Grassland productivity is limited by multiple nutrients

Philip A. Fay[1*], Suzanne M. Prober[2], W. Stanley Harpole[3-6], Johannes M.H. Knops[7], Jonathan D. Bakker[8], Elizabeth T. Borer[9], Eric M. Lind[9], Andrew S. MacDougall[10], Eric W. Seabloom[9], Peter D. Wragg[9], Peter B. Adler[11], Dana M. Blumenthal[12], Yvonne M. Buckley[13], Chengjin Chu[14], Elsa E. Cleland[15], Scott L Collins[16], Kendi F. Davies[17], Guozhen Du[14], Xiaohui Feng[18], Jennifer Firn[19], Daniel S Gruner[20], Nicole Hagenah[21], Yann Hautier[22], Robert W. Heckman[23], Virginia L. Jin[24] Kevin P. Kirkman[21], Julia Klein[25], Laura M. Ladwig[16], Qi Li[26], Rebecca L. McCulley[27], Brett A. Melbourne[17], Charles E. Mitchell[23], Joslin L Moore[28], John W. Morgan[29], Anita C. Risch[30], Martin Schütz[30], Carly J Stevens[31], David A. Wedin[32], Louie H. Yang[33]

1. USDA-ARS Grassland Soil and Water Research Lab, Temple, TX, 76502 USA
2. CSIRO Land and Water Flagship, Private Bag 5, Wembley, WA 6913, Australia
3. Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA, 50011 USA
4. German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, D-04103 Leipzig, Germany
5. Department of Physiological Diversity, Helmholtz Center for Environmental Research – UFZ, Permoserstr. 15, 04318 Leipzig, Germany
6. Institute of Biology, Martin Luther University Halle-Wittenberg, Am Kirchtor 1, 06108 Halle (Saale), Germany
7. School of Biological Sciences, University of Nebraska, Lincoln, NE, 68588 USA
8. School of Environmental and Forest Sciences, University of Washington, Seattle, WA, 98195 USA
9. Department of Ecology, Evolution, and Behavior, University of MN, St. Paul, MN, 55108 USA
10. Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1
11. Department of Wildland Resources and the Ecology Center, Utah State University, Logan, UT, 84322 USA
12. USDA-ARS Rangeland Resources Research Unit, Fort Collins, CO 80526 USA
13. School of Natural Sciences, Zoology, Trinity Centre for Biodiversity Research, Trinity College Dublin, Dublin 2, Ireland
14. Research Station of Alpine Meadow and Wetland Ecosystems, Lanzhou University, Lanzhou, 730000, China.
15. Ecology, Behavior & Evolution Section, University of California, San Diego, La Jolla, CA, 92093 USA
16. Department of Biology, MSC03-2020, University of New Mexico, Albuquerque, NM, 88003 USA
17. Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO, 80309 USA
18. Department of Plant Biology, University of Illinois, Urbana, IL, 61801 USA
19. School of Earth, Environment and Biological Sciences, Queensland University of Technology, Brisbane, Queensland 4001, Australia
20. Department of Entomology, University of Maryland, College Park, MD, 20742 USA
21. School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, 3209, South Africa.
22. Ecology and Biodiversity Group, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, Netherlands

23. Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599 USA
24. USDA-ARS Agroecosystem Management Research Unit, Lincoln, NE, 68538 USA.
25. Department of Forest, Rangeland, and Watershed Stewardship, Colorado State University, Fort Collins, CO, 80523 USA
26. Key Laboratory of Adaptation and Evolution of Plateau Biota, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, 810008, China
27. Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY, 40546 USA
28. School of Biological Sciences, Monash University, Victoria 3800, Australia.
29. Department of Botany, La Trobe University, Bundoora 3083, Victoria, Australia
30. Swiss Federal Institute for Forest, Snow and Landscape Research, Community Ecology, 8903 Birmensdorf, Switzerland
31. Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK
32. School of Natural Resources, University of Nebraska, Lincoln, NE, 68583 USA
33. Department of Entomology and Nematology, University of California, Davis, CA, 95616 USA.

