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

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Abstract

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 four continents. We find specific leaf area (SLA; leaf area per unit mass), a commonly measured morphological trait to infer shifts between plant growth strategies, did not respond to up to four years of soil nutrient additions. Leaf nitrogen, phosphorus and potassium concentrations did increase 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 interspecific trait variation was a significant predictor of leaf nitrogen and potassium, but not of leaf phosphorus concentration. Climatic conditions and pre-treatment soil nutrient levels also accounted for significant amounts of variation in the leaf traits measured. Overall, we find that leaf morphological traits such as SLA are not appropriate indicators of plant response to anthropogenic perturbations in grasslands.

Text: Biodiversity loss is accelerating at an alarming rate, particularly in grasslands due to eutrophication linked to agricultural intensification and industrial pollution[1], and altered trophic level interactions such as reduced consumption by native hervivores[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 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

97 part of the 'Holy Grail'[6,10] set of traits, to predict plant-animal interactions[11],
98 community composition and ecosystem function in response to perturbations[12].
99 Ecology's focus on leaf traits is based on strong eco-physiological evidence
100 that leaves represent important investment strategies for plant growth and survival.
101 Plants invest photosynthate and mineral nutrients in the construction of leaves, which
102 capture light to produce more photosynthate[13,14]. Leaf traits such as specific leaf area
103 (SLA) and leaf nutrient concentrations are typically used as comparative measures of
104 how plants capitalize on these investments. SLA, measured as leaf area per unit mass,
105 represents a trade-off between surface area for capturing photons and thickness
106 related to structural adaptations for water conservation and herbivore defence.
107 Indeed, leaf traits correlate across a continuum of fast to slow returns-on-investment,
108 known as the leaf economic spectrum (LES)[14-16].
109 Fast-growing species, which are adept at resource acquisition and tend to
110 dominate in regions with high rainfall levels and soils where resource availability is
111 not limiting, are hypothesized to have higher SLAs and leaf nutrient
112 concentrations[10,17]. High SLA is associated with lower costs of leaf construction, and
113 higher rates of herbivory as tissue becomes more palatable[6]. Additionally, higher
114 species turnover and palatability are also positively correlated with leaf nitrogen (N),
115 phosphorus (P), and potassium (K) concentrations[14-16]. By contrast, slower-growing
116 species, which exhibit resource conservation, are hypothesized to have lower SLAs
117 and leaf nutrient concentrations[14-17]. As a result, slow-growing species are less
118 palatable to herbivores, while having a longer leaf life span.
119 Trade-offs between leaf traits discovered in the LES were shaped over
120 evolutionary timeframes as successful trait combinations are selected for and
121 unfavourable combinations are selected against. LES relationships were built from

122 comparative relationships among leaves collected across biomes ranging from tundra
123 to tropical forests[14]. However, the extent to which rapid changes in structuring forces
124 such as soil nutrient availability and reduced herbivory result in predictable shifts in
125 trait values within a biome, like grasslands, remains equivocal[6]. Indeed, in agriculture
126 the growth-dilution effect postulates that leaf nutrient concentrations may not increase
127 in response to fertiliser because increased plant growth outpaces nutrient
128 accumulation in tissue[18]

129 SLA and leaf nutrient concentrations are commonly used as surrogate
130 measures of broad-scale biogeographical differences[12]. However, leaf trait responses
131 of individual species are also influenced by short-term local-scale abiotic and biotic
132 factors. Climatic and edaphic conditions interact with fertilization and changes in
133 natural disturbance regimes to sculpt community composition and ultimately
134 ecosystem functioning[5,10,11,19,20]. Given the complex sets of interactions that may
135 explain leaf trait responses to short-term environmental change, a modelling approach
136 is necessary to discern interactions that may otherwise be missed when using
137 traditional bi-variate analyses[21,22].

138 In a global experimental test, we quantified how leaf traits in grasslands
139 change in response to the addition of soil nutrients (i.e., N, P and K) and the exclusion
140 of vertebrate herbivores. We sampled leaf traits from the Nutrient Network (NutNet)[23]
141 cross-continental distributed experiment established at 27 sites (Fig. 1, Supplementary
142 Table 1). This experimental network allowed us to test how commonly measured leaf
143 traits respond to environmental change across grasslands. At the majority of sites, we
144 sampled leaf traits after three to four years of treatment (five sites after two years and
145 22 of the 27 sites after three to four years; see Supplementary Table 1 for detailed
146 information on each site).

