

Research



Cite this article: Han Z-Y, Wieczynski DJ, Yammine A, Gibert JP. 2023 Temperature and nutrients drive eco-phenotypic dynamics in a microbial food web. *Proc. R. Soc. B* **290**: 20222263.

<https://doi.org/10.1098/rspb.2022.2263>

Received: 8 November 2022

Accepted: 3 January 2023

Subject Category:

Ecology

Subject Areas:

ecology

Keywords:

global warming, food-web temperature responses, eutrophication, anthropogenic impacts, communities, trait-based ecology

Author for correspondence:

Ze-Yi Han

e-mail: zeyihanw@gmail.com

Electronic supplementary material is available online at <https://doi.org/10.6084/m9.figshare.c.6387924>.

Temperature and nutrients drive eco-phenotypic dynamics in a microbial food web

Ze-Yi Han, Daniel J. Wieczynski, Andrea Yammine and Jean P. Gibert

Department of Biology, Duke University, Durham, NC, USA

Z-YH, 0000-0001-5552-8636; DJW, 0000-0003-4090-2677; JPG, 0000-0002-5083-6418

Anthropogenic increases in temperature and nutrient loads will likely impact food web structure and stability. Although their independent effects have been reasonably well studied, their joint effects—particularly on coupled ecological and phenotypic dynamics—remain poorly understood. Here we experimentally manipulated temperature and nutrient levels in microbial food webs and used time-series analysis to quantify the strength of reciprocal effects between ecological and phenotypic dynamics across trophic levels. We found that (1) joint—often interactive—effects of temperature and nutrients on ecological dynamics are more common at higher trophic levels, (2) temperature and nutrients interact to shift the relative strength of top-down versus bottom-up control, and (3) rapid phenotypic change mediates observed ecological responses to changes in temperature and nutrients. Our results uncover how feedback between ecological and phenotypic dynamics mediate food web responses to environmental change. This suggests important but previously unknown ways that temperature and nutrients might jointly control the rapid eco-phenotypic feedback that determine food web dynamics in a changing world.

1. Introduction

Understanding how rapid global climate change (GCC) will affect the structure and dynamics of communities is a pressing goal of ecology [1,2]. Increasing temperatures associated with GCC influence the metabolism of individuals [3–5], which strengthens species interactions [6–8], alters community structure [9–11] and affects ecosystem function [12]. Additionally, increasing nutrient loads from agricultural run-offs can result in eutrophication and destabilize natural communities [13–15], often leading to species loss [16]. Warming and eutrophication can independently and jointly impact food web structure and stability [17,18]. Counterintuitively, simultaneous increases in temperature and nutrient load can produce outcomes that are qualitatively different from the combined negative effects of each variable on its own [19,20]. These non-additive (interactive) outcomes are still poorly understood, but central to honing our understanding of GCC impacts on food web structure and dynamics in a highly anthropogenized world.

The mechanisms through which warming and increasing nutrient loads independently influence food webs are relatively well understood [10,21,22], specifically regarding their impacts on the relative strength of bottom-up and top-down effects [19,20,23]. For example, warming can increase predation pressure [24], thus decreasing resource biomass, while increasing the proportion of top predators [7,23,25]. Alternatively, warming can increase metabolic demands while reducing conversion efficiency [26], leading to predator starvation at high temperatures, loss of top predators [27] and reduced food chain length [28]. Unlike warming, increasing nutrient loads tend to increase bottom-up effects, resulting in unstable dynamics and species loss (i.e. paradox of enrichment, [13,29]), often leading to top-heavy, unstable food webs [29]. Eutrophication resulting from increasing nutrient loads can also change consumer trophic position, leading to changes in species interactions and food web structure [30].

While warming and nutrients can independently influence food webs, they can also have non-additive (interactive) effects when acting in unison [18–20], but these are much less well understood. Although temperature increases are typically considered to be destabilizing [6], at low temperatures, small temperature increases can cause consumer starvation, stabilizing nutrient-induced instabilities [19,20]. However, at high temperatures, increasing nutrient loads can counter warming-induced consumer starvation by increasing carrying capacity and predator attack rates [19]. Warming also weakens nutrient-induced increases in community biomass (negative interactive effects, [18]), in turn influencing food web structure [31], species richness and community composition [32].

In addition to their effects on entire food webs, temperature and nutrients can both determine the physiology and morphology of organisms [18,33]. For example, higher temperatures often result in smaller sizes [34,35], while nutrient enrichment leads to larger organisms [36,37]. Additionally, temperature and nutrients can interactively affect body size: size increases with higher nutrient loads at low temperatures, but decreases at high temperature [18]. Although body size is often considered a response variable, it also has well-known effects on population growth and species interactions [38–41], so rapid body size responses to temperature, nutrients or both, may have consequences for food web structure and dynamics in warmer climates [42–44]. But it is still unclear how rapid, environmentally induced shifts in body size might influence ecological dynamics as they unfold.

Here, we address how temperature and nutrients influence feedback between species' ecological dynamics and rapid changes in their body sizes (phenotypic dynamics) in a tractable microbial food web. We describe observed food web and body size responses to temperature and nutrients across trophic levels and also study the mechanisms of these responses. Specifically, we ask: (1) do temperature and nutrients independently or interactively influence ecological dynamics in this microbial food web? (2) Do these effects alter the relative importance of top-down versus bottom-up control? (3) Do these effects vary across trophic levels? (4) Does body size just passively respond to temperature and nutrients, as suggested elsewhere (e.g. [18,20]), or does this body size response also play a role in determining how the food web itself responds to environmental change? To address these questions, we manipulate nutrient levels and temperature in a microbial food web composed of a complex bacterial community, a bacterivorous protist (*Tetrahymena pyriformis*), and an omnivorous top predator (*Euplotes* sp.), then track changes in the population densities of all organisms and the body sizes of the protists over time. We use time-series analysis to evaluate the relative strength of top-down versus bottom-up processes across trophic levels as well as whether and how the observed ecological and phenotypic dynamics influence one another across temperature and nutrient treatments. Our results reveal complex but quantifiable temperature and nutrient effects on food web dynamics that vary predictably across trophic levels by altering the relative strength of trait-mediated bottom-up and top-down effects.

2. Methods

(a) Culture care

Euplotes sp. and *T. pyriformis* stock cultures were acquired from Carolina Biological Supply (Burlington, NC, USA) and grown

in laboratory conditions for a year prior to this experimental work. Both species were kept in autoclaved liquid protist medium (Carolina Biological Supply), supplemented with one autoclaved wheat seed as a carbon source [45]. Protists were fed a mixture of pond bacteria collected from an ephemeral pond at Duke Forest (Wilbur/Gate 9 pond, Lat 36.013914, Long -78.979720) and composed of thousands of bacterial species, as described elsewhere [46]. We maintained all cultures on a 16:8 light:dark cycle at 22°C and 65% humidity in AL-22L2 Percival growth chambers (Perry, IA, USA).

(b) Experimental design

Microcosms were set up in autoclaved 250 ml borosilicate jars filled with 200 ml of protist media. We manipulated temperature and nutrient loads by imposing two temperature levels (22°C/25°C) and two nutrient levels (normal protist media concentration plus one wheat seed, i.e. high nutrients, or half concentration plus half a wheat seed, i.e. low nutrients, as done in [47] in a factorial design with four treatments and six replicates per treatment. Day/night cycle and humidity levels mimicked rearing conditions and were kept constant. We inoculated 2 ml of bacterial communities from the stock culture, the same used to rear the protists. The intermediate consumer species, *T. pyriformis*, only preys on bacteria and was introduced at a starting density of 37 individual (ind) ml⁻¹. The omnivorous consumer species, *Euplotes* sp., preys on both bacteria and the intermediate consumer *T. pyriformis* and was introduced at a starting density of 0.24 ind ml⁻¹ in all microcosms. We recorded the density of both protist species once per day through fluid imaging (FlowCam; Yokogawa Fluid Imaging Technologies, Portland, ME, USA), Monday through Friday, for 16 days. Fluid imaging generates individual cell raster images that were used to quantify changes in protist size (measured as cell area, in µm²) over time. Bacteria density was quantified as optical density at a wavelength of 600 nm (OD₆₀₀), using a BioTEK Epoch-2 microplate spectrophotometer (Winooski, VT, USA).

