

1 **Diversity-dependent temporal divergence of ecosystem functioning in experimental ecosystems**

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43 **The effects of biodiversity on ecosystem functioning generally increase over time but the underlying processes**
44 **remain unclear. Using 26 long-term grassland and forest experimental ecosystems we demonstrate that**
45 **biodiversity-ecosystem functioning relationships strengthen mainly by greater increases in functioning in**
46 **high-diversity communities in grasslands and forests. In grasslands, biodiversity effects also strengthen due to**
47 **decreases in functioning in low-diversity communities. Contrasting trends across grasslands are associated**
48 **with differences in soil characteristics.**

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65 More than two decades of research have revealed that biodiversity is a significant driver of ecosystem functioning^{1,2}.
66 Positive biodiversity effects on ecosystem functioning have been found in grassland and forest biodiversity
67 experiments^{3,4} with growing evidence showing that biodiversity–ecosystem functioning relationships may become
68 stronger over time^{5,6,7}. Moreover, several recent studies suggest that long-term biodiversity effects in experiments
69 better mirror natural conditions than short-term studies and likely help explain biodiversity–ecosystem functioning
70 relationships in real-world ecosystems^{8,9,10,11}.

71 Temporal increases in plant diversity effects on ecosystem functioning may result from an increase in
72 functioning in high-diversity communities⁷, a decrease in functioning in low-diversity communities¹² or both.
73 However, it remains unknown which of the above trends drive temporal increases in diversity effects on ecosystem
74 functioning, whether these trends are consistent across experiments and ecosystems, and if not, whether context-
75 dependency in temporal trends may be attributed to site conditions. For instance, soil characteristics likely influence
76 the biodiversity–ecosystem functioning relationship^{10,13,14} and may influence temporal trajectories as well, but
77 whether or not they do so is unclear.

78 Understanding temporal trends of biodiversity effects on ecosystem functioning is critical for providing
79 insights into biodiversity–ecosystem functioning relationships^{9,16} and predicting the potential consequences that
80 progressive biodiversity change^{18,19} and management^{20,21} have on ecosystem functioning and service provisioning
81 over time. Further, examining these temporal trends is fundamental for guiding research on understanding the
82 underlying mechanisms, e.g. a variety of niche-differentiation processes such as complementary resource use and
83 facilitation, which can have positive effects on the functioning of high-diversity communities^{6,17}, and the impact of
84 pest and diseases, which can have negative effects on the functioning of low-diversity communities⁹.

85 In this study, we examined temporal shifts in biodiversity effects on ecosystem functioning in terrestrial
86 ecosystems, specifically plant diversity effects on plant aboveground biomass in grassland and on basal area in
87 forest experimental ecosystems. We used data from 26 long-term biodiversity experiments that manipulated plant-
88 species richness in grasslands and forests (14 and 12 experiments, respectively; Supplementary Table 1). We
89 investigated whether the strength of the biodiversity–ecosystem functioning relationship increases with time and
90 whether temporal divergence across plant richness levels is driven by an increase in function in high-diversity
91 communities, a decrease in function in low-diversity communities, or a combination of both. Finally, if temporal

92 trends differed across experiments, we assessed the potential role of soil characteristics in shaping these temporal
93 trends.

94 In grasslands, the relationship between plant species richness and plant aboveground biomass was positive
95 and became significantly stronger over time (Supplementary Table 2, Fig. 1a). Temporal divergence across plant
96 richness levels was observed in 10 out of 14 grassland experiments (Supplementary Fig. 1). Although temporal
97 divergence was frequently associated with more diverse communities showing stronger increases in plant
98 aboveground biomass over time (Fig. 1a), the temporal increase of diversity effects was not determined by a
99 consistent trend across studies (see variance components in Supplementary Table 2): temporal divergence was
100 driven by a decrease in function in low-diversity communities in one experiment, by an increase in function in high-
101 diversity communities in six experiments, or a combination of both in three experiments (Supplementary Fig. 1).