147 At each site, three blocks of ten 5 m x 5 m plots were established, and two
148 experiments initiated: 1) a full factorial nutrient addition experiment, including the
149 addition of all factorial combinations of N, P and $K_{+\mu}$, where the subscript +μ refers to
150 the inclusion of ten other micronutrients in the first application year as part of the K
151 addition treatment (see Borer et al.[23] and Methods for more detail), and 2) a
152 combination full nutrient addition ($NPK_{+\mu}$ addition) and herbivore exclusion
153 experiment where fences were built to exclude vertebrate herbivores that were larger
154 in weight than 50 g (for more details see Methods).
155 Relative cover was visually estimated before the experiment began and prior
156 to the leaf harvest period, when leaf traits were collected from the three to five most
157 dominant species in each plot. Overall, 243 species were sampled across the 27 sites,
158 including grasses, forbs and legumes, and 2664 leaf samples were measured for leaf
159 area, leaf dry weight, and leaf N, P and K concentrations[24]. Overall the sampled
160 species accounted for 26% of the total vegetation cover at the time when leaves were
161 collected. The effect sizes of the mean leaf trait values for all species in response to
162 the experimental treatments were estimated using multilevel regression models in a
163 hierarchical Bayesian framework using integrated nested Laplace approximation[25],
164 where the random effect structure included block nested in site nested in species. SLA
165 values were log-transformed to meet assumptions of normality in the multilevel
166 regression model.

167 **Results and discussion**

168 We found that SLA did not increase consistently with the treatments. We did,
169 however, find evidence of a small but significant increase in SLA in the NP (mean
170 log(SLA) = 8.79 mm^2/g) and NPK fertiliser treatments (mean log(SLA) = 8.81
171 mm^2/g) compared to the control (mean log(SLA) = 8.69 mm^2/g), suggesting

simultaneous increases in availability of N and P 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 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 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.

N, P and K leaf concentrations increased significantly when the corresponding nutrients were applied as fertiliser (Fig. 2). Previous NutNet studies have found multiple-nutrient constraints on aboveground net primary production, including increased vegetation cover and biomass[29]. Leaf N concentration also increased in leaves with $PK_{+\mu}$ fertilization (Fig. 2b), a likely reflection of the increased availability of N in soils[30] and the importance of other nutrient limitations for increasing plant N uptake. Leaf P showed the opposite trend to leaf N and decreased in concentration when either N or $NK_{+\mu}$ were applied as fertiliser (Fig. 2c). This trend likely reflects the limited availability of phosphate to plants, because of its high affinity to soil particles[31], as otherwise we may have found an increase in Leaf P when limitations were lifted by the addition of other essential nutrients[26]. Leaf K concentration showed the highest variation associated with ‘species’ random effects (~60%, Fig. 3). The

196 fencing treatment did not significantly alter leaf nutrient concentrations only when
197 soil nutrient addition was combined with the fencing treatment (Fig. 2).
198 Our findings of an increase in leaf nutrient concentrations in response to the
199 fertiliser treatments could be explained by intraspecific trait variation (increases
200 shown by the same species over time) and by interspecific changes in dominant
201 species following the application of treatments. After treatment initiation, changes in
202 dominant species were observed at some study sites, whereas little change was
203 observed at other sites. This difference is important because increases in leaf nutrient
204 concentrations could be explained by two mechanisms: 1. current species increase
205 their uptake of nutrients (i.e. intraspecific trait variation)[32] and 2. new species are
206 recruited into the dominant class (i.e. interspecific trait variation) as the increased
207 nutrient availability favours their growth and establishment[33]. Therefore, we evaluated
208 the effects of temporal species turnover on leaf trait responses. We estimated
209 temporal species turnover using Bray Curtis dissimilarity for the three to five most
210 dominant species in each plot comparing pretreatment species composition with
211 composition when the leaf traits were measured, two to four years later.
212 Given the global extent of our study sites and the high amounts of variation in
213 leaf traits found at the site level, particularly for SLA (Fig. 3), we also evaluated the
214 effects of climatic conditions and pre-treatment soil nutrient levels. We used
215 structural equation models to examine the influence of these additional possible
216 drivers (see supplementary material for details on model development including
217 Supplementary Fig. 1 to 3). Because we did not find evidence of a leaf trait response
218 to the fencing treatments, we did not further evaluate these treatments, only the
219 nutrient addition treatments. Overall, the R^2 values for each of the leaf nutrient trait
220 response variables were high, indicating a strong explanatory power of the models;

leaf K had the highest R^2 value and SLA the lowest (leaf N, R^2= 0.53; leaf P, R^2= 0.32; leaf K, R^2= 0.55; SLA, R^2 = 0.11).

