1   **General destabilizing effects of eutrophication on grassland productivity at multiple**

2   **spatial scales**

3   Yann Hautier[1]*, Pengfei Zhang[1-4], Michel Loreau[5], Kevin R. Wilcox[6], Eric W. Seabloom[7],

4   Elizabeth T. Borer[7], Jarrett E.K. Byrnes[8], Sally E. Koerner[9], Kimberly J. Komatsu[10], Jonathan

5   S. Lefcheck[11], Andy Hector[12], Peter B. Adler[13], Juan Alberti[14], Carlos A. Arnillas[15], Jonathan

6   D. Bakker[16], Lars A. Brudvig[17], Miguel N. Bugalho[18], Marc Cadotte[19], Maria C. Caldeira[20],

7   Oliver Carroll[21], Mick Crawley[22], Scott L. Collins[23], Pedro Daleo[14], Laura E. Dee[24], Nico

8   Eisenhauer[25,26], Anu Eskelinen[25,27,28], Philip A. Fay[29], Benjamin Gilbert[30], Amandine

9   Hansar[31], Forest Isbell[7], Johannes M. H. Knops[32], Andrew S. MacDougall[21], Rebecca L.

10 McCulley[33], Joslin L. Moore[34], John W. Morgan[35], Akira S. Mori[36], Pablo L. Peri[37], Edwin T.

11 Pos[1], Sally A. Power[38], Jodi N. Price[39], Peter B. Reich[38,40], Anita C. Risch[41], Christiane

12 Roscher[25,42], Mahesh Sankaran[43], Martin Schütz[41], Melinda Smith[44,45], Carly Stevens[46], Pedro

13 M. Tognetti[47], Risto Virtanen[28], Glenda M. Wardle[48], Peter A. Wilfahrt[7], and Shaopeng

14 Wang[49]*

15

16 *Corresponding authors: y.hautier@uu.nl, shaopeng.wang@pku.edu.cn

17

18 [1]Ecology and Biodiversity Group, Department of Biology, Utrecht University, Padualaan 8,

19 3584 CH Utrecht, The Netherlands. [2]State Key Laboratory of Grassland and Agro-

20 Ecosystems, School of Life Science, Lanzhou University, Gansu Province, 730000, P. R.

21 China. [3]Institute of Eco-Environmental Forensics of Shandong University, Shandong

22 Province, 266237, P. R. China. [4]Ministry of Justice Hub for Research & Practice in Eco-

23 Environmental Forensics, Shandong Province, 266237, P. R. China. [5]Centre for Biodiversity

24 Theory and Modelling, Theoretical and Experimental Ecology Station, CNRS, 2 route du

25 CNRS, 09200 Moulis, France. [6]Department of Ecosystem Science and Management,

26 University of Wyoming, Laramie, WY, USA. [7]Department of Ecology, Evolution, and

27 Behavior, University of MN, St. Paul, MN, 55108 USA. [8]Department of Biology, University

28 of Massachusetts Boston, Boston, MA 02125. [9]Department of Biology, University of North

29 Carolina Greensboro, Greensboro, NC, USA. [10]Smithsonian Environmental Research Center,

30 Edgewater, MD 21037 USA. [11]Tennenbaum Marine Observatories Network, MarineGEO,

31 Smithsonian Environmental Research Center, Edgewater, MD 21037, USA. [12]University of

32 Oxford Department of Plant Sciences, OX1 3RB, UK. [13]Department of Wildland Resources

33 and the Ecology Center, Utah State University, Logan, UT, 84322, USA. [14]Instituto de

34 Investigaciones Marinas y Costeras (IIMyC), FCEyN, UNMdP-CONICET, CC 1260 Correo

Central, B7600WAG, Mar del Plata, Argentina. [15]Department of Physical and Environmental Sciences, University of Toronto at Scarborough, Scarborough, Canada. [16]School of Environmental and Forest Sciences, University of Washington, Seattle, WA 98195-4115 USA. [17]Department of Plant Biology and Program in Ecology, Evolutionary Biology, and Behavior, Michigan State University, East Lansing, MI, USA. [18]Centre for Applied Ecology "Prof. Baeta Neves" (CEABN-InBIO), School of Agriculture, University of Lisbon, Portugal. [19]Department of Biological Sciences, University of Toronto at Scarborough, Scarborough, Canada. [20]Forest Research Centre, School of Agriculture, University of Lisbon, Portugal. [21]Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada N1G2W1. [22]Life Sciences, Imperial College London, Silwood Park, Ascot, SL5 7PY, UK. [23]University of New Mexico, Department of Biology, Albuquerque, NM 87131 USA. [24]Department of Ecology and Evolutionary Biology, University of Colorado at Boulder, 1560 30th Street, Boulder, Colorado 80309-0450 USA. [25]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany. [26]Leipzig University, Institute of Biology, Deutscher Platz 5e, 04103 Leipzig, Germany. [27]Department of Physiological Diversity, Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany. [28]Department of Ecology and Genetics, University of Oulu, Oulu, Finland. [29]USDA-ARS Grassland, Soil, and Water Research Laboratory, Temple, Texas 76502 USA. [30]Department of Ecology and Evolutionary Biology, University of Toronto, Canada M5S3B2. [31]Centre de recherche en écologie expérimentale et prédictive (CEREEP-Ecotron IleDeFrance), Département de biologie, Ecole normale supérieure, CNRS, PSL University, 77140, St-Pierre-les-Nemours, France. [32]Department of Heatth and Environmental Sciences, Xi'an Jiaotong liverpool University, Suzhou, Jiangsu, 214123, China. [33]University of Kentucky, Plant & Soil Science, 1405 Veterans Drive, Lexington KY 40546-0312. [34]School of Biological Sciences, Monash University, Clayton Campus, Victoria 3800, Australia. [35]Department of Ecology, Environment & Evolution, La Trobe University, Bundoora 3086, Australia. [36]Graduate School of Environment and Information Sciences, Yokohama National University, 79-7 Tokiwadai, Hodogaya, Yokohama, Kanagawa, 240-8501, Japan. [37]INTA (National Institute of Agricultural Research)- UNPA (Southern Patagonia National University)-CONICET. Santa Cruz, Argentina. [38]Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, New South Wales 2751, Australia. [39]Institute of Land, Water and Society, Charles Sturt University, Albury, NSW, Australia, 2640. [40]Department of Forest Resources, University of Minnesota, Saint Paul, MN, USA. [41]Swiss Federal Institute for Forest, Snow and Landscape Research

2

69   WSL, Zuercherstrasse 111, 8903 Birmensdorf, Switzerland. [42]UFZ, Helmholtz Centre for

70   Environmental Research, Physiological Diversity, Permoserstrasse 15, 04318 Leipzig,

71   Germany. [43]Ecology & Evolution Group, National Centre for Biological Sciences, TIFR,

72   Bangalore, Karnataka 560065, India (and0 School of Biiology, University of Leeds, Leeds

73   LS2 9JT, UK. [44]Department of Biology, Colorado State University, Fort Collins, CO, 80523.

74   [45]Graduate Degree Program in Ecology, Colorado State University, Fort Collins, CO, 80523.

