

Leaf nutrients, not specific leaf area, are consistent indicators of elevated nutrient inputs

Jennifer Firm ^{1*}, James M. McGree¹, Eric Harvey ², Habacuc Flores-Moreno ³, Martin Schütz⁴, Yvonne M. Buckley ⁵, Elizabeth T. Borer ³, Eric W. Seabloom ³, Kimberly J. La Pierre ⁶, Andrew M. MacDougall⁷, Suzanne M. Prober⁸, Carly J. Stevens⁹, Lauren L. Sullivan³, Erica Porter¹, Emma Ladouceur ^{10,11}, Charlotte Allen¹, Karine H. Moromizato¹, John W. Morgan¹², W. Stanley Harpole ^{10,11,13}, Yann Hautier ¹⁴, Nico Eisenhauer ^{10,15}, Justin P. Wright¹⁶, Peter B. Adler¹⁷, Carlos Alberto Arnillas¹⁸, Jonathan D. Bakker¹⁹, Lori Biederman²⁰, Arthur A. D. Broadbent^{9,21}, Cynthia S. Brown²², Miguel N. Bugalho²³, Maria C. Caldeira²⁴, Elsa E. Cleland²⁵, Anne Ebeling²⁶, Philip A. Fay ²⁷, Nicole Hagenah²⁸, Andrew R. Kleinbasselink²⁹, Rachel Mitchell³⁰, Joslin L. Moore³¹, Carla Nogueira²⁴, Pablo Luis Peri³², Christiane Roscher^{10,11}, Melinda D. Smith ³³, Peter D. Wragg³ and Anita C. Risch ⁴

Leaf traits are frequently measured in ecology to provide a ‘common currency’ for predicting how anthropogenic pressures impact ecosystem function. Here, we test whether leaf traits consistently respond to experimental treatments across 27 globally distributed grassland sites across 4 continents. We find that specific leaf area (leaf area per unit mass)—a commonly measured morphological trait inferring shifts between plant growth strategies—did not respond to up to four years of soil nutrient additions. Leaf nitrogen, phosphorus and potassium concentrations increased in response to the addition of each respective soil nutrient. We found few significant changes in leaf traits when vertebrate herbivores were excluded in the short-term. Leaf nitrogen and potassium concentrations were positively correlated with species turnover, suggesting that interspecific trait variation was a significant predictor of leaf nitrogen and potassium, but not of leaf phosphorus concentration. Climatic conditions and pretreatment soil nutrient levels also accounted for significant amounts of variation in the leaf traits measured. Overall, we find that leaf morphological traits, such as specific leaf area, are not appropriate indicators of plant response to anthropogenic perturbations in grasslands.

Biodiversity loss is accelerating at an alarming rate, particularly in grasslands due to eutrophication linked to agricultural intensification and industrial pollution¹, and altered trophic-level interactions such as reduced consumption by native

herbivores^{2,3}. These anthropogenic pressures also impact species composition, potentially selecting for species with particular traits, and thereby affecting ecosystem function^{4,5}. Functionally relevant traits, rather than species richness, have been increasingly used as

¹Queensland University of Technology, Brisbane 4000 Queensland, Australia. ²Département de Sciences Biologiques, Université de Montréal, Montréal, Quebec, Canada. ³Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, MN, USA. ⁴Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf, Switzerland. ⁵School of Natural Sciences, Zoology, Trinity College Dublin, Dublin, Ireland. ⁶Smithsonian Environmental Research Center, Edgewater, MD, USA. ⁷Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada. ⁸CSIRO Land and Water, Floreat, Western Australia, Australia. ⁹Lancaster Environment Centre, Lancaster University, Lancaster, UK. ¹⁰German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany. ¹¹Department of Physiological Diversity, Helmholtz Center for Environmental Research, Leipzig, Germany. ¹²Department of Ecology, Environment and Evolution, La Trobe University, Melbourne, Victoria, Australia. ¹³Institute of Biology, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany. ¹⁴Ecology and Biodiversity Group, Department of Biology, Utrecht University, Utrecht, the Netherlands. ¹⁵Institute of Biology, Leipzig University, Leipzig, Germany. ¹⁶Department of Biology, Duke University, Durham, NC, USA. ¹⁷Department of Wildland Resources/Ecology Center, Utah State University, Logan, UT, USA. ¹⁸Department of Physical and Environmental Sciences, University of Toronto Scarborough, Toronto, Ontario, Canada. ¹⁹School of Environmental and Forest Sciences, University of Washington, Seattle, WA, USA. ²⁰Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA, USA. ²¹School of Earth and Environmental Sciences, Michael Smith Building, The University of Manchester, Manchester, UK. ²²Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO, USA. ²³Centre for Applied Ecology (CEABN-INBIO), School of Agriculture, University of Lisbon, Lisbon, Portugal. ²⁴Forest Research Centre, School of Agriculture, University of Lisbon, Lisbon, Portugal. ²⁵Division of Biological Sciences, University of California San Diego, La Jolla, CA, USA. ²⁶Institute of Ecology and Evolution, University of Jena, Jena, Germany. ²⁷Agricultural Research Service, United States Department of Agriculture, Grassland Soil and Water Research Laboratory, Temple, TX, USA. ²⁸Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa. ²⁹Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA, USA. ³⁰School of Earth Sciences and Environmental Sustainability, Northern Arizona University, Flagstaff, AZ, USA. ³¹School of Biological Sciences, Monash University, Melbourne, Victoria, Australia. ³²Department of Forestry, Agriculture and Water, National University-INTA-CONICET, Rio Gallegos, Santa Cruz, Patagonia, Argentina. ³³Department of Biology, Graduate Degree Program in Ecology, Colorado State University, Fort Collins, CO, USA. *e-mail: jennifer.firm@qut.edu.au

a 'common currency' to assess the consequences of biodiversity loss^{6,7} on ecosystem functioning^{8,9}. Leaf traits are commonly used, and considered as part of the 'Holy Grail'^{6,10} set of traits, to predict plant–animal interactions¹¹, community composition and ecosystem function in response to perturbations¹².

