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

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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 uncover 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.

54 INTRODUCTION

55 Understanding how rapid global climate change (GCC) will affect the structure and dynamics of
56 communities is a pressing goal of ecology (Karl 2003; Barbour & Gibert 2021). Increasing
57 temperatures associated with GCC influence the metabolism of individuals (Gillooly *et al.* 2002;
58 Brown *et al.* 2004; Clarke 2006), which strengthens species interactions (Barton *et al.* 2009;
59 O'Connor 2009; Gounand *et al.* 2016), alters community structure (Bartley *et al.* 2019; Gibert
60 2019; Gauzens *et al.* 2020), and affects ecosystem function (Dossena *et al.* 2012). Additionally,
61 increasing nutrient loads from agricultural runoffs can result in eutrophication and destabilize
62 natural communities (Rosenzweig 1971; Fussmann 2000; Hautier *et al.* 2014), often leading to
63 species loss (Hautier *et al.* 2009). Warming and eutrophication can independently and jointly
64 impact food web structure and stability (Malcolm *et al.* 2006; Tabi *et al.* 2019).
65 Counterintuitively, simultaneous increases in temperature and nutrient load can produce
66 outcomes that are qualitatively different from the combined negative effects of each variable on
67 its own (Binzer *et al.* 2012, 2016). These non-additive (interactive) outcomes are still poorly
68 understood, but central to honing our understanding of GCC impacts on food web structure and
69 dynamics in a highly anthropogenized world.
70

71 The mechanisms through which warming and increasing nutrient loads independently influence
72 food webs are relatively well understood (McClelland & Valiela 1998; Carlier *et al.* 2008; Gibert
73 2019), specifically regarding their impacts on the relative strength of bottom-up and top-down
74 effects (Binzer *et al.* 2012, 2016; Shurin *et al.* 2012). For example, warming can increase
75 predation pressure (Kratina *et al.* 2012), thus decreasing resource biomass, while increasing the
76 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 (Gibert *et al.* 2022a), and reduced food chain length (Petchey *et al.* 1999). Unlike warming, increasing nutrient loads tend to increase bottom-up effects, resulting in unstable dynamics and species loss (i.e., paradox of enrichment, Rip and McCann, 2011; Rosenzweig, 1971), often leading to 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 unison (Binzer *et al.* 2012, 2016; Tabi *et al.* 2019), but these are much less well understood. Although temperature increases are typically considered to be destabilizing (Barton *et al.* 2009), at low temperatures, small temperature increases can cause consumer starvation, stabilizing nutrient-induced instabilities (Binzer *et al.* 2012, 2016). However, at high temperatures, increasing nutrient loads can counter 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.* 2016a).

In addition to their effects on entire food webs, temperature and nutrients can both determine the physiology and morphology 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 leads to larger organisms (Irwin *et al.* 2006; Marañón *et al.* 2013). Additionally, temperature and nutrients can interactively affect body size: size increases with higher nutrient loads at low temperatures, but decreases 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 and species interactions (Fenchel 1974; Savage *et al.* 2004; Ferenc & Sheppard 2020; Gibert *et al.* 2022b), 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). 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 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 just passively respond to temperature and nutrients, as suggested elsewhere (e.g., Binzer et al 2016, Tabi et al 2019), 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

123 over time. We use time-series analysis to evaluate the relative strength of top-down vs. bottom-
124 up processes across trophic levels as well as whether and how the observed ecological and
125 phenotypic dynamics influence one another across temperature and nutrient treatments. Our
126 results reveal complex but quantifiable temperature and nutrient effects on food web dynamics
127 that vary predictably across trophic levels by altering the relative strength of trait-mediated
128 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, as done in
147 (Gibert *et al.* 2017) in a factorial design with 4 treatments and 6 replicates per treatment.
148 Day/night cycle and humidity levels mimicked rearing conditions and were kept constant. We
149 inoculated 2 ml of bacterial communities from the stock culture, the same used to rear the
150 protists. The intermediate consumer species, *T. pyriformis*, only preys on bacteria and was
151 introduced at a starting density of 37 ind/ml. The omnivorous consumer species, *Euplotes sp.*,
152 preys on both bacteria and the intermediate consumer *T. pyriformis* and was introduced at a
153 starting density of 0.24 ind/ml in all microcosms. We recorded the density of both protist species
154 once per day through fluid imaging (FlowCam; Yokogawa Fluid Imaging Technologies,
155 Portland, ME, USA), Monday through Friday, for 16 days. Fluid imaging generates individual
156 cell raster images that were used to quantify changes in protist size (measured as cell area, in
157 μm^2) over time. Bacteria density was quantified as optical density at a wavelength of 600nm
158 (OD600), using a BioTEK Epoch-2 microplate spectrophotometer (Winooski, VT, USA).
159
160 *Statistical analysis*
161 To test for possible effects of temperature and nutrients on ecological dynamics, we fitted
162 Generalized Additive Mixed Models (GAMM) to time series of species density (OD600 for
163 bacteria) and protist body size, across all treatments, using the 'mgcv' package v1.8-31 (Gibert *et*
164 *al.* 2017) in R v4.0.2 (R Core Team 2020). To control for temporal autocorrelation, we used an
165 Autoregressive Moving Average (ARMA) correlation structure in the GAMMs using the 'nlme'
166 R package v3.1-148. To account for repeated sampling within each replicate, we included
167 replicates as a random intercept in the model. We compared models with additive and/or
168 interactive temperature and nutrient effects, as well as different ARMA correlation structures,