75   [46]Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ. [47]IFEVA-

76   Facultad de Agronomia, Universidad de Buenos Aires - CONICET, Av San Martin 4453

77   C1417DSE, Ciudad Autonoma de Buenos Aires, Argentina. [48]School of Life and

78   Environmental Sciences, University of Sydney, Sydney, New South Wales 2006, Australia.

79   [49]Institute of Ecology, College of Urban and Environmental Science, and Key Laboratory for

80   Earth Surface Processes of the Ministry of Education, Peking University, 100871, Beijing,

81   China.

82

83   **Eutrophication is a widespread environmental change that usually reduces the stabilizing**

84   **effect of plant diversity on productivity in local communities. Whether this effect is scale**

85   **dependent remains to be elucidated. Here, we determined the relationship between plant**

86   **diversity and temporal stability of productivity for 243 plant communities from 42**

87   **grasslands across the globe and quantified the effect of chronic fertilization on these**

88   **relationships. Unfertilized local communities with more plant species exhibited greater**

89   **asynchronous dynamics among species in response to natural environmental fluctuations,**

90   **resulting in greater local stability (alpha stability). Moreover, neighbourhood**

91   **communities that had greater spatial variation in plant species composition within sites**

92   **(higher beta diversity) had greater spatial asynchrony of productivity among**

93   **communities, resulting in greater stability at the larger scale (gamma stability).**

94   **Importantly, fertilization consistently weakened the contribution of plant diversity to**

95   **both of these stabilizing mechanisms, thus diminishing the positive effect of biodiversity**

96   **on stability at differing spatial scales. Our findings suggest that preserving grassland**

97   **functional stability requires conservation of plant diversity within and among ecological**

98   **communities. Furthermore, our results demonstrate the threat of increased ecosystem**

99   **eutrophication to the stable provisioning of grassland services across spatial scales under**

100   **current and future environmental conditions.**

101

102　Humans are altering global nutrient cycles via combustion of fossil fuels and fertilizer

103　application[1]. We have more than doubled pre-industrial rates of nitrogen (N) and phosphorus

104　(P) supply to terrestrial ecosystems[2]. Terrestrial N and P inputs are predicted to reach levels

105　that are three to four times pre-industrial rates by 2050[3]. This pervasive global eutrophication

106　will have dramatic consequences on the structure and functioning of terrestrial and aquatic

107　ecosystems[3]. In grasslands, nutrient enrichment usually increases primary productivity, but

108　reduces plant diversity and alters the ability of ecosystems to reliably provide functions and

109　services for humanity[4-7].

110

111　Concerns that eutrophication compromises both the diversity and stability of ecosystems have

112　led to a growing number of theoretical and empirical studies investigating how these ecosystem

113　responses may be mechanistically linked[4,6,8-11]. These studies, have repeatedly shown that the

114　positive effect of plant species richness on the temporal stability of community productivity in

115　ambient (unfertilized) conditions is usually reduced with fertilization[4-6]. However, these studies

116　have primarily focused on plant responses at relatively small scales (i.e., within single local

117　communities). Whether fertilization reduces the positive effect of diversity on temporal

118　stability at larger scales (i.e. among neighbouring local communities) remains unclear. Filling

119　this knowledge gap is important because the stable provision of ecosystem services is critical

120　for society[12]. This is especially true, given an increasing concern for large variability of

121　environmental conditions due to multiple anthropogenic influences including eutrophication

122　and climate change[13].

123

124　A recent theoretical framework allows the quantification of the processes that determine the

125　stability of ecosystem functioning at scales beyond the single local community (Fig. 1)[14-16].

126　Stability at any given scale is defined as the temporal mean of primary productivity divided by

127　its standard deviation[17]. Higher local scale community stability (*alpha stability*), can result

128　from two main processes. First, a higher average temporal stability of all species in the

129　community (*species stability*) can stabilize community productivity due to lower variation in

130　individual species abundances from year to year (Fig. 1b). Second, more asynchronous

131　temporal dynamics among species in response to environmental fluctuations (*species*

132　*asynchrony*) can stabilize community productivity because declines in the abundance of some

133　species through time are compensated for by increases in other species (Fig. 1c). Higher

134　stability at the larger scale (*gamma stability*) can result from higher alpha stability and more

135　asynchronous dynamics across local communities (*spatial asynchrony*) (Fig. 1d). Thus, the

136   stabilizing effect of spatial asynchrony on productivity at the larger scale (spatial insurance

137   hypothesis)[14,18] mirrors the stabilizing effect of species asynchrony on productivity at the local

138   scale (species or local insurance hypothesis)[8,16,19,20]. Higher species asynchrony and species

139   stability can result from higher local species diversity through higher *species richness*[9,21,22],

140   higher *species evenness*[8], or both (e.g. higher values of diversity indices - such as the *Shannon*

141   index - that combines the two[23]) (Fig. 1e). Higher spatial asynchrony can result from greater

142   local species diversity or higher variation in species composition among communities (*beta*

143   *diversity*)[16].

144

145   According to this framework, fertilization can affect the links between diversity, asynchrony

146   and stability across spatial scales (Fig. 1e, Table 1). At the local scale, fertilization can decrease

147   niche dimensionality, and favour a few dominant plant species by affecting the competitive

148   balance among species, potentially reducing the insurance effects of local diversity[7,22]. At the

149   larger scale, fertilization can reduce spatial heterogeneity in community composition, and

150   decrease variations among local plant community structure, potentially reducing the spatial

151   insurance effect of beta diversity[16]. Moreover, fertilization often reduces plant diversity which

152   could in turn reduce asynchrony and stability at multiple scales[4,9,17,24]. However, the role of

153   fertilization in mediating the functional consequences of biodiversity changes (variations in the

154   number, abundance and identities of species) and compensatory mechanisms (variation and

155   compensation in species responses) that can affect the stable provisioning of ecosystem

156   functions at larger spatial scales remains to be elucidated[25].

157

158   To our knowledge, only one recent study has assessed the effect of nutrient enrichment on

159   stability within and among interconnected communities in a temperate grassland[26]. By adding

160   different nitrogen treatments to communities in ten blocks spread out within a single site, that

161   study found that five years of chronic nitrogen addition reduced alpha stability through a

162   decline in species asynchrony, but had no effect on spatial asynchrony. However, these

163   conclusions were based on a single grassland site manipulating a single nutrient, with the

164   implicit assumption that the relationship between diversity and stability was unaffected by

165   eutrophication. This argues for multisite comparative studies assessing the generality of the

166   mechanistic links between these ecosystem responses to eutrophication.

