Temperature and nutrients drive complex 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 is 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

Type: Letter

Number of words: abstract (150 words), main text (3983 words), boxes (0 words)

Number of figures: 5

Number of references: 48

Number of tables: 0

Number of boxes: 0

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. Though their independent effects have been 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 and causality of reciprocal effects between ecological and phenotypic dynamics across species. We found that i) temperature and nutrients have more complex effects on ecological dynamics at higher trophic levels, ii) temperature and nutrients interact antagonistically to shift the relative strength of top-down vs. bottom-up control, iii) phenotypic dynamics have stronger impacts on ecological dynamics than vice versa (especially at higher temperature), and iv) 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.

INTRODUCTION

Understanding how rapid global climate change (GCC) will affect the structure and dynamics of communities is a pressing goal of ecology (Karl 2003). Increasing temperatures associated with GCC influence the metabolism of individuals (Clarke 2006), which strengthens species interactions (Barton *et al.* 2009; O'Connor 2009; Gounand *et al.* 2016), alters community structure (Bartley *et al.* 2019; Gibert 2019; Gauzens *et al.* 2020), and ecosystem function (Dossena *et al.* 2012). Additionally, increasing nutrient loads from agricultural runoffs can result in eutrophication and destabilize natural communities (Rosenzweig 1971; Fussmann 2000; Hautier *et al.* 2014), often leading to species loss (Hautier *et al.* 2009). Warming and eutrophication may therefore independently and jointly impact food web structure and stability (Malcolm *et al.* 2006; Tabi *et al.* 2019). Counterintuitively, however, 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 (Binzer *et al.* 2012, 2016). These non-additive (interactive) outcomes are still poorly understood, but central to honing our predictions about 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 (McClelland & Valiela 1998; Carlier *et al.* 2008; Gibert 2019), specifically regarding their impacts on the relative strength of bottom-up and top-down effects (Binzer *et al.* 2012, 2016; Shurin *et al.* 2012). For example, warming can increase predation pressure (Kratina *et al.* 2012), thus decreasing resource biomass, while increasing the proportion of top predators (Vasseur & McCann 2005; O'Connor 2009; Shurin *et al.* 2012). Alternatively, warming can increase metabolic demands while reducing conversion efficiency

(Barneche *et al.* 2021), leading to predator starvation at high temperatures, loss of top predators, and reduced food chain length (Petchey *et al.* 1999)Unlike warming, increasing nutrient loads tend to increase bottom-up effects, often resulting in unstable dynamics and species loss (i.e., paradox of enrichment, Rip and McCann, 2011; Rosenzweig, 1971). Increasing nutrient loads can alter consumer-resources interactions, sometimes resulting in top-heavy, unstable food webs (Rip & McCann 2011). Eutrophication resulting from increasing nutrient loads can also change consumer trophic position, leading to changes in species interactions and food web structure (Lee *et al.* 2021).

While warming and nutrients can independently influence food webs, they can also have non-additive (interactive) effects when acting together (Binzer *et al.* 2012, 2016; Tabi *et al.* 2019). For example, at low temperatures, small temperature increases can stabilize nutrient-induced instabilities (Binzer *et al.* 2012, 2016), although temperature increases are themselves considered destabilizing because they strengthen consumer-resource interactions (Barton *et al.* 2009). At high temperatures, increasing nutrient loads can counteract warming-induced consumer starvation by increasing carrying capacity and predator attack rates (Binzer *et al.* 2012). Warming also weakens nutrient-induced increases in community biomass (negative interactive effects, Tabi et al., 2019), in turn influencing food web structure (Sentis *et al.* 2014), species richness, and community composition (Wang *et al.* 2016).

Temperature and nutrients can both determine the physiological and morphological traits of organisms (Rosenblatt & Schmitz 2016; Tabi *et al.* 2019). For example, higher temperatures often result in smaller sizes (Atkinson 1995; Atkinson *et al.* 2003), while nutrient enrichment

110 leads to larger organisms (Irwin *et al.* 2006; Marañón *et al.* 2013). Additionally, temperature and
111 nutrients can interactively affect body size, where body size may increase with nutrients at low
112 temperature but decrease at high temperature (Tabi *et al.* 2019). Although body size is often
113 considered a response variable, its strong influences on population growth (Fenchel 1974;
114 Savage *et al.* 2004) and species interactions (Ferenc & Sheppard 2020) mean that rapid
115 phenotypic responses to temperature, nutrients, or both, may have consequences for food web
116 structure and dynamics in warmer climates (Brose *et al.* 2012; Bernhardt *et al.* 2018; Wieczynski
117 *et al.* 2021). However, whether body size only responds to environmental conditions, whether
118 rapid changes in body size influences how environmental conditions affect food webs, or
119 whether shifts in body size in a species can influence body size responses to environmental
120 conditions in the species they interact with, are not well understood.
121
122 Here, we study how temperature and nutrients influence the feedbacks between ecological and
123 body size dynamics in a tractable microbial food web by first describing observed food web and
124 body size responses to temperature and nutrients across trophic levels, then studying the
125 mechanisms of response. Specifically, we ask: 1) do temperature and nutrients independently or
126 interactively influence ecological dynamics within microbial food webs?, 2) do these effects alter
127 the relative importance of top-down vs. bottom-up control?, 3) do these effects vary across
128 trophic levels?, and 4) is body size only responding to temperature and nutrients, as suggested
129 elsewhere (e.g., Binzer et al 2016, Tabi et al 2019), or does that response play a role in
130 determining how the food web itself responds to environmental change. To address these
131 questions, we manipulate nutrient inputs and temperature in a microbial food web composed of
132 bacteria, a bacterivorous protist (*Tetrahymena pyriformis*), and an omnivorous top predator

