

Eutrophication weakens stabilizing effects of diversity in natural grasslands

Yann Hautier^{1,2}, Eric W. Seabloom¹, Elizabeth T. Borer¹, Peter B. Adler³, W. Stanley Harpole⁴, Helmut Hillebrand⁵, Eric M. Lind¹, Andrew S. MacDougall⁶, Carly J. Stevens⁷, Jonathan D. Bakker⁸, Yvonne M. Buckley^{9,10}, Chengjin Chu¹¹, Scott L. Collins¹², Pedro Daleo¹³, Ellen I. Damschen¹⁴, Kendi F. Davies¹⁵, Philip A. Fay¹⁶, Jennifer Firm¹⁷, Daniel S. Gruner¹⁸, Virginia L. Jin¹⁹, Julia A. Klein²⁰, Johannes M. H. Knops²¹, Kimberly J. La Pierre²², Wei Li²³, Rebecca L. McCulley²⁴, Brett A. Melbourne¹⁵, Joslin L. Moore^{25,26}, Lydia R. O'Halloran²⁷, Suzanne M. Prober²⁸, Anita C. Risch²⁹, Mahesh Sankaran^{30,31}, Martin Schuetz²⁹ & Andy Hector³²

Studies of experimental grassland communities^{1–7} have demonstrated that plant diversity can stabilize productivity through species asynchrony, in which decreases in the biomass of some species are compensated for by increases in others^{1,2}. However, it remains unknown whether these findings are relevant to natural ecosystems, especially those for which species diversity is threatened by anthropogenic global change^{8–11}. Here we analyse diversity–stability relationships from 41 grasslands on five continents and examine how these relationships are affected by chronic fertilization, one of the strongest drivers of species loss globally⁸. Unmanipulated communities with more species had greater species asynchrony, resulting in more stable biomass production, generalizing a result from biodiversity experiments to real-world grasslands. However, fertilization weakened the positive effect of diversity on stability. Contrary to expectations, this was not due to species loss after eutrophication but rather to an increase in the temporal variation of productivity in combination with a decrease in species asynchrony in diverse communities. Our results demonstrate separate and synergistic effects of diversity and eutrophication on stability, emphasizing the need to understand how drivers of global change interactively affect the reliable provisioning of ecosystem services in real-world systems.

Rapid declines in plant diversity have prompted concern over the consequences for the stability of ecosystem functioning and the reliable provisioning of ecological services^{7,12,13}. The first attempts to address this concern used observational studies of natural variation in diversity^{14,15} and were limited in their ability to separate effects of diversity from other confounding factors¹⁶. In response, more recent studies have directly manipulated diversity in experimentally established communities (that is, biodiversity experiments) to assess its impact on ecosystem functioning, particularly above-ground net primary production (ANPP)^{1–7}. Numerous biodiversity experiments have shown that greater species diversity promotes a greater stability of productivity over time^{2,3,6}, with asynchronous response of species to environmental fluctuations as an important underlying mechanism^{1,2}. There would be no stabilizing

effect if species fluctuated in perfect synchrony. However, asynchrony in species response to environmental fluctuations causes declines in the biomass of some species to be compensated for by increases in others, thus buffering temporal fluctuation in the productivity of the whole community. Species asynchrony can increase the stability of aggregate functions in species-rich communities, because compensatory effects are more likely to occur when the species pool is larger and more diverse^{17–19}.

Biodiversity experiments, in turn, have their own limitations, and their relevance to natural grassland ecosystems is debated^{11,12,16}. For example, experimental gradients of diversity are usually assembled randomly from a local species pool, whereas in natural systems composition and diversity are influenced by a variety of factors including nutrient availability, climatic conditions and anthropogenic land use^{9–11}. It is also likely that diversity is not the only, or even the primary, driver of the stability of ANPP^{20,21}; however, few experiments have simultaneously addressed changes in both biodiversity and other aspects of global change.

In natural grasslands the situation is likely to be complex, because anthropogenic impacts such as climate change and eutrophication are likely to change diversity—with potential consequences for stability—as well as having their own direct effects on stability^{4,13,22,23}. In particular, anthropogenic increases in nutrient inputs into grasslands (through direct organic and inorganic fertilization and atmospheric deposition) affect the structure and functioning of natural ecosystems worldwide^{8,24,25}. For instance, nutrient enrichment usually increases productivity and reduces plant diversity^{24,25}. However, the effect of eutrophication on the stability of productivity in natural grasslands remains unclear. On the basis of theory and results limited to single-site experiments^{22,23}, we expect eutrophication to reduce the stability of productivity, because the well-known negative effects of nutrient enrichment on diversity^{24,25} could in turn reduce species asynchrony and stability^{1,6,14,22,26}. However, eutrophication may have additional impacts on stability that are independent of any changes in diversity. The temporal stability of ANPP is the ratio of the temporal mean to the temporal standard deviation, so an increase in stability can result from an increase in the mean, a decrease

¹Department of Ecology, Evolution, and Behavior, University of Minnesota, St Paul, Minnesota 55108, USA. ²Institute of Evolutionary Biology and Environmental Studies, University of Zurich, 8057 Zurich, Switzerland. ³Department of Wildland Resources and the Ecology Center, Utah State University, Logan, Utah 84322, USA. ⁴Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, Iowa 50011, USA. ⁵Institute for Chemistry and Biology of the Marine Environment, Carl-von-Ossietzky University Oldenburg, D-26111 Oldenburg, Germany. ⁶Department of Integrative Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada. ⁷Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK. ⁸School of Environmental and Forest Sciences, University of Washington, Seattle, Washington 98195, USA. ⁹Australian Research Council Centre of Excellence for Environmental Decisions, School of Biological Sciences, The University of Queensland, Queensland 4072, Australia. ¹⁰School of Natural Sciences, Department of Zoology, Trinity College Dublin, Dublin 2, Ireland. ¹¹State Key Laboratory of Grassland and Agro-Ecosystems, Research Station of Alpine Meadow and Wetland Ecosystems, School of Life Sciences, Lanzhou University, Lanzhou 730000, China. ¹²Department of Biology, MSC03-2020, University of New Mexico, Albuquerque, New Mexico 87131, USA. ¹³Instituto de Investigaciones Marinas y Costeras (IIMyC) (CONICET-UNMdP), Mar del Plata 7600, Argentina. ¹⁴Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706, USA. ¹⁵Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, Colorado 80309, USA. ¹⁶United States Department of Agriculture Agricultural Research Service, Grassland Soil and Water Research Lab, Temple, Texas 76502, USA. ¹⁷Queensland University of Technology, School of Biological Sciences, Brisbane 4000, Australia. ¹⁸Department of Entomology, University of Maryland, College Park, Maryland 20742, USA. ¹⁹United States Department of Agriculture Agricultural Research Service, Agroecosystem Management Research Unit, Lincoln, Nebraska 68583, USA. ²⁰Department of Forest, Rangeland and Watershed Stewardship, Colorado State University, Fort Collins, Colorado 80523, USA. ²¹School of Biological Sciences, University of Nebraska, Lincoln, Nebraska 68588, USA. ²²Berkeley Initiative for Global Change Biology, University of California, Berkeley, California 94720, USA. ²³Yunnan Academy of Biodiversity, Southwest Forestry University, Kunming 650224, China. ²⁴Department of Plant and Soil Sciences, University of Kentucky, Lexington, Kentucky 40546, USA. ²⁵Australian Research Centre for Urban Ecology, Melbourne, c/o School of Botany, University of Melbourne, Victoria 3010, Australia. ²⁶School of Biological Sciences, Monash University, Victoria 3800, Australia. ²⁷Department of Zoology, Oregon State University, Corvallis, Oregon 97331, USA. ²⁸CSIRO Ecosystem Sciences, Wembley, WA 6913, Australia. ²⁹Swiss Federal Institute for Forest, Snow and Landscape Research, 8903 Birmensdorf, Switzerland. ³⁰School of Biology, University of Leeds, Leeds LS2 9JT, UK. ³¹National Centre for Biological Sciences, GKVK Campus, Bangalore 560065, India. ³²Department of Plant Sciences, University of Oxford, Oxford OX1 3RB, UK.