167

168   Here, we use a coordinated, multi-site and multi-year nutrient enrichment experiment (+/-

169   chronic nitrogen, phosphorus and potassium addition, Nutrient Network[27]) to assess the scale

dependence of fertilization impacts on plant diversity and stability. Treatments were randomly assigned to 25 m² plots and were replicated in three blocks at most sites (Extended Data Table 1). Samples were collected in 1 m² subplots across 243 communities from 42 grassland sites on six continents and followed a standardized protocol at all sites[27]. We selected these sites as they contained between four to nine years of experimental duration (hereafter 'period of experimental duration') and three blocks per site, excluding additional blocks from sites that had more than three (Extended Data Table 1). Sites spanned a broad range of seasonal variation in precipitation and temperature (Extended Data Fig.1) and a wide range of grassland types (Extended Data Table 1). In our analysis, we treated each 1 m² subplot as a 'community' and the replicated subplots within a site as the 'larger scale' *sensu* Whittaker[28]. We computed diversity, asynchrony, and stability within a community (local 'alpha' scale) and across the three replicated communities within a site (larger 'gamma' scale) (see Methods). We then used bivariate analysis and structural equation modelling (SEM)[29] to assess fertilizer impacts and disentangle the relative contributions of diversity and asynchrony to stability (Fig. 1e).

Results and Discussion

Analyses of variance revealed the negative effects of nutrient inputs on biodiversity and stability at the two scales investigated, consistent with recent findings from a single site[26]. Fertilization consistently reduced species richness, alpha and gamma stability but had no effect on beta diversity (Extended Data Fig.2). Bivariate analyses further revealed the negative effects of nutrient inputs on biodiversity-stability relationships at the two scales investigated (Fig. 2). Relationships were generally consistent across the different periods of experimental duration considered (Extended Data Table 2). Under ambient (unfertilized) conditions, species richness was positively associated with alpha and gamma stability (Fig. 2a, b), but fertilization weakened the positive effect of species richness on stability at the two scales (Fig. 2c, d). Fertilization reduced local stability of grassland functioning by increasing temporal variability in species-rich communities (Extended Data Fig.3). Similarly, high beta diversity (variation in species composition among communities) was positively associated with spatial asynchrony and gamma stability under ambient conditions (Fig. 2e, f), but again fertilization weakened the positive effect of beta diversity on spatial asynchrony and gamma stability (Fig. 2g, h). These results remained when accounting for variation in climate using residual regression (Extended Data Fig.4), when using local diversity indices accounting for species abundance (Extended Data Fig.5), and when data were divided into overlapping intervals of four years (Extended Data Fig.6). Our results extend previous evidence of the negative impact of fertilization on the

6

diversity-stability relationship obtained within local plots and over shorter experimental periods[4,6,26]. Importantly, they show that these negative effects propagate from within to among communities. To our knowledge, our study is the first to report the negative impacts of fertilization on the relationships of beta diversity with spatial asynchrony and gamma stability.

To understand the relative role of local vs. larger scale community properties in determining asynchrony and stability at different spatial scales, we conducted SEM analyses including all measures in a single causal model (Fig. 3, Extended Data Fig.7, Extended Data Table 3). Under ambient conditions, SEM revealed that higher plant species richness contributed to greater alpha and gamma stability largely through higher asynchronous dynamics among species (species asynchrony, standardized path coefficient = 0.39), and not necessarily through greater species stability (standardized path coefficient = 0.01) (Fig. 3a, Extended Data Fig.8a, b). The positive association between species richness and alpha stability is consistent with existing experimental[17,24] and shorter-term observational evidence[4,30,31]. Our results confirm that the stabilizing effects of species richness in naturally-assembled grassland communities is largely driven by species asynchrony, but not species stability[4,6,22,26]. In addition, they show that the positive impact of species richness on the stability of community productivity via species asynchrony in turn leads to greater stability of productivity at the larger spatial scale.

While correlated with species richness, higher beta diversity also contributed to greater gamma stability through an independent pathway, namely via higher asynchronous dynamics among local communities (spatial asynchrony, standardized path coefficient = 0.20, Fig. 3a). While theoretical studies have suggested a role for beta diversity in driving spatial asynchrony[15,16], previous empirical studies conducted along a nitrogen gradient at a single site[26] or across 62 sites with non-standardized protocols[21] did not find an association between these two variables. Here, we show that the presence of different species among local communities is linked to higher variation in dynamics among them, demonstrating the stabilizing role of beta diversity at larger spatial scales through spatial asynchrony. This also indicates the need for multi-site replication with standardized treatments and protocols to detect such effects.

Importantly, fertilization acted to destabilize productivity at the local and larger spatial scale through several mechanisms (Fig. 3, Table 2). At the local scale, fertilization weakened the positive effects of plant species richness on alpha and gamma stability (Fig. 2a, c and b, d) via a combination of two processes (Fig. 3b, Extended Data Fig.8c, d). First, the positive

7

relationship between species richness and species asynchrony in the control communities (standardized path coefficient = 0.39, Fig. 3a), was weaker in the fertilized communities (standardized path coefficient = 0.20, Fig. 3b). Moreover, this general positive effect of richness on asynchrony was counteracted by a second stronger negative relationship of richness with species stability (standardized path coefficient = -0.37). Such negative effect of fertilization on species stability was not observed under ambient conditions and could be due to shifts in functional composition in species-rich communities from more stable conservative species to less stable exploitative species in a temporally variable environment[32,33]. Together, these two effects explain the overall weaker alpha stability at higher richness with fertilization. We did not find evidence that the loss of diversity caused by fertilization (an average of -1.8 ± 0.5 species m^{-2}, Extended Data Fig.2a, Extended Data Fig.9a) was related to the decline of alpha stability, confirming results from other studies[5,6] and earlier Nutrient Network results[4] obtained over shorter time periods. This could be because the negative feedback of the loss of richness caused by fertilization on stability requires a longer experimental duration, or greater loss of plant diversity, to manifest[9,34]. Another possible explanation is that fertilization may have a direct positive effect on stability, by increasing community biomass (t = 2.41, d.f. = 326, P = 0.016) and enhancing stability via overyielding effects[35], a formal test that would require monocultures.

At the larger scale, fertilization reduced the strength of the relationship between beta diversity and gamma stability by reducing the strength of the relationship between beta diversity and spatial asynchrony (standardized path coefficient = 0.20 in Fig. 3a vs. standardized path coefficient = 0.03 in Fig. 3b). This result provides evidence that fertilization can reduce the stabilizing role of spatial asynchrony among initially dissimilar communities. We did not find evidence that this was due to a negative feedback of changes in beta diversity caused by fertilization on gamma stability (Extended Data Fig.2b, Extended Data Fig.9b). The positive relationship between beta diversity and spatial asynchrony, and the negative impact of fertilization on that relationship, suggests that the spatial insurance effect caused by variation in species composition among local communities may be disrupted in a eutrophic world.

Our results support the idea that asynchronous dynamics among species in species-rich communities play a stabilizing role and show that this effect propagates to larger spatial scales[21,26]. Furthermore, our study is the first to report the positive association between beta diversity and gamma stability through spatial asynchrony in real-world grasslands.

