Multiple nutrient interactions govern the global grassland biomass – precipitation relationship

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Significance: Understanding how multiple interacting nutrients regulate the global relationship between mean annual precipitation and aboveground biomass is crucial to forecast how eutrophication and precipitation changes will alter ecosystem function. We fertilized with nitrogen, phosphorus, and potassium plus micronutrients in all combinations in 71 grasslands representing a global precipitation gradient. The grassland biomass-precipitation relationship became steeper with increased number of added nutrients. The largest increase occurred in grasslands where biomass was synergistically co-limited by nitrogen and phosphorus. We found little evidence that variation in plant species diversity mediated changes in the biomass – precipitation relationship. Multiple nutrient co-limitation, particularly by nitrogen and phosphorus, is a defining feature of grassland biomass-precipitation relationships, and crucial to predicting grassland responses to global change. (120 words, limit 120)

Ecosystems are experiencing changing global patterns of mean annual precipitation (MAP) and enrichment with multiple nutrients that potentially co-limit biomass production. In grasslands, mean aboveground biomass increases with MAP, but the impact of enrichment with multiple co-limiting nutrients on the biomass-MAP relationship in grasslands is unclear. We propose a 'Multiple Nutrient Co-limitation' hypothesis. Inputs of a greater number of nutrients and stronger interactions among co-limiting nutrients will cause a steeper biomass-MAP relationship and increase mediation of this relationship by changes in plant community diversity. We measured aboveground biomass production and species diversity across 71 grassland sites on six continents where we fertilized with one, two, or three nutrients (nitrogen, phosphorus, and potassium with micronutrients) in all combinations to understand how single nutrients and subadditive, additive, and synergistic nutrient co-limitation altered the grassland biomass-MAP relationship. As hypothesized, fertilizing with one, two, or three nutrients progressively steepened the biomass-MAP relationship. The slope of the biomass – MAP relationship was steepest in sites where nitrogen and phosphorus synergistically co-limited biomass production. Unexpectedly, we found little evidence for mediation of the biomass – MAP relationship by plant community diversity because relationships of species richness, evenness and beta diversity to MAP and to biomass were weak or opposing. Site-level properties including baseline biomass production, climate, soils, and management explained little variation in biomass-MAP relationships. These findings reveal multiple nutrient co-limitation as a defining feature of the global grassland biomass-MAP relationship. This critical new insight will improve predictions of grassland productivity and ecosystem services. (246 words, limit 250)

Climate change is changing mean annual precipitation (MAP) across the globe (1). Concurrently, terrestrial ecosystems are increasingly enriched with multiple elemental nutrients (2) including nitrogen (N), phosphorus (P), and potassium (K), which frequently co-limit plant aboveground biomass production (3-13), a major component of ecosystem primary productivity (14). In grasslands, aboveground biomass increases with increasing ecosystem MAP - the biomass-MAP relationship (15-23). Theory predicts greater nutrient limitation of biomass production with increasing MAP (17, 19), reflecting higher demand for nutrients to maintain plant carbon metabolism and water balance (13, 24, 25). Thus, enrichment with limiting nutrients should result in a steeper biomass-MAP relationship (Figure 1) (26-28). However, to what extent stronger interactions among co-limiting nutrients increase the steepness of the biomass-MAP relationship is poorly understood. Clarifying the role of multiple nutrient interactions is necessary to forecast how interacting global change drivers – climate change and nutrient enrichment – will affect global patterns in energy flow, primary productivity, and ecosystem services. These processes are critical to societal efforts to mitigate and adapt to the impacts of global change drivers (2, 29, 30).

We propose a new framework to evaluate how interactions among multiple co-limiting nutrients influence the global grassland biomass-MAP relationship - the 'Multiple Nutrient Co-limitation' Hypothesis - holding that the slope of the biomass-MAP relationship: 1) increases with the number of added nutrients and attendant reduction in multiple nutrient limitation, and 2) increases with the strength of interaction among co-limiting nutrients (10). This idea extends previous concepts of the controls on the grassland biomass-MAP relationship (17, 19, 26-28) by explicitly accounting for the effects of the predominant interactive forms of nutrient co-limitation (Figure 1). This idea also builds on previous findings of widespread globally-averaged multiple nutrient co-limitation of productivity in grasslands (12, 31) and other ecosystems (10, 32).

Multiple nutrient co-limitation is revealed when ecosystems are fertilized with nutrients individually and in combination and can take several forms (Figure 1). Synergistic co-limitation of aboveground biomass is present when the response to multiple nutrients is greater than the sum of the single nutrient responses. Additive co-limitation occurs when the multiple nutrient response equals the sum of the single nutrient responses, and sub-additive co-limitation occurs when the response is less than the sum of the single nutrient responses. Limitation by a single nutrient or by none of the applied nutrients are also possible. Therefore, fertilizing with multiple nutrients should cause the maximum increase in slope where co-limitation is synergistic (Figure 1), and lesser increases in slope are expected where co-limitation is additive, sub-additive, or where limitation is by a single nutrient. The effects of these forms of nutrient limitation on the steepness of the global grassland biomass-MAP relationship have not been evaluated, primarily because the multiple nutrient enrichment experiments needed to directly test these effects in grasslands spanning a globally relevant range of MAP have only recently become available (33).

Variation in plant community diversity among sites and in response to fertilization could mediate single and multiple nutrient effects on the grassland biomass-MAP relationship because of the central role of plant diversity in biomass production (34-40). We predict greater mediation of the biomass- MAP relationship by plant community diversity in synergistically co-limited grasslands than in grasslands with weaker co-limitation or single limitation. With increasing MAP, sites should increase in species richness (41-45) and favor faster-growing, more productive species (46) which may also have higher nutrient requirements (27, 47). At the same time, with increasing MAP communities may also be more susceptible to the synergistic effects of adding multiple limiting nutrients, including amplified species loss (28, 48, 49), stronger dominance (50-53), or other deviations from the regional species pool (54, 55). Resolving whether the form and strength of nutrient co-limitation alters plant diversity feedbacks on the global grassland biomass-MAP relationship is crucial for forecasting how climate change and eutrophication impact the provision of biomass-related ecosystem services in grasslands.

Here we test the Multiple Nutrient Co-limitation hypothesis by analyzing the relationship of site mean aboveground biomass production (hereafter, "biomass") to site MAP across 71 sites in a global multiple nutrient fertilization experiment, the Nutrient Network (56). The sites were distributed across six continents and spanned 167 to 1,823 mm y^{-1} MAP, -3.3 to 24.1 °C mean annual temperature, and 0 to 4,241 m elevation (Supplemental Table 1). The sites represented native and planted grasslands, a wide range of edaphic properties and management practices, and much of the climate envelope of the grassland biome. Thus, we evaluated controls on the biomass-MAP relationship emergent across realistic sources of complexity in grassland ecosystem structure and function.

All sites performed a standardized yearly fertilization treatment for at least four years and up to maximum 15 years (Supplemental Table 1). N, P, and K were applied to 5 m x 5 m plots in a randomized block design with at least three replicates. In year 1, micronutrients were also added to plots receiving K (Kμ). Sites performed yearly standardized sampling of peak live aboveground biomass, a widely used estimate of aboveground net primary production in grasslands and other herbaceous-dominated ecosystems (14). Plant species composition was concurrently sampled to evaluate whether changes in species richness (effective species richness, eH), evenness, and beta diversity caused by fertilization mediated changes in the biomass-MAP relationship (Abbreviated Methods). Site MAP was computed from annual precipitation during the years in which biomass and diversity were sampled (Extended Methods).

Our analysis addressed three primary research questions about grassland biomass – MAP – nutrient interactions: 1) Does the biomass-MAP relationship become steeper with increased number of added nutrients? 2) Does the steepness of the biomass-MAP relationship increase with the strength of co-limiting nutrient interactions (Figure 1)? 3) Does mediation of the biomass-MAP relationship by community diversity increase with the strength of co-limiting nutrient interactions?

Results

1) Does the biomass-MAP relationship become steeper with increased number of added nutrients?

As hypothesized, fertilization increased the slope of the biomass-MAP relationship, and this increase was more pronounced when greater numbers of nutrients were added (Figure 2A; MAP x # Nutrients $p < 0.0001$, Table 1). Fertilization with all three nutrients - N, P, and Kµ together - increased the slope of the biomass-MAP relationship by 46% compared to the slope in unfertilized plots (Figure 2A, Supplemental Table 3). The increase in slope was smaller, 28%, for fertilization with pairs of nutrients (NP, NKµ, or PKµ), and smallest, 15%, for fertilization with single nutrients (N, P, or Kµ alone; Figure 2A inset).

Fertilizing with greater numbers of nutrients increased the slope of the biomass-MAP relationship primarily because of responses to N and P, and not in response to added Kµ (Figure 2B, Supplemental Table 3). Fertilizing with N alone increased the slope by 19% compared to unfertilized plots (Figure 2B inset, MAP x N $p < 0.0001$, Table 1), while fertilizing with P alone increased the slope by only 8% (MAP x P $p = 0.037$, Table 1). In contrast, fertilizing with N and P together increased the slope 30%. N and P did not interact with MAP to influence biomass (MAP x N x P $p = 0.83$, Table 1, Figure 1). Therefore, N and P together caused an additive increase in the steepness of the biomass-MAP relationship (Figure 1). Fertilizing with Kµ together with N and P caused slight increases in the slope of the biomass-MAP relationship (Figure 2B, Supplemental Table 3) but there was no statistical support for an interaction of Kµ with MAP, N, or P to affect the slope ($0.06 < p < 0.86$, Table 1).

The additive effects of fertilizing with N and P together on the biomass-MAP relationship contrasted with the effects of N and P addition on across-site mean aboveground biomass (Figure 2C). Fertilizing with N and P together increased mean aboveground biomass 41%, compared to 23% for N alone and 8% for P alone (Figure 2C), indicating a synergistic global average response to combined N and P fertilization (N x P $p = 0.006$, Table 1). This synergistic response in global mean biomass did not translate to the expected synergistic increase in steepness of the global biomass-MAP relationships (Figure 1) because fertilizing with both N and P synergistically increased the intercept while additively increasing the slope (Supplemental Table 3, Figure 2B). Kµ did not increase the across-site mean aboveground biomass alone or in interaction with N or P ($0.37 < p < 0.77$, Table 1). These findings support the hypothesis that increasing numbers of nutrients, and particularly fertilizing with N and P together, increases the steepness of the grassland biomass-MAP relationship.

2) *Does the steepness of the biomass-MAP relationship increase with the strength of co-limiting nutrient interactions?*

As hypothesized, the strength of interactions among co-limiting nutrients strongly influenced the slope of the biomass-MAP relationship. As predicted, sites classified as synergistically co-limited by N and P (17 sites, Extended Methods Table 3) had the steepest biomass-MAP relationships (slopes 0.79 to 0.97, adjusted R^2 0.46 to 0.48, Figure 3D) of the four forms of nutrient limitation we identified (Supplemental Table 3). In contrast, aboveground biomass was uncorrelated with MAP for sites classified as not limited by N or P (12 sites, Figure 3A) across all treatments or for any treatment individually (adjusted R^2 -0.09 to 0.01, Supplemental Table 3). Biomass-MAP relationships for sites with single nutrient limitation (15 for N, 3 for P) and for sites with additive NP co-limitation (20 sites; Extended Methods Table 3), fell between these extremes in both steepness (Slopes: Single 0.27 to 0.38; Additive 0.29 to 0.39) and in amount of biomass variation explained by MAP (R^2: Single 0.30 to 0.38;

Additive 0.18 to 0.29). We could not evaluate the biomass-MAP relationship for two sites classified as subadditive and two sites classified as having negative responses (Supplemental Table 2). These findings indicate that the strength of nutrient co-limitation plays a major role in shaping the global grassland biomass-MAP relationship.

We evaluated two alternate explanations for differing biomass-MAP relationships. First, sites with synergistic NP co-limitation may exhibit a steeper biomass-MAP relationship than sites with other forms of limitation because they spanned a lower range of MAP (Figure 3 A – D), where primary production is increasingly controlled by precipitation inputs (57). However, after excluding sites of all limitation forms with MAP greater than the highest MAP for synergistic sites (1,013 mm y^{-1}), biomass-MAP relationships remained steepest in the synergistic co-limitation sites (Supplemental Figure 1, Supplemental Table 3). Also, the biomass-MAP relationships of unfertilized plots of synergistic sites did not differ from unfertilized plots in sites with other limitation forms ($F = 1.0$, $p = 0.40$). Second, site-level factors including management, latitude, elevation, mean annual temperature and soil total nutrient contents or texture could contribute to varying biomass-MAP relationships. However, none differed in occurrence or magnitude among limitation categories (Supplemental Table 4). Thus, our results suggest that the form of nutrient limitation strongly affects the steepness of the global biomass-MAP relationship.