in the standard deviation, or both. Because eutrophication is expected to increase productivity it may have a stabilizing effect by increasing the temporal mean. However, there is also the potential for effects of eutrophication on stability through changes in the temporal standard deviation, but these are less well understood. We therefore require a better picture of how drivers of global change affect ecosystem stability both through changes in diversity and through other routes. Here we compare the relationship between diversity and stability found in grassland biodiversity experiments with those in fertilized and unfertilized plots in natural grasslands. We also assess the effects of eutrophication on the diversity–stability relationship both through changes in diversity and through other routes.

We evaluated the relationships between species diversity, species asynchrony and stability of ANPP across 41 naturally assembled grassland ecosystems on five continents (Extended Data Fig. 1 and Extended Data Table 1), using data from the Nutrient Network (NutNet; <http://www.nutnet.org>) collaborative experiment^{27,28}. We used standardized methods to assess plant diversity and ANPP at each site in both unmanipulated controls and experimentally fertilized plots in a well-replicated design. We quantified diversity as the average plant species richness in standard 1-m² plots over a three-year period. Stability can take a variety of meanings in the ecological literature^{29,30}; here we focus on temporal stability of community-level, above-ground live plant biomass from all species in a plot (a measure of ANPP) over three years. We define

temporal stability for each plot as the temporal mean of ANPP divided by its temporal variability—that is, the temporal standard deviation over a common period (see Methods).

Stability of ANPP was positively associated with plant diversity in the unmanipulated communities (Fig. 1a). Using a hierarchical sampling design and statistical model we found that stability increased with diversity consistently within and among sites, resulting in parallel relationships (coloured and black lines, respectively, in Fig. 1a). The consistent relationship between diversity and stability is concordant with experimental results obtained in grasslands across Europe¹ and with experiments and observations at single locations^{2,3,6,21,26}. We used multiple regression to evaluate the influence of plant diversity and key biotic and abiotic factors on stability in our 41 grasslands. Stability was still associated with diversity after using covariates to control for differences in average site productivity and climatic conditions including annual trends, seasonality and extreme or limiting environmental factors (Extended Data Tables 1 and 2). Together these results demonstrate that temporal stability of ANPP was positively related to variation in plant diversity in our 41 naturally assembled grassland ecosystems.

We determined the role of species asynchrony as a mechanism promoting stability, by using a community-wide measure that allowed direct comparison between communities with different numbers of species^{17–19}. Because the biomass of individual plant species was available at few sites, we used estimates based on our three-year record of the percentage

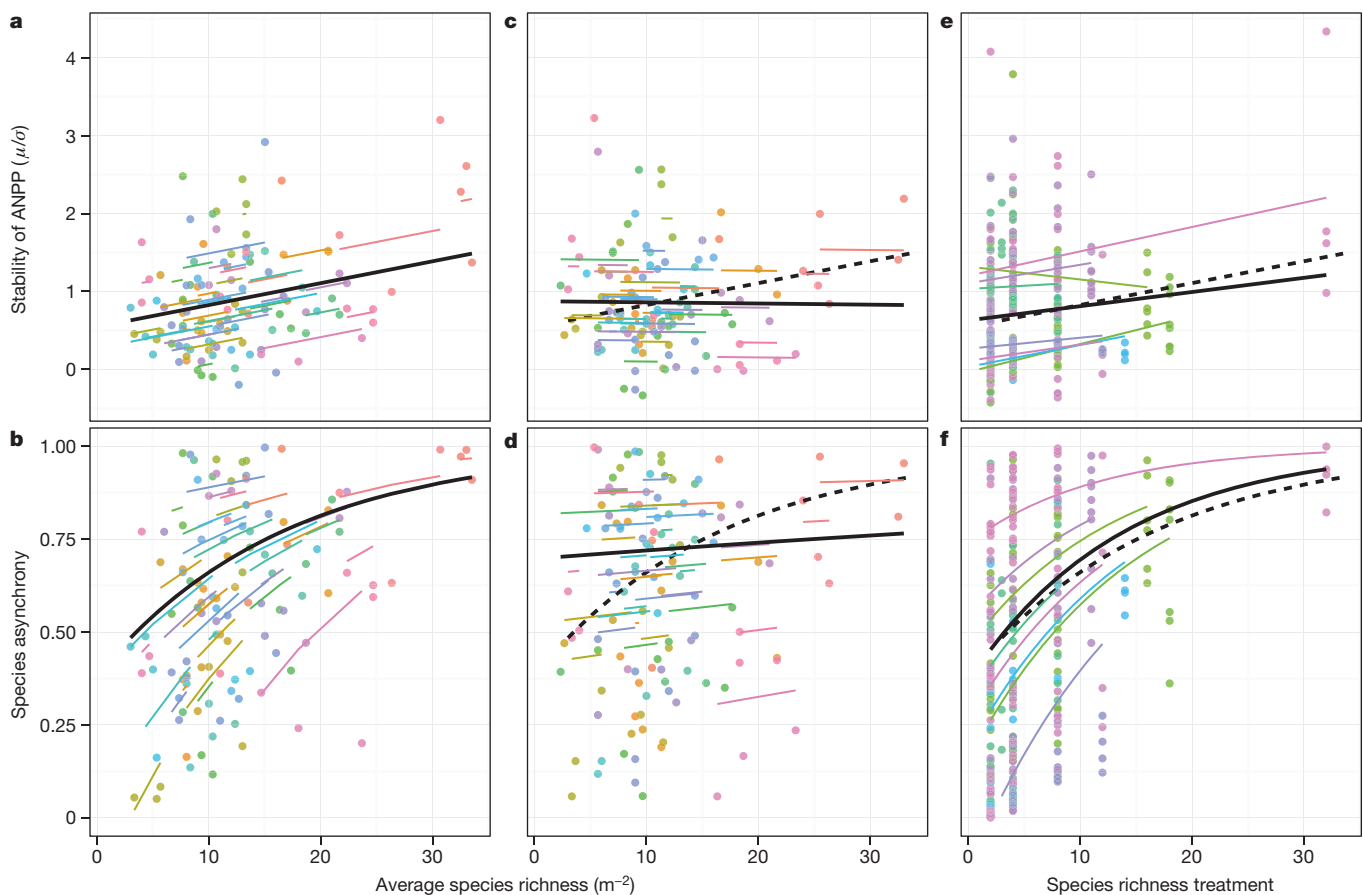


Figure 1 | Relationships of temporal stability of ANPP (upper row) and species asynchrony (lower row) with species diversity. a–d, Unmanipulated (a, b) and fertilized (c, d) communities of the Nutrient Network. e, f, The BIODEPTH network of grassland biodiversity experiments. Relationships of temporal stability of ANPP (temporal mean/temporal standard deviation; natural log transformed for analysis) of 41 grassland sites of the Nutrient Network were positive in the unmanipulated communities (a, b) (slopes and 95% confidence intervals: 0.028 (0.006 to 0.050) and 0.060 (0.023 to 0.097)), but not detectible in the fertilized communities (c, d) (−0.001 (−0.025 to 0.022) and 0.008 (−0.031 to 0.047)). (e, f) Relationships in the BIODEPTH network