8

Importantly, fertilization reduced the contribution of biodiversity to these stabilizing mechanisms at both scales, diminishing the local and spatial insurance of biodiversity on stability. Such diminished insurance effects lead to a reduced ecosystem stability at larger scales. Future climate will be characterised by more variability including more frequent extreme events[13]. Our results indicate that preserving ecosystem stability across spatial scales in a changing world requires conserving biodiversity within and among local communities. Moreover, policies and management procedures that prevent and mitigate eutrophication are needed to safeguard the positive effects of biodiversity on stability at multiple scales.

Methods

Study sites and experimental design

The study sites are part of the Nutrient Network (NutNet) experiment (Extended Data Table 1; http://nutnet.org/)[27]. Plots at each site are 5 × 5 m separated by at least 1 m. All sites included in the analyses presented here included unmanipulated plots and fertilized plots with nitrogen (N), phosphorus (P) and potassium and micronutrients (K) added in combination (NPK+). N, P and K were applied annually before the beginning of the growing season at rates of 10 gm^{-2} y^{-1}. N was supplied as time-release urea ($(NH_2)_2CO$) or ammonium nitrate (NH_4NO_3). P was supplied as triple super phosphate ($Ca(H_2PO_4)_2$), and K as potassium sulphate (K_2SO_4). In addition, a micronutrient mix (Fe, S, Mg, Mn, Cu, Zn, B and Mo) was applied at 100 gm^{-2} y^{-1} to the K-addition plots, once at the start of the experiment but not in subsequent years to avoid toxicity. Treatments were randomly assigned to the 25 m^2 plots and were replicated in three blocks at most sites (some sites had fewer/more blocks or were fully randomised). Sampling was done in 1 m^2 subplots and followed a standardized protocol at all sites[27].

Site selection

Data were retrieved on 1 May 2020. To keep a constant number of communities per site and treatment, we used three blocks per site, excluding additional blocks from sites that had more than three (Extended Data Table 1). Sites spanned a broad envelope of seasonal variation in precipitation and temperature (Extended Data Fig.1) and represent a wide range of grassland types including alpine, desert and semi-arid grasslands, prairies, old fields, pastures, savanna, tundra and shrub-steppe (Extended Data Table 1).

Stability and asynchrony measurements are sensitive to taxonomic inconsistencies. We adjusted the taxonomy to ensure consistent naming over time within sites. This was usually

306   done by aggregating taxa at the genus level when individuals were not identified to species in

307   all years. Taxa are however referred to as "species".

308   We selected sites that had a minimum of four years, and up to nine years of post-treatment data.

309   Treatment application started at most sites in 2008, but some sites started later resulting in a

310   lower number of sites with increasing duration of the study, from 42 sites with four years of

311   post-treatment duration to 15 sites with nine years of duration (Extended Data Table 1). Longer

312   time series currently exist but for a limited number of sites within our selection criteria.

313

314   *Primary productivity and cover*

315   We used above-ground live biomass as a measure of primary productivity, which is an effective

316   estimator of above-ground net primary production in herbaceous vegetation[36]. Primary

317   productivity was estimated annually by clipping at ground level all aboveground live biomass

318   from two 0.1 m^2 (10 x 100 cm) quadrats per subplot. For shrubs and subshrubs, leaves and

319   current year's woody growth were collected. Biomass was dried to constant mass at 60°C and

320   weighed to the nearest 0.01 g. Areal percent cover of each species was measured concurrently

321   with primary productivity in one 1 x 1m subplot in which no destructive sampling occurred.

322   Cover was visually estimated annually to the nearest percent independently for each species,

323   so that total summed cover can exceed 100% for multilayer canopies. Cover and primary

324   productivity were estimated twice during the year at some sites with strongly seasonal

325   communities. This allowed to assemble a complete list of species and to follow management

326   procedures typical of those sites. For those sites the maximum cover of each species and total

327   biomass were used in the analyses.

328

329   *Diversity, asynchrony and stability across spatial scales*

330   We quantified local scale and larger scale diversity indices across the three replicated 1-m^2

331   subplots for each site, treatment and duration period using cover data[37,38]. In our analysis, we

332   treated each subplot as a 'community' and the collective subplots as the 'larger scale' *sensu*

333   Whittaker[28]. Local scale diversity indices (species richness, species evenness, Shannon and

334   Simpson) were measured for each community and averaged across the three communities for

335   each treatment at each site resulting in one single value per treatment and site. Species richness

336   is the average number of plant species. Shannon is the average of Shannon–Weaver indices[39].

337   Species evenness is the average of the ratio of the Shannon–Weaver index and the natural

338   logarithm of average species richness (i.e. Pielou's evenness[40]). Simpson is the average of

339   inverse Simpson indices[41]. Due to strong correlation between species richness and other

10

common local diversity indices (Shannon: r = 0.90 (95% CIs = 0.87 – 0.92), Simpson: r = 0.88
(0.86 – 0.91), Pielou's evenness: r = 0.62 (0.55 – 0.68), with df = 324 for each), we used species
richness as a single, general proxy for those variables in our models. Results using these
diversity indices did not differ quantitatively from those presented in the main text using
species richness (Extended Data Fig.5), suggesting that fertilization modulate diversity effects
largely through species richness. Following theoretical models[15,16], we quantified abundance-
based gamma diversity as the inverse Simpson index over the three subplots for each treatment
at each site and abundance-based beta diversity as the multiplicative partitioning of abundance-
based gamma diversity: abundance-based beta equals the abundance-based gamma over
Simpson[28,42], resulting in one single beta diversity value per treatment and site. We used
abundance-based beta diversity index because it is directly linked to ecosystem stability in
theoretical models[15,16] and thus directly comparable to theories. We used the R functions
'diversity', 'specnumber', and 'vegdist' from the vegan package[43] to calculate Shannon-
Weaver, Simpson and species richness indices within and across replicated plots.

Stability at multiple scales was determined both without detrending and after detrending data.
For each species within communities, we detrended by using species-level linear models of
percent cover over years. We used the residuals from each regression as detrended standard
deviations to calculate detrended stability[17]. Results using detrended stability did not differ
quantitatively from those presented in the main text without detrending. Stability was defined
by the temporal invariability of biomass (for alpha and gamma stability) or cover (for species
stability and species asynchrony), calculated as the ratio of temporal mean to standard
deviation[14,17]. Gamma stability represents the temporal invariability of the total biomass of
three plots with the same treatment, alpha stability represents the temporal invariability of
community biomass averaged across three plots per treatment and per site, and species stability
represents the temporal invariability of species cover averaged across all species and the three
plots per treatment[14]. The mathematical formula are:

$$Species\ stability = \frac{\sum_{i,k} m_{i,k}}{\sum_{i,k} \sqrt{w_{ii,kk}}}$$

$$Alpha\ stability = \frac{\sum_k \mu_k}{\sum_k \sqrt{v_{kk}}}$$

$$Gamma\ stability = \frac{\sum_k \mu_k}{\sqrt{\sum_{k,l} v_{kl}}}$$

where $m_{i,k}$ and $w_{ii,kk}$ denote the temporal mean and variance of the cover of species i in subplot
k; μ_k and v_{kk} denotes the temporal mean and variance of community biomass in subplot k,
and v_{kl} denotes the covariance in community biomass between subplot k and l. We then define

372 species asynchrony as the variance-weighted correlation across species, and spatial asynchrony

373 as the variance-weighted correlation across plots:

374 $$Species\ asynchrony = \frac{\sum_{i,k}\sqrt{w_{ii,kk}}}{\sum_k\sqrt{\sum_{ij,kl}w_{ij,kl}}}$$

375 $$Spatial\ asynchrony = \frac{\sum_k\sqrt{v_{kk}}}{\sqrt{\sum_{k,l}v_{kl}}}$$

376 where $w_{ij,kl}$ denotes the covariance in species cover between species i in subplot k and

377 species j in subplot l.

