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

Ze-Yi Han[1], Daniel J. Wieczynski[1], Andrea Yammine[1], Jean P. Gibert[1]
[1]Department of Biology, Duke University, Durham, North Carolina, USA

Statement of authorship: ZH and JPG conceived the study; ZH and JPG designed the experimental work. ZH collected the data with support from AY. ZH analyzed the data with support from DJW and JPG; ZH wrote the first version of the manuscript; all authors contributed substantially to subsequent versions.

Data accessibility statement: All annotated code and data are available at our dedicated repository (https://github.com/ZeYiHan/Nut_Temp_Pheno_Eco).

Running title: Consequences of thermal asymmetries

Keywords: Global warming, Food-web temperature responses, Eutrophication, Anthropogenic impacts, Communities, Trait-based Ecology

To whom correspondence should be addressed: Ze-Yi Han, Department of Biology, Duke University, Durham, NC, USA, 402-405-3888, zeyihanw@gmail.com

ABSTRACT

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 i) joint –often interactive– effects of temperature and nutrients on ecological dynamics are more common at higher trophic levels, ii) temperature and nutrients interact to shift the relative strength of top-down vs. bottom-up control, and iii) rapid phenotypic change mediates observed ecological responses to changes in temperature and nutrients. Our results expose how feedbacks 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 feedbacks that determine food web dynamics in a changing world.

53 INTRODUCTION

54 Understanding how rapid global climate change (GCC) will affect the structure and dynamics of
55 communities is a pressing goal of ecology (Karl 2003; Barbour & Gibert 2021). Increasing
56 temperatures associated with GCC influence the metabolism of individuals (Gillooly *et al.* 2002;
57 Brown *et al.* 2004; Clarke 2006), which strengthens species interactions (Barton *et al.* 2009;
58 O'Connor 2009; Gounand *et al.* 2016), alters community structure (Bartley *et al.* 2019; Gibert
59 2019; Gauzens *et al.* 2020), and affects ecosystem function (Dossena *et al.* 2012). Additionally,
60 increasing nutrient loads from agricultural runoffs can result in eutrophication and destabilize
61 natural communities (Rosenzweig 1971; Fussmann 2000; Hautier *et al.* 2014), often leading to
62 species loss (Hautier *et al.* 2009). Warming and eutrophication can independently and jointly
63 impact food web structure and stability (Malcolm *et al.* 2006; Tabi *et al.* 2019).
64 Counterintuitively, simultaneous increases in temperature and nutrient load can produce
65 outcomes that are qualitatively different from the combined negative effects of each variable on
66 its own (Binzer *et al.* 2012, 2016). These non-additive (interactive) outcomes are still poorly
67 understood, but central to honing our understanding of GCC impacts on food web structure and
68 dynamics in a highly anthropogenized world.
69
70 The mechanisms through which warming and increasing nutrient loads independently influence
71 food webs are relatively well understood (McClelland & Valiela 1998; Carlier *et al.* 2008; Gibert
72 2019), specifically regarding their impacts on the relative strength of bottom-up and top-down
73 effects (Binzer *et al.* 2012, 2016; Shurin *et al.* 2012). For example, warming can increase
74 predation pressure (Kratina *et al.* 2012), thus decreasing resource biomass, while increasing the
75 proportion of top predators (Vasseur & McCann 2005; O'Connor 2009; Shurin *et al.* 2012).

76 Alternatively, warming can increase metabolic demands while reducing conversion efficiency
77 (Barneche *et al.* 2021), leading to predator starvation at high temperatures, loss of top predators
78 (Gibert *et al.* 2022a), and reduced food chain length (Petchey *et al.* 1999). Unlike warming,
79 increasing nutrient loads tend to increase bottom-up effects, resulting in unstable dynamics and
80 species loss (i.e., paradox of enrichment, Rip and McCann, 2011; Rosenzweig, 1971), often
81 leading to top-heavy, unstable food webs (Rip & McCann 2011). Eutrophication resulting from
82 increasing nutrient loads can also change consumer trophic position, leading to changes in
83 species interactions and food web structure (Lee *et al.* 2021).
84
85 While warming and nutrients can independently influence food webs, they can also have non-
86 additive (interactive) effects when acting together (Binzer *et al.* 2012, 2016; Tabi *et al.* 2019),
87 but these are much less well understood. For example, although temperature increases are
88 typically considered to be destabilizing (Barton *et al.* 2009), at low temperatures, small
89 temperature increases can cause consumer starvation, stabilizing nutrient-induced instabilities
90 (Binzer *et al.* 2012, 2016). However, at high temperatures, increasing nutrient loads can counter
91 warming-induced consumer starvation by increasing carrying capacity and predator attack rates
92 (Binzer *et al.* 2012). Warming also weakens nutrient-induced increases in community biomass
93 (negative interactive effects, Tabi et al., 2019), in turn influencing food web structure (Sentis *et*
94 *al.* 2014), species richness, and community composition (Wang *et al.* 2016a).
95
96 In addition to their effects on entire food webs, temperature and nutrients can both determine the
97 physiology and morphology of organisms (Rosenblatt & Schmitz 2016; Tabi *et al.* 2019). For
98 example, higher temperatures often result in smaller sizes (Atkinson 1995; Atkinson *et al.* 2003),

while nutrient enrichment leads to larger organisms (Irwin *et al.* 2006; Marañón *et al.* 2013). Additionally, temperature and nutrients can interactively affect body size, which increases with increasing nutrients at low temperature but has the opposite effect at high temperature (Tabi *et al.* 2019). Although body size is often considered a response variable, it also has well-known effects on population growth (Fenchel 1974; Savage *et al.* 2004; Gibert *et al.* 2022b) and species interactions (Ferenc & Sheppard 2020), so rapid body size responses to temperature, nutrients, or both, may have consequences for food web structure and dynamics in warmer climates (Brose *et al.* 2012; Bernhardt *et al.* 2018; Wieczynski *et al.* 2021). However, whether body size only responds to environmental conditions, whether rapid changes in body size mediate how environmental conditions affect food webs, or whether shifts in body size influence body size responses to environmental conditions in another species, are not well understood.

