

## Grassland productivity is limited by multiple nutrients

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**Terrestrial ecosystem productivity is widely accepted to be nutrient limited<sup>1</sup>. Although nitrogen is deemed a key determinant of aboveground net primary production<sup>2,3</sup>, the prevalence of co-limitation by nitrogen and phosphorus is increasingly recognized<sup>4-8</sup>. However, the extent to which terrestrial productivity is co-limited by nutrients other than nitrogen and phosphorus, such as potassium and micronutrients, has remained unclear. Here, we report results from a standardized factorial nutrient addition experiment, in which we added nitrogen (N), phosphorus (P), and potassium combined with a selection of micronutrients ( $K_{+\mu}$ ), alone or in concert, to 42 grasslands spanning five continents, and monitored aboveground net primary production. Nutrient availability limited productivity at 31 of the 42 grassland sites studied. Pairwise combinations of N, P, and  $K_{+\mu}$  co-limited aboveground net primary production at 24 of the 42 sites, and nitrogen limitation peaked in cool, high latitude sites. Our findings indicate widespread variation in the combination of nutrients that limit aboveground grassland productivity, and highlight the importance of potassium and micronutrients, and an apparent absence of limitation by the nutrients we considered at some sites. This variation in nutrient limitation must be considered when assessing the ecosystem-scale consequences of nutrient enrichment.**

1 Terrestrial ecosystem productivity is widely accepted to be nutrient limited<sup>1</sup>, and many  
2 studies have focused on limitation by a single nutrient, nitrogen (N)<sup>2,3</sup>. In grasslands, and in  
3 other systems, the role of additional nutrients is increasingly recognized. A recent meta-analysis  
4 of 1400 N and phosphorus (P) fertilization studies<sup>4</sup> showed limitation of terrestrial productivity  
5 by both N and P. Furthermore, these nutrients often were synergistically co-limiting, where  
6 together they limited productivity more than the sum of their individual limitations. This meta-  
7 analysis is the most comprehensive assessment of ecosystem nutrient limitation to date.  
8 However, the global extent and magnitude of multiple limitation by nutrients other than N and P  
9 remains poorly understood in natural systems<sup>4,5,9</sup>, including grasslands<sup>3,10</sup>, a critically endangered  
10 biome that accounts for approximately one-third of Earth's terrestrial net primary production<sup>11</sup>.  
11 More importantly, multiple nutrient limitation has not been experimentally tested in grasslands  
12 on a global scale using a standardized experimental approach. The potential for synergistic co-  
13 limitation of grasslands by multiple nutrients, or conversely, failing to account for the absence of  
14 single or multiple nutrient limitation means we may misestimate the magnitude and extent of  
15 nutrient limitation of terrestrial net primary productivity.

16 Humans now produce more reactive N than is produced from all natural terrestrial  
17 sources, primarily as fertilizers, industrial products, and through fossil fuel combustion<sup>12,13</sup>.  
18 Anthropogenic increases in atmospheric N will result in further terrestrial N deposition, altering  
19 ecosystem function<sup>12,14-17</sup> and potentially increasing limitation by other nutrients, such as P,  
20 potassium (K), or trace elements<sup>16</sup>. K occurs in high concentrations in plant tissues<sup>18</sup> and its  
21 uptake is correlated with that of other nutrients<sup>19,20</sup>. Our understanding of limitation by nutrients  
22 other than N in grasslands lags that of agro-ecosystems, where the importance of P, K, and  
23 micronutrients is better understood<sup>20</sup>.

Here we report the frequency, magnitude, and global extent of nutrient limitation of grassland ANPP by N, P, and K combined with micronutrients ( $K_{+\mu}$ ) at 42 grassland sites in the Nutrient Network (NutNet; Supplementary Material and <sup>21</sup>). All sites conducted the same standardized N-P- $K_{+\mu}$  addition experiment, the definitive test for nutrient limitation. This approach overcomes major constraints of previous meta-analyses<sup>4</sup>, differing nutrient addition rates and methodologies and the rarity of factorial nutrient treatments in the underlying studies<sup>5</sup>.