102 The context-dependency underlying biodiversity–ecosystem functioning relationships in grasslands was
103 strongly associated with variation in soil characteristics across experiments (Supplementary Table 3). Soils
104 influenced biodiversity–ecosystem functioning relationships in two ways. First, the interaction between soil
105 characteristics related to soil texture and pH and plant species richness shaped the overall richness effect (significant
106 richness \times soil PC2 interaction; Supplementary Table 3 and Supplementary Figs. 2 and 3). Second, soil
107 characteristics, such as cation-exchange capacity (CEC), soil organic carbon (C), water content at wilting point, and
108 bulk density contributed to driving temporal divergence (significant richness \times time \times soil PC1 interaction;
109 Supplementary Table 3, Fig. 2, and Supplementary Fig. 2). Temporal divergence driven by an increase in function in
110 high-diversity communities was associated with studies located in areas with higher CEC, soil organic C, water
111 content, and lower bulk density, while a decrease in function in low-diversity communities was associated with the
112 inverse pattern, e.g. lower soil organic C (Fig. 2).

113 The general increase of the biodiversity–ecosystem functioning relationship through time was due to
114 contrasting trajectories across grassland studies, showing the importance of context-dependency of the biodiversity–
115 ecosystem functioning relationship in this ecosystem. Our analyses reveal that soil characteristics contribute to
116 strengthening plant species richness effects on ecosystem functioning in general¹³ and through time¹¹ in multiple
117 ways. First, variability in ecosystem functioning across plant species richness levels was generally lower in
118 experiments with sandy soils. Second, temporal divergence was explained by stronger increases in ecosystem

119 function in high-diversity than in low-diversity communities in experimental sites with higher soil organic C,
120 whereas temporal divergence in experimental sites with low soil organic C was explained by a decrease in
121 ecosystem function in low-diversity communities. Therefore, the influence of resource availability on plant-plant
122 interactions as well as multi-trophic interactions¹⁹ may underlie temporal changes of biodiversity effects^{10,13} and
123 related mechanisms^{14,22}. It is also likely that other abiotic and biotic factors play a role in shaping the biodiversity–
124 ecosystem functioning relationship through time. For instance, most of the grassland biodiversity experiments are
125 perennial–dominated (more than 75% of the species were perennial), except for BIODDEPTH Greece and Portugal
126 sites (less than 30% of the species were perennial), where there was no evidence of temporal divergence. Grassland
127 experiments dominated by annual plants may be strongly affected by processes related to recruitment, such as seed
128 availability (either from their own plot or surrounding plots) and microsites²³. Recruitment may influence diversity
129 effects in grasslands, mainly due to changes in plant density rather than changes in plant size²⁴.

130 In forests, plant richness effects on periodic annual increment of basal area were consistently positive
131 across studies (see variance components in Supplementary Table 2, Fig. 1b, Supplementary Fig. 4), and, in contrast
132 to grasslands, we did not find evidence that they changed over time (neither time nor richness \times time were
133 significant; Supplementary Table 2, Fig. 1b). Consequently, the temporal divergence of total basal area among tree
134 species richness levels depended on consistently positive diversity effects on periodic annual increment of basal
135 area. (Supplementary Tables 2 and 4, Fig. 1c, and Supplementary Fig. 5). The absence of context-dependency in
136 forests could not be explained by overall differences in soil characteristics between forest and grassland studies,
137 which are located along similar soil gradients (Supplementary Figs. 6 and 7) that exhibit moderate differences in soil
138 cation-exchange capacity (p-value = 0.06) and pH (p-value = 0.02; Supplementary Fig. 8).

139 Our results show that positive tree diversity effects started early and accumulated through time. Thus,
140 mechanisms associated with positive biodiversity effects on ecosystem functioning like complementarity may play a
141 key role even during the early stages of community assembly²⁵. Decreases in ecosystem functioning in forests, e.g.
142 due to tree mortality, appear to be offset by higher growth of surviving trees. This differs from grasslands, in which
143 community-level biomass is highly dependent on plant density²⁴. Temporal divergence may continue to increase not
144 only due to cumulative processes (detected in our study), but also due to strengthening of competitive interactions²⁶.
145 The importance of niche partitioning over time also may increase at smaller spatial scales²⁷, and thus may require

146 longer to be detected at the plot level. Data availability from long-term studies and from more diverse forest systems
147 remain one of the main challenges for understanding temporal dynamics in forest experimental ecosystems. For
148 example, the longest-running forest biodiversity experiments in this study usually had communities with only one
149 and two species. Moreover, longer and multi-generation forest experiments may provide a better understanding of
150 the effects that pathogen and herbivore attacks and the accumulation of soil pathogens may have on biodiversity
151 effects through time. It is possible that temporal dynamics of biodiversity effects in forest ecosystems become
152 increasingly similar to those of grasslands when compared at similar stages in terms of generations of the study
153 organisms or under different soil characteristics, e.g. sites with lower CEC and higher pH (Supplementary Fig. 8).

