

Biodiversity increases and decreases ecosystem stability

Frank Pennekamp^{1*}, Mikael Pontarp^{1,2}, Andrea Tabi¹, Florian Altermatt^{1,3}, Roman Alther^{1,3}, Yves Choffat¹, Emanuel A. Fronhofer^{1,3,5}, Pravin Ganesanandamoorthy^{1,3}, Aurélie Garnier¹, Jason I. Griffiths⁴, Suzanne Greene^{1,6}, Katherine Horgan¹, Thomas M. Massie¹, Elvira Mächler^{1,3}, Gian Marco Palamara^{1,7}, Mathew Seymour^{3,8} & Owen L. Petchey¹

Losses and gains in species diversity affect ecological stability^{1–7} and the sustainability of ecosystem functions and services^{8–13}. Experiments and models have revealed positive, negative and no effects of diversity on individual components of stability, such as temporal variability, resistance and resilience^{2,3,6,11,12,14}. How these stability components covary remains poorly understood¹⁵. Similarly, the effects of diversity on overall ecosystem stability¹⁶, which is conceptually akin to ecosystem multifunctionality^{17,18}, remain unknown. Here we studied communities of aquatic ciliates to understand how temporal variability, resistance and overall ecosystem stability responded to diversity (that is, species richness) in a large experiment involving 690 micro-ecosystems sampled 19 times over 40 days, resulting in 12,939 samplings. Species richness increased temporal stability but decreased resistance to warming. Thus, two stability components covaried negatively along the diversity gradient. Previous biodiversity manipulation studies rarely reported such negative covariation despite general predictions of the negative effects of diversity on individual stability components³. Integrating our findings with the ecosystem multifunctionality concept revealed hump- and U-shaped effects of diversity on overall ecosystem stability. That is, biodiversity can increase overall ecosystem stability when biodiversity is low, and decrease it when biodiversity is high, or the opposite with a U-shaped relationship. The effects of diversity on ecosystem multifunctionality would also be hump- or U-shaped if diversity had positive effects on some functions and negative effects on others. Linking the ecosystem multifunctionality concept and ecosystem stability can transform the perceived effects of diversity on ecological stability and may help to translate this science into policy-relevant information.

Ecological stability consists of numerous components, including temporal variability, resistance to environmental change and rate of recovery from disturbance^{1,2,16}. Effects of species losses and gains on these components are of considerable interest, not least because of the potential effects on ecosystem functioning and therefore on the sustainable delivery of ecosystem services^{1–13}. A growing number of experimental studies reveal stabilizing effects of diversity on individual stability components. In particular, higher diversity often—but not always—reduces temporal variability of biomass production¹³. Positive effects of diversity on resistance are common, although neutral and negative effects on resistance and resilience also occur^{9,13,19,20}. Although the assessment of individual components of stability is essential, a more integrative approach to ecological stability could lead to clearer conceptual understanding¹⁵ and might help to improve policy guidance concerning ecological stability¹⁶.

Analogous to ecosystem multifunctionality^{17,18}, a more integrative approach considers variation in multiple stability components and the often-ignored covariation among stability components. The nature

of this covariation is of paramount importance, as it defines whether diversity has consistent effects on multiple stability components, or whether some stability components increase with diversity while others decrease. However, the nature, prevalence and implications of negative covariation between stability components along diversity gradients are almost completely overlooked, including the ensuing possibility for non-monotonic effects of diversity on overall ecosystem stability.

We first describe our experimental findings of how biodiversity affects the intrinsic stability of ecosystems and their resistance to warming. Temperature is a highly relevant disturbance owing to its importance for biological processes and its great variability across space and time. However, our findings equally apply to and have implications for other environmental changes that could result in opposing effects on stability components, such as flooding¹² or chemical stress²¹. We then review other evidence for negative covariation in effects of diversity on stability and potential mechanisms. Finally, we analyse overall ecosystem stability, a concept that embraces the covariation between stability components and their weighting, and show the plausibility of previously overlooked non-monotonic (hump- and U-shaped) effects of diversity on overall ecosystem stability.

We performed a factorial manipulation of the diversity and composition of competing species (1–6 species, 53 unique community compositions) and temperature (six constant levels, modelled as a linear predictor) in microbial communities of bacterial consumers, and recorded community biomass dynamics over time. For each replicate we then calculated two stability components: resistance (that is, the total biomass at T minus the total biomass at 15 °C divided by T minus 15 °C, in which T is the temperature of the replicate) and the temporal stability of biomass (the inverse of the coefficient of variation of community biomass). Although these stability indices are widely used by empiricists, they should not be mistaken for mathematical definitions—such as asymptotic resilience—that are more precise but also more restrictive²².

Increased species richness caused greater temporal stability of total biomass (Fig. 1a) (linear mixed-effects model of log inverse coefficient of variation: richness effect size 0.33 ± 0.065 (mean \pm s.e.m.)) at all temperatures (Extended Data Fig. 1). Total biomass increased during the first week of the experiment and then declined over the next five weeks and total biomass was higher in more species-rich communities (Fig. 1b, c and Extended Data Table 1). The effect size for log(richness) was 0.05 ± 0.0096 mg ml⁻¹ per log(species richness) (mean \pm s.e.m.).

By contrast, increased species richness decreased resistance of total biomass to warming (Fig. 1a) (negative effect of log(richness) in a linear model, effect size of -0.006 ± 0.0018 mg per °C per log(species richness) (mean \pm s.e.m.). Richness negatively affected resistance measured on both absolute and relative scales (Extended Data Fig. 2). This effect was

¹Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland. ²Department of Ecology and Environmental Science, Umeå University, Umeå, Sweden.

³Department of Aquatic Ecology, Eawag: Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland. ⁴Department of Mathematics, University of Utah, Salt Lake City, UT, USA. ⁵Present address: ISEM, Université de Montpellier, CNRS, IRD, EPHE, Montpellier, France. ⁶Present address: MIT Center for Transportation & Logistics, Massachusetts Institute of Technology, Cambridge, MA, USA. ⁷Present address: Department of Systems Analysis, Integrated Assessment and Modelling, Eawag: Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland. ⁸Present address: Molecular Ecology and Fisheries Genetics Laboratory, School of Biological Sciences, Bangor University, Bangor, UK. *e-mail: frank.pennekamp@ieu.uzh.ch

