



Herbivory and eutrophication mediate grassland plant nutrient responses across a global climatic gradient

T. MICHAEL ANDERSON ^{1,21} DANIEL M. GRIFFITH,² JAMES B. GRACE ³ ERIC M. LIND,⁴ PETER B. ADLER,⁵ LORI A. BIEDERMAN,⁶ DANA M. BLUMENTHAL,⁷ PEDRO DALEO,⁸ JENNIFER FIRN,⁹ NICOLE HAGENAH,^{10,A} W. STANLEY HARPOLE,^{11,12,13} ANDREW S. MACDOUGALL,¹⁴ REBECCA L. MCCULLEY,¹⁵ SUZANNE M. PROBER,¹⁶ ANITA C. RISCH,¹⁷ MAHESH SANKARAN,^{18,19} MARTIN SCHÜTZ,¹⁷ ERIC W. SEABLOOM ⁴ CARLY J. STEVENS,²⁰ LAUREN L. SULLIVAN,⁴ PETER D. WRAGG,⁴ AND ELIZABETH T. BORER⁴

¹Department of Biology, Wake Forest University, Winston-Salem, North Carolina 27109 USA

²Department of Forest Ecosystems and Society, Oregon State University, Corvallis, Oregon 97333 USA

³US Geological Survey Wetland and Aquatic Research Center, 700 Cajundome Blvd, Lafayette, Louisiana 70506 USA

⁴Department of Ecology, Evolution, and Behavior, University of MN, St. Paul, Minnesota 55108 USA

⁵Department of Wildland Resources and the Ecology Center, Utah State University, Logan, Utah 84322 USA

⁶Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, Iowa 50011 USA

⁷USDA-ARS Rangeland Resources & Systems Research Unit, Fort Collins, Colorado 80526 USA

⁸Instituto de Investigaciones Marinas y Costeras (IIMyC), UNMdP, CONICET, Mar del Plata, Argentina

⁹School of Earth, Environment and Biological Sciences, Queensland University of Technology (QUT), Brisbane, Queensland 4001 Australia

¹⁰School of Life Sciences, University of KwaZulu-Natal, Scottsville, South Africa

¹¹Helmholtz Center for Environmental Research – UFZ, Department of Physiological Diversity, Permoserstrasse 15, 04318 Leipzig, Germany

¹²German Centre for Integrative Biodiversity Research (iDiv), Deutscher Platz 5e, Leipzig 04103 Germany

¹³Martin Luther University Halle-Wittenberg, am Kirchtor 1, Halle (Saale) 06108 Germany

¹⁴Department of Integrative Biology, University of Guelph, Guelph, Ontario N1G 2W1 Canada

¹⁵Department of Plant and Soil Sciences, University of Kentucky, Lexington, Kentucky 40546 USA

¹⁶CSIRO Land and Water, Private Bag 5, Wembley, Western Australia 6913 Australia

¹⁷Swiss Federal Institute for Forest, Snow and Landscape Research, Community Ecology, Birmensdorf 8903 Switzerland

¹⁸Centre for Biological Sciences, TIFR, Bangalore 560065, India

¹⁹School of Biology, University of Leeds, Leeds LS2 9JT UK

²⁰Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ UK

Abstract. Plant stoichiometry, the relative concentration of elements, is a key regulator of ecosystem functioning and is also being altered by human activities. In this paper we sought to understand the global drivers of plant stoichiometry and compare the relative contribution of climatic vs. anthropogenic effects. We addressed this goal by measuring plant elemental (C, N, P and K) responses to eutrophication and vertebrate herbivore exclusion at eighteen sites on six continents. Across sites, climate and atmospheric N deposition emerged as strong predictors of plot-level tissue nutrients, mediated by biomass and plant chemistry. Within sites, fertilization increased total plant nutrient pools, but results were contingent on soil fertility and the proportion of grass biomass relative to other functional types. Total plant nutrient pools diverged strongly in response to herbivore exclusion when fertilized; responses were largest in ungrazed plots at low rainfall, whereas herbivore grazing dampened the plant community nutrient responses to fertilization. Our study highlights (1) the importance of climate in determining plant nutrient concentrations mediated through effects on plant biomass, (2) that eutrophication affects grassland nutrient pools via both soil and atmospheric pathways and (3) that interactions among soils, herbivores and eutrophication drive plant nutrient responses at small scales, especially at water-limited sites.

Key words: climate; eutrophication; fencing; fertilizer; grasses; herbivores; N deposition; Nutrient Network (NutNet); nutrients; solar insolation; stoichiometry.

INTRODUCTION

The relative concentration of elements in living tissues, i.e. ecological stoichiometry, is a fundamental organismal property regulating processes from cell metabolism to growth and reproduction (Sternner and Elser 2002). In plants, nutrient concentrations influence primary production, herbivore

consumption and decomposition, thus regulating ecosystem energy flow (Elser et al. 2010). Macronutrients, such as N and P, play essential roles in cellular and metabolic processes – N is a major constituent of photosynthetic enzymes (i.e., RuBisCO) and P is in high demand by ribosomal RNA during growth and development (Elser et al. 2003). Consequently, N and P are widely acknowledged as the elements that limit primary productivity (Güsewell 2004). However, recent studies suggest that less well-studied elements, such as K, may also limit or co-limit global plant productivity (e.g., Fay et al. 2015).

Across terrestrial ecosystems, plant nutrient concentrations vary predictably with latitude, with %N and %P decreasing towards the tropics (Reich and Oleksyn 2004,

Manuscript received 11 October 2017; revised 2 December 2017; accepted 20 December 2017. Corresponding Editor: Joseph B. Yavitt.

^ACurrent address: Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa

²¹E-mail: anderstm@wfu.edu

Borer et al. 2013). Nutrient supply rates can directly change plant tissue chemistry (Bracken et al. 2015) and, thus, one hypothesis to explain the latitudinal gradient in plant tissue chemistry is based on changing nutrient supply rates with latitude. Specifically, this “nutrient supply hypothesis” suggests that higher temperatures near the tropics promote greater carbon fixation per unit enzyme thus diluting N, while tropical soils are highly weathered thus also limiting P availability (Reich and Oleksyn 2004). However, the alteration of nutrient supply can occur by several major pathways. First, soil fertility, rates of decomposition and microbial processes can vary within and across sites in ways that alter the rate of nutrient supply to plants. A second major source of altered nutrient supply is via anthropogenic eutrophication, which itself can occur by two major pathways, including atmospheric deposition and agricultural application of fertilizers. Atmospheric nutrient deposition is a large, landscape-level phenomenon, while fertilization and agricultural runoff can vary across regions as well at finer scales depending on the location of point sources and watershed topography.

Recent theory has attempted to explicitly link temperature to plant stoichiometry across global gradients (e.g., Kerkhoff et al. 2005, Allen and Gillooly 2009). However, the convergence in tissue %N and %P across global latitudinal gradients and similar scaling of %N and %P (i.e., the slope of the $\log N \sim \log P$ regression) across freshwater, marine and terrestrial ecosystems, leads to the alternative hypothesis that growing season length or solar radiation reaching earth’s surface (insolation) is responsible for global variation in producer nutrient concentrations (Borer et al. 2013).