154 In conclusion, our results show a consistent temporal divergence of ecosystem functioning across plant
155 diversity levels in both grassland and forest experimental ecosystems. In grasslands, temporal divergence was the
156 result of a variety of patterns, all ultimately causing an increase in biodiversity effects over time. In forests, by
157 contrast, temporal divergence was not detected when ecosystem functioning was measured as a rate (periodic annual
158 increment of basal area) but rather as an amount (total basal area). Therefore, the increasing strength of the
159 biodiversity–ecosystem functioning relationship in forests was related to an increase in function of high-diversity
160 communities driven by a consistent positive effect of high-diversity communities on periodic annual increment of
161 basal area. Temporal divergence in ecosystem functioning found in our analysis may have multiple implications for
162 the provisioning of vital ecosystem services in managed ecosystems. For instance, we need to determine other
163 potential biotic and abiotic factors that drive either an increase of ecosystem function in high-diversity communities
164 or a decrease in low-diversity communities over time. Such mechanistic understanding is fundamental as low-
165 diversity plant communities are widely used in productive landscapes^{20,21}. Overall, our results support the
166 importance of management practices that reinforce the functional and structural complexity of ecosystems at
167 different spatial and temporal scales²⁰ and, crucially, either attenuate decreases in function in grasslands or increase
168 function in grassland and forest ecosystems.

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172 **Methods**

173 **Data acquisition and description**

174 Long-term experiments that have manipulated plant species richness in grasslands and forests were identified using
175 published meta-analyses, review papers on related topics and experimental platforms for biodiversity research
176 (Supplementary methods). Long-term experiments were included if: a) plant species richness was directly
177 manipulated through sowing or planting and included monocultures of all species present in the mixtures, b) raw
178 data at least at the plot level were available, c) aboveground plant biomass (in grassland) or basal area (in forest)
179 data from at least three points in time from different years were available, and d) the experiment was conducted for
180 at least 3 years in grasslands and 5 years in forests. For forests, the required experimental duration was higher than
181 for grasslands because the establishment of tree-dominated experimental studies and the biodiversity effects on
182 ecosystem functions are expected to take longer in forests.

183 Data from 26 long-term biodiversity experiments met these criteria (Supplementary Table 1) including 12 forest
184 experiments (370 plots, $n = 1,887$ measurements (plot by age combination) across experiments) and 14 grassland
185 experiments (1,045 plots, $n = 7886$ measurements (plot by age combination) across experiments). Annual peak
186 aboveground biomass (g/m^2) and basal area (m^2/ha) were used in grassland and forests, respectively. In forests, we
187 included two types of ecosystem functions: – periodic annual increment of basal area – is a rate and is therefore
188 more comparable to annual peak aboveground biomass in grasslands (see Supplementary methods) and – total basal
189 area – is an amount that captures cumulative tree growth. Both measures were used to quantify ecosystem
190 functioning following the definition in Hooper³, i.e. ecosystem functioning includes ecosystem properties such as
191 process rates and the size of the compartments.

192 **Temporal divergence**

193 We used linear mixed-effect models to assess the temporal dynamics of ecosystem function among plant species
194 richness levels using either plant aboveground biomass in grassland or basal area in forest experiments. We fitted a
195 separate model for grassland experiments using annual peak aboveground biomass and two separate models for
196 forest experiments, one using total basal area and the other using periodic annual increment of basal area. The initial
197 model included plant species richness, time, and the interaction between richness and time as fixed effects in both

198 grassland and forest experiments. We then simplified models by excluding non-significant fixed effects and
199 interactions (p-value > 0.1). Plant richness was the sown or planted richness (natural logarithm), and time was
200 experimental age in years (natural logarithm). The natural logarithm transformation was used based on the
201 expectation of fast, initial increases in ecosystem function, followed by constant growth in the later years of the
202 experiment. Using a random slope and intercept structure, random effects were included for: study, study × richness,
203 study × time, study × richness × time interaction, and a term for plot within study for grasslands and for total basal
204 area in forests. The random structure for periodic annual increment of basal area included study, study × richness
205 interaction, and a term for plot within study. We accounted for repeated measurements within plots by using a first-
206 order autoregressive covariance structure, which fitted the data better than a compound symmetry covariance
207 structure based on the Akaike information criterion. The best covariance structure was first-order autoregressive.
208 Models were fitted with asreml function in the asreml package in R, and the results were extracted using the
209 test.asreml function in the pascal package in R. Analyses were run in R version 3.2.4²⁹.