Here, we study how temperature and nutrients influence feedbacks 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 vs. bottom-up control? 3) Do these effects vary across trophic levels? And, 4) does body size only respond to temperature and nutrients, as suggested elsewhere (e.g., Binzer et al 2016, Tabi et al 2019), or does that response 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*

122 *pyriformis*), and an omnivorous top predator (*Euplotes sp.*), then track changes in the population
123 densities of all organisms and the body sizes of the protists over time. We use time-series
124 analysis to evaluate the relative strength of top-down vs. bottom-up processes across trophic
125 levels as well as whether and how the observed ecological and phenotypic dynamics influence
126 one another across temperature and nutrient treatments. Our results reveal complex temperature
127 and nutrient effects on food web dynamics that vary predictably across trophic levels by altering
128 the relative strength of trait-mediated bottom-up and top-down effects.
129
130 METHODS
131 *Culture care*
132 *Euplotes sp.* and *Tetrahymena pyriformis* stock cultures were acquired from Carolina Biological
133 Supply (Burlington, NC, USA) and grown in laboratory conditions for a year prior to this
134 experimental work. Both species were kept in autoclaved liquid protist medium (Carolina
135 Biological Supply), supplemented with one autoclaved wheat seed as a carbon source (Altermatt
136 et al 2014). Protists were fed a mixture of pond bacteria collected from an ephemeral pond at
137 Duke Forest (Wilbur/Gate 9 pond, Lat 36.013914, -Long 78.979720) and composed of thousands
138 of bacterial species, as described elsewhere (Rocca *et al.* 2022). We maintained all cultures on a
139 16:8 light:dark cycle at 22°C and 65% humidity in AL-22L2 Percival growth chambers (Perry,
140 IA, USA).
141
142 *Experimental design*
143 Microcosms were set up in autoclaved 250mL borosilicate jars filled with 200mL of protist
144 media. We manipulated temperature and nutrient loads by imposing two temperature levels

145 (22°C/25°C) and two nutrient levels (normal protist media concentration plus one wheat seed –
146 i.e., high nutrients–, or half concentration plus half a wheat seed, –i.e., low nutrients) in a
147 factorial design with 4 treatments and 6 replicates per treatment. Day/night cycle and humidity
148 levels mimicked rearing conditions and were kept constant. We inoculated the bacterial
149 communities from 2 ml of stock culture, the same used to rear the protists. The intermediate
150 consumer species, *T. pyriformis*, was introduced at a starting density of 37 ind/ml, and the larger
151 protists species, *Euplotes sp.*, at a starting density of 0.24 ind/ml in all microcosms. We recorded
152 the density of both protist species through fluid imaging (FlowCam; Yokogawa Fluid Imaging
153 Technologies, Portland, ME, USA), Monday through Friday, for 16 days. Fluid imaging
154 generates individual cell raster images that were used to quantify changes in protist size
155 (measured as cell area, in μm^2) over time. Bacteria density was quantified as optical density at a
156 wavelength of 600nm (OD600), using a BioTEK Epoch-2 microplate spectrophotometer
157 (Winooski, VT, USA).
158
159 *Statistical analysis*
160 To test for possible effects of temperature and nutrients on ecological dynamics, we fitted
161 Generalized Additive Mixed Models (GAMM) to time series of species density (OD600 for
162 bacteria) and protist body size, across all treatments, using the 'mgcv' package v1.8-31 (Wood
163 2011) in R v4.0.2 (R Core Team 2020). To control for temporal autocorrelation, we used an
164 Autoregressive Moving Average (ARMA) correlation structure in the GAMMs using the 'nlme'
165 R package (version 3.1-148). To account for repeated sampling within each replicate, we
166 included replicates as a random intercept in the model. We compared models with additive
167 and/or interactive temperature and nutrients effects, as well as different ARMA correlation

168 structures, using AICc (Appendix I Table S1-2). We discarded *T. pyriformis* phenotypic data
169 from days in which fewer than 10 individuals were measured (i.e., after populations collapsed).
170
171 *Characterizing ecological dynamics*
172 To better understand which aspects of the food web dynamics were most influenced by
173 temperature and nutrients, we characterized multiple aspects of the observed ecological
174 dynamics of the bacterial community and the protists across treatments. Specifically, we
175 quantified: 1) initial growth rate (day^{-1}) as $[\ln(\mathrm{Nf}) - \ln(\mathrm{Ni})]/\mathrm{time}$ for early-dynamics data, (up
176 to day 1 for *T. pyriformis* & bacteria and day 8 for *Euplotes sp.*, due to much slower growth), 2)
177 maximum density (individual/ml for protists and OD_{600} for bacteria), using the highest average
178 daily population density across replicates but within treatments for the protists, and the top three
179 observed OD600 values for each replicate from each treatment for the bacteria (because their
180 ecological dynamics remained relatively stable), 3) the coefficient of variation (CV = standard
181 deviation/mean) of the temporal population dynamics within treatments (typically used as a
182 measure of stability (McCann 2012)), 4) the time to population collapse in days (only *T.*
183 *pyriformis*), and 5) the time to population peak in days (only *Euplotes sp.*).
184
185 *Quantifying top-down/bottom-up effects and eco-phenotypic feedbacks*
186 To understand the mechanisms through which temperature and nutrients affected the observed
187 food web dynamics, we quantified the reciprocal effects of ecological dynamics and body size on
188 each other using Convergent Cross Mapping (CCM) (Sugihara *et al.* 2012). The CCM algorithm
189 has now been used multiple times across ecological systems and taxa reliably to estimate the
190 strength of causal effects between variables for which time-series are available (Matsuzaki *et al.*

2018; Wang *et al.* 2018; Rogers *et al.* 2020; Barraquand *et al.* 2021; Doi *et al.* 2021; Abidha *et al.* 2022; Gibert *et al.* 2022b) and we followed this specialized literature to infer causation in our data (see Appendix II Fig. S1-15). In a nutshell, 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 (Sugihara *et al.* 2012). The larger the causal effect of X on Y, the better the ability of Y to predict X, as Y contains 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 (Sugihara *et al.* 2012). 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 (Sugihara *et al.* 2012). 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; Sugihara et al., 2012).

We used a modified CCM algorithm that allows for replication in the time series through the R package 'multispatialCCM' (Version 1.0, Clark *et al.* 2015). The package can be used to detect causality between short replicated time series, as in our case. 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

214 the ‘approx’, ‘spline’, and ‘smooth.spline’ functions in base R (see Appendix 2). To increase the
215 robustness of our inference, we then performed CCM in each of these time series and averaged
216 the estimation skill from our CCM analysis across these three separate interpolated time-series.
217 That said, CCM results based on each independent smoothing technique held qualitatively
218 (Appendix II Fig. 1-12), which corroborates the robustness of our inference. Additionally, we
219 only used CCM results that showed convergence in cross mapping skill (ρ) with increasing
220 library size (indicating causality) to focus only on likely causal effects between species
221 ecological and phenotypic dynamics (Appendix II Fig. 13-15).
222
223 Based on previous literature (Sugihara *et al.* 2012; Matsuzaki *et al.* 2018; Barraquand *et al.*
224 2021), we interpreted the cross-mapping skill (ρ) as the magnitude of the effect of one variable
225 on another, whenever convergence was present. We calculated this cross-mapping skill between
226 all predator and prey densities, between densities and trait dynamics (except the effect of protist
227 densities on bacterial traits as we lack phenotypic data for the bacteria community), and between
228 protist trait dynamics. The effect of prey density on predator density was thus considered as
229 representing bottom-up control, and the effect of predator density on prey density as top-down
230 control (dubbed “eco-eco” effects, for simplicity). The effects of change in body size on
231 ecological dynamics (density) where dubbed ‘pheno-eco’ effects, and the effects of density on
232 body size dynamics as ‘eco-pheno’ effects. Reciprocal effects of changes in predator and prey
233 protist body sizes were dubbed ‘pheno-pheno’ effects.
234
235 RESULTS
236 *General Ecological and Phenotypic dynamics*