169 using AICc (Appendix I Table S1-2). We discarded *T. pyriformis* phenotypic data from days in
170 which fewer than 10 individuals were measured (i.e., after populations collapsed).
171
172 *Characterizing ecological dynamics*
173 To better understand which aspects of the food web dynamics were most influenced by
174 temperature and nutrients, we characterized multiple aspects of the observed ecological
175 dynamics of the bacterial community and the protists across treatments. Specifically, we
176 quantified: 1) initial growth rate (day^{-1}) as $[\ln(Nf) - \ln(Ni)]/time$ for early-dynamics data, (up
177 to day 1 for *T. pyriformis* & bacteria and day 8 for *Euplotes sp.*, due to much slower growth), 2)
178 maximum density (individual/ml for protists and OD_{600} for bacteria), measured as average
179 density across replicates on the day with the highest average density, 3) the coefficient of
180 variation (CV = standard deviation/mean) of the temporal population dynamics within treatments
181 (typically used as a measure of stability (McCann 2012)), 4) the time to population collapse in
182 days (only *T. pyriformis*), and 5) the time to population peak in days (only *Euplotes sp.*). Last,
183 we calculated the effect sizes of the significant effects of temperature, nutrients, and their
184 interaction using the function eta_squared() in package 'effectsize' v0.7 (Ben-Shachar *et al.*
185 2020).
186
187 *Quantifying top-down/bottom-up effects and eco-phenotypic feedbacks*
188 To understand the mechanisms through which temperature and nutrients affected the observed
189 food web dynamics, we quantified the reciprocal effects of ecological dynamics and body size on
190 each other using Convergent Cross Mapping (CCM) (Sugihara *et al.* 2012). The CCM algorithm
191 has now been used multiple times across ecological systems and taxa reliably to estimate the

192 strength of causal effects between variables for which time-series are available (Matsuzaki *et al.*
193 2018; Wang *et al.* 2018; Rogers *et al.* 2020; Barraquand *et al.* 2021; Doi *et al.* 2021; Abidha *et*
194 *al.* 2022; Gibert *et al.* 2022b) and we followed this specialized literature to infer causation in our
195 data (see Appendix II Fig. S1-15). In a nutshell, CCM quantifies the strength of causation of one
196 dynamical variable onto another by measuring the extent to which the time series of one variable
197 can reliably estimate the state of another variable (Sugihara *et al.* 2012). The larger the causal
198 effect of X on Y, the better the ability of Y to predict X, as Y contains more information about X
199 (by virtue of being 'forced' by X). Meanwhile, a variable X that does not influence a variable Y
200 cannot be predicted from the dynamics of Y, as no information regarding X is contained in Y
201 (Sugihara *et al.* 2012). The CCM algorithm yields a 'cross-mapping estimation skill' in the form
202 of a correlation coefficient (ρ) between observed and predicted points in the time-series
203 (Sugihara *et al.* 2012). The larger this number, the larger the dependence of one variable on the
204 other. Whether there is a cause-effect relationship between two dynamical variables, as opposed
205 to simple correlation, further depends on whether the cross-mapping estimation skill increases
206 with the length of the time series used for this estimation (called the 'library size'). Whenever
207 such an increase is observed, a causal effect of a variable on another one is likely (i.e.
208 convergence; Sugihara et al., 2012).
209
210 We used a modified CCM algorithm that allows for replication in the time series through the R
211 package 'multispatialCCM' (Version 1.0, Clark *et al.* 2015). The package can be used to detect
212 causality between shorter but highly replicated time series, like ours. Our time series lacked data
213 on days 5, 6 and 12, 13 across all replicates. However, the CCM algorithm does not allow for
214 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 Appendix 2). 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 (Appendix II Fig. 1-12), which 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 (Appendix II Fig. 13-15).

Based on previous literature (Sugihara *et al.* 2012; Matsuzaki *et al.* 2018; Barraquand *et al.* 2021), 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) where 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.

RESULTS

238 *General Ecological and Phenotypic dynamics*

239 Overall, bacterial density rapidly increased to carrying capacity (Fig. 1a, light grey), the
240 intermediate predator *T. pyriformis* increased rapidly, then decreased (Fig. 1a, dark grey), while
241 the omnivorous predator *Euplotes sp.* increased almost monotonically to carrying capacity (Fig.
242 1a, black), ultimately resulting in a one protist + bacterial community state. Temperature and
243 nutrients affected all three species and led to significantly different dynamics across treatments
244 (Fig. 1b-d, Appendix I Table S2). The body sizes of both protists changed rapidly over time (Fig.
245 1e, f) and responded to both temperature and nutrients (Fig. 1e, f, Appendix I Table S2).