210 **Effects of soil characteristics on temporal divergence**

211 To explore the variation in temporal trends among grassland studies, an additional model was tested that included
212 species richness, time, soil characteristics, and their interactions (Supplementary methods). Because a consistent set
213 of soil variables was not available across studies, we used data from SoilGrids250³⁰ to provide a general and
214 consistent description of the study area. However, these data are proxies for site-specific quantitative information
215 and need to be interpreted with caution. The soil characteristics were used to perform a principal component
216 analysis, in which the first and second axes explained 48 and 40% of the variation across grassland experiments,
217 respectively (Supplementary Fig. 2). We did not analyze the effects of soil characteristics in forest experiments
218 because we did not find evidence of multiple trends underlying the temporal divergence (Supplementary Table 2,
219 Fig. 1b). To compare the potential differences in the range of soil characteristics between experimental ecosystems,
220 we performed an additional principal component analysis including both forest and grassland studies
221 (Supplementary methods and Supplementary Figs. 6, 7, and 8).

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224 **Data availability**

225 The data that support the findings of this study are available from the authors upon request.

226 **Code availability**

227 R code of linear mixed-effects models is provided in the Supplementary methods section.

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229 **References**

- 230 1. Isbell F, *et al.* *Nature* **546**, 65 - 72 (2017).
- 231 2. Tilman D, Isbell F, Cowles JM. *Annu Rev Ecol Evol Syst* **45**, 471 - 493 (2014).
- 232 3. Hooper DU, *et al.* *Ecological Monographs* **75**, 3 - 35 (2005).
- 233 4. Balvanera P, *et al.* *Ecology Letters* **9**, 1146 - 1156 (2006).
- 234 5. Reich PB, *et al.* *Science* **336**, 589 - 592 (2012).
- 235 6. Cardinale BJ, *et al.* *PNAS* **104**, 18123 - 18128 (2007).
- 236 7. Ewel JJ, Celis G, Schreeg L. *Biotropica* **47**, 162 - 171 (2015).
- 237 8. Flombaum P, Sala OE. *PNAS* **105**, 6087 - 6090 (2008).
- 238 9. Eisenhauer N, *et al.* *Journal of Vegetation Science* **27**, 1061 - 1070 (2016).
- 239 10. Grace JB, *et al.* *Nature* **529**, 390 - 393 (2016).
- 240 11. Hautier Y, *et al.* *Nature* **508**, 521 - 525 (2014).
- 241 12. Marquard E, *et al.* *Plos One* **8**, e75599 (2013).
- 242 13. Fridley JD. *Oecologia* **132**, 271 - 277 (2002).
- 243 14. Boyden S, Binkley D, Senock R. *Ecology* **86**, 992 - 1001 (2005).
- 244 15. De Deyn GB. *Oikos* **126**, 497 - 507 (2017).

- 245 16. Forrester DI, Bauhus J. *Curr Forestry Rep* **2**, 45 - 61 (2016).
- 246 17. Fargione J, *et al.* *Proc R Soc B* **274**, 871 - 876 (2007).
- 247 18. Newbold T, *et al.* *Nature* **520**, 45 - 50 (2015).
- 248 19. Urban MC. *Science* **348**, 571 - 573 (2015).
- 249 20. Paquette A, Messier C. *Fron Ecol Environ* **8**, 27 - 34 (2010).
- 250 21. Tilman D, *et al.* *Science* **292**, 281 - 284 (2001).
- 251 22. Craven D, *et al.* *Philosophical Transactions B* **371**, 1 - 8 (2016).
- 252 23. Eriksson O, Ehrlén J. *Oecologia* **91**, 360 - 364 (1992).
- 253 24. Marquard E, Weigelt A, Roscher C, Gubsch M, Lipowsky A, Schmid B. *Journal of Ecology* **97**, 696 - 704
254 (2009).
- 255 25. Williams LJ, Paquette A, Cavender-Bares J, Messier C, Reich PB. *Nature Ecology and Evolution* **1**, 1 -7
256 (2017).
- 257 26. Ewel JJ, Mazzarino MJ. *PNAS* **105**, 18836 - 18841 (2008).
- 258 27. Potvin C, Dutilleul P. *Ecology* **90**, 321 - 327 (2009).
- 259 28. Binkley D, Senock R, Bird S, Cole TG. *Forest Ecology and Management* **182**, 93 - 102 (2003).
- 260 29. R Development Core Team (2015).
- 261 30. Hengl T, *et al.* *Plos One* **12**, e0169748 (2017).