(c) Statistical analysis

To test for possible effects of temperature and nutrients on ecological dynamics, we fitted generalized additive mixed models (GAMM) to time series of species density (OD₆₀₀ for bacteria) and protist body size, across all treatments, using the 'mgcv' package v. 1.8-31 [48] in R v. 4.0.2 [49]. To control for temporal autocorrelation, we used an autoregressive moving average (ARMA) correlation structure in the GAMMs using the 'nlme' R package v. 3.1-148. To account for repeated sampling within each replicate, we included replicates as a random intercept in the model. We compared models with additive and/or interactive temperature and nutrient effects, as well as different ARMA correlation structures, using AICc (electronic supplementary material, appendix I table S1–S2). We discarded *T. pyriformis* phenotypic data from days in which fewer than 10 individuals were measured (i.e. after populations collapsed).

(d) Characterizing ecological dynamics

To better understand which aspects of the food web dynamics were most influenced by temperature and nutrients, we characterized multiple aspects of the observed ecological dynamics of the bacterial community and the protists across treatments. Specifically, we quantified: (1) initial growth rate (day⁻¹) as $[\ln(N_f) - \ln(N_i)]/\text{time}$ for early dynamics data, (up to day 1 for *T. pyriformis* and bacteria and day 8 for *Euplotes* sp., due to much slower growth), (2) maximum density (ind ml⁻¹ for protists and OD₆₀₀ for bacteria), measured as average density across replicates on the day with the highest average density, (3) the coefficient of variation (CV = standard deviation/mean)

of the temporal population dynamics within treatments (typically used as a measure of stability [50]), (4) the time to population collapse in days (only *T. pyriformis*) and (5) the time to population peak in days (only *Euplotes* sp.). Last, we calculated the effect sizes of the significant effects of temperature, nutrients, and their interaction using the function `eta_squared()` in package 'effectsize' v. 0.7 [51].

(e) Quantifying top-down/bottom-up effects and eco-phenotypic feedback

To understand the mechanisms through which temperature and nutrients affected the observed food web dynamics, we quantified the reciprocal effects of ecological dynamics and body size on each other using convergent cross mapping (CCM) [52]. The CCM algorithm has now been used multiple times across ecological systems and taxa reliably to estimate the strength of causal effects between variables for which time-series are available [41,53–58] and we followed this specialized literature to infer causation in our data (see electronic supplementary material, appendix II figure S1–S15). Concisely, CCM quantifies the strength of causation of one dynamical variable onto another by measuring the extent to which the time series of one variable can reliably estimate the state of another variable [52]. The larger the causal effect of *X* on *Y*, the better the ability of *Y* to predict *X*, as *Y* contains more information about *X* (by virtue of being 'forced' by *X*). Meanwhile, a variable *X* that does not influence a variable *Y* cannot be predicted from the dynamics of *Y*, as no information regarding *X* is contained in *Y* [52]. The CCM algorithm yields a 'cross-mapping estimation skill' in the form of a correlation coefficient (ρ) between observed and predicted points in the time-series [52]. The larger this number, the larger the dependence of one variable on the other. Whether there is a cause–effect relationship between two dynamical variables, as opposed to simple correlation, further depends on whether the cross-mapping estimation skill increases with the length of the time series used for this estimation (called the 'library size'). Whenever such an increase is observed, a causal effect of a variable on another one is likely (i.e. convergence; [52]).

We used a modified CCM algorithm that allows for replication in the time series through the R package 'multispatialCCM' (v. 1.0, [59]). The package can be used to detect causality between shorter but highly replicated time series, like ours. Our time series lacked data on days 5, 6 and 12, 13 across all replicates. However, the CCM algorithm does not allow for missing time points. To resolve this issue we interpolated the time series data for each replicate using three methods: linear interpolation, spline interpolation and smooth spline interpolation using the 'approx', 'spline' and 'smooth.spline' functions in base R (see electronic supplementary material, appendix S2). To increase the robustness of our inference, we then performed CCM in each of these time series and averaged the estimation skill from our CCM analysis across these three separate interpolated time series. That said, CCM results based on each independent smoothing technique held qualitatively (electronic supplementary material, appendix II figure S1–S12), corroborates the robustness of our inference. Additionally, we only used CCM results that showed convergence in cross mapping skill (ρ) with increasing library size (indicating causality) to focus only on likely causal effects between species ecological and phenotypic dynamics (electronic supplementary material, appendix II figure S13–S15).

Based on previous literature [52,53,56], we interpreted the cross-mapping skill (ρ) as the magnitude of the effect of one variable on another, whenever convergence was present. We calculated this cross-mapping skill between all predator and prey densities, between densities and trait dynamics (except the effect of protist densities on bacterial traits as we lack

phenotypic data for the bacteria community), and between protist trait dynamics. The effect of prey density on predator density was thus considered as representing bottom-up control, and the effect of predator density on prey density as top-down control (dubbed 'eco-eco' effects, for simplicity). The effects of change in body size on ecological dynamics (density) were dubbed 'pheno-eco' effects, and the effects of density on body size dynamics as 'eco-pheno' effects. Reciprocal effects of changes in predator and prey protist body sizes were dubbed 'pheno-pheno' effects.

3. Results

(a) General ecological and phenotypic dynamics

Overall, bacterial density rapidly increased to carrying capacity (figure 1a, light grey), the intermediate predator *T. pyriformis* increased rapidly, then decreased (figure 1a, dark grey), while the omnivorous predator *Euplotes* sp. increased almost monotonically to carrying capacity (figure 1a, black), ultimately resulting in a one protist + bacterial community state. Temperature and nutrients affected all three species and led to significantly different dynamics across treatments (figure 1b–d, electronic supplementary material, appendix I table S2). The body sizes of both protists changed rapidly over time (figure 1e,f) and responded to both temperature and nutrients (figure 1e,f, electronic supplementary material, appendix I table S2).

(b) Temperature and nutrient effects changed across trophic levels in systematic ways

We found that both temperature and nutrients significantly and independently affected bacterial dynamics: higher temperature decreased bacterial maximum density ($T_B = -0.007$, $p = 0.004$, figure 2d), nutrients increased bacterial maximum density ($N_B = 0.014$, $p = 5.09 \times 10^{-6}$, figure 2d), and nutrients also increased bacterial population density CV ($N_B = 0.2$, $p = 8.6 \times 10^{-5}$, figure 2g).

Temperature independently and solely affected *T. pyriformis* initial growth rate and time to population collapse, while temperature and nutrients independently and interactively affected its maximum density and density CV. Temperature strongly increased initial growth rate in *T. pyriformis* ($T = 0.50$, $p = 1.16 \times 10^{-6}$, figure 2b) and accelerated the time to collapse ($T = -2.8$, $p = 1.6 \times 10^{-5}$; figure 2j). The maximum density of *T. pyriformis* increased with nutrients across temperature but only at high nutrient levels, while at low nutrient levels, higher temperature decreased *T. pyriformis* maximum density ($T = -975.3$, $p < 0.02$; $N = 1002.2$, $p = 0.01$; $N \times T = 1526.7$, $p < 0.01$, figure 2e). The density CV of *T. pyriformis* was also interactively influenced by nutrients and temperature such that at high temperature, *T. pyriformis* CV was high across nutrient levels, but at low temperatures, *T. pyriformis* CV decreased with increasing nutrients ($T = 0.2$, $p < 0.006$; $N = -0.19$, $p < 0.01$; $N \times T = 0.2$, $p = 0.045$, figure 2h).