Resolving the mechanisms that control plant stoichiometry within and across ecosystems has important implications for understanding food web structure. Across ecosystems, producer tissue %N and %P are strong predictors of the ratio of herbivore to producer (H:P) biomass and herbivore consumption rates (Cebrian et al. 2009, Hillebrand et al. 2009). On the other hand, herbivores alter plant stoichiometry directly by changing nutrient concentrations in re-growing tissues, or indirectly, by influencing plant growth rates, resource supply, or species composition (Hobbie 1992, Bardgett and Wardle 2003, Piñeiro et al. 2010, Cherif and Loreau 2013). Herbivore influences on nutrient cycling and plant nutrient concentrations have significance because humans are reducing native, large-bodied herbivore populations throughout the world’s grassland and savannas (e.g., Craigie et al. 2010, Ceballos et al. 2015, Ripple et al. 2015).

Another way that humans are altering Earth’s biogeochemical cycles is through intensified agricultural practices and atmospheric nutrient deposition (e.g., Vitousek et al. 1997, Stevens et al. 2004). Global anthropogenic sources of N applied in fertilizers is $\sim 77.4 \pm 4.6$ Tg/yr (Table 2 in Potter et al. 2010), while atmospheric N deposition, derived from local fertilizer and industrial sources, has nearly doubled from pre-industrial levels (from ~ 22 Tg N/yr to ~ 39 Tg N/yr) and is projected to double again by 2100 (Intergovernmental Panel on Climate Change 2000; Krishnamurthy et al. 2007). Anthropogenic nutrient inputs impact plant communities by altering plant growth rates, tissue stoichiometry, rates of herbivory and community composition (e.g., Pardo et al. 2011). In grasslands and savannas, which

cover >25% of the terrestrial biosphere (Scholes and Archer 1997, Asner et al. 2004), eutrophication and herbivore loss are occurring concomitantly, with important, and potentially interacting, consequences for ecosystem function and biodiversity (Borer et al. 2014a, Hautier et al. 2014).

One goal of our work was to analyze the strength of local-scale disturbances, such as eutrophication and the loss of large herbivores, on controlling plant stoichiometry within the context of broad-scale climate factors. Studies investigating total nutrient stocks have largely focused on C and N and typically within single ecosystems (e.g., Schuman et al. 1999, Green and Detling 2000), with few studies seeking controls across global extents (but see Wang et al. 2010). Consequently, the goal of our study was to compare the relative contributions of (1) global climate factors, including temperature, precipitation, solar radiation and N deposition (e.g., Stevens et al. 2015), (2) herbivory and (3) nutrient supply on concentrations of nutrients at the plant- (e.g., nutrient concentrations, g per g tissue) and community-level (e.g., on an areal basis, g/m^2). As herbivore and fertilization effects may depend on climate and background nutrient availability, we also tested for statistical interactions among eutrophication, herbivory, climate and variation in soil fertility across a global range of sites.

In our first analysis, we experimentally manipulated nutrient supply (NPK fertilization) and herbivory (fencing) and used an analysis of covariance to ask how the relative quantity of nutrients (C, N, P and K) in the standing biomass (measured in g/m^2 , hereafter “total plant nutrients”) varied across a global climate gradient (model one). For model one, we predicted that total plant nutrients would increase under eutrophication and that the magnitude of this effect would increase with rainfall. However, we also predicted that the response would be amplified by fencing at high rainfall sites, because we expected the abundance of nutrient-rich, palatable plants would increase where herbivores were excluded and water was abundant. Thus, we expected an interaction between eutrophication and herbivory across sites. This prediction results from work demonstrating that herbivores can selectively eliminate nutrient-rich, palatable plant species and herbivore-induced changes in species composition are greater at mesic compared to arid sites (e.g., Olf and Ritchie 1998, Chase et al. 2000). On the other hand, if total plant nutrients across sites is controlled largely by herbivore effects on biomass, instead of species composition (i.e., plant species turnover), then herbivore exclusion may lead to greater relative impacts of eutrophication at arid sites, where herbivore effects on biomass are the largest (e.g., Chase et al. 2000).

Subsequently, we analyzed the network of hypothesized direct and indirect effects of climate, herbivory and nutrient supply on total plant nutrient responses (the sum of N, P and K) at multiple spatial scales by combining site- and plot-level predictors in a multi-level structural equation model (SEM, model two). For model two (the multi-level SEM), we decomposed the responses of total plant nutrients into the two alternative pathways of changes in plant tissue nutrient concentration and those driven by changes in plant biomass. We expected concentrations of N, P and K to decrease with increasing temperature across sites, as reported elsewhere (e.g., Reich and Oleksyn 2004, Borer et al. 2013). Within sites, we predicted that fertilization and

herbivory would have strong direct effects on nutrient responses through their effects on plant tissue chemistry and plant biomass (Ferraro and Oesterheld 2002, Anderson et al. 2013), but that these effects would depend on background soil nutrient supply, i.e., the largest responses were expected in plots with low soil nutrients. Finally, in terms of their strength in controlling variation in total plant nutrient pools, we predicted that plot-level drivers (e.g., herbivores, eutrophication and resource supply) would be comparable in magnitude to broad-scale, site-level drivers (e.g., rainfall, temperature, insolation and N-deposition).

METHODS

Site location and study design

Our study was conducted at 20 sites in Africa, Asia, Australia, Europe, North and South America (Fig. 1) as part of the Nutrient Network (NutNet; Borer et al. 2014b). Sites spanned a gradient of mean annual precipitation (MAP) from 305 to 2315 mm/yr (Appendix S1: Table S1), but all were in grass-dominated plant communities. At each site, fully factorial combinations of nutrient addition \times fencing were randomly assigned to 25-m² plots arranged in 3–6 blocks within sites from 2007 to 2011 (Appendix S1: Table S1). Within each 25-m² plot, randomly chosen 1-m² quadrats were selected in one of four sectors for continuous data collection. In nutrient addition plots, NPK was added annually at the onset of the growing season as a combination of nutrients at the following rates: slow release urea (10 g N m⁻² yr⁻¹), triple super phosphate (10 g P m⁻² yr⁻¹), and potassium sulfate (10 g K m⁻² yr⁻¹). In year one only, 100 g/m² of a micronutrient mix (Fe, S, Mg, Mn, Cu, Zn, B and Mo) was applied in the nutrient addition plots. In herbivore exclusion plots, fences were constructed of heavy gauge wire to heights of 120–180 cm designed to exclude mammalian herbivores >50 g. To exclude small mammals, most sites included a 1 cm wire mesh secured at the base of each fence to a height of 90 cm. NutNet sites include all combinations of N, P and K nutrient addition in the absence of fences (e.g.,

Fay et al. 2015), but here we chose to focus only on the factorial NPK \times fencing treatments given our emphasis on herbivore-nutrient interactions.

Sample collection and nutrient analyses

Plant biomass and tissue concentration of key elements (C, N, P, and K) (Elser et al. 2003, Fay et al. 2015) were sampled 3–5 yr after the establishment of the experiment at 16 sites and after either 1 or 2 yr at the remaining four sites (Appendix S1: Table S1). Aboveground plant biomass was clipped to ground-level in two replicate 10 \times 100 cm strips, sorted by functional type (grass, forb, legume and woody plants), dried and weighed to the nearest 0.1 g. Samples were transported to Wake Forest University where they were air-dried and ground in an UDY belt-drive sample mill, dried again at 65°C for 48 h and analyzed, by functional type, on a Bruker near infrared spectrophotometer (NIRS) (Bruker Optics, Ettlingen, Germany). Reflectance data from each sample were averaged from triplicate measurements between wavelengths of 781–2778 nm (12,800–3,600 per cm) at 16 nanometer resolution with the rotating cup method, except for samples <3 g, in which case samples were analyzed in a stationary vial (~19% of samples).