Ecology's focus on leaf traits is based on strong ecophysiological evidence that leaves represent important investment strategies for plant growth and survival. Plants invest photosynthate and mineral nutrients in the construction of leaves, which capture light to produce more photosynthate^{13,14}. Leaf traits, such as specific leaf area (SLA) and leaf nutrient concentrations, are typically used as comparative measures of how plants capitalize on these investments. SLA, measured as the leaf area per unit mass, represents a trade-off between surface area for capturing photons and thickness related to structural adaptations for water conservation and herbivore defence. Indeed, leaf traits correlate across a continuum of fast to slow returns on investment, known as the leaf economic spectrum (LES)^{14–16}.

Fast-growing species, which are adept at resource acquisition and tend to dominate in regions with high rainfall levels and soils where resource availability is not limiting, are hypothesized to have higher SLAs and leaf nutrient concentrations^{10,17}. High SLA is associated with lower costs of leaf construction and higher rates of herbivory as tissue becomes more palatable⁶. Additionally, higher species turnover and palatability are also positively correlated with leaf nitrogen, phosphorus and potassium concentrations^{14–16}. In contrast, slower-growing species, which exhibit resource conservation, are hypothesized to have lower SLAs and leaf nutrient concentrations^{14–17}. As a result, slow-growing species are less palatable to herbivores, while having a longer leaf life span.

Trade-offs between leaf traits discovered in the LES were shaped over evolutionary timeframes, as successful trait combinations are selected for and unfavourable combinations are selected against. LES relationships were built from comparative relationships among leaves collected across biomes ranging from tundra to tropical forests¹⁴. However, the extent to which rapid changes in structuring forces such as soil nutrient availability and reduced herbivory result in predictable shifts in trait values within a biome, such as grasslands, remains equivocal⁶. Indeed, in agriculture, the growth-dilution effect postulates that leaf nutrient concentrations may not increase in response to fertilizer because increased plant growth outpaces nutrient accumulation in tissue¹⁸.

SLA and leaf nutrient concentrations are commonly used as surrogate measures of broad-scale biogeographical differences¹². However, leaf trait responses of individual species are also influenced by short-term local-scale abiotic and biotic factors. Climatic and edaphic conditions interact with fertilization and changes in natural disturbance regimes to sculpt community composition and, ultimately, ecosystem functioning^{5,10,11,19,20}. Given the complex sets of interactions that may explain leaf trait responses to short-term environmental change, a modelling approach is necessary to discern interactions that may otherwise be missed when using traditional bivariate analyses^{21,22}.

In a global experimental test, we quantified how leaf traits in grasslands change in response to the addition of soil nutrients (that is, nitrogen, phosphorus and potassium) and the exclusion of vertebrate herbivores. We sampled leaf traits from the Nutrient Network (NutNet)²³ cross-continental distributed experiment established at 27 sites (Fig. 1 and Supplementary Table 1). This experimental network allowed us to test how commonly measured leaf traits respond to environmental change across grasslands. At the majority of sites, we sampled leaf traits after 3–4 years of treatment (5 sites after 2 years and 22 of the 27 sites after 3–4 years; see Supplementary Table 1 for detailed information on each site).

At each site, 3 blocks of 10 5-m × 5-m plots were established, and 2 experiments were initiated: (1) a full factorial nutrient addition

experiment, including the addition of all factorial combinations of nitrogen, phosphorus and K_{+μ}, where the subscript '+μ' refers to the inclusion of ten other micronutrients in the first application year as part of the potassium addition treatment (see Borer et al.²³ and Methods for more details); and (2) a combination full nutrient addition (NPK_{+μ} addition) and herbivore exclusion experiment where fences were built to exclude vertebrate herbivores that were larger in weight than 50 g (for more details, see Methods).

Relative cover was visually estimated before the experiment began and before the leaf harvest period, when leaf traits were collected from the three to five most dominant species in each plot. Overall, 243 species were sampled across the 27 sites, including grasses, forbs and legumes, and 2,664 leaf samples were measured for leaf area, leaf dry weight, and leaf nitrogen, phosphorus and potassium concentrations²⁴. Overall the sampled species accounted for 26% of the total vegetation cover at the time when the leaves were collected. The effect sizes of the mean leaf trait values for all species in response to the experimental treatments were estimated using multilevel regression models in a hierarchical Bayesian framework using integrated nested Laplace approximation²⁵, where the random effect structure included block nested in site nested in species. SLA values were log transformed to meet assumptions of normality in the multilevel regression model.