133 (*Euplotes sp.*), then track the changes in population densities and body sizes of over time. We
134 use state-of-the-art time-series analysis to evaluate the relative strength of top-down vs. bottom-
135 up processes across trophic levels, as well as whether and how the observed ecological and
136 phenotypic dynamics influence one another. Our results reveal complex temperature and nutrient
137 effects on food web dynamics that vary predictably across trophic levels by altering the relative
138 strength of trait-mediated bottom-up and top-down effects.
139
140
141 METHODS
142 *Culture care*
143 *Euplotes sp.* and *Tetrahymena pyriformis* stock cultures were acquired from Carolina Biological
144 Supply (Burlington, NC, USA). Both species were kept in autoclaved liquid protist medium
145 (Carolina Biological Supply), supplemented with one autoclaved wheat seed as a carbon source
146 (Altermatt et al 2014). Protists were fed a mixture of pond bacteria collected from an ephemeral
147 pond at Duke Forest (Wilbur/Gate 9 pond, Lat 36.013914, -Long 78.979720). We maintained all
148 cultures on a 16:8 light:dark cycle at 22°C and 65% humidity in AL-22L2 Percival growth
149 chambers (Perry, IA, USA).
150
151 *Experimental design*
152 Microcosms were set up in autoclaved 250mL borosilicate jars filled with 200mL of protist
153 media. We manipulated temperature and nutrient loads by imposing two temperature levels
154 (22°C/25°C) and two nutrient levels (normal protist media concentration plus one wheat seed –
155 i.e., high nutrients–, or half concentration plus half a wheat seed, –i.e., low nutrients) in a

156 factorial design with 4 treatments and 6 replicates per treatment. Day/night cycle and humidity
157 levels mimicked rearing conditions and were kept constant. We inoculated the bacterial
158 communities from 2 ml of stock culture, the same used to rear the protists. The intermediate
159 consumer species, *T. pyriformis*, was introduced at a starting density of 37 ind/ml, and the larger
160 protists species, *Euplotes sp.*, at a starting density of 0.24 ind/ml in all microcosms. We recorded
161 the density of both protist species through fluid imaging (FlowCam; Yokogawa Fluid Imaging
162 Technologies, Portland, ME, USA), Monday through Friday, for 16 days. Fluid imaging
163 generates individual cell raster images that were used to quantify changes in protist size
164 (measured as cell area, in μm^2) over time. Bacteria density was quantified as optical density at a
165 wavelength of 600nm (OD600), using a BioTEK Epoch-2 microplate spectrophotometer
166 (Winooski, VT, USA).
167
168 *Data analysis*
169 To test for possible effects of temperature and nutrients on ecological dynamics, we fitted
170 Generalized Additive Mixed Models (GAMM) to time series of species density (OD600 for
171 bacteria) and protist body size, across all treatments, using the 'mgcv' package (version 1.8-
172 31,(Wood 2011)) in R (version 4.0.2, R Core Team 2020). To control for temporal
173 autocorrelation, we used an Autoregressive Moving Average (ARMA) correlation structure in
174 the GAMM models using the 'nlme' R package (version 3.1-148). We compared models with
175 additive and/or interactive effects temperature and nutrients effects, as well as different ARMA
176 correlation structures, using AICc (Appendix I Table S1-2). We discarded *T. pyriformis*
177 phenotypic data from days on which less than 10 individuals were measured after populations
178 collapsed.

179

180 *Characterization of ecological dynamics*

181 To assess environmental effects on microcosm food web dynamics, we characterized multiple
182 aspects of the population dynamics of bacteria and protists and their responses to environmental
183 conditions. Specifically, we quantified: 1) initial growth rate (day^{-1}) as $[\ln(Nf) -$
184 $\ln(Ni)]/time$ for early-dynamics data, (up to day 1 for *T. pyriformis* & bacteria and day 8 for
185 *Euplotes sp.*, due to much slower growth), 2) maximum density (individual/ml for protists and
186 OD_{600} for bacteria), using the highest average daily population density across replicates but
187 within treatments for protists, and the top three observed OD600 values for each replicate from
188 each treatment for bacteria because their population dynamics remained relatively stable, 3) the
189 coefficient of variation (CV = standard deviation/mean) of the temporal population dynamics
190 within treatments (typically used as a measure of stability (McCann 2012)), 4) the time to
191 population collapse in days (only *T. pyriformis*), and 5) the time to population peak in days (only
192 *Euplotes sp.*).