Overall, bacterial density rapidly increased to carrying capacity (Fig. 1a, light grey), *T. pyriformis* increased rapidly, then decreased (Fig. 1a, dark grey), while *Euplotes sp.* increased almost monotonically to carrying capacity (Fig. 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 (Fig. 1b-d, Appendix I Table S2). The body sizes of both protists changed rapidly over time (Fig. 1e, f) and responded to both temperature and nutrients (Fig. 1e, f, Appendix I Table S2).

Temperature and nutrient effects changed across trophic levels in systematic ways

Temperature significantly affected a larger number of dynamical descriptors than nutrients (Fig. 2, 3). Individual temperature effects were more frequent in the upper trophic levels than in the bacterial community (Fig. 3), as were interactions between temperature and nutrients (Fig. 3). In particular, higher temperature alone i) had no significant effect on the intrinsic growth rate of the bacteria (Fig a, Temperature effect (T from now on) = -0.003, $p = 0.28$), ii) strongly increased initial growth rate in *T. pyriformis* ($T = 0.50$ $p = 1.16\times10^{-6}$, Fig. 2b), iii) decreased the maximum density of bacteria and *T. pyriformis* ($T_B = -0.005$, $p = 0.004$; $T_T = -975.3$, $p < 0.02$; Fig. 2d-e) but increased that of *Euplotes sp.* ($T_E = 39.83$, $p < 0.03$, Fig. 2f), iv) increased the variability in *T. pyriformis* density (i.e. CV, $T = 0.2$, $p < 0.006$, Fig. 2h) but decreased that of *Euplotes sp.* ($T = -0.08$, $p < 0.02$, Fig. 2i), v) accelerated the time to collapse in *T. pyriformis* ($T = -2.8$, $p = 1.6\times10^{-5}$; Fig. 2j), and vi) accelerated the time to peak density in *Euplotes sp.* ($T = -2.2$, $p < 0.02$; Fig. 2k).

Higher nutrients alone i) decreased the initial growth rate of *Euplotes sp.* (Nutrient effect (N from now on) = -0.09, $p = 5\times10^{-4}$ Fig. 2c), ii) increased maximum density of all species (N_B= 0.01, $p = 2\times10^{-10}$; N_T = 1002.2, p = 0.01; N_E = 60.67, $p < 0.002$, Fig. 2d-f), and iii) increased the CV of bacteria and *Euplotes sp.* (N_B = 0.2, $p = 8.6\times10^{-5}$, Fig. 2g; N_E = 0.16, $p = 2.9\times10^{-5}$, Fig. 2i) while decreasing that of *T. pyriformis* (N_T = -0.19, $p < 0.01$, Fig. 2h). Interestingly, increasing either temperature or nutrients had opposite effects on the CVs of *T. pyriformis* and *Euplotes sp.*, indicating antagonistic additive effects of temperature and nutrients on food web stability.

Temperature and nutrients had complex interactive effects on ecological dynamics of both protist consumers. Nutrients had a negative effect on the initial growth rate of *Euplotes sp.* at low temperature, but a positive effect at high temperature (N×T = 0.13, p < 10^-3; Fig. 2c). The maximum density of *T. pyriformis* increased with nutrients, but this effect was stronger at high temperature (N×T = 1526.7, $p < 0.01$, Fig. 2e). Nutrients also had i) a negative effect on *T. pyriformis* population variability (CV) at low temperature but no effect at high temperature (N×T = 0.2, p = 0.045, Fig. 2h) and ii) a positive effect on *Euplotes sp.* CV that was stronger at low temperature (N×T = -0.12, $p < 0.01$, Fig. 2i). Additional model stats can be found in Appendix I Table S3. Taken together, these results imply that temperature and nutrients individually and interactively influence species ecological dynamics at all trophic levels, with more of both types of effects occurring at higher trophic levels (Fig. 3).

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 (Fig. 4a). Top-down and bottom-up effects between the two

protists were slightly stronger in the warmer temperature (Fig. 4a, yellow lines). Importantly, temperature and nutrients showed interactive effects on both top-down and bottom-up effects (Fig. 4a). For example, while the top-down effects of protists on bacteria (Fig.4a, green and purple dashed lines) were generally stronger than the bottom-up effects of the bacteria on the protists (Fig. 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 (Fig.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 (Fig. 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 (Fig. 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 5a). To visualize these changes, we present CCM cross-mapping skill value at the largest library size as links between species pairs (Fig. 5a). At low nutrient levels, higher temperature led to stronger species interactions (Fig. 5a, bottom left and right) while at high nutrient levels, interaction strengths were generally stronger and temperature only had a small effect (Fig. 5a, top left and right). Low nutrients and high temperatures led to the strongest interactions among all three species (Fig. 5a, bottom right).

Temperature and nutrients altered the reciprocal effects of ecological and phenotypic dynamics

304 Protist size not only responded to temperature and nutrients, but also played an important role in
305 determining the effects of temperature and nutrients on ecological dynamics (Fig. 4b-c). The
306 bidirectional effects between the body-size dynamics of *Euplotes sp.* and the ecological
307 dynamics of *T. pyriformis* were the strongest overall across treatments (Fig. 4b-c). The body size
308 dynamics of the omnivorous predator, *Euplotes sp.*, but not those of *T. pyriformis,* had relatively
309 strong causal effects on the ecological dynamics of all species, including its own (Fig. 4c).
310 Specifically, at low temperature, increasing nutrient level strengthened the effect of *Euplotes sp.*
311 phenotypic dynamics on its own ecological dynamics and those of *T. pyriformis*, but weakened
312 these effects on the ecological dynamics of bacteria (Fig. 4c). Yet, at the warmer temperature,
313 increasing nutrient levels weakened the effects of *Euplotes sp.* phenotypic dynamics on the
314 ecological dynamics of all species, including itself (Fig. 4c), indicating that plastic changes in
315 top predator body size can mediate how food web dynamics respond to temperature and
316 nutrients.
317
318 Trait on trait effects among the two predators (Fig. 4d, pheno-pheno) were weaker than their
319 pheno-eco or eco-pheno counterparts. Interestingly, pheno-pheno causal effects were only
320 observed at the higher temperature (Fig. 4d). Increasing nutrient levels increased the pheno-
321 pheno effects of *T. pyriformis* on *Euplotes sp.* but decreased those of *Euplotes sp*. on *T.*
322 *pyriformis* (Fig. 4d).
323
324 Overall, we also found stronger effects between ecological and phenotypic dynamics at higher
325 temperatures, especially in the low nutrient treatment (Fig. 5b, bottom right), consistent with
326 results from the top-down vs bottom-up effects (Fig 5a). Overall, changes in body size seem to