NIRS works by linking spectral reflectance data from each sample to calibration data collected on a subset of the samples using traditional wet chemical analysis (i.e., the “known” calibration samples). The spectral data were used to identify the subset of samples (20% of the total sample number), that were submitted for wet chemical analysis (Appendix S2: Supplemental Methods). The calibration samples were analyzed for total C, N, P, and K at either North Carolina State University or Kansas State University using Dumas combustion (C and N), flame atomic absorption or ICP Spectrometry after nitric-perchloride digestion (K) or the colorimetric industrial method 334-74W/B after sulfuric acid/hydrogen peroxide digestion (P). The resulting dataset was further subdivided into a calibration (model development) and validation (test set; 10%) subset using an algorithmic experimental design approach (Appendix S2: Supplemental Methods).

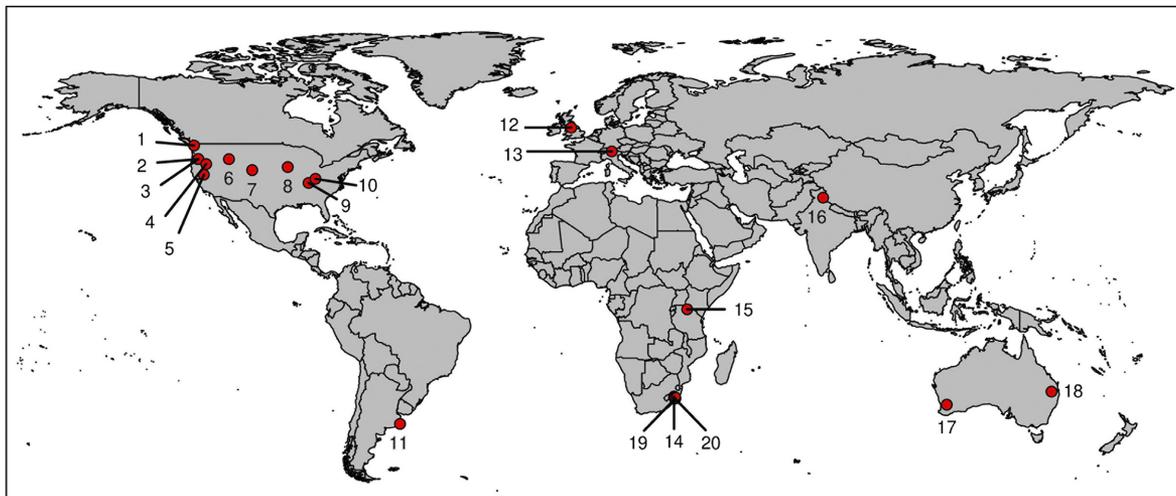


FIG. 1. Global map showing the distribution of the 20 NutNet sites across 6 continents represented in our analysis. See Appendix S1: Table S1 for the specific site information.

We measured the total nutrients in soils as indices of soil fertility and soil nutrient supply (separate from estimates of nutrient deposition). Soils were collected 2–4 yr post treatment (mean = 3.4 ± 0.8 yr; Appendix S1: Table S1) in each subplot to 10 cm (approximately 250 g soil), bagged, air-dried and sent to the University of Nebraska for archiving and total %C and %N analysis via dry combustion gas chromatography (Dumas method, COSTECH ESC 4010 Element Analyzer). As our interests were in soil fertility and its interaction with treatments, we focused on soil %N, as it is a widely acknowledged indicator of soil fertility and a major plant-limiting nutrient (e.g., Elser et al. 2007). Soils were unavailable from three sites (Appendix S1: Table S1) and soil %N was imputed for these sites because of the relatively strong relationship between soil N and major climate factors (Appendix S2: Supplemental Methods). Further details on sampling methodology are at http://www.nutnet.org/exp_protocol.

Data analysis

Effect of fertilization on total plot nutrients in grazed vs. ungrazed grassland (model one).—In our first model, we analyzed the effects of eutrophication and herbivore exclusion on total plot nutrients within the context of global environmental variation. To analyze plot-level responses to herbivores and eutrophication, we asked if the relative responses of total plant nutrients to fertilization depended on climate or soil fertility, and if the presence of herbivores altered the relationship. We focused on three climate factors with strong conceptual and empirical links to plant stoichiometry: temperature, rainfall, and solar insolation (Appendix S1: Table S1). For each site, we extracted mean annual temperature (MAT, °C) and mean annual precipitation (MAP, mm/yr) from WorldClim (<http://worldclim.org/>; Hijmans et al. 2005). For solar insolation (INS, kWh m⁻² d⁻¹), we extracted average annual data (1983–2005) from the NASA Surface meteorology and Solar Energy database (<http://eosweb.larc.nasa.gov/sse/>). Soil fertility at the plot-level was represented in the models by soil %N as described above.

For this analysis, all plots subjected to fencing (FENCE; fenced vs. control; $n = 18$, Appendix S1: Table S1) and fertilizer addition treatments (NPK; fertilized vs. control, $n = 20$, Appendix S1: Table S1) were included (2 fencing levels \times 2 NPK addition levels = 4 treatment combinations per block). Plot-level estimates of each nutrient (C, N, P and K) were obtained by summing, for all functional types in a plot, the product of their tissue nutrient concentration (% dry weight) and biomass in g/m², yielding the total plot nutrient content in aboveground biomass (g/m²) for each element. We then quantified the relative effects of nutrient addition by calculating log response ratios (LRR) within blocks at each site: $\log\left(\frac{\text{total nutrient content in fertilized}}{\text{total nutrient content in control}}\right)$. LRR for each block and site were plotted against INS, MAP, MAT and soil %N for both fenced and unfenced treatments. An analysis of covariance using the “lm” command in R (R Core Team 2017) was used to determine if the slopes of the LRR \sim environmental predictors were different for levels of FENCE.

To identify the best model, we followed a model selection procedure based on Akaike’s Information Criterion modified

for small sample sizes (AICc; Burnham and Anderson 2002). Candidate models included all main effects plus environment by treatment interactions. Models were selected as best fits to the data when ΔAICc values were <2 below that of other models. For models within a 1 ΔAIC unit of each other, the model with the fewest parameters was selected as the best model. After identifying the best model (see below), we tested for the significance of terms in the accepted model using type III sums of squares using the ANOVA command in R-package “car” (Fox and Weisberg 2011).