Results and discussion

We found that SLA did not increase consistently with the treatments. We did, however, find evidence of a small but significant increase in SLA in the NP (mean log[SLA] = 8.79 mm² g⁻¹) and NPK fertilizer treatments (mean log[SLA] = 8.81 mm² g⁻¹) compared with the control (mean log[SLA] = 8.69 mm² g⁻¹), suggesting that simultaneous increases in the availability of nitrogen and phosphorus may be necessary to find consistent increases in SLA in grasslands (Fig. 2a)²⁶. When we considered the variation explained by the random effects in the model, SLA showed the highest variability of any of the measured leaf traits at the site level (Fig. 3; ~75% of the variation in SLA in response to treatments was explained among sites), suggesting that variation in SLA may be explained by other local abiotic and biotic factors not included in these models. These results provide a new mechanistic understanding of previous NutNet studies, which found that plant aboveground biomass increased in response to nutrient enrichment and fencing treatments, with the highest increase being recorded in the fencing treatments after just three years^{27,28}. Our results indicate that this increase in plant biomass is not explained by an increase in SLA, but instead may be explained by the number of leaves, stems and other structural elements produced.

Nitrogen, phosphorus and potassium leaf concentrations increased significantly when the corresponding nutrients were applied as fertilizer (Fig. 2). Previous NutNet studies have found multiple-nutrient constraints on aboveground net primary production, including increased vegetation cover and biomass²⁹. The leaf nitrogen concentration also increased in leaves with PK_{+μ} fertilization (Fig. 2b)—a probable reflection of the increased availability of nitrogen in soils³⁰ and the importance of other nutrient limitations for increasing plant nitrogen uptake. Leaf phosphorus showed the opposite trend to leaf nitrogen, decreasing in concentration when either nitrogen or NK_{+μ} were applied as fertilizer (Fig. 2c). This trend probably reflects the limited availability of phosphate to plants, because of its high affinity to soil particles³¹, as otherwise we may have found an increase in leaf phosphorus when limitations were lifted by the addition of other essential nutrients²⁶. The leaf potassium concentration showed the highest variation associated with 'species' random effects (~60%; Fig. 3). The fencing treatment did not significantly alter leaf nutrient concentrations only when soil nutrient addition was combined with the fencing treatment (Fig. 2).

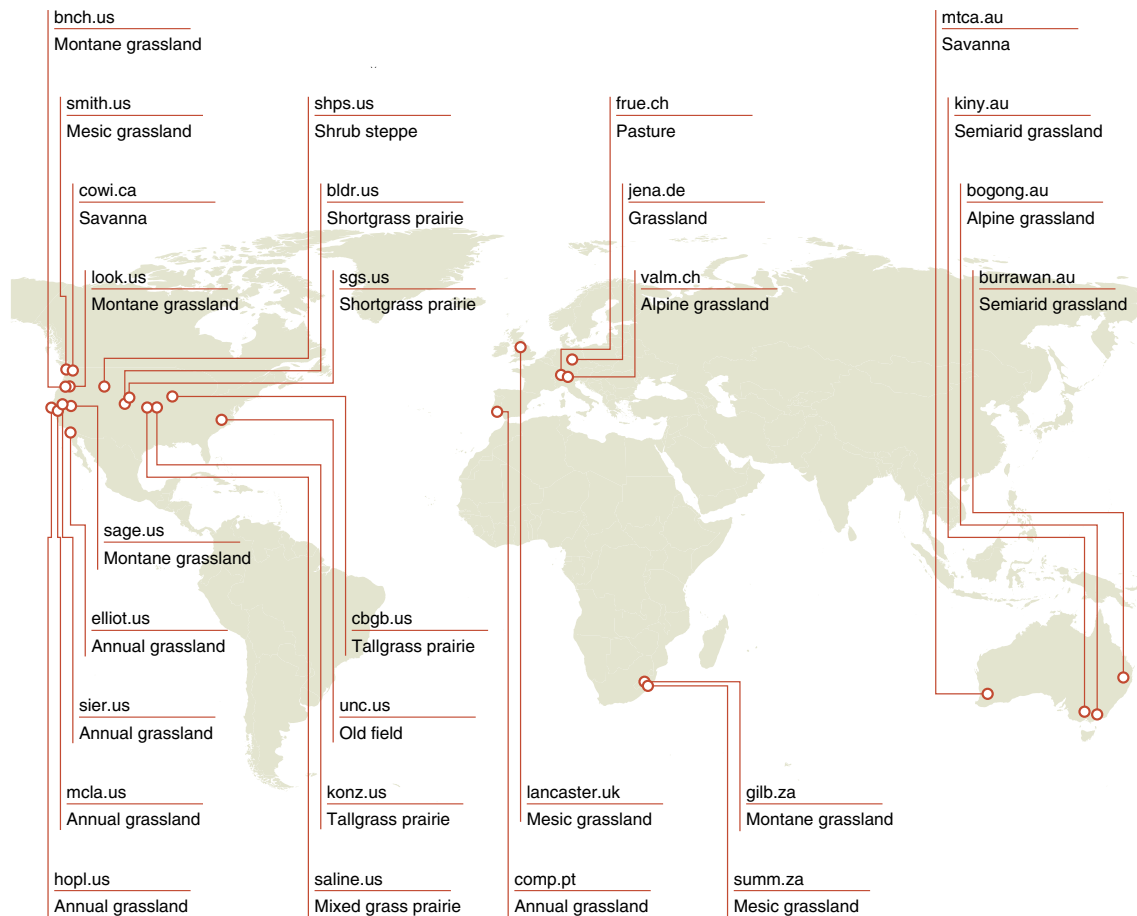


Fig. 1 | Map showing the locations of the 27 NutNet experimental sites where leaf trait information was collected using a standardized protocol. Sites span four continents and eight countries (au, Australia; ca, Canada; ch, Switzerland; de, Germany; pt, Portugal; uk, United Kingdom; us, United States; za, South Africa). Diagram by Evidently So.