were positive (0.018 (0.003 to 0.039) and 0.073 (0.053 to 0.093)). Species asynchrony varied from zero (perfect synchrony) to one (perfect asynchrony). Species richness values for the Nutrient Network are average values over the three years of post-treatment data. Points are values for individual plots ($n = 117$ for Nutrient Network, $n = 480$ for BIODEPTH). Black lines are the back-transformed fixed-effect linear regression slopes between sites from the mixed-effects model; coloured lines show patterns within sites. Dashed lines show regression slopes between sites in the unmanipulated communities of the Nutrient Network. Colours correspond to the ‘colour code’ column in Extended Data Table 1.

cover of individual plant species in each plot (see Methods). Our analysis of potential stability mechanisms showed that species asynchrony was positively related to plant diversity (Fig. 1b) and stability (Fig. 2a), consistent with theory on the stabilizing effects of species asynchrony in species-rich communities. Greater stability at higher diversity can also result from an increase in the temporal mean of ANPP with diversity (a ‘performance-enhancing effect’ that results in a higher ratio of the temporal mean to the temporal variation)^{1,17}. Consistent with earlier NutNet analyses²⁷, we found that the temporal mean of ANPP was not related to plant diversity (Fig. 3a). Although it is an indirect test, our result provides no support for a performance-enhancing effect in stabilizing higher-diversity communities in our study. Instead, we found stronger support for a decrease in temporal variation of ANPP (measured by the standard deviation) with diversity (Fig. 3a). In other words, greater stability at higher diversity resulted because diversity decreased the temporal variation of ANPP relative to its mean, resulting in a more stable mean-to-variance ratio.

To compare our results from naturally assembled grasslands with results from artificially assembled biodiversity experiments, we calculated values of species asynchrony from the BIODEPTH experiment¹. BIODEPTH—a pan-European network of grassland biodiversity experiments—was conducted at eight field sites with a comparable hierarchical design, plot size and measurements (see Methods). Our results are comparable to those from BIODEPTH because both studies use the same three-year experimental duration and cover a similar range of diversity levels (although, by design, biodiversity experiments feature many more low-diversity communities than observational surveys). We found that the sign and slope of the overall relationships between diversity and stability and between diversity and asynchrony from our global multisite study were comparable to those from the BIODEPTH network of grassland biodiversity experiments (Fig. 1e, f; compare the solid and dashed lines).

We tested the impact of eutrophication on temporal stability and species asynchrony by using data from NutNet plots that were fertilized for three years with a combination of nitrogen, phosphorus, potassium and micronutrients (see Methods). Fertilization weakened the positive effect of diversity on stability and species asynchrony (Fig. 1c, d; compare the solid and dashed lines). We expected this result on the basis of theory, because nutrient enrichment often reduces diversity^{24,25}, which could in turn reduce species asynchrony and stability^{1,6,14,22,26}. However, although fertilization reduced diversity by an average of 1.3 species (95% confidence interval 0.7–1.9) per site (corresponding to a reduction

of diversity from 2.0% to 16.9% relative to average levels in the control plots ranging from 4.4 to 32.3 species per square metre (Extended Data Table 1)), counter to expectations this loss of diversity did not lead to a reduction of stability through a decrease in species asynchrony (Extended Data Fig. 2). Instead, the lower slope of the diversity–stability relationship in the fertilized communities (Fig. 1c) can be explained by a combination of two factors. First, fertilization increased the temporal variation of ANPP in diverse communities in comparison with unmanipulated communities (Fig. 3a, b; compare the dashed lines). Because fertilization generally increased mean productivity in comparison with unmanipulated communities (Fig. 3a, b; compare the solid lines), this increased variation weakened the positive effect of diversity on stability in comparison with unmanipulated communities. Second, fertilization resulted in a decrease in species asynchrony in diverse communities compared with unmanipulated communities (Fig. 1d; compare the dashed and solid lines). Because fertilization did not alter the positive relationship between species asynchrony and stability (Fig. 2b), this decrease in species asynchrony resulted in decreased stability in diverse communities compared with unmanipulated communities. In total, the results of our fertilization experiment did not show the expected destabilizing effects of diversity loss. Instead, eutrophication affected stability directly through a combination of diversity-dependent effects on species asynchrony and on the temporal variation of productivity. These direct effects of eutrophication on the diversity–stability relationship could not have been predicted from studies of natural or experimental diversity gradients.

The results of our observational study of naturally assembled grassland communities are consistent with a stabilizing effect of asynchronous responses of species to environmental fluctuations in more diverse plant communities—a result previously restricted to biodiversity experiments¹ and observational studies at single locations^{21,26}. However, the drivers of global change causing a loss of diversity may have additional effects on stability. The results of our fertilization experiment demonstrate impacts on stability that were not caused by changes in diversity but came about through effects of eutrophication on both the temporal variation in production and on species asynchrony. However, although the effects of fertilization on stability were not caused by species loss, the changes in species asynchrony and temporal variation that were responsible were both affected by levels of community diversity. Predicting the effects of drivers of global change therefore requires a better understanding of both their direct effects on ecosystem stability and their indirect effects through changes in diversity. Our results indicate that although

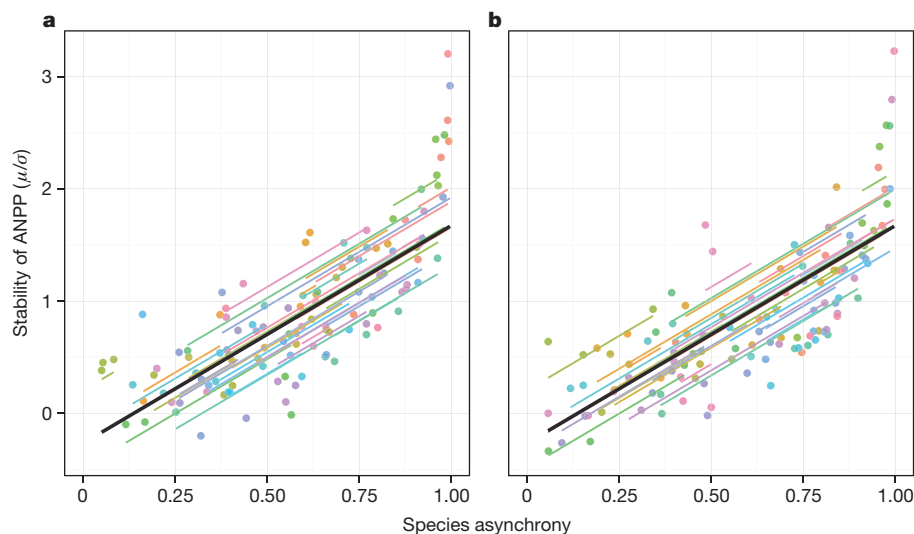


Figure 2 | Relationships between temporal stability of ANPP (natural log transformed) and species asynchrony in 41 grassland sites of the Nutrient Network. **a**, Unmanipulated communities; **b**, fertilized communities. The temporal stability was greatest in plots in which species fluctuations were asynchronous in both the unmanipulated (slope and 95% confidence intervals:

1.93 (1.70 to 2.16)) and fertilized communities of 41 grassland sites of the Nutrient Network (1.90 (1.58 to 2.21)). Points are values for individual plots ($n = 117$). Colours correspond to the ‘colour code’ column in Extended Data Table 1.

