TITLE: Herbivory and eutrophication modulate grassland plant nutrient responses across a global climatic gradient

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ABSTRACT

Plant stoichiometry, a key regulator of ecosystem functioning, is being altered by human activities. We measured plant elemental responses (C, N, P and K) to eutrophication and vertebrate herbivore removal at eighteen sites on six continents. Nutrient pools diverged strongly in response to herbivore removal when fertilized; response ratios were highest in fenced, ungrazed plots at low rainfall, whereas herbivore grazing dampened the community nutrient responses to eutrophication. Across sites, climate and atmospheric N deposition emerged as strong predictors of plot-level nutrients, mediated by biomass and plant chemistry. Within sites, fertilization increased nutrient pools, but results were contingent on soil fertility and the proportion of the biomass that was grass relative to other functional types. Our study highlights (i) the role of climate in determining plant nutrient concentrations through effects on plant biomass, (ii) that eutrophication affects grassland nutrient pools via both soil and atmospheric pathways and (iii) interactions among soils, herbivores and eutrophication drive plant nutrient responses at small scales, especially at water-limited sites.

INTRODUCTION

The relative concentration of elements in living tissues, i.e. stoichiometry, is a fundamental organismal property regulating processes from cell metabolism to growth and reproduction

(Sterner & Elser 2002). In plants, nutrient concentrations influence primary production, herbivore consumption and decomposition, thus dictating 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 (Gusewell 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 & Oleksyn 2004, Borer et al. 2013). Nutrient supply rates can directly change plant tissue chemistry (Bracken et al. 2014) and one hypothesis to explain the latitudinal gradient in plant tissue chemistry is based on changing nutrient supply rates with latitude. Specifically, the '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 limiting P availability (Reich & Oleksyn 2004). And indeed recent theory has attempted to link temperature to plant stoichiometry across global gradients (*e.g.*, Kerkoff et al. 2005, Allen & 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 ~ 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 which 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 (Ruess & McNaughton 1987, Hobbie 1992, Bardgett & Wardle 2003, Piñeiro et al. 2010, Cherif & Loreau 2013). Herbivore influences on nutrient cycling and plant nutrient concentrations is of elevated significance because humans are reducing native, large-bodied herbivore populations throughout the world’s grassland and savannas (*e.g.*, Craigie et al. 2010, Cebellos 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 ~ 77.4 ± 4.6 Tg year^{-1} (Potter et al. 2010, Table 2), while atmospheric N deposition, derived from local fertilizer and industrial sources, has nearly doubled from pre-industrial levels (from ~ 22 Tg N yr^{-1} to ~ 39 Tg N yr^{-1}) and is projected to double again by 2100 (IPCC 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 & 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, 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 & Detling 2000), with few studies seeking controls across global extents (but see Wang et al. 2010). Consequently, a specific goal of our study was to compare the contribution of global climate factors, including temperature, precipitation, solar radiation and N deposition (*e.g.*, Stevens et al. 2015), to variation in herbivory and nutrient supply on nutrient concentrations at the plant- (*e.g.*, nutrient concentrations, g g^{-1} tissue) and community-level (*e.g.*, on an areal basis, g m^{-2}). As herbivore and fertilization effects may depend on climate and nutrient supply, 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’ nutrients) varied across a global climate gradient (model one). Subsequently, we analyzed the network of direct and indirect effects of climate, herbivores and resource 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 one, we predicted an interaction between eutrophication and herbivory across sites: specifically, that the relative effects of eutrophication would increase with rainfall, but the response would be amplified by increased abundance of nutrient-rich palatable plants at higher rainfall sites when herbivores were removed. This prediction results from work demonstrating herbivore effects on plant species turnover are greater at mesic compared to arid sites (*e.g.*, Chase et al. 2000). On the other hand, if nutrient availability across sites results largely from herbivore effects on biomass instead of plant turnover, then herbivore loss may lead to greater impacts of eutrophication at arid sites, where herbivore effects on biomass are the largest (*e.g.*, Chase et al. 2000).