3) *Does mediation of the biomass-MAP relationship by community diversity increase with the strength of co-limiting nutrient interactions?*

Contrary to our hypothesis, community diversity played little role in mediating biomass-MAP relationship for any form of nutrient limitation. SEMs relating biomass to MAP, the # of nutrients added, and diversity metrics fit adequately for all sites combined and for each limitation form ($p > 0.63$, Table 2). MAP was the largest driver of aboveground biomass in each limitation form (Figure 4A, 5). The total effect of MAP on aboveground biomass (Supplemental Table 5) ranged from 0.238 to 0.674, which was 2 to 10 – fold larger than total nutrient effects (0.021 to 0.293). Direct effects of MAP and nutrients accounted for nearly all the total effects (Figure 4B). Mediation of MAP effects on biomass by community diversity was near 0 (Figure 4C), despite large decreases in effective species richness (eH) and evenness and increased beta diversity (βplot) averaged across all sites (Supplemental Figure 2). SEMs also included mediation of nutrient effects on biomass by community diversity. These were also near 0, with one exception. Single nutrient-limited sites displayed a small community diversity - mediated effect of nutrients on aboveground biomass (0.057, $p = 0.00$, Figure 4C), representing a positive feedback on aboveground biomass mediated by the combined effects of eH, evenness and βplot.

Mediation of MAP and nutrient effects on aboveground biomass by community diversity was small because either paths linking MAP and nutrients to the diversity variables (eH, evenness, and βplot) were not resolved (Figure 5), or in other cases paths to and from multiple diversity variables were resolved but one diversity variable offset another. For example, community mediation of MAP effects on aboveground biomass in single limitation sites was absent ($p = 0.60$) because mediation by eH was negative ($-0.165 \times 0.197 = -0.032$) but mediation by evenness positive ($-0.213 \times -0.189 = 0.040$). These findings indicate that in sites with multiple nutrient co-limitation, aboveground biomass is predominantly controlled by MAP and whether N, P or both were added.

Discussion

Anthropogenic global changes are concurrently enriching ecosystems with multiple potentially limiting nutrients and causing long-term changes in MAP (1, 2). These changes will have significant consequences for aboveground biomass production, a key component of primary productivity, global carbon cycling and many ecosystem services. Our findings largely supported the Multiple Nutrient Co-limitation hypothesis. They demonstrate that the global grassland biomass-MAP relationship is governed by whether plant aboveground biomass production is limited by one or multiple nutrients (question 1), and the strength of interaction among co-limiting nutrients (question 2), with the steepest biomass-MAP relationship where grassland biomass production was synergistically co-limited by N and P. However, we found little evidence for mediation of the biomass-MAP relationship by community diversity changes (question 3), represented by the combined effects of eH, evenness, or βplot, because of weak or offsetting effects on biomass among these measures of species diversity. These findings provide robust experimental support for the long-held principle that nutrient availability increasingly limits primary productivity across spatial gradients of increasing mean water availability (17, 19), and extend that principle by revealing the key roles for the number and identity of limiting nutrients and the strength of their interactions.

It is well established that the form of nutrient limitation – single limitation, additive co-limitation, synergistic co-limitation, or no nutrient limitation – is an important determinant of site-level biomass production (9, 10, 12, 31, 49). Our findings provide robust, global-scale experimental support for the prediction that the effects of supply single nutrients and multiple interacting nutrients extend to the biomass-MAP relationship in grasslands (17, 19). This finding builds on existing concepts (3-6, 11) by making clear how grasslands varying in number of limiting nutrients and strength of co-limitation nutrients, in particular N and P, predicted the increases in slope of biomass-MAP relationships (Figures 1,3, and 4). Steeper biomass-MAP relationships are consistent with at least two identified mechanisms; multiple nutrient fertilization causes greater temporal variance in biomass production (31) and steeper within-site temporal biomass-precipitation relationships (58). The present findings align with our previous findings of widespread site level synergistic co-limitation of grassland biomass production by N and P (12, 31). Here we consider more sites than in earlier analyses (12, 31), extending their generality. Furthermore, with many sites now fertilized for over a decade (vs. 4 to 7 years), more forms of nutrient interactions can be resolved. We continue to find little evidence for across-site average limitation by Kμ, alone or in interaction with N and P, although individual sites may continue to be Kμ limited (12).

In sites where N and P individually or jointly limited biomass production , fertilizing with these nutrients increased the coupling of biomass production to MAP, as expected if fertilizing alleviates nutrient limitations and water availability becomes the primary control on biomass production (16, 19, 22, 23, 59, 60). The unfertilized control plots represented the baseline biomass-MAP relationship for these sites. This baseline relationship was relatively weak, with slope of ~0.30 and explaining about 20% of the variation. In contrast, continental scale studies found considerably stronger grassland biomass-MAP relationships with slopes of ~ 0.60 to that explained 50% to 95% of the variation in biomass (59). The relatively weak baseline biomass-MAP relationship we found may be unsurprising for sites spanning multiple continents, large differences in plant species assemblages, and varying management, soils, latitude, and aspects of climate other than MAP (Supplemental Table 1). Notably, we found that fertilizing synergistically co-limited grasslands with NP resulted in stronger biomass-MAP relationships (Figure 3, Supplemental Table 3) than in the continental scale studies (59), suggesting biomass production in synergistically co-limited grasslands fertilized with N and P was more strongly coupled to mean water availability than observed in several major world grassland regions.

Our findings ran counter to our prediction that plant community change would mediate the biomass-MAP relationship (34, 61, 62). We found no evidence that combined changes in effective species richness, evenness, or plot β diversity mediated the biomass-MAP relationship despite synergistic decreases averaged across sites in all three measures of species diversity in plots fertilized with both N and P (Supplemental Figure 2) and other evidence that fertilization reduces compositional stability (55, 63) and increase sensitivity to precipitation (64). Several underlying mechanisms may explain this finding. Fertilization effects on species diversity within sites may be poor predictors of responses across larger spatial gradients (65) because the MAP gradient encompasses large, potentially non-linear diversity changes (45) while within-site responses are limited by the local species pool. In addition, longer time periods may be required to detect plant community mediation (34, 61). Biomass and diversity responses increased through 11 years of NPK fertilization treatment (66), so community mediation may emerge when more sites accumulate more years of fertilization (39). Finally, we did not consider abundance-weighted metrics (40) or functional group changes (39). However, biomass gains following fertilization can be explained by species that persist following fertilization rather than by replacement (51-53, 66). Further analysis of plant compositional dynamics in grasslands differing in form and strength of single and multiple nutrient limitation is a promising area for future research.

Our findings point to a critical need for better understanding of edaphic mechanisms causing single and multiple nutrient limitation in grasslands. Because water is necessary for biogeochemical processes, mechanisms likely center on ways that water availability influences nutrient availability (67, 68). The strength of interaction in response to inputs of co-limiting nutrients may depend on alignment of the timing and amount of available water with the timing and threshold amounts determining microbial processing, biogeochemical cycling, and plant uptake of both nutrients (69, 70). For example, Vázquez*, et al.* (71) found that synergistic increases in aboveground biomass production resulted in part from enhanced N and P uptake and retention. Water availability can also interact with soil parent material to control nutrient availability (72, 73) and stoichiometric coupling of the elements we manipulated with others that we did not manipulate in factorial combination with N and P such as calcium or magnesium (74). A comprehensive spatial model incorporating mechanistic drivers of single and multiple nutrient limitation is needed to link with productivity models to predict global scale responses of ecosystem productivity to changing precipitation and eutrophication.

The core finding of this study is that the steepness of the grassland biomass-MAP relationship depends on the number of limiting nutrients and the strength of interactions among co-limiting nutrients, as predicted by the Multiple Nutrient Co-limitation hypothesis. Specifically, biomass-MAP relationships were weakest in grasslands not limited by N or P and were steepest where biomass production was synergistically co-limited by N and P. This critical, globally relevant insight on the regulation of grassland productivity can be exploited to predict the interactive effects of eutrophication and hydrologic intensification on grassland productivity and related ecosystem services. Applying this insight will require predicting the number and strength of interaction among multiple limiting nutrients across the world's grasslands, and developing a more general understanding of magnitude and extent to which plant community change mediates grassland productivity – precipitation relationships.

Abbreviated Methods

Each site applied the Nutrient Network standard experimental protocol (56). Nitrogen (N), phosphorus (P), and potassium (K; in year 1, applications also included micronutrients) were applied to 30 to 50 5 m x 5m plots per site in a factorial design, which allowed application of a rubric (Extended Methods Table 3) to classify the form of nutrient limitation (Figure 1) at each site and to interpret changes in slope of the biomass-MAP relationship across all sites and by each form of nutrient limitation. Each nutrient was applied at 10 g m^{-2} yr^{-1}, a rate expected to exceed plant demand (56). Sites included in this study were fertilized for 4 to 15 years (Supplemental Table 1), yielding 18,361 total experimental plot-years. Each site used network protocols to measure peak aboveground live biomass production (in g m^{-2} y^{-1}) and the percent cover of each plant species (56) in each plot. From species cover we derived three diversity metrics: the effective number of species (e^H), representing species richness if all species were equally abundant (75); Whitaker's beta (βplot), the ratio of site level species richness to plot-level species richness (76), and Evenness (E), describing the distribution of species relative abundances and the inverse of dominance. Preparation of the biomass and diversity data is detailed in Extended Methods.

We focused on site MAP as the predictor of aboveground biomass after screening 31 other potential site metrics of precipitation, temperature, and evaporative demand. None provided better model fit than MAP based on AIC values from linear mixed models relating the fertilization treatments and their interactions with each candidate variable to aboveground biomass (Extended Methods Table 2). For most (59) sites MAP was derived from weather station measured precipitation summed from harvest to harvest for the selected years of biomass and cover data (Extended Methods). For the rest we determined MAP from downscaled precipitation estimates from CRU (citation) or BIOCLIM (77) (Extended Methods Tables 1, 2). Precipitation from CRU and BIOCLIM were highly correlated and unbiased estimates of measured precipitation for sites where we had both sources ($R^2 > 0.95$, slopes ~1.0, Extended Methods Figures 1,2).

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The data and code used in this paper will be deposited in DRYAD on acceptance.

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Siddharth Bharath				x		x			
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Figure 1. Conceptualized changes in the global mean aboveground biomass - mean annual precipitation (MAP) relationship. Responses (here scaled in arbitrary linear units) depend on the number of nutrients limiting aboveground biomass and the form of interactions among co-limiting nutrients – additive, sub-additive, or synergistic. The predicted increase in slope of the grassland biomass-MAP relationship is defined by the mean response of biomass to fertilization with putatively limiting nutrients A and B singly and together (AB). Limitation forms are generalized from (8). See Extended Methods Table 3 for the assignment rubric and expansion of non-additive (subadditive, synergistic) forms.

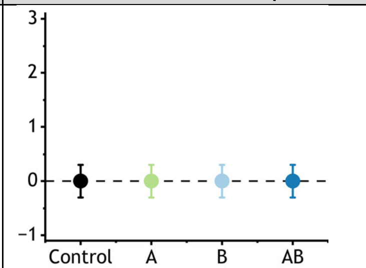
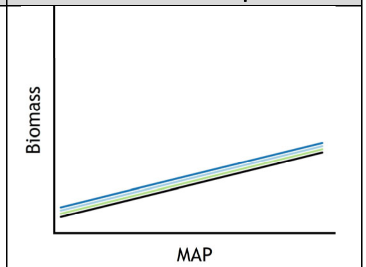
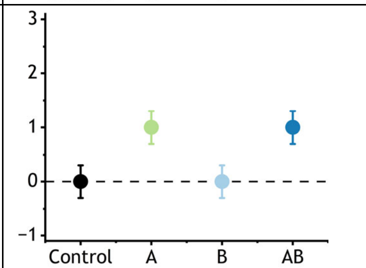
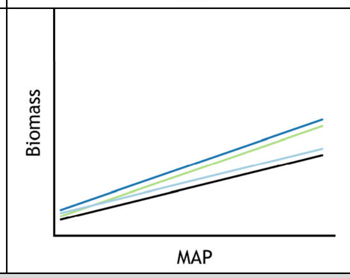
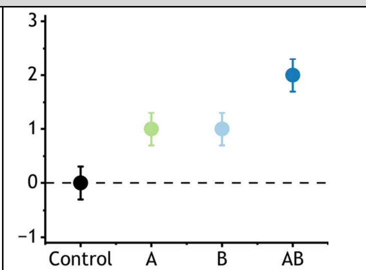
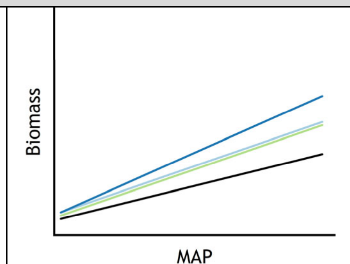
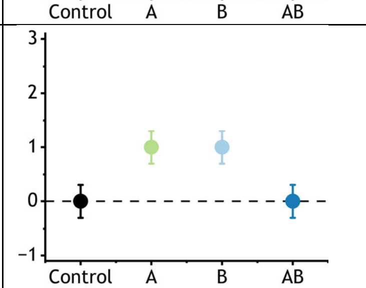
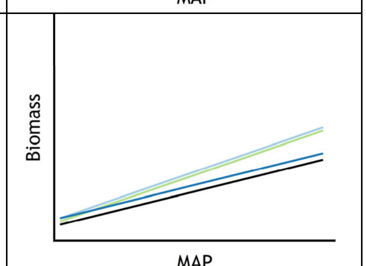
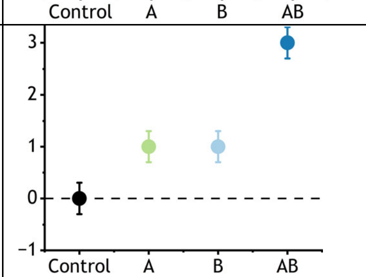
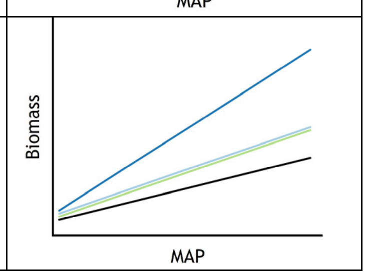
Form of Nutrient Limitation	Definition	Mean Biomass Response	Predicted Biomass-MAP Relationships
No Limitation	Biomass does not increase with A or B, alone or together.		
Single	Biomass increases only when one nutrient is added (A) is added, with or without the other (B).		
Multiple Nutrient Co-limitation			
Additive	Biomass increase with A and B together (AB) is the sum of increases with A and B singly.		
Subadditive	Biomass increase with A and B together is less than the sum of A and B singly. At least one single nutrient must increase biomass.		
Synergistic	Biomass increase with AB together is greater than the sum of A and B singly. None, one, or both single nutrients may contribute.		