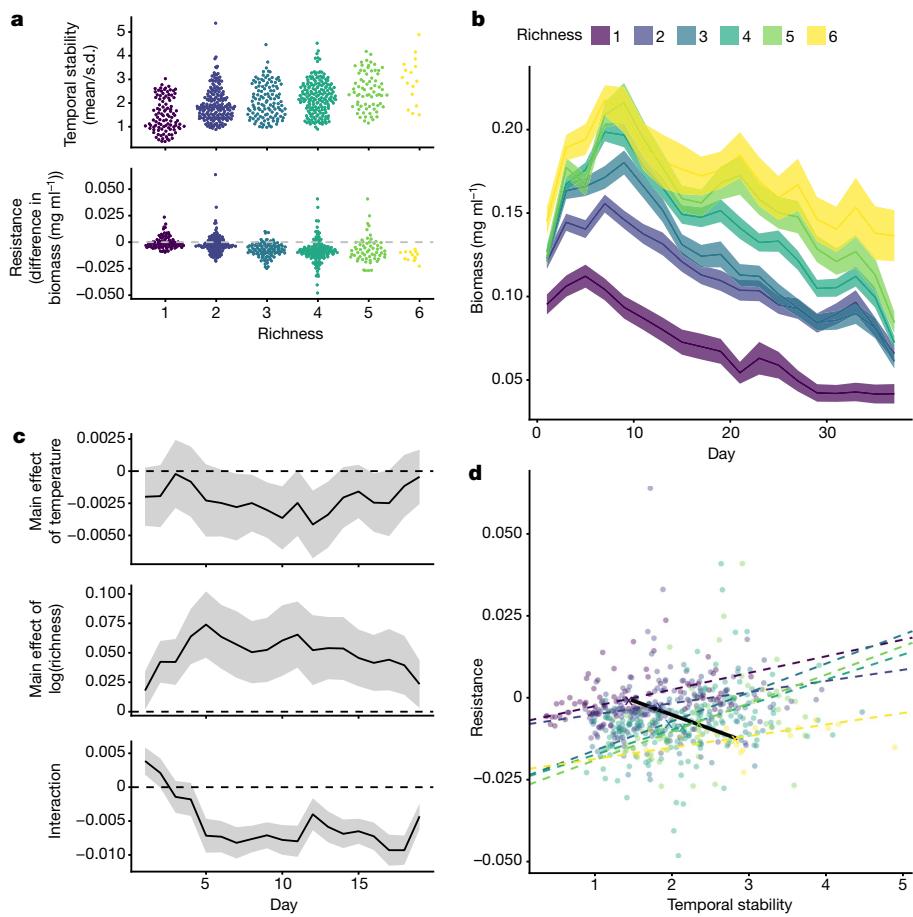


Fig. 1 | Biomass and stability in experimental microbial communities. **a**, Richness increased temporal stability (top, $n = 681$ independent microcosms), but decreased resistance (bottom, $n = 567$ independent microcosms). The dashed line indicates zero resistance. **b**, Average (mean \pm s.e.m.) biomass for each diversity level ($n = 12,939$ microcosm \times day combinations). **c**, Temporal dynamics of effect sizes (and 95% confidence intervals) of a linear mixed-effects model of total biomass showed, on average, a negative effect of temperature on biomass (top), a positive effect of diversity on biomass (middle) and a more negative effect of temperature at higher richness on biomass (persistent negative interaction term; bottom) ($n = 681$ independent microcosms per day). The dashed lines indicate zero effect. **d**, Resistance and temporal stability ($n = 567$ independent microcosms) covaried negatively across richness (solid line through centroid of each richness level, $n = 6$ independent richness levels), but positively within richness levels (dashed lines).

corroborated in analyses of total biomass by a negative interaction term between temperature and richness, which persisted through the experiment, except during the first days (Fig. 1c) ($\log(\text{richness}) \times \text{temperature}$ interaction of $-0.0053 \pm 0.00051 \text{ mg ml}^{-1}$ per $^{\circ}\text{C}$ per $\log(\text{species richness})$), despite large variation in dynamics of total biomass (Fig. 1b). This negative interaction reflects a stronger negative effect of temperature on total biomass (that is, lower resistance) in richer communities (that is, a richness-dependent response of total biomass to temperature).

Therefore, temporal stability and resistance were negatively correlated across the species richness gradient (Fig. 1d, reduced major axis regression analysis with a slope of -0.009 and a 95% confidence interval of -0.0178 to -0.0051). Niche complementarity, statistical averaging, low overall response diversity and possibly lower response diversity in more diverse communities were probably causes of the opposite effects of richness on temporal stability (Extended Data Fig. 3). The two stability components were, however, positively correlated within any single level of species richness (Fig. 1d and Extended Data Table 2).

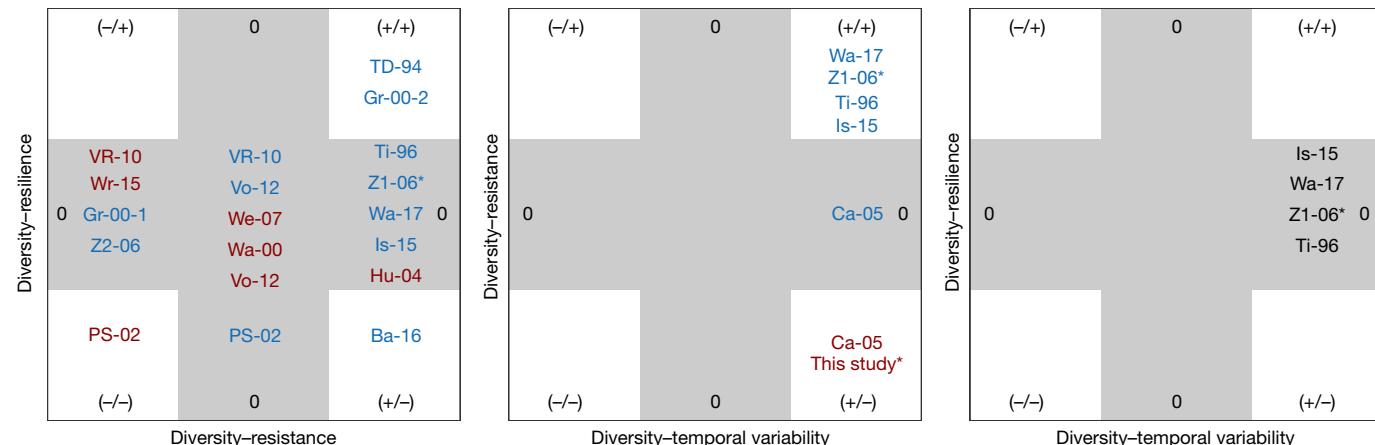


Fig. 2 | Positive, negative and neutral relationships among resistance, resilience and temporal variability in empirical studies with diversity manipulation. The 30 bivariate relationships were reported by 17 independent studies (in addition to this study). Detailed information about individual studies (for example, code VR-10) is provided in Extended Data Tables 3, 4. Light-grey regions indicate no covariation. Relative positions

within regions are arbitrary and do not indicate relative strengths of relationships. Different colours indicate the effect of diversity on absolute (red) or relative (blue) resistance, whereas temporal stability and resilience are shown in black. *Studies that examine intrinsic stability alongside response to an environmental change.

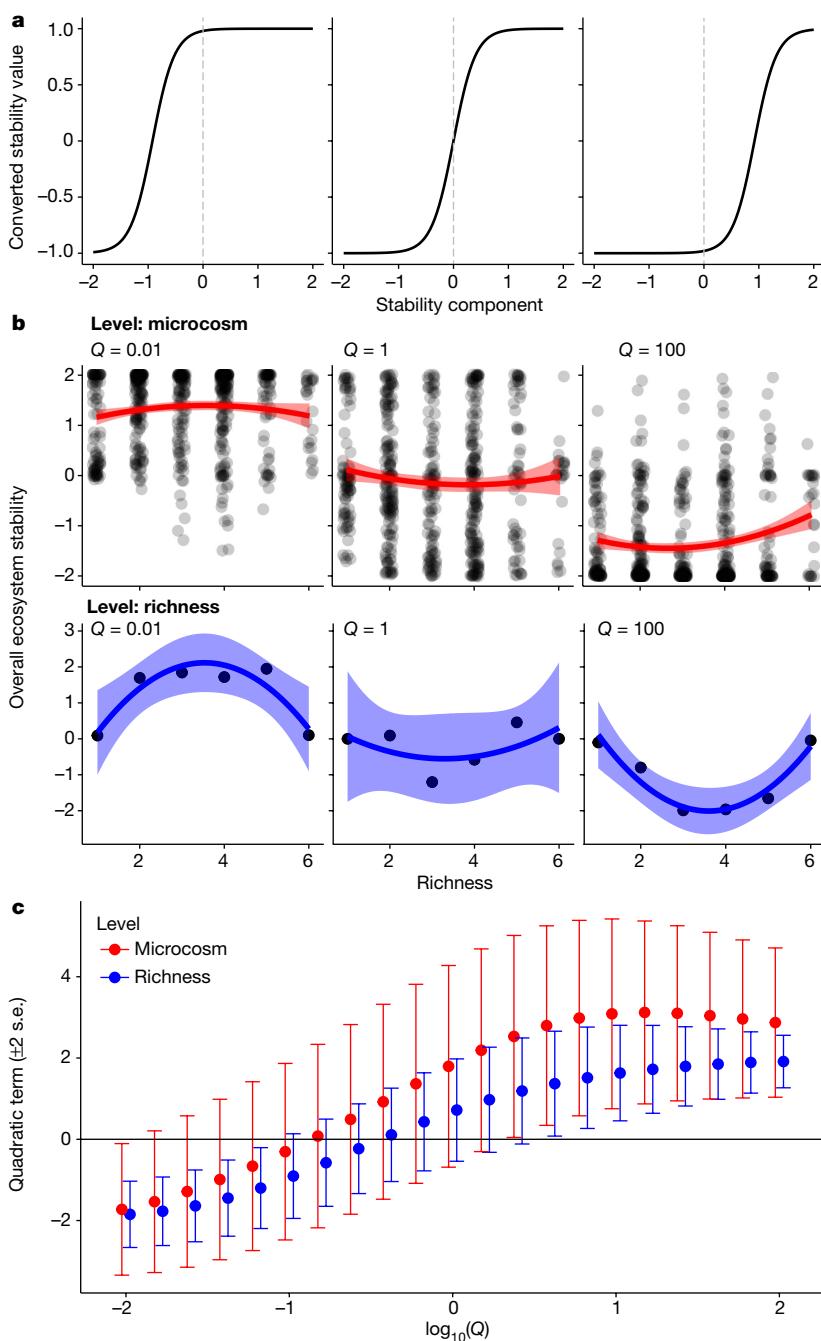


Fig. 3 | Hump- and U-shaped diversity–stability relationships. **a**, The intercept of the generalized logistic to convert measured stability components into a common currency varies with the parameter Q . **b**, The non-aggregated ($n = 567$ independent microcosms) or aggregated ($n = 6$ richness levels) data exhibits hump- to flat- to U-shaped diversity–stability relationships as Q varies. Lines show the fit of a quadratic model and the 95% confidence interval (bands). **c**, The variation from hump-shaped to U-shaped relationship depends smoothly on Q —that is, the position of the threshold (quantified by the quadratic term of a regression with mean (dot) and 95% confidence intervals (bars)).