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273 **Author contributions**

274 N.E conceived the idea; N.E and N.G-R developed the idea; A.H, B.W, C.P, C.Pot, C.R, D.F, D.P, D.T, F.M, H.A,
275 H.E, J.E, J.J, J.K, J.P. JvR, P.R contributed experimental data, N.G-R assembled the data; N.G-R and D.C analysed
276 the data with the input from F.I, J.K, and A.H; N.G-R wrote the paper with substantial input from all authors.

277 **Competing interests**

278 The authors have no competing interests.

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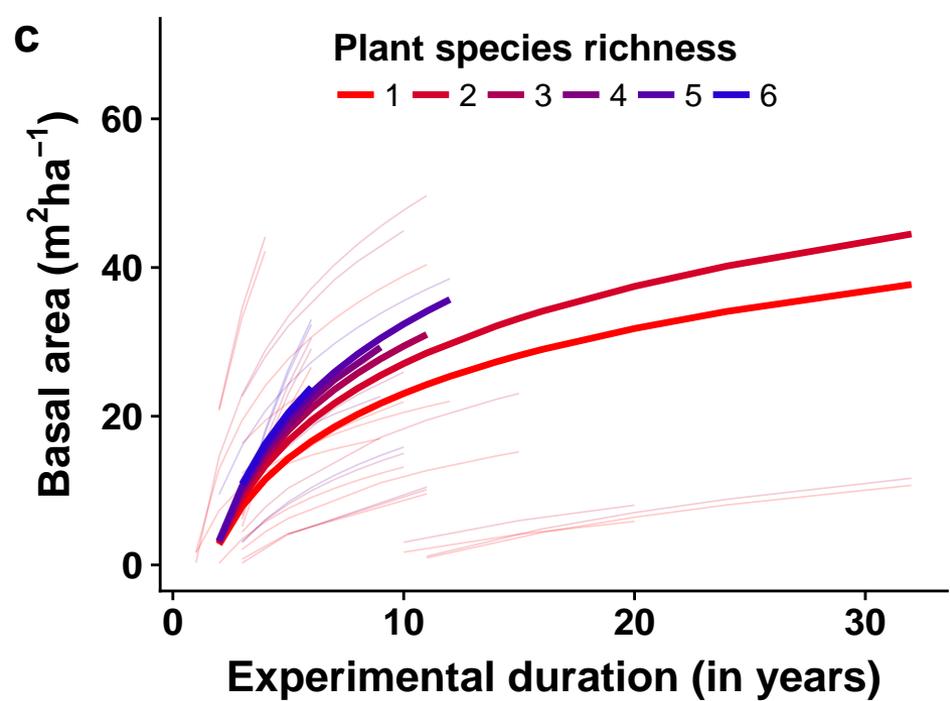
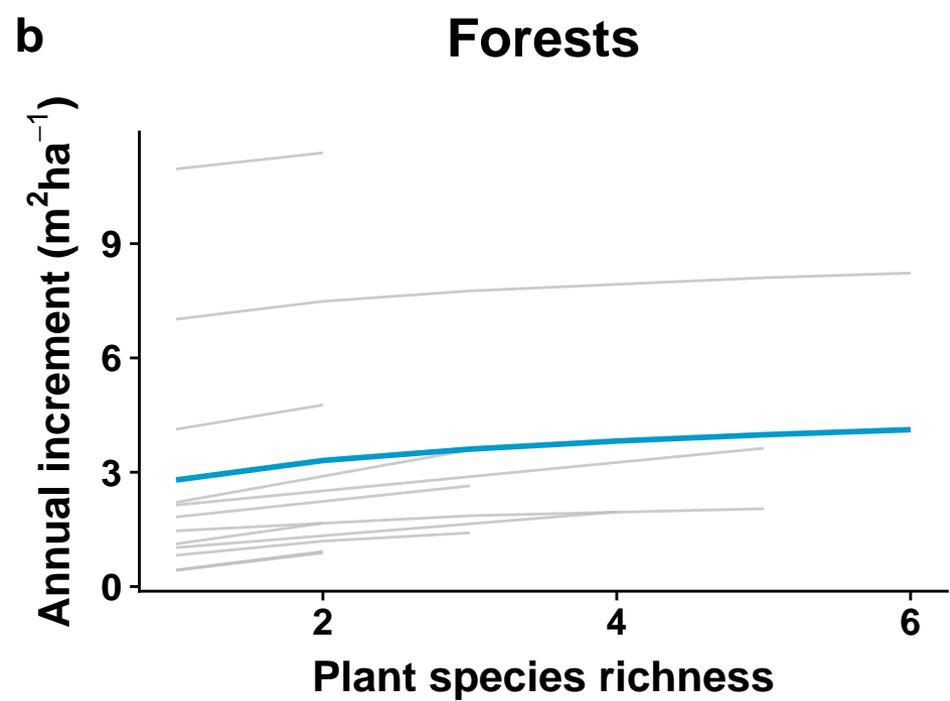
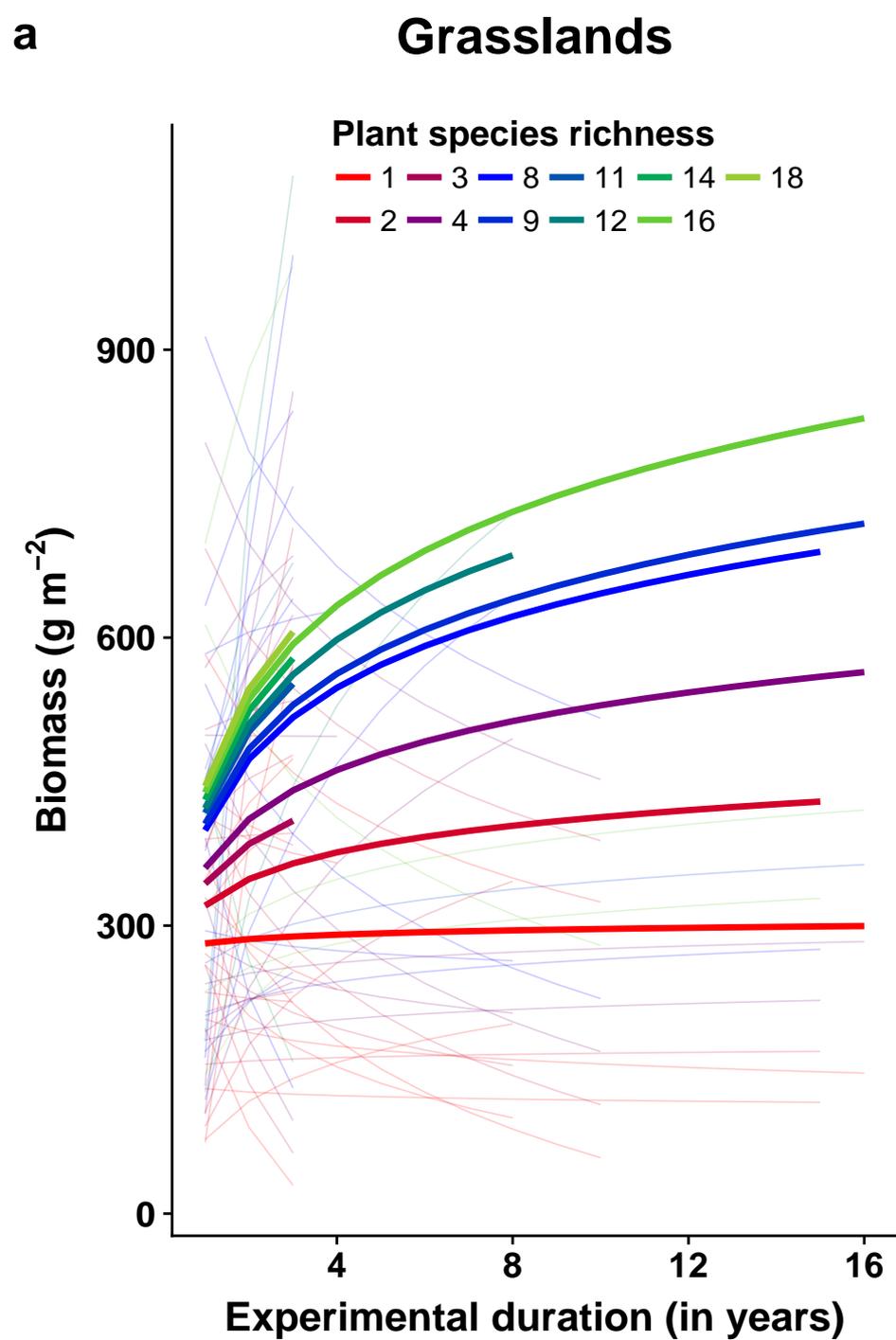