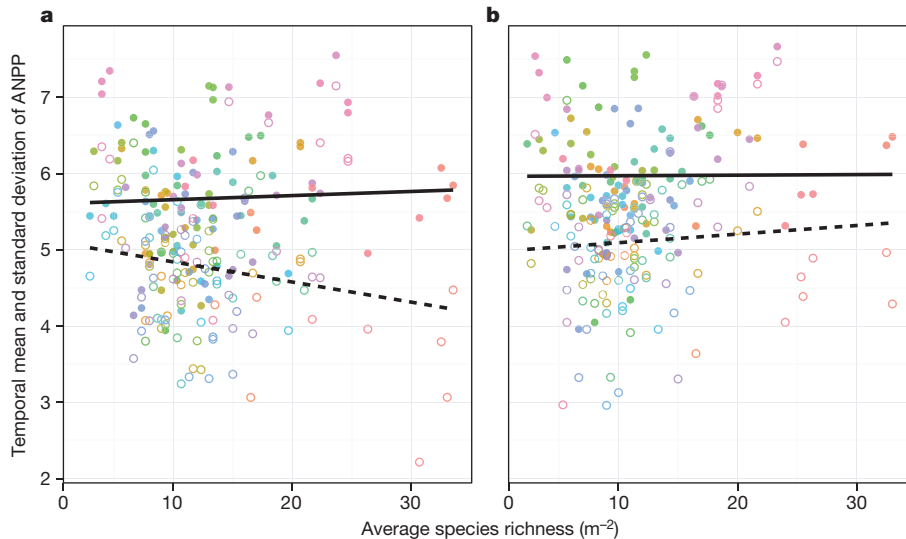


Figure 3 | Relationships of temporal mean and standard deviation of ANPP (natural log transformed) to species diversity in 41 grassland sites of the Nutrient Network. a, Unmanipulated communities; b, fertilized communities. Temporal mean (μ ; solid line and filled symbols) was not related to species diversity (slope and 95% confidence intervals: 0.01 (−0.02 to 0.03)) in the unmanipulated communities (a), and standard deviation (σ ; dashed line and open symbols) was negatively related to species diversity (−0.03 (−0.05 to −0.01)), suggesting that greater stability at higher diversity in the

eutrophication is intended to increase average levels of productivity it can also affect its temporal stability. Sustainable management of grassland ecosystems therefore requires a better understanding of the complex interrelationships between diversity, productivity and stability and how they are affected by fertilization.

METHODS SUMMARY

The 41 sites are part of the Nutrient Network Global Research Cooperative²⁸ (Extended Data Fig. 1 and Extended Data Table 1) (see NutNet's website). Experimental plots included untreated controls and plots with nitrogen, phosphorus and potassium and micronutrients added in combination (NPK). The analyses presented here include all sites with the first three years of post-treatment community-level ANPP ($\text{g m}^{-2} \text{yr}^{-1}$) and species-level ANPP estimates based on percentage cover.

We also examined data from BIODEPTH, a consortium of coordinated biodiversity experiments that manipulated plant diversity at eight European grassland sites¹. We analysed community and species-level ANPP for the three main years of this project⁸.

Ecosystem temporal stability was defined for each plot as μ/σ , where μ is the temporal mean of ecosystem-level ANPP and σ its temporal standard deviation over the three-year period. Species asynchrony was measured for each plot as $1 - \phi_b$, where ϕ_b is species synchrony and is calculated as $1 - \frac{\sigma^2}{\left(\sum_{i=1}^S \sigma_i\right)^2}$,

where σ_i is the temporal standard deviation of species i in a plot with S species over the three years¹⁸. Thus, stability and species asynchrony are related such that higher levels of species asynchrony are associated with greater stability of the community as a whole¹⁴.

We modelled relationships with linear mixed-effects models by using the lme function from the nlme library in R2.15.1. To improve normality, the ecosystem temporal stability and community-wide species asynchrony were log-transformed before analyses. Sites and blocks nested within sites for the NutNet data and sites and species composition nested within sites for BIODEPTH were treated as random effects, allowing both the intercepts and slopes of regression versus diversity to vary between sites if supported by model selection.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 11 October 2013; accepted 10 January 2014.

Published online 16 February 2014.

1. Hector, A. *et al.* General stabilizing effects of plant diversity on grassland productivity through population asynchrony and overyielding. *Ecology* **91**, 2213–2220 (2010).

unmanipulated communities (Fig. 1a) resulted from a decrease in temporal variation. Both temporal mean (slope and 95% confidence intervals 0.01 (−0.02 to 0.03)) and standard deviation (slope and 95% confidence intervals 0.01 (−0.02 to 0.04)) were not related to species diversity in the fertilized communities (b). Fertilization increased the temporal variation in diverse communities compared with unmanipulated communities, resulting in a reduced positive effect of diversity on stability in fertilized communities (Fig. 1c). Points are values for individual plots ($n = 117$).

- Isbell, F. I., Polley, H. W. & Wilsey, B. J. Biodiversity, productivity and the temporal stability of productivity: patterns and processes. *Ecol. Lett.* **12**, 443–451 (2009).
- Tilman, D., Reich, P. B. & Knops, J. M. H. Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature* **441**, 629–632 (2006).
- Reich, P. B. Elevated CO₂ reduces losses of plant diversity caused by nitrogen deposition. *Science* **326**, 1399–1402 (2009).
- Naeem, S., Thompson, L. J., Lawler, S. P., Lawton, J. H. & Woodfin, R. M. Declining biodiversity can alter the performance of ecosystems. *Nature* **368**, 734–737 (1994).
- Bezemer, T. M. & van der Putten, W. H. Ecology: diversity and stability in plant communities. *Nature* **446**, E6–E7 (2007).
- Cardinale, B. J. Biodiversity loss and its impact on humanity. *Nature* **486**, 59–67 (2012).
- Vitousek, P. M., Mooney, H. A., Lubchenco, J. & Melillo, J. M. Human domination of Earth's ecosystems. *Science* **277**, 494–499 (1997).
- Selmants, P. C., Zavaleta, E. S., Pasari, J. R. & Hernandez, D. L. Realistic plant species losses reduce invasion resistance in a California serpentine grassland. *J. Ecol.* **100**, 723–731 (2012).
- Zavaleta, E. S. & Hulvey, K. B. Realistic species losses disproportionately reduce grassland resistance to biological invaders. *Science* **306**, 1175–1177 (2004).
- Srivastava, D. S. & Vellend, M. Biodiversity–ecosystem function research: is it relevant to conservation? *Annu. Rev. Ecol. Evol. Syst.* **36**, 267–294 (2005).
- Loreau, M. *et al.* Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science* **294**, 804–808 (2001).
- Ives, A. R. & Carpenter, S. R. Stability and diversity of ecosystems. *Science* **317**, 58–62 (2007).
- Tilman, D. & Downing, J. A. Biodiversity and stability in grasslands. *Nature* **367**, 165–175 (1994).
- McNaughton, S. J. Stability and diversity of ecological communities. *Nature* **274**, 251–253 (1978).
- Huston, M. A. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia* **110**, 449–460 (1997).
- Yachi, S. & Loreau, M. Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. *Proc. Natl Acad. Sci. USA* **96**, 1463–1468 (1999).
- Loreau, M. & de Mazancourt, C. Species synchrony and its drivers: neutral and nonneutral community dynamics in fluctuating environments. *Am. Nat.* **172**, E48–E66 (2008).
- Loreau, M. *From Populations to Ecosystems: Theoretical Foundations for a New Ecological Synthesis* (Princeton Univ. Press, 2010).
- Sankaran, M. & McNaughton, S. J. Determinants of biodiversity regulate compositional stability of communities. *Nature* **401**, 691–693 (1999).
- Bai, Y., Han, X., Wu, J., Chen, Z. & Li, L. Ecosystem stability and compensatory effects in the inner Mongolia grassland. *Nature* **431**, 181–184 (2004).
- Yang, Z. L., van Ruijven, J. & Du, G. Z. The effects of long-term fertilization on the temporal stability of alpine meadow communities. *Plant Soil* **345**, 315–324 (2011).
- Yang, H. J. *et al.* Diversity-dependent stability under mowing and nutrient addition: evidence from a 7-year grassland experiment. *Ecol. Lett.* **15**, 619–626 (2012).