Sites were located on five continents (Supplementary Figure 1) and spanned a 23-fold range in ANPP (Supplementary Figure 2A), over 25° in absolute latitude (actual latitudes 54° N to 37° S), nearly 3,500 m in elevation, and wide ranges in mean annual precipitation (260 – 1900 mm), mean annual temperature (0.3 – 22° C; Supplementary Figure 1), soil texture, and pre-treatment soil N, P, and K pools (Supplementary Table 1). The sites included both native and previously cultivated grassland, and some sites were managed with burning, grazing, or other practices. Thus, we were able to evaluate single and multiple-nutrient limitation of ANPP at global, continental, and site spatial extents, and as influenced by management, climate, and soils.

N, P, and  $K_{+\mu}$  were factorially applied annually to replicated 5 m<sup>2</sup> plots at the beginning of each site's growing season at rates commonly used in grassland fertilization experiments<sup>22,23</sup>. The  $K_{+\mu}$  treatment included a micronutrient mix in the first treatment year only, to avoid micronutrient toxicity. Nutrient limitation of ANPP was quantified by harvesting current year standing crop biomass for 3 to 5 years. and computing the log response ratio (LRR), the natural logarithm of the ratio of treatment plot to control plot ANPP, a metric commonly used in meta-analyses<sup>24</sup>

Multiple nutrient limitation of ANPP often occurs in the form of co-limitation<sup>5</sup>. Co-limitation is synergistic when the response to multiple nutrients is greater than the sum of the responses to each nutrient added individually, additive if the multiple-nutrient response equals the sum of the individual nutrient responses, and sub-additive if less than the sum of the single-nutrient responses<sup>5</sup>. Across all sites and years, the combined addition of N and P increased ANPP by an average of 40% over controls (LRR= 0.34), compared to increases of only 18% (LRR = 0.16) for N individually and 9% for P individually (LRR = 0.09;  $p = 0.03$ , Figure 1A, Supplementary Figure 3). This provides clear evidence for globally-averaged synergistic co-limitation of ANPP by N and P in these grasslands. Synergistic co-limitation by N and P across sites and years emerged from frequent occurrence of NP co-limitation at the site level. Some form of NP co-limitation averaging 67% (LRR = 0.51) occurred at 60% of the sites, including sites on all five continents, with synergistic NP co-limitation at 13 sites and sub-additive co-limitation at 12 sites, (Supplementary Table 3). Globally-averaged synergistic co-limitation of grassland ANPP by N and P contradicts the long-held perception that N is the predominant nutrient limiting grassland productivity<sup>3,10</sup> and highlights a critical role for P.

There was no globally-averaged single or multiple-nutrient limitation involving  $K_{+\mu}$  ( $p > 0.32$ , Supplementary Table 2). However, this did not preclude frequent site-level single and multiple-nutrient  $K_{+\mu}$  limitation of grassland ANPP. ANPP was limited at 3 sites by  $K_{+\mu}$  alone and co-limited by  $NK_{+\mu}$  at 18 sites, both averaging 48% (LRR = 0.39; Figure 1A, Supplementary Table 3). Additionally, ANPP was co-limited by  $PK_{+\mu}$  at 9 sites by an average of 52% (LRR = 0.42), approaching the magnitude of ANPP limitation at sites where N (57%) or P (54%) individually limited ANPP (Figure 1A). Site-level  $NK_{+\mu}$  and  $PK_{+\mu}$  co-limitation occurred in synergistic, additive, and sub-additive forms, and in total, single or multiple-nutrient limitation

involving  $K_{+μ}$  occurred at 24 sites again occurring on all five continents (Supplementary Table 3). This finding represents the broadest assessment to date of potential  $K_{+μ}$  limitation of grassland ANPP, and suggests that single- and multiple-nutrient K limitation, potentially augmented by micronutrient addition during year 1, occurs more frequently than previously recognized<sup>20,25</sup>.