For model two (the multi-level SEM), we decomposed the response of total plant nutrients into those due to changes in tissue nutrient concentration and those driven by changes in plant biomass. We expected concentrations of N, P and K to decrease with 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 chemistry and plant biomass, but that these effects would depend on background resource supply, i.e., the largest responses in low soil nutrient plots. Finally, in terms of their strength in controlling variation in total plant nutrient pools, we predicted that plot-level (*e.g.*, herbivores, eutrophication and resource supply) would be comparable in magnitude to broad-scale climatic drivers (*e.g.*, rainfall, temperature, insolation and N-deposition).

METHODS

Site location and study design

Our study was conducted at 18 sites in Africa, Asia, Australia, Europe, North and South America (Fig. 1) as part of the Nutrient Network (NutNet; Borer et al. 2014b). Sites represented a range of habitat types and spanned a gradient of mean annual precipitation (MAP) from 305 to 2315 mm yr^{-1} (Table S1 in Supporting Information), but all were located in grass-dominated plant communities. At each site, fully factorial combinations of nutrient addition and fencing were randomly assigned to 25-m^2 plots arranged in 1 - 5 blocks within sites from 2007 - 2012 (Table S1). Within each 25-m^2 plot, randomly chosen 1-m^2 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 m^{-2} 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 those treatments are ignored 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 years after the establishment of the experiment at 16 sites and after either 1 or 2 years at the remaining two sites (Table S1). Aboveground plant biomass was clipped to ground-level in 2 replicate 10 x 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 ground in an UDY belt-drive sample mill, dried 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 ground sample were averaged from triplicate measurements between wavelengths of 781 – 2778 nm (12,800 – 3,600 cm^{-1}) 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).

Spectral data were used to identify a subset of samples (20 % of the total sample number), stratified by site, for wet chemical analysis, i.e., the ‘known’ calibration samples (Appendix S1). Samples were analyzed for total C, N, P, and K at either North Carolina State University or Kansas State University using standard analytical methods. The resulting dataset was further subdivided into a calibration (model development) and validation (test set; 10%) subset using an algorithmic experimental design approach (Appendix S1).

Soils were collected 2 - 4 years post treatment (mean = 3.4 ± 0.8 years; 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 (Table S1) and soil %N was imputed for these sites because of the relatively strong relationship between soil N and major climate factors (Appendix S1). 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 removal 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 plot nutrient content 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 (Table S1). For each site, we extracted mean annual temperature (MAT, ^{o}C), variable BIO1, and mean annual precipitation (MAP, mm yr^{-1}), BIO12, from BioClim (Hijmans et al. 2005). For solar insolation (INS, kWh m^{-2} day^{-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 versus control) and fertilizer addition treatments (NPK; fertilized versus control) were included (2 fencing levels x 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 m^{-2}, yielding the total nutrient content in aboveground biomass (g m^{-2}) for each element. We then quantified the relative effects of nutrient addition by calculating log response ratios (LRR) within blocks at each site: $\log(\frac{total\ nutrient\ content\ in\ NPK\ addition}{total\ nutrient\ content\ in\ control})$. 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 was used to determine if the slopes of the LRR ~ environmental predictors were different for levels of FENCE.

To identify the best model, we followed a model selection procedure on the basis Akaike's Information Criteria 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 final, accepted model. After identifying the most suitable model (see below), we tested for interactions among NPK addition, fencing and environmental predictors using type III sums of squares using the *Anova* command in R-package 'car'.

Structural equation model of total plot NPK (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. For this analysis, rather than analyze each nutrient separately, total N, P, and K were summed on an areal basis (g m^{-2}) to create a single nutrient variable that was modeled as the response. As the total plot nutrient content 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.*, g nutrient g $plant^{-1}$), (2) direct effects due to plant community biomass (g m^{-2}) or (3) indirect effects due to variation in the abundance of functional types (*i.e.*, grass vs. forb) among sites (Figure S1 and 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. The %grass variable was logit transformed prior to analyses to meet model assumptions. External predictors were MAT, MAP, INS, atmospheric N deposition, 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 S1). Due to the hierarchical nature of the data (*i.e.*, some predictors existed only at the site level while others existed for individual plots; Figure S1) we analyzed each response variable in a piecewise fashion using a multi-level approach (*e.g.*, Gelman & Hill 2007; Appendix S1). Note that the final response variable in the SEM, total standing NPK, 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 NPK were computed analytically rather than estimated. These computed parameters represent the contributions to variations in total standing NPK derived from variation in component variables (Appendix S1).