Figure 2. Aboveground biomass responses to fertilization with single nitrogen (N), phosphorus (P), and potassium with year 1 micronutrients (Kµ). A) the aboveground biomass – mean annual precipitation (MAP) relationship by number of nutrients added. Inset shows the percent increase in linear regression slope relative to unfertilized controls. B) the aboveground biomass-MAP relationships for factorial combinations of N, P, and Kµ fertilization. Inset shows the percent increase in linear regression slopes relative to unfertilized controls for N, P, and NP treatments averaged across levels of Kµ. Nutrient treatments are color-coded as in Panel C. C) Across-site mean ± standard error of aboveground biomass for factorial combinations of N, P, and Kµ fertilization. The linear regression equations are presented in Supplemental Table 3.

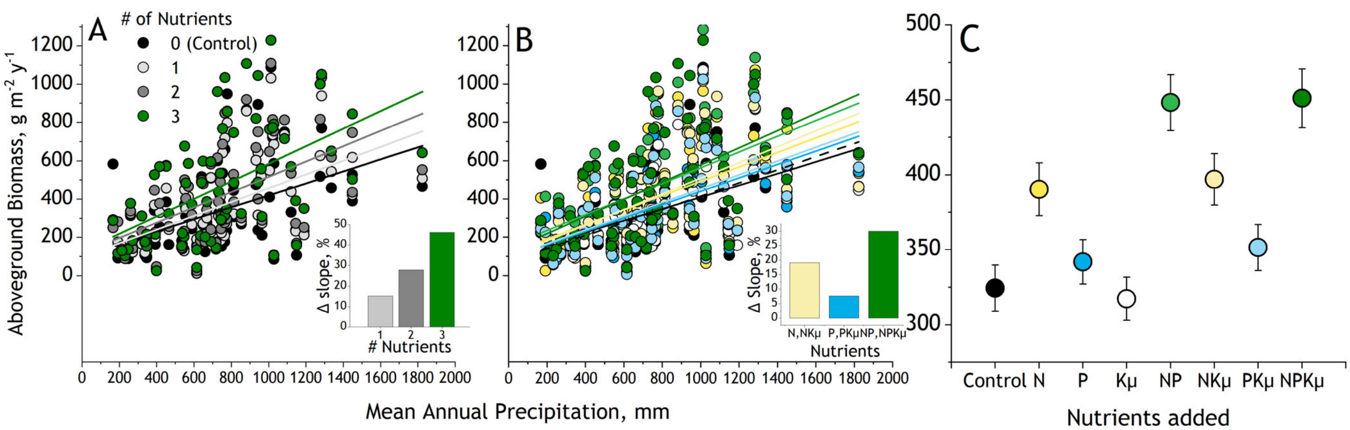


Table 1. Linear Mixed model F statistics and p-values for aboveground biomass, effective species richness (eH), Evenness, and plot β.

	Aboveground biomass		**eH**		**Evenness**		**plotß**	
Mixed model effects	**F(dfs)**	**p-value**	**F(dfs)**	**p-value**	**F(dfs)**	**p-value**	**F(dfs)**	**p-value**
				# of Nutrients				
# Nutrients	69.9(3,140)	**< 0.0001**	29.3(3,185)	**< 0.0001**	20.0(3,203)	**< 0.0001**	25.0(3,187)	**< 0.0001**
MAP	27.3(1,69)	**< 0.0001**	0.1(1,70)	0.8123	0.1(1,70)	0.7999	0.0(1,70)	0.9374
MAP x # Nutrients	8.7(3,150)	**< 0.0001**	1.8(3,195)	0.1520	1.1(3,226)	0.3480	2.3(3,200)	0.0805
				Factorial Nutrient Combinations				
N	253.0(1,452)	**< 0.0001**	99.7(1,504)	**< 0.0001**	70.8(1,461)	**< 0.0001**	93.1(1,467)	**< 0.0001**
P	58.3(1,452)	**< 0.0001**	18.1(1,504)	**< 0.0001**	6.9(1,461)	**0.0089**	11.1(1,467)	**0.0009**
N*P	7.8(1,452)	**0.0056**	1.6(1,504)	0.2128	5.4(1,461)	**0.0211**	4.4(1,467)	**0.0359**
Kμ	0.4(1,452)	0.5043	4.8(1,504)	**0.0285**	1.7(1,461)	0.1900	3.0(1,467)	0.0864
N*Kμ	0.1(1,452)	0.7727	0.0(1,504)	0.9422	0.1(1,461)	0.8034	0.0(1,467)	0.8408
P*Kμ	0.4(1,452)	0.5416	0.0(1,504)	0.8773	0.6(1,461)	0.4536	0.1(1,467)	0.7816
N*P*Kμ	0.8(1,452)	0.3655	0.0(1,504)	0.8363	0.0(1,461)	0.9206	0.3(1,467)	0.5915
MAP	27.5(1,69)	**< 0.0001**	0.0(1,69)	0.8700	0.1(1,69)	0.7467	0.0(1,70)	0.8532
MAP*N	20.7(1,475)	**< 0.0001**	0.0(1,531)	0.8743	3.5(1,499)	0.0637	5.2(1,498)	**0.0235**
MAP*P	4.4(1,475)	**0.0367**	7.8(1,531)	**0.0054**	0.4(1,499)	0.5097	0.7(1,498)	0.4058
MAP*N*P	0.0(1,475)	0.8270	1.4(1,531)	0.2310	0.0(1,499)	0.9873	0.4(1,498)	0.5338
MAP*Kμ	3.4(1,475)	0.0641	0.6(1,531)	0.4405	0.1(1,499)	0.8151	0.1(1,498)	0.7696
MAP*N*Kμ	0.2(1,475)	0.6817	3.1(1,531)	0.0782	3.2(1,499)	0.0749	0.6(1,498)	0.4565
MAP*P*Kμ	0.0(1,475)	0.8620	0.5(1,531)	0.4856	0.2(1,499)	0.6627	1.2(1,498)	0.2688
MAP*N*P*Kμ	0.2(1,475)	0.6918	0.0(1,531)	0.8398	0.6(1,499)	0.4323	0.4(1,498)	0.5341

Figure 3. Aboveground biomass in relation to mean annual precipitation for sites classified by form of response to nitrogen (N) and phosphorus (P) fertilization. Treatments are averaged across levels of Kμ fertilization. See Supplemental Table 3 for linear regression equations. For panel C, there is one relatively low biomass site at high (1800 mm) MAP (Figure 3C; burren.ie); if this site is omitted the biomass-MAP slopes becomes steeper (slopes of 0.40 to 0.58; R^2 0.31 to 0.33), but still less steep than for synergistic co-limitation sites (panel D). Upper panels are kernel-smoothed site MAP distributions for each response classification.

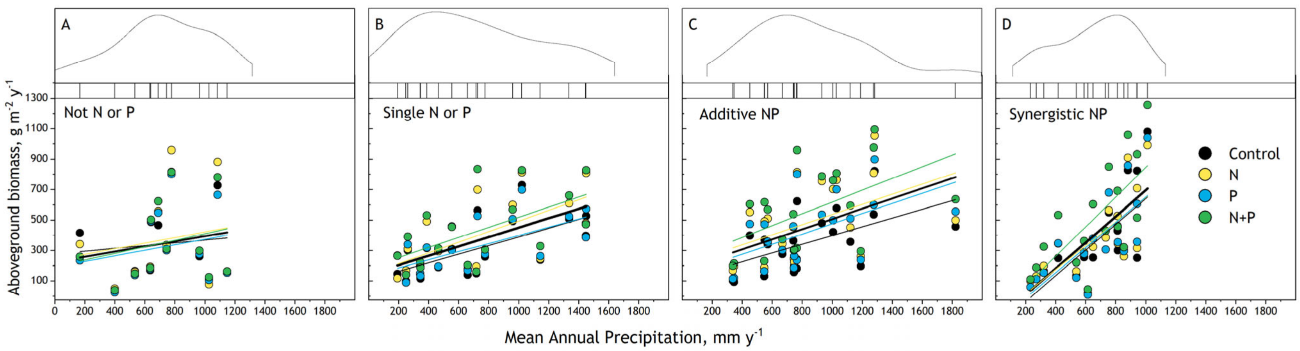


Table 2. Fit statistics for structural equation models fit to all sites and to sites classified by the four forms of nutrient limitation. $\chi 2$: Chi-square test. RMSEA: Root mean square error. PCF: Probability of close fit.

	Model Fit		
	$\chi 2$ (p-value)	**RMSEA**	**PCF**
Kind of Limitation		**Thresholds**	
	p > 0.05	**< 0.06**	**> 0.05**
All	1.73 (0.6311)	0.0000	0.9711
No Limitation	0.69 (0.9521)	0.0000	0.9737
Single	0.26 (0.8779)	0.0000	0.9340
Additive	0.07 (0.9955)	0.0000	0.9983
Synergistic	0.41 (0.9377)	0.0000	0.9719

Figure 4. Summary of the effects identified in structural equation models of nutrient addition and mean annual precipitation (MAP) on aboveground biomass production for grassland sites assigned to four forms of nutrient limitation (Figure 5, Supplemental Table 5). A) Total (direct + indirect) effects of nutrients and MAP from the models fit to each form of nutrient limitation. The left nodes represent total effects of MAP and # of nutrients across all sites, and the right nodes represent total # nutrients and MAP effects for sites in four limitation forms. The links between nodes depicts variation in total effects among the limitation forms. The total effect of MAP on aboveground biomass generally increases relative to total nutrient effects from no limitation (None) to synergistic co-limitation. Panels B and C partition the total effects of nutrient addition and MAP on biomass. B) Standardized direct effects of MAP and nutrient addition. C) Standardized indirect effects of MAP and nutrient addition on biomass mediated by the combined effects of effective species richness, species evenness, and beta diversity.

