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

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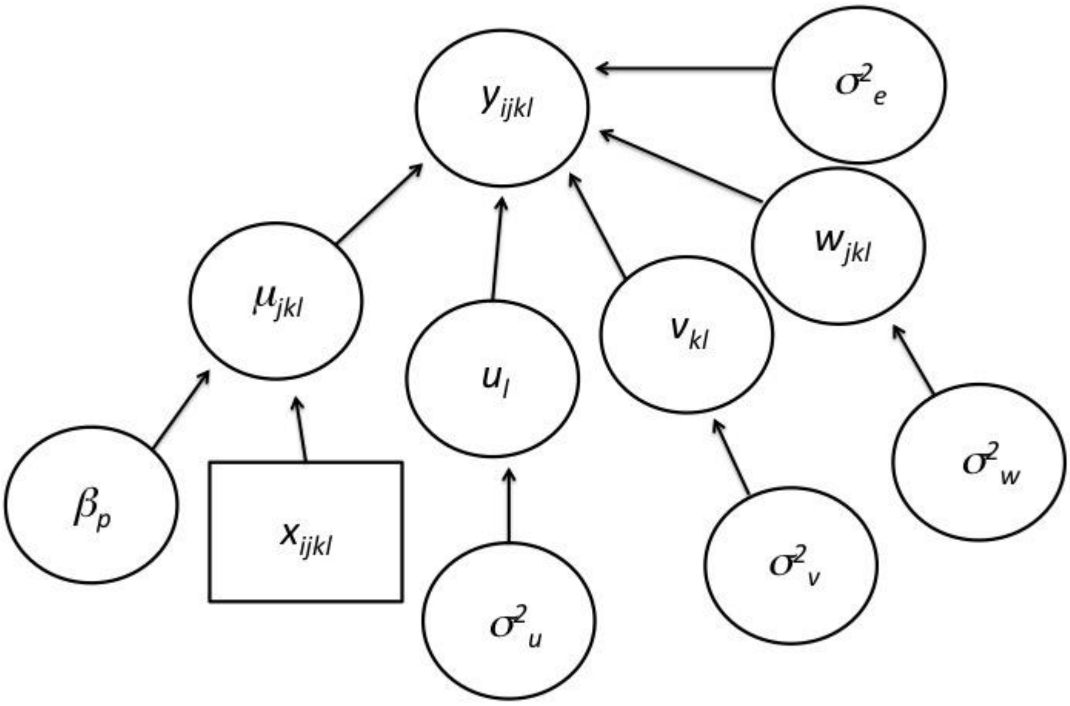

Fig. 1: Directed acyclic graph (DAG) used to represent the multilevel regression models in a hierarchical Bayesian framework for the overall model networks that were developed for both the nutrient addition experiment, and the nutrient addition and herbivore exclusion experiment.

Structural Equation Modelling

Description of the model processes stages

The difficulty in building meaningful meta-models increases with the number of predictors involved because the number of potential links among variables increases exponentially. As a consequence, drawing a causal link between any two variables can have implications that are challenging to predict based on *a priori* knowledge (e.g., indirect effects). To reduce this level of complexity, we separated our predictors into two layers; the first one representing the experimental treatments, which are the core of the present study, and the second one representing external abiotic factors related to initial edaphic conditions, temperature and precipitation (see Methods section of the main text). We first built a meta-model that included effects from only the experimental treatments (see Supplementary Fig. 2) that we tested using structural equation modelling, and then as a second step, from the knowledge gained from the first step, we built a second meta-model that integrated the effects of external abiotic factors (see Supplementary Fig. 3). This sequential approach allowed us to gain sufficient insight into the system to reach a level of confidence and complexity in the final model that would otherwise have been difficult to achieve.

