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Long term impacts of species loss on community properties across contrasting ecosystems

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Biodiversity loss can heavily impact the functioning of ecosystems, and improving our understanding of how ecosystems will respond to biodiversity decline is among the main challenges in ecology¹⁻⁴. However, several important aspects of the longer-term effects of biodiversity loss on ecosystems remain unresolved, including how these effects depend on environmental context⁵⁻⁷. Here, we analyzed data from the world's longest running across-ecosystem biodiversity manipulation experiment, which has been set up on 30 lake islands in Sweden that vary greatly in productivity and soil fertility due to differences in fire history^{8,9}. We tested the effects of environmental context on how plant species loss affects two fundamental community attributes over 20 years: plant community biomass and temporal variability. In contrast to findings from artificially assembled communities¹⁰⁻¹², we found that the effects of species loss on community

26 biomass decreased over time; this decrease was strongest on the least productive and
 27 fertile islands. Species loss generally also increased temporal variability and these effects
 28 were greatest on the most productive and fertile islands. Our findings highlight that the
 29 ecosystem-level consequences of biodiversity loss are not constant across ecosystems and
 30 that understanding and forecasting these consequences necessitates taking into account
 31 the overarching role of environmental context.

32 Biodiversity experiments have often shown that diverse communities are more efficient
 33 in capturing resources, and therefore produce more biomass than species-poor communities^{1,2}.
 34 Some experiments have also shown that effects of plant diversity on biomass production
 35 increase over time as complementarity of resource use among species increases¹⁰⁻¹². This has
 36 led to suggestions that ecosystem-level consequences of biodiversity loss might be stronger
 37 than predicted from short-term experiments. However, the available evidence emerges from
 38 artificially and randomly assembled communities^{10,12}. It remains untested how effects of
 39 species loss develop over time in natural ecosystems, and – importantly – how long-term
 40 effects of biodiversity loss vary among ecosystems. This is despite growing evidence that the
 41 strength of relationships between plant diversity and productivity can vary with environmental
 42 conditions, notably soil resource availability^{6,13}. If soil resource availability is an important
 43 driver of plant diversity–productivity relationships, then variation in soil resources could have
 44 important consequences for how effects of species loss change over time and in the long term.

45 The diversity of plant communities can also buffer temporal variability in response to
 46 external perturbations and fluctuations in environmental conditions^{14,15}. Greater temporal
 47 invariability (i.e., the consistency of biomass production over time or $1/(\text{coefficient of}$
 48 $\text{variation (CV)})$, also often referred to as ‘temporal stability’¹⁴⁻¹⁷) in more diverse
 49 communities is commonly ascribed to greater temporal complementarity between species
 50 resulting from higher asynchrony of species responses to environmental fluctuations^{14,18}.

However, uncertainty remains as to whether there are generalizable relationships between plant diversity and temporal invariability in ecosystem functioning^{16,19,20}. Although there is recent evidence that addition of nitrogen can moderate species diversity effects on temporal invariability of community biomass²¹, empirical tests of how these relationships vary with environmental context in natural ecosystems are scarce⁷.

We examined effects of environmental context on impacts of species loss over time using data from the longest running across-ecosystem biodiversity manipulation experiment in existence, set up in 1996 across a post-fire chronosequence consisting of 30 forested lake islands in northern Sweden^{9,22}. Here, the main disturbance is wildfire with large islands burning more often than smaller ones, creating a successional gradient across the islands. Large islands have greater soil fertility and supply rates of available soil nutrients and are more productive relative to smaller ones²² (Extended Data Table 1). In line with prior work on this system^{8,9,22}, we divided the islands into three size classes of ten islands each: ‘large’, ‘medium’ and ‘small’⁸. For each island, plots were established comprising a full-factorial combination of three dwarf shrub species removals (removal of *Vaccinium myrtillus*, *V. vitis-idaea* and *Empetrum hermaphroditum*) (see Methods). These species account for >98% of vascular plant biomass in the understory layer⁹. The ecosystems we studied have comparatively low plant alpha-diversity, as is characteristic of extensive areas of the boreal zone worldwide, and are therefore likely to be especially vulnerable to species loss. Our study design enables us to explore impacts of all three-way combinations of the same species across widely contrasting environments.

For each plot we took measurements of the biomass of each shrub species annually from 1996 to 2016 (see Methods), which as expected revealed generally lower biomass in single- and two-species plots than in three-species plots. However, effects of removal of certain (combinations of) species on total plant biomass strongly differed among island size classes

(Fig. 1, Extended Data Table 1). It could be predicted that species loss effects are larger on smaller islands due to lower soil resource availability and greater resource partitioning among species²³. This would constrain the extent to which the remaining species could compensate for lost species. However, our data instead show that effects of species loss across different-sized islands are strongly species dependent (Fig. 1). Biomass of individual species was also strongly affected by plant species removal, with these effects also often varying with island size (Extended Data Fig. 1 and Table 1). These species-specific effects are partly explicable by different dominance patterns of the three shrub species among island size classes⁹ which are in turn associated with interspecific differences in nutrient acquisition strategies²⁴.

