

Eutrophication weakens stabilizing effects of diversity in natural grasslands

Yann Hautier^{1,2*}, Eric W. Seabloom¹, Elizabeth T. Borer¹, Peter Adler³, W. Stanley Harpole⁴, Helmut Hillebrand⁵, Eric M. Lind¹, Andrew S. MacDougall⁶, Carly Stevens⁷, Jonathan D. Bakker⁸, Yvonne Buckley^{9,10}, Chengjin Chu¹¹, Scott L. Collins¹², Pedro Daleo¹³, Ellen I. Damschen¹⁴, Kendi Davies¹⁵, Philip A. Fay¹⁶, Jennifer Firn¹⁷, Daniel S. Gruner¹⁸, Virginia L. Jin¹⁹, Julia A. Klein²⁰, Johannes M. H. Knops²¹, Kimberly J. La Pierre²², Wei Li²³, Rebecca L. McCulley²⁴, Brett Melbourne¹⁴, Joslin L. Moore²⁵, Lydia R. O'Halloran²⁶, Suzanne M. Prober²⁷, Anita C. Risch²⁸, Mahesh Sankaran^{29,30}, Martin Schuetz²⁸, Andy Hector³¹

¹ Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, MN, USA.

² Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland.

³ Department of Wildland Resources and the Ecology Center, Utah State University, Logan, UT, USA.

⁴ Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA, USA.

⁵ Institute for Chemistry and Biology of the Marine Environment, Carl-von-Ossietzky University Oldenburg, Germany.

⁶ Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada.

⁷ Lancaster Environment Center, Lancaster University, Lancaster, UK.

⁸ School of Environmental and Forest Sciences, University of Washington, Seattle, WA, USA.

⁹ ARC Centre of Excellence for Environmental Decisions, School of Biological Sciences, The University of Queensland, Queensland, Australia.

¹⁰ School of Natural Sciences, Department of Zoology, Trinity College Dublin, Dublin 2, Ireland

¹¹ State Key Laboratory of Grassland and Agro-Ecosystems, Research Station of Alpine Meadow and Wetland Ecosystems, School of Life Sciences, Lanzhou University, Lanzhou, China.

¹² Biology, MSC03-2020, University of New Mexico, Albuquerque, NM 87131, USA.

¹³ Instituto de Investigaciones Marinas y Costeras (IIMyC), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

¹⁴ Department of Zoology, University of Wisconsin, Madison, WI, USA.

¹⁵ Department of Ecology and Evolutionary Biology, University of Colorado, Boulder CO, USA.

¹⁶ USDA-ARS Grassland Soil and Water Research Lab, Temple, TX, USA.

¹⁷ Queensland University of Technology, School of Biological Sciences, Brisbane, Australia.

¹⁸ Department of Entomology, University of Maryland, College Park MD, USA.

¹⁹ USDA-ARS Agroecosystem Management Research Unit, Lincoln, NE, USA.

²⁰ Dept. Forest, Rangeland & Watershed Stewardship, Colorado State University, Fort Collins, CO, USA.

²¹ School of Biological Sciences, University of Nebraska, Lincoln, NE, USA.

²² Department of Integrative Biology, University of California, Berkeley, CA, USA.

²³ Yunnan Academy of Biodiversity, Southwest Forestry University, Kunming, China.

²⁴ Department of Plant & Soil Sciences, University of Kentucky, Lexington, KY, USA.

²⁵ Australian Research Centre for Urban Ecology, Melbourne, c/o School of Botany, University of Melbourne, Victoria, Australia.

²⁶ Department of Zoology, Oregon State University, Corvallis, OR 97331, USA.

²⁷ CSIRO Ecosystem Sciences, Wembley WA, Australia.

²⁸ Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf, Switzerland.

²⁹ School of Biology, University of Leeds, Leeds, UK.

³⁰ National Centre for Biological Sciences, GKVK Campus, Bangalore, India.

³¹ Department of Plant Sciences, University of Oxford, UK.

*To whom correspondence should be addressed. Email: hauti001@umn.edu

49 **Studies of experimental grassland communities¹⁻⁷ have demonstrated that plant diversity**
50 **can stabilize productivity through species asynchrony, where decreases in the biomass of**
51 **some species are compensated by increases in others^{1,2}. However, it remains unknown**
52 **whether these findings are relevant to natural ecosystems, especially those where species**
53 **diversity is threatened by anthropogenic global change⁸⁻¹¹. Here we analyze diversity-**
54 **stability relationships from 41 grasslands on five continents and ask how these**
55 **relationships are affected by chronic fertilization, one of the strongest drivers of species loss**
56 **globally⁸. Unmanipulated communities with more species had greater species asynchrony**
57 **resulting in more stable biomass production, generalizing a result from biodiversity**
58 **experiments to real-world grasslands. However, fertilization weakened the positive effect of**
59 **diversity on stability. Counter to expectations, this was not due to species loss following**
60 **eutrophication but rather to an increase in the temporal variation of productivity in**
61 **combination with a decrease in species asynchrony in diverse communities. Our results**
62 **demonstrate separate and synergistic effects of diversity and eutrophication on stability,**
63 **emphasizing the need to understand how global change drivers interactively affect the**
64 **reliable provisioning of ecosystem services in real-world systems.**

65

66 **One Sentence Summary:** Experimental eutrophication weakens stabilizing effects of plant
67 diversity on the productivity of natural grasslands.