In both SEM analytical steps, we started with the relevant initial meta-model and used modification indices to standardize our decisions of adding missing paths to the model. We used the "modindices" function in the lavaan package, 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)[1]. We used the modification indices in a stepwise approach, adding only one path at a time, until no modification indices were higher than 2. Modification indices can be constructed between any two variables in the model, and thus we only added a suggested path when it made ecological sense to do so (e.g., a path suggesting that nitrogen addition is caused by leaf nutrient concentration is not a sensible consideration ecologically). Once this incremental approach was finished, we scanned the path regressions and pruned all non-significant ones (based on $p < 0.05$), generating a final more parsimonious candidate model. We then compared all candidate models using the Akaike information criterion[2]. This general approach ensured that, starting from the simplified meta-model, any important paths (i.e., with modification indices higher than two) between two variables would be considered and that the final selected model would represent a satisfactory information-parsimony trade-off.

For all models, we corrected for the nested experimental design by including a stratified independent design with blocks nested within sites as stratified variables. Using the lavaan.survey package[1], we extracted a robust test statistic, the pseudo maximum likelihood (PML), for each model[1].

Initial step – experimental treatments only

Our initial meta-model was built based on expectations from the experimental treatments (Supplementary Fig. 2), because of the results found using the Bayesian multilevel regressions. We predicted that nutrient additions would affect the leaf nutrient concentrations and SLA directly, showing evidence of plasticity in trait expression, or through an effect on temporal species turnover, suggesting that community-level processes dominate observed effects on leaf traits ([3-6] and see Supplementary Fig. 2). Temporal turnover was calculated as the Bray-Curtis dissimilarity in each plot at each site between time t0 and time x+n, which corresponded to the time of the leaf trait measurement.

We started with the meta-model (Supplementary Fig. 3) and followed the incremental process outlined above, which led to the creation of 3 candidate models, from which we identified the best model with an AICc difference >13 compared with the closest model and an AICc weight of 1 (Supplementary Fig. 3). The selected model showed a very good model-data fit (PML = 5.75, 15 model degrees of freedom and $p = 0.98$). The model showed positive effects of each soil nutrient addition on the leaf nutrient concentrations, while only phosphorus affected plant species temporal turnover. It is noteworthy that none of the treatments had detectable impacts on SLA (Supplementary Fig. 4).

Final step – integration with external abiotic predictors

Based on the insights gained during the initial step when determining the effects of the experimental treatments on the leaf traits, we built a final meta-model and integrated the effects of external abiotic factors (Supplementary Fig. 3). In this model, we assumed that if SLA was not affected by the experimental treatments then it was likely more sensitive to external abiotic factors (Supplementary Fig. 3). We also assumed that the initial soil nutrient content would affect the leaf nutrient concentrations and that temperature- and precipitation-related variables would likely influence leaf nutrients via an effect on plant species turnover (Supplementary Fig. 3). This latter assumption is a simplification that allowed us to build a final meta-model that was not saturated, while integrating all predictors. Given our general approach with the modification indices, we believe that it is more appropriate to start with a simplified model, assuming that all important paths (i.e., modification indices higher than 2) will be identified during the incremental process rather than starting with a saturated model where there is no space for path addition and where we have to make *ad hoc* decisions on which path to remove. The selected best model had an AICc difference >5 with respect to the closest model and an AICc weight of 0.77. Using the lavaan.survey package, we extracted a robust test statistic (PML = 23.35, 32 model degrees of freedom, and P = 0.867), indicating a good model-data fit. The results from the incremental process starting with the meta-model shown in Supplementary Fig. 3 are presented in Supplementary Fig. 5 and in the Results section of the main text in Fig. 4.

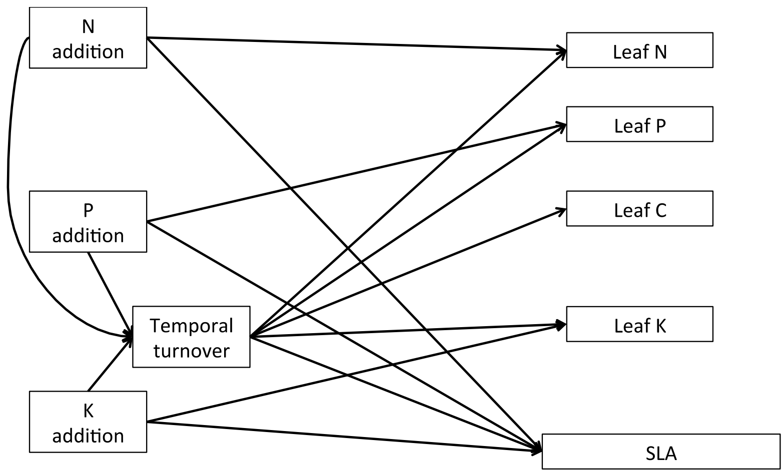

Fig. 2. Meta-model including only effects from the experimental treatments.

