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How and why plant ionomes vary across North American grasslands and its implications for herbivore abundance

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Abstract. Plant elemental content can vary up to 1,000-fold across grasslands, with implications for the herbivores the plants feed. We contrast the regulation, in grasses and forbs, of 12 elements essential to plants and animals (henceforth plant-essential), 7 essential to animals but not plants (animal-essential) and 6 with no known metabolic function (nonessential). Four hypotheses accounted for up to two thirds of the variation in grass and forb ionomes across 54 North American grasslands. Consistent with the supply-side hypothesis, the plant-essential ionome of both forbs and grasses tracked soil availability. Grass ionomes were more likely to harvest even nonessential elements like Cd and Sr. Consistent with the grazing hypothesis, cattle-grazed grasslands also accumulated a handful of metals like Cu and Cr. Consistent with the NP-catalysis hypothesis, increases in the macronutrients N and P in grasses were associated with higher densities of cofactors like Zn and Cu. The plant-essential elements of forbs, in contrast, consistently varied as per the nutrient-dilution hypothesis—there was a decrease in elemental parts per million with increasing local carbohydrate production. Combined, these data fit a working hypothesis that grasses maintain lower elemental densities and survive on nutrient-poor patches by opportunistically harvesting soil nutrients. In contrast, nutrient-rich forbs use episodes of high precipitation and temperature to build new carbohydrate biomass, raising leaves higher to compete for light, but diluting the nutrient content in every bite of tissue. Herbivores of forbs may thus be particularly prone to increases in $p\mathrm{CO}_2$ via nutrient dilution.

Key words: *biogeochemistry; grazing; herbivores; ionomics; nutrient dilution; plant biomass; plant chemistry; precipitation.*

INTRODUCTION

Plant form, biomass, and productivity vary systematically across Earth in biomes like desert scrub, grasslands, and forest (Odum 1959). These plant variables in turn shape the form, abundance, and composition of the food webs they support (MacArthur 1972, Kaspari and Weiser 2012). Less understood is the ecosystem-level variation in the 25+ chemical elements that make up plant tissues (Marschner 1995, Frausto da Silva and Williams 2001, Sterner and Elser 2002). This distribution of all the inorganic elements in plant tissue, from aluminum to zinc—ranked by parts per million (ppm)—is its *ionome* (Salt et al. 2008).

Ionomics (and its cousin, ecological stoichiometry [Sterner and Elser 2002]) link the plants at the base of green food webs to the nutritional ecology of the rest of that web via the common currency of elemental composition. The distribution of the 16 elements deemed essential for all plants—that is, for which shortfalls can be experimentally linked to declines in performance (Sharma et al. 2013)—arises from decisions and constraints of the plant (Salt et al. 2008). Those same elements are required by plant herbivores, setting up potential interactions between the plant's metabolic performance and its vulnerability to herbivory (Arms et al. 1974, Kaspari 2020). Still other elements reside in plant tissues that are not metabolically required by plants but are essential to their animal herbivores and mutualists (e.g., Na, Cr, Li, and Co [McDowell 2003]) or have little to no evidence of metabolic function (e.g., Al, Ba, Sr, and Pb [Frausto da Silva and Williams 2001]). Here we use a continental data set across 54 North American grasslands to ask how and why plant ionomes vary. These hypotheses comprise a mix of strategic mechanisms linked to plant fitness and proximate mechanisms associated with the physiology of uptake.

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Four hypotheses for variation in plant ionomes across ecosystems

Fig. 1 summarizes four hypotheses for the regulation of plant ionomes. The supply-side hypothesis assumes that gradients of soil elemental availability constrain their inclusion in a plant's ionome (Marschner 1995, BassiriRad 2005, Stevens et al. 2015). Dedicated root pores and transporters then target and harvest available essential elements (like N, P, K, Mg, and Mn [BassiriRad 2005, Robinson 2005, Sharma 2006, Han et al. 2011]). Soil moisture too can bring ions within reach of root transporters through diffusion and mass flow (Cataldo and Wildung 1978). Supply-side mechanisms can work for essential and nonessential elements alike, and can even allow the uptake of toxic, or neutral elements into the plant ionome (Cataldo and Wildung 1978), especially if those ions mimic the chemical properties of essential ions (e.g., strontium for calcium, cadmium for Fe [Cataldo and Wildung 1978]). Here we evaluate two predictions of the supply-side hypothesis: (1) a positive relationship between a site's soil ppm for an element and the average ppm of that element in plant tissue, and (2) an increase in an element's ppm with precipitation.