68

69 Rapid declines in plant diversity have prompted concern over its consequences for the stability of
70 ecosystem functioning and the reliable provisioning of ecological services^{7,12,13}. The first
71 attempts to address this concern used observational studies of natural variation in diversity^{14,15}

72 and were limited in their ability to separate effects of diversity from other confounding factors¹⁶.
73 In response, more recent studies have directly manipulated diversity using experimentally
74 established communities (i.e. biodiversity experiments) to assess its impact on ecosystem
75 functioning, particularly aboveground net primary production (ANPP)¹⁻⁷. Numerous biodiversity
76 experiments have demonstrated that greater species diversity promotes greater stability of
77 productivity through time^{2,3,6} with asynchronous response of species to environmental
78 fluctuations as an important underlying mechanism^{1,2}. There would be no stabilizing effect if
79 species fluctuated in perfect synchrony. However, asynchrony in species response to
80 environmental fluctuations causes declines in the biomass of some species to be compensated by
81 increases in others, thus buffering temporal fluctuation in the productivity of the whole
82 community. Species asynchrony can increase the stability of aggregate functions in species-rich
83 communities because compensatory effects are more likely to occur when the species pool is
84 larger and more diverse¹⁷⁻¹⁹.

85

86 Biodiversity experiments, in turn, have their own limitations and their relevance to natural
87 grassland ecosystems is debated^{11,12,16}. For example, experimental gradients of diversity are
88 usually assembled randomly from a local species pool, whereas in natural systems composition
89 and diversity are influenced by a variety of factors including nutrient availability, climatic
90 conditions and anthropogenic land-use⁹⁻¹¹. It is also likely that diversity may not be the only, or
91 even the primary, driver of the stability of ANPP^{20,21} but few experiments have addressed
92 changes in both biodiversity and other aspects of global change simultaneously.

93

94 In natural grasslands, the situation is likely to be complex since anthropogenic impacts like
95 climate change and eutrophication are likely to change diversity - with potential consequences
96 for stability - as well as having their own direct effects on stability^{4,13,22,23}. In particular,
97 anthropogenic increases in nutrient inputs into grasslands (via direct organic and inorganic
98 fertilization and atmospheric deposition) affect the structure and functioning of natural
99 ecosystems worldwide^{8,24,25}. For instance, nutrient enrichment usually increases productivity and
100 reduces plant diversity^{24,25}. However, the effect of eutrophication on the stability of productivity
101 in natural grasslands remains unclear. Based on theory and results limited to single-site
102 experiments^{22,23}, we expect eutrophication to reduce the stability of productivity since the well-
103 known negative effects of nutrient enrichment on diversity^{24,25} could in turn reduce species
104 asynchrony and stability^{1,6,14,22,26}. However, eutrophication may have additional impacts on
105 stability independent of any changes in diversity. The temporal stability of ANPP is the ratio of
106 the temporal mean to the temporal standard deviation; hence an increase in stability can result
107 from an increase in the mean, a decrease in the standard deviation, or both. Since eutrophication
108 is expected to increase productivity it may have a stabilizing effect by increasing the temporal
109 mean. However, there is also the potential for effects of eutrophication on stability via changes in
110 the temporal standard deviation but these are less well understood. We therefore require a better
111 picture of how global change drivers affect ecosystem stability both via changes in diversity and
112 via other routes. Here, we compare the relationship between diversity and stability found in
113 grassland biodiversity experiments with those in fertilized and unfertilized plots in natural
114 grasslands. We also assess the effects of eutrophication on the diversity-stability relationship
115 both through changes in diversity and via other routes.

116

117 We evaluated the relationships between species diversity, species asynchrony and stability of
118 ANPP across 41 naturally-assembled grassland ecosystems on five continents (Extended Data
119 Fig. 1, Extended Data Table 1) using data from the Nutrient Network (NutNet, nutnet.org)
120 collaborative experiment^{27,28}. We used standardized methods to assess plant diversity and ANPP
121 at each site in both unmanipulated controls and experimentally fertilized plots in a well-
122 replicated design. We quantified diversity as the average plant species richness in standard 1 m²
123 plots over a three-year period. Stability can take a variety of meanings in the ecological
124 literature^{29,30}; here we focus on temporal stability of community-level, aboveground live plant
125 biomass from all species in a plot (a measure of ANPP) over three years. We define temporal
126 stability for each plot as the temporal mean of ANPP divided by its temporal variability – that is
127 the temporal standard deviation over a common time period (see Methods).