Structural equation model of total plot nutrient pools (model two).—In our second analysis, we used structural equation modeling (SEM) to quantify system-level influences of climate, soil fertility, herbivory and eutrophication on total plot nutrients (Grace et al. 2010). As the total plot nutrient content in plants is a product of multiple direct and indirect sources, our a priori model was driven by variation in three sources: (1) direct effects due to plant chemistry (i.e., nutrient concentration per g plant), (2) direct effects due to plant community biomass (g/m²) or (3) indirect effects due to variation in the abundance of functional types (i.e., grass vs. forb) among sites (Appendix S3: Fig. S1 and Appendix S1: Table S2). Due to the dominance of grasses across the sites and their important functional role, percent grass biomass (“%grass”) was included to account for functional type turnover among sites. External predictors were MAT, MAP, INS, atmospheric N deposition (N-DEP; Stevens et al. 2015), soil %N and the two treatment variables, NPK and FENCE, as discrete binomial predictors. In addition, we included a “grazing index” that accounted for site-level variation in herbivore abundance and diversity (Appendix S2: Supplemental Methods). Due to the hierarchical nature of the data, some predictors existed only at the site level ($n = 18$, Appendix S1: Table S1) while others existed for individual plots ($n = 175$; Appendix S3: Fig. S1). Consequently, we analyzed each response variable in a piecewise fashion using a multi-level approach (e.g., Gelman and Hill 2007, Appendix S2: Supplemental Methods). In the plot level model, we assumed a beta error distribution for the %grass due to the proportional nature of the data and modeled the response with the “glmmTMB” command in the *glmmTMB* package (Magnusson et al. 2017). We assumed Gaussian error distributions for other response variables and used the “lmer” command from the *lme4* package (Bates et al. 2015) with site as a random factor for the plot-level and “lm” for the site level model (Appendix S2: Supplemental Methods). Models were trimmed via AICc model selection using the “AICctab” from the *bbmle* package (Bolker and the R Development Core Team 2017) or the “stepAIC” command from the *MASS* package (Venables and Ripley 2002). Note that the final response variable in the SEM, total plot nutrient content in plants, is a mathematical product of the quantity of plant material in a plot and the nutrient concentration in plant tissue. For this reason, standardized path coefficients connecting plot biomass and plant chemistry to total standing nutrients were computed analytically rather than estimated. These computed parameters represent the contributions to variations in total standing nutrients derived from variation in component variables (Appendix S2: Supplemental Methods). We first conducted the analysis separately for each of

the elements (C, N, P and K). However, our SEM modelling revealed similar responses for N, P and K (Appendix S3: Figs. S3–S5); consequently, to increase the generality of our model results, we summed these nutrients on an areal basis (g/m^2) to create a single nutrient variable that was modeled as the response.

RESULTS

Effects of eutrophication in unfenced (grazed) vs. fenced (ungrazed) grassland (model one)

The ANCOVA analysis demonstrated that element responses to eutrophication across a global gradient in rainfall depended on the experimental exclusion of herbivores. For each of the elements analyzed (C, N, P and K), the best model identified by AICc included an interaction between MAP and FENCE on element log response ratio (LRR) under fertilization (Appendix S1: Table S3). All elements showed a consistently strong negative relationship between the LRR and MAP inside fenced plots, meaning that, in the absence of herbivores, the strongest effects of fertilization was at arid sites and there was no effect at mesic sites (Fig. 2). The presence of herbivores counteracted the strong effects of fertilization at dry sites, demonstrated by the flat relationship between MAP and LRR in grazed plots across a gradient of MAP ($P > 0.1$ for hypotheses that slopes and intercepts were non-zero in linear models for all elements in the fenced treatments). No other model was similar in its fit with LRR across sites (Appendix S1: Table S3) and the final

coefficient of determination (R^2) for the models were between 26% (for plot P) and 32% (for plot N).

SE model results of total plot NPK (model two)

For the SEM, our initial overall hypothesis was that herbivore exclusion and eutrophication would alter total grassland nutrients by influencing plant chemistry and plant biomass, and that their effects would be similar in magnitude. Except for a somewhat weaker coefficient of determination for whole plant [P] ($R^2 = 0.26$) compared to [N] and [K] ($R^2 = 0.52$ and 0.62 , respectively), the nutrients showed similar responses when analyzed separately (Appendix S3: Figs. S3–S5; Appendix S1: Table S4). Therefore, we focus our results on the summed NPK response variable at the plant and plot level. The final SE model had coefficients of determination (R^2) of 0.33 for %grass, 0.49 for total plot biomass and 0.58 for total plant NPK (Fig. 3). Here, we present standardized path coefficients for the final SE model (both standardized and unstandardized coefficients are presented in Appendix S1: Tables S5 and S6). The model results support the interpretation that there are strong direct effects of climate variables, especially MAT and INS, on %grass and plot biomass, and somewhat weaker influences of climate on plant chemistry (plant NPK). MAT influences on %grass and biomass were positive (1.09 and 1.05), while plant NPK decreased with INS (-0.69). The only direct influence of MAP in the model was a positive effect on plant NPK (0.37). INS had relatively strong negative influences on both %grass (-0.90) and plot biomass (-1.0). After

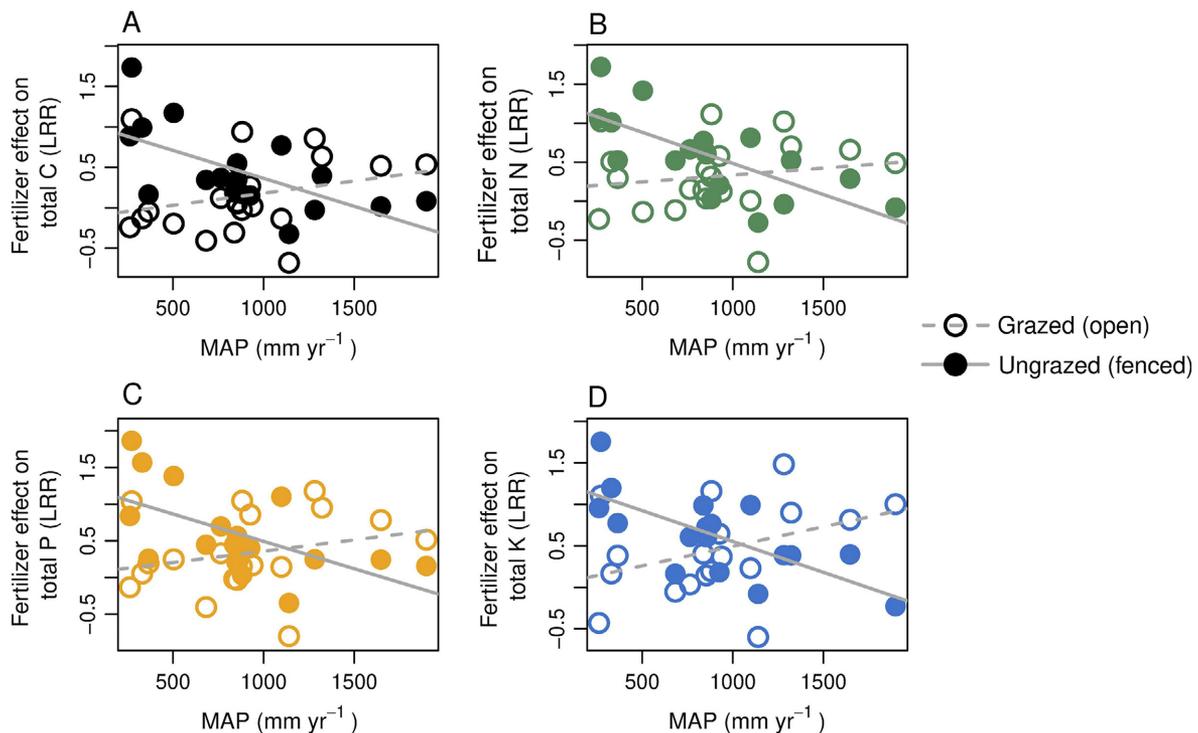


FIG. 2. Effect of fertilizer addition, expressed as a log response ratio (LRR: $\log[\text{NPK addition/control}]$) on total plot carbon (A), nitrogen (B), phosphorous (C) and potassium (D) plotted against mean annual rainfall from grassland plots from 18 sites inside (closed circles, solid lines) and outside (open circles dashed lines) fences. Solid and dashed lines represent significantly different fencing \times MAP interaction across sites (see Appendix S1: Table S3).