378 These two asynchrony indices quantify the incoherence in the temporal dynamics of species

379 cover and community biomass, respectively, which serve as scaling factors to link stability

380 metrics across scales[14] (Fig. 1). To improve normality, stability and asynchrony measures were

381 logarithm transformed before analyses. We used the R function 'var.partition' to calculate

382 asynchrony and stability across spatial scales[14].

383

384 *Climate data*

385 Precipitation and temperature seasonality were estimated for each site using the long-term

386 coefficient of variation of precipitation (MAP_VAR) and temperature (MAT_VAR)

387 respectively derived from the WorldClim Global Climate database (version 1.4;

388 http://www.worldclim.org/)[44].

389

390 *Analyses*

391 All analyses were conducted in R 4.0.2[45] with N=42 for each analysis unless specified. First,

392 we used analysis of variance (ANOVA) to determine the effect of fertilization and period of

393 experimental duration on biodiversity and stability at the two scales investigated. Models

394 including an autocorrelation structure with a first-order autoregressive model (AR(1)), where

395 observations are expected to be correlated from one year to the next, gave substantial

396 improvement in model fit when compared with models lacking autocorrelation structure.

397 Second, we used bivariate analyses and linear models to test the effect of fertilization and

398 period of experimental duration on biodiversity-stability relationships at the two scales

399 investigated. Again, models including an autocorrelation structure gave substantial

400 improvement in model fit (Extended Data Table 2)[46]. We ran similar models based on nutrient-

401 induced changes in diversity, stability and asynchrony. For each site, relative changes in

402 biodiversity, stability and asynchrony at the two scales considered were calculated as the

403 natural logarithm of the ratio between the variable in the fertilized and unmanipulated plots

(Extended Data Fig.9). Because plant diversity, asynchronous dynamics and temporal stability may be jointly controlled by inter-annual climate variability[22], we ran similar analyses on the residuals of models that included the coefficient of variation among years for each of temperature and precipitation. Results of our analyses controlling for inter-annual climate variability did not differ qualitatively from the results presented in the text (Extended Data Fig.4). Additionally, to test for temporal trends in stability and diversity responses to fertilization, we used data on overlapping intervals of four consecutive years. Results of our analyses using temporal trends did not differ qualitatively from the results presented in the text (Extended Data Fig.6). Inference was based on 95% confidence intervals.

Second, we used structural equation modelling (SEM)[29] with linear models, to evaluate multiple hypothesis related to key predictions from theories (Table 1). The path model shown in Fig. 1e was evaluated for each treatment (control and fertilized) and we ran separate SEMs for each period of experimental duration (from 4 to 9 years of duration). We generated a summary SEM by performing a meta-analysis of the standardized coefficients across all durations for each treatment. We then tested whether the path coefficients for each model differed by treatment by testing for a model-wide interaction with the `treatment` factor. A positive interaction for a given path implied that effects of one variable on the other are significantly different between fertilized and unfertilized treatments. We used the R functions 'psem' to fit separate piecewise SEMs[49] for each duration and combined the path coefficients from those models using the 'metagen' function[50].

Data availability

Code availability

References

1   Erisman, J. W. *et al.* Consequences of human modification of the global nitrogen cycle. *Philosophical Transactions of the Royal Society B-Biological Sciences* **368** (2013).
2   Galloway, J. N. The global nitrogen cycle: Past, present and future. *Science in China Series C-Life Sciences* **48**, 669-677 (2005).

13

439   3   Tilman, D., D'Antonio, Dobson, Simberloff, D. & etal. Forecasting agriculturally driven global
440   environmental change. *Science* **292**, 281-284 (2001).
441   4   Hautier, Y. *et al.* Eutrophication weakens stabilizing effects of diversity in natural grasslands.
442   *Nature* **508**, 521-525 (2014).
443   5   Xu, Z. W. *et al.* Environmental changes drive the temporal stability of semi-arid natural
444   grasslands through altering species asynchrony. *J. Ecol.* **103**, 1308-1316 (2015).
445   6   Zhang, Y. H. *et al.* Nitrogen enrichment weakens ecosystem stability through decreased
446   species asynchrony and population stability in a temperate grassland. *Global Change Biology*
447   **22**, 1445-1455 (2016).
448   7   Harpole, W. S. *et al.* Addition of multiple limiting resources reduces grassland diversity.
449   *Nature* **537**, 93-96 (2016).
450   8   Thibaut, L. M. & Connolly, S. R. Understanding diversity-stability relationships: towards a
451   unified model of portfolio effects. *Ecol. Lett.* **16**, 140-150 (2013).
452   9   Hautier, Y. *et al.* Anthropogenic environmental changes affect ecosystem stability via
453   biodiversity. *Science* **348**, 336-340 (2015).
454   10 Koerner, S. E. *et al.* Nutrient additions cause divergence of tallgrass prairie plant
455   communities resulting in loss of ecosystem stability. *J. Ecol.* **104**, 1478-1487 (2016).
456   11 Yang, H. J. *et al.* Diversity-dependent stability under mowing and nutrient addition: evidence
457   from a 7-year grassland experiment. *Ecol. Lett.* **15**, 619-626 (2012).
458   12 Millennium Ecosystem Assessment. *Ecosystems and Human Well-being: Synthesis*. (Island
459   Press, 2005).
460   13 Shukla, P. R. *et al.* in *Climate Change and Land: an IPCC special report on climate change,*
461   *desertification, land degradation, sustainable land management, food security, and*
462   *greenhouse gas fluxes in terrestrial ecosystems*   (IPCC 2019).
463   14 Wang, S. P., Lamy, T., Hallett, L. M. & Loreau, M. Stability and synchrony across ecological
464   hierarchies in heterogeneous metacommunities: linking theory to data. *Ecography* **42**, 1200-
465   1211 (2019).
466   15 Wang, S. P. & Loreau, M. Ecosystem stability in space: alpha, beta and gamma variability.
467   *Ecol. Lett.* **17**, 891-901 (2014).
468   16 Wang, S. P. & Loreau, M. Biodiversity and ecosystem stability across scales in
469   metacommunities. *Ecol. Lett.* **19**, 510-518 (2016).
470   17 Tilman, D., Reich, P. B. & Knops, J. M. H. Biodiversity and ecosystem stability in a decade-
471   long grassland experiment. *Nature* **441**, 629-632 (2006).
472   18 Loreau, M., Mouquet, N. & Gonzalez, A. Biodiversity as spatial insurance in heterogeneous
473   landscapes. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 12765-12770 (2003).
474   19 Lamy, T. *et al.* Species insurance trumps spatial insurance in stabilizing biomass of a marine
475   macroalgal metacommunity. *Ecology* **100** (2019).
476   20 Loreau, M. & de Mazancourt, C. Biodiversity and ecosystem stability: a synthesis of
477   underlying mechanisms. *Ecol. Lett.* **16**, 106-115 (2013).
478   21 Wilcox, K. R. *et al.* Asynchrony among local communities stabilises ecosystem function of
479   metacommunities. *Ecol. Lett.* **20**, 1534-1545 (2017).
480   22 Gilbert, B. *et al.* Climate and local environment structure asynchrony and the stability of
481   primary production in grasslands. *Global Ecology and Biogeography* (2020).
482   23 Zhang, Y., Loreau, M., He, N., Zhang, G. & Han, X. Mowing exacerbates the loss of ecosystem
483   stability under nitrogen enrichment in a temperate grassland. *Functional Ecology* **31**, 1637-
484   1646 (2017).
485   24 Hector, A. *et al.* General stabilizing effects of plant diversity on grassland productivity
486   through population asynchrony and overyielding. *Ecology* **91**, 2213-2220 (2010).
487   25 Mori, A. S., Isbell, F. & Seidl, R. beta-Diversity, Community Assembly, and Ecosystem
488   Functioning. *Trends in Ecology & Evolution* **33**, 549-564 (2018).