128
129 Stability of ANPP was positively associated with plant diversity in the unmanipulated
130 communities (Fig. 1a). Using a hierarchical sampling design and statistical model we found that
131 stability increased with diversity consistently within and among sites resulting in parallel
132 relationships (colored and black lines respectively in Fig. 1a). The consistent relationship
133 between diversity and stability is concordant with experimental results obtained in grasslands
134 across Europe¹ and experiments and observations at single locations^{2,3,6,21,26}. We used multiple
135 regression to evaluate the influence of plant diversity and key biotic and abiotic factors, on
136 stability in our 41 grasslands. Stability was still associated with diversity after using covariates to
137 control for differences in average site productivity and climatic conditions including annual
138 trends, seasonality and extreme or limiting environmental factors (Extended Data Table 1 and 2).

139 Together these results demonstrate that temporal stability of ANPP was positively related to
140 variation in plant diversity in our 41 naturally-assembled grassland ecosystems.

141
142 We determined the role of species asynchrony as a mechanism promoting stability by using a
143 community-wide measure that allows direct comparison among communities with different
144 species numbers¹⁷⁻¹⁹. Because the biomass of individual plant species was available at few sites,
145 we used estimates based on our three-year record of the percent cover of individual plant species
146 in each plot (see Methods). Our analysis of potential stability mechanisms showed that species
147 asynchrony was positively related to plant diversity (Fig. 1b) and stability (Fig. 2a), consistent
148 with theory on the stabilizing effects of species asynchrony in species-rich communities. Greater
149 stability at higher diversity can also result from an increase in the temporal mean of ANPP with
150 diversity (a ‘performance-enhancing effect’ that results in a higher ratio of the temporal mean
151 relative to the temporal variation)^{1,17}. Consistent with earlier NutNet analyses²⁷, we found that
152 the temporal mean of ANPP was not related to plant diversity (Fig. 3a). Although it is an indirect
153 test, our result provides no support for a performance-enhancing effect in stabilizing higher
154 diversity communities in our study. Instead, we found stronger support for a decrease in temporal
155 variation of ANPP (measured by the standard deviation) with diversity (Fig. 3a). In other words
156 greater stability at higher diversity resulted because diversity decreased the temporal variation of
157 ANPP relative to its mean resulting in a more stable mean-variance ratio.

158
159 To compare our results from naturally-assembled grasslands to results from artificially-
160 assembled biodiversity experiments, we calculated values of species asynchrony from the
161 BIODDEPTH experiment¹. BIODDEPTH is a pan-European network of grassland biodiversity

162 experiments conducted at eight field sites with a comparable hierarchical design, plot size and
163 measurements (see Methods). Our results are comparable to BIODDEPTH because both studies
164 use the same three-year experimental duration and cover a similar range of diversity levels
165 (although by design, biodiversity experiments feature many more low diversity communities
166 than observational surveys). We found that the sign and slope of the overall relationships
167 between diversity and stability and between diversity and asynchrony from our global multisite
168 study were comparable to those from the BIODDEPTH network of grassland biodiversity
169 experiments (compare the solid and dashed lines in Fig 1e and 1f).

170

171 We tested the impact of eutrophication on temporal stability and species asynchrony by using
172 data from NutNet plots that were fertilized for three years with a combination of nitrogen
173 phosphorus, potassium and micronutrients (see Methods). Fertilization weakened the positive
174 effect of diversity on stability and species asynchrony (compare the solid and dashed lines in Fig.
175 1c and d). We expected this result based on theory since nutrient enrichment often reduces
176 diversity^{24,25}, which could in turn reduce species asynchrony and stability^{1,6,14,22,26}. However,
177 while fertilization reduced diversity by an average of 1.3 species [95% CI = 0.7 – 1.9] per site
178 (corresponding to a reduction of diversity from 2.0% to 16.9% relative to average levels in the
179 control plots ranging from 4.4 to 32.3 species/m² (Extended Data Table 1)), counter to
180 expectations this loss of diversity did not lead to a reduction of stability through a decrease in
181 species asynchrony (Extended Data Fig. 2). Instead, the reduced slope of the diversity-stability
182 relationship in the fertilized communities (Fig. 1c) can be explained by a combination of two
183 factors. First, fertilization increased the temporal variation of ANPP in diverse communities
184 compared to unmanipulated communities (compare the dashed lines in Fig. 3a and b). Because

185 fertilization generally increased mean productivity compared to unmanipulated communities
186 (compare the solid lines in Fig. 3a and b), this increased variation weakened the positive effect of
187 diversity on stability compared to unmanipulated communities. Second, fertilization resulted in a
188 decrease in species asynchrony in diverse communities compared to unmanipulated communities
189 (compare the dashed and solid lines in Fig 1d). Because fertilization did not alter the positive
190 relationship between species asynchrony and stability (Fig. 2b), this decrease in species
191 asynchrony resulted in decreased stability in diverse communities compared to unmanipulated
192 communities. In total, the results of our fertilization experiment did not show the expected
193 destabilizing effects of diversity loss. Instead, eutrophication affected stability directly via a
194 combination of diversity-dependent effects on species asynchrony and on the temporal variation
195 of productivity. These direct effects of eutrophication on the diversity-stability relationship could
196 not have been anticipated from studies of natural or experimental diversity gradients.