That is, composition variation without changes in species richness resulted in positively covarying temporal stability and resistance.

Next, we examined studies (including our own) that measured multiple stability components across diversity gradients based on a previously published review¹⁶ (Fig. 2 and Extended Data Tables 3, 4). In total 7 out of 30 comparisons showed positive covariance, 20 studies showed no covariance, and 3 studies showed negative covariance. Notably, our study identifies negative covariation between resistance and temporal variability caused by intrinsic dynamics only. Although infrequently reported, negative covariation is disproportionately important, because it complicates conclusions about and practical implications of the effects of diversity on stability. Furthermore, these studies may be unrepresentative of the true prevalence of negative covariation, because it may be overlooked, because of publication bias towards positive diversity–stability relationships³ or if the scale of analysis masks such covariation, for example, within richness versus across richness.

A general mechanistic understanding of why different studies find different correlations would be a major step forward. Of the 30 pairs of

stability components, only 7 were accompanied by quantitative analyses of mechanism for both diversity–stability relationships (Extended Data Table 4). Response diversity was implicated in five of these seven studies. Indeed, response diversity has been identified as an important driver of the resilience of ecological systems^{23,24}, and correlation among effect (that is, high biomass production) and response (for example, response to an environmental driver) traits—at least in the absence of strong interspecific interactions—might predict covariance among stability components²³. None of the studies involved manipulation of mechanism.

Negative covariation also raises the potential for non-monotonic effects of diversity on overall ecosystem stability. Ecosystem multifunctionality is the simultaneous consideration of several ecosystem functions. Similarly, overall ecosystem stability considers multiple stability components simultaneously¹⁶ (see Extended Data Fig. 4 for clarification of terms and concepts). We first calculated overall ecosystem stability as the sum of the standardized individual components of ecosystem stability²⁵. For the results of our experiment, in which one stability component increases linearly with diversity and another decreases linearly

(Fig. 1a), overall ecosystem stability is invariant with species richness, because the two standardized components perfectly balance each other.

We further explored the implications of the negative covariation among stability components. Existing methods for calculating ecosystem multifunctionality include a mathematical function for converting values of each ecosystem function into a common currency. Following previous studies²⁶, we assumed a logistic shape conversion function, and converted observed stability components into the common currency, with parameter values chosen to ensure that the range of the data includes the lower and upper asymptote. Here we present the influence of the value of the intercept (Q) of the conversion function (Fig. 3a), which is similar to varying the threshold value when calculating ecosystem multifunctionality with the threshold approach²⁷.

The opposing stability–diversity relationships from our experiment, coupled with logistic conversion functions, produced a hump-shaped relationship between overall ecosystem stability and diversity at low values of Q , a flat relationship at intermediate values and a U-shaped relationship at higher values (Fig. 3b, c). Effects of richness on overall ecosystem stability were weaker at the replicate level than the richness level (Fig. 3b), in part because of the relatively large variability among the replicates within richness levels and also because of positive covariance of temporal stability and resistance within diversity levels (Fig. 1d).

Finding hump- and U-shaped diversity–stability relationships fundamentally alters the importance of biodiversity, from it being unimportant (variation in biodiversity has no consequence) to it being state-dependent. The hump-shaped and U-shaped relationships occurred because measured stability components (for example, resistance) cross the threshold of the conversion function at different levels of species richness (see Methods). Therefore, negatively covarying stability components and nonlinear conversion functions can produce a non-monotonic effect of diversity on overall ecosystem stability. Furthermore, we expect the same to apply to ecosystem multifunctionality when individual ecosystem functions negatively covary along a diversity gradient.

The chosen mathematical conversion function and its parameter values are therefore important. Ecosystem multifunctionality–diversity relationships can be negative, neutral or positive depending on the chosen threshold value²⁷. Similarly, different weightings of ecosystem functions can alter which species and interactions are considered important for multifunctionality²⁶. Other types and parameterizations of conversion functions might conceivably result in other diversity–stability relationships. For instance, weighting one ecosystem function (or stability component) highly would lead to a multifunctionality and/or overall ecosystem stability measure that is essentially univariate. We view the choice and parameterization of conversion functions as an opportunity to tailor estimates of ecosystem multifunctionality and overall ecosystem stability to their policy and decision-making applications^{16,26,28,29}. These conversion functions and estimates of overall ecosystem stability can then transform the perceived importance of biodiversity change for the sustainable delivery of multiple ecosystem services and may help to translate the results of scientific studies into actionable information.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at <https://doi.org/10.1038/s41586-018-0627-8>.

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Additional information

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METHODS

Data reporting. No statistical methods were used to predetermine sample size. The experiments were randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

Experimental methods. The experiment was conceived and designed to research relationships between biodiversity and the ecosystem, with a focus on questions on environmental gradients and effects on temporal changes and/or stability, including the one addressed in this Letter. We factorially manipulated temperature (15, 17, 19, 21, 23 and 25 °C) and species richness (1–6 species of bacterivorous ciliates: *Colpidium striatum*, *Dexiostoma campylum*, *Loxocephalus* sp., *Paramecium caudatum*, *Spirostomum teres* and *Tetrahymena thermophila*) in 690 microcosms (250-ml Duran bottles). There were two incubators at each temperature. Manipulating temperature with a replicated gradient is recommended to harness the power of a regression design, while still allowing us to test for a nonlinear temperature effect³⁰. During previous experiments, the temperature of liquid in similar microcosms varied around the set-point temperature by 0.1 °C. Long-term ciliate cultures were kept at 15 °C, and warming usually decreases their carrying capacities but increases growth rates³¹. For *Colpidium*, temperatures above 20 °C represent a stress that decreases population growth rate, whereas the other species are still within their tolerated thermal range.

To start the experiment, ciliates were grown to their respective carrying capacities at 20 °C in bottles containing 1 l of bacterized medium. Throughout the experiments, medium consisted of protist pellets (Carolina Biological Supplies) at a concentration of 0.055 g l⁻¹ of Chalkley's medium in which the bacterium *Serratia fonticola* was grown as the resource for all ciliate consumers. Two autoclaved wheat seeds were added to each bottle for slow nutrient release³².

Monocultures (species richness = 1) were initiated at a density of 3 individuals per ml in a total of 100 ml medium. Multispecies communities (containing 2 to 6 species) were initiated with a total of 40 ml of ciliate culture topped up with 60 ml of fresh medium (100 ml culture in total). The 40-ml cultures were assembled by adding a fixed fraction (that is, 20 ml for two species, 13.33 ml for three species and so on) of each species at their specific carrying capacity, adopting a substitutive design. Because the number of possible species compositions exceeded the number of feasible experimental units, we used all possible compositions only for the monocultures, and for two- and six-species communities. For all other levels, species compositions were selected randomly from the set of all possible compositions such that all species occurred the same number of times, resulting in a total of 53 different compositions. Each level of species richness and composition was replicated at least twice, including an additional replicate for the monoculture, and three additional replicates for the six-species community resulting in 115 experimental units per temperature.