327 mediate the effect of temperature and nutrients on ecological dynamics (Fig. 5b), and these
328 effects seem stronger at higher temperatures but low nutrient levels.
329
330 DISCUSSION
331 Our results reveal complex but systematic effects of temperature and nutrients on the ecological
332 and phenotypic dynamics of a microbial food web. We show that temperature and nutrients can
333 each independently influence different aspects of food web dynamics (Fig. 1, 2), while their joint
334 effects get increasingly complex at higher trophic levels (Fig. 2, 3). We also found that changes
335 in the relative strength of top-down and bottom-up effects likely drive observed responses to
336 temperature and nutrients (Fig. 4, 5) and that rapid changes in body size mediate these effects
337 (Fig. 4, 5). Stronger species interactions at low nutrient levels and high temperature coupled with
338 phenotypic dynamics having more and stronger effects in both ecological and phenotypic change
339 at low nutrients and high temperature, suggest that phenotypic change may mediate the
340 temperature response of ecological dynamics, perhaps strengthening species interactions (Fig 4,
341 5). These results therefore suggest that body size not only responds to shifts in environmental
342 conditions, but also plays a role in determining ecological responses to such shifts (Fig. 5).
343 Evaluating feedbacks between ecological and phenotypic dynamics may therefore be integral to
344 understanding food web responses to environmental change.
345
346 *Increasingly complex effects of temperature and nutrients at higher trophic levels*
347 Our results reveal the pervasive effects of temperature and nutrients across all species, but also
348 how these effects are more numerous and increasingly complex (temperature-nutrient
349 interactions) at higher trophic levels (Fig. 2, 3). The effects of temperature and nutrients on

350 dietary preferences, species interactions, and foraging behavior may help explain why these
351 interactive effects may be more likely among consumer species (Fig. 1d; Fig. 2c).
352
353 Because energy enters at the bottom of a food web, basal species may be more strongly
354 influenced by direct effects of nutrients while species at higher trophic levels may be more
355 strongly affected by a combination of temperature and nutrients (Petchey *et al.* 1999; Voigt *et al.*
356 2003). 6/21/22 11:24:00 AM Some of these effects may be explained through the transitivity of
357 causality (Sugihara *et al.* 2012) as *Euplotes sp.* preys on *T. pyriformis* which in turn preys on the
358 bacterial community. So *Euplotes sp.* will be causally, albeit indirectly, linked to bacteria, and
359 also to the effects that temperature and nutrients have on bacteria. In ecological terms, as energy
360 flows from the basal species to the consumers, the temperature and nutrient effects that act on
361 basal species could be integrated by consumers across trophic levels, leading to additional
362 indirect temperature and nutrient effects on consumers at the top of the food web. Moreover,
363 these effects may be exaggerated in omnivores due to the complex nature of their trophic
364 interactions. As temperature and nutrients change species interactions and foraging behaviors
365 (Sentis *et al.* 2014), omnivorous consumers may shift diets between basal and intermediate
366 resources (Sentis *et al.* 2014), altering the strength/prevalence of competitive vs. predatory
367 interactions (Vandermeer 2006) and leading to climate-driven food web rewiring (Bartley *et al.*
368 2019; Barbour & Gibert 2021). Whether the joint effects of temperature and nutrients become
369 increasingly complex at higher trophic levels in food webs with a higher prevalence of
370 omnivory, however, is not known, but a promising avenue for future research.
371
372 *Top-down control, bottom-up control, and food web stability*

373 Consistent with our results (Fig. 2d), theory predicts that higher nutrient loads should increase
374 energy flux in food webs, through increases in basal species density (Rip & McCann 2011;
375 Shurin *et al.* 2012). Theory also predicts that increasing energy flux can be destabilizing, leading
376 to increasing oscillations in density (Rosenzweig 1971; Rip & McCann 2011), but increasing
377 temperatures should stabilize oscillations by weakening top-down effects (Vasseur & McCann
378 2005; Binzer *et al.* 2016; Tabi *et al.* 2019). We found that the interactive effects of temperature
379 and nutrients have divergent impacts on stability—with temperature stabilizing, and nutrients
380 destabilizing *Euplotes sp.* densities, but the opposite being true for *T. pyriformis* (Fig. 2h-i). We
381 also observed that top-down control on bacteria by both consumers was much stronger than
382 bottom-up effects across treatments (Fig. 4a). Temperature and nutrients had different but
383 interactive effects on top-down controls by the two predators even though bottom-up effects
384 remained unaffected by the treatments (Fig. 4a). These results thus indicate that changes in the
385 strength of top-down effects between basal resources and consumers—instead of bottom-up
386 effects—could be the dominant mechanism through which higher temperature may stabilize
387 instabilities caused by nutrients (Binzer *et al.* 2012, 2016).
388
389 *Phenotypes mediate temperature and nutrient effects on food web dynamics*
390 Our results indicate that phenotypic dynamics play a larger role in mediating environmental
391 impacts on food web dynamics than previously thought (Fig. 4, 5). Previous studies showed that
392 predator-prey body size ratios significantly influence temperature and nutrients effects (Binzer *et*
393 *al.* 2012; Gibert & DeLong 2014) and that body size responds to changes in nutrients,
394 temperature, and ecological dynamics in specific ways (Tabi *et al.* 2019). Our results support—
395 but also extend—those results by showing that 1) phenotypic effects on ecological dynamics can