Our findings of an increase in leaf nutrient concentrations in response to the fertilizer treatments could be explained by intra-specific trait variation (increases shown by the same species over time) and by interspecific changes in dominant species following the application of treatments. After treatment initiation, changes in dominant species were observed at some study sites, whereas little change was observed at other sites. This difference is important because increases in leaf nutrient concentrations could be explained by two mechanisms: (1) current species increase their uptake of nutrients (that is, intraspecific trait variation)³²; and (2) new species are recruited into the dominant class (that is, interspecific trait variation) as the increased nutrient availability favours their growth and establishment³³. Therefore, we evaluated the effects of temporal species turnover on leaf trait responses. We estimated temporal species turnover using Bray–Curtis dissimilarity for the three to five most dominant species in each plot, comparing pretreatment species composition with composition when the leaf traits were measured, two to four years later.

Given the global extent of our study sites and the high amounts of variation in leaf traits found at the site level, particularly for SLA (Fig. 3), we also evaluated the effects of climatic conditions and pre-treatment soil nutrient levels. We used structural equation models to examine the influence of these additional possible drivers (see Supplementary Materials for details on model development, including Supplementary Figs. 2–4). Because we did not find evidence of a leaf trait response to the fencing treatments, we did not further evaluate these treatments, focusing instead on the nutrient addition

treatments. Overall, the R^2 values (coefficients of determination) for each of the leaf nutrient trait response variables were high, indicating a strong explanatory power of the models; leaf potassium had the highest R^2 value and SLA the lowest (leaf nitrogen, $R^2 = 0.53$; leaf phosphorus, $R^2 = 0.32$; leaf potassium, $R^2 = 0.55$; SLA, $R^2 = 0.11$).

All leaf traits varied with climatic and edaphic conditions (Fig. 4 and Supplementary Fig. 5). The nutrient addition treatments explained considerable amounts of variation in the leaf nutrient contents but not in SLA. Species temporal turnover was positively correlated with leaf nitrogen and potassium contents, but significant correlations were not found with the leaf phosphorus content or SLA. This result shows that a portion of the increase in the leaf nitrogen and potassium contents was explained by interspecific variation, suggesting some selection effect of the addition of these nutrients on species composition, whereas the positive response of leaf phosphorus was explained by intraspecific trait variation. These findings corroborate other studies that have also found that considerable amounts of variation in leaf chemical traits are explained by intraspecific variation³². The duration of the nutrient addition treatments (represented as ‘Year’ in Fig. 4 and Supplementary Fig. 5) was also positively correlated with species temporal turnover, suggesting that sites with longer treatment durations had higher species turnover. Covariances among the leaf nutrient contents were high in the structural equation model, but SLA showed the lowest covariation with all leaf nutrient contents (Supplementary Table 2).

Before trait-based ecological studies can scale the responses of leaf traits from individuals to communities and ecosystems¹⁰,