The NP-catalysis hypothesis (Fig. 1) builds on the assumption that organisms pursue an optimal balance of elements to meet metabolic demands (Sterner and Elser 2002, Salt et al. 2008). It posits a common shortfall of two ionomic workhorses—nitrogen and phosphorus—so that as those elements increase in tissue, they also increase demand for colimiting cofactors in the metabolome (e.g., Mg, Fe, Zn [Frausto da Silva and Williams 2001]). In this way, fertilizing soils with N and P can induce deficiencies of colimiting Mg, Ca, and Fe (Foy et al. 1978). NP catalysis is not isolated to plants: when fed algae with enhanced P content, the aquatic herbivore *Daphnia* developed higher ionomic concentrations of a variety of other elements, like Fe, Mg, S, N, S, and Zn (Jeyasingh et al. 2020). Here we evaluate the prediction that an increase in tissue N and P is associated with an increase in other inorganic, plant-essential elements.

The nutrient-dilution hypothesis (Fig. 1) generates ionomic variation in plant-tissue ppm via the factors that constrain net primary production (NPP, $g\ C{\cdot}m^{-2}{\cdot}yr^{-1}$). It posits that releasing constraints on photosynthesis—without changing the underlying supply of available minerals—can dilute the concentration of those minerals in plant tissue with extra carbohydrates (CHOs [Fan et al. 2008, McLauchlan et al. 2010, Loladze 2014, Ziska et al. 2016, Welti et al. 2020*b*). As NPP is limited by temperature, precipitation, and atmospheric CO_2 (Rosenzweig 1968, La Pierre et al. 2011, Welti et al. 2020*b*), variation in the supplies of all three can thus drive temporal and spatial variation in plant ionomes. For example, consistent with nutrient dilution, droughts that inhibit CHO fixation can concentrate plant N and P and other essential elements (La Pierre et al. 2011; Ozment et al., 2021). A review of 24 studies found CO_2 fertilization decreased tissue concentration of Ca, Co, Cu, Fe, I, K, Mg, Mn, N, Na, P, S, Se, and/or Zn in 85 of 115 species studied (Welti et al. 2020*b*); all 24 studies revealed CO_2-driven nutrient dilution for at least some plant taxa. Moreover, as plants (and plant mass) grow, the increase in structural CHOs can in turn decrease the tissue ppm of N and P (Elser et al. 2010, La Pierre et al. 2011). This balance between mineral and carbohydrate availability underlies the frequent use of ratios (i.e., C:N, C:P) in ecological stoichiometry (Sterner and Elser 2002). Here we evaluate the prediction that an element's ppm in plant tissue decreases with indicators of plant investment in CHOs, including carbon ppm, total biomass, and precipitation.

In the grazing hypothesis (Fig. 1), large grazers boost plant nutrient ppm using two of the mechanisms just reviewed. First, grazers can enhance plant nutrient supplies via application of nutrient-rich urine and feces directly to the foliage or indirectly via the soil and roots (Benes et al. 1996, Hempson et al. 2015). Second, by reducing the stature of herbs and grasses—and their resulting CHO-based support tissue (Elser et al. 2010)—grazing can return a mature plant to the stature of a young shoot, with the plant translocating belowground resources to promote regrowth (McNaughton 1984, McNaughton et al. 1997). Thus, grazers can both enhance nutrients (supply side) and replace tall, CHO-rich vegetation with short ionomically rich vegetation (reversing nutrient dilution). Such grazing compensation can produce nutrient-rich, long-lasting grazing lawns (Woodroffe et al. 1990, McNaughton et al. 1997, Hempson et al. 2015). Here we evaluate the prediction that grasslands exposed to cattle grazing will increase an element's ppm in plant tissue.

We are unaware of any studies of terrestrial communities that explore how plant ionomes covary simultaneously with the mechanisms outlined above. Grasslands constitute about 40% of terrestrial biomes (Hoekstra et al. 2005) as native prairie, pasture, and converted cropland. We thus use these four hypotheses to account for variation in grassland ionomes across North America. We contrast the responses of two common functional groups—grasses and forbs—as their underlying baseline chemistry differs systematically, with forbs averaging twice the ppm of grasses for elements like N, S, and Na, and which are frequently consumed by a different guild of herbivores (Jonas and Joern 2008, Walters 2011, Joern et al. 2012). In contrast to similar studies of ionomes across species (Han et al. 2011) we use clip plots that pool the ionomes of coexisting species. In doing so the ionomes of common species are more represented, as they would be when grazed.