193

194 *Quantifying top-down/bottom-up effects and feedbacks between ecological and body size*
195 *dynamics*

196 To understand the mechanisms through which temperature and nutrients affected the observed
197 food web dynamics, we quantified the reciprocal effects of ecological and body size dynamics
198 using Convergent Cross Mapping (CCM) from the R package 'rEDM' (version 1.5.0; Park et al
199 2020). CCM quantifies the strength of causation of one dynamical variable onto another by
200 measuring the extent to which the time series of one variable X can reliably estimate the state of
201 another variable Y in a future time step (Sugihara *et al.* 2012). The higher the ability of X to

202 predict Y, the higher the causal effect of Y on X. Meanwhile, a variable Y that does not
203 influence a variable X cannot be predicted from the dynamics of X (Sugihara *et al.* 2012). This
204 procedure yields a 'predictability' value that can be interpreted as a correlation coefficient
205 between past and future states of the two dynamical variables (Sugihara *et al.* 2012). Whether
206 there is a cause-effect relationship between two dynamical variables, as opposed to simple
207 correlation, further depends on whether the average predictability value increases with library
208 size (i.e., number of data points taken into account to run the analysis, Sugihara et al., 2012). We
209 took this into account to infer causation in our data (see Appendix II Fig. S1-14).
210
211 CCM is often applied to relatively long time series (Sugihara *et al.* 2012). However, the
212 dynamics in our system occurred much too fast (Fig. 1), precluding us from collecting longer
213 time series. To resolve this issue, we used CCM on the GAMM model predictions of changes in
214 density (for all species) and body size (for protists) with time, for each treatment. These GAMM
215 model predictions considered the effects of each treatment as a whole—i.e., each combination of
216 nutrient and temperature levels—because they more closely reproduced the observed dynamics
217 (Appendix I Table S1). We refer to these GAMM predictions as 'time-series' in the context of
218 CCM analyses. Using CCM on the density time series, we calculated the predictability of
219 changes in prey density from changes predator density (i.e., bottom-up control, as predator
220 density predicting prey density means changes in prey density causes changes in predator
221 densities, Sugihara et al., 2012) and the predictability of changes in predator density from
222 changes in prey (i.e., top-down control). We dubbed these "eco-eco" effects, for simplicity. To
223 assess feedbacks between body size and ecological dynamics, we calculated the predictability of
224 changes in protist body size from changes in species density (i.e., causal effects of changes in

225 body size on densities, or 'pheno-eco' effects), and vice versa ('eco-pheno' effects). Last, we
226 calculated the predictability of changes in predator and prey body sizes on each other ('pheno-
227 pheno' effects).
228
229
230 RESULTS
231 *General Ecological and Phenotypic dynamics*
232 Overall, bacterial density increased to carrying capacity early (Fig. 1a, light grey), *T. pyriformis*
233 increased rapidly, then decreased (Fig. 1a, dark grey), while *Euplotes sp.* increased almost
234 monotonically to carrying capacity (Fig. 1a, black), ultimately resulting in a two-species state.
235 Temperature and nutrients affected all three species and led to significantly different dynamics
236 across treatments (Fig. 1b-d, Appendix I Table S2). The body sizes of both protists changed
237 rapidly over time (Fig. 1e, f) and responded to both temperature and nutrients (Fig. 1e, f,
238 Appendix I Table S2).
239
240 *Temperature and nutrient effects change across trophic levels in systematic ways*
241 Temperature significantly affected a larger number of dynamical descriptors than nutrients (Fig.
242 2, 3). Individual temperature effects were more frequent in the upper trophic levels than in
243 bacteria (Fig. 3), as were interactions between temperature and nutrients (Fig. 3). In particular,
244 higher temperature alone i) strongly increased initial growth rate in Tetrahymena (T = 0.50025, p
245 = 1.16×10^{-6}, Fig. 2b), ii) decreased the maximum density of bacteria and *T. pyriformis* (T_B = -
246 0.004917, p = 0.00419; T_T = -975.3, p < 0.0155; Fig. 2d-e) but increased that of *Euplotes sp.* (T_E
247 = 39.83, p = 0.02668, Fig. 2f), iii) increased the variability in *T. pyriformis* density (i.e. CV, T =