489	26	Zhang, Y. H. *et al.* Nitrogen addition does not reduce the role of spatial asynchrony in
490		stabilising grassland communities. *Ecol. Lett.* **22**, 563-571 (2019).
491	27	Borer, E. T. *et al.* Finding generality in ecology: a model for globally distributed experiments.
492		*Methods in Ecology and Evolution* **5**, 63-73 (2013).
493	28	Whittaker, R. H. Evolution and measurement of species diversity. *Taxon*, 213–225 (1972).
494	29	Grace, J. B. *et al.* Guidelines for a graph-theoretic implementation of structural equation
495		modeling. *Ecosphere* **3** (2012).
496	30	Bai, Y., Han, X., Wu, J., Chen, Z. & Li, L. Ecosystem stability and compenatory effects in the
497		inner Mongolia grassland. *Nature* **431**, 181-184 (2004).
498	31	Tilman, D. Biodiversity: population versus ecosystem stability. *Ecology* **77**, 350-353 (1996).
499	32	Polley, H. W., Isbell, F. I. & Wilsey, B. J. Plant functional traits improve diversity-based
500		predictions of temporal stability of grassland productivity. *Oikos* **122**, 1275-1282 (2013).
501	33	Majekova, M., de Bello, F., Dolezal, J. & Leps, J. Plant functional traits as determinants of
502		population stability. *Ecology* **95**, 2369-2374 (2014).
503	34	Isbell, F. *et al.* Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem
504		productivity. *Proc. Natl. Acad. Sci. U. S. A.* **110**, 11911-11916 (2013).
505	35	de Mazancourt, C. *et al.* Predicting ecosystem stability from community composition and
506		biodiversity. *Ecol. Lett.* **16**, 617-625 (2013).
507	36	Oesterheld, M. & McNaughton, S. J. (eds O. E. Sala, R. B. Jackson, H. A. Mooney, & R. W.
508		Howarth) 151–157 (Springer, 2000).
509	37	Tuomisto, H. An updated consumer's guide to evenness and related indices. *Oikos* **121**,
510		1203-1218 (2012).
511	38	Jost, L. *et al.* Partitioning diversity for conservation analyses. *Diversity and Distributions* **16**,
512		65-76 (2010).
513	39	Shannon, C. E. A MATHEMATICAL THEORY OF COMMUNICATION. *Bell System Technical*
514		*Journal* **27**, 379-423 (1948).
515	40	Pielou, E. C. MEASUREMENT OF DIVERSITY IN DIFFERENT TYPES OF BIOLOGICAL
516		COLLECTIONS. *Journal of Theoretical Biology* **13**, 131-& (1966).
517	41	Simpson, E. H. MEASUREMENT OF DIVERSITY. *Nature* **163**, 688-688 (1949).
518	42	Olszewski, T. D. A unified mathematical framework for the measurement of richness and
519		evenness within and among multiple communities. *Oikos* **104**, 377-387 (2004).
520	43	Dixon, P. VEGAN, a package of R functions for community ecology. *J. Veg. Sci.* **14**, 927-930
521		(2003).
522	44	Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G. & Jarvis, A. Very high resolution
523		interpolated climate surfaces for global land areas. *International Journal of Climatology* **25**,
524		1965-1978 (2005).
525	45	A language and environment for statistical computing (R Foundation for Statistical
526		Computing, Vienna, Austria, 2018).
527	46	Pinheiro, J. C. & Bates, D. M. Mixed-Effects Models in S and S-Plus. *Spinger-Verlag: New York*
528		(2000).
529	47	Trikalinos, T. A. & Olkin, I. Meta-analysis of effect sizes reported at multiple time points: A
530		multivariate approach. *Clinical Trials* **9**, 610-620 (2012).
531	48	Hedges, L. V. & Olkin, I. *Statistical methods for meta-analysis*. (Academic Press, 1985).
532	49	Lefcheck, J. S. PIECEWISESEM: Piecewise structural equation modelling in R for ecology,
533		evolution, and systematics. *Methods in Ecology and Evolution* **7**, 573-579 (2016).
534	50	Viechtbauer, W. Conducting Meta-Analyses in R with the metafor Package. *Journal of*
535		*Statistical Software* **36**, 1-48 (2010).
536	51	Loreau, M. & de Mazancourt, C. Species synchrony and its drivers: Neutral and nonneutral
537		community dynamics in fluctuating environments. *American Naturalist* **172**, E48-E66 (2008).

52   Mellin, C., Bradshaw, C. J. A., Fordham, D. A. & Caley, M. J. Strong but opposing beta-diversity-stability relationships in coral reef fish communities. *Proceedings of the Royal Society B-Biological Sciences* **281** (2014).

Author Contributions

YH, PZ, KRW, ML and SW developed and framed research questions. YH and SW analysed the data with help from PZ, KRW, EWS, JEKB, SEK, KJK and JSL. YH wrote the paper with contributions and input from all authors. EWS and ETB are Nutrient Network coordinators. The author contribution matrix is provided as Extended data table 4.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information is available for this paper.

Materials & Correspondence

Correspondence and requests for materials should be addressed to y.hautier@uu.nl and shaopeng.wang@pku.edu.cn.