197
198 In conclusion, the results of our observational study of naturally-assembled grassland
199 communities are consistent with a stabilizing effect of asynchronous species responses to
200 environmental fluctuations in more diverse plant communities – a result previously restricted to
201 biodiversity experiments¹ and observational studies at single locations^{21,26}. However, the global
202 change drivers causing loss of diversity may have additional effects on stability. The results of
203 our fertilization experiment demonstrate impacts on stability that were not caused by changes in
204 diversity but came about through effects of eutrophication on both the temporal variation in
205 production and on species asynchrony. However, while the effects of fertilization on stability
206 were not caused by species loss, the changes in species asynchrony and temporal variation that
207 were responsible were both affected by levels of community diversity. Predicting the effects of

208 global change drivers therefore requires a better understanding of their direct effects on
209 ecosystem stability as well as their indirect effects through changes in diversity. Our results point
210 out that while eutrophication is intended to increase average levels of productivity it can also
211 impact its temporal stability. Therefore, sustainable management of grassland ecosystems
212 requires a better understanding of the complex inter-relationships between diversity, productivity
213 and stability and how they are impacted by fertilization.

214

215 **Methods summary**

216

217 The 41 sites are part of the Nutrient Network Global Research Cooperative²⁸ (Extended Data
218 Fig. 1, Extended Data Table 1, <http://nutnet.org/>). Experimental plots included untreated controls
219 and plots with nitrogen, phosphorus and potassium and micronutrients added in combination
220 (NPK). The analyses presented here include all sites with the first three years of post-treatment
221 community-level ANPP ($\text{g m}^{-2} \text{yr}^{-1}$) and species-level ANPP estimates based on percent cover
222 (see Methods).

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

226 Ecosystem temporal stability was defined for each plot as μ/σ where μ is the temporal mean of
227 ecosystem-level ANPP and σ its temporal standard deviation over the three year period. Species
228 asynchrony was measured for each plot as $1 - \varphi_b$ where φ_b is species synchrony and calculated as
229 follows: $1 - \varphi_b = 1 - \frac{\sigma^2}{(\sum_{i=1}^S \sigma_i)^2}$, where σ_i is the temporal standard deviation of species i in a plot
230 with S species over the three years¹⁸. Thus, stability and species asynchrony are related such that

231 higher levels of species asynchrony are associated with greater stability of the community as a
232 whole¹⁴.

233 We modeled relationships with linear mixed-effects models using the lme function from the
234 nlme library in R 2.15.1. To improve normality, ecosystem temporal stability and community-
235 wide species asynchrony were log transformed before analyses. Sites and blocks nested within
236 sites for the NutNet data and sites and species composition nested within sites for BIODDEPTH
237 were treated as random effects, allowing both the intercepts and slopes of the regression vs.
238 diversity to vary among sites if supported by model selection.

239

240 **Full Methods** and any associated references are available in the online version of the paper at
241 www.nature.com/nature.

242

243 **References and notes**

- 244 1 Hector, A. *et al.* General stabilizing effects of plant diversity on grassland productivity through
245 population asynchrony andoveryielding. *Ecology* **91**, 2213-2220, (2010).
- 246 2 Isbell, F. I., Polley, H. W. & Wilsey, B. J. Biodiversity, productivity and the temporal stability of
247 productivity: patterns and processes. *Ecol. Lett.* **12**, 443-451, (2009).
- 248 3 Tilman, D., Reich, P. B. & Knops, J. M. H. Biodiversity and ecosystem stability in a decade-long
249 grassland experiment. *Nature* **441**, 629-632, (2006).
- 250 4 Reich, P. B. Elevated CO₂ Reduces Losses of Plant Diversity Caused by Nitrogen Deposition.
251 *Science* **326**, 1399-1402, (2009).
- 252 5 Naeem, S., Thompson, L. J., Lawler, S. P., Lawton, J. H. & Woodfin, R. M. Declining biodiversity
253 can alter the performance of ecosystems. *Nature* **368**, 734-737, (1994).

254 6 Bezemer, T. M. & van der Putten, W. H. Ecology - Diversity and stability in plant communities.
255 *Nature* **446**, E6-E7, (2007).

256 7 Cardinale, B. J. Biodiversity loss and its impact on humanity. *Nature* **489**, 326-326, (2012).