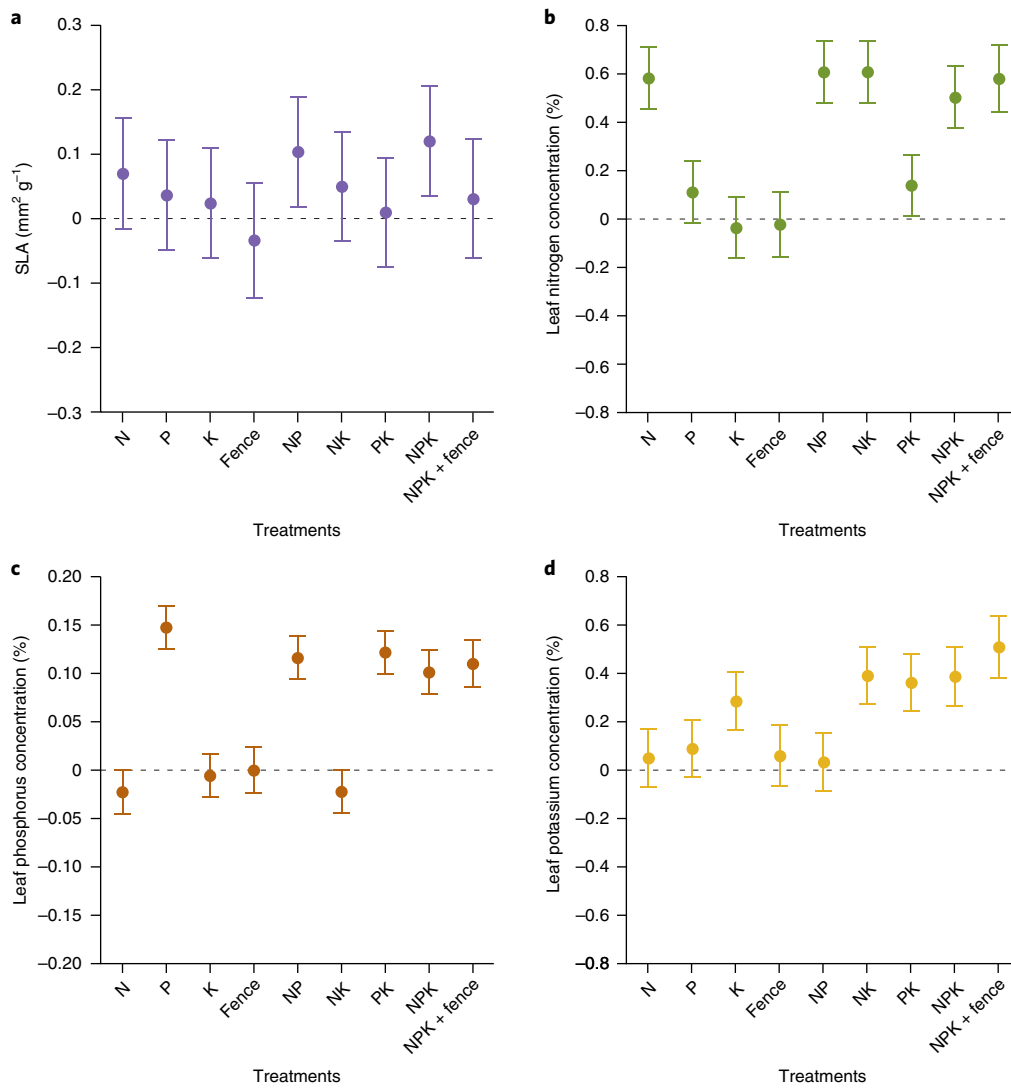


Fig. 2 | Comparison of effect estimates. Error bars represent 95% credible intervals, and the reference line at 0 represents the intercepts of the models that were the control treatments for SLA (**a**; log effect sizes shown), leaf nitrogen concentration (**b**), leaf phosphorus concentration (**c**) and leaf potassium concentration (**d**) from Bayesian hierarchical models fit with INLA with a random-effects structure of species, site and block. Intercept values (8.7 ± 0.6 for **a**; 2.7 ± 0.3 for **b**; 0.2 ± 0.04 for **c**; and 1.9 ± 0.30 for **d**) represent leaf trait estimates in the control treatments. Diagram by Evidently So.

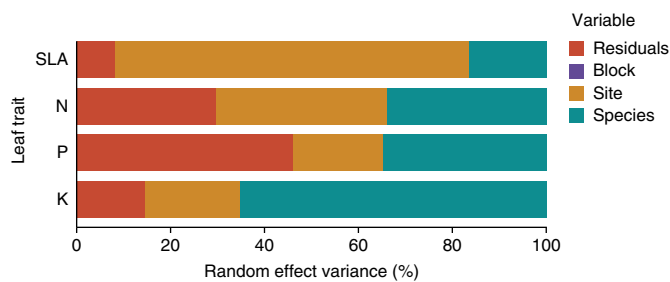


Fig. 3 | Percentage of variation explained by the random effects of block nested in site nested in species plus residual variation from the Bayesian hierarchical models fit with INLA for SLA, leaf nitrogen concentration, leaf phosphorus concentration and leaf potassium concentration. Response effects are shown in Fig. 2. Diagram by Evidently So.

a more definitive understanding of when, where and how to interpret changes in plant trait values is needed. This includes how to match plant traits to appropriate environmental conditions depending

on the characteristics of specific ecosystems. This necessitates testing plant trait responses in experimental studies, particularly in relation to local and short-term environmental changes or disturbances⁶. We found, using a global common experimental test of leaf trait responses, that leaf nutrient concentrations responded consistently to short-term nutrient additions, and this response is explained by both changes in dominant species and the ability of current dominant species to take up more nutrients when available. The SLA of the dominant species did not increase consistently in response to short-term nutrient addition treatments. Our findings corroborate a recent meta-analysis that found higher intraspecific variation in leaf traits than in morphological traits such as SLA³². Based on these findings, if species composition within treatment plots continues to turn over, we may find a clearer response in SLA.

In contrast with expectations, we found little evidence of a consistent short-term increase in SLA or leaf nutrient concentrations to reduced vertebrate herbivory (fencing treatment). The lack of a consistent response to the fencing treatment might be due to variation in vertebrate herbivore pressure at these globally distributed grassland sites. The majority of previous studies that have found a