246

247 *Temperature and nutrient effects changed across trophic levels in systematic ways*

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

252

253 Temperature independently and solely affected *T. pyriformis* initial growth rate and time to
254 population collapse, while temperature and nutrients independently and interactively affected its
255 maximum density and density CV. Temperature strongly increased initial growth rate in *T.*
256 *pyriformis* (T = 0.50 p = 1.16×10^{-6}, Fig. 2b) and accelerated the time to collapse (T = -2.8, p =
257 1.6×10^{-5}; Fig. 2j). The maximum density of *T. pyriformis* increased with nutrients across
258 temperature but only at high nutrient levels, while at low nutrient levels, higher temperature
259 decreased *T. pyriformis* maximum density (T = -975.3, p < 0.02; N = 1002.2, p = 0.01; N×T =
260 1526.7, p < 0.01, Fig. 2e). The density CV of *T. pyriformis* was also interactively influenced by

261 nutrients and temperature such that at high temperature, *T. pyriformis* CV was high across
262 nutrient levels, but at low temperatures, *T. pyriformis* CV decreased with increasing nutrients ($T = 0.2$, $p < 0.006$; $N = -0.19$, $p < 0.01$; $N \times T = 0.2$, $p = 0.045$, Fig. 2h).
264
265 Temperature and nutrient levels independently influenced the maximum density and time to the
266 population peak of *Euplotes sp.* and interactively affected its initial growth rate and density CV.
267 Both temperature and nutrients increased the maximum density of *Euplotes sp*. ($T = 39.83$, $p <$
268 0.03; $N = 60.67$, $p < 0.002$, Fig. 2f). Temperature alone accelerated *Euplotes* time to peak density
269 ($T = -2.2$, $p < 0.02$; Fig. 2k). Higher initial growth rates were observed at higher temperatures,
270 while higher nutrient levels led to large decreases in initial growth rates at low temperatures but a
271 small increase in initial growth rates at high temperatures ($N = -0.09$, $p = 5\times10^{-4}$, $N\times T = 0.13$, p
272 $< 10^{-3}$, Fig. 2c). In general, temperature decreased the density CV of *Euplotes sp.* while higher
273 nutrients increased its CV, more so in lower temperature than in higher temperature ($T = -0.08$, p
274 < 0.02, $N_E = 0.16$, $p = 2.9\times10^{-5}$, $N\times T = -0.12$, $p < 0.01$, Fig. 2i). Additional model stats can be
275 found in Appendix I Table S3.
276
277 We found significant temperature and nutrient interactions only among the predators, with the
278 total effect size of the interaction terms increasing with species trophic level (Fig. 3a). Bacterial
279 population dynamics were mainly influenced by nutrients and the intermediate predator received
280 the most temperature effects (Fig. 3a). These results can be better understood by decomposed
281 total effect sizes of nutrients and temperatures on initial growth rates, maximum density, and
282 density CV. We found no interactive effects of nutrients and temperature on bacteria initial
283 growth rates, but increasingly complex temperature and nutrient effects on species initial growth

284 at higher trophic levels (Fig. 3b). Interestingly, we also found decreasing effect sizes of
285 temperature, nutrients, and their interaction on species maximum density at higher trophic levels
286 (Fig. 3b). However, increasing effect sizes of these effects with increasing trophic level were
287 observed for species density CV (Fig. 3d). These results imply that the maximum density of
288 species at higher trophic levels was less affected by the changes in temperature and nutrients,
289 while the stability of those populations were more sensitive to such changes (Fig. 3).
290
291 *Temperature and nutrients influence top-down and bottom-up effects*
292 Results from CCM analyses indicated that the relative strength of bottom-up and top-down
293 effects changed across treatments (Fig. 4a). Top-down and bottom-up effects between the two
294 protists were slightly stronger in the warmer temperature (Fig. 4a, yellow lines). Importantly,
295 temperature and nutrients showed interactive effects on both top-down and bottom-up effects
296 (Fig. 4a). For example, while the top-down effects of protists on bacteria (Fig.4a, green and
297 purple dashed lines) were generally stronger than the bottom-up effects of the bacteria on the
298 protists (Fig. 4a green and purple solid lines), temperature and nutrients interactively –but
299 differentially– shifted the magnitude of the top-down effects of the two protist predators on
300 bacteria (Fig.4a, open dots and dashed lines). Indeed, the top-down effect of *T. pyriformis* on
301 bacteria decreased in the high-nutrient treatment, but higher temperature strengthened this effect
302 (Fig. 4a purple dashed line). Meanwhile, high nutrients increased the top-down effect of *Euplotes*
303 *sp.* on bacteria in the lower temperature but decreased it at higher temperature (Fig. 4a green
304 dashed line).
305