*To whom correspondence should be addressed (philip.fay@ars.usda.gov)

Terrestrial ecosystem productivity is widely accepted to be nutrient limited[1]. Although nitrogen is deemed a key determinant of aboveground net primary production[2,3], the prevalence of co-limitation by nitrogen and phosphorus is increasingly recognized[4-8]. 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[1], and many

2   studies have focused on limitation by a single nutrient, nitrogen (N)[2,3]. 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[4] 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[4,5,9], including grasslands[3,10], a critically endangered

10   biome that accounts for approximately one-third of Earth's terrestrial net primary production[11].

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[12,13].

18   Anthropogenic increases in atmospheric N will result in further terrestrial N deposition, altering

19   ecosystem function[12,14-17] and potentially increasing limitation by other nutrients, such as P,

20   potassium (K), or trace elements[16]. K occurs in high concentrations in plant tissues[18] and its

21   uptake is correlated with that of other nutrients[19,20]. 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[20].

24   Here we report the frequency, magnitude, and global extent of nutrient limitation of

25   grassland ANPP by N, P, and K combined with micronutrients ($K_{+\mu}$) at 42 grassland sites in the

26   Nutrient Network (NutNet; Supplementary Material and [21]). All sites conducted the same

27   standardized N-P-$K_{+\mu}$ addition experiment, the definitive test for nutrient limitation. This

28   approach overcomes major constraints of previous meta-analyses[4], differing nutrient addition

29   rates and methodologies and the rarity of factorial nutrient treatments in the underlying studies[5].

30   Sites were located on five continents (Supplementary Figure 1) and spanned a 23-fold

31   range in ANPP (Supplementary Figure 2A), over 25° in absolute latitude (actual latitudes 54° N

32   to 37° S), nearly 3,500 m in elevation, and wide ranges in mean annual precipitation (260 – 1900

33   mm), mean annual temperature (0.3 – 22° C; Supplementary Figure 1), soil texture, and pre-

34   treatment soil N, P, and K pools (Supplementary Table 1). The sites included both native and

35   previously cultivated grassland, and some sites were managed with burning, grazing, or other

36   practices. Thus, we were able to evaluate single and multiple-nutrient limitation of ANPP at

37   global, continental, and site spatial extents, and as influenced by management, climate, and soils.

38   N, P, and $K_{+\mu}$ were factorially applied annually to replicated 5 m^2 plots at the beginning

39   of each site's growing season at rates commonly used in grassland fertilization experiments[22,23].

40   The $K_{+\mu}$ treatment included a micronutrient mix in the first treatment year only, to avoid

41   micronutrient toxicity. Nutrient limitation of ANPP was quantified by harvesting current year

42   standing crop biomass for 3 to 5 years. and computing the log response ratio (LRR), the natural

43   logarithm of the ratio of treatment plot to control plot ANPP, a metric commonly used in meta-

44   analyses[24]

45   Multiple nutrient limitation of ANPP often occurs in the form of co-limitation[5]. Co-

46   limitation is synergistic when the response to multiple nutrients is greater than the sum of the

47   responses to each nutrient added individually, additive if the multiple-nutrient response equals

48   the sum of the individual nutrient responses, and sub-additive if less than the sum of the single-

49   nutrient responses[5]. Across all sites and years, the combined addition of N and P increased

50   ANPP by an average of 40% over controls (LRR= 0.34), compared to increases of only 18%

51   (LRR = 0.16) for N individually and 9% for P individually (LRR = 0.09; p = 0.03, Figure 1A,

52   Supplementary Figure 3). This provides clear evidence for globally-averaged synergistic co-

53   limitation of ANPP by N and P in these grasslands. Synergistic co-limitation by N and P across

54   sites and years emerged from frequent occurrence of NP co-limitation at the site level. Some

55   form of NP co-limitation averaging 67% (LRR = 0.51) occurred at 60% of the sites, including

56   sites on all five continents, with synergistic NP co-limitation at 13 sites and sub-additive co-

57   limitation at 12 sites, (Supplementary Table 3). Globally-averaged synergistic co-limitation of

58   grassland ANPP by N and P contradicts the long-held perception that N is the predominant

59   nutrient limiting grassland productivity[3,10] and highlights a critical role for P.