396 be strong, especially among top predators, 2) changes in the prey ecological dynamics may
397 strongly drive changes in predator size, and 3) interactions involving phenotypic dynamics (eco-
398 pheno, pheno-eco, or pheno-pheno) vary across environmental conditions (Fig. 4b-d, Fig. 5).
399
400 *Caveats*
401 A caveat of this study is that, despite uniquely detailed, long, and well-replicated time-series, our
402 time-series have a few small gaps due to lack of sampling on weekends. To address this issue,
403 we interpolated these missing datapoints using three different methods, but, despite being
404 generally robust, some variation remained between these results and the CCM inference of
405 species interactions (Appendix II Figure 1-12). All three methods showed that bottom-up and
406 top-down effects between the protist predators were the strongest and remained strong across all
407 treatments. Moreover, top-down controls from both protists predators to bacteria were always
408 stronger than bottom-up effects (Appendix II Figure 13-15 Eco-Eco panels). Last, the effects of
409 *Euplotes sp.* body size on its own ecological dynamics and that of the *T. pyriformis* and bacteria
410 were consistent across interpolation methods (Appendix II Figure 13-15 Eco-Pheno, Pheno-Eco
411 and Pheno-Pheno panels). As CCM and related methods grow in use (Wang *et al.* 2016b;
412 Matsuzaki *et al.* 2018; Barraquand *et al.* 2021; Doi *et al.* 2021; Kitayama *et al.* 2021), there is a
413 real need for these tools to be robust even in the face of imperfect data, including missing
414 timepoints. Our results therefore underline the needs to better understand how missing data may
415 affect CCM and other time-series analyses and how to best predict missing data for analysis.
416
417 *Concluding remarks*

Rapid phenotypic change has been suggested as a main driver of food web rewiring in future climates (Barbour & Gibert 2021), but little experimental evidence exists. Here, our results show that strong feedbacks 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.

ACKNOWLEDGMENTS

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 Number DE-SC0020362 to JPG.

Figures

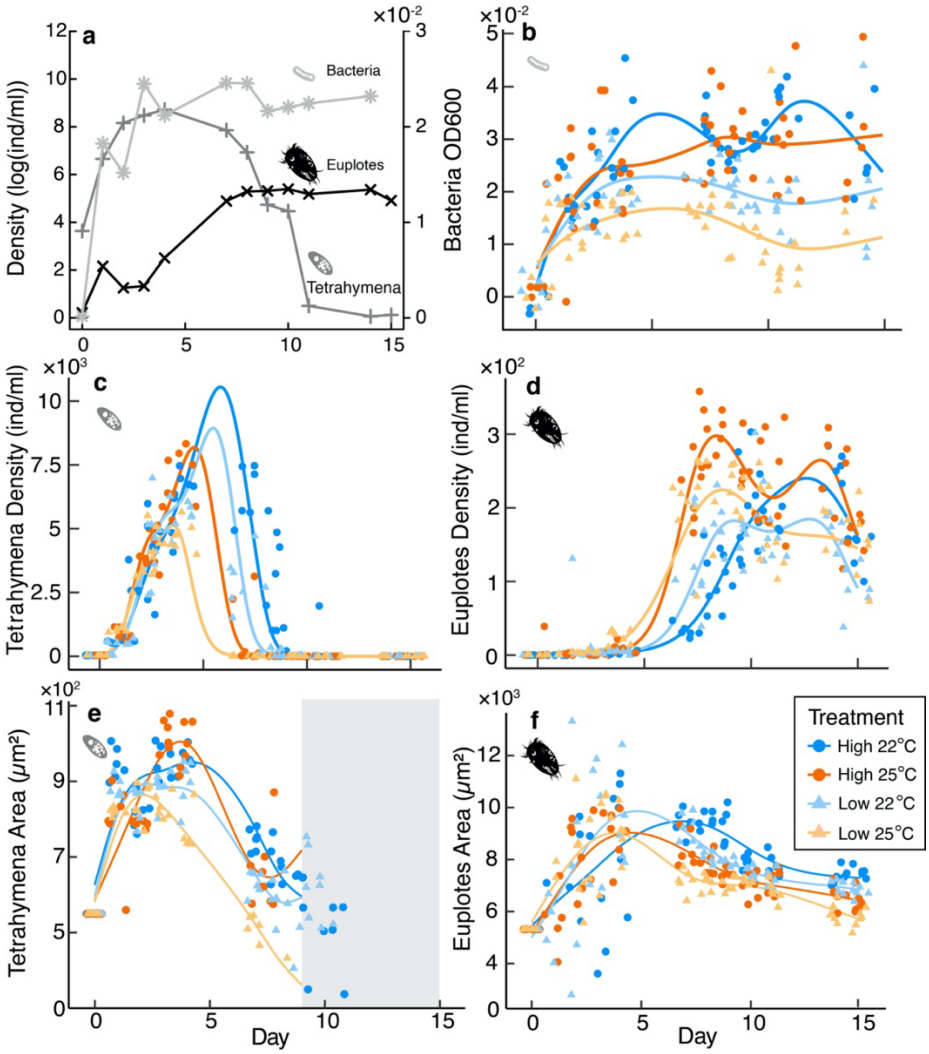

Figure 1. Ecological and phenotypic dynamics within experimental microcosms. (a) Average population densities across treatments. (b-d) Density dynamics of bacteria, *T. pyriformis*, and *Euplotes sp*. (e-f) Changes in the body size (measured as the area of the cell in microm^2) for both protists over time. *T. pyriformis* were effectively extinct by day 9. In (b–f), dots are the empirical measurements and lines are GAMM fits. The greyed area in (e) indicates days for which data were insufficient to estimate trait distributions.