248 0.20033, p = 0.00554, Fig. 3h) but decreased that of *Euplotes sp.* (T = -0.07689, p = 0.01692,
249 Fig. 3i), iv) accelerated the time to collapse in *T. pyriformis* (T = -2.7576, p = 1.59×10^{-5}; Fig. 2j),
250 and v) accelerated the time to peak density in *Euplotes sp.* (T = -2.1667, p = 0.0196; Fig. 2k).
251 Higher nutrients alone i) decreased the initial growth rate of *Euplotes sp.* (N = -0.087, p =
252 5.02×10^{-4} Fig. 2c), ii) increased maximum density of all species (N_B= 0.012717, p = $2.03\times10^{-}$
253 ^{10}; N_T = 1002.2, p = 0.0132; N_E = 60.67, p = 0.00157, Fig. 2d-f), and iii) increased the CV of
254 bacteria and *Euplotes sp.* (N_B = 0.17521, p = 8.54×10^{-5}, Fig. 2g; N_E = 0.15853, p = 2.94×10^{-5},
255 Fig. 2i) while decreasing that of *T. pyriformis* (N_T = -0.18727, p = 0.00875, Fig. 2h).
256 Interestingly, increased temperature and nutrients had opposite effects on the CV of *T. pyriformis*
257 and *Euplotes sp.*, indicating antagonistic additive effects of temperature and nutrients on food
258 web stability.
259
260 Temperature and nutrients had complex interactive effects on ecological dynamics of both protist
261 consumers. Nutrients had a negative effect on the initial growth rate of *Euplotes sp.* at low
262 temperature, but a positive effect at high temperature (N×T = 0.12621, p = 0.000391; Fig. 2c).
263 The maximum density of *T. pyriformis* increased with nutrients, but this effect was stronger at
264 high temperature (N×T = 1526.7, p = 0.0083, Fig. 2e). Nutrients also had i) a negative effect on
265 *T. pyriformis* population variability (CV) at low temperature but no effect at high temperature
266 (N×T = 0.19492, p = 0.04500, Fig. 2h), and ii) a positive effect on *Euplotes sp.* CV that was
267 stronger at low temperature (N×T = -0.12045, p = 0.00913, Fig. 2i). Additional model stats can
268 be found in Appendix I Table S3. Taken together, these results imply that temperature and
269 nutrients individually and interactively influence population dynamics at all trophic levels, with
270 more of both types of effects occurring at higher trophic levels (Fig. 3).

Temperature and nutrients influence top-down and bottom-up effects

Using CCM analysis, we estimated changes in the relative strength of bottom-up and top-down ecological effects across treatments (Fig. 4a). In general, top-down and bottom-up effects between the top predators were the strongest (Fig. 4a, yellow). Top-down effects involving bacteria were the most strongly influenced by temperature and nutrients (Fig.4a, green & purple dashed lines). Importantly, temperature and nutrients showed interactive effects on top-down and bottom-up control. Most notably, top-down control by both protists on bacteria decreased with nutrients at low temperature but increased with nutrients at high temperatures (Fig.4a, open dots and dashed lines).

The most dominant species interactions in the overall food web also changed according to shifts in the strength of top-down and bottom-up control between species pairs (Figure 5a). To be conservative and increase the chance of picking up biologically relevant effects, we only present effects whose CCM predictability value was above 0.75 in Figure 5 (Appendix III Table S1). Specifically, increasing nutrients weakened top-down control from *T. pyriformis* to bacteria at low temperatures (Fig. 5a left panel, dashed purple line), but strengthened top-down control from both protists to bacteria at high temperatures (Fig. 5a right panel, dashed lines). In other words, top-down effects were strongest when temperature and nutrients were both low, or both high, suggesting that shifts in the relative importance of top-down and bottom-up effects across trophic levels explain some effects of temperature and nutrients on the overall food web.

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

294 Protist size not only responded to temperature and nutrients, but also played an important role in
295 determining the effects of temperature and nutrients on ecological dynamics (Fig. 4b-c). On
296 average, higher temperature generally led to stronger eco-pheno effects (mean CCM at 22°C =
297 0.55, mean CCM at 25°C = 0.66, Fig. 4b, Appendix II Table S1) and pheno-eco interactions (size
298 driving ecological dynamics, mean CCM at 22°C = 0.71, mean CCM at 25°C = 0.79, Fig. 4c,
299 Appendix II Table S1), indicating stronger coupling between ecological and phenotypic
300 dynamics at higher temperatures. Pheno-eco effects were generally stronger (14 strong effects,
301 i.e., CCM predictability > 0.75, Fig. 4b) than eco-pheno effects (11 strong effects, Fig. 4c),
302 suggesting stronger effects of body size on ecological dynamics than the other way around. For
303 example, *Euplotes sp.* size had stronger effects on its own population dynamics than the opposite
304 (Fig. 4b-c, black dotted lines).
305
306 Both eco-pheno and pheno-eco effects generally responded interactively to changes in
307 temperature and nutrients. For instance, *T. pyriformis* eco-pheno effects on *Euplotes. sp* (yellow
308 solid lines in Fig.4b) decreased with nutrients at low temperature but remained relatively
309 unchanged at high temperature. Also, nutrients increased the effect of *T. pyriformis* size on
310 bacterial dynamics and this pattern was more exaggerated at higher temperature (Fig. 4c, dashed
311 purple lines). Meanwhile, nutrients decreased the effects of *Euplotes sp.* size on bacteria
312 dynamics at low temperature and this pattern was reversed at high temperature (Fig. 4c, dashed
313 green lines). In 22 out of the 25 eco-pheno or pheno-eco interactions, we found strong support
314 for causation (Appendix II Fig. S1-14).
315