257 8 Vitousek, P. M., Mooney, H. A., Lubchenco, J. & Melillo, J. M. Human domination of Earth's
258 ecosystems. *Science* **277**, 494-499, (1997).

259 9 Selmants, P. C., Zavaleta, E. S., Pasari, J. R. & Hernandez, D. L. Realistic plant species losses
260 reduce invasion resistance in a California serpentine grassland. *J. Ecol.* **100**, 723-731, (2012).

261 10 Zavaleta, E. S. & Hulvey, K. B. Realistic species losses disproportionately reduce grassland
262 resistance to biological invaders. *Science* **306**, 1175-1177, (2004).

263 11 Srivastava, D. S. & Vellend, M. Biodiversity-ecosystem function research: Is it relevant to
264 conservation? *Annual Review of Ecology, Evolution and Systematics* **36**, 267-294, (2005).

265 12 Loreau, M. *et al.* Ecology - Biodiversity and ecosystem functioning: Current knowledge and
266 future challenges. *Science* **294**, 804-808, (2001).

267 13 Ives, A. R. & Carpenter, S. R. Stability and diversity of ecosystems. *Science* **317**, 58-62, (2007).

268 14 Tilman, D. & Downing, J. A. Biodiversity and stability in grasslands. *Nature* **367**, 165-175, (1994).

269 15 McNaughton, S. J. Stability and diversity of ecological communities. *Nature* **274**, 251-253,
270 (1978).

271 16 Huston, M. A. Hidden treatments in ecological experiments: Re-evaluating the ecosystem
272 function of biodiversity. *Oecologia* **110**, 449-460, (1997).

273 17 Yachi, S. & Loreau, M. Biodiversity and ecosystem productivity in a fluctuating environment: The
274 insurance hypothesis. *Proc. Natl. Acad. Sci. U. S. A.* **96**, 1463-1468, (1999).

275 18 Loreau, M. & de Mazancourt, C. Species synchrony and its drivers: Neutral and nonneutral
276 community dynamics in fluctuating environments. *American Naturalist* **172**, E48-E66, (2008).

277 19 Loreau, M. *From Populations to Ecosystems: Theoretical Foundations for a New Ecological*
278 *Synthesis*. (Princeton University Press, 2010).

279 20 Sankaran, M. & McNaughton, S. J. Determinants of biodiversity regulate compositional stability
280 of communities. *Nature* **401**, 691 - 693, (1999).

281 21 Bai, Y., Han, X., Wu, J., Chen, Z. & Li, L. Ecosystem stability and compensatory effects in the inner
282 Mongolia grassland. *Nature* **431**, 181-184, (2004).

283 22 Yang, Z. L., van Ruijven, J. & Du, G. Z. The effects of long-term fertilization on the temporal
284 stability of alpine meadow communities. *Plant and Soil* **345**, 315-324, (2011).

285 23 Yang, H. J. *et al.* Diversity-dependent stability under mowing and nutrient addition: evidence
286 from a 7-year grassland experiment. *Ecol. Lett.* **15**, 619-626, (2012).

287 24 Stevens, C. J., Dise, N. B., Mountford, J. O. & Gowing, D. J. Impact of nitrogen deposition on the
288 species richness of grasslands. *Science* **303**, 1876-1879, (2004).

289 25 Hautier, Y., Niklaus, P. A. & Hector, A. Competition for Light Causes Plant Biodiversity Loss After
290 Eutrophication. *Science* **324**, 636-638, (2009).

291 26 Tilman, D. Biodiversity: population versus ecosystem stability. *Ecology* **77**, 350-353, (1996).

292 27 Adler, P. B. *et al.* Productivity Is a Poor Predictor of Plant Species Richness. *Science* **333**, 1750-
293 1753, (2011).

294 28 Borer, E. T. *et al.* Finding generality in ecology: a model for globally distributed experiments.
295 *Methods Ecol. Evol.*, (2013).

296 29 May, R. M. *Stability and complexity in model ecosystems*. (Princeton University Press, 1973).

297 30 Pimm, S. L. The complexity and stability of ecosystems. *Nature* **307**, 669-674, (1984).

298

299 **Supplementary Data Information** is linked to the online version of the paper at
300 www.nature.com/nature.

301
302 **Acknowledgments** The research leading to these results has received funding from the European
303 Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 298935 to Y.
304 Hautier (with A. Hector and E. Seabloom). This work was generated using data from the
305 Nutrient Network (<http://nutnet.org>) experiment, funded at the site-scale by individual
306 researchers. Coordination and data management have been supported by funding to E. Borer and
307 E. Seabloom from the National Science Foundation Research Coordination Network (NSF-DEB-
308 1042132), the Long Term Ecological Research program (NSF-DEB-1234162 to Cedar Creek as
309 well as other LTER sites), and the Institute on the Environment at the University of Minnesota
310 (DG-0001-13). We also thank the Minnesota Supercomputer Institute for hosting project data
311 and the Institute on the Environment for hosting Network meetings. We are grateful to Rita S. L.
312 Veiga, Forest Isbell, Raphael K. Didham, Heather McGinness and Michael O'Brien for
313 suggestions that improved the manuscript.