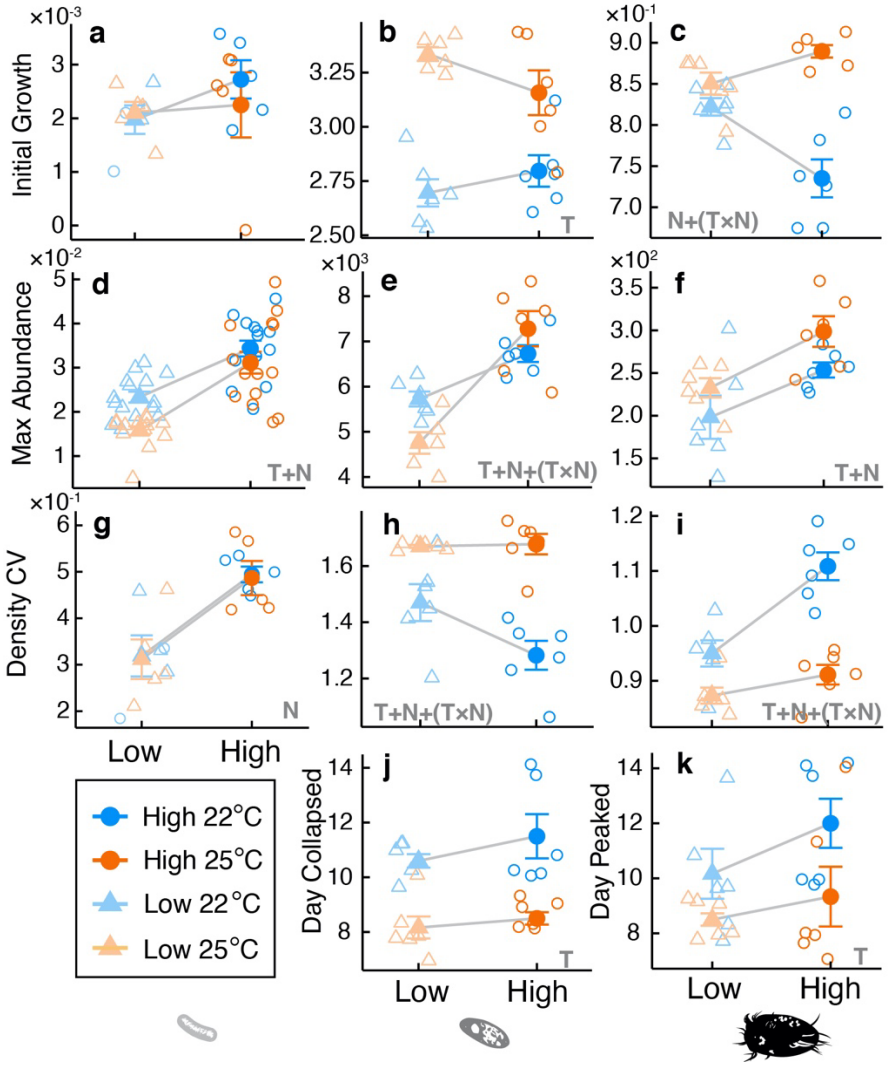

Figure 2. Additive and interactive effects of temperature and nutrients on descriptors of population dynamics. Open circles and triangles represent data points from each replicate. Solid shapes represent means across 6 replicates. Grey letters T, N, and (T×N) represent statistically significant effects from temperature, nutrients, and interactive effects between temperature and nutrients, respectively.

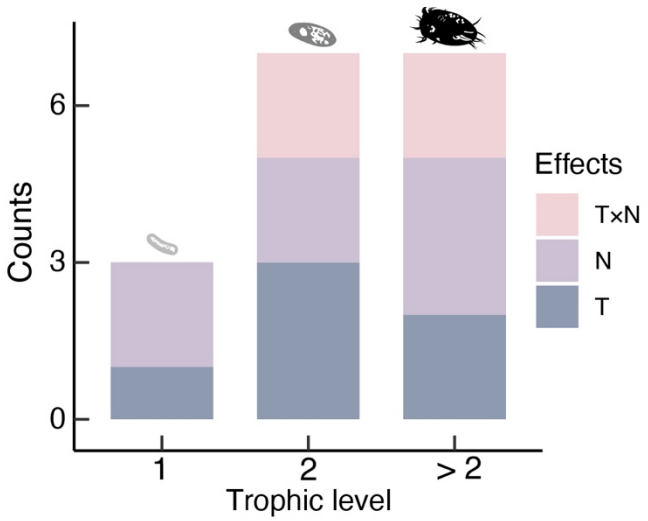

Fig. 3. Significant temperature and nutrient effects on ecological dynamics (i.e., initial growth, maximum abundance, and density CV). T, N, and T×N denote effects of temperature, nutrients, and interactive effects of temperature and nutrients, respectively.

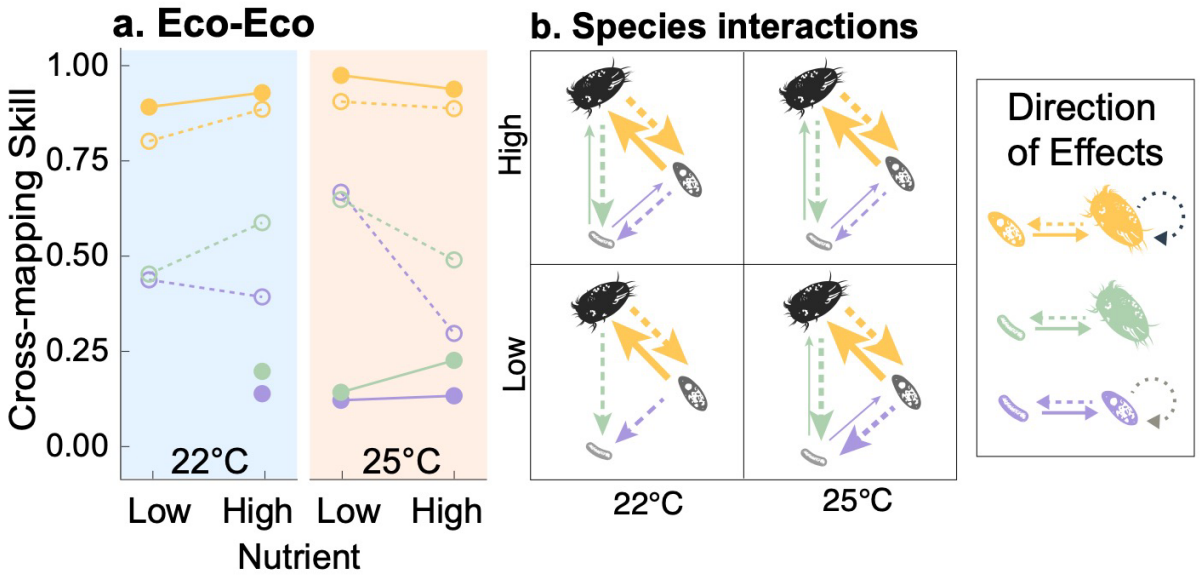

Figure 4. Cross-mapping skill (ρ) of species ecological dynamics across treatments for each species pair. (a) Shows CCM estimation skill of one species' effect on another. Solid circles and

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.