316 Pheno-pheno effects between protists (Fig. 4d) were relatively weaker than pheno-eco or eco-
317 pheno effects. In general *T. pyriformis* size more strongly affected *Euplotes sp*. size than the
318 other way around (Fig. 4d). Additionally, temperature generally increased body size effects
319 between protists and we observed the strongest phenotypic coupling between protists when
320 temperature and nutrients were both high (Fig. 4d), indicating that higher temperatures may
321 increase the role of phenotypic dynamics in consumer-resource interactions.
322
323 Overall, these results indicate that the effect of temperature and nutrients on ecological dynamics
324 may be mediated by changes in body size (Fig. 5). Temperature and nutrients showed negative
325 interactions on strong eco-eco effects (CCM predictability > 0.75), leading to stronger eco-eco
326 effects when temperature and nutrients were both low or both high (Fig. 5a). However, the
327 number of strong causal effects between ecological and phenotypic dynamics increased with
328 temperature but were weakened by nutrients (Fig. 5b).
329
330
331 DISCUSSION
332 Our results reveal complex but systematic effects of temperature and nutrients on the ecological
333 and phenotypic dynamics of a microbial food web. We show that temperature and nutrients can
334 each independently influence different aspects of food web dynamics (Fig. 1, 2), while their joint
335 effects get increasingly complex at higher trophic levels (Fig. 2, 3). We also found that changes
336 in the relative strength of top-down and bottom-up effects likely drive observed responses to
337 temperature and nutrients (Fig. 4, 5), and that rapid changes in body size mediate these effects
338 (Fig. 4, 5). These results suggest that body size not only responds to shifts in environmental

conditions, but also plays a role in determining ecological responses to such shifts. Evaluating feedbacks between ecological and phenotypic dynamics is therefore integral to understanding food web responses to environmental change.

Increasingly complex effects of temperature and nutrients at higher trophic levels

Our results reveal the pervasive effects of temperature and nutrients across all species, but these effects are more numerous and increasingly complex (temperature-nutrient interactions) at higher trophic levels (Fig. 2, 3). The effects of temperature and nutrients on dietary preference, species interactions, and foraging behavior may help explain why these interactive effects may be more likely among consumer species (Fig. 1d; Fig. 2c).

Because energy enters at the bottom of a food web, basal species are more likely to be influenced by direct effects of nutrients while species at higher trophic levels may be more strongly affected by temperature (Petchey *et al.* 1999; Voigt *et al.* 2003)8/12/21 11:03:00 AM. As energy flows from the basal species to the consumers, the temperature and nutrient effects that act on basal species could be integrated by consumers, leading to additional indirect temperature and nutrient effects on consumers. Additionally, these effects may be exaggerated in omnivores due to the complex nature of their trophic interactions. As temperature and nutrients change species interactions and foraging behaviors (Sentis *et al.* 2014), omnivorous consumers may shift diets between basal and intermediate resources (Sentis *et al.* 2014), altering the strength/prevalence of competitive vs. predatory interactions (Vandermeer 2006), thus rewiring food webs (Bartley *et al.* 2019). Taken together, this may indicate why the joint effects of temperature and nutrients may be more complex at higher trophic levels in food webs with higher prevalence of omnivory.

362

363 *Top-down control, bottom-up control, and food web stability*

364 Consistent with our results (Fig. 2d), theory predicts that higher nutrient loads should increase
365 energy flux in food webs, increasing basal species density (Rip & McCann 2011; Shurin *et al.*
366 2012). Theory also predicts that increasing energy flux can destabilize population
367 oscillations(Rosenzweig 1971; Rip & McCann 2011), but increasing temperatures should
368 stabilize oscillations by weakening top-down effects (Vasseur & McCann 2005; Binzer *et al.*
369 2016; Tabi *et al.* 2019). We found that temperature and nutrients interact to have divergent
370 impacts on population stability—with temperature stabilizing, and nutrients destabilizing
371 *Euplotes sp.* densities, but the opposite being true for *T. pyriformis* (Fig. 2h-i). We also observed
372 that top-down control of bacteria by both consumers was strongest when temperature and
373 nutrients were both high (and both low), while bottom-up effects were relatively invariant across
374 treatments (Fig. 4a, Fig. 5). Interestingly, these shifts in top-down control across environments
375 (Fig. 4a) coincided with significant changes in population stability that varied across trophic
376 levels (Fig. 2i). These results thus indicate that changes in the strength of top-down effects
377 between basal resources and consumers—instead of bottom-up effects—could be the dominant
378 mechanism through which higher temperature stabilize unstable fluctuations caused by nutrients.

379

380 *Phenotypes mediate temperature and nutrients effects on food web dynamics*

381 Our results indicate that phenotypic dynamics play a larger role in mediating environmental
382 impacts on food web dynamics than previously thought (Fig. 5, Appendix III Fig. S1). Previous
383 studies showed that predator-prey body size ratios significantly influence temperature and
384 nutrients effects (Binzer *et al.* 2012) and that body size responds to changes in nutrients,

temperature, and ecological dynamics in specific ways (Tabi *et al.* 2019). Our results support—but also extend—those results by showing that 1) phenotypic effects on ecological dynamics may be stronger than the other way around, as also recently reported elsewhere (Gibert *et al.* 2021), 2) changes in prey size may more strongly drive changes in predator size than the other way around, and 3) interactions involving phenotypic dynamics (eco-pheno, pheno-eco, or pheno-pheno) vary in complex ways across environmental conditions, but seem to be generally stronger at higher temperature (Fig. 4b-d, Fig. 5). 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, we found that strong feedbacks between ecological and phenotypic dynamics vary with temperature and nutrients, suggesting that rapid phenotypic change influences food web responses to changing environmental conditions. 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.