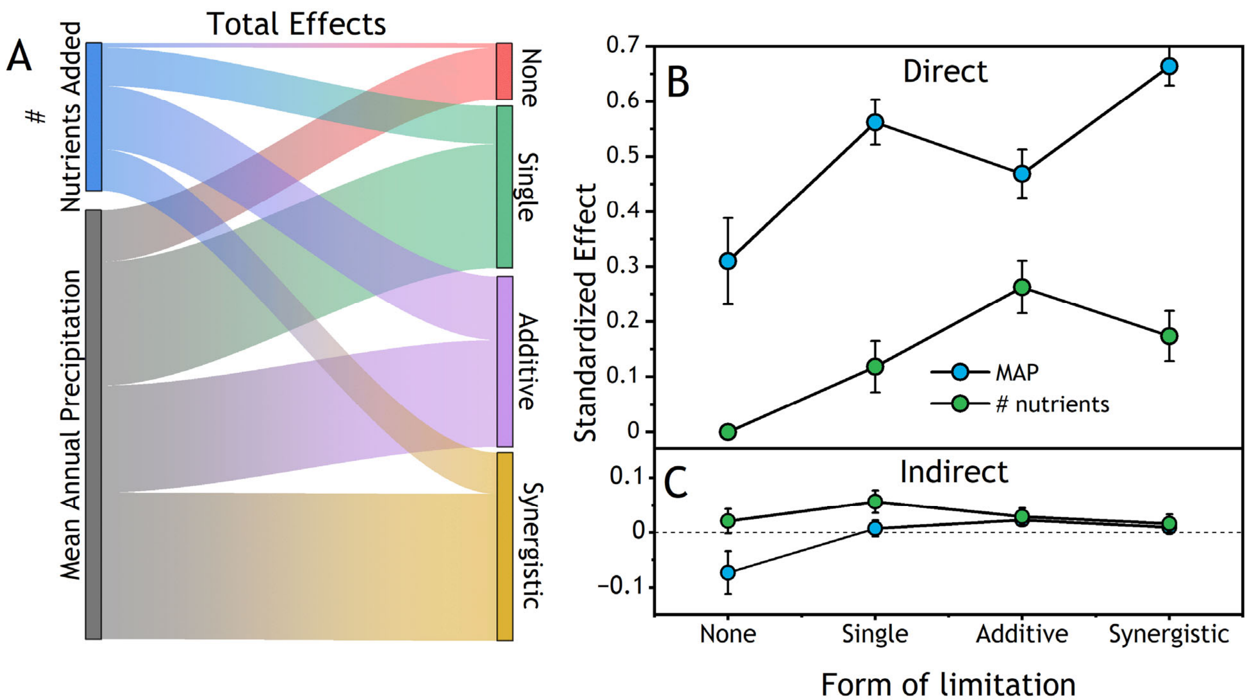
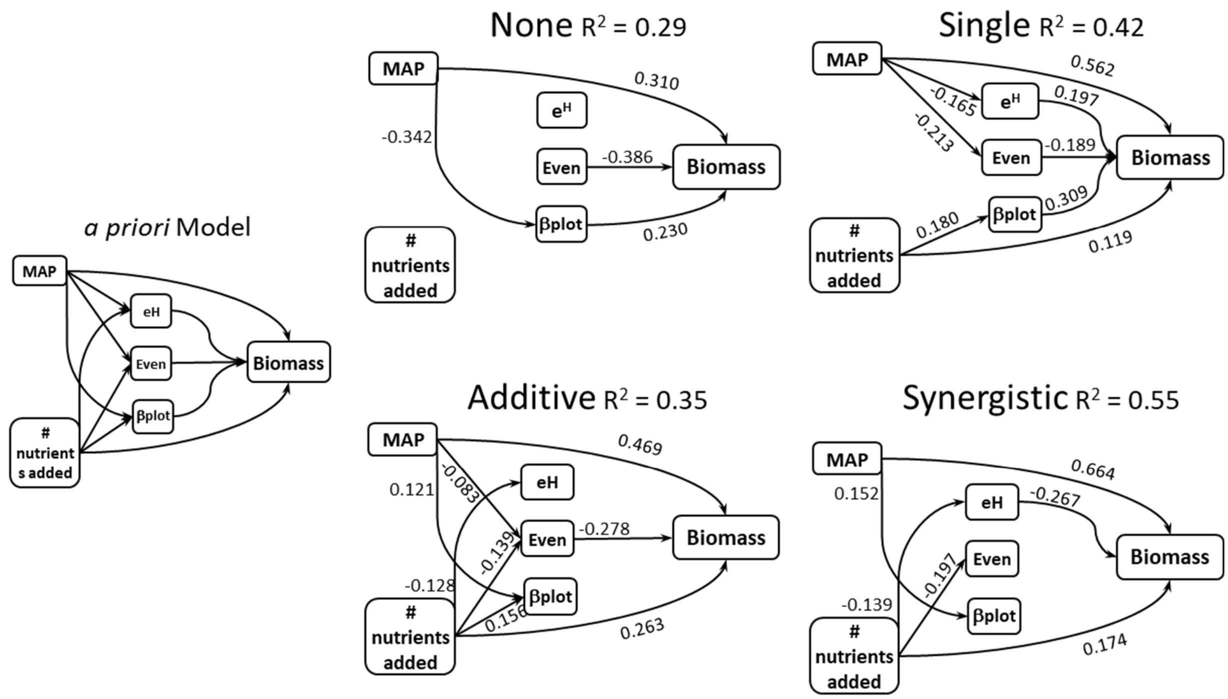


Figure 5. Structural equation models relating aboveground biomass (Biomass) to mean annual precipitation (MAP), #of nutrients added, effective species richness (e^H), evenness (Even), and beta diversity (βplot). The *a priori* model was fit separately to sites classified as not limited by N or P (None, 12 sites), limited by N or P (Single, 18 sites), additively co-limited by N and P (Additive, 20 sites) and synergistically co-limited by N and P (Synergistic, 17 sites). Depicted paths indicate statistically significant direct effects. Non-significant paths are not shown. See Table 2 for fit statistics, Figure 4 for summaries of total, direct, and indirect effects of MAP and # of nutrients added, and Supplementary Table 5 for effect p-values.



Supplemental Table 1. Biophysical properties of the study sites, sorted from lowest to highest mean annual precipitation (MAP). MAT: mean annual temperature (MAT). See Extended Methods for explanation of MAP source.

Site Code	Site Name (1)	Continent	Latitude, Longitude, °	Elevation, m	MAT, ºC	MAP, mm	Unfertilized biomass, g m^{-2}	MAP Source
ethass.au	Ethabuka (South Site) (6, G)	Australia	-23.6, 138.4	104	24.0	167	583.5	Station
ethamc.au	Ethabuka (Main Camp) (6, B)	Australia	-23.8, 138.5	104	24.1	192	91.1	Station
sevi.us	Sevilleta LTER (15)	N. Amer.	34.4, -106.7	1600	13.1	214	138.8	Station
hart.us	Hart Mountain (5)	N. Amer.	42.7, -119.5	1508	7.7	232	88.6	Station
potrok.ar	Potrok Aike (6, M)	S. Amer.	-51.9, -70.4	160	6.6	249	87.5	BIOCLIM
elliot.us	Elliott Chaparral (12)	N. Amer.	32.9, -117.1	200	17.7	261	313.3	Station
shps.us	Sheep Experimental Station (5, G)	N. Amer.	44.3, -112.2	1661	5.3	272	127.8	Station
mtca.au	Mt. Caroline (13)	Australia	-31.8, 117.6	285	17.7	321	175.5	Station
sgs.us	Shortgrass Steppe LTER (14)	N. Amer.	40.8, -104.8	1650	8.9	338	105.5	Station
msla.us	Missoula (4)	N. Amer.	46.7, -114.0	1169	7.3	344	156.9	Station
msla_2.us	Missoula - MPG Ranch (4)	N. Amer.	46.7, -114.0	1188	7.3	346	98.6	Station
msla_3.us	Missoula - MPG Ranch - 3 (4)	N. Amer.	46.7, -114.0	1158	7.3	346	92.6	Station
sedg.us	Sedgwick Reserve UCNRS (10)	N. Amer.	34.7, -120.0	550	15.6	389	340.1	Station
kiny.au	Kinypanial (10)	Australia	-36.2, 143.8	90	15.6	393	163.4	Station
kibber.in	Kibber (Spiti) (5)	Asia	32.3, 78.0	4241	-1.5	400	37.1	BIOCLIM
ping.au	Pingelly Paddock (8, G, C)	Australia	-32.5, 117.0	338	16.3	417	254.8	Station
badlau.de	Bad Lauchstaedt (7, M, C)	Europe	51.4, 11.9	120	9.3	451	384.9	Station
cdpt.us	Cedar Point Biological Station (14)	N. Amer.	41.2, -101.6	965	9.6	466	161.3	Station
kilp.fi	KilpisjÃ¤rvi (7, G)	Europe	69.1, 20.9	700	-3.3	535	181.9	Station
saana.fi	Saana (8)	Europe	69.0, 20.8	600	-2.6	538	144.1	Station
bldr.us	Boulder South Campus (8, M)	N. Amer.	40.0, -105.2	1633	9.9	547	136.1	Station
smith.us	Smith Prairie (9)	N. Amer.	48.2, -122.6	63	10.2	550	372.3	Station
msum.us	Minnesota State Univ. Morehead (5, B)	N. Amer.	46.9, -96.5	311	5.0	556	309.3	BIOCLIM
jena.de	JeNut (9, M)	Europe	50.9, 11.5	320	8.6	571	334.6	Station
saline.us	Saline Experimental Range (8)	N. Amer.	39.1, -99.1	555	12.1	590	244.0	Station
ahth.is	Audkuluheidi Heath (4, G)	Europe	65.1, -19.7	470	0.8	615	158.7	BIOCLIM
amlr.is	Audkuluheidi Melur (4, G)	Europe	65.1, -19.7	470	0.8	615	12.1	BIOCLIM

Site Code	Site Name (1)	Continent	Latitude, Longitude, °	Elevation, m	MAT, ºC	MAP, mm	Unfertilized biomass, g m^{-2}	MAP Source
burrawan.au	Burrawan (11)	Australia	-27.7, 151.1	425	18.2	635	199.1	Station
cereep.fr	CEREEP - Ecotron IDF (8, G, C)	Europe	48.3, 2.7	83	10.8	641	517.2	CRU
comp.pt	Companhia das Lezirias (9, G, C)	Europe	38.8, -8.8	20	16.6	650	262.9	Station
sage.us	Sagehen Creek UCNRS (6)	N. Amer.	39.4, -120.2	1920	5.8	660	123.7	Station
mcla.us	Mclaughlin UCNRS (13)	N. Amer.	38.9, -122.4	642	14.0	669	276.7	Station
hero.uk	Heronsbrook (Silwood Park) (5)	Europe	51.4, -0.6	60	10.2	690	508.9	Station
rook.uk	Rookery (Silwood Park) (5)	Europe	51.4, -0.6	60	10.1	690	180.2	Station
doane.us	Doane College (7, B, C)	N. Amer.	40.7, -96.9	418	10.6	718	137.4	Station
lake.us	Lakeside Laboratory (4, B, C)	N. Amer.	43.4, -95.2	452	7.3	726	599.9	BIOCLIM
azitwo.cn	Azi Two (4)	Asia	33.6, 101.5	3500	-1.1	733	325.5	BIOCLIM
yarra.au	Yarramundi (7, M, C)	Australia	-33.6, 150.7	19	17.3	742	378.3	Station
bayr.de	Bayreuth (5, M, C)	Europe	49.9, 11.6	340	8.5	745	163.1	BIOCLIM
sereng.tz	Serengeti (4)	Africa	-2.4, 34.9	1536	21.9	745	298.8	CRU
nilla.au	Nillahcootie (6, G, C)	Australia	-36.9, 146.0	280	13.8	748	152.4	Station
ukul.za	Ukulinga (11, M)	Africa	-29.7, 30.4	842	17.7	755	534.3	Station
cdcr.us	Cedar Creek LTER (14)	N. Amer.	45.4, -93.2	270	6.3	764	176.4	Station
koffler.ca	Koffler Reserve (11, M)	N. Amer.	44.0, -79.5	301	6.3	767	660.0	Station
hopl.us	Hopland REC (13)	N. Amer.	39.0, -123.1	598	13.2	776	235.0	Station
pape.de	Papenburg (6, C)	Europe	53.1, 7.5	0	9.1	779	948.8	CRU
konz.us	Konza LTER (11, B)	N. Amer.	39.1, -96.6	440	12.1	813	444.8	Station
sier.us	Sierra Foothills REC (13)	N. Amer.	39.2, -121.3	197	16.3	814	306.7	Station
bnch.us	Bunchgrass (Andrews LTER) (14)	N. Amer.	44.3, -122.0	1318	6.8	855	269.0	CRU
temple.us	USDA ARS Temple, TX (11)	N. Amer.	31.0, -97.4	184	19.4	883	865.7	Station
cowi.ca	Cowichan (14)	N. Amer.	48.8, -123.6	50	10.4	932	475.4	Station
gilb.za	Mt Gilboa (4, B)	Africa	-29.3, 30.3	1748	14.1	943	237.7	BIOCLIM
bnbt.us	Benedictine Bottoms (5, B)	N. Amer.	39.6, -95.1	240	12.4	944	893.4	BIOCLIM
kbs.us	Kellogg Biological Station LTER (6)	N. Amer.	42.4, -85.4	288	8.8	961	541.6	Station
valm.ch	Val Mustair (12)	Europe	46.6, 10.4	2320	0.1	965	211.8	Station
cbgb.us	Chichaqua Bottoms (12, B, C)	N. Amer.	41.8, -93.4	274	9.3	1006	411.1	Station

Site Code	Site Name (1)	Continent	Latitude, Longitude, °	Elevation, m	MAT, ºC	MAP, mm	Unfertilized biomass, g m^{-2}	MAP Source
trel.us	Trelease (9)	N. Amer.	40.1, -88.8	200	11.1	1013	1088.7	Station
marc.ar	Mar Chiquita (11)	S. Amer.	-37.7, -57.4	6	14.3	1022	719.8	Station
sava.us	Savannah River (5)	N. Amer.	33.3, -81.7	71	17.4	1028	85.4	Station
chilcas.ar	Las Chilcas (9)	S. Amer.	-36.3, -58.3	15	15.1	1029	586.2	Station
pinj.au	Pinjarra Hills (4, G, C)	Australia	-27.5, 152.9	38	20.0	1085	748.9	BIOCLIM
unc.us	Duke Forest (4, C)	N. Amer.	36.0, -79.0	141	14.9	1121	330.6	Station
lagoas.br	Tres Lagoas (7, B, C)	S. Amer.	-21.0, -51.8	279	23.2	1145	240.1	BIOCLIM
lancaster.uk	Lancaster (9, G)	Europe	54.0, -2.6	202	8.0	1150	106.7	Station
look.us	Lookout (Andrews LTER) (14)	N. Amer.	44.2, -122.1	1500	6.9	1190	233.7	CRU
hall.us	Hall's Prairie (7, M)	N. Amer.	36.9, -86.7	194	13.8	1277	518.6	Station
frue.ch	Fruebuel (7, G, C)	Europe	47.1, 8.5	995	7.0	1284	770.6	Station
spin.us	Spindletop (14, G, C)	N. Amer.	38.1, -84.5	271	12.5	1337	457.8	Station
arch.us	Archbold Biological Station (7, B)	N. Amer.	27.2, -81.2	8	22.7	1449	389.0	Station
bogong.au	Bogong (14)	Australia	-36.9, 147.3	1760	6.0	1450	527.2	Station
burren.ie	Slieve Carran (5, G)	Europe	53.1, -9.0	104	9.8	1824	466.1	Station

(1) Number of years of aboveground biomass data analyzed. B: burned, C: cultivated grassland, G: grazed, M: unspecified management.