We then tested whether the strength of effects of species loss on community biomass increases over time^{10,12}, and if the magnitude of this increase is greater on small islands, as expected if plants on small (unproductive) islands were to suffer most from a temporal decrease in resource complementarity. Artificially and randomly assembled biodiversity experiments have shown that complementarity increases over time, for example through increasing nitrogen retention in more diverse communities²⁵. We found that effects of species removal on total plant biomass varied through time (Fig. 1, Extended Data Table 1), but against expectations the strength of the effects of species loss on plant biomass actually decreased. The decrease over time in the amount of variation explained by species richness did not depend on island size (Fig. 2, Extended Data Table 2). However, the amount of variation explained by removal treatments also decreased, and this was strongest for small islands (Extended Data Fig. 2 and Table 2). Polynomial regressions revealed some nonlinearity in these temporal patterns (See Supplementary Information). To further examine context-dependency of impacts of species loss, we also tested how commonly used quantitative measures of biodiversity effects on plant biomass changed over time, and how these temporal changes varied with island size. These analyses indicated that the temporal

101 decrease in effects of species loss could largely be explained by changes in species
102 complementarity (see Supplementary Information).

103 Contrary to the results of highly controlled randomly assembled communities^{10,12}, we
104 show with long-term data derived from manipulations of low-diverse, naturally-assembled
105 communities that while biodiversity loss does significantly reduce plant biomass, this effect
106 does not necessarily increase over time. This could occur through mitigation of effects of
107 species loss by compensatory responses of remaining species²⁶, and with this mitigation
108 strengthening over time. Importantly, our findings highlight that such compensatory responses
109 depend greatly on interactions between species identity and environmental context. This
110 indicates that ecosystems are resilient to species loss if other species are able to occupy the
111 newly available niche space. In our low-diversity boreal ecosystem, the shape of the resilience
112 pattern would be determined by the growth responses of the remaining species. In other
113 systems, colonization rates of species from external pools may be equally or even more
114 important. One possible reason why previous experiments using controlled, randomly
115 assembled communities have often found effects of biodiversity to increase over time is that
116 they constrain species' niche widths and do not allow species colonization of species from
117 external pools.

118 Finally, we tested whether species loss increases the temporal variance (or reduces 1/CV
119 or invariability) in community biomass, and if this increase is strongest for small islands, on
120 the basis that in low-productivity systems the species remaining following biodiversity loss
121 would be less capable of maintaining complementary interactions under temporally
122 fluctuating conditions²⁷. Asynchronous responses among species are particularly important in
123 maintaining biomass production over time, especially in low-productivity systems²¹. In partial
124 support of this, temporal variability of total plant community biomass differed significantly
125 among species removal treatments, and with these effects differing with island size, for the

most recent 10 years of the experiment (Fig. 3a-c, Extended Data Table 3). Contrary to our expectation, we found that relative to the three-species mixtures (i.e., plots with no species removals), temporal invariability (i.e., $1/CV$) was significantly less in at least three reduced-diversity treatments on medium and large islands, and only one one-species treatment on small islands. Across all removals, we also found significant positive relationships between realized species richness and temporal invariability in community biomass but which were independent of island size (Fig. 3d-e, Extended Data Table 3). When analyzed for the initial years of our experiment (when there was an overall downward trend in biomass; see Methods) or for the full experimental period, effects of species removal on temporal variability were less pronounced (Supplementary Table 8, Supplementary Figures 11-12). However, our findings support theoretical work²⁸ proposing that negative effects of species loss on the consistency of biomass production over time depend strongly on which species are lost and the compensatory dynamics of remaining species over time, and highlight that these effects are mediated by environmental context and associated differences in plant dominance.

Biodiversity loss is well known to have important consequences for the structure and productivity of plant communities and associated ecosystem functions¹⁻⁴. Most available empirical evidence is from studies under highly controlled conditions in which plant diversity has been varied through random draws from species pools, but our results show that different patterns may occur when species are removed from natural ecosystems^{10,12}. Moreover, we show that long-term effects of diversity loss are not consistent and can differ greatly across contrasting ecosystems. While human impacts often cause loss of biodiversity across contrasting types of ecosystems²⁹, our results reveal that this loss of biodiversity affects the functioning of different ecosystems in contrasting ways. The loss of biodiversity in natural ecosystems under global change is not a random process, and we show that for a type of low diversity system that is globally widespread but little studied from a diversity-functioning

perspective, it matters not only which species are lost and to what extent the remaining species are able to exploit the available resources, but also from which ecosystems they are lost. In acknowledging the need for testing the generality of our findings to other systems including more-diverse systems that have been much more widely studied, we emphasize that forecasting consequences of biodiversity loss for the functioning of natural ecosystems necessitates taking into account when and how environmental context moderates the effects of this loss.