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

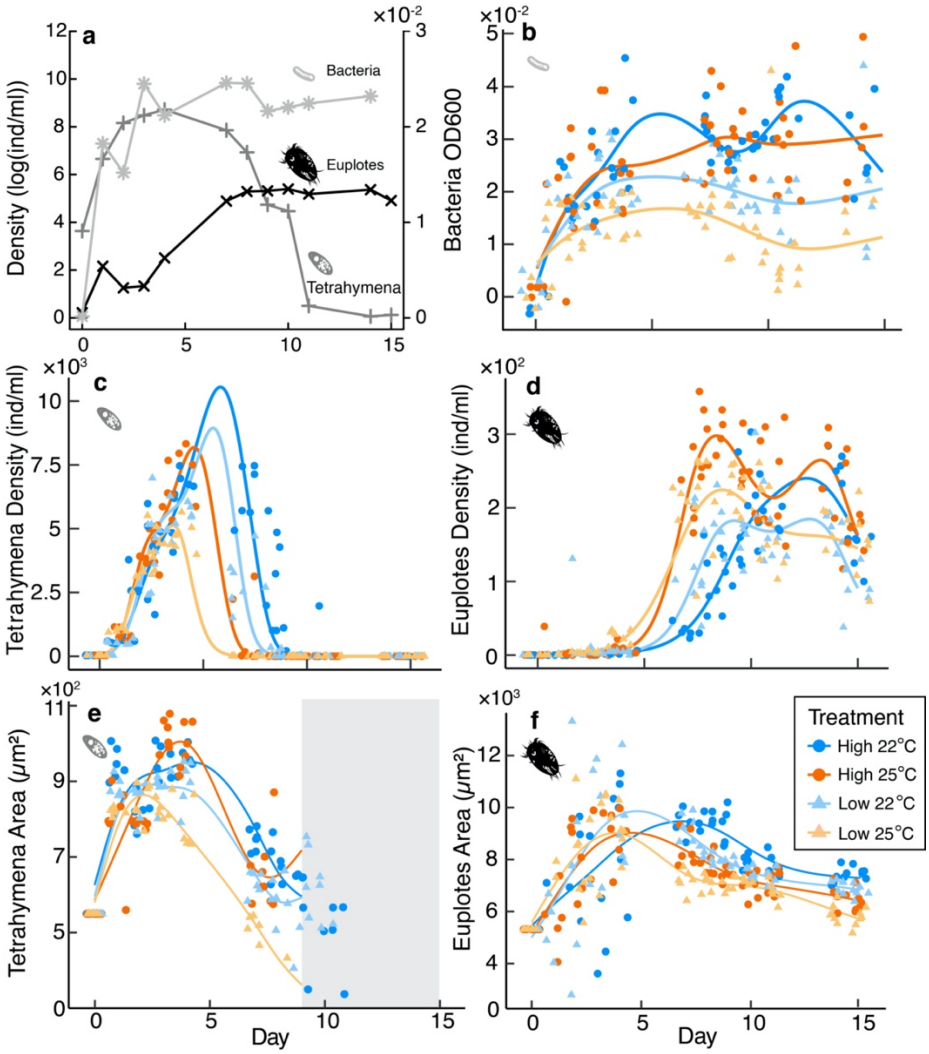

Figure 1. Population and phenotypic dynamics within experimental microcosms. (a) The average population density across treatments shows the general population dynamics of each species. (b-d) show GAMM smoothed population dynamics of bacteria, *T. pyriformis* and *Euplotes sp.*. (e-f) show the changes in the area (body size) of both protists body over time. *T. pyriformis* were effectively extinct by day 9. Greyed area indicates days for which data are insufficient to estimate trait distributions.