24. Stevens, C. J., Dise, N. B., Mountford, J. O. & Gowing, D. J. Impact of nitrogen deposition on the species richness of grasslands. *Science* **303**, 1876–1879 (2004).
25. Hautier, Y., Niklaus, P. A. & Hector, A. Competition for light causes plant biodiversity loss after eutrophication. *Science* **324**, 636–638 (2009).
26. Tilman, D. Biodiversity: population versus ecosystem stability. *Ecology* **77**, 350–353 (1996).
27. Adler, P. B. *et al.* Productivity is a poor predictor of plant species richness. *Science* **333**, 1750–1753 (2011).
28. Borer, E. T. *et al.* Finding generality in ecology: a model for globally distributed experiments. *Methods Ecol. Evol.* **5**, 65–73 (2014).
29. May, R. M. *Stability and Complexity in Model Ecosystems* (Princeton Univ. Press, 1973).
30. Pimm, S. L. The complexity and stability of ecosystems. *Nature* **307**, 669–674 (1984).

Acknowledgements The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 298935 to Y.H. (with A.H. and E.W.S.). This work was generated using data from the Nutrient Network (<http://www.nutnet.org>) experiment, funded at the site-scale by individual researchers. Coordination and data management have been

supported by funding to E.T.B. and E.W.S. from the National Science Foundation Research Coordination Network (NSF-DEB-1042132), the Long Term Ecological Research (LTER) programme (NSF-DEB-1234162 to Cedar Creek as well as other LTER sites), and the Institute on the Environment at the University of Minnesota (DG-0001-13). We also thank the Minnesota Supercomputer Institute for hosting project data, and the Institute on the Environment for hosting Network meetings. We thank R. S. L. Veiga, F. Isbell, R. K. Didham, H. McGinness and M. O'Brien for suggestions that improved the manuscript.

Author Contributions E.W.S., E.T.B., W.S.H. and E.M.L. are Nutrient Network coordinators. Y.H. and A.H. developed and framed research questions. Y.H., E.W.S., E.T.B., P.B.A., W.S.H., H.H., A.S.M.D., C.J.S., J.D.B., Y.M.B., C.C., S.L.C., E.I.D., K.F.D., P.A.F., J.F., D.S.G., V.L.J., J.A.K., J.M.H.K., K.J.L., W.L., R.L.McC., B.A.M., J.L.M., S.M.P., A.C.R., M.S., M.S. and A.H. collected the data used in this analysis. Y.H. and A.H. analysed the data. E.W.S., E.T.B., H.H., E.M.L., P.D., K.J.L., J.L.M., L.R.O. and M.S. contributed to data analyses. Y.H. and A.H. wrote the paper with input from all authors.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to Y.H. (hauti001@umn.edu).

METHODS

Site selection and experimental design. The 41 study sites are part of the Nutrient Network (NutNet) Global Research Cooperative (Extended Data Fig. 1 and Extended Data Table 1) (see also NutNet's website). See ref. 28 for a complete description of site selection, methods and measurements. To be as representative as possible of realistic grassland ecosystems, our analyses included sites covering a wide range of grassland habitats (for example alpine grassland, prairie, pasture, shrub steppe, savanna and old field). Thus, the between-site variation across NutNet sites captures a globally relevant gradient of fine-scale (1-m²) and site-level variation in factors including above-ground biomass, species richness, land-use history and environmental variables (Extended Data Table 1). In some sites, human land use (grazing, burning and mowing as part of the traditional site management) is currently or has been recently carried on (Extended Data Table 1). However, our analyses were robust to land-use history: effects of species richness were similar after we removed 13 sites with strong anthropogenic influence.

All sites included in the analyses presented here included control plots and plots with nitrogen (N), phosphorus (P) and potassium and micronutrients (K) added in combination (NPK) (details are given below).

Treatments were randomly assigned to the 25-m² plots and were replicated in three blocks at most sites, although the number of blocks ranged from one to six between sites (Extended Data Table 1). Treatments and sampling followed a standardized protocol at all sites, detailed in ref. 28. Treatment application started at most sites in 2008, although eight sites started in 2009 and two in 2010. For this study we included all sites with three year of post-treatment data collection. We used data collected during the first three-years of post-treatment data collection so that our results would be independent of the time since the start of treatment application. All of our sites had three years of post-treatment data, although three sites had discontinuous data collection (Extended Data Table 1). Longer time series currently exist for only a limited number of sites, but the results were qualitatively the same when extended to four and five years.

Climate data. We quantified precipitation and temperature data using the WorldClim Global Climate database³¹ (version 1.4; <http://www.worldclim.org/>). A principal component analysis (PCA) was used to reduce the number of climatic variables, many of which were collinear, resulting in a subset of bioclimatic variables representing annual trends (mean annual temperature (°C) and precipitation (mm)), seasonality (mean annual range in temperature, standard deviation in temperature, coefficient of variation of precipitation) and extreme or limiting environmental factors (mean temperature during the wettest four months).

Fertilization. Nitrogen (N), phosphorus (P) and potassium (K) were applied annually to fertilized plots, before the beginning of the growing season, at relatively high rates: 10 g m⁻² y⁻¹. These rates are comparable to those for other grassland experiments that seek to alter diversity³². N was supplied as time-release urea ((NH₂)₂CO) or ammonium nitrate (NH₄NO₃) (the form of N did not have differential effects on production²⁸). P was supplied as triple super phosphate (Ca(H₂PO₄)₂), and K as potassium sulphate (K₂SO₄). In addition, a micronutrient mix (Fe, S, Mg, Mn, Cu, Zn, B and Mo) was applied at 100 g m⁻² y⁻¹ to the K-addition plots, once at the start of the experiment but not in subsequent years to avoid toxicity.