306 The strength of species interactions in the food web, overall, also changed according to shifts in
307 the strength of top-down and bottom-up control between species pairs (Figure 4b). To visualize
308 these changes, we present CCM cross-mapping skill value at the largest library size as links
309 between species pairs (Fig. 4b). At low nutrient levels, higher temperature led to a larger number
310 of stronger species interactions (Fig. 4b, bottom left and right) while at high nutrient levels,
311 interaction strengths were generally stronger and temperature only had a small effect (Fig. 4b,
312 top left and right). Low nutrients and high temperatures led to the strongest interactions among
313 all three species (Fig. 4b, bottom right).
314
315 *Temperature and nutrients altered the reciprocal effects of ecological and phenotypic dynamics*
316 Protist size not only responded to temperature and nutrients, but also played an important role in
317 determining the effects of temperature and nutrients on ecological dynamics (Fig. 5a-c). The
318 bidirectional effects between the body-size dynamics of *Euplotes sp.* and the ecological
319 dynamics of *T. pyriformis* were the strongest overall across treatments (Fig. 5a-c). The body size
320 dynamics of the omnivorous predator, *Euplotes sp.*, but not those of *T. pyriformis,* had relatively
321 strong causal effects on the ecological dynamics of all species, including its own ecological
322 dynamics (Fig. 5b). Specifically, at low temperature, increasing nutrient level strengthened the
323 effect of *Euplotes sp.* phenotypic dynamics on its own ecological dynamics and those of *T.*
324 *pyriformis*, but weakened these effects on the ecological dynamics of bacteria (Fig. 5b). Yet, at
325 the warmer temperature, increasing nutrient levels weakened the effects of *Euplotes sp.*
326 phenotypic dynamics on the ecological dynamics of all species, including itself (Fig. 5b),
327 indicating that plastic changes in top predator body size can mediate how food web dynamics
328 respond to temperature and nutrients.

329

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

335

336 Overall, we also found a larger number of stronger effects between ecological and phenotypic
337 dynamics at higher temperatures, especially in the low nutrient treatment (Fig. 5d, bottom right),
338 consistent with results from the top-down vs bottom-up effects (Fig 4b). Additionally, changes in
339 body size, especially those of the omnivorous predator, seem to mediate the effect of temperature
340 and nutrients on ecological dynamics (Fig. 5d).

341

342 DISCUSSION

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

352 change may mediate the temperature response of ecological dynamics, perhaps strengthening
353 species interactions (Fig 4, 5). These results therefore suggest that body size not only responds to
354 shifts in environmental conditions, but also plays a role in determining ecological responses to
355 such shifts (Fig. 5). Evaluating feedbacks between ecological and phenotypic dynamics may
356 therefore be integral to understanding food web responses to environmental change.
357
358 *Increasingly complex effects of temperature and nutrients at higher trophic levels*
359 Our results reveal the pervasive effects of temperature and nutrients within a microbial food web,
360 but also show how these effects are more numerous and increasingly complex (i.e., larger effect
361 sizes of temperature-nutrient interactions) at higher trophic levels (Fig. 2, 3). Because energy
362 enters at the bottom of a food web, basal species may be more strongly influenced by direct
363 effects of nutrients, while species at higher trophic levels may be more strongly affected by a
364 combination of temperature and nutrient treatments (Petchey *et al.* 1999; Voigt *et al.* 2003).
365 Additionally, 1/5/23 5:11:00 PMthe effects of temperature and nutrients on dietary preferences,
366 species interactions, and foraging behavior, may further explain why these interactive effects are
367 stronger and more numerous among consumer species (Fig. 1d; Fig. 2c). Indeed, as temperature
368 and nutrients change species interactions and foraging behaviors (Sentis *et al.* 2014), omnivorous
369 consumers may shift diets between basal and intermediate resources (Sentis *et al.* 2014), leading
370 to climate-driven food web rewiring (Bartley *et al.* 2019; Barbour & Gibert 2021). This dietary
371 shift might also affect how much energy top consumers receive from basal species vs
372 intermediate consumers, and therefore, how temperature and nutrients indirectly influence top
373 predator dynamics. In such case, causal effects of both environmental stressors are likely
374 transitive (e.g. as temperature affects bacteria, and because *Euplotes sp.* preys on bacteria, the