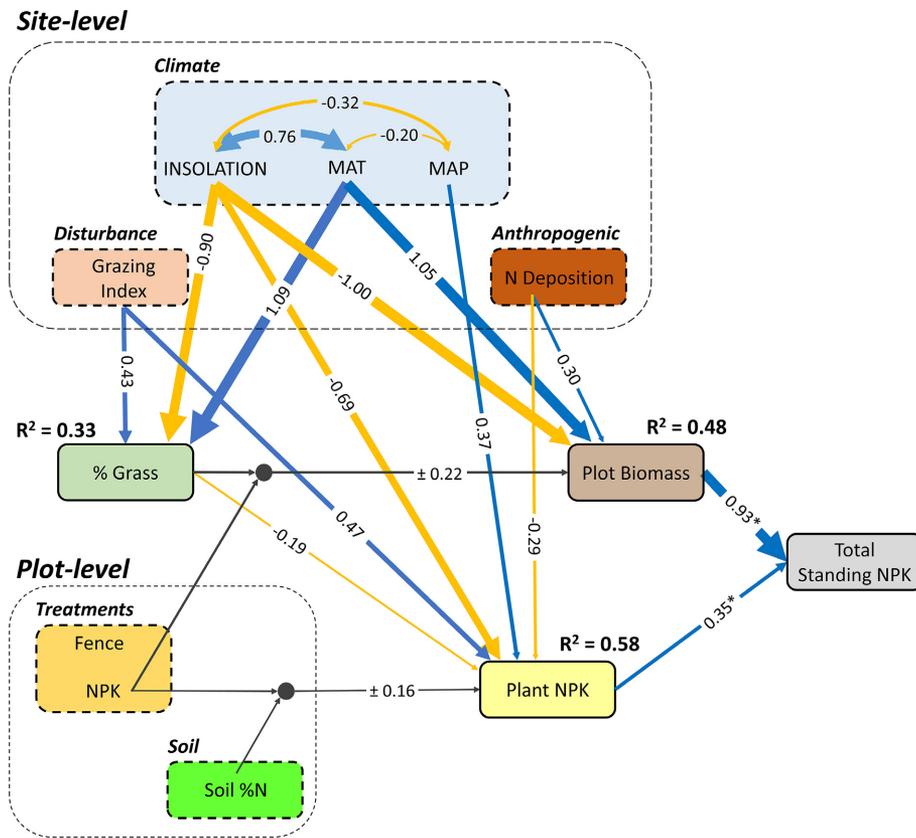


FIG. 3. Final structural equation model results. The figure depicts direct and indirect effects of predictors (climate [insolation, MAT and MAP], grazing index, N-deposition, treatment effects [fences and NPK addition] and soil %N) on response variables (%grass, plot biomass [g/m²], plant NPK [sum of N, P and K in % dry weight] and total standing NPK [sum of N, P and K in g/m²]). Response variables are shown in solid and predictor variables are shown in bold-dashed boxes. Predictors of a similar type are grouped within bold-dashed boxes to facilitate interpretation (such as climate variables and treatments). The hierarchical structure of the model is represented by thin-dashed boxes which enclose predictors into site- and plot-level groups. Interaction effects are depicted with grey arrows pointing to a solid grey dot which points at the response variable. Curved arrows represent correlations while straight arrows imply causal effects of one variable on another. Numbers on arrows are standardized path strengths with arrow widths proportional their values; blue arrows show positive and orange negative relationships. Bold numbers associated with the response variables are total variance explained (R^2) by all significant predictors. Note that total standing NPK in a plot is a mathematical product of the plot biomass and plant nutrient concentration; therefore, these path coefficients (labeled with “*” in the diagram) are computed rather than estimated and they represent the sensitivity of variations in total standing NPK derived from variation in component variables. See Appendix S1: Table S6 for standardized total effects of predictors on the response variables.

accounting for climate effects, N deposition increased plot biomass (0.30) and decreased plant NPK (-0.29). The reduction in plant NPK with N-DEP, together with the increase in plant C with N-DEP when elements were analyzed separately (e.g., path coefficient = 0.41; Appendix S3: Fig. S2), suggests a growth-induced nutrient dilution in plant tissues due to increased C:nutrient ratios.

While fencing was not significant in the final SE model, the grazing index was positively related to %grass (0.43) and plant NPK (0.47) at the site-level. At the plot-level, there was a relatively weak response of plant NPK to an interaction between NPK fertilizer and soil %N (0.16; Fig. 3) and a somewhat stronger response of plot biomass to an interaction between NPK fertilization and %grass (0.22; Fig. 3). For plant NPK, the interaction arose from a positive response of plant NPK to the soil fertility gradient (soil % N) in unfertilized plots and a negative response in fertilized plots (Fig. 4). For the plot biomass, the interaction arose from a positive relationship between %grass in plots and total biomass in the absence of NPK fertilization and a negative relationship for plots fertilized with NPK (Fig. 5).

However, inspection of the relationship demonstrates that the interaction is driven by a large biomass response at low %grass in fertilized plots and a relatively stable response of high %grass plots to fertilization (Fig. 5).

After computing standardized coefficients, plot biomass had 2.7 times the influence on the variance in total standing NPK compared to plant NPK (0.93 vs. 0.35). In terms of total effects on total standing NPK (i.e., direct + indirect effects), INS had the strongest negative effect (-1.11), which was mediated by strong negative influences on biomass and plant NPK, which in turn had positive relationships with standing NPK (Appendix S1: Table S6). These negative effects of INS on NPK were offset by a weak positive (0.06) effect of INS that was mediated by %grass and plant NPK. MAT had the strongest positive effect on plot standing NPK content (0.90), which was mediated by its strong positive association with plot biomass that was offset by a weaker negative effect of MAT that was mediated by %grass (Appendix S1: Table S6). MAP had a weaker positive effect on total standing NPK (0.13), which was mediated by its positive effects on plant NPK.