METHODS

Sampling occurred between April and August of 2017 across 54 North American grassland sites (see Appendix S1: Table S1, and map in Welti et al. [2020*a*]).

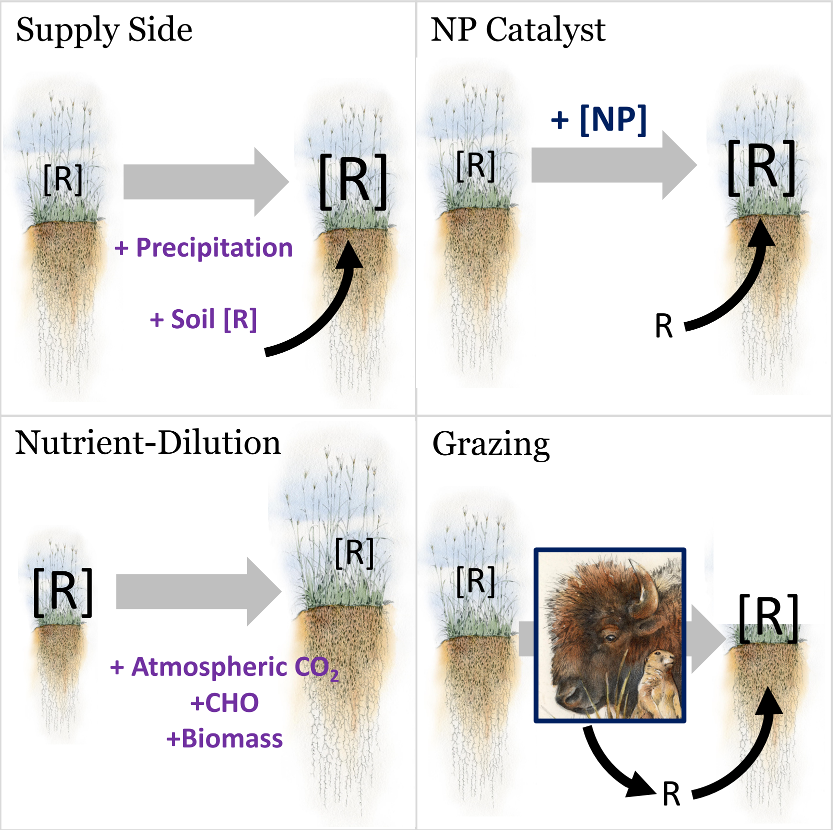

FIG. 1. Four hypotheses for gradients in the concentration (parts per million [ppm]) of nutrients in plant tissue [R]. Supply side posits that the availability of nutrients in soil, and/or the mass flow of those nutrients via precipitation constrains [R]. NP catalyst posits that any increase in the limiting macronutrients N and P increases metabolic demand and uptake for colimiting R. Nutrient dilution posits that any driver enhancing the production or amount of carbohydrates, without changes in nutrient [R], will decrease the tissue ppm of R. Grazing posits that loss of aboveground plant tissue will result in compensatory increases [R] from belowground storage, or through direct fertilization by urine and feces.

Sites included native and restored grasslands managed by universities, the U.S. Forest Service, the Nature Conservancy, the U.S. Department of Agriculture (USDSA), the National Science Foundation Long-Term Ecological Research (NSF LTER) Network, and the Audubon Society. Nine of these grasslands were grazed by cattle.

At each site, five plots (each 1 m^2) were set up—arranged in a 100 m × 30 m rectangle with one central plot—and either one or two 0.1 × 1 m metal frames per plot were used to sample all aboveground plant tissue, separated into live grass, live forb, and standing dead/litter (two frames were used per plot when plant cover was more sparse). Soil was collected from each control plot in two pooled 2.5 cm wide × 5 cm deep soil cores. Soils and plants were dried at 60°C until they reached a constant weight. Soils were pooled and mixed into one sample; grasses and forb samples were weighed for biomass and ground.