Although single and multiple-nutrient limitation of ANPP was widespread, there were nonetheless 15 sites with no evidence for multiple nutrient limitation, and at 12 of the 15 also no evidence for single-nutrient limitation (Supplementary Table 3). The presence and magnitude of nutrient limitation may depend on site climate, soil development, or fertility<sup>26,27</sup>. For these reasons, greater site-level limitation of ANPP by one nutrient may correlate with greater limitation by one or more additional nutrients. Indeed, site-level individual nutrient limitation of ANPP increased with limitation by other individual nutrients ( $R^2$  0.08 – 0.15; Figure 2A,B,D), and ANPP limitation by nutrient pairs increased with that of a third individual nutrient ( $R^2$  0.07 – 0.21; Figure 2C,E,F). These correlations suggest that site-level attributes may predict the magnitude of single and multiple nutrient limitation of ANPP.

Sites differed in various potential qualitative and quantitative predictors of the presence and magnitude of nutrient limitation of ANPP (Supplementary Table 1). We found no evidence that nutrient limitation differed among the qualitative predictors continent, history of management with burning, grazing, and previous cultivation, and predominant soil texture (Supplementary Figure 4, Supplementary Table 4). Quantitative predictors were examined at the 19 longest running sites, where estimates of site mean nutrient limitation should best correspond to site mean climate and soil attributes. N limitation increased with decreasing site mean annual temperature (MAT;  $R^2 = 0.40$ ,  $p = 0.002$ , Figure 3A) and peaked at temperate latitude sites

( $\sim 45^\circ$ ;  $R^2 = 0.38$ ,  $p = 0.009$ , Figure 3B) with lower MAT ( $R^2 = 0.66$ ,  $p < 0.0001$ , Figure 3B inset). Limitation of ANPP by other single or multiple nutrient combinations was not correlated with soil texture fractions, pre-treatment soil nutrient contents, soil pH, or climate variables ( $p > 0.05$ , data not shown). These findings suggest that N limitation predominated at cooler temperate latitudes, as others have predicted<sup>26</sup>, and validate the power of these experiments to detect spatial trends in ecosystem nutrient limitation.

The magnitude of single or multiple-nutrient limitation of grassland ANPP may increase through time (Supplementary Figure 2B). To isolate the temporal signal of nutrient addition effects on ANPP, we calculated new ANPP response ratios using the pre-treatment year (i.e., year 0) ANPP for each treatment plot rather than the control plot, to control for plot-to-plot spatial variation in ANPP. For 37 sites with no missing ANPP data in years 1-3, the ANPP response to nutrient addition increased through time in all treatments, ANPP increased each year more in treatments containing N ( $p < 0.005$ , Figure 4, Supplementary Table 2) compared to the P,  $K_{+\mu}$ , and  $PK_{+\mu}$  treatments. Thus, the larger global-averaged increase in ANPP with N than P or  $K_{+\mu}$  (Figure 1A) can be explained partly by both smaller and later manifestation of P and  $K_{+\mu}$  effects. The full extent of nutrient limitation of grassland ANPP, particularly for nutrients other than N, may require additional years to emerge.

Multiple nutrient limitation of ANPP in these grasslands was spatially and temporally varied, often included K and micronutrients, but sometimes none of the nutrients we considered. Thus, while N was an important limiting nutrient in many grasslands, P and  $K_{+\mu}$  were also limiting in many instances, both alone and in combination with each other and with N. These estimates for globally-averaged nutrient limitation in these grasslands are conservative because

nutrient limitation increased in successive treatment years. The magnitude of nutrient limitation may require revision upward if the increases continue in future years.

Multiple nutrient limitation in grasslands, especially synergistic co-limitation, makes the consequences of anthropogenic nutrient inputs in these and other systems more difficult to predict. For example, nutrient co-limitation may actually restrict the impacts of single nutrient inputs such as N deposition<sup>17</sup>, but ecosystems may respond more rapidly than expected to subsequent inputs of the co-limiting nutrient. Considering only one of the co-limiting nutrients would risk reaching erroneous conclusions about the consequences of future inputs for biodiversity or ecosystem goods/services provision<sup>14,15,28,29</sup>. It is imperative to consider multiple nutrient constraints on primary productivity in grasslands and other ecosystems.