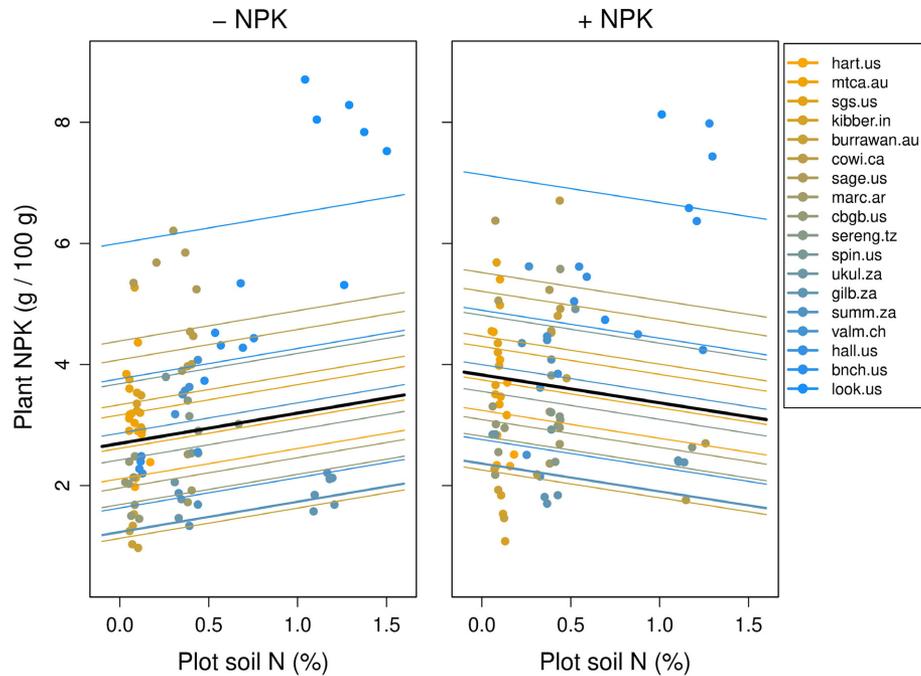


FIG. 4. Relationship between plot soil fertility (soil %N) and plant NPK (% dry weight) in control plots (left panel) and experimentally fertilized plots (right panel) across 18 sites that separated samples by plant functional type (grass, forb and legume). Points are replicate plots within sites and are labelled in order from low (orange) to high (blue) precipitation; for site information and identities see Appendix S1: Table S1. Lines show random intercepts fit to individual site with the mean interaction response (either $-NPK$ or $+NPK$) shown as the bold, black line.

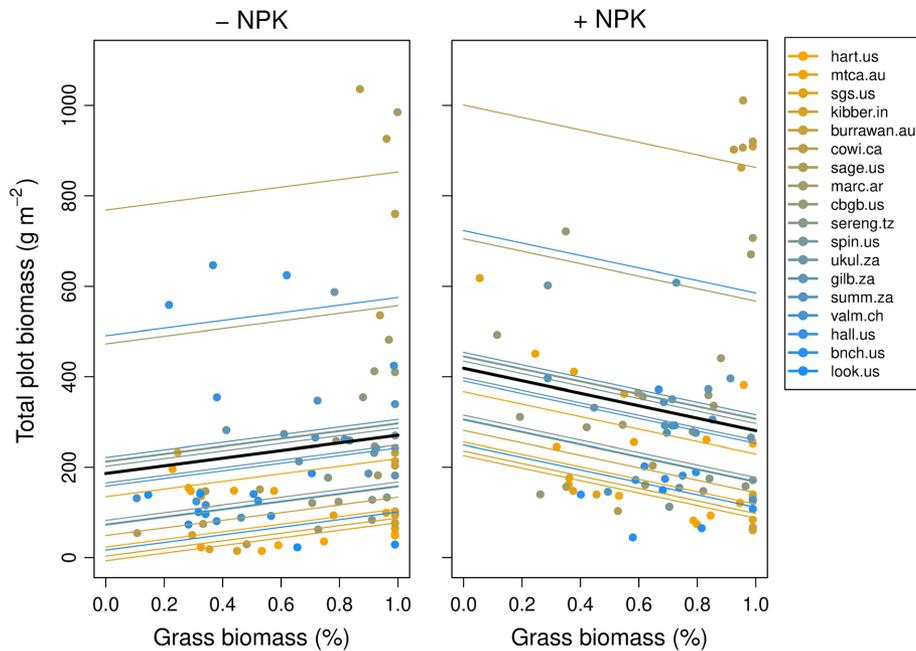


FIG. 5. Relationship between grass abundance (%) and total plot biomass ($g\ m^{-2}$) in control plots (left panel) and experimentally fertilized plots (right panel) across 18 sites that separated samples by plant functional type (grass, forb and legume). Points are replicate plots within sites and are labelled in order from low (orange) to high (blue) precipitation; for site information and identities see Appendix S1: Table S1. Lines show random intercepts fit to individual site with the mean interaction response (either $-NPK$ or $+NPK$) shown as the bold, black line.

Atmospheric nitrogen deposition (N-DEP) was somewhat unique in the model in that it had positive effects on biomass that were offset by negative effects on plant nutrient concentrations (Appendix S1: Table S6). For example, the

increase in total standing NPK due to greater biomass ($0.30 * 0.93 = 0.28$), was offset by a decrease in total standing NPK due to lower plant NPK ($-0.29 * 0.35 = -0.10$), which dampened the overall positive influence of N

deposition on community nutrient pools. Herbivore abundance, as measured by the grazing index, increased total standing NPK by increasing plant NPK (Fig. 3). However, these were offset by a positive relationship between the grazer index on %grass, which reduced plant NPK; the result was a moderate overall increase in total standing NPK (0.14; Appendix S1: Table S6). Finally, the effects of nutrient additions depended on the background plant community (% grass) and underlying resource availability (soil %N). However, the total strength of fertilization, which includes interactions with both %grass and soil %N, accounted for a consistent positive effect on total standing plant NPK (0.26).

DISCUSSION

Across our global sampling of grassland sites, climate variation best explained broad-scale patterns of plant nutrient concentrations, but these effects were modified locally by eutrophication and herbivory. For example, the ratio of total plant nutrients in fertilized vs. unfertilized plots showed a consistently strong negative relationship with MAP in ungrazed plots, whereas LRRs were not different from zero across a global precipitation gradient in the presence of herbivores (Fig. 2). These results contrast with our initial predictions that total plant nutrients would increase under eutrophication at mesic sites and suggest instead that herbivore effects on element standing stocks are dominated by their consumptive effects on biomass, rather than their effects on plant species compositional turnover that increase with precipitation (e.g., Chase et al. 2000, Anderson 2008). However, our results are consistent with studies showing consumers have their greatest proportional effect (e.g., on productivity) in arid relative to mesic sites (Olf and Ritchie 1998, Chase et al. 2000). Our results are novel in that we demonstrate how these influences translate into vegetation nutrient stocks across global variation in climate.

Another implication of these results is that, across a global range of sites, herbivory compensated for plot-level nutrient production after experimental eutrophication. Because plant nutritional quality acts as a key regulator of decomposition and carbon storage (e.g., Cebrian 1999), the outcome of our experiment suggests that the continued loss of large herbivores from ecosystems (e.g., Ripple et al. 2015) will further compound effects of anthropogenic eutrophication on ecosystem processes.

In the final SE model, insolation (INS) and mean annual temperature (MAT) provided the greatest explanatory power of global variation in total standing quantities of NPK in vegetation. Our findings support the hypothesis that insolation and temperature are major drivers of global variation in plant nutrients across the earth's surface (Reich and Oleksyn 2004, Borer et al. 2013). Our explicit test of the insolation hypothesis found strong support (Fig. 3, Appendix S1: Table S5), with influences that were mediated via multiple mechanisms, including %grass, biomass and plant chemistry. Our results suggest that the latitudinal decline in NPK observed in grassland plants (e.g., Reich and Oleksyn 2004, He et al. 2008, Borer et al. 2013) arises from direct effects of MAP and INS on plant nutrient concentrations at the plant-scale (i.e., paths from MAP and INS to plant NPK in Fig. 3)

and indirect effects, mediated by biomass, at the plot-scale (i.e., path from INS and MAT to plot biomass in Fig. 3).

Because of the 2.7 times greater sensitivity of total standing NPK to plant biomass compared to plant chemistry (i.e., tissue nutrient quantity as opposed to concentrations - standardized path coefficients of 0.93 compared to 0.35), the factors with the largest influence on plant biomass have the greatest impact on total nutrient flows in grasslands. Perhaps not unexpected on its own, this is surprising given that much of the research on plant stoichiometry has focused on patterns of variation in plant-level chemistry at global (e.g., Reich and Oleksyn 2004, Craine et al. 2005, Borer et al. 2013), regional (e.g., He et al. 2008, Zhang et al. 2012), functional type (e.g., Han et al. 2011) or phylogenetic (Stock and Verboom 2012) scales while ignoring the consequences of variation in plant biomass for total plant nutrient pools. Even though changes in plant composition can modify nutrient content on a mass basis, such as the strong effects of legumes on %N (Spehn et al. 2002), our results suggest that such influences are relatively small compared to processes that influence primary production.