One subsample of soil, ground grasses, and ground forbs from each site was analyzed by Cornell Nutrient Analysis Laboratory for ppm elemental chemistry (Al, As, B, Ba, C, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, N, Na, Ni, P, Pb, S, Si, Sr, Ti, and Zn) using hot plate digestion and inductively coupled plasma atomic emission spectroscopy.[5] C and N were measured with a CN elemental analyzer. With one exception, we used reviews of plant (Sharma 2006) and animal (McDowell 2003) ionomics to classify elements as metabolically essential for plants and animals (C, N, K, Ca, P, Mg, S, Fe, Mn, B, Zn, Cu, Mo; henceforth plant-essential), for just animals (Si, Na, Cr, Li, Ni, As, Co; henceforth animal-essential), and those elements with rare, or no known metabolic function (Pb, Al, Ba, Sr, Ti, Cd; henceforth nonessential). Contra McDowell (2003), we classified Pb (lead) as nonessential based on the limited evidence presented.

For climate data, we downloaded mean monthly precipitation PRISM data (PRISM Climate Group, Oregon State University).[6] This data set is gridded with a spatial resolution of 4 km. PRISM uses approximately 13,000 stations for the precipitation interpolation (Daly et al. 2008). We used these data to calculate the average annual precipitation of a site.

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

[6] http://prism.oregonstate.edu

Statistics

We used generalized linear models, selected using Akaike's information criterion, corrected (AIC_c) (Burnham and Anderson 2002) to evaluate the contributions of the drivers—supply side (soil ppm, precipitation), NP catalyst (ppm of N + P in plant tissue), nutrient dilution (ppm carbon in plant tissue, total plant biomass in a clip plot, precipitation), and grazing (presence/absence cattle grazing)—to each of the 25 elements and across the 54 grasslands. Grazing was indicated by an ordinal 1 vs. 0 in the model; soil and plant chemistry were $\log_{10}$-transformed, and were screened for low collinearity (variance inflation factor <2.0).

For each element, we used the MuMIn package (Bartoń 2018) in R version 4.0.1 (R Development Core Team 2019) to identify all possible models with a $\Delta AIC_c < 2$ relative to the best model (Burnham and Anderson 2002). Where more than one model met that criteria, we first defaulted to the null hypothesis (only intercept significant) if it existed within the top models ($\Delta AIC_c < 2$), then to the model with the fewest parameters, then, if there was more than one model that fit that criteria, the model with the lowest *P* value. Our goal was to tally evidence for each of the hypotheses for both nutrient-poor grass tissue and relatively rich forb tissue, across the three classes of element function.

RESULTS

The 54 grasslands varied 50-fold in their total dry biomass, from 2 g 0.1 m^{-2} in a Florida coastal prairie to 95 g 0.1 m^{-2} in a Colorado montane meadow. Grasses averaged about twice the biomass of forbs (15.7 vs. 8.5 g 0.1 m^{-2}), but the fraction of forb biomass varied from 0 g (none recorded) in southern sandy prairies to 10:1 forb:grass biomass in Colorado montane meadows.

The average plant and soil ionome covaried based on element functional class

To explore how the three classes of elements covaried with each other and with soil availability, we calculated the average ppm of all 25 elements across all the soils, grasses and forbs of all 54 grasslands. The average ppm of the three element classes covaried with soil availability (Fig. 2, Appendix S1: Table S2) but in different ways. The mean ppm of the plant-essential elements varied 10,000-fold and roughly tracked soil availability in both grasses ($\text{grass} = 131.8\text{soil}^{0.58}$) and forbs ($\text{forb} = 269\text{soil}^{0.61}$). Five elements—N, K, P, B, and Mo—averaged ppm in plant tissues well above the average of the soil they grew in (Fig. 2a, b). Forbs were roughly twice as nutrient dense as grasses on average (Appendix S1: Table S3; Fig. 2c—note data are plotted on a $\log_{10}$ scale).

In contrast, animal-essential elements (that are not essential to plants) almost always averaged lower concentrations in plant tissue than soil. Na was the one exception, having higher concentrations in plant tissues than soil. Other animal-essential elements that were uncommon in soil were even more so in plant tissue ($\text{grass} = 0.00\text{soil}^{3.0}$, $\text{forb} = 0.00\text{soil}^{2.7}$; Fig. 2d, e). The ppm of all nonessential nutrients except Sr were lower in plant tissue and increased with the element's average soil availability ($\text{grass} = 0.02\text{soil}^{0.76}$, $\text{forb} = 0.08\text{soil}^{0.74}$; Fig. 2g, h). Both animal-essential and nonessential elements were found in similar densities in grasses and forbs (animal-essential: $\text{forb} = 0.79\text{grass}^{0.88}$, plant-essential, $\text{forb} = 4.0\text{grass}^{1.0}$; Fig. 2f, i).