60   There was no globally-averaged single or multiple-nutrient limitation involving $K_{+\mu}$ (p >

61   0.32, Supplementary Table 2). However, this did not preclude frequent site-level single and

62   multiple-nutrient $K_{+\mu}$ limitation of grassland ANPP. ANPP was limited at 3 sites by $K_{+\mu}$ alone

63   and co-limited by $NK_{+\mu}$ at 18 sites, both averaging 48% (LRR = 0.39; Figure 1A, Supplementary

64   Table 3). Additionally, ANPP was co-limited by $PK_{+\mu}$ at 9 sites by an average of 52% (LRR =

65   0.42), approaching the magnitude of ANPP limitation at sites where N (57%) or P (54%)

66   individually limited ANPP (Figure 1A). Site-level $NK_{+\mu}$ and $PK_{+\mu}$ co-limitation occurred in

67   synergistic, additive, and sub-additive forms, and in total, single or multiple-nutrient limitation

68 involving $K_{+\mu}$ occurred at 24 sites again occurring on all five continents (Supplementary Table

69 3). This finding represents the broadest assessment to date of potential $K_{+\mu}$ limitation of

70 grassland ANPP, and suggests that single- and multiple-nutrient K limitation, potentially

71 augmented by micronutrient addition during year 1, occurs more frequently than previously

72 recognized[20,25].

73   Although single and multiple-nutrient limitation of ANPP was widespread, there were

74 nonetheless 15 sites with no evidence for multiple nutrient limitation, and at 12 of the 15 also no

75 evidence for single-nutrient limitation (Supplementary Table 3). The presence and magnitude of

76 nutrient limitation may depend on site climate, soil development, or fertility[26,27]. For these

77 reasons, greater site-level limitation of ANPP by one nutrient may correlate with greater

78 limitation by one or more additional nutrients. Indeed, site-level individual nutrient limitation of

79 ANPP increased with limitation by other individual nutrients (R^2 0.08 – 0.15; Figure 2A,B,D),

80 and ANPP limitation by nutrient pairs increased with that of a third individual nutrient (R^2 0.07 –

81 0.21; Figure 2C,E,F). These correlations suggest that site-level attributes may predict the

82 magnitude of single and multiple nutrient limitation of ANPP.

83   Sites differed in various potential qualitative and quantitative predictors of the presence

84 and magnitude of nutrient limitation of ANPP (Supplementary Table 1). We found no evidence

85 that nutrient limitation differed among the qualitative predictors continent, history of

86 management with burning, grazing, and previous cultivation, and predominant soil texture

87 (Supplementary Figure 4, Supplementary Table 4). Quantitative predictors were examined at the

88 19 longest running sites, where estimates of site mean nutrient limitation should best correspond

89 to site mean climate and soil attributes. N limitation increased with decreasing site mean annual

90 temperature (MAT; $R^2 = 0.40$, p = 0.002, Figure 3A) and peaked at temperate latitude sites

6

91   (~45°; $R^2 = 0.38$, p = 0.009, Figure 3B) with lower MAT ($R^2 = 0.66$, p < 0.0001, Figure 3B

92   inset). Limitation of ANPP by other single or multiple nutrient combinations was not correlated

93   with soil texture fractions, pre-treatment soil nutrient contents, soil pH, or climate variables (p >

94   0.05, data not shown). These findings suggest that N limitation predominated at cooler

95   temperate latitudes, as others have predicted[26], and validate the power of these experiments to

96   detect spatial trends in ecosystem nutrient limitation.

97   The magnitude of single or multiple-nutrient limitation of grassland ANPP may increase

98   through time (Supplementary Figure 2B). To isolate the temporal signal of nutrient addition

99   effects on ANPP, we calculated new ANPP response ratios using the pre-treatment year (i.e.,

100 year 0) ANPP for each treatment plot rather than the control plot, to control for plot-to-plot

101 spatial variation in ANPP. For 37 sites with no missing ANPP data in years 1-3, the ANPP

102 response to nutrient addition increased through time in all treatments, ANPP increased each year

103 more in treatments containing N (p < 0.005, Figure 4, Supplementary Table 2) compared to the

104 P, $K_{+\mu}$, and $PK_{+\mu}$ treatments. Thus, the larger global-averaged increase in ANPP with N than P or

105 $K_{+\mu}$ (Figure 1A) can be explained partly by both smaller and later manifestation of P and $K_{+\mu}$

106 effects. The full extent of nutrient limitation of grassland ANPP, particularly for nutrients other

107 than N, may require additional years to emerge.