571 **Table 1. Hypotheses related to key predictions from theories relating biodiversity,**

572 **asynchrony and stability within and among interconnected communities.**

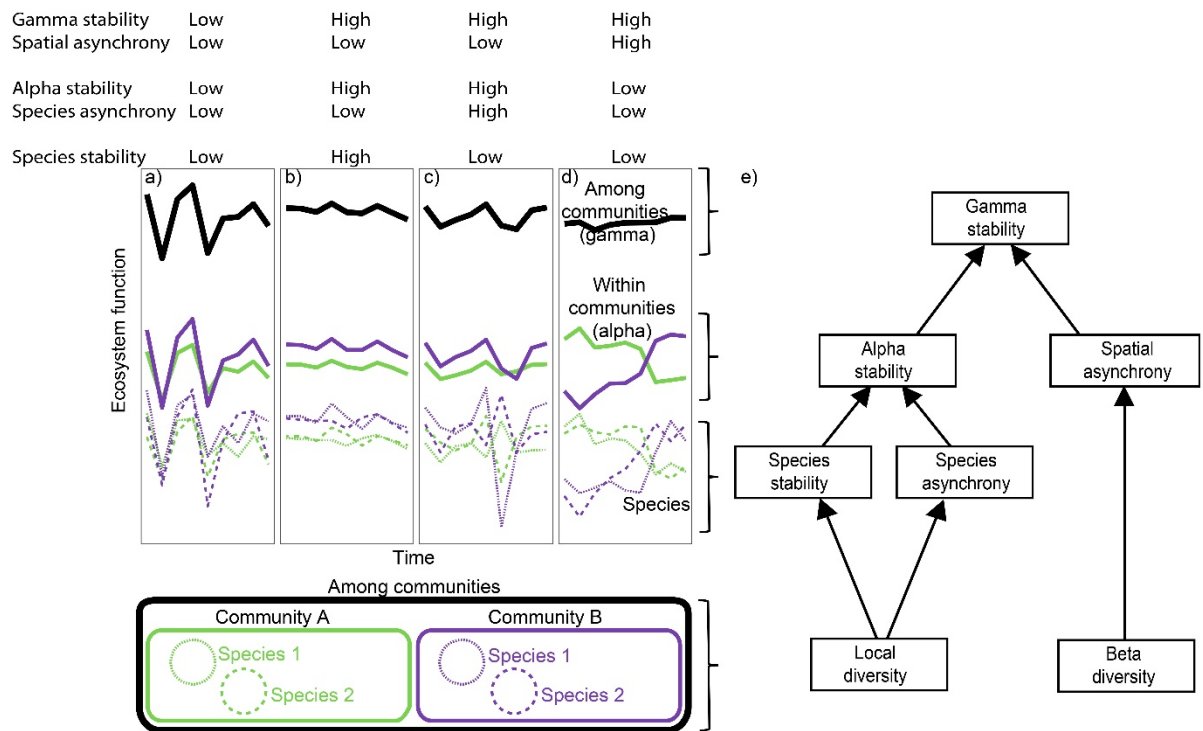| Pathway	Hypotheses and mechanisms	References
Within communities		
Species richness -> species stability	Higher plant richness within a community either increases or decreases the temporal stability of species abundances within the community by either decreasing or increasing variation in individual species abundances from year to year.	[8]
Species richness -> species asynchrony	Higher plant richness within a community provides greater likelihood for asynchronous fluctuations among species to compensate one another when the number of species is higher.	[51]
Species stability -> alpha stability	Higher temporal stability of species abundances within the community increases the temporal stability of community productivity due to lower variation in individual species abundances from year to year	[8,14]
Species asynchrony -> alpha stability	Higher species asynchronous responses to environmental fluctuations within the community increases the temporal stability of community productivity because declines in the abundance of some species are compensated for by increases in others, thus buffering temporal fluctuation in the abundance of the whole community (species or local insurance hypothesis).	[8,14,19,20]
Among communities		
Beta diversity -> spatial asynchrony	Higher variation and dissimilarity in species composition among communities increase asynchronous community responses to environmental fluctuations.	[16]
Alpha stability -> gamma stability	Higher temporal stability of local communities cascades to larger scales and increase the temporal stability of total ecosystem function at the landscape level	[15]
Spatial asynchrony -> gamma stability	Higher asynchronous community responses to environmental fluctuations increase temporal stability of productivity at the larger scale because declines in the productivity of some communities are compensated for by increases in others, thus buffering temporal fluctuation in the productivity of interconnected local communities (spatial insurance hypothesis).	[15,18]

573

574

Table 2. Summary of meta-analysis results showing tests for differences of model paths
between the unmanipulated control and fertilized conditions, including Cochrane Q
statistics for the treatment effect (unmanipulated control versus fertilized condition) with
associated degrees of freedom and p-values.

Pathway	Cochrane Q statistics	d.f.	P-value
Within communities			
Species richness -> species stability	36.52	1	<0.001
Species richness -> species asynchrony	3.44	1	0.064
Species stability -> alpha stability	0.09	1	0.77
Species asynchrony -> alpha stability	7.15	1	0.008
Among communities			
Beta diversity -> spatial asynchrony	4.52	1	0.034
Alpha stability -> gamma stability	5.27	1	0.022
Spatial asynchrony -> gamma stability	0.11	1	0.74

|				
Gamma stability	Low	High	High	High
Spatial asynchrony	Low	Low	Low	High
Alpha stability	Low	High	High	Low
Species asynchrony	Low	Low	High	Low
Species stability	Low	High	Low	Low