RESULTS

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

The ANCOVA analysis (*i.e.*, LRRs) demonstrated that element responses to eutrophication across a global gradient in rainfall depended on the experimental removal of herbivores. For each of the elements analyzed (C, N, P and K), the top model identified by AICc included an interaction between MAP and FENCE on element LRR under fertilization (Table S3). All elements showed a consistently strong negative relationship between the LRR and MAP inside ungrazed, fenced plots, meaning that the strongest effects of increasing nutrient supply were at arid sites in the absence of herbivores (Fig. 2). The presence of herbivores counteracted the strong effects of NPK addition 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 a linear models for all elements in the ungrazed treatments). No other model was similar in its fit with LRR across sites (Table S3) and the final coefficient of variations (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 removal and eutrophication would alter total grassland nutrients by influencing plant chemistry and plant composition, and that their effects would be similar in magnitude. The final SE model had coefficients of determination (R^2) of 0.33 for percent grass, 0.49 for total plot biomass and 0.67 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 Tables S4 and S5). 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 (1.06 and 1.08), but plant NPK decreased with MAT (-0.63). The only direct influence of MAP in the model was a positive effect on plant NPK (0.50). INS had relatively strong negative influences on both %grass (-0.88) and plot biomass (-1.0). After accounting for climate effects, N deposition increased plot biomass (0.29) and decreased plant NPK (-0.37), likely because of growth-induced nutrient dilution in plant tissues.

While fencing was not significant in the final SE model, the grazer index was positively related to %grass (0.42) and plant NPK (0.22) 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 (Fig. 3) and a somewhat stronger response of plot biomass to an interaction between NPK fertilization and %grass (0.19; 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. 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 effect (-0.91), which was mediated by a strong negative influence on plot biomass and a strong positive relationship between biomass and standing NPK (Table S5). MAT had the strongest positive effect on plot standing NPK content (0.72), which was mediated by its strong positive association with plot biomass (Table S5). MAP had a weaker positive effect on total standing NPK (0.18), which was mediated by its positive effects on plant NPK.

MAT and N deposition were unique in the model in that both predictors had positive effects on biomass that were offset by negative effects on plant nutrient concentrations (Table S5). For example, for N deposition, the increase in total standing NPK due to greater biomass (0.27) was offset by a decrease in total standing NPK due to lower plant NPK (-0.13), 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 weak overall increase in total standing NPK (0.05; Table S5). Finally, the effects of nutrient additions depended on the background plant community (%grass) and underlying resource availability (soil %N). However, the total strength of eutrophication, which includes interactions with both %grass and soil %N, accounted for a consistent positive effect on total standing NPK (0.23).

DISCUSSION

Across our global sampling of grassland sites, climate variation best explained broad-scale patterns of nutrient concentrations, but these effects were modified locally by eutrophication and herbivory. For example, elements showed a consistently strong negative relationship between the LRR and MAP in ungrazed, fenced plots, whereas response ratios were flat and not different from zero across a global precipitation gradient in the presence of herbivores (Fig. 2). These results contrast with our initial predictions and suggest that herbivore effects on element standing stocks are dominated by their consumptive effects on biomass, rather than their effects on plant species compositional turnover, which 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 (Olff & Ritchie 1998, Chase et al. 2000).

Another implication of these results is that, across a global range of sites, herbivory fully 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, INS and MAT provided the greatest explanatory power of global variation in total standing quantities of NPK in vegetation. Our findings are consistent with the hypothesis that solar irradiance and temperature are major drivers of global variation in plant nutrients across the earth's surface (Borer et al. 2013). Our explicit test of the solar insolation hypothesis found strong support (Figure 3, Table S5), although the major influence was through negative effects on biomass and %grass rather than on plant chemistry *per se*. Our results suggest that the latitudinal decline in NPK observed in grassland plants (*e.g.*, Reich & Oleksyn 2004, He et al. 2008, Borer et al. 2013) arises from direct effects of MAT and MAP on plant nutrient concentrations at the plant-scale (*i.e.*, paths from MAT and MAP to plant NPK in Fig. 3) and indirect effects, mediated by biomass, at the plot-scale (*i.e.*, path from insolation and MAT to plot biomass in Fig. 3).

