and M. Kaspari. 2020a. Salty, mild, and low plant biomass grasslands increase top-heaviness of invertebrate trophic pyramids. Global Ecology and Biogeography 29:1474–1485.

Welti, E. A. R., R. M. Prather, N. J. Sanders, K. M. deBeurs, and M. Kaspari. 2020b. Bottom-up when it is not top-down: Predators and plants control biomass of grassland arthropods. Journal of Animal Ecology 89:1286–1294.

Welti, E. A. R., K. A. Roeder, K. M. de Beurs, A. Joern, and M. Kaspari. 2020c. Nutrient dilution and climate cycles underlie declines in a dominant insect herbivore. Proceedings of the National Academy of Sciences of the United States of America 117:7271–7275.

Welti, E. A. R., N. J. Sanders, K. M. de Beurs, and M. Kaspari. 2019. A distributed experiment demonstrates widespread sodium limitation in grassland food webs. Ecology 100: e02600.

White, T. 1978. The importance of a relative shortage of food in animal ecology. Oecologia 33:71–86.

Wolf, O., and W. D. Jeschke. 1987. Modeling of sodium and potassium flows via phloem and xylem in the shoot of salt-stressed barley. Journal of Plant Physiology 128:371–386.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.3453/suppinfo

OPEN RESEARCH

Raw data and effect sizes of invertebrates and plant biomass (Prather et al. 2021) are deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.qnk98sfgr).