375 temperature effects on bacteria and *Euplotes sp.* are indirectly but casually linked, Sugihara *et al.*
376 2012).
377
378 This transitivity of causality (where omnivorous consumers are indirectly affected by
379 temperature and nutrients that first acted on the basal and intermediate species) could also
380 explain why the population variability (CV) of the top (omnivorous) predator is more often
381 jointly influenced by temperature and nutrients than at lower trophic levels. Moreover, maximum
382 density is more likely to reflect changes in nutrient availability, and these effects should wane
383 across trophic levels, as energy is lost to energy conversion between consumers and prey across
384 trophic levels. And this could explain the decrease of temperature and nutrient effects on species
385 maximum density at higher trophic levels. Whether the joint effects of temperature and nutrients
386 change in more natural food webs as reported here, however, is not known, but is a promising
387 avenue for future research.
388
389 *Top-down control, bottom-up control, and food web stability*
390 Consistent with our results (Fig. 2d), theory predicts that higher nutrient loads should increase
391 energy flux in food webs, through increases in basal species density (Rip & McCann 2011;
392 Shurin *et al.* 2012). Theory also predicts that increasing energy flux can be destabilizing, leading
393 to increasing oscillations in density (Rosenzweig 1971; Rip & McCann 2011), but increasing
394 temperatures should stabilize oscillations by weakening top-down effects (Vasseur & McCann
395 2005; Binzer *et al.* 2016; Tabi *et al.* 2019). We found that the interactive effects of temperature
396 and nutrients have divergent impacts on stability—with temperature stabilizing, and nutrients
397 destabilizing *Euplotes sp.* densities, but the opposite being true for *T. pyriformis* (Fig. 2h-i).

398 Temperature and nutrients had different but interactive effects on top-down controls by the two
399 predators even though bottom-up effects remained unaffected by the treatments (Fig. 4a). These
400 results thus indicate that changes in the strength of top-down effects between basal resources and
401 consumers—instead of bottom-up effects—could be the dominant mechanism through which
402 higher temperatures may stabilize instabilities caused by nutrients (Binzer *et al.* 2012, 2016).
403
404 We also observed that top-down control on bacteria by both consumers was much stronger than
405 bottom-up effects across treatments (Fig. 4a) while the top-down and bottom-up controls are
406 both strong and tightly coupled between the two protists predators. The dominant top-down
407 effects on the bacterial community could be explained by the short generation time of all species
408 in the system and the fast turnover rate of the bacteria relative to the protists. In addition to the
409 potential stabilizing effects of top-down control, these results also indicate that top-down control
410 on basal resources might have stronger effects in systems with high turnover rates, such as
411 aquatic systems.
412
413 *Phenotypes mediate temperature and nutrient effects on food web dynamics*
414 Our results indicate that phenotypic dynamics play a larger role in mediating environmental
415 impacts on food web dynamics than previously thought (Fig. 4, 5). Previous studies showed that
416 predator-prey body size ratios significantly influence temperature and nutrients effects (Binzer *et*
417 *al.* 2012; Gibert & DeLong 2014) and that body size responds to changes in nutrients,
418 temperature, and ecological dynamics in specific ways (Tabi *et al.* 2019). A recent study showed
419 that the body size of a species can affect its own ecological dynamics (Gibert *et al.* 2022b),
420 which we have also shown here as tight coupling between the phenotypic and ecological

421 dynamics of *Euplotes sp.* (Fig. 5). Our results support—but also extend—previous findings by
422 showing that 1) phenotypic effects on ecological dynamics can be strong, especially among top
423 predators, 2) changes in the prey ecological dynamics may strongly drive changes in predator
424 size, and 3) interactions involving phenotypic dynamics (eco-pheno, pheno-eco, or pheno-pheno)
425 vary across environmental conditions (Fig. 4b-d, Fig. 5).
426
427 *Caveats*
428 One caveat of this study is that we only tracked the dynamics of the bacterial community as a
429 whole, as we lack information on how each individual species responded to temperature or
430 nutrients. Recent work has shown that the composition of bacterial communities changes under
431 joint temperature and nutrient loads, and that predation by protists mediates these responses
432 (Thurman *et al.* 2010; Rocca *et al.* 2022). Coupling an experiment like ours with 16S amplicon
433 sequencing to keep track of bacterial dynamics would thus be an exciting avenue for future
434 research that should deepen the understanding generated by our current study as to how
435 microbial food webs will respond to rapid environmental change.
436
437 Another caveat is that, despite uniquely detailed, long, and well-replicated time-series, our time-
438 series have a few small gaps due to lack of sampling on weekends. To address this issue, we
439 interpolated these missing datapoints using three different methods, but, despite being generally
440 robust, some variation remained between these results and the CCM inference of species
441 interactions (Appendix II Figure 1-12). All three methods showed that bottom-up and top-down
442 effects between the protist predators were the strongest and remained strong across all
443 treatments. Moreover, top-down controls from both protists predators to bacteria were always

444 stronger than bottom-up effects (Appendix II Figure 13-15 Eco-Eco panels). Last, the effects of
445 *Euplotes sp.* body size on its own ecological dynamics and that of the *T. pyriformis* and bacteria
446 were consistent across interpolation methods (Appendix II Figure 13-15 Eco-Pheno, Pheno-Eco
447 and Pheno-Pheno panels). As CCM and related methods grow in use (Wang *et al.* 2016b;
448 Matsuzaki *et al.* 2018; Barraquand *et al.* 2021; Doi *et al.* 2021; Kitayama *et al.* 2021), there is a
449 real need for these tools to be robust even in the face of imperfect data, including missing
450 timepoints. Our results therefore underline the needs to better understand how missing data may
451 affect CCM and other time-series analyses and how to best predict missing data for analysis.
452
453 Last, because CCM analysis quantifies the effects of one time series on another but not on itself,
454 we were unable measure density dependence within each species. Therefore, while our research
455 showed the potential of using CCM in understanding complex casual effects between species
456 ecological and phenotypic dynamics, we also notice the importance of combining time series
457 analyses with other, possibly experimental, methods to measure causality for intra-specific
458 interactions.
459
460 *Concluding remarks*
461 Rapid phenotypic change has been suggested as a main driver of food web rewiring in future
462 climates (Barbour & Gibert 2021), but little experimental evidence exists. Here, our results show
463 that strong feedbacks between ecological and phenotypic dynamics depend on environmental
464 conditions (temperature and nutrients), suggesting that rapid phenotypic change influences food
465 web responses to environmental change. Moreover, the joint effects of temperature and nutrients
466 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.