Species richness and cover. Diversity was quantified as the average plant species richness in standard 1-m² plots over the three years of post-treatment data for the analyses. We used species richness as a measure of diversity because species asynchrony in response to environmental fluctuations is the basis for functional compensation between species and stability theory³³; decreases in the functioning of some species are partly or wholly compensated for by increases in other species. Cover was estimated independently for each species, so that total summed cover can exceed 100% for multilayer canopies. To better match theory, percentage cover was converted to biomass estimates for each species by assuming that the proportion of total cover for each species was equivalent to its proportion of total above-ground biomass³⁴, because we did not have direct measures of biomass for each individual species. Our results were independent of the measure chosen; results of our analyses using percentage cover data did not differ qualitatively from the results presented in the text using estimated biomass data for species, based on percentage cover.

Productivity. We used above-ground live biomass as a measure of primary productivity, which is an effective estimator of above-ground net primary production (ANPP) in herbaceous vegetation^{35,36}. At some sites with strongly seasonal communities, cover and biomass were estimated twice during the year to assemble a

complete list of species, and the summed biomass of each species was used in the analyses (Extended Data Table 1). However, our results were retained when we performed analyses excluding these sites.

BIODEPTH. The BIODEPTH data used in our analysis are available online (<http://www.esapubs.org/archive/ecol/E091/155/>) from Ecological Archives¹.

BIODEPTH comprised a consortium of eight coordinated biodiversity experiments that manipulated plant diversity at different European grassland sites^{1,37,38}. The analyses presented here use data on net above-ground biomass production (g m⁻² yr⁻¹) of species from the experimental plots at each of the eight BIODEPTH field sites for the three main years of the project^{1,38}. The data set comprises information on 480 plots, each containing between 1 and 32 species. In total this produces 1,934 data points per year, with each data point reporting the biomass of a species in an individual plot. Each monoculture or species mixture was replicated in two identical plots (with a few exceptions: five plant assemblages were replicated four times³⁸). Monocultures were removed from the analysis to produce a more comparable range of species richness.

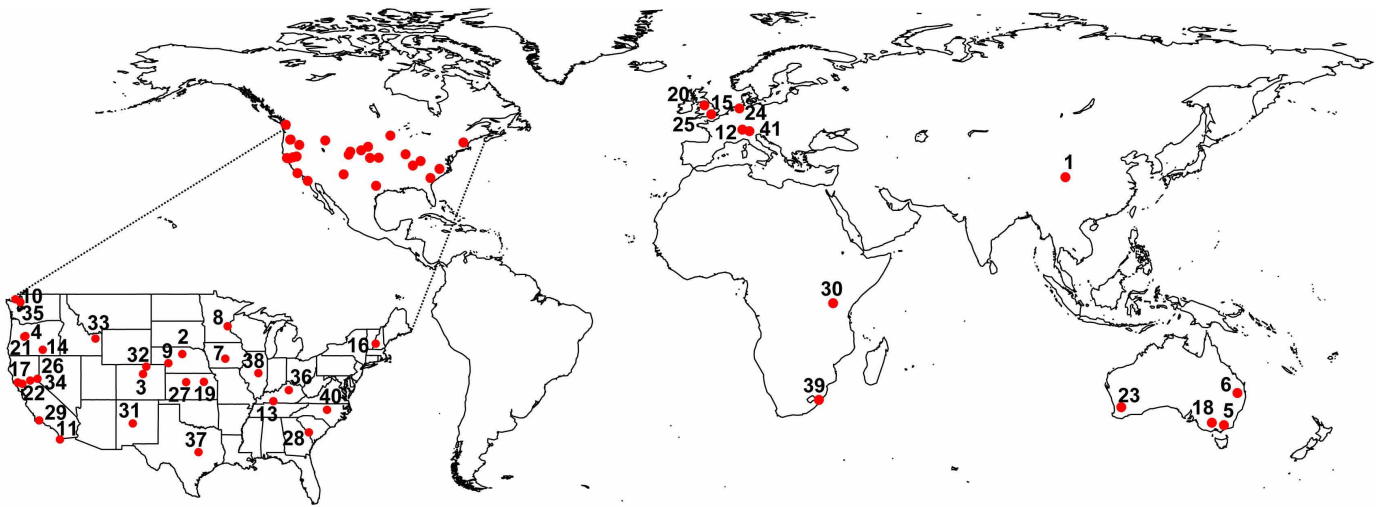
Stability. Ecosystem temporal stability was defined for each plot as μ/σ , where μ is the temporal mean of ecosystem-level ANPP and σ is its temporal standard deviation over the three-year period.

Asynchrony. Species asynchrony was measured for each plot as $1 - \phi_b$, where ϕ_b is species synchrony and is calculated as $1 - \phi_b = 1 - \frac{\sigma^2}{\left(\sum_{i=1}^S \sigma_i\right)^2}$, where σ^2 is the

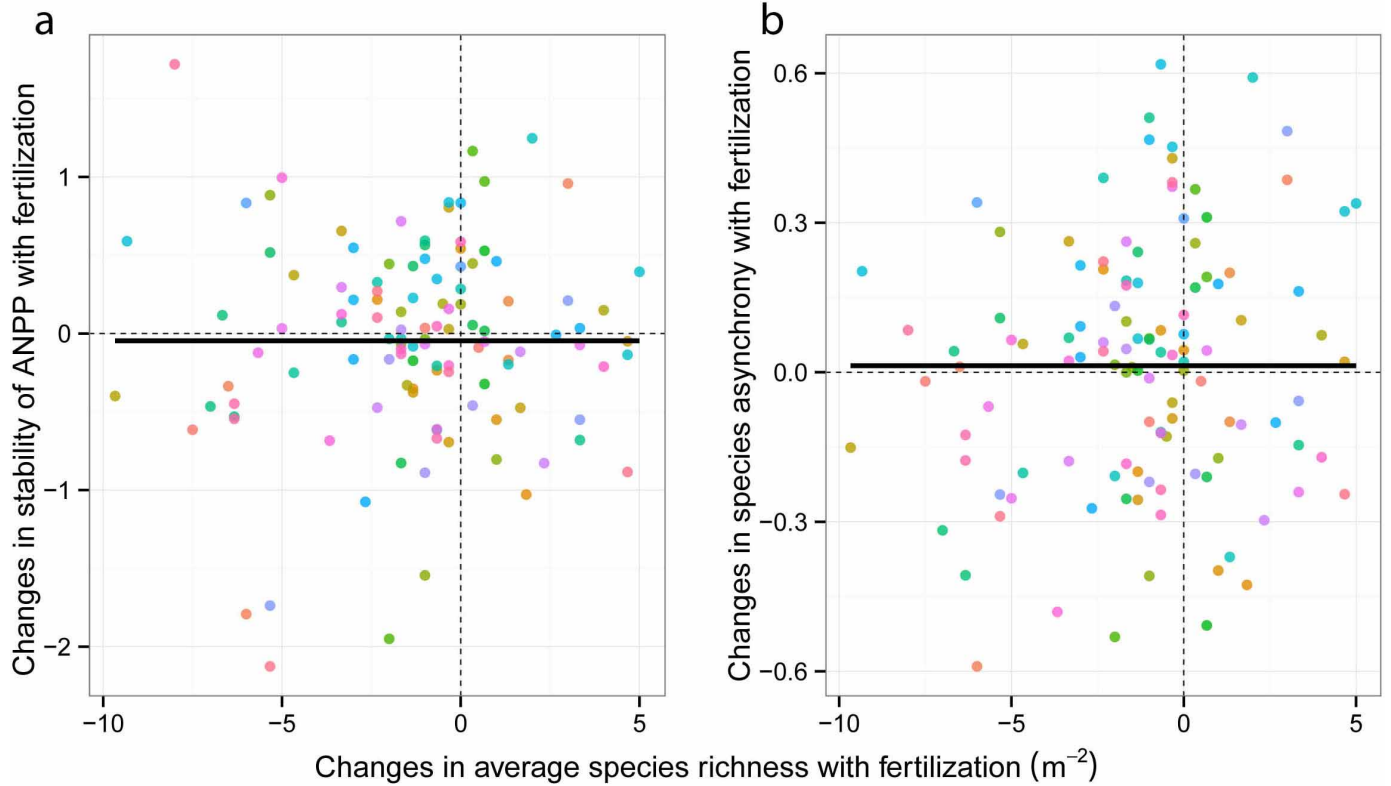
temporal variance in ecosystem function and σ_i is the temporal standard deviation in function of species i in a plot with S species over the three years¹⁸. Thus, stability and species asynchrony are related such that higher levels of species asynchrony are associated with greater stability of the community as a whole^{18,39,40}.