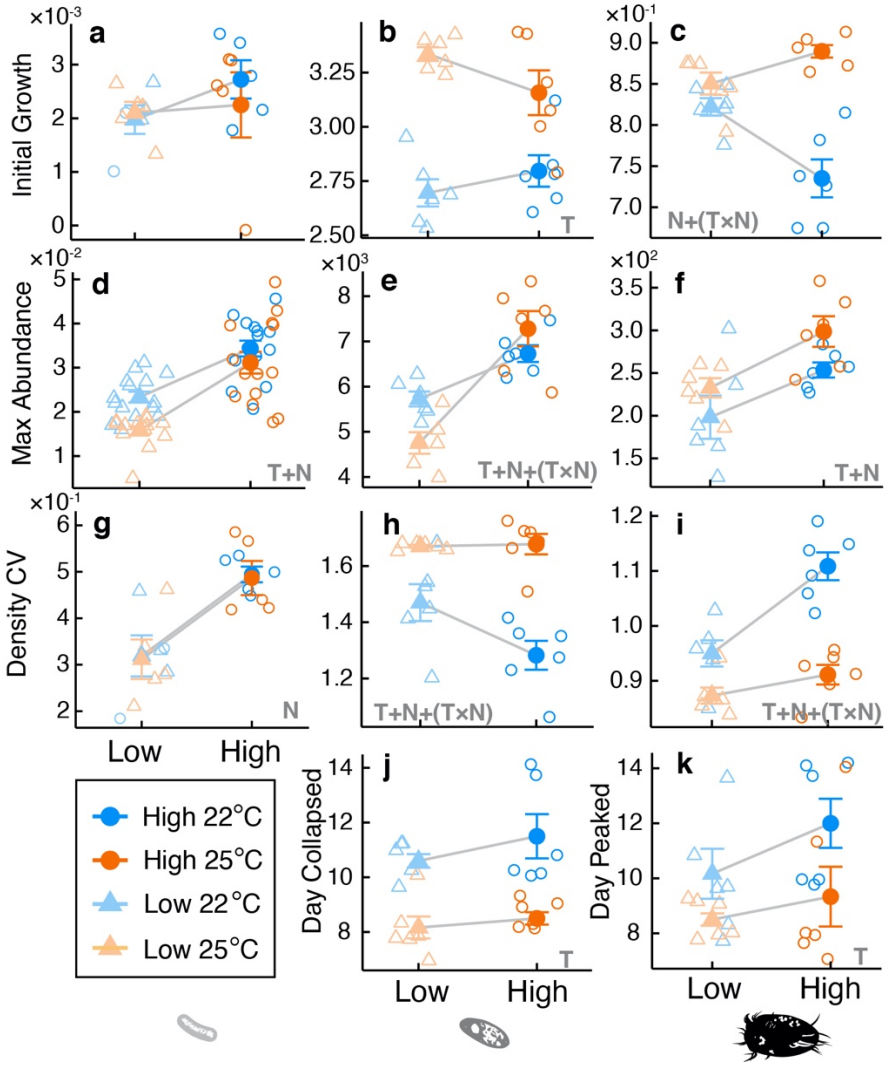

Figure 2. Interactive plot of effects of temperature and nutrients on descriptors of population dynamics. Open circles and triangles represent data points from each replicate. Solid shapes are the mean across 6 replicates. Grey letters T, N, and (T×N) represent statistically significant effects from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

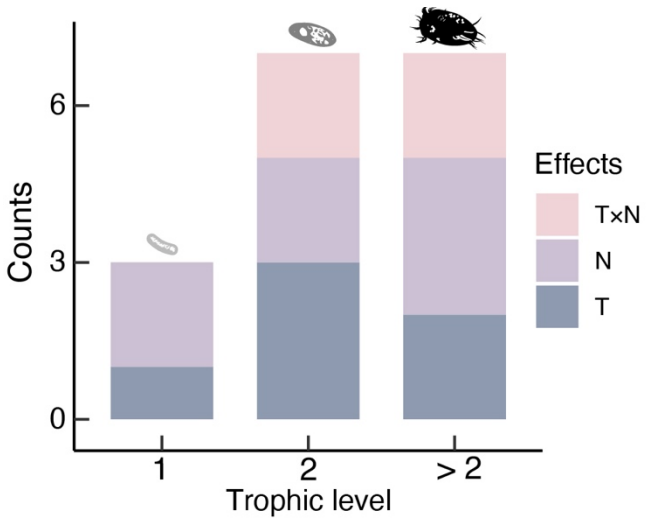

Fig. 3. Significant temperature and nutrients effects on ecological dynamics. T, N, and T×N denote effects of temperature, nutrients, and interactive effects of temperature and nutrients, respectively.