**Full Methods** and any associated references are available in the online version of the paper at [www.nature.com/nature](http://www.nature.com/nature).

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## **Methods**

### ***Study system***

The rationale, goals, and experimental protocols used at grasslands in the Nutrient Network (NutNet) are described in Borer et al.<sup>21</sup>, and are summarized here. This study used aboveground net primary productivity (ANPP) data from a standardized nutrient addition experiment conducted at 42 NutNet sites in eight countries (Australia, Canada, China, Switzerland, Tanzania, United Kingdom, USA, South Africa) on five continents (Australia [N =



4], Africa [N = 4], Europe [N = 5], Asia [N = 1], North America [N = 28]; Supplementary Figure 1, Table 1).

The grassland types included alpine/montane grasslands, shortgrass, mixed, and tallgrass prairies, desert and semi-arid grasslands, old fields and pastures, savanna and shrub-steppe, and annual grasslands. Sites span wide ranges of mean annual precipitation (MAP; 252 - 1898 mm  $y^{-1}$ ), mean annual temperature (MAT; 0.3 - 22.1 °C; Supplementary Figure 1), elevation (50 - 3500 m), and absolute latitude (over 25°, actual latitudes 54° N to 37° S, Supplementary Table 1). Site climate data were derived from the WorldClim database<sup>30</sup> (version 1.4.). Local investigators classified sites as to management practice (burned, grazed, other management) and native or anthropogenic (restored grassland and agricultural pasture) origin. Sites collected pre-treatment soil nutrient content and texture data.

At each site, N, P, and K were added in full factorial combination (8 treatments including control plots) to 5 x 5 m plots in a randomized complete blocks design with N=3 for most sites (range 2-6). This experimental scale is well-suited for the relatively short-statured herbaceous vegetation in grasslands. Micronutrients (6% Ca, 3% Mg, 12% S, 0.1% B, 1% Cu, 17% Fe, 2.5% Mn, 0.05% Mo, and 1% Zn) were added with K only in year 1 to avoid possible micronutrient toxicity. Nutrient additions began at most sites in 2008 (N = 28), and additional sites began in 2009 (N = 9), 2010 (N = 3) and 2011 (N = 2).

### ***Sampling***

Total ANPP ( $g\ m^{-2}\ yr^{-1}$ ) was estimated annually from clip samples of current year peak aboveground biomass from two 0.1  $m^2$  quadrats per plot. Samples were dried to constant mass at 60°C.

## *Statistical Analyses*

We tested for nutrient treatment effects on ANPP using linear mixed models in SAS/STAT v 9.2. First, we tested for variation among sites in nutrient main and interactive effects and their variation with treatment year (Equation 1):

$$\ln(\text{ANPP})_{ijkl} = \mu + \text{nutrients}_i + \text{nutrients}(\text{site})_{ij} + \text{nutrients}(\text{year})_{ik} + \text{nutrients}(\text{site} \times \text{year})_{ijk} + e_{ijkl}. \quad (1)$$

where *nutrients* refers to the eight factorial combinations of added N, P, and K. Nutrient treatments (i) were nested within site (j), within year (k), and within site x year (jk). Year was not treated as a repeated effect because each site experienced each year differently, and thus both the year effects and the covariance among years were not the same at each site. There were 42 sites with three years of data, 33 sites with four years, and 22 sites with five years. Model (1) was tested using all available treatment years, and with only the first three treatment years to ascertain whether any nutrient x year interactions were caused by sites added later with fewer years of nutrient addition.

We tested whether ANPP responses to nutrient treatments varied with continent, management, dominant soil texture, and origin by replacing the site term in Eq. 1 with each of the site classification and soils variables, one at a time. Europe and Asia were combined in tests of continent because of the single Asian site.