References

- 1 Hector, A. *et al.* Plant diversity and productivity experiments in European grasslands. *Science* **286**, 1123-1127 (1999).
- 2 Hooper, D. U. *et al.* A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature* **486**, 105-108 (2012).
- 3 Isbell, F. *et al.* Linking the influence and dependence of people on biodiversity across scales. *Nature* **546**, 65-72 (2017).
- 4 Duffy, E., Godwin, C. M. & Cardinale, B. J. Biodiversity effects in the wild are common and as strong as key drivers of productivity. *Nature* **549**, 261-264 (2017).
- 5 Craven, D. *et al.* Plant diversity effects on grassland productivity are robust to both nutrient enrichment and drought. *Phil. Trans. R. Soc. B* **371**, 20150277 (2016).
- 6 Fridley, J. D. Resource availability dominates and alters the relationship between species diversity and ecosystem productivity in experimental plant communities. *Oecologia* **132**, 271-277 (2002).
- 7 Gross, K. *et al.* Species richness and the temporal stability of biomass production: A new analysis of recent biodiversity experiments. *Am. Nat.* **183**, 1-12 (2014).

- 175 8 Wardle, D. A., Hörnberg, G., Zackrisson, O., Kalela-Brundin, M. & Coomes, D. A.
176 Long-term effects of wildfire on ecosystem properties across an island area gradient.
177 *Science* **300**, 972-975 (2003).
- 178 9 Wardle, D. A. & Zackrisson, O. Effects of species and functional group loss on island
179 ecosystem properties. *Nature* **435**, 806-810 (2005).
- 180 10 Cardinale, B. J. *et al.* Impacts of plant diversity on biomass production increase
181 through time because of species complementarity. *Proc. Natl. Acad. Sci. U.S.A.* **104**,
182 18123-18128 (2007).
- 183 11 Guerrero-Ramirez, N. R. *et al.* Diversity-dependent temporal divergence of ecosystem
184 functioning in experimental ecosystems. *Nat. Ecol. Evol.* **1**, 1639–1642 (2017).
- 185 12 Reich, P. B. *et al.* Impacts of biodiversity loss escalate through time as redundancy
186 fades. *Science* **336**, 589-592 (2012).
- 187 13 Reich, P. B. *et al.* Plant diversity enhances ecosystem responses to elevated CO₂ and
188 nitrogen deposition. *Nature* **410**, 809-812 (2001).
- 189 14 Isbell, F. I., Polley, H. W. & Wilsey, B. J. Biodiversity, productivity and the temporal
190 stability of productivity: patterns and processes. *Ecol. Lett.* **12**, 443-451 (2009).
- 191 15 Tilman, D., Reich, P. B. & Knops, J. M. H. Biodiversity and ecosystem stability in a
192 decade-long grassland experiment. *Nature* **441**, 629-632 (2006).
- 193 16 Bezemer, T. M. & van der Putten, W. H. Diversity and stability in plant communities.
194 *Nature* **446**, E6-E7 (2007).
- 195 17 Cardinale, B. J. *et al.* Biodiversity simultaneously enhances the production and
196 stability of community biomass, but the effects are independent. *Ecology* **94**, 1697-
197 1707 (2013).