Figure 1. Conceptual figure illustrating the non-exclusive processes by which species stability, species asynchrony and spatial asynchrony may contribute to stabilize functioning (such as productivity) within (alpha stability) and among communities (gamma stability). a) Low stability and asynchrony of species within communities result in low alpha stability that in turn results in low gamma stability under low degree of asynchronous dynamics among communities (spatial asynchrony). Relatively high alpha and gamma stability may result from b) high species stability and c) high species asynchrony. d) Relatively high gamma stability may additionally result from high spatial asynchrony. e) Path analysis used to assess the relationship of local and beta diversity with the mechanisms promoting stability at multiple spatial scales under unmanipulated control or fertilized condition. Note that species names belong to a given community, they could or could not be the same species among communities. Adapted from Wilcox et al.[21] and Mellin et al.[52].
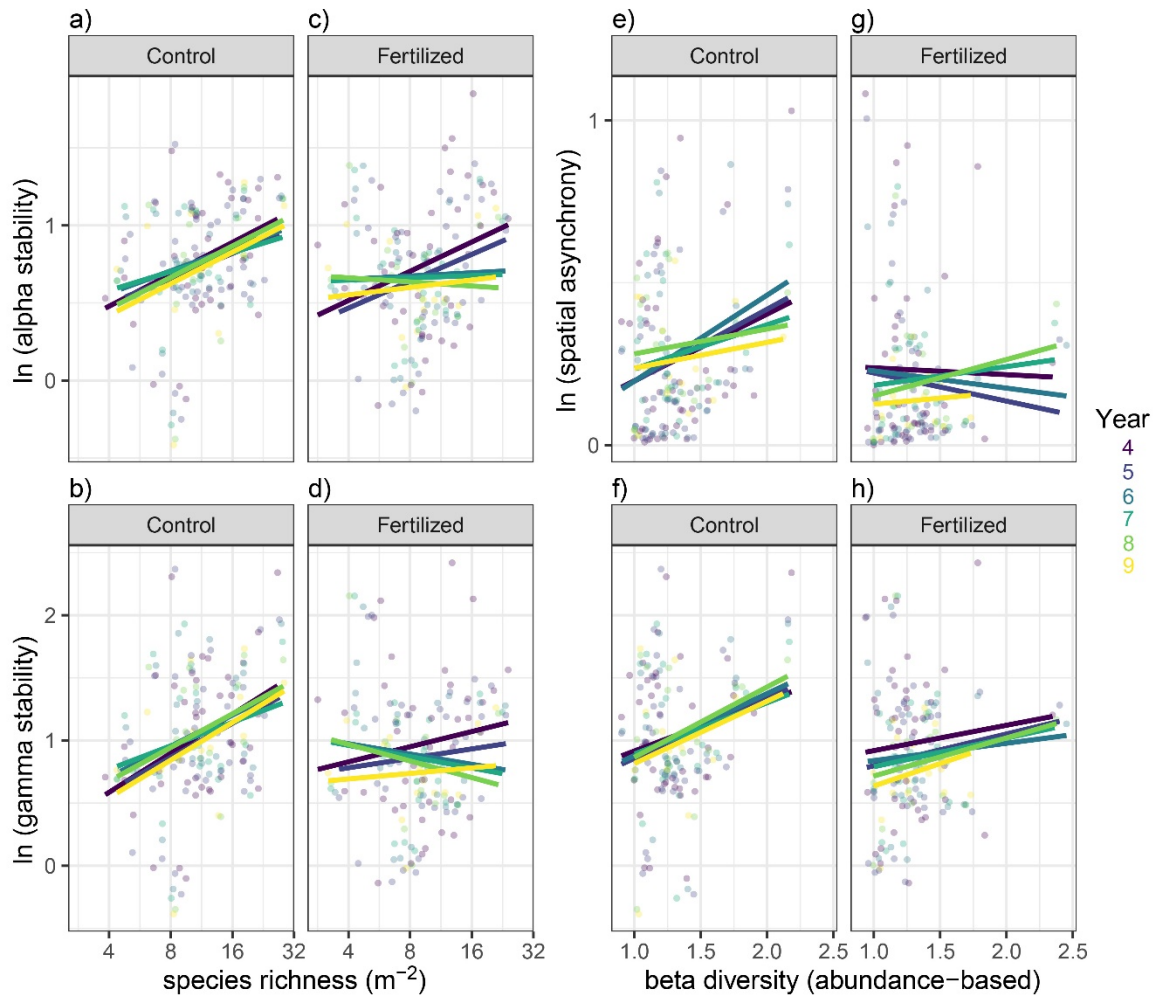
Figure 2. Impact of fertilization on biodiversity-stability relationships across spatial scales. Stability was measured as the temporal mean of primary productivity divided by its temporal standard deviation. Relationships were generally consistent among the periods of experimental duration considered (Extended Data Table 2). Species richness was positively associated with a) alpha (slope and 95% CIs across time = 0.17 (0.08 – 0.26)) and b) gamma stability (0.27 (0.15 – 0.39)) in the unmanipulated communities, but unrelated to c) alpha (0.01 (-0.07 – 0.10)) and d) gamma stability (-0.02 (-0.09 – 0.14)) in the fertilized communities. Beta diversity was positively related to e) spatial asynchrony (0.18 (0.06 – 0.30)) and f) gamma stability (0.47 (0.19 – 0.74)) in the unmanipulated communities, but unrelated to g) spatial asynchrony (-0.01 (-0.13 – 0.12)) and h) gamma stability (0.21 (-0.07 – 0.50)) in the fertilized communities. Note the scale of y-axis differ across panels and this needs to be considered when visually inspecting slopes.
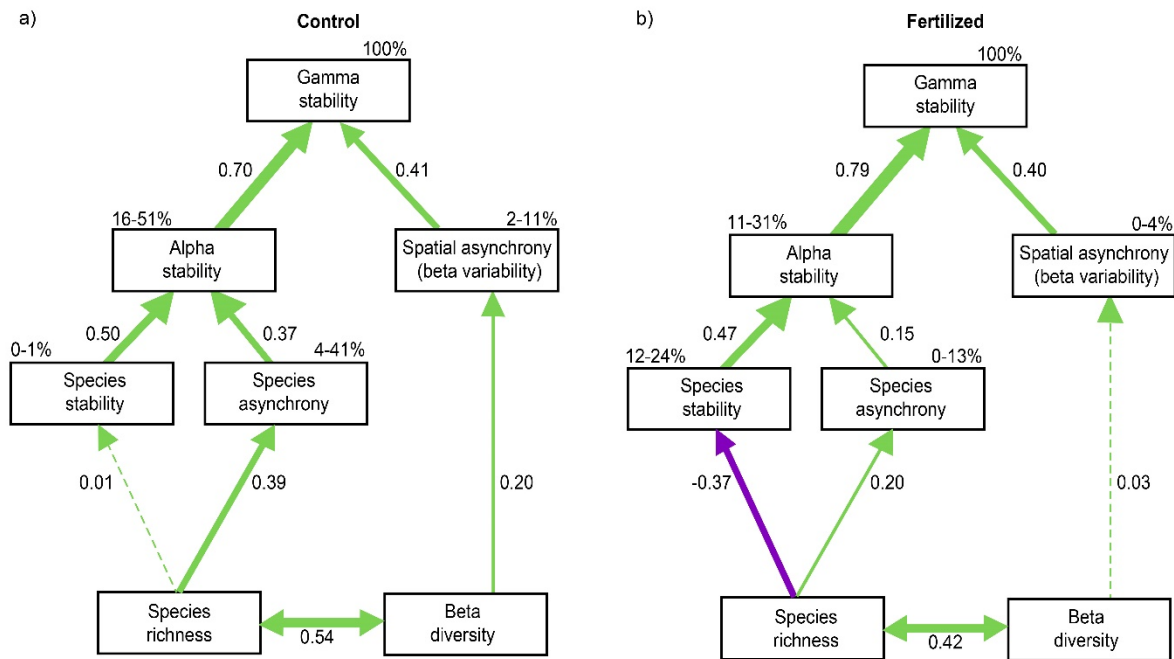
Figure 3. Summary of meta-analysis results showing the direct and indirect pathways through which biodiversity, asynchrony and stability at multiple spatial scales determines gamma stability under a) unmanipulated control or b) fertilized condition. Boxes represent measured variables and arrows represent relationships among variables. Numbers next to the arrows are averaged effect sizes as standardised path coefficients. Solid green and pink arrows represent significant (P ≤ 0.05) positive and negative coefficients, respectively, and dashed green and pink arrows represent non-significant coefficients. Widths of paths are scaled by standardized path coefficients. Percentages next to endogenous variables indicate the range of variance explained by the model (R^2) across period of experimental duration.