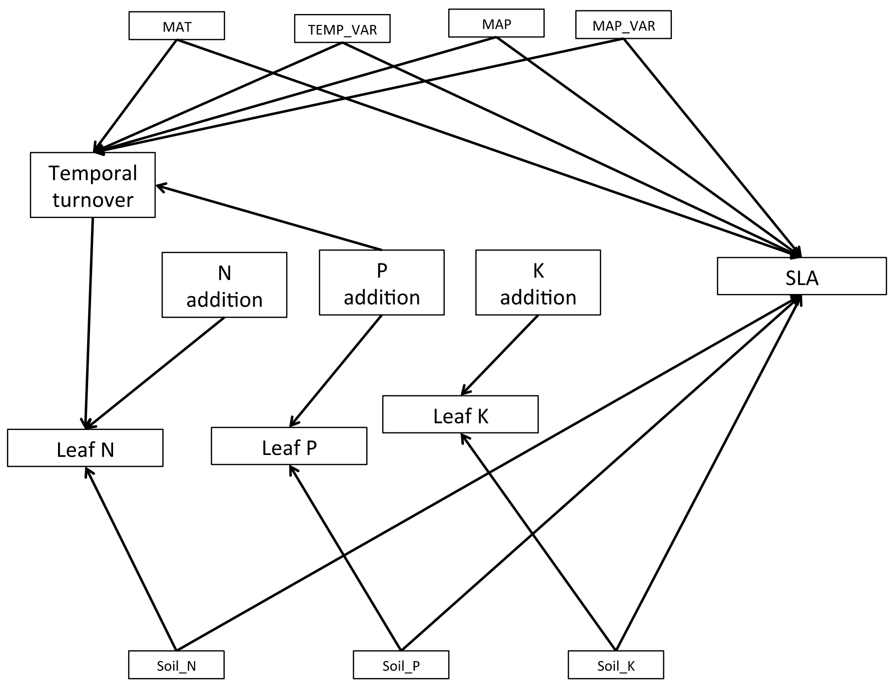

Fig. 3. Meta-model including effects from both the experimental treatments and external abiotic factors. MAT: mean annual temperature, TEMP_VAR: annual variation in temperature, MAP: mean annual precipitation, MAP_VAR: annual

variation in precipitation, soil_N: initial soil nitrogen content, soil_P: initial soil phosphorus content, soil_K: initial soil potassium content.