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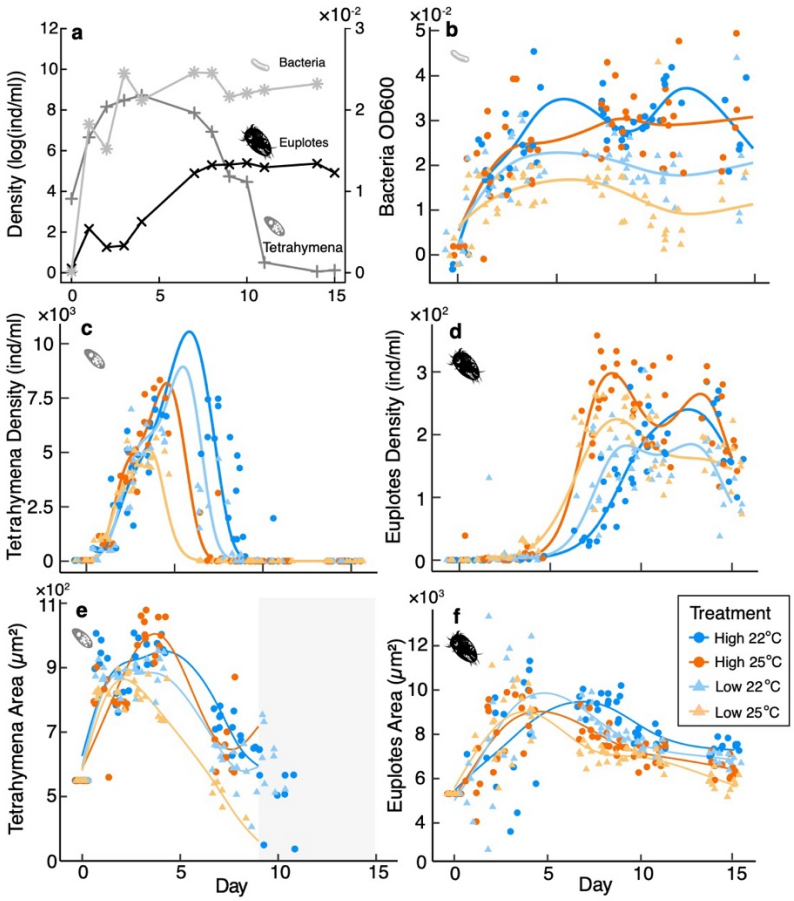
Figures