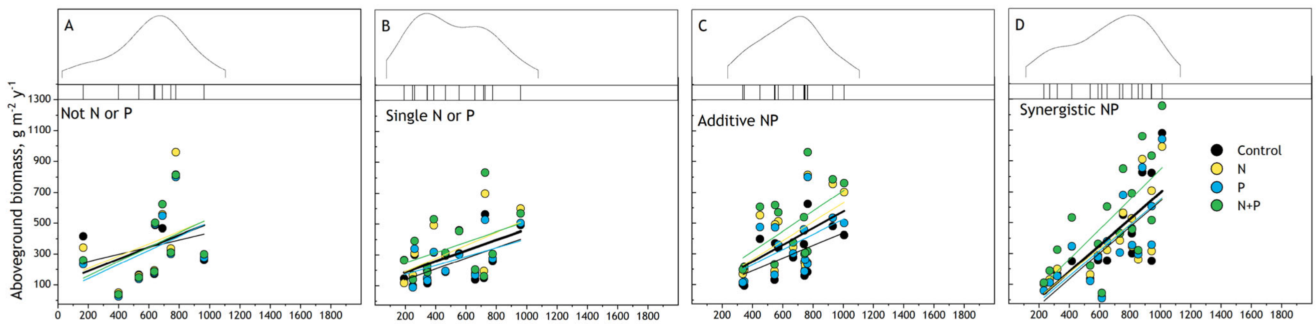
Supplemental Table 2. Classification of sites into forms of nutrient limitation. Significant effects shown in bold font. Significance of treatment mean effects is determined by the Linear Mixed Model p-value for N or P main effects. P-values for single treatment effects denote whether those effects are significantly different from 0.

	N & P Main Effects				NxP Interaction	Single Treatment Effects					
Site	**N**	**p-value**	**P**	**p-value**	**p-value**	**N**	**p-value**	**P**	**p-value**	**NP**	**p-value**
Superadditive											
amlr.is	**188.1**	0.0001	**65.7**	0.0224	0.0036	50.5	0.2908	-28.5	0.5471	**243.7**	<0.0001
comp.pt	**45.1**	0.0000	**71.5**	0.0000	0.0180	26.9	0.1242	**51.3**	0.0045	**137.8**	<0.0001
ping.au	**47.3**	0.0000	**48.1**	0.0000	0.0376	**38.0**	0.0032	**38.8**	0.0026	**113.6**	<0.0001
gilb.za	**36.9**	0.0000	**55.3**	0.0000	0.0096	**25.8**	0.0129	**43.4**	0.0001	**107.4**	<0.0001
mtca.au	**65.0**	0.0000	**32.1**	0.0000	0.0000	**21.3**	0.0525	-6.2	0.5717	**98.5**	<0.0001
saana.fi	**49.9**	0.0000	**16.5**	0.0141	0.0003	**20.1**	0.0526	-9.3	0.3665	**65.8**	<0.0001
konz.us	**36.5**	0.0000	**20.6**	0.0000	0.0020	**22.6**	0.0012	7.8	0.2525	**60.9**	<0.0001
shps.us	**37.0**	0.0000	**19.0**	0.0142	0.0009	8.1	0.4689	-7.7	0.4917	**55.3**	<0.0001
ukul.za	**15.7**	0.0453	**37.3**	0.0000	0.0917	2.8	0.8161	**23.2**	0.0601	**55.3**	<0.0001
sier.us	**22.2**	0.0000	**25.1**	0.0000	0.0668	**15.1**	0.0303	**18.0**	0.0104	**51.2**	<0.0001
saline.us	**22.1**	0.0005	**17.8**	0.0036	0.0847	12.2	0.1658	8.0	0.3584	**41.9**	<0.0001
azitwo.cn	**33.4**	0.0000	**6.4**	0.0366	0.0321	**25.9**	<0.0001	-0.2	0.9685	**40.6**	<0.0001
temple.us	**16.9**	0.0000	**10.3**	0.0080	0.0777	**10.1**	0.0774	3.7	0.5100	**28.0**	<0.0001
trel.us	6.0	0.2900	**10.9**	0.0638	0.0129	-8.3	0.2942	-3.7	0.6347	**16.3**	0.0408
bnbt.us	16.9	0.1198	-1.1	0.9113	0.0090	-10.6	0.4253	**-26.3**	0.0484	13.6	0.2985
hart.us	**31.7**	0.0498	-15.1	0.2343	0.0430	-0.8	0.9626	**-41.1**	0.0248	10.1	0.5680
bnch.us	-2.0	0.6964	**9.9**	0.0759	0.0425	**-12.7**	0.0867	-1.4	0.8534	7.3	0.3231
Additive											
msla_3.us	**94.3**	0.0000	**16.7**	0.0212	0.7633	**111.5**	<0.0001	**29.4**	0.0643	**134.2**	<0.0001
sgs.us	**68.3**	0.0000	**14.9**	0.0125	0.3425	**65.1**	<0.0001	12.3	0.2624	**92.2**	<0.0001
bayr.de	**59.0**	0.0000	**20.3**	0.0041	0.3107	**55.6**	<0.0001	17.4	0.1479	**90.1**	<0.0001
hall.us	**57.4**	0.0000	**17.2**	0.0455	0.3567	**50.9**	0.0011	11.7	0.4358	**82.3**	<0.0001
cbgb.us	**59.7**	0.0000	**12.8**	0.0130	0.8973	**66.0**	<0.0001	**17.9**	0.0637	**82.1**	<0.0001
bldr.us	**42.6**	0.0026	**23.4**	0.0583	0.7341	**42.6**	0.0449	23.4	0.2579	**75.9**	0.0009
cdcr.us	**33.9**	0.0000	**30.4**	0.0000	0.2675	**33.6**	<0.0001	**30.1**	<0.0001	**74.5**	<0.0001
jena.de	**54.1**	0.0000	**9.4**	0.0868	0.4286	**50.5**	<0.0001	6.4	0.5029	**67.7**	<0.0001

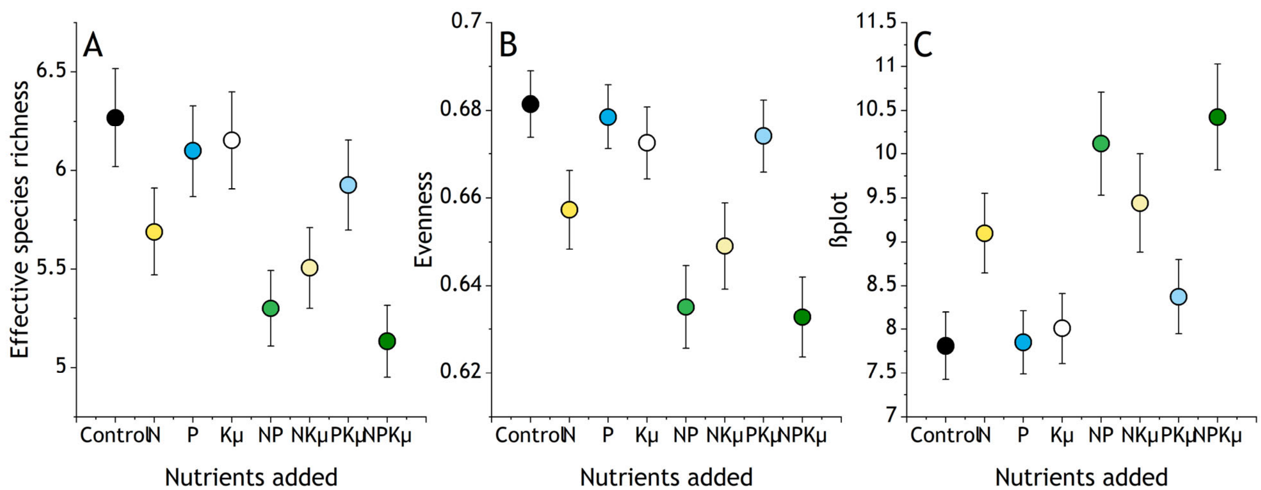
	N & P Main Effects				NxP Interaction	Single Treatment Effects					
Site	**N**	**p-value**	**P**	**p-value**	**p-value**	**N**	**p-value**	**P**	**p-value**	**NP**	**p-value**
Additive, continued											
smith.us	**31.7**	0.0000	**26.8**	0.0000	0.5667	**32.8**	0.0001	**28.0**	0.0009	**67.3**	<0.0001
unc.us	**20.4**	0.0861	**37.4**	0.0053	0.9433	25.8	0.2013	**43.2**	0.0370	**67.0**	0.0021
cowi.ca	**52.1**	0.0000	**6.9**	0.0175	0.5076	**57.5**	<0.0001	**11.3**	0.0317	**64.0**	<0.0001
nilla.au	**16.9**	0.0956	**35.3**	0.0025	0.6320	14.1	0.3869	**32.3**	0.0540	**57.4**	0.0014
koffler.ca	**24.3**	0.0000	**22.6**	0.0000	0.6840	**30.1**	0.0006	**28.4**	0.0012	**53.7**	<0.0001
badlau.de	**32.7**	0.0000	**13.8**	0.0204	0.7033	**38.5**	0.0003	**19.1**	0.0550	**52.3**	<0.0001
look.us	**27.7**	0.0011	**16.9**	0.0329	0.8652	**32.1**	0.0136	21.1	0.1014	**50.1**	0.0002
yarra.au	**20.2**	0.0016	**21.9**	0.0007	0.8585	**24.1**	0.0168	**25.8**	0.0107	**47.3**	<0.0001
chilcas.ar	**22.9**	0.0014	**12.3**	0.0636	0.3847	**31.9**	0.0042	**20.9**	0.0552	**39.6**	0.0005
burren.ie	**12.3**	0.0329	**24.7**	0.0001	0.5057	9.5	0.2833	**21.7**	0.0167	**39.4**	<0.0001
mcla.us	**24.3**	0.0000	**9.0**	0.0774	0.9213	**24.9**	0.0024	9.5	0.2363	**35.5**	<0.0001
frue.ch	**24.8**	0.0000	**6.3**	0.0993	0.6146	**28.1**	<0.0001	9.3	0.1285	**33.1**	<0.0001
Single N											
sedg.us	**60.9**	0.0000	5.6	0.1748	0.2748	**55.6**	<0.0001	1.4	0.8493	**68.5**	<0.0001
cdpt.us	**59.7**	0.0000	5.0	0.2204	0.4732	**56.8**	<0.0001	2.6	0.7182	**66.9**	<0.0001
msla_2.us	**53.2**	0.0000	4.7	0.6285	0.4479	**67.3**	0.0005	16.2	0.3802	**63.8**	0.0009
bogong.au	**49.1**	0.0000	4.9	0.2153	0.6227	**53.6**	<0.0001	8.6	0.2213	**57.4**	<0.0001
msla.us	**41.1**	0.0004	9.5	0.3177	0.7820	**39.7**	0.0160	8.3	0.6078	**54.2**	0.0012
sage.us	**24.7**	0.0538	18.6	0.1298	0.9519	28.2	0.1534	22.1	0.2611	**48.7**	0.0175
potrok.ar	**61.2**	0.0504	-7.9	0.7083	0.8896	53.3	0.1748	-13.7	0.7170	47.0	0.2272
msum.us	**49.5**	0.0000	-1.5	0.8153	0.8679	**47.4**	0.0002	-3.3	0.7774	**46.8**	0.0002
doane.us	**41.4**	0.0135	0.3	0.9801	0.2706	**64.7**	0.0129	19.7	0.4232	**45.9**	0.0696
lake.us	**38.1**	0.0057	7.6	0.4858	0.2819	23.9	0.1724	-4.7	0.7834	**45.7**	0.0136
spin.us	**23.3**	0.0000	5.8	0.1540	0.4200	**20.0**	0.0021	2.8	0.6589	**30.0**	<0.0001
arch.us	**21.9**	0.0037	-1.8	0.7746	0.9688	**21.4**	0.0379	-2.3	0.8179	**19.6**	0.0559
hopl.us	**14.3**	0.0029	2.9	0.5194	0.4600	**18.4**	0.0084	6.7	0.3288	**17.9**	0.0101
kbs.us	**17.1**	0.0005	-1.8	0.6810	0.3185	**22.1**	0.0015	2.8	0.6770	**15.4**	0.0243
marc.ar	**14.8**	0.0154	-1.1	0.8400	0.5951	11.4	0.1735	-4.3	0.6041	13.3	0.1120