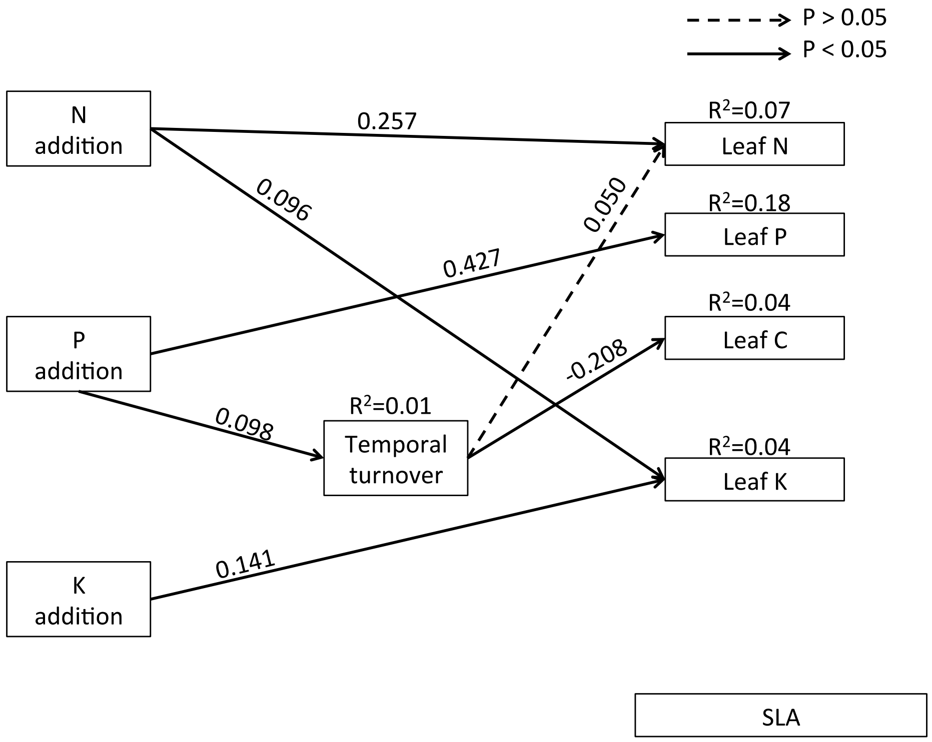

Fig. 4. Final model from the initial step including experimental treatments only. Path values are standardized coefficients.

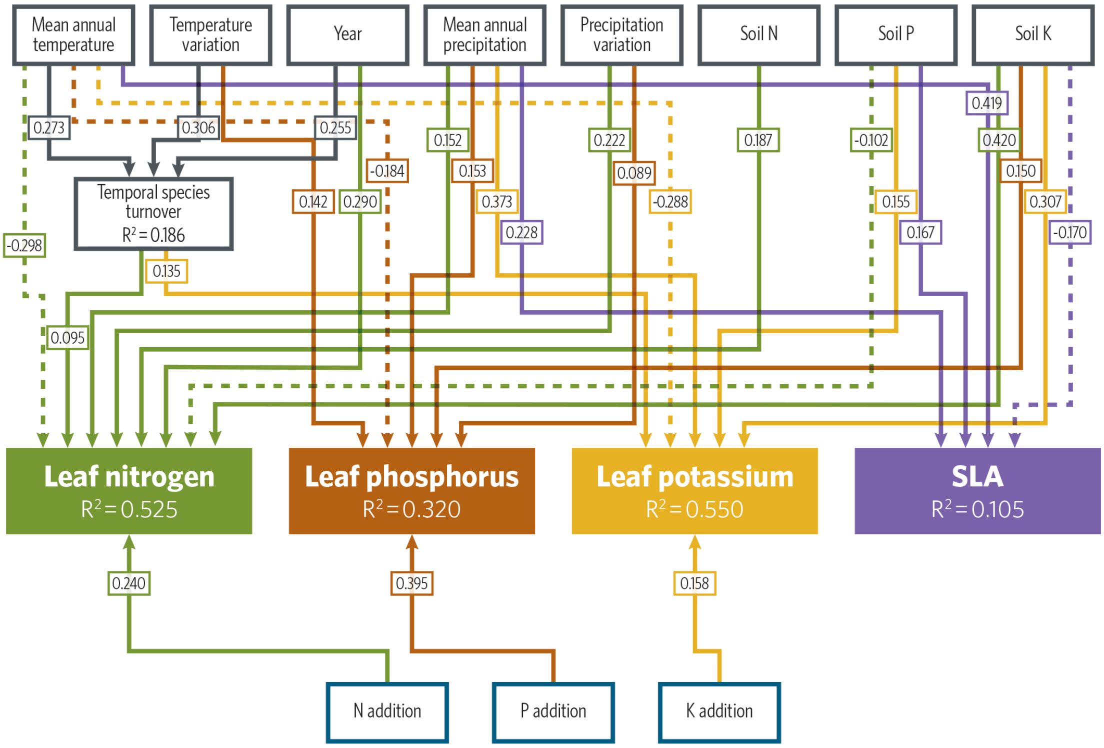

Fig. 5. Structural equation model diagram representing connections between leaf traits, experimental nutrient addition treatments, site-level average climatic and pre-treatment edaphic conditions, as well as species turnover. Values in boxes represent correlations and R^2 values. Only significant connections are shown. Diagram by Evidently So. Please follow this link to see an interactive visualisation of this figure: http://evidentlyso.com.au/clients/qut/functionalTraits0120/