Temperature and nutrient levels independently influenced the maximum density and time to the population peak of *Euplotes* sp. and interactively affected its initial growth rate and density CV. Both temperature and nutrients increased the maximum density of *Euplotes* sp. ($T = 39.83$, $p < 0.03$; $N = 60.67$, $p < 0.002$, figure 2f). Temperature alone accelerated *Euplotes* time to peak density ($T = -2.2$, $p < 0.02$;

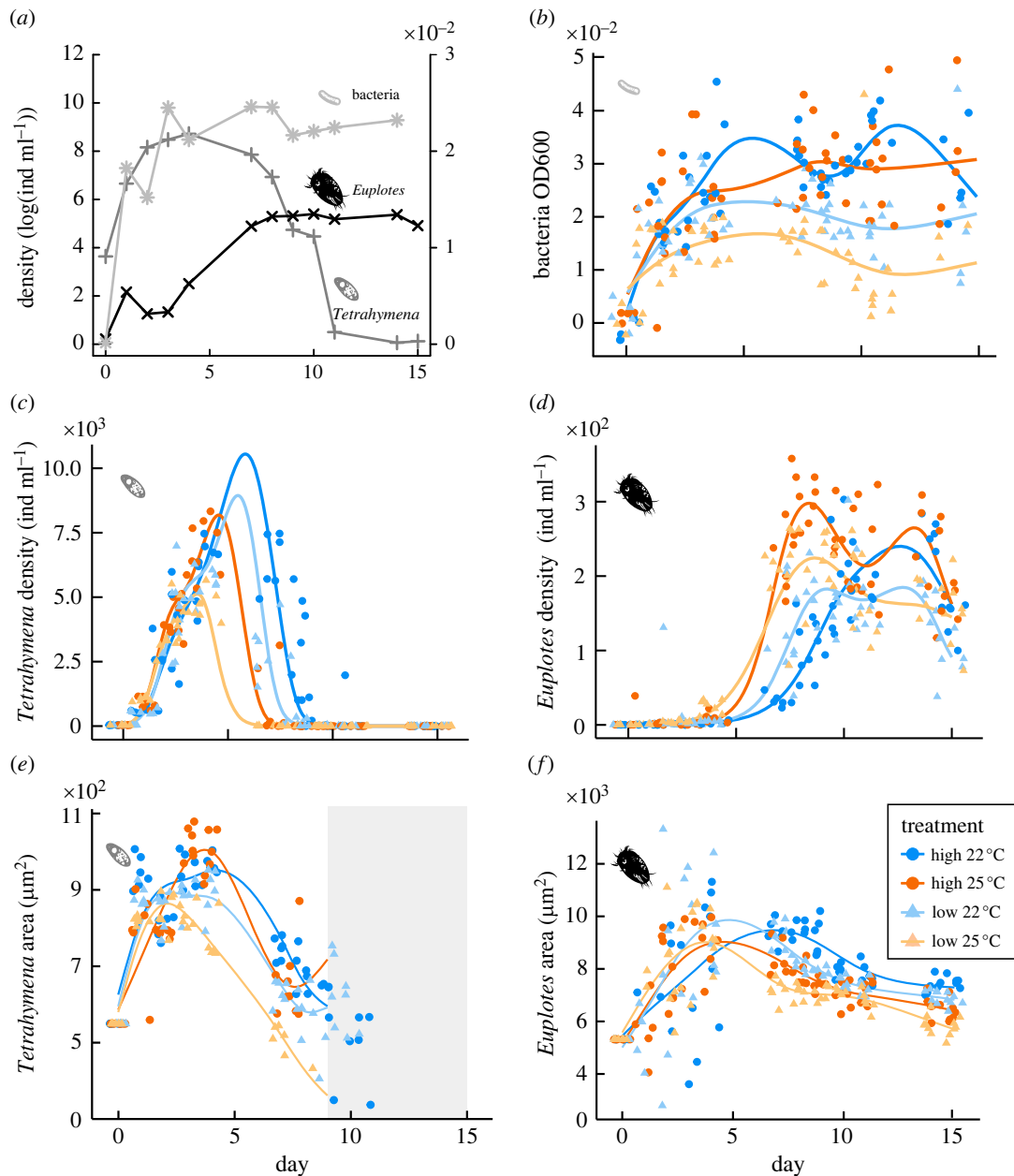


Figure 1. Ecological and phenotypic dynamics within experimental microcosms. (a) Average population densities across treatments. (b–d) Density dynamics of bacteria, *T. pyriformis*, and *Euplotes* sp. (e,f) Changes in the body size (measured as the area of the cell in square micrometres) for both protists over time. *T. pyriformis* was effectively extinct by day 9. In (b–f), dots are the empirical measurements and lines are GAMM fits. The grey area in (e) indicates days for which data were insufficient to estimate trait distributions.

figure 2k). Higher initial growth rates were observed at higher temperatures, while higher nutrient levels led to large decreases in initial growth rates at low temperatures but a small increase in initial growth rates at high temperatures ($N = -0.09$, $p = 5 \times 10^{-4}$, $N \times T = 0.13$, $p < 10^{-3}$, figure 2c). In general, temperature decreased the density CV of *Euplotes* sp. while higher nutrients increased its CV, more so at lower temperature than at higher temperature ($T = -0.08$, $p < 0.02$, $N_E = 0.16$, $p = 2.9 \times 10^{-5}$, $N \times T = -0.12$, $p < 0.01$, figure 2i). Additional model stats can be found in electronic supplementary material, appendix I table S3.

We found significant temperature and nutrient interactions only among the predators, with the total effect size of the interaction terms increasing with species trophic level (figure 3a). Bacterial population dynamics were mainly influenced by nutrients and the intermediate predator received the

most temperature effects (figure 3a). These results can be better understood by decomposed total effect sizes of nutrients and temperatures on initial growth rates, maximum density, and density CV. We found no interactive effects of nutrients and temperature on bacteria initial growth rates, but increasingly complex temperature and nutrient effects on species initial growth at higher trophic levels (figure 3b). Interestingly, we also found decreasing effect sizes of temperature, nutrients, and their interaction on species maximum density at higher trophic levels (figure 3b). However, increasing effect sizes of these effects with increasing trophic level were observed for species density CV (figure 3d). These results imply that the maximum density of species at higher trophic levels was less affected by the changes in temperature and nutrients, while the stability of those populations were more sensitive to such changes (figure 3).