- 198 18 Morin, X., Fahse, L., de Mazancourt, C., Scherer-Lorenzen, M. & Bugmann, H.
 199 Temporal stability in forest productivity increases with tree diversity due to
 200 asynchrony in species dynamics. *Ecol. Lett.* **17**, 1526-1535 (2014).
- 201 19 van Ruijven, J. & Berendse, F. Contrasting effects of diversity on the temporal
 202 stability of plant populations. *Oikos* **116**, 1323-1330 (2007).
- 203 20 de Mazancourt, C. *et al.* Predicting ecosystem stability from community composition
 204 and biodiversity. *Ecol. Lett.* **16**, 617-625 (2013).
- 205 21 Hautier, Y. *et al.* Eutrophication weakens stabilizing effects of diversity in natural
 206 grasslands. *Nature* **508**, 521-525 (2014).
- 207 22 Wardle, D. A. *et al.* Linking vegetation change, carbon sequestration and biodiversity:
 208 insights from island ecosystems in a long-term natural experiment. *J. Ecol.* **100**, 16-30
 209 (2012).
- 210 23 Huston, M. A. & Deangelis, D. L. Competition and coexistence: The effects of
 211 resource transport and supply rates. *Am. Nat.* **144**, 954-977 (1994).
- 212 24 Gundale, M. J., Hyodo, F., Nilsson, M. C. & Wardle, D. A. Nitrogen niches revealed
 213 through species and functional group removal in a boreal shrub community. *Ecology*
 214 **93**, 1695-1706 (2012).
- 215 25 Fargione, J. *et al.* From selection to complementarity: shifts in the causes of
 216 biodiversity-productivity relationships in a long-term biodiversity experiment. *Proc.*
 217 *Royal Soc. B* **274**, 871-876 (2007).
- 218 26 Symstad, A. J. & Tilman, D. Diversity loss, recruitment limitation, and ecosystem
 219 functioning: lessons learned from a removal experiment. *Oikos* **92**, 424-435 (2001).
- 220 27 Loreau, M. Biodiversity and ecosystem functioning: recent theoretical advances. *Oikos*
 221 **91**, 3-17 (2000).

28 Fowler, M. S., Laakso, J., Kaitala, V., Ruokolainen, L. & Ranta, E. Species dynamics
 alter community diversity-biomass stability relationships. *Ecol. Lett.* **15**, 1387-1396
 (2012).

29 Suding, K. N. *et al.* Functional- and abundance-based mechanisms explain diversity
 loss due to N fertilization. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 4387-4392 (2005).

Supplementary Information is available in the online version of the paper.

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 the first draft of the manuscript, and all authors contributed to manuscript completion and
 revision.

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FIGURE LEGENDS

Figure 1. Effects of plant species removal on temporal plant biomass patterns. Shown are temporal patterns (1999-2016; years 3-20) of total plant biomass (g/m^2) for a) large, b) medium, and c) small islands. Species codes refer to the plant species remaining (i.e., not removed): M = *Vaccinium myrtillus*, V = *Vaccinium vitis-idaea*, E = *Empetrum hermaphroditum*. Thick, dark-colored lines show mean values per treatment ($n = 10$ islands per size class except for 'E' treatments on large islands ($n = 8$), 'E' treatments on medium islands ($n = 5$), 'M+E' treatments on medium islands ($n = 8$), 'E' treatments on small islands ($n = 9$), 'M' treatments on small islands ($n = 8$), and 'M+E' treatments on small islands ($n = 9$). Thin, light-colored lines show values for individual plots. Within island size classes, removal treatments with the same letters are not significantly different across the duration of the study. Treatment effects were tested using linear mixed models fitted by a Restricted Maximum Likelihood method, and we used contrast analyses to test across-year differences between removal treatments (see Methods for details).

Figure 2. Effects of species richness on plant biomass decreases over time. Temporal patterns (1999-2016; years 3-20) of the proportion of variance explained in total plant biomass by realized species richness, for large, medium and small islands. The proportion of variance explained (also called effect size) was calculated using marginal R^2 values ($R^2_{\text{GLMM}(m)}$) for linear mixed models ($n = 10$ islands per size class). Effects of island size, year, and their interactions were tested using linear mixed models, and contrasts were applied to compare slopes among island size classes for temporal changes in the amount of variance explained (See Extended Data Table 2 for details). Linear regressions were fitted for each island size class (dotted lines). P-values denote slopes significantly different from zero.

267

268 **Figure 3. Effects of species removal and species richness on temporal biomass**269 **invariability.** A-C) Effects of plant species removals on temporal invariability (1/(coefficient

270 of variation or CV)) in community biomass (2007-2016; years 11-20) for large, medium, and

271 small islands. Species codes refer to the plant species remaining (i.e., not removed): M =

272 *Vaccinium myrtillus*, V = *Vaccinium vitis-idaea*, E = *Empetrum hermaphroditum*. Bar graphs273 show mean values $\pm$ s.e. (n = 10 islands per size class, except for 'E' treatments on large

274 islands (n = 8), 'E' treatments on medium islands (n = 5), 'M+E' treatments on medium

275 islands (n = 8), 'E' treatments on small islands (n = 9), 'M' treatments on small islands (n =

276 8), and 'M+E' treatments on small islands (n = 9)). Dots indicate values for individual islands.

277 Data were analyzed using linear mixed models (see Methods for details). Within island size

278 classes, removal treatments with the same letters are not significantly different across years

279 (Tukey posthoc tests, $p < 0.05$). D-F) Relationship between mean realized species richness

280 and temporal invariability (2007-2016) for large, medium and small islands. Dots indicate

281 individual plots (large islands: n = 68; medium islands: n = 63; small islands: n = 65). Insets

282 show results from linear models testing for the effects of species richness on temporal

283 invariability (see Methods for model details). Significant p -values (<0.05) are shown as bold284 numbers. R^2 values indicate the strength of the relationship.