484

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

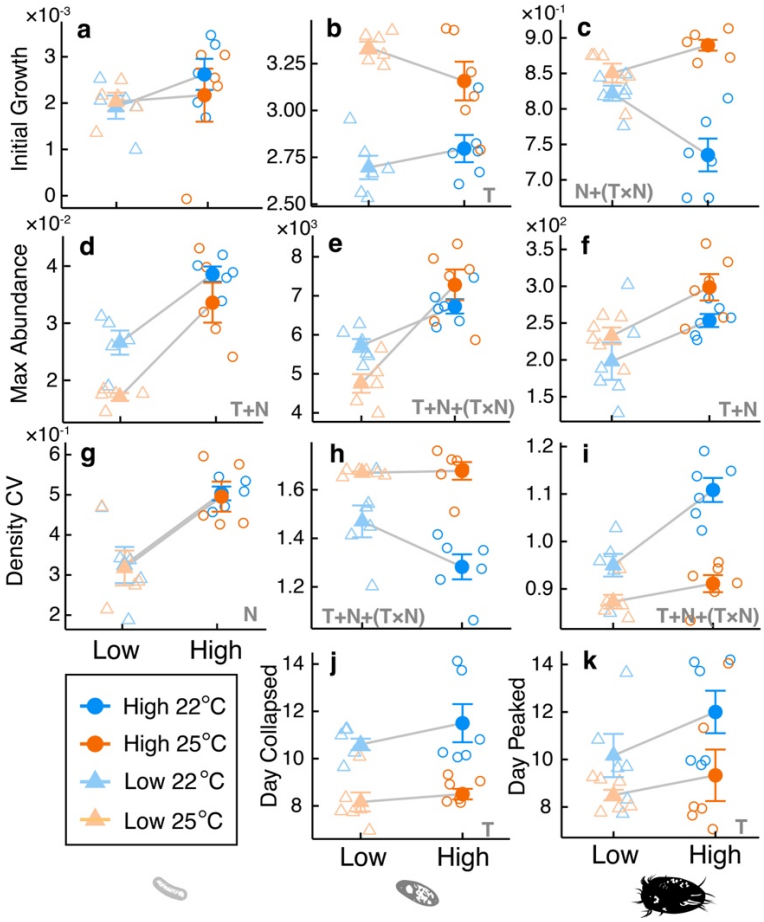


Figure 2. Additive and interactive effects of temperature and nutrients on descriptors of population dynamics. Open circles and triangles represent data points from each replicate. Solid shapes represent means across 6 replicates. Grey letters T, N, and (T×N) represent statistically significant effects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

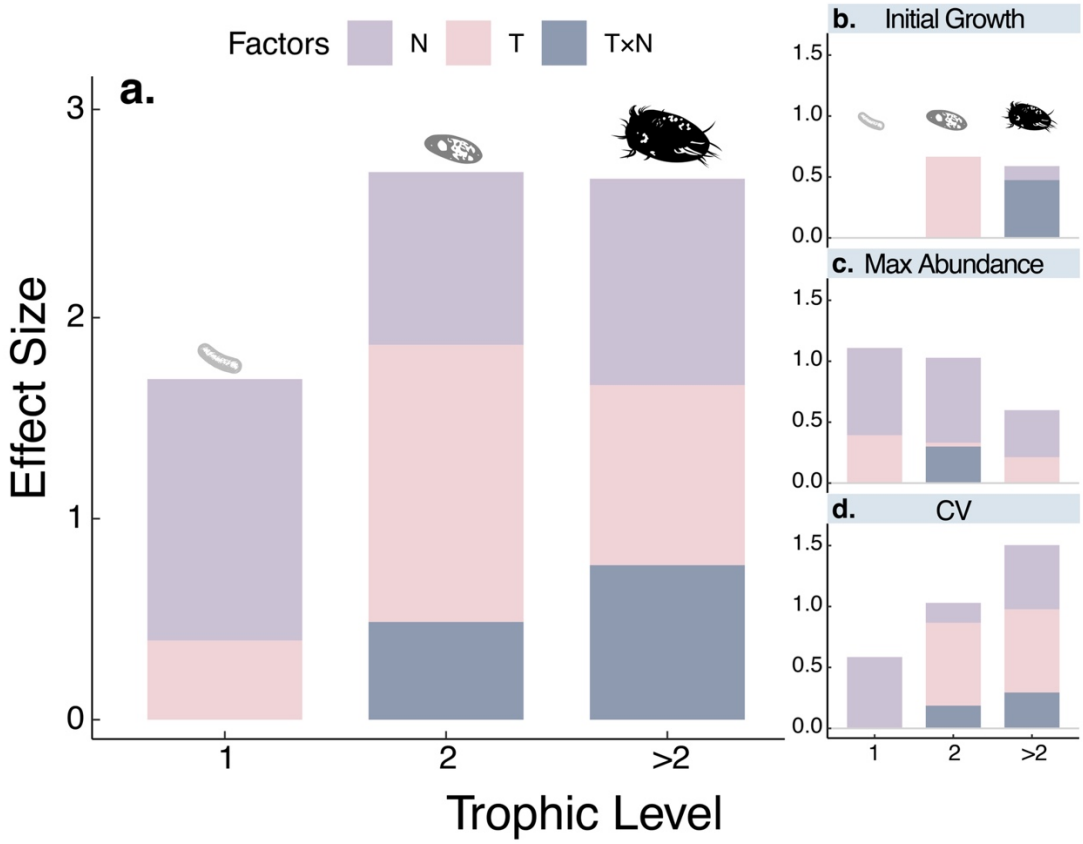


Fig. 3. The effect sizes of significant temperature and nutrient effects on ecological dynamics (i.e., initial growth, maximum density, and density CV). T, N, and T×N denote the effects of temperature, nutrients, and interactive effects of temperature and nutrients, respectively.