Another clear pattern that emerged from our study is that anthropogenic eutrophication has complex effects on nutrient availability across environmental gradients. First, eutrophication has two pathways by which it can alter plant nutrients, one atmospheric and the other by anthropogenic fertilizers applied to soil. Stevens et al. (2015) showed that N deposition was a strong predictor of grassland primary production, better even than soil N. Our results demonstrate both pathways (N-deposition and fertilization) have influences on total standing NPK, but that they are complex. In the case of N deposition, two offsetting paths, one mediated by positive effects on plot biomass and the other by negative effects on plant tissue NPK, result in an overall positive effect on total plot NPK (Appendix S1: Table S5). In the case of fertilization, the response is complicated by interactions with soil nutrients (reduction of plant NPK at fertile sites) and %grass (greater biomass responses when fertilized in low grass plots).

While the strong link to climate is consistent with the growth rate hypothesis (Elser et al. 2003, 2007), it is difficult to separate effects of nutrient availability insofar as temperature and moisture modify decomposition, chemical weathering and other factors that drive plant nutrient availability (O'Halloran et al. 2013). Studies suggest that plants do not simply reflect the nutrient availability of a site, but instead that plant chemistry is determined by a complex balance of taxonomic identity, competition and resource supply rates (e.g., Craine et al. 2005, He et al. 2008, Kraft et al. 2008). Indeed, our SEM analysis demonstrated that when the effects of eutrophication were present, the specific nature of the outcome often interacted with background soil fertility or composition of the plant community. Finally, the lack of a mean annual precipitation (MAP) effect on plot biomass was surprising, but is consistent with the fact that across 42 NutNet sites MAP had no effect on plot primary productivity (e.g., O'Halloran et al. 2013), whereas atmospheric deposition significantly increased site level primary production (Stevens et al. 2015).

CONCLUSION

Our study highlights the importance of global climate gradients in creating across-site variation in nutrients at the plant- and plot-level. Solar insolation, mean annual temperature and mean annual precipitation emerged as major drivers of among-site variation in grassland nutrient pools through both direct and indirect effects on plant chemistry and biomass. However, eutrophication was a key driver of plant nutrient responses within sites. Moreover, herbivores dampen the effects of eutrophication on nutrient standing stocks through their consumption, especially at sites with lower precipitation. Consequently, continued loss of herbivore diversity and increased eutrophication may disproportionately increase total plant nutrients in dry areas. In the absence of the diversity-promoting effects of herbivores (e.g., Borer et al. 2014a, Yang et al. 2015) arid sites may become further destabilized by nitrogen addition (Hautier et al. 2014) leading to impacted rates of nutrient cycling in these regions. We suggest that a full understanding of nutrient dynamics and energy flow in savanna and grassland ecosystems requires a hierarchical and multivariate approach to the various ecological drivers. Finally, we recommend that efforts to map the global distribution of nutrients in grassland forage (e.g., Wang et al. 2010) should include climate, eutrophication and herbivore distributions.

ACKNOWLEDGMENTS

A Wake Forest University pilot research grant to TM Anderson provided funding for lab analysis of nutrient concentrations. Jesse Nippert at KSU was instrumental in facilitating lab analysis. Data collection in Serengeti was supported by NSF-DEB 1145861 to TMA. This work was generated using data from the Nutrient Network (<http://www.nutnet.org>) experiment, funded at the site-scale by individual researchers. Coordination and data management have been supported by funding to E. Borer and E. Seabloom from the National Science Foundation Research Coordination Network (NSF-DEB-1042132) and Long Term Ecological Research (NSF-DEB-1234162 to Cedar Creek LTER) programs, and the Institute on the Environment (DG-0001-13). We also thank the Minnesota Supercomputer Institute for hosting project data and the Institute on the Environment at UMN and sDiv at the University of Leipzig for hosting Network meetings. A.S.M. was funded by NSERC and the University of Guelph's CFREF project "Food from Thought". JBG was supported by the USGS Ecosystems and Climate and Land use Change Programs. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

LITERATURE CITED

- Allen, A. P., and J. F. Gillooly. 2009. Towards an integration of ecological stoichiometry and the metabolic theory of ecology to better understand nutrient cycling. *Ecology Letters* 12:369–384.
- Anderson, T. M. 2008. Plant compositional change over time increases with rainfall in Serengeti grasslands. *Oikos* 117:675–682.
- Anderson, T. M., B. B. Kumordzi, W. Fokkema, H. V. Fox, and H. Olf. 2013. Distinct physiological responses underlie defoliation tolerance in African lawn and bunch grasses. *International Journal of Plant Sciences* 174:769–778.
- Asner, G. P., A. J. Elmore, L. P. Olander, R. E. Martin, and A. T. Harris. 2004. Grazing systems, ecosystem responses, and global change. *Annual Review of Environment and Resources* 29: 261–299.
- Bardgett, R.D. and D. A. Wardle. 2003. Herbivore-mediated linkages between aboveground and belowground communities. *Ecology* 84:2258–2268.
- Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67:1–48.
- Bolker, B., & the R Development Core Team 2017. *bbmle: Tools for General Maximum Likelihood Estimation*. R package version 1.0.19. <https://CRAN.R-project.org/package=bbmle>
- Borer, E. T., et al. 2013. Global biogeography of autotroph chemistry: Is insolation a driving force? *Oikos* 122:1121–1130.
- Borer, E. T., et al. 2014a. Herbivores and nutrients control grassland plant diversity via light limitation. *Nature* 508:517–520.
- Borer, E. T., W. S. Harpole, P. B. Adler, E. M. Lind, J. L. Orrock, E. W. Seabloom, and M. D. Smith. 2014b. Finding generality in ecology: a model for globally distributed experiments. *Methods in Ecology and Evolution* 5:65–73.
- Bracken, M. E., et al. 2015. Signatures of nutrient limitation and co-limitation: responses of autotroph internal nutrient concentrations to nitrogen and phosphorus additions. *Oikos* 124:113–121.
- Burnham, K. P., and D. R. Anderson. 2002. *Model selection and multimodel inference: a practical information-theoretic approach*. Second edition. Springer-Verlag, New York.
- Ceballos, G., P. R. Ehrlich, A. D. Barnosky, A. García, R. M. Pringle, and T. M. Palmer. 2015. Accelerated modern human-induced species losses: entering the sixth mass extinction. *Science Advances* 1:e1400253.
- Cebrian, J. 1999. Patterns in the fate of production in plant communities. *American Naturalist* 154:449–468.
- Cebrian, J., J. B. Shurin, E. T. Borer, B. J. Cardinale, J. T. Ngai, M. D. Smith, and W. F. Fagan. 2009. Producer nutritional quality controls ecosystem trophic structure. *PLoS ONE* 4:e4929.
- Chase, J. M., M. A. Leibold, A. L. Downing, and J. B. Shurin. 2000. The effects of productivity, herbivory, and plant species turnover in grassland food webs. *Ecology* 81:2485–2497.
- Cherif, M., and M. Loreau. 2013. Plant–herbivore–decomposer stoichiometric mismatches and nutrient cycling in ecosystems. *Proceedings of the Royal Society of London – Series B* 280: 20122453.
- Craigie, I. D., J. E. Baillie, A. Balmford, C. Carbone, B. Collen, R. E. Green, and J. M. Hutton. 2010. Large mammal population declines in Africa's protected areas. *Biological Conservation* 143:2221–2228.
- Craine, J. M., W. G. Lee, W. J. Bond, R. J. Williams, and L. C. Johnson. 2005. Environmental constraints on a global relationship among leaf and root traits of grasses. *Ecology* 86:12–19.
- Elser, J. J., et al. 2003. Growth rate–stoichiometry couplings in diverse biota. *Ecology Letters* 6:936–943.
- 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.
- Elser, J. J., W. F. Fagan, A. J. Kerkhoff, N. G. Swenson, and B. J. Enquist. 2010. Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. *New Phytologist* 186:593–608.
- Fay, P. A., et al. 2015. Grassland productivity limited by multiple nutrients. *Nature Plants* 1:15080.
- Ferraro, D. O., and M. Oesterheld. 2002. Effect of defoliation on grass growth. A quantitative review. *Oikos* 98:125–133.
- Fox, J., and S. Weisberg 2011. *An R companion to applied regression*. Second edition. Sage, Thousand Oaks, California, USA. <http://socserv.socsci.mcmaster.ca/jfox/Books/Companion>
- Gelman, A., and J. Hill. 2007. *Data analysis using regression and multilevel/hierarchical models*. Cambridge University Press, New York, New York, USA.
- Grace, J. B., T. M. Anderson, H. Olf, and S. M. Scheiner. 2010. On the specification of structural equation models for ecological systems. *Ecological Monographs* 80:67–87.