285

METHODS

Study system. This study was conducted along a post-fire chronosequence consisting of 30 forested islands in lakes Hornavan and Uddjaure in northern Sweden (65°55'N to 66°09'N; 17°43'E to 17°55'E). The islands are all of the same geologic age, and experience the same macroclimate. As such, mean annual precipitation is 750 mm, of which 350 mm falls during the growing season (May to September), and the mean annual temperature is +0.5 °C (+13 °C in July and -14 °C in January). The only major extrinsic factor that varies among the islands is the frequency of wildfire, with larger islands burning more frequently than smaller islands because they have a greater probability of being struck by lightning^{8,30}. Previous work has shown that as islands become smaller and the time since fire increases, the availability of nutrients (notably nitrogen and phosphorus) decreases; this leads to impairment of the rates of decomposition and nutrient fluxes, and lower plant biomass and productivity^{8,22,30-32}. Consistent with previous studies in this system^{8,22,30-32}, these islands were divided into three size classes with 10 islands per class: large (>1.0 ha), medium (0.1–1.0 ha), and small (<0.1 ha), with a mean time since last major fire of about 585, 2180 and 3250 years at the start of the experiment, respectively⁸. This replicate number was necessary for correctly identifying important differences among islands size classes³⁰. The overstory vegetation is dominated by *Betula pubescens*, *Pinus sylvestris*, and *Picea abies*, and the ground layer vegetation consists of the ericaceous dwarf shrubs *Vaccinium myrtillus*, *V. vitis-idaea*, and *Empetrum hermaphroditum* and feather mosses. Extended Data Table 4 shows a selection of ecosystem properties for each of the three island size classes.

Experimental design. For this study we focused on the dwarf shrub vegetation, and used a 'removal experiment' approach because this is a powerful tool for investigating the effects of

local, non-random losses of biotic components and species interactions in natural ecosystems³³. We established 8 experimental plots on each of the 30 islands, each representing a different dwarf shrub species removal treatment, i.e., full factorial removal of *V. myrtillus*, *V. vitis-idaea*, and *E. hermaphroditum*⁹. One of these plots involved removal of all shrub species and was not considered in this study. The level of species richness we manipulated is typical of the diversity of understory vegetation that characterizes boreal forests at the spatial scale of our plots. As such, our system is representative for low-diversity communities for which ecosystem functioning is especially vulnerable to species loss. These three shrub species dominate the ericaceous shrub layer in large, medium and small islands, respectively, and collectively account for $\pm 98\%$ of vascular plant biomass in the understory⁸ (see Supplementary Table 9 for details on productivity and leaf traits for each of the shrub species across the island size gradient). All plots were 55 cm $\times$ 55 cm, but only the inner 45 cm $\times$ 45 cm were ever measured. All plots were located at similar distances from the shore for each island, regardless of island size, to prevent edge and microclimatic effects from confounding the results^{8,9}. The experiment was established in August 1996 and has been maintained annually ever since. Shrub removal treatments were conducted and maintained through annual physical removal of vegetation. It is recognized that vegetation removals impose initial disturbance effects, but these are likely to be transient and of minimal importance after the initial years^{34,35}. As such, in a separate 14-year experiment performed on the dwarf shrub community on all 30 islands and in plots that are adjacent to the plots used in this study, and that involved experimental disturbance treatments with greater disturbance than the removals performed in this study³⁶, it was shown that disturbance legacies of vegetation removal are mostly gone within 3-6 years. Further details of the removal experiment reported here are presented in Ref. ⁹.

Every August from 1996 until 2016 inclusive, the total cover of shrub species was assessed in each plot by point quadrant analysis, by determining the total number of times the vegetation of that species was intercepted by a total of 100 downwardly projecting points. The total number of intercepts for each species was then converted to biomass per unit area through equations previously developed by destructively sampling calibration plots⁸. For each of the three shrub species, the total number of point intercepts is very closely correlated with aboveground standing biomass, with R^2 values consistently above 0.90⁸. During the first 10 years of the experiment, we observed an overall downward trend in plant community biomass across island sizes. This trend was not related to the removal treatments; it also occurred in the control plots (Fig. 1). There are two plausible explanations. First, it could have been a function of where we positioned the plots when the experiment was set up in 1996, as we always placed our plots in well-developed vegetation. As part of their natural population dynamics, long-lived dwarf shrub species in boreal forest tend to move around over time³⁷. This means that well-developed shrub patches may well decline in biomass over time while less developed shrub patches may well aggrade to become well developed patches in the future. Second, over the course of the experiment plant biomass was likely to have turned over only about three times^{9,38}, and if during one of the cycles there is high (or low) biomass, then this high (or low) biomass situation will persist for some years. It is plausible that if there were environmental conditions that promoted high biomass in all plots at the start of the experiment then that effect would have a legacy that would persist several years into the experiment. We emphasize that this downward trend is a non-biased pattern that has no bearing on conclusions that we can draw from this study.