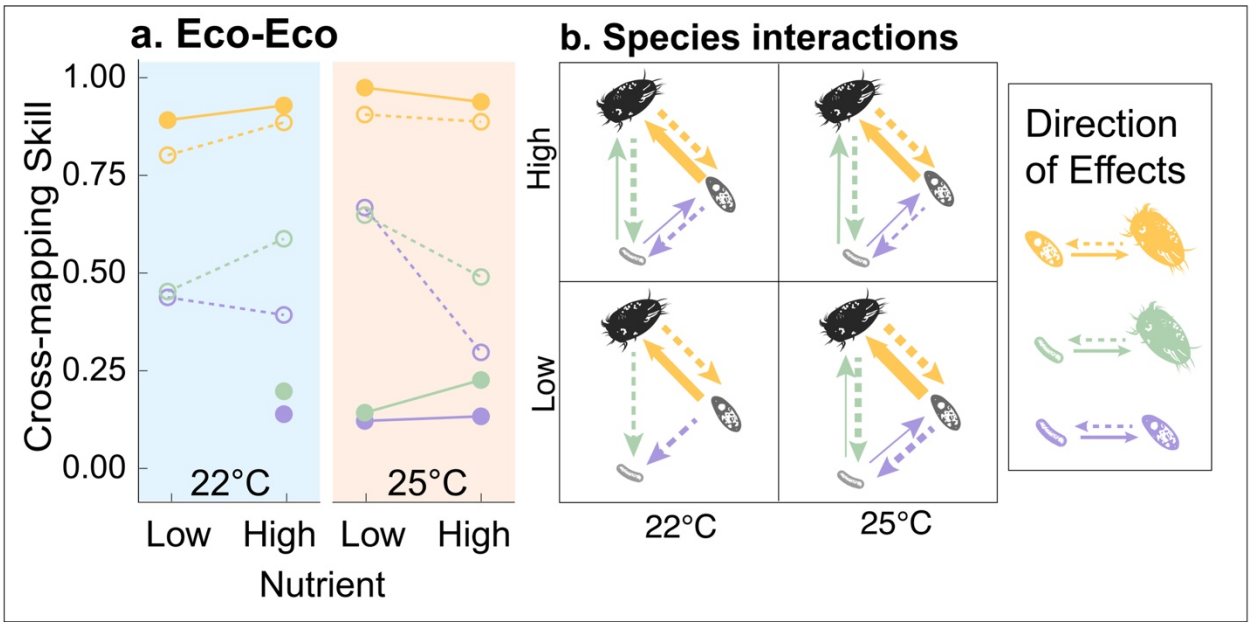


Figure 4. Cross-mapping skill (ρ) of species ecological dynamics across treatments for each species pair. (a) Shows CCM estimation skill of one species' effect on another. Solid circles and solid lines represent bottom-up effects and open circles and dashed lines represent top-down effects. (b) Shows 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.

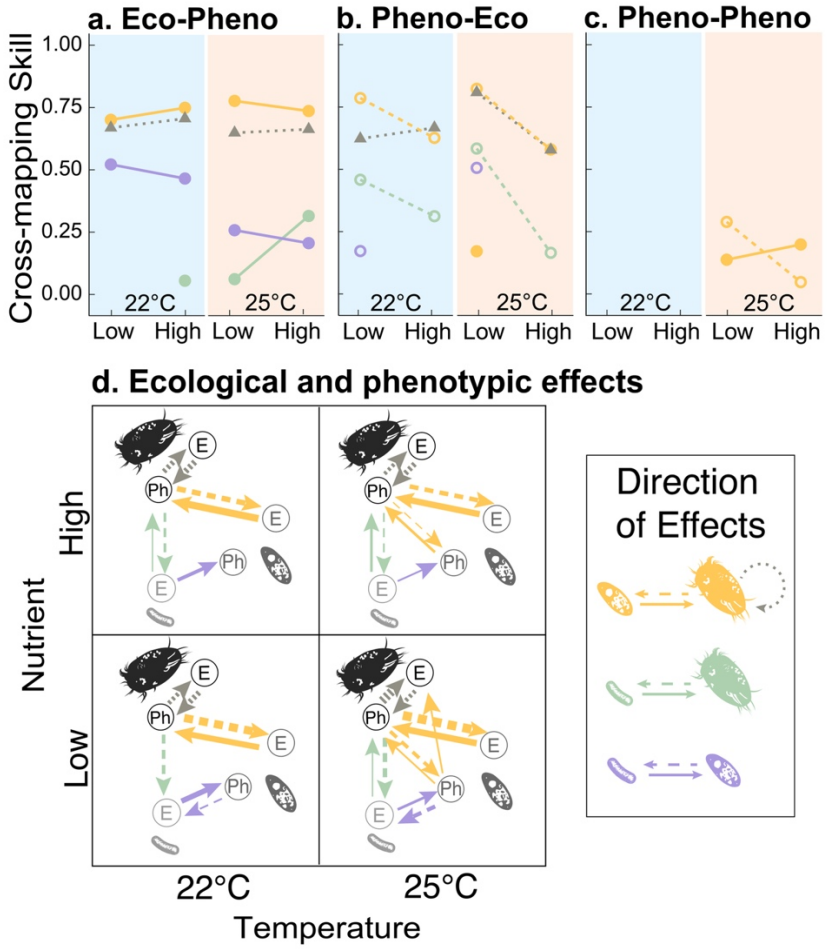


Figure 5. Interactions between phenotypic (Ph) and ecological (E) dynamics. (a-c) Show CCM estimation skill of density on traits (eco-pheno), traits on density (pheno-eco), and trait on trait (pheno-pheno). Circles represent CCM estimation skill of one species' effect on another species, while triangles represent intraspecific effects. Dashed and solid lines represent top-down and bottom-up effects respectively. Grey dotted lines represent intraspecific effects of *Euplotes 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. Color code as in Fig. 4a.

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