Four hypotheses combine to account for grass and forb ionome variation

The four hypotheses varied in their ability to account for the 10- to 100-fold variation in grass and forb ionomes. Grass models accounted for 0 (Si) to 62% (K and S) with r^2s averaging 33% (Table 1). Models for forb ionomes accounted for 0 (Si, Cd) to 64% (K) across 50 grasslands (four sites yielded too little forb tissue for analysis) averaging 27% of the variation (Table 1). The four hypotheses were generally better at accounting for continental variation in plant-essential elements (mean r^2 = 41% and 32% for forbs and grasses) than for animal-essential and nonessential elements (mean r^2 = 27% and 25%, and r^2 = 24% and 21%, respectively).

Supply side.—Soil availability and precipitation frequently constrained tissue concentrations, particularly for the 12 plant-essential elements (Table 1, Appendix S1: Tables S4, S5). Both grasses and forbs tracked soil availability of K, Ca, P, Mg, S, Mn, and Zn. B and Cu tissue ppm did not. Grass, but not forb, tissues tracked the availability of N and Mo. Among seven animal-essentials, only Na content of both forbs and grasses tracked availability via increases in precipitation. Grasses, but not forbs, tended to mirror the availability of five of six nonessential elements (Table 1).

NP catalysis.—The ppm of N + P in plant tissue was commonly correlated with other plant-essential elements for grasses but not forbs. Grass ionome concentrations of K, Ca, Mg, S, Fe, Mn, Zn, and Cu, covaried positively with NP; in forbs, only Mo increased with the NP in tissue. Animal-essential Ni and Co increased with NP in both grasses and forbs, with Cr also enhanced in forbs. Nonessential elements rarely tracked NP; we recorded such associations only in Al, and then only for grasses.

Nutrient dilution.—Drivers associated with increased plant production or biomass—C ppm, plant biomass, and precipitation—were associated with element depletion in forbs far more than grasses. Ten of 12 Plant-Essential nutrients—all but Co and Mo—declined with increasing C ppm in forbs, and this was true for all but one animal-essential (Si), and four of six nonessential