Analyses. We modelled the relationships with linear mixed-effects models by using the lme function from the nlme library⁴¹ in R 2.15.1 (ref. 42). To improve normality, the temporal stability of ANPP, community-wide species asynchrony, temporal mean of ANPP and temporal standard deviation of ANPP were log-transformed before analyses. Changes in diversity, stability and asynchrony were calculated as the average difference per block between the fertilized and unmanipulated plots of the Nutrient Network. Sites and blocks nested within sites for the NutNet data and sites and species composition nested within sites for BIODEPTH were treated as random effects allowing both the intercepts and slopes of regression versus diversity to vary between sites if supported by model selection. For the fixed-by-random-effects interactions, we used a model-selection approach based on minimization of BIC⁴¹, in which we compared models with and without a given random effect to determine which level of variation was required in the model. In every case, model selection for NutNet data retained variation between sites but excluded variation due to blocks, whereas model selection for BIODEPTH data retained variation between sites and species composition. Inference for the fixed effects was based on 95% confidence intervals.

- Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G. & Jarvis, A. Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* **25**, 1965–1978 (2005).
- Harpole, W. S. & Tilman, D. Grassland species loss resulting from reduced niche dimension. *Nature* **446**, 791–793 (2007).
- McNaughton, S. J. Diversity and stability of ecological communities: a comment on the role of empiricism in ecology. *Am. Nat.* **111**, 515–525 (1977).
- Fargione, J. *et al.* From selection to complementarity: shifts in the causes of biodiversity–productivity relationships in a long-term biodiversity experiment. *Proc. R. Soc. Lond. B* **274**, 871–876 (2007).
- Lauenroth, W. K., Hunt, H. W., Swift, D. M. & Singh, J. S. Estimating aboveground net primary production in grasslands: a simulation approach. *Ecol. Modell.* **33**, 297–314 (1986).
- Oesterheld, M. & McNaughton, S. J. in *Methods in Ecosystem Science* (eds Sala, O. E., Jackson, R. B., Mooney, H. A. & Howarth, R. W.) 151–157 (Springer, 2000).
- Hector, A. *et al.* Plant diversity and productivity experiments in European grasslands. *Science* **286**, 1123–1127 (1999).
- Spehn, E. M. *et al.* Ecosystem effects of biodiversity manipulations in European grasslands. *Ecol. Monogr.* **75**, 37–63 (2005).
- de Mazancourt, C. *et al.* Predicting ecosystem stability from community composition and biodiversity. *Ecol. Lett.* **16**, 617–625 (2013).
- Loreau, M. & de Mazancourt, C. Biodiversity and ecosystem stability: a synthesis of underlying mechanisms. *Ecol. Lett.* **16**, 106–115 (2013).
- Pinheiro, J. C. & Bates, D. M. *Mixed-Effects Models in S and S-Plus* (Springer, 2000).
- R Development Core Team. *A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, 2012).



Extended Data Figure 1 | Locations of the 41 Nutrient Network sites included in this study. Numbers correspond to the 'site code' column in Extended Data Table 1.



Extended Data Figure 2 | Effect of fertilization-induced changes in diversity on changes in stability of ANPP and changes in species asynchrony.

a, Changes in stability of ANPP (slope and 95% confidence intervals: 0.009 (−0.048 to 0.030)); **b**, changes in species asynchrony (0.012 (−0.004 to 0.027)). Neither was related to changes in species richness caused by fertilization.

Flat lines represent the overall non-significant mean effects. Nutrient-induced changes were calculated as the average difference per block between fertilized and unmanipulated Nutrient Network plots. Colours correspond to the 'colour code' column in Extended Data Table 1. Points are values for individual plots ($n = 117$).

Extended Data Table 1 | Additional information on the 41 Nutrient Network study sites