314
315 **Author contributions** E.S., E.T.B., W.S.H. and E.L. are Nutrient Network coordinators. Y.H.
316 and A.H. developed and framed research questions. Y.H., E.S., E.T.B., P.A., W.S.H., H.H.,
317 A.S.MD., C.S., J.D.B., Y.B., C.C., S.L.C., E.I.D., K.D., P.A.F., J.F., D.S.G., V.L.J., J.A.K.,
318 J.M.H.K., K.J.L., W.L., R.L.MC., B.M., J.L.M., S.M.P., A.C.R., M.S., M.S. and A.H. collected
319 the data being used in this analysis. Y.H. and A.H. analyzed the data. E.S., E.T.B., H.H., E.L.,
320 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
321 paper with input from all authors.

322

323 **Author information** Reprints and permissions information is available at
324 www.nature.com/reprints. The authors declare no competing financial interests. Readers are
325 welcome to comment on the online version of this article at www.nature.com/nature.
326 Correspondence and requests for materials should be addressed to Y.H. (hauti001@umn.edu).
327

328 **Fig. 1. Relationships of temporal stability of ANPP (upper row) and species asynchrony**
329 **(lower row) with species diversity in the unmanipulated (a-b) and fertilized communities (c-**
330 **d) of the Nutrient Network, and the BIODDEPTH network of grassland biodiversity**
331 **experiments (e-f).** Relationships of temporal stability of ANPP (temporal mean/temporal
332 standard deviation; natural log transformed for analysis) of 41 grassland sites of the Nutrient
333 Network were positive in the unmanipulated communities (**a-b**) (slopes and 95% CIs = 0.028
334 [0.006 – 0.050] and 0.060 [0.023 – 0.097]), but not detectible in the fertilized communities (**c-d**)
335 (-0.001 [-0.025 – 0.022] and 0.008 [-0.031 – 0.047]). Relationships in the BIODDEPTH network
336 were positive (**e-f**) (0.018 [0.003 – 0.039] and 0.073 [0.053 – 0.093]). Species asynchrony varies
337 from zero (perfect synchrony) to one (perfect asynchrony). Species richness values for the
338 Nutrient Network are average values over the three years of post-treatment data. Points are
339 values for individual plots (n=117 for Nutrient Network, n=480 for BIODDEPTH). Black lines are
340 the back-transformed fixed-effect linear regression slopes among sites from the mixed-effects
341 model, while colored lines show patterns within sites. Dashed lines report regression slopes
342 among sites in the unmanipulated communities of the Nutrient Network. Colours correspond to
343 the “Colour code” column in Extended Data Table 1.

344

345 **Fig. 2. Relationships of temporal stability of ANPP (natural log transformed) and species**
346 **asynchrony in the unmanipulated (a) and fertilized communities of 41 grassland sites of the**
347 **Nutrient Network (b).** The temporal stability was greatest in plots where species fluctuations
348 were asynchronised in both the unmanipulated (slope and 95% CIs = 1.93 [1.70 – 2.16]) and
349 fertilized communities of 41 grassland sites of the Nutrient Network (1.90 [1.58 – 2.21]). Points

350 are values for individual plots (n=117). Colours correspond to the “Colour code” column in
351 Extended Data Table 1.

352

353 **Fig. 3. Relationships of temporal mean and standard deviation of ANPP (natural log**
354 **transformed) with species diversity in the unmanipulated (a) and fertilized communities (b)**
355 **of 41 grassland sites of the Nutrient Network.** Temporal mean was not related to species
356 diversity (slope and 95% CIs = 0.01 [-0.02 – 0.03]) in the unmanipulated communities (a) while
357 standard deviation was negatively related to species diversity (-0.03 [-0.05 – -0.01]), suggesting
358 that greater stability at higher diversity in the unmanipulated communities (Fig. 1a) resulted from
359 a decrease in temporal variation. Both temporal mean (slope and 95% CIs = 0.01 [-0.02 – 0.03])
360 and standard deviation (slope and 95% CIs = 0.01 [-0.02 – 0.04]) were not related to species
361 diversity in the fertilized communities (b). Fertilization increased the temporal variation in
362 diverse communities compared to unmanipulated communities resulting in reduced positive
363 effect of diversity on stability in fertilized communities (Fig. 1c). Points are values for individual
364 plots (n=117).