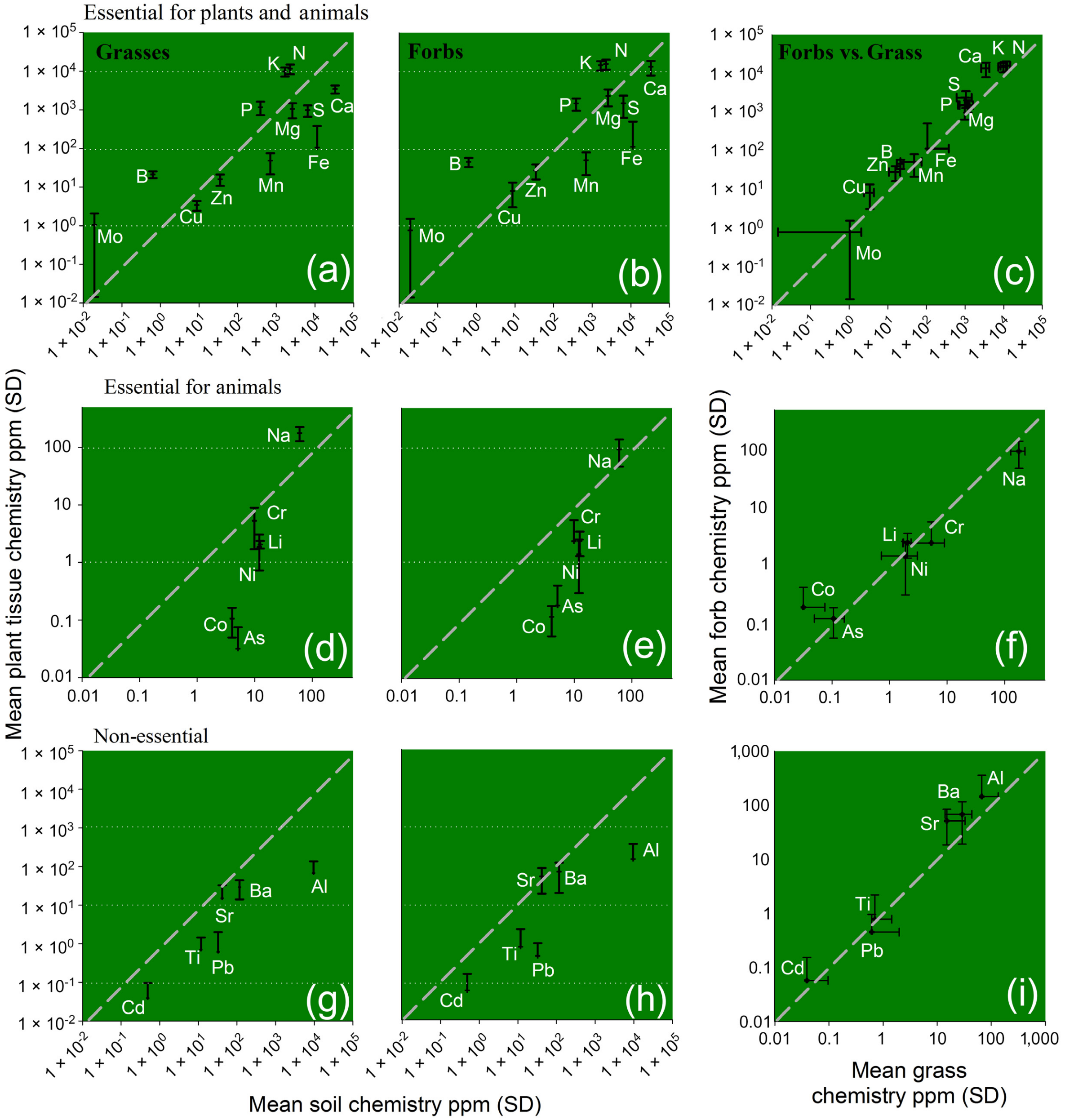

FIG. 2. In the top tier, (a) and (b) contrast the parts per million of elements—essential to plants and animals—in the soil vs. in the grasses and forbs sampled by clip plots from that soil; (c) contrasts the average ionomes of the same grasses and forbs for that functional group of elements. The middle tier—(d), (e), and (f)—does the same for elements essential to animals but not plants. The bottom tier—(g), (h), and (i)—does the same for elements with no known metabolic function in plants and animals.

elements. Nutrient dilution was less common in the grass ionome, occurring in only three plant-essential elements (S, B, and Mo). But like the forbs, four animal-essential elements (Na, Cr, Li, Co) were depleted with increases in carbon availability, but no nonessential elements were.

Grazing.—The nine grasslands grazed by cattle showed enhanced densities of three plant-essential elements in forbs (B, Zn, Cu) and one (Fe) in grasses. Grazing enhanced animal-essential Cr in both grasses and forbs, and two more (Ni and Co) in grasses. Grazing had little effect on the densities of nonessential elements in either grasses or forbs.

DISCUSSION

Grasslands comprise 40% of Earth's terrestrial surface, and those grasses and forbs provide food for

Table 1. Summary of responses of the ionomes of grasses and forbs from 54 North American grasslands (see Appendix S1: Tables S4, S5 for Akaike's information criterion, corrected informed regressions).

	Grass					Forb				
	r^2	+Supply	+NP	+CHO	+Grazing	r^2	+Supply	+NP	+CHO	+Grazing
Plant+										
N	17	+	. . .			9		. . .	–	
K	62	+	+			64	+		–	
Ca	53	+	+			47	+		–	
P	42	+	. . .			32	+	. . .	–	
Mg	21	+	+			27	+		–	
S	62	+	+	–		51			–	
Fe	49		+		+	40			–	
Mn	14	+	+			10	+		–	
B	45			–		48			–	+
Zn	41	+	+			29	+		–	+
Cu	61	+	+			6				+
Mo	30			–		17		+		
Animal										
Na	42	+		–		17	+		–	
Cr	40			–	+	57		+	–	+
Li	16			–		11			–	
Ni	35		+		+	26		+	–	
Si	0	. . .				0	. . .			
As	19					18			–	
Co	36		+	–	+	45		+	–	
NE										
Al	29		+		+	46			–	
Pb	32	+				29	+			
Ba	36	+				7			–	
Sr	13	+				13			–	
Ti	29	+				33			–	
Cd	5	+				0				

Notes: Elements essential for plants and animals are labeled Plant+; those essential for animals but not plants are labeled Animal; those with no clearly understood biological function are labeled NE. r^2 represents percentage of variation accounted for by best model. Ellipses (. . .) represent hypotheses not tested.