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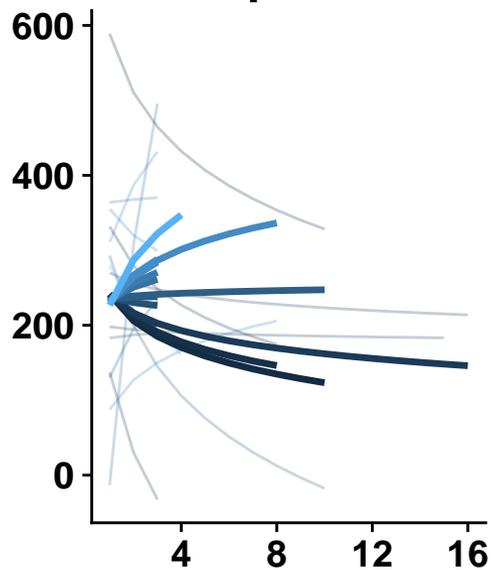
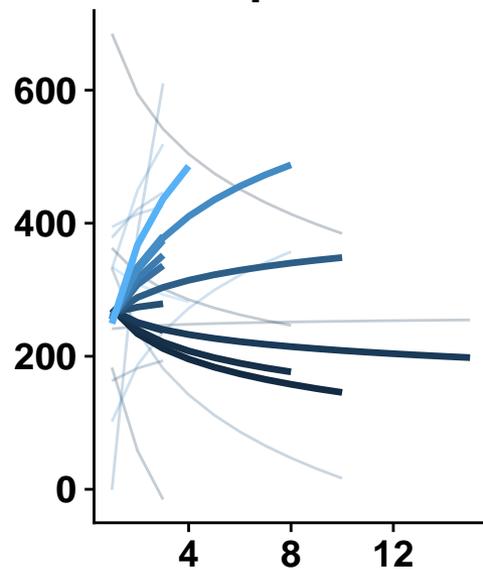
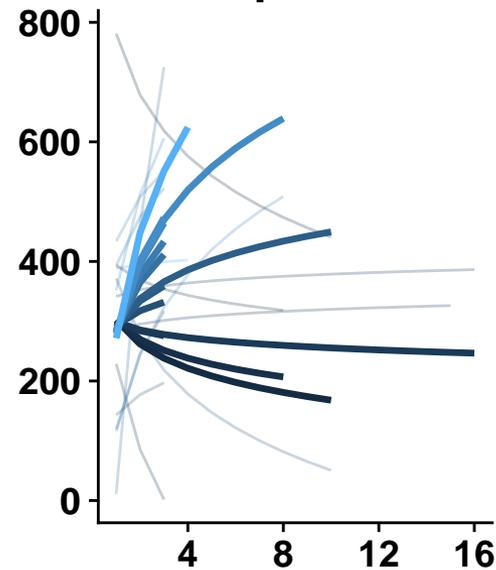
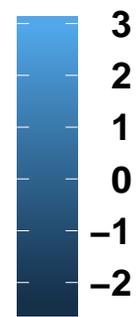
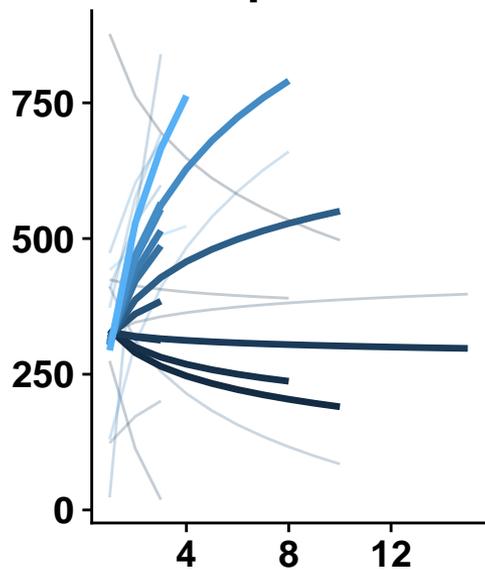
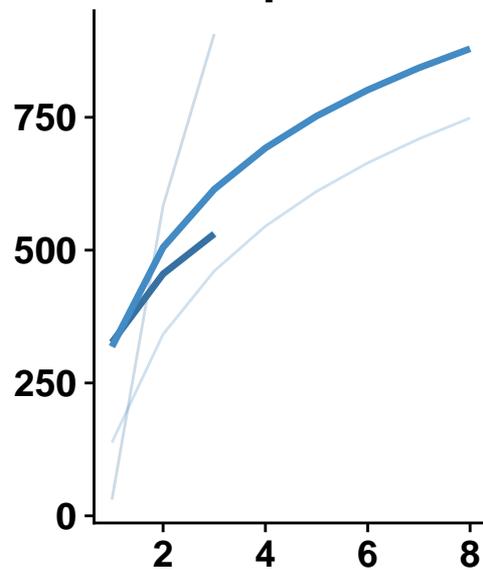
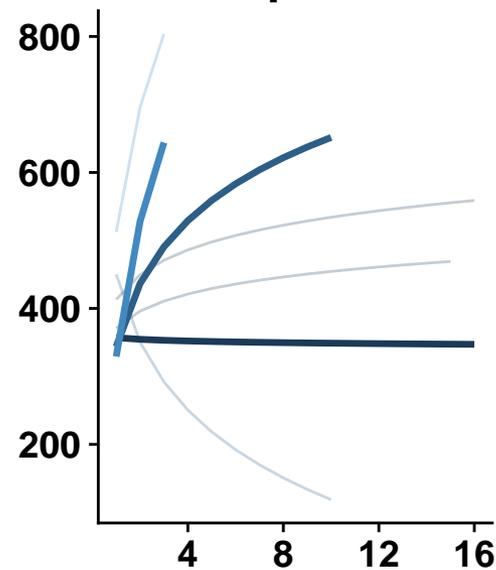
289 **Figures Legends**