## *Calculation of nutrient effect sizes*

Effects sizes of the nutrient treatments were calculated and graphed as natural-log response ratios (LRR). LRRs were calculated in two ways; first, by comparing the ANPP of each

treatment plot to the ANPP of the control plot in the same block (2), to isolate treatment effects;  
 and second, by compared treatment plot ANPP to that of its pre-treatment (i.e., year 0) ANPP  
 (3). This isolated the temporal signal of nutrient addition responses.

$$\text{LRR} = \ln[\text{ANPP}_{\text{trt}}/\text{ANPP}_{\text{control}}] \quad (2)$$

$$\text{LRR} = \ln[\text{ANPP}_{\text{trt}}(\text{year X})/\text{ANPP}_{\text{trt}}(\text{year 0})] \quad (3)$$

We estimated a critical threshold LRR (4) for categorizing the significance of site LRRs. An  
 LRR was considered statistically significant if it exceeded a value corresponding to the critical  
 Z-score (1.65) at  $p = 0.05$ , computed as:

$$\text{Threshold LRR} = 1/7 * (\text{LRR}_N + \text{LRR}_P + \text{LRR}_K + \dots \text{LRR}_{\text{NPK}})/\text{pooled SD.} \quad (4)$$

#### *Correlations of site variables with nutrient effects*

We used linear regression analyses to test whether LRRs for each single nutrient and  
 nutrient pair were predicted by site latitude (absolute value), elevation, mean annual precipitation  
 (MAP), mean temperature annual (MAT), potential evapotranspiration (PET), soil fractions of  
 sand, silt, and clay, pre-treatment soil N, P, and K contents, and soil pH. We presented  
 regressions only where  $p < 0.05$ , and fit polynomial curves where indicated by evaluation of  
 residuals.

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**Supplementary Information** is linked to the online version of the paper at [www.nature.com/nature](http://www.nature.com/nature).

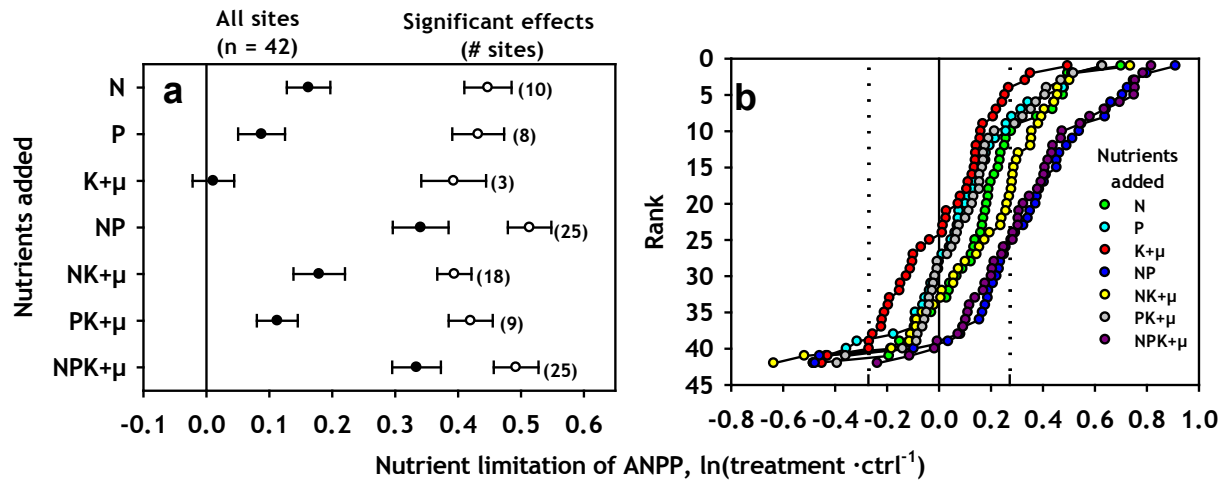
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This work was generated using data from the Nutrient Network (<http://www.nutnet.org>) experiment.