Table 1

Description of the 27 sites including habitat type; latitude (from -90 [S] TO +90 [N] in decimal degrees); longitude (from -180 [W] to +180 [E)]in decimal degrees); experimental year when leaves were collected at each site, mean elevation (m); MAT (mean annual temperature, °C)**;** Temperature variation (temperature seasonality calculated as the standard deviation of temperature x 100); MAP (mean annual precipitation, mm); variation in mean annual precipitation (precipitation seasonality calculated as the coefficient of variation of precipitation); N (pre-treatment soil nitrogen in percent by mass); P (pre-treatment soil phosphorus in ppm); K (pre-treatment soil potassium in ppm); current domestic grazing (based on biomass consumed estimated qualitatively or comparing inside and outside of grazing exclosures); current ratio of native to exotic herbivores (all mammals); years since last domestic grazing at the time the experiment was established. ma-not available.

Site code	Country	Grassland type	Experimental year when leaves were collected	elevation	latitude	longitude	MAT	Temp. variation	MAP	MAP variation	Soil N (%)	Soil P (ppm)	Soil K (ppm)	Current Native: Exotic Herbivores	Years since llast domestic grazing
bldr.us	USA	shortgrass prairie	3	1633	39.9720	-105.2335	9.7	79.52	425	42	0.09	20	78	2:0	Never
bnch.us	USA	montane	4	1318	44.2766	-121.9680	5.5	60.55	1647	65	0.68	15	87	2:0	96
bogong.au	Australia	alpine	2	1760	-36.874	147.254	5.7	47.59	1592	26	0.51	71	211	0:4	NA
burrawan.au	Australia	semiarid	3	425	-27.7348	151.1395	18.4	50.49	683	36	0.11	35	70	4:2	0
cbgb.us	USA	tallgrass prairie	3	275	41.7850	-93.3853	9	108.46	855	46	0.06	72	95	3:0	20
comp.pt	Portugal	annual	2	200	38	-8	16.5	49.77	554	61	0.14	52	74	1:0	1
cowi.ca	Canada	old field	4	50	48.46	-123.38	9.8	40.44	764	64	0.43	47	88	3:0	6
elliot.us	USA	annual	3	200	32.875	-117.052243	17.2	35.93	331	87	0.17	15	327	3:0	40

frue.ch	Switzerland	pasture	3	995	47.1131	8.5418	6.5	59.89	1355	23	0.44	57	161	3:0	2
gilb.za	South Africa	montane	2	1748	-29.2842	30.2917	13.1	34.19	926	67	1.26	17	108	3:0	Never
hopl.us	USA	annual	4	598	39.0127	-123.0603	12.3	52.78	1127	87	NA	NA	NA	2:0	23
jena.de	Germany	Mesic	2	320	50.9333	11.53333	8	62.51	610	27	0.55	155	1340	1:0	Never
kiny.au	Australia	semiarid	4	90	-36.2	143.75	15.5	49.26	426	21	0.09	9	315	1:1	2
konz.us	USA	tallgrass prairie	4	440	39.0708	-96.5828	11.9	99.32	877	50	NA	NA	NA	2:0	38
lancaster.uk	UK	mesic	3	180	53.9856	-2.6284	8	45.42	1322	23	1.55	21	90	2:0	0
look.us	USA	montane	4	1500	44.2051	-122.1284	4.8	58.66	1898	65	1.14	75	181	3:0	86
mcla.us	USA	annual	4	642	38.8642	-122.4064	13.5	59.94	867	88	NA	NA	NA	2:0	24
mtca.au	Australia	savanna	3	285	-31.7821	117.6108	17.3	52.55	330	55	NA	NA	NA	1:1	0, removed September 2015