All leaf traits varied with climatic and edaphic conditions (Fig. 4 and Supplementary Fig. 4). 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 considerable amounts of variation in leaf chemical traits are explained by intraspecific variation[32]. The duration of the nutrient addition treatments (represented as year in Fig 4 and Supplementary Fig. 4) was also positively correlated with species temporal turnover, suggesting that sites with longer treatment durations had higher species turnover. Co-variances among the leaf nutrient contents were high in the structural equation model, but SLA showed the lowest co-variation 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 ecosystems[10], 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 disturbances[6]. 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 nutrients than in morphological traits such as SLA[32]. Based on these findings, if species composition within treatment plots continues to turn over, we may find a clearer response in SLA.

Contrary to 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 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 consistent increase in SLA and leaf nutrient concentrations with the exclusion of vertebrate herbivores focused on the impacts of cattle and sheep[5,35-37], whose grazing pressure tends to be higher and known for selectivity of plant tissue for increased palatability and nutrition[38]. Here, only eight 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[11,27,39]; where 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

271 indicator of plant resource utilization strategies at biogeographical-scales [19]. However,
272 a global-scale experimental test demonstrated that SLA is not a consistent indicator of
273 the short-term response of plants to increased soil nutrients or the exclusion of
274 vertebrate herbivores.

275 Broad-scale biogeographical trait relationships, such as the worldwide leaf
276 economic spectrum[14], do not necessarily correlate as plant functional responses to
277 short-term disturbance and changing abiotic conditions. Our results show that changes
278 in individual traits, in the same species or because of species turnover, do not
279 necessarily represent a 'common currency' for comparing ecosystem-level responses
280 in grasslands to anthropogenic perturbations. When it comes to dominant plant
281 species, leaf nutrients are responsive to elevated soil nutrients, even across sites
282 characterized by very different climatic and edaphic conditions, and are potentially
283 more consistent plant functional response traits than SLA, particularly in the short-
284 term.

285 **Methods**

286 *Network of experimental sites*

287 The 27 study sites are part of the Nutrient Network, a cooperative globally
288 distributed experiment (Fig. 1 and Table S1 in Supporting Information,
289 http://www.nutnet.org/). Each experimental site had a randomized block design, and
290 at most sites, three replicate blocks divided of ten 5 m x 5 m plots were established,
291 resulting in a total of 30 plots per site.

292 We quantified climatic variables (mean annual temperature, mean annual
293 precipitation, temperature variation which is a measure of seasonality (calculate as the
294 standard deviation * 100), precipitation variation which is a measure of seasonality
295 (calculated as the coefficient of variation) for each site using modelled values sourced

296 from the WorldClim Global Climate database (version 1.4;
297 http://www.worldclim.org). The sites included in this study represented a wide range
298 of climatic conditions with mean annual temperatures ranging from 0.3 ºC (alpine
299 grassland in Switzerland) to 18.4 ºC (semi-arid C_4 perennial grassland in Australia)
300 and mean annual precipitation ranging from 262 mm (shrub steppe in the USA) to
301 1898 mm (montane grassland in the USA).
302 *Nutrient addition experiment*
303 In this experiment, we established a set of nutrient addition treatments that
304 included a full factorial combination of three essential plant macronutrients (N, P,
305 $K_{+\mu}$), including a control. The following rates of nutrients, obtained from the same
306 chemical sources, were applied at all sites: 10 g N m^{-2} yr^{-1} as timed-release urea, 10 g
307 P m^{-2} yr^{-1} as triple super phosphate, and 10 g K m^{-2} yr^{-1} as potassium sulphate plus a
308 once-off addition (100 g m^{-2} yr^{-1}) of macro- and micro-nutrients (i.e., Fe, S, Mg, Mn,
309 Cu, Zn, B, Mo, Ca). At all sites, N, P, and K fertilisers were applied annually,
310 whereas micro-nutrients were applied once at the start of the study to avoid toxicity
311 and only in treatments that included K. Sites entered the NutNet in different years
312 (2007-2014) and usually measured leaf traits after 3-4 years of nutrient addition
313 (Table S2). Note that ammonium nitrate was used in 2007 at some sites before
314 switching to urea because of increasing difficulty in sourcing ammonium nitrate
315 globally. At a subset of these sites, we tested whether this one-year addition of
316 ammonium nitrate would influence the outcomes of the plant community responses
317 and found no significant effect of nitrogen source[23].
318 To quantify soil nutrients during the pre-treatment year, we first removed the
319 litter and vegetation from the soil surface and then collected two soil cores (2.5 cm in
320 diameter and 10 cm deep) from each plot. The plot subsamples were composited,