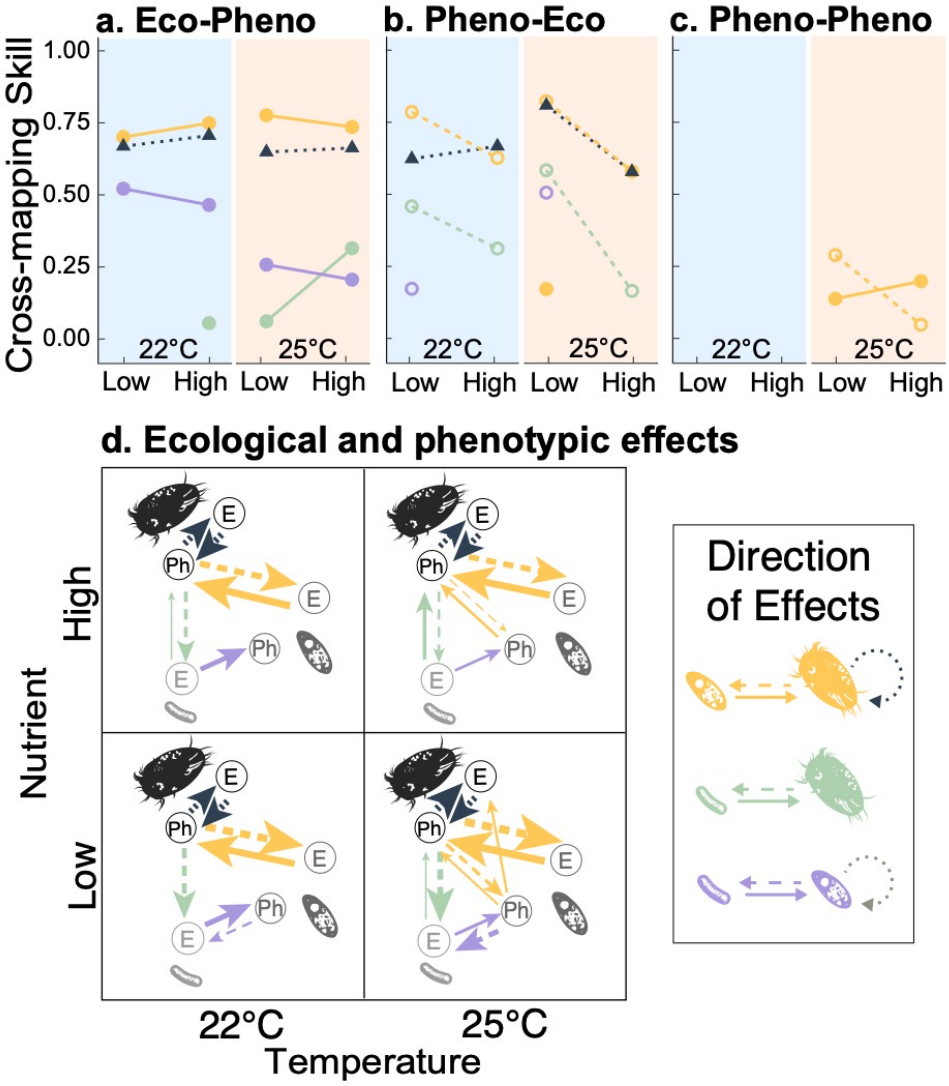

Figure 5. Interactions between phenotypic (Ph) and ecological (E) dynamics. (a-c) Show CCM estimation skill of density on traits (eco-pheno), traits on density (pheno-eco), and trait on trait (pheno-pheno). Circles represent CCM estimation skill of one species' effect on another species,

while triangles represent intraspecific effects. Dashed and solid lines represent top-down and bottom-up effects respectively. Dotted lines represent intraspecific effects. In (d), line widths are proportional to the CCM skill. Color code as in Fig. 4a.

References:

Abidha, C.A., Amoako, Y.A., Nyamekye, R.K., Bedu-Addo, G., Grziwotz, F., Mockenhaupt, F.P., *et al.* (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.

Atkinson, D. (1995). Effects of temperature on the size of aquatic ectotherms: Exceptions to the general rule. *J. Therm. Biol.*, 20, 61–74.

Atkinson, D., Ciotti, B.J. & Montagnes, D.J.S. (2003). Protists decrease in size linearly with temperature: *ca* . 2.5% °C $^{-1}$. *Proc. R. Soc. Lond. B Biol. Sci.*, 270, 2605–2611.

Barbour, M.A. & Gibert, J.P. (2021). Genetic and plastic rewiring of food webs under climate change. *J. Anim. Ecol.*, 1365-2656.13541.

Barneche, D.R., Hulatt, C.J., Dossena, M., Padfield, D., Woodward, G., Trimmer, M., *et al.* (2021). Warming impairs trophic transfer efficiency in a long-term field experiment. *Nature*.

Barraquand, F., Picoche, C., Detto, M. & Hartig, F. (2021). Inferring species interactions using Granger causality and convergent cross mapping. *Theor. Ecol.*, 14, 87–105.

Bartley, T.J., McCann, K.S., Bieg, C., Cazelles, K., Granados, M., Guzzo, M.M., *et al.* (2019). Food web rewiring in a changing world. *Nat. Ecol. Evol.*, 3, 345–354.

Barton, B.T., Beckerman, A.P. & Schmitz, O.J. (2009). Climate warming strengthens indirect interactions in an old-field food web. *Ecology*, 90, 2346–2351.

Bernhardt, J.R., Sunday, J.M. & O'Connor, M.I. (2018). Metabolic Theory and the Temperature-Size Rule Explain the Temperature Dependence of Population Carrying Capacity. *Am. Nat.*, 192, 687–697.

Binzer, A., Guill, C., Brose, U. & Rall, B.C. (2012). The dynamics of food chains under climate change and nutrient enrichment. *Philos. Trans. R. Soc. B Biol. Sci.*, 367, 2935–2944.

Binzer, A., Guill, C., Rall, B.C. & Brose, U. (2016). Interactive effects of warming, eutrophication and size structure: impacts on biodiversity and food-web structure. *Glob. Change Biol.*, 22, 220–227.

Brose, U., Dunne, J.A., Montoya, J.M., Petchey, O.L., Schneider, F.D. & Jacob, U. (2012). Climate change in size-structured ecosystems. *Philos. Trans. R. Soc. B Biol. Sci.*, 367, 2903–2912.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. & West, G.B. (2004). Toward a metabolic theory of ecology. *Ecology*, 85, 1771–1789.

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.

Clark, A.T., Ye, H., Isbell, F., Deyle, E.R., Cowles, J., Tilman, G.D., *et al.* (2015). Spatial convergent cross mapping to detect causal relationships from short time series. *Ecology*, 96, 1174–1181.

Clarke, A. (2006). Temperature and the metabolic theory of ecology. *Funct. Ecol.*, 20, 405–412.

Doi, H., Yasuhara, M. & Ushio, M. (2021). Causal analysis of the temperature impact on deep-sea biodiversity. *Biol. Lett.*, 17, 20200666.

Dossena, M., Yvon-Durocher, G., Grey, J., Montoya, J.M., Perkins, D.M., Trimmer, M., *et al.* (2012). Warming alters community size structure and ecosystem functioning. *Proc. R. Soc. B Biol. Sci.*, 279, 3011–3019.

Fenchel, T. (1974). Intrinsic rate of natural increase: The relationship with body size. *Oecologia*, 14, 317–326.

Ferenc, V. & Sheppard, C.S. (2020). The stronger, the better – trait hierarchy is driving alien species interaction. *Oikos*, 129, 1455–1467.