sage.us	USA	montane	4	1920	39.43	-120.24	5.7	65.39	882	69	0.39	28	212	6:0	12
saline.us	USA	mixedgrass prairie	4	440	39.05	-99.1	11.8	100.33	607	53	NA	NA	NA	2:0	0
sgs.us	USA	shortgrass prairie	4	1650	40.8166	-104.7666	8.4	84.82	365	59	0.09	74	263	9:0	8
shps.us	USA	shrub steppe	4	910	44.2429	-112.1983	5.5	95.57	262	37	0.22	29	616	2:1	0
sier.us	USA	annual	4	197	39.2355	-121.2836	15.6	64.7	935	84	0.21	22	163	2:0	2
smith.us	USA	mesic	4	62	48.2065	-122.6247	9.8	42.14	597	36	0.57	64	195	1:1	unknown
summ.za	South Africa	mesic	2	679	-29.8116	30.7157	18.2	25.51	939	55	0.32	13	88	2:1	>14
unc.us	USA	old field	4	141	36.0082	-79.0204	14.6	76.18	1163	11	0.19	38	109	1:0	unknown
valm.ch	Germany	alpine	3	2320	46.6313	10.3722	0.3	54.23	1098	29	0.43			4:0	40

Table 2: Co-variances between leaf traits based on the structural equation model.

	Leaf P	Leaf K	SLA
Leaf N	0.401	0.341	0.192
Leaf P		0.462	0.224
Leaf K			0.153

Table 3. Details of author contributions. The rubric for authorship roles is shown at the end of this table

Co-author name	Institution	Developed and framed research question(s)	Analysed data	Contributed to data analyses	Wrote the paper	Contributed to paper writing	Site coordinator	Nutrient Network coordinator
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James McGree	Queensland University of Technology (QUT), Brisbane, QLD, Australia		x			x		
Eric Harvey	Department of Ecology and Evolutionary Biology, University Of Toronto, Toronto Ontario Canada M5S 3B2.	x	x			x		
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Martin Schütz	Swiss Federal Institute for Forest, Snow and Landscape Research,	x		x		x	x	

	Community Ecology							
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Elizabeth T. Borer	Dept. of Ecology, Evolution, and Behavior, University of MN, St. Paul, MN 55108					x	x	x
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Charlotte Allen	Queensland University of Technology (QUT), Brisbane, QLD, Australia			x		x		
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Justin Wright	Duke University					x	x	
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Rubric item	Example contribution meriting a checked box
Developed and framed research question(s)	Originated idea for current analysis of Nutrient Network data; contributed significantly to framing the ideas in this analysis at early stage of manuscript preparations
Analyzed data	Generated models (conceptual, statistical and/or mathematical), figures, tables, etc.
Contributed to data analyses	Provided comments, suggestions, and code for data analysis
Wrote the paper	Wrote the majority of at least one of the sections of the paper
Contributed to paper writing	Provided suggestions such as restructuring ideas, text, and citations linking to new literature areas, copy editing
Site level coordinator	Coordinated data collection, proofing, and submission of data for at least one site implementing the experiment
Nutrient Network Coordinators	Contributed substantially (i.e., more than 300 hours per year) to network level activities such as management of network data, recruiting and assisting new sites, finding funding for network level management activities.

References

1 Rosseel, Y. lavaan: An R package for structural equation modelling,. *Journal of Statistical Software* **48**, 1-36 (2012).

2 Burnham, K. P. & Anderson, D. R. *Model selection and mult-model inference: a practical information-theoretic* (Springer, 2002).

3 Funk, J. L. *et al.* Revisiting the Holy Grail: using plant functional traits to understand ecological processes. *Biological Reviews* **1153-1176**, 1-18 (2017).

4 Firn, J., Schuetz, M., Nguyen, H. & Risch, A. C. Herbivores sculpt leaf traits differently in grasslands depending on life form and land-use histories. *Ecology* **98**, 239-252 (2017).

5 Firn, J., Prober, S. M. & Buckley, Y. M. Plastic traits of an exotic grass contribute to its abundance but are not always favourable. *PLoS One* **7**, e35870 (2012).

6 Cingolani, A. M., Posse, G. & Collantes, M. B. Plant functional traits, herbivore selectivity and response to sheep grazing in Patagonian steppe grasslands. *Journal of Applied Ecology* **42**, 50-59 (2005).