290 **Figure 1. Ecosystem functioning in grassland and forest experimental ecosystems.** In grasslands, trajectories of
291 aboveground biomass (g m^{-2}) among plant species richness levels diverge over time (a). In forests, significant plant
292 species richness effects on periodic annual increment of basal area ($\text{m}^2 \text{ha}^{-1}$) are consistent over time (b). The
293 consistent positive effect of high-diversity communities on periodic annual increment of basal area may explain the
294 temporal divergence in total basal area among plant species richness levels (c). For panels a and c, lines are mixed-
295 effects model fits for each plant species richness level within each study (thin lines) or across all studies (thick
296 lines). For panel b, lines are mixed-effects model fits for each study (gray lines) or across studies (blue line). For
297 grasslands, aboveground biomass was significant affected by species richness ($F_{1,5754.7} = 14.21$, p-value <0.001) and
298 the species richness \times time interaction ($F_{1,5754.7} = 8.53$, p-value <0.01). For forests, periodic annual increment of
299 basal area was significantly affected by species richness ($F_{1,1433.1} = 10.07$, p-value <0.01), and total basal area was
300 significantly affected by time ($F_{1,291.9} = 24.32$, p-value <0.001) and the species richness \times time interaction ($F_{1,291.9} =$
301 18.39 , p-value <0.001). See extended information in Supplementary Tables 2 and 4. Data from 14 grassland (1,045
302 plots $n = 7,886$ measurements (plot by age combination)) and from 12 forest experimental ecosystems were entered
303 in the analyses (370 plots, $n = 1,887$ measurements (plot by age combination)).

304

305 **Figure 2. Influence of soil characteristics on temporal divergence in grasslands.** Lines are mixed-effects model
306 fits for each plant species richness level and soil characteristics within each study (thin lines) or across all studies
307 (thick lines). Plots only show temporal trajectories of plant species richness levels present in at least two
308 experimental grasslands. Soil characteristics are based on a principal component analysis; the first principal axis
309 (Soil PC1) explained 48% of variation where positive values were associated with higher cation-exchange capacity,
310 soil organic carbon content, and volumetric water content at wilting point and lower soil bulk density. See extended
311 information in Supplementary Table 3 and Supplementary Fig. 2.



1 species**2 species****4 species****Soil PC1****8 species****12 species****16 species****Experimental duration (in years)**