We sampled each experimental unit every day for the first 7 days, then 3 times per week for the following 50 days and a final sampling 7 days later, resulting in time series of 27 time points over a period of 57 days. We used video sampling techniques to count and measure individual ciliates in all communities³³. For sampling, microcosms were taken out of the incubator, gently stirred to homogenize the culture and a sample was pipetted into a counting chamber. The counting chamber was covered with a lid and a 5-s long video was taken under the microscope. The videos were subsequently processed with the R package BEMOVI³⁴.

We derived community biomass by summing the biovolume of all individuals of a given species in a given community and multiplying biovolume with a constant density equal to water (that is, 1 g cm⁻³). For each community, this resulted in a time series of community biomass. To avoid analysing monoculture time series while starting at a low fraction of the carrying capacity (3 individuals per ml)—compared to our multi-species culture species, which started between 7 and 20% carrying capacity (depending on richness)—we aligned the monoculture time series such that they started at the day on which they crossed 20% of their expected carrying capacity. Results regarding the effect of richness on temporal stability are qualitatively robust to exclusion of monoculture data (Extended Data Fig. 1c).

The temporal stability of each replicate community was calculated as the inverse of the coefficient of temporal variation in community biomass (mean community biomass divided by the standard deviation of community biomass). Resistance was measured for each replicate as the absolute difference between total biomass in the replicate and the mean total biomass at 15 °C, divided by the temperature difference, with dimensions of milligrams (per ml) per degree Celsius. Resistance values close to 0 indicate high resistance, whereas negative values indicate lower resistance. We also quantified relative resistance as the difference between total biomass in the replicate and the mean total biomass at 15 °C divided by the mean total biomass at 15 °C (that is, the loss proportional to the mean biomass at 15 °C).

We did not quantify resilience, because in a constant environment temporal variability is in principle closely related to resilience³⁵. To understand the potential drivers and mechanisms, we investigated the role of statistical averaging and species asynchrony. Statistical averaging is assessed by the scaling relationship between the species mean biomass and species variance in biomass³⁶. Synchrony was assessed

by a previously published metric³⁷ that calculates the average correlation between the biomass of each species and the total biomass of all other species in the group. We used the R package codyn³⁸ to calculate asynchrony.

Analyses. Analysis of total biomass was performed using linear mixed-effects models³⁹, with temperature (linear, centred), species richness (log-linear) and their interaction as fixed effects (Extended Data Table 1a). Microcosm identity nested in composition was included as a random factor to account for the repeated measurements. Heterogeneity of residuals was accounted for by estimating a composition-specific variance. AIC comparison consistently identified as most parsimonious a model with a linear effect of temperature, as opposed to when temperature was a factor (ΔAIC of the model with a nonlinear temperature term was greater than 2 in all comparisons). A simpler analysis of the temporal average of total biomass of each individual microcosm to test the effect of richness, temperature and their interaction—including a random effect for species composition—yielded quantitatively and qualitatively similar results (see Extended Data Table 1b). Figure 1c shows the estimated effects of linear mixed-effects models fitted separately for each day with temperature (linear, centred), species richness (log-linear) and their interaction as fixed effects and composition as a random effect. Analyses of temporal stability and resistance did not contain repeated measures and therefore only accounted for variation in composition with a random effect. We used the R package nlme for all linear mixed-effects models⁴⁰. To calculate associations between resistance and temporal stability among and within richness levels, we calculated reduced major axis regressions using the R package lmodel2⁴¹ as both variables potentially contain measurement errors. The significance of reduced major axis regression slopes deviating from zero was assessed by one-tailed permutation tests. All analyses were done in R—the statistical computing environment⁴².

Review of empirical studies. On the basis of the previously published review¹⁶, we obtained a set of 17 independent studies of resilience, resistance and temporal variability of ecosystem functions in response to direct or indirect experimental manipulations of diversity^{8,9,12,13,20,21,43–52}. Direct manipulations were defined as changing diversity by adding different sets of species to an experimental plot, jar or other unit, whereas indirect manipulations induced variation in diversity through the experimental treatment, such as fertilization. We only included studies that performed experimental manipulations.

To analyse whether specific mechanisms lead to covariation, we noted the type of mechanism proposed for each of the individual diversity–stability components (Extended Data Table 4). Furthermore, we assessed whether a quantitative or verbal argument was provided (or the mechanisms were not addressed at all) and synthesized the available evidence by vote counting.

Calculating overall ecosystem stability. An interactive website (https://frankpennekamp.shinyapps.io/Overall_ecosystem_stability_demo/) describes the calculation of ecosystem multifunctionality (also known as overall ecosystem functioning) or overall ecosystem stability and illustrates the following. The calculation requires that values of an ecosystem function (for example, biomass production) or of a stability component (for example, resistance to temperature) are converted into a common currency. The threshold approach uses a step mathematical function⁵³, the averaging approach uses a linear mathematical function (and both equalize relative contributions of different ecosystem functions and/or stability components)²⁵; a principal component approach uses a specific linear mathematical function for each ecosystem function or stability component⁵⁴; and step-like mathematical functions have previously been proposed²⁶ with more or less gradual changes from the lower to higher value. The generalized logistic function (also known as the Richard's function) is flexible enough to give a wide range of shapes of conversion functions. If x is the measured variable, and Y is the converted variable, the generalized logistic function is:

$$Y = A + \frac{K-A}{(C+Qe^{-Bx})^{1/v}}$$

in which A is the lower asymptote, K is the upper asymptote and B is the gradient. v affects the symmetry and the value of $Y(0)$. Q affects the value of $Y(0)$, that is, it shifts the function horizontally. C is typically set to 1. x is the value of the measured ecosystem function or stability component.

Overall ecosystem stability (that is, the aggregate measure of multiple stability components) is then the sum of the standardized and converted stability components: $\text{OES} = f(z(\text{res})) + f(z(\text{ts}))$ in which 'res' is the measured resistance, 'ts' is the measured temporal stability, the function $z()$ subtracts the mean and divides by the standard deviation, and $f()$ is the generalized logistic function. The parameters of $f()$ were $A = -1$, $K = 1$, $B = 5$, $v = 1$, $C = 1$ and Q was varied from 10^{-2} to 10^2 . These values were chosen to produce converted stability measures that span the range from A to K and to have a relatively threshold-like change from A to K .

Standardization before summation results in overall ecosystem stability with a mean of 0, emphasizing that the units of valuation here are arbitrary (although they generally need not be). Standardization also suggests equal weights for

different stability components; weighting of functions needs to be further considered and may be specified according to the specific use cases⁵⁵. Differential weightings, if desired and justified, can be incorporated into the conversion functions. Suggestions regarding the choice of conversion functions for managed systems can be found in two previous studies^{26,28}.

Unimodal relationships can result from negative covariation among two stability components. We also took into consideration how more than two components affect the unimodal pattern. Although the unimodal relationship is most pronounced when equal numbers of positive and negative relationships are considered, a unimodal relationship will persist as long as there is at least one opposing stability component (see Extended Data Fig. 5).