Earth's herbivores, from aphids to elephants. Across North America the elemental ppm per random bite of plant tissue varies orders of magnitude; some elements essential for animals, like Na, vary 1,000-fold across grasslands (Appendix S1: Tables S4, S5). Here we show that up to 2/3 of ionome variation can be attributable to four mechanisms, all with drivers that are changing in systematic ways in the Anthropocene. Grass ionomic variability from site to site most reflects the supply of nutrients in the soil; and increases in the macronutrients N and P are associated with an increase of seven of eight other plant-essential elements. The 10-fold greater geographical variability in forb ionomes depends less on soil availability (six vs. nine plant-essential nutrients) but is far more likely to decline with correlates of the quantity of carbohydrates fixed, the process called nutrient dilution. Although there were some key similarities—animal-essential nutrients not required by plants appeared to be mostly shunned by plant transporters—a repeated, unexpected, pattern was the divergent modes of ionome regulation among grasses and forbs, with implications for the grassland herbivores that frequently fall into corresponding functional groups: gramivore, forbivore, and mixed feeder (Joern et al. 2012, Welti et al. 2019*a*).

Plant ionomes broadly reflect soil availability but show selection

Our data suggest three functional explanations for the geography of plant ionomes. First, the average ionome of grasses and forbs roughly mirrors the availability of these elements in the soil. Frausto da Silva and Williams (2001) suggest this reflects the opportunistic nature of the evolution of life: building tissues from elements most readily at hand. However, plants accumulate five elements—N, P, K, B, and Mo—above that predicted by soil levels, and these elements are thus key prospects for nutrient limitation (Liebig 1855). Experimental additions of N, P, and K are already known to enhance grassland biomass (e.g., Harpole and Tilman [2007], Firn et al. [2019]). The ecological role of boron (a key structural component of cell walls) and Mo (a co-factor in enzymes processing nitrogen [Sharma 2006]), have been less well explored, even though both are regularly added in micronutrient cocktails (Borer et al. 2014).

That grass and forb transporters avoid animal-essential nutrients makes sense if accumulation would increase the risk of herbivory (Blair-West et al. 1968). The one exception, Na, is accumulated in grasses and forbs, especially in wetter environments. As Na is increasingly shown to limit animal populations (Jones and Hanson 1985, Welti et al. 2019*b*), one working hypothesis is that Na accumulation by plants, added to exudates, works to lure and manipulate the behavior of mutualists like pollinators and ant guards (Kaspari 2020, Kaspari et al. 2020).

. . .that may in turn, reflect higher nutrient use efficiency in grasses

A number of our results are consistent with a grasses' higher nutrient use efficiency—the ability to survive in nutrient-poor environments (Vitousek 1982)—compared to forbs. Grasses have a higher ratio of CHOs to plant-essential elements (Fig. 2c; see also Han et al. [2011], for an analysis at the species level). If forbs, in contrast, specialize on high-nutrient patches compared to grasses, this can alter the balance of competition for nutrients, water, and light (Harpole et al. 2016). In this scenario, forbs use their richer ionome to build leaves full of photosynthetic machinery (and, perhaps, defensive compounds [Rosenthal and Janzen 1979]). Then, a lifting of constraints to photosynthesis brought by, say, increased rainfall, are best invested in carbon skeletons that lift those leaves above the grassland canopy so as best to compete for light. The added CHOs needed to support taller competitors (Elser et al. 2010, La Pierre et al. 2011) may thus underlie the role of nutrient dilution as a dominant driver of the ionomes of grassland forbs (Table 1). It would also help account for the 10-fold more variation in forb vs. grass biomass across 54 sites as forbs differentially respond to variation in ecosystem productivity.

In the same vein, grass root transport systems appear to be less discriminating, perhaps toward scrounging every available ion in nutrient-poor soils. The accumulation of nonessential elements by grasses makes little functional sense, and often is attributed to nonessential ions mimicking the chemical properties of essential ions (e.g., strontium for calcium, cadmium for Fe [Cataldo and Wildung 1978]). Discriminating against harmless (if not helpful) elements like Sr may be relatively more costly if it interferes with uptake of that element's useful chemical doppelgänger, Ca. Low ppm of plant-essential nutrients itself—as in grasses compared to forbs—can also inhibit the ability for plant roots to discriminate against nonessential elements such as Pb (Foy et al. 1978), likely through the inability to build effective root transporters.