365

366 **Methods**

367

368 **Site selection and experimental design.** The 41 study sites are part of the Nutrient Network
369 (NutNet) Global Research Cooperative (Extended Data Fig. 1, Extended Data Table 1,
370 <http://nutnet.org/>). See Borer *et al.*²⁸ for a complete description of site selection, methods, and
371 measurements. To be as representative as possible of realistic grassland ecosystems, our analyses
372 included sites covering a wide range of grassland habitats (e.g. alpine grassland, prairie, pasture,
373 shrub steppe, savanna, old field). Thus, the among-site variation across NutNet sites captures a
374 globally-relevant gradient of fine-scale (1 m²) and site-level variation in factors including
375 aboveground biomass, species richness, land-use history, and environmental variables (Extended
376 Data Table 1). In some sites, human land-use (grazing, burning and mowing as part of the
377 traditional site management) is currently or has been recently carried on (Extended Data Table
378 1). However, our analyses were robust to land-use history: effects of species richness were
379 similar after we removed 13 sites with strong anthropogenic influence.

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

383 Treatments were randomly assigned to the 25 m² plots, and were replicated in three blocks at
384 most sites, although the number of blocks ranged from one to six among sites (Extended Data
385 Table 1). Treatments and sampling followed a standardized protocol at all sites, detailed in Borer
386 *et al.*²⁸. Treatment application started at most sites in 2008, though eight sites started in 2009 and
387 two sites in 2010. For this study, we included all sites with three-years of post-treatment data
388 collection. We used data collected during the first three-years of post-treatment data collection so

389 that our results are independent of the time since the start of treatment application. All of our
390 sites had three years of post-treatment data, though 3 sites had discontinuous data collection
391 (Extended Data Table 1). Longer time series currently exist for only a limited number of sites,
392 but the results were qualitatively the same when extended to 4 and 5 years.

393

394 **Climate data.** We quantified precipitation and temperature data using the WorldClim Global
395 Climate database³¹ (version 1.4; <http://www.worldclim.org/>). A Principal Component Analysis
396 (PCA) was used to reduce the number of climatic variables, many of which were collinear,
397 resulting in a subset of bioclimatic variables representing annual trends: mean annual
398 temperature (°C) and precipitation (mm); seasonality: mean annual range in temperature,
399 standard deviation in temperature, coefficient of variation of precipitation; and extreme or
400 limiting environmental factors: mean temperature during the wettest 4 months.

401

402 **Fertilization.** Nitrogen (N), phosphorus (P) and potassium (K) were applied annually to
403 fertilized plots, prior to the beginning of the growing season, at relatively high rates, $10 \text{ g m}^{-2} \text{ y}^{-1}$.
404 These rates are comparable to other grassland experiments that aim to alter diversity³². N was
405 supplied as time release urea $(\text{NH}_2)_2\text{CO}$ or ammonium nitrate NH_4NO_3 (the N form did not have
406 differential effects on production²⁸). P was supplied as triple super phosphate $\text{Ca}(\text{H}_2\text{PO}_4)_2$ and K
407 as potassium sulfate K_2SO_4 . In addition, $100 \text{ g m}^{-2} \text{ y}^{-1}$ of a micronutrient mix (Fe, S, Mg, Mn,
408 Cu, Zn, B, Mo) was applied to the K-addition plots once at the start of the experiment but not in
409 the following years to avoid toxicity.

410

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

423

424 **Productivity.** We used aboveground live biomass as a measure of primary productivity; an
425 effective estimator of aboveground net primary production (ANPP) in herbaceous vegetation^{35,36}.
426 At some sites with strongly seasonal communities, cover and biomass were estimated twice
427 during the year to assemble a complete list of species and the summed biomass of each species
428 was used in the analyses (Extended Data Table 1). However, our results were retained when we
429 performed analyses excluding these sites.

430

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

433 BIODEPTH comprised a consortium of eight coordinated biodiversity experiments that
434 manipulated plant diversity at different European grassland sites^{1,37,38}. The analyses presented
435 here use data on net aboveground biomass production ($\text{g m}^{-2} \text{ year}^{-1}$) of species from the
436 experimental plots at each of the eight BIODEPTH field sites for the three main years of the
437 project^{1,38}. The dataset comprises information on 480 plots each containing between 1 and 32
438 species. In total this produces 1934 data points per year, with each data point reporting the
439 biomass of a species in an individual plot. Each monoculture or species mixture was replicated in
440 two identical plots (with a few exceptions: five plant assemblages were replicated 4 times³⁸).
441 Monocultures were removed from the analysis to produce a more comparable range of species
442 richness.

443

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

446

447 **Asynchrony.** Species asynchrony was measured for each plot as $1 - \varphi_b$, where φ_b is species
448 synchrony and calculated as follows: $1 - \varphi_b = 1 - \frac{\sigma^2}{(\sum_{i=1}^S \sigma_i)^2}$, where σ^2 is the temporal variance
449 in ecosystem function and σ_i is the temporal standard deviation in function of species i in a plot
450 with S species over the three years¹⁸. Thus, stability and species asynchrony are related such that
451 higher levels of species asynchrony are associated with greater stability of the community as a
452 whole^{18,39,40}.