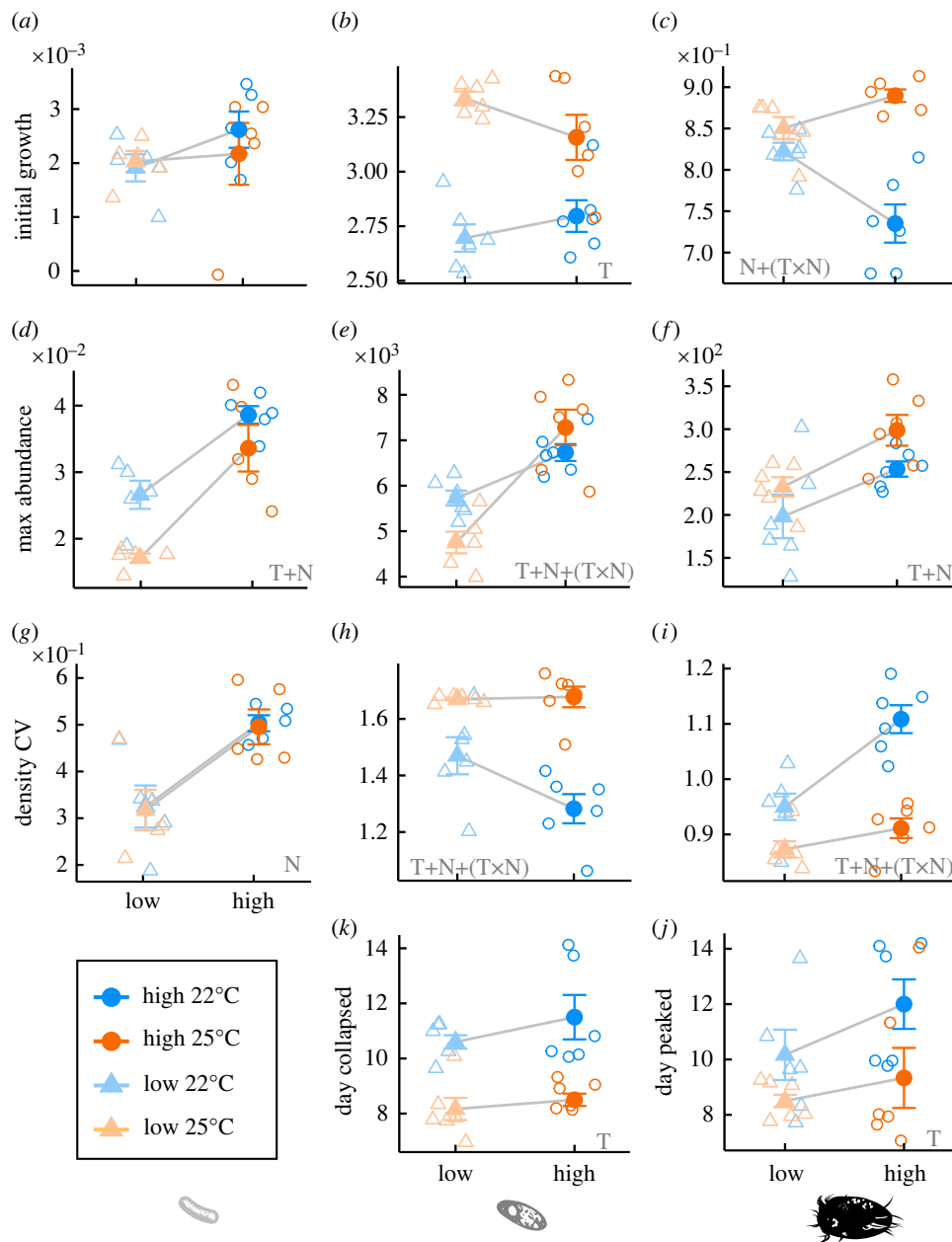


Figure 2. Additive and interactive effects of temperature and nutrients on descriptors of population dynamics. Open circles and triangles represent data points from each replicate. Solid shapes represent means across six replicates. Grey letters T, N and (T × N) represent statistically significant effects from temperature, nutrients and interactive effects between temperature and nutrients, respectively.

(c) Temperature and nutrients influence top-down and bottom-up effects

Results from CCM analyses indicated that the relative strength of bottom-up and top-down effects changed across treatments (figure 4a). Top-down and bottom-up effects between the two protists were slightly stronger in the warmer temperature (figure 4a, yellow lines). Importantly, temperature and nutrients showed interactive effects on both top-down and bottom-up effects (figure 4a). For example, while the top-down effects of protists on bacteria (figure 4a, green and purple dashed lines) were generally stronger than the bottom-up effects of the bacteria on the protists (figure 4a green and purple solid lines), temperature and nutrients interactively—but differentially—shifted the magnitude of the top-down effects of the two protist predators on bacteria (figure 4a, open dots and dashed lines). Indeed, the top-down effect of *T. pyriformis* on bacteria decreased in the

high-nutrient treatment, but higher temperature strengthened this effect (figure 4a purple dashed line). Meanwhile, high nutrients increased the top-down effect of *Euplotes* sp. on bacteria in the lower temperature but decreased it at higher temperature (figure 4a green dashed line).

The strength of species interactions in the food web, overall, also changed according to shifts in the strength of top-down and bottom-up control between species pairs (figure 4b). To visualize these changes, we present CCM cross-mapping skill value at the largest library size as links between species pairs (figure 4b). At low nutrient levels, higher temperature led to a larger number of stronger species interactions (figure 4b, bottom left and right) while at high nutrient levels, interaction strengths were generally stronger and temperature only had a small effect (figure 4b, top left and right). Low nutrients and high temperatures led to the strongest interactions among all three species (figure 4b, bottom right).

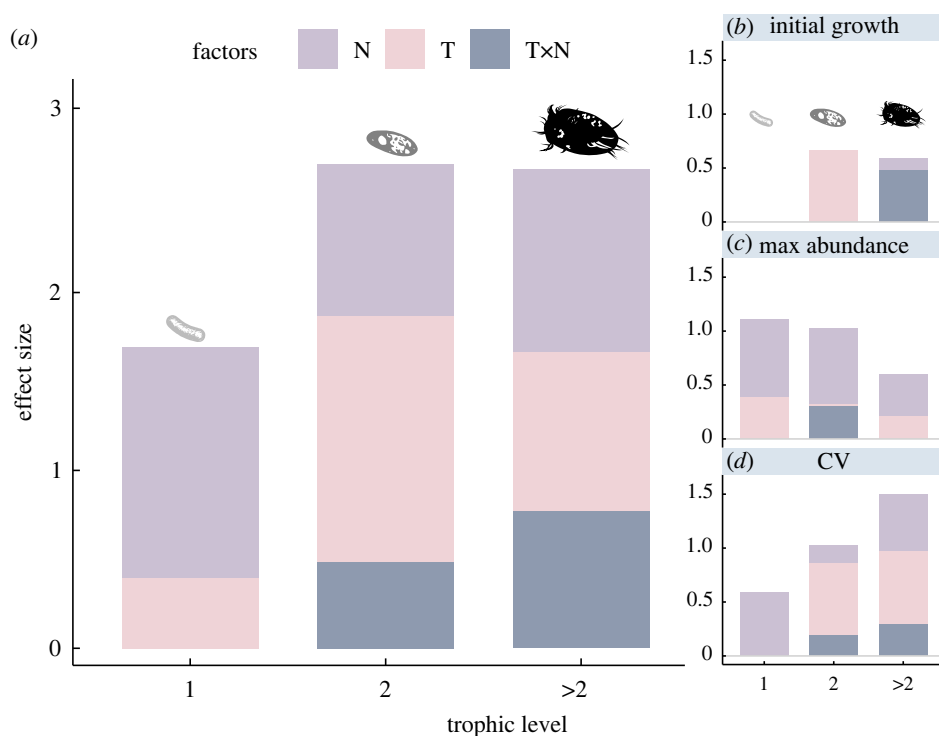


Figure 3. The effect sizes of significant temperature and nutrient effects on ecological dynamics (i.e. initial growth, maximum density and density CV). T, N and T×N denote the effects of temperature, nutrients and interactive effects of temperature and nutrients, respectively.