Third, if grasses specialize in generating more biomass with less nutrients, their ionome economy may be more set up to follow the NP-catalysis hypothesis, harvesting metals like K, Ca, Mg, Mn, Zn, and Cu (Table 1) on an "as needed" basis when N and P supplies increase. Note that our evidence for NP catalysis in grasses cannot simply be attributed to more overall fertile soils; increases in K, Ca, Mg, Mn, Zn, and Cu occurred with NP in tissue on top of increases predicted by the supply side hypothesis. In contrast, Mo was the only plant-essential element in forbs that behaved consistent with NP catalysis; Mo is a co-factor for processing nitrogen compounds (Sharma 2006).

Implications for herbivores

Nutrient dilution can occur in any system in which carbohydrate production is enhanced without a change in underlying biogeochemistry of the soil or aerosols. Nutrient dilution reduces ionomic concentrations of animal-essential elements in both grasses and forbs (see also Fan et al. [2008], Welti et al. [2020*b*]). Although evidence for nutrient dilution with increasing $p\mathrm{CO}_2$ has been demonstrated via CO_2 enrichment (Robinson et al. 2012), temperature and precipitation can drive the 10- to 100-fold geographic and interannual variation in biomass production across grasslands, and can play the same role in generating variation in plant ionomes (Anderson et al. 2018, Welti et al. 2020*b*). Our evidence for nutrient dilution across the plant ionome, especially for forbs, and for animal-essential elements for both grasses and forbs, suggest rich possibilities for the regulation of herbivore populations (Welti et al. 2020*a*, *b*). This is especially true given the role of micronutrient "cocktails" in enhancing invertebrate abundance (Kaspari et al. 2017), especially their role of catalyzing NP fertilization in grasslands (Prather et al. 2020).

Caveats and future work

One of the challenges in the emerging field of ecological stoichiometry/ionomics is our imperfect knowledge of the role of each element in organism performance (McDowell 2003, Sharma 2006). "Trace" elements are difficult to manipulate and may show nonlinear, even unimodal effects on plant performance (Lyu et al. 2017). Moreover, as the difference between essential and nonessential is based on the absence of experimentally induced performance, it is easy to imagine how an element critical to herbivore resistance, evaluated in a sterile lab, may fail its "essentiality exam." As most of what we know about essentiality has focused on agronomic studies of growth and yield, there are doubtless holes in our understanding—and great opportunity for collaboration—between ecological stoichiometry and ionomics.

Likewise, our focus on aboveground biomass and chemistry in response to climate, grazing, and soil availability misses the belowground picture of roots, mycorrhizae, and microbes. For example, an alternative to our working hypothesis for the high incidence of nutrient dilution in forbs (i.e., that they have lower nutrient use efficiency) would invoke the increased nutrient stores in the roots of grasses, allowing them to maintain constant leaf tissue ppm in times of enhanced productivity (but see Welti et al. [2020*b*]).

A second working hypothesis that can account for the apparent nutrient dilution of P in P-rich forbs (Table 1) reflects interactions with microbial competitors. Cellular titers of P increase with an organism's growth rate (Elser et al. 1996), suggesting that soil bacteria are effective competitors with plants for P (Kaspari and Yanoviak 2008). Higher rates of NPP may thus boost the quantities of sugary root exudates (Kaspari et al. 2020), in turn promoting bacterial biomass and reducing the P available to plants (Blair 1997). Both field and lab studies that take into account belowground interactions will likely further improve the application of ionomics (Table 1).

A related, unanswered question from our clip-plot–based study is how these elements are distributed within the plant. This is especially germane to the effects on the local herbivore community. Consider the scenario that nutrient dilution in a clip plot results from increases in CHO-rich stems, but leaf ionomes remain unchanged (La Pierre et al. 2016). Grasshoppers that eat only leaves may not experience any change in diet, whereas large grazers that eat grasses and forbs by the mouthful would experience nutrient dilution. Studies of the nutrient content of plant parts is a logical next step in the ionomics of the world's grasslands.

The makeup of plant ionomes has striking implications for the fitness of plants and their herbivores. This is especially true as the drivers embedded in our hypotheses are changing. Soil ionomes are altered by changes in atmospheric deposition (Stevens et al. 2004), erosion (Quinton et al. 2010), and levels of cattle grazing (Sanderson et al. 2020). Changing patterns of precipitation and $p\mathrm{CO_2}$ can in turn alter a grassland's balance of CHOs to other elements (La Pierre et al. 2016, Knapp et al. 2017, Gherardi and Sala 2019, Welti et al. 2020*b*). This snapshot experiment across 54 grasslands captures mechanisms operating as they are, but provides a roadmap to future changes in grassland ionomes tracking ongoing changes in the Anthropocene.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.3459/suppinfo

OPEN RESEARCH

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