- Green, R. A., and J. K. Detling. 2000. Defoliation-induced enhancement of total aboveground nitrogen yield of grasses. *Oikos* 91:280–284.
- Güsewell, S. 2004. N: P ratios in terrestrial plants: variation and functional significance. *New Phytologist* 164:243–266.
- 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.
- Hautier, Y., et al. 2014. Eutrophication weakens stabilizing effects of diversity in natural grasslands. *Nature* 508:521–525.
- He, J. S., L. Wang, D. F. Flynn, X. Wang, W. Ma, and J. Fang. 2008. Leaf nitrogen: phosphorus stoichiometry across Chinese grassland biomes. *Oecologia* 155:301–310.
- Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25:1965–1978.
- Hillebrand, H., et al. 2009. Herbivore metabolism and stoichiometry each constrain herbivory at different organizational scales across ecosystems. *Ecology Letters* 12:516–527.
- Hobbie, S. E. 1992. Effects of plant species on nutrient cycling. *Trends in Ecology and Evolution* 7:336–339.
- Intergovernmental Panel on Climate Change. 2000. special report on emission scenarios, edited by N. Nakicenovic et al., 599. Cambridge University Press, New York.
- Kerkhoff, A. J., B. J. Enquist, J. J. Elser, and W. F. Fagan. 2005. Plant allometry, stoichiometry and the temperature-dependence of primary productivity. *Global Ecology and Biogeography* 14:585–598.
- Kraft, N. J., R. Valencia, and D. D. Ackerly. 2008. Functional traits and niche-based tree community assembly in an Amazonian forest. *Science* 322:580–582.
- Krishnamurthy, A., J. K. Moore, C. S. Zender, and C. Luo. 2007. Effects of atmospheric inorganic nitrogen deposition on ocean biogeochemistry. *Journal of Geophysical Research: Biogeosciences* 112:G2.
- Magnusson, A., H. Skaug, A. Nielsen, C. Berg, K. Kristensen, M. Maechler, van Benthem K., B. Bolker, and M. Brooks. 2017. *glmmTMB: Generalized Linear Mixed Models using Template Model Builder*. R package version 0.1.1. <https://CRAN.R-project.org/package=glmmTMB>
- O'Halloran, L. R., et al. 2013. Regional contingencies in the relationship between aboveground biomass and litter in the world's grasslands. *PLoS ONE* 8:e54988.
- Olf, H., and M. E. Ritchie. 1998. Effects of herbivores on grassland plant diversity. *Trends in Ecology and Evolution* 13:261–265.
- Pardo, L. H., et al. 2011. Effects of nitrogen deposition and empirical nitrogen critical loads for ecoregions of the United States. *Ecological Applications* 21:3049–3082.
- Piñeiro, G., J. M. Paruelo, M. Oesterheld, and E. G. Jobbágy. 2010. Pathways of grazing effects on soil organic carbon and nitrogen. *Rangeland Ecology and Management* 63:109–119.
- Potter, P., N. Ramankutty, E. M. Bennett, and S. D. Donner. 2010. Characterizing the spatial patterns of global fertilizer application and manure production. *Earth Interactions* 14:1–22.
- R Core Team 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Reich, P. B., and J. Oleksyn. 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences of the United States of America* 101:11001–11006.
- Ripple, W. J., et al. 2015. Collapse of the world's largest herbivores. *Science Advances* 1:e1400103.
- Scholes, R. J., and S. R. Archer. 1997. Tree-grass interactions in savannas. *Annual Review of Ecology and Systematics* 1997:517–544.
- Schuman, G. E., J. D. Reeder, J. T. Manley, R. H. Hart, and W. A. Manley. 1999. Impact of grazing management on the carbon and nitrogen balance of a mixed-grass rangeland. *Ecological Applications* 9:65–71.
- Spehn, E. M., et al. 2002. The role of legumes as a component of biodiversity in a cross-European study of grassland biomass nitrogen. *Oikos*, 98:205–218.
- 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.
- Stevens, C. J., N. B. Dise, J. O. Mountford, and D. J. Gowing. 2004. Impact of nitrogen deposition on the species richness of grasslands. *Science* 303:1876–1879.
- Stevens, C. J., et al. 2015. Anthropogenic nitrogen deposition predicts local grassland primary production worldwide. *Ecology* 96:1459–1465.
- Stock, W. D., and G. A. Verboom. 2012. Phylogenetic ecology of foliar N and P concentrations and N: P ratios across Mediterranean-type ecosystems. *Global Ecology and Biogeography* 21: 1147–1156.
- Venables, W. N., and B. D. Ripley. 2002. *Modern applied statistics with S*. Fourth edition. Springer, New York.
- Vitousek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo. 1997. Human domination of Earth's ecosystems. *Science* 277:494–499.
- Wang, Y. P., R. M. Law, and B. Pak. 2010. A global model of carbon, nitrogen and phosphorus cycles for the terrestrial biosphere. *Biogeosciences* 7:2261–2282.
- Yang, Z., Y. Hautier, E. T. Borer, C. Zhang, and G. Du. 2015. Abundance-and functional-based mechanisms of plant diversity loss with fertilization in the presence and absence of herbivores. *Oecologia* 179:261–270.
- Zhang, S. B., J. L. Zhang, J. W. Slik, and K. F. Cao. 2012. Leaf element concentrations of terrestrial plants across China are influenced by taxonomy and the environment. *Global Ecology and Biogeography* 21:809–818.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at <http://onlinelibrary.wiley.com/doi/10.1002/ecy.2175/supinfo>

DATA AVAILABILITY

Data associated with this study are available from the Dryad Data Repository: <https://doi.org/10.5061/dryad.mq56q>