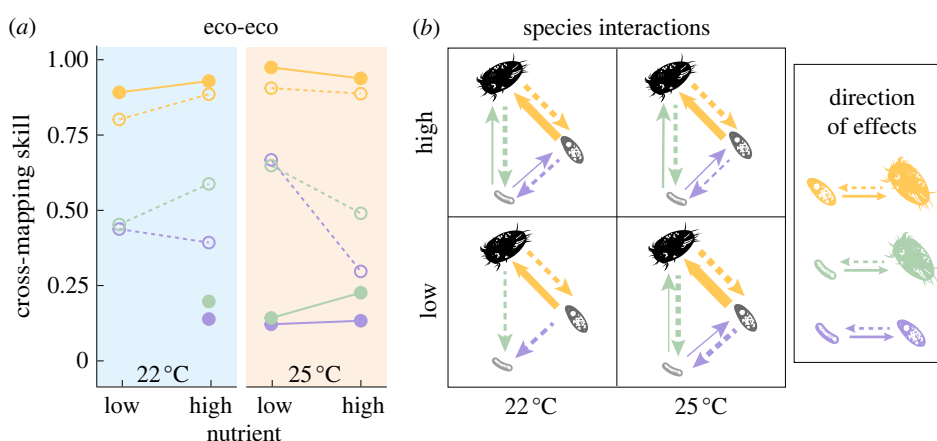


Figure 4. Cross-mapping skill (ρ) of species ecological dynamics across treatments for each species pair. (a) Shows CCM estimation skill of one species' effect on another. Solid circles and solid lines represent bottom-up effects and open circles and dashed lines represent top-down effects. (b) Shows all causal species interactions for all species considered. Line widths are proportional to the magnitude of the CCM skill while the size of the arrowheads is fixed.

(d) Temperature and nutrients altered the reciprocal effects of ecological and phenotypic dynamics

Protist size not only responded to temperature and nutrients, but also played an important role in determining the effects of temperature and nutrients on ecological dynamics (figure 5a–c). The bidirectional effects between the body-size dynamics of *Euplotes* sp. and the ecological dynamics of *T. pyriformis* were the strongest overall across treatments (figure 5a–c). The body size dynamics of the omnivorous predator, *Euplotes* sp., but not those of *T. pyriformis*, had relatively strong causal effects on the ecological dynamics of all species, including its own ecological dynamics (figure 5b). Specifically, at low temperature, increasing nutrient level strengthened the effect of *Euplotes* sp. phenotypic dynamics on its own ecological dynamics and those of *T. pyriformis*, but weakened these effects on the ecological dynamics of

bacteria (figure 5b). Yet, at the warmer temperature, increasing nutrient levels weakened the effects of *Euplotes* sp. phenotypic dynamics on the ecological dynamics of all species, including itself (figure 5b), indicating that plastic changes in top predator body size can mediate how food web dynamics respond to temperature and nutrients.

Trait on trait effects among the two predators (figure 5c, pheno-pheno) were weaker than their pheno-eco or eco-pheno counterparts. Interestingly, pheno-pheno causal effects were only observed at the higher temperature (figure 5c). Increasing nutrient levels increased the pheno-pheno effects of *T. pyriformis* on *Euplotes* sp. but decreased those of *Euplotes* sp. on *T. pyriformis* (figure 5c).

Overall, we also found a larger number of stronger effects between ecological and phenotypic dynamics at higher temperatures, especially in the low nutrient treatment (figure 5d, bottom right), consistent with results from the top-down

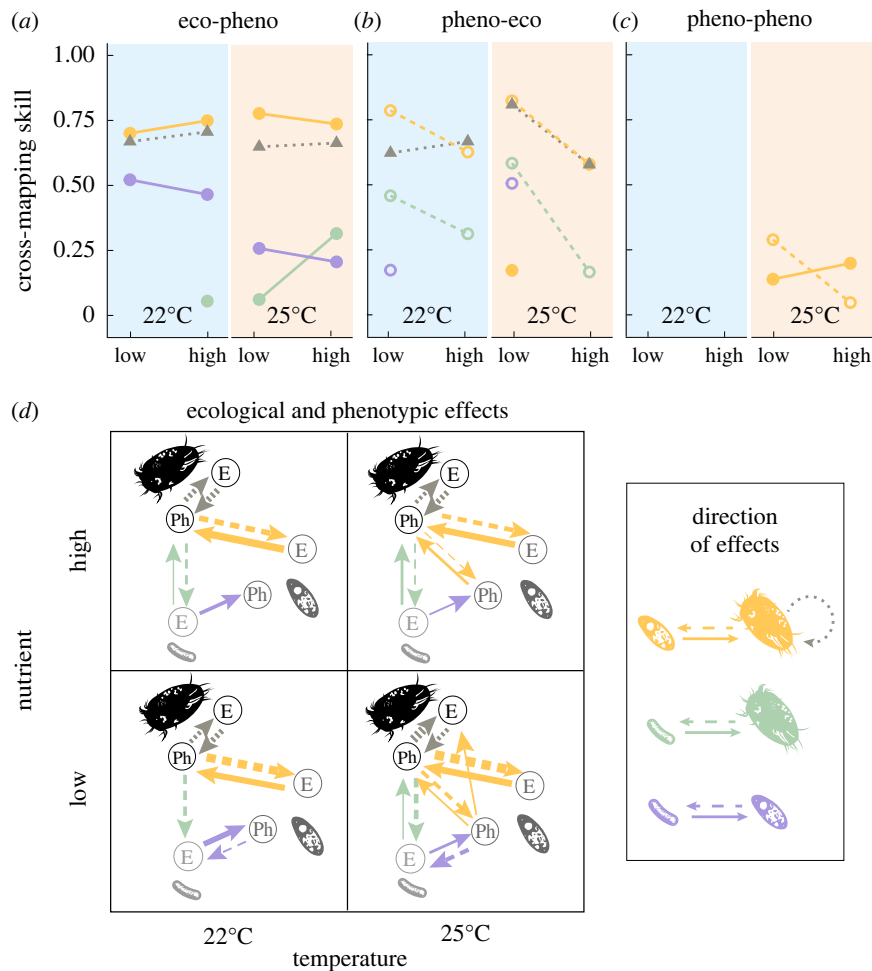


Figure 5. Interactions between phenotypic (Ph) and ecological (E) dynamics. (a–c) Show CCM estimation skill of density on traits (eco-pheno), traits on density (pheno-eco) and trait on trait (pheno-pheno). Circles represent CCM estimation skill of one species' effect on another species, while triangles represent intraspecific effects. Dashed and solid lines represent top-down and bottom-up effects respectively. Grey dotted lines represent intraspecific effects of *Euplates* sp. phenotypic dynamics on its own ecological dynamics, and vice versa. In (d), line widths are proportional to the CCM skill while the size of the arrowheads is fixed. Colour code as in figure 4a.

versus bottom-up effects (figure 4b). Additionally, changes in body size, especially those of the omnivorous predator, seem to mediate the effect of temperature and nutrients on ecological dynamics (figure 5d).

4. Discussion

Our results reveal complex but quantifiable and systematic effects of temperature and nutrients on the ecological and phenotypic dynamics of a microbial food web. We show that temperature and nutrients can each independently influence different aspects of food web dynamics (figures 1 and 2), while their joint effects get increasingly complex at higher trophic levels (figures 2 and 3). We also found that changes in the relative strength of top-down and bottom-up effects likely drive observed responses to temperature and nutrients (figures 4 and 5) and that rapid changes in body size mediate these effects (figures 4 and 5). Stronger species interactions at low nutrient levels and high temperature coupled with phenotypic dynamics having more and stronger effects in both ecological and phenotypic change at low nutrients and high temperature, suggest that phenotypic change may mediate the temperature response of ecological dynamics, perhaps strengthening species interactions (figures 4 and 5). These

results, therefore, suggest that body size not only responds to shifts in environmental conditions but also plays a role in determining ecological responses to such shifts (figure 5). Evaluating feedback between ecological and phenotypic dynamics may therefore be integral to understanding food web responses to environmental change.