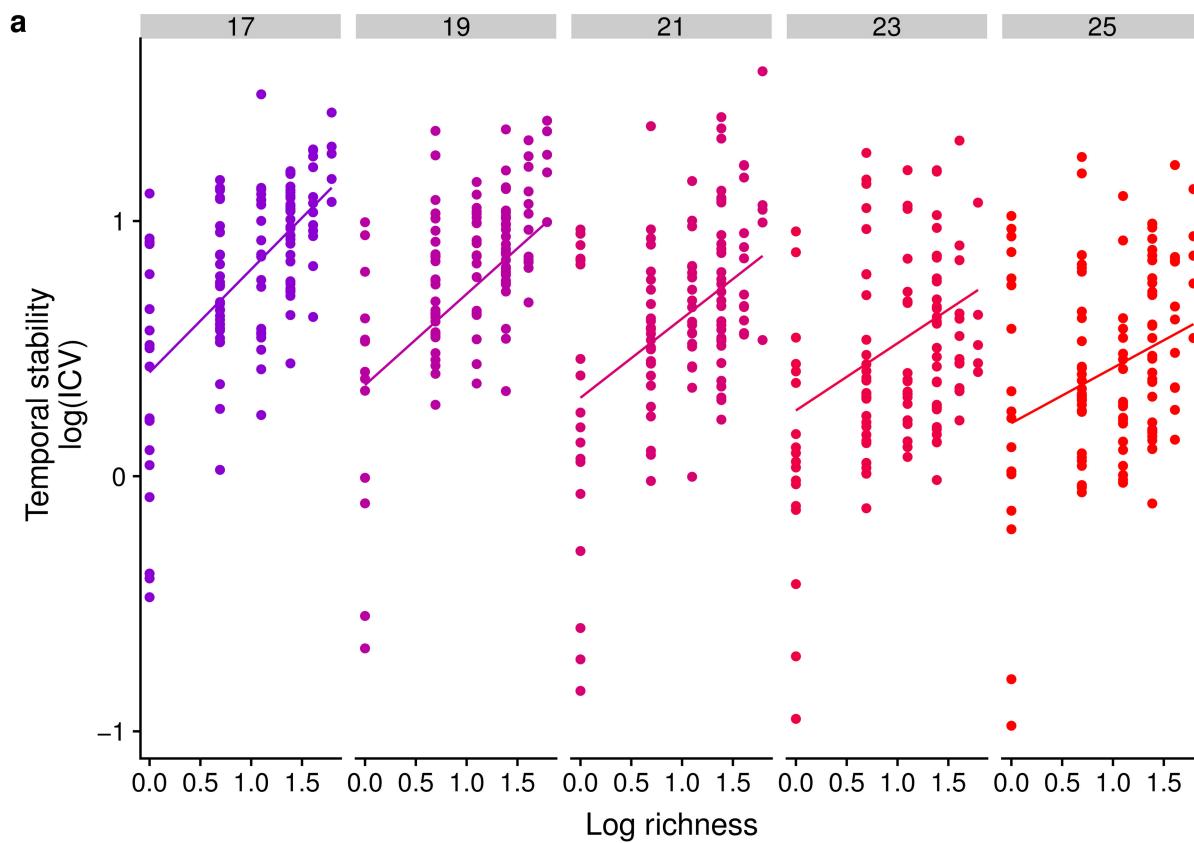
Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Code availability. Code to reproduce the analyses and figures is accessible at Github (https://github.com/pennekampster/Code_and_data_OverallEcosystemStability) with the identifier (<https://doi.org/10.5281/zenodo.1345557>).

Data availability

The experimental data that support the findings of this study are available at Github (https://github.com/pennekampster/Code_and_data_OverallEcosystemStability) with the identifier (<https://doi.org/10.5281/zenodo.1345557>). Source Data for Figs. 1–3 are provided in the online version of the paper.

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**b**

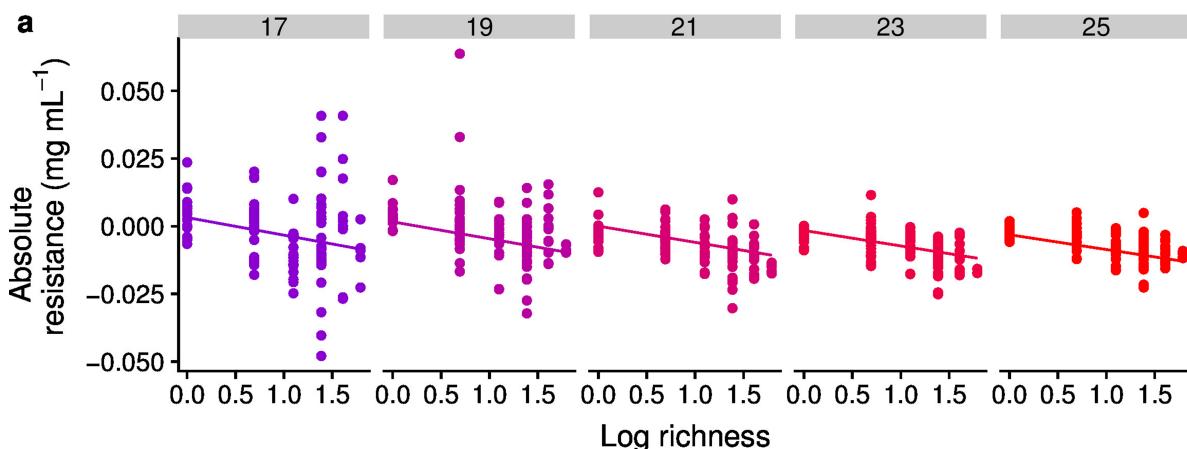
	Estimate	CI (lower)	CI (upper)	Std.Error	DF	t-value	p-value
Intercept	0.3320	0.1897	0.4744	0.07250	626	4.58	0.000006
Richness	0.3335	0.2038	0.4631	0.06459	51	5.16	0.000004
Temperature	-0.0249	-0.0360	-0.0137	0.00569	626	-4.37	0.000015
Rich. x temp.	-0.0234	-0.0334	-0.0134	0.00508	626	-4.61	0.000005

c

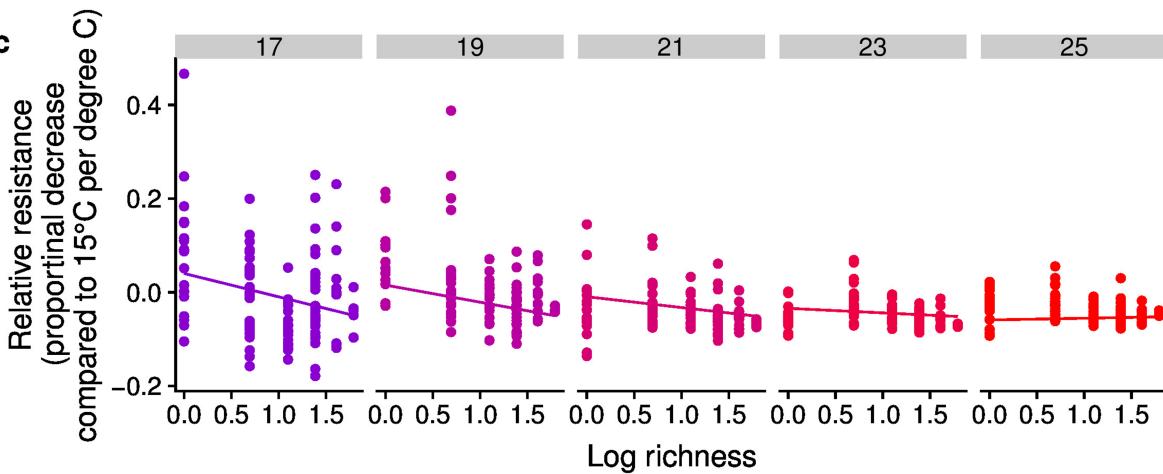
	Estimate	CI (lower)	CI (upper)	Std.Error	DF	t-value	p-value
Intercept	0.3937	0.2163	0.57118	0.09032	531	4.36	0.000016
Richness	0.2842	0.1319	0.43648	0.07562	45	3.76	0.000491
Temperature	-0.0317	-0.0506	-0.01272	0.00965	531	-3.28	0.001095
Rich. x temp.	-0.0181	-0.0337	-0.00247	0.00794	531	-2.28	0.023283

Extended Data Fig. 1 | Richness increased temporal stability across temperatures. **a**, The stabilizing effect of richness was present across all temperatures, although temperature had a negative effect on mean stability. The inverse coefficient of variation (ICV) is calculated as the mean biomass divided by the standard deviation of biomass. **b**, Results of the linear mixed-effects model of log richness, temperature and their interaction on temporal stability supporting the positive effects of richness

and the negative effect of temperature on temporal stability ($n = 681$ independent microcosms). **c**, Results of the same analysis as in **b** but without the monocultures. Results are qualitatively the same, indicating that the relationship between richness and temporal stability is not driven only by the monocultures ($n = 580$ independent microcosms). CI, 95% confidence interval; DF, degrees of freedom; Std.Error, standard error of the estimate.