	N & P Main Effects				NxP Interaction	Single Treatment Effects					
Site	**N**	**p-value**	**P**	**p-value**	**p-value**	**N**	**p-value**	**P**	**p-value**	**NP**	**p-value**
Single P											
ethamc.au	-6.6	0.8525	**103.4**	0.0709	0.8107	-21.1	0.7636	80.6	0.2568	83.3	0.2417
lagoas.br	14.1	0.2467	**22.4**	0.0793	0.3533	3.0	0.8675	10.9	0.5436	**37.6**	0.0421
elliot.us	9.3	0.1126	**19.8**	0.0016	0.2446	2.7	0.7617	12.8	0.1448	**29.9**	0.0009
Not N or P											
hero.uk	16.0	0.1018	14.4	0.1371	0.8669	19.1	0.1985	17.5	0.2392	**33.1**	0.0309
valm.ch	7.3	0.2750	8.2	0.2244	0.7784	5.6	0.5645	6.5	0.5064	15.9	0.1049
sava.us	-7.0	0.7248	20.7	0.3690	0.1996	-31.8	0.2478	-7.6	0.7784	10.6	0.6962
burrawan.au	8.2	0.3595	-0.2	0.9815	0.6096	13.0	0.3137	4.5	0.7302	8.2	0.5270
pinj.au	19.2	0.1257	-10.2	0.3416	0.8149	21.1	0.2102	-8.5	0.6095	7.1	0.6707
cereep.fr	2.0	0.6183	1.2	0.7645	0.9594	2.2	0.6977	1.4	0.8042	3.2	0.5729
lancaster.uk	2.6	0.8036	-1.0	0.9235	0.7622	-0.5	0.9690	-4.0	0.7782	1.5	0.9143
pape.de	9.6	0.3478	-9.0	0.3346	0.4279	17.5	0.2287	-1.8	0.8975	-0.3	0.9842
sereng.tz	3.7	0.6453	-6.9	0.3663	0.9283	4.3	0.6971	-6.4	0.5633	-3.5	0.7513
kibber.in	30.0	0.4131	-29.6	0.2782	0.8260	17.7	0.6702	-38.7	0.3559	-8.0	0.8475
kilp.fi	1.9	0.8329	-11.0	0.1852	0.5989	-2.6	0.8234	-15.2	0.1914	-9.2	0.4275
ethass.au	-7.5	0.7661	-35.2	0.1067	0.5645	-17.2	0.5371	-43.6	0.1224	-38.1	0.1758
Subadditive											
kiny.au	5.3	0.4625	**21.1**	0.0076	0.0480	**23.7**	0.0557	**40.9**	0.0012	**30.0**	0.0162
sevi.us	-1.8	0.8356	10.7	0.2740	0.0689	18.5	0.2223	**32.3**	0.0484	9.5	0.5178
Negative											
rook.uk	**-21.0**	0.0116	**26.0**	0.0134	0.0014	8.9	0.5415	**63.8**	0.0002	-0.7	0.9644
ahth.is	8.4	0.4323	**-28.8**	0.0030	0.1235	23.1	0.1036	-16.7	0.2322	**-24.5**	0.0863

Supplemental Figure 1. Aboveground biomass in relation to mean annual precipitation (MAP) for sites with less 1014 mm y^{-1} MAP classified by form of response to nitrogen (N) and phosphorus (P) fertilization. N and P treatments are averaged across levels of Kµ fertilization. See Figure 4 for plots containing all sites, and Supplemental Table 3 for linear regression equations.



Supplemental Figure 2. Species diversity metrics (means ± SE) for the eight factorial nutrient treatments averaged across all 71 sites.



Supplemental Table 3. Equations and coefficients of determination (adjusted R^2) for linear regressions relating aboveground biomass to MAP in Figures 2, 3, and Supplemental Figure 1. Equation form: Aboveground biomass (AGB) = Slope(SE) * MAP + Intercept(SE). Concatenated refers to regressions fit across all treatments.

All Sites and Limitation Forms - Figure 2			
	# Nutrients Added		**Factorial Treatments**
0	AGB = 0.312(0.074) * MAP + 107.5(60.2) R2 = 0.19	Control	AGB = 0.307(0.072) * MAP + 103.3(58.8) R2 = 0.21
1	AGB = 0.360(0.068) * MAP + 97.0(55.2) R2 = 0.28	N	AGB = 0.376(0.080) * MAP + 117.1(64.8) R2 = 0.25
2	AGB = 0.399(0.075) * MAP + 117.0(60.8) R2 = 0.28	P	AGB = 0.350(0.067) * MAP + 91.2(54.6) R2 = 0.29
3	AGB = 0.457(0.089) * MAP + 127.6(71.8) R2 = 0.27	Kµ	AGB = 0.343(0.063) * MAP + 72.9(51.6) R2 = 0.30
		NP	AGB = 0.409(0.088) * MAP + 154.7(71.5) R2 = 0.24
		NKµ	AGB = 0.410(0.074) * MAP + 100.1(60.6) R2 = 0.31
		PKµ	AGB = 0.360(0.069) * MAP + 94.6(56.4) R2 = 0.29
		NPKµ	AGB = 0.449(0.090) * MAP + 129.6(73.4) R2 = 0.27
	All MAP - Figure 3		**Sites < 1013 MAP - Supplemental Figure 1**
No limitation			
All	AGB = 0.169(0.132) * MAP + 228.0(103.4) R2 = 0.01		AGB = 0.390(0.172) * MAP + 111.2(112.6) R2 = 0.11
Control	AGB = 0.092(0.264) * MAP + 277.0(207.7) R2 = -0.09		AGB = 0.237(0.366) * MAP + 202.5(239.8) R2 = -0.08
N	AGB = 0.185(0.319) * MAP + 237.3(281.0) R2 = -0.06		AGB = 0.399(0.418) * MAP + 127.2(273.8) R2 = -0.01
P	AGB = 0.183(0.289) * MAP + 191.5(203.7) R2 = -0.05		AGB = 0.459(0.354) * MAP + 49.1(231.5) R2 = 0.08
NP	AGB = 0.216(0.278) * MAP + 194.2(218.3) R2 = -0.04		AGB = 0.464(0.366) * MAP + 65.8(239.4) R2 = 0.07
Single N or P			
All	AGB = 0.309(0.049) * MAP + 141.8(40.4) R2 = 0.39		AGB = 0.348(0.090) * MAP + 118.4(50.6) R2 = 0.21
Control	AGB = 0.285(0.085) * MAP + 108.1(70.3) R2 = 0.38		AGB = 0.346(0.151) * MAP + 70.3(84.7) R2 = 0.26
N	AGB = 0.380(0.105) * MAP + 143.8(87.2) R2 = 0.37		AGB = 0.422(0.192) * MAP + 107.4(107.6) R2 = 0.24
P	AGB =0.268(0.080) * MAP + 131.3(66.6) R2 = 0.37		AGB =0.287(0.148) * MAP + 116.7(83.2) R2 = 0.19
NP	AGB = 0.331(0.114) * MAP + 187.4(94.9) R2 = 0.30		AGB = 0.338(0.231) * MAP + 179.2(129.3) R2 = 0.09
Additive NP			
All	AGB = 0.334(0.068) * MAP + 173.1(62.1) R2 = 0.23		AGB = 0.546(0.131) * MAP + 30.8(89.2) R2 = 0.23
Control	AGB = 0.287(0.104) * MAP + 111.3(98.4) R2 = 0.29		AGB = 0.413(0.197) * MAP + 23.9(134.6) R2 = 0.21
N	AGB = 0.332(0.149) * MAP + 208.5(133.7) R2 = 0.18		AGB = 0.615(0.272) * MAP + 16.7(185.7) R2 = 0.24
P	AGB = 0.333(0.120) * MAP + 142.7(110.0) R2 = 0.26		AGB = 0.493(0.244) * MAP + 32.1(166.9) R2 = 0.19
NP	AGB = 0.388(0.181) * MAP + 232.9(138.4) R2 = 0.23		AGB = 0.661(0.294) * MAP + 50.6(201.0) R2 = 0.24
Synergistic NP			
All	AGB = 0.886(0.112) * MAP + -161.4(79.4) R2 = 0.46		AGB = 0.886(0.112) * MAP + -161.4(79.4) R2 = 0.46
Control	AGB = 0.843(0.220) * MAP + -202.8(186.5) R2 = 0.46		AGB = 0.843(0.220) * MAP + -202.8(186.5) R2 = 0.46
N	AGB = 0.786(0.205) * MAP + -140.2(148.6) R2 = 0.46		AGB = 0.786(0.205) * MAP + -140.2(148.6) R2 = 0.46
P	AGB = 0.828(0.206) * MAP + -178.8(146.4) R2 = 0.48		AGB = 0.828(0.206) * MAP + -178.8(146.4) R2 = 0.48
NP	AGB = 0.970(0.287) * MAP + -126.9(182.5) R2 = 0.48		AGB = 0.970(0.287) * MAP + -126.9(182.5) R2 = 0.48

Supplemental Table 4. Means and standard errors (SE) of site characteristics management, mean annual temperature (MAT), geographic position, and soil properties for sites categorized by form of nutrient limitation. F and p-value are for tests for differences between limitation forms, detailed in Extended Methods.

Site Characteristic	F	p-value	No Limitation		Single		Additive		Superadditive	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE
Anthropogenic	0.89	0.4489	0.25	0.13	0.21	0.10	0.35	0.11	0.12	0.08
Burned	1.28	0.2874	0.00	0.00	0.32	0.11	0.05	0.05	0.18	0.10
Grazed	1.16	0.3329	0.42	0.15	0.16	0.09	0.15	0.08	0.24	0.11
Managed	0.70	0.5583	0.25	0.13	0.37	0.11	0.50	0.11	0.35	0.12
MAT	0.30	0.8260	11.26	2.68	12.21	1.41	10.38	0.73	10.75	1.67
Latitude	0.87	0.4625	39.12	5.17	38.32	1.82	43.54	1.32	40.54	2.70
Elevation	0.72	0.5420	815.00	374.33	560.68	132.09	503.45	125.00	829.76	214.62
% N	0.39	0.7622	0.36	0.12	0.23	0.04	0.30	0.08	0.34	0.09
% K	0.13	0.9410	204.56	60.52	236.15	32.59	195.24	55.48	224.31	56.89
% P	1.85	0.1506	69.89	18.38	46.08	16.66	48.06	8.02	26.08	5.37
% Clay	0.54	0.6554	15.50	3.88	14.21	2.21	12.28	2.41	17.96	4.95
% Sand	1.15	0.3434	62.36	9.15	58.47	6.58	54.74	7.31	42.41	7.64
% Silt	1.51	0.2309	22.04	5.75	27.26	4.90	32.91	5.59	39.57	5.41

Supplemental Table 5. Standardized direct, indirect, and total effects of mean annual precipitation (MAP), # of nutrients added, effective species richness (eH), evenness, and Whitaker's beta diversity (*β*plot) on aboveground biomass for all sites and for sites categorized by form of limitation by nitrogen and phosphorus.

Kind of Limitation	Predictor	Standardized Effects on Aboveground Biomass					
		Direct	p value	Indirect	p value	Total	p value
All	MAP	0.508	< 0.0001	0.005	0.0708	0.512	< 0.0001
	# Nutrients	0.156	< 0.0001	0.025	0.0003	0.181	< 0.0001
	eH	- -	- -	- -	- -	0.000	- -
	Evenness	-0.088	0.0072	- -	- -	-0.088	0.0072
	ßplot	0.124	0.0001	- -	- -	0.124	0.0001
No Limitation	MAP	0.311	< 0.0001	-0.073	0.0599	0.238	0.0010
	# Nutrients	- -	- -	0.021	0.3445	0.021	0.3445
	eH	0.107	0.2608	- -	- -	0.107	0.2608
	Evenness	-0.386	0.0006	- -	- -	-0.386	0.0006
	ßplot	0.230	0.0252	- -	- -	0.230	0.0252
Single	MAP	0.562	< 0.0001	0.008	0.5950	0.570	< 0.0001
	# Nutrients	0.119	0.0107	0.057	0.0058	0.175	0.0004
	eH	0.197	0.0087	- -	- -	0.197	0.0087
	Evenness	-0.189	0.0053	- -	- -	-0.189	0.0053
	ßplot	0.309	< 0.0001	- -	- -	0.309	< 0.0001
Additive	MAP	0.469	< 0.0001	0.023	0.0790	0.492	< 0.0001
	# Nutrients	0.263	< 0.0001	0.029	0.0606	0.293	< 0.0001
	eH	0.072	0.2835	- -	- -	0.072	0.2835
	Evenness	-0.279	< 0.0001	- -	- -	-0.279	< 0.0001
	ßplot	- -	- -	- -	- -	0.000	- -
Synergistic	MAP	0.664	< 0.0001	0.010	0.2004	0.674	< 0.0001
	# Nutrients	0.174	0.0001	0.017	0.3248	0.191	< 0.0001
	eH	-0.267	< 0.0001	- -	- -	-0.267	< 0.0001
	Evenness	0.104	0.0740	- -	- -	0.104	0.0740
	ßplot	- -	- -	- -	- -	0.000	- -

Extended Methods

Data Preparation

We obtained the June 2023 version of the Nutrient Network biomass and plant cover dataset, *comb-by-plot-clim-soil-diversity-2023-06-28*. Preparation of the dataset was performed in SAS 9.4.