(a) Increasingly complex effects of temperature and nutrients at higher trophic levels

Our results reveal the pervasive effects of temperature and nutrients within a microbial food web, but also show how these effects are more numerous and increasingly complex (i.e. larger effect sizes of temperature–nutrient interactions) at higher trophic levels (figures 2 and 3). Because energy enters at the bottom of a food web, basal species may be more strongly influenced by direct effects of nutrients, while species at higher trophic levels may be more strongly affected by a combination of temperature and nutrient treatments [28,60]. Additionally, the effects of temperature and nutrients on dietary preferences, species interactions, and foraging behaviour, may further explain why these interactive effects are stronger and more numerous among consumer species (figures 1d; 2c). Indeed, as temperature

and nutrients change species interactions and foraging behaviors [31], omnivorous consumers may shift diets between basal and intermediate resources [31], leading to climate-driven food web rewiring [2,9]. This dietary shift might also affect how much energy top consumers receive from basal species versus intermediate consumers, and therefore, how temperature and nutrients indirectly influence top predator dynamics. In such cases, causal effects of both environmental stressors are likely transitive (e.g. as temperature affects bacteria, and because *Euplotes* sp. preys on bacteria, the temperature effects on bacteria and *Euplotes* sp. are indirectly but causally linked, [52]).

This transitivity of causality (where omnivorous consumers are indirectly affected by temperature and nutrients that first acted on the basal and intermediate species) could also explain why the population variability (CV) of the top (omnivorous) predator is more often jointly influenced by temperature and nutrients than at lower trophic levels. Moreover, maximum density is more likely to reflect changes in nutrient availability, and these effects should wane across trophic levels, as energy is lost to energy conversion between consumers and prey across trophic levels. And this could explain the decrease in temperature and nutrient effects on species maximum density at higher trophic levels. Whether the joint effects of temperature and nutrients change in more natural food webs as reported here, however, is not known, but is a promising avenue for future research.

(b) Top-down control, bottom-up control and food web stability

Consistent with our results (figure 2*d*), theory predicts that higher nutrient loads should increase energy flux in food webs, through increases in basal species density [23,29]. Theory also predicts that increasing energy flux can be destabilizing, leading to increasing oscillations in density [13,29], but increasing temperatures should stabilize oscillations by weakening top-down effects [18,20,25]. We found that the interactive effects of temperature and nutrients have divergent impacts on stability—with temperature stabilizing, and nutrients destabilizing *Euplotes* sp. densities, but the opposite being true for *T. pyriformis* (figure 2*h,i*). Temperature and nutrients had different but interactive effects on top-down controls by the two predators even though bottom-up effects remained unaffected by the treatments (figure 4*a*). These results thus indicate that changes in the strength of top-down effects between basal resources and consumers—instead of bottom-up effects—could be the dominant mechanism through which higher temperatures may stabilize instabilities caused by nutrients [19,20].

We also observed that top-down control on bacteria by both consumers was much stronger than bottom-up effects across treatments (figure 4*a*) while the top-down and bottom-up controls are both strong and tightly coupled between the two protist predators. The dominant top-down effects on the bacterial community could be explained by the short generation time of all species in the system and the fast turnover rate of the bacteria relative to the protists. In addition to the potential stabilizing effects of top-down control, these results also indicate that top-down control on basal resources might have stronger effects in systems with high turnover rates, such as aquatic systems.

(c) Phenotypes mediate temperature and nutrient effects on food web dynamics

Our results indicate that phenotypic dynamics play a larger role in mediating environmental impacts on food web dynamics than previously thought (figures 4 and 5). Previous studies showed that predator–prey body size ratios significantly influence temperature and nutrients effects [19,61] and that body size responds to changes in nutrients, temperature and ecological dynamics in specific ways [18]. A recent study showed that the body size of a species can affect its own ecological dynamics [41], which we have also shown here as tight coupling between the phenotypic and ecological dynamics of *Euplotes* sp. (figure 5). Our results support—but also extend—previous findings by showing that (1) phenotypic effects on ecological dynamics can be strong, especially among top predators, (2) changes in the prey ecological dynamics may strongly drive changes in predator size and (3) interactions involving phenotypic dynamics (eco-pheno, pheno-eco or pheno-pheno) vary across environmental conditions (figures 4*b–d*, 5).

(d) Caveats

One caveat of this study is that we only tracked the dynamics of the bacterial community as a whole, as we lack information on how each individual species responded to temperature or nutrients. Recent work has shown that the composition of bacterial communities changes under joint temperature and nutrient loads, and that predation by protists mediates these responses [46,62]. Coupling an experiment like ours with 16S amplicon sequencing to keep track of bacterial dynamics would thus be an exciting avenue for future research that should deepen the understanding generated by our current study as to how microbial food webs will respond to rapid environmental change.

Another caveat is that, despite uniquely detailed, long, and well-replicated time series, our time series have a few small gaps due to lack of sampling on weekends. To address this issue, we interpolated these missing datapoints using three different methods, but, despite being generally robust, some variation remained between these results and the CCM inference of species interactions (electronic supplementary material, appendix II figure S1–S12). All three methods showed that bottom-up and top-down effects between the protist predators were the strongest and remained strong across all treatments. Moreover, top-down controls from both protists predators to bacteria were always stronger than bottom-up effects (electronic supplementary material, appendix II figure S13–S15 eco-eco panels). Last, the effects of *Euplotes* sp. body size on its own ecological dynamics and that of the *T. pyriformis* and bacteria were consistent across interpolation methods (electronic supplementary material, appendix II figure S13–S15 eco-pheno, pheno-eco and pheno-pheno panels). As CCM and related methods grow in use [32,53,56,57,63], there is a real need for these tools to be robust even in the face of imperfect data, including missing time points. Our results, therefore, underline the need to better understand how missing data may affect CCM and other time-series analyses and how to best predict missing data for analysis.

Last, because CCM analysis quantifies the effects of one time series on another but not on itself, we were unable

to measure density dependence within each species. Therefore, while our research showed the potential of using CCM in understanding complex casual effects between species ecological and phenotypic dynamics, we also notice the importance of combining time-series analyses with other, possibly experimental, methods to measure causality for intra-specific interactions.

5. Concluding remarks

Rapid phenotypic change has been suggested as a main driver of food web rewiring in future climates [2], but little experimental evidence exists. Here, our results show that strong feedback between ecological and phenotypic dynamics depend on environmental conditions (temperature and nutrients), suggesting that rapid phenotypic change influences food web responses to environmental change. Moreover, the joint effects of temperature and nutrients do not equally affect all members of the community, as higher trophic levels are more likely to experience both independent and joint effects. Together, our results emphasize the need to

incorporate phenotypic dynamics in future studies of food web responses to warming and eutrophication in a changing world and show how shifts in distinct environmental stressors can have complex but systematic effects on food web dynamics.

Data accessibility. All annotated code and data are available at our dedicated repository (https://github.com/ZeYiHan/Nut_Temp_Pheno_Eco) and Zenodo (<https://doi.org/10.5281/zenodo.7474517>).

The data are provided in the electronic supplementary material [64].

Authors' contributions. Z.-Y.H.: conceptualization, data curation, formal analysis, investigation, visualization, writing—original draft, writing—review and editing; D.J.W.: formal analysis, writing—review and editing; A.Y.: investigation, writing—review and editing; J.P.G.: conceptualization, formal analysis, funding acquisition, resources, supervision, visualization, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

Funding. This work was supported by a U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomic Science Program Grant under award no. DE-SC0020362 to J.P.G.