Data analyses. To account for the initial disturbance effects of the removal treatments⁹, the first three years of data (1996-1999) were excluded from the data analyses. Further, in all

analyses we only used plots where at the start of the experiment (i.e., after implementation of the removal treatments) at least one of the three shrub species was present. This resulted in the *a priori* exclusion of 13 out of a total of 210 plots across all islands (large islands: 2 plots where *V. myrtillus* and *V. vitis-idea* were removed; medium islands: 5 plots where *V. myrtillus* and *V. vitis-idea* were removed and two plots where *V. vitis-idea* was removed; small islands: 1 plot where *V. myrtillus* and *V. vitis-idea* were removed, 1 plots where *V. vitis-idea* was removed, and two plots where *V. vitis-idea* and *E. hermaphroditum* were removed).

Effects of plant species removal, island size class, and their interaction on total plant biomass and biomass of each of the three shrub species were tested using linear mixed models (LMMs) fitted by a Restricted Maximum Likelihood method. Island identity was included as a random effect to account for the repeated measurements across time and plots were nested within island identity to allow treatments comparison within each island separately. We fitted a first-order auto-regressive variance structure¹¹. To further explore the effects of species removal on total and plant species-specific biomass, we ran LMMs separately for each island size class and used contrast analyses to test across-year differences between removal treatments. To evaluate potential effects of the overall downward trend in plant biomass during the first 10 years of the experiment, we ran all LMMs separately for 1999-2006, 2007-2016, and 1999-2016. These separate analyses showed that effects of species removal and island size were largely consistent across these different stages of the experiment (see Extended Data Table 1).

To test how the effects of species loss on plant community biomass changed over time, we calculated the proportion of variance explained by the species removal treatment and by species richness (to better account for species lost over the course of the experiment) separately for each year of the experiment (1999-2016) and for each island size class (small, medium, large). For each plot, the proportion of variance explained (also called effect size)

was calculated using marginal R^2 values ($R^2_{\text{GLMM}(m)}$) for LMMs³⁹, with species removals or realized species richness (i.e., the number of shrub species present across the entire experimental period³⁵) considered as fixed factors and island identity as a random factor. The values of the marginal $R^2_{\text{GLMM}(m)}$ describe the proportion of variance explained by fixed factors alone. We then tested how $R^2_{\text{GLMM}(m)}$ changed over time using LMMs with island size class and year as fixed factors and year as a continuous variable. Contrasts on the interaction between island size class and year were used to test if the slopes of regressions between year and $R^2_{\text{GLMM}(m)}$ differed between island size classes. Finally, we used polynomial models to test for non-linear patterns. In these models, we employed LMMs with island size class and the second-order polynomial of year as fixed factors and year as a continuous variable. To compare if polynomial models varied among island size classes, we compared models by grouping island size classes two-by-two and tested if the effect of the grouping variable was significant.

Further, to better understand the underlying mechanisms of the effects of species loss on total and plant-species specific biomass and how they change over time, we used two approaches for comparing biomass in multi-species communities relative to their component monocultures⁴⁰; in our study monocultures consisted of the three treatments in which only one of the three species was not removed. These measures were calculated separately for three-species mixtures (i.e., plots for which no removals were performed) and for each of the three possible two-species mixtures (i.e., plots for which one of the three species was removed). First, we used the additive partitioning approach to separate net biodiversity effects into complementarity and selection effects⁴¹. Net biodiversity effects measure the deviation of biomass in any given multi-species community from its expected value based on biomass of its component species grown in monoculture. Complementarity effects measure the average relative species biomass in multi-species communities relative to the expected biomass based

on the weighted-average biomass of their component monocultures. Selection effects measure the effects of species with higher or lower than average biomass in monocultures on total community biomass. Second, we calculated two measures of complementarity that are independent of absolute biomass values: relative yield totals (RYT) and transgressive overyielding. The relative yield of a species measures its biomass in a multi-species community as a proportion of its biomass in monoculture, and RYT of a multi-species community is the sum of the relative yield of all component species⁴². Meanwhile, transgressive overyielding measures whether multi-species communities obtain higher biomass than the monoculture of its most productive component species. Transgressive overyielding was calculated as D_{Max} following Ref. ⁴³. We then tested how each of the measures of biodiversity effects that we calculated changed over time using LMMs and polynomial models as described above for $R^2_{GLMM(m)}$, and with individual islands ($n = 10$ for each of the three island size classes) used as units of replication. We fitted a first-order autoregressive variance structure to account for temporal pseudoreplication through repeated measurements on each island¹¹. Measures of biodiversity effects could not be calculated whenever calculations included denominator values of zero, and there were also some cases ($< 1\%$) in which very low denominator biomasses resulted in extremely high values of biodiversity measures; these extreme values were removed from the analyses following the outlier labelling rule with a conservative tuning parameter of $g = 2.2$ ⁴⁴.