**b**

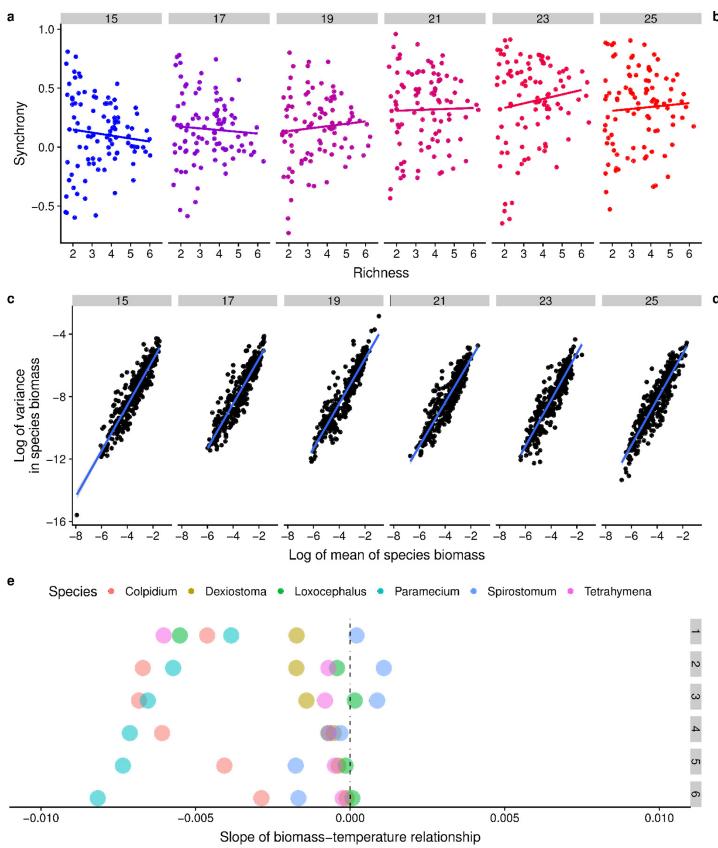
	Estimate	CI (lower)	CI (upper)	Std.Error	DF	t-value	p-value
Intercept	0.000812	-0.003137	0.004760	0.002010	512	0.404	0.686407
Richness	-0.006115	-0.009710	-0.002520	0.001791	51	-3.415	0.001258
Temperature	-0.000796	-0.001215	-0.000376	0.000214	512	-3.726	0.000216
Rich. x temp.	0.000133	-0.000241	0.000507	0.000190	512	0.697	0.486295

c**d**

	Estimate	CI (lower)	CI (upper)	Std.Error	DF	t-value	p-value
Intercept	0.00284	-0.02027	0.02595	0.01176	512	0.241	0.80951
Richness	-0.02980	-0.05085	-0.00875	0.01048	51	-2.842	0.00643
Temperature	-0.01241	-0.01544	-0.00937	0.00155	512	-8.023	< 0.000001
Rich. x temp.	0.00670	0.00399	0.00941	0.00138	512	4.858	0.000002

Extended Data Fig. 2 | The effect of richness on absolute and proportional resistance. **a, c,** Richness decreased resistance, regardless of whether it was measured on an absolute or proportional scale.

b, d, Results of linear mixed-effects models of richness, temperature and their interaction on absolute and proportional richness ($n = 567$ independent microcosms).

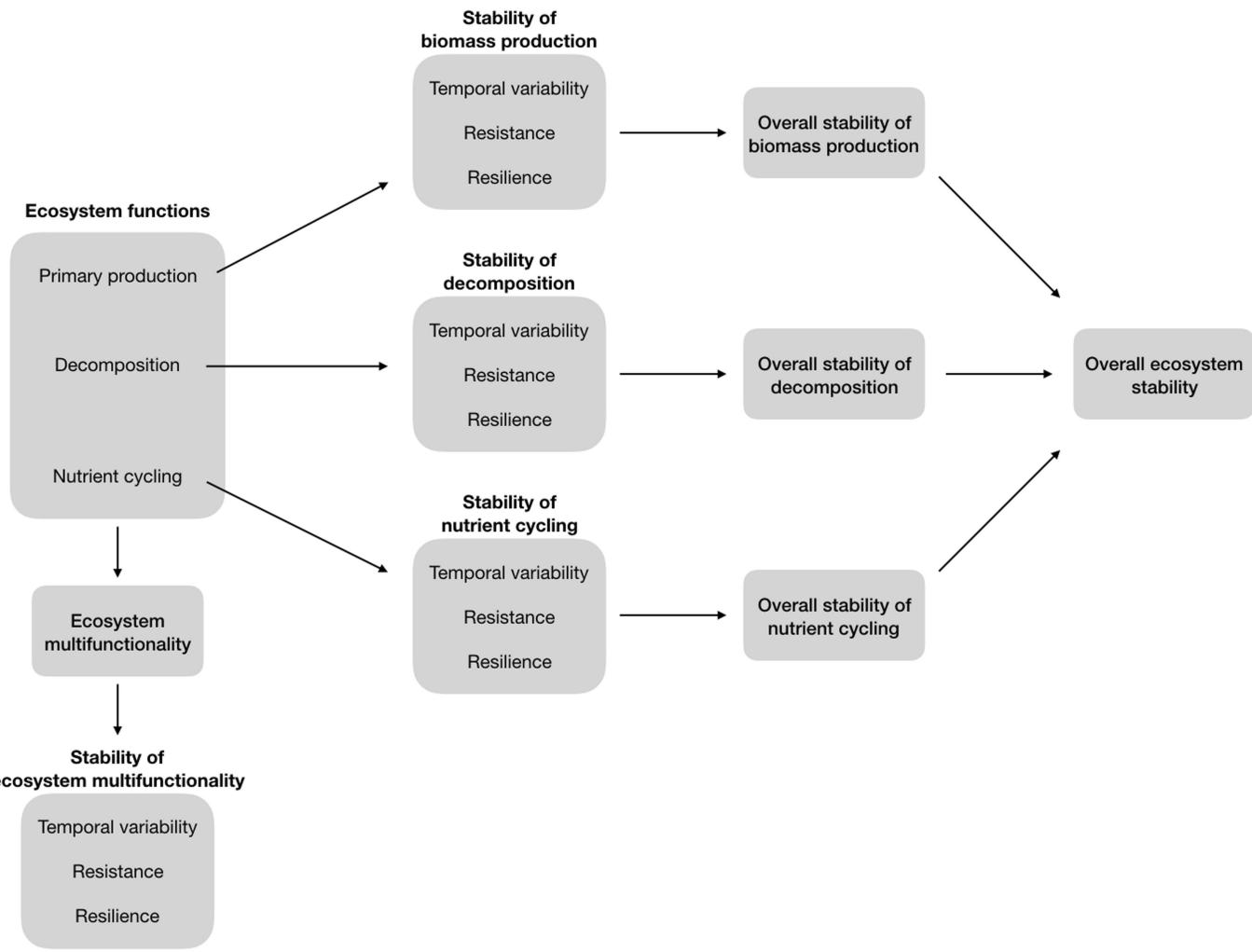


Extended Data Fig. 3 | Niche complementarity and low response diversity probably caused the negative covariance of stability components. Niche complementarity and the resulting increase in total biomass with richness tended to increase temporal stability (Fig. 1). **a, b**, We found little evidence for an effect of population asynchrony on temporal stability (linear mixed-effects model with composition as random effect and log richness and temperature as fixed effects; $n = 681$ independent microcosms). **c, d**, By contrast, statistical averaging contributed to stabilization (linear regression between mean species biomass and the variance of species biomass; $n = 2,077$ species mean-variance biomass observations). **e**, Low response diversity was inferred

	Estimate	CI (lower)	CI (upper)	Std.Error	DF	t-value	p-value
Intercept	0.18940	-0.04873	0.4275	0.12122	531	1.562	0.1188
Richness	0.00666	-0.01117	0.0245	0.00908	531	0.734	0.4635
Temperature	0.04958	-0.15500	0.2542	0.10157	45	0.488	0.6278
Rich. x temp.	0.01821	0.00354	0.0329	0.00747	531	2.439	0.0151