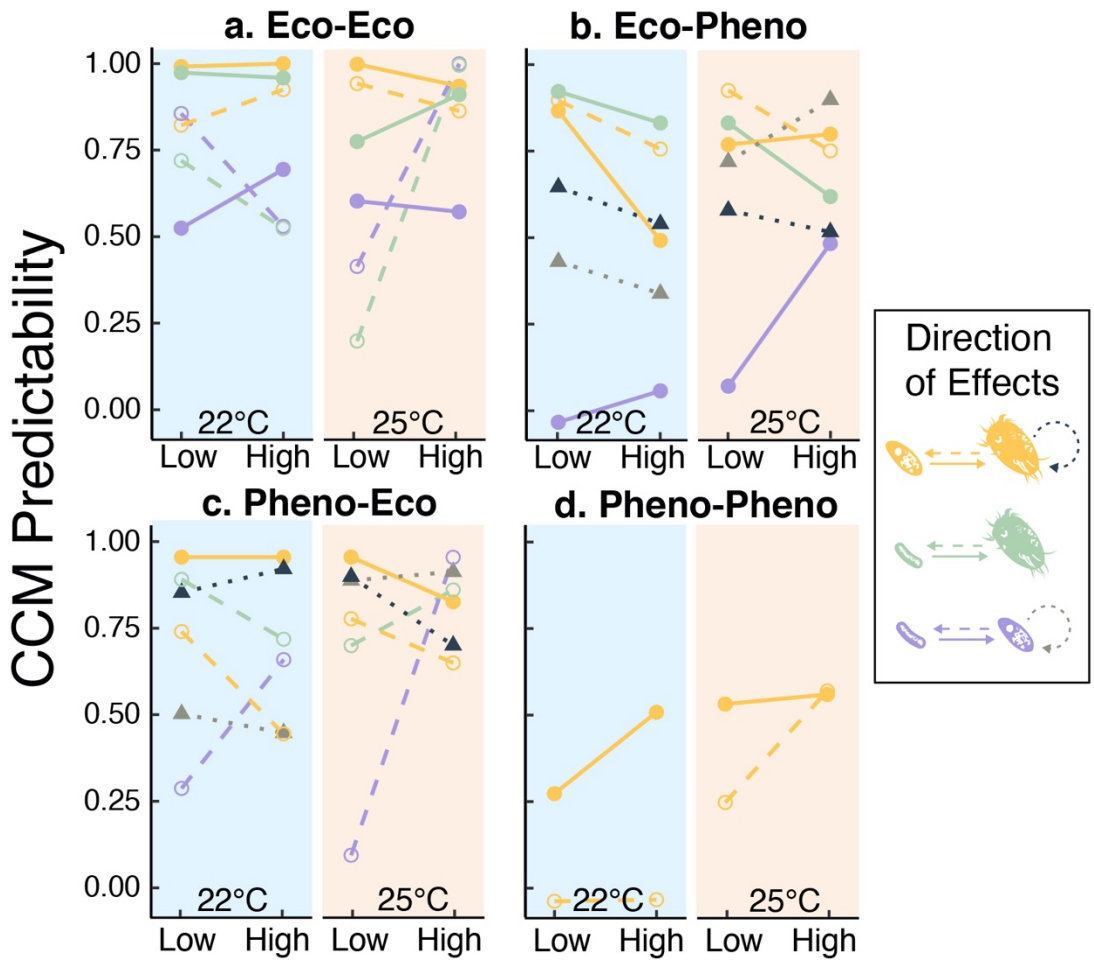

Figure 4. CCM predictabilities ecological and phenotypic dynamics across treatments for each species pair. Circles represents CCM predictability of one species' effect on another species, while the triangles representing intraspecific. Solid circles and solid lines represent CCM predictability of bottom-up effects and open circles and dashed lines represent top-down effects. Interactions with CCM predictability higher than 0.75 are considered to be strong interactions.

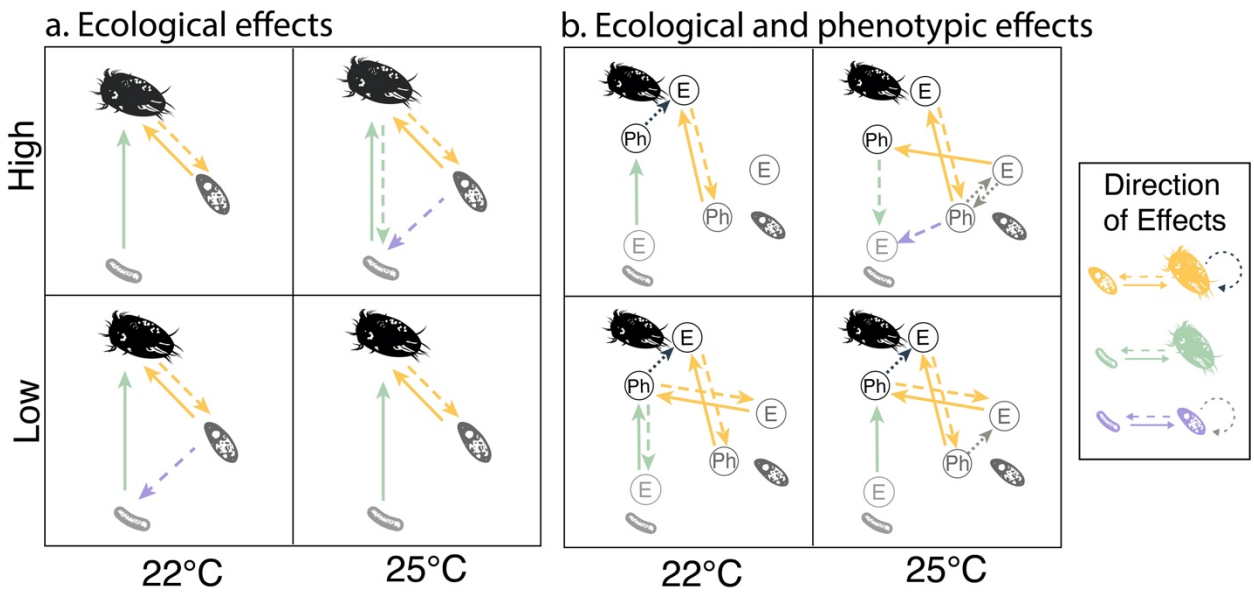

Figure 5. Strong effects (i.e., CCM predictability higher than 0.75) within food webs across treatments. Panel (a) shows strong eco-eco effects (Fig. 4a). Panel (b) shows all strong interactions between phenotypic (Ph) and ecological (E) dynamics (pheno-eco, eco-pheno and pheno-pheno effects; Fig. 4b-d). Dashed and solid lines represent top-down and bottom-up effects respectively. Dotted lines represent intraspecific effects. Color code is the same as in Fig. 4a.

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