homogenized, and air-dried. The Ecosystems Analysis Laboratory at the University of Nebraska assayed the soils to determine C (%) and N (%) using dry combustion GC analysis (COSTECH ESC 4010 Elemental Analyzer, Costech Analytical Technologies, Valencia, California, USA). Extractable soil P and K and soil pH were assayed at A&L Analytical Laboratory (Memphis, TN). 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 herbivores[23]. 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 method[40], where cover is estimated to the nearest 1% within one 1-m^2 sub-plot 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

345 because we wanted to capture the full range of spatial variation in composition and
346 responses to the treatments, including species turnover.

347 *Leaf trait collection and trait analyses*

348 For each species selected for leaf trait analysis in each plot, we randomly
349 selected five fully developed leaves with little to no signs of herbivore damage from
350 five mature individuals. Sampling followed the standardized protocols detailed by
351 Cornelissen et al.[24]. All leaves from each species in each plot were combined to
352 measure leaf area. Depending on the resources available at each site, leaf area (mm^2)
353 was measured using various leaf area meters or using a flatbed scanner (Epson
354 perfection V300) and image analysis software ImageJ; [41]. Thereafter, all leaves were
355 dried at 60 ºC for 48 h and then weighed (dry weight; g). SLA was calculated as leaf
356 area divided by dry weight. SLA was calculated for all five leaves collected from each
357 species in each plot at every site.

358 Dried leaves were then ground, bulked per plot and per species and analysed for
359 leaf nutrient concentrations. The leaf nitrogen content was determined using a LECO
360 TruMac, which is based on a combustion technique that uses thermal conductivity
361 relative to pure gas; the leaf nitrogen content is determined and is considered accurate
362 to within 1%. The leaf potassium, and phosphorus concentrations were determined
363 using laser ablation ICPMS after Duodu et al.[42] with the following exceptions: the
364 internal standard was not added but was measured C, the most abundant naturally
365 occurring element was used, and no extra pulverizing was performed beyond that
366 required for C and N analysis, which consisted of placing a sample and a 2-mm-
367 diameter tungsten carbide ball inside 2-mm plastic centrifuge vials, followed by
368 grinding for 15 min using a TissueLyser©. Leaves (approximately 0.2 g) were
369 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 were placed inside the laser chamber. A New Wave 193-nm excimer laser with a True-line cell was connected to an Agilent 8800 ICPMS. The laser beam was 65 microns in diameter and was rastered across a length of approximately 500 microns for approximately 50 seconds, five times per sample with a 30-second 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 NIST NBS peach leaves[43], and NIST NBS spinach[44] was used as a monitoring standard; these were analysed every three samples (15 rasters) for moderately close sample-standard bracketing. The average and standard deviation of each element in each sample were calculated and reported after the method presented by Longerich et al.[45] using Iloite data reduction software.[46]

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 (v. 3.3.2) [47]. The default priors in INLA were used for all analyses, which included the normal distribution specified as N (mean, precision), fixed effects: intercept = N (0,0), slopes = N (0,0.001), and variances modelled as log-precision with priors of log-gamma (1, 5e-5), which was specified as log-gamma (shape, inverse-scale). 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 of leaf trait (i.e., specific leaf area, leaf N, P and K concentrations), where y_{ijkl} denoted the response, and $\boldsymbol{x}_{jk} = (x_{1jk}, x_{2jk},..., x_{pjk})$ denoted the *i*th observation from the *j*th block at the *k*th site of the *l*th plant species (Fig. M1). Specific leaf area was log transformed to meet assumptions of normality. Models were constructed as follows:

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

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

$$\mu_{jkl} = \beta_0 + \beta_1 x_{1jk} + \beta_2 x_{2jkl} + ... + \beta_p x_{pjkl},$$