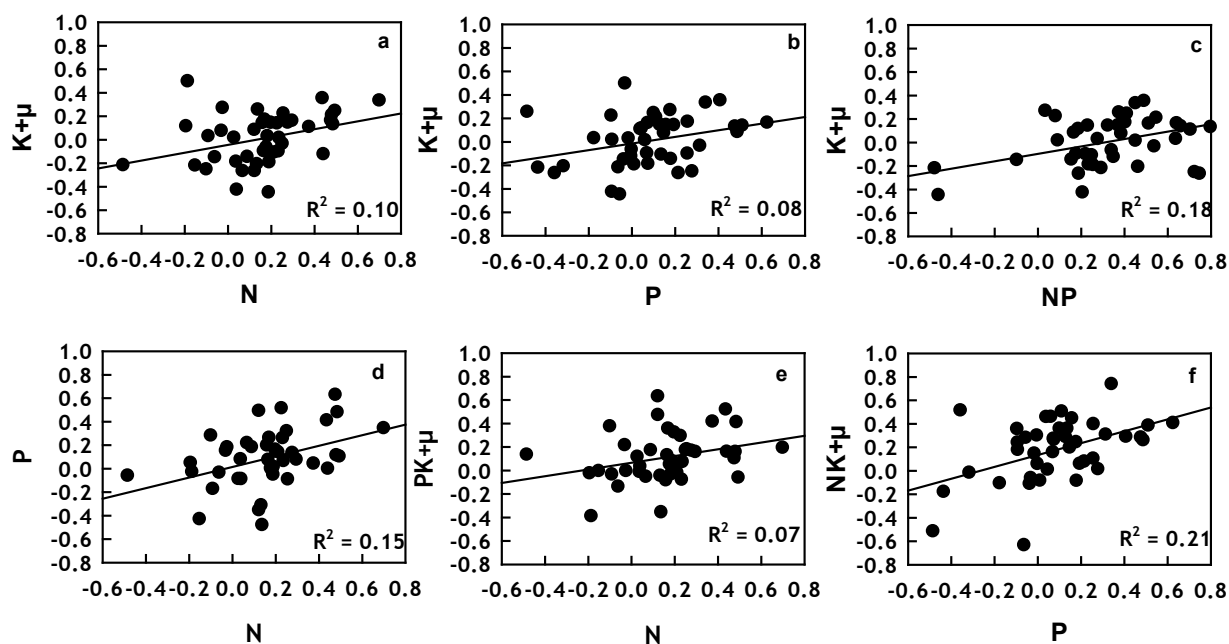
**Author Information** The data reported in this paper are available through <http://www.nutnet.org>. Reprints and permissions information is available at [www.nature.com/reprints](http://www.nature.com/reprints). The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at [www.nature.com/nature](http://www.nature.com/nature). Correspondence and requests for materials should be addressed to P.A.F. ([philip.fay@ars.usda.gov](mailto:philip.fay@ars.usda.gov)).

Figure 1



**Figure 1.** Nutrient limitation of aboveground net primary productivity (ANPP; log response ratio,  $\ln[\text{treatment} \cdot \text{control}^{-1}]$ ,) by nitrogen (N), phosphorus (P), and potassium plus year 1 micronutrients (K+ $\mu$ ). a) Mean  $\pm$  SE over all years available at each site. Means for all sites are closed symbols. Means of sites where individual nutrient treatments had significant positive effects (defined in panel b) are open symbols. b) Site nutrient limitation of ANPP ranked by the magnitude of limitation, averaged over all available years. Vertical dashed lines mark the effect sizes approximating a 0.05 significance level for negative and positive nutrient effects on ANPP. Note that sites rank in a different order in each nutrient treatment.