Finally, to test how species loss affects temporal invariability in plant community biomass, we calculated the inverse coefficient of variation ($1/CV$), i.e., the mean biomass over the study period divided by the standard deviation for that period¹⁴⁻¹⁷. The inverse of coefficient of variation provides a widely used standardized measure of invariability that is comparable across ecosystems, and that is commonly referred to as ‘temporal stability’⁷. We focused on temporal invariability for the most recent ten-year period, i.e., 2007-2016. This is

in line with the ten year period used in other comparable analyses of temporal invariability¹⁵, and was done because up to 2007 there was a general decline in total plant community biomass, whereas over the last ten years of the experiment there was no apparent directional change in total community biomass (Fig. 1). However, we also calculated temporal invariability for 1999-2006 and for 1999-2016. For each period, effects of plant species removal and island size class on temporal invariability were then tested using LMMs with island identity as random factor. Within island size classes, contrast analyses were used to test differences between species removal treatments. Finally, relationships between realized species richness and temporal invariability were tested using linear regression. Similar to the biomass data, all analyses for invariability were ran separately for 1999-2006, 2007-2016, and 1999-2016.

References

- 30 Wardle, D. A., Zackrisson, O., Hörnberg, G. & Gallet, C. The influence of island area on ecosystem properties. *Science* **277**, 1296-1299 (1997).
- 31 Wardle, D. A., Walker, L. R. & Bardgett, R. D. Ecosystem properties and forest decline in contrasting long-term chronosequences. *Science* **305**, 509-513 (2004).
- 32 Clemmensen, K. E. *et al.* Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science* **339**, 1615-1618 (2013).
- 33 Díaz, S., Symstad, A. J., Chapin, F. S., Wardle, D. A. & Huenneke, L. F. Functional diversity revealed by removal experiments. *Trends Ecol. Evol.* **18**, 140-146 (2003).
- 34 Coomes, D. A. & Grubb, P. J. Impacts of root competition in forests and woodlands: A theoretical framework and review of experiments. *Ecol. Monogr.* **70**, 171-207 (2000).

- 459 35 McGrady-Steed, J., Harris, P. M. & Morin, P. J. Biodiversity regulates ecosystem
460 predictability. *Nature* **390**, 162-165 (1997).
- 461 36 Wardle, D. A. & Jonsson, M. Long-term resilience of above- and belowground
462 ecosystem components among contrasting ecosystems. *Ecology* **95**, 1836-1849 (2014).
- 463 37 Flower-Ellis, J. G. K. *Age Structure and Dynamics in Stands of Bilberry (Vaccinium*
464 *myrtillus L.)* (Royal College of Forestry, Stockholm, Department of Forest Ecology
465 and Forest Soils, Research Note No. 9, Stockholm, 1971).
- 466 38 Wardle, D. A. *et al.* Drivers of inter-year variability of plant production and
467 decomposers across contrasting island ecosystems. *Ecology* **93**, 521-531 (2012).
- 468 39 Nakagawa, S. & Schielzeth, H. A general and simple method for obtaining R^2 from
469 generalized linear mixed-effects models. *Methods Ecol. Evol.* **4**, 133-142 (2013).
- 470 40 Roscher, C. *et al.* Overyielding in experimental grassland communities - irrespective
471 of species pool or spatial scale. *Ecol. Lett.* **8**, 576-577 (2005).
- 472 41 Loreau, M. & Hector, A. Partitioning selection and complementarity in biodiversity
473 experiments. *Nature* **412**, 72-76 (2001).
- 474 42 Hector, A. The effect of diversity on productivity: detecting the role of species
475 complementarity. *Oikos* **82**, 597-599 (1998).
- 476 43 Loreau, M. Separating sampling and other effects in biodiversity experiments. *Oikos*
477 **82**, 600-602 (1998).
- 478 44 Hoaglin, D. C. & Iglewicz, B. Fine-tuning some resistant rules for outlier labeling. *J.*
479 *Am. Stat. Assoc.* **82**, 1147-1149 (1987).
- 480 45 Lagerström, A., Esberg, C., Wardle, D. A. & Giesler, R. Soil phosphorus and
481 microbial response to a long-term wildfire chronosequence in northern Sweden.
482 *Biogeochemistry* **95**, 199-213 (2009).
- 483

484 **Data availability statement.** The datasets generated during and/or analysed during the
485 current study are available from the corresponding author on reasonable request.