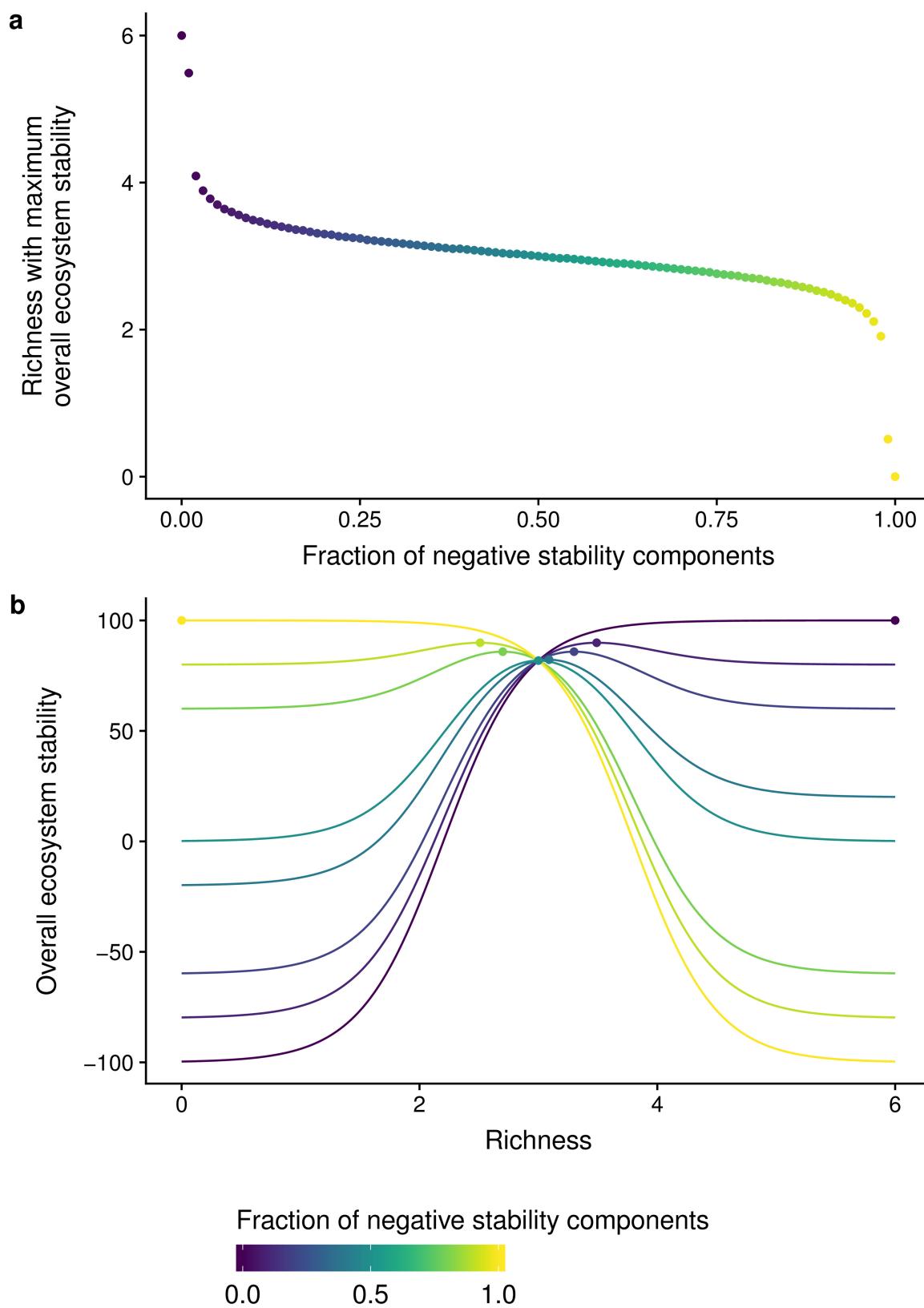
	Estimate	CI (lower)	CI (upper)	Std. Error	t value	Pr(> t)
Intercept	-2.58856	-2.69474	-2.48238	0.0541	-47.810	< 0.000001
Log species mean biomass	1.44577	1.41858	1.47297	0.0139	104.261	< 0.000001
Temperature	0.04280	0.01195	0.07366	0.0157	2.721	0.00657
Log species mean biomass x temperature	0.00139	-0.00645	0.00923	0.0040	0.348	0.72772

because the biomass of most species decreased or was unaffected by temperature (linear regression between temperature and species biomass; $n = 972$ species biomass \times temperature observations). Consequently, when there were more species, there was greater total biomass and greater temporal stability, but a greater biomass loss, with temperature increase. Therefore, niche complementarity (that is, effect diversity) probably not only caused a positive effect of diversity on temporal stability but also had a negative effect of diversity on resistance in the absence of high response diversity. However, this explanation cannot apply within richness levels, for which positive covariance among stability components was found.



Extended Data Fig. 4 | Overview of terms and the concept of overall ecosystem stability. Measured ecosystem functions (left-most top box) can each have multiple components of stability (for example, temporal variability, resistance and resilience of biomass production), each of which can be combined into a measure of overall stability. When—as in our study—there is only one ecosystem function, this overall stability of a

specific function is also the overall ecosystem stability. In studies of more than one ecosystem function, the overall stability of several functions could be combined to give overall ecosystem stability. Alternatively, one could first calculate ecosystem multifunctionality and then measure its stability components.



Extended Data Fig. 5 | The effect of aggregating more than two stability components into overall ecosystem stability. The fraction of stability components with a negative sign influences whether or not a unimodal pattern will result for a total of 100 stability components. **a**, A unimodal

relationship between diversity and OES will result if at least one stability component is negative. **b**, However, the strength of the pattern depends on the relative balance of positive and negative relationships.

Extended Data Table 1 | Richness increased, whereas temperature decreased, biomass production

a

	Estimate	CI (lower)	CI (upper)	Std.Error	DF	t-value	p-value
Intercept	0.07286	0.05182	0.093890	0.010731	12258	6.79	< 0.000001
Richness	0.04977	0.03058	0.068958	0.009559	51	5.21	0.000003
Temperature	-0.00205	-0.00315	-0.000948	0.000561	626	-3.65	0.000281
Rich. x temp.	-0.00534	-0.00634	-0.004345	0.000507	626	-10.54	< 0.000001

b

	Estimate	CI (lower)	CI (upper)	Std.Error	DF	t-value	p-value
Intercept	0.07323	0.05206	0.094407	0.010782	626	6.79	< 0.000001
Richness	0.04959	0.03031	0.068872	0.009603	51	5.16	0.000004
Temperature	-0.00220	-0.00351	-0.000892	0.000667	626	-3.30	0.00102
Rich. x temp.	-0.00547	-0.00664	-0.004296	0.000596	626	-9.18	< 0.000001

a, Results of the linear mixed-effects model of richness, temperature and their interaction on the temporal dynamics of biomass ($n=12,939$ microcosm \times day combinations). **b**, Results of the linear mixed-effects model of richness, temperature and their interaction with the time series aggregated to the average biomass for each microcosm ($n=681$ independent microcosms).

Extended Data Table 2 | Positive temporal stability–resistance relationships within richness levels

richness	Intercept	Slope	P–perm (1-tailed)	N
1	−0.00760617400951036	0.00506367932695562	0.01	84
2	−0.00843466390669893	0.00339781153057296	0.02	148
3	−0.0247877029765781	0.00880722901581866	0.02	100
4	−0.0249323685277533	0.00762486814778502	0.01	150
5	−0.027634192253533	0.0086737833813624	0.01	60
6	−0.0220565962582076	0.00349526254239345	0.02	25

Reduced major axis regression analyses revealed positive relationships between temporal stability and resistance within each of the six richness levels (the *P* values were based on one-tailed permutation tests (*P*-perm); *N* indicates the sample size for each richness level).

Extended Data Table 3 | Overview of studies used for literature survey

Code	Description	Reference
Ca-05	Diversity manipulation of grassland plants (Portuguese BIODEPTH site) analysing temporal variability and resistance to natural perturbation (frost and low precipitation) of biomass production.	43
PS-02	Diversity manipulation of grassland plants (Swiss BIODEPTH site) analysing resistance and resilience of biomass production to experimental drought perturbation.	9
Z2-06	Diversity manipulation of unicellular algae analysing resistance and resilience of biomass production to experimental cold perturbation.	44
Z1-06 *	Diversity manipulation of unicellular algae analysing temporal variability and resistance to experimental cold perturbation of biomass production.	20
Is-15	Forty-six diversity manipulations of grassland plants analysing temporal variability, resistance, and resilience to natural perturbations (drought and wet events) of biomass production.	13
VR-10	Diversity manipulation of plants, analysing resistance and resilience to natural perturbation (drought) of biomass production.	45
Ba-16	Diversity manipulation of diatoms, analysing resistance and resilience to perturbation (chemical stressor, atrazine) of biomass production.	21
Gr-00-1	Diversity manipulation by fumigation of soil microorganisms, analysing resilience to perturbation (heating) and resistance to perturbation (heating) of respiration.	46
Gr-00-2	Diversity manipulation by fumigation of soil microorganisms, analysing resilience to perturbation (chemical stress) and resistance to perturbation (chemical stress, CuSO ₄) of respiration.	46
TD-94	Diversity manipulation by fertilisation of plants, analysing resistance and resilience of biomass production to natural perturbation (drought).	8
Wa-00	Diversity manipulation of plants, analysing resistance and resilience to experimental perturbation (drought) of various ecosystem functions.	47
Hu-04	Analysis of diverse studies of marine ecosystems, some of which examined diversity-resistance and some diversity-resilience relationships for various ecosystem functions.	48
Vo-12	Diversity manipulation of grassland plants. Analysis of resistance and resilience of biomass production to drought perturbation.	49
Wr-15	Diversity manipulation of grassland plants. Analysis of temporal variability and resilience of biomass production to drought perturbation.	12
We-07	Manipulation of soil microbial diversity by serial dilution. Analysis of resistance and resilience of denitrification and nitrite oxidation to a temperature perturbation.	50
Wa-17	Diversity manipulation of grassland plants. Analysis of temporal variability and resilience of biomass production to drought perturbation.	51
Ti-96	Diversity manipulation of grassland plants via fertilisation. Analysis of temporal variability, resilience and resistance of biomass production to natural drought perturbation.	52