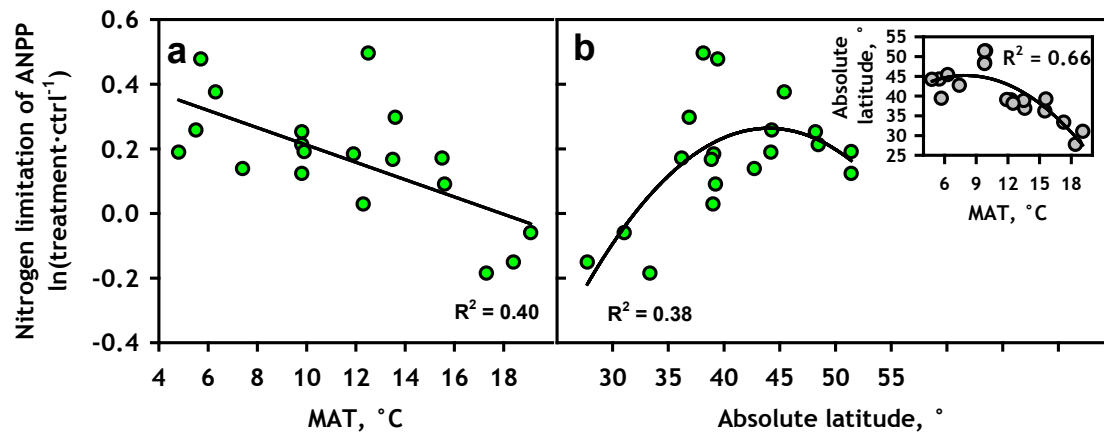
Figure 2



**Figure 2.** Correlations of nutrient limitation of aboveground net primary productivity (ANPP; log response ratios,  $\ln[\text{treatment} \cdot \text{control}^{-1}]$ ) among single and paired nutrients. a,b,d) Correlations among single nutrients. c,e,f) Correlations of nutrient pairs with the third nutrient. Coefficients of determination ( $R^2$ ) from linear regression ( $0.002 \leq P \leq 0.05$ ).

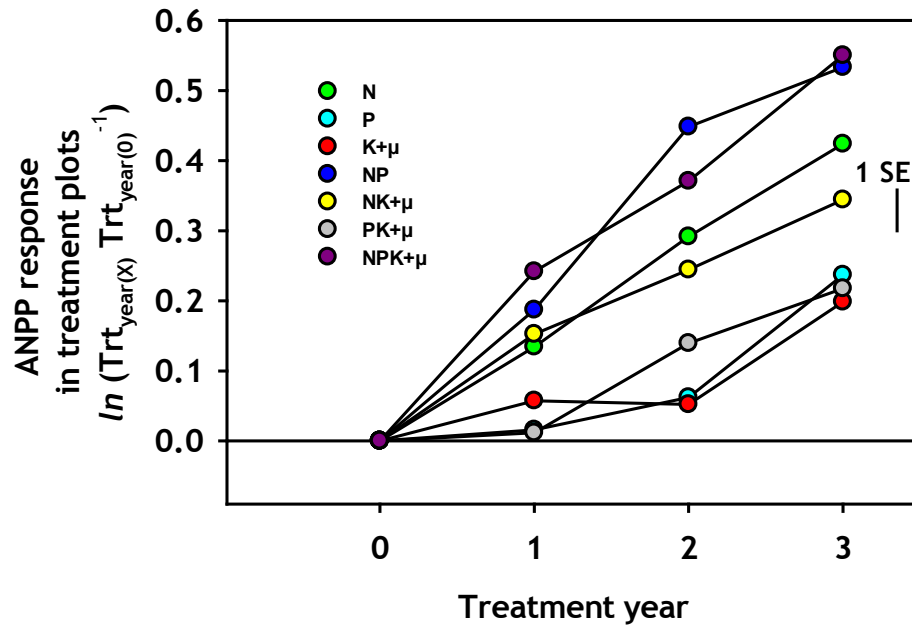


**Figure 3**



**Figure 3.** Predictors of nitrogen (N) limitation of aboveground net primary productivity (ANPP; log response ratio,  $\ln[\text{treatment} \cdot \text{control}^{-1}]$ ). a) site mean annual temperature (MAT), b) site latitude, in degrees away from the equator, and inset) MAT relationship with site latitude. N limitation values are means of five continuous years of nutrient treatment from 19 sites.

Figure 4



**Figure 4.** Aboveground net primary productivity (ANPP) responses to factorial nitrogen (N), phosphorus (P) and potassium plus year 1 micronutrient ( $K_{+\mu}$ ) treatments by year in 37 grasslands with three continuous years of ANPP data. Data represent the mean natural log response ratio of ANPP in the treatment year to ANPP in the pre-treatment year in the same plot, which controls for plot-to-plot variation.