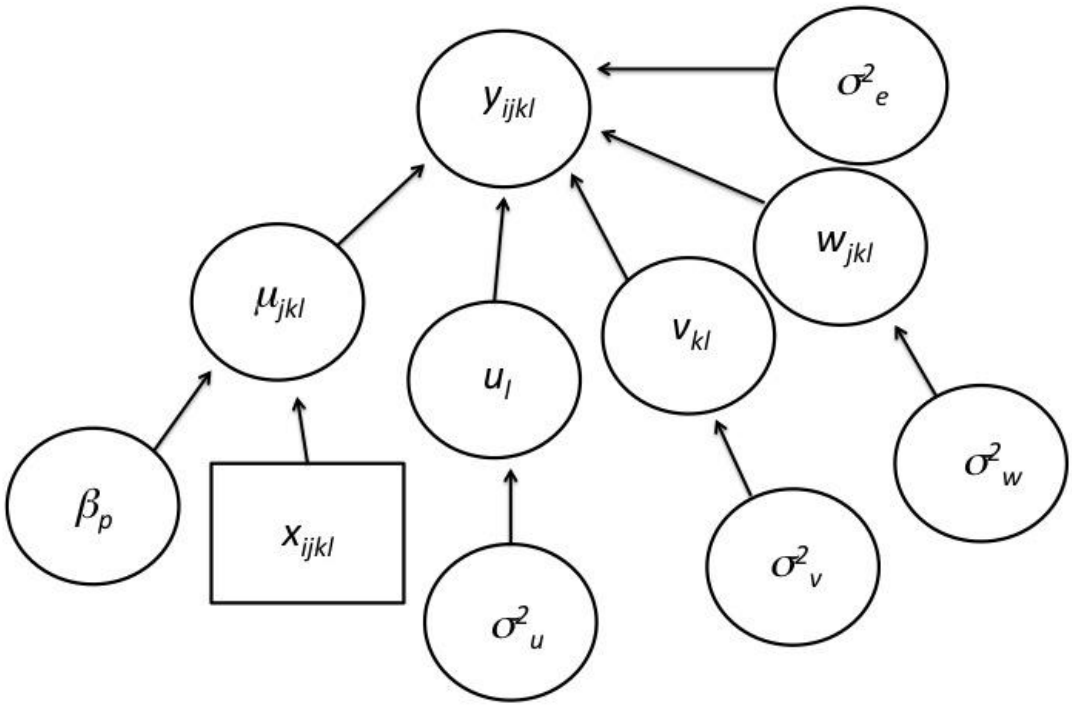
$$u_l \sim N(0, \sigma^2_u),$$

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

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

$$e_{ijkl} \sim N(0, \sigma^2_e) \text{ such that } \sigma^2_u + \sigma^2_v + \sigma^2_w + \sigma^2_e = \sigma^2,$$

where μ_{jkl} 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, i.e., x_{pjkl}. In addition, u_l is the random effect associated with the *l*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*.



412

413 Fig. M1: Directed acyclic graph (DAG) used to represent the multilevel regression
414 models in a hierarchical Bayesian framework for the overall model networks that
415 were developed for both the nutrient addition experiment, and the nutrient addition
416 and herbivore exclusion experiment.

417 Once a model was fit, residual plots were inspected for any potential
418 relationships in the data that may not have been captured by the model (residuals were
419 calculated as the observed value of the data minus the posterior mean prediction).
420 Plots of the cross-validated probability integral transform (PIT[48]) for each model were
421 also inspected. PIT values provide estimates of the probability that the prediction is
422 less than or equal to the corresponding observed data point, conditional on all other
423 data. A histogram and normal quantile-quantile plot of these values were used to
424 assess the calibration of out-of-sample predictions[49]. If the residual and PIT plots
425 were reasonable, then it was concluded that the model provided a satisfactory fit to
426 the data.

Structural equation models

We began with an initial meta-model (Supplementary Fig. 2) 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 indices[50] to standardize our decisions of adding missing paths to the model. We used the "modindices" function in the lavaan package[50], 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-square 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 19th model. Among the 19 competing models, 13 had a significant model-data fit (estimated by maximum likelihood[50]). To optimize the information-parsimony trade-off, we compared those 13 models using the Akaike information criterion[51].

The selected best model had an AICc difference > 5 with respect to the closest model and an AICc 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 degrees of freedom, and P = 0.867), indicating a good model-data fit. All analyses were run using R 3.3.2.

Data availability: The data that support the findings of this study are available from the corresponding author upon request.

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Competing interests

The authors declare no competing financial interests.

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