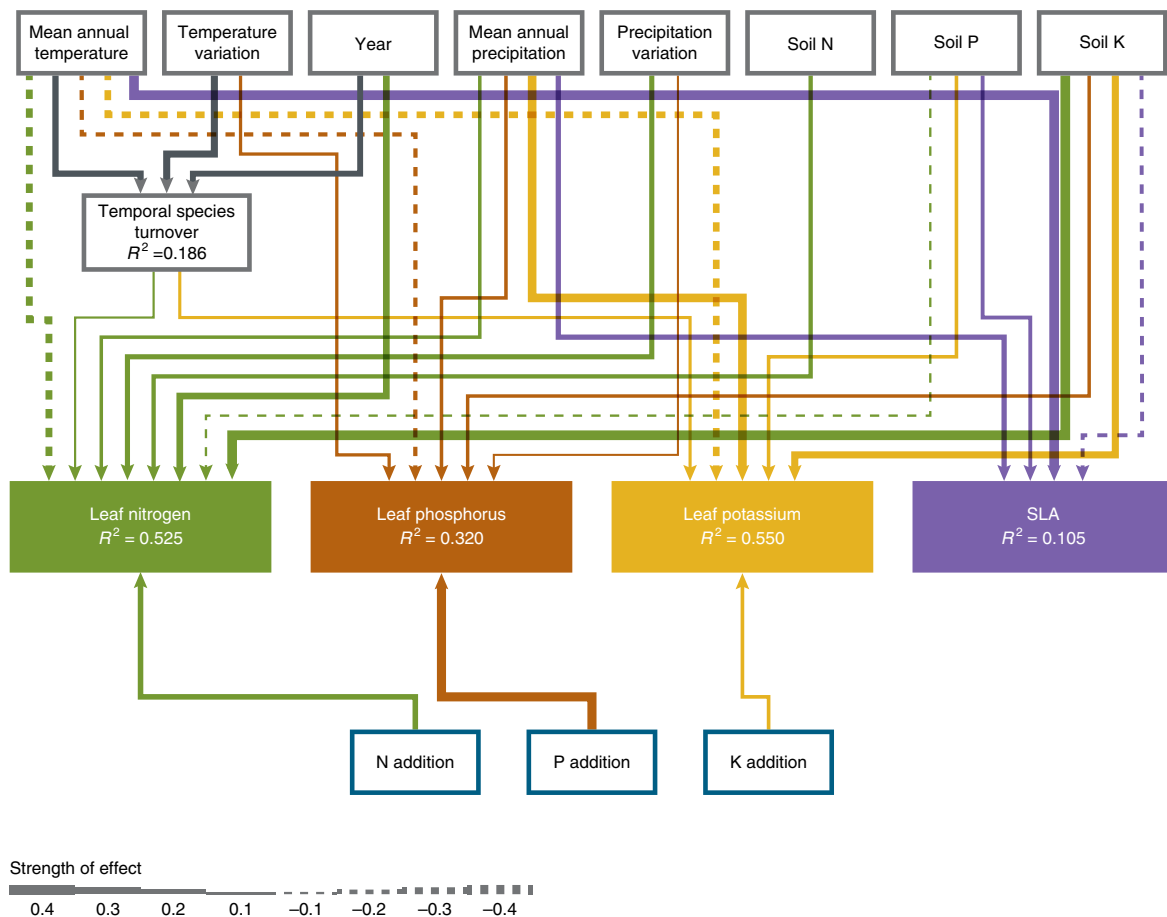


Fig. 4 | Structural equation model diagram representing connections between leaf traits, experimental nutrient addition treatments, and site-level average climatic and pretreatment edaphic conditions, as well as species turnover. The width of the connections represents estimates of the standardized path coefficients, with solid lines representing a positive relationship and dashed lines a negative relationship. Only significant connections are shown. Path coefficients are presented in Supplementary Fig. 4. Diagram by Evidently So.

consistent increase in SLA and leaf nutrient concentrations with the exclusion of vertebrate herbivores focused on the impacts of cattle and sheep^{5,34–36}, whose grazing pressure tends to be higher and known for the selectivity of plant tissue for increased palatability and nutrition³⁷. Here, only 8 of our 27 grasslands included a recent or current history of domestic grazing. Other studies that have excluded wild herbivores have found the strongest increases in SLA and leaf nutrient concentrations when invertebrate herbivores were also excluded^{11,27,38}, whereas in this experiment we only excluded vertebrate herbivores.

Our findings have implications for how leaf traits are used to infer responses to local-scale environmental perturbations within grassland ecosystems. SLA should be interpreted carefully when used as a predictor of functional response to environmental change within grasslands. SLA has been found to be a reliable indicator of plant resource utilization strategies at biogeographical scales¹⁹. However, a global-scale experimental test demonstrated that SLA is not a consistent indicator of the short-term response of plants to increased soil nutrients or the exclusion of vertebrate herbivores.

Broad-scale biogeographical trait relationships, such as the worldwide LES¹⁴, do not necessarily correlate as plant functional responses to short-term disturbance and changing abiotic conditions. Our results show that changes in individual traits—in the same species or because of species turnover—do not necessarily represent a ‘common currency’ for comparing ecosystem-level responses in grasslands to anthropogenic perturbations. When it comes to dominant plant species, leaf nutrients are responsive to

elevated soil nutrients, even across sites characterized by very different climatic and edaphic conditions, and are potentially more consistent plant functional response traits than SLA, particularly in the short term.

Methods

Network of experimental sites. The 27 study sites are part of NutNet—a cooperative globally distributed experiment (Fig. 1 and Supplementary Table 1; <http://www.nutnet.org/>). Each experimental site had a randomized block design, and at most sites, 3 replicate blocks divided into 10 5-m × 5-m plots were established, resulting in a total of 30 plots per site.

We quantified climatic variables (mean annual temperature, mean annual precipitation, temperature variation (a measure of seasonality calculated as the standard deviation × 100) and precipitation variation (a measure of seasonality calculated as the coefficient of variation)) for each site using modelled values sourced from the WorldClim Global Climate database (version 1.4; <http://www.worldclim.org>). The sites included in this study represented a wide range of climatic conditions with mean annual temperatures ranging from 0.3 °C (alpine grassland in Switzerland) to 18.4 °C (semi-arid C₄ perennial grassland in Australia), and mean annual precipitation ranging from 262 mm (shrub steppe in the USA) to 1,898 mm (montane grassland in the USA).