Site	Site code	Colour code	Continent	Country	Latitude	Longitude	Years*	Blocks†	Land-use history	Habitat‡	Number of biomass data collection per year	MAT‡	MAP§	MAT Range	Temp SD¶	MAP VAR#	Temp Wet††	Average species richness in the unmanipulated plot	Percentage of species lost with fertilization
azi.cn	1		Asia	China	33.7	101.9	2008, 2009, 2011	3	Managed	Alpine grassland	1	2	667	15.9	70.6	87	9.9	32.3	2.0
bart.us	2		North America	USA	42.2	-99.7	2008, 2009, 2011	3	Natural	Mixed-grass prairie	1	8.7	597	14.5	103.3	58	19.4	14.7	4.6
bdt.us	3		North America	USA	40	-105.2	2009, 2010, 2011	2	Natural	Short-grass prairie	1	9.7	425	15.5	79.5	42	13.3	9.1	7.6
bnch.us	4		North America	USA	44.3	-122	2008, 2009, 2010	3	Natural	Montane grassland	1	5.5	1647	12.2	60.6	65	-1.1	10.1	6.9
boqng.au	5		Australia	Australia	-36.9	147.3	2010, 2011, 2012	3	Natural	Alpine grassland	1	5.7	1592	9.8	47.6	26	0.2	20.0	3.3
burrawan.au	6		Australia	Australia	-27.7	151.1	2009, 2010, 2011	3	Grazed	Semi-arid grassland	1	18.4	683	14.3	50.5	36	24.4	11.3	6.1
cbg.us	7		North America	USA	-93.4	41.8	2008, 2009, 2010	6	Burned Anthropogenic	Tall-grass prairie	1	9.0	855	11.8	108.5	46	20.1	8.4	8.3
cdt.us	8		North America	USA	45.4	-93.2	2008, 2009, 2010	5	Natural	Tall-grass prairie	1	6.3	750	11.7	116.2	51	20.3	9.2	7.6
cdp.us	9		North America	USA	41.2	-101.6	2008, 2009, 2011	6	Natural	Short-grass prairie	1	9.5	445	15.2	99.5	59	19.7	11.1	6.2
cow.ca	10		North America	Canada	48.5	-123.4	2008, 2009, 2010	3	Natural	Old field	1	9.8	764	7.4	40.4	64	5.3	5.2	14.2
elli.us	11		North America	USA	32.9	-117.1	2009, 2010, 2011	3	Natural	Annual grassland	1	17.2	331	12.1	35.9	87	13.3	11.6	5.9
frue.ch	12		Europe	Switzerland	47.1	8.5	2009, 2010, 2011	3	Managed Grazed Anthropogenic	Pasture	2	6.5	1355	7.2	59.9	23	14.1	13.1	5.2
hdt.us	13		North America	USA	36.9	-86.7	2008, 2009, 2010	3	Managed	Tall-grass prairie	2	13.6	1282	12.6	84.7	14	13.6	7.8	9.0
hart.us	14		North America	USA	42.7	-119.5	2008, 2009, 2010	3	Natural	Shrub steppe	1	7.4	272	16.1	69.2	25	10	10.2	8.8
hero.uk	15		Europe	UK	51.4	-0.6	2008, 2009, 2010	3	Natural	Mesic grassland	1	9.9	692	7.9	52.2	14	4.1	18.3	4.1
hnr.us	16		North America	USA	43.4	-72.1	2008, 2009, 2010	3	Anthropogenic	Old field	1	6.4	1033	12.4	96.5	11	18.4	8.1	8.6
hopl.us	17		North America	USA	39	-123.1	2008, 2009, 2010	3	Managed Burned	Annual grassland	1	12.3	1127	15.9	52.8	87	6.7	17.8	3.8
kiny.au	18		Australia	Australia	-36.2	143.8	2008, 2009, 2010	3	Natural	Semi-arid grassland	1	15.5	426	12.8	49.3	21	12.2	12.7	5.4
kortz.us	19		North America	USA	39.1	-96.6	2008, 2009, 2010	3	Managed Burned	Tall-grass prairie	2	11.9	877	12.6	99.3	50	21.9	15.3	4.4
lancaster.uk	20		Europe	UK	54	-2.6	2009, 2010, 2011	3	Managed Grazed	Mesic grassland	2	8	1322	6.9	45.4	23	3.3	10.3	6.7
look.us	21		North America	USA	44.2	-122.1	2008, 2009, 2010	3	Natural	Montane grassland	1	4.8	1886	11.7	58.7	65	-1.4	7.8	9.1
mda.us	22		North America	USA	38.9	-122.4	2008, 2009, 2010	3	Natural	Annual grassland	1	13.5	867	15.2	59.9	88	6.5	8.1	8.6
mtca.au	23		Australia	Australia	-31.8	117.6	2009, 2010, 2011	4	Grazed	Savanna	1	17.3	330	13.9	52.6	55	11.9	14.0	4.8
paspe.de	24		Europe	Germany	53.1	7.5	2008, 2009, 2010	1	Natural	Old field	1	8.9	783	7.2	56.9	17	16	8.6	13.0
rook.uk	25		Europe	UK	51.4	-0.6	2008, 2009, 2010	3	Natural	Mesic grassland	1	9.8	706	7.9	52.4	15	4	10.5	6.6
sage.us	26		North America	USA	39.4	-120.2	2008, 2009, 2010	3	Natural	Montane grassland	1	5.7	882	16.1	65.4	69	-2.2	13.7	5.0
saline.us	27		North America	USA	39.1	-99.1	2008, 2009, 2010	3	Natural	Mixed-grass prairie	2	11.8	607	14.6	100.3	53	22.1	10.0	6.9
sava.us	28		North America	USA	33.3	-81.7	2008, 2009, 2010	2	Natural	Savanna	1	17.3	1194	14	69.5	19	26	9.1	7.6
sedg.us	29		North America	USA	34.7	-120	2008, 2009, 2010	3	Natural	Annual grassland	1	14.9	521	13.5	39.6	93	10.6	11.3	6.1
seerng.tz	30		Africa	Tanzania	34.5	-2.3	2009, 2010, 2011	3	Natural	Savanna	1	22.1	854	11.3	6.9	54	22.7	12.9	5.3
sevi.us	31		North America	USA	34.4	-106.7	2008, 2009, 2010	1	Natural	Desert grassland	2	12.6	252	18.9	78.8	62	21.8	15.8	4.3
sgs.us	32		North America	USA	40.8	-104.8	2008, 2009, 2010	3	Natural	Short-grass prairie	2	8.4	355	15.9	84.8	59	17.1	8.3	8.5
shps.us	33		North America	USA	44.2	-112.2	2008, 2009, 2010	4	Managed Grazed	Shrub steppe	1	5.5	282	14.2	95.6	37	15	15.1	4.5
sier.us	34		North America	USA	39.2	-121.3	2008, 2009, 2010	5	Natural	Annual grassland	1	15.6	935	13.8	64.7	84	8.2	9.1	7.6
smth.us	35		North America	USA	48.2	-122.6	2008, 2009, 2010	3	Natural	Old field	1	9.8	597	8.5	42.1	36	5	20.4	3.3
spn.us	36		North America	USA	38.1	-84.5	2008, 2009, 2010	3	Managed Grazed Anthropogenic	Pasture	1	12.5	1140	11.9	85.6	15	12.1	9.5	7.3
temple.us	37		North America	USA	31	-97.3	2008, 2009, 2010	3	Natural	Tall-grass prairie	2	19.1	871	12.6	73.8	27	23.1	19.8	3.4
trei.us	38		North America	USA	40.1	-88.8	2009, 2010, 2011	3	Natural	Tall-grass prairie	1	11	982	11.9	98.7	24	21.1	4.5	16.9
ukul.za	39		Africa	South Africa	-29.7	30.4	2010, 2011, 2012	3	Managed	Mesic grassland	2	18.1	880	12.8	29.3	62	21.3	22.3	3.0
unc.us	40		North America	USA	36	-79	2008, 2009, 2010	3	Anthropogenic	Old field	1	14.6	1163	13.3	76.2	11	24.2	10.9	6.3
valm.ch	41		Europe	Switzerland	46.6	10.4	2008, 2009, 2011	3	Natural	Alpine grassland	1	0.3	1098	5.9	54.2	29	7.1	26.4	2.5

* Years of data collection used in the analyses.
 † Number of blocks in each site.
 ‡ Mean annual temperature (°C)
 § Mean annual precipitation (mm)
 || Mean annual range in temperature (°C)
 ¶ Standard deviation in temperature.
 # Coefficient of variation of precipitation.
 ☆ Mean temperature during the wettest four months (°C).

Extended Data Table 2 | Multiple regression evaluating the influence of plant diversity and key biotic and abiotic factors, productivity and climate, on stability of ANPP in our 41 grasslands

	numDF	denDF	F-value	p-value
(Intercept)	1	75	142.06	<.0001
Average site productivity	1	33	5.44	0.030
MAT‡	1	33	7.35	0.011
MAP§	1	33	0.09	0.76
MAT Range	1	33	1.28	0.27
Temp SD¶	1	33	0.06	0.81
MAP VAR#	1	33	1.18	0.29
Temp Wet☆	1	33	1.72	0.20
Species diversity	1	75	4.88	0.030

‡ Mean annual temperature (°C).

§ Mean annual precipitation (mm).

|| Mean annual range in temperature (°C).

¶ Standard deviation in temperature.

Coefficient of variation of precipitation.

☆ Mean temperature during the wettest four months (°C).