As a consequence of the 2.7 times greater sensitivity of total standing NPK to plant biomass compared to plant chemistry (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.*, Craine et al. 2002, Reich & Oleksyn 2004, Borer et al. 2013), regional (*e.g.*, He et al. 2006, Zhang et al. 2012), functional type (*e.g.*, Han et al. 2011) or phylogenetic (Stock & Verboom 2012) scales while ignoring the consequences of variation in plant biomass for nutrient availability. 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 fertilization. Our results demonstrate both pathways have offsetting influences on total NPK availability which are mediated by positive effects on plot biomass and negative effects on the concentration of NPK in plants. In the case of N deposition, the offsetting paths result in a positive effect on total plot NPK (Table S5), but in the case of fertilization the response is further complicated by the interaction with soil nutrients (reduction of plant NPK at fertile sites).

While the strong link to climate is consistent with the growth rate hypothesis, 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. 2012). Studies (*e.g.*, Craine et al. 2005, Kraft et al. 2008, He et al. 2009) 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. 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 MAP effect on plot biomass was surprising, but is consistent with regional contingencies in the effects of MAP on biomass across grassland sites (*e.g.*, O'Halloran et al. 2013) and the fact that across 42 NutNet sites MAP had no effect on plot ANPP, whereas atmospheric deposition significantly increased site level ANPP (Stevens et al. 2015).

Conclusion

Our study highlights the importance of global climate gradients in establishing across site variation in nutrients at the plant- and plot-level. In particular, solar insolation, MAT and MAP emerged as major drivers of among-site variation in grassland nutrient pools through direct effects on plant chemistry and indirect effects on plant biomass. However, consistent responses to eutrophication within sites was a key driver of plant nutrient responses. Moreover, herbivores dampen the effects of eutrophication on nutrient standing stocks through their consumption, especially at sites where water is limiting. Consequently, continued loss of herbivore diversity and increased eutrophication may disproportionally increase standing 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 future efforts to map the global distribution of nutrients in grassland forage (*e.g.*, Wang et al. 2010) should include climate, eutrophication and herbivore distributions in future models.

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Figure Legends

Figure 1. Global map showing the distribution of the 18 NutNet sites across 5 continents represented in our analysis. See Table S1 for the specific site information.

Figure 2. Effect of fertilizer addition, expressed as a log response ratio (LRR: log [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 x MAP interaction across sites (see Table S4 in Supplementary Results).

Figure 3. Final structural equation model results. The figure depicts direct and indirect effects of predictors (climate [insolation, MAT and MAP], grazing index, treatment effects [fences and NPK addition] and soil %N) on response variables (% grass, plot biomass [g m^{-2}], plant NPK [sum of N, P and K in % dry weight] and total standing NPK [sum of N, P and K in g m^{-2}]). 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 boxed 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 with arrow widths proportional to the standardized path coefficient (shown on each arrow). 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 Table S5 in Supplementary Results for standardized total effects of predictors on the response variables.

Figure 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. Points are replicate plots within sites and are labelled in order from low (orange) to high (blue) precipitation; for site information and identities see 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.

Figure 5. Relationship between logit transformed grass abundance (%) and total plot biomass (g m^{-2}) in control plots (left panel) and experimentally fertilized plots (right panel) across 18 sites. Points are replicate plots within sites and are labelled in order from low (orange) to high (blue) precipitation; for site information and identities see 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.

Figure 1.