108   Multiple nutrient limitation of ANPP in these grasslands was spatially and temporally

109 varied, often included K and micronutrients, but sometimes none of the nutrients we considered.

110 Thus, while N was an important limiting nutrient in many grasslands, P and $K_{+\mu}$ were also

111 limiting in many instances, both alone and in combination with each other and with N. These

112 estimates for globally-averaged nutrient limitation in these grasslands are conservative because

7

113   nutrient limitation increased in successive treatment years. The magnitude of nutrient limitation

114   may require revision upward if the increases continue in future years.

115   Multiple nutrient limitation in grasslands, especially synergistic co-limitation, makes the

116   consequences of anthropogenic nutrient inputs in these and other systems more difficult to

117   predict. For example, nutrient co-limitation may actually restrict the impacts of single nutrient

118   inputs such as N deposition[17], but ecosystems may respond more rapidly than expected to

119   subsequent inputs of the co-limiting nutrient. Considering only one of the co-limiting nutrients

120   would risk reaching erroneous conclusions about the consequences of future inputs for

121   biodiversity or ecosystem goods/services provision[14,15,28,29]. It is imperative to consider multiple

122   nutrient constraints on primary productivity in grasslands and other ecosystems.

123   **Full Methods** and any associated references are available in the online version of the paper at

124   www.nature.com/nature.

127

128   **Methods**

129   *Study system*

130   The rationale, goals, and experimental protocols used at grasslands in the Nutrient

131   Network (NutNet) are described in Borer et al.[21], and are summarized here. This study used

132   aboveground net primary productivity (ANPP) data from a standardized nutrient addition

133   experiment conducted at 42 NutNet sites in eight countries (Australia, Canada, China,

134   Switzerland, Tanzania, United Kingdom, USA, South Africa) on five continents (Australia [N =

8

135   4], Africa [N = 4], Europe [N = 5], Asia [N = 1], North America [N = 28]; Supplementary Figure

136   1, Table 1).

137   The grassland types included alpine/montane grasslands, shortgrass, mixed, and tallgrass

138   prairies, desert and semi-arid grasslands, old fields and pastures, savanna and shrub-steppe, and

139   annual grasslands. Sites span wide ranges of mean annual precipitation (MAP; 252 - 1898 mm

140   y^{-1}), mean annual temperature (MAT; 0.3 - 22.1 °C; Supplementary Figure 1), elevation (50 -

141   3500 m), and absolute latitude (over 25°, actual latitudes 54° N to 37° S, Supplementary Table

142   1). Site climate data were derived from the WorldClim database[30] (version 1.4,). Local

143   investigators classified sites as to management practice (burned, grazed, other management) and

144   native or anthropogenic (restored grassland and agricultural pasture) origin. Sites collected pre-

145   treatment soil nutrient content and texture data.

146   At each site, N, P, and K were added in full factorial combination (8 treatments including

147   control plots) to 5 x 5 m plots in a randomized complete blocks design with N=3 for most sites

148   (range 2-6). This experimental scale is well-suited for the relatively short-statured herbaceous

149   vegetation in grasslands. Micronutrients (6% Ca, 3% Mg, 12% S, 0.1% B, 1% Cu, 17% Fe,

150   2.5% Mn, 0.05% Mo, and 1% Zn) were added with K only in year 1 to avoid possible

151   micronutrient toxicity. Nutrient additions began at most sites in 2008 (N = 28), and additional

152   sites began in 2009 (N = 9), 2010 (N = 3) and 2011 (N = 2).

153   *Sampling*

154   Total ANPP ($g\ m^{-2}\ yr^{-1}$) was estimated annually from clip samples of current year peak

155   aboveground biomass from two 0.1 m^2 quadrats per plot. Samples were dried to constant mass at

156   60°C.