The individual study code, a short description and the reference for each study^{8,9,12,13,20,21,43-52} is shown.

*Studies that examine intrinsic stability alongside response to a disturbance.

Extended Data Table 4 | Putative mechanisms and type of evidence for bivariate diversity–stability relationships

Code	diversity_stability_relation	direction	mechanism1	mechanism2	evidence1	evidence2	scale
Ba-16	resistance/resilience	positive/negative	low response diversity	selection effect	signals of mechanisms	signals of mechanisms	relative
Ca-05	resistance/temporal_var	negative/positive	selection effect	increased evenness, synchrony, statistical averaging	signals of mechanisms	signals of mechanisms	absolute
Ca-05	resistance/temporal_var	neutral/positive	selection effect	increased evenness, synchrony, statistical averaging	signals of mechanisms	signals of mechanisms	relative
Gr-00-1	resistance/resilience	negative/neutral	not stated	not stated	missing	missing	relative
Gr-00-2	resistance/resilience	positive/positive	insurance effect	insurance effect	verbal	verbal	relative
Hu-04	resistance/resilience	positive/neutral	trade-offs in response diversity among stressors	not a sampling effect	signals of mechanisms	signals of mechanisms	absolute
Is-15	resistance/resilience	positive/neutral	not stated	not stated	missing	missing	relative
Is-15	resistance/temporal_var	positive/positive	not stated	not stated	missing	missing	relative
Is-15	resilience/temporal_var	neutral/positive	not stated	not stated	missing	missing	
PS-02	resistance/resilience	negative/negative	high response diversity; evenness	species composition	signals of mechanisms	signals of mechanisms	absolute
PS-02	resistance/resilience	neutral/negative	high response diversity; evenness	species composition	signals of mechanisms	signals of mechanisms	relative
TD-94	resistance/resilience	positive/positive	high response diversity	not stated	verbal	missing	relative
Ti-96	resistance/resilience	positive/neutral	high response diversity	not stated	verbal	missing	relative
Ti-96	resistance/temporal_var	positive/positive	high response diversity	high response diversity; competition	verbal	signals of mechanisms	relative
Ti-96	resilience/temporal_var	neutral/positive	not stated	high response diversity; competition	missing	signals of mechanisms	
Vo-12	resistance/resilience	neutral/neutral	not stated	not stated	missing	missing	absolute
Vo-12	resistance/resilience	neutral/neutral	not stated	not stated	missing	missing	relative
VR-10	resistance/resilience	negative/neutral	high response diversity	not stated	signals of mechanisms	missing	absolute
VR-10	resistance/resilience	neutral/neutral	high response diversity	not stated	signals of mechanisms	missing	relative
Wa-00	resistance/resilience	neutral/neutral	species composition	species composition	verbal	verbal	absolute
Wa-17	resistance/resilience	positive/neutral	insurance effect	insurance effect	verbal	verbal	relative
Wa-17	resistance/temporal_var	positive/positive	insurance effect	insurance effect	verbal	verbal	relative
Wa-17	resilience/temporal_var	neutral/positive	insurance effect	insurance effect	verbal	verbal	
We-07	resistance/resilience	neutral/neutral	high response diversity	high response diversity	verbal	verbal	absolute
Wr-15	resistance/resilience	negative/neutral	selection effect; community composition	not stated	signals of mechanisms	missing	absolute
Z1-06 *	resistance/resilience	positive/neutral	high response diversity	not stated	verbal	missing	relative
Z1-06 *	resistance/temporal_var	positive/positive	high response diversity	statistical averaging	verbal	signals of mechanisms	relative
Z1-06 *	resilience/temporal_var	neutral/positive	not stated	statistical averaging	missing	signals of mechanisms	
Z2-06	resistance/resilience	negative/neutral	low response diversity	not stated	signals of mechanisms	missing	relative

All reported bivariate diversity–stability relationships are listed. The nature of the covariation, the putative mechanism for each stability measure (for example, response diversity, evenness or statistical averaging), as well as the type of evidence provided (that is, verbal arguments, signals of mechanisms (that is, statistical evidence), manipulation of mechanisms in experiments or missing) are included.

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- An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
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- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated
- Clearly defined error bars
State explicitly what error bars represent (e.g. SD, SE, CI)

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Software and code

Policy information about [availability of computer code](#)

Data collection

Video processing and analysis was done using the freely available R package BEMOVI (version 1.0.2).

Data analysis

All statistical analyses were done using the statistical computing environment R (version 3.5). We used the following R packages for analyses: nlme (version 3.1.137), lmodel2 (version 1.7.3), codyn (version 2.0.0). All custom analysis scripts are available in the Github repository (DOI: 10.5281/zenodo.1345557).

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- A list of figures that have associated raw data
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The experimental data that support the findings of this study is available at Github (https://github.com/pennekampster/Code_and_data_OverallEcosystemStability) with the identifier (DOI: 10.5281/zenodo.1345557). Source data for figures 1-3 are provided with the paper.

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Ecological, evolutionary & environmental sciences study design

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Study description

We factorially manipulated temperature (15, 17, 19, 21, 23 and 25 °C) and species richness (1 to 6 species) in 690 independent microcosms (= experimental jars). There were two incubators at each temperature and at least two replicates of the 53 different composition treatments.

Research sample

We sampled the biomass of bacterivorous ciliate communities with different compositions of the following species: Colpidium striatum, Dexiostoma campylum, Loxocephalus sp., Paramecium caudatum, Spirostomum teres, and Tetrahymena thermophila.

Sampling strategy

For sampling, microcosms were taken out of the incubator, gently stirred to homogenize the culture and a sample was pipetted into a counting chamber. One video was taken per microcosm.

Data collection

We used video sampling techniques to count and measure individual ciliates in all communities. Data was collected by all coauthors except AT and JG.

Timing and spatial scale

We sampled each experimental unit every day for the first 7 days, then 3 times per week for the following 50 days and a final sampling 7 days later, resulting in time series of 27 time points over a 57-days period. This sampling is adequate to characterize the biomass dynamics over time and is both longer and more frequent than similar studies using protist microcosms.

Data exclusions

10 microcosms with contaminations were excluded prior to the analysis.

Reproducibility

Each treatment combination was successfully replicated at least twice, with replicates being true biological replicates indicating biological variation.

Randomization

Experimental units (jars) were randomly assigned to treatments. During the experiment, experimental units were kept in incubators and positions within incubators changed at random between samplings.

Blinding

No blinding was applied, however, as data was collected via automated video analysis no systematic observer effects are expected.

Did the study involve field work?

Yes No

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Unique biological materials
<input checked="" type="checkbox"/>	<input type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Unique biological materials

Policy information about [availability of materials](#)

Obtaining unique materials All unique biological materials (i.e. ciliate strains) are readily available from the authors.

Animals and other organisms

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Laboratory animals

We used the following six ciliate species: Colpidium striatum, Dexiostoma campylum, Loxocephalus sp., Paramecium caudatum, Spirostomum teres, and Tetrahymena thermophila. Stocks of these ciliates are kept for many thousands of generations in the lab at 15° C under conditions similar to the monocultures used in the experiment.

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve field-collected samples.