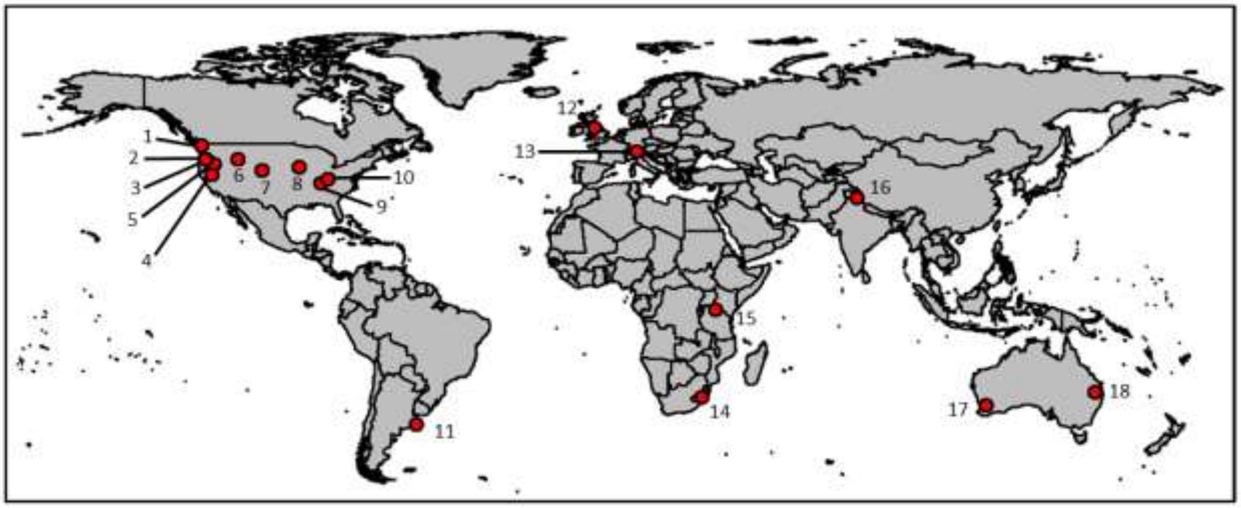


Figure 2.