157 *Statistical Analyses*

158   We tested for nutrient treatment effects on ANPP using linear mixed models in

159 SAS/STAT v 9.2. First, we tested for variation among sites in nutrient main and interactive

160 effects and their variation with treatment year (Equation 1):

161      $$\mathbf{ln(ANPP)}_{ijkl} = \mathbf{\mu} + \mathbf{\textit{nutrients}}_i + \mathbf{\textit{nutrients}(site)}_{ij} + \mathbf{nutrients(year)}_{ik}$$

162      $$+ \mathbf{nutrients(site \ x \ year)}_{ijk} + \mathbf{e}_{ijkl}. \quad \textbf{(1)}$$

163 where <u>*nutrients*</u> refers to the eight factorial combinations of added N, P, and K. Nutrient

164 treatments (i) were nested within site (j), within year (k), and within site x year (jk). Year was

165 not treated as a repeated effect because each site experienced each year differently, and thus both

166 the year effects and the covariance among years were not the same at each site. There were 42

167 sites with three years of data, 33 sites with four years, and 22 sites with five years. Model (1) was

168 tested using all available treatment years, and with only the first three treatment years to

169 ascertain whether any nutrient x year interactions were caused by sites added later with fewer

170 years of nutrient addition.

171   We tested whether ANPP responses to nutrient treatments varied with continent,

172 management, dominant soil texture, and origin by replacing the site term in Eq. 1 with each of

173 the site classification and soils variables, one at a time. Europe and Asia were combined in tests

174 of continent because of the single Asian site.

175 *Calculation of nutrient effect sizes*

176   Effects sizes of the nutrient treatments were calculated and graphed as natural-log

177 response ratios (LRR). LRRs were calculated in two ways; first, by comparing the ANPP of each

178   treatment plot to the ANPP of the control plot in the same block (2), to isolate treatment effects;

179   and second, by compared treatment plot ANPP to that of its pre-treatment (i.e., year 0) ANPP

180   (3). This isolated the temporal signal of nutrient addition responses.

181   **LRR = ln[ANPPtrt/ANPPcontrol]   (2)**

182   **LRR = ln[ANPPtrt(year X)/ANPPtrt(year 0)]   (3)**

183   We estimated a critical threshold LRR (4) for categorizing the significance of site LRRs. An

184   LRR was considered statistically significant if it exceeded a value corresponding to the critical

185   Z-score (1.65) at p = 0.05, computed as:

186   **Threshold LRR=   1/7 * (LRR_N + LRR_P + LRR_K + …. LRR_{NPK})/pooled SD. (4)**

187   *Correlations of site variables with nutrient effects*

188   We used linear regression analyses to test whether LRRs for each single nutrient and

189   nutrient pair were predicted by site latitude (absolute value), elevation, mean annual precipitation

190   (MAP), mean temperature annual (MAT), potential evapotranspiration (PET), soil fractions of

191   sand, silt, and clay, pre-treatment soil N, P, and K contents, and soil pH. We presented

192   regressions only where p < 0.05, and fit polynomial curves where indicated by evaluation of

193   residuals.

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270 **Author Contributions** P.A.F. wrote the manuscript, drafted the figures, and led the data

271 analysis; E.M.L., developed the database; S.M.P. and W.S.H. contributed to data analysis;

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273 development and data interpretation. All co-authors contributed data and manuscript editing.

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

275 experiment.