EXTENDED DATA

Extended Data Table 1. Effects of species removal and island size, and their interactions, on total and species-specific plant biomass. Data were analyzed using linear mixed models, and were analyzed for 1999-2016, and separately for 1999-2006 and 2007-2016 ($n = 10$ islands per size class) (see Methods for details). Significant p -values (<0.05) are shown as bold numbers. Results from contrast analyses testing across-year differences in total and plant species-specific biomass between removal treatments for large, medium, and small islands are indicated in Fig. 1 and Extended Data Fig. 1 (1999-2016) and in Supplementary Table 10 (1999-2006 and 2007-2016). For the analyses of total biomass all seven species removal treatments were used; for each of the analyses for *Vaccinium myrtillus*, *V. vitis-idaea*, and *Empetrum hermaphroditum*, and only the treatments from which those species were not removed were used.

Extended Data Table 2. Effects of species removal and island size, and their interactions on the amount of variance explained ($R^2_{\text{GLMM}(m)}$) by species removal treatment and by realized species richness. The proportion of variance explained (also called effect size) was calculated using marginal R^2 values ($R^2_{\text{GLMM}(m)}$) for linear mixed models ($n = 10$ islands per size class). Data were analyzed using linear mixed models, and contrasts were applied to compare slopes among island size classes for temporal changes in the amount of variance explained. Significant p -values (<0.05) are shown as bold numbers. See Methods for model details.

Extended Data Table 3. Effects of species removal or realized species richness, and island size, and their interactions on temporal invariability. Data were analyzed using linear models testing for the effects of A) species removal, island size (small, medium, large) and their interactions; and B) realized species richness, island size and their interactions on temporal invariability ($1/(\text{coefficient of$

variation or CV)) in plant community biomass; for the period 2007-2016 ($n = 10$ islands per size class). Significant p -values (<0.05) are shown as bold numbers.

Extended Data Table 4. Selected ecosystem properties across the island size gradient. Data shown are mean values $\pm$ standard errors ($n = 10$ islands per size class). Within each row, numbers followed by the same letter are not statistically significant at $p = 0.05$ (Tukey's test following one-way ANOVA). Data from Ref. ^{8,22,30-31,45}

Extended Data Figure 1. Effects of plant species removal on temporal biomass patterns (1999-2016, years 3-20) of individual species biomass. Data show individual species biomass (g/m^2) for *Vaccinium myrtillus* (A-C), *V. vitis-idaea* (D-F), and *Empetrum hermaphroditum* (G-I) for large, medium, and small islands. Species codes refer to the plant species remaining after removal: M = *Vaccinium myrtillus*, V = *Vaccinium vitis-idaea*, E = *Empetrum hermaphroditum*. Thick, dark-colored lines show mean values per treatment ($n = 10$ islands per size class, except for 'E' treatments on large islands ($n = 8$), 'E' treatments on medium islands ($n = 5$), 'M+E' treatments on medium islands ($n = 8$), 'E' treatments on small islands ($n = 9$), 'M' treatments on small islands ($n = 8$), and 'M+E' treatments on small islands ($n = 9$). Thin, light-colored lines show values for individual plots. Within island size classes, removal treatments with the same letters are not significantly different across years through the duration of the study. Treatment effects were tested using linear mixed models fitted by a Restricted Maximum Likelihood method, and we used contrast analyses to test across-year differences between removal treatments (see Methods for details).

Extended Data Figure 2. Temporal patterns (1999-2016; years 3-20) of the proportion of variance in total plant biomass explained by the species removal treatment for large, medium, and small islands. The proportion of variance explained (also called effect size) was calculated using marginal R^2 values ($R^2_{\text{GLMM}(m)}$) for linear mixed models ($n = 10$ islands per size class). Linear

537 regressions were fit for each island size class (dotted lines). We used linear mixed models to test how
538 $R^2_{\text{GLMM}(m)}$ changed over time with island size class and year as fixed factors and year as a continuous
539 variable. Contrasts on the interaction between island size class and year were used to test if the slopes
540 of regressions between year and $R^2_{\text{GLMM}(m)}$ differed between island size classes. Significant
541 differences in slopes among island size classes at $\alpha = 0.05$ are indicated in the panel. Asterisks denote
542 slopes significantly different from zero: *** = $p < 0.001$, ** = $p < 0.01$.