References

- Karl TR. 2003 Modern global climate change. *Science* **302**, 1719–1723. (doi:10.1126/science.1090228)
- Barbour MA, Gibert JP. 2021 Genetic and plastic rewiring of food webs under climate change. *J. Anim. Ecol.* **90**, 1814–1830. (doi:10.1111/1365-2656.13541)
- Gillooly JF, Charnov EL, West GB, Savage VM, Brown JH. 2002 Effects of size and temperature on developmental time. *Nature* **417**, 70–73. (doi:10.1038/417070a)
- Brown JH, Gillooly JF, Allen AP, Savage VM, West GB. 2004 Toward a metabolic theory of ecology. *Ecology* **85**, 1771–1789. (doi:10.1890/03-9000)
- Clarke A. 2006 Temperature and the metabolic theory of ecology. *Funct. Ecol.* **20**, 405–412. (doi:10.1111/j.1365-2435.2006.01109.x)
- Barton BT, Beckerman AP, Schmitz OJ. 2009 Climate warming strengthens indirect interactions in an old-field food web. *Ecology* **90**, 2346–2351. (doi:10.1890/08-2254.1)
- O'Connor MI. 2009 Warming strengthens an herbivore–plant interaction. *Ecology* **90**, 388–398. (doi:10.1890/08-0034.1)
- Gounand I, Kéfi S, Mouquet N, Gravel D. 2016 Trait selection during food web assembly: the roles of interactions and temperature. *Theor. Ecol.* **9**, 417–429. (doi:10.1007/s12080-016-0299-7)
- Bartley TJ, McCann KS, Bieg C, Cazelles K, Granados M, Guzzo MM, Macdougall AS, Tunney TD, Mcmeans BC. 2019 Food web rewiring in a changing world. *Nat. Ecol. Evol.* **3**, 345–354. (doi:10.1038/s41559-018-0772-3)
- Gibert JP. 2019 Temperature directly and indirectly influences food web structure. *Sci. Rep.* **9**, 5312. (doi:10.1038/s41598-019-41783-0)
- Gauzens B, Rall BC, Mendonça V, Vinagre C, Brose U. 2020 Biodiversity of intertidal food webs in response to warming across latitudes. *Nat. Clim. Change* **10**, 264–269. (doi:10.1038/s41558-020-0698-z)
- Dossena M, Yvon-Durocher G, Grey J, Montoya JM, Perkins DM, Trimmer M, Woodward G. 2012 Warming alters community size structure and ecosystem functioning. *Proc. R. Soc. B Biol. Sci.* **279**, 3011–3019. (doi:10.1098/rspb.2012.0394)
- Rosenzweig ML. 1971 Paradox of enrichment: destabilization of exploitation ecosystems in ecological time. *Science* **171**, 385. (doi:10.1126/science.171.3969.385)
- Fussmann GF. 2000 Crossing the Hopf bifurcation in a live predator–prey system. *Science* **290**, 1358–1360. (doi:10.1126/science.290.5495.1358)
- Hautier Y *et al.* 2014 Eutrophication weakens stabilizing effects of diversity in natural grasslands. *Nature* **508**, 521–525. (doi:10.1038/nature13014)
- Hautier Y, Niklaus PA, Hector A. 2009 Competition for light causes plant biodiversity loss after eutrophication. *Science* **324**, 636–638. (doi:10.1126/science.1169640)
- Malcolm JR, Liu C, Neilson RP, Hansen L, Hannah L. 2006 Global warming and extinctions of endemic species from biodiversity hotspots. *Conserv. Biol.* **20**, 538–548. (doi:10.1111/j.1523-1739.2006.00364.x)
- Tabi A, Petchey OL, Pennekamp F. 2019 Warming reduces the effects of enrichment on stability and functioning across levels of organisation in an aquatic microbial ecosystem. *Ecol. Lett.* **22**, 1061–1071. (doi:10.1111/ele.13262)
- Binzer A, Guill C, Brose U, Rall BC. 2012 The dynamics of food chains under climate change and nutrient enrichment. *Phil. Trans. R. Soc. B* **367**, 2935–2944. (doi:10.1098/rstb.2012.0230)
- Binzer A, Guill C, Rall BC, Brose U. 2016 Interactive effects of warming, eutrophication and size structure: impacts on biodiversity and food-web structure. *Glob. Change Biol.* **22**, 220–227. (doi:10.1111/gcb.13086)
- McClelland J, Valiela I. 1998 Changes in food web structure under the influence of increased anthropogenic nitrogen inputs to estuaries. *Mar. Ecol. Prog. Ser.* **168**, 259–271. (doi:10.3354/meps168259)
- Carlier A, Riera P, Amouroux J-M, Bodiou J-Y, Desmalades M, Grémare A. 2008 Food web structure of two Mediterranean lagoons under varying degree of eutrophication. *J. Sea Res.* **60**, 264–275. (doi:10.1016/j.seares.2008.10.006)
- Shurin JB, Clasen JL, Greig HS, Kratina P, Thompson PL. 2012 Warming shifts top-down and bottom-up control of pond food web structure and function. *Phil. Trans. R. Soc. B* **367**, 3008–3017. (doi:10.1098/rstb.2012.0243)
- Kratina P, Greig HS, Thompson PL, Carvalho-Pereira TSA, Shurin JB. 2012 Warming modifies trophic cascades and eutrophication in experimental freshwater communities. *Ecology* **93**, 1421–1430. (doi:10.1890/11-1595.1)
- Vasseur DA, McCann KS. 2005 A mechanistic approach for modeling temperature-dependent consumer–resource dynamics. *Am. Nat.* **166**, 184–198. (doi:10.1086/431285)
- Barneche DR, Hulatt CJ, Dossena M, Padfield D, Woodward G, Trimmer M, Yvon-Durocher G. 2021 Warming impairs trophic transfer efficiency in a long-term field experiment. *Nature* **592**, 76–79. (doi:10.1038/s41586-021-03352-2)
- Gibert JP, Grady JM, Dell AI. 2022 Food web consequences of thermal asymmetries. *Funct. Ecol.* **36**, 1887–1899. (doi:10.1111/1365-2435.14091)
- Petchey OL, McPhearson PT, Casey TM, Morin PJ. 1999 Environmental warming alters food-web structure and ecosystem function. *Nature* **402**, 69–72. (doi:10.1038/47023)