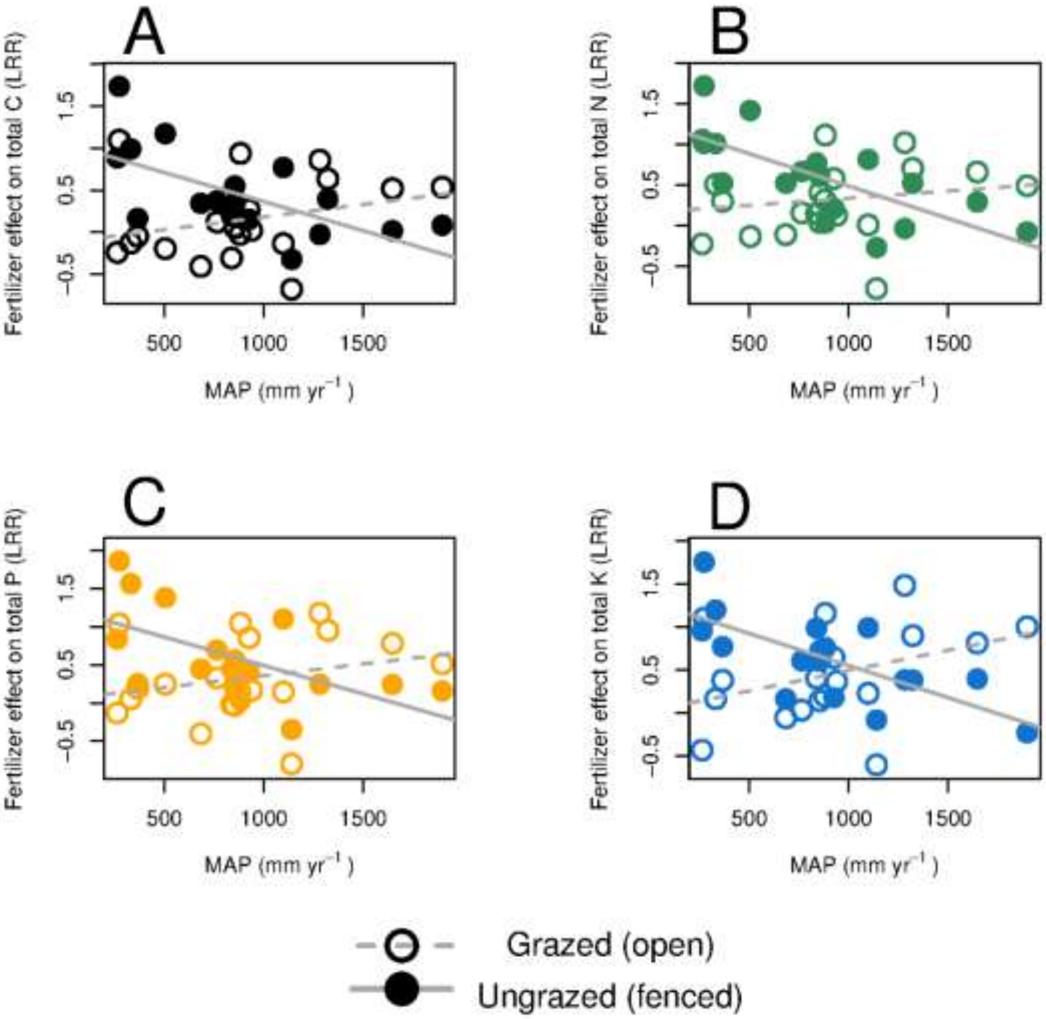

A
B
C
D
Fertilizer effect on total C (LRR)
Fertilizer effect on total N (LRR)
Fertilizer effect on total P (LRR)
Fertilizer effect on total K (LRR)
-0.5
0.5
1.5
500
1000
1500
MAP (mm yr^{-1})
Grazed (open)
Ungrazed (fenced)

Figure 3.