Nutrient addition experiment. In this experiment, we established a set of nutrient addition treatments that included a full factorial combination of three essential plant macronutrients (nitrogen, phosphorus and K_{sp}), including a control. The following rates of nutrients, obtained from the same chemical sources, were applied at all sites: 10 gN m⁻² yr⁻¹ as timed-release urea; 10 gP m⁻² yr⁻¹ as triple super phosphate; and 10 gK m⁻² yr⁻¹ as potassium sulphate plus a one-off addition (100 g m⁻² yr⁻¹) of macro- and micronutrients (that is, Fe, S, Mg, Mn, Cu, Zn, B, Mo and Ca). At all sites, nitrogen, phosphorus and potassium fertilizers were applied annually, whereas micronutrients were applied once at the start of the

study to avoid toxicity, and only in treatments that included potassium. Sites entered the NutNet in different years (2007–2014), and leaf traits were usually measured after 3–4 years of nutrient addition (Supplementary Table 2). Note that ammonium nitrate was used in 2007 at some sites before switching to urea because of the increasing difficulty in sourcing ammonium nitrate globally. At a subset of these sites, we tested whether this one-year addition of ammonium nitrate would influence the outcomes of the plant community responses, and found no significant effect of the nitrogen source²³.

To quantify soil nutrients during the pretreatment year, we first removed the litter and vegetation from the soil surface and then collected two soil cores (2.5 cm in diameter and 10-cm deep) from each plot. The plot subsamples were composited, homogenized and air dried. Soils were assayed at the Ecosystems Analysis Laboratory at the University of Nebraska to determine carbon (%) and nitrogen (%) using dry combustion GC analysis (Costech ESC 4010 Elemental Analyzer; Costech Analytical Technologies). Extractable soil phosphorus and potassium and soil pH were assayed at A&L Analytical Laboratories. Soil pH was measured using a 1:1 soil-to-water slurry.

Nutrient addition and herbivore exclusion experiment. The vertebrate herbivore exclusion treatment was established by fencing two plots within each of the blocks. We designed the fences to exclude large aboveground mammalian herbivores, including ungulates, across a diverse range of grasslands characterized by different herbivores²³. At most sites, the height of the fences was 180 cm, and the fence design included wire mesh (1-cm holes) across the first 90 cm in addition to a 30-cm outward-facing flange stapled to the ground to exclude burrowing animals; climbing and subterranean animals could potentially have accessed these plots.

Cover sampling within treatment plots. At peak biomass, species areal cover was visually estimated using a modified Daubenmire method³⁹, where cover was estimated to the nearest 1% within a 1-m² subplot in each plot. Cover was estimated independently for each species, so the total summed cover may have exceeded 100% for multilayer canopies. In the year when leaf traits were measured at each site (usually after three years of treatment), we used the cover data to identify the top three to five species (although the eight most dominant species were sampled at one site) in each plot to measure leaf traits. We chose to identify the most dominant species in each plot rather than across each site because we wanted to capture the full range of spatial variation in composition and responses to the treatments, including species turnover.

Leaf trait collection and trait analyses. For each species selected for leaf trait analysis in each plot, we randomly selected five fully developed leaves with little to no signs of herbivore damage from five mature individuals. Sampling followed the standardized protocols detailed by Cornelissen et al.²⁴. All leaves from each species in each plot were combined to measure leaf area. Depending on the resources available at each site, leaf area (mm²) was measured using various leaf area meters or using a flatbed scanner (Epson perfection V300) and the image analysis software ImageJ⁴⁰. Thereafter, all leaves were dried at 60 °C for 48 h and then weighed (dry weight; g). SLA was calculated as the leaf area divided by the dry weight. SLA was calculated for all five leaves collected from each species in each plot at every site.

Dried leaves were then ground, bulked per plot and per species, and analysed for leaf nutrient concentrations. The leaf nitrogen content was determined using a LECO TruMac, which is based on a combustion technique that uses thermal conductivity relative to pure gas. This is considered accurate to within 1%. The leaf potassium and phosphorus concentrations were determined using laser ablation inductively coupled plasma mass spectrometry after Duodu et al.⁴¹ with the following exceptions: the internal standard was not added, but carbon was measured; the most abundant naturally occurring element was used; and no extra pulverizing was performed beyond that required for carbon and nitrogen analysis, which consisted of placing a sample and a 2-mm-diameter tungsten carbide ball inside 2-mm plastic centrifuge vials, followed by grinding for 15 min using a TissueLyser. Leaves (approximately 0.2 g) were compressed in a hydraulic dye, which produced a pellet approximately 5-mm across and 2-mm tall. These pellets were glued to a plastic tray in groups of ~100 and placed inside the laser chamber. A New Wave 193-nm excimer laser with a TrueLine cell was connected to an Agilent 8800 inductively coupled plasma mass spectrometer. The laser beam was 65 µm in diameter and rastered across a length of approximately 500 µm for approximately 50 s, 5 times per sample with a 30-s washout or background between rasters. The laser fluence at the laser exit was approximately 2 J cm⁻² and the repetition rate was 7 Hz. The reference material was National Institute of Standards and Technology National Bureau of Standards peach leaves⁴², and National Institute of Standards and Technology National Bureau of Standards spinach⁴³ was used as a monitoring standard; these were analysed every 3 samples (15 rasters) for moderately close sample-standard bracketing. The average and standard deviation of each element in each sample were calculated and reported following the method presented by Longerich et al.⁴⁴ using Iloite data reduction software⁴⁵.