29. Rip JMK, McCann KS. 2011 Cross-ecosystem differences in stability and the principle of energy flux: cross-ecosystem differences in stability. *Ecol. Lett.* **14**, 733–740. (doi:10.1111/j.1461-0248.2011.01636.x)
30. Lee GH, Vonk JA, Verdonschot RCM, Kraak MHS, Verdonschot PFM, Huisman J. 2021 Eutrophication induces shifts in the trophic position of invertebrates in aquatic food webs. *Ecology* **102**, e03275.
31. Sentis A, Hemptinne J-L, Brodeur J. 2014 Towards a mechanistic understanding of temperature and enrichment effects on species interaction strength, omnivory and food-web structure. *Ecol. Lett.* **17**, 785–793. (doi:10.1111/ele.12281)
32. Wang J, Pan F, Soininen J, Heino J, Shen J. 2016 Nutrient enrichment modifies temperature–biodiversity relationships in large-scale field experiments. *Nat. Commun.* **7**, 13960. (doi:10.1038/ncomms13960)
33. Rosenblatt AE, Schmitz OJ. 2016 Climate change, nutrition, and bottom-up and top-down food web processes. *Trends Ecol. Evol.* **31**, 965–975. (doi:10.1016/j.tree.2016.09.009)
34. Atkinson D. 1995 Effects of temperature on the size of aquatic ectotherms: exceptions to the general rule. *J. Therm. Biol.* **20**, 61–74. (doi:10.1016/0306-4565(94)00028-H)
35. Atkinson D, Ciotti BJ, Montagnes DJS. 2003 Protists decrease in size linearly with temperature: ca. 2.5% °C⁻¹. *Proc. R. Soc. Lond. B* **270**, 2605–2611. (doi:10.1098/rspb.2003.2538)
36. Irwin AJ, Finkel ZV, Schofield OME, Falkowski PG. 2006 Scaling-up from nutrient physiology to the size-structure of phytoplankton communities. *J. Plankton Res.* **28**, 459–471. (doi:10.1093/plankt/fbi148)
37. Marañón E, Cermeño P, López-Sandoval DC, Rodríguez-Ramos T, Sobrino C, Huete-Ortega M, Blanco JM, Rodríguez J. 2013 Unimodal size scaling of phytoplankton growth and the size dependence of nutrient uptake and use. *Ecol. Lett.* **16**, 371–379. (doi:10.1111/ele.12052)
38. Fencl T. 1974 Intrinsic rate of natural increase: the relationship with body size. *Oecologia* **14**, 317–326. (doi:10.1007/BF00384576)
39. Savage VM, Gillooly JF, Brown JH, West GB, Charnov EL. 2004 Effects of body size and temperature on population growth. *Am. Nat.* **163**, 429–441. (doi:10.1086/381872)
40. Ferenc V, Sheppard CS. 2020 The stronger, the better—trait hierarchy is driving alien species interaction. *Oikos* **129**, 1455–1467. (doi:10.1111/oik.07338)
41. Gibert JP, Han Z, Wiczyński DJ, Votzke S, Yammine A. 2022 Feedbacks between size and density determine rapid eco-phenotypic dynamics. *Funct. Ecol.* **36**, 1668–1680. (doi:10.1111/1365-2435.14070)
42. Brose U, Dunne JA, Montoya JM, Petchey OL, Schneider FD, Jacob U. 2012 Climate change in size-structured ecosystems. *Phil. Trans. R. Soc. B* **367**, 2903–2912. (doi:10.1098/rstb.2012.0232)
43. Bernhardt JR, Sunday JM, O'Connor MI. 2018 Metabolic theory and the temperature–size rule explain the temperature dependence of population carrying capacity. *Am. Nat.* **192**, 687–697. (doi:10.1086/700114)
44. Wiczyński DJ, Singla P, Doan A, Singleton A, Han Z-Y, Votzke S, Yammine A, Gibert JP. 2021 Linking species traits and demography to explain complex temperature responses across levels of organization. *Proc. Natl Acad. Sci. USA* **118**, e2104863118. (doi:10.1073/pnas.2104863118)
45. Altermatt et al. 2015 Big answers from small worlds: a user's guide for protist microcosms as a model system in ecology and evolution. *Methods Ecol. Evol.* **6**, 218–231. (doi:10.1111/2041-210X.12312)
46. Rocca JD, Yammine A, Simonin M, Gibert JP. 2022 Protist predation influences the temperature response of bacterial communities. *Front. Microbiol.* **13**, 847964. (doi:10.3389/fmicb.2022.847964)
47. Gibert JP, Allen RL, Hruska RJ, DeLong JP. 2017 The ecological consequences of environmentally induced phenotypic changes. *Ecol. Lett.* **20**, 997–1003. (doi:10.1111/ele.12797)
48. Wood SN. 2011 Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models: estimation of semiparametric generalized linear models. *J. R. Stat. Soc. Ser. B Stat. Methodol.* **73**, 3–36. (doi:10.1111/j.1467-9868.2010.00749.x)
49. R Core Team. 2020 *R: a language and for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. <https://www.R-project.org/>.
50. McCann KS. 2012 *Food webs. Monographs in population biology*. Princeton, NJ: Princeton University Press.
51. Ben-Shachar M, Lüdtke D, Makowski D. 2020 effectsize: Estimation of effect size indices and standardized parameters. *J. Open Source Softw.* **5**, 2815. (doi:10.21105/joss.02815)
52. Sugihara G, May R, Ye H, Hsieh C-H, Deyle E, Fogarty M, Munch S. 2012 Detecting causality in complex ecosystems. *Science* **338**, 496–500. (doi:10.1126/science.1227079)
53. Matsuzaki SS, Suzuki K, Kadoya T, Nakagawa M, Takamura N. 2018 Bottom-up linkages between primary production, zooplankton, and fish in a shallow, hypereutrophic lake. *Ecology* **99**, 2025–2036. (doi:10.1002/ecy.2414)
54. Wang Y, Yang J, Chen Y, De Maeyer P, Li Z, Duan W. 2018 Detecting the causal effect of soil moisture on precipitation using convergent cross mapping. *Sci. Rep.* **8**, 12171. (doi:10.1038/s41598-018-30669-2)
55. Rogers TL, Munch SB, Stewart SD, Palkovacs EP, Giron-Nava A, Matsuzaki SS, Symons CC. 2020 Trophic control changes with season and nutrient loading in lakes. *Ecol. Lett.* **23**, 1287–1297. (doi:10.1111/ele.13532)
56. Barraquand F, Picoche C, Detto M, Hartig F. 2021 Inferring species interactions using Granger causality and convergent cross mapping. *Theor. Ecol.* **14**, 87–105. (doi:10.1007/s12080-020-00482-7)
57. Doi H, Yasuhara M, Ushio M. 2021 Causal analysis of the temperature impact on deep-sea biodiversity. *Biol. Lett.* **17**, 20200666. (doi:10.1098/rsbl.2020.0666)
58. Abidha CA, Amoako YA, Nyamekye RK, Bedu-Addo G, Grziwotz F, Mockenhaupt FP, Telschow A, Danquah I. 2022 Fasting blood glucose in a Ghanaian adult is causally affected by malaria parasite load: a mechanistic case study using convergent cross mapping. *Malar. J.* **21**, 93. (doi:10.1186/s12936-022-04076-y)
59. Clark AT, Ye H, Isbell F, Deyle ER, Cowles J, Tilman GD, Sugihara G. 2015 Spatial convergent cross mapping to detect causal relationships from short time series. *Ecology* **96**, 1174–1181. (doi:10.1890/14-1479.1)
60. Voigt W et al. 2003 Trophic levels are differentially sensitive to climate. *Ecology* **84**, 2444–2453. (doi:10.1890/02-0266)
61. Gibert JP, DeLong JP. 2014 Temperature alters food web body-size structure. *Biol. Lett.* **10**, 20140473. (doi:10.1098/rsbl.2014.0473)
62. Thurman J, Parry JD, Hill PJ, Laybourn-Parry J. 2010 The filter-feeding ciliates *Colpidium striatum* and *Tetrahymena pyriformis* display selective feeding behaviours in the presence of mixed, equally-sized, bacterial prey. *Protist* **161**, 577–588. (doi:10.1016/j.protis.2010.04.001)
63. Kitayama K, Ushio M, Aiba S. 2021 Temperature is a dominant driver of distinct annual seasonality of leaf litter production of equatorial tropical rain forests. *J. Ecol.* **109**, 727–736. (doi:10.1111/1365-2745.13500)
64. Han Z-Y, Wiczyński DJ, Yammine A, Gibert JP. 2023 Temperature and nutrients drive eco-phenotypic dynamics in a microbial food web. Figshare. (doi:10.6084/m9.figshare.c.6387924)