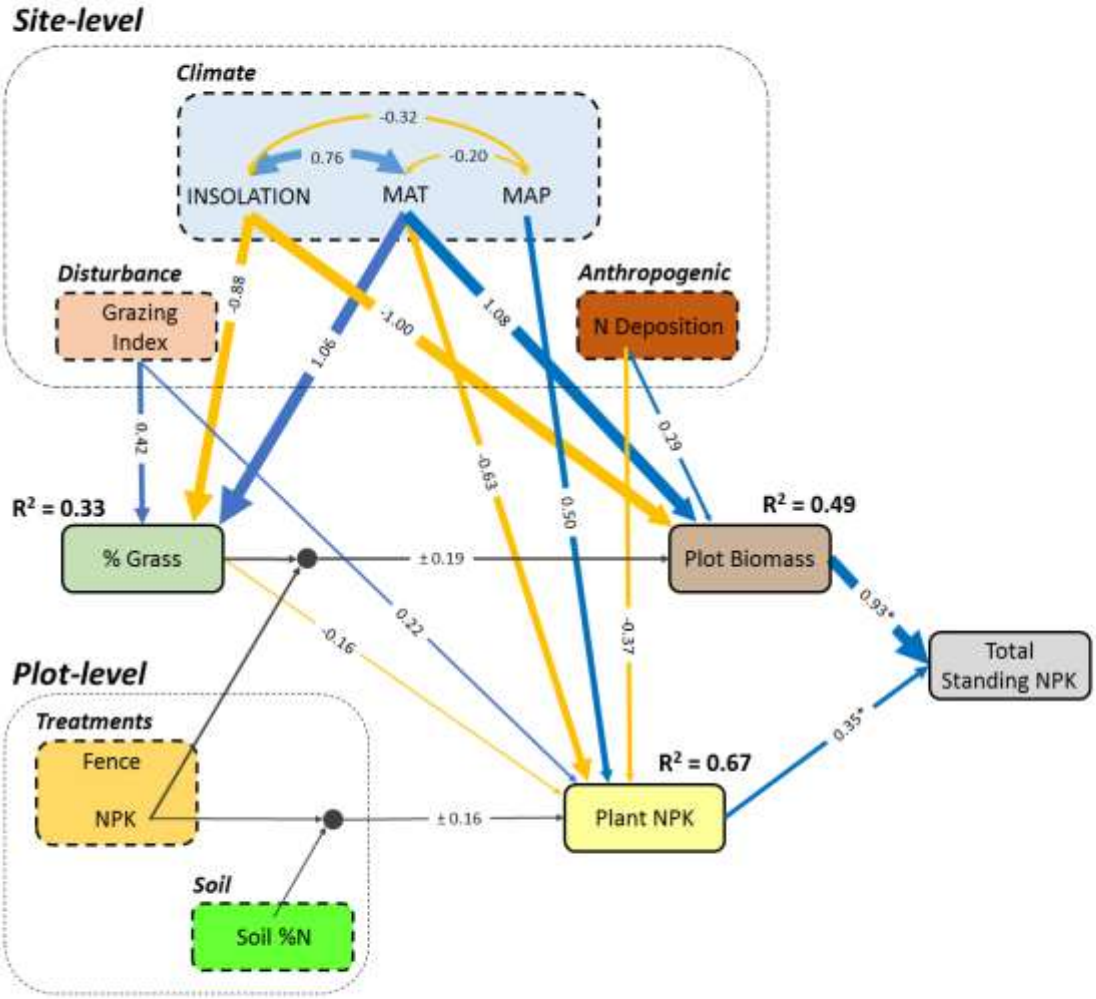
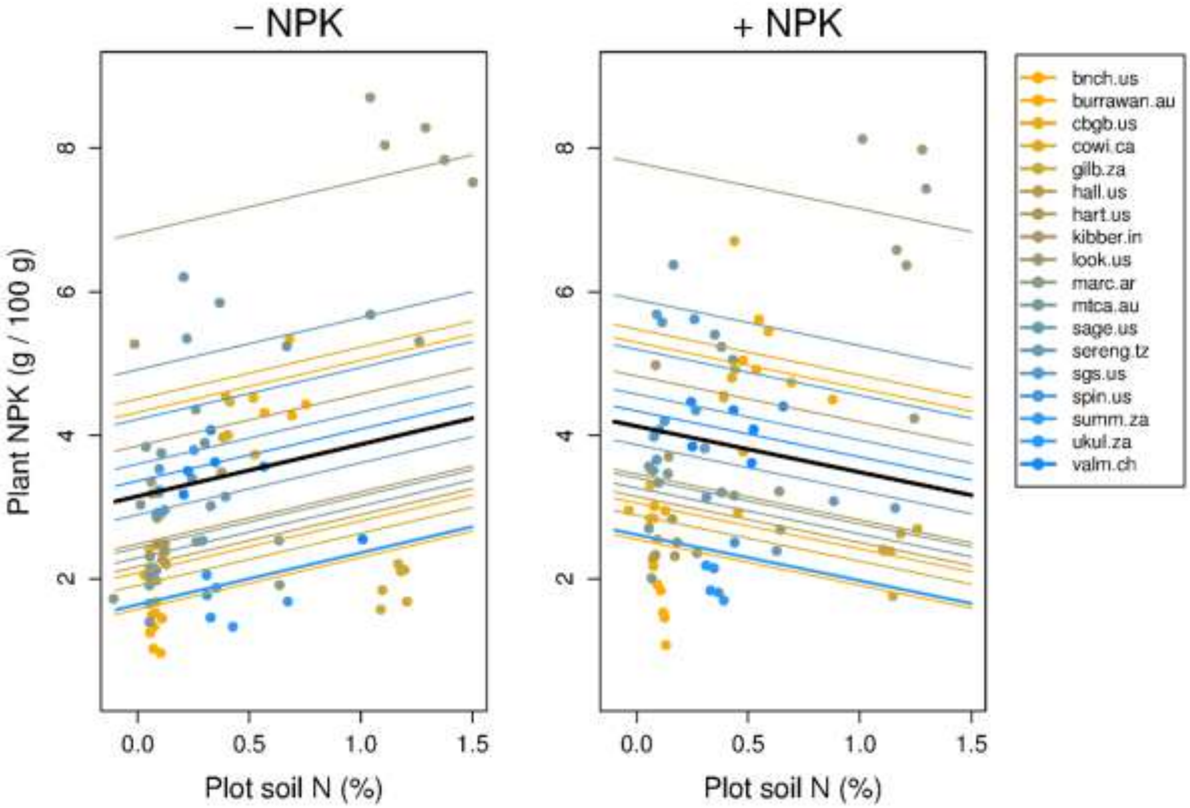

Site-level
Climate
-0.32
0.76
-0.20
INSOLATION
MAT
MAP
Disturbance
Grazing Index
Anthropogenic
N Deposition
-0.88
-1.00
1.08
1.06
0.29
0.42
-0.63
0.50
R² = 0.33
R² = 0.49
% Grass
±0.19
Plot Biomass
0.93*
-0.16
0.22
-0.37
Total Standing NPK
Plot-level
Treatments
Fence
NPK
±0.16
0.35*
R² = 0.67
Plant NPK
Soil
Soil %N

Figure 4.