Site selection: We selected 71 out of 130 total Nutrient Network sites (Supplemental Table 1). Sites were included if they conducted the full factorial NPKμ fertilization design for at least four years. We excluded pre-treatment years, sites with only observational data which did not conduct the fertilization experiment, and sites which only conducted the herbivory fence x NPKμ experiment, which does not include the factorial nutrient treatments.

Aboveground biomass calculation: We determined total live aboveground biomass (‘aboveground biomass’) in g m^{-2} y^{-1} by summing three biomass fractions in the dataset: vascular_live_mass, nonvascular_live_mass, and unsorted_live_mass. Three sites which otherwise met the inclusion criteria did not sort biomass to these fractions in one or more years. For those sites the variable total unsorted_mass (which includes an unquantified amount of dead mass) was assigned to aboveground biomass.

Dataset Repair: Structural errors in the dataset were corrected, mostly deleting occasional treatment years for a site without both aboveground biomass values and total cover and the diversity variables derived from cover.

Outlier identification: We filtered aboveground biomass for outliers in two steps. First, visual inspection of frequency distribution and Q-Q plots (Proc Univariate) identified four large aboveground biomass values as candidate outliers. Second, we fit a linear mixed model (Proc Mixed) to aboveground biomass as a function of nutrient treatment, year, and their interaction. The ‘influence’ option produced Cook’s D statistic, which confirmed the highest three aboveground biomass values had high influence on model fit and likely represented unusual predictor-response combinations. These three values were set to missing. The frequency distribution of total cover did not indicate any initial outlier candidates and no further outlier analysis was performed.

Imputation of missing values: After outlier identification, the dataset contained 20,743 observations with 24 missing values for aboveground biomass and 46 missing values for total cover. Missing values were filled with averages of the available values from the same site, treatment, and year.

Data reduction: Biomass and diversity observations were reduced to produce three dataset forms used in analyses described below. Form 1: averaged by site, nutrient treatment, and year, across blocks within each site; Form 2: averaged by site and nutrient treatment, across blocks and years; and Form 3: averaged by site, N treatment, and P treatment, across levels of Kμ, blocks, and years. Then, the biomass, diversity, and precipitation variables in each dataset form were centered and scaled (mean = 0, standard deviation = 1) for statistical analysis. Means and standard errors in original units are used in graphs, which were prepared in OriginPro 10.0.5.157.

Site Climate Data

Our primary objective was to evaluate the role of site mean annual precipitation in grassland responses to fertilization with single and multiple nutrients. We also screened a larger set of site level climate variables related to mean precipitation supply and evaporative demand. We proceeded in two main steps.

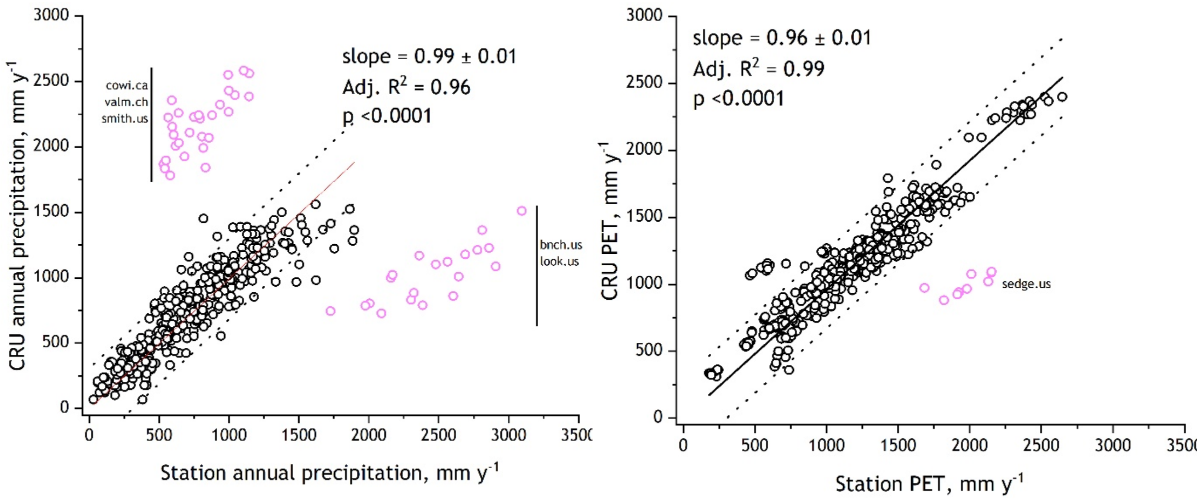
First, we assembled a precipitation dataset to determine site mean annual precipitation (MAP) and mean annual potential evapotranspiration (MPET) during the selected years of biomass/cover data for each site. The primary source was measured monthly precipitation and air temperature from weather stations in the Global Historic Climatology Network (GHCN) database. We chose weather stations confirmed by site PIs to suitably represent their site's climate. Monthly PET was computed using the Hargreaves method (1). Annual precipitation and potential evapotranspiration (PET) were summed on a harvest-year basis from the month following the previous harvest to the month of the current harvest. This dataset was an updated version of the dataset compiled by Bharath et al. (2). Site MAP and MPET based on these data are denoted 'Station' in Extended Methods Table 1.

Some sites and years were not present in the GHCN database. For these, we used annual precipitation and PET from the CRU TS v 4.1 dataset (Climatic Research Unit, University of East Anglia, and NCAS https://crudata.uea.ac.uk/cru/data/hrg/), compiled by Siddharth Bharath. CRU precipitation data are on an annual timestep values on a 0.5° x 0.5° grid derived from observed weather. Sites where MAP and mPET are partially or wholly derived from CRU data are denoted 'CRU' in Extended Methods Table 1.

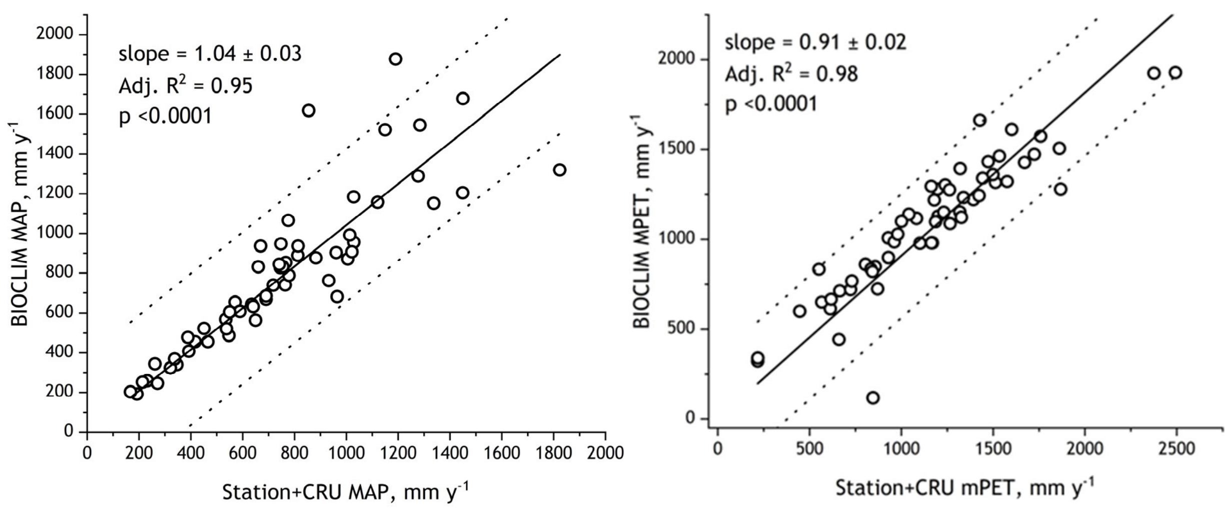
The combination of Station + CRU precipitation and PET data provided values for the selected years with biomass and cover data for 59 of the 71 selected sites (Extended Methods Table 1). We checked the comparability of CRU and Station -derived MAP and MPET for these 59 sites with bivariate plots of sites and years where both sources were available (Extended Methods Figure 1). For most sites CRU calendar year annual precipitation and PET are highly correlated ($R^2 > 0.96$) and unbiased (slopes near 1.0) representations of Station derived harvest year values. For a few sites (magenta data points) CRU either overestimated (cowi.ca, valm.ch, smith.us, which are in topographically complex areas) or underestimated (bnch.us, look.us) Station values. In each case we retained the source with the lower values.

For 12 remaining sites without Station or CRU data we used long-term MAP and mPET derived from the *BIOCLIM* database (3) which was available for all sites in the biomass/diversity dataset. We again used bivariate plots (Extended Methods Figure 2) to compare *BIOCLIM* MAP and MPET with average MAP and MPET values derived from Station + CRU for the 59 sites with both. *BIOCLIM* MAP and MPET were highly correlated ($R^2 > 0.95$) and unbiased (slope = 1.04) representations of Station + CRU MAP and were a small underestimation of Station + CRU mPET (slope = 0.91). Combining Station, CRU, and *BIOCLIM* precipitation and PET data did not introduce any substantive bias that might influence fertilization effects on biomass-MAP relationships.

Extended Methods Figure 1. Comparison of station measured with CRU dataset values for annual precipitation (left) and potential evapotranspiration (PET, right). Solid lines and statistics are for linear regression fits. Dotted lines indicate 95% confidence intervals. Data points in magenta are for the sites where CRU either overestimated or underestimated station values; in these cases we retained the source with the lower values.



Extended Methods Figure 2. Comparison of mean annual precipitation (MAP, left) and mean annual potential evapotranspiration (MPET, right) when sourced from Station+CRU (horizontal axes) or *BIOCLIM* (vertical axes). Solid lines and statistics are for linear regression fits. Dotted lines indicate 95% confidence intervals.



Extended Methods Table 1. Summary of precipitation/PET data source correspondence with experimental aboveground biomass/diversity data years.

Obs	Site	Data Source	# aboveground biomass years	# Station + CRU data years
1	cdcr.us	Station	14	14
2	sgs.us	Station	14	14
3	cowi.ca	Station	14	13
4	hopl.us	Station	13	13
5	mtca.au	Station	13	13
6	sier.us	Station	13	13
7	spin.us	Station	14	13
8	cbgb.us	Station	12	12
9	elliot.us	Station	12	12
10	mcla.us	Station	13	12
11	bogong.au	Station	14	11
12	burrawan.au	Station	11	11
13	cdpt.us	Station	14	11
14	konz.us	Station	11	11
15	sevi.us	Station	15	11
16	temple.us	Station	11	11
17	kiny.au	Station	10	10
18	sedg.us	Station	10	10
19	bnch.us	CRU	14	9
20	koffler.ca	Station	11	9
21	lancaster.uk	Station	9	9
22	look.us	CRU	14	9
23	smith.us	Station	9	9
24	trel.us	Station	9	9
25	ukul.za	Station	11	9
26	valm.ch	Station	12	9
27	bldr.us	Station	8	8
28	jena.de	Station	9	8
29	ping.au	Station	8	8
30	saline.us	Station	8	8
31	doane.us	Station	7	7
32	frue.ch	Station	7	7
33	hall.us	Station	7	7
34	kilp.fi	Station	7	7
35	marc.ar	Station	11	7
36	saana.fi	Station	8	7
37	yarra.au	Station	7	7

38	arch.us	Station	7	6
39	badlau.de	Station	7	6
40	comp.pt	Station	9	6
41	ethamc.au	Station	6	6
42	ethass.au	Station	6	6
43	nilla.au	Station	6	6
44	pape.de	CRU	6	6
45	sage.us	Station	6	6
46	chilcas.ar	Station	9	5
47	hart.us	Station	5	5
48	hero.uk	Station	5	5
49	kbs.us	Station	6	5
50	rook.uk	Station	5	5
51	sava.us	Station	5	5
52	shps.us	Station	5	5
53	burren.ie	Station	5	4
54	cereep.fr	CRU	8	4
55	msla.us	Station	4	4
56	msla_2.us	Station	4	4
57	msla_3.us	Station	4	4
58	sereng.tz	CRU	4	4
59	unc.us	Station	4	4
60	bayr.de	BIOCLIM	5	3
61	gilb.za	BIOCLIM	4	3
62	pinj.au	BIOCLIM	4	3
63	ahth.is	BIOCLIM	4	0
64	amlr.is	BIOCLIM	4	0
65	azitwo.cn	BIOCLIM	4	0
66	bnbt.us	BIOCLIM	5	0
67	kibber.in	BIOCLIM	5	0
68	lagoas.br	BIOCLIM	8	0
69	lake.us	BIOCLIM	4	0
70	msum.us	BIOCLIM	5	0
71	potrok.ar	BIOCLIM	6	0

For the second step in the processes we screened climate variables for their contribution to model fit in a linear mixed model testing how the factorial NPK fertilization treatments and each climate variable independently and jointly predicted aboveground biomass.

We devised linear mixed models of the general form:

$$\text{aboveground biomass} = \mu + \textit{nutrients} + \textit{nutrients*precip} + \textit{error}. \qquad \text{(Equation 1)}$$

Nutrients refers to the eight factorial combinations of added N, P, and Kµ, and *precip* refers to climate variables tested one at a time. Random effects Site, block*site, and nutrients*site were also fit.