276 **Author Information** The data reported in this paper are available through

277 http://www.nutnet.org. Reprints and permissions information is available at

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280 Correspondence and requests for materials should be addressed to P.A.F.
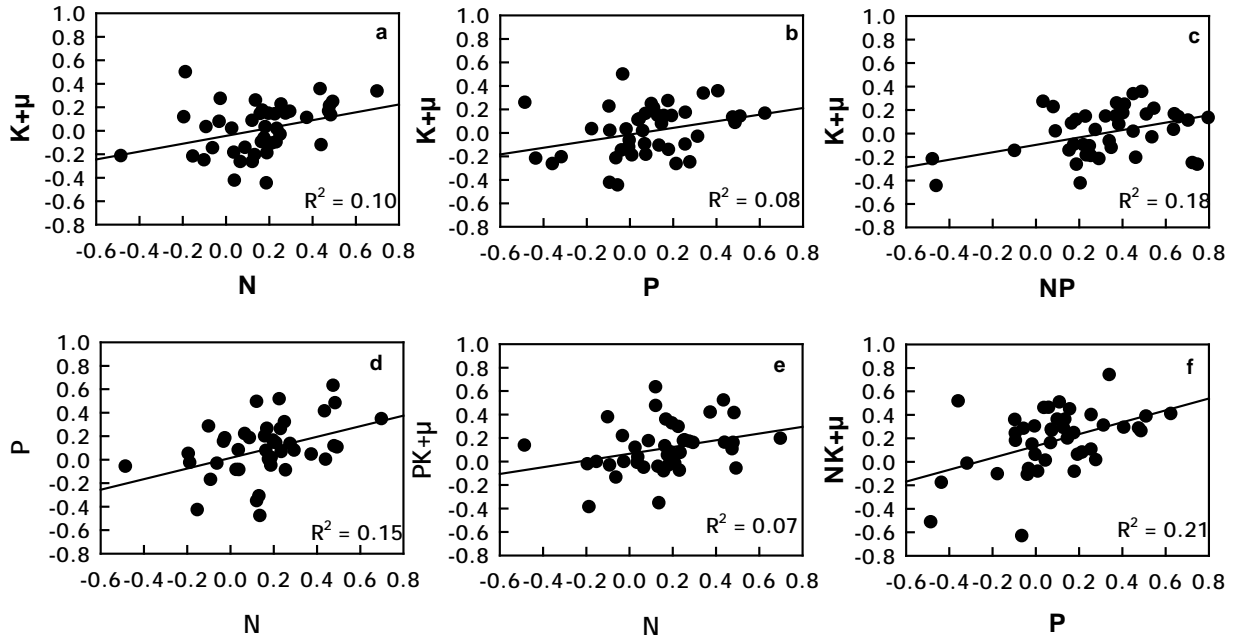
281 (philip.fay@ars.usda.gov).

Figure 1

282
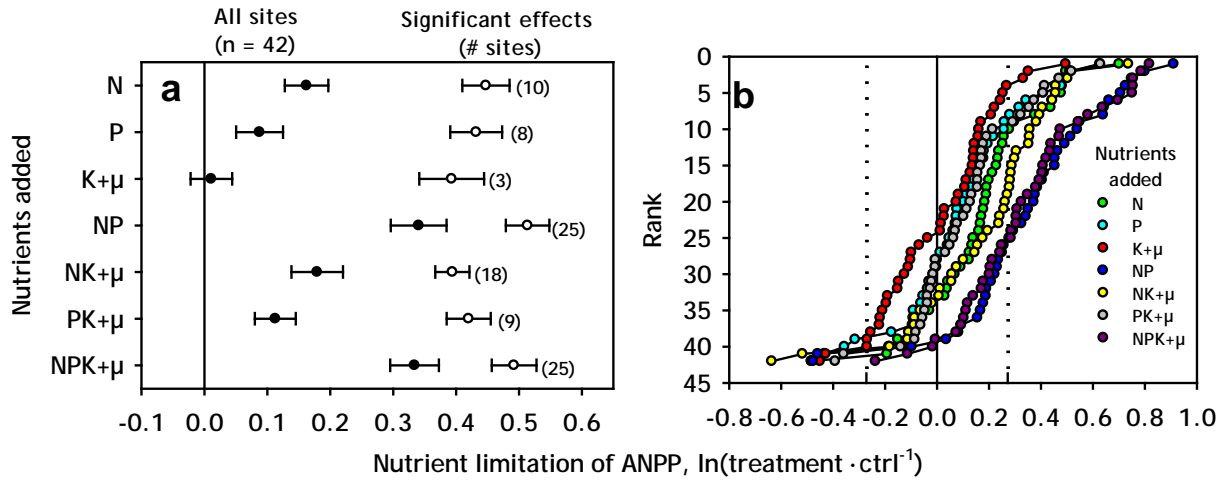
Figure 1. Nutrient limitation of aboveground net primary productivity (ANPP; log response

ratio, ln[treatment · control^{-1}],) by nitrogen (N), phosphorus (P), and potassium plus year 1

micronutrients (K$_{+\mu}$). a) Mean ± 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

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Figure 2. Correlations of nutrient limitation of aboveground net primary productivity (ANPP;

log response ratios, *ln*[treatment · control^{-1}]) among single and paired nutrients. a,b,d)