Fussmann, G.F. (2000). Crossing the Hopf Bifurcation in a Live Predator-Prey System. *Science*, 290, 1358–1360.

Gauzens, B., Rall, B.C., Mendonça, V., Vinagre, C. & Brose, U. (2020). Biodiversity of intertidal food webs in response to warming across latitudes. *Nat. Clim. Change*, 10, 264–269.

Gibert, J.P. (2019). Temperature directly and indirectly influences food web structure. *Sci. Rep.*, 9, 5312.

Gibert, J.P. & DeLong, J.P. (2014). Temperature alters food web body-size structure. *Biol. Lett.*, 10, 20140473.

Gibert, J.P., Grady, J.M. & Dell, A.I. (2022a). Food web consequences of thermal asymmetries. *Funct. Ecol.*, 1365-2435.14091.

Gibert, J.P., Han, Z., Wieczynski, D.J., Votzke, S. & Yammine, A. (2022b). Feedbacks between size and density determine rapid eco-phenotypic dynamics. *Funct. Ecol.*, 1365-2435.14070.

Gillooly, James.F., Charnov, E.L., West, G.B., Savage, V.M. & Brown, J.H. (2002). Effects of size and temperature on developmental time. *Nature*, 417, 70–73.

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.

Hautier, Y., Niklaus, P.A. & Hector, A. (2009). Competition for Light Causes Plant Biodiversity Loss After Eutrophication. *Science*, 324, 636–638.

Hautier, Y., Seabloom, E.W., Borer, E.T., Adler, P.B., Harpole, W.S., Hillebrand, H., *et al.* (2014). Eutrophication weakens stabilizing effects of diversity in natural grasslands. *Nature*, 508, 521–525.

Irwin, A.J., Finkel, Z.V., Schofield, O.M.E. & Falkowski, P.G. (2006). Scaling-up from nutrient physiology to the size-structure of phytoplankton communities. *J. Plankton Res.*, 28, 459–471.

Karl, T.R. (2003). Modern Global Climate Change. *Science*, 302, 1719–1723.

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.

Kratina, P., Greig, H.S., Thompson, P.L., Carvalho-Pereira, T.S.A. & Shurin, J.B. (2012). Warming modifies trophic cascades and eutrophication in experimental freshwater communities. *Ecology*, 93, 1421–1430.

Lee, G.H., Vonk, J.A., Verdonschot, R.C.M., Kraak, M.H.S., Verdonschot, P.F.M. & Huisman, J. (2021). Eutrophication induces shifts in the trophic position of invertebrates in aquatic food webs. *Ecology*, 102.

Malcolm, J.R., Liu, C., Neilson, R.P., Hansen, L. & Hannah, L. (2006). Global Warming and Extinctions of Endemic Species from Biodiversity Hotspots. *Conserv. Biol.*, 20, 538–548.

Marañón, E., Cermeño, P., López-Sandoval, D.C., Rodríguez-Ramos, T., Sobrino, C., Huete-Ortega, M., *et al.* (2013). Unimodal size scaling of phytoplankton growth and the size dependence of nutrient uptake and use. *Ecol. Lett.*, 16, 371–379.

Matsuzaki, S.S., 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.

McCann, K.S. (2012). *Food webs*. Monographs in population biology. Princeton University Press, Princeton, N.J.

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.

O'Connor, M.I. (2009). Warming strengthens an herbivore–plant interaction. *Ecology*, 90, 388–398.

Petchey, O.L., McPhearson, P.T., Casey, T.M. & Morin, P.J. (1999). Environmental warming alters food-web structure and ecosystem function. *Nature*, 402, 69–72.

Rip, J.M.K. & McCann, K.S. (2011). Cross-ecosystem differences in stability and the principle of energy flux: Cross-ecosystem differences in stability. *Ecol. Lett.*, 14, 733–740.

Rocca, J.D., Yammine, A., Simonin, M. & Gibert, J.P. (2022). Protist Predation Influences the Temperature Response of Bacterial Communities. *Front. Microbiol.*, 13, 847964.

Rogers, T.L., Munch, S.B., Stewart, S.D., Palkovacs, E.P., Giron-Nava, A., Matsuzaki, S.S., *et al.* (2020). Trophic control changes with season and nutrient loading in lakes. *Ecol. Lett.*, 23, 1287–1297.

Rosenblatt, A.E. & Schmitz, O.J. (2016). Climate Change, Nutrition, and Bottom-Up and Top-Down Food Web Processes. *Trends Ecol. Evol.*, 31, 965–975.

Rosenzweig, M.L. (1971). Paradox of Enrichment: Destabilization of Exploitation Ecosystems in Ecological Time. *Science*, 171, 385.

Savage, V.M., Gillooly, J.F., Brown, J.H., West, G.B. & Charnov, E.L. (2004). Effects of Body Size and Temperature on Population Growth. *Am. Nat.*, 163, 429–441.

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.

Shurin, J.B., Clasen, J.L., Greig, H.S., Kratina, P. & Thompson, P.L. (2012). Warming shifts top-down and bottom-up control of pond food web structure and function. *Philos. Trans. R. Soc. B Biol. Sci.*, 367, 3008–3017.

Sugihara, G., May, R., Ye, H., Hsieh, C. -h., Deyle, E., Fogarty, M., *et al.* (2012). Detecting Causality in Complex Ecosystems. *Science*, 338, 496–500.

Tabi, A., Petchey, O.L. & 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.

Vandermeer, J. (2006). Omnivory and the stability of food webs. *J. Theor. Biol.*, 238, 497–504.

Vasseur, D.A. & McCann, K.S. (2005). A Mechanistic Approach for Modeling Temperature-Dependent Consumer-Resource Dynamics. *Am. Nat.*, 166, 184–198.

Voigt, W., Perner, J., Davis, A.J., Eggers, T., Schumacher, J., Bährmann, R., *et al.* (2003). Trophic levels are differentially sensitive to climate. *Ecology*, 84, 2444–2453.

Wang, J., Pan, F., Soininen, J., Heino, J. & Shen, J. (2016a). Nutrient enrichment modifies temperature-biodiversity relationships in large-scale field experiments. *Nat. Commun.*, 7, 13960.

Wang, J., Pan, F., Soininen, J., Heino, J. & Shen, J. (2016b). Nutrient enrichment modifies temperature-biodiversity relationships in large-scale field experiments. *Nat. Commun.*, 7, 13960.

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.

Wieczynski, D., Singla, P., Doan, A., Singleton, A., Han, Z., Yammine, A., *et al.* (2021). *Simple traits predict complex temperature responses across scales* (preprint). In Review.

Wood, S.N. (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.