We applied equation 1 to perform a simple model selection process to test 32 candidate site-level mean climate values using the Form 2 dataset. From the precipitation dataset we tested site-level MAP, MPET, MPET – MAP, and MPET*MAP^{-1}. We also tested variables describing mean annual, quarterly, and monthly precipitation, temperature, and temperature or evaporative demand metrics relative to precipitation included in the Nutrient Network dataset, mostly from WORLDCLIM. (Extended Methods Table 2). Linear mixed models were fit in Proc Mixed in SAS STAT version 15.3 coded as:

```
Proc mixed data=Form2 method=reml covtest ic;
Class nutrients site block;
Model aboveground biomass= nutrients * precip/ddfm=kr;                    (Model 1)
        Random site   block*site    n*p*k*site;
```

We fit Model 1 with no climate variable and including each climate variable one at a time. Models were ranked by their AIC score (Extended Methods Table 2). MAP from the precipitation dataset yielded the lowest AIC value (2026.6), which was lower than all other tested climate variables and lower than the model fit with no climate variable (AIC = 2041). The other climate variables were not considered further.

Extended Methods Table 2. Akaike's Information Criterion (AIC) for candidate climate variables describing mean site precipitation (MAP), temperature, potential evapotranspiration (PET), and temperature or PET relative to precipitation. Models are reported in order of increasing AIC within categories. The model with MAP_mm had the lowest AIC of all tested. A model with no climate variable had AIC of 2041, indicating that many climate variables yielded worse model fit than models with no climate variable.

Climate Variable	Definition	AIC
	Precipitation	
MAP_mm	Mean Annual Precipitation (Precipitation Dataset)	2026.6
MAP_V2	Mean Annual Precipitation (WorldClim)	2045.6
MAP_DRY_Q_v2	Precipitation of Driest Quarter (WorldClim)	2049.2
MAP_DRY_M_v2	Precipitation of Driest Month (WorldClim)	2050.2
MAP_WARM_Q_v2	Precipitation of Warmest Quarter (WorldClim)	2052.2
MAP_WET_Q_v2	Precipitation of Wettest Quarter (WorldClim)	2061.0
MAP_WET_M_v2	Precipitation of Wettest Month (WorldClim)	2063.2
MAP_COLD_Q_v2	Precipitation of Coldest Quarter (WorldClim)	2071.5
MAP_VAR_v2	Precipitation Seasonality (Coefficient of Variation) (WorldClim)	2072.0
	Temperature	
TEMP_WARM_Q_v2	Mean Temperature of Warmest Quarter (WorldClim)	2063.0
ISO_v2	Isothermality (Diurnal Range/Annual Range) (* 100) (WorldClim)	2063.7
TEMP_VAR_v2	Temperature Seasonality (standard deviation *100) (WorldClim)	2066.8
MAT_v2	Annual Mean Temperature (BIOCLIM)	2067.1
TEMP_WET_Q_v2	Mean Temperature of Wettest Quarter (WorldClim)	2067.3
MAX_TEMP_v2	Max Temperature of Warmest Month (WorldClim)	2067.4
MAT_RANGE_v2	Mean Diurnal Range (Mean of monthly (max temp - min temp)) (WorldClim)	2067.4

Climate Variable	Definition	AIC
TEMP_Cold_Q_v2	Mean Temperature of Coldest Quarter (WorldClim)	2069.6
MIN_TEMP_v2	Min Temperature of Coldest Month (WorldClim)	2069.9
ANN_TEMP_RANGE_v2	Temperature Annual Range (WorldClim)	2070.2
TEMP_DRY_Q_v2	Mean Temperature of Driest Quarter (WorldClim)	2072.2
	Evaporative demand indices relative to precipitation	
Maxv2_MAPmm	MAX_v2 * MAP_mm^{-1}	2045.5
MPETmm_MAPmm	MPET_mm * MAP_mm^{-1}	2048.0
defic_mm	MPET_mm - MAP_mm	2052.6
MATv2_MAPmm	MAX_TEMP_v2 * MAP_mm^{-1}	2058.0
RAIN_PET	Rainfall - potential evapotranspiration	2059.4
TtoP_COLDQ	TEMP_COLD_Q_V2 * MAP_COLD_Q_V2^{-1}	2061.7
TtoP_WETQ	TEMP_WET_Q_V2 * MAP_WET_Q_V2^{-1}	2061.7
MPET_mm	Mean Annual Potential Evapotranspiration (Precipitation Dataset)	2071.3
AI	Aridity Index (MAP / Mean annual PET) (CGIAR)	2072.8
PET	Potential Evapo-transpiration (mm yr^{-1}) (CGIAR)	2074.5
TtoP_WARMQ	TEMP_WARM_Q_V2 * MAP_WARM_Q_V2^{-1}	2075.1
TtoP_DRYQ	TEMP_DRY_Q_V2 * MAP_DRY_Q_V2^{-1}	2077.6

Statistical Procedures

The general approach was to apply linear mixed models and structural equation models to answer the three research questions.

Question 1. Does the biomass-MAP relationship become steeper with increased number of added nutrients?

We applied two variants of Model 1 to the Form 2 dataset to test how fertilizing with combinations of N, P, and K affected the biomass-MAP relationship. In one the *nutrients* term was the number of nutrients applied: 0 = Control, 1 for N, P, or Kµ applied singly, 2 for nutrient pairs, and 3 for the NPKµ treatment. In the other the *nutrients* term was the factorial combinations: Control, N, P, Kµ, N*P, N*Kµ, P*Kµ, and N*P*Kµ. These models provided tests of nutrient main (across-site) effects and nutrient x linear MAP interactions. We did not test for non-linear MAP effects in this or subsequent analyses because preliminary analysis did not suggest the presence of non-linear forms, and because a linear form is the simplest test of the hypotheses.

Question 2. Does the steepness of the biomass-MAP relationship increase with the strength of co-limiting nutrient interactions

Assignment of Nutrient Limitation Forms to Sites: We developed a rubric (Extended Methods Table 3) to assign each site to a form of nutrient limitation (Figure 1). The rubric identifies the limitation forms None, Single, Additive, Sub-additive, and Synergistic defined by the site-level response to fertilization with factorial combinations of N and P. We did not consider the Kμ fertilization treatment because the analyses for Question 1 indicated that Kμ did not interact with N, P, or MAP to influence aboveground biomass. Negative responses to fertilization are possible and occurred at two sites which were not considered further. The nutrient limitation categories correspond to those in (4), except we combine Harpole's three sub-forms of synergistic colimitation.

Nutrient limitation categories were defined by application of a linear mixed model (Model 2) to each site using the Form 1 dataset.

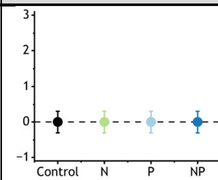
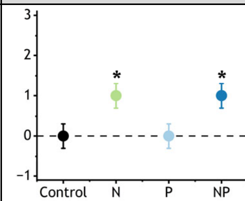
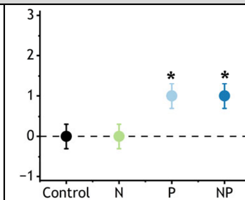
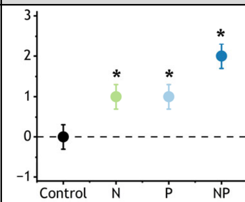
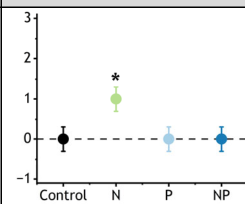
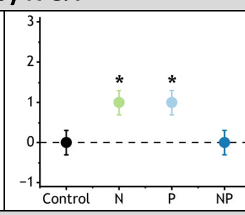
$$\text{aboveground biomass} = \mu + \textit{nutrients} + \textit{nutrients*year\_trt} + \textit{error}. \qquad \text{(Model 2)}$$

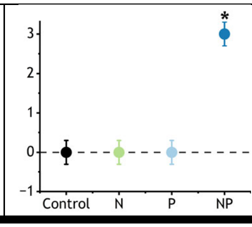
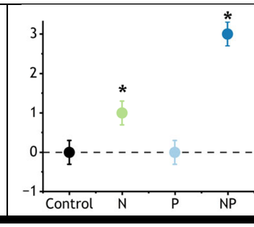
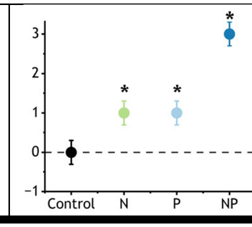
where *nutrients* represents N and P main effects and the N x P interaction effect. Model 2 includes year x nutrient interactions fit with ar(1) covariance structure to account for this source of variability in site-level aboveground biomass, though nutrient interactions with year were not part of the categorization rubric. Model 2 was coded as:

```
proc mixed data=Form1 method=reml covtest ic; by site_code;
class N P site_code block year_trt ;
model aboveground biomass= N P N x P N x year_trt P x year_trt N x P x year_trt/ddfm=kr;
random block;
repeated/subject=block*N*P type=ar(1);
lsmeans N*P/diff=control('0' '0');
```

Categorization was based on two factors: 1) which nutrient effects (N and P main effects, N x P interaction) were statistically significant defined as $p < 0.10$, and 2) which mean individual nutrient treatment differences, produced by the lsmeans statement, were significantly greater than 0 (lsmeans diff $p < 0.10$). SAS code applying the rubric is deposited in the XXXX repository.

Extended Methods Table 3. Rubric to apply the conceptual framework of Figure 1 to identify the form of nutrient limitation expressed at each site from responses to fertilization with nitrogen (N), phosphorus (P) or both (NP), relative to unfertilized controls. Subadditive and synergistic co-limitation can occur with different patterns of individual nutrient responses. Asterisks denote significant responses to fertilization with N, P, or both (NP).

<table>
<tr><th colspan="2">Mixed Model Effects</th><th>Relationships among Means</th><th colspan="3">Form of Nutrient Limitation</th></tr>
<tr><td rowspan="6">Additive Forms:
N x P interaction: p > 0.10
N + P = NP</td><td></td><td></td><td colspan="3">No Limitation by N or P</td></tr>
<tr><td>No main effects for N or P (p > 0.10)</td><td>N, P, NP = 0</td><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td colspan="3">Single Limitation by N or by P</td></tr>
<tr><td>Significant main effect for one nutrient (p < 0.10) and not the other.</td><td>$\overline{X}$ (N, NP) > 0
$\overline{X}$ (None, P) = 0
-or-
$\overline{X}$ (P, NP) > 0
$\overline{X}$ (None, N) = 0</td><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td colspan="3">Additive co-limitation by N & P</td></tr>
<tr><td>Significant main effect for N and P (p < 0.10)</td><td>$\overline{X}$(N, NP) > 0
$\overline{X}$(P, NP) > 0
$\overline{X}$(N, P) < NP</td><td></td><td></td><td></td></tr>
<tr><td rowspan="3">Non-Additive Forms:
N x P interaction p < 0.10
N + P ≠ NP</td><td></td><td></td><td colspan="3">Subadditive co-limitation by N & P</td></tr>
<tr><td>None, one (N or P) or two (N and P) significant main effects
(p < 0.10)</td><td>N and/or P > 0
NP ≥ 0
NP ≤ (N+P)</td><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td colspan="3">Synergistic co-limitation by N & P</td></tr>
</table>

	None, one (N or P) or two (N and P) significant main effects ($p < 0.10$)	N *and/or* P ≥ 0 NP > 0 NP $>$ (N+P)			

Next, we refit Model 1 separately to the sites in each nutrient limitation category using the Form 3 dataset. This analysis confirmed the categorization of the sites if the N, P, and NxP effects and interactions with MAP matched those expected from the rubric.

For questions 1 and 2, slopes of biomass-MAP relationships were computed from linear regressions fit to site-level treatment means in OriginPro 10.0.5.157.

Question 3. Does mediation of the biomass-MAP relationship by community diversity increase with the strength of co-limiting nutrient interactions?

We fit structural equation models to the form 3 dataset using Proc Calis to identify the joint effects of the number of nutrients applied (0 = Control, 1 = N or P, 2 = NP), site MAP, and diversity variables effective species richness (expH), Evenness € and plot beta diversity (βplot) jointly related to aboveground biomass. We first fit an *a priori* model containing paths representing hypothesized relationships among MAP, number of nutrients, the diversity variables, and aboveground biomass. Correlations among the diversity variables were included, but for clarity are not shown in Figure 5. Correlation of 0 was specified between MAP and number of nutrients because nutrient treatment was independent of site MAP. The *a priori* model was fit to all sites combined and yielded adequate fit (Table 2), so the model was then fit to each nutrient limitation category.

1. P. Droogers, R. G. Allen, Estimating Reference Evapotranspiration Under Inaccurate Data Conditions. *Irrigation and Drainage Systems* **16**, 33-45 (2002).
2. S. Bharath *et al.*, Nutrient addition increases grassland sensitivity to droughts. *Ecology* **101**, e02981 (2020).
3. S. E. Fick, R. J. Hijmans, WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology* **37**, 4302-4315 (2017).
4. W. S. Harpole *et al.*, Nutrient co-limitation of primary producer communities. *Ecol. Lett.* **14**, 852-862 (2011).