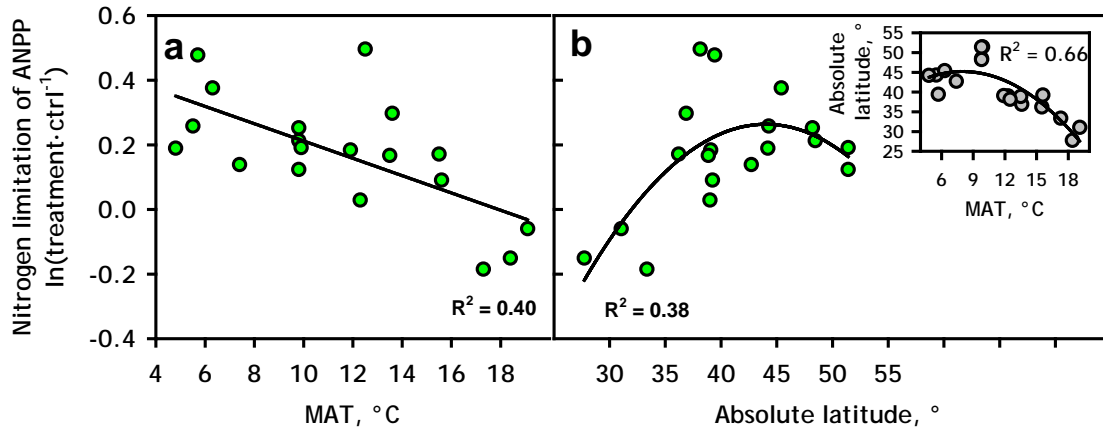Correlations among single nutrients. c,e,f) Correlations of nutrient pairs with the third nutrient.

Coefficients of determination (R^2) from linear regression ($0.002 \leq P \leq 0.05$).

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Figure 3

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301 **Figure 3.** Predictors of nitrogen (N) limitation of aboveground net primary productivity (ANPP;
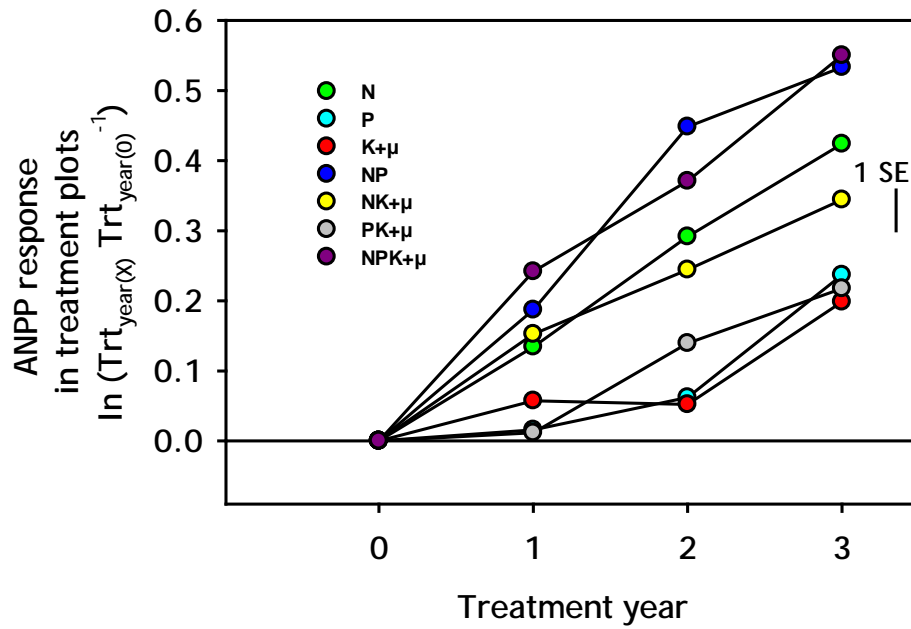
302 log response ratio, *ln*[treatment · control^{-1}]). a) site mean annual temperature (MAT), b) site

303 latitude, in degrees away from the equator, and inset) MAT relationship with site latitude. N

304 limitation values are means of five continuous years of nutrient treatment from 19 sites.

305

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.