Data analyses. *Hierarchical Bayesian multilevel regression models.* We developed multilevel regression models in a hierarchical Bayesian framework. All analyses were run using the integrated nested Laplace approximation (INLA)²⁵ interfaced

with the R statistical computing package (version 3.3.2)⁴⁶. Bayesian modelling requires the specification of prior information on parameters through probability distributions. For this analysis, vague priors were considered via normal distributions, specified as (mean, precision), for the fixed effects: intercept = (0,0) and slopes = (0,0.001) and the variances were modelled as log precisions with log gamma priors with shape and inverse-scale parameters of 1 and 5 × 10⁻⁵ respectively. The random effect structure was constructed to reflect the design of the experiment, and its structure was fixed for all models, regardless of whether each component explained a significant source of variability.

We ran separate models for each leaf trait (that is, SLA, and leaf nitrogen, phosphorus and potassium concentrations), where y_{ijkl} denoted the response, and $x_{jk} = (x_{1jk}, x_{2jk}, \dots, x_{pjk})$ denoted the i th observation from the j th block at the k th site of the l th plant species (Supplementary Fig. 1). SLA was log transformed to meet assumptions of normality. Models were constructed as follows:

$$y_{ijkl} \sim N(\mu_{ijkl}, \sigma^2),$$

where $y_{ijkl} = \mu_{ijkl} + u_i + v_{kl} + w_{jkl} + e_{ijkl}$

$$\mu_{ijkl} = \beta_0 + \beta_1 x_{1jk} + \beta_2 x_{2jk} + \dots + \beta_p x_{pjk},$$

$$u_i \sim N(0, \sigma_u^2),$$

$$v_{kl} \sim N(0, \sigma_v^2),$$

$$w_{jkl} \sim N(0, \sigma_w^2) \text{ and}$$

$$e_{ijkl} \sim N(0, \sigma_e^2) \text{ such that } \sigma_u^2 + \sigma_v^2 + \sigma_w^2 + \sigma_e^2 = \sigma^2$$

where μ_{ijkl} is the fixed effects associated with species l and block j at site k , β_0 is an estimate of the model intercept, and β_p represents the slope estimates for each linear predictor (that is, x_{pjk}). In addition, u_i is the random effect associated with the i th species, v_{kl} is the random effect associated with the k th site (within species l), w_{jkl} is the random effect associated with the j th block (within species l and site k) and e_{ijkl} is the residual error associated with the i th response of block j at site k for species l .

Once a model was fit, residual plots were inspected for any potential relationships in the data that may not have been captured by the model (residuals were calculated as the observed value of the data minus the posterior mean prediction). Plots of the cross-validated probability integral transform (PIT)⁴⁷ for each model were also inspected. PIT values provide estimates of the probability that the prediction is less than or equal to the corresponding observed data point, conditional on all other data. A histogram and normal quantile–quantile plot of these values were used to assess the calibration of out-of-sample predictions⁴⁸. If the residual and PIT plots were reasonable, it was concluded that the model provided a satisfactory fit to the data.

Structural equation models. We began with an initial meta-model (Supplementary Fig. 3) based on a priori expert knowledge and the literature. To correct for the nested experimental design, we included a stratified independent design with blocks nested within sites as stratified variables. We used modification indices⁴⁹ to standardize our decisions of adding missing paths to the model. We used the ‘modindices’ function in the lavaan package⁴⁹, which provides a list of all missing path regressions between two variables in the model, as well as the expected effect of the addition on the model-data fit (chi-squared value). We used the modification indices in a stepwise approach, adding ecologically sound paths one at a time, until no modification indices were higher than 2. This incremental process led to the creation of 18 different models. We then scanned path regressions and pruned all non-significant ones (based on $P < 0.05$), generating a final nineteenth model. Among the 19 competing models, 13 had a significant model-data fit (estimated by maximum likelihood⁴⁹). To optimize the information–parsimony trade-off, we compared those 13 models using the Akaike information criterion⁵⁰.

The selected best model had a small-sample-size-corrected AIC difference > 5 with respect to the closest model, and a small-sample-size-corrected AIC weight of 0.77. To correct for the nested experimental design, we included a stratified independent design with blocks nested within sites as stratified variables. Using the lavaan.survey package, we extracted a robust test statistic (pseudo-maximum likelihood = 23.35; 32 model d.f.; $P = 0.867$), indicating a good model-data fit. All analyses were run using R version 3.3.2.

Data availability

The data that support the findings of this study are available in the Dryad Digital Repository with the identifier <https://doi.org/10.5061/dryad.qp25093>.

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Author contributions

A.C.R., E.H., J.F., M.Sc., S.M.P. and Y.M.B. developed and framed the research question(s). E.H., H.F., J.F. and J.M. analysed the data. A.C.R., A.M.M., C.A., E.L., E.P. K.H.M. and M.Sc. contributed to the data analysis. J.F. wrote the manuscript with contributions from all other authors. A.C.R., A.E., A.M.M., A.R.K., C.A.A., C.J.S., C.N., C.R., C.S.B., E.B., E.C., E.S., J.D.B., J.F., J.L.M., J.W., J.W.M., K.J.L.P., L.B., L.S., M.C.C., M.N.B., M.Sc., M.Sm., N.E., N.H., P.A.F., P.B.A., P.D.W., P.L.P., R.M., S.M.P., W.S.H., Y.H. and Y.M.B. are site coordinators. E.S., E.B., M.Sm. and W.S.H. are Nutrient Network coordinators.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to J.F.