453

454 **Analyses.** We modeled the relationships with linear mixed-effects models using the lme function
455 from the nlme library⁴¹ in R 2.15.1⁴². To improve normality, temporal stability of ANPP,

456 community-wide species asynchrony, temporal mean of ANPP and temporal standard deviation
457 of ANPP were log transformed before analyses. Changes in diversity, stability and asynchrony
458 were calculated as the average difference per block between the fertilized and unmanipulated
459 plots of the Nutrient Network. Sites and blocks nested within sites for the NutNet data and sites
460 and species composition nested within sites for BIODDEPTH, were treated as random effects
461 allowing both the intercepts and slopes of the regression vs. diversity to vary among sites if
462 supported by model selection. For the fixed-by-random-effects interactions, we used a model-
463 selection approach based on minimization of BIC⁴¹, in which we compared models with and
464 without a given random effect to determine which level of variation was required in the model.
465 In every case, model-selection for NutNet data retained variation between sites, but excluded
466 variation due to blocks, while model-selection for BIODDEPTH data retained variation between
467 sites and species composition. Inference for the fixed effects was based on 95% CIs.

468

469 31 Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G. & Jarvis, A. Very high resolution
470 interpolated climate surfaces for global land areas. *International Journal of Climatology* **25**,
471 1965-1978, (2005).

472 32 Harpole, W. S. & Tilman, D. Grassland species loss resulting from reduced niche dimension.
473 *Nature* **446**, 791-793, (2007).

474 33 McNaughton, S. J. Diversity and stability of ecological communities: a comment on the role of
475 empiricism in ecology. *American Naturalist* **111**, 515-525, (1977).

476 34 Fargione, J. *et al.* From selection to complementarity: shifts in the causes of biodiversity-
477 productivity relationships in a long-term biodiversity experiment. *Proceedings Of The Royal*
478 *Society B-Biological Sciences* **274**, 871-876, (2007).

479 35 Lauenroth, W. K., Hunt, H. W., Swift, D. M. & Singh, J. S. Estimating aboveground net primary
480 production in grasslands: A simulation approach. *Ecological Modelling* **33**, 297-314, (1986).

481 36 Oesterheld, M. & McNaughton, S. J. in *Methods in Ecosystem Science* (eds O. E. Sala, R. B.
482 Jackson, H. A. Mooney, & R. W. Howarth) 151–157 (Springer, 2000).

483 37 Hector, A. *et al.* Plant diversity and productivity experiments in European grasslands. *Science*
484 **286**, 1123-1127, (1999).

485 38 Spehn, E. M. *et al.* Ecosystem effects of biodiversity manipulations in European grasslands.
486 *Ecological Monographs* **75**, 37-63, (2005).

487 39 de Mazancourt, C. *et al.* Predicting ecosystem stability from community composition and
488 biodiversity. *Ecol. Lett.* **16**, 617-625, (2013).

489 40 Loreau, M. & de Mazancourt, C. Biodiversity and ecosystem stability: a synthesis of underlying
490 mechanisms. *Ecol. Lett.* **16**, 106-115, (2013).

491 41 Pinheiro, J. C. & Bates, D. M. Mixed-Effects Models in S and S-Plus. *Springer-Verlag: New York*,
492 (2000).

493 42 R Development Core Team. A language and environment for statistical computing (R Foundation
494 for Statistical Computing, Vienna, Austria, 2012).

495

496 **Extended Data Figure 1 | Locations of the 41 Nutrient Network sites included in this study.**

497 Numbers correspond to the “Site code” column in Extended Data Table 1.

498

499 **Extended Data Figure 2 | Effect of fertilization-induced changes in diversity on changes in**

500 **stability of ANPP (a) and changes in species asynchrony (b).** Changes in stability (a) (slope

501 and 95% CIs = 0.009 [-0.048 – 0.030]) and changes in species asynchrony (b) (0.012 [-0.004 –

502 0.027]) were not related to changes in species richness caused by fertilization. Flat lines
503 represent the overall non-significant mean effects. Nutrient-induced changes were calculated as
504 the average difference per block between fertilized and unmanipulated Nutrient Network plots.
505 Colours correspond to the “Colour code” column in Extended Data Table 1.

506

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

508 *Years of data collection used in the analyses.

509 †Number of blocks in each site.

510 ‡Mean annual temperature (°C)

511 §Mean annual precipitation (mm)

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

513 ¶Standard deviation in temperature

514 #Coefficient of variation of precipitation

515 ☆Mean temperature during wettest 4 months (°C).

516

517 **Extended Data Table 2 | Multiple regression evaluating the influence of plant diversity and** 518 **key biotic and abiotic factors, productivity and climate, on stability of ANPP in our 41** 519 **grasslands.**

520 ‡Mean annual temperature (°C)

521 §Mean annual precipitation (mm)

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

523 ¶Standard deviation in temperature

524 #Coefficient of variation of precipitation

525 ☆Mean temperature during wettest 4 months (°C).

526