– NPK
+ NPK
Plant NPK (g / 100 g)
2
4
6
8
0.0
0.5
1.0
1.5
Plot soil N (%)
bnch.us
burrawan.au
cbgb.us
cowi.ca
gilb.za
hall.us
hart.us
kibber.in
look.us
marc.ar
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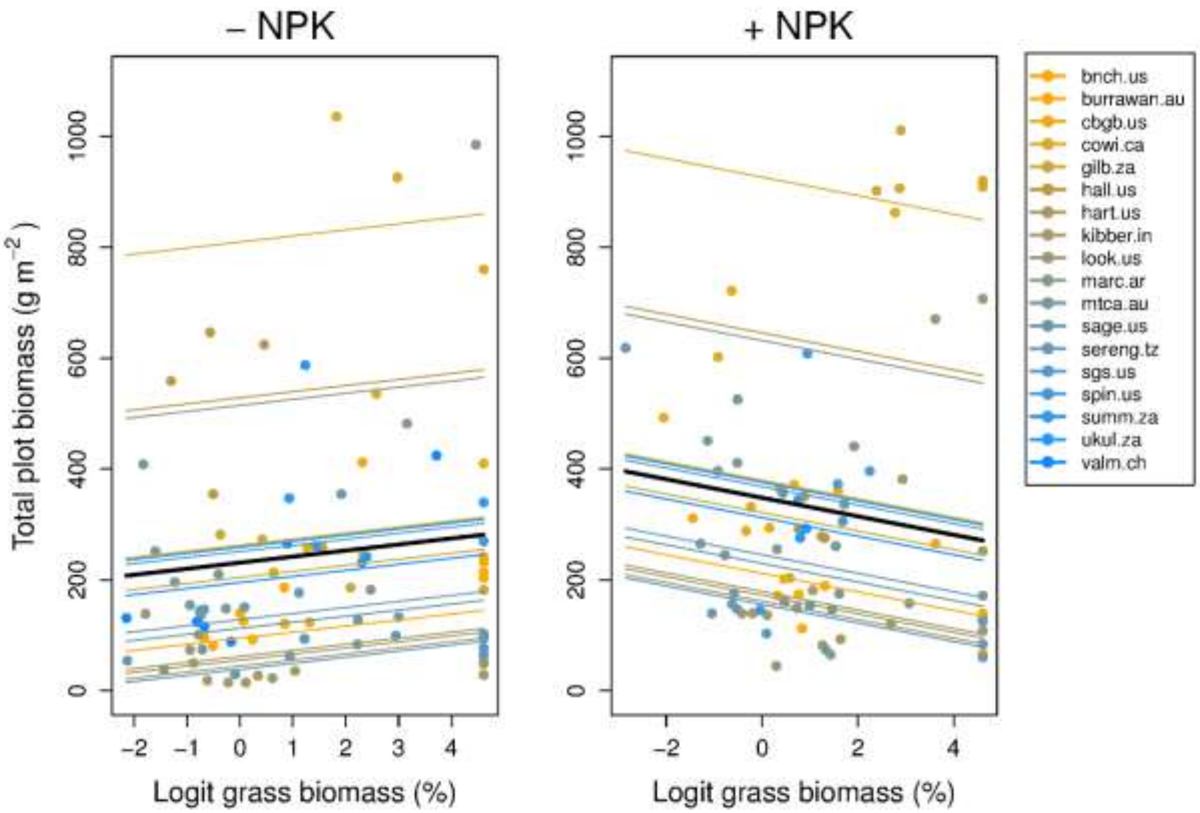
ukul.za